Supporting information for:

A Chiroptical Molecular Sensor for Ferrocene

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1) Synthetic procedures and characterization

General Experimental. All commercially available reagents and solvents were purchased from Sigma-Aldrich, Fluka and Alfa Aesar, and used as received. THF (Na, benzophenone) and CH_2Cl_2 (CaH_2) were dried and distilled before use. Analytical thin layer chromatography was performed on chromophore loaded, commercially available silica gel plates. Flash chromatography was carried out using silica gel (pore size 60 Å, 230-400 Mesh). ^{1}H- and ^{13}C-NMR spectra were recorded from solutions in $CDCl_3$, d_6-DMSO or CD_3CN on 200, 300 or 500 MHz spectrometers using the solvent residual proton signal or tetramethylsilane as the internal standard. 2D ROESY NMR experiments were performed applying the following parameters: pulse programme: roesyph from Bruker library, T= 300 K, 1024x256 data points, NS= 128, D1= 1 s, spin-lock (mixing) time= 300 ms. Spectra were processed with Topspin 2.1.Samples for the mass spectrometry were analysed as FIA-ESI/MS with an ion trap mass spectrometer ThermoScientific LCQ FLEETin the following conditions: spray voltage=4.5 kV, capillar temperature=220°C, capillar voltage=19 V, tube lens=120 V, mass interval=50-2000 Da, mode=positive ions. The UV/Vis spectroscopic studies were recorded using commercially-available spectrophotometers, with a scanning speed of 400 nm/min at room temperature. CD spectroscopy was performed using a commercially-available spectropolarimeter. CD spectra were recorded at 25 °C and with the following parameters: scanning speed= 50 nm/min, data pitch= 0.5 nm, response= 4 seconds, band width= 1 nm. The spectra were then background corrected. Cyclic voltammetry were recorded on BASi PWR-3 power module and EF-1085 C-3 cell stand, equipped with a glassy carbon electrode (diameter 2.0 mm) as working electrode; an Ag/AgCl/NaCl (3 M NaCl, saturated with AgCl) reference electrode and a platinum wire as auxiliary electrode, both obtained from BASi, were used.

The overall synthesis for the compounds of interest is shown in Scheme S1. Compound (*R*)-**3** was synthesized according to ref. S1. $Pd(en)(NO_3)_2$ was prepared according to reference S2.

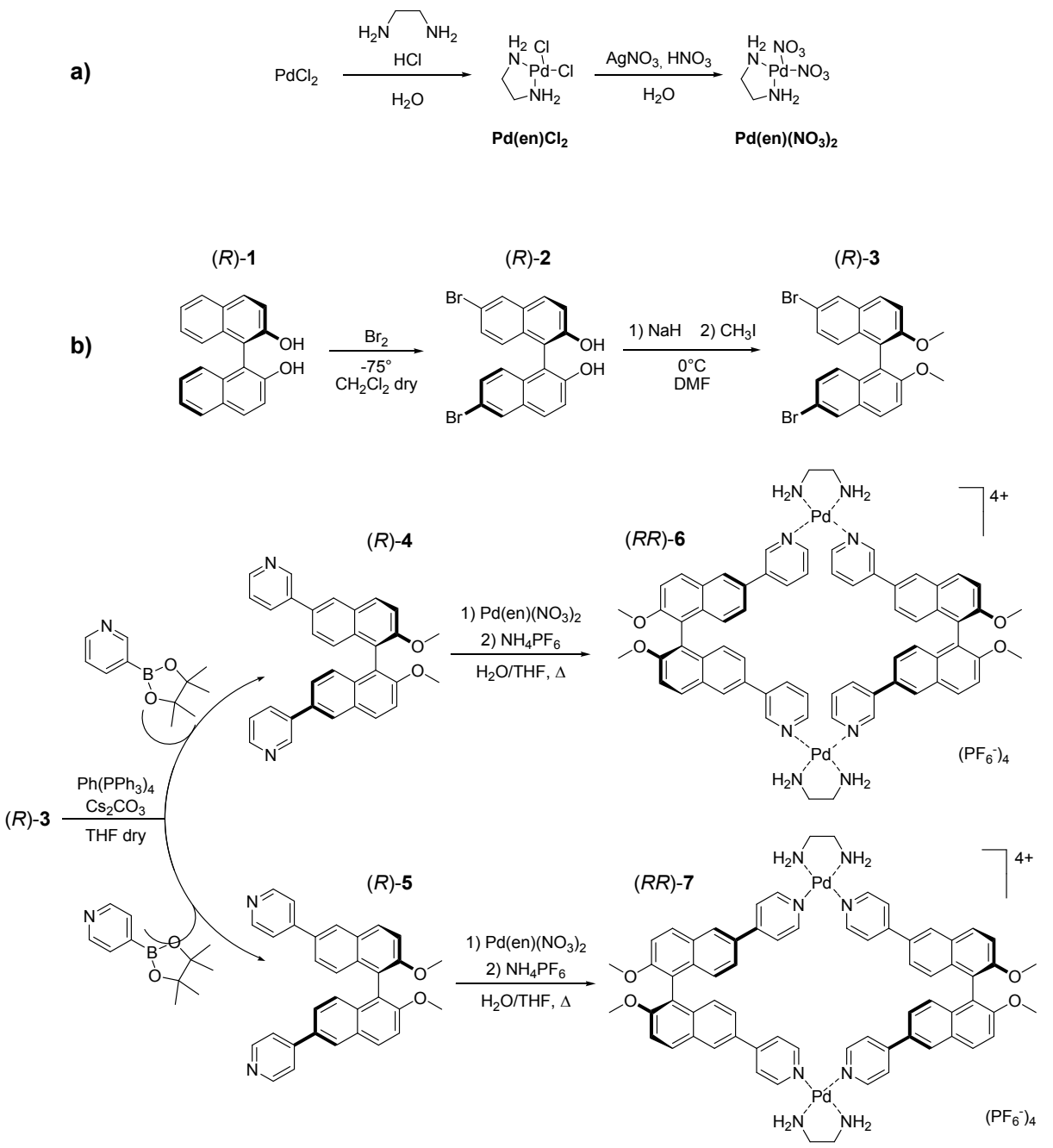


Figure S1. Scheme for the synthesis of compound [Pd(en)$(NO_3)_2$] (a) and metal-linked macrocycles (*RR*)-**6** and (b) (*RR*)-**7**.

Compound (*R*)-**4.** Cs_2CO_3 (0.403 g, 1.24 mmol, 4 eq) and 3-pyridineboronic acid pinacol ester (0.151 g, 0.74 mmol, 2.5 eq) were added to a solution of compound (*R*)-**3** (0.139 g, 0.3 mmol, 1 eq) in dry THF (40 mL), keeping N_2 atmosphere and continuous stirring. After 15 min, $Pd(PPh_3)_4$ (0.075 g, 0.06 mmol, 0.2 eq) was added to the round-bottom flask and the dark yellow solution was stirred overnight under reflux. The reaction was monitored by TLC (AcOEt:Hexane:Et_3N 6:3:1) and the mixture was quenched with H_2O (150 mL), extracted with AcOEt (3x 150 mL) and the yellow organic phase dried (Na_2SO_4). The solution was

filtered and concentrated *in vacuo*, and the crude product was purified by flash chromatography (AcOEt:hexanes:Et_3N 6:3:1) to yield compound (*R*)-**4** (0.14 g, 0.29 mmol, 96%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, 200 MHz, 25 °C) δ = 8.95 (s, 2H; pyridine), 8.59 (m, 2H; pyridine), 8.10 (d, 2H; binaphthyl), 8.08 (d, 2H; binaphthyl), 7.95 (s, 2H; pyridine), 7.54 (d, 2H; binaphthyl), 7.48 (m, 2H; pyridine), 7.37 (dd, 2H; binaphthyl), 7.26 (d, 2H; binaphthyl), 3.82 (s, 6H; -OCH_3).^{13}C-NMR ($CDCl_3$, 75 MHz, 25 °C) δ = 155.4 (C_{quat}), 148.3 (CH), 148.1 (CH), 136.5 (C_{quat}), 134.2 (CH), 133.4 (C_{quat}), 132.7 (C_{quat}), 129.9 (CH), 129.2 (C_{quat}), 126.2 (CH), 126.05 125.5 (CH), 123.5 (CH), 119.1 (C_{quat}), 114.7 (CH), 56.7 (CH_3).

Compound (*R*)-**5.** Cs_2CO_3 (0.389 g, 1.2 mmol, 4 eq) and 4-pyridineboronic acid pinacol ester (0.154 g, 0.75 mmol, 2.5 eq) were added to a solution of compound (*R*)-**3** (0.137 g, 0.3 mmol, 1 eq) in dry THF (40 mL), keeping N_2 atmosphere and continuous stirring. After 15 min, $Pd(PPh_3)_4$ (0.077 g, 0.07 mmol, 0.2 eq) was added to the round-bottom flask and the dark yellow solution was stirred overnight under reflux. The end of the reaction was monitored by TLC (AcOEt:Hexane:Et_3N 6:3:1) and the mixture was quenched with H_2O (150 mL), extracted with AcOEt (3x 150 mL) and the yellow organic phase dried (Na_2SO_4). The solution was filtered and concentrated in vacuo, and the crude product was purified by flash chromatography (AcOEt:Hexane:Et_3N 6:3:1) to yield compound (*R*)-**5** (0.13 g, 0.27 mmol, 90%) as a yellow solid. ^{1}H-NMR ($CDCl_3$, 200 MHz, 25 °C) δ = 8.67 (d, 4H; pyridine), 8.19 (s, 2H; binaphthyl), 8.11 (d, 2H; binaphthyl), 7.61 (d, 4H; pyridine), 7.57-7.50 (m, 4H; binaphthyl), 7.27 (d, 2H; binaphthyl), 3.83 (s, 6H; -OCH_3). ^{13}C-NMR ($CDCl_3$, 75 MHz, 25 °C) δ = 155.7 (C_{quat}), 150.0 (CH), 148.2 (C_{quat}), 134.0 (C_{quat}), 132.9 (C_{quat}), 130.2 (CH), 129.0 (C_{quat}), 126.5 (CH), 126.0 (CH), 125.0 (CH), 121.5 (CH), 119.0 (C_{quat}), 114.6 (CH), 56.7 (CH_3).

Compound (*RR*)-**6.** A suspension of $Pd(en)(NO_3)_2$(0.034g, 0.12 mmol, 1 eq) in H_2O (10 mL) was stirred at room temperature.A solution of compound (*R*)-**4** (0.051 g, 0.11 mmol, 1 eq) in THF (5 mL) was added and the reaction mixture was then heated at 70°C for 30 minutes. The solution is clear and yellow. An aqueous solution of NH_4PF_6 (1M, 2.5 mL) was added dropwise and the solution became turbid. A dark agglomerate was formed. After additional 15 min stirring, the solution was filtered to yield compound (*RR*)-**6** (0.05 g, 0.03 mmol, 56%), as a light brown solid. ^{1}H-NMR (d_6-DMSO, 200 MHz, 25 °C) δ = 9.49 (s, 4H; pyridine), 8.95 (s, 4H; pyridine), 8.45 (s, 4H; binaphthyl), 8.39 (d, 4H; pyridine), 8.15 (d, 4H; binaphthyl), 7.79-7.72 (m, 8H; binaphthyl), 7.50 (d, 4H; pyridine), 6.98 (d, 2H; binaphthyl),

5.60 (broad peak, 8H; -NH_2), 3.78 (s, 12H; -OCH_3), 2.71 (broad singlet, 8H; -CH_2-). ^{13}C-NMR (CD_3CN, 75 MHz, 25 °C) δ = 156.0 (C_{quat}), 149.8 (CH), 149.5 (CH), 139.2 (C_{quat}), 138.3 (CH), 133.6 (C_{quat}), 130.4 (CH), 129.7 (C_{quat}), 129.0 (C_{quat}), 127.2 (CH), 126.8 (CH), 125.6 (CH), 124.7 (CH), 118.3 (C_{quat}), 115.0 (CH), 56.1 (CH_3), 46.7 (CH_2). ESI-MS *m/z* = 1704.11 [M-PF_6]$^+$.

