Electronic Supplemental Information

Metastable Doubly Threaded [3]Rotaxanes With a Large Macrocycle

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Materials and Methods

Materials

All reagents were purchased from Sigma-Aldrich unless otherwise stated. All chemicals were used as received without further purification unless otherwise stated. Solvents for chromatography were purchased from Fisher-Scientific. Deuterated solvents and 3,5-dihydroxybenzyl alcohol were purchased from ACROS Organics. 4-Bromo-4'-tert-butylbiphenyl was purchased from TCI chemicals. *p*-Toluenesulfonyl chloride was purchased from Alfa Aesar. Iron(II) bistriflimide[1] and 2,6-bisbenzimidazolylpyridine ligands[2] were prepared following literature procedures. Tetrahydrofuran (THF) was dried over sodium and benzophenone. Dichloromethane was distilled over calcium hydride before use. Dimethylformamide (DMF) was dried with activated molecular sieves before use. Thin layer chromatography plates (1000 micron) were purchased from Analtech.

Instrumentation

Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS). MALDI-TOF was measured by a Bruker Ultraflextreme MALDI-TOF-TOF spectrometer in linear (or reflectance) mode using dithranol as matrix and sodium trifluoroacetate or silver trifluoroacetate as ionizer (or no ionizer).

Nuclear Magnetic Resonance Spectroscopy (NMR). Room Temperature Nuclear Magnetic Resonance Spectroscopy was performed using a Bruker Ascend Avance III 500 MHz spectrometer, a Bruker Avance II+ 500 MHz spectrometer, or a Bruker DRX 400 MHz spectrometer at the University of Chicago NMR facilities. ^{1}H NMR spectra were referenced to the residual protonated solvent signal and $^{13}C\{^{1}H\}$ NMR spectra were referenced to the deuterated solvent carbon resonance signal.

Diffusion-Ordered NMR Spectroscopy (DOSY NMR). All DOSY-NMR experiments were performed on dilute solutions in 5mm NMR tubes at a constant temperature of 25°C using the 500 MHz Bruker AVANCE II^{+} 500 MHz Tesla NMR. Diffusion measurements were obtained using the 2D Bruker pulse program *stebpgp1s*, which includes a stimulated echo, bipolar gradient pulses, and one spoil gradient. The corresponding 1D pulse sequence stebpgp1s1d was used to optimize the parameters D20 ("big delta", the major diffusion delay) and P30 ("little delta", the diffusion gradient length), in accord with manufacturer-recommended methods.[3] The 2D data were acquired with a linear array of 16 or 32 diffusion gradient strengths (GPZ6 values) from 5% to 95%.

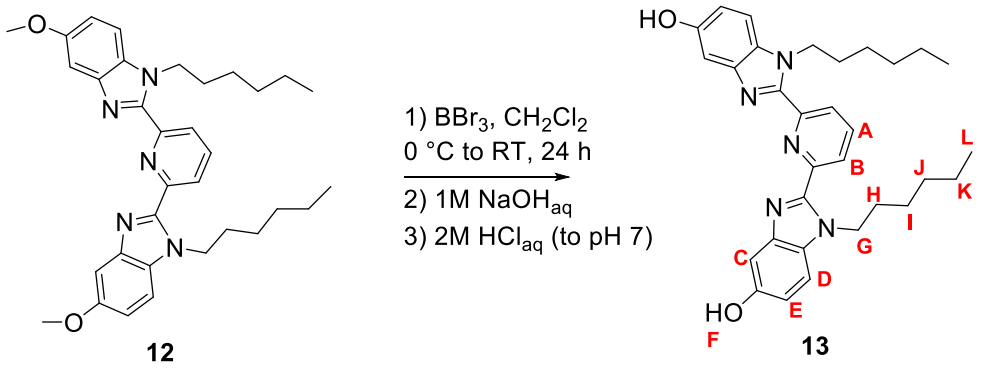
NMR Slippage Kinetic Experiments. Kinetic experiments were performed in Shigemi Tubes purchased from Wilmad-Labglass in $CDCl_3$ (1mM) using a Bruker AVANCE III HD 500 MHz spectrometer at the NMR facilities at the University of Chicago.

Gel Permeation Chromatography (GPC). GPC measurements were performed utilizing the Soft Matter Characterization Facility at the University of Chicago. Measurements were conducted at 25°C using 3:1 THF:DMF as eluent (flow rate = 1 mL/min), using a Shimadzu autosampler, Shimadzu HPLC LC20-AD pump, 2 Agilent PLgel 5 um MIXED-D + guard SEC columns, and a Wyatt Optilab T-rEX differential refractive index detector.

UV-Vis Spectrometry. UV-Vis spectrometry was measured using a Shimadzu UV-3600 Plus UV-Vis-NIR spectrophotomer and a 1 cm width quartz cuvette.

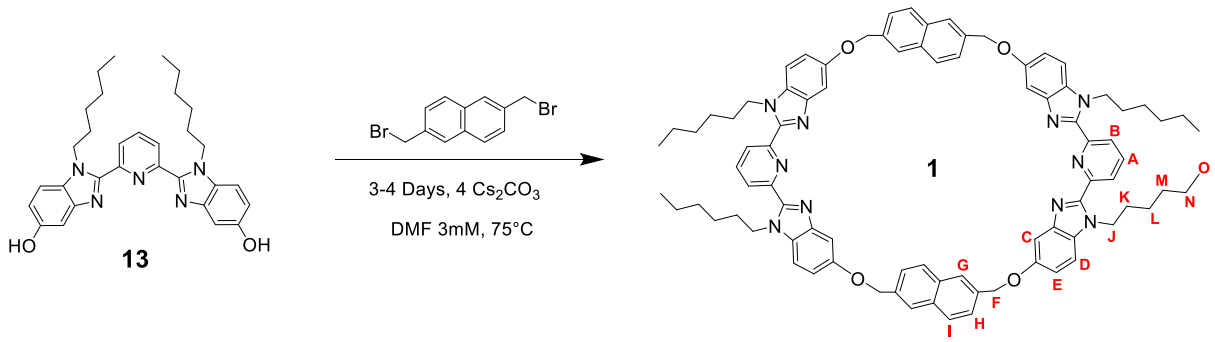
Synthesis of Macrocycle and Thread Components

Synthesis of Macrocycle Component (**1**)



Scheme S1. Synthesis of **13**.

A 500mL RBF flask was purged with Ar and charged with **12**[2] (14 g, 25.9 mmol), a stir bar and DCM (250 mL). The mixture was stirred, cooled to 0 °C and BBr_3 (15 mL, 39.6 g, 158 mmol) was added dropwise over 5 min resulting in a deep red colored mixture. The reaction was warmed to RT and stirred for a total of 24 h. The reaction mixture was poured slowly into a 4L beaker containing 1M aq. NaOH (1.5 L) and vigorously stirred until the color changed from red to yellow. The pH of the suspension was then adjusted to 7 using a 2M aq HCl solution. Upon reducing the pH of the solution, a pale yellow precipitate formed, which was subsequently collected by vacuum filtration and purified by recrystallization (chloroform/methanol) to yield **13**, a white solid, in 79% yield (10.5g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.19 (s, 2H, F), 8.22 (d, J = 8.8 Hz, 2H, B), 8.16 (t, J = 8.8 Hz, 1H, A), 7.48 (d, J = 8.7 Hz, 2H, E), 7.04 (s, 2H, C), 6.84 (dd, J = 8.7, 2.3 Hz, 2H, D), 4.68 (t, J = 7.3 Hz, 4H, G), 1.62 (t, J = 7.2 Hz, 4H, H), 1.01-0.93 (m, 12H, I+J+K), 0.55 (m, 6H, L). ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6) δ 153.57, 149.70, 149.46, 143.27, 138.41, 130.01, 124.76, 113.47, 111.13, 103.68, 45.69, 30.45, 29.46, 25.50, 21.83, 13.52. MALDI-MS: 619.7 ([M]+Ag^+).

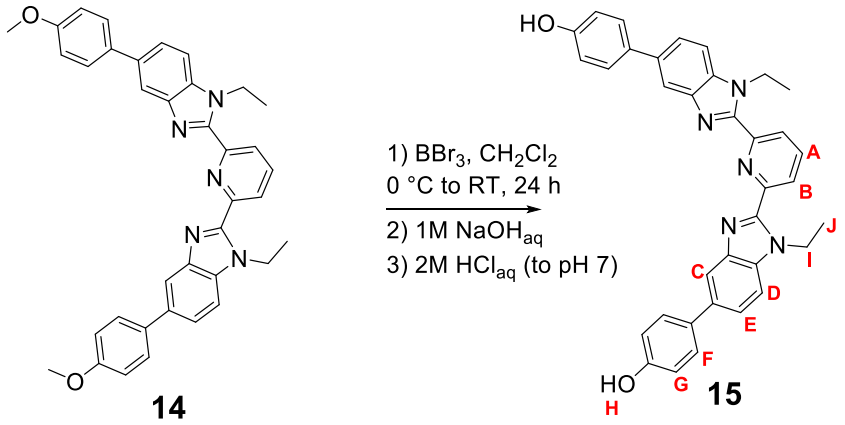


Scheme S2. Synthesis of **1**.

A 2-necked 1 L RBF was charged with **13** (1.0 g, 1.95 mmol), Cs_2CO_3 (2.6 g, 7.8 mmol, 4 eq.) and DMF (440 mL) under an Ar atmosphere. The mixture was heated to 75 °C and stirred while a DMF (220 mL) solution of 2,6-bis(bromomethyl)naphthalene (0.61, 1.95 mmol) was added dropwise (at an approximate rate of one drop every 10 s) over 3 d. When all the solution was added the reaction was stirred for a further 24 h at 75 °C. After this time (total reaction time 4 d) the reaction mixture was cooled to RT and the solvent was removed under reduced pressure. The residue was washed in

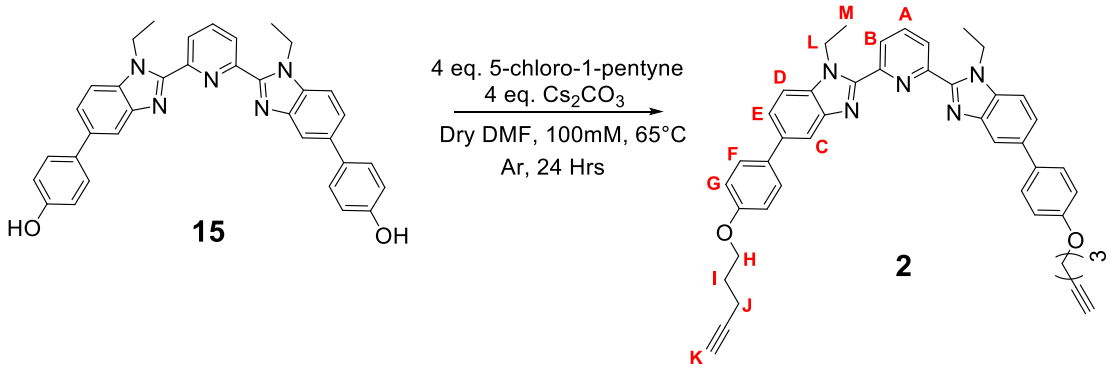
hot $CHCl_3$ (4 × 100 mL) and the insoluble material (salts) was removed by filtration. The filtrate was collected and the solvent removed under reduced pressure. The resulting material was purified using column chromatography (TEA treated silica gel, chloroform/methanol gradient as eluent) followed by recrystallization (chloroform/methanol mixture) to yield white crystals of **1** in 26% yield (0.670g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, J = 8.0 Hz, 4H, B), 7.79 (t, J = 8.0 Hz, 2H, A), 7.77 (s, 4H, C), 7.71 (d, J = 8.5 Hz, 4H, H), 7.50 (d, J = 8.5 Hz, 4H, I), 7.16 (m, 8H, G+D), 6.96 (dd, J = 8.9, 2.4 Hz, 4H, E), 5.44 (s, 8H, F), 4.44 (t, J = 7.5 Hz, 8H, J), 1.52 (m, 8H, K), 0.89 (m, 24H, L+M+N), 0.49 (m, 12H, O). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 154.48, 150.17, 149.83, 143.29, 137.70, 135.42, 132.90, 131.19, 128.49, 125.66, 125.23, 124.99, 114.88, 110.73, 104.80, 70.46, 44.85, 31.15, 30.04, 26.32, 22.41, 13.77. MALDI-MS: 1435.9 ([M]+Ag^+).

Synthesis of Thread Component (**2**)



Scheme S3. Synthesis of **15**.

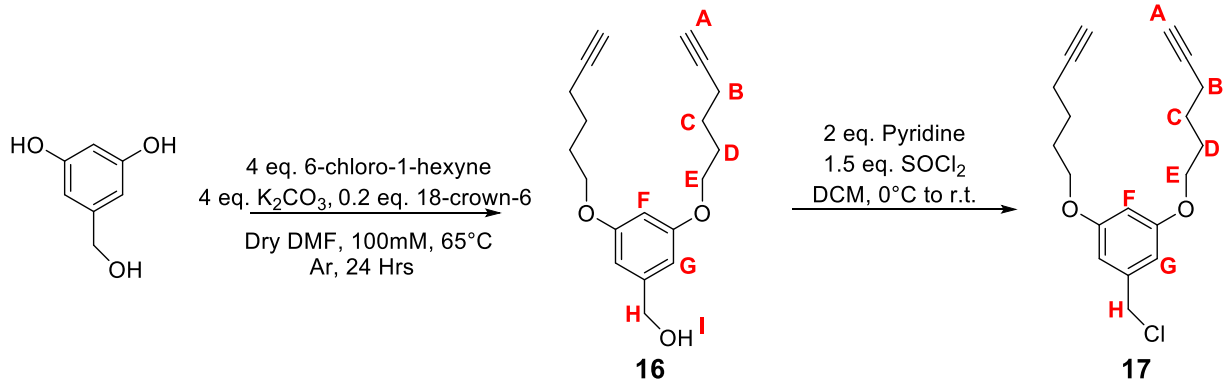
A 1L RBF flask was purged with Ar and charged with **14**[2] (20 g, 34.5 mmol), a stir bar and DCM (350 mL). The mixture was stirred, cooled to 0 °C and BBr_3 (19.6 mL, 207 mmol) was added dropwise over 10 min resulting in a deep red colored mixture. The reaction was warmed to RT and stirred for a total of 24 h. The reaction mixture was poured slowly into a 4L beaker containing 1M aq. NaOH (1.5 L) and vigorously stirred until the color changed from red to yellow. The pH of the suspension was then adjusted to 7 using a 2M aq HCl solution. Upon reducing the pH of the solution, a pale yellow precipitate formed, which was subsequently collected by vacuum filtration and purified by recrystallization (chloroform/methanol) to yield **15**, a white solid, in 90% yield (17.1g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.67 (s, 2H, H), 8.38 (d, *J*=7.8 Hz, 2H, B), 8.20 (t, *J*=7.8 Hz, 1H, A), 7.92 (s, 2H, C), 7.71 (d, *J* = 8.5 Hz, 2H, D), 7.56 (m, 6H, E+F), 6.97-6.86 (m, 4H, G), 4.83 (q, *J* = 7.1 Hz, 4H, I), 1.30 (t, *J* = 7.1 Hz, 6H, J). ^{13}C {^{1}H} NMR (101 MHz, DMSO-d_6) δ 156.88, 149.63, 149.59, 143.04, 138.65, 135.45, 134.82, 131.54, 127.91, 125.50, 122.41, 116.58, 115.79, 111.25, 15.39. MALDI-MS: 552.1 ([M]+H^+).



Scheme S4. Synthesis of **2**.

15 (2g, 3.62mmol) and Cs_2CO_3 (4.73 g, 14.5mmol, 4eq) were added to a 100mL RBF which was purged with Ar. To this mixture was added DMF (36 mL) and 5-chloro-1-pentyne (1.48 g, 1.53 mL, 14.5 mmol, 4 eq.). The subsequent mixed was stirred at 65°C for 24 h. After this time the solvent was removed under reduced pressure leaving a yellow residue. This residue was washed with chloroform (4 × 150 mL) and filtered. The filtrate was collected and the solvent removed under reduced pressure. The resulting material was purified using column chromatography (TEA treated silica gel, chloroform to 5% methanol in chloroform gradient as eluent, followed by recrystallization (chloroform/methanol mixture) to yield off-white crystals of **2** in 72% yield (1.73g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.37 (d, *J*=7.9Hz, 2H, B), 8.08 (t, *J*=7.9Hz, 1H, A), 8.03 (s, 2H, C), 7.65-7.56 (m, 6H, E+F), 7.52 (d, J = 8.4 Hz, 2H, D), 7.06-6.99 (m, 4H, G), 4.83 (q, *J*=7.2 Hz, 4H, L), 4.14 (t, J = 6.1 Hz, 4H, H), 2.45 (m, 4H, J), 2.09-2.01 (m, 4H, I), 1.99 (t, J =2.6 Hz, 2H, K), 1.40 (t, J =7.2 Hz, 6H, M). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 158.33, 150.48, 150.09, 143.63, 138.22, 136.32, 135.26, 134.45, 128.50, 125.79, 123.27, 118.21, 115.02, 110.45, 83.61, 69.03, 66.35, 40.05, 28.34, 15.62, 15.33. MALDI-MS: 684.3 ([M]+Na^+).

Synthesis of Thread Component (**3**)

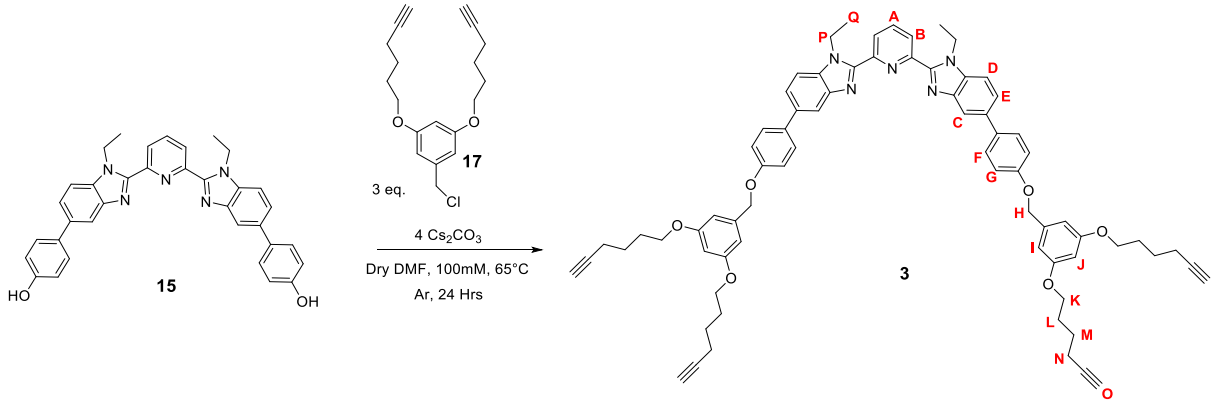


Scheme S5. Synthesis of **17**.

3,5-Dihydroxybenzyl alcohol (2.0g, 14.2mmol), K_2CO_3 (7.89g, 57mmol), and 18-crown-6 (0.75g, 2.8mmol) were added to a 100mL RBF. The reaction chamber was purged with argon and DMF (28.5mL) was added via cannula. The reaction mixture was heated to 65°C and 6-chloro-1-hexyne (4.6mL, 57mmol) was injected via syringe. The reaction mixture was allowed to stir at 65°C overnight and the following day cooled to room temperature. The reaction mixture was diluted with

75mL ether and washed 1x100mL 1M NaOH and 2x100mL H_2O. The organic layer was isolated and the solvent was removed under vacuum to yield a yellow oil that was purified via column chromatography (25% hexanes in chloroform to pure chloroform gradient) to yield **16** as a light yellow oil in 70% yield (3.0g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (bs, 2H, G), 6.37 (bs, 1H, F), 4.61 (d, J = 5.9 Hz, 2H, H), 3.97 (t, J = 6.8 Hz, 4H, E), 2.27 (td, J = 7.0, 2.6 Hz, 4H, B), 1.97 (t, J = 2.6 Hz, 2H, A), 1.90 (m, 4H, D), 1.71 (m, 4H, C). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.40, 143.40, 105.17, 100.58, 84.18, 68.79, 67.41, 65.32, 28.31, 25.10, 18.21. MALDI-MS: 322.9 ([M]+Na^+).

16 (2.05g, 6.8mmol) was added to a 100mL RBF with stir bar followed pyridine (1.10mL, 13.6mmol). The reaction chamber was purged with argon and DCM (27.3ml) was added via cannula. The reaction mixture was cooled to 0°C and $SOCl_2$ (0.74mL, 10.2mmol) was injected dropwise. The reaction mixture was allowed to stir overnight and slowly warm to room temperature. 50mL H_2O and 25mL DCM were then added to the crude reaction mixture and the organic layer was isolated, washed 3 x 75mL H_2O/brine, dried over magnesium sulfate and finally solvent was removed under vacuum. Column chromatography (50% hexanes in chloroform to pure chloroform gradient) gave **17** as a colorless oil in 62% yield (1.34g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.52 (bd, J = 2.2 Hz, 2H, G), 6.39 (bt, J = 2.2 Hz, 1H, F), 4.50 (s, 2H, H), 3.97 (t, J = 6.8 Hz, 4H, E), 2.28 (td, J = 7.0, 2.6 Hz, 4H, B), 1.97 (t, J = 2.6 Hz, 2H, A), 1.90 (m, 4H, D), 1.72 (m, 4H, C). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.37, 139.49, 107.06, 101.37, 84.32, 68.82, 67.46, 46.45, 28.28, 25.09, 18.22. MALDI-MS: 341.1 ([M]+Na^+).



Scheme S6. Synthesis of **3**.

15 (253mg, 0.45mmol) were added to a 25mL RBF followed by **17** (440mg, 1.35mmol) and Cs_2CO_3 (600mg, 1.8mmol, 4eq.). The reaction chamber was purged with Ar and DMF (4.6mL) was syringed into reaction vessel. The reaction mixture was allowed to stir under Ar at 65°C for 24 hours. Solvent was then removed under vacuum and the crude solid was boiled in chloroform and consequently filtered (4x50mL). The filtrate was collected and the solvent was removed under reduced pressure. The resulting material was purified using column chromatography (TEA treated silica gel, chloroform to 5% methanol in chloroform gradient) followed by recrystallization (chloroform/methanol mixture) to yield **3** as off-white crystals in 90% yield (0.46g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J*=7.8Hz, 2H, B), 8.08 (t, *J*=7.8Hz, 1H, A), 8.05 (s, 2H, C), 7.66-7.57 (m, 6H, D+E), 7.52 (d, J = 8.4 Hz, 2H, F), 7.09 (d, J = 8.7 Hz, 4H, G), 6.62 (bd, J = 2.2 Hz, 4H, I), 6.42 (bt, J = 2.2 Hz, 2H, J), 5.06 (s, 4H, H), 4.83 (q, *J*=7.2 Hz, 4H, P), 4.00 (t, J = 6.2 Hz, 8H, K), 2.28 (td, J = 7.0, 2.6 Hz, 8H, N), 1.98 (t, J = 2.6 Hz, 4H, O), 1.96-1.87 (m, 8H, L), 1.79-1.68 (m, 8H, M), 1.40 (t, J = 7.2 Hz, 6H, Q). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.52, 158.21, 150.49, 150.08, 143.63, 139.49, 138.23, 136.26, 135.29, 134.67, 128.51, 125.80, 123.28, 118.22, 115.38, 110.46, 105.81, 100.89, 84.20, 70.24, 68.80, 67.47, 40.06, 28.36, 25.16, 18.27, 15.62. MALDI-MS: 1,224.4 ([M]+Ag^+).

Fe(II) Assembly of Pseudo[3]rotaxane 1:2_2:Fe(II)$_2$

UV/Vis titration of **1**

A 80μM solution of macrocycle **1** was prepared using 3:2 chloroform:acetonitrile as the solvent. This solution was used to prepare a 1.2 mM solution of $Zn(NTf_2)_2$ to ensure the macrocycle concentration remains constant throughout the titration. 2.5 mL (200μmol) of the macrocycle solution was added to a 1 cm quartz cuvette and the UV Vis spectrum was taken. Subsequently 30 μL (36 nmol) of the prepared $Zn(NTf_2)_2$ solution was added to the cuvette and the UV Vis spectrum was taken after 5 min. This process was repeated until an excess (>4mol equiv.) of the $Zn(NTf_2)_2$ was added.

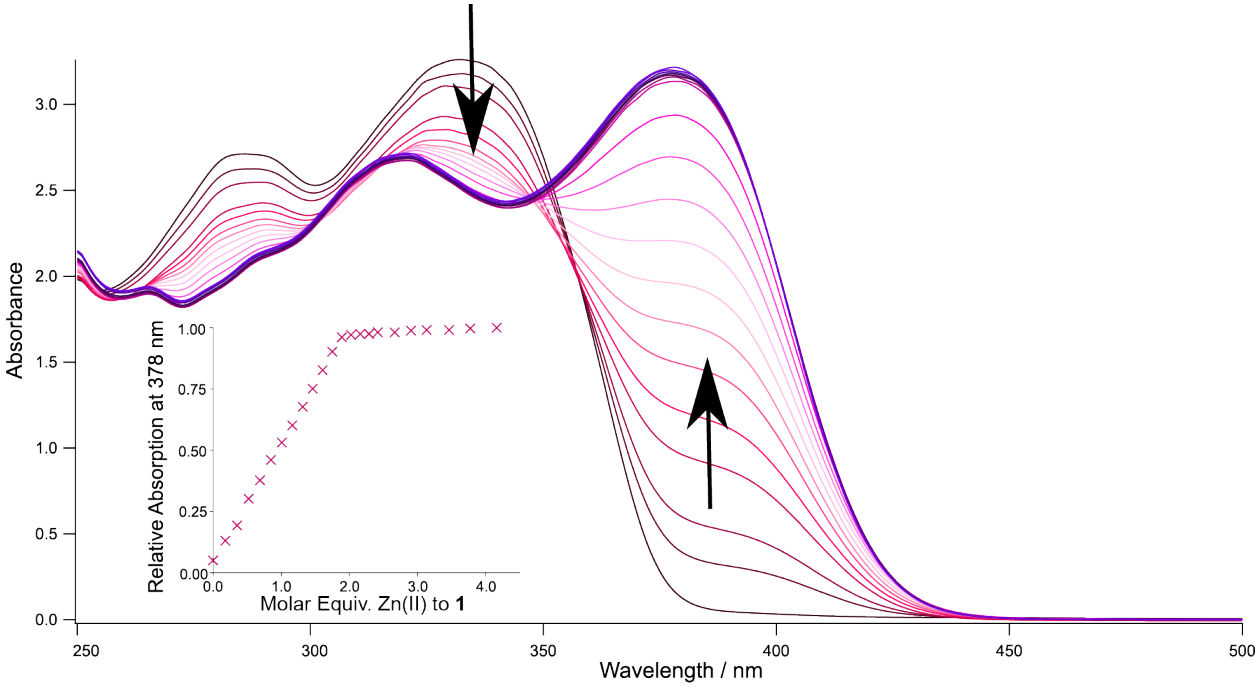
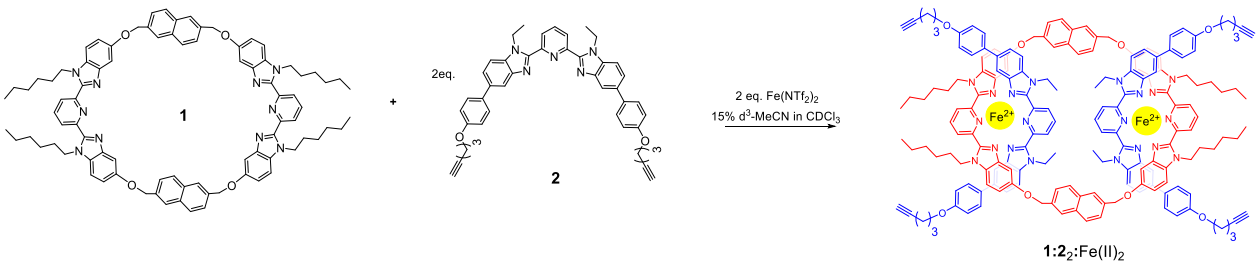


Figure S1. UV/Vis titration of **1** with Zn^{2+}, inset shows absorbance at 378nm.

Assembly of Pseudo[3]rotaxane **1:2$_2$:**Fe(II)$_2$ from **1** and **2**

Dissolved 20.2mg of **1** in 1.5mL $CDCl_3$**.** Titrated thread stock solution (30mM) of **2** into solution of **1** until an exact 2:1 (**2**:**1**) ratio was formed (done by monitoring both the N-CH_2 peaks on the alkyl groups of the bip ligands). The mixture was then diluted to a total volume to 3mL $CDCl_3$ (5 mM **1**). A stock solution of $Fe(NTF_2)_2$ (30mM in 2:1 $CDCl_3$:d_3-MeCN) was added until no free Bip peak appeared at ~2 equiv. of metal ion. The solvent was removed under vacuum resulting in a dark purple solid that was redissolved in 2mL dry 15% MeCN in $CHCl_3$, bubbled with argon for 1 min, and allowed to stir under Ar at 45°C for 1 day to allow equilibration. Solvent was then removed under vacuum and ^{1}H-NMR was recorded using 15% d_3-MeCN in $CDCl_3$.



Scheme S7. Formation of **1:2**$_2$**:**Fe(II)$_2$.

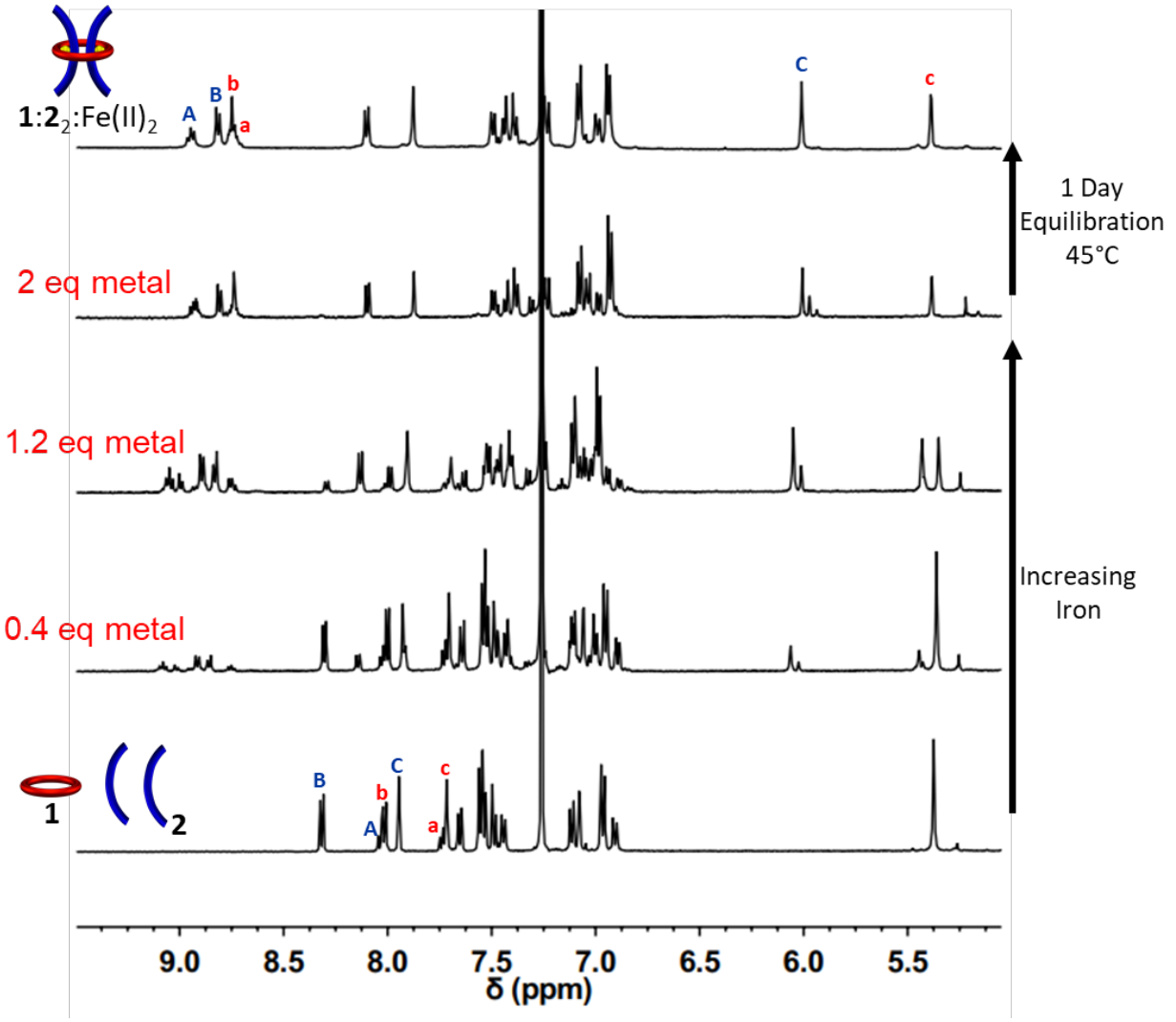


Figure S2. Partial ^{1}H-NMR overlay (500 MHz, 25°C, Solvent: 0, 3, 9, 15, 15% d_3-MeCN in $CDCl_3$ increasing upwards) of metal addition and 1 day equilibration. ^{1}H assignments from Schemes S2 and S4.

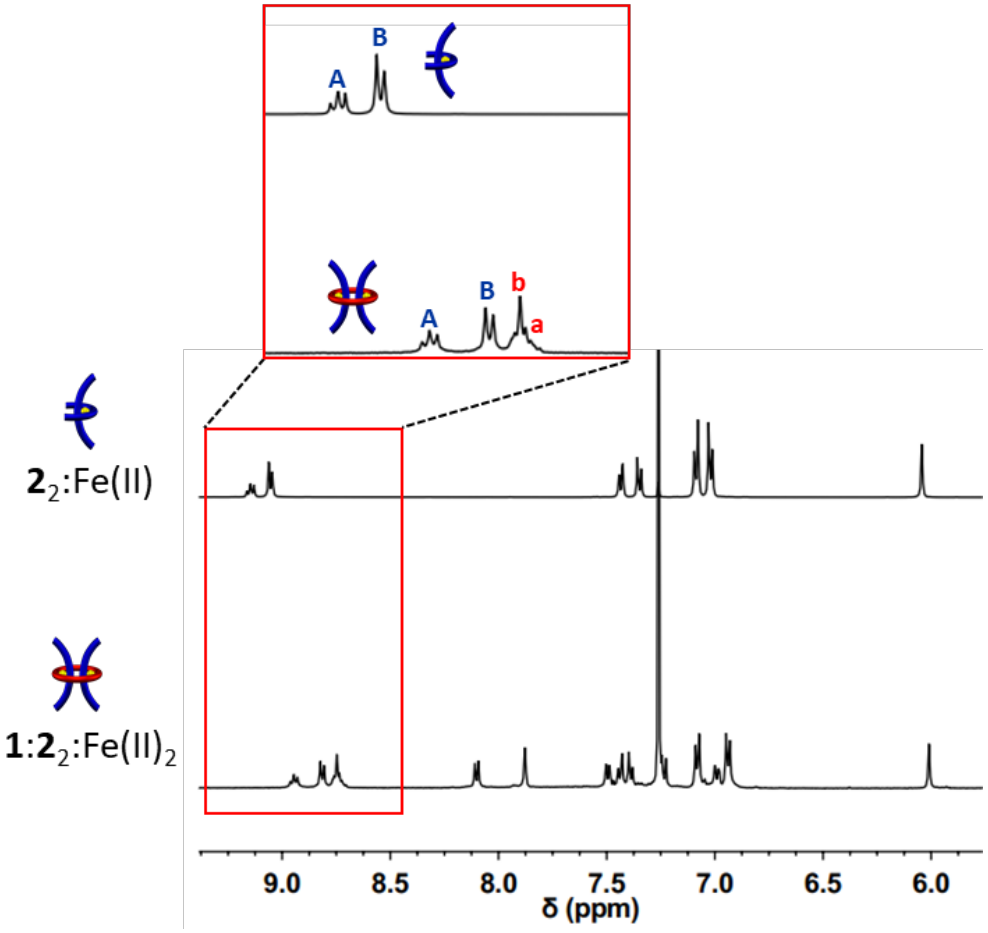


Figure S3. Partial ^{1}H-NMR overlay (500 MHz, 25°C, 15% d_3-MeCN in $CDCl_3$) of $\mathbf{2}_2$**:**Fe(II) (top) and $\mathbf{1}$**:**$\mathbf{2}_2$**:**Fe(II)$_2$ (bottom) with expansion showing diagnostic pyridyl peaks. ^{1}H assignments from Schemes S2 and S4.

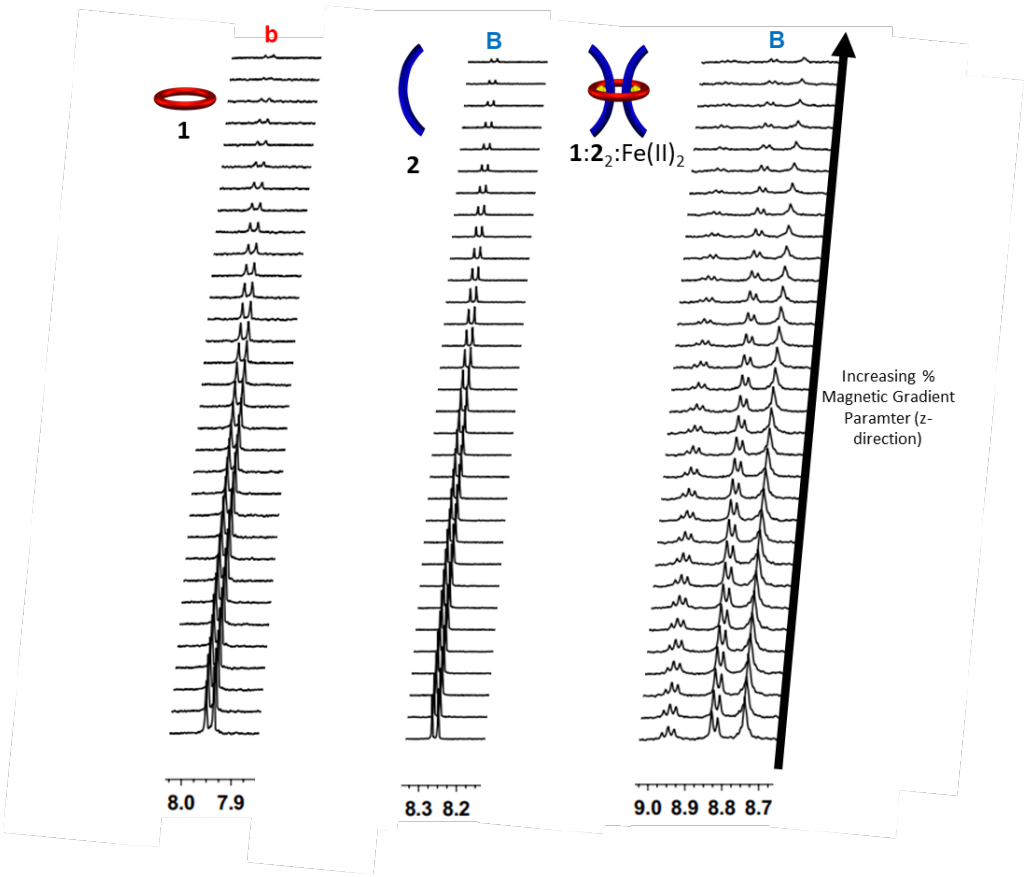


Figure S4. Partial ^{1}H-NMR overlay (5mM, 500 MHz, 25°C, 15% d_3-MeCN in $CDCl_3$) of increasing diffusion gradient strength from 5% (bottom) to 95% (top) for a) **1**, b) **2**, c) **1:2$_2$:**Fe(II)$_2$. ^{1}H assignments from Schemes S2 and S4 and Figure S2.

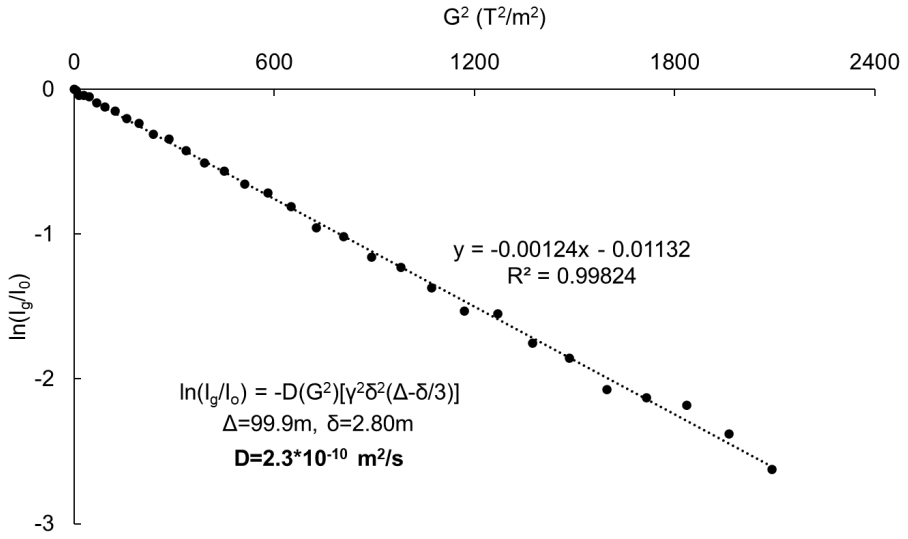


Figure S5. Plot for the calculation of diffusion coefficient of **1**.

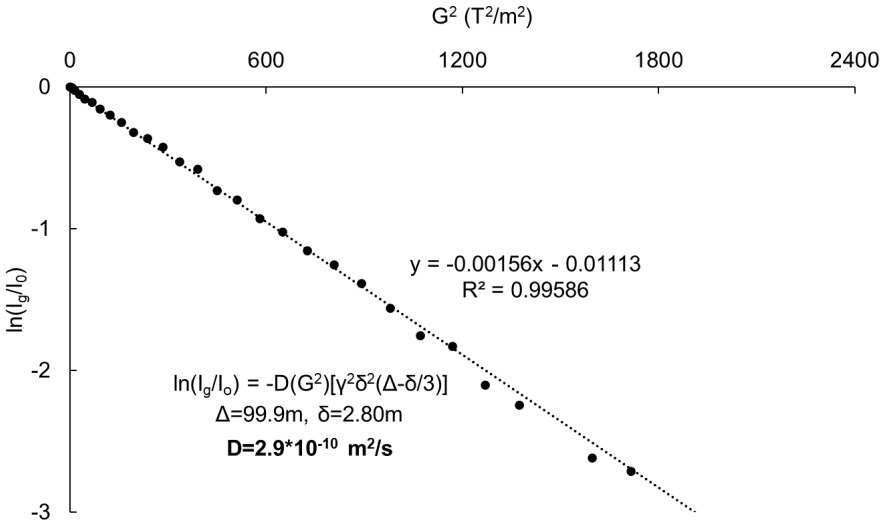


Figure S6. Plot for the calculation of diffusion coefficient of **2**.

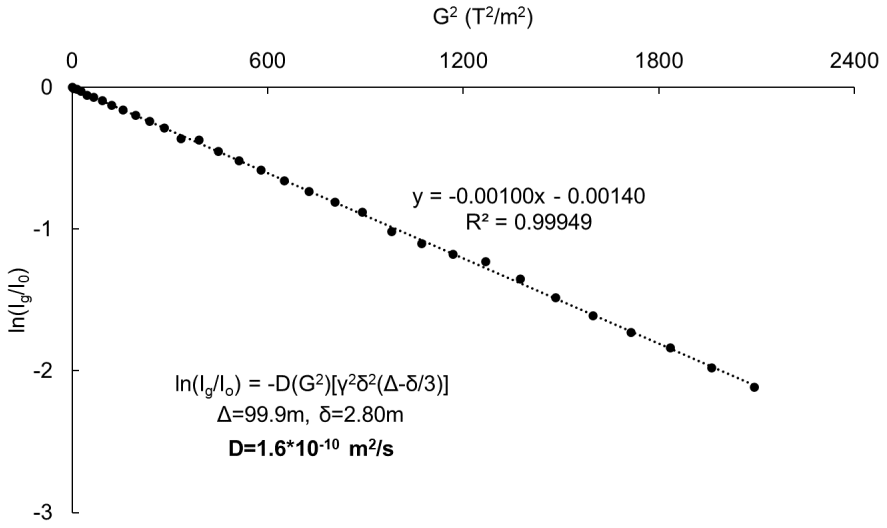
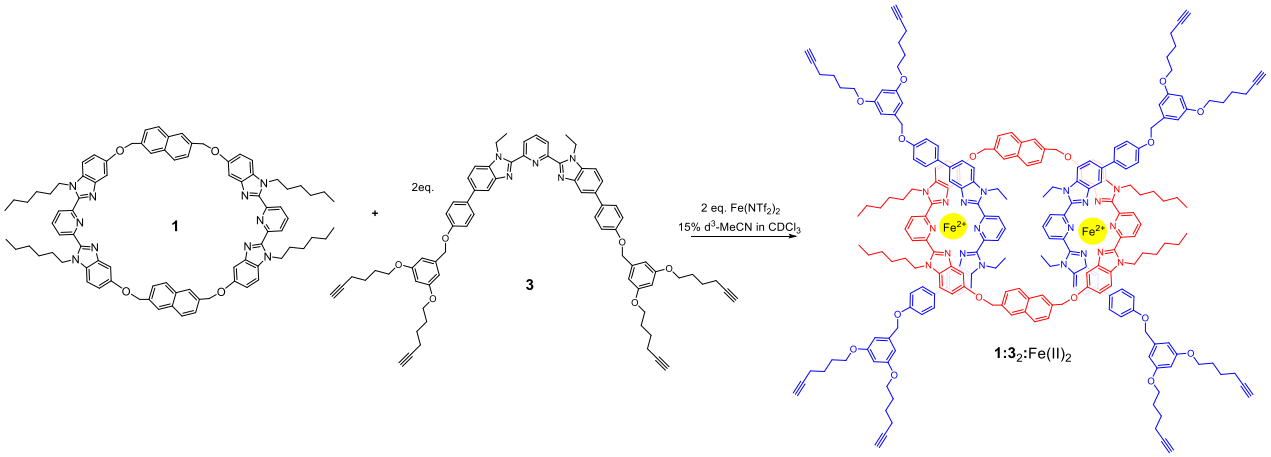


Figure S7. Plot for the calculation of diffusion coefficient of **1:2**$_2$**:**Fe(II)$_2$.

Fe(II) Assembly of Pseudo[3]rotaxane 1:3$_2$:Fe(II)$_2$

Assembly of Pseudo[3]rotaxane **1:3$_2$:**Fe(II)$_2$ from **1** and **3**

Dissolved 26.5mg of **1** in 1.5mL $CDCl_3$**.** Titrated thread stock solution (30mM) of **3** into solution of **1** until an exact 2:1 (**3**:**1**) ratio was formed (done by monitoring both the N-CH_2 peaks on the alkyl groups of the bip ligands). The mixture was then diluted to a total volume to 4mL $CDCl_3$ (5 mM **1**). A stock solution of $Fe(NTF_2)_2$ (30mM in 2:1 $CDCl_3$:d_3-MeCN) was added until no free Bip peak appeared at ~2 equiv. of metal ion. The solvent was removed under vacuum resulting in a dark purple solid that was redissolved in 2mL dry 15% MeCN in $CHCl_3$, bubbled with argon for 1 min, and allowed to stir under Ar at 45°C for 2 days to allow equilibration. Solvent was then removed under vacuum and ^{1}H-NMR was recorded using 15% d_3-MeCN in $CDCl_3$.



Scheme S8. Formation of **1:3$_2$:**Fe(II)$_2$.

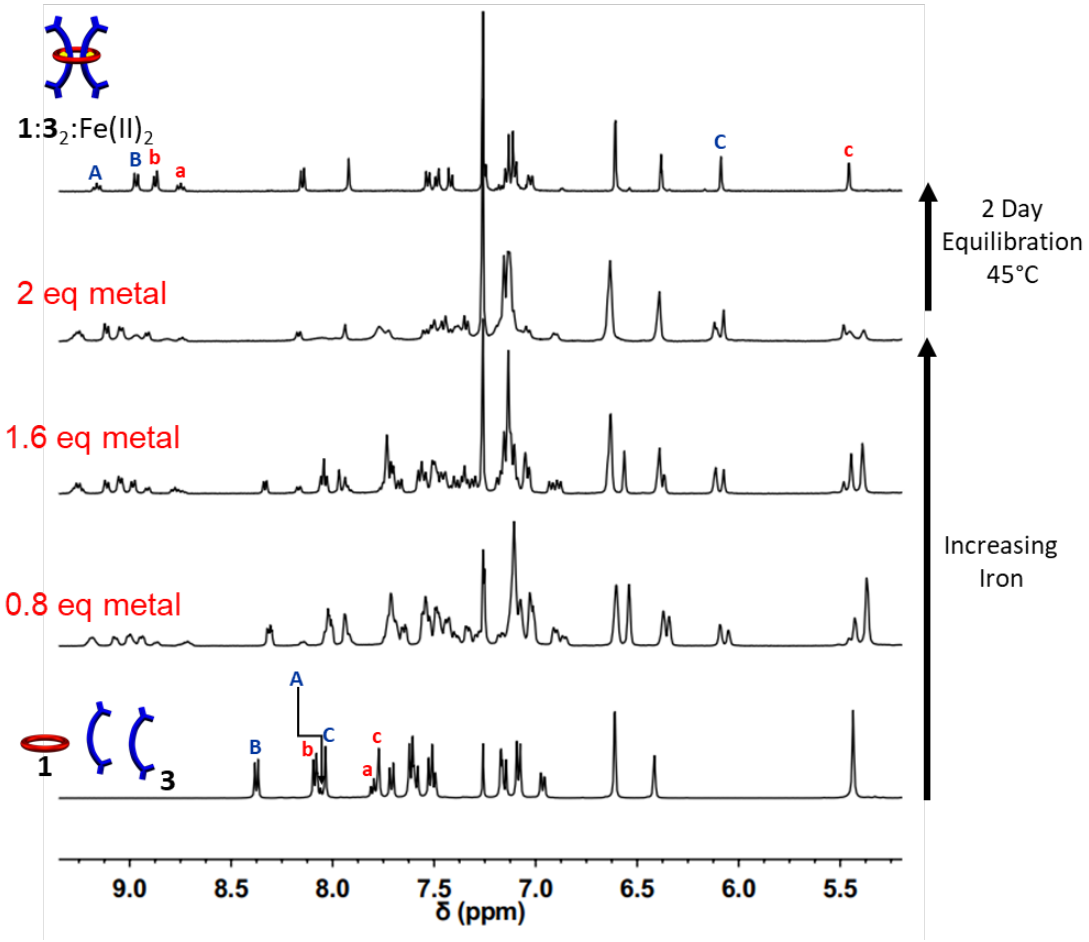


Figure S8. Partial ^{1}H-NMR overlay (500 MHz, 25°C, Solvent: 0, 6, 12, 15, 15% d_3-MeCN in $CDCl_3$ increasing upwards) of metal addition and equilibration. ^{1}H assignments from Schemes S2 and S6.

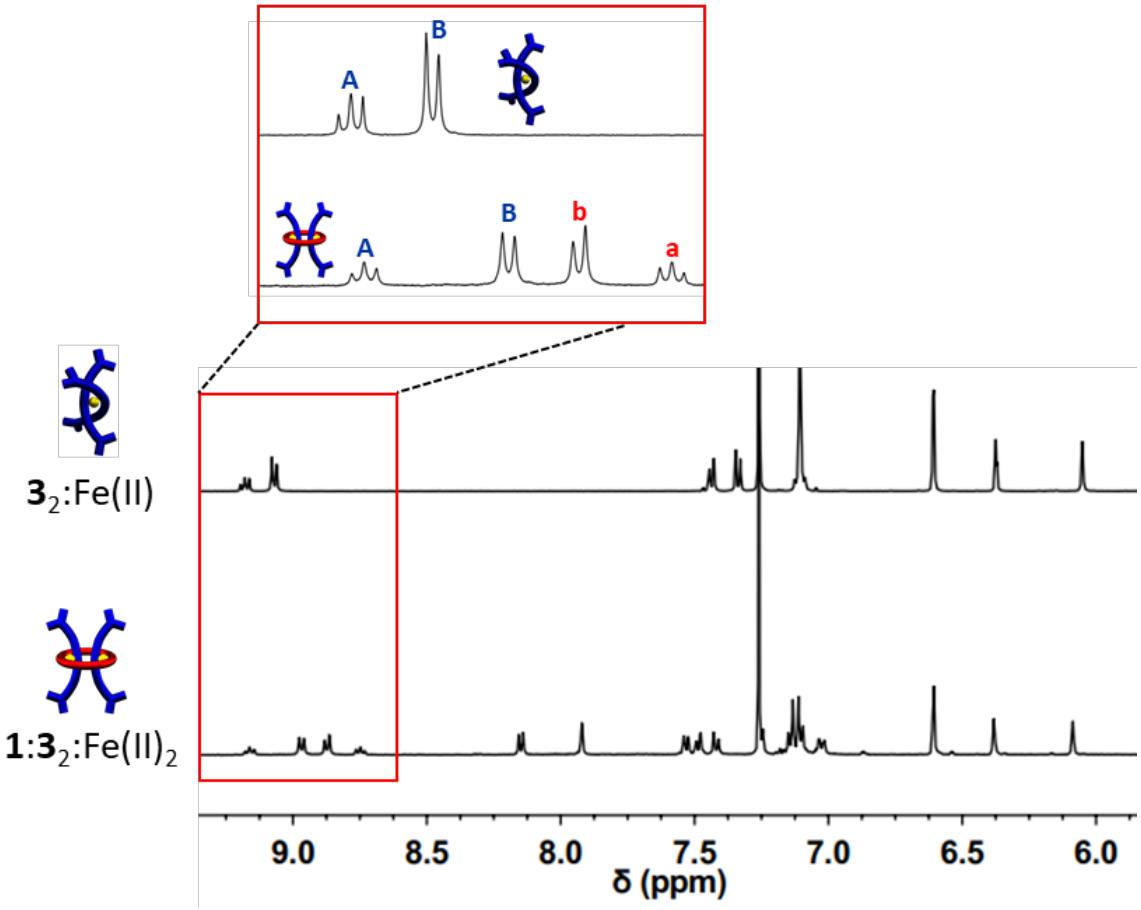


Figure S9. Partial ^{1}H-NMR overlay (500 MHz, 25°C, 15% d_3-MeCN in $CDCl_3$) of **3**$_2$:Fe(II) (top) and **1:3**$_2$:Fe(II)$_2$ (bottom) with expansion showing diagnostic pyridyl peaks. ^{1}H assignments from Schemes S2 and S6.

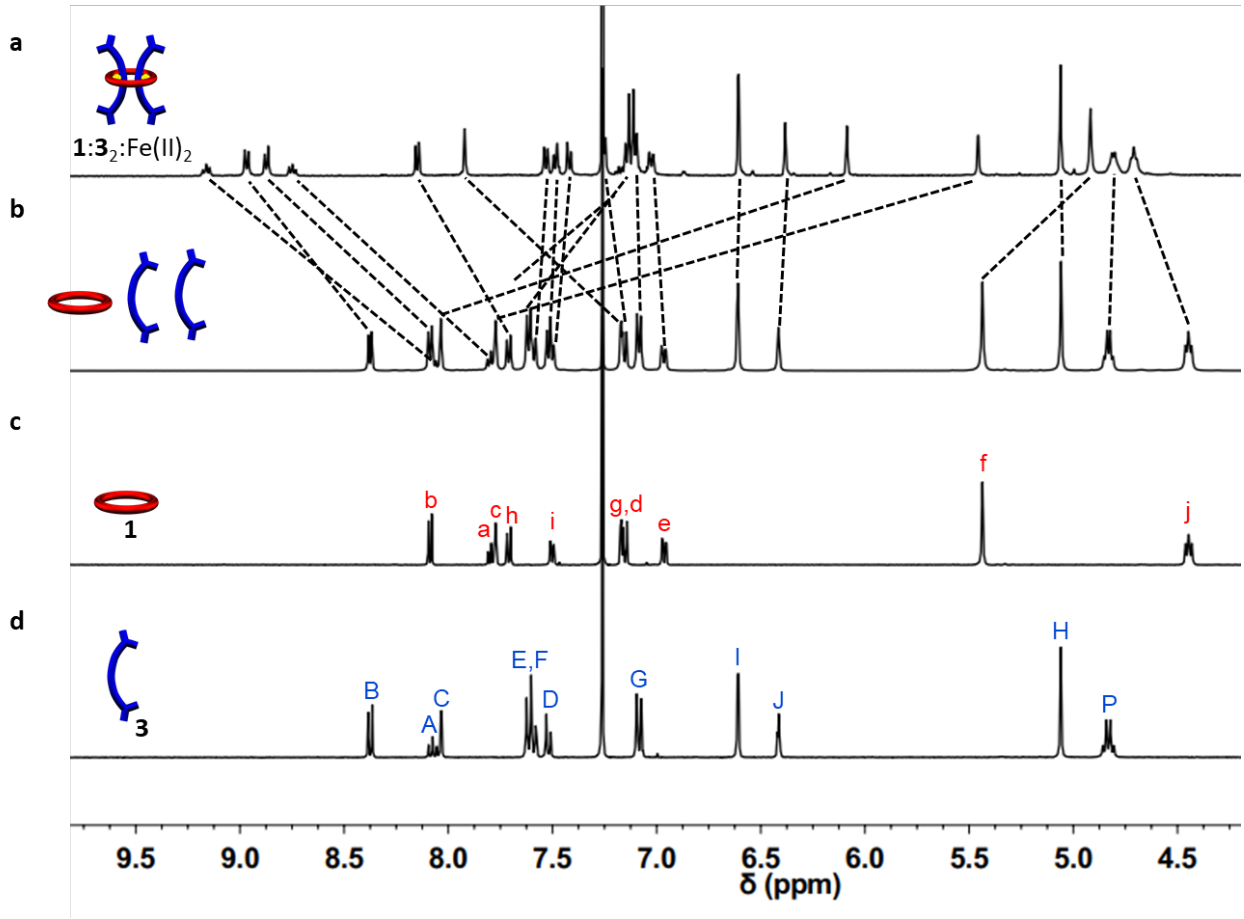
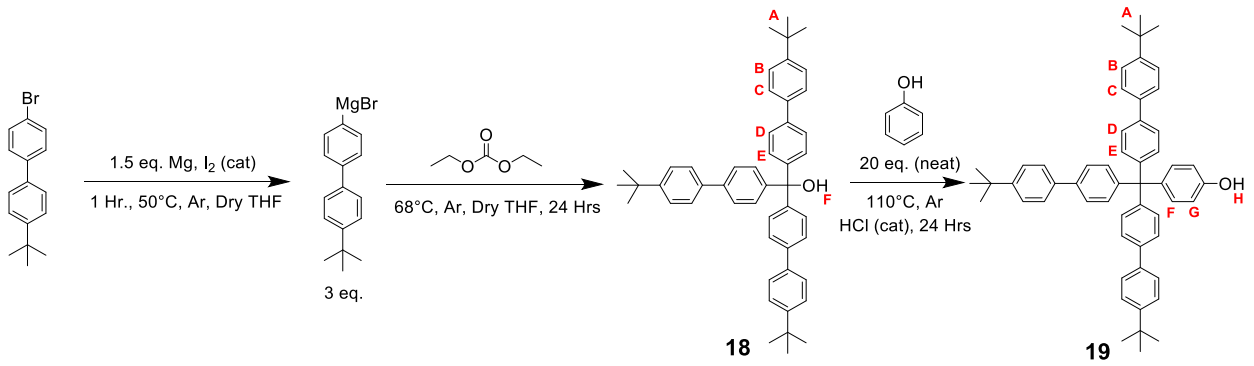


Figure S10. Partial ^{1}H-NMR overlay (500 MHz, 25°C)of a) **1:3$_2$:**Fe(II)$_2$ (15% d$_3$-MeCN in $CDCl_3$), b) 2:1 mixture of **3**:**1** ($CDCl_3$), c) **1** ($CDCl_3$) and d) **3** ($CDCl_3$). ^{1}H assignments from Schemes S2 and S6.

Synthesis of Stopper Group Components 4 + 5

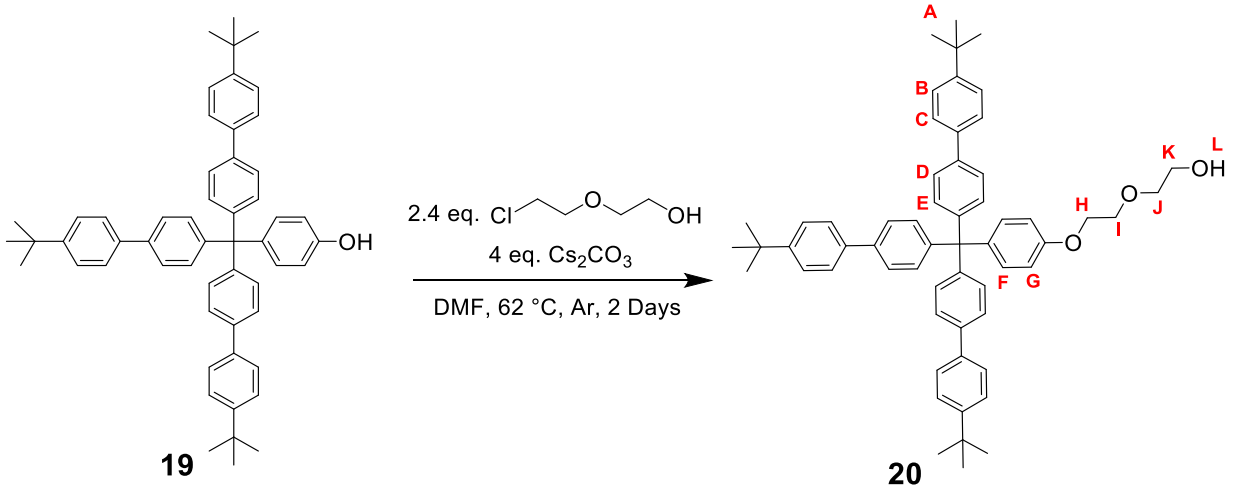
Synthesis of **4**



Scheme S9. Synthesis of **19**.

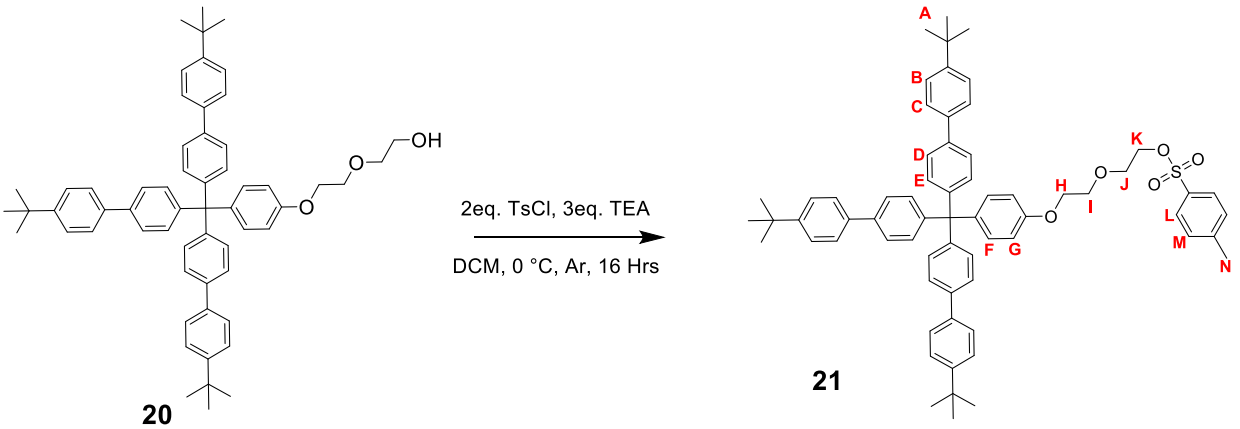
An oven dried 3-neck 500 mL RBF was charged with Mg turnings (1.67 g, 68.6 mmol), I_2(10 mg) and THF (30 mL) under an Ar atmosphere and stirred at 50 °C. A solution of 4-bromo-4'-tert-butylbiphenyl (13.21 g 45.7 mmol) in THF (60mL) was prepared in an addition funnel and added to the RBF over 15 min. Once the bromide was completely added the reaction was stirred under reflux for an additional 90 min. After this time the reaction mixture was cooled to RT and a solution of diethyl carbonate (1.85 mL, 15.2 mmol) in THF (7 mL) was added. The reaction was then heated under reflux for 18 h. After this time the reaction was cooled to RT, quenched with 1M HCl (60 mL) and filtered. To the filtrate was added hexanes (2 × 75 mL) and H_2O (50 mL). The organic layer was washed with brine (2 × 200 mL), dried with $MgSO_4$ and subsequently concentrated under reduced pressure to yield an off-white solid, **18**, in a 71% yield (7.1g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59-7.55 (d overlap, J = 8.4 Hz, 12H, D+B), 7.47 (d, J = 8.4 Hz, 6H, E), 7.43 (d, J = 8.4 Hz, 6H, C), 1.37 (s, 27H, A). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 150.47, 145.70, 140.10, 137.86, 128.46, 126.87, 126.69, 125.86, 81.88, 34.68, 31.52. MALDI-MS: 679.6 ([M]+Na^+) and 639.7 ([M] – OH^-).

To an argon filled 100 mL RBF was added **18** (4.50 g, 6.8 mmol), phenol (14 mL of an 89% phenol solution in H_2O) and conc. HCl (1mL, 12M). The reaction mixture was stirred and heated at 110 °C for 22 h. The reaction mixture was subsequently cooled to RT, diluted with toluene (~200 mL) and washed with 1x200mL 1M NaOH followed by 2x200mL H_2O. Dried organic phase over magnesium sulfate and removed solvent under reduced pressure resulting in a yellow crude solid. Boiled solid in 200mL hexanes and filtered (3 times) resulting in an off-white solid in 79% yield (5.0g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, J = 8.5 Hz, 6H, D), 7.51 (d, J = 8.5 Hz, 6H, B), 7.45 (d, J = 8.5 Hz, 6H, E), 7.32 (d, J = 8.5 Hz, 6H, C), 7.18 (d, J = 8.8 Hz, 2H, F), 6.77 (d, J = 8.8 Hz, 2H, G), 4.73 (s, 1H, H), 1.37 (s, 27H, A). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 153.64, 150.30, 145.91, 139.41, 138.54, 137.83, 132.59, 131.58, 126.73, 126.07, 125.82, 114.47, 63.79, 34.67, 31.52. MALDI-MS: 841.1 ([M]+Ag^+).



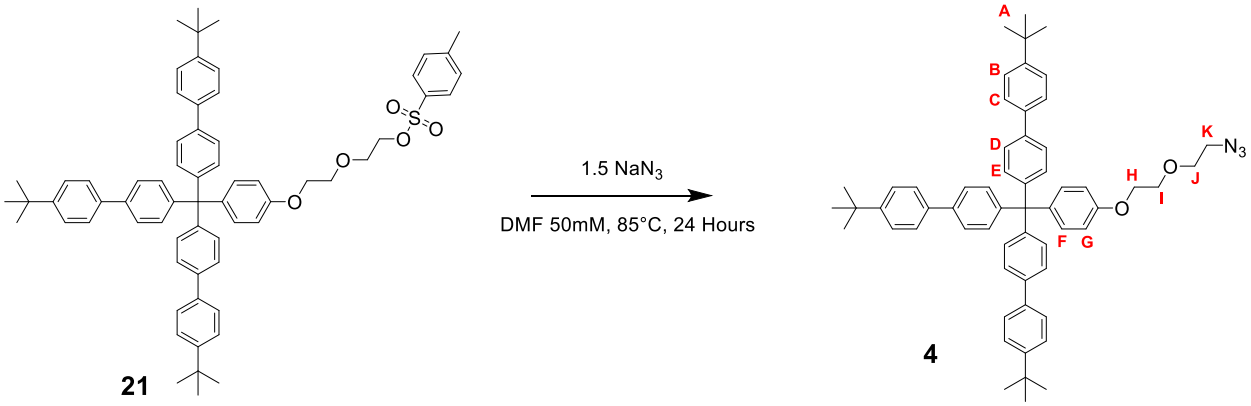
Scheme S10. Synthesis of **20**.

A 200 mL RBF was charged with **19** (5.0 g, 6.8 mmol), Cs_2CO_3 (8.9 g, 27 mmol, 4eq.) and DMF (68 mL, reaction concentration 100 mM) in an argon atmosphere. The reaction was stirred and 2-(2-chloroethoxy)ethanol (0.86 mL, 8.2mmol, 1.2eq.) was added dropwise and the reaction was heated at 62 °C for 18 h. A further portion of 2-(2-chloroethoxy)ethanol (0.86 mL, 8.2mmol, 1.2eq) was added and the reaction was stirred for a further 24 h. The reaction was cooled to RT and the volatiles were removed under vacuum. To the resulting off-white solid was added hot $CHCl_3$ (3 × 250 mL) and insoluble material was removed by filtration. The solvent was removed from the filtrate under reduced pressure resulting in an off-white residue. Purification of desired product **20** was achieved by column chromatography (silica gel, eluent gradient of 100% $CHCl_3$ to 95:5 $CHCl_3$:MeOH) followed by recrystallization (chloroform/hexane layering). 89% yield (5.0g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, J = 8.5 Hz, 6H, D), 7.50 (d, J = 8.5 Hz, 6H, B), 7.45 (d, J = 8.5 Hz, 6H, E), 7.31 (d, J = 8.5 Hz, 6H, C), 7.21 (d, J = 8.9 Hz, 2H, F), 6.85 (d, J = 8.9 Hz, 2H, G), 4.14 (m, 2H, K), 3.88 (m, 2H, H), 3.76 (m, 2H, J), 3.68 (m, 2H, I), 2.10 (t, J = 6.2 Hz, 1H, L), 1.35 (s, 27H, A). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 156.81, 150.28, 145.90, 139.50, 138.52, 137.82, 132.39, 131.57, 126.72, 126.06, 125.80, 113.56, 72.68, 69.84, 67.40, 63.78, 61.94, 34.65, 31.50. MALDI-MS: 929.1 ([M]+Ag^+).



Scheme S11. Synthesis of **21**.

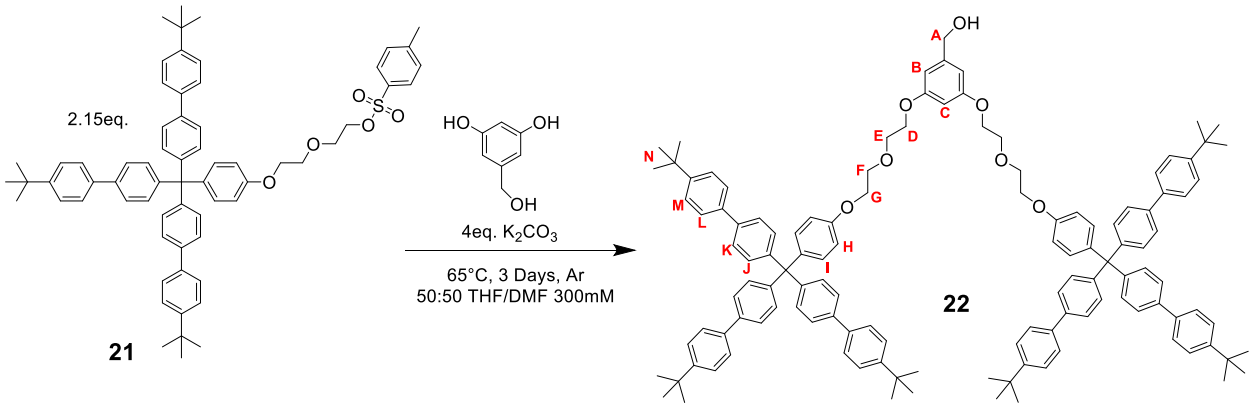
A 100 mL RBF was charged with **20** (5 g, 6.08 mmol), *p*-toluenesulfonyl chloride (2.30 g, 12.2 mmol, 2 eq.) and DCM (41 mL, reaction conc. = 150 mM). The reaction was stirred, cooled with an ice bath and NEt_3 (2.55 mL, 18.2 mmol, 3 eq.) was added dropwise and the reaction was allowed to warm to RT. After 18 h the reaction mixture was diluted with 40mL CH_2Cl_2 and H_2O (100 mL) was added. The organic layer was removed and dried with $MgSO_4$, filtered and the solvent reduced under reduced pressure. The resulting material was purified using column chromatography (silica gel, eluent gradient 1:1 hexanes:$CHCl_3$ to 95:5 $CHCl_3$:MeOH followed by precipitation (chloroform/hexane layering) to yield **21** as a white solid in 78% yield (4.6g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 8.3Hz, 2H, M), 7.55 (d, J = 8.5 Hz, 6H, D), 7.51 (d, J = 8.5 Hz, 6H, B), 7.45 (d, J = 8.5 Hz, 6H, E), 7.31 (d, J = 8.5 Hz, 6H, C), 7.28 (d, J = 8.3 Hz, 2H, L), 7.21 (d, J = 8.9 Hz, 2H, F), 6.85 (d, J = 8.9 Hz, 2H, G) 4.19 (m, 2H, K), 4.03 (m, 2H, H), 3.77 (m, 4H, I+J), 2.37 (s, 3H, N), 1.35 (s, 27H, A). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 156.76, 150.26, 145.88, 144.87, 139.42, 138.49, 137.78, 133.06, 132.34, 131.55, 129.91, 128.10, 126.69, 126.05, 125.80, 113.50, 69.98, 69.38, 68.96, 67.34, 63.76, 34.63, 31.49, 21.72. MALDI-MS: 1083.5 ([M]+Ag^+).



Scheme S12. Synthesis of **4**.

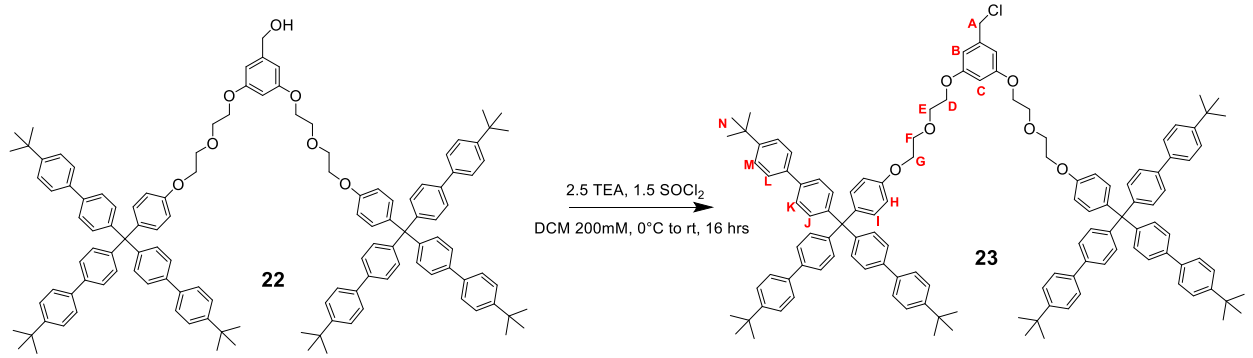
An Ar-filled 50 mL RBF was charged with **21** (400 mg, 0.41 mmol), NaN_3 (40 mg, 1.15 mmol, 1.5 eq.) and DMF (8.2 mL, reaction concentration 50 mM). The reaction mixture was heated at 85 °C and stirred for 18 h. After this time the solvent was removed under reduced pressure resulting in an off-white solid that was washed with hot $CHCl_3$ (4 × 15 mL) and filtered. The filtrate was collected and the solvent removed under reduced pressure resulting in an off-white residue. Purification using column chromatography (standard silica gel, eluent gradient 1:3 $CHCl_3$:Hexanes to 100 % $CHCl_3$) resulted in a white solid, **4**, in 90% yield (0.31g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, J = 8.2 Hz, 6H, D), 7.51 (d, J = 8.2 Hz, 6H, B), 7.45 (d, J = 8.2 Hz, 6H, E), 7.31 (d, J = 8.2 Hz, 6H, C), 7.21 (d, J = 8.6 Hz, 2H, F), 6.85 (d, J = 8.6 Hz, 2H, G), 4.15 (t, J = 4.8 Hz, 2H, H), 3.87 (t, J = 4.8 Hz, 2H, I), 3.75 (t, J = 5.0 Hz, 2H, J), 3.42 (t, J = 5.0 Hz, 2H, K), 1.36 (s, 27H, A). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 156.85, 150.27, 145.93, 139.43, 138.51, 137.83, 132.37, 131.58, 126.72, 131.58, 126.72, 126.05, 125.81, 113.59, 70.36, 69.95, 67.46, 63.79, 50.86, 34.66, 31.51. MALDI-MS: 954.1 ([M]+Ag^+) and 926.2 ([M]+Ag^+ -N_2).

Synthesis of **5**



Scheme S13. Synthesis of **22**.

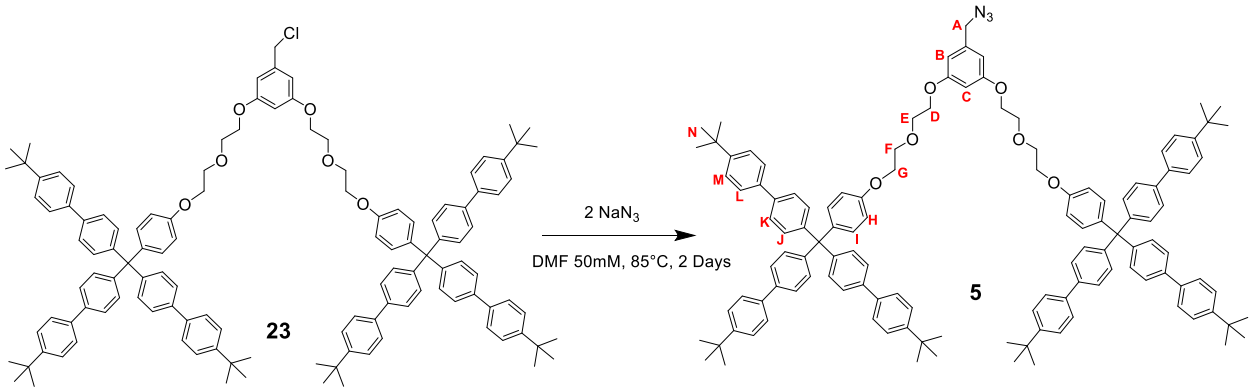
An Ar-filled 100 mL RBF was charged with **21** (4.5g, 4.6mmol, 2.15eq.), K_2CO_3 (1.18 g 8.5mmol, 4eq.) and 3,5-dihydroxybenzyl alcohol (300 mg, 2.1mmol, 1eq.). To the reaction vessel was added THF/DMF (1:1 ratio, 15 mL) and the reaction was stirred at 65 °C for 3 d. The solvent was cooled to RT and the solvent was removed under vacuum. To the resulting off-white solid was added hot $CHCl_3$ (4 × 200 mL) and the insoluble material was removed by filtration. The filtrate was collected and the solvent removed under reduced pressure. The resulting material was purified using column chromatography (silica gel, eluent gradient of 12:88 ethyl acetate:hexanes to 7:3 $CHCl_3$:hexanes to $CHCl_3$.) followed by recrystallization (chloroform/methanol layering) resulting in **22** as a white solid in 50% yield (1.88g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 8.5 Hz, 12H, K), 7.49 (d, J = 8.5 Hz, 12H, M), 7.44 (d, J = 8.5 Hz, 12H, J), 7.30 (d, J = 8.5 Hz, 12H, L), 7.19 (d, J = 8.9 Hz, 4H, I), 6.83 (d, J = 8.9 Hz, 4H, H), 6.53 (d, J = 2.2 Hz, 2H, B), 6.44 (t, J = 2.3 Hz, 1H, C), 4.58 (d, J = 6.3 Hz, 2H, A), 4.13 (m, 8H, D+G), 3.90 (m, 8H, E+F), 1.35 (s, 54H, N). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 160.46, 157.25, 150.72, 146.39, 144.26, 139.56, 138.71, 137.89, 132.44, 131.70, 126.87, 126.35, 126.13, 113.89, 105.63, 100.81, 70.22, 67.96, 65.32, 64.07, 60.65, 34.80, 31.49, 14.42. MALDI-MS: 1854.6 ([M]+Ag^+).



Scheme S14. Synthesis of **23.**

A 5 mL RBF was charged with **22** (0.24 g, 0.14 mmol) and purged with Ar. CH_2Cl_2 (0.7 mL) was added to the reaction vessel, which was subsequently cooled to 0°C. The solution was vigorously stirred while NEt_3 (48 µL, 0.35 mmol, 2.5 eq.) and $SOCl_2$ (15 µL, 0.21mmol, 1.5eq.) were added dropwise. The reaction mixture was stirred at RT for a total of 18 h before dilution with additional

CH_2Cl_2 (25 mL). The resulting mixture was washed with H_2O (2 × 25 mL) and sat. $NaHCO_3$ (1 × 25 mL). The organic layer was dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure resulting in an off-white residue. Purification by column chromatography (SiO_2, eluent gradient from 1:3 $CHCl_3$:hexanes to 100% $CHCl_3$) followed by recrystallization (chloroform/methanol layering) resulted in the isolation of **23** as a white solid in 80% yield (0.19g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, J = 8.5 Hz, 12H, K), 7.52 (d, J = 8.5 Hz, 12H, M), 7.46 (d, J = 8.5 Hz, 12H, J), 7.33 (d, J = 8.5 Hz, 12H, L), 7.23 (d, J = 8.9 Hz, 4H, I), 6.86 (d, J = 8.9 Hz, 4H, H), 6.57 (d, J = 2.2 Hz, 2H, B), 6.49 (t, J = 2.2 Hz, 1H, C), 4.47 (s, 2H, A), 4.15 (m, 8H, D+G), 3.94 (m, 8H, E+F), 1.37 (s, 54H, N). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 160.15, 156.90, 150.27, 145.94, 139.57, 139.35, 138.50, 137.83, 132.35, 131.59, 126.72, 126.05, 125.81, 113.60, 107.64, 101.81, 70.10, 69.97, 67.76, 67.48, 63.79, 46.40, 34.66, 31.51. MALDI-MS: 1873.0 ([M]+Ag^+).

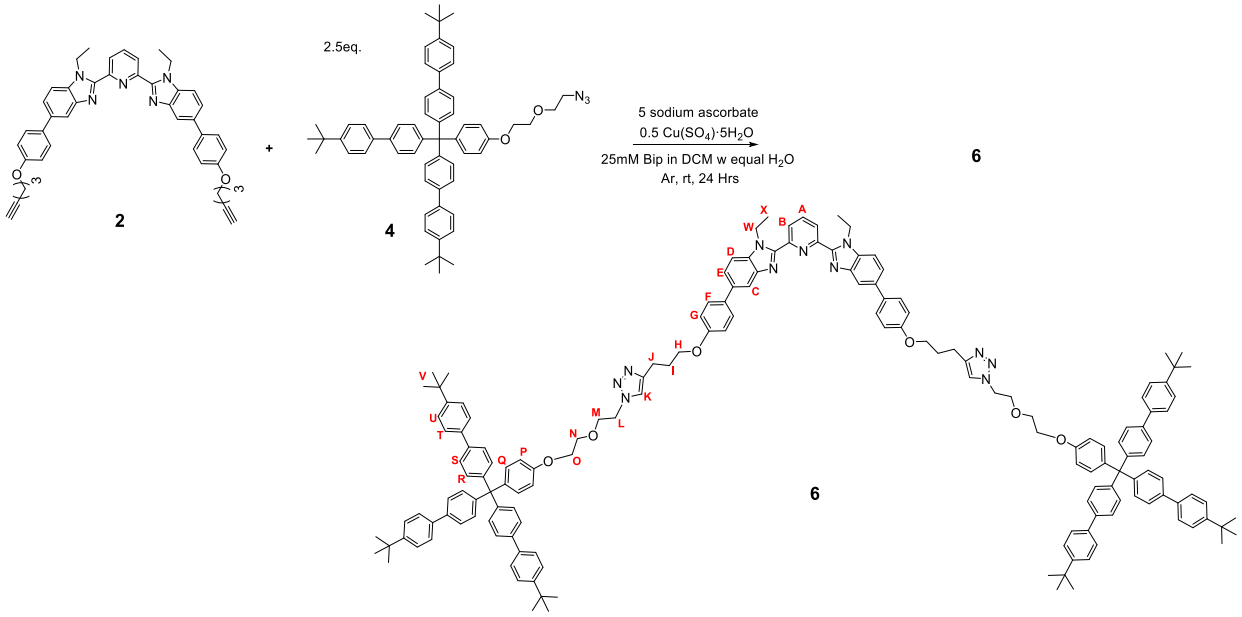


Scheme S15. Synthesis of **5**.

An Ar-purged 10 mL RBF was charged with **23** (0.24 g, 0.14mmol) NaN_3 (9mg, 0.14 mmol, 1eq.) and DMF (2.7 mL, reaction conc = 50 mM). The reaction was stirred for 18h at 85 °C and another portion of NaN_3 (9 mg, 0.14 mmol) was added, and the reaction was stirred for a further 18 h (total reaction time 36 h). The solvent was then removed under reduced pressure and the mixture washed with hot $CHCl_3$ (4 × 15 mL) and filtered. The filtrate was collected and the solvent removed under reduced pressure resulting in an off-white residue. Purification of **5** was achieved using column chromatography (silica gel, eluent gradient of 12:88 to 20:80 ethyl acetate:hexanes followed by 7:3 $CHCl_3$:hexanes to 100% $CHCl_3$) resulting in a white solid in 72% yield (0.17g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, J = 8.4 Hz, 12H, K), 7.52 (d, J = 8.4 Hz, 12H, M), 7.46 (d, J = 8.4 Hz, 12H, J), 7.33 (d, J = 8.4 Hz, 12H, L), 7.23 (d, J = 8.9 Hz, 4H, I), 6.86 (d, J = 8.9 Hz, 4H, H), 6.50 (bs, 3H, B+C), 4.23 (s, 2H, A), 4.15 (m, 8H, D+G), 3.93 (m, 8H, E+F), 1.37 (s, 54H, N). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 160.29, 156.88, 150.26, 145.92, 139.33, 138.49, 137.82, 137.63, 132.34, 131.58, 126.72, 126.05, 125.80, 113.57, 107.15, 101.53, 70.09, 69.96, 67.72, 67.45, 63.77, 54.92, 34.65, 31.50. MALDI-MS: 1879.5 ([M]+Ag^+) and 1851.9 ([M]+Ag^+ -N_2).

Attempted Synthesis of [3]Rotaxane 7

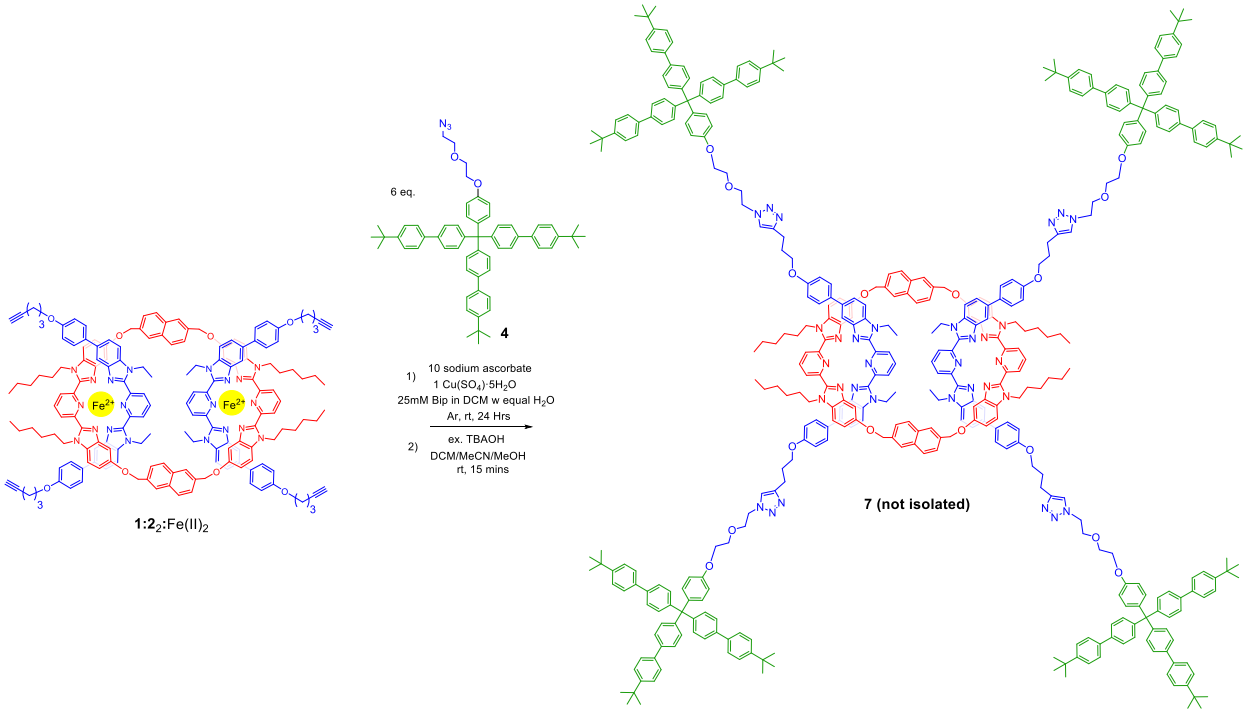
Synthesis of Dumbbell Component (**6**)



Scheme S16. Synthesis of **6**.

4 (45 mg, 0.053mmol, 2.5eq.), **2** (14.5 mg, 0.021 mmol) and sodium ascorbate (20.9 mg, 0.1 mmol, 5 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.90 mL, conc of **2** = 25 mM), H_2O (0.8 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (100 mM, 0.010mmol, 0.5eq, (25 mol% per alkyne)). The reaction was stirred vigorously for 18 h at RT. After this time the reaction mixture was diluted with CH_2Cl_2 and H_2O (10 mL each). The organic layer was taken and washed with H_2O (2 × 5 mL). Removal of the organic solvent resulted in a light brown crude solid, which was purified using preparative thin layer chromatography (SiO_2, eluent = 95:5 $CHCl_3$:MeOH) followed by recrystallization (chloroform/methanol layering) to result in a white solid, **6** in 86% yield (44 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (d, J = 7.9Hz, 2H, B), 8.06-8.01 (m, 3H, A+C), 7.57– 7.45 (m, 34H, S+U+D+E+F+K), 7.43 (d, J = 8.5 Hz, 12H, R), 7.31 (d, J = 8.5 Hz, 12H, T), 7.23 (d, J = 8.9 Hz, 4H, Q), 6.96 (d, J = 8.7 Hz, 4H, G), 6.82 (d, J = 8.9 Hz, 8H, P), 4.80 (q, J = 7.2 Hz, 4H, W), 4.54 (t, J = 5.0 Hz, 4H, H), 4.08 (m, 4H, L), 4.03 (t, J = 6.2 Hz, 4H, O), 3.93 (t, J = 5.0 Hz, 4H, N), 3.80 (m, 4H, M), 2.92 (t, J = 7.5 Hz, 4H, J), 2.19 (m, 4H, I), 1.37 (t, J =7.2 Hz, 6H, X), 1.34 (s, 54H, V). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 158.45, 156.79, 156.74, 150.48, 150.32, 150.10, 147.29, 145.89, 143.64, 139.61, 138.54, 138.26, 137.79, 136.39, 135.26, 134.33, 132.44, 131.57, 128.50, 126.72, 126.08, 125.83, 123.33, 122.42, 118.22, 115.02, 113.52, 110.46, 69.97, 67.26, 67.08, 63.80, 50.33, 40.08, 34.67, 31.50, 29.86, 22.33, 15.64. MALDI-MS: 2,484.2 ([M]+Ag^+).

Attempted Synthesis of **7** by Stoppering **1:2**$_2$:Fe(II)$_2$ With **4**



Scheme S17. Attempted stoppering and demetallation of doubly threaded [3]rotaxane **7**.

1:2$_2$: Fe(II)$_2$ (8.2 mg, 0.0021mmol), **4** (10.6 mg, 0.012 mmol, 6eq) and sodium ascorbate (4.1 mg, 0.021 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.34 mL, conc of alkyne = 25 mM), H_2O (0.24 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (21 mM, 0.0021mmol, 1eq, (25 mol% per alkyne)). The reaction was stirred vigorously for 18 h at RT. After this time the reaction mixture was diluted with CH_2Cl_2 and H_2O (5 mL each). The organic layer was taken and washed with H_2O (2 × 5 mL). Removal of the organic solvent resulted in a purple solid that was washed 3x with 5mL of 10% chloroform in hexanes to help remove leftover stopper group. The crude purple solid was redissolved in 5mL of 50:50 dichloromethane and acetonitrile and stirred slowly at room temperature. 2mL of tetrabutylammonium hydroxide solution (1M in MeOH) were added dropwise to the stirring solution resulting in a rapid color change from purple to light brown and precipitation of demetallated product. After 15 minutes of stirring an offwhite solid was filtered off and washed with methanol (2x 10mL). The frit was then washed with 2mL of $CDCl_3$ to redissolve the demetallated product and transferred to an NMR tube for analysis.

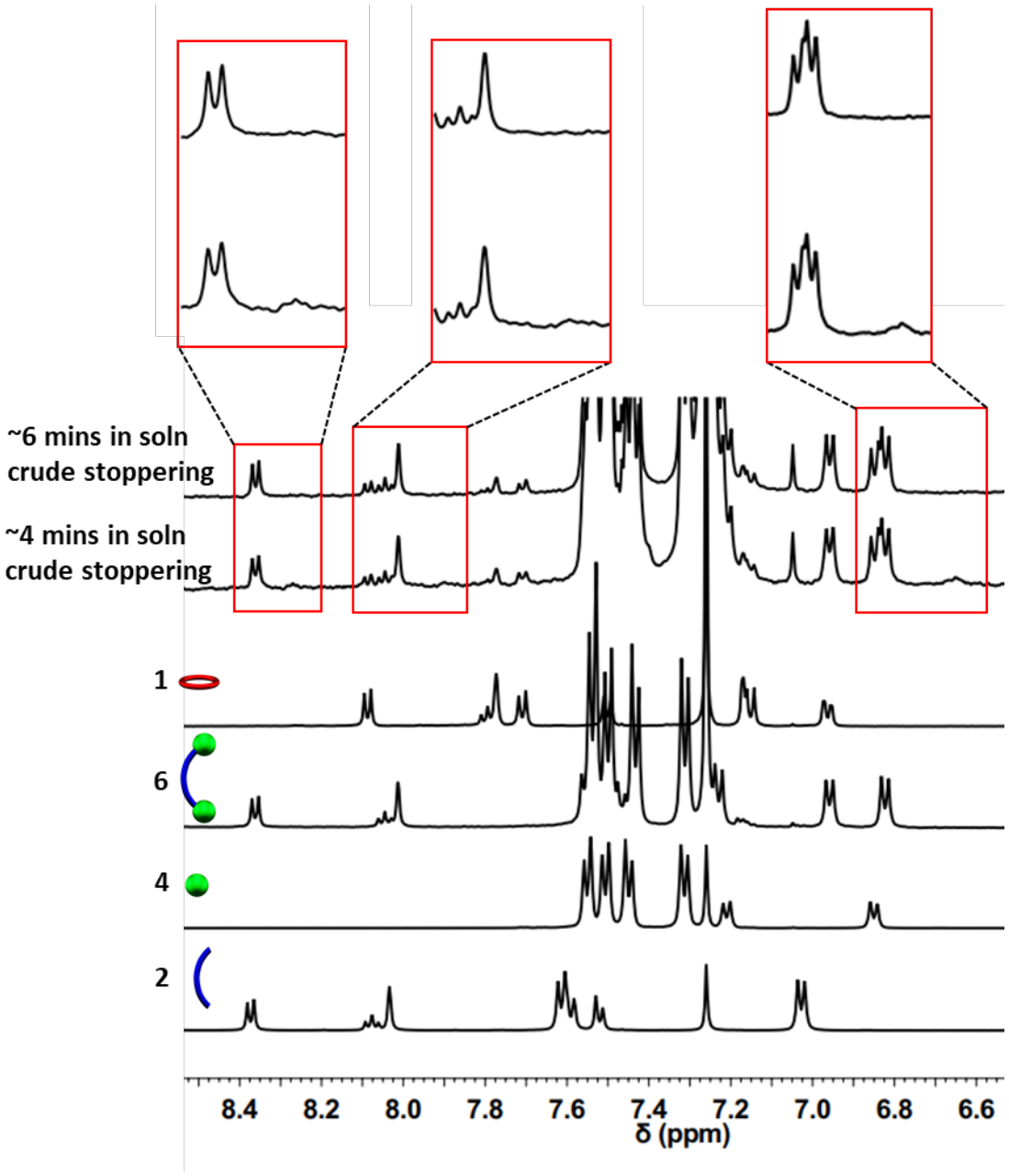


Figure S11. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of the demetallated crude reaction mixture from the stoppering of **1:2**$_2$:Fe(II)$_2$ with **4**. Top two NMR spectra correspond to crude reaction mixture at indicated time in solution. Bottom four spectra correspond to indicated components and starting materials for comparison.

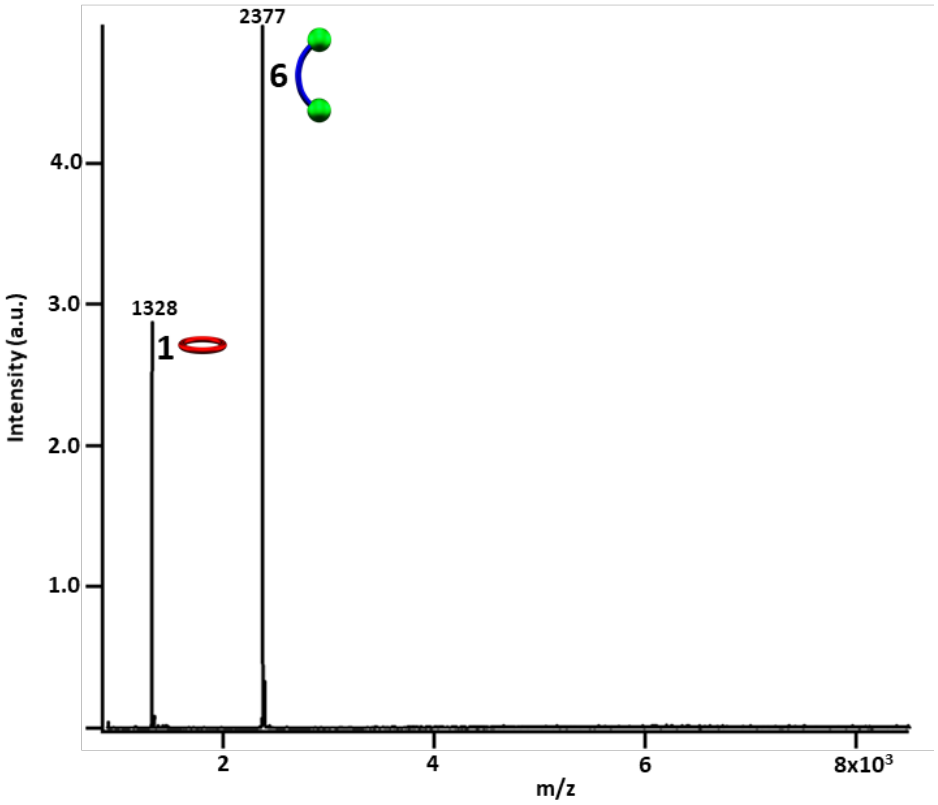
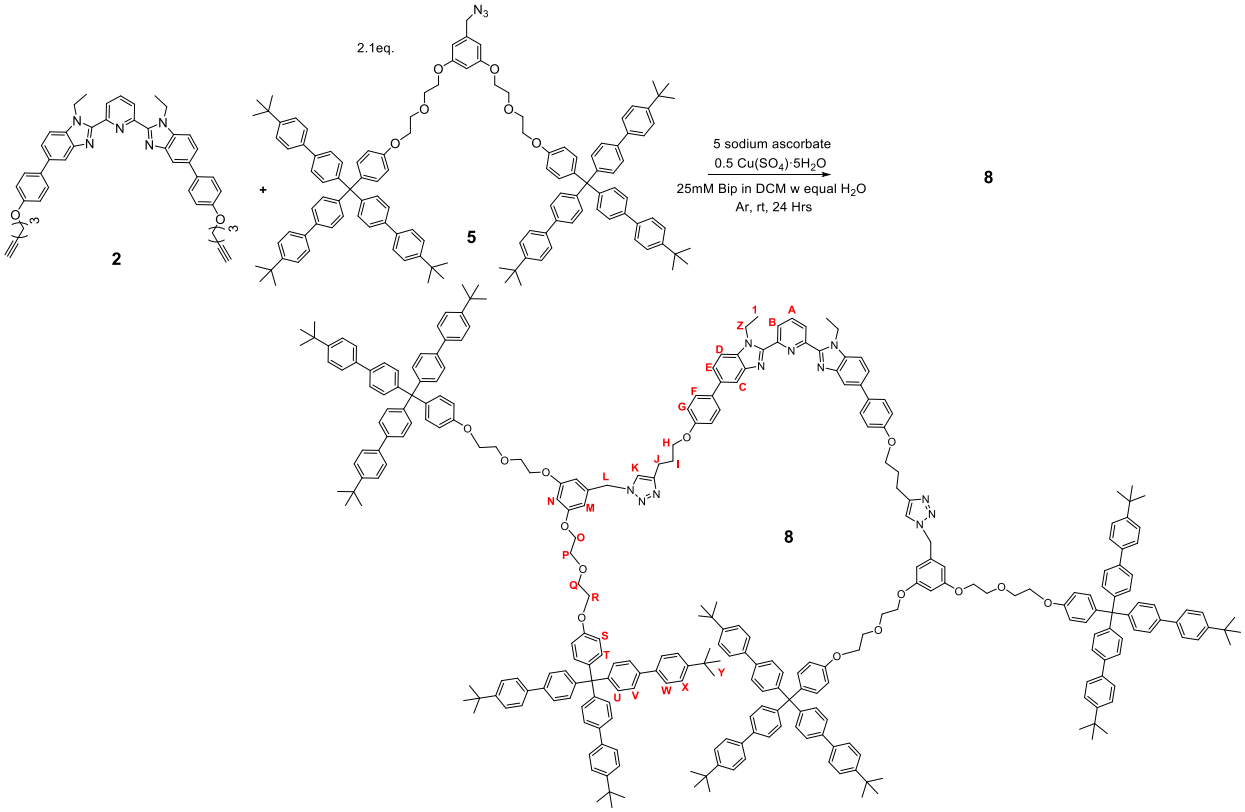


Figure S12. Maldi-TOF analysis (Dithranol, no salt) of the of the demetallated crude reaction mixture from the stoppering of **1**:**2**$_2$:Fe(II)$_2$ with **4**.

Synthesis, Purification, and Characterization of [3]Rotaxane 9

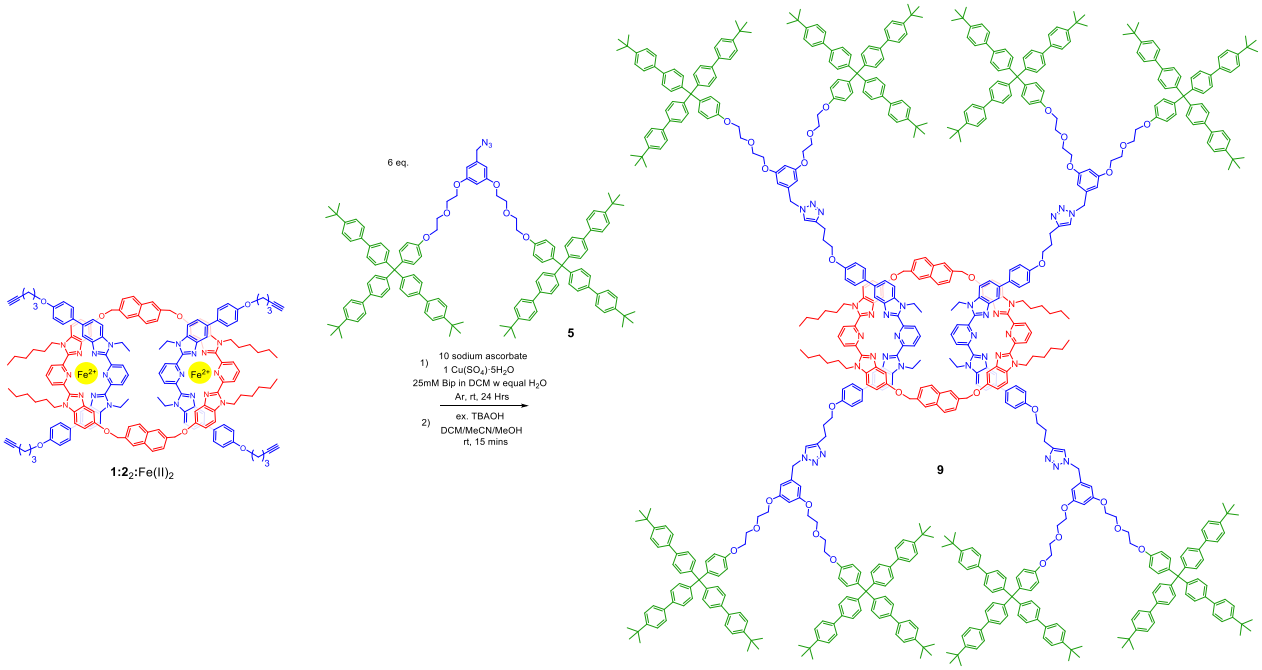
Synthesis of Dumbbell Component (**8**)



Scheme S18. Synthesis of **8**.

5 (60 mg, 0.034mmol, 2.1eq.), **2** (11 mg, 0.016 mmol) and sodium ascorbate (16 mg, 0.08 mmol, 5 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.63 mL, conc of **2** = 25 mM), H_2O (0.53 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (80 mM, 0.008mmol, 0.5eq, (25mol% per alkyne)). The reaction was stirred vigorously for 18 h at RT. After this time the reaction mixture was diluted with CH_2Cl_2 and H_2O (10 mL each). The organic layer was taken and washed with H_2O (2 × 5 mL). Removal of organic solvent resulted in a light brown crude solid, which was purified using preparative thin layer chromatography (SiO_2, eluent = 95:5 $CHCl_3$:MeOH) followed by recrystallization (chloroform/methanol layering) resulting in a white solid, **8** in 88% yield (60 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (d, J = 7.9Hz, 2H, B), 8.02 (m, 3H, A+C), 7.58 – 7.48 (m, 56H, V+X+D+E+F), 7.43 (d, J = 8.2 Hz, 24H, U), 7.30 (d, J = 8.2 Hz, 24H, W), 7.21 (s, 2H, K), 7.19 (d, J = 8.5 Hz, 8H, T), 6.96 (d, J = 8.3 Hz, 4H, G), 6.82 (d, J = 8.5 Hz, 8H, S), 6.48 (s, 2H, N), 6.40 (d, J = 2.2 Hz, 4H, M), 5.35 (s, 4H, L), 4.79 (q, J = 7.2 Hz, 4H, Z), 4.12-4.02 (m, 20H, O+R+H), 3.89-3.85 (m, 16H, P+Q), 2.90 (t, J = 7.2 Hz, 4H, J), 2.17 (t, J = 7.1 Hz, 4H, I), 1.37 (t, J =7.2 Hz, 6H, 1), 1.34 (s, 108H, Y). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 160.51, 158.40, 156.88, 150.49, 150.28, 150.09, 147.86, 145.92, 143.65, 139.36, 138.50, 138.25, 137.81, 137.05, 136.38, 135.26, 134.34, 132.35, 131.58, 128.51, 126.71, 126.52, 126.05, 125.81, 123.32, 121.05, 118.22, 115.00, 113.57, 110.47, 107.14, 101.66, 70.10, 69.91, 67.76, 67.44, 67.10, 63.77, 54.17, 40.07, 34.66, 31.50, 29.03, 22.41, 15.64. MALDI-MS: 4,334.9 ([M]+Ag^+).

Stoppering of 1:**2**$_2$:Fe(II)$_2$ with **5**



Scheme S19. Stoppering and demetallation of doubly threaded [3]rotaxane **9**.

1:2$_2$:Fe(II)$_2$ (20.0 mg, 0.0051mmol), **5** (54.2 mg, 0.030 mmol, 6eq) and sodium ascorbate (10.1 mg, 0.051 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.82 mL, conc of alkyne = 25 mM), H_2O (0.72 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (51mM, 0.0051mmol, 1eq, (25mol% per alkyne)). The reaction was stirred vigorously for 18 h at RT. The reaction mixture was then diluted with CH_2Cl_2 and H_2O (5 mL each). The organic layer was collected and washed with H_2O (2 × 5 mL). Removal of organic solvent resulted in a purple solid that was washed 3x with 5mL of 10% chloroform in hexanes to help remove leftover stopper group. The crude purple solid was redissolved in 5mL of 50:50 dichloromethane and acetonitrile and stirred slowly at room temperature. 1mL of tetrabutylammonium hydroxide solution (1M in MeOH) was added dropwise to the stirring solution resulting in a rapid color change from purple to light brown and precipitation of demetallated product. After 15 minutes of stirring, an off-white solid was filtered off and washed with methanol (2x 10mL). The frit was then washed with 5mL of $CHCl_3$ to redissolve the demetallated product and washed once with 5mL water. Solvent was removed under reduced pressure resulting in an off-white residue. Purification of **9** was achieved using preparative thin layer chromatography (SiO_2, eluent = 94:6 $CHCl_3$:MeOH, lowest Rf band taken (R_f=0.1-0.3) as [3]R product, macrocycle byproduct R_f=0.4, dumbbell byproduct R_f=0.65-0.75) followed by precipitation from cold methanol to result in an off-white solid, **9** in 75% isolated yield (37.4 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (d, J = 7.8Hz, 4H), 7.96-7.88 (m, 8H), 7.81 (t, J = 7.8Hz, 2H), 7.67 (s, 4H), 7.55-7.45 (m, 106H), 7.42 (d, J = 8.5 Hz, 48H), 7.37 (d, J = 8.9 Hz, 8H), 7.31-7.28 (m 52H), 7.24 (s, 2H), 7.21-7.15 (m, 20H), 6.86-6.75 (m, 28H), 6.62 (d, J = 8.6 Hz, 8H), 6.49 (s, 4H), 6.37 (d, J = 2.0 Hz, 8H), 5.32 (bs, 4H), 5.18 (s, 4H), 4.59 (bq, J = 7.2 Hz, 8H), 4.27 (bt, 8H) 4.12-4.00 (m, 32H), 3.90-3.78 (m, 32H), 3.48 (bt, 8H), 2.39 (bt, 8H), 1.62 (bt, 8H), 1.36 (m, 8H), 1.33 (s, 216H), 1.18 (t, J = 7.2H, 12H), 0.90-0.70 (m, 24H), 0.47 (t, J = 6.9Hz, 12H). ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 160.48, 158.19, 156.85, 154.42, 150.25, 150.18, 150.09, 149.90, 149.66, 147.73, 145.90, 143.50, 143.28, 139.32, 138.47, 137.95, 137.79, 137.33, 136.13, 135.82, 135.13, 133.80, 132.76, 132.33, 131.56, 131.27, 130.14, 130.05, 129.87, 128.40, 128.12, 126.70, 126.04, 125.80, 125.33,

125.08, 123.08, 120.84, 117.85, 114.74, 114.06, 113.54, 110.90, 110.41, 107.11, 105.06, 101.44, 70.71, 70.06, 69.90, 67.73, 67.38, 66.88, 63.75, 56.12, 53.83, 44.65, 39.86, 36.04, 34.64, 31.49, 29.84, 27.34, 26.17, 22.37, 15.52, 13.91. MALDI-MS: 9,782.2 ([M]+H⁺), 5,554.5 ([M]+H⁺-DB(**7**)), 4,226.7 ([M]+H⁺-MC(**1**)-DB(**7**)).

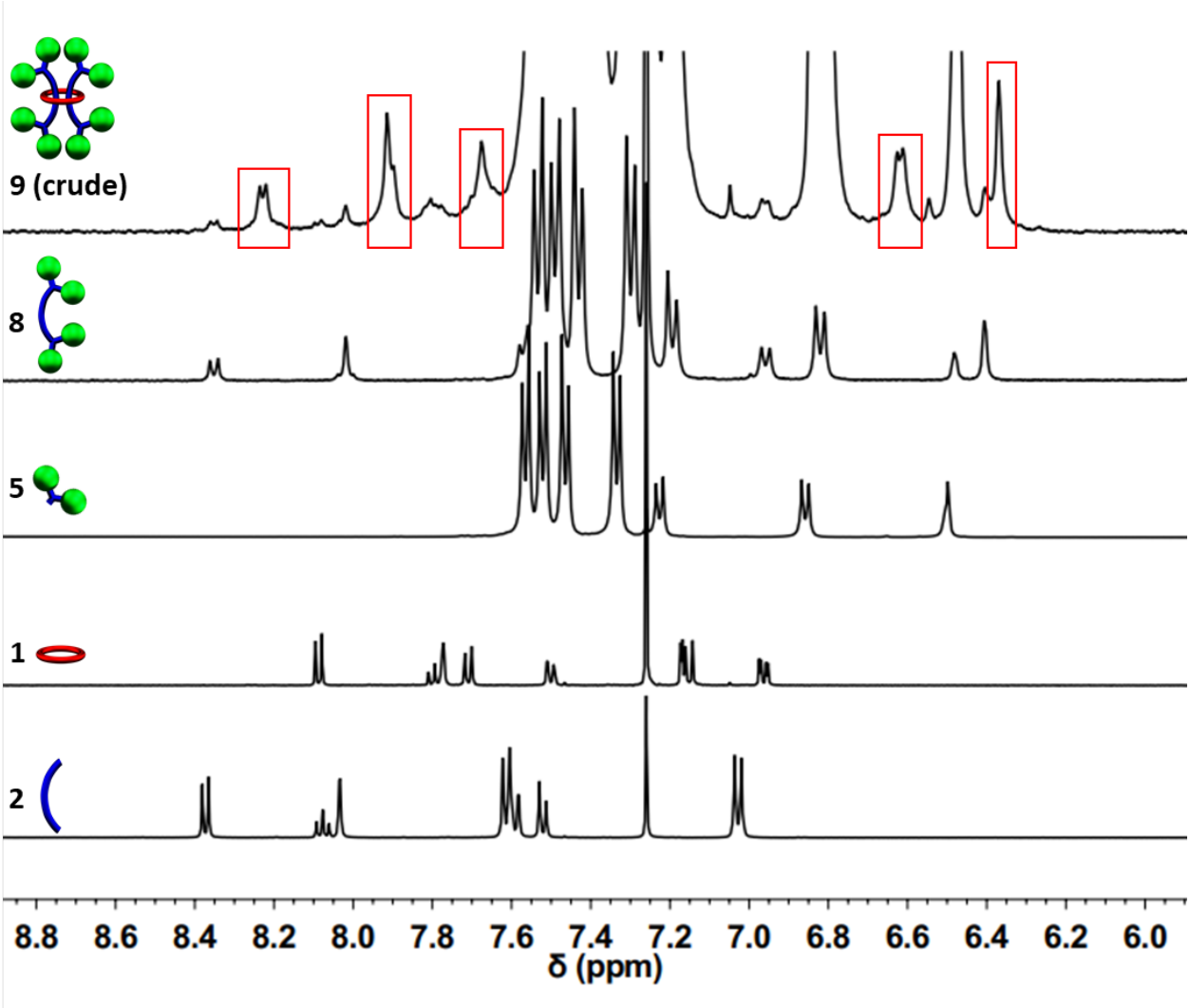


Figure S13. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of crude **9**. Top NMR spectrum corresponds to the crude demetallated reaction mixture of **9**. Bottom four spectra correspond to indicated components and starting materials for comparison. Upfield shifted peaks from interlocked product outlined in red.

