Electronic Supplementary Information

Competitive binding for triggering a fluorescence response in a hydrazodicarboxamide-based [2]rotaxane

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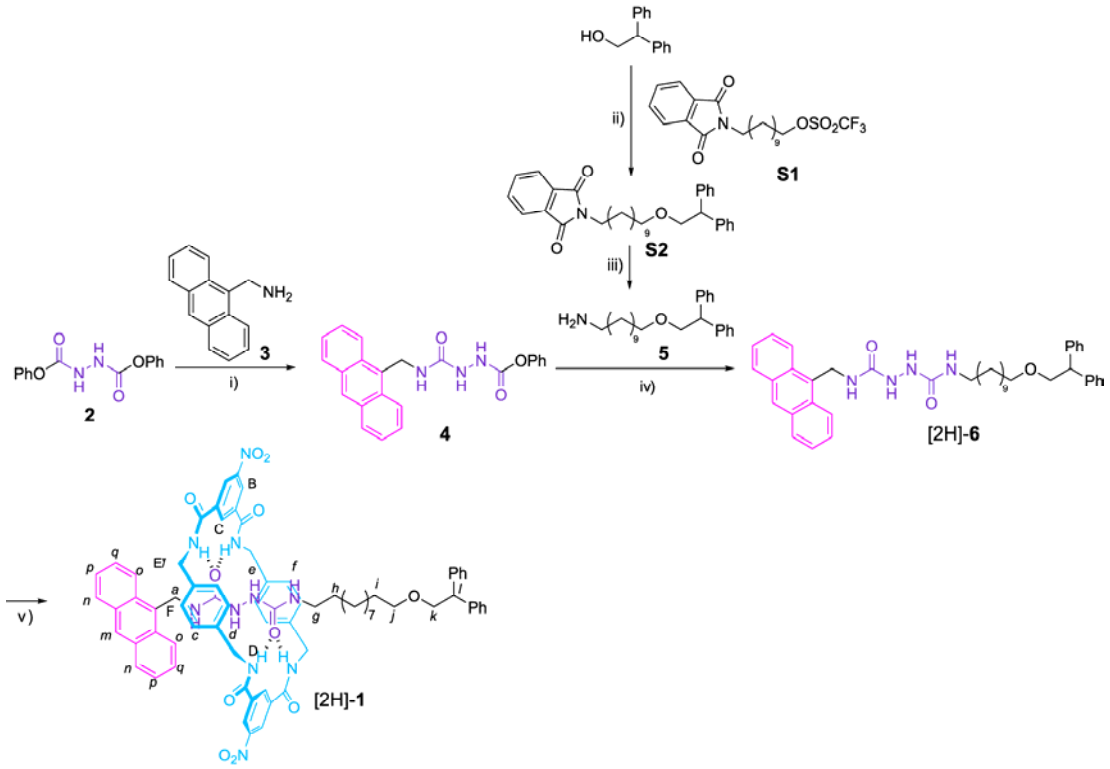
1. General Experimental Section

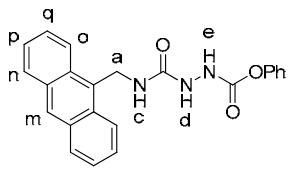
Unless stated otherwise, all reagents were purchased from Aldrich Chemicals and used without further purification. HPLC grade solvents (Scharlab) were nitrogen saturated and were dried and deoxygenated using an Innovative Technology Inc. Pure-Solv 400 Solvent Purification System. Column chromatography was carried out using silica gel (60 Å, 70-200 μm, SDS) as stationary phase, and TLC was performed on precoated silica gel on aluminum cards (0.25 mm thick, with fluorescent indicator 254 nm, Fluka) and observed under UV light. All melting points were determined on a Kofler hot-plate melting point apparatus and are uncorrected. ^{1}H- and ^{13}C NMR spectra were recorded at 298 K on a Bruker Avance 300, 400 and 600 MHz instruments. ^{1}H NMR chemical shifts are reported relative to Me_4Si and were referenced via residual proton resonances of the corresponding deuterated solvent whereas ^{13}C NMR spectra are reported relative to Me_4Si using the carbon signals of the deuterated solvent. Signals in the ^{1}H and ^{13}C NMR spectra of the synthesized compounds were assigned with the aid of DEPT or APT, or two-dimensional NMR experiments (COSY, HMQC and HMBC). Abbreviations of coupling patterns are as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet. Mass spectra were recorded with a HPLC/MS TOF 6220 mass spectrometer.

2. Synthesