Electronic Supplemental Information

Balancing Ring And Stopper Group Size to Control the Stability of Doubly Threaded [3]Rotaxanes

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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.

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^{o}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.

Synthesis of Macrocycle Components

Macrocycle Components ($\mathbf{1}_{40\text{-}48}$)

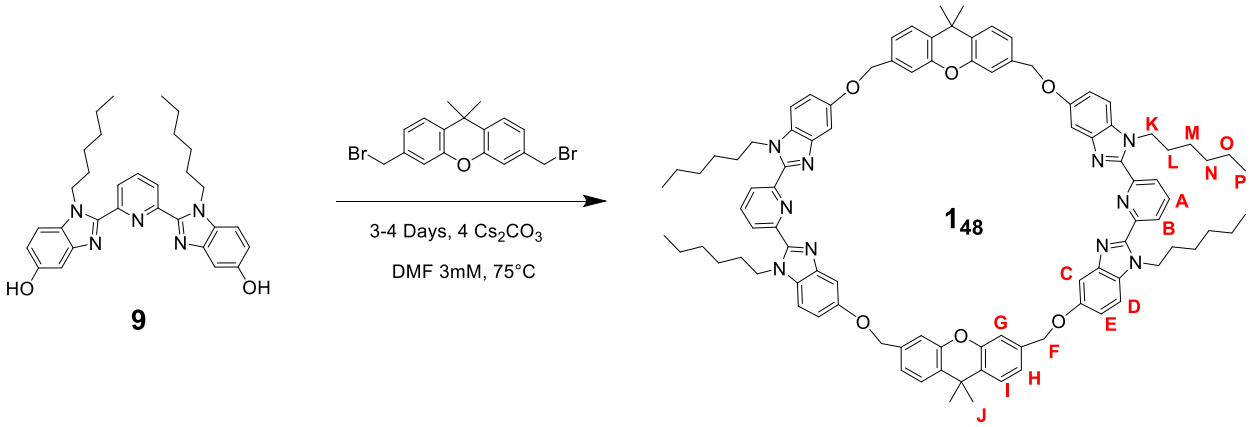

Figure S1. Synthesis of $\mathbf{1}_{48}$.

A 2-necked 1 L RBF was charged with **9**[3] (1.5 g, 2.94 mmol), Cs_2CO_3 (3.82 g, 11.8 mmol) and DMF (620mL) under an Ar atmosphere. The mixture was heated to 75 °C and stirred while a DMF (310 mL) solution of 3,6-bis(bromomethyl)-9,9-dimethyl-9H-xanthene[4] (1.16g, 2.94 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 $\mathbf{1}_{48}$ in 21% yield (0.435g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (d, J = 7.9 Hz, 4H, B), 7.87 (t, J = 7.9 Hz, 2H, A), 7.35 (d, J = 8.0 Hz, 4H, H), 7.25-7.21 (m, 8H, C+I), 7.11 (d, J = 8.1 Hz, 4H, D), 7.06 (s, 4H, G), 7.00 (dd, J = 8.9, 2.3 Hz, 4H, E), 5.24 (s, 8H, F), 4.54 (t, J = 7.5 Hz, 8H, K), 1.62 (m, 8H, L), 1.58 (s, 12H, J), 1.00 (m, 24H, M+N+O), 0.54 (m, 12H, P). ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.07, 150.55, 150.31, 150.06, 143.47, 137.86, 137.47, 131.24, 129.28, 126.60, 125.16, 121.39, 114.81, 114.77, 110.74, 104.42, 70.00, 44.95, 33.90, 32.61, 31.19, 30.10, 26.36, 22.42, 13.82. MALDI-MS: 1599.6 ([M]+Ag^+).

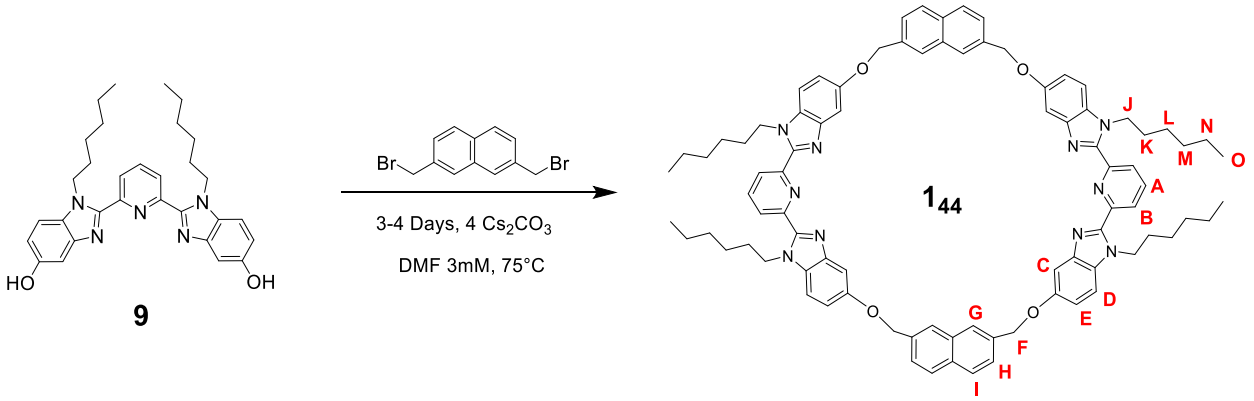

Figure S2. Synthesis of $\mathbf{1}_{44}$.

A 2-necked 1 L RBF was charged with **9**[3] (1.0 g, 1.95 mmol), Cs_2CO_3 (2.6 g, 7.8 mmol) and DMF (440 mL) under an Ar atmosphere. The mixture was heated to 75 °C and stirred while a DMF (220 mL) solution of 2,7-bis(bromomethyl)naphthalene (0.61g, 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 $\mathbf{1_{44}}$ in 20% yield (0.255g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 7.9 Hz, 4H, B), 7.81-7.75 (m, 10H, A+C+I), 7.53 (d, J = 8.0 Hz, 4H, H), 7.24 (s, 4H, G), 7.19 (d, J = 8.9 Hz, 4H, D), 7.01 (dd, J = 8.9, 2.3 Hz, 4H, E), 5.42 (s, 8H), F, 4.48 (t, J = 7.5 Hz, 8H, J), 1.55 (m, 8H, K), 0.89 (m, 24H, L+M+N), 0.49 (m, 12H, O). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.97, 150.20, 149.98, 143.41, 137.73, 135.83, 133.38, 132.64, 131.25, 128.44, 125.66, 125.22, 124.76, 114.70, 110.78, 104.72, 70.72, 44.89, 31.18, 29.85, 26.35, 22.43, 13.79. MALDI-MS: 1436.1 ([M]+Ag^+).

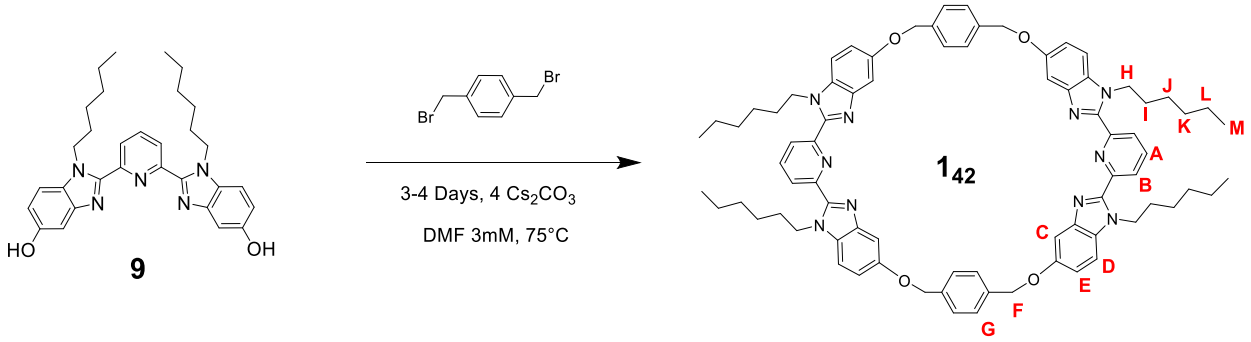

Figure S3. Synthesis of $\mathbf{1_{42}}$.

1 L RBF was charged with **9**[3] (1.0 g, 1.95 mmol), Cs_2CO_3 (2.6 g, 7.8 mmol) and DMF (440 mL) under an Ar atmosphere. The mixture was heated to 75 °C and stirred while a DMF (220 mL) solution of α,α′-Dibromo-p-xylene (0.51g, 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 $\mathbf{1_{42}}$ in 11% yield (0.133g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 7.9 Hz, 4H, B), 7.90 (t, J = 7.9 Hz, 2H, A), 7.37 (s, 8H, G), 7.21-7.15 (m, 8H, C+D), 6.93 (dd, J = 8.8, 2.4 Hz, 4H, E), 5.30 (s, 8H, F), 4.48 (t, J = 7.5 Hz, 8H, H), 1.55 (m, 8H, I), 0.89 (m, 24H, J+K+L), 0.49 (m, 12H, M). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.64, 150.14, 150.02, 143.39, 137.84, 137.26, 131.26, 126.98, 125.14, 114.71, 110.69, 104.77, 70.06, 44.86, 31.16, 30.04, 26.35, 22.44, 13.74. MALDI-MS: 1228.9 ([M]+H^+).

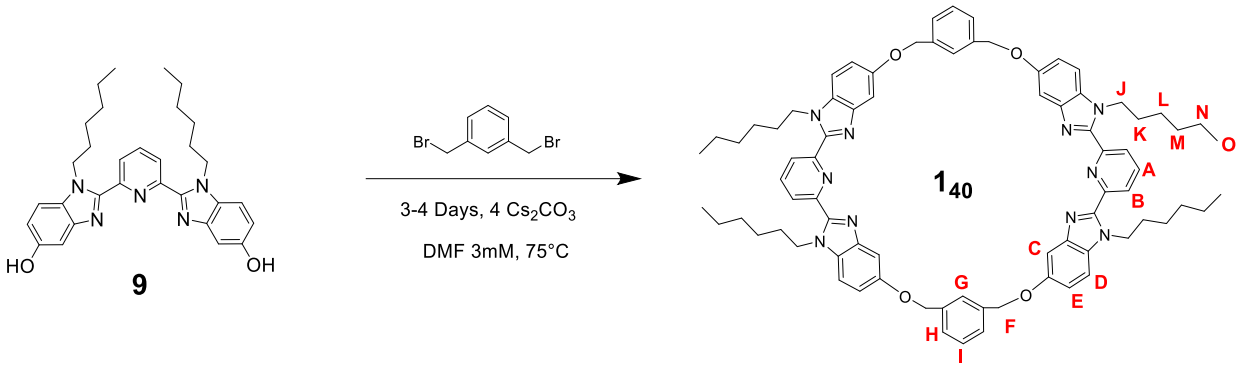

Figure S4. Synthesis of **1**$_{40}$.

1 L RBF was charged with **9**[3] (1.0 g, 1.95 mmol), Cs_2CO_3 (2.6 g, 7.8 mmol) and DMF (440 mL) under an Ar atmosphere. The mixture was heated to 75 °C and stirred while a DMF (220 mL) solution of α,α′-Dibromo-m-xylene (0.51g, 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**$_{40}$ in 7% yield (0.085g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, J = 7.9Hz, 4H, B), 7.92 (t, J = 7.9Hz, 2H, A), 7.46 (s, 2H, G), 7.29 (m, 6H, H+I), 7.24 (bs, 4H, C), 7.11 (d, J = 8.8Hz, 4H, D), 6.86 (dd, J = 8.8, 2.1Hz, 4H, E), 5.24 (s, 8H, F), 4.42 (t, J = 7.2Hz, 8H, J), 1.52 (bm, 8H, K), 0.88 (bm, 24H, L+M+N), 0.51 (d, J = 6.7Hz, 12H, O). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.94, 150.20, 150.17, 143.40, 138.17, 137.90, 131.37, 128.95, 126.40, 126.00, 125.09, 115.17, 110.57, 105.66, 71.09, 44.84, 31.20, 30.05, 26.29, 22.40, 13.78. MALDI-MS: 1228.7 ([M]+H$^+$).

Fe(II) Assembly of Pseudo[3]rotaxanes $\mathbf{1}_{40\text{-}48}$:$\mathbf{2}_2$:Fe(II)$_2$

Assembly of Pseudo[3]rotaxane $\mathbf{1}_{48}$:$\mathbf{2}_2$:Fe(II)$_2$ from $\mathbf{1}_{48}$ and **2**

Dissolved 25.1mg of $\mathbf{1}_{48}$ in 1.5mL $CDCl_3$**.** Titrated thread stock solution (30mM) of **2** into solution of $\mathbf{1}_{48}$ until an exact 2:1 (**2**:$\mathbf{1}_{48}$) 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 3.5mL $CDCl_3$ (5 mM $\mathbf{1}_{48}$). A stock solution of $Fe(NTF_2)_2$ (30mM in 1:1 $CDCl_3$: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 $CDCl_3$.

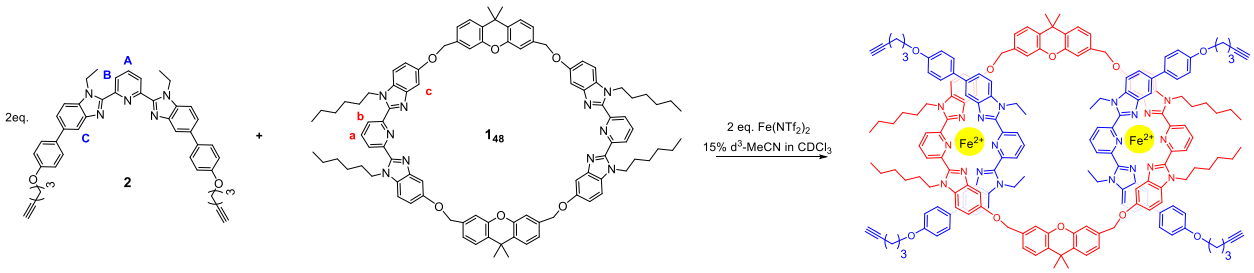

Figure S5. Formation of $\mathbf{1}_{48}$:$\mathbf{2}_2$:Fe(II)$_2$.

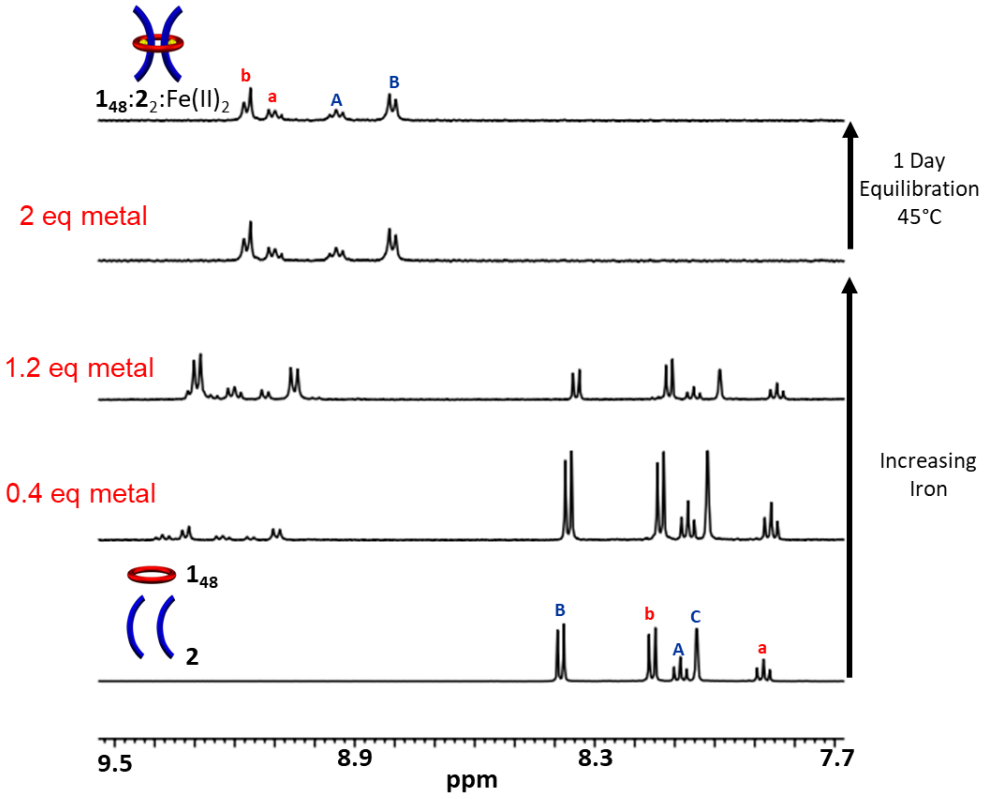

Figure S6. Partial ^{1}H-NMR overlay (500 MHz, 25°C, Solvent: 0, 3, 9, 15, 15% d_3-MeCN in $CDCl_3$ increasing upwards) of metal addition and 1 day equilibration. ^{1}H assignments from Figure S9.

Assembly of Pseudo[3]rotaxane **1_{44}:2_2**:Fe(II)$_2$ from **1_{44}** and **2**

Dissolved 23.2mg of **1_{44}** in 1.5mL $CDCl_3$**.** Titrated thread stock solution (30mM) of **2** into solution of **1_{44}** until an exact 2:1 (**2**:**1_{44}**) 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 3.5mL $CDCl_3$ (5 mM **1_{44}**). A stock solution of $Fe(NTF_2)_2$ (30mM in 1:1 $CDCl_3$: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 $CDCl_3$.

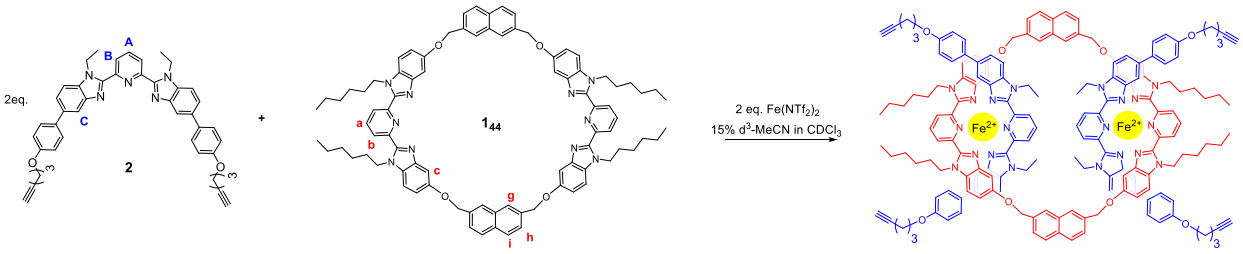

Figure S7. Formation of **1_{44}:2_2**:Fe(II)$_2$.

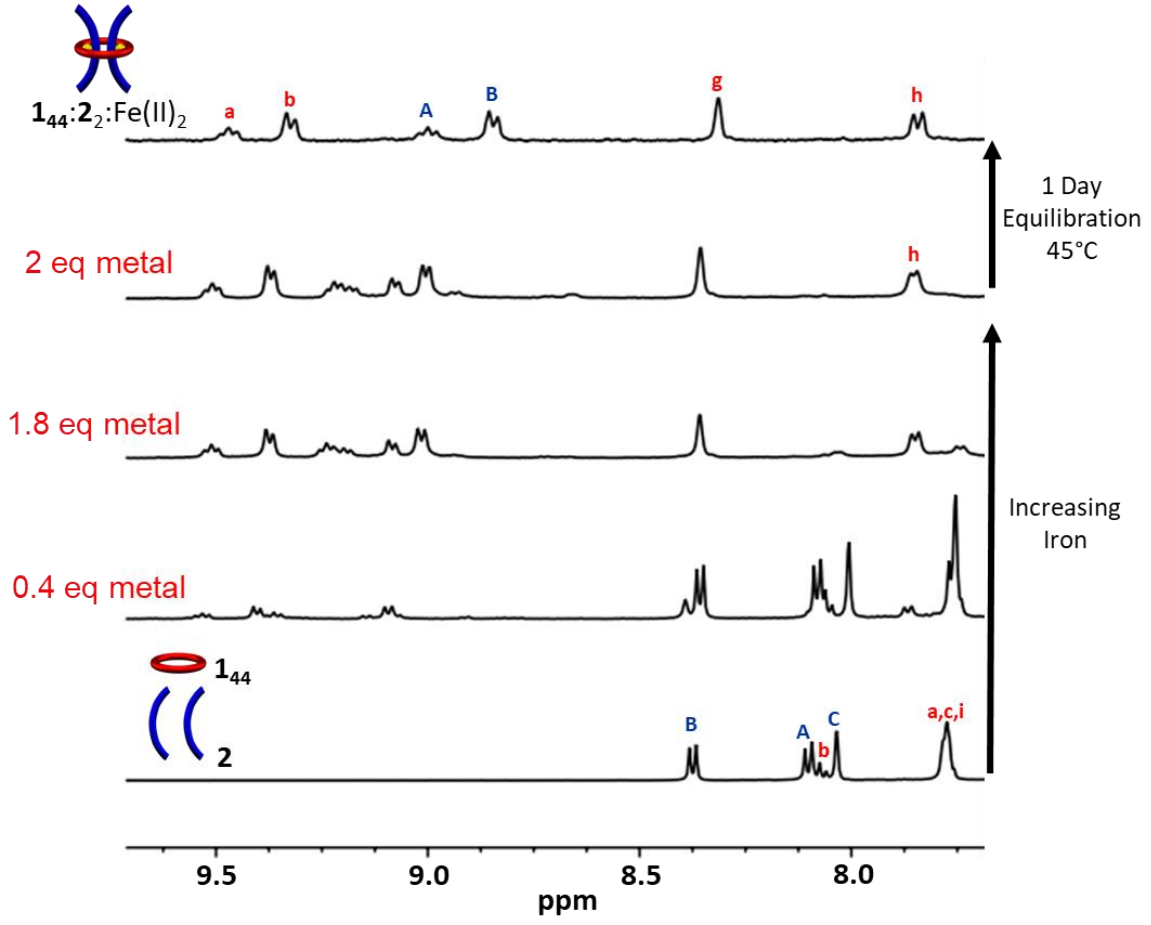

Figure S8. Partial ^{1}H-NMR overlay (500 MHz, 25°C, Solvent: 0, 3, 12, 15, 15% d_3-MeCN in $CDCl_3$ increasing upwards) of metal addition and 1 day equilibration. ^{1}H assignments from Figure S11.

Assembly of Pseudo[3]rotaxane **1_{42}:2_2**:Fe(II)$_2$ from **1_{42}** and **2**

Dissolved 24.8mg of **1_{42}** in 1.5mL $CDCl_3$**.** Titrated thread stock solution (30mM) of **2** into solution of **1_{42}** until an exact 2:1 (**2**:**1_{42}**) 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_{42}**). A stock solution of Fe$(NTF_2)_2$ (30mM in 1:1 $CDCl_3$: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 $CDCl_3$.

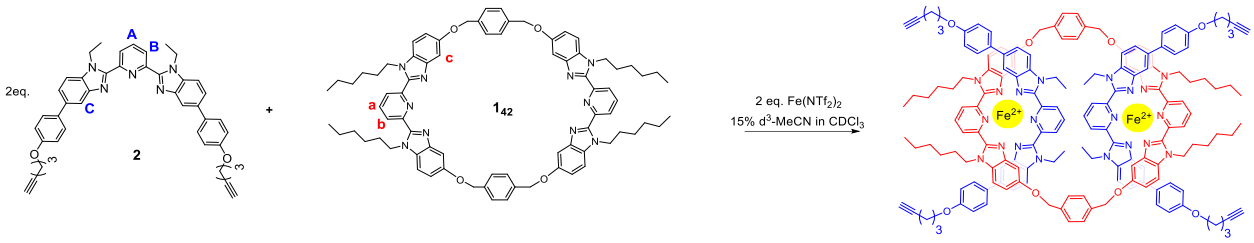

Figure S9. Formation of **1_{42}:2_2**:Fe(II)$_2$.

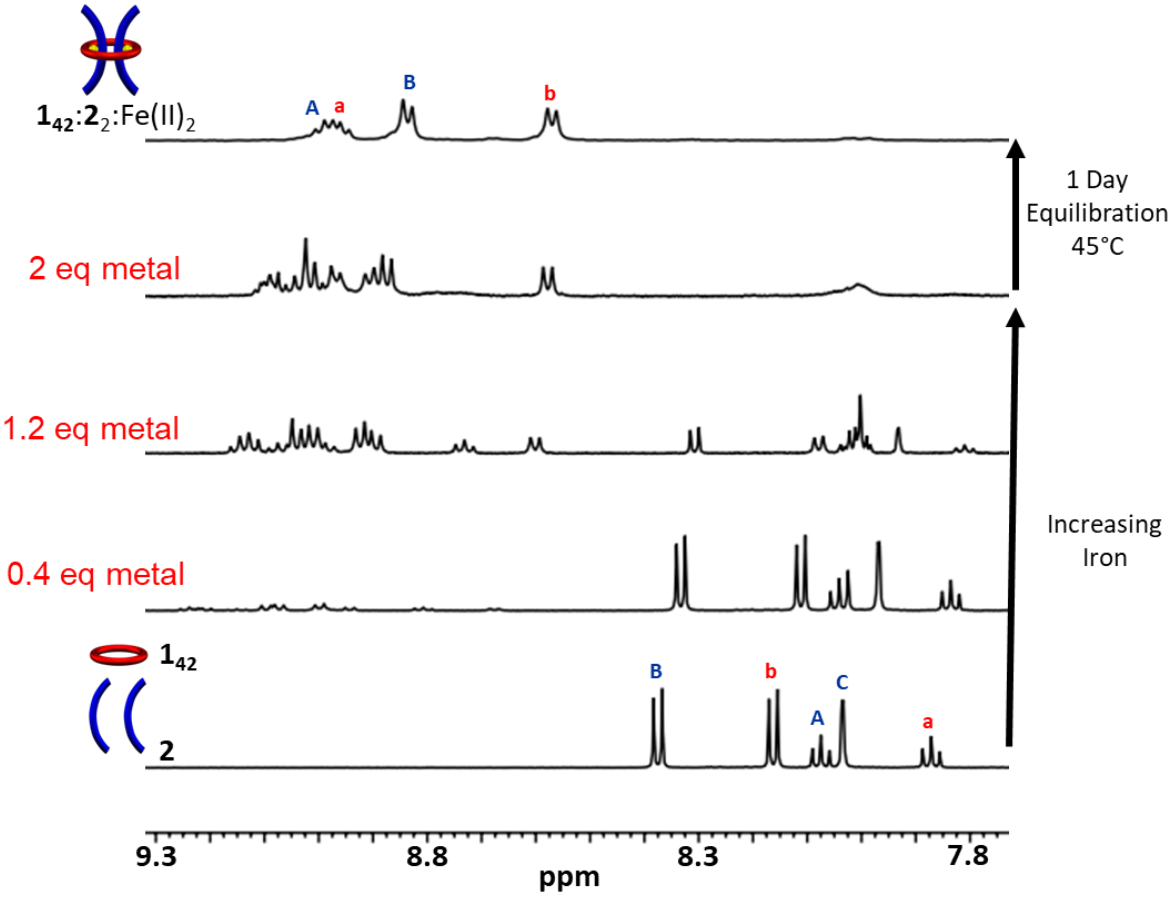

Figure S10. Partial ^{1}H-NMR overlay (500 MHz, 25°C, Solvent: 0, 3, 9, 15, 15% d_3-MeCN in $CDCl_3$ increasing upwards) of metal addition and 1 day equilibration. ^{1}H assignments from Figure S13.

Assembly of Pseudo[3]rotaxane **1_{40}:2_2**:Fe(II)$_2$ from **1_{40}** and **2**

Dissolved 16.8mg of **1_{40}** in 1.0mL $CDCl_3$**.** Titrated thread stock solution (30mM) of **2** into solution of **1_{40}** until an exact 2:1 (**2**:**1_{40}**) 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_{40}**). A stock solution of $Fe(NTF_2)_2$ (30mM in 1:1 $CDCl_3$: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 1mL 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 $CDCl_3$.

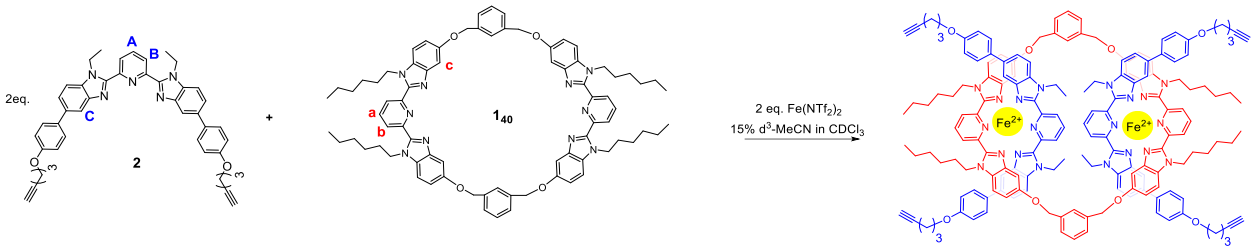

Figure S11. Formation of **1_{40}:2_2**:Fe(II)$_2$.

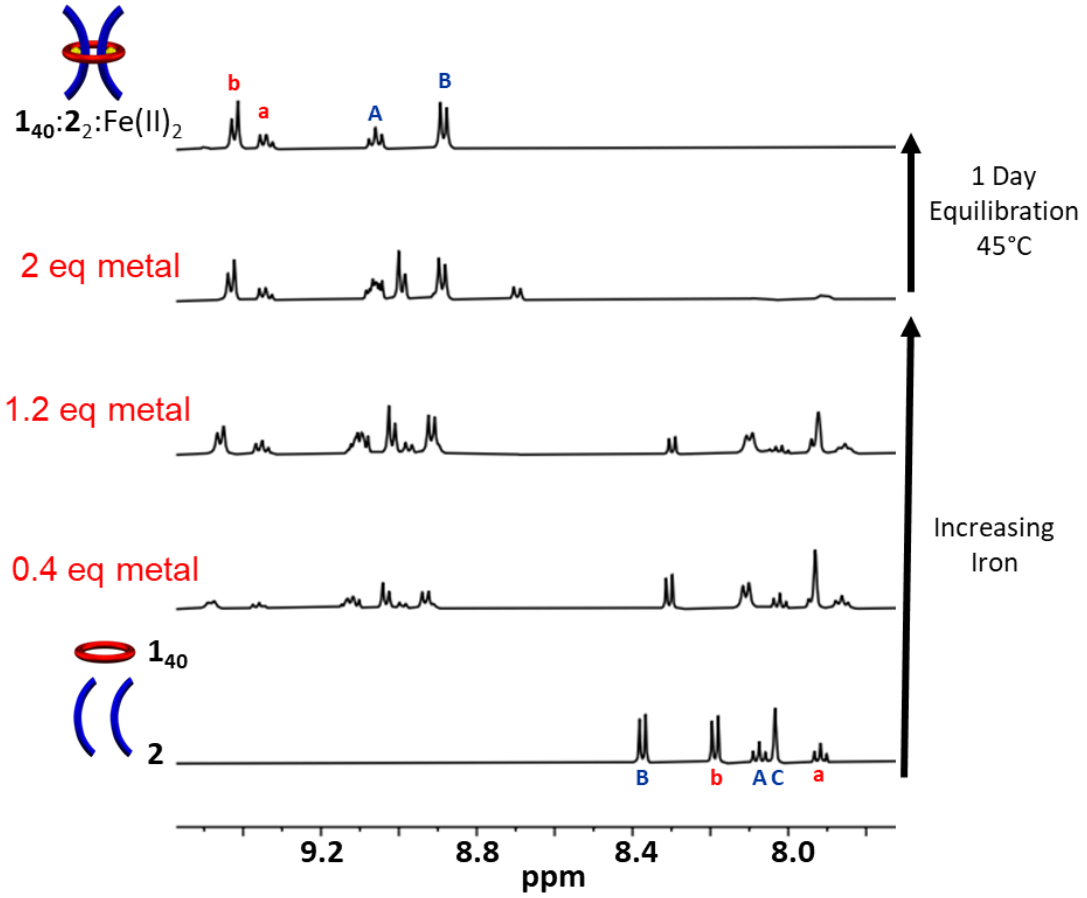

Figure S12. Partial ^{1}H-NMR overlay (500 MHz, 25°C, Solvent: 0, 3, 9, 15, 15% d_3-MeCN in $CDCl_3$ increasing upwards) of metal addition and 1 day equilibration. ^{1}H assignments from Figure S15.

Stoppering of Pseudo[3]rotaxanes $\mathbf{1}_{40\text{-}48}$:$\mathbf{2}_2$:Fe(II)$_2$

Stoppering With Stopper Component **3**

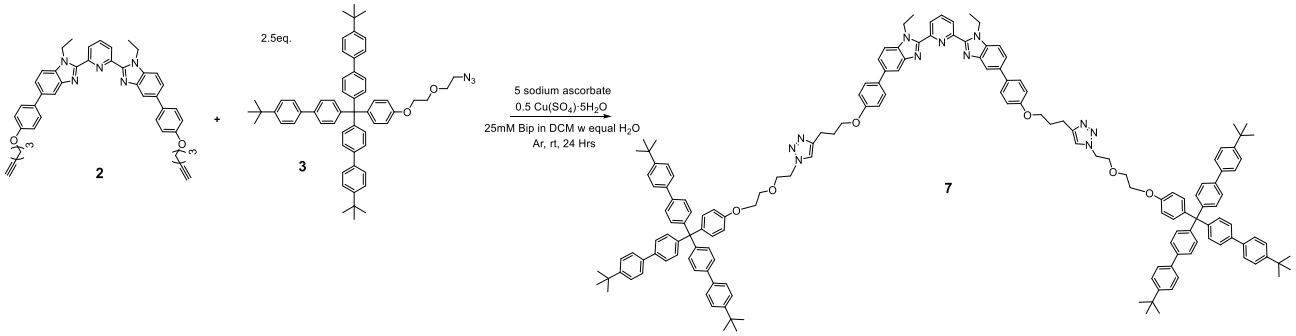

Figure S13. Synthesis of dumbbell component **7**.[3]

Reference dumbbell component **7** was synthesized following a previously published procedure.[3]

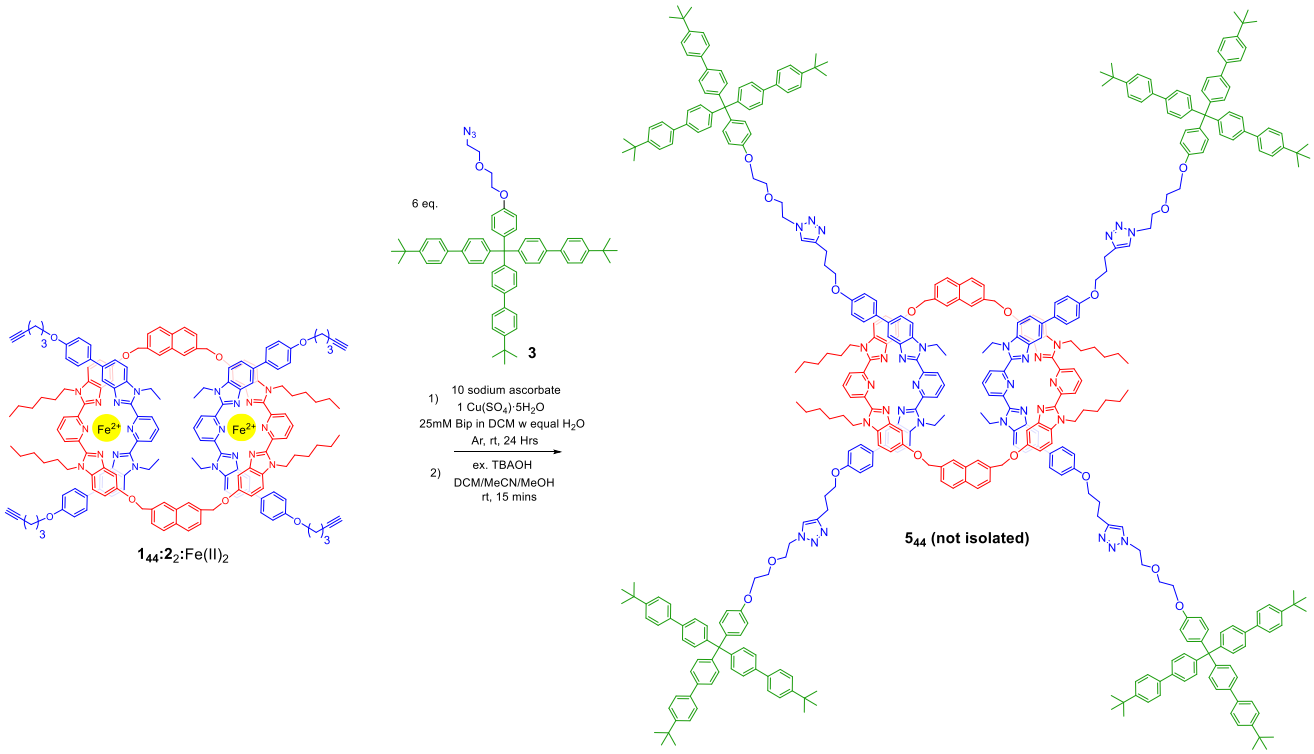

Figure S14. Attempted stoppering and demetallation of $\mathbf{1}_{44}$:$\mathbf{2}_2$:Fe(II)$_2$ with **3** to make $\mathbf{5}_{44}$.

$\mathbf{1}_{44}$:$\mathbf{2}_2$:Fe(II)$_2$ (8.8 mg, 0.0022mmol), **3** (11.4 mg, 0.013 mmol, 6eq) and sodium ascorbate (4.4 mg, 0.022 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.35 mL, conc of alkyne = 25 mM), H_2O (0.25 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (22 mM, 0.0022mmol, 1eq, (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 organic solvent resulted in a purple solid that was washed 3x with 5mL of 5% chloroform in hexanes to help remove leftover stopper group. The crude purple solid was redissolved in 4mL 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) to remove excess base. The frit was then washed with 2mL of $CDCl_3$ to redissolve the demetallated product and transferred to an NMR tube for immediate analysis.

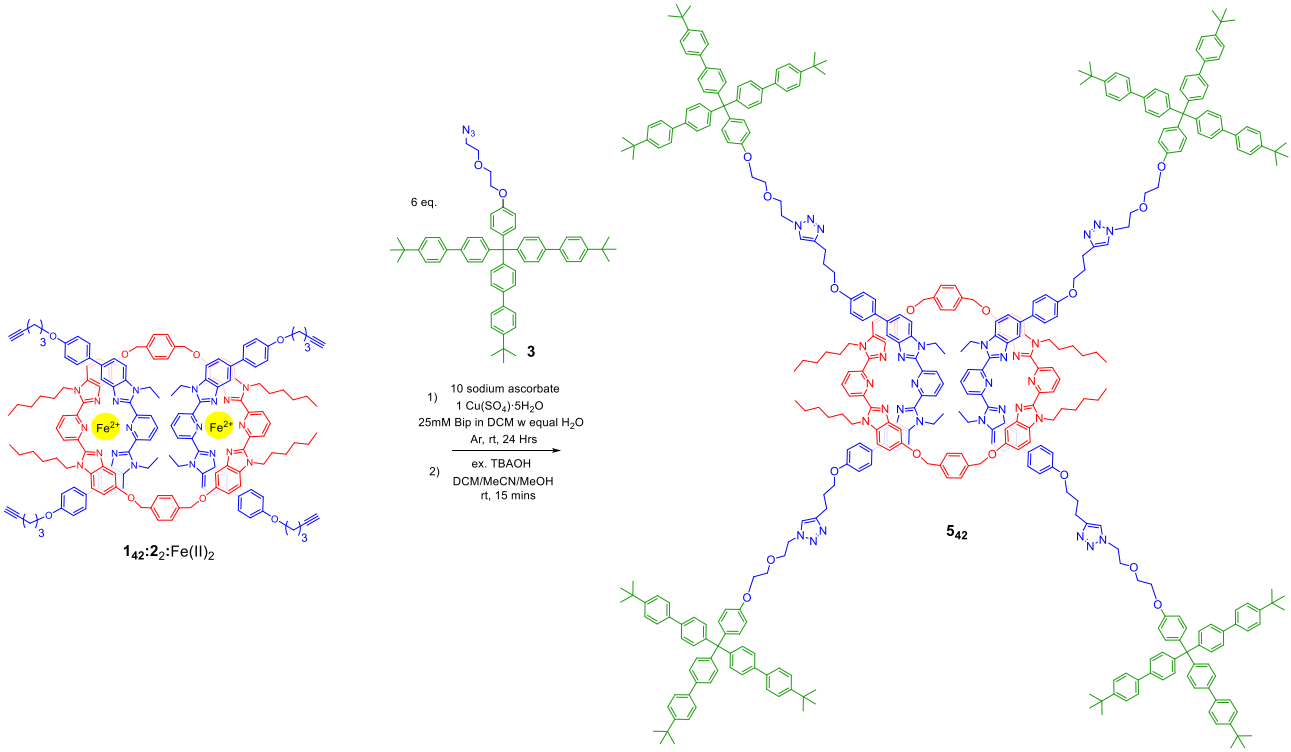

Figure S15. Stoppering and demetallation of $\mathbf{1}_{42}$:$\mathbf{2}_2$:Fe(II)$_2$ with **3** to make $\mathbf{5}_{42}$.

$\mathbf{1}_{42}$:$\mathbf{2}_2$:Fe(II)$_2$ (20.9 mg, 0.0055mmol), **3** (27.7 mg, 0.033 mmol, 6eq) and sodium ascorbate (10.6 mg, 0.055 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.85 mL, conc of alkyne = 25 mM), H_2O (0.75 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (55 mM, 0.0055mmol, 1eq, (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 organic solvent resulted in a purple solid that was washed 3x with 10mL of 5% 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) to remove excess base. 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 $\mathbf{5}_{42}$ was achieved using preparative thin layer chromatography (SiO_2, eluent = 94:6 $CHCl_3$:MeOH, lowest Rf band taken (R_f=0.1-0.35) as [3]R product, macrocycle byproduct R_f=0.45, dumbbell byproduct R_f=0.60-0.70) followed by precipitation from cold methanol to result in an off-white solid, $\mathbf{5}_{42}$ in 34% isolated yield (11.1 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.30 (d, J = 7.9Hz, 4H), 7.98 (d, J = 7.7Hz, 4H), 7.98-7.80 (m, 6H), 7.63 (t, J = 7.7Hz, 2H), 7.55-7.45 (m, 56H), 7.42 (d, J = 8.0Hz, 24H), 7.36 (d, J = 8.2Hz, 8H), 7.34-7.28 (m, 36H), 7.20 (s, 4H), 7.18 (d, J = 8.0 Hz, 8H), 6.79-6.74 (m, 8H), 6.72-6.65 (m, 8H), 5.15 (bs, 8H), 4.72 (bq, 8H), 4.32-4.20 (m, 16H), 3.83 (bt, 8H), 3.73 (bt, 8H), 3.64 (bt, 8H), 3.54 (bt, 8H), 2.67 (bt, 8H), 1.93 (bt, 8H), 1.40 (m, 8H), 1.33 (s, 108H),

1.26 (bt, 12H), 0.85-0.72 (m, 24H), 0.58 (t, J = 7.1Hz, 12H). MALDI-MS: 5981.1 ([M]+H$^+$), 3605.8 ([M]+H$^+$-DB(**7**)), 2378.1 ([M]+H$^+$-MC(**1**$_{42}$)-DB(**7**)).

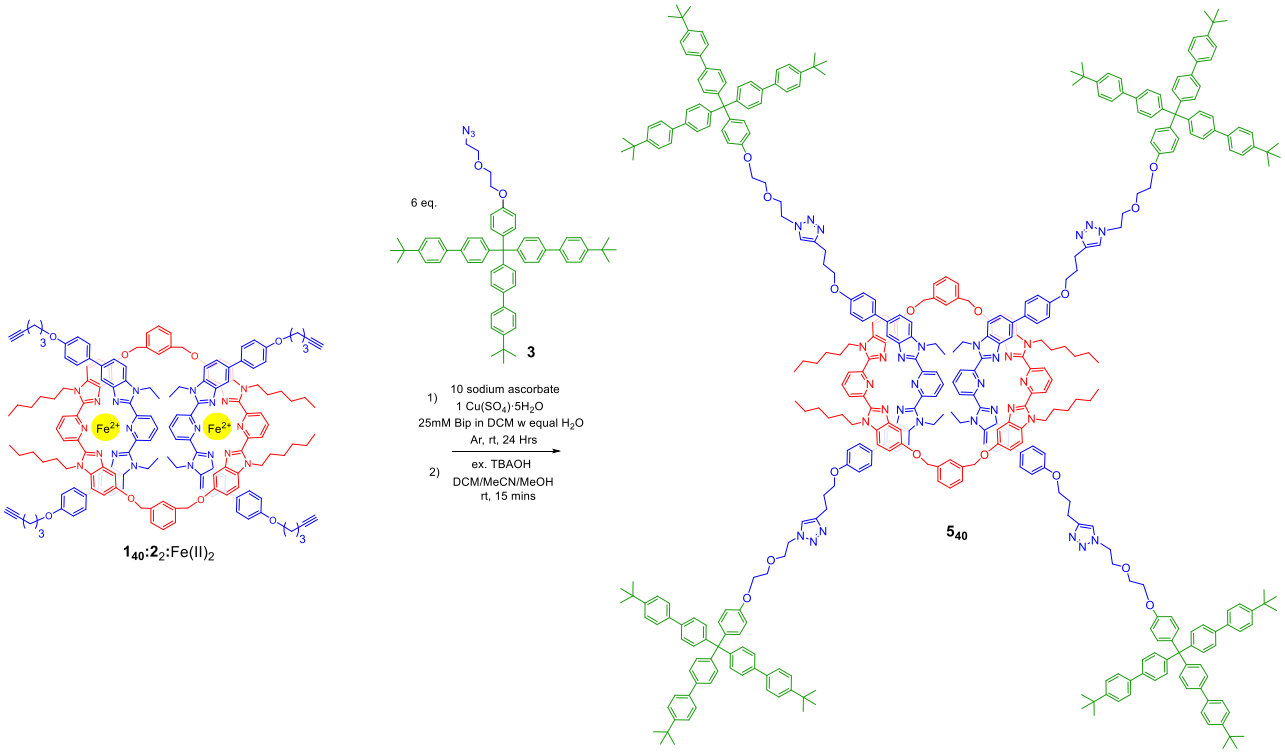

Figure S16. Stoppering and demetallation of **1**$_{40}$:**2**$_2$:Fe(II)$_2$ with **3** to make **5**$_{40}$.

1$_{40}$**:2**$_2$**:**Fe(II)$_2$ (20.1 mg, 0.0053mmol), **3** (26.0 mg, 0.032 mmol, 6eq) and sodium ascorbate (10.2 mg, 0.053 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.80 mL, conc of alkyne = 25 mM), H_2O (0.70 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (53 mM, 0.0053mmol, 1eq, (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 organic solvent resulted in a purple solid that was washed 3x with 10mL of 5% 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) to remove excess base. 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 **5**$_{40}$ was achieved using preparative thin layer chromatography (SiO_2, eluent = 94:6 $CHCl_3$:MeOH, lowest Rf band taken (R_f=0.1-0.35) as [3]R product, macrocycle byproduct R_f=0.45, dumbbell byproduct R_f=0.60-0.70) followed by precipitation from cold methanol to result in an off-white solid, **5**$_{40}$ in 42% isolated yield (13.2 mg). ^{1}HNMR (500 MHz, $CDCl_3$) δ 8.33 (d, J = 7.8Hz, 4H), 8.01 (d, J = 7.7Hz, 4H), 7.97 (t, J = 7.8Hz, 2H), 7.91 (s, 4H), 7.77 (t, J = 7.7Hz, 2H), 7.55-7.45 (m, 56H), 7.42 (d, J = 8.0Hz, 24H), 7.36 (d, J = 8.3Hz, 8H), 7.34-7.28 (m, 36H), 7.22 (s, 4H), 7.15 (d, J = 8.4 Hz, 8H), 6.88 (d, J = 8.8 Hz, 4H), 6.78-6.69 (m, 12H), 6.63 (d, J = 8.4 Hz, 8H), 5.00 (bs, 8H), 4.73 (bq, 8H), 4.31 (bt, 8H), 4.22 (bt, 8H), 3.77 (bm, 16H), 3.60 (bt, 8H), 3.47 (bt, 8H), 2.69 (bt, 8H), 1.95 (bt, 8H), 1.44 (m, 8H), 1.33 (s, 108H), 1.26 (bt, 12H), 0.89-0.75 (m, 24H), 0.58 (t, J = 7.0Hz, 12H). MALDI-MS: 5981.9 ([M]+H$^+$), 3605.1 ([M]+H$^+$-DB(**7**)), 2377.9 ([M]+H$^+$-MC(**1**$_{40}$)-DB(**7**)).

Stoppering With Stopper Component **4**

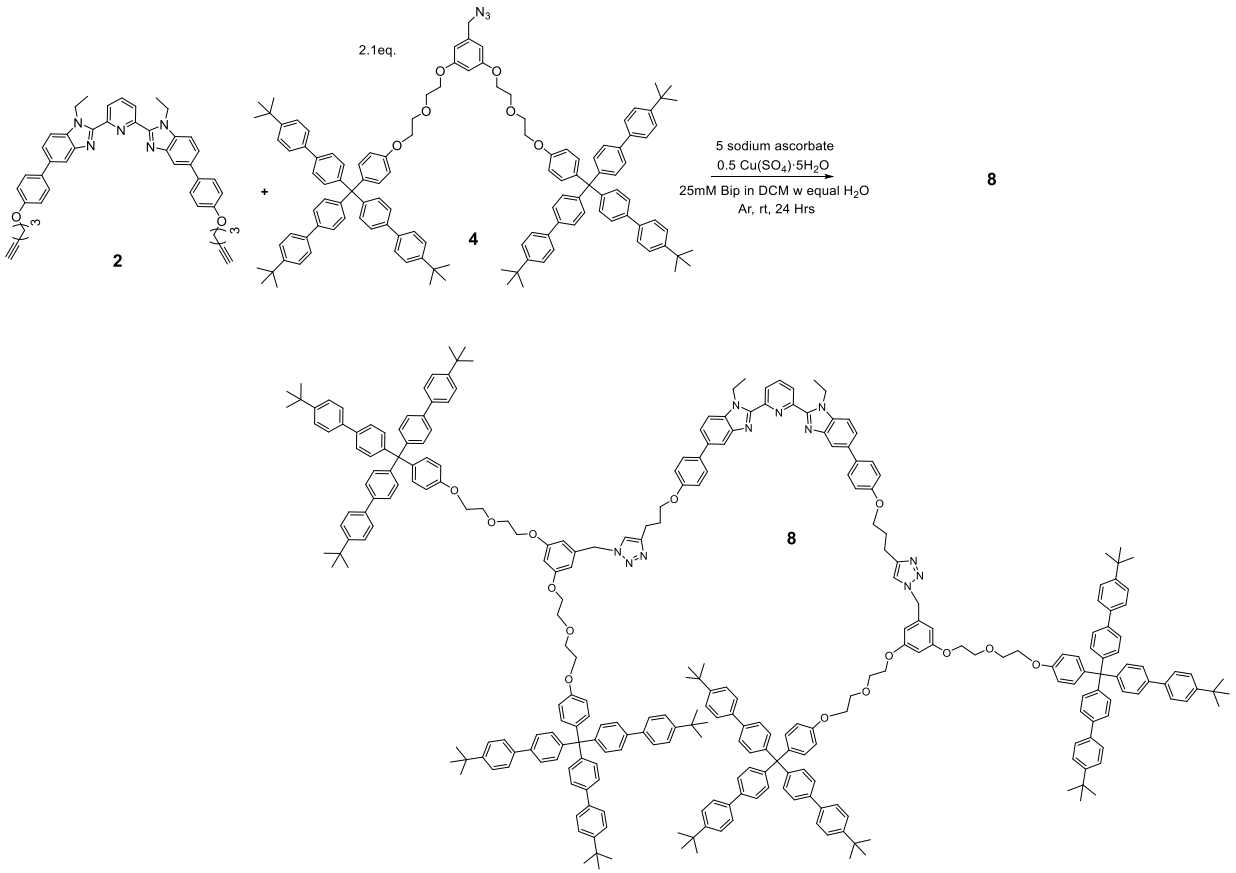

Figure S17. Synthesis of dumbbell component **8**. (ref)

Reference dumbbell component **8** was synthesized following a previously published procedure.[3]

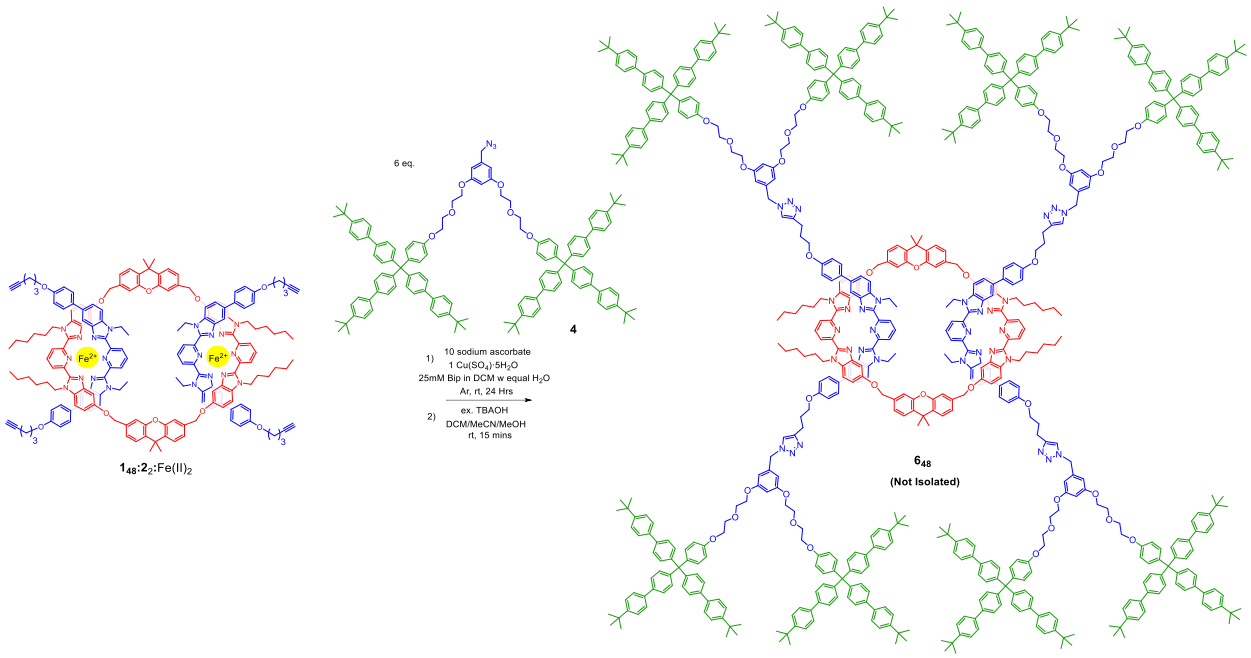

Figure S18. Attempted stoppering and demetallation of $\mathbf{1}_{44}$:$\mathbf{2}_2$:Fe(II)$_2$ with **4** to make $\mathbf{6}_{48}$.

$\mathbf{1}_{48}$:$\mathbf{2}_2$:Fe(II)$_2$ (11.0 mg, 0.0027 mmol), **4** (28.6 mg, 0.016 mmol, 6eq) and sodium ascorbate (5.2 mg, 0.027 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.43 mL, conc of alkyne = 25 mM), H_2O (0.33 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (27mM, 0.0027mmol, 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 3mL of 50:50 dichloromethane and acetonitrile and stirred slowly at room temperature. 0.5mL 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 2mL of $CDCl_3$ to redissolve the demetallated product and analyzed immediately via 1H-NMR spectroscopy and MALDI-TOF mass spectrometry.

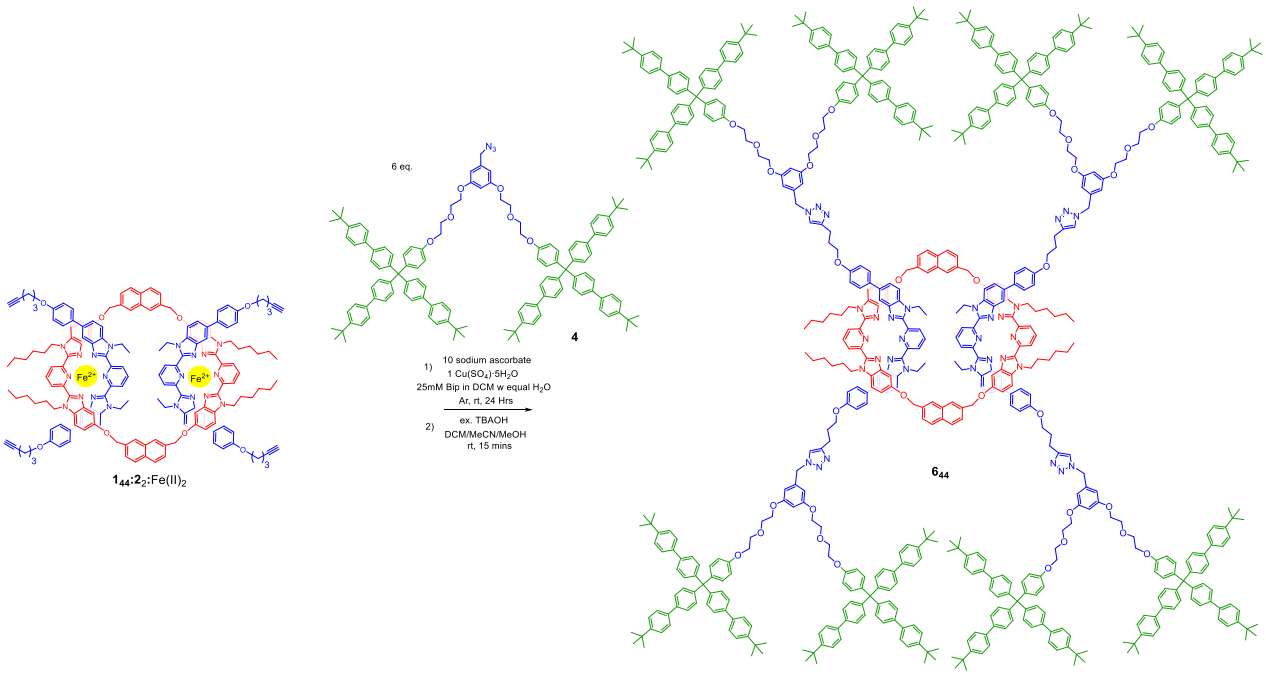

Figure S19. Stoppering and demetallation of $\mathbf{1_{44}}$:$\mathbf{2_2}$:Fe(II)$_2$ with **4** to make $\mathbf{6_{44}}$.

$\mathbf{1_{44}}$:$\mathbf{2_2}$:Fe(II)$_2$ (15.8 mg, 0.004 mmol), **4** (43.1 mg, 0.024 mmol, 6eq) and sodium ascorbate (8.0 mg, 0.04 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.65 mL, conc of alkyne = 25 mM), H_2O (0.55 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (40mM, 0.004mmol, 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 $\mathbf{6_{44}}$ was achieved using preparative thin layer chromatography (SiO_2, eluent = 94:6 $CHCl_3$:MeOH, lowest Rf band taken (R_f=0.15-0.30) 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, $\mathbf{6_{44}}$ in 75% isolated yield (29.5 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.25 (d, J = 7.8Hz, 4H), 7.98 (d, J = 7.8Hz, 4H), 7.91 (s, 4H), 7.85 (t, J = 7.9Hz, 2H), 7.67 (t, J = 7.9Hz, 2H), 7.63 (d, J = 8.5Hz, 4H), 7.55-7.45 (m, 108H), 7.42 (d, J = 8.5Hz, 48H), 7.37 (d, J = 8.6 Hz, 8H), 7.31-7.28 (m, 52H), 7.21-7.15 (m, 20H), 6.84-6.70 (m, 28H), 6.66 (d, J = 8.6 Hz, 8H), 6.47 (bs, 4H), 6.34 (bd, 8H), 5.29 (bs, 8H), 5.15 (s, 8H), 4.62 (bq, J = 7.2 8H), 4.30 (bt, 8H) 4.12-4.00 (m, 32H), 3.88-3.76 (m, 32H), 3.58 (bt, 8H), 2.47 (bt, 8H), 1.70 (bt, 8H), 1.36 (m, 8H), 1.33 (s, 216H), 1.18 (t, J = 7.2Hz, 12H), 0.89 -0.69 (m, 24H), 0.50 (t, J = 7.0Hz, 12H). MALDI-MS: 9,782.3 ([M]+H^+), 5,554.5 ([M]+H^+-DB(**8**)), 4,226.9 ([M]+H^+-MC($\mathbf{1_{44}}$)-DB(**8**)).