NMR Analysis of **9**

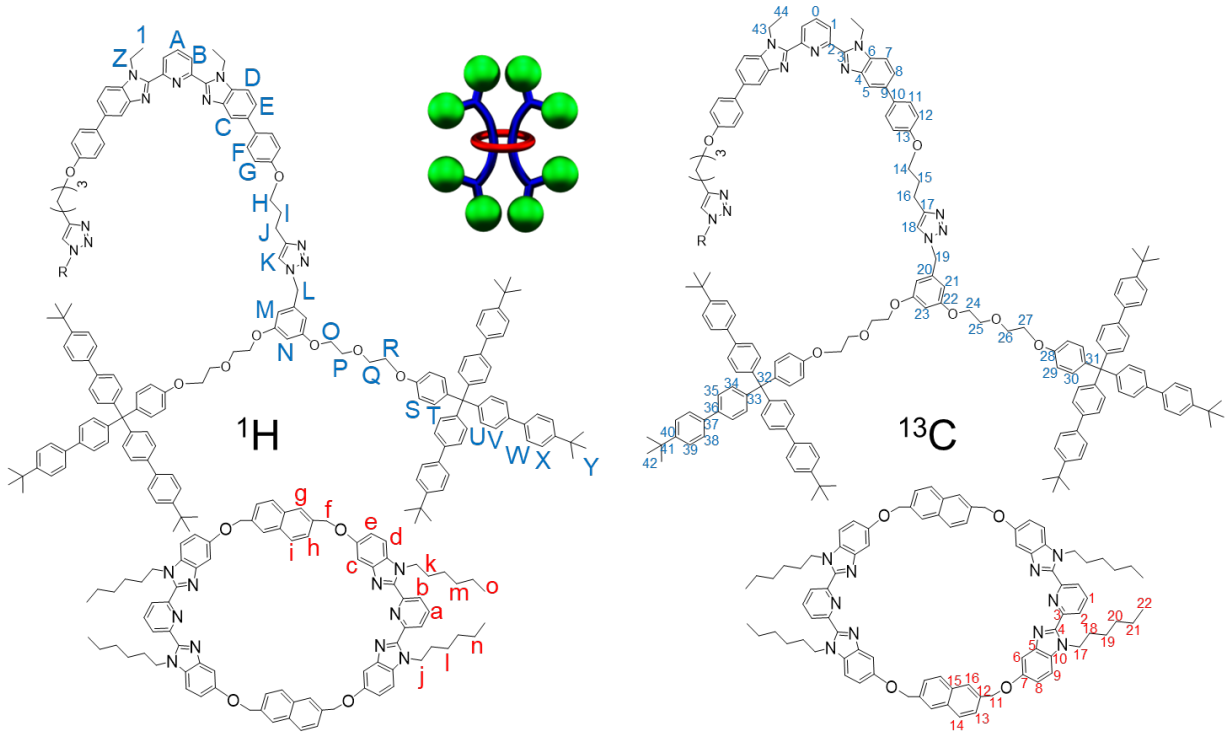


Figure S14. ^{1}H and ^{13}C-NMR labeling assignments of **9**.

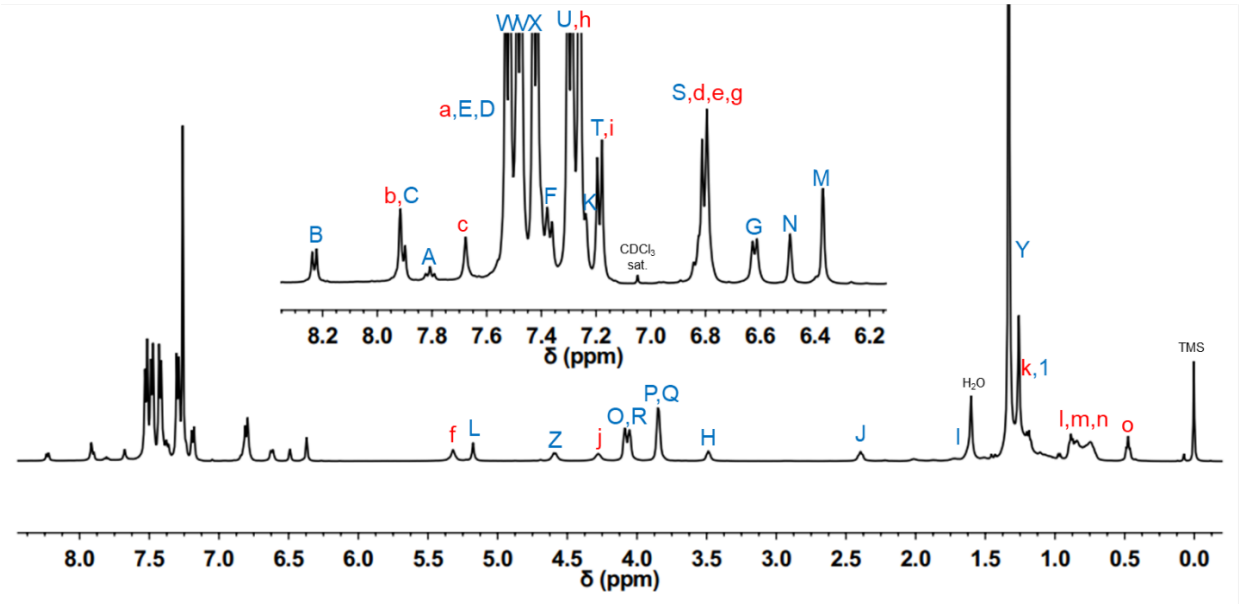


Figure S15. ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of doubly threaded [3]rotaxane **9**. Peak assignments correspond to those given in Figure S14.

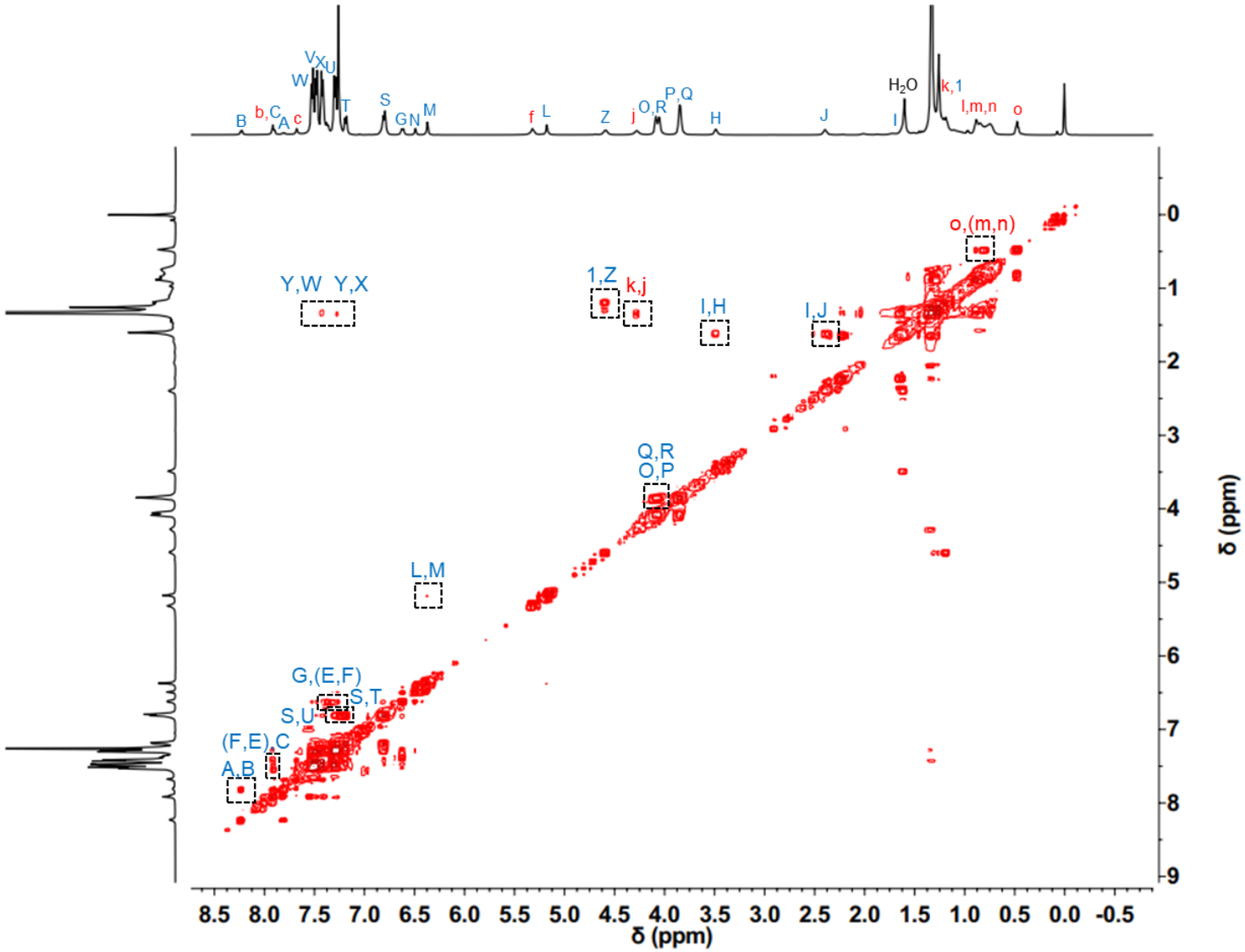


Figure S16. Full ^{1}H-^{1}H COSY (5mM, 500 MHz, 25°C, $CDCl_3$) of **9**. Select ^{1}H annotations correspond to labels in Figure S14.

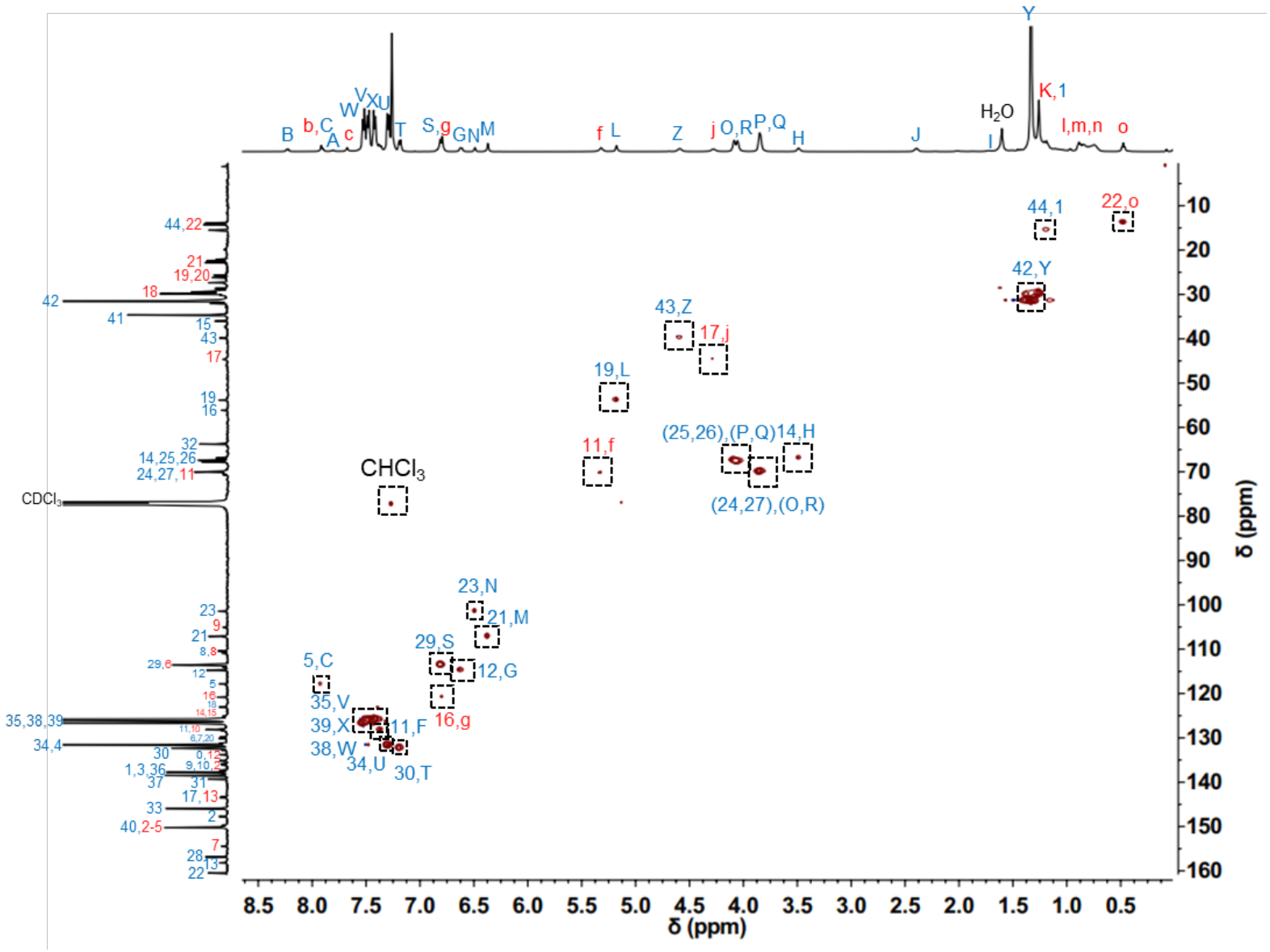


Figure S17. Full HSQC (5mM, 500 MHz, 25°C, $CDCl_3$) of **9**. Full ^{13}C and select ^{1}H annotations correspond to labels in Figure S14.

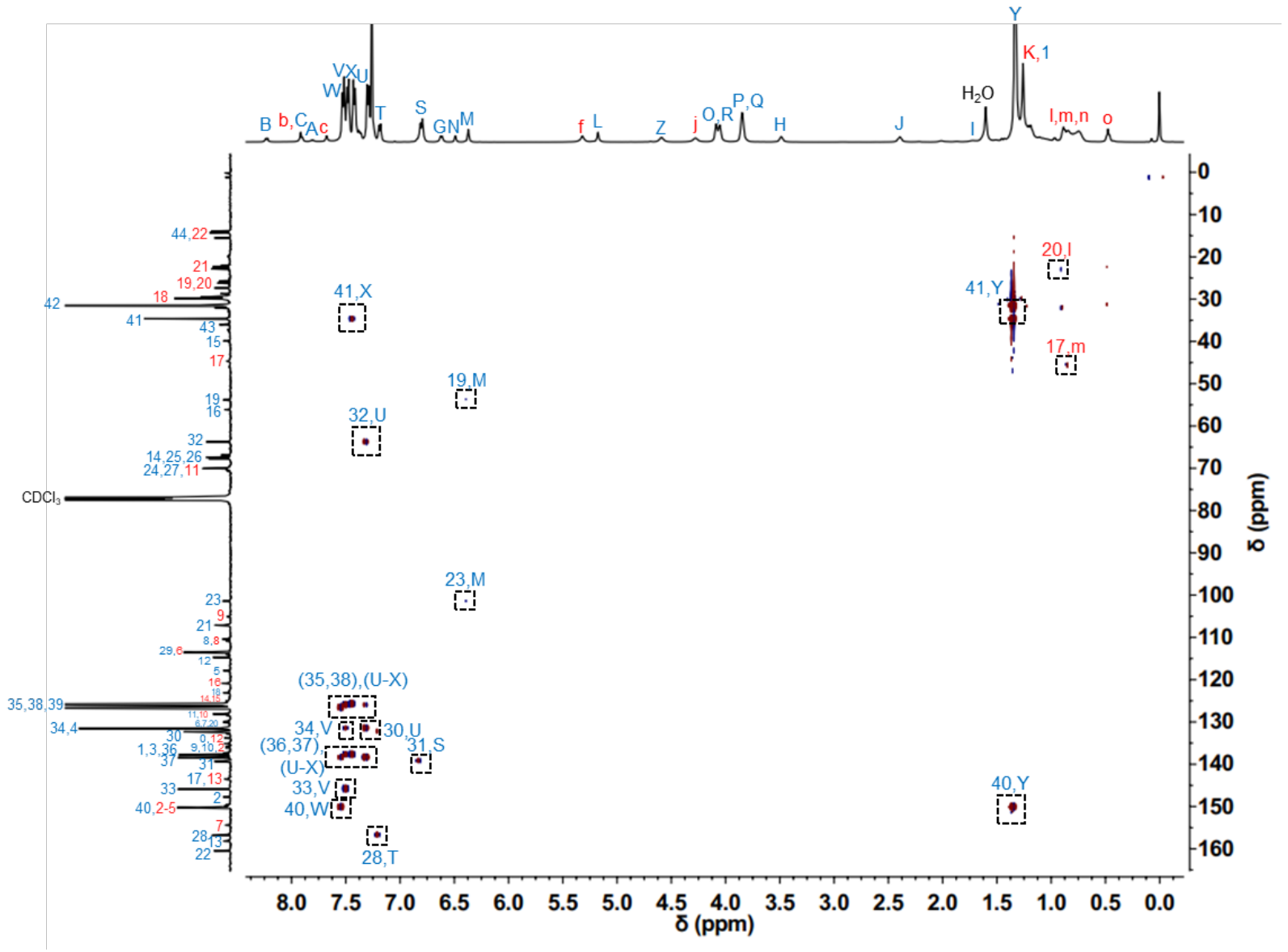


Figure S18. Full HMBC (5mM, 500 MHz, 25°C, $CDCl_3$) of **9**. Full ^{13}C and select ^{1}H annotations correspond to labels in Figure S14.

DOSY Analysis

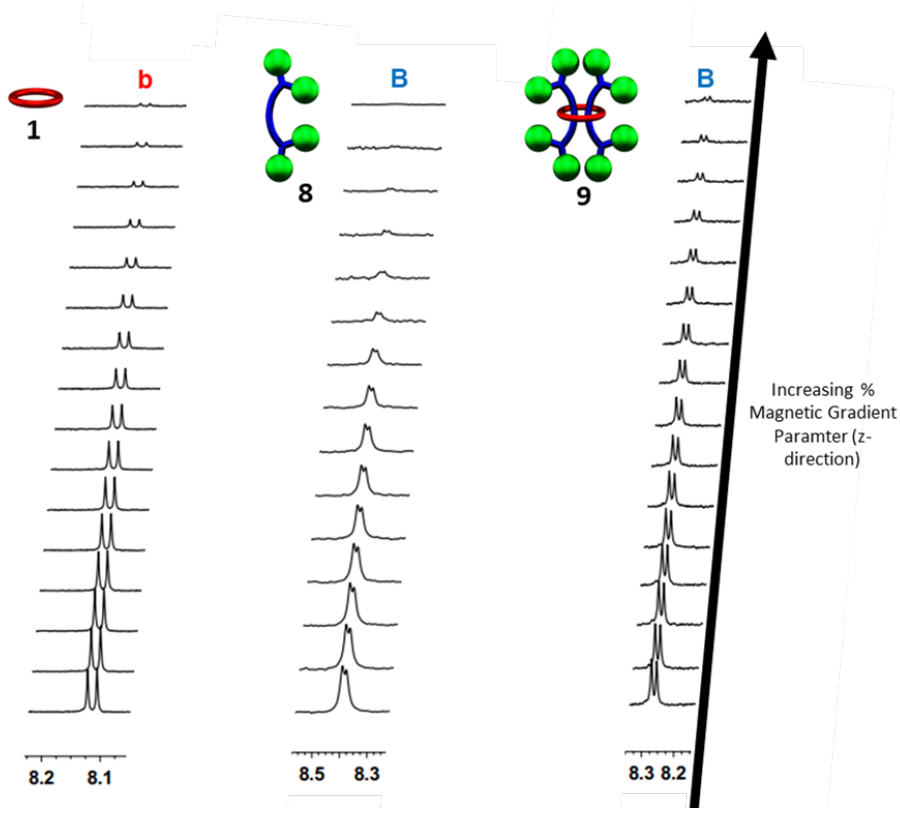


Figure S19. Partial ^{1}H-NMR overlay (1mM, 500 MHz, 25°C, $CDCl_3$) of increasing diffusion gradient strength from 5% (bottom) to 95% (top) for a) **1**, b) **8**, c) **9**. ^{1}H assignments from Figure S14.

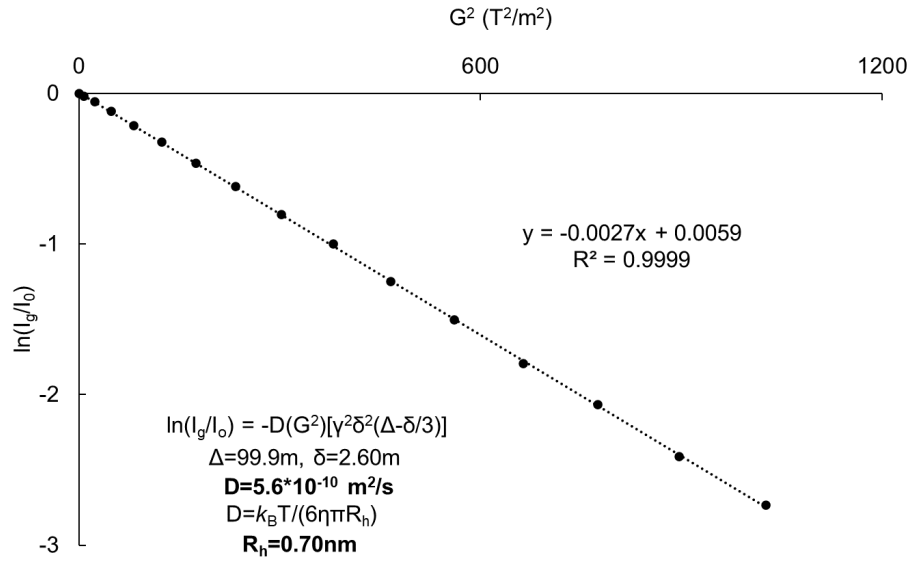


Figure S20. Plot for the calculation of diffusion coefficient of **1**.

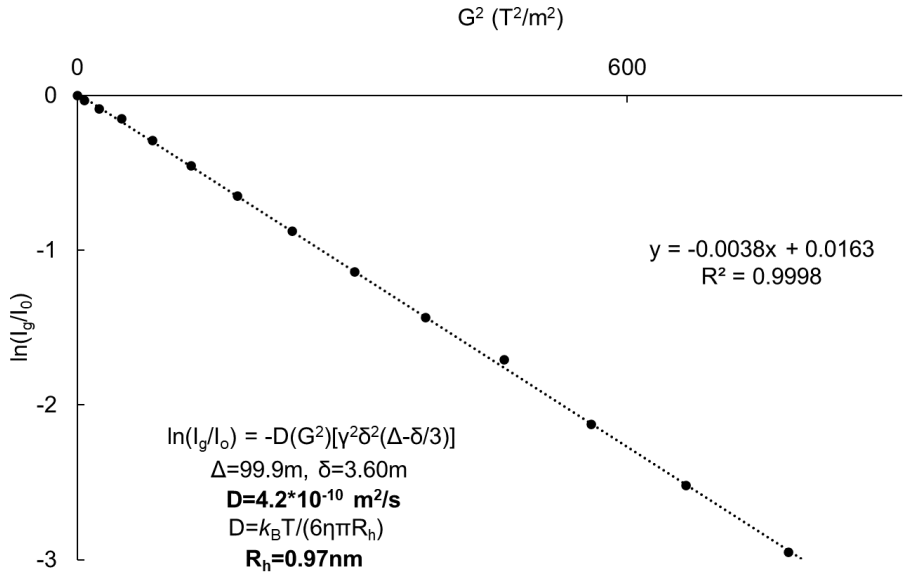


Figure S21. Plot for the calculation of diffusion coefficient of **8**.

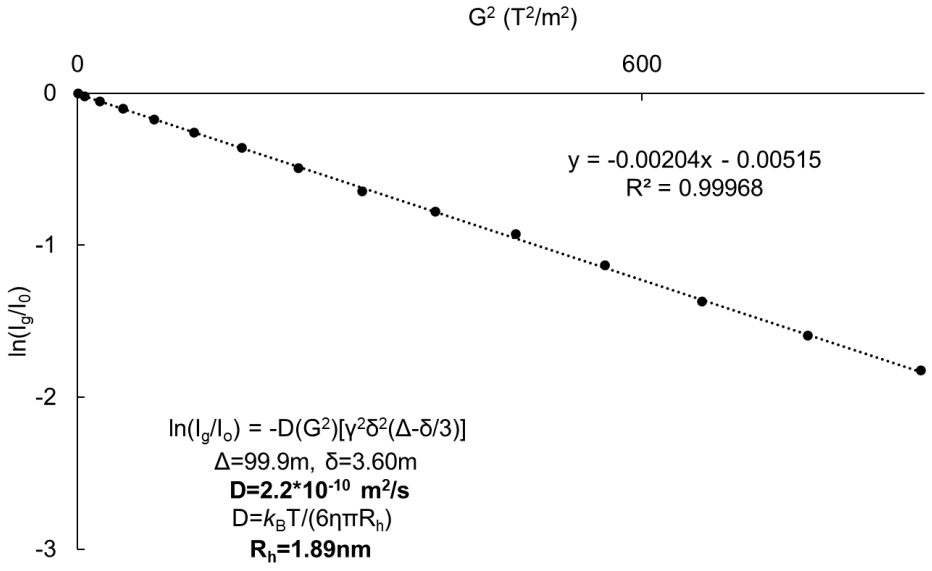


Figure S22. Plot for the calculation of diffusion coefficient of **9**.

^{1}H-^{1}H NOESY Analysis

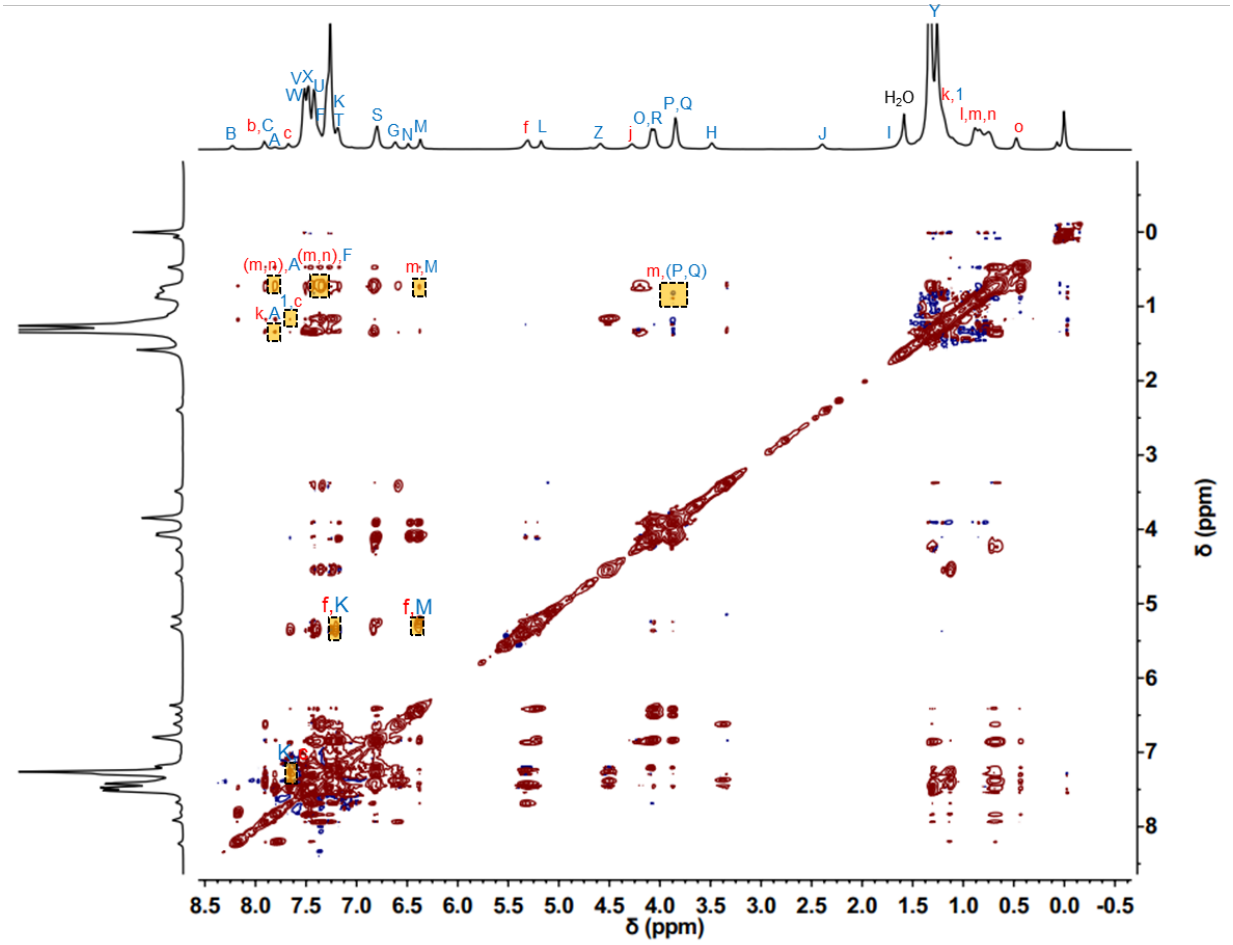


Figure S23. Full ^{1}H-^{1}H NOESY spectrum (1mM, 500 MHz, 5°C, $CDCl_3$) of doubly threaded [3]rotaxane **9.** Select ^{1}H annotations correspond to labels in Figure S14.

