Supporting Information

Cyclodextrin-based Pseudorotaxanes: Easily Conjugatable Scaffolds for Synthesizing Hyperpolarized Xenon-129 Magnetic Resonance Imaging Agents

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Experimental Section

1. Reagents

Substrates, including 1-ethylimidazole, 1,8-dibromooctane, 1,10-dibromodecane, 1,5-dibromopentane, cucurbit[6]uril, cucurbit[7]uril, cucurbit[8]uril, α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin 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 N₂.

2. Instrumentation

NMR spectra were obtained using a 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. Isothermal calorimetry was performed using TA Instruments TAM III™ instrument.
3. Synthesis of guest molecules

3.1. Synthesis of $\text{C}_{10}$ diethylimidazolium guest, 1

1-Ethylimidazole (1.942 mL, 20 mmol) and 1,10-dibromodecane (1.792 mL, 8 mmol) were dissolved in toluene (15 mL) and refluxed for 12 hours. Once complete, the toluene was decanted, and the crude product was washed with diethyl ether (3 x 20 mL) and dried under vacuum until a white solid product formed (2.20 g, 56% yield). Spectral data matched the reported values.\(^1\)

\(^1\)H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.34 (s, $J = 1.7$ Hz, 2H), 7.85 (dd, $J = 5.8$, 1.9 Hz, 4H), 4.20 (dq, $J = 14.3$, 7.3 Hz, 8H), 1.79 (p, $J = 7.3$ Hz, 4H), 1.43 (t, $J = 7.3$ Hz, 6H), 1.25 (s, 12H).

\(^{13}\)C NMR (101 MHz, DMSO-$d_6$) $\delta$ 136.1, 122.8, 122.6, 49.2, 44.6, 29.8, 29.2, 28.8, 25.9, 15.5.

HRMS ESI (m/z): [M+] calculated for $\text{C}_{20}\text{H}_{36}\text{N}_4^{2+}$ 166.1467, observed 166.1455.
Figure S1. $^1$H NMR of Guest 1
Figure S2. $^{13}$C NMR of Guest 1
3.2. Synthesis of C₈ diethylimidazolium guest, 2

1-Ethylimidazole (1.06 mL, 11 mmol) and 1,8-dibromooctane (808.7 µL, 4.4 mmol) were dissolved in toluene (15 mL) and refluxed for 12 hrs. Once complete, the toluene was decanted, and the crude product was washed with diethyl ether (3 x 20 mL) and dried under vacuum until a white solid product formed (0.51 g, 38% yield). Spectral data matched the reported values.¹

¹H NMR (400 MHz, DMSO-d₆) δ 9.40 (s, 2H), 7.86 (d, J = 1.7 Hz, 4H), 4.11 (m, 8H), 1.79 (p, J = 7.2 Hz, 4H), 1.42 (t, J = 7.3 Hz, 6H), 1.12 (m, 8H).

¹³C NMR (101 MHz, DMSO-d₆) δ 136.2, 122.8, 122.6, 49.2, 49.6, 29.7, 28.6, 25.8, 15.6.

HRMS ESI (m/z): [M+] calculated for C₁₈H₃₂N₄²⁺ 152.1308, observed 152.1298.
Figure S3. $^1$H NMR of Guest 2
Figure S4. $^{13}$C NMR of Guest 2
4. Synthesis of pseudorotaxanes using cucurbit[n]urils and bars