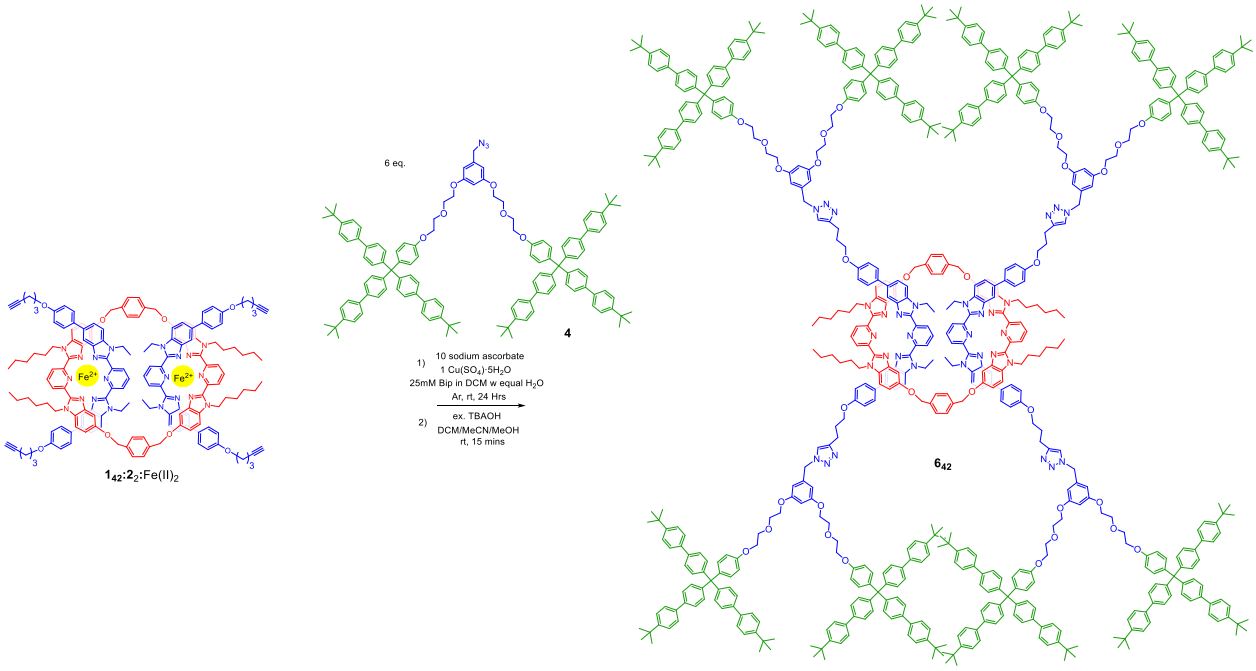

Figure S20. Stoppering and demetallation of **1_{42}**:**2_2**:Fe(II)$_2$ with **4** to make **6_{42}**.

1_{42}:2_2:Fe(II)$_2$ (18.2 mg, 0.0047 mmol), **4** (50.1 mg, 0.029 mmol, 6eq) and sodium ascorbate (9.3 mg, 0.047 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.76 mL, conc of alkyne = 25 mM), H_2O (0.66 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (47mM, 0.0047mmol, 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 **6_{42}** 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, **6_{42}** in 73% isolated yield (33.5 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.28 (d, J = 7.8Hz, 4H), 7.92 (d, J = 7.8Hz, 4H), 7.90-7.84 (m, 6H), 7.56-7.44 (m, 106H), 7.42 (d, J = 8.5 Hz, 48H), 7.32-7.28 (m, 56H), 7.22-7.18 (m, 28H), 6.98 (s, 4H), 6.81 (d, J = 8.9 Hz, 16H), 6.76-6.70 (m, 8H), 6.53 (d, J = 8.2 Hz, 8H), 6.48 (bs, 4H), 6.41 (bd, J = 2.0 Hz, 8H), 5.25 (bs, 8H), 5.14 (bs, 8H), 4.71 (bq, 8H), 4.25 (bt, 8H), 4.12-4.02 (m, 32H), 3.89-3.79 (m, 32H), 3.53 (bt, 8H), 2.49 (bt, 8H), 1.73 (bt, 8H), 1.36 (m, 8H), 1.33 (s, 216H), 1.26 (t, J = 7.2H, 12H), 0.90-0.70 (m, 24H), 0.47 (t, J = 6.9Hz, 12H). MALDI-MS: 9,682.1 ([M]+H^+), 5,455.8 ([M]+H^+-DB(**8**)), 4,226.8 ([M]+H^+-MC(**1_{42}**)-DB(**8**)).

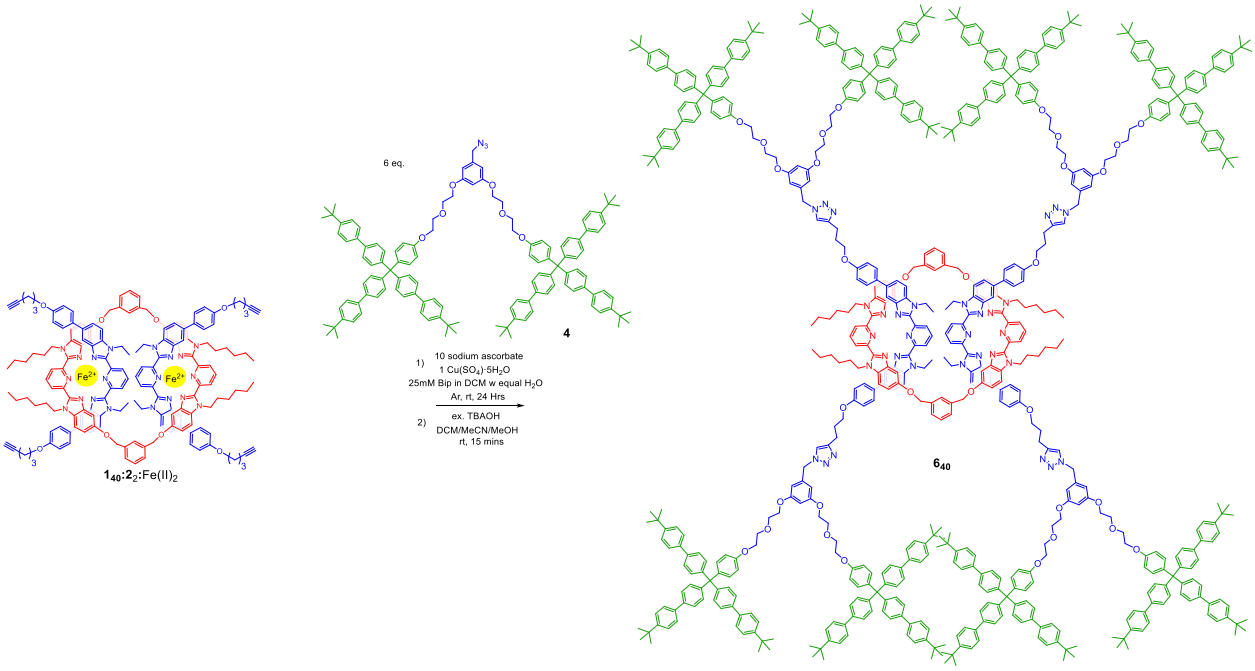

Figure S21. Stoppering and demetallation of $\mathbf{1}_{40}$:$\mathbf{2}_2$:Fe(II)$_2$ with **4** to make $\mathbf{6}_{40}$.

$\mathbf{1}_{42}$:$\mathbf{2}_2$:Fe(II)$_2$ (18.2 mg, 0.0047 mmol), **4** (51.7 mg, 0.030 mmol, 6eq) and sodium ascorbate (9.5 mg, 0.048 mmol, 10 eq) were added to a 4mL glass vial purged with Ar. To this mixture was added CH_2Cl_2 (0.75 mL, conc of alkyne = 25 mM), H_2O (0.65 mL), and 100µL of an aqueous stock solution of $Cu(SO_4)\cdot 5H_2O$ (47mM, 0.0047mmol, 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 $\mathbf{6}_{40}$ 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, $\mathbf{6}_{40}$ in 70% isolated yield (31.7 mg). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.30 (d, J = 7.8Hz, 4H), 7.96 (d, J = 7.8Hz, 4H), 7.91 (t, J = 7.8Hz, 2H), 7.85 (s, 4H), 7.73 (t, J = 7.8Hz, 2H), 7.55-7.44 (m, 104H), 7.42 (d, J = 8.5 Hz, 48H), 7.34 (d, J = 8.1Hz, 8H), 7.32-7.28 (m, 50H), 7.22-7.16 (m, 26H), 7.08 (s, 4H), 6.88-6.74 (m, 24H), 6.53 (d, J = 7.8 Hz, 8H), 6.48 (bs, 4H), 6.41 (bd, 8H), 5.21 (bs, 8H), 5.00 (bs, 8H), 4.68 (bq, 8H), 4.25 (bt, 8H), 4.08 (bt, 16H), 4.01 (bt, 16H), 3.88-3.77 (m, 32H), 3.55 (bt, 8H), 2.49 (bt, 8H), 1.77 (bt, 8H), 1.38 (m, 8H), 1.33 (s, 216H), 1.26 (t, 12H), 0.92-0.75 (m, 24H), 0.57 (t, J = 7.2Hz, 12H). MALDI-MS: 9,682.8 ([M]+H$^+$), 5,456.6 ([M]+H$^+$-DB(**8**)), 4,227.5 ([M]+H$^+$-MC($\mathbf{1}_{40}$)-DB(**8**)).

Attempted Characterization of [3]Rotaxanes 5_{44} and 6_{48}

Attempted characterization of [3]Rotaxane $\mathbf{6_{48}}$

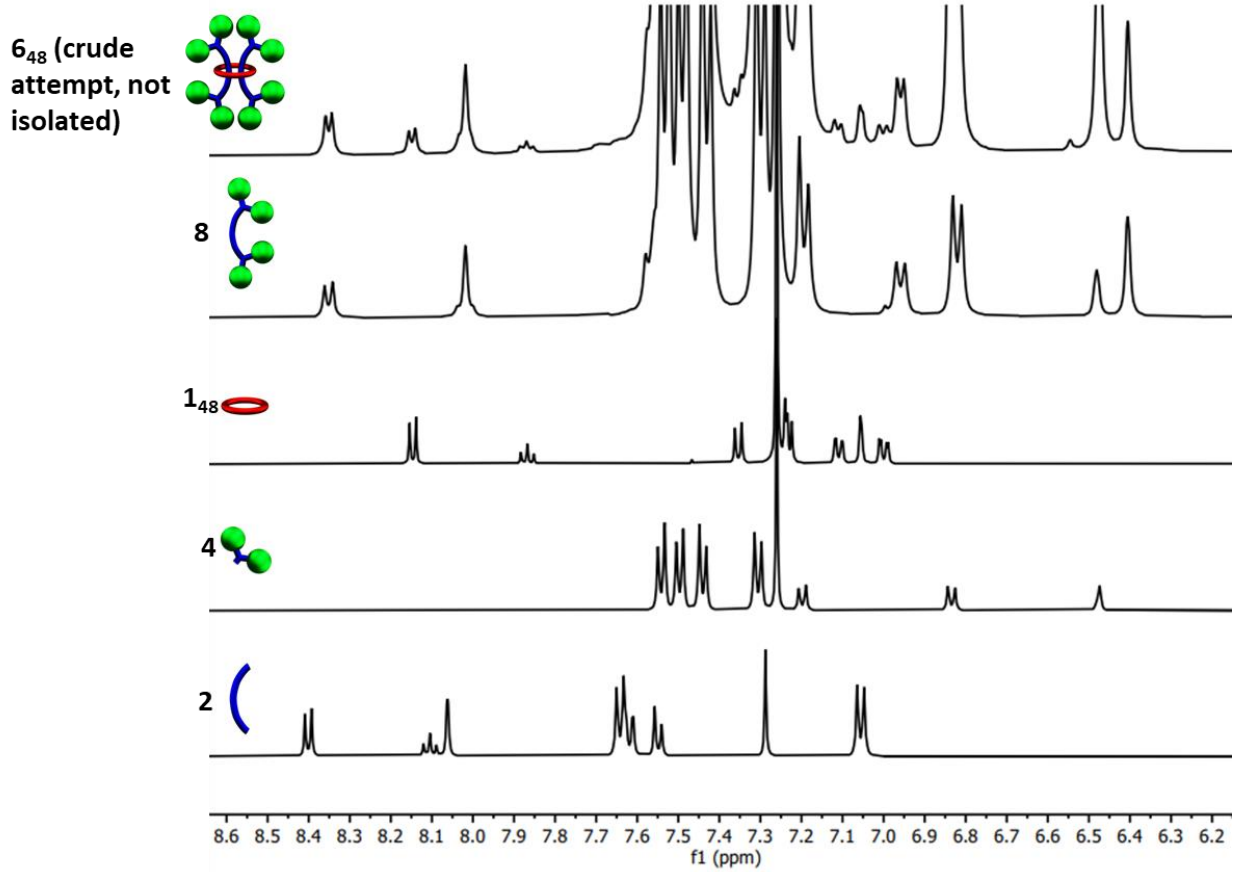

Figure S22. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of the demetallated crude reaction mixture from the stoppering of $\mathbf{1_{48}}$**:**$\mathbf{2_2}$**:**$Fe(II)_2$ with **4**. Top NMR spectrum corresponds to crude reaction mixture and bottom four spectra correspond to indicated components and starting materials for comparison.

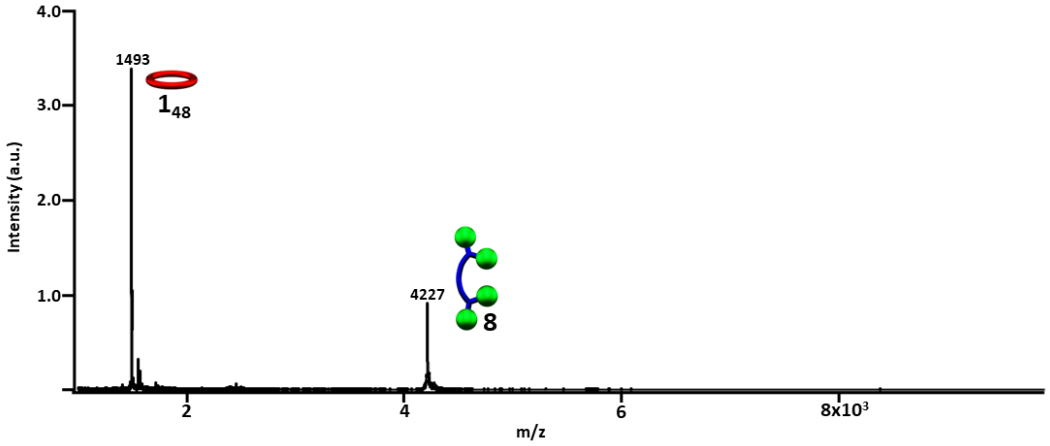

Figure S23. Maldi-TOF analysis (Dithranol, no salt) of the of the demetallated crude reaction mixture from the stoppering of $\mathbf{1_{48}}$**:**$\mathbf{2_2}$**:**$Fe(II)_2$ with **4**.

Attempted characterization of [3]Rotaxane **5**$_{44}$

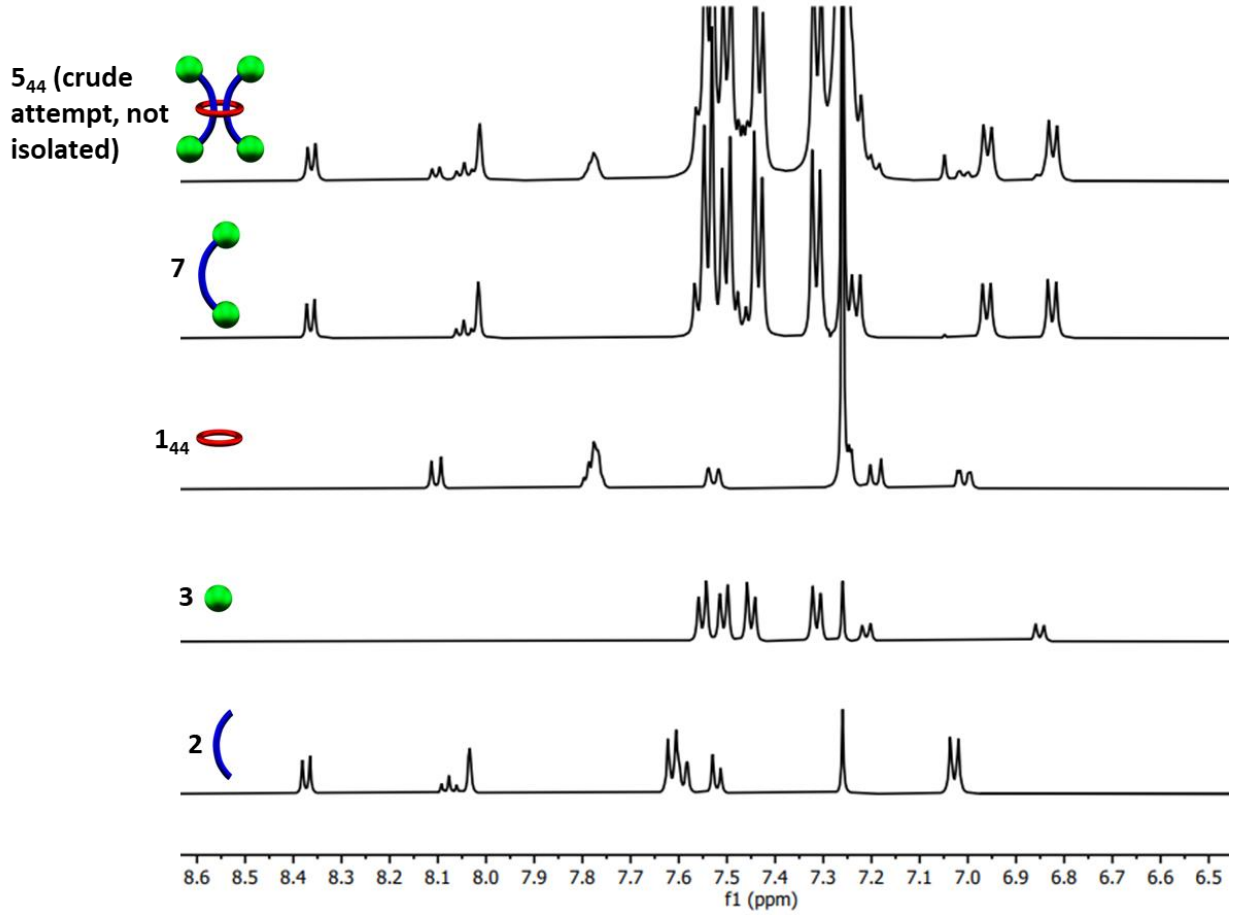

Figure S24. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of the demetallated crude reaction mixture from the stoppering of **1**$_{44}$**:2**$_2$**:**Fe(II)$_2$ with **3**. Top NMR spectrum corresponds to crude reaction mixture and bottom four spectra correspond to indicated components and starting materials for comparison.

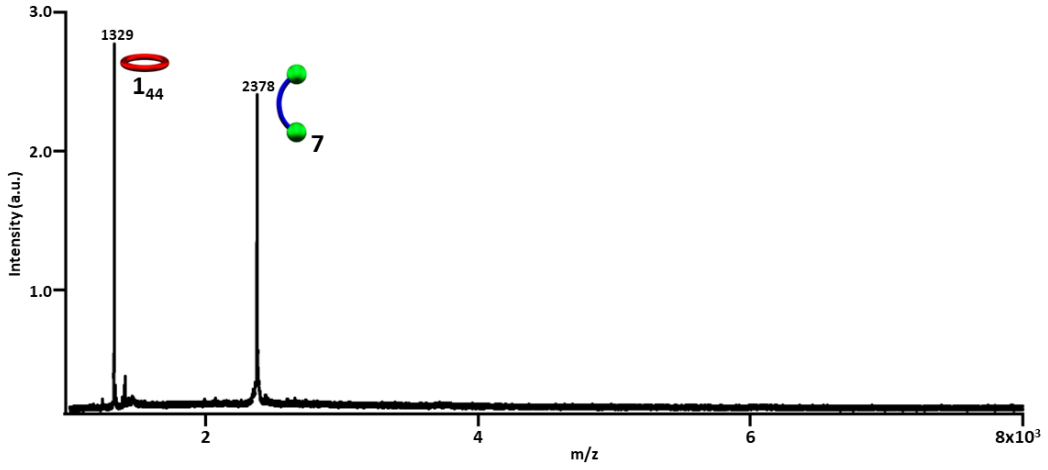

Figure S25. Maldi-TOF analysis (Dithranol, no salt) of the of the demetallated crude reaction mixture from the stoppering of **1**$_{44}$**:2**$_2$**:**Fe(II)$_2$ with **3**.

Crude ^{1}H-NMR Characterization of [3]Rotaxanes $5_{40\text{-}42}$ and $6_{40\text{-}44}$

Crude ^{1}H-NMR of [3]Rotaxanes $\mathbf{6}_{40\text{-}44}$

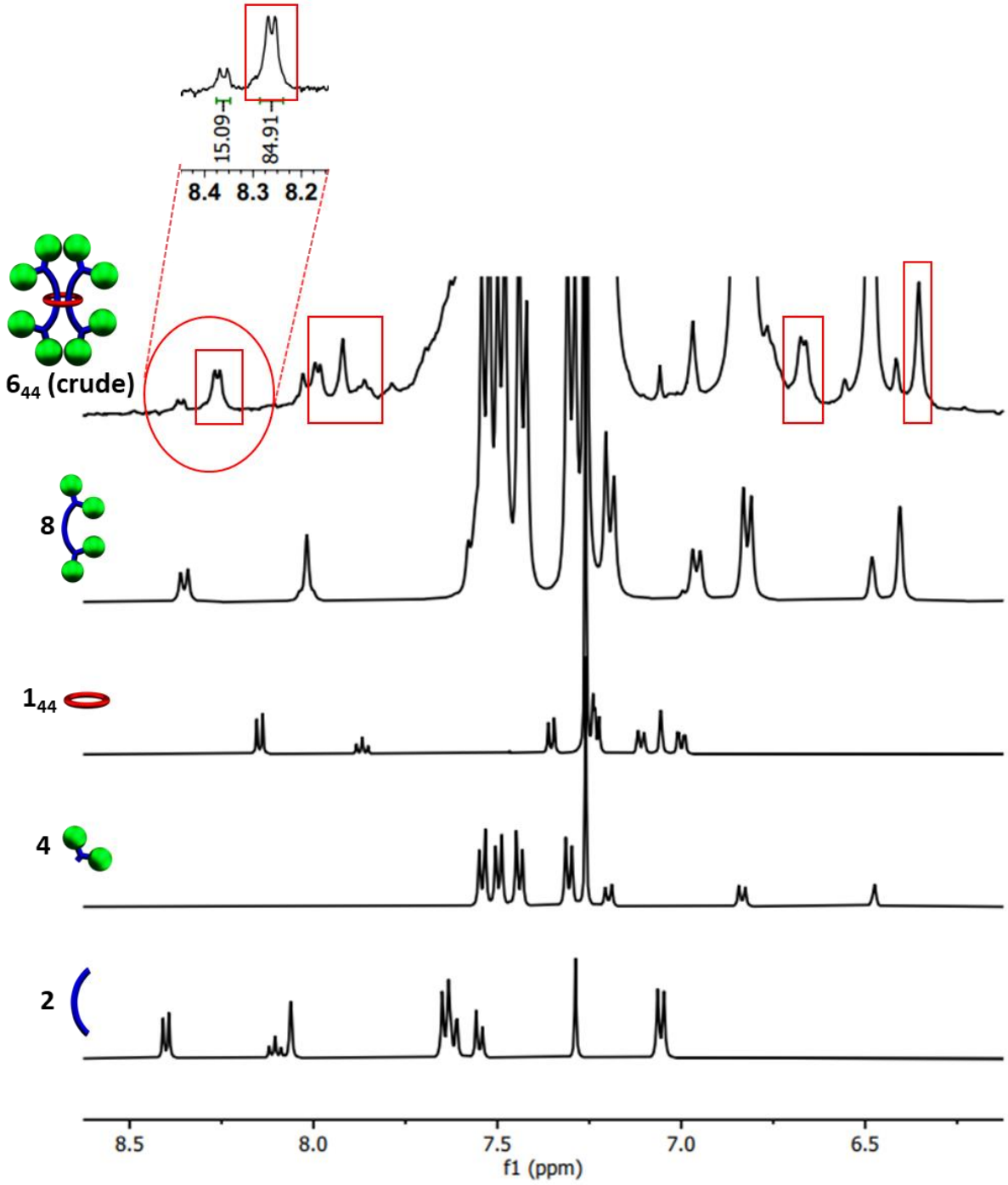

Figure S26. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of the demetallated crude reaction mixture from the stoppering of $\mathbf{1}_{44}$**:2**$_2$**:**Fe(II)$_2$ with **4**. Top NMR spectrum corresponds to crude reaction mixture and bottom four spectra correspond to indicated components and starting materials for comparison. Red boxes indicate interlocked product and expansion provides integral for crude interlocked conversion.

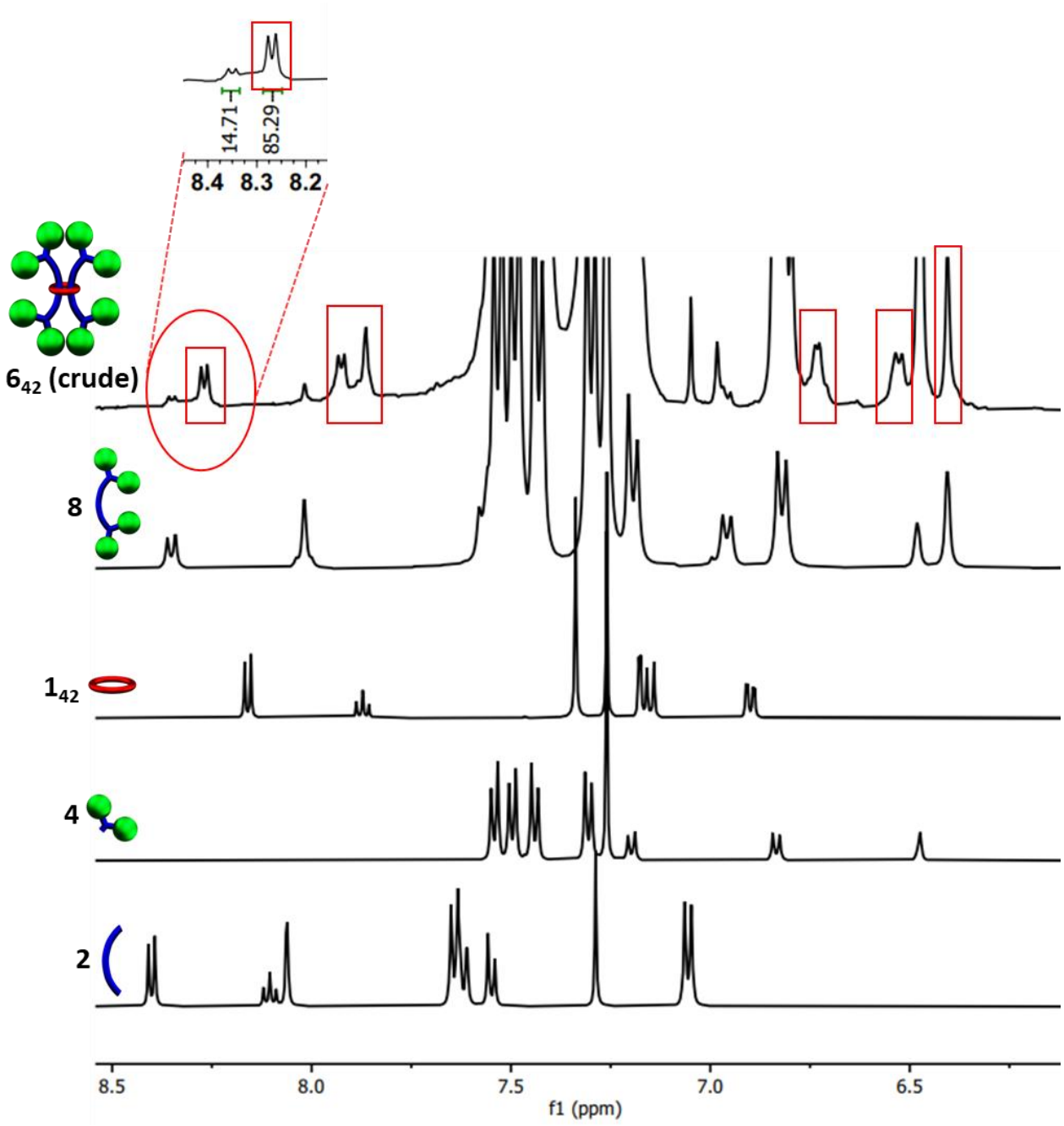

Figure S27. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of the demetallated crude reaction mixture from the stoppering of $\mathbf{1}_{42}$:$\mathbf{2}_2$:Fe(II)$_2$ with **4**. Top NMR spectrum corresponds to crude reaction mixture and bottom four spectra correspond to indicated components and starting materials for comparison. Red boxes indicate interlocked product and expansion provides integral for crude interlocked conversion.