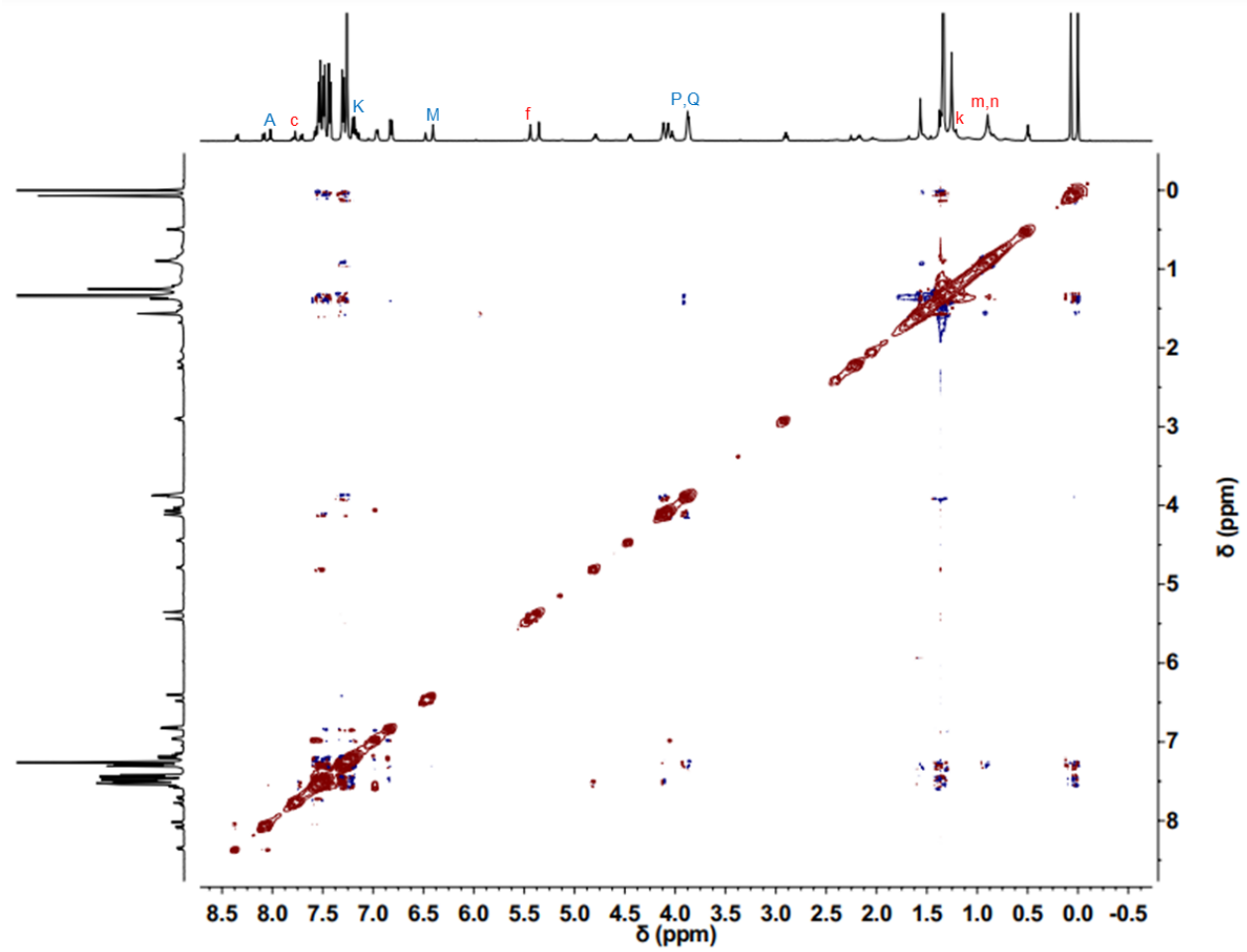


Figure S24. Full NOESY spectrum(1mM, 500 MHz, 5°C, $CDCl_3$) of 2:1 solution of **8**:**1** (noninterlocked control). Select ^1H annotations correspond to labels in Figure S14.

^{1}H-^{1}H NOESY Molecular Simulation Details

Simulations were carried out using the OpenMM[4] molecular dynamics package. The OPLS-AA force field[5] was used with a 1.5 nm cutoff radius for Columbic and Leonard-Jones interactions. Simulations were conducted with implicit solvent with non-periodic boundary conditions using a Langevin integrator at 298K with a 2 fs time step. To more clearly resolve the thread-macrocycle interactions, a tension force of 200 pN was applied independently to each dumbbell to maintain a parallel, nearly-linear conformation. A total of $2x10^{7}$ timesteps were performed, leading to a simulation time of 40 ns.

Most components could be adequately described via analogy using existing OPLS atom types and parameters. However, a dihedral potential for rotation around the bi-aryl pyridine benzimidazole bond, which greatly affects the shape of Bip containing molecules, was not available, custom parameters taken from previous work were employed.[6] The alkyl chains on the Bip moieties differ slightly from those reported in the experimental section, but these were judged insignificant to the interaction in question.

The reported structures were identified and visualized with the Visual Molecular Dynamics (VMD) software.[7] Conformations were attributed to NOEs 1, 2, 4, 5, 6, 8, 9 and 10 when the distance between the relevant hydrogens was below 0.5 nm. No conformations meeting this criterion could be identified for NOE's 3 and 7 in this simulation.

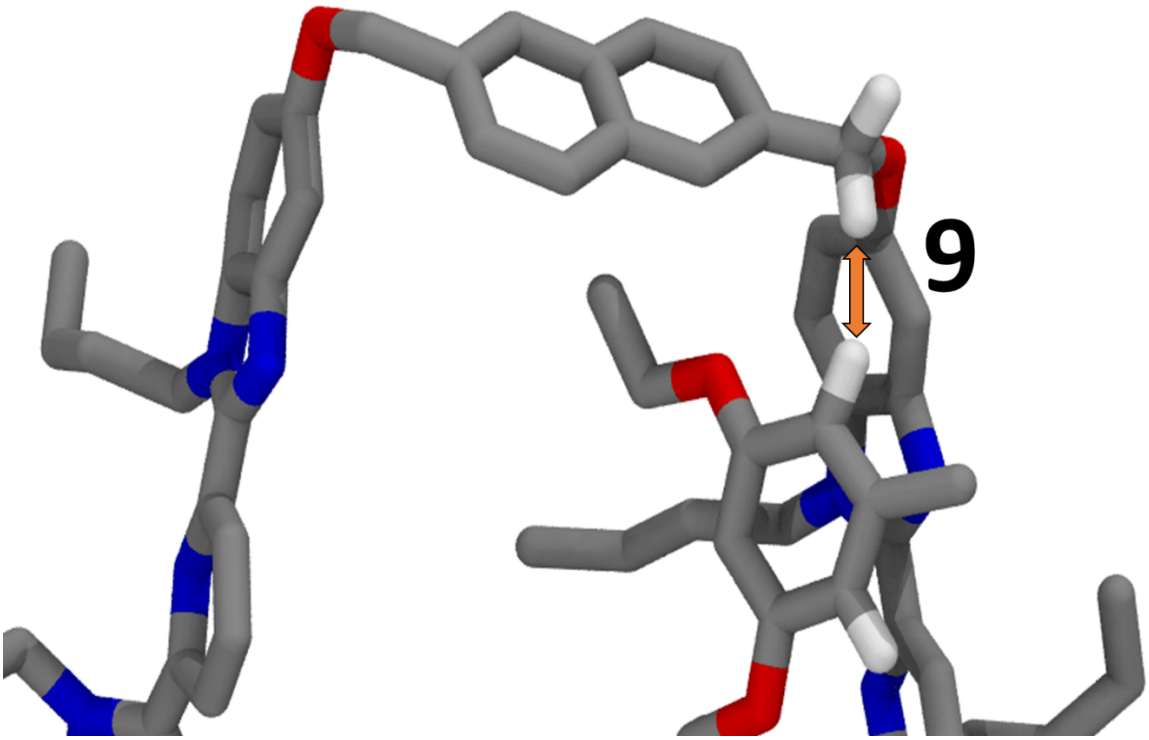


Figure S25. All-atom implicit-solvent model render of NOE 9.

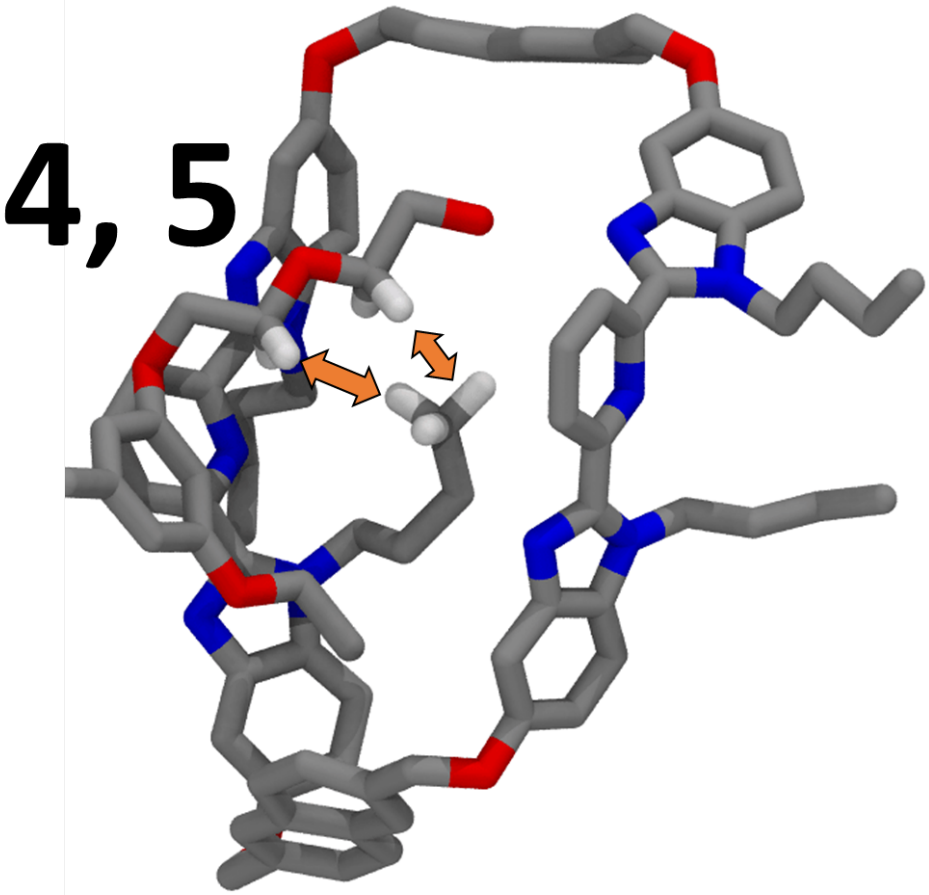


Figure S26. All-atom implicit-solvent model render of NOEs 4+5.

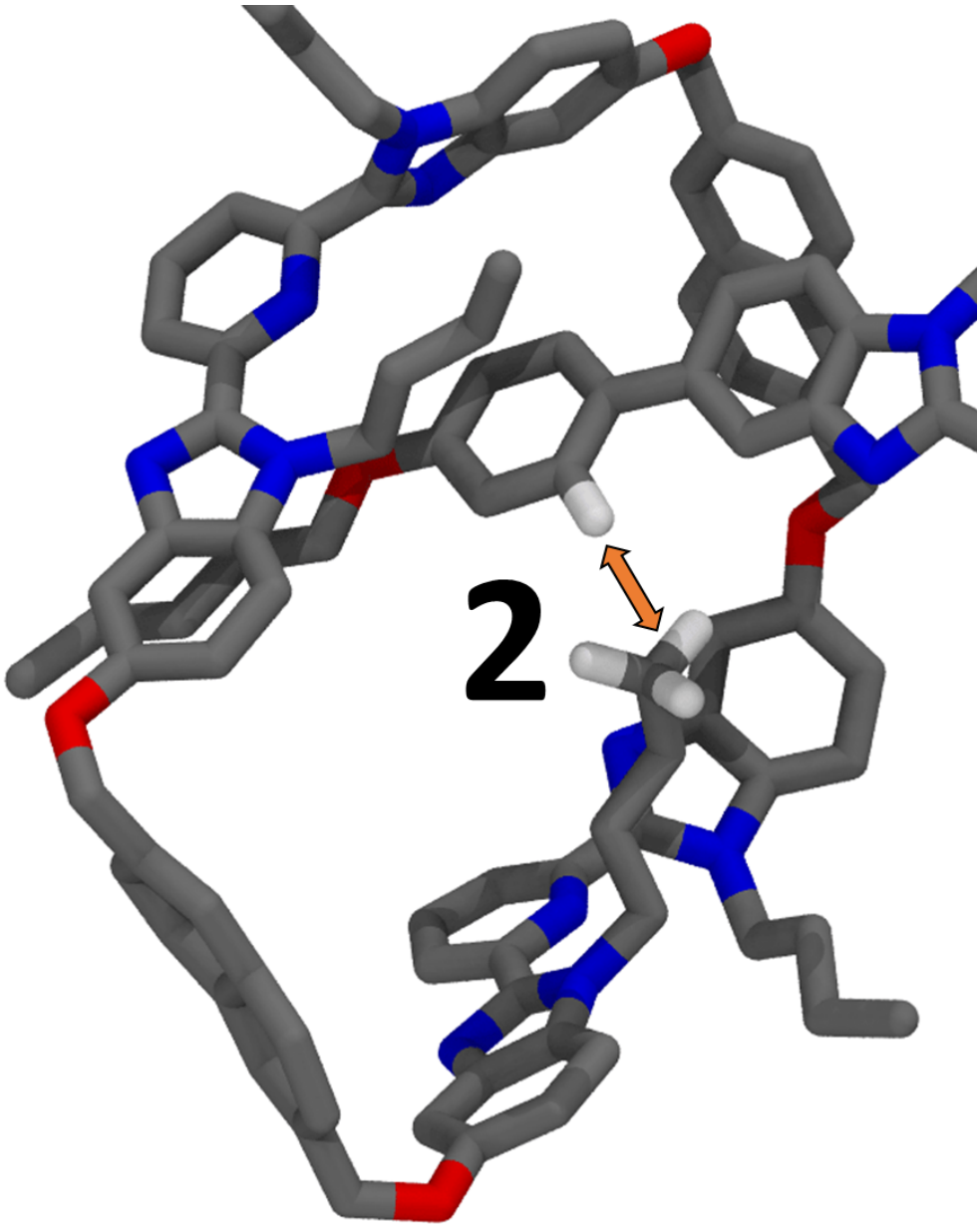


Figure S27. All-atom implicit-solvent model render of NOE 2.

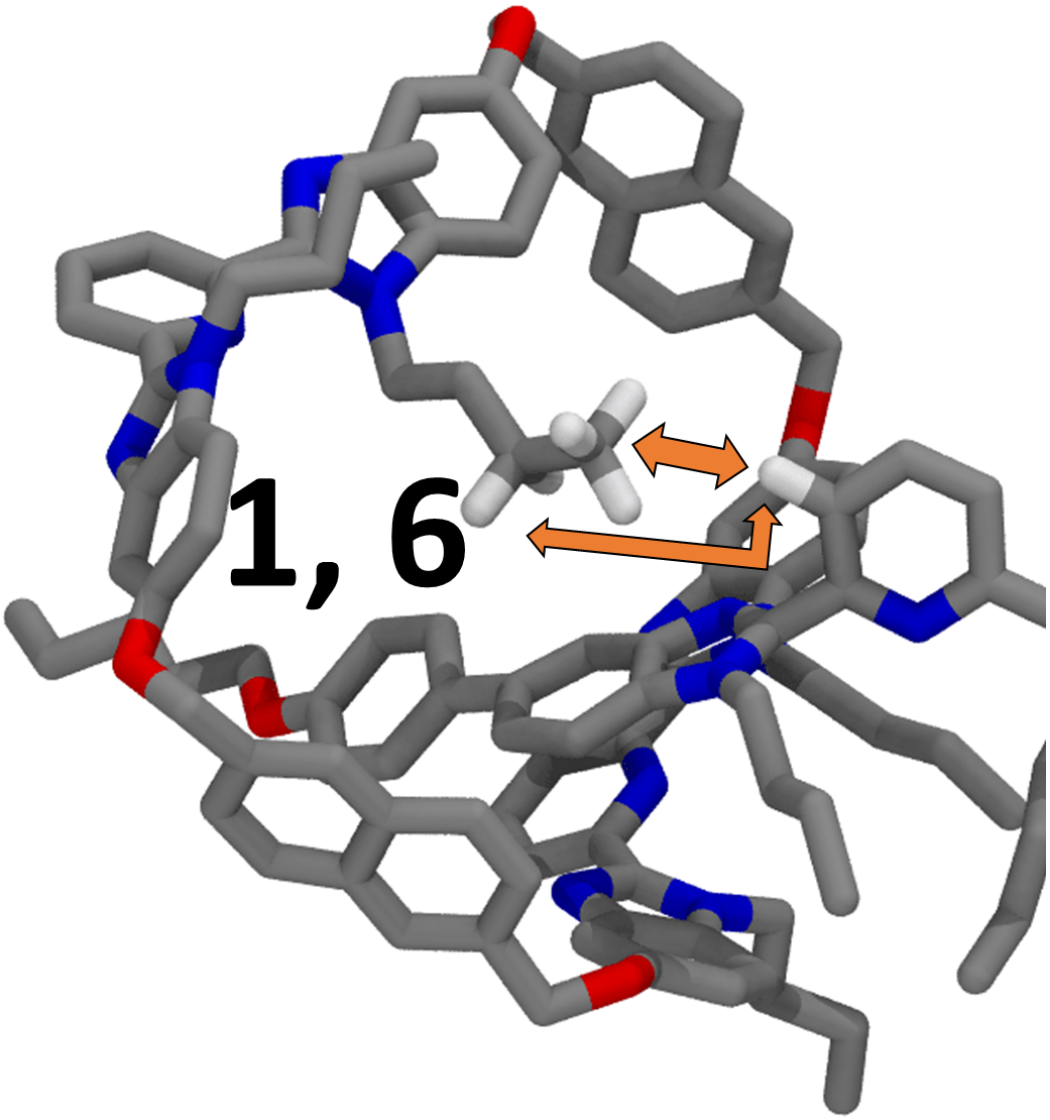


Figure S28. All-atom implicit-solvent model render of NOEs 1+6.

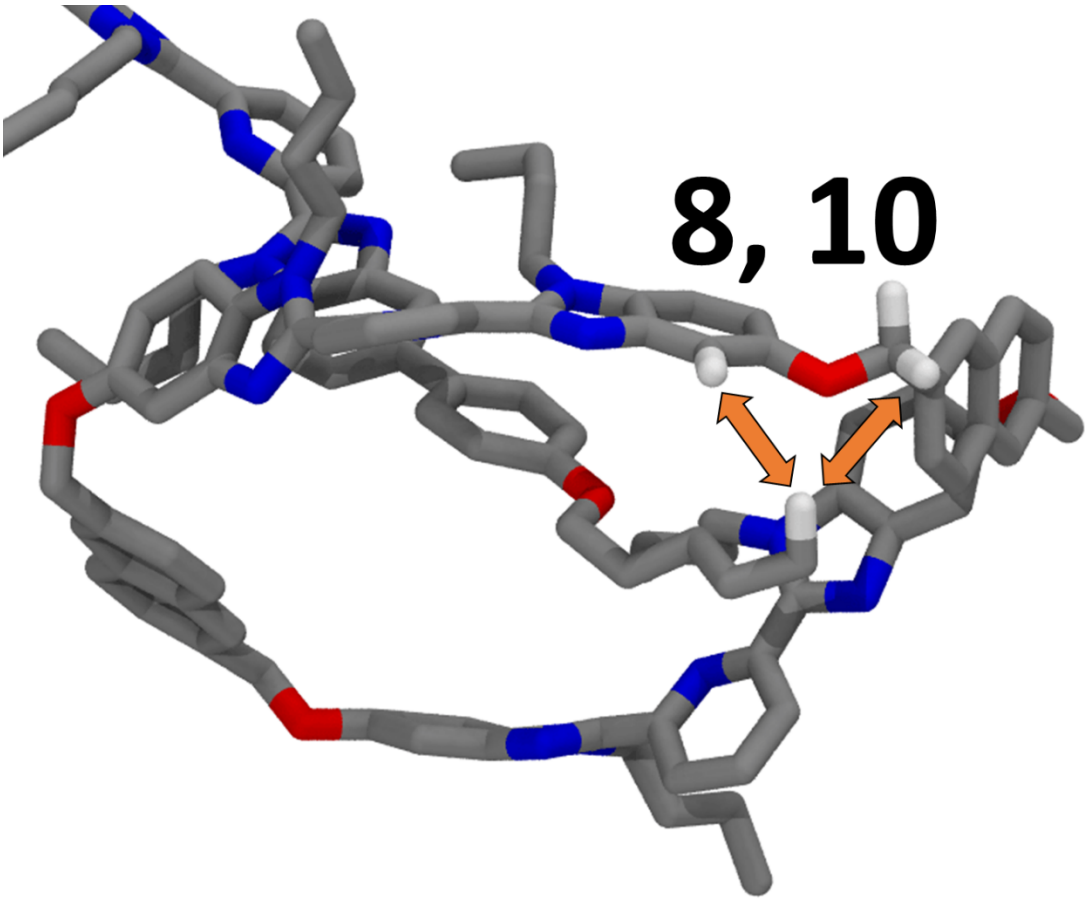
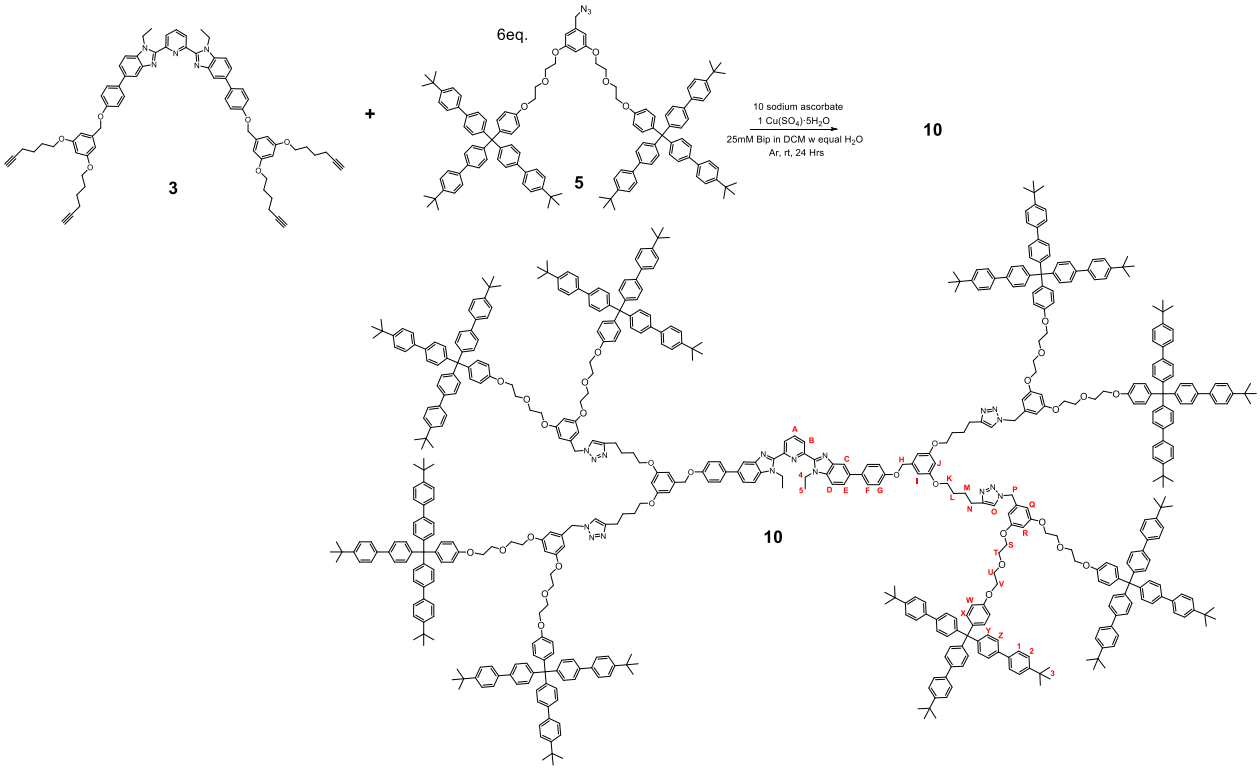


Figure S29. All-atom implicit-solvent model render of NOEs 8+10.

Stoppering of 1:3$_2$:Fe(II)$_2$, Demetallation, and Characterization of 11

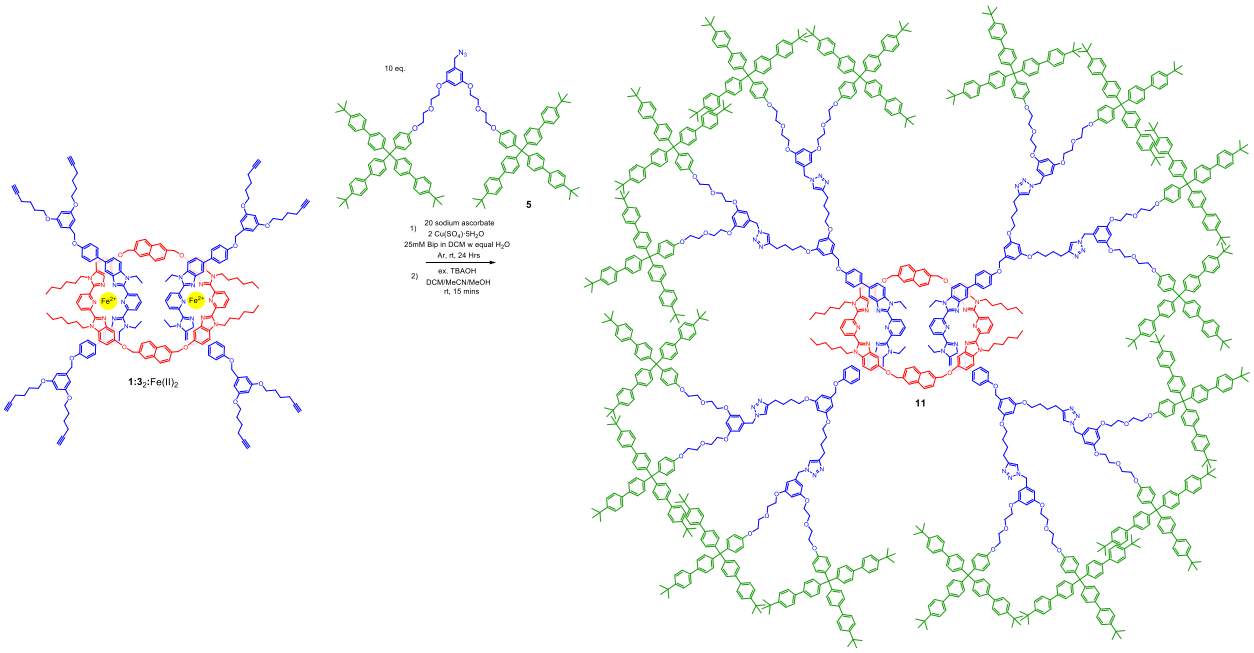
Synthesis of Dumbbell Component (**10**)



Scheme S20. Synthesis of **10**.

6 (51mg, 0.028mmol, 6eq.) was added to a 4mL glass vial followed by **3** (5.3mg, 0.0047mmol), sodium ascorbate (9.3mg, 0.047mmol, 10eq.), and the reaction chamber was purged with Ar. 200µL DCM (25mM **3**), 100µL H_2O, and finally 100µL of stock $Cu(SO_4)\cdot 5H_2O$ in H_2O solution (1.2mg, 0.0047mmol, 1.0eq, (25mol% per alkyne)) were added to reaction mixture. The reaction mixture was left to stir overnight and then diluted with 10mL DCM and 10mL H_2O. The organic layer was isolated and washed with 2x5mL H_2O before the organic solvent was removed under reduced pressure resulting in a light brown crude solid. The solid was purified using preparative thin layer chromatography (5% MeOH in $CHCl_3$ as eluent) followed by recrystallization (chloroform/methanol layering) resulting in **10** as white solid in 82% yield (32 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (d, J = 7.9Hz, 2H, A), 8.01 (m, 3H, B+C), 7.57 (d, J = 8.6 Hz, 4H, F), 7.56 – 7.44 (m, 100H, D+E+Z+2), 7.42 (d, J = 8.4 Hz, 48H, Y), 7.28 (d, J = 8.4 Hz, 48H, 1), 7.18 (d, J = 8.9 Hz, 16H, X), 7.15 (s, 4H, O), 7.04 (d, J = 8.3 Hz, 4H, G), 6.80 (d, J = 8.9 Hz, 16H, W), 6.55 (bd, J = 1.8 Hz, 4H, I), 6.46 (bt, J = 2.2 Hz, 4H, R), 6.38 (bd, J = 2.2 Hz, 8H, Q), 6.34 (bt, J = 1.8 Hz, 2H, J), 5.31 (s, 8H, P), 4.97 (s, 4H, H), 4.77 (q, J = 7.1 Hz, 4H, 4), 4.09 (t, J = 5.6 Hz, 16H, V), 4.05 (q, J = 5.6 Hz, 16H, S), 3.92 (bt, J = 6.9 Hz, 8H, K), 3.89-3.81 (m, 32H, T+U), 2.72 (t, J = 6.9 Hz, 8H, N), 1.79 (bs, 16H, L+M), 1.34 (s, 216H, 3), 0.88 (t, J =7.2 Hz, 6H, 5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.52, 160.49, 158.26, 156.87, 150.47, 150.27, 150.09, 148.43, 145.92, 143.64, 139.40, 139.35, 138.49, 137.80, 137.13, 136.32, 135.29, 134.63, 132.34, 131.57, 130.16, 129.87, 128.52, 126.71, 126.04, 125.81, 123.33, 120.83, 118.23, 115.37, 113.58, 110.48, 107.07, 105.85, 101.63, 100.85, 70.25, 70.08, 69.90, 67.74, 67.43, 63.77, 54.09, 40.07, 34.65, 31.50, 29.85, 28.93, 26.05, 25.56, 15.63. MALDI-MS: 8,197.3 ([M]+Na^+).

Stoppering of 1:$\mathbf{3}_2$:Fe(II)$_2$ with **5**



Scheme S21. Stoppering and demetallation of doubly threaded [3]rotaxane **11**.