Compound (*RR*)-**7**. A suspension of compound Pd(en)(NO_3)$_2$(0.034g, 0.12 mmol, 1 eq) in water (10 mL) was stirred at room temperature. A solution of compound (*R*)-**5** (0.052 g, 0.11 mmol, 1 eq) in THF (5 mL) was added and the reaction mixture was then heated at 70 °C for 30 minutes. The solution is clear and yellow. An aqueous solution of NH_4PF_6 (1M, 2.5 mL) was added dropwise and the solution became turbid. A dark agglomerate was formed. After additional 15 minutes stirring, the solution was filtered to yield compound (*RR*)-**7** (0.07 g, 0.04mmol, 72%), as a dark green solid. ^{1}H-NMR (d_6-DMSO, 200 MHz, 25 °C) δ = 8.76 (d, 8H; pyridine), 8.67 (s, 4H; binaphthyl), 8.23 (d, 4H; binaphthyl), 8.02 (d, 8H; pyridine), 7.72 (d, 4H; binaphthyl), 7.55 (d, 2H; binaphthyl), 6.79 (d, 4H; binaphthyl), 5.65 (broad peak, 8H; -NH_2), 3.76 (s, 12H; -OCH_3), 2.71 (broad singlet, 8H; -CH_2-). ^{13}C-NMR (CD_3CN, 75 MHz, 25 °C) δ = 156.7 (C_{quat}), 151.5 (CH), 151.4 (C_{quat}), 134.6 (C_{quat}), 131.0 (CH), 129.5 (C_{quat}), 128.8 (C_{quat}), 127.9 (CH), 125.7 (CH), 124.4 (CH), 123.6 (CH), 118.3 (C_{quat}), 115.1 (CH), 56.1 (CH_3), 46.7 (CH_2). ESI-MS (m/z)=1704.31 ([*M*-PF_6]$^+$).

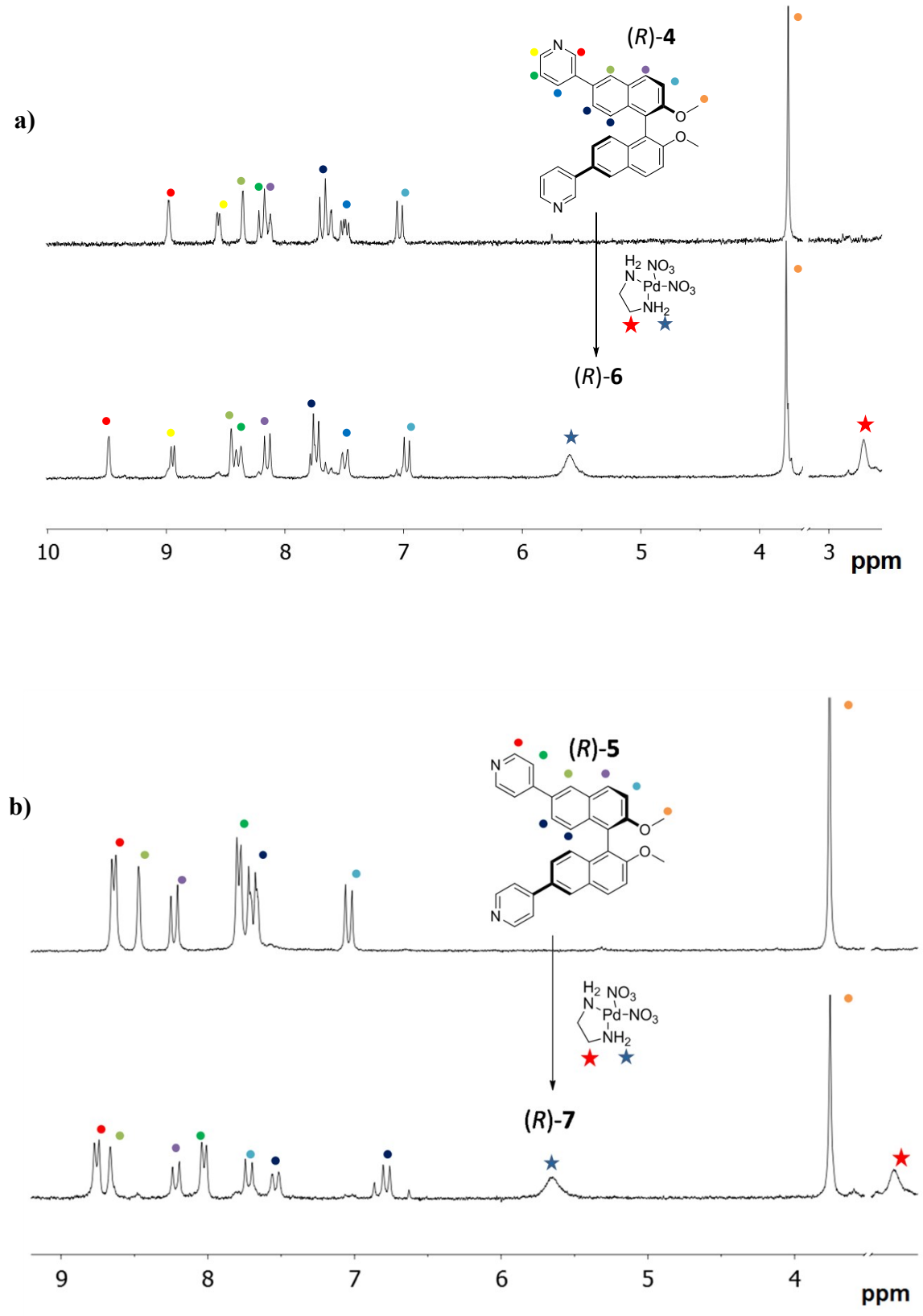


Figure S2.a) Comparison between the ^{1}H-NMR spectra of the ligand (*R*)-**4** and the complex (*RR*)-**6** in DMSO-d_6; b) comparison between the ^{1}H-NMR spectra of the ligand (*R*)-**5** and the complex (*RR*)-**7** in DMSO-d_6.

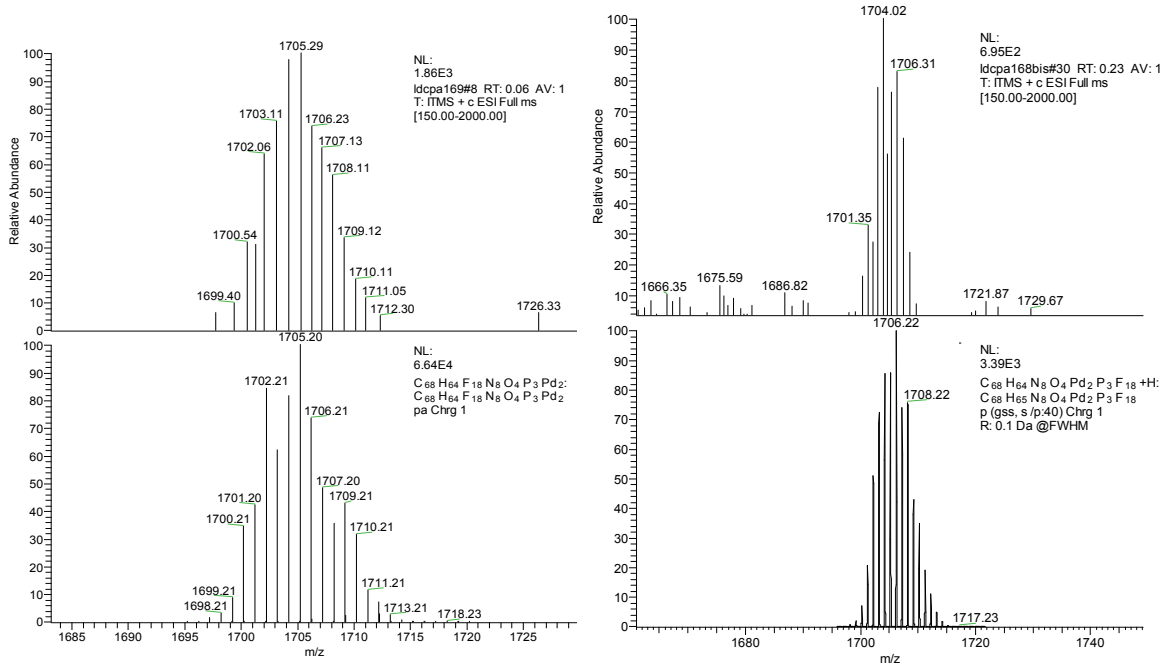


Figure S3. Experimental (top) and theoretical (bottom) isotopic assets for the quasi molecular ions $[M\text{-}PF_6]^+$ of compounds (*RR*)-**6** on the left and (*RR*)-**7** on the right.

2) Additional titrations and procedures

General procedure for the NMR, UV, CD and CV titration experiments. The titration experiments (UV/Vis, Circular Dichroism (CD) and ^{1}H-NMR) were conducted as follows. To a stock solution of the host molecule (solution A) in pure CH_3CN or CH_3CN:H_2O mixtures (UV/Vis spectroscopic grade or HPLC grade; CD_3CN and D_2O in the case of ^{1}H-NMR) were added several aliquots of the guest compound (solution B). Solution B is formed by the guest molecule at higher concentration dissolved in solution A, in order to maintain the ligand always at the same, constant concentration. *Cyclic voltammetry (CV) experiments* were performed in a mixture of CH_3CN:H_2O 7:3 with tetrabutylammonium hexafluorophosphate (0.1 M) as the supporting electrolyte, and scan speed of 100 mV/s; potential range investigated was from -200mV to +900 mV (vs Ag/AgCl/3M NaCl). Solutions were deaerated with a nitrogen stream (5 min) before use. In this case, incrementing aliquots of concentrated *guest* solution were added to a diluted solution of *host* (1.3×10^{-4} M in the voltammetric cell) in order to avoid diluting effect.

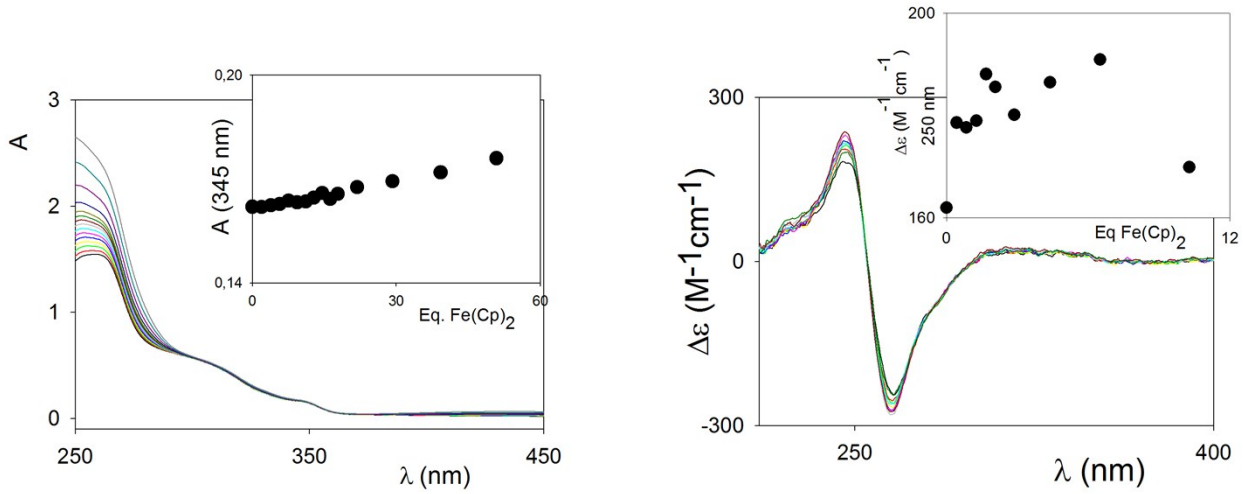


Figure S4. Left: UV/Vis titration in MeCN of compound (*RR*)-**6** (1×10^{-5} M) with increasing amounts of ferrocene. Right: CD titration in MeCN of compound (*RR*)-**6** (1×10^{-5} M) with increasing amounts of ferrocene.