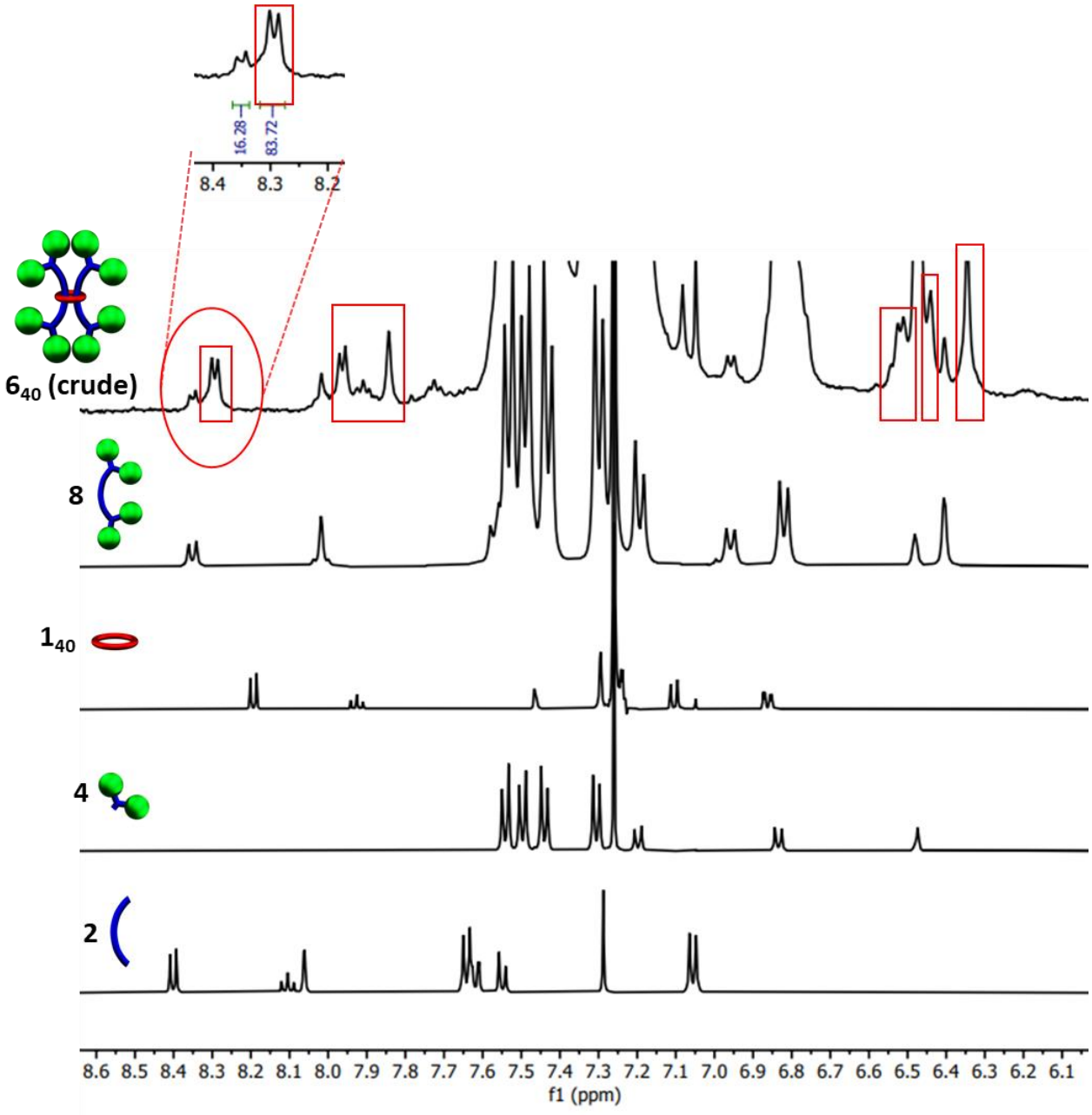

Figure S28. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of the demetallated crude reaction mixture from the stoppering of $\mathbf{1}_{40}$**:**$\mathbf{2}_2$**:**Fe(II)$_2$ with **4**. Top NMR spectrum corresponds to crude reaction mixture and bottom four spectra correspond to indicated components and starting materials for comparison. Red boxes indicate interlocked product and expansion provides integral for crude interlocked conversion.

Crude ^{1}H-NMR of [3]Rotaxanes $\mathbf{5}_{40\text{-}42}$

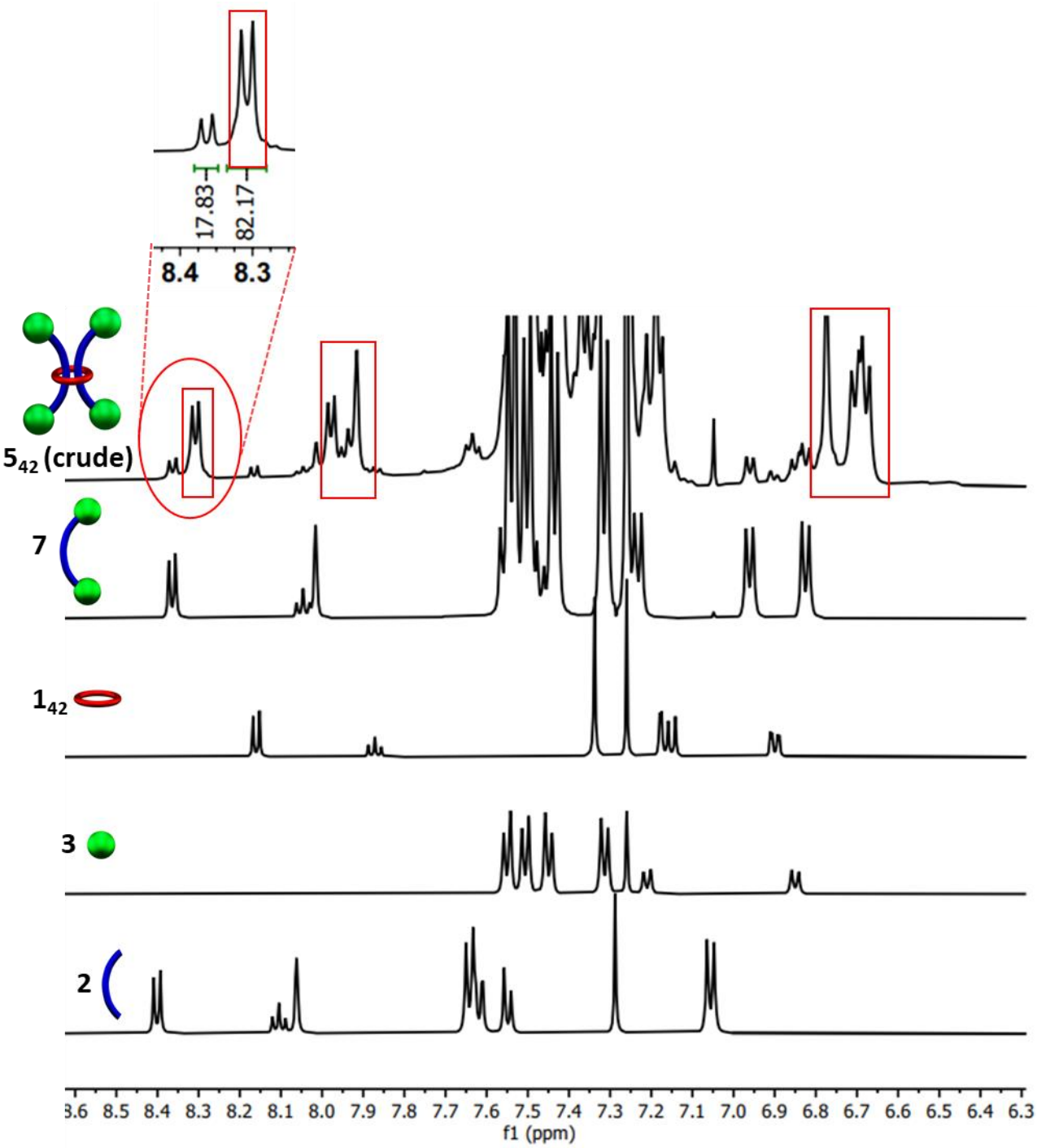

Figure S29. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of the demetallated crude reaction mixture from the stoppering of $\mathbf{1}_{42}$:$\mathbf{2}_2$:$Fe(II)_2$ with **3**. Top NMR spectrum corresponds to crude reaction mixture and bottom four spectra correspond to indicated components and starting materials for comparison.

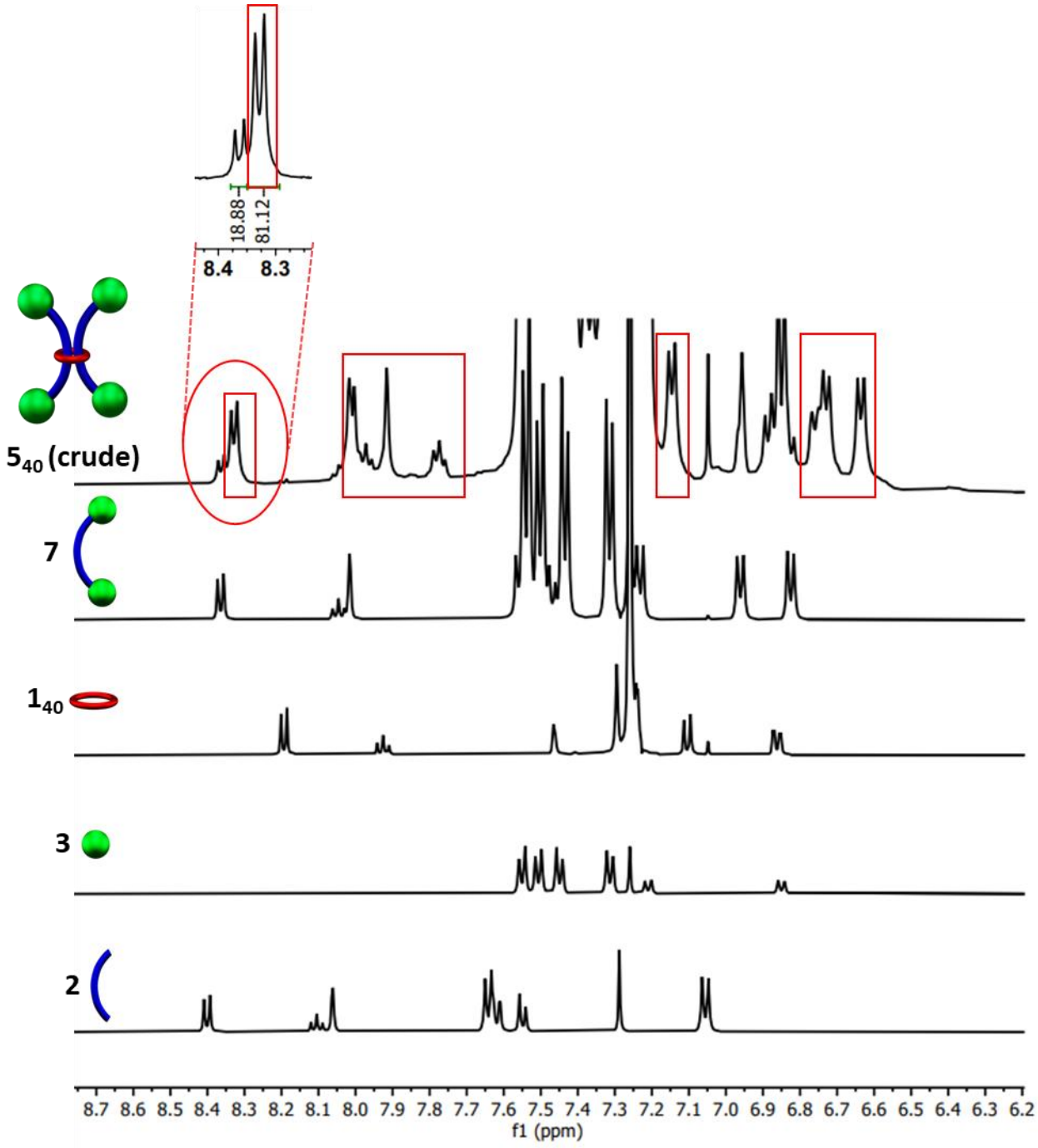

Figure S30. Partial ^{1}H-NMR overlay (500 MHz, 25°C, $CDCl_3$) of the demetallated crude reaction mixture from the stoppering of $\mathbf{1_{40}}$**:2**$_2$**:**Fe(II)$_2$ with **3**. Top NMR spectrum corresponds to crude reaction mixture and bottom four spectra correspond to indicated components and starting materials for comparison.

MALDI-TOF Characterization of [3]Rotaxanes $5_{40\text{-}42}$ and $6_{40\text{-}44}$

MALDI-TOF Analysis of [3]Rotaxanes $\mathbf{6_{40\text{-}44}}$

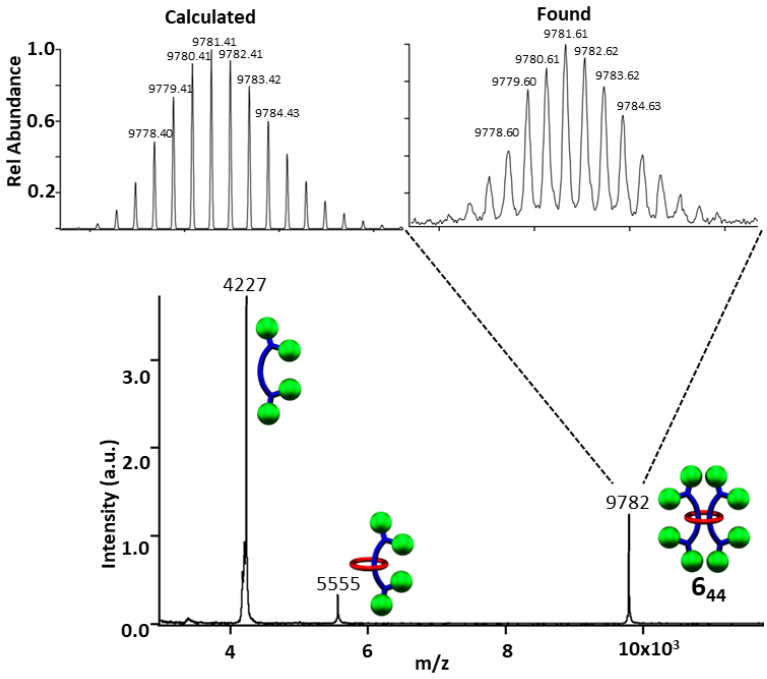

Figure S31. Maldi-TOF analysis (Dithranol, no salt) of purified [3]rotaxane $\mathbf{6_{44}}$ with expansion showing reflection mode analysis of the [3]rotaxane peak.

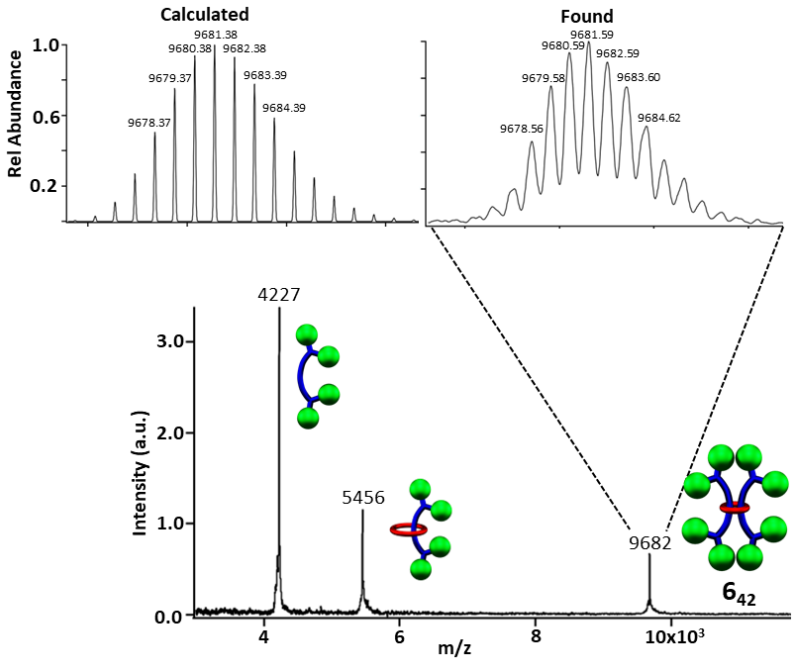

Figure S32. Maldi-TOF analysis (Dithranol, no salt) of purified [3]rotaxane $\mathbf{6_{42}}$ with expansion showing reflection mode analysis of the [3]rotaxane peak.

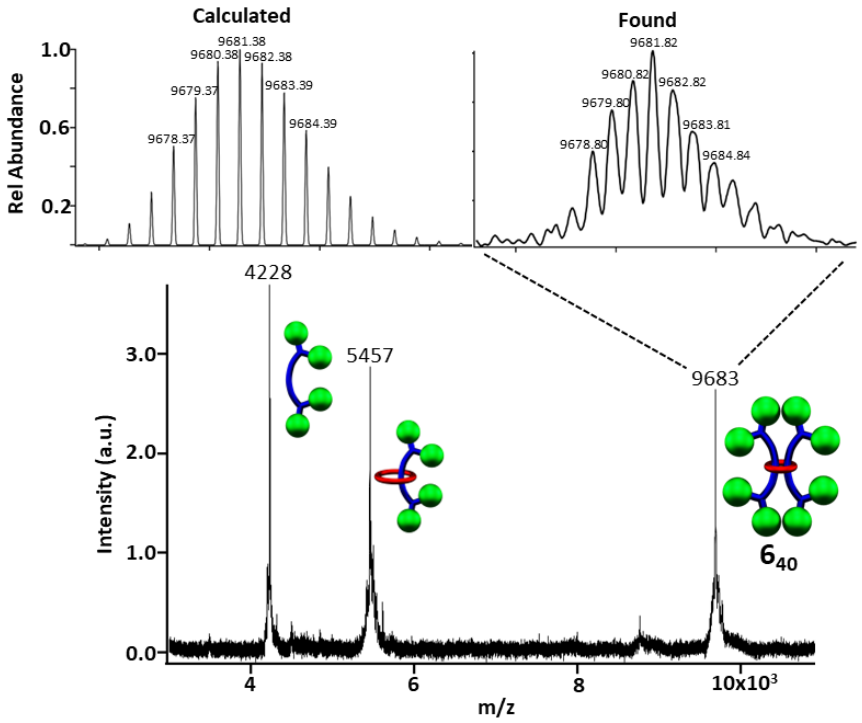

Figure S33. Maldi-TOF analysis (Dithranol, no salt) of purified [3]rotaxane $\mathbf{6_{40}}$ with expansion showing reflection mode analysis of the [3]rotaxane peak.

MALDI-TOF Analysis of [3]Rotaxanes $\mathbf{5_{40\text{-}42}}$

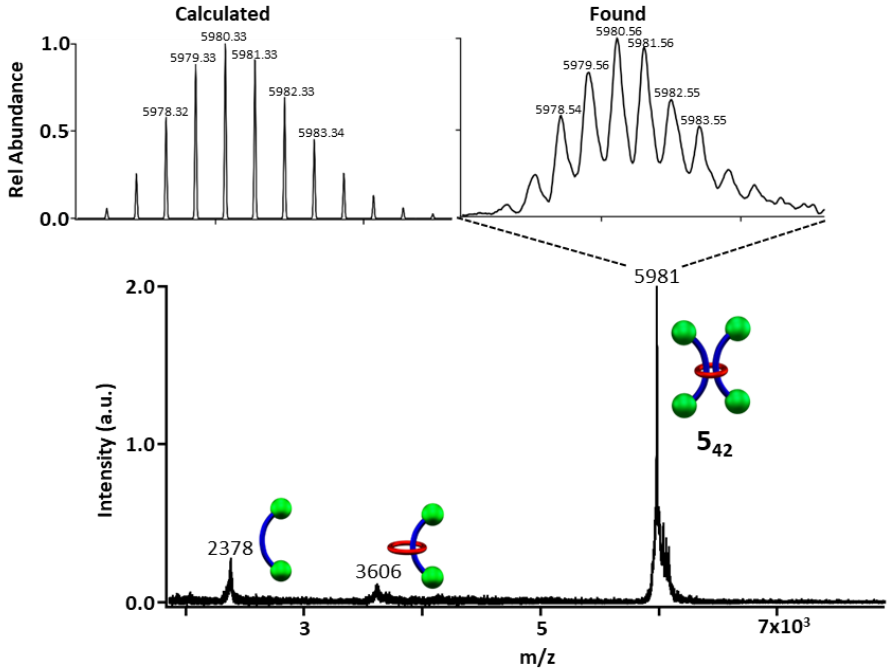

Figure S34. Maldi-TOF analysis (Dithranol, no salt) of purified [3]rotaxane $\mathbf{5_{42}}$ with expansion showing reflection mode analysis of the [3]rotaxane peak.

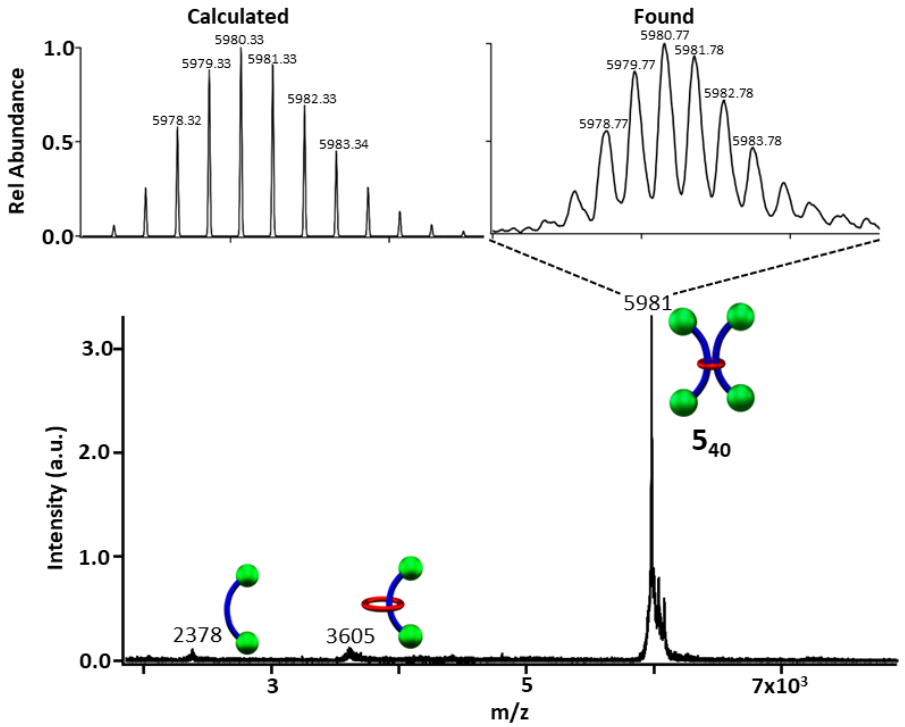

Figure S35. Maldi-TOF analysis (Dithranol, no salt) of purified [3]rotaxane $\mathbf{5_{40}}$ with expansion showing reflection mode analysis of the [3]rotaxane peak.

SEC Analysis of [3]Rotaxanes $5_{40\text{-}42}$ and $6_{40\text{-}44}$

SEC Analysis of [3]Rotaxanes $\mathbf{6}_{40\text{-}44}$

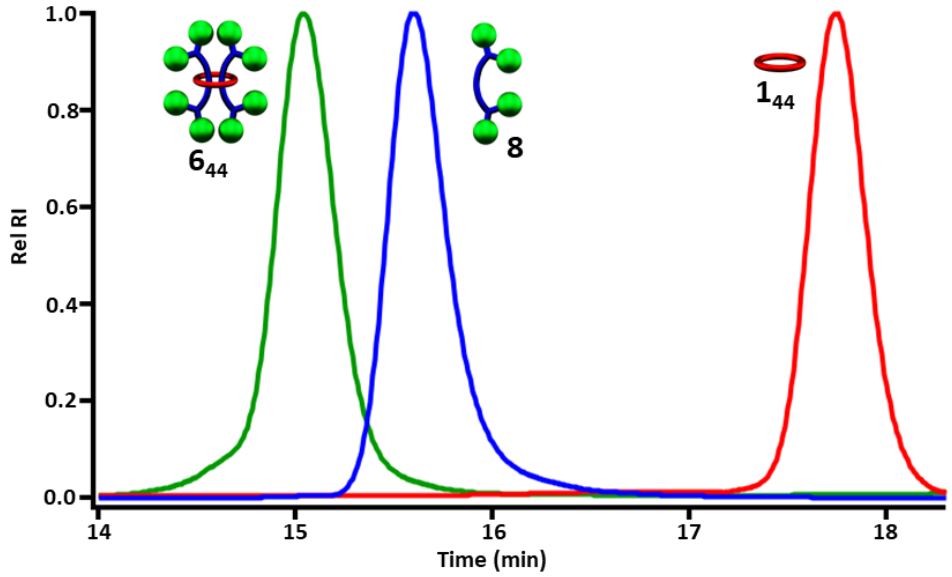

Figure S36. GPC chromatogram of (eluent 3:1 THF:DMF) of purified $\mathbf{6}_{44}$, **8**, and $\mathbf{1}_{44}$ at 25°C.

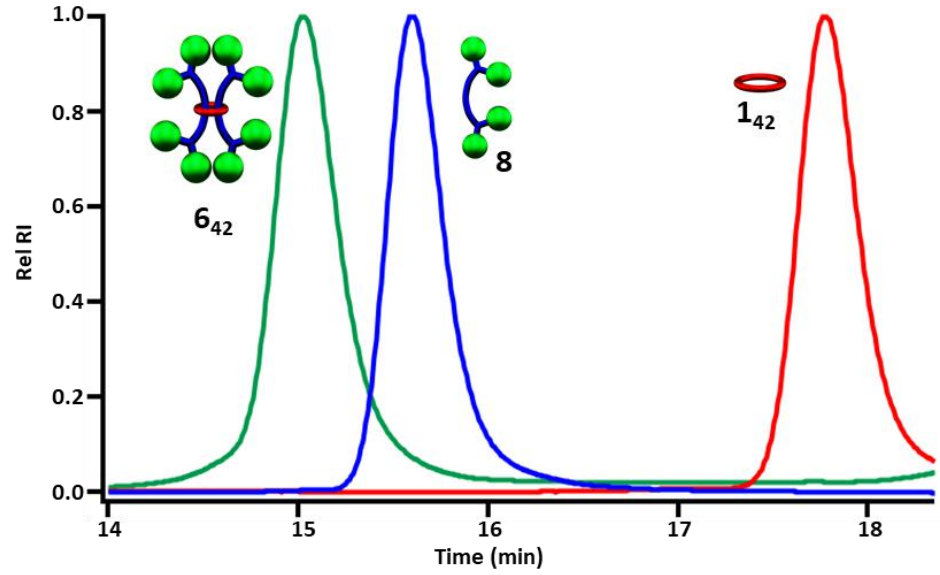

Figure S37. GPC chromatogram of (eluent 3:1 THF:DMF) of purified $\mathbf{6}_{42}$, **8**, and $\mathbf{1}_{42}$ at 25°C.

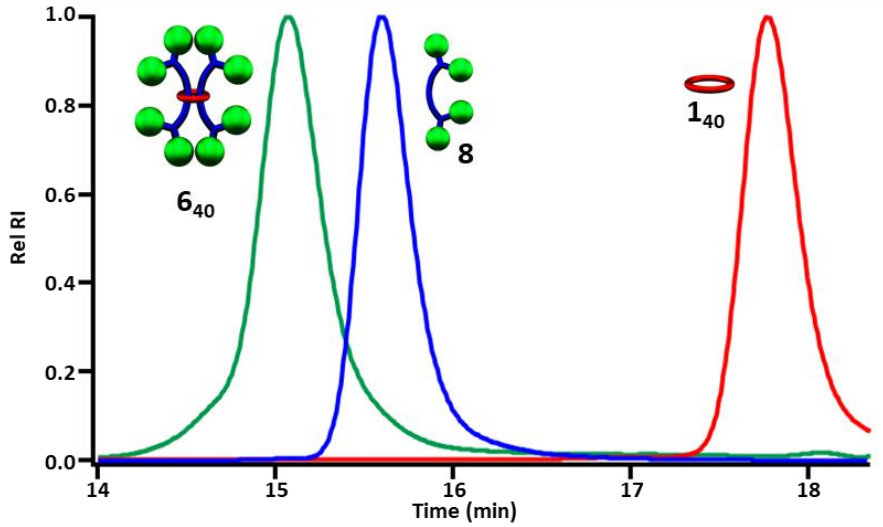

Figure S38. GPC chromatogram of (eluent 3:1 THF:DMF) of purified $\mathbf{6}_{40}$, **8**, and $\mathbf{1}_{40}$ at 25°C.

SEC Analysis of [3]Rotaxanes $\mathbf{5}_{40\text{-}42}$

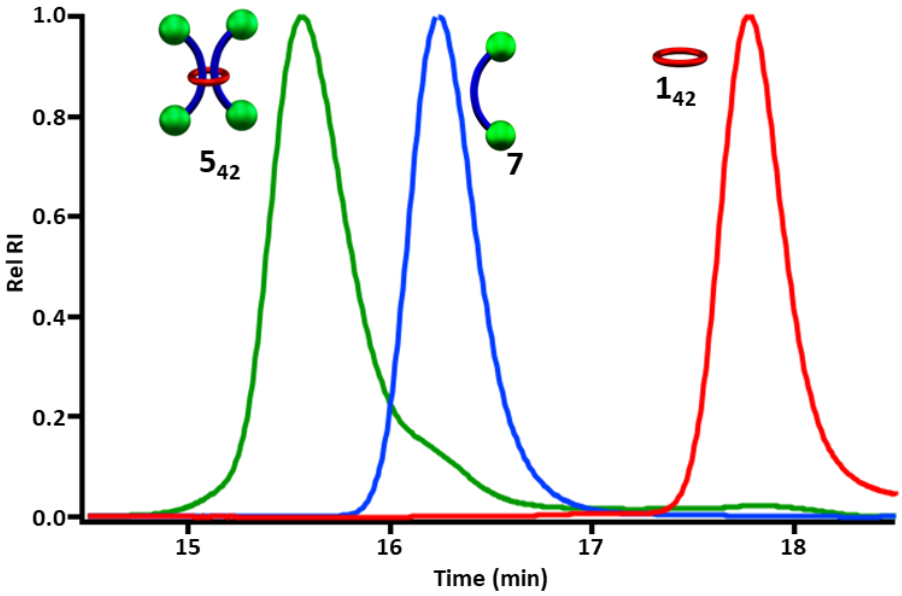

Figure S39. GPC chromatogram of (eluent 3:1 THF:DMF) of purified $\mathbf{5}_{42}$, **8**, and $\mathbf{1}_{42}$ at 25°C. Samples spent approximately 3 hours in solution between purification and sample prep resulting in a small amount of noninterlocked product observed in the baseline.

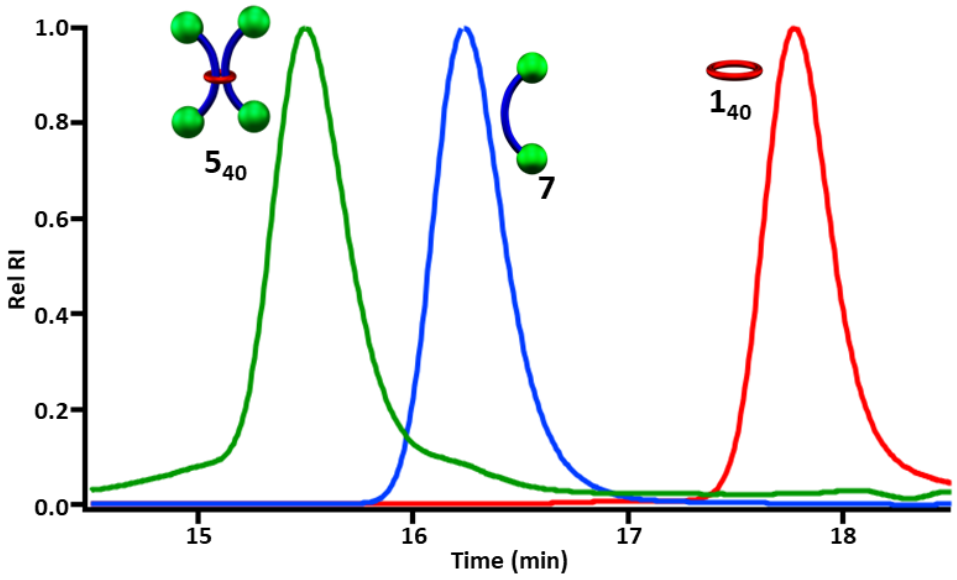

Figure S40. GPC chromatogram of (eluent 3:1 THF:DMF) of purified $\mathbf{5}_{40}$, **8**, and $\mathbf{1}_{40}$ at 25°C. Samples spent approximately 3 hours in solution between purification and sample prep resulting in a small amount of noninterlocked product observed in the baseline.

^{1}H and ^{1}H-^{1}H COSY NMR Characterization of [3]Rotaxanes $5_{40\text{-}42}$ and $6_{40\text{-}44}$

^{1}H and ^{1}H-^{1}H COSY NMR Characterization of [3]Rotaxanes $\mathbf{6_{40\text{-}44}}$

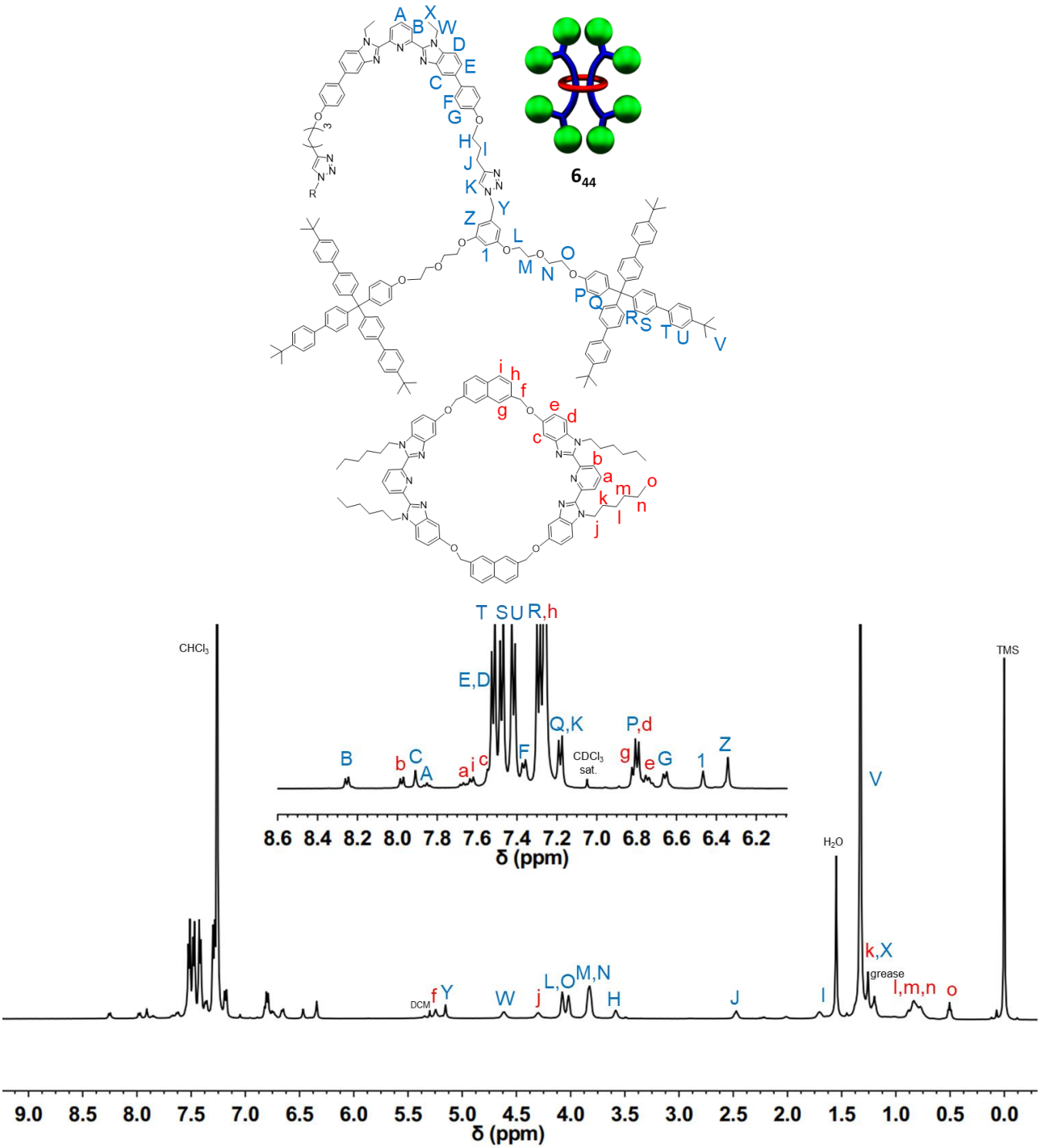

Figure S41. Full ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of doubly threaded [3]rotaxane $\mathbf{6_{44}}$. Peak assignments correspond to those given in top of figure.

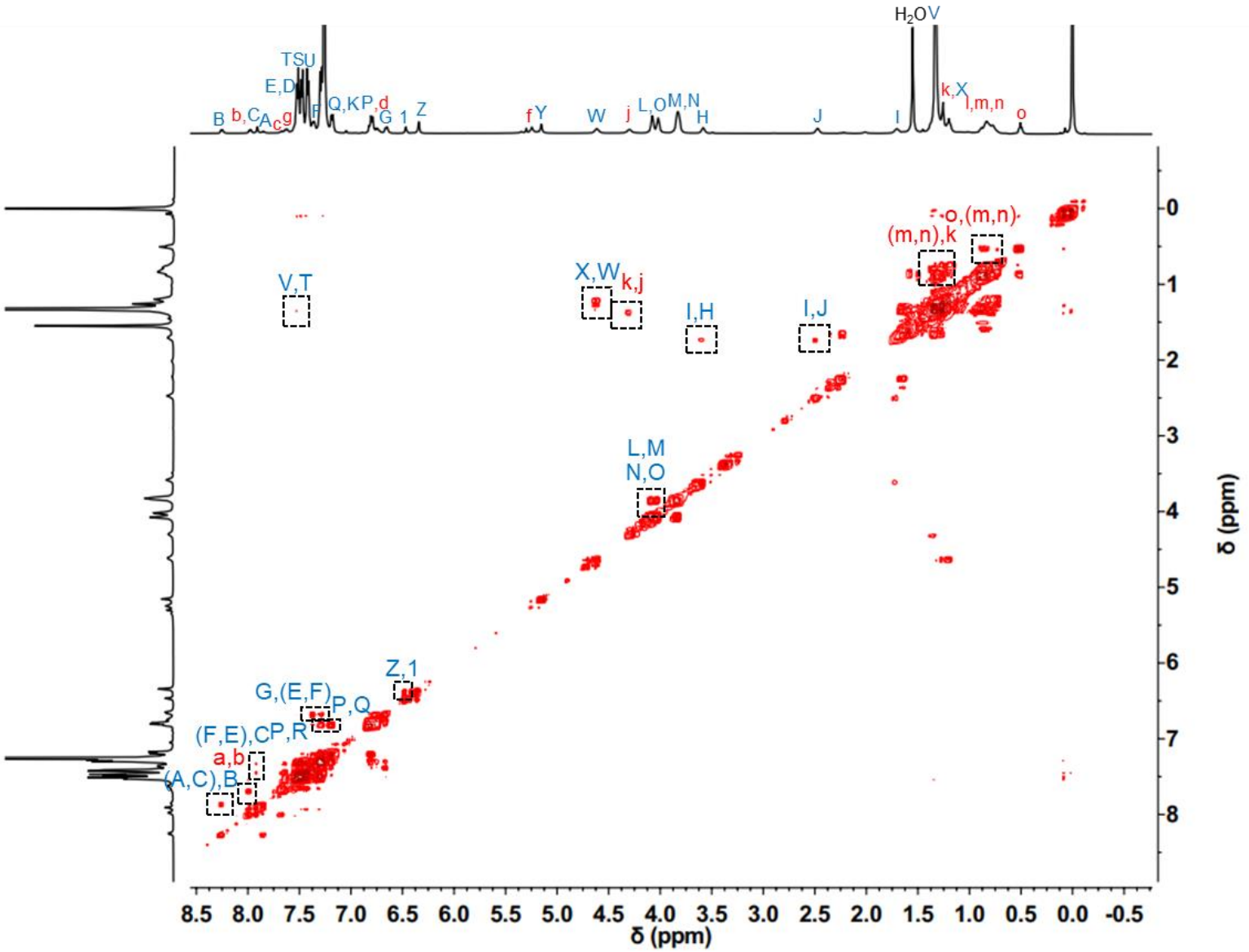

Figure S42. Full ^{1}H-^{1}H COSY (5mM, 500 MHz, 25°C, $CDCl_3$) of $\mathbf{6_{44}}$. Select ^{1}H annotations correspond to labels in Figure S41.

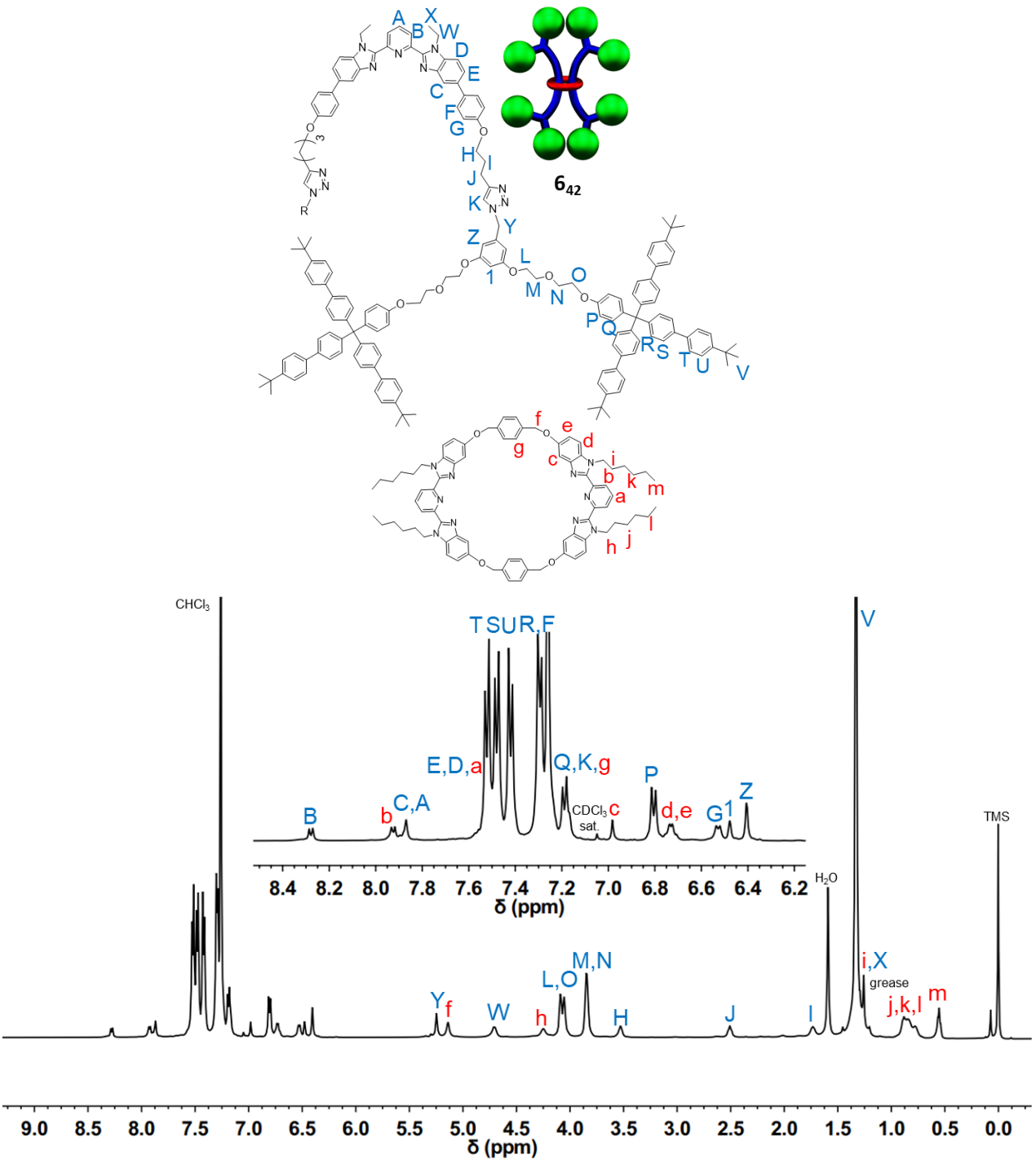

Figure S43. Full ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of doubly threaded [3]rotaxane **6**$_{42}$. Peak assignments correspond to those given in top of figure.

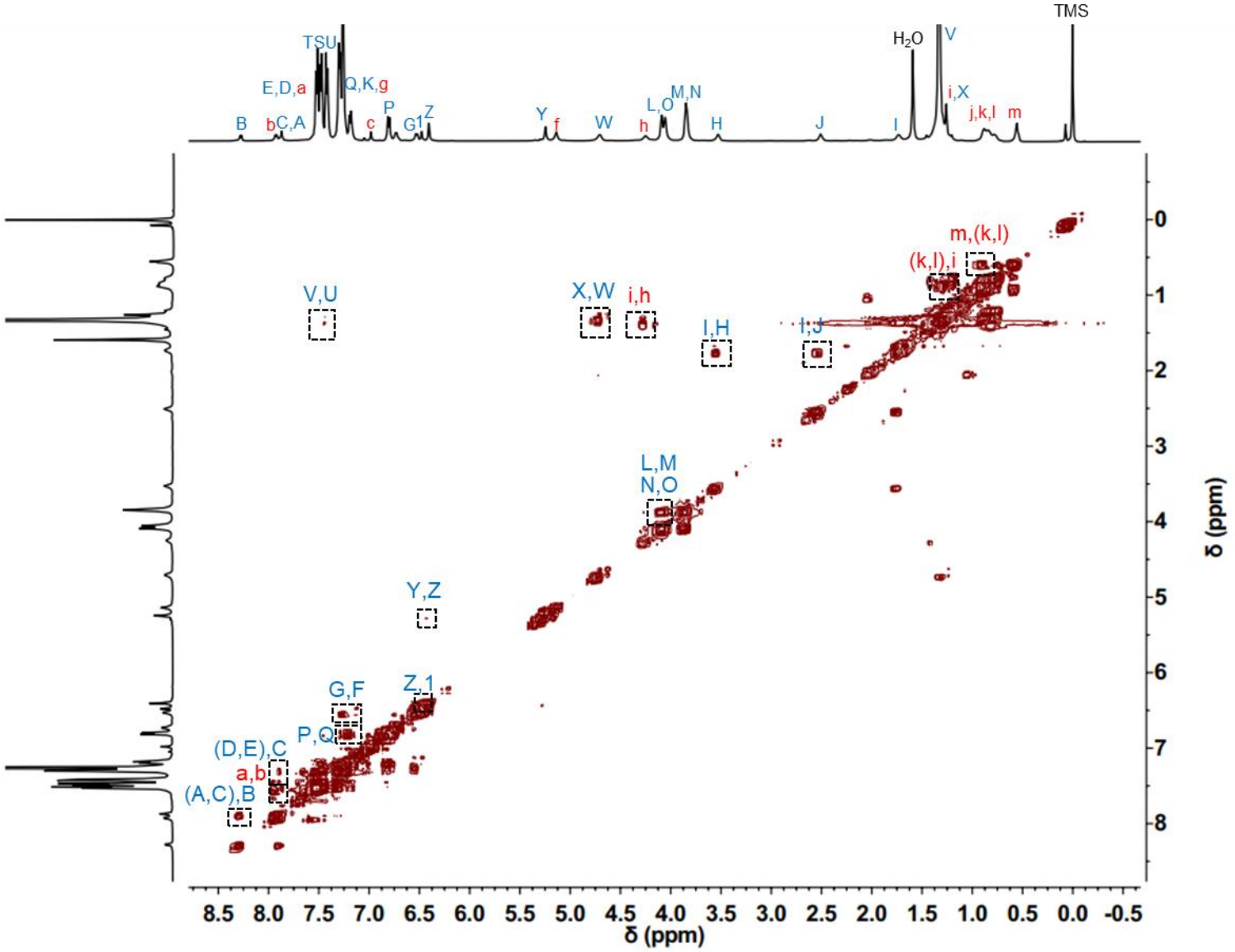

Figure S44. Full ^{1}H-^{1}H COSY (5mM, 500 MHz, 25°C, $CDCl_3$) of **6**$_{42}$. Select ^{1}H annotations correspond to labels in Figure S43.

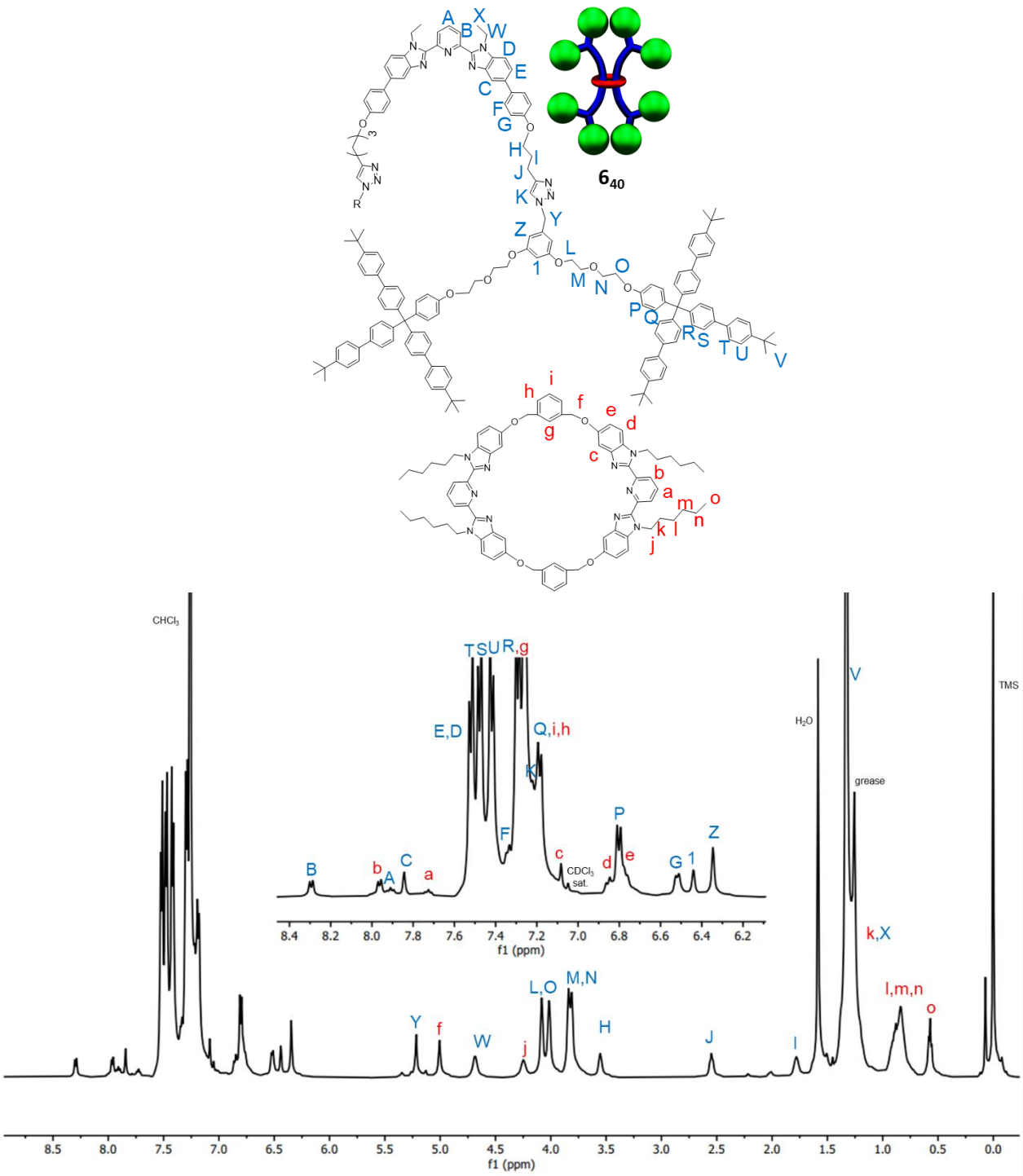

Figure S45. Full ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of doubly threaded [3]rotaxane **6_{40}**. Peak assignments correspond to those given in top of figure.

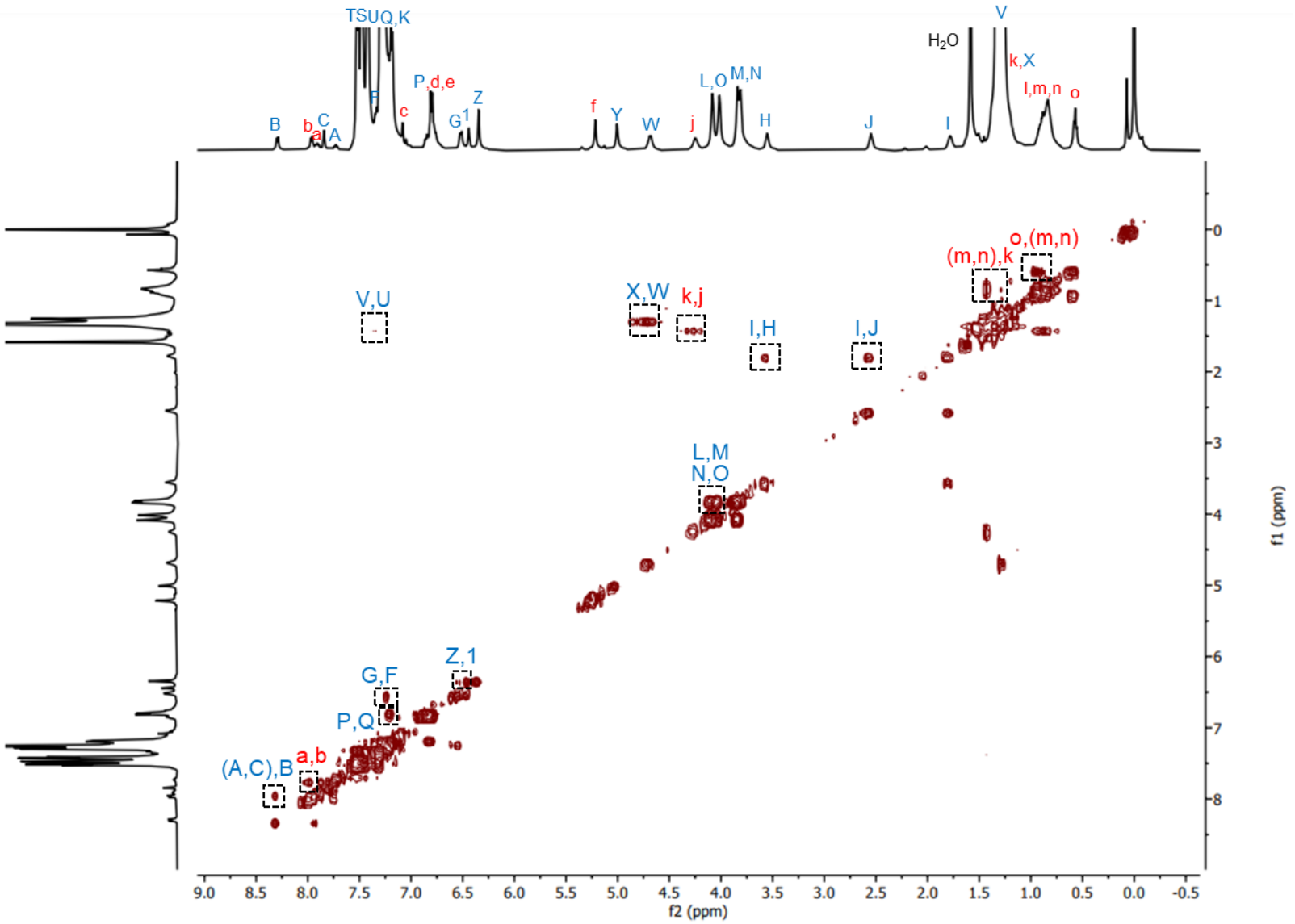

Figure S46. Full ^{1}H-^{1}H COSY (5mM, 500 MHz, 25°C, $CDCl_3$) of $\mathbf{6}_{40}$. Select ^{1}H annotations correspond to labels in Figure S45.

[1]H and [1]H-[1]H COSY NMR Characterization of [3]Rotaxanes **5**$_{40\text{-}42}$

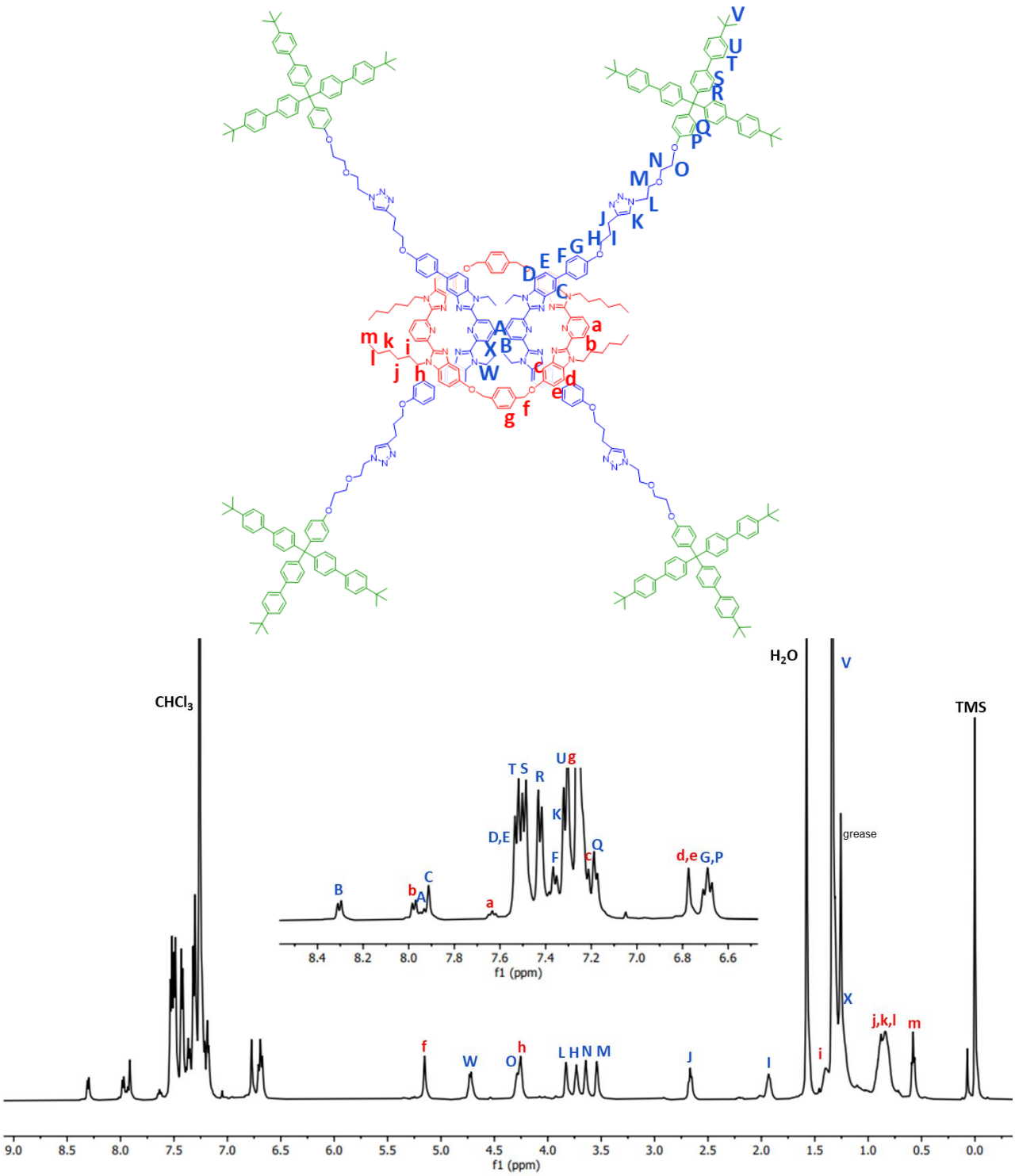

Figure S47. Full [1]H-NMR (500 MHz, 25°C, $CDCl_3$) of doubly threaded [3]rotaxane **5**$_{42}$. Peak assignments correspond to those given in top of figure.

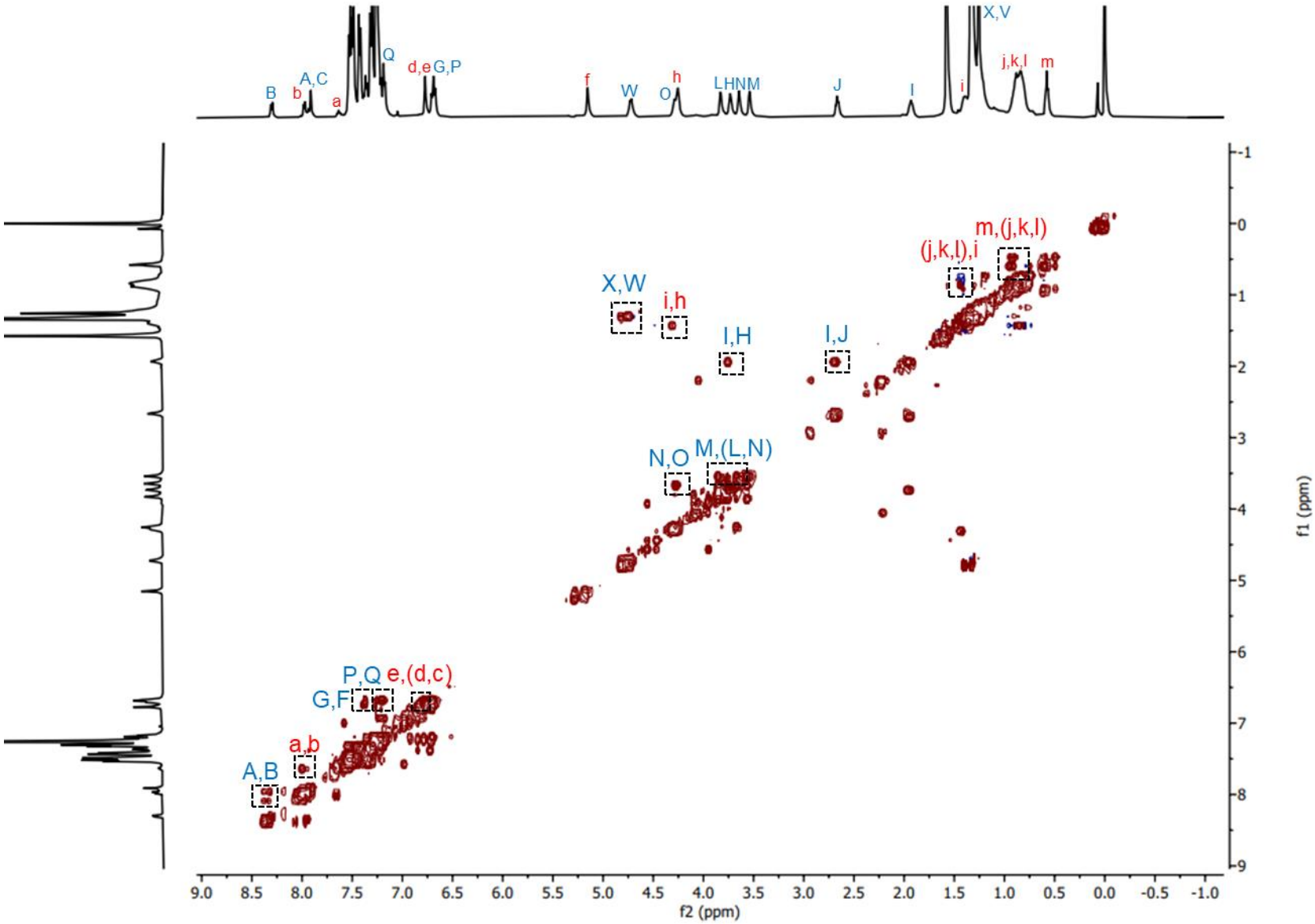

Figure S48. Full ^{1}H-^{1}H COSY (5mM, 500 MHz, 25°C, $CDCl_3$) of $\mathbf{5_{42}}$. Select ^{1}H annotations correspond to labels in Figure S47.

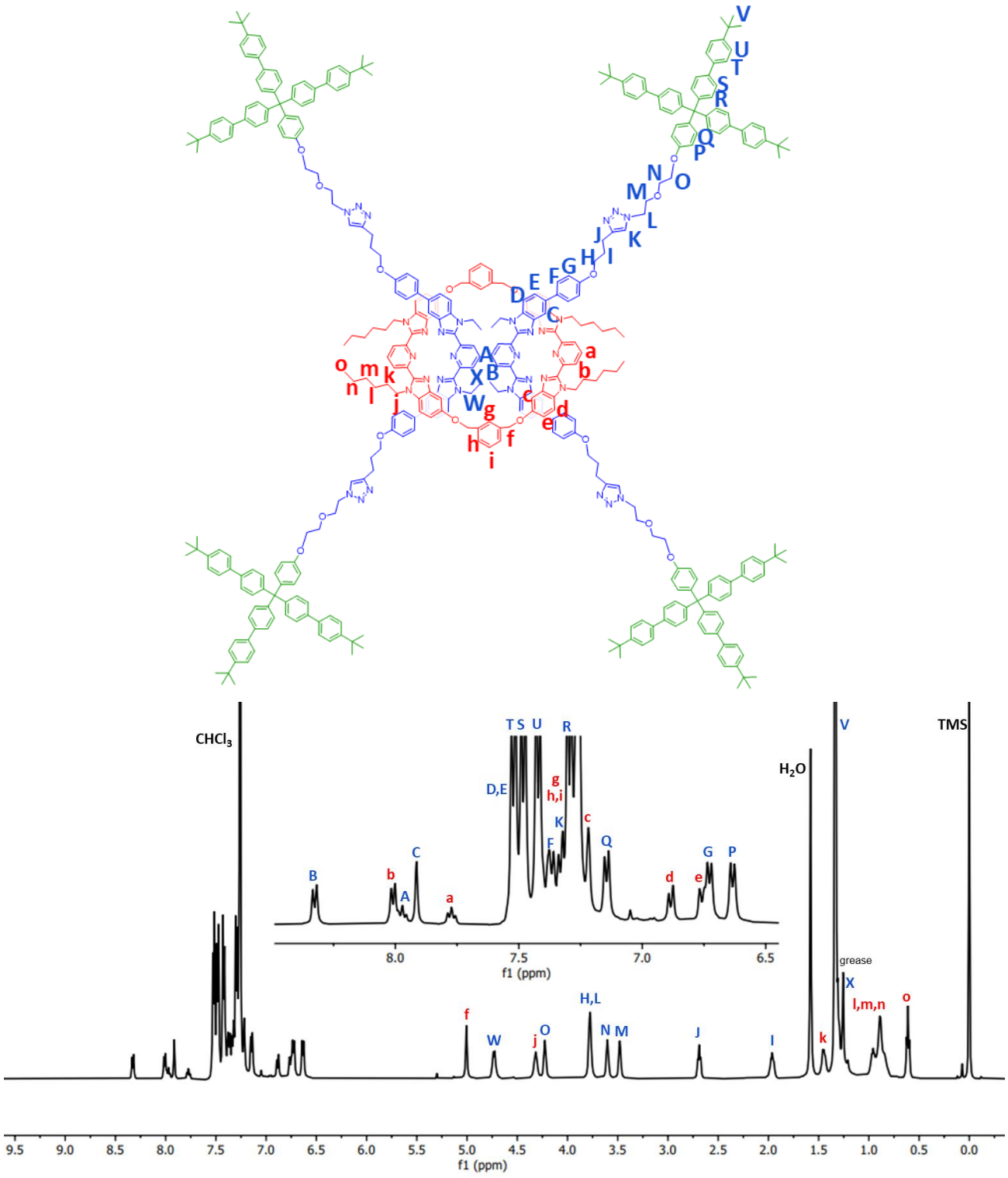

Figure S49. Full ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of doubly threaded [3]rotaxane **5$_{40}$**. Peak assignments correspond to those given in top of figure.

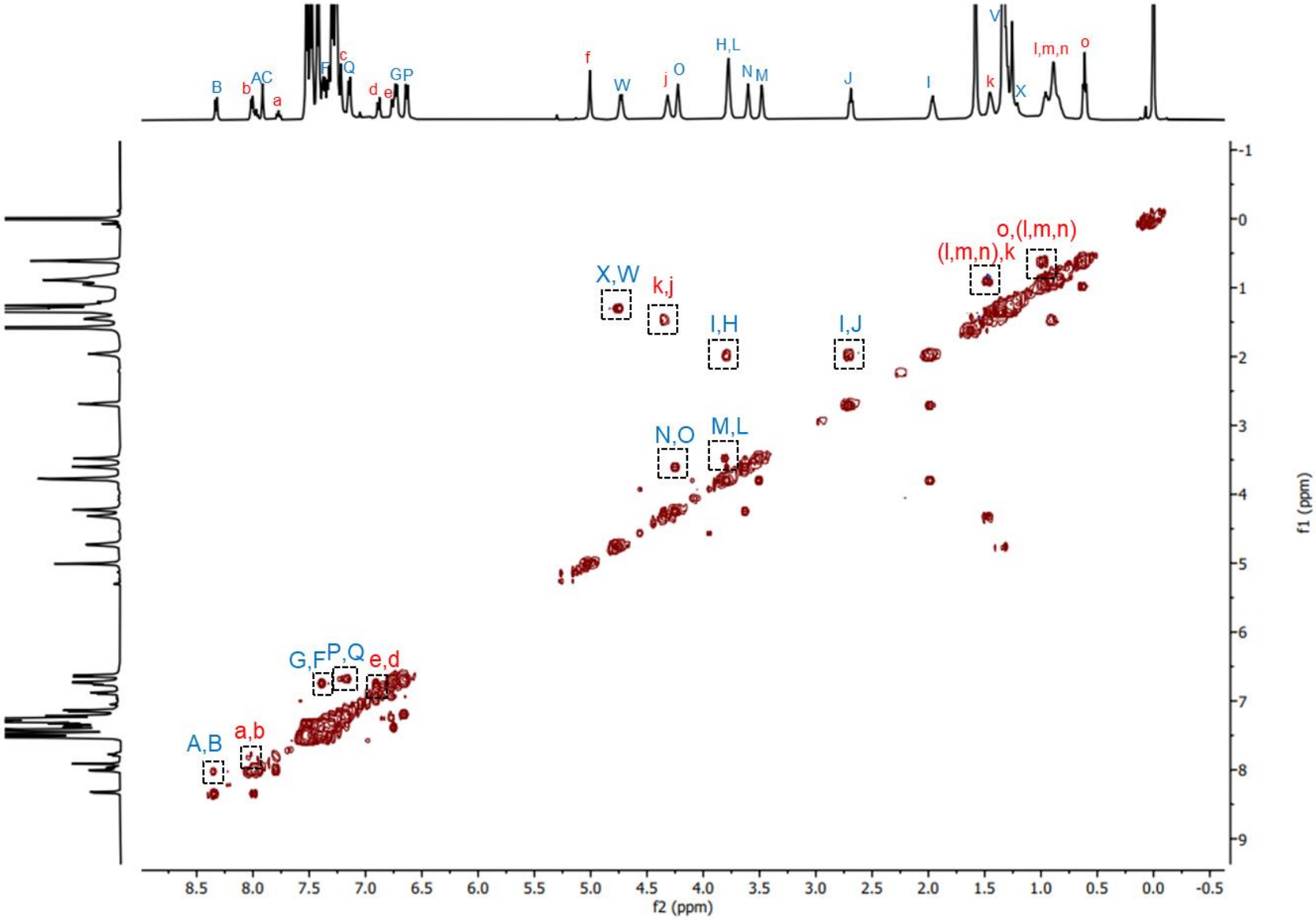

Figure S50. Full ^{1}H-^{1}H COSY (5mM, 500 MHz, 25°C, $CDCl_3$) of $\mathbf{5_{40}}$. Select ^{1}H annotations correspond to labels in Figure S49.

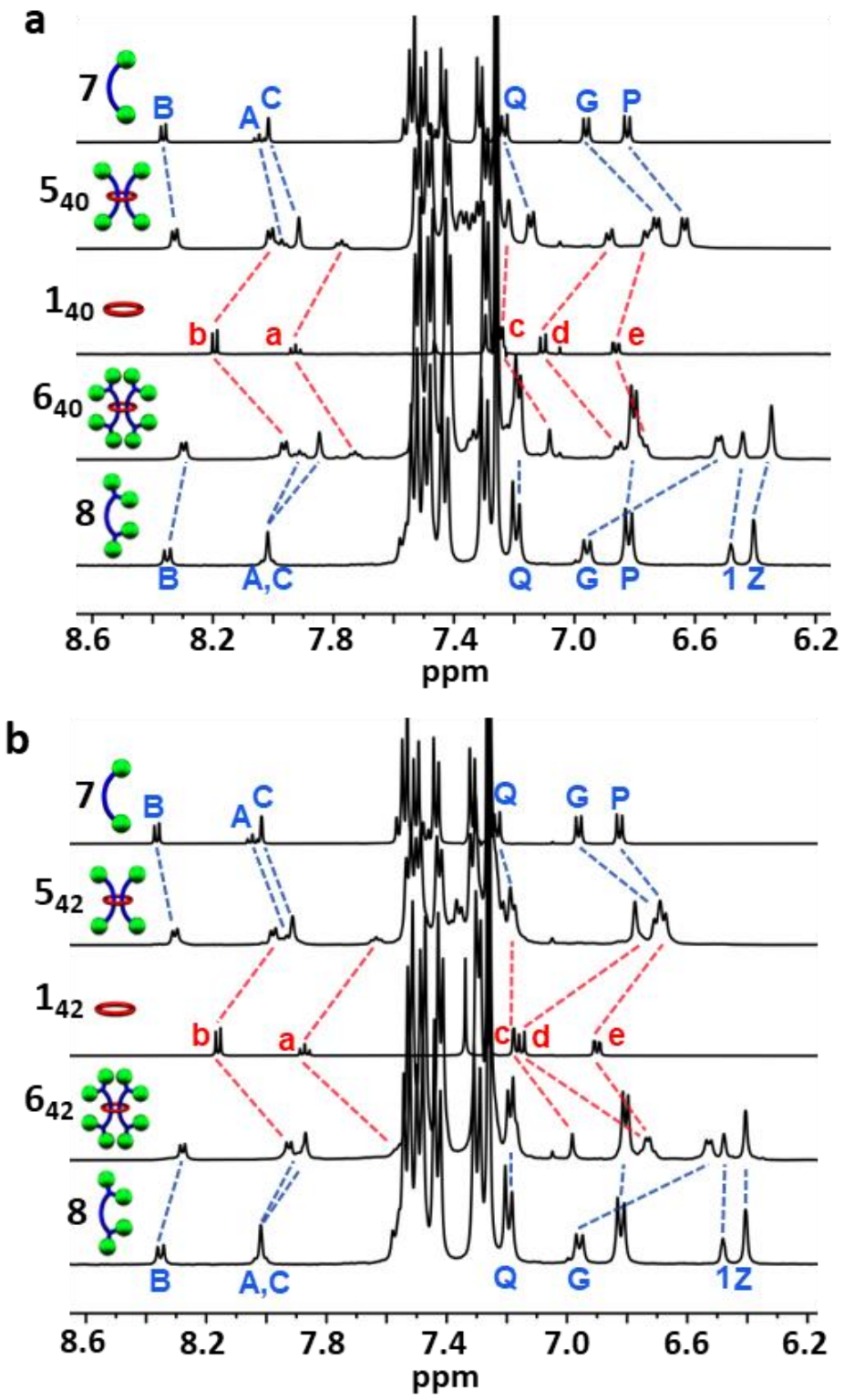

Figure S51. Partial ^{1}H-NMR Overlay (500 mHz, $CDCl_3$, 25°C) of a) [3]rotaxanes **5**$_{40}$ and **6**$_{40}$ and their free components **1**$_{40}$, **7**, and **8** and b) [3]rotaxanes **5**$_{42}$ and **6**$_{42}$ and their free components **1**$_{42}$, **7**, and **8.**

^{1}H-^{1}H NOESY NMR Characterization of [3]Rotaxanes $\mathbf{6}_{40\text{-}44}$

^{1}H-^{1}H NOESY NMR Characterization of [3]Rotaxanes $\mathbf{6}_{40\text{-}44}$

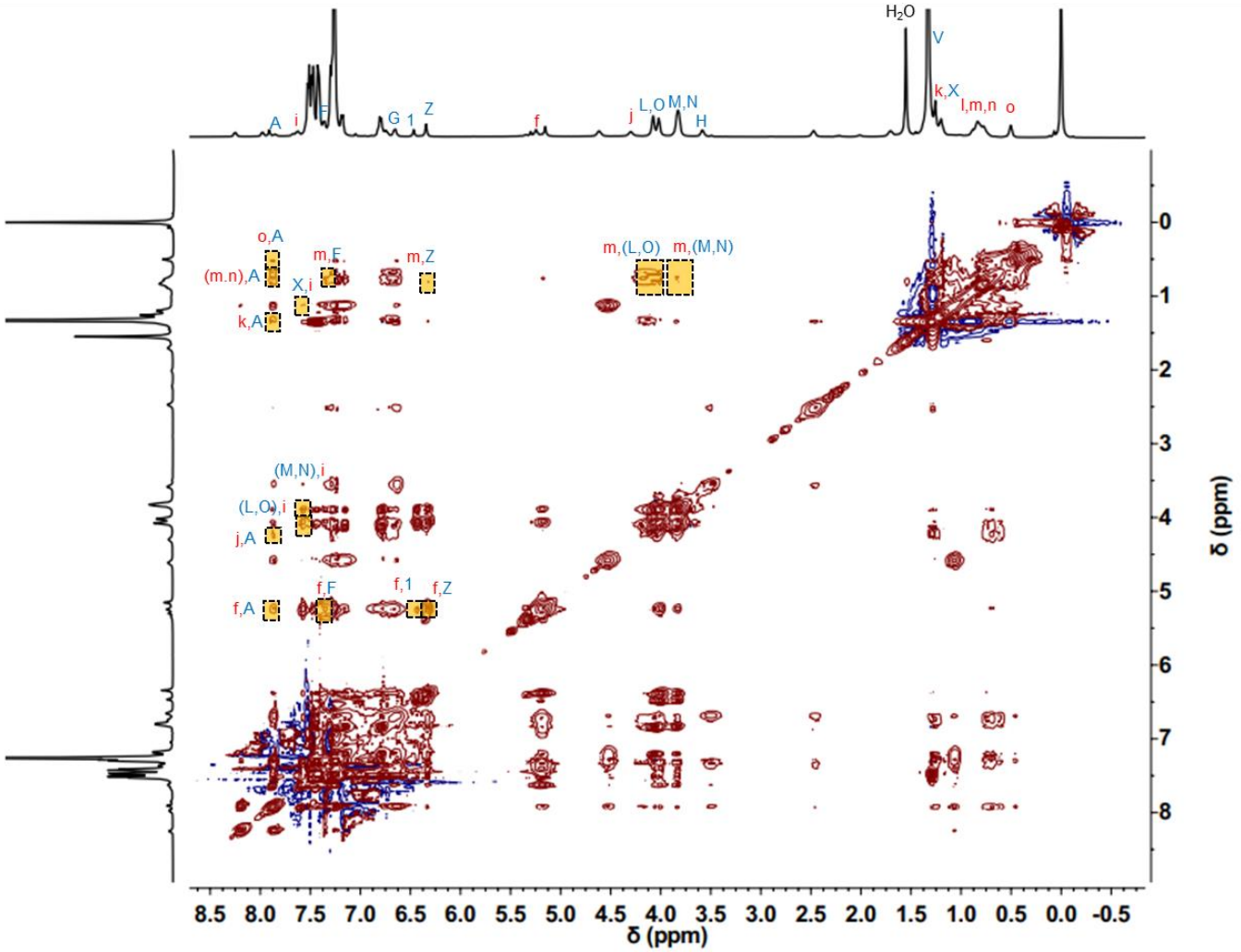

Figure S52. Full ^{1}H-^{1}H NOESY (1mM, 500 MHz, 5°C, $CDCl_3$) of $\mathbf{6}_{44}$. Select ^{1}H annotations correspond to labels in Figure S41.

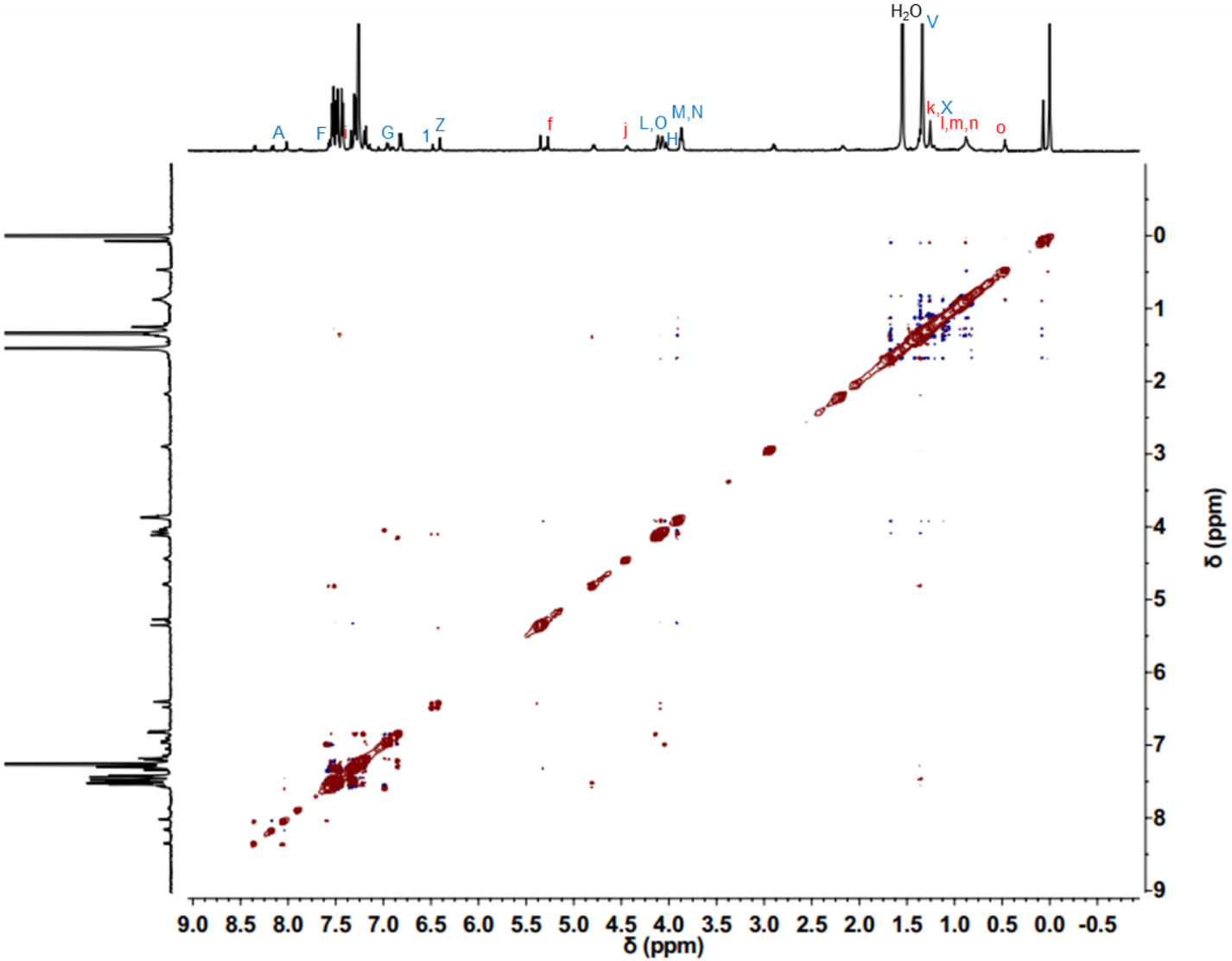

Figure S53. Full NOESY spectrum (1mM, 500 MHz, 5°C, $CDCl_3$) of 2:1 solution of **8**:**1**$_{44}$ (noninterlocked control). Select ^{1}H annotations correspond to labels in Figure S41.

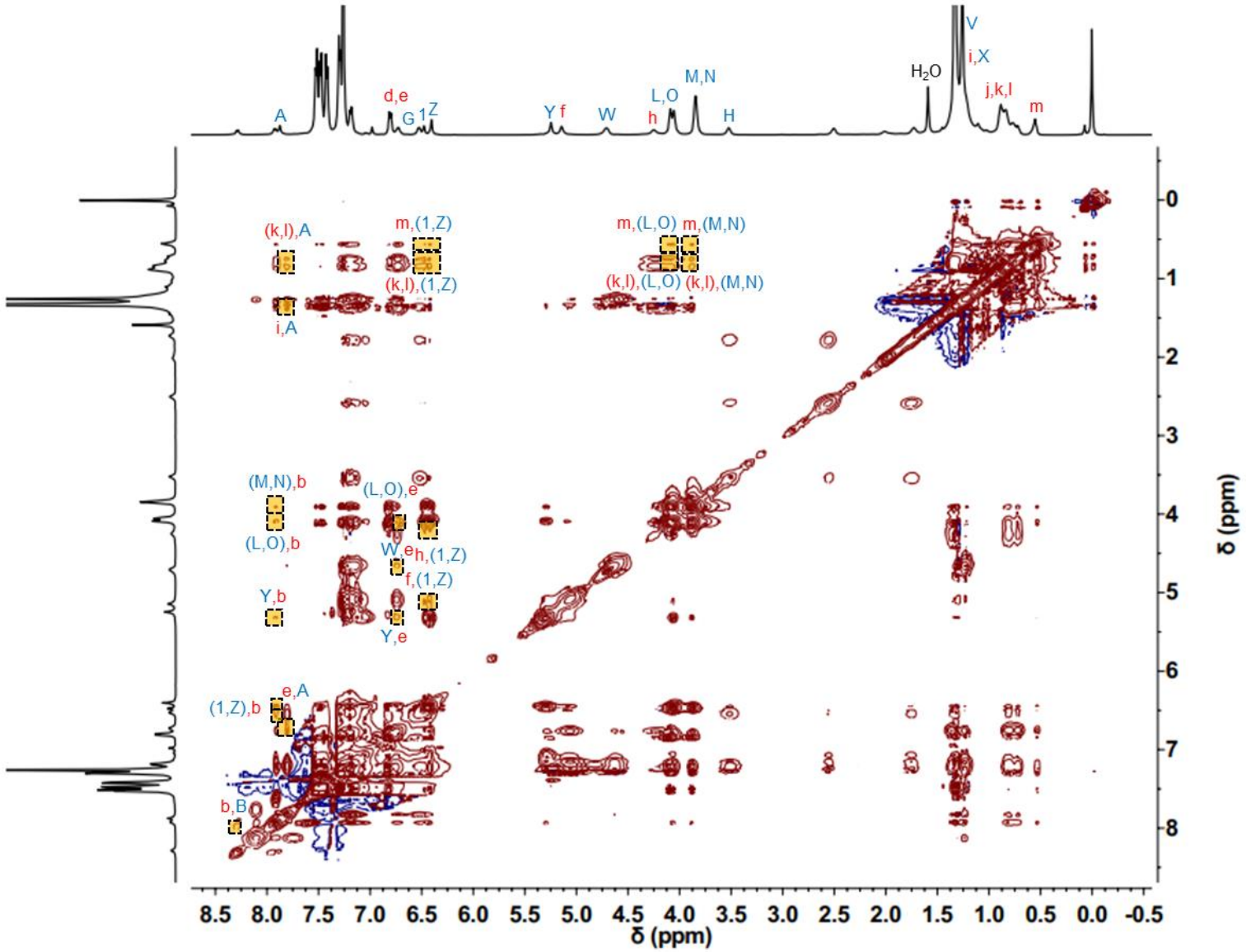

Figure S54. Full ^{1}H-^{1}H NOESY (1mM, 500 MHz, 5°C, $CDCl_3$) of $\mathbf{6_{42}}$. Select ^{1}H annotations correspond to labels in Figure S43.

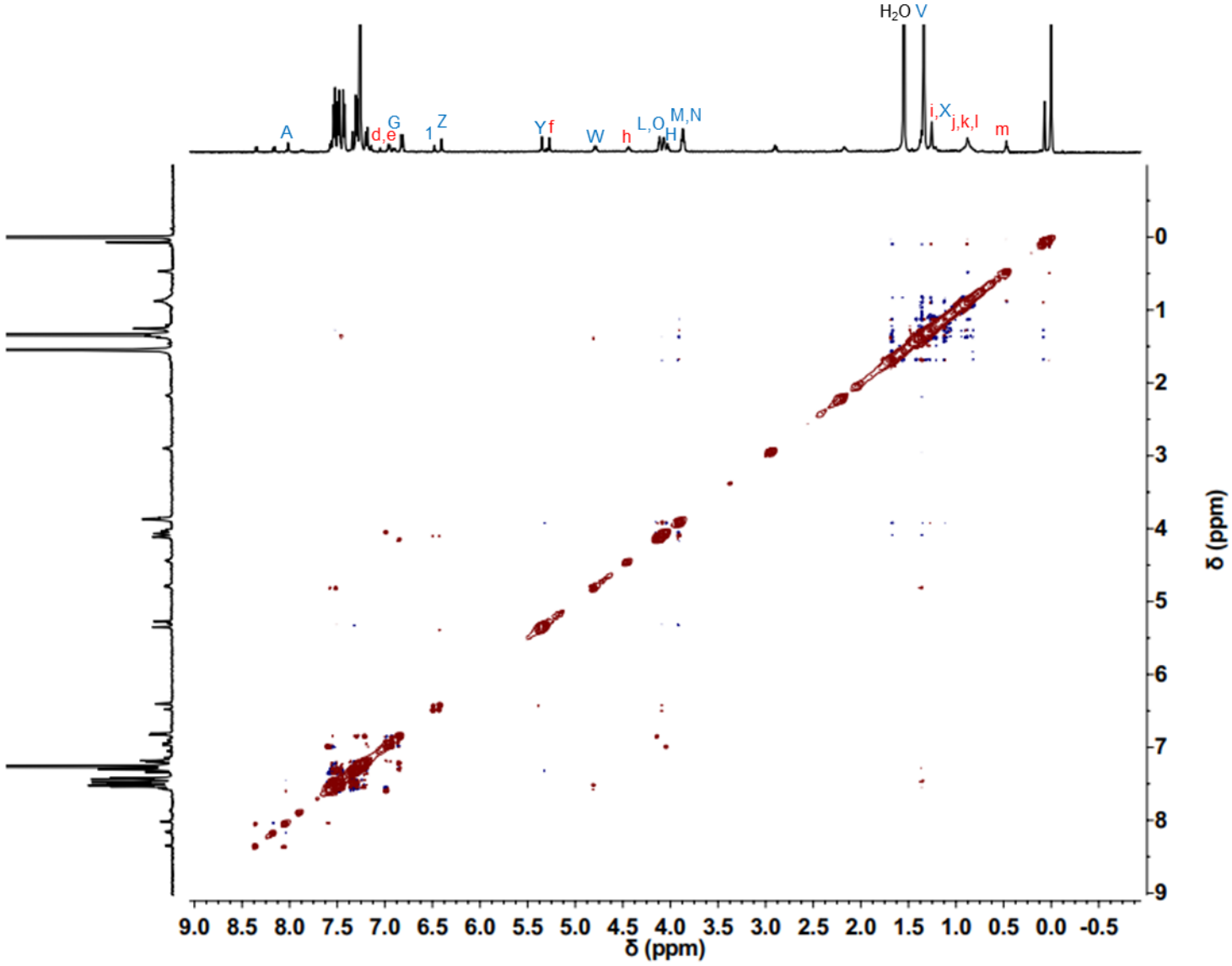

Figure S55. Full NOESY spectrum (1mM, 500 MHz, 5°C, $CDCl_3$) of 2:1 solution of **8**:**1**$_{42}$ (noninterlocked control). Select ^{1}H annotations correspond to labels in Figure S43.