1:$\mathbf{3}_2$:Fe(II)$_2$ (17.2 mg, 0.0036mmol), **5** (63.5 mg, 0.036 mmol, 10eq) and sodium ascorbate (13.8 mg, 0.036 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.62 mL, conc of alkyne = 25 mM), H_2O (0.52 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (72mM, 0.0072mmol, 2eq, (25mol% per alkyne)). The reaction was stirred vigorously for 18 h at RT. After this time the reaction mixture was diluted with CH_2Cl_2 and H_2O (5 mL each). The organic layer was taken and washed with H_2O (2 × 5 mL). Removal of the organic solvent resulted in a purple solid that was washed 3x with 5mL of 10% chloroform in hexanes to help remove leftover stopper group. The crude purple solid was redissolved in 5mL of 50:50 dichloromethane and acetonitrile and stirred slowly at room temperature. 1mL of tetrabutylammonium hydroxide solution (1M in MeOH) was added dropwise to the stirring solution resulting in a rapid color change from purple to light brown and precipitation of the demetallated product. After 15 minutes of stirring, the off-white solid was filtered off and washed with methanol (2x 10mL). The frit was then washed with 5mL of $CHCl_3$ to redissolve the demetallated product and washed once with 5mL water. Solvent was removed under reduced pressure resulting in an off-white residue. Purification of **11** was achieved using preparative thin layer chromatography (SiO_2, eluent = 94:6 $CHCl_3$:MeOH, lowest Rf band taken (R_f=0.15-0.3) as [3]R product, macrocycle byproduct R_f=0.35, dumbbell byproduct R_f=0.70-0.80) followed by precipitation from cold methanol resulting in an off-white solid, **11** in 65% isolated yield (41.1 mg). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (bd, J = 8.5 Hz, 4H), 7.85 (bd, J = 8.4 Hz, 4H), 7.73 (s, 4H), 7.69 (s, 4H), 7.53-7.32 (m, 308H), 7.29 (d, J = 8.2 Hz, 96H), 7.20-7.14 (m, 52H), 6.98 (bd, 4H), 6.82-6.74 (m, 44H), 6.56 (bd, J = 8.4 Hz, 8H), 6.46-6.40 (m, 16H), 6.38-6.31 (m, 20H), 5.36-5.25 (m, 24H), 4.7(bd, 8H), 4.55 (bd, 8H), 4.36 (bd, 8H), 4.10-3.97 (m, 64H), 3.94-3.78 (m, 80H), 2.70 (m, 16H), 1.72 (m, 16H), 1.36 (s, 512H), 1.28 (bt, 8H), 0.96-0.72 (m, 52H), 0.46 (bt, 12H). MALDI-MS: 17,733.2 ([M]+H$^+$), 9,531.5 ([M]+H$^+$-DB(**10**)), 8,203.1 ([M]+H$^+$-MC(**1**)-DB(**10**)).

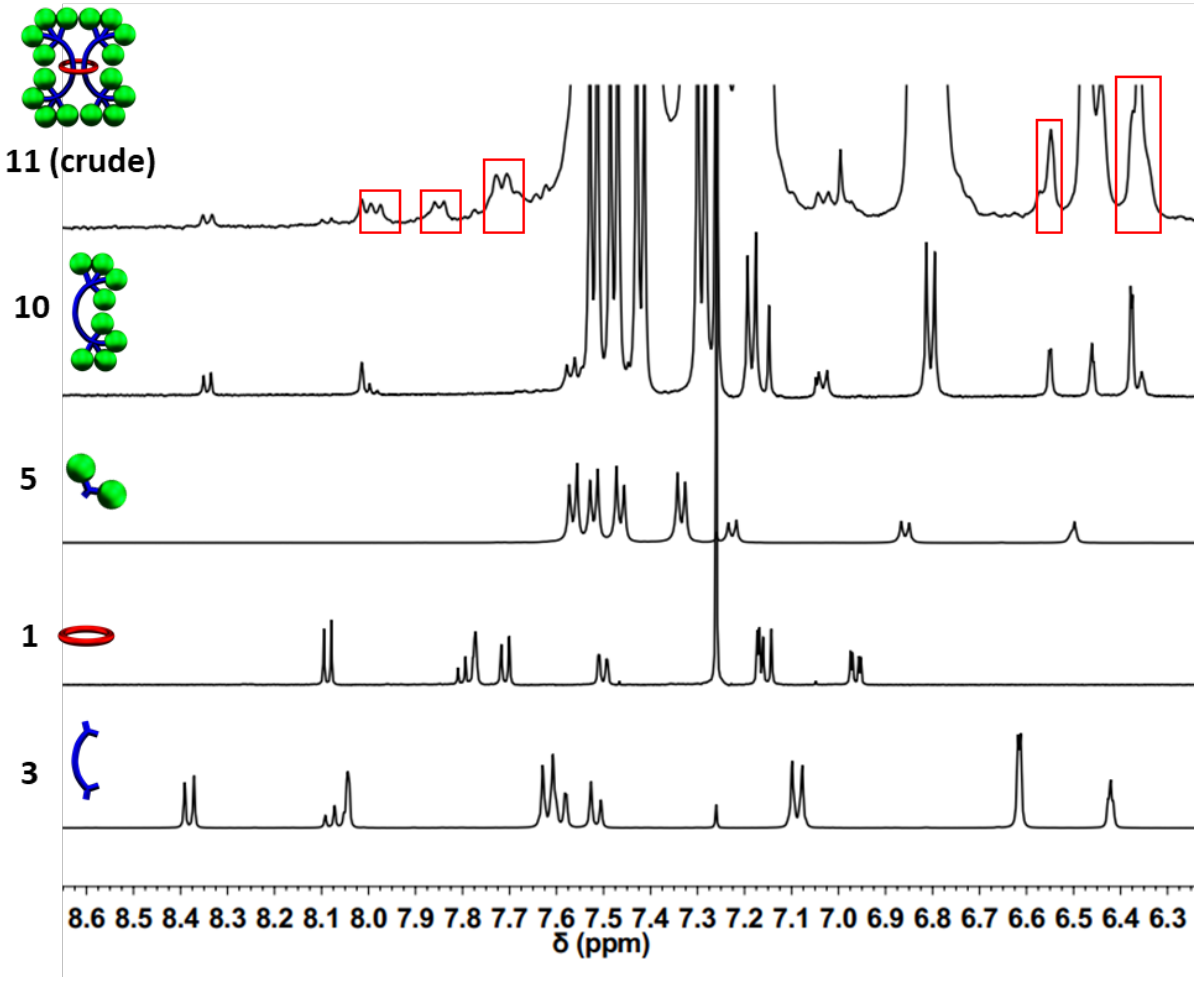


Figure S30. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of crude **11**. Top NMR spectrum corresponds to the crude demetallated reaction mixture of **11**. Bottom four spectra correspond to indicated components and starting materials for comparison. Upfield shifted peaks from interlocked product outlined in red.

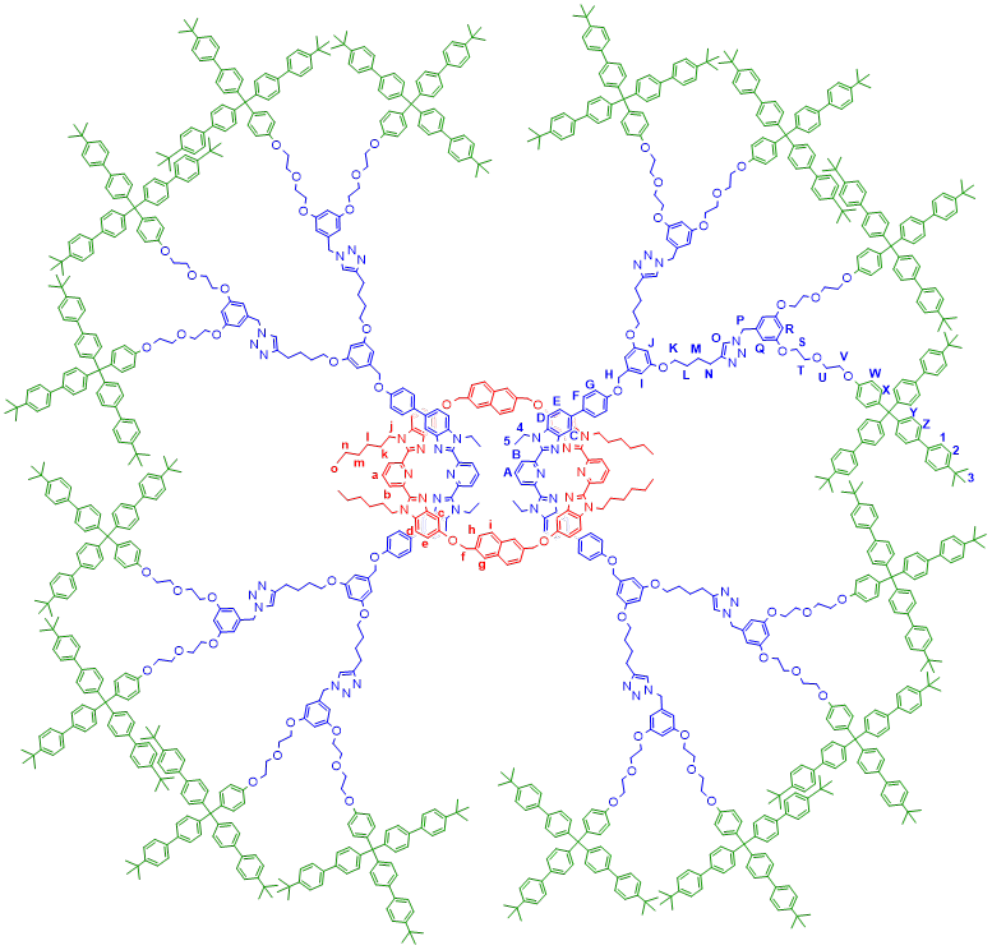


Figure S31. ^{1}H labeling assignments of **11**.

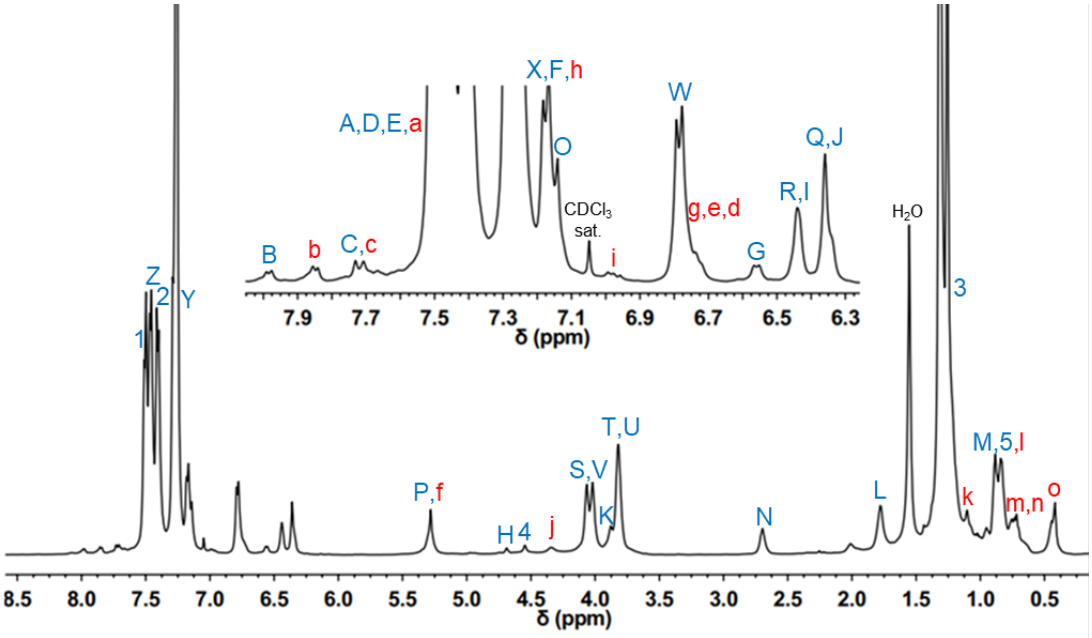


Figure S32. Full ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of doubly threaded [3]rotaxane **11**.

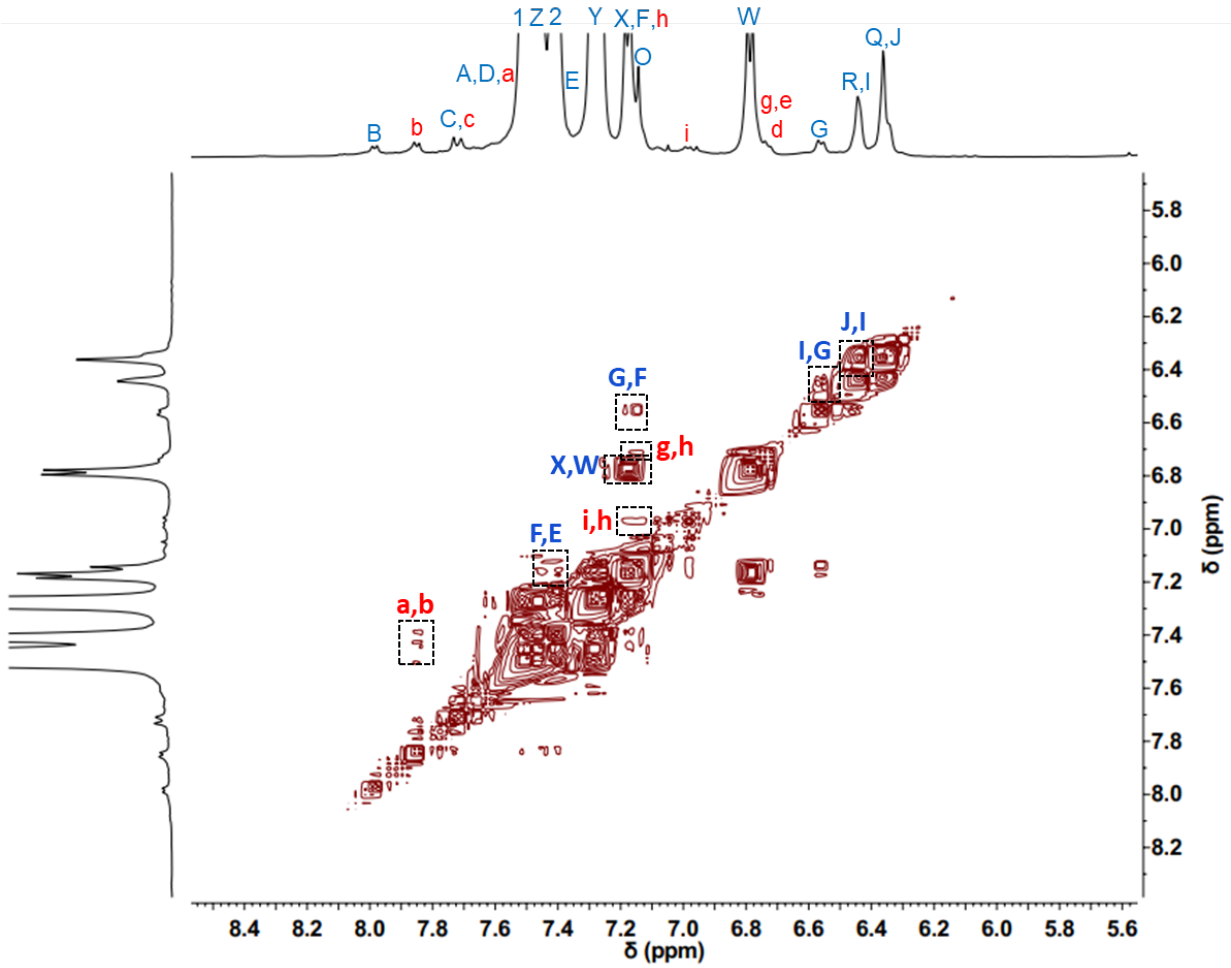


Figure S33. Partial ^{1}H-^{1}H COSY (5mM, 500 MHz, 25°C, $CDCl_3$) of doubly threaded [3]rotaxane **11**. Peak assignments correspond to those given in Figure S31.

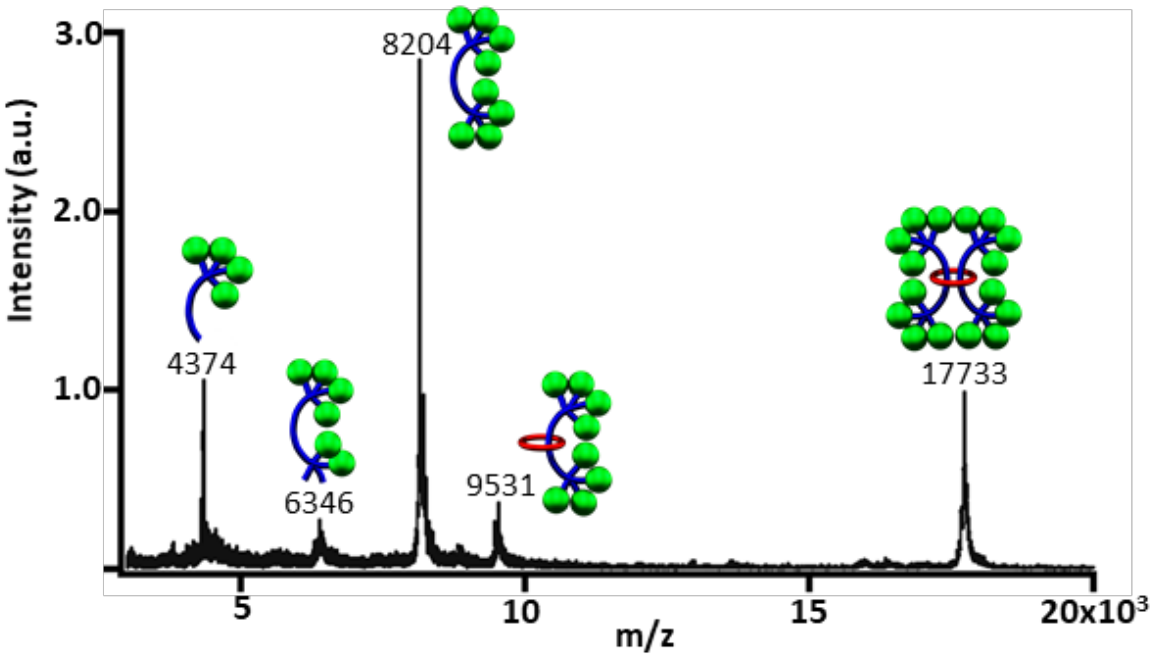


Figure S34. Maldi-TOF MS (Dithranol, no salt) of purified **11**.

Dethreading Kinetics of [3]Rotaxanes 9 and 11

Initial Observations

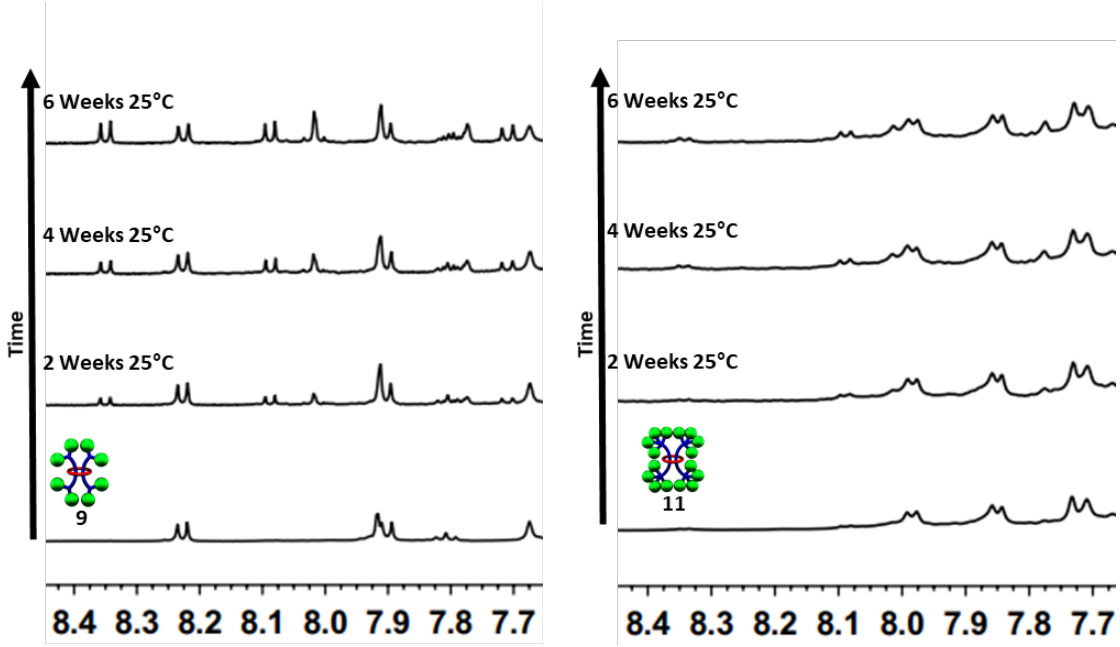


Figure S35. Partial ^{1}H-NMR overlay (1mM, 500 MHz, 25°C, $CDCl_3$) of initial room temperature slippage observations of **9** and **11**.

Kinetic Slippage Experiments of **9**

Kinetic experiments were modeled after work by Sauvage and coworkers.[8] A 1mM sample of fresh **9** was prepared in a 5 mm Bruker Shigemi NMR tube. This tube was placed in an oil bath at 35°C and the ^{1}H-NMR spectrum was recorded every 24 hours for 5 days and then after another 48 hours for a seventh day. The tube was then heated to 40°C and the same ^{1}H-NMR acquisition process was repeated. The tube was then heated to 45°C and the same ^{1}H-NMR acquisition process was repeated. In total, the [3]rotaxane was heated for three weeks. This process was repeated in triplicate.

The resulting kinetic data was analyzed according to the following method. The downfield doublet corresponding to the proton labeled B in the dumbbell of the [3]rotaxane (Fig S14) was used to determine the amount of [3]R left in each sample as the shift between free and interlocked species was diagnostic and clean of other peaks. The free B doublet is in the region 8.34-8.38ppm and the interlocked B doublet is in the region 8.21-8.25ppm. Let C_0 be the initial concentration of [3]rotaxane and C be the concentration of [3]rotaxane at timepoint *t*. The absolute integral intensity of B that is interlocked ($I_{3[R]}$) divided by the sum of the absolute integral intensities of B that is free and interlocked (I_{DB} + $I_{3[R]}$) multiplied by 100% gives the percent of [3]R remaining in the sample. Dividing this percent rotaxane remaining by the initial rotaxane percent present is equivalent to C/C_0 for each timepoint of slippage. Following first-order kinetics and standard Eyring/Arrhenius methods the thermodynamic and kinetic parameters were determined.

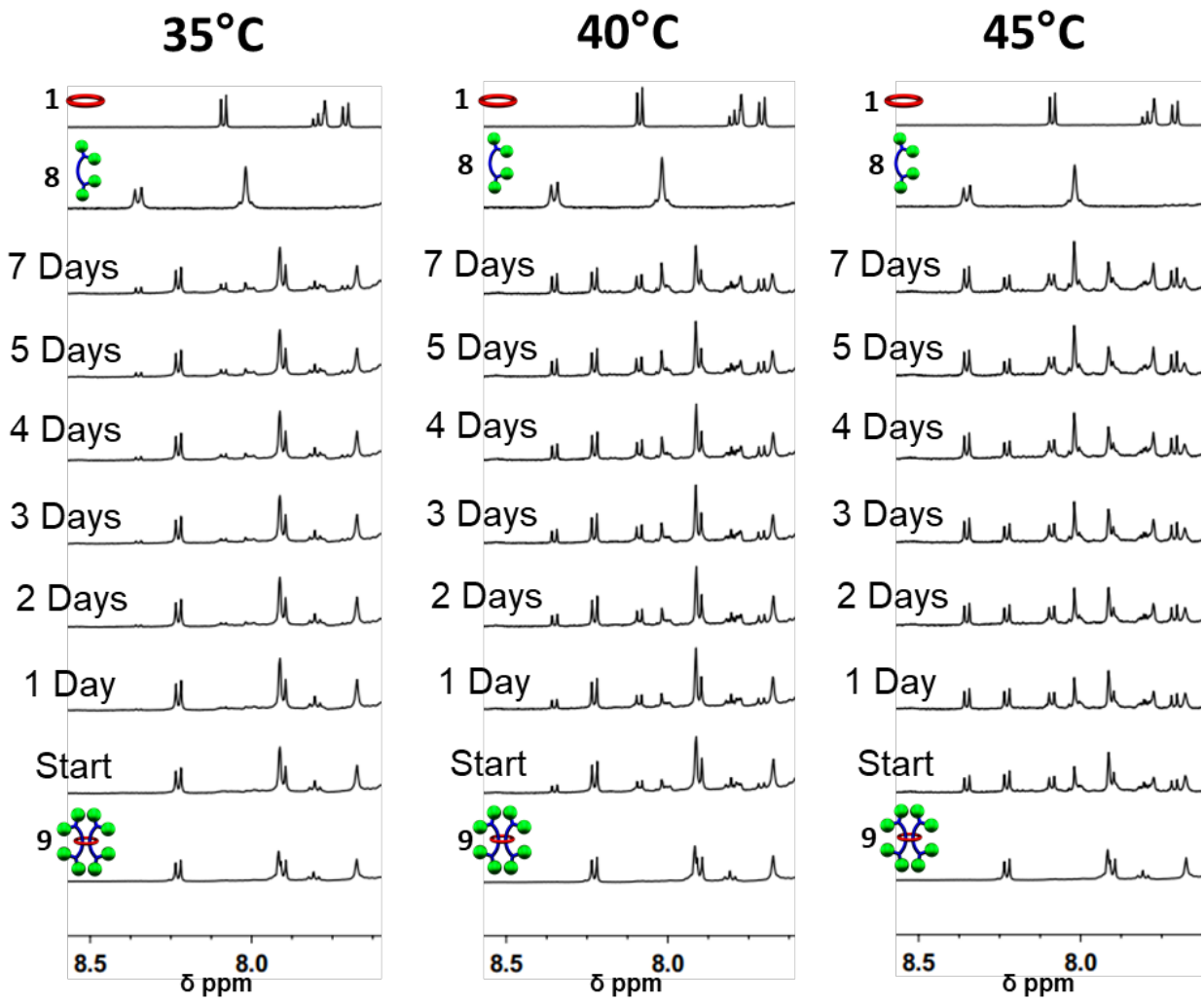


Figure S36. Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of trial 1 of 3-week kinetic slippage experiments of **9**.

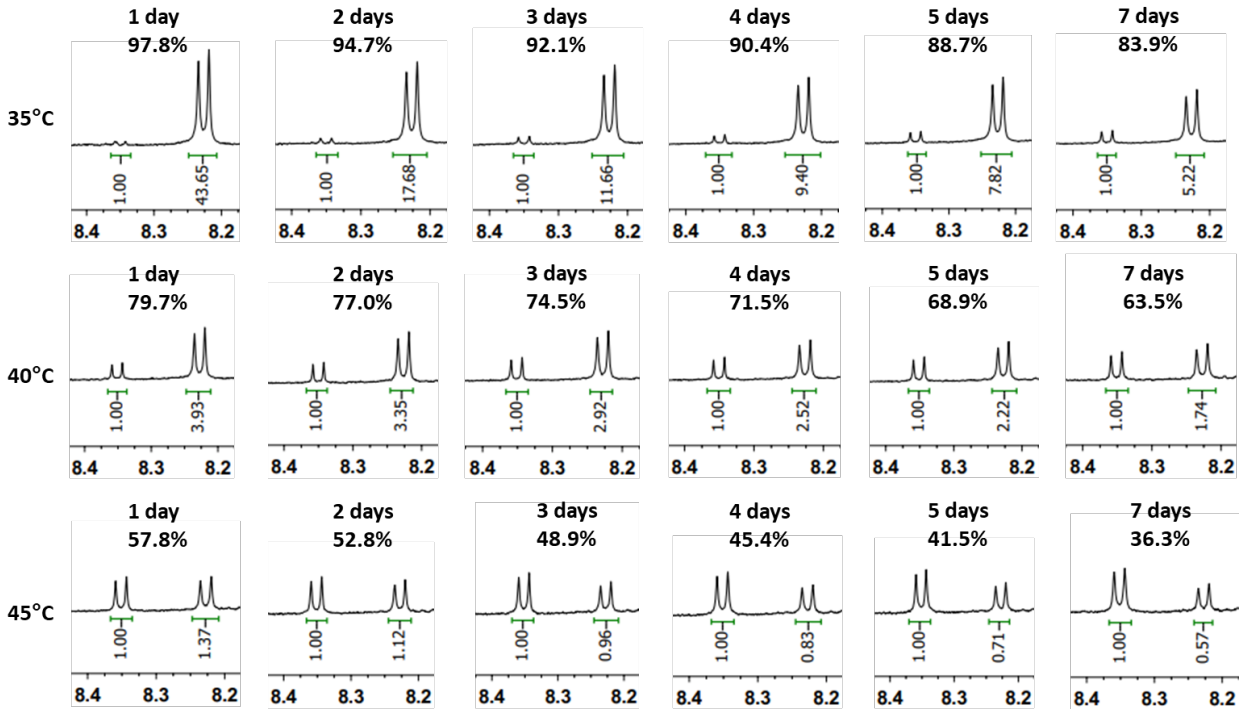


Figure S37. ^{1}H-NMR analysis of trial 1 of slippage experiments of **9.** The free B doublet is in the region 8.34-8.38ppm and slowly increases while the interlocked B doublet is in the region 8.21-8.25ppm and slowly decreases during the 3-week experiment (See Fig S14 for ^{1}H assignments).