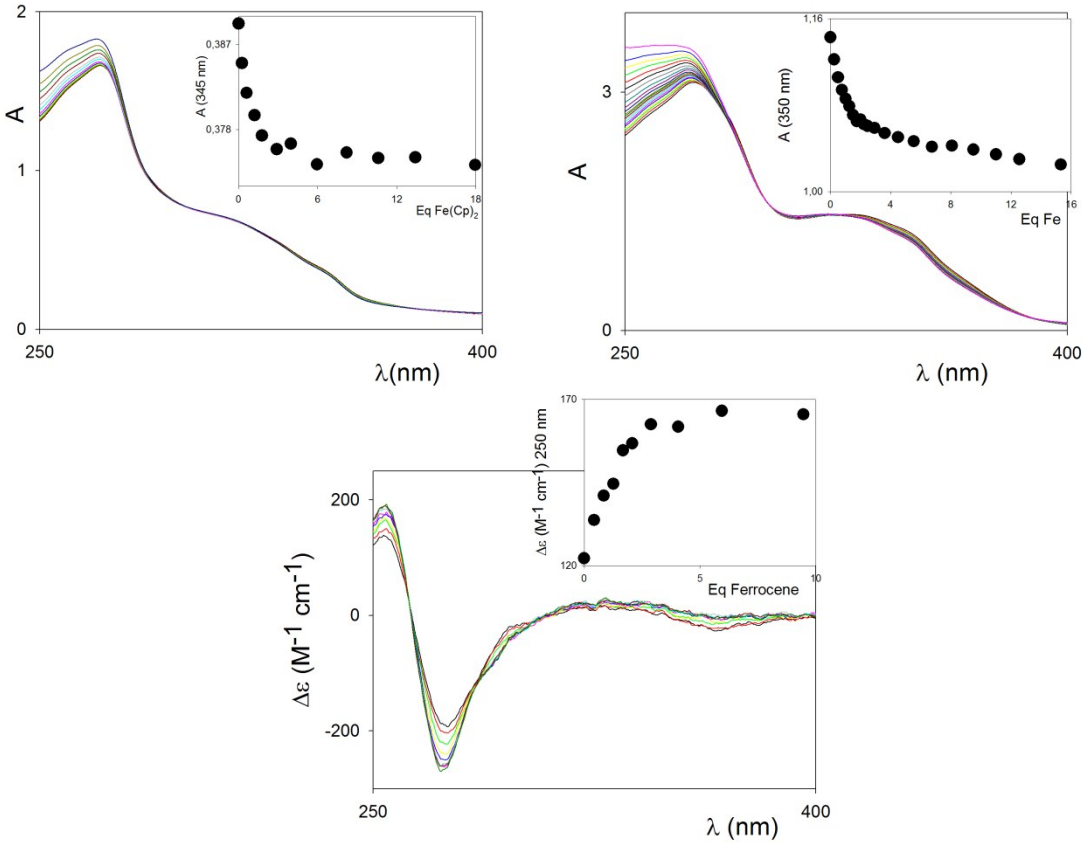


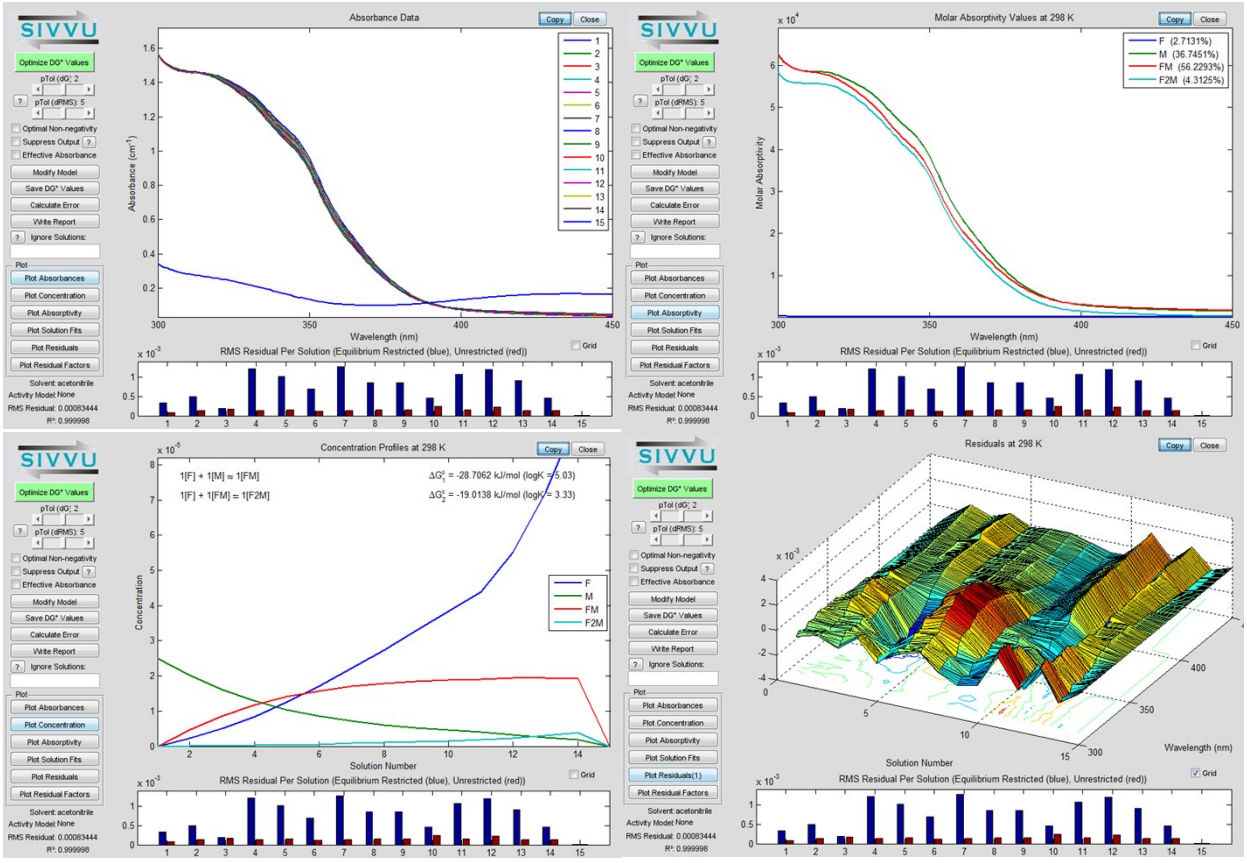
Figure S5. Top: UV-Vis titration spectra in different solvents mixtures of compound (*RR*)-**7** with increasing amounts of ferrocene: left) 1x10^{-5} M of macrocycle in MeCN:H_2O 7:3; right) 2,5x10^{-5} M in MeCN:H_2O 9:1; and bottom:CD titration of (*RR*)-**7**(1x10^{-5} M)in MeCN:H_2O 7:3.

Table S1. Binding energies and association constants at 298 K for the multiple equilibriums found in the titration of (*RR*)-**7** with ferrocene obtained using SIVVU (see Table S2-S4).

Entry	Solvent	ε[a]	$\Delta G_{11}°$ (kJM^{-1})	$\Delta G_{12}°$ (kJ M^{-1})	ΔG_{tot} (kJM^{-1})	*Log* K_{11} (kcal M^{-1})	*Log* K_{12} (kcal M^{-1})
1	MeCN	35,9	-28,7	-19	-47,7	4,41	2,71
2	MeCN:H_2O 9:1	39,4	-30,9	-16,2	-47,1	4,79	2,22
3	MeCN:H_2O 7:3	46,8	-35,4	-17,2	-52,2	5,58	2,39

[a] Dielectric constant. Data taken form ref. S3.

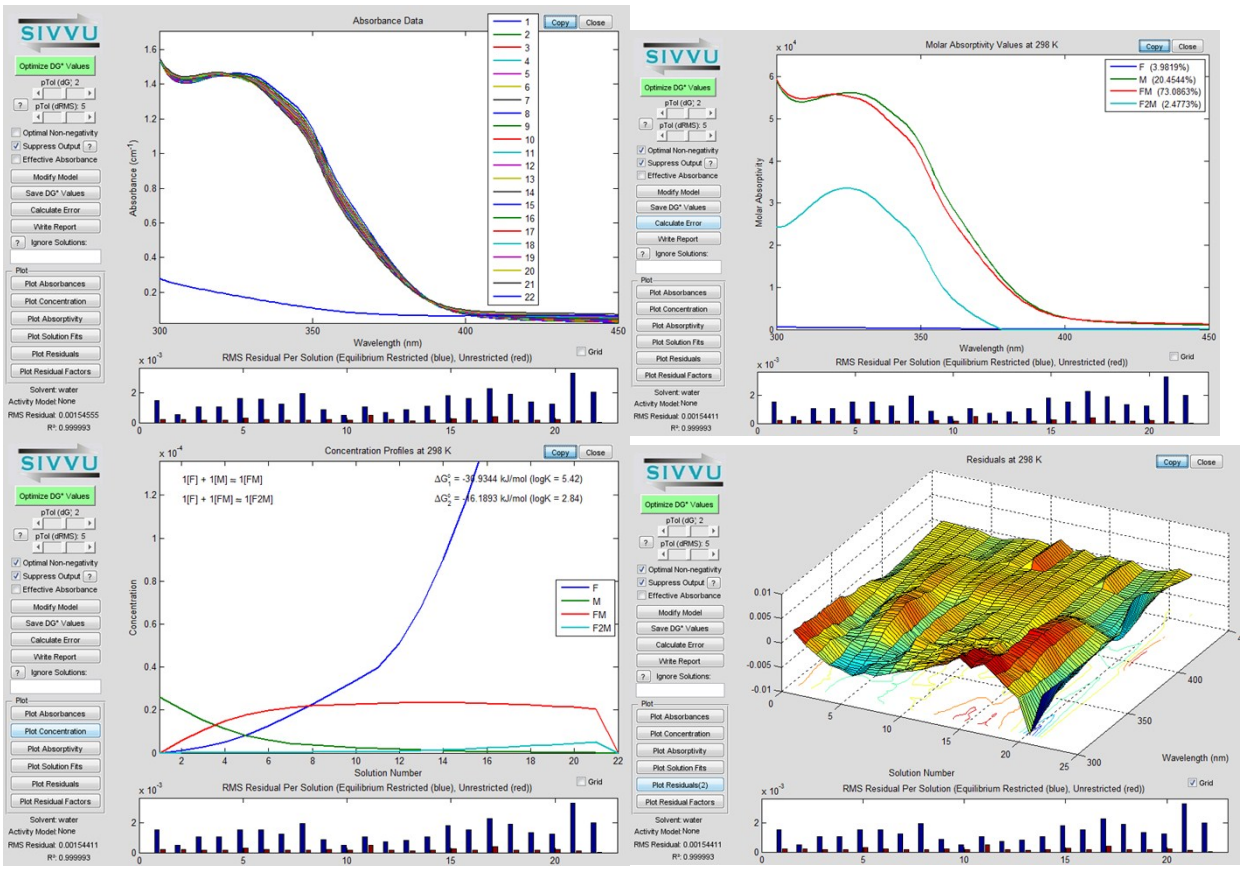
Table S2. Fit of titration in entry 1 Table S1 (24.9 µM Macrocycle (*RR*)-**7** in solvent of 100% MeCN) was titrated with 0 –5 equivalents of ferrocene at 298 K. A 1.3 mM solution of ferrocene alone is also included.



Optimization Summary:
Data at 298 K
Non-negativity was enforced with optimization (not truncation).
Activity Coefficients Model: None.
Species with Fixed Molar Absorptivity Curves: None.
Solutions ignored: None.
Optimized Values (kJ/mol): $\Delta G°_1$ = -28.7(±0.3); $\Delta G°_2$ = -19(±2);
Equilibrium Restricted RMS Residual (4 chemical factors): 0.00083444
Unrestricted RMS Residual (4 mathematical factors): 0.00015618
Restricted Data Reconstruction (4 chemical factors): 99.9376%
Unrestricted Data Reconstruction (4 mathematical factors): 99.9423%
Remaining Error Imbedded in Absorbance Values: 0.00050319
R^2: 99.9998%

The standard deviations of the ΔG°values are estimated by re-optimizing the model 40 times, each with half of the absorbance data randomly ignored (30% of the solutions and 30% percent of the wavelengths).