10 mM stock solutions of cucurbit[n]uril (n= 6, 7, 8) and the guests were prepared using D$_2$O as the solvent. Using the above stock solutions, 0.25 mL of the host and 0.25 mL of the guest were mixed inside a GC vial. The threading of the guest into the host was achieved by heating the solution inside the GC vial to 85 °C for 3 min. This solution was then cooled to room temperature and transferred to an NMR tube. While the initial CB solutions were cloudy, the final pseudorotaxane solutions were clear and homogeneous. $^1$H NMR spectra were then acquired at 300 K. $^1$H NMR was performed to see the movement of the alkyl protons in the guest and the movement of the guests after the inclusion complexes were formed.
Figure S5. $^1$H NMR of 2$\subset$CB6
Figure S6. $^1$H NMR (magnified) of $2\text{C}B6$ in the region of the guest protons
Figure S7. $^1$H NMR of 1<sub>CB6</sub>
Figure S8. $^1$H NMR (magnified) of 1@CB6 in the region of guest protons
Figure S9. $^1$H NMR of 2cCB7
Figure S10. $^1$H NMR (magnified) of 2–CB7 in the region of guest protons
Figure S11. $^1$H NMR of 1-CB7
Figure S12. $^1$H NMR (magnified) of 1⊂CB7 in the region of guest protons
Figure S13. $^1$H NMR of 2–CB8
Figure S14. $^1$H NMR (magnified) of 2¢CB8 in the region of guest protons
Figure S15. $^1$H NMR of 1$\subset$CB8
**Figure S16.** $^1$H NMR (magnified) of 1⊂CB8 in the region of guest protons
5. Synthesis of Pseudorotaxanes using α, β, and γ-CDs

10 mM stock solutions of cyclodextrins (α, β, γ) and the guests were prepared using D2O as the solvent. Using the above stock solutions, 0.25 mL of the host and 0.25 mL of the guest were mixed inside a GC vial. The threading of the guest into the host was achieved by heating the solution inside the GC vial to 40 °C for 3 min. This solution was then cooled to room temperature and transferred to a NMR tube. The final solutions containing the pseudorotaxanes were clear and homogeneous. All $^1$H NMR spectra were acquired at 300 K. $^1$H NMR was performed to see the movement of the alkyl protons in the guest and the movement of the cyclodextrins’ H-3 proton and H-5 protons, which are present inside the cavity of the CDs.
Figure S17. $^1$H NMR of $2\alpha$-CD
Figure S18. $^1$H NMR (magnified) of 2α-CD in the region of guest protons.
Figure S19. $^1$H NMR of 1α-CD
Figure S20. $^1$H NMR of 2$\beta$-CD
Figure S21. $^1$H NMR of 1$\subset$β-CD
Figure S22. $^1$H NMR (magnified) of $1\beta$-CD in the region of guest protons
Figure S23. $^1$H NMR of 2γ-CD
Figure S24. $^1$H NMR (magnified) of 2⊂γ-CD in the region of guest protons
Figure S25. $^1$H NMR (magnified) of 2γ-CD in the region of H-3 proton of γ-CD
Figure S26. $^1$H NMR of 1γ-CD
Figure S27. $^1$H NMR (magnified) of 1γ-CD in the region of guest protons
Figure S28. $^1$H NMR (magnified) of 1⊂γ-CD in the region of H-3 proton of γ-CD
6. Determination of association constants for host cyclodextrins and guests

The NMR titration experiments were conducted per the following procedures. The host CD concentrations were kept constant while the guest concentration was increased periodically. Stock solutions of 2 mM host (cyclodextrin) and 200 mM guest were prepared. A series of NMR samples were prepared in 5 mm NMR tubes. The series ranged from 0.111 mM to 200 mM. All samples were then heated for 3 min at 40 °C to facilitate the threading of the guest in to the host. All samples were cooled to room temperature before the $^1$H NMR experiments. All $^1$H NMR were performed at 300 K. The H-3 proton of the host CD was monitored. Data was processed using an Excel spreadsheet using the equation shown below. The plots are shown below. The data were also evaluated using the “OpenDataFit” website, which is an online free fitting program found at supramolecular.org. The two different analysis methods provided similar results.