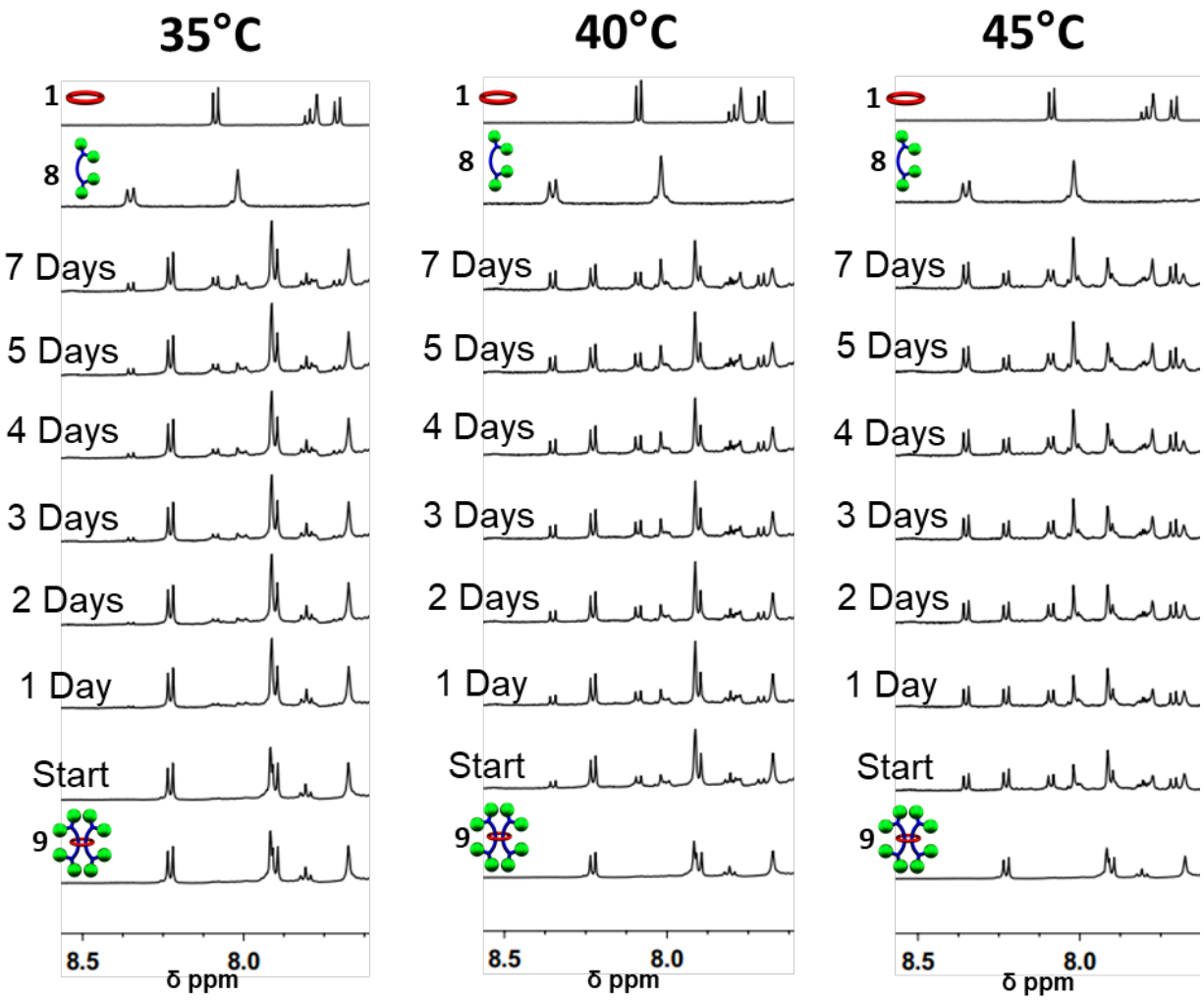


Figure S38. Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of trial 2 of 3-week kinetic slippage experiments of **9**.

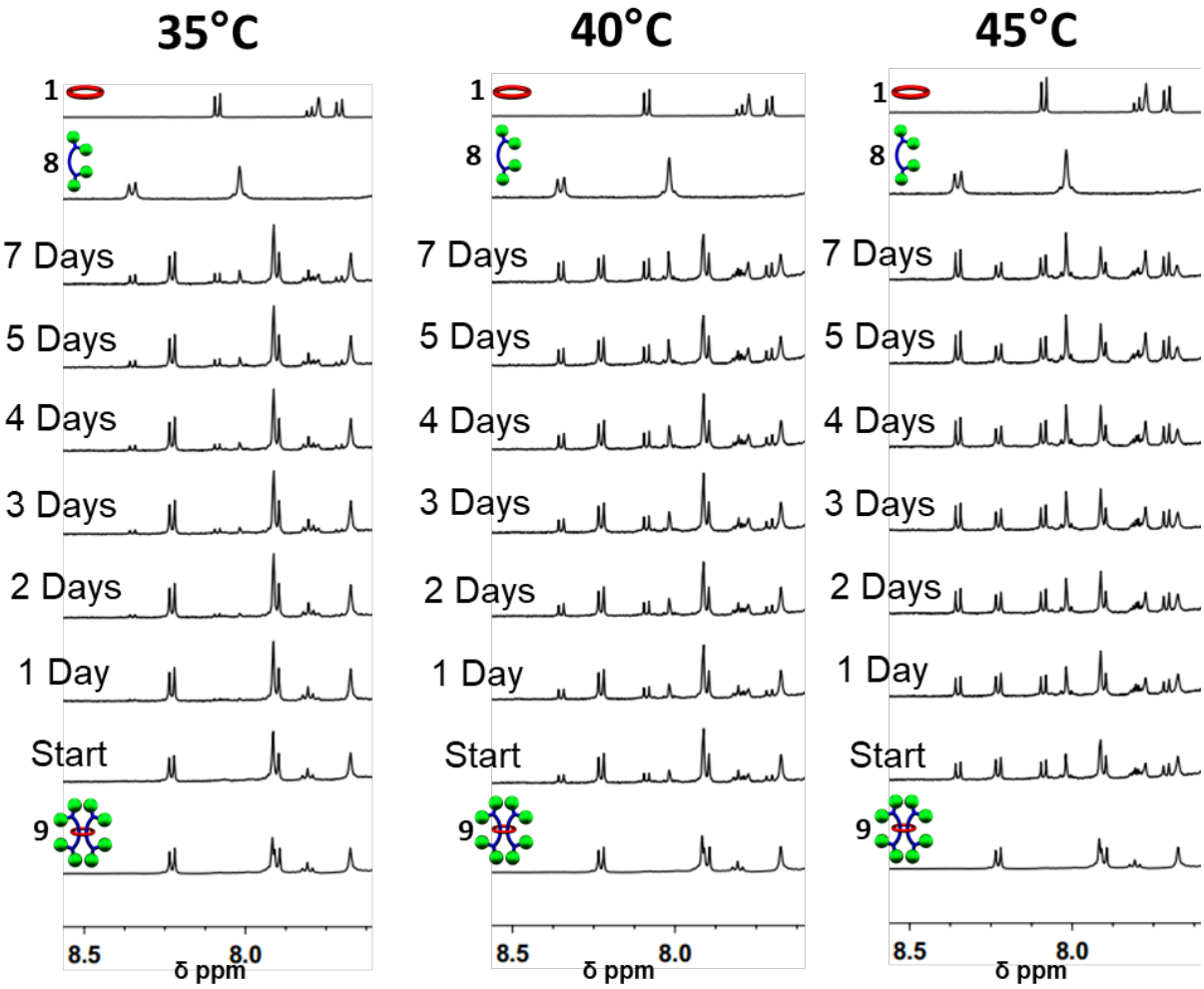


Figure S39. Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of trial 3 of 3-week kinetic slippage experiments of **9**.

Kinetic Slippage Experiments of **11**

Kinetic experiments were conducted similarly to **9** above. A 1mM sample of fresh **11** was prepared in a 5 mm Bruker Shigemi NMR tube. This tube was placed in an oil bath at 35°C and the ^{1}H-NMR spectrum was recorded after 2 days, 4 days, 5 days, and 7 days. The tube was then heated to 40°C and the same ^{1}H-NMR acquisition process was repeated. The tube was then heated to 45°C and the same ^{1}H-NMR acquisition process was repeated. In total, the [3]rotaxane was heated for three weeks. This process was repeated in triplicate. The resulting kinetic data was analyzed following a similar procedure to **9** above. The downfield doublets corresponding to the proton labeled B in the free dumbbell (**10**) and b of the [3]rotaxane **11** (Fig S31) was used to determine the amount of [3]R left in each sample as the shift between free and interlocked species was diagnostic and clean of other peaks. The free B doublet was in the region 8.32-8.37ppm and the interlocked b doublet was in the region 7.82-7.88ppm.

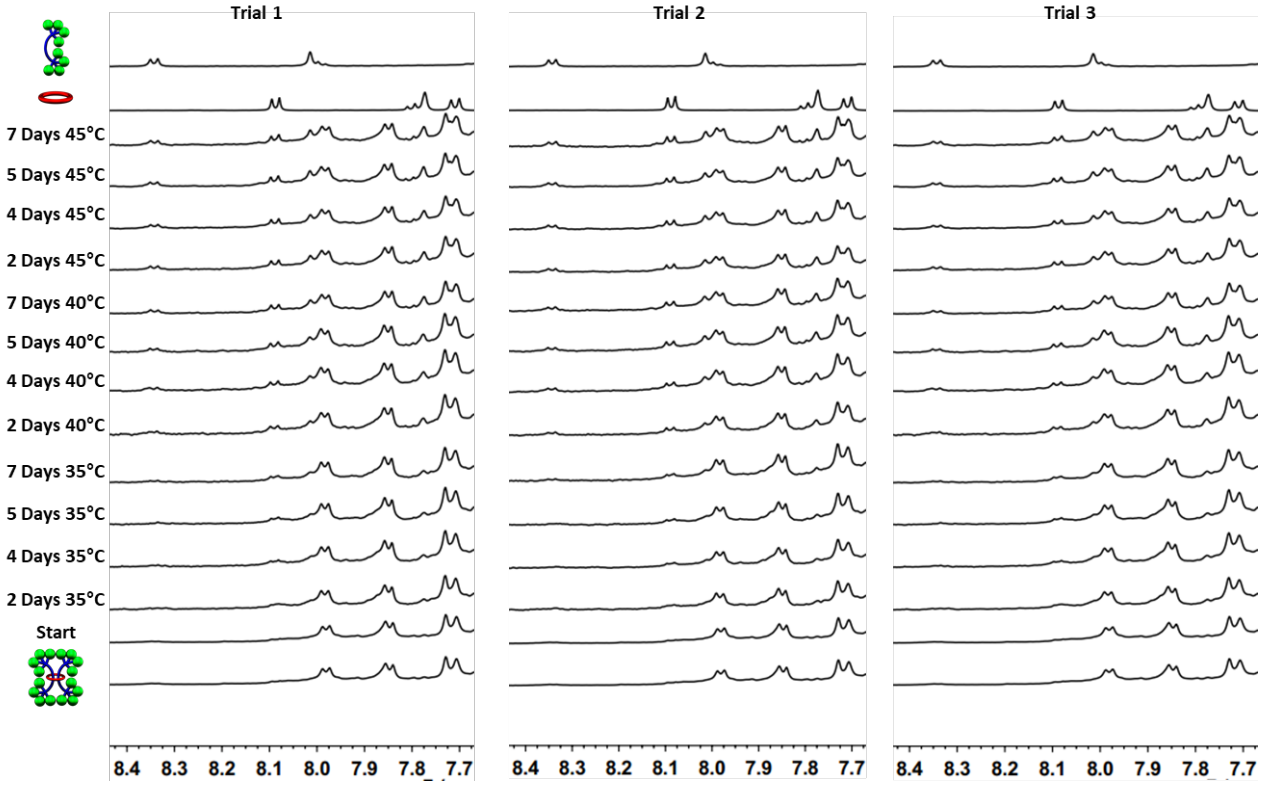


Figure S40. Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of 3 trials of 3-week kinetic slippage experiments of **11**. Time and temperature between measurements indicated on left side.

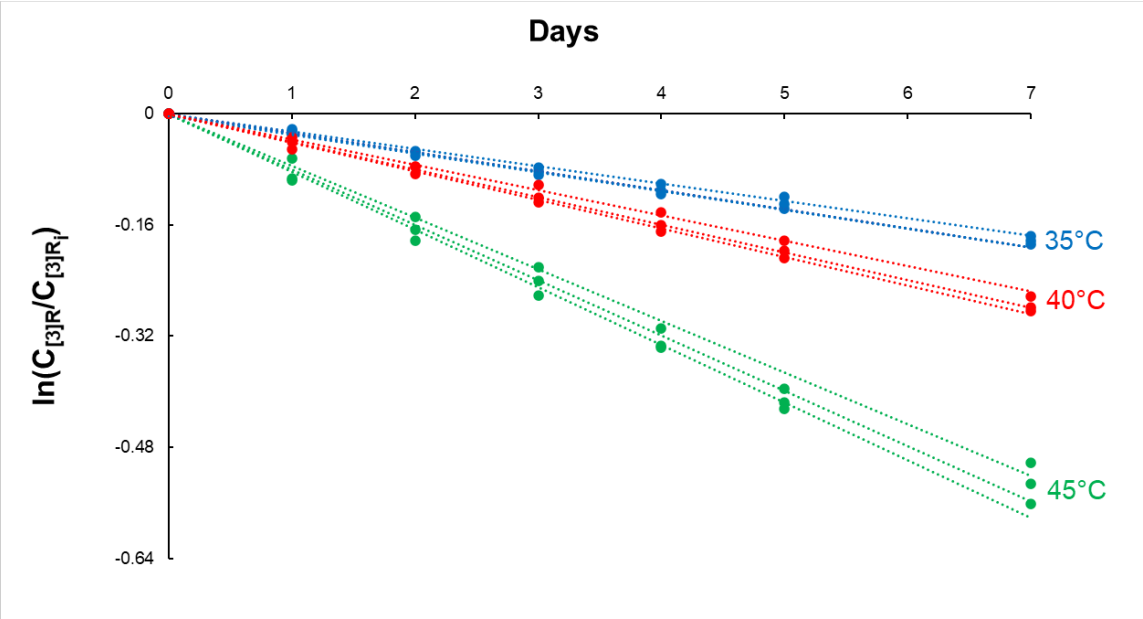


Figure S41. Kinetic first-order plot of three trials of slippage of **9** at three different temperatures.

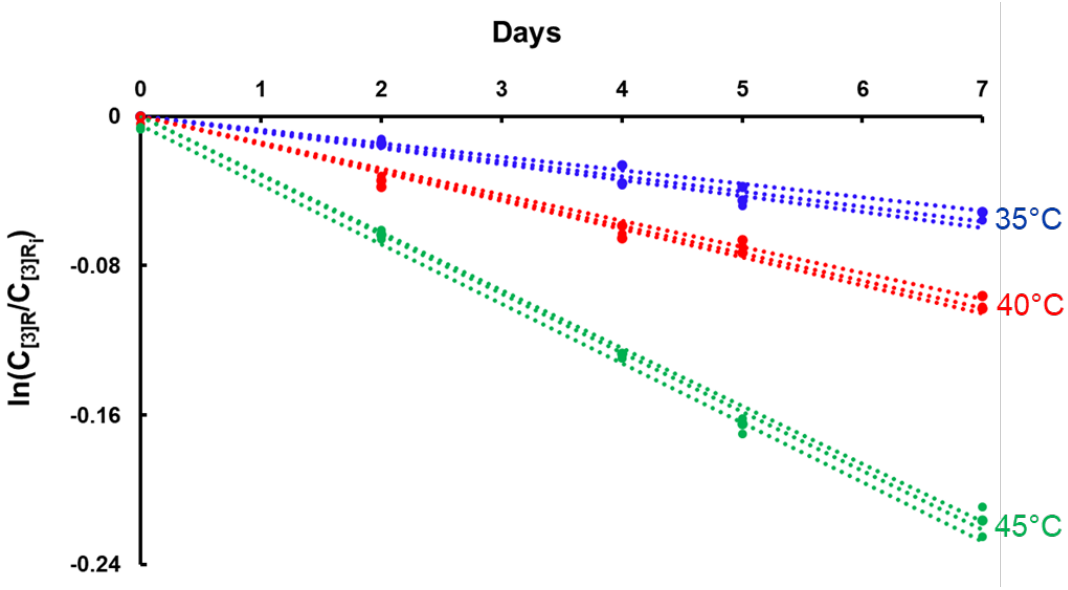


Figure S42. Kinetic first-order plot of three trials of slippage of **11** at three different temperatures.

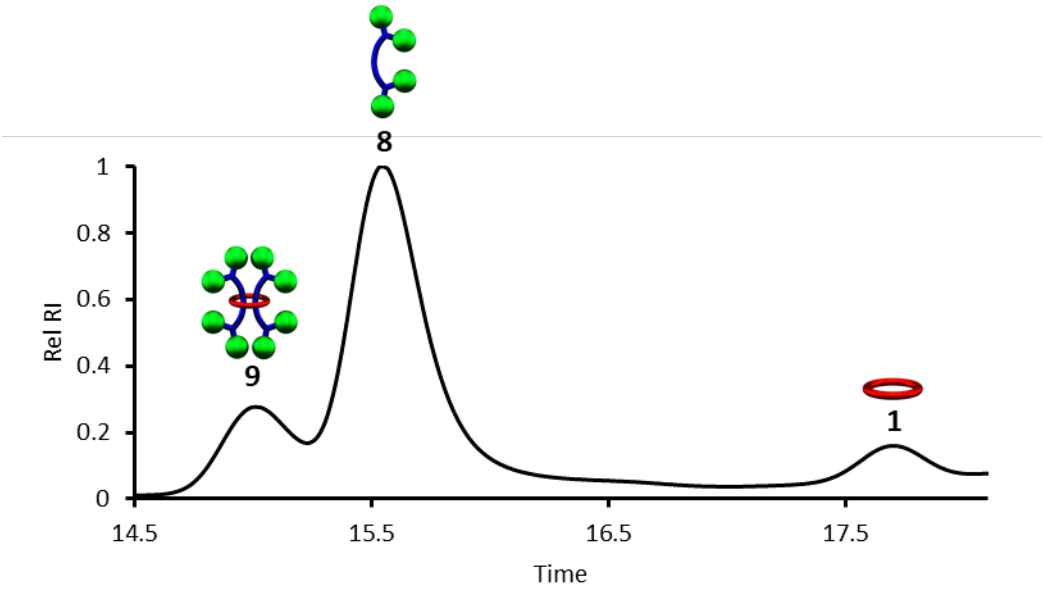


Figure S43. GPC (3:1 THF:DMF as eluent, 25°C) trace of **9** after the 3-week slippage experiment showing the presence of both **8** and **1** .

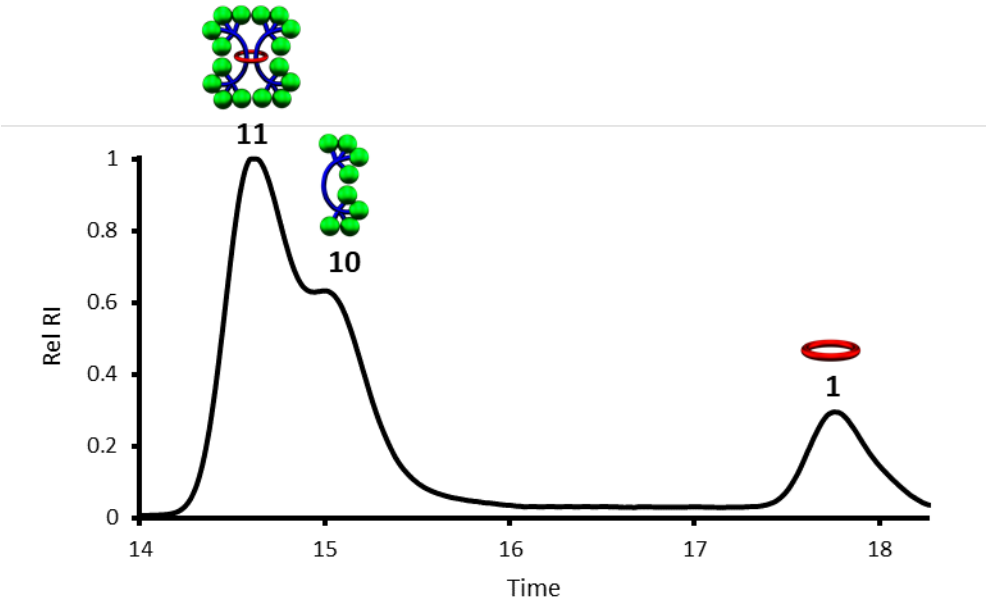


Figure S44. GPC (3:1 THF:DMF as eluent, 25°C) trace of **11** after the 3-week slippage experiment showing the presence of both **10** and **1** .

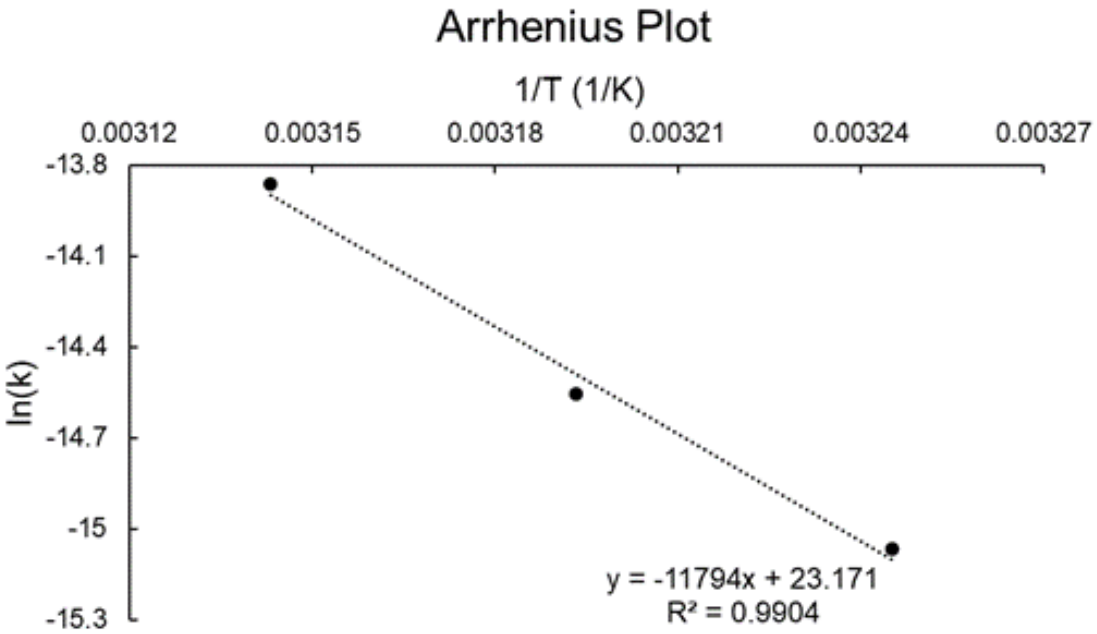


Figure S45. Arrhenius plot of the slippage of **9**.

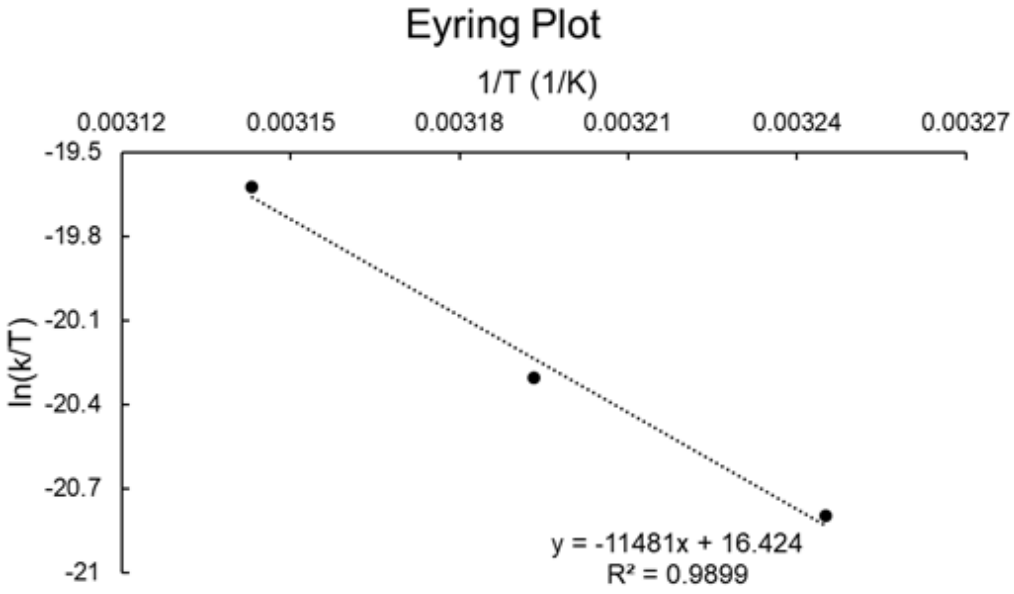


Figure S46. Eyring plot of the slippage of **9**.

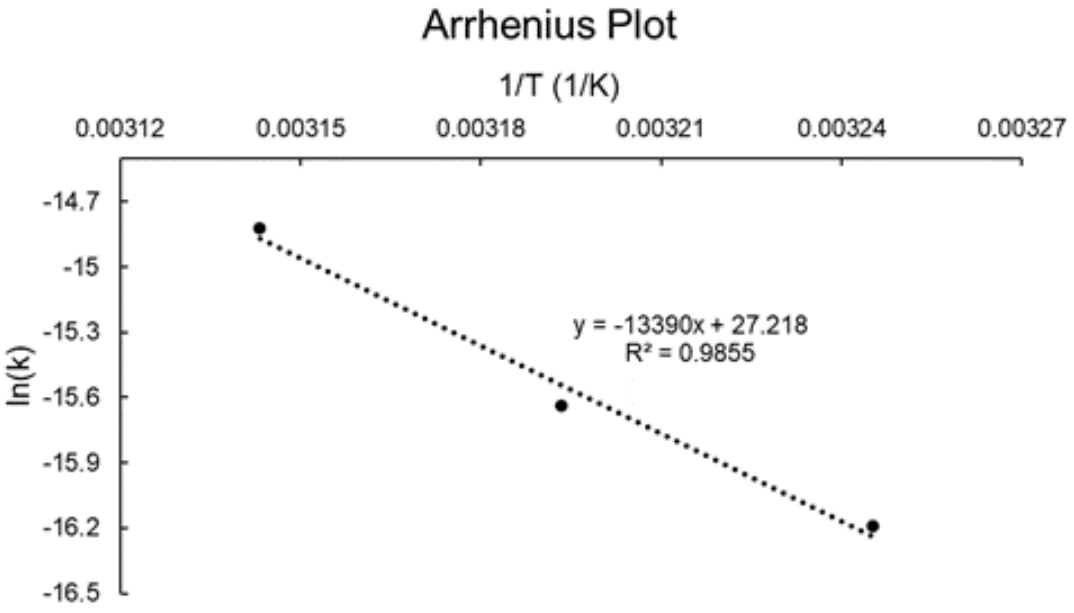


Figure S47. Arrhenius plot of the slippage of **11**.

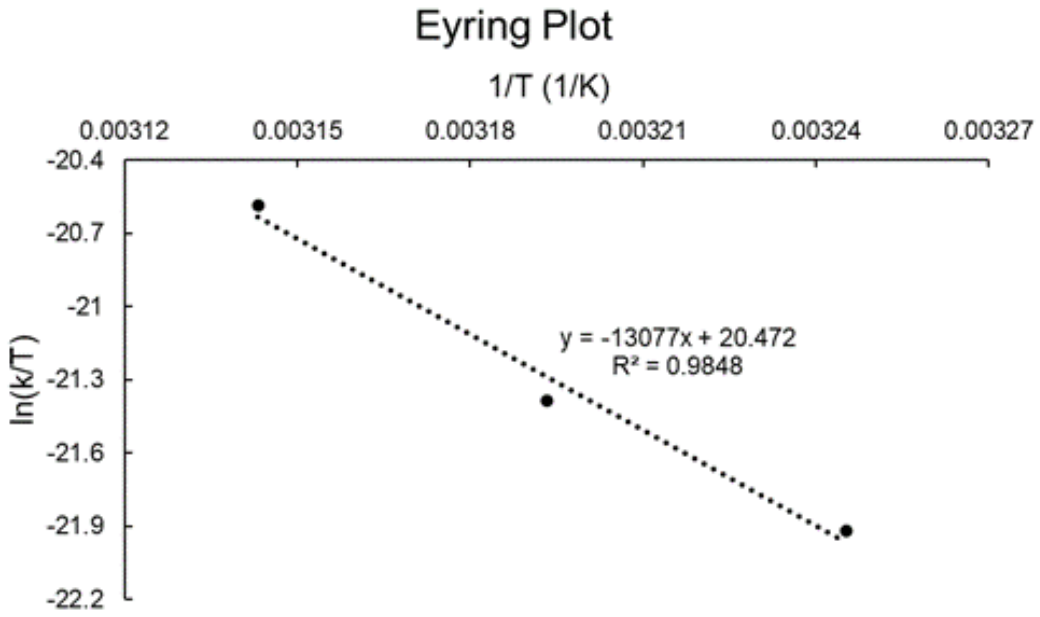


Figure S48. Eyring plot of the slippage of **11**.

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