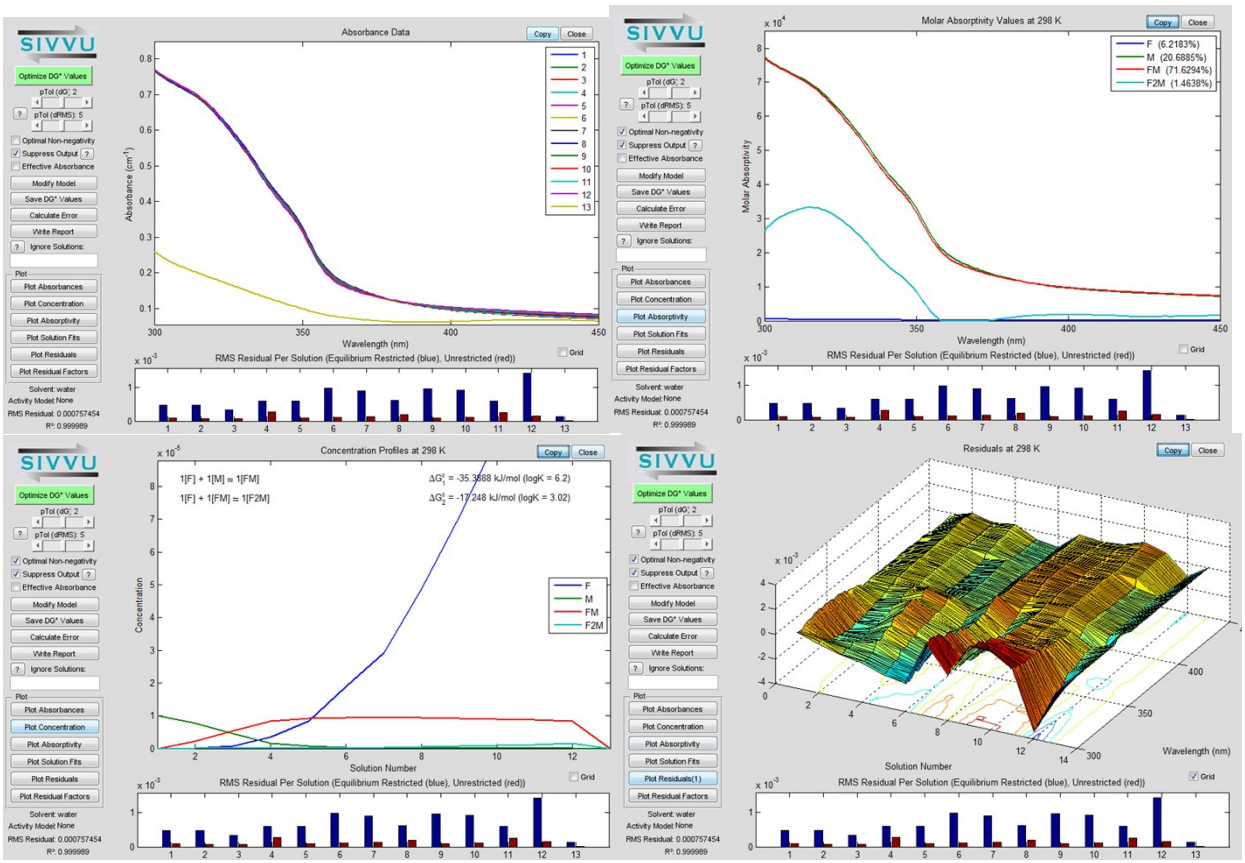
Table S3. Fit of titration in entry 2 Table S1 (26 µM Macrocycle (*RR*)-**7** in solvent of 90% MeCN and 10% water) with 0 – 15 equivalents of ferrocene at 298 K. A 0.511 mM solution of ferrocene alone is also included.



Optimization Summary:
Data at 298 K
Non-negativity was enforced with optimization (not truncation).
Activity Coefficients Model: None.
Species with Fixed Molar Absorptivity Curves: None.
Solutions ignored: None.
Optimized Values (kJ/mol): $\Delta G°_1$ = -30.9(±0.4); $\Delta G°_2$ = -16.2(±0.3);
Equilibrium Restricted RMS Residual (4 chemical factors): 0.0015441
Unrestricted RMS Residual (4 mathematical factors): 0.00022314
Restricted Data Reconstruction (4 chemical factors): 99.9106%
Unrestricted Data Reconstruction (4 mathematical factors): 99.917%
Remaining Error Imbedded in Absorbance Values: 0.0007279
R^2: 99.9993%

The standard deviations of the ΔG°values are estimated by re-optimizing the model 40 times, each with half of the absorbance data randomly ignored (30% of the solutions and 30% percent of the wavelengths).

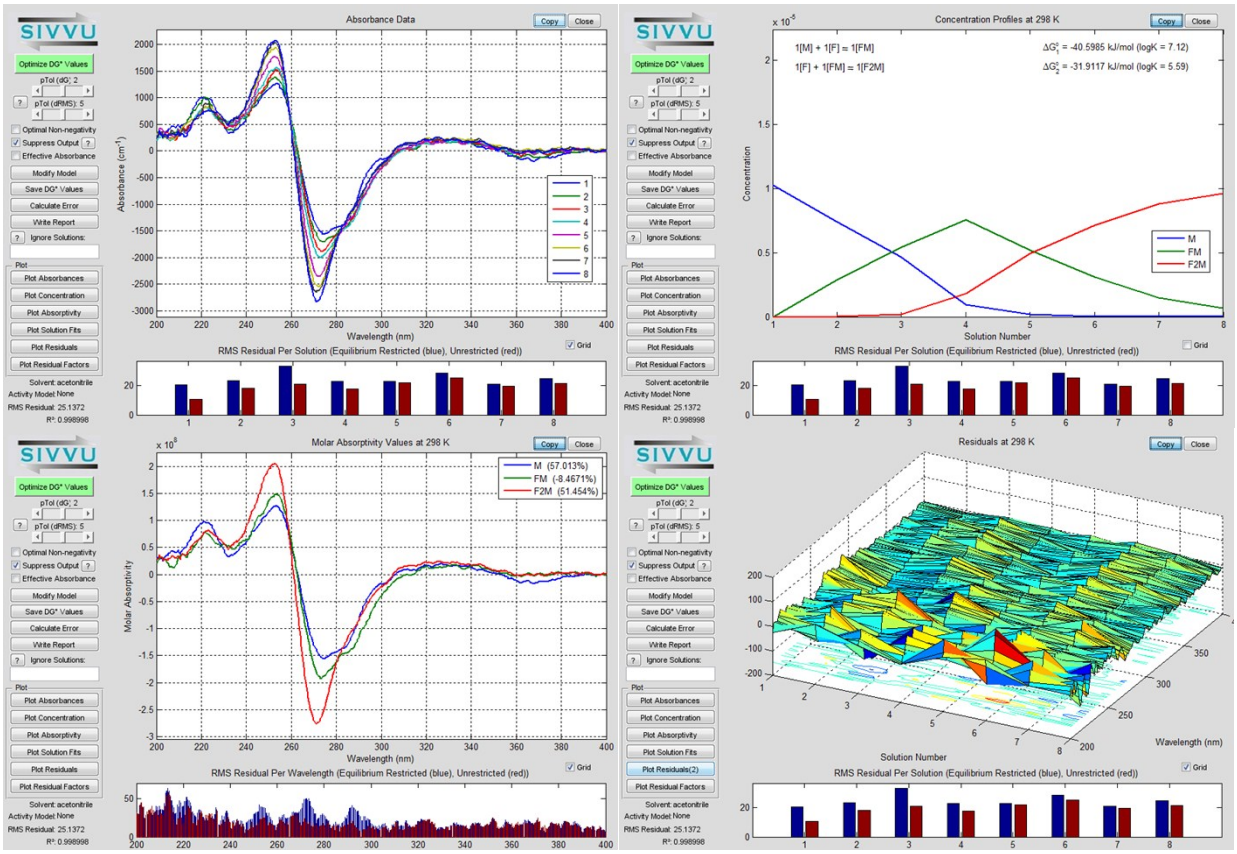
Table S4. Fit of titration in entry 3Table S1 (9.95 µM Macrocycle (*RR*)-**7**in solvent of 70% MeCN and 30% water) with 0 – 18 equivalents of ferrocene at 298 K. A 0.538 mM solution of ferrocene alone is also included.



Optimization Summary:
Data at 298 K
Non-negativity was enforced with optimization (not truncation).
Activity Coefficients Model: None.
Species with Fixed Molar Absorptivity Curves: None.
Solutions ignored: None.
Optimized Values (kJ/mol): $\Delta G°_1$ = -35.4(±0.6); $\Delta G°_2$ = -17.2(±0.1);
Equilibrium Restricted RMS Residual (4 chemical factors): 0.00075745
Unrestricted RMS Residual (4 mathematical factors): 0.00014401
Restricted Data Reconstruction (4 chemical factors): 99.8901%
Unrestricted Data Reconstruction (4 mathematical factors): 99.909%
Remaining Error Imbedded in Absorbance Values: 0.00050497
R^2: 99.9989%

The standard deviations of the ΔG° values are estimated by re-optimizing the model 40 times, each with half of the absorbance data randomly ignored (30% of the solutions and 30% percent of the wavelengths).

Table S5. Fit of titration of a 10.3 µM solution of Macrocycle (*RR*)-**7**in MeCN with 0 – 5.5 equivalents of ferrocene at 298 K.



Optimization Summary:
Data at 298 K
Non-negativity was not enforced.
Activity Coefficients Model: None.
Species with Fixed Molar Absorptivity Curves: None.
Solutions ignored: None.
Optimized Values (kJ/mol): $\Delta G°_1$ = -41(±3); $\Delta G°_2$ = -31.9(±0.6);
Equilibrium Restricted RMS Residual (3 chemical factors): 25.1372
Unrestricted RMS Residual (3 mathematical factors): 19.9598
Restricted Data Reconstruction (3 chemical factors): 95.2171%
Unrestricted Data Reconstruction (3 mathematical factors): 95.4065%
Remaining Error Imbedded in Absorbance Values: 19.4712
R^2: 99.8998%

The standard deviations of the ΔG° values are estimated by re-optimizing the model 40 times, each with half of the absorbance data randomly ignored (30% of the solutions and 30% of the wavelengths).

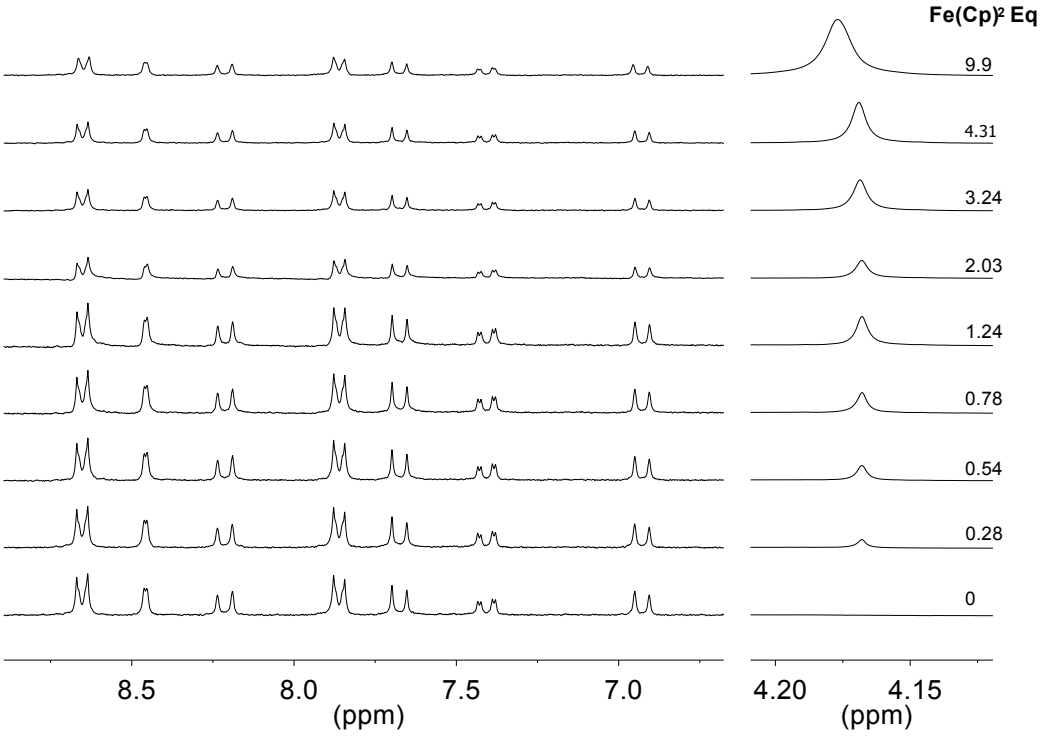


Figure S6. ^{1}H-NMR titration (200 MHz, CD_3CN:D_2O 7:3) of compound (*RR*)-**7** 1x10^{-3} M.

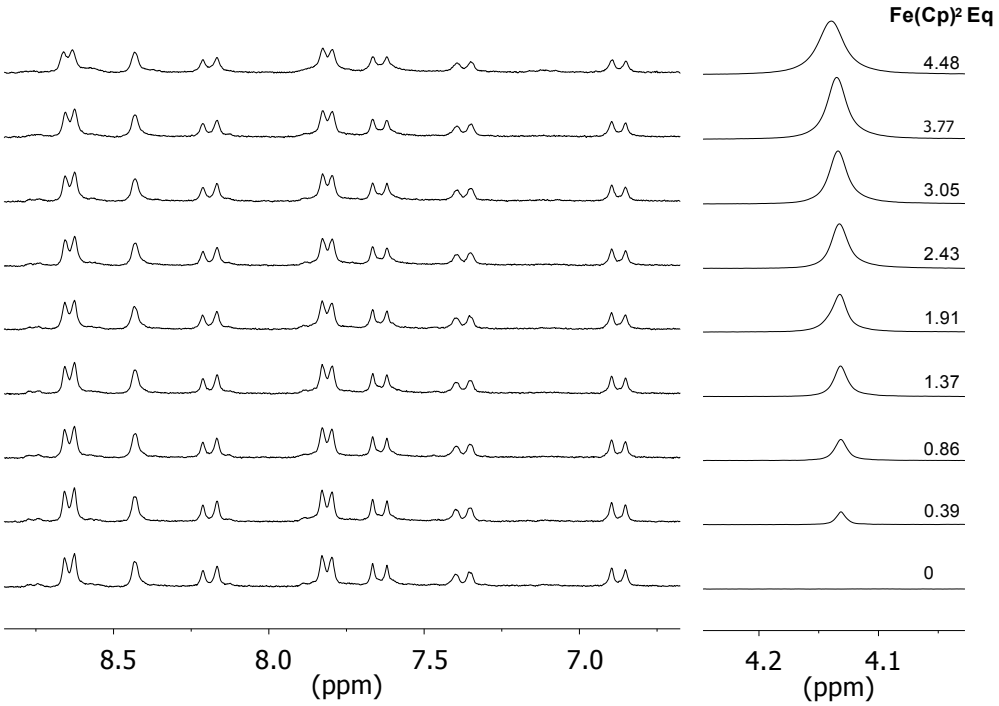


Figure S7. ^{1}H-NMR titration (200 MHz, CD_3CN) of compound (*RR*)-**7** 1x10^{-3} M.

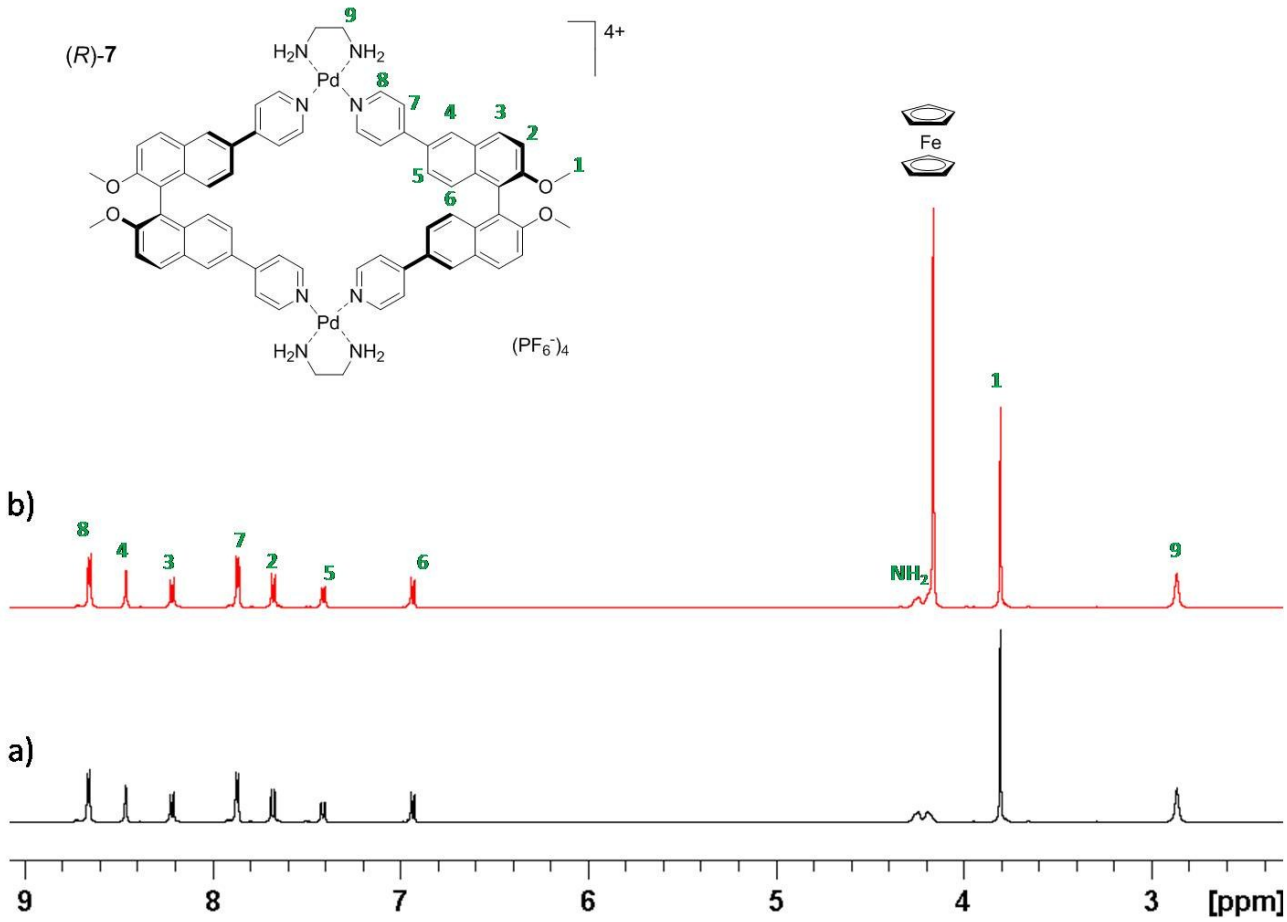


Figure S8. ^{1}H NMR spectra (500 MHz, CD_3CN) of compound (*RR*)-**7** alone (a, black trace, 5.5 mM) and in the presence of ferrocene (b, red trace, 16 mM).

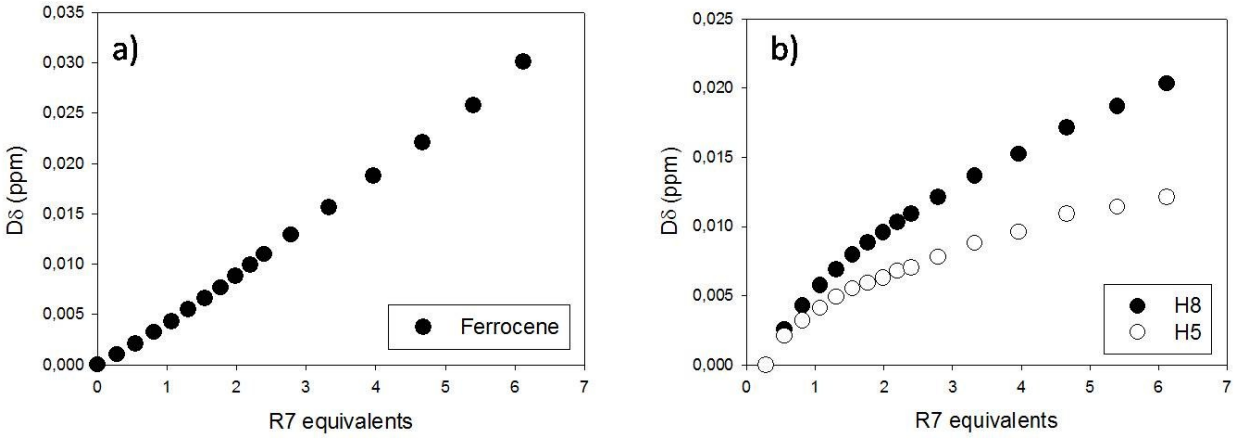


Figure S9. Plots of the shifts of the ^{1}H NMR peaks of ferrocene (a) and of hydrogens 8 and 5 of compound (*RR*)-**7** (b) for the titration shown in Figure 3 main text.

Table S5. Outcome of the fitting of data from the ^{1}H NMR peaks of ferrocene (Figure S9) using the open access program BindFit (www.supramolecular.org). Model 2:1 binding (species at constant concentration:species at variable concentration, ferrocene:macrocycle, respectively)

K_{11}	K_{21}	K_{11} error (%)	K_{21} error (%)	SSR			
0,142173937	64585,6346	4,11042066	3,24260059	4,1554E-07			
Datapoints fitted	**Params fitted**	**H coeffs**	**HG coeffs**	**H2G coeffs**	**Raw coeffs 1**	**Raw coeffs 2**	**Raw coeffs 3**
17	4	4,15653	-40,2809993	4,26520904	4,15653	-40,2809993	4,26520904

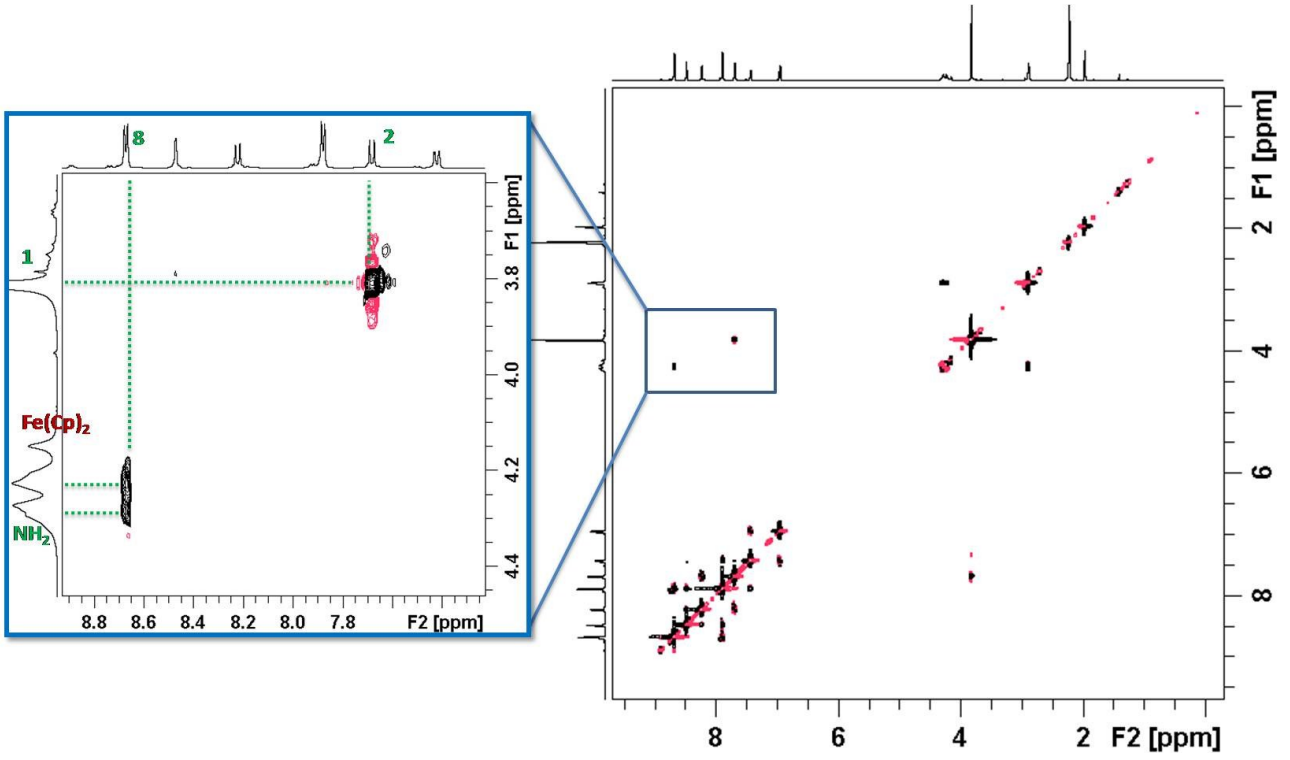


Figure S10. 2D ROESY NMR spectrum of a mixture of ferrocene (2.4 mM) and (*RR*)-**7** (14.7 mM) in CD_3CN.

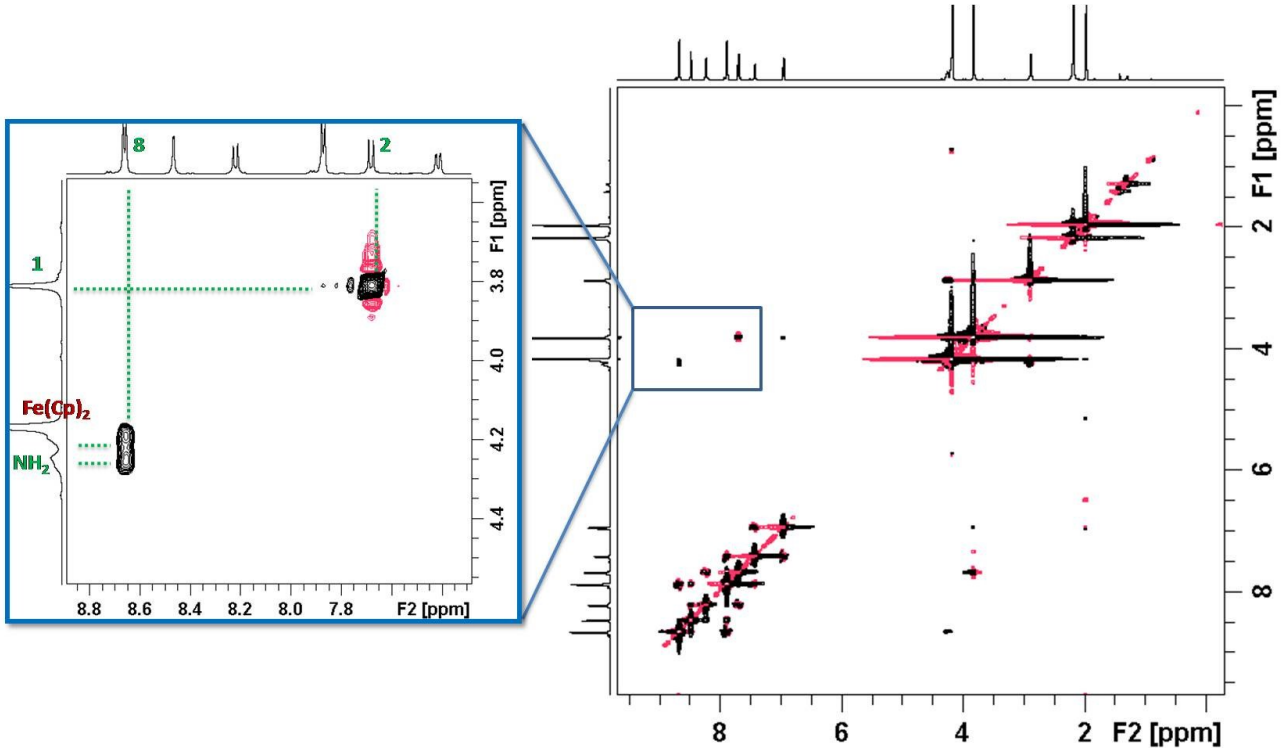


Figure S11.2D ROESY NMR spectrum of a mixture of ferrocene (16.1 mM) and (*RR*)-**7** (5.6 mM) in CD_3CN.

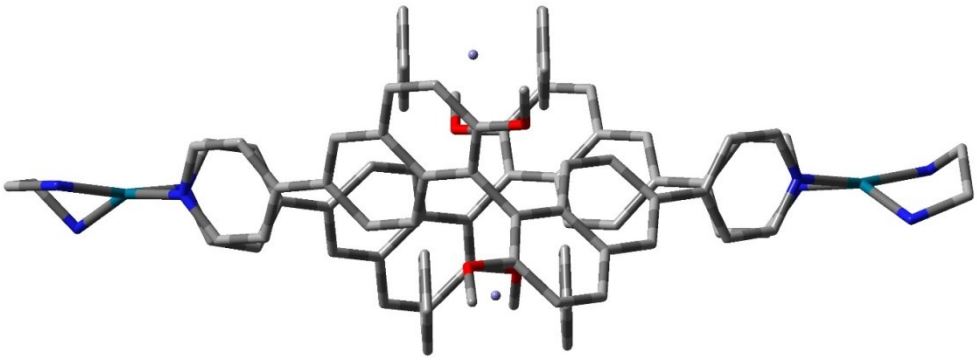


Figure S12. Modelling of the 1:2 host guest complex between (*RR*)-**7** and ferrocene.

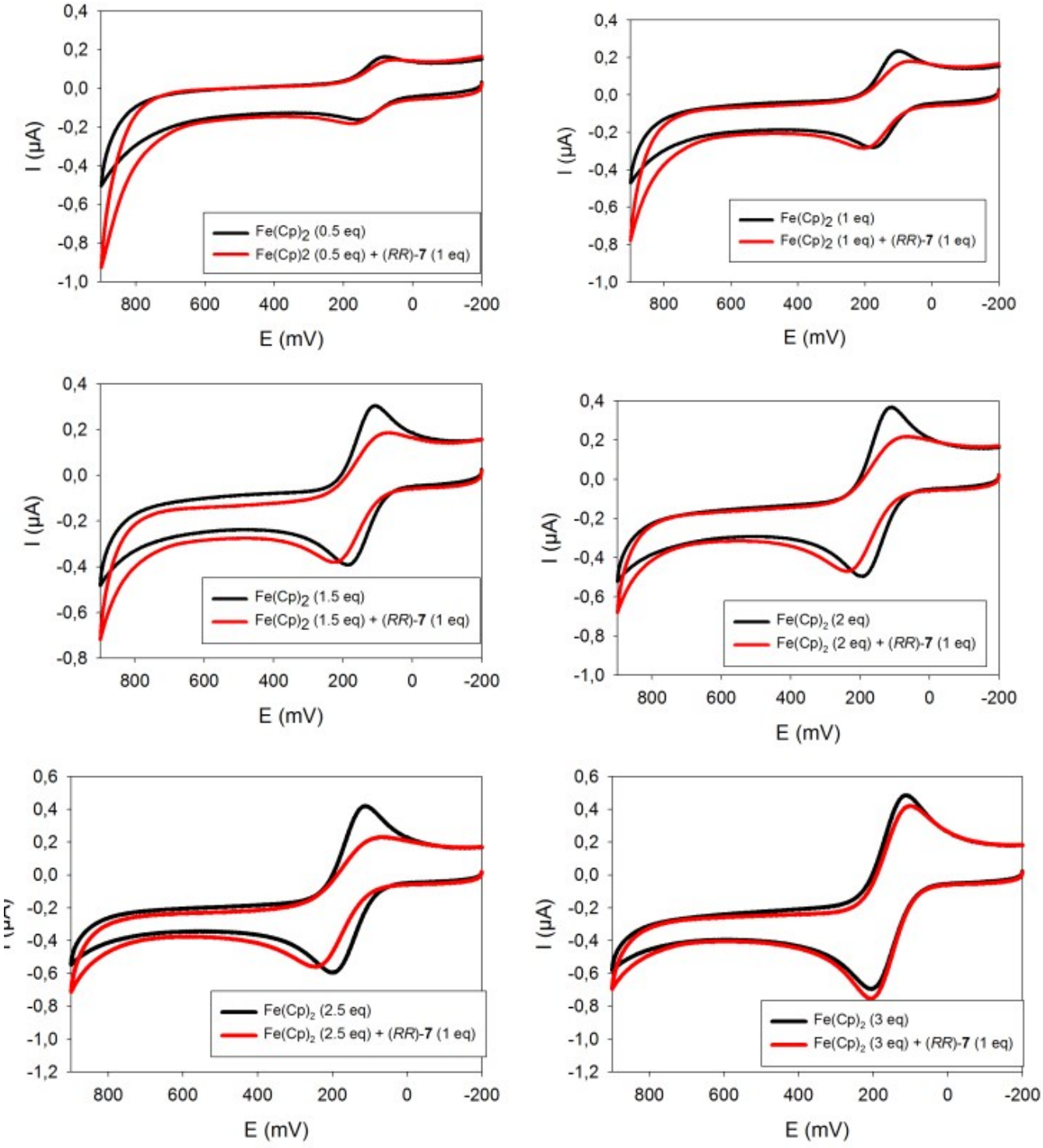
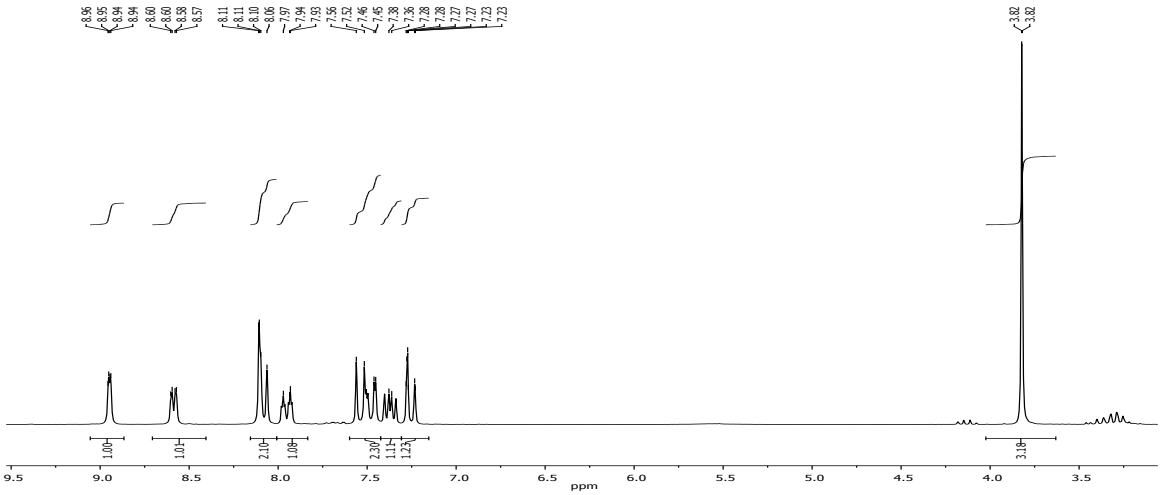


Figure S13. Comparisons between the Cyclic Voltammetry curves of ferrocene alone and in the presence of compound (*RR*)-**7** (1.3×10^{-4} M)in CH_3CN:H_2O 7:3.

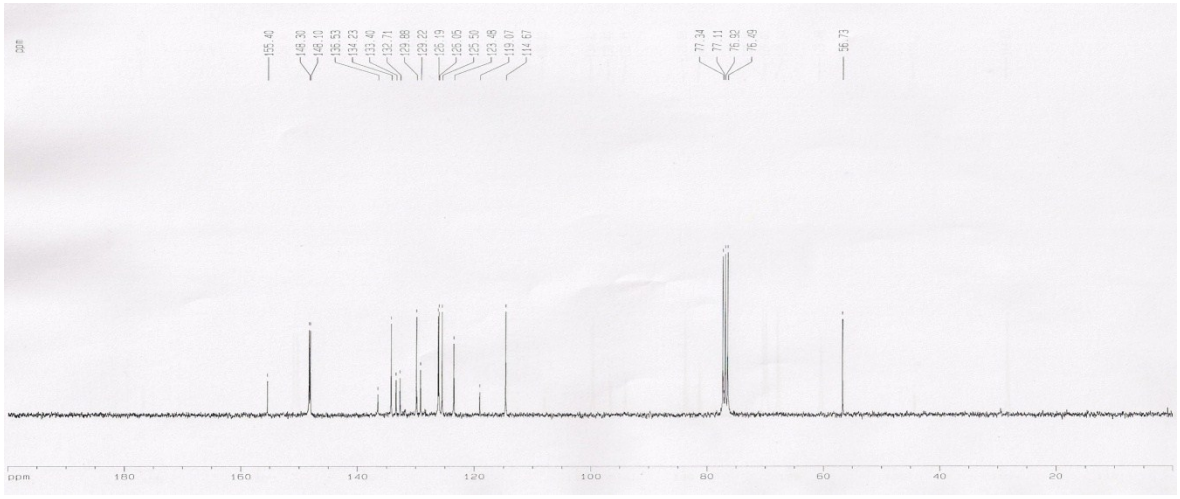
Copies of NMR and Mass Spectra of Newly Synthesized Compounds

Compound (R)-**4**.

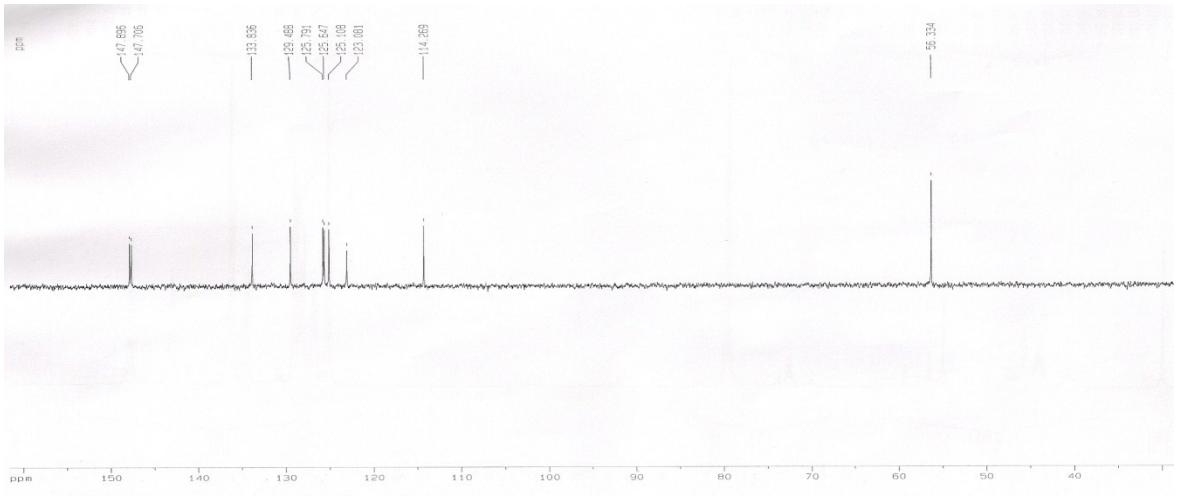
^{1}H NMR ($CDCl_3$, 200 MHz)



^{13}C NMR ($CDCl_3$, 75 MHz)