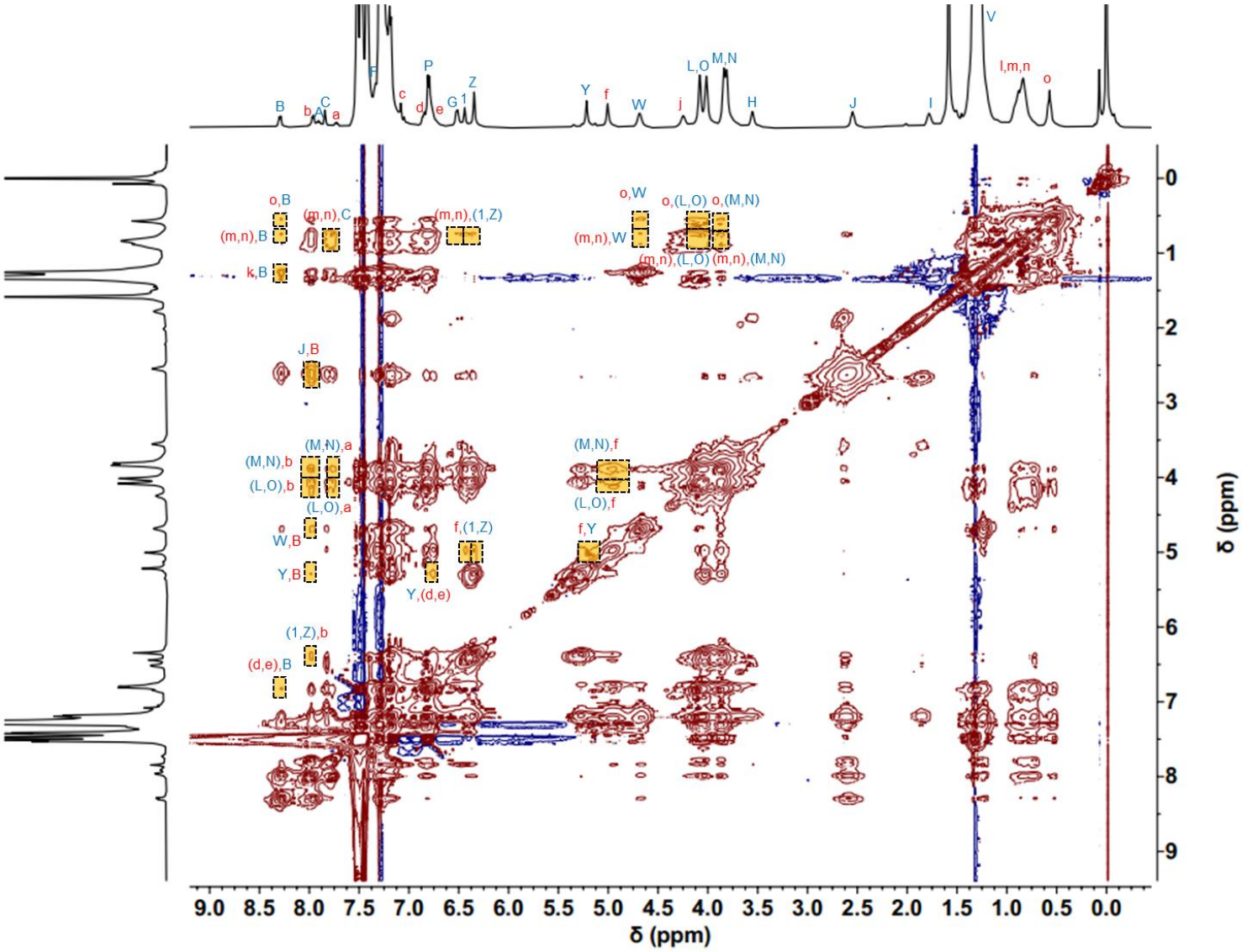

Figure S56. Full ^{1}H-^{1}H NOESY (1mM, 500 MHz, 5°C, $CDCl_3$) of $\mathbf{6_{40}}$. Select ^{1}H annotations correspond to labels in Figure S45.

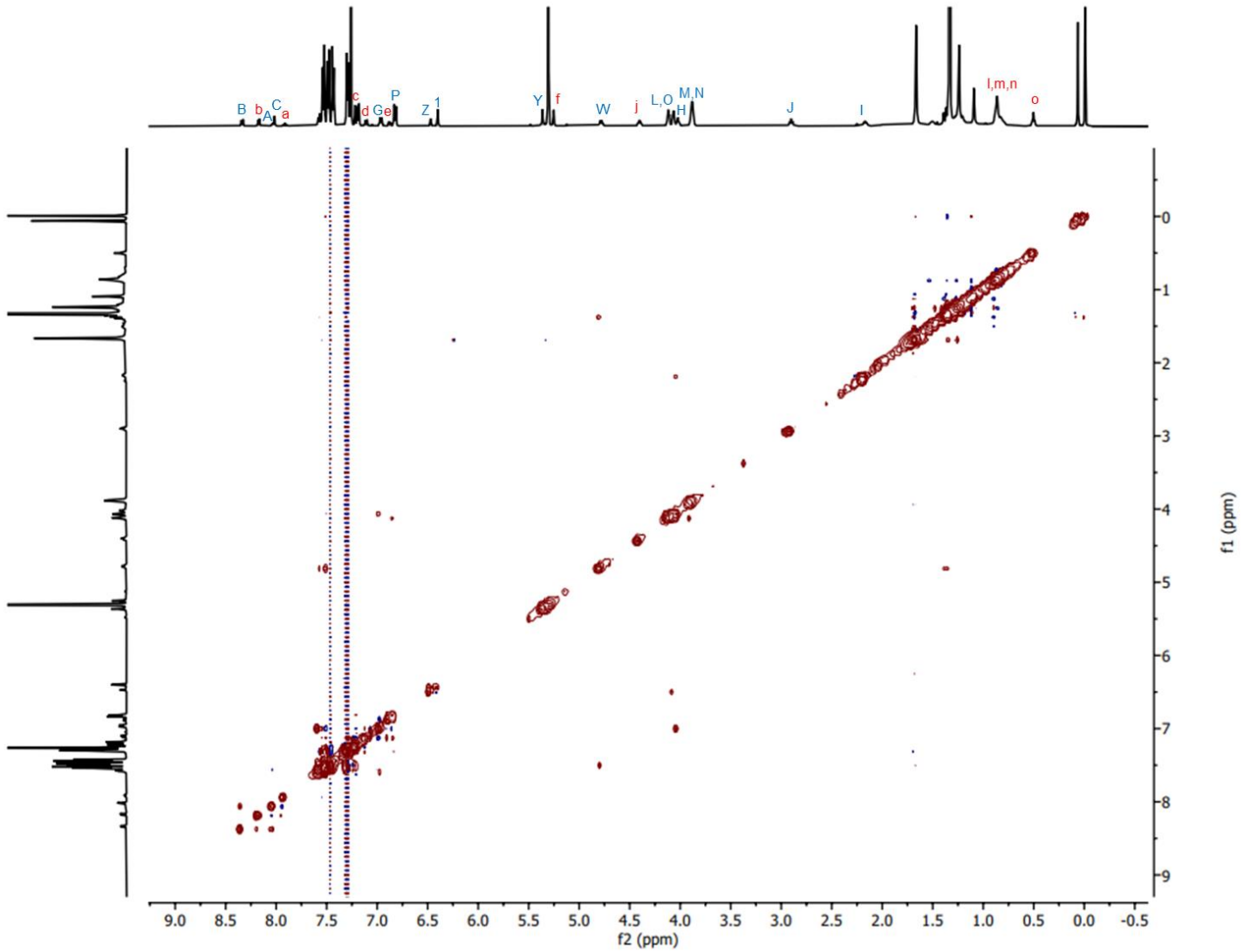

Figure S57. Full NOESY spectrum (1mM, 500 MHz, 5°C, $CDCl_3$) of 2:1 solution of **8**:**1**$_{40}$ (noninterlocked control). Select ^{1}H annotations correspond to labels in Figure S45.

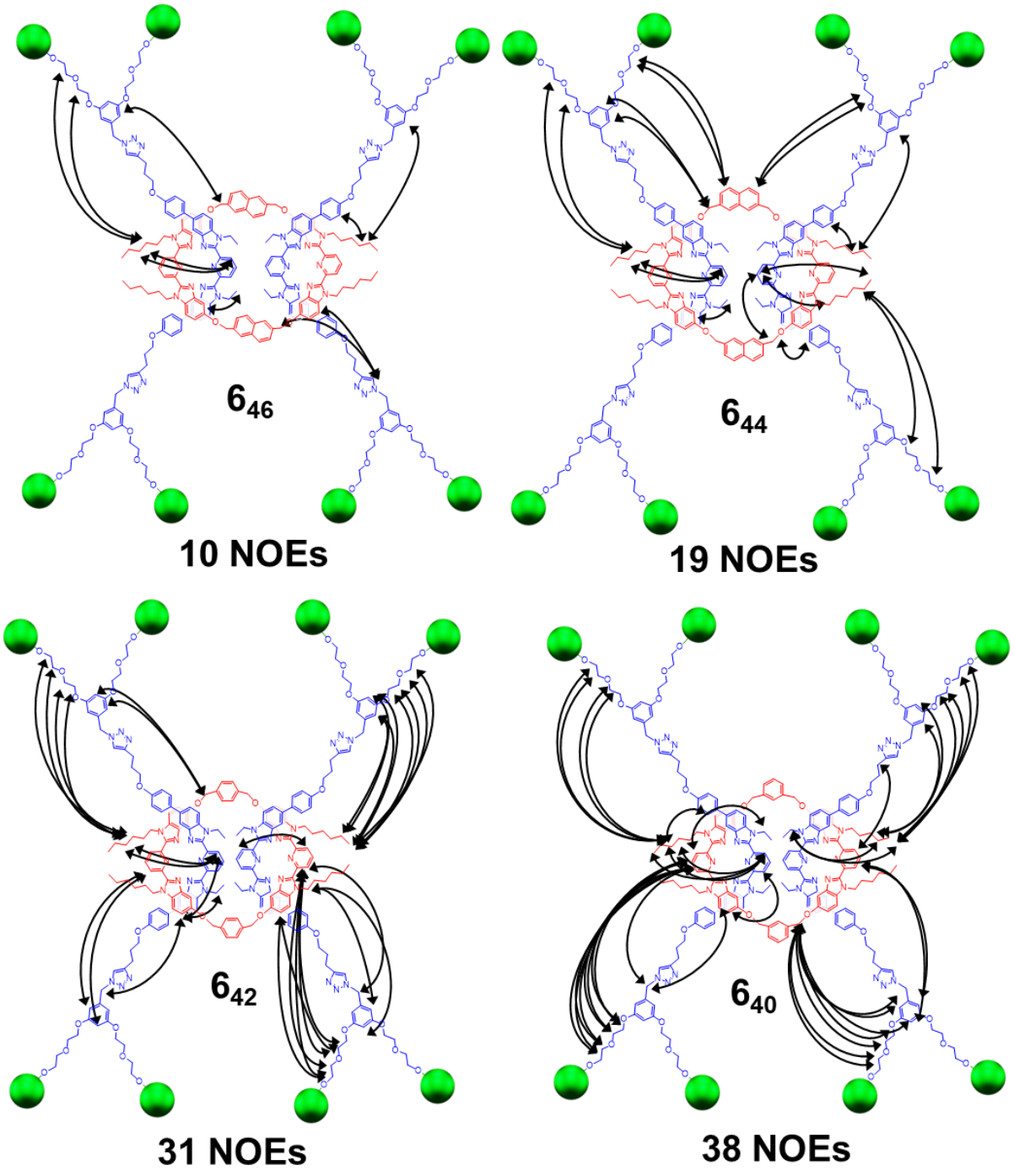

Figure S58. Chemical structure of $\mathbf{6_{46}}$, $\mathbf{6_{44}}$, $\mathbf{6_{42}}$, and $\mathbf{6_{40}}$ with arrows showing intercomponent NOEs found in each [3]rotaxane.

Slippage Analysis of [3]Rotaxanes $\mathbf{5}_{40\text{-}42}$ and $\mathbf{6}_{40\text{-}44}$

Kinetic experiments were modeled after previously published work detailing the slippage of $\mathbf{6}_{46}$ which was based on initial work by Sauvage and coworkers.[5] A 1mM sample of freshly purified [3]rotaxane $\mathbf{6}_{40}$, $\mathbf{6}_{42}$, $\mathbf{6}_{44}$, $\mathbf{5}_{40}$, or $\mathbf{5}_{42}$ was prepared in a 5 mm Bruker Shigemi NMR tube. This tube was sealed, stored at room temperature (25°C), and its ^{1}H-NMR spectrum was recorded after 3 days, 7 days, 10 days, 14 days, 17 days, and finally 21 days. For the rotaxane $\mathbf{5}_{42}$ a quicker sampling time of 4 hours of slippage and then once a day for 1 week was used.

The resulting kinetic data was analyzed according to the following method. The downfield doublet corresponding to the proton labeled B in the dumbbell of each [3]rotaxane (See Fig 3) 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 8.22-8.34ppm (slight shift depending on specific [3]rotaxane, see Fig 4 + 5). 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 standard first-order kinetics the kinetic parameters were determined.

Slippage Analysis of of [3]Rotaxanes **6**$_{44}$ and **5**$_{40\text{-}42}$

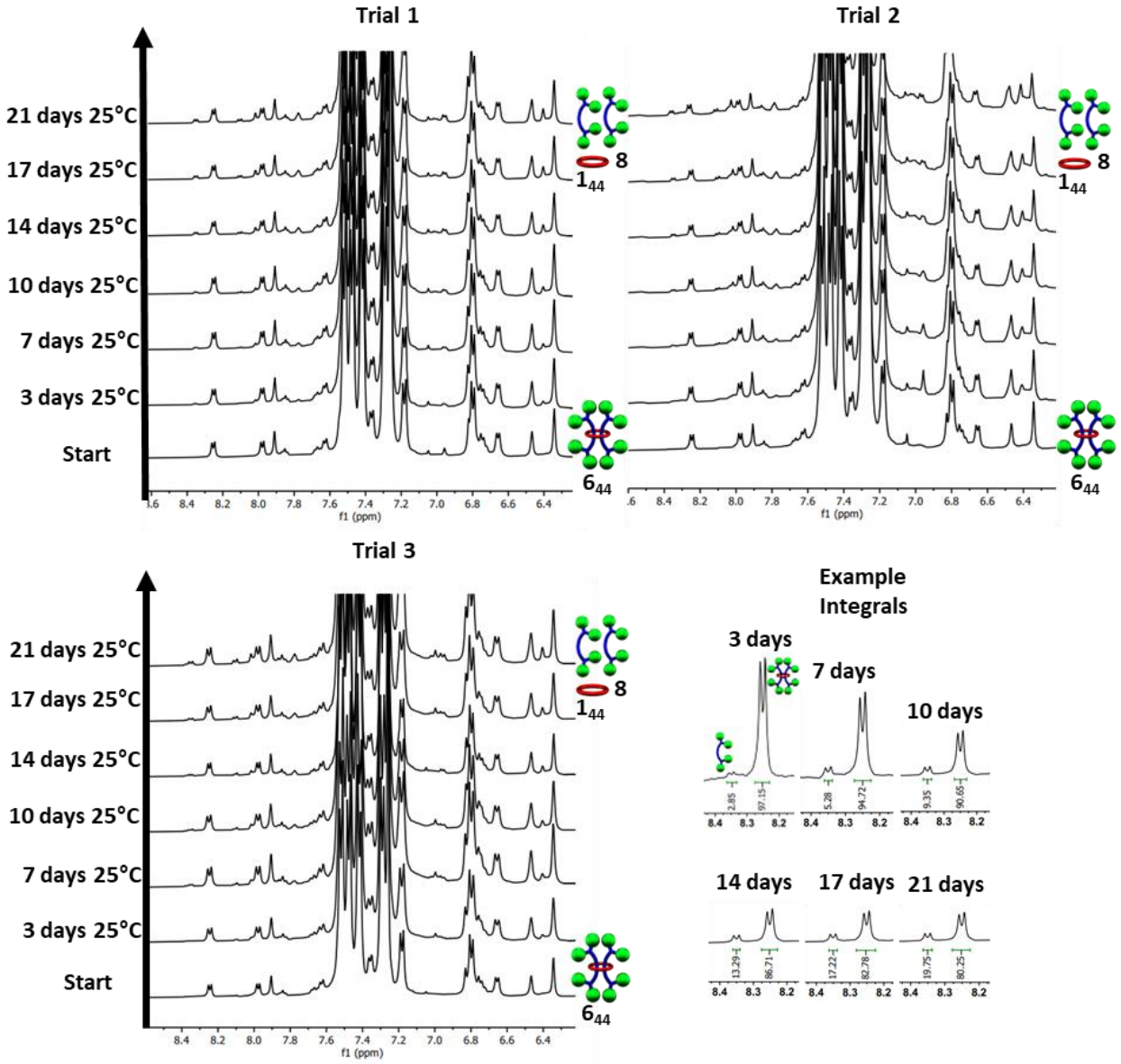

Figure S59. Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of 3 trials of 3-week room temperature slippage experiments of **6**$_{44}$. Examples integrals in bottom right corner of figure display integral values used in trial 1 of kinetic measurements.

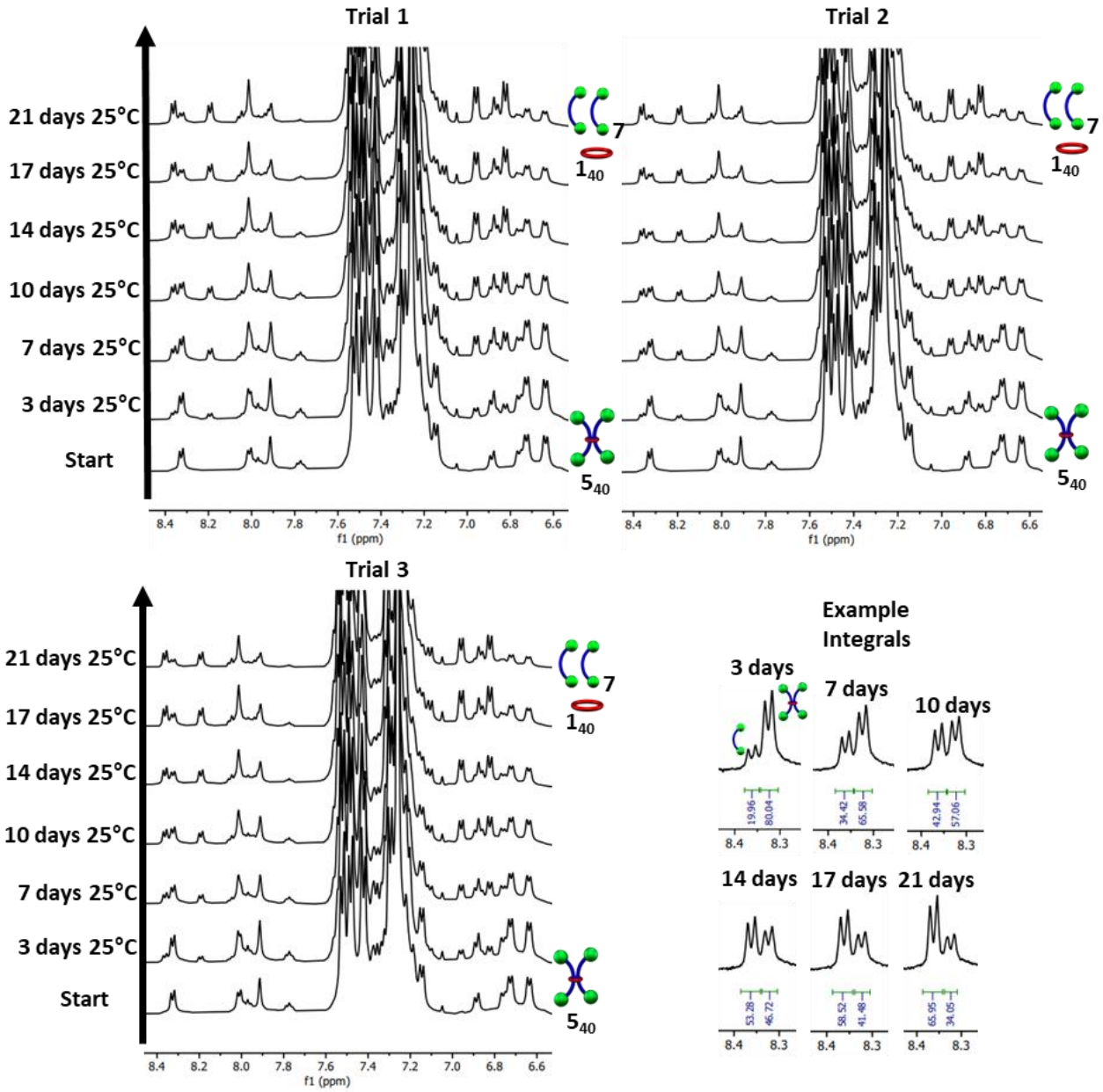

Figure S60. Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of 3 trials of 3-week room temperature slippage experiments of **5₄₀**. Examples integrals in bottom right corner of figure display integral values used in trial 1 of kinetic measurements.

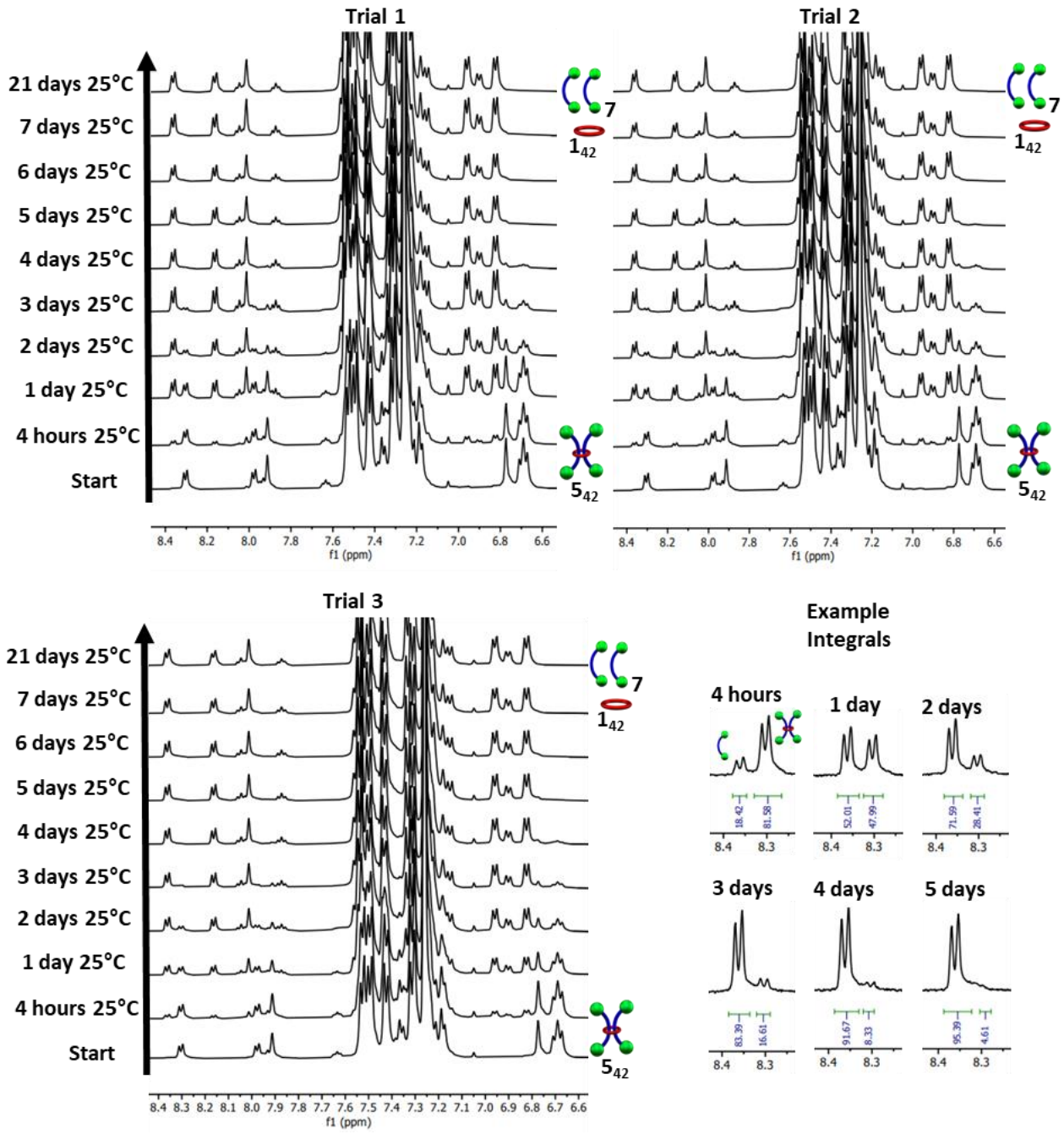

Figure S61. Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of 3 trials of 3-week room temperature slippage experiments of $\mathbf{5_{42}}$. Examples integrals in bottom right corner of figure display integral values used in trial 1 of kinetic measurements.

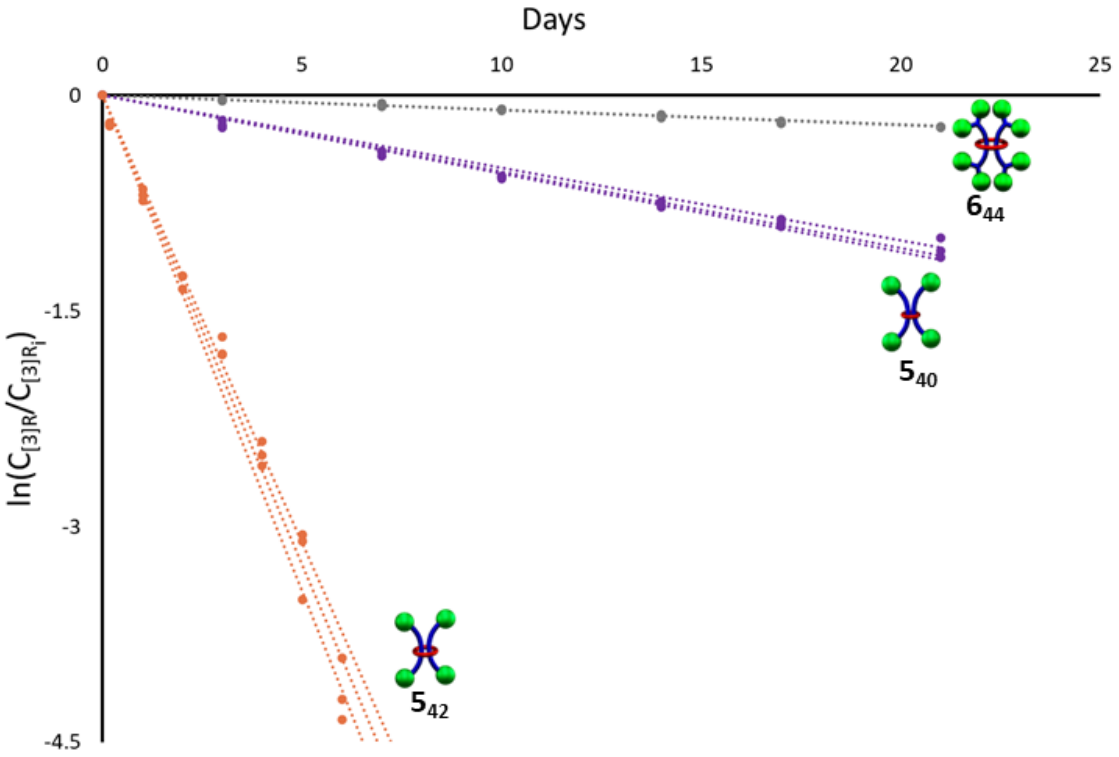

Figure S62. Kinetic first-order plot of three trials of slippage of each [3]rotaxane $\mathbf{6}_{44}$ and $\mathbf{5}_{40\text{-}42}$.

Slippage Analysis of of [3]Rotaxanes **6**$_{40\text{-}42}$

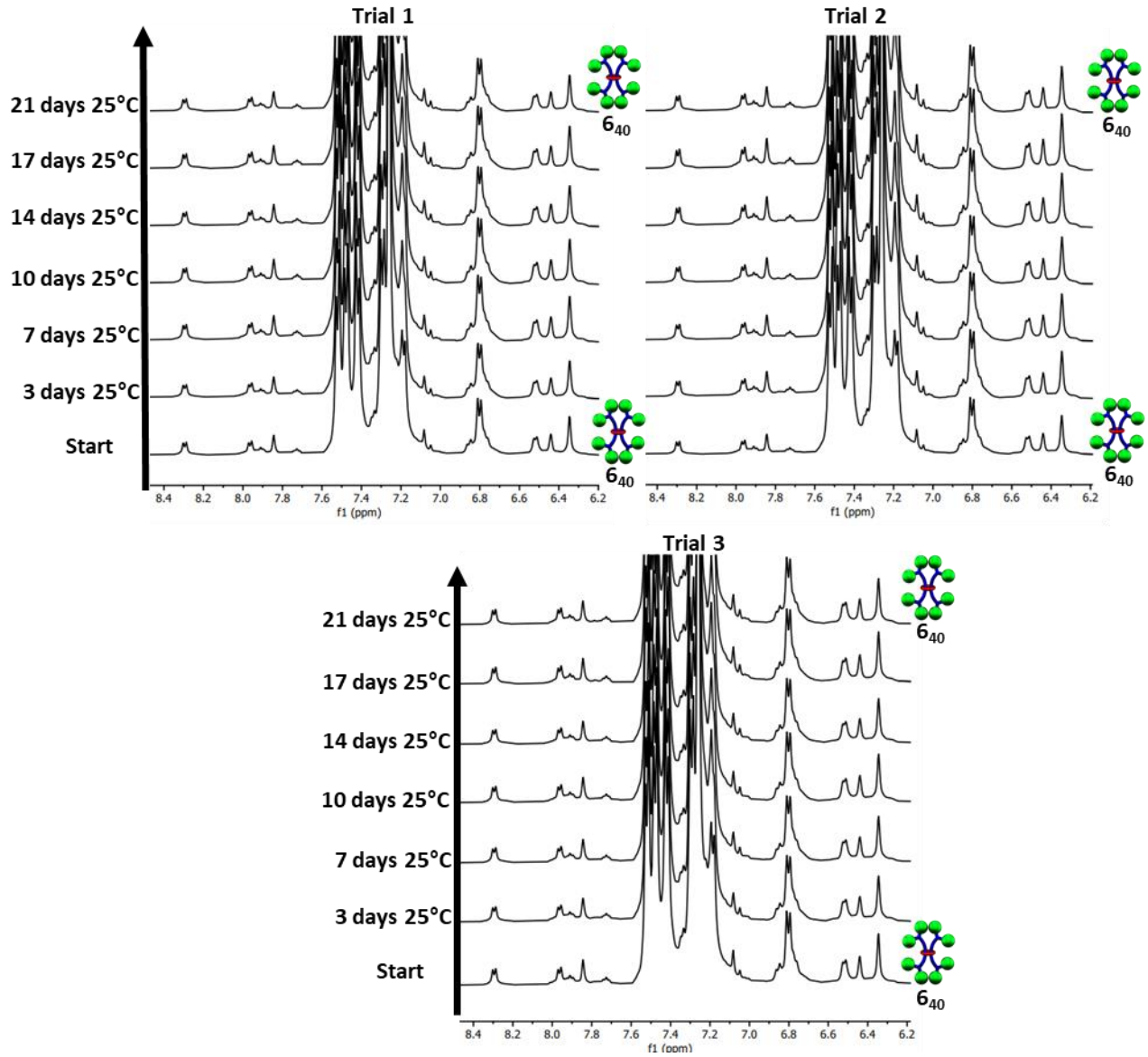

Figure S63. Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of 3 trials of 3-week room temperature slippage experiments of **6**$_{40}$. Time between measurements indicated on left side.

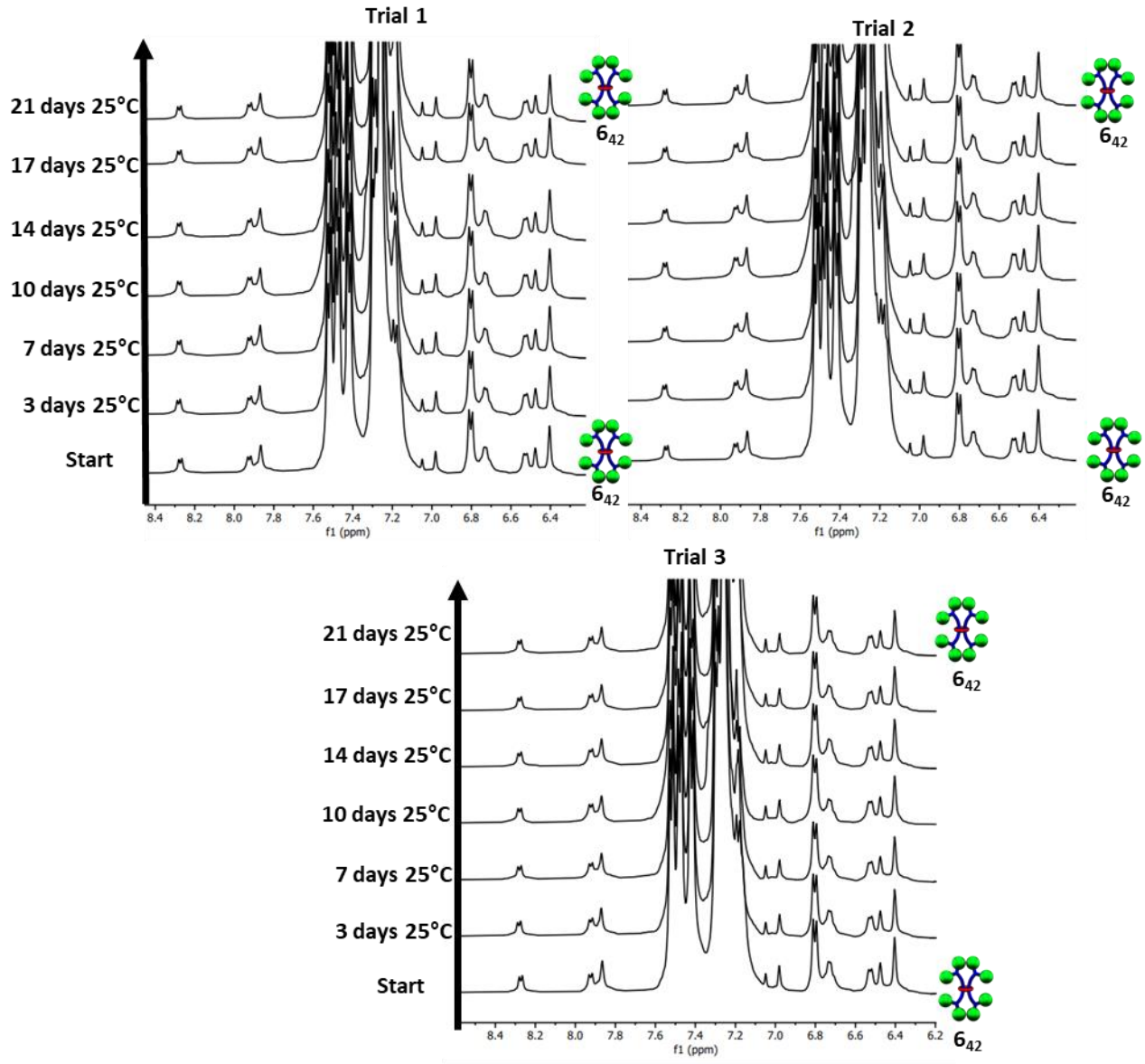

Figure S64 Partial ^{1}H-NMR overlay (1mM, 500 MHz, $CDCl_3$, 25°C) of 3 trials of 3-week room temperature slippage experiments of $\mathbf{6_{42}}$. Time between measurements indicated on left side.

^{1}H and ^{13}C NMR Spectra of Ring Components $\mathbf{1}_{40\text{-}48}$

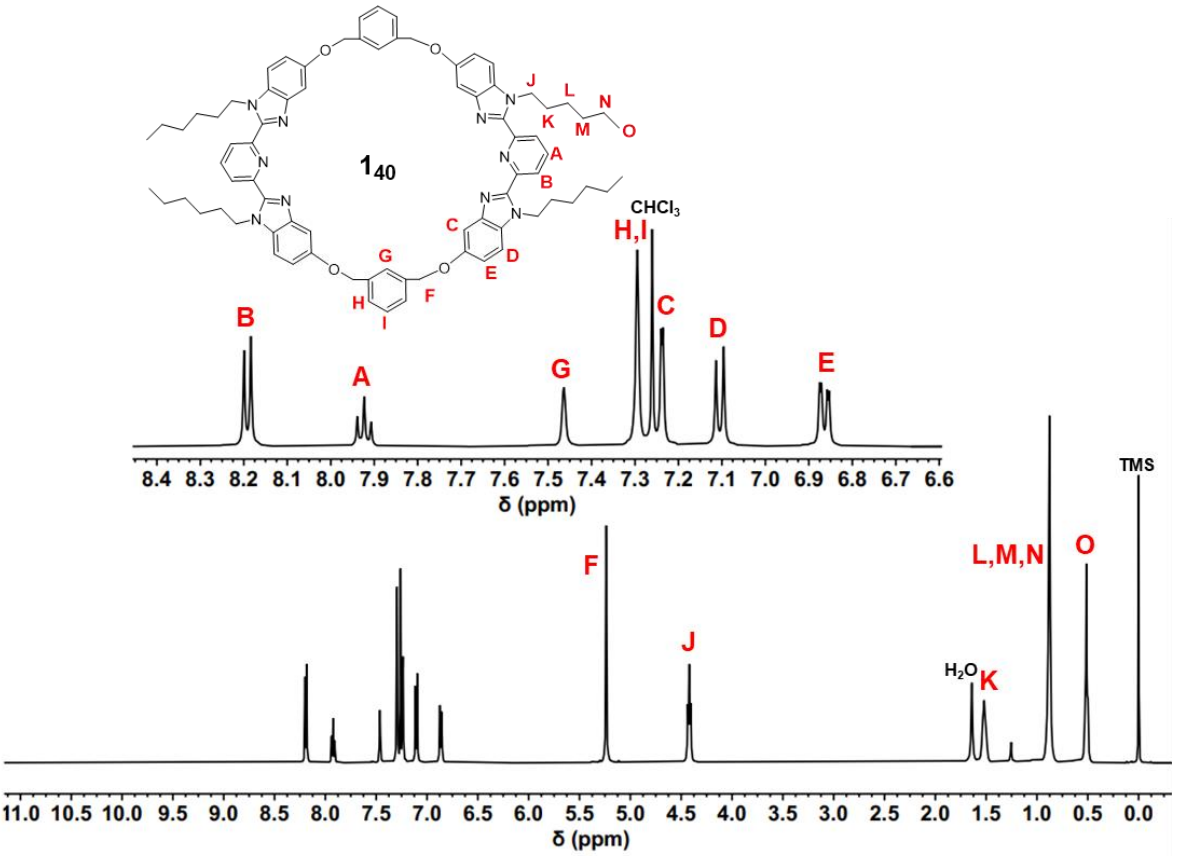

Figure S65. ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of ring component $\mathbf{1}_{40}$.

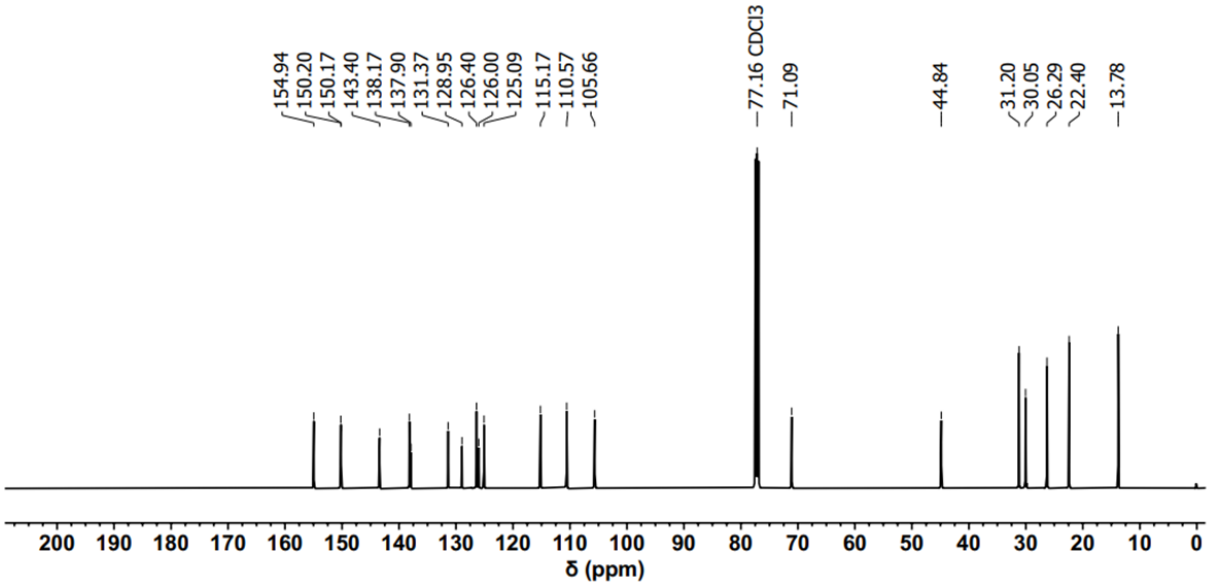

Figure S66. ^{13}C-NMR (126 MHz, 25°C, $CDCl_3$) of ring component $\mathbf{1}_{40}$.

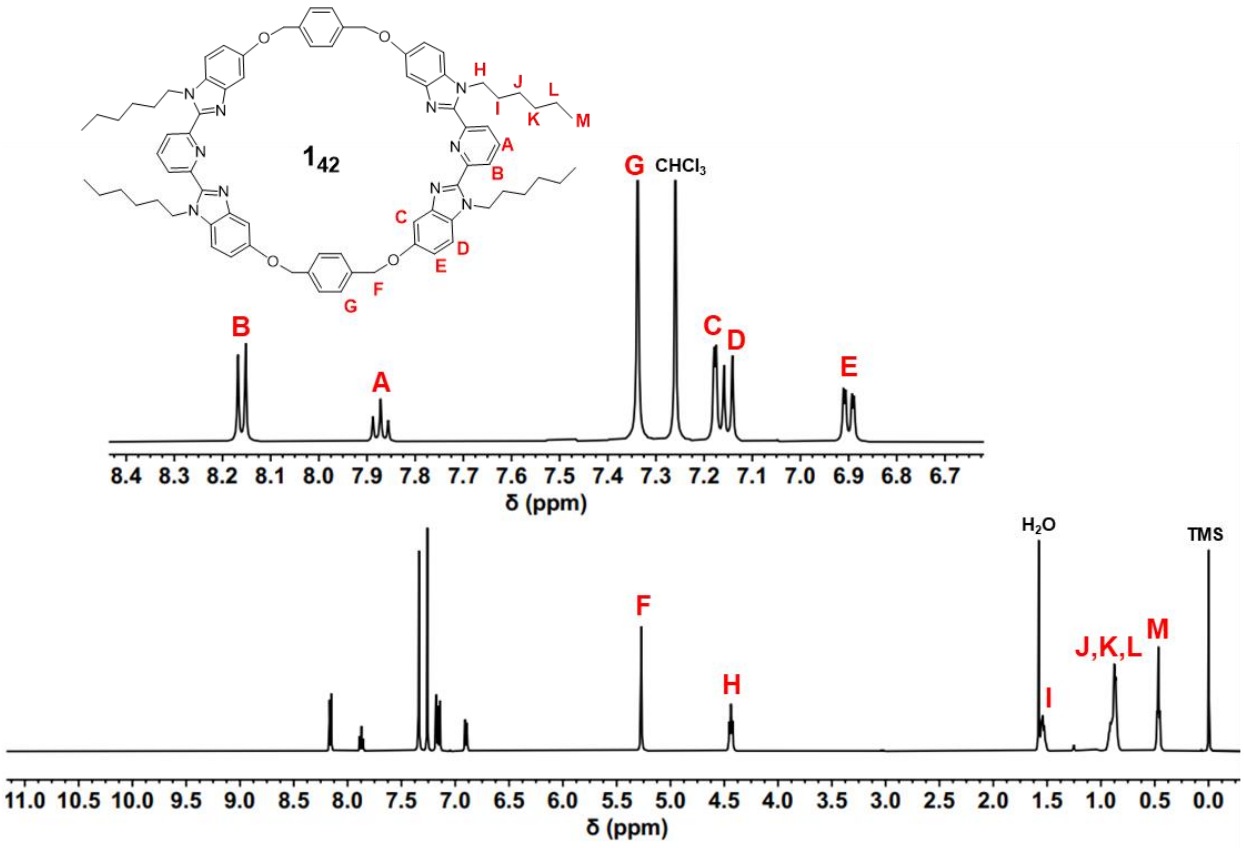

Figure S67. ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of ring component $\mathbf{1_{42}}$.

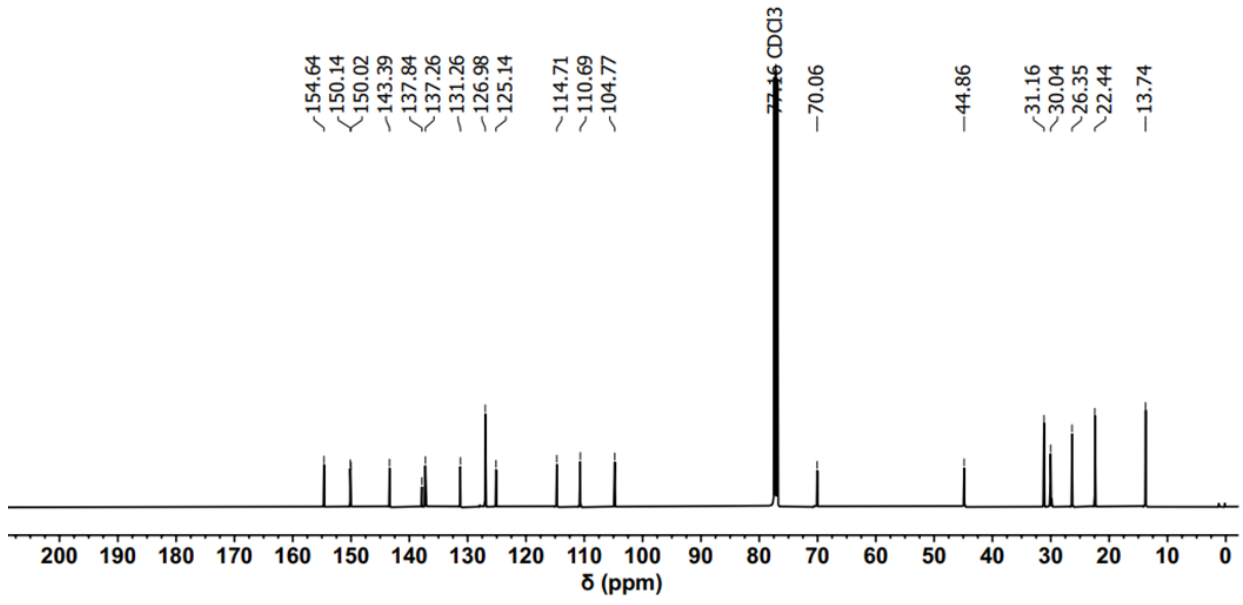

Figure S68. ^{13}C-NMR (126 MHz, 25°C, $CDCl_3$) of ring component $\mathbf{1_{42}}$.

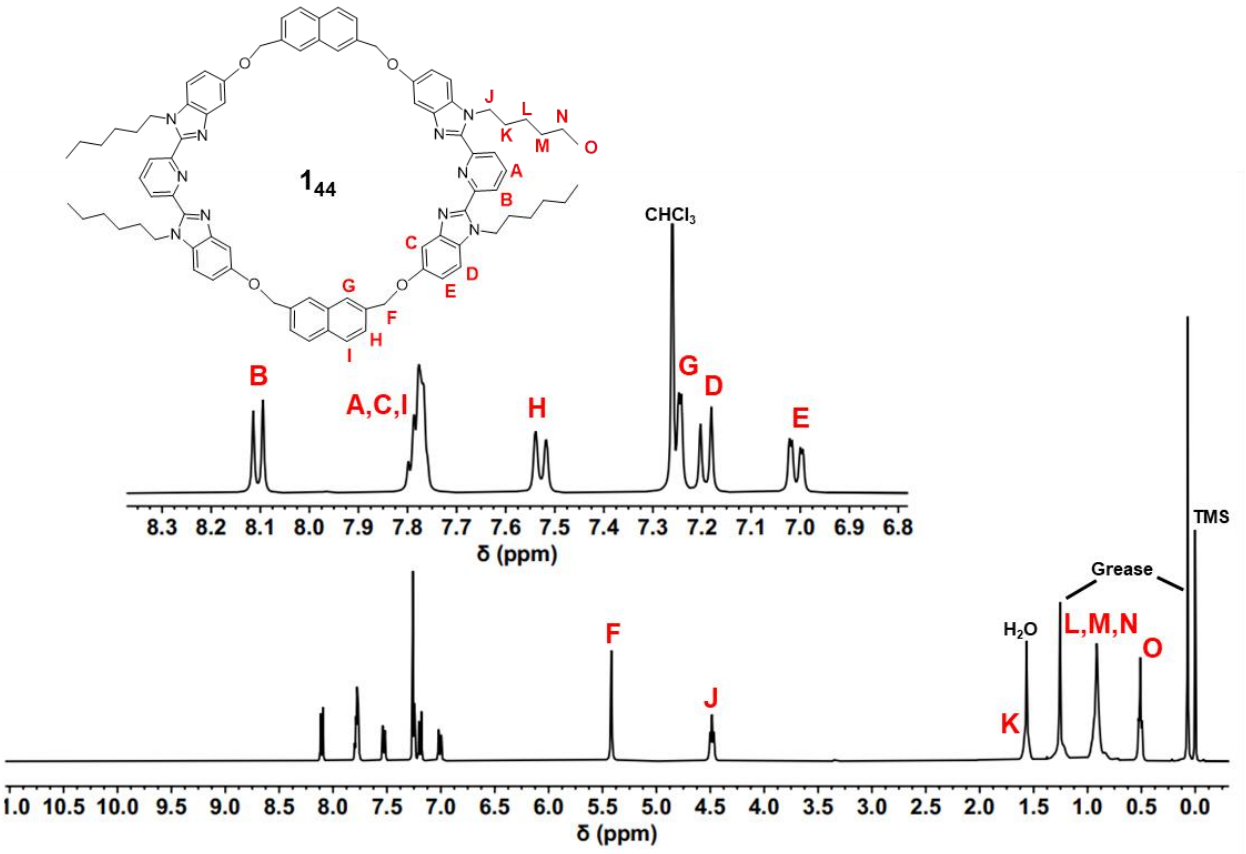

Figure S69. ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of ring component $\mathbf{1_{44}}$.

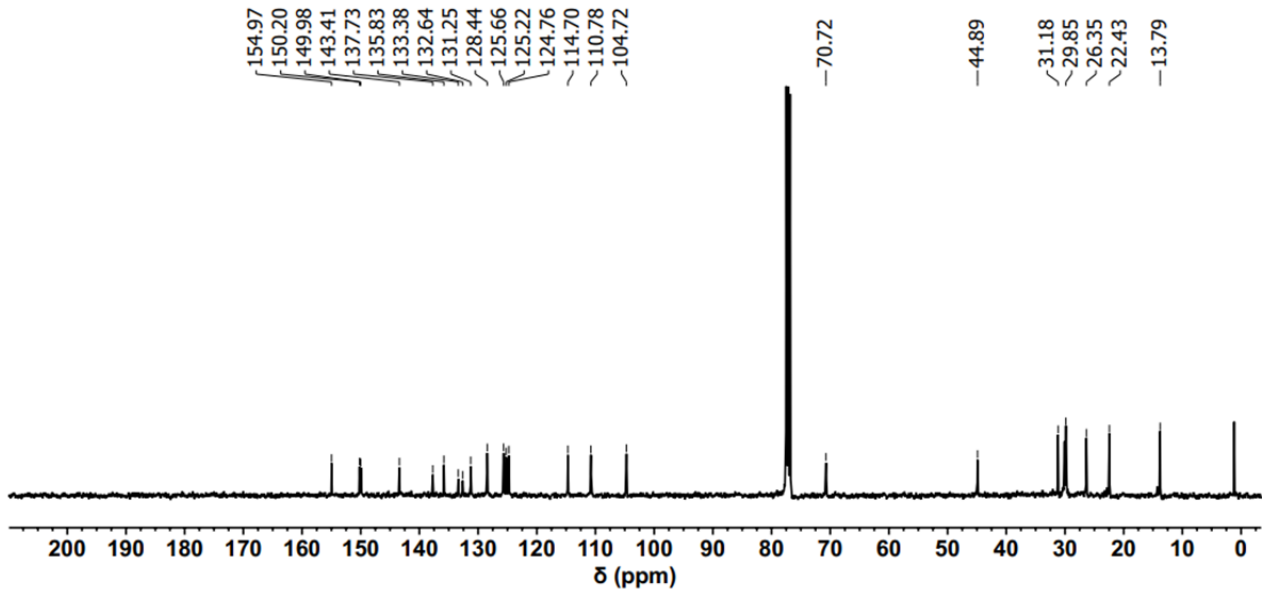

Figure S70. ^{13}C-NMR (126 MHz, 25°C, $CDCl_3$) of ring component $\mathbf{1_{44}}$.

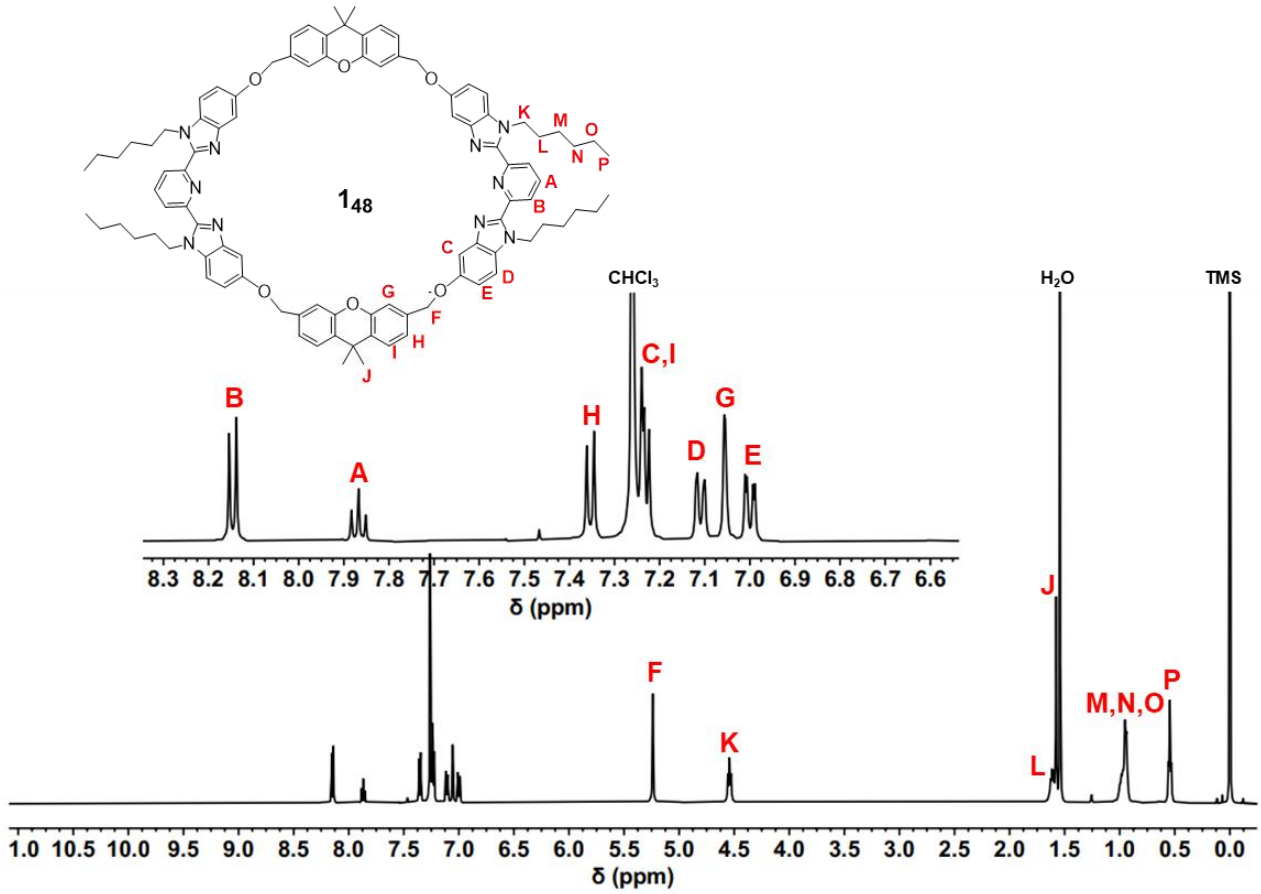

Figure S71. ^{1}H-NMR (500 MHz, 25°C, $CDCl_3$) of ring component **1$_{48}$**.

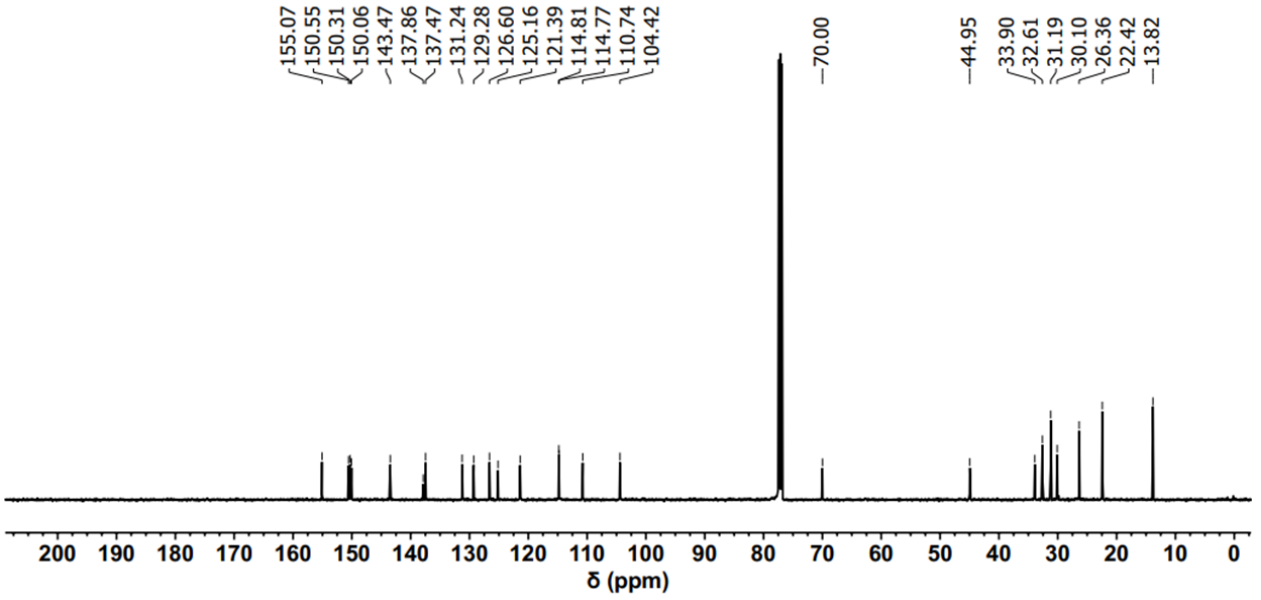

Figure S72. ^{13}C-NMR (126 MHz, 25°C, $CDCl_3$) of ring component **1$_{48}$**.

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