The following equation was used to calculate the association constant:

$$
\Delta \delta = \left( \frac{\Delta \delta_{\text{max}}}{2[H_0]} \right) + \left\{ [H_0] + [G_0] + \frac{1}{K} - \left( \left( [H_0] + [G_0] + \frac{1}{K} \right)^2 - 4[H_0][G_0] \right) \right\}^{1/2}
$$

where $\Delta \delta =$ change in chemical shift after the addition of the guest, $[H_0] =$ initial host concentration, $[G_0] =$ initial concentration, and $K =$ association constant. $\Delta \delta$ was plotted as a function of the guest concentration.
Figure S29. $^1$H-NMR stacked plot of γ-cyclodextrin and varying guest (1)
Figure S30. 1:1 Binding curve for the association of 1 with γ-CD.

Figure S31. 1:1 Binding curve for the association of 1 with γ-CD.
Figure S32. 1:1 Binding curve for the association of 2 with γ-CD.

Figure S33. 1:1 Binding curve for the association of 2 with γ-CD.
7. Mass spectroscopic studies

All compounds were prepared in the deionized water at a concentration of 200 µM for both guest and host molecules separately and as a mixture. Then they were diluted to 20 µM (each) in a solution of methanol/water (50/50) to produce the standards for mass spectrometric analysis. The final solutions were infused into a Thermo Scientific LTQ Orbitrap XL™ (Waltham, MA, USA) mass spectrometer at a rate of 10 µL/min using electrospray ionization source in a positive mode. The rest of the ionization and ion optics parameters were as follows: sheath gas 15, auxiliary gas 3, spray voltage 5 kV, capillary temperature 275 °C, capillary voltage 47 V, tube lens 225 V, multipole 00 offset -5.5 V, lens 0 -6.0 V, multipole 0 offset -5.75 V, lens 1 -10.0 V, gate lens -46.0 V, multiple 1 offset -19.5 V, multipole RF amplitude 400.0 V, front lens -6.75 V. The mass spectra were collected using full scan mode with resolution of 30000 in the range between 100 and 2000 amu. The spectra were averaged over at least 50 micro scans with 10.0 ms maximum injection time and 2.0x10^5 ions for AGC target settings.
Figure S34. Mass spectrum of α-CD

ESI+ ACD_25july2017 #1-11 | RT: 0.00-0.16 | AV: 11 | NL: 7.59E6
T: FTMS + p ESI Full ms [315.00-2000.00]
Figure S35. Mass spectrum of β-CD
Figure S36. Mass spectrum of $\gamma$-CD.

**ESI+** GCD 25july2017 #1-23  RT: 0.01-0.32  AV: 21  NL: 3.19E6  T: FTMS + p ESI Full ms [100.00-2000.00]

Chemical Formula: C$_{38}$H$_{66}$N$_6$O$_{10}$$^+$
Exact Mass: 1310.4118
Figure S37. 1\textsuperscript{17}C-CD (ESI+, 5 nL/min, FS, 20 uM/20 uM) – m/z 1707.6307

Isotope Simulation

**Chemical Formula:** C\textsubscript{68}H\textsubscript{116}Br\textsubscript{1}N\textsubscript{4}O\textsubscript{40} 

**Charge:** 1

**Data:** FTMS + p ESI Full ms [100.00-2000.00] 

**NL:** 3.65E4

**ESI+**

_GCD-10C_1-

1_26July2017_2#4-92

RT: 0.05-1.45  AV: 89 T: 

FTMS + p ESI Full ms [100.00-2000.00]

**NL:** 2.15E5

C\textsubscript{68}H\textsubscript{116}Br\textsubscript{1}N\textsubscript{4}O\textsubscript{40} Chrg 1
Figure S38. 3-\gamma-CD (ESI+, 5 uL/min, FS, 20 uM/20 uM) – m/z 1679.5996

Isotope Simulation

NL:
4.95E5
ESI+
_GCD-8C_1-
1_27july2017_1#1-75
RT: 0.01-1.18 AV: 75 T:
FTMS + p ESI Full ms
[150.00-2000.00]

NL:
2.20E5
c_{66} h_{112} br_{1} n_{4} o_{40}:
C_{66} H_{112} Br_{1} N_{4} O_{40}
pa Chrg 1
Figure S39. Lce-CD (ESI+, 5 uL/min, FS, 20 uM/20 uM) – m/z 1707.6307
Figure S40. 3α-Cd-CD (ESI+, 5 uL/min, FS, 20 μM/20 μM) – m/z 638.2887

Chemical Formula: C₅₄H₉₂N₄O₃₀

Isotope Simulation

NL: 1.07E6
ESI+
_ACD-8C_1-
L_28july2017_1#2-105
RT: 0.66-1.66  AV: 64 T:
FTMS + p ESI Full ms
[100.00-2000.00]

NL: 5.07E5
C₅₄H₉₂N₄O₃₀:
pa Chrg 2
Figure S41. 1β-CD (ESI+, 5 uL/min, FS, 20 uM/20 uM) – m/z 733.3298
8. Determination of association constants for guest probes and γ-CD using Isothermal Titration Calorimetry (ITC)

A 1000 mM solution of 1 and 100 mM solutions of γ-CD were made in water and plasma. Once the solutions were made, the guest solution was loaded into the micro-syringe and the γ-CD solution was placed in the sample ampoule (2 ml). Lower concentrations of the host and guest did not provide sufficient heat flow for reliable ITC analysis. For the titration, a 1 µl/S rate and 21 injections were chosen. Except for the first injection, which was 3 µl, all the other injections were 10 µl injections. Each titration was performed for 20 minutes with a 5-minute baseline. For data analysis, blank titrations were performed with addition of guest 1 to DI water and plasma without the γ-CD. The data from the blank titration were used to subtract the heat of dilutions. Once the Raw heat data was obtained, the data was processed using the online software, AFFINImeter (https://www.affinimeter.com/).
Figure S42. ITC of 1 with γ-CD in water
Figure S43. ITC of 1 with γ-CD in bovine plasma
**Figure S44.** Analysis of ITC binding data for 1 with γ-CD in water

Association Constant of 1−γ-CD = 1.00 ± 0.0327 x 10^4 M⁻¹
Figure S45. Analysis of ITC binding data for 1 with γ-CD in bovine plasma

Association Constant of 1<γ-CD (plasma) = 1.0114 ± 0.0592 x 10^2 M⁻¹
9. Computational studies

A series of computational studies were performed to measure the cavity size of the pseudorotaxanes using the Spartan 16 computational chemistry package. A first minimization was performed using molecular mechanics with the MMFF force field in the gas phase. Once the minima were obtained, the same force field was used to run the simulation in water as the solvation source. After that, the semi-empirical PM3 force field was used to minimize the structure in the gas phase. This was followed using an energy minimization (PM3) using external water molecules (One water molecule of water was used per oxygen atom in the host.) to mimic the hydrophobic and hydrophilic interactions of the guest to the hydrophobic cavity of the host.
Table S1. Calculated binding energies for pseudorotaxanes

| Host  | Guest | ΔE (kcal/mol) | ΔE (kcal/mol) |
|-------|-------|---------------|---------------|
|       |       | Guest-1       | Guest-2       |
|       |       | ΔE (kcal/mol) | ΔE (kcal/mol) |
|       |       | α-CD          | -132.23       | -108.96       |
|       |       | β-CD          | -448.94       | -433.7        |
|       |       | γ-CD          | -778.93       | -722.71       |
|       |       | CB[6]         | -198.98       | -174.29       |
|       |       | CB[7]         | -234.64       | -210.69       |
|       |       | CB[8]         | -370.23       | -338.92       |

Molecular modeling calculations were performed using Spartan 16 for all complexes as guests encapsulated in the hosts to estimate the stabilization energy obtained by complexation (which provides an indication of the relative binding constants). Above ΔE values are given in kcal/mol. The following equation was used to obtain the final values:

$$\Delta E = E_{(\text{complex})} - E_{(\text{host})} - E_{(\text{Guest})}$$

External water molecules (one water molecule per oxygen in the host molecule) were used to mimic the solvent environment for both the hosts and the host-guest complexes.
Table S2. Computed distances between guest and host for 2-CB6

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C32, C47    | 8.725            |
| C31, C47    | 7.318            |
| **C20, C47**| **7.369**        |
| C34, C47    | 6.039            |
| C18, C47    | 6.557            |
| **C17, C47**| **5.805**        |
| C19, C47    | 6.511            |
| C15, C47    | 5.832            |

Bold distances represent the atoms that are inside the host cavity.
**Table S3.** Computed distances between guest and host for 1⊂CB6

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C32, C47    | 7.802            |
| C31, C47    | 6.976            |
| **C20, C47**| **5.740**        |
| C34, C47    | 5.322            |
| C18, C47    | 4.389            |
| C17, C47    | 4.884            |
| C16, C47    | 4.766            |
| C15, C47    | 6.154            |
| C57, C47    | 6.473            |
| C13, C47    | 8.001            |

**bold distances represent the atoms that are inside the host cavity**
Table S5. Computed distances between guest and host for 2⊂CB7

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C14, C58    | 7.802            |
| **C13, C58** | **6.976**        |
| C11, C58    | 5.740            |
| C9, C58     | 5.322            |
| C4, C58     | 4.389            |
| **C5, C58** | **4.884**        |
| C3, C58     | 4.766            |
| C1, C58     | 6.154            |

*bold distances represent the atoms that are inside the host cavity*
**Table S6.** Computed distances between guest and host for $1 \subset$ CB7

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C1, C47     | 7.726            |
| **C3, C47** | **6.481**        |
| C5, C47     | 6.161            |
| C7, C47     | 5.075            |
| C9, C47     | 5.333            |
| **C11, C47**| **5.256**        |
| C13, C47    | 6.071            |
| C14, C47    | 6.712            |
| C25, C47    | 8.012            |
| C26, C47    | 8.876            |

*Bold distances represent the atoms that are inside the host cavity*
Table S7. Computed distances between guest and host for 2 CB8

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C25, C71    | 7.809            |
| C24, C71    | 6.389            |
| C19, C71    | **6.084**        |
| C14, C71    | 5.255            |
| C4, C71     | 5.808            |
| C3, C71     | 5.340            |
| C2, C71     | 6.566            |
| C1, C71     | 6.593            |

**Bold** distances represent the atoms that are inside the host cavity.
**Table S8.** Computed distances between guest and host for 1⊂CB8

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C40, C20    | 8.521            |
| C39, C20    | 7.202            |
| C25, C20    | 7.066            |
| **C24, C20**| **5.895**        |
| C19, C20    | 6.276            |
| **C14, C20**| **5.438**        |
| C4, C20     | 6.455            |
| **C3, C20** | **6.089**        |
| C2, C20     | 7.453            |
| C1, C20     | 7.586            |

*bold distances represent the atoms that are inside the host cavity*
**Table S9.** Computed distances between guest and host for $2\alpha$-CD

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C6, C10     | 6.903            |
| **C5, C10** | **5.739**        |
| C4, C10     | 6.250            |
| C3, C10     | 5.493            |
| C2, C10     | 6.404            |
| C7, C10     | 6.458            |
| **C18, C10**| **7.366**        |
| C19, C10    | 7.791            |

**bold** distances represent the atoms that are inside the host cavity.
**Table S10.** Computed distances between guest and host for 1α-CD

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C32, C13    | 8.006            |
| C21, C13    | 7.552            |
| C2, C13     | 6.093            |
| C3, C13     | 5.953            |
| C4, C13     | 4.690            |
| C5, C13     | 5.155            |
| C6, C13     | 4.398            |
| C7, C13     | 5.408            |
| C18, C13    | 5.257            |
| C19, C13    | 6.569            |

Bold distances represent the atoms that are inside the host cavity.
Table S11. Computed distances between guest and host for 2β-CD

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C13, C30    | 6.811            |
| C12, C30    | 6.102            |
| **C11, C30**| **5.812**        |
| C5, C30     | 5.383            |
| C4, C30     | 5.423            |
| **C3, C30** | **5.094**        |
| C2, C30     | 5.709            |
| C1, C30     | 5.740            |

*bold distances represent the atoms that are inside the host cavity*
**Table S12.** Computed distances between guest and host for 1-β-CD

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C35, C59    | 7.385            |
| C34, C59    | 6.2783           |
| C13, C59    | **6.664**        |
| C12, C59    | 5.892            |
| C11, C59    | 6.763            |
| C5, C59     | 6.527            |
| C4, C59     | 7.629            |
| C3, C59     | **7.812**        |
| C2, C59     | 8.992            |
| C1, C59     | 9.554            |

*bold distances represent the atoms that are inside the host cavity*
**Table S13.** Computed distances between guest and host for 2γ-CD

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C2, C19     | 7.907            |
| **C3, C19** | **7.947**        |
| C4, C19     | 9.127            |
| C7, C19     | 9.408            |
| C9, C19     | 10.558           |
| C23, C19    | 10.887           |
| C25, C19    | 12.338           |
| C33, C19    | 12.767           |

**bold** distances represent the atoms that are inside the host cavity
Table S14. Computed distances between guest and host for 1\textsubscript{γ}-CD

| Atom labels | C-C distance (Å) |
|-------------|------------------|
| C2, C19     | 9.347            |
| C3, C19     | 8.737            |
| C4, C19     | 9.309            |
| C7, C19     | 9.223            |
| C9, C19     | 10.032           |
| C23, C19    | 10.344           |
| C25, C19    | 11.233           |
| C33, C19    | 11.926           |
| C45, C19    | 12.772           |
| C46, C19    | 13.599           |

*bold distances represent the atoms that are inside the host cavity*
10. **Xenon NMR studies**

Naturally abundant $^{129}$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 (N2) source, to facilitate the flow of HP $^{129}$Xe gas from the Tedlar bag into the glass-fritted cell containing rotaxane solution.

Following polarization of $^{129}$Xe gas, 2.5 mL of solution (50 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 129Xe 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 mM.

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 rotaxane molecules, two different pre-pulse trains were used: the first consisted of 16-30 ms 3-lobe sinc pulses, while the second consisted of 16-30 ms sinusoidal pulses, both with 0 ms pulse intervals. To acquire each depletion spectrum, 37 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 -150 ppm to 30 ppm, where 0 ppm represents dissolved-phase $^{129}$Xe, with a predetermined step before each of the subsequent excitation pulses. Offset frequency steps were 176.7 Hz, which correspond to 5 ppm at 3.0 T.

**Figure S46.** HyperCEST pulse sequence

The pulse diagrams shown above were used to prepare HyperCEST depletion spectra using different saturation pre-pulse trains including 3-lobe sinc pulses (A) and sinusoidal pulses (B). Here, $f_j$ represents the frequency of saturation pulses during FID acquisition number j. All spoiler gradients along x, y, z axis are also illustrated.
**Figure S47.** Apparatus for obtain HP Xe spectra.

**Figure S48.** Custom-made coil and sample chamber.

The image above illustrates the continuous flow of HP $^{129}\text{Xe}$ gas throughout the glass-fritted vessel. As depicted, once the flow of HP $^{129}\text{Xe}$ reaches the fine glass-fritted disc, numerous microbubbles are produced, thereby causing dissolution of HP $^{129}\text{Xe}$ 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 pseudorotaxane complex.
11. Cavity volume calculations

Commercially available MOE\textsuperscript{4} software was used to calculate the free space in the host after the guest was threaded inside the CD or CB cavity, using the Assisted Model Building with Energy Refinement (AMBER) force field for energy minimizations and molecular dynamics. A custom-made active site volume script provided from MOE was used in the process. The script computationally filled the void space in the pseudorotaxane with spheres having a volume of 2.75 Å\textsuperscript{3}. The volume of the cavity was calculated as the sum of the volumes of the spheres. This method is analogous to Rebek’s technique for calculating the volume of a cavitand.\textsuperscript{4}
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