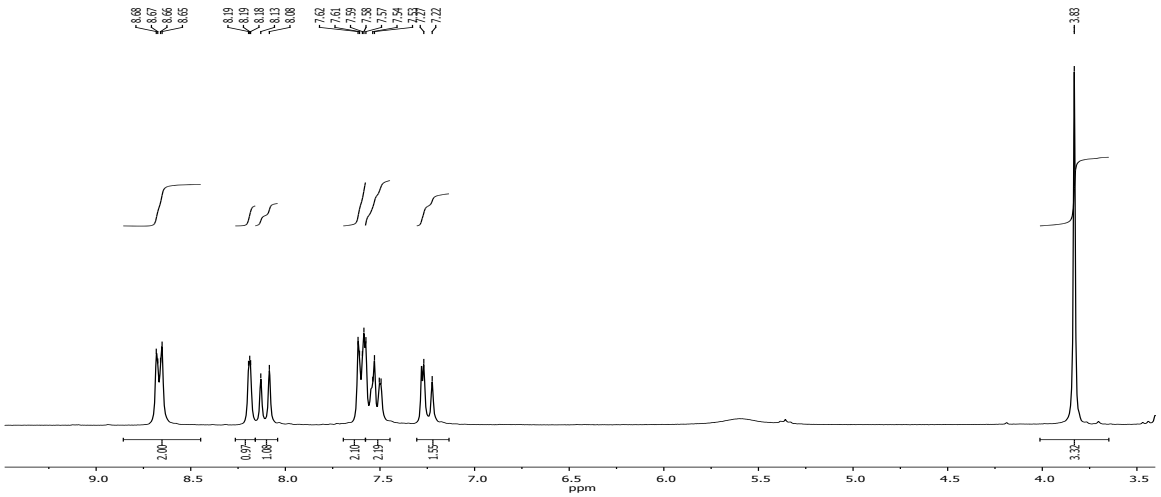


^{13}C NMR DEPT ($CDCl_3$, 75 MHz)

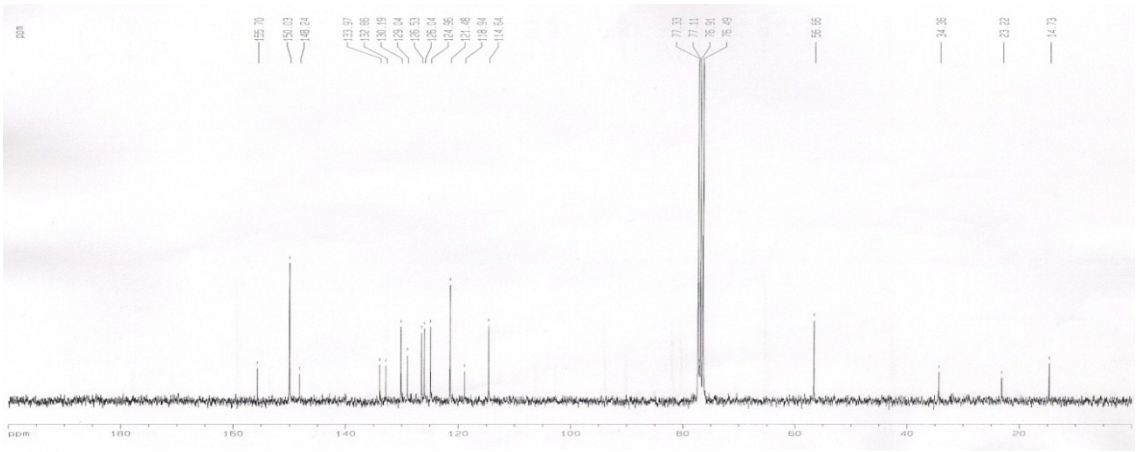


Compound (R)-**5**.

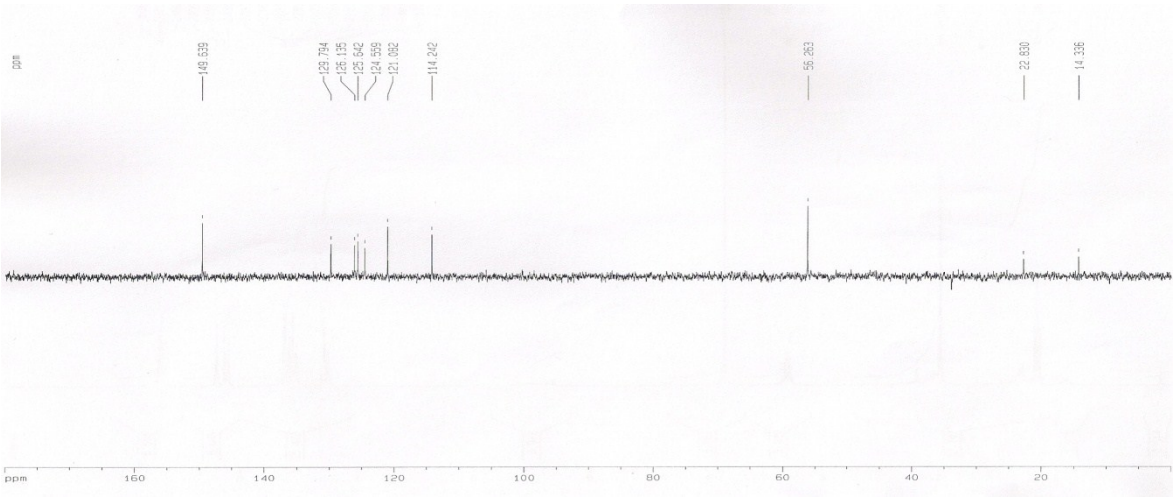
^{1}H NMR ($CDCl_3$, 200 MHz)



^{13}C NMR ($CDCl_3$, 75 MHz)

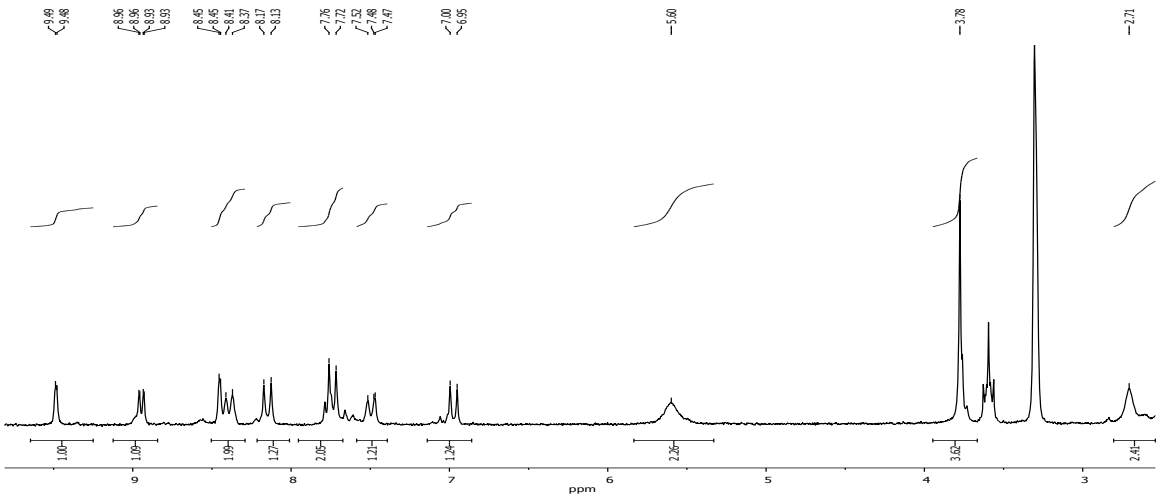


^{13}C NMR DEPT ($CDCl_3$, 75 MHz)

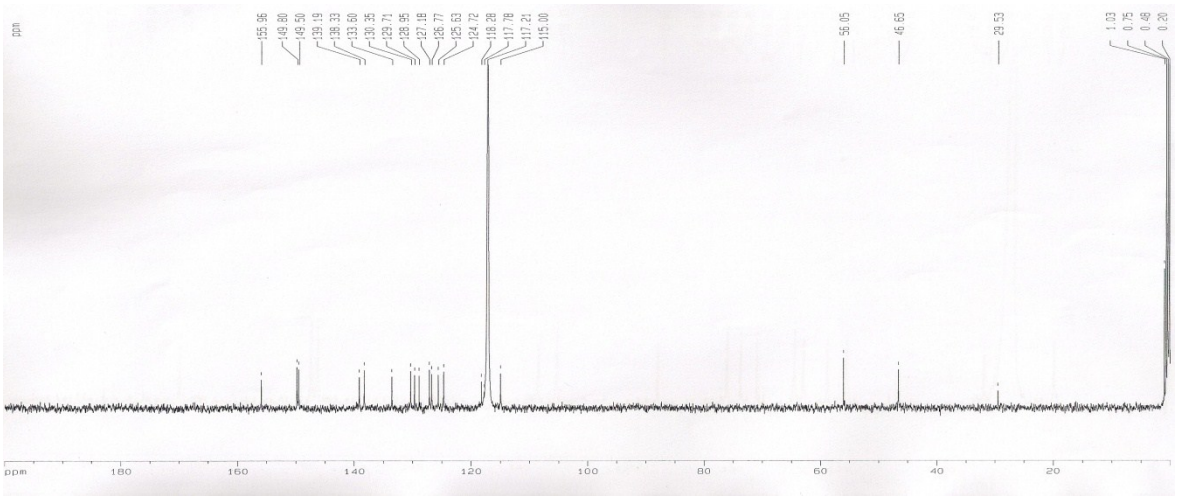


Compound (RR)-**6**.

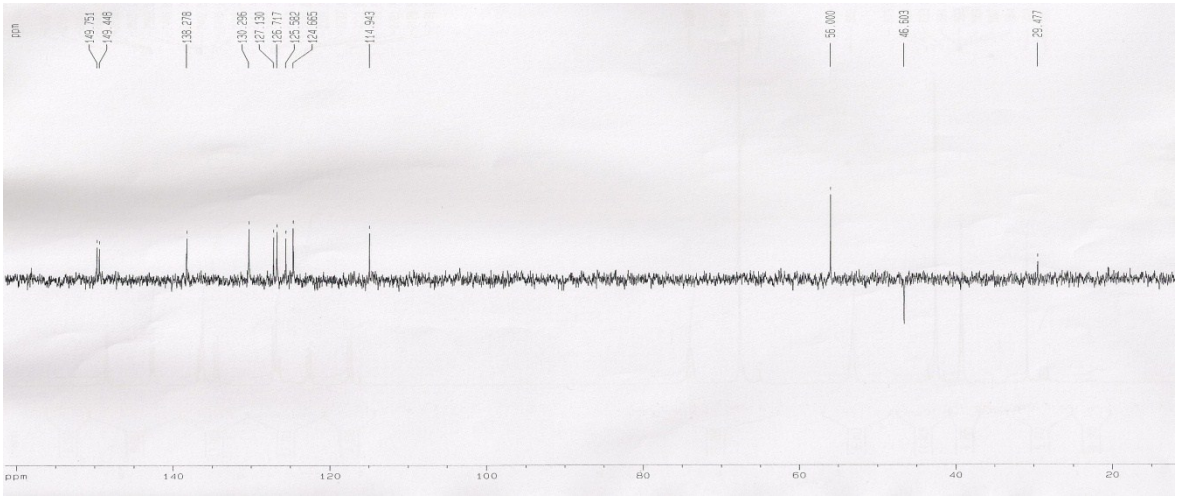
^{1}H NMR (DMSO-d_6, 200 MHz)



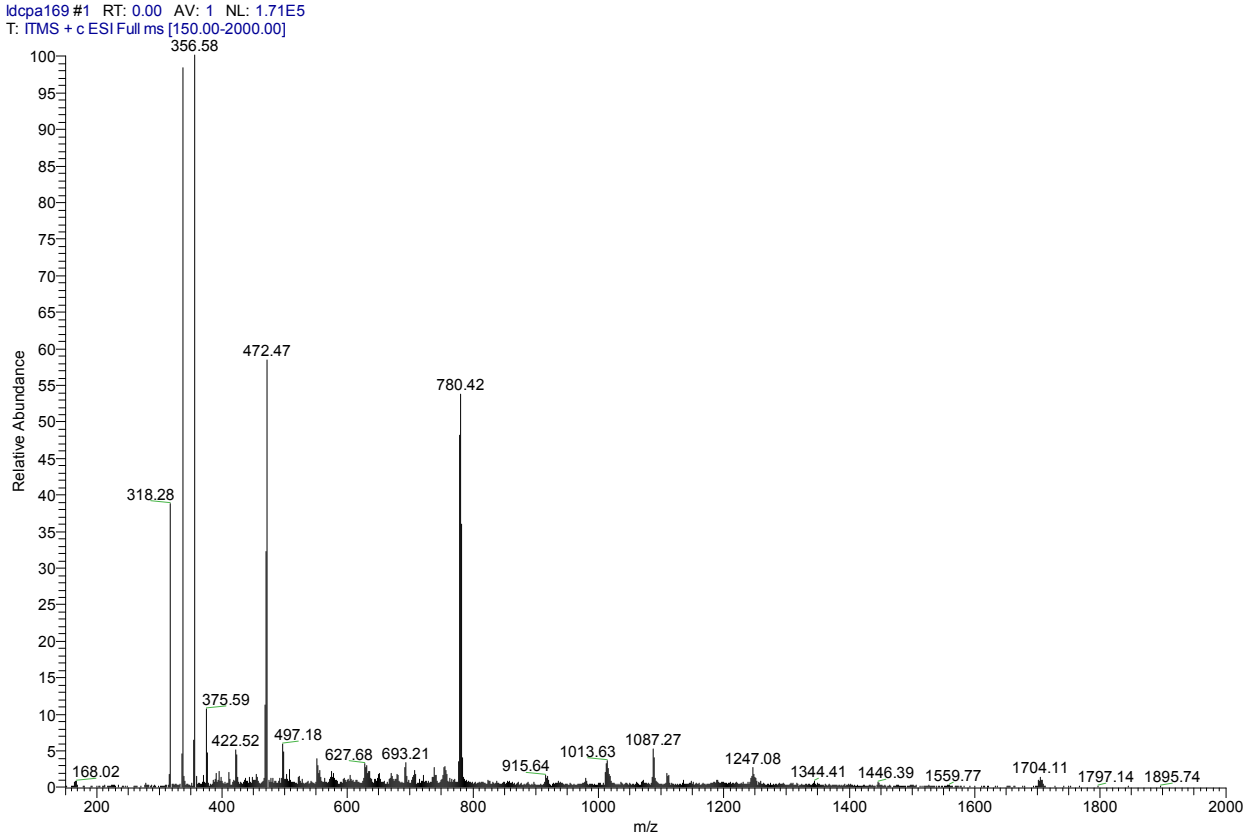
^{13}C NMR (CD_3CN, 75 MHz)



^{13}C NMR DEPT (CD_3CN, 75 MHz)

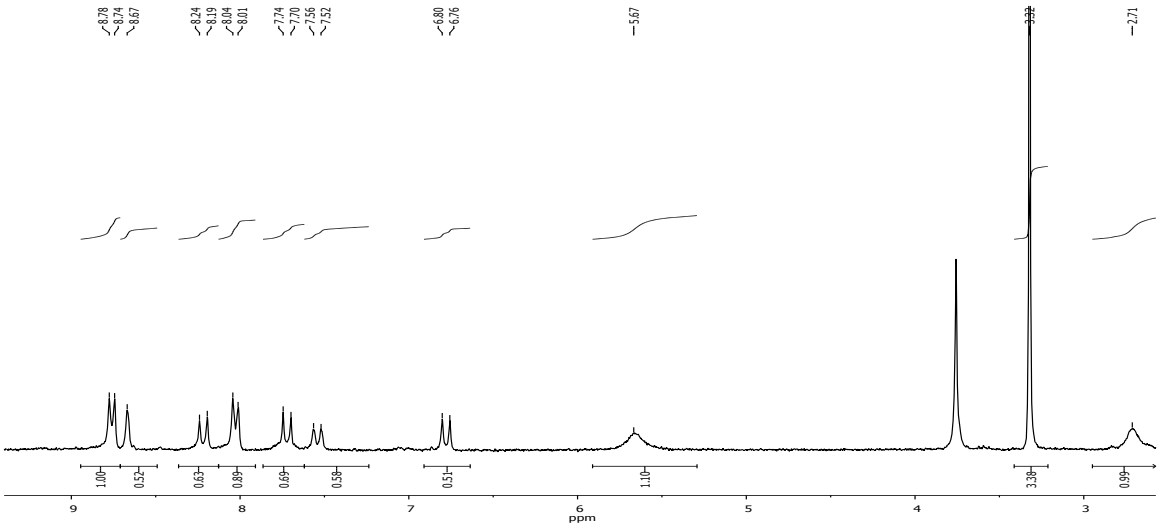


ESI-MS

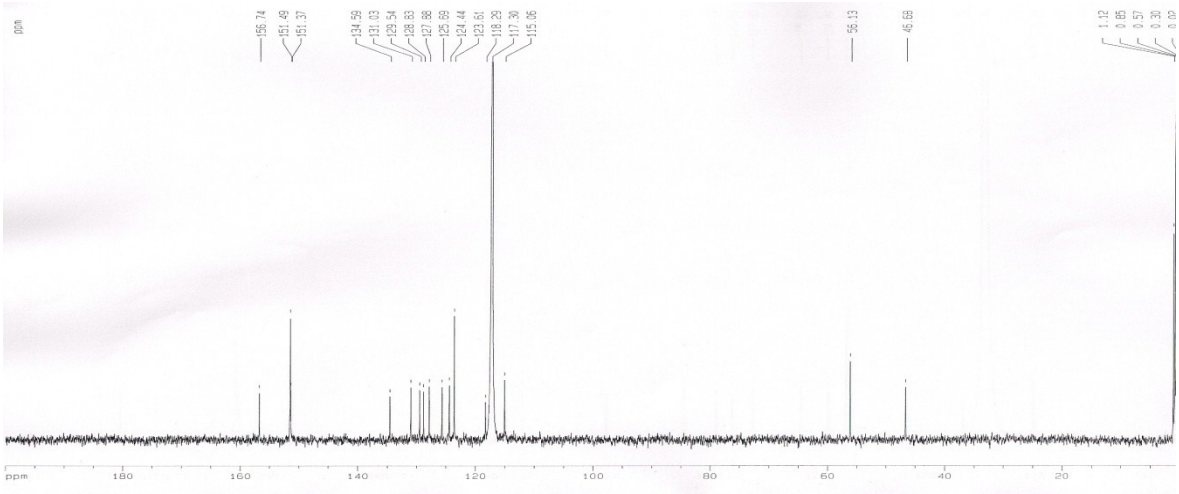


Compound (RR)-**7**.

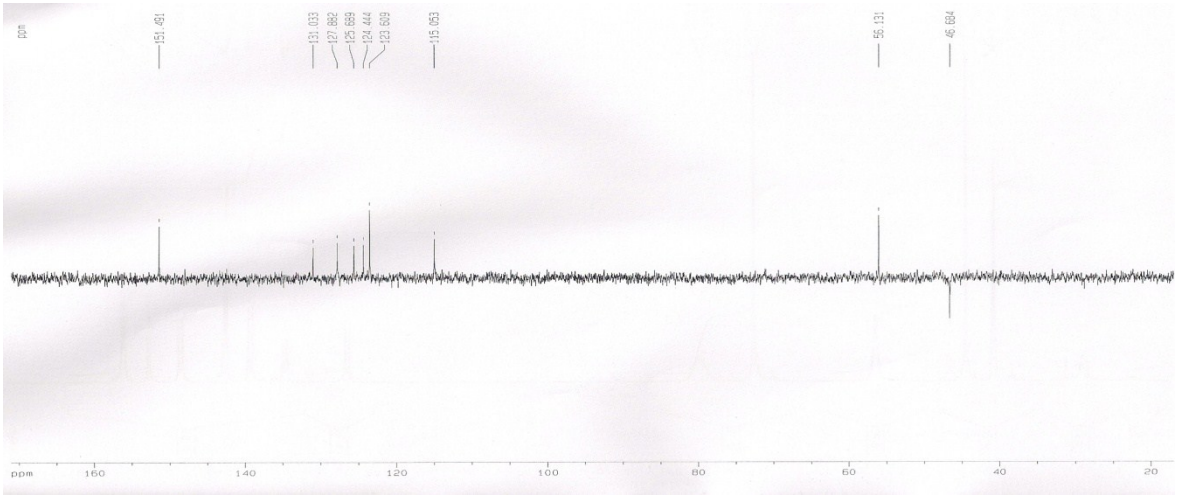
^{1}H NMR (DMSO-d_6, 200 MHz)



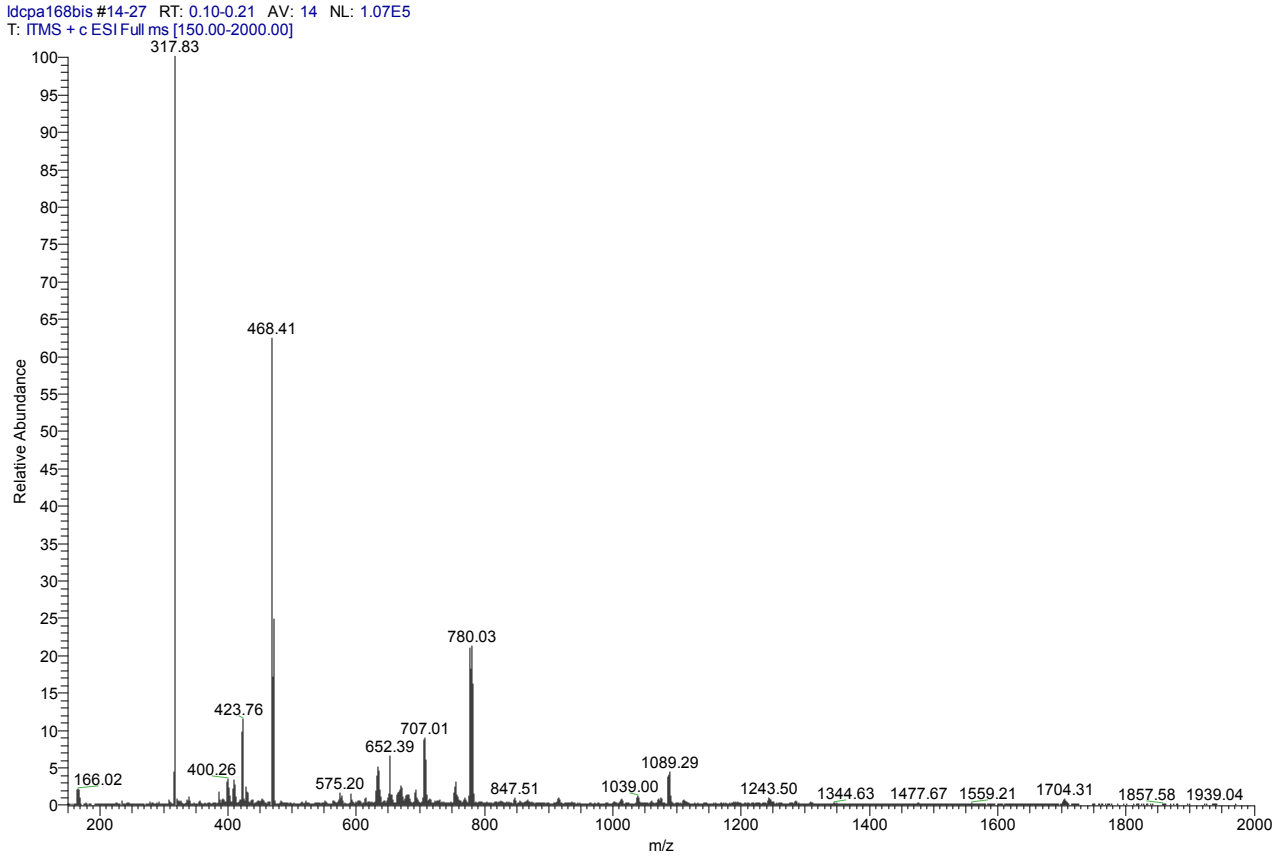
^{13}C NMR (CD_3CN, 75 MHz)



^{13}C NMR DEPT (CD_3CN, 75 MHz)



ESI-MS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Relative Abundance
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423.76
707.01
652.39
1089.29
166.02
400.26
575.20
847.51
1039.00
1243.50
1344.63
1477.67
1559.21
1704.31
1857.58
1939.04

References

S1. M. Agnes, A. Sorrenti, D. Pasini, K. Wurst and D. B. Amabilino, *CrystEngComm*, 2014, **16**, 10131-10138.

S2. a) K. Gilg, T. Mayer, N. Ghaschghaie and P. Klüfers, *Dalton Trans.*, 2009, 7934. b) M. Morell Cerda`, B. Costisella and B. Lippert, *Inorg. Chim. Acta,* 2006, **359**, 1485. c) H. D. K. Drew, F. W. Pinkard, G. H. Preston and W. J. Wardlaw, *J. Chem. Soc.,* 1932, 1895.

S3. L. G. Gagliardi, C. B. Castells, C.Rafols, M. Roses and Elisabeth Bosch, *J. Chem. Eng. Data*, 2007, **52**, 1103-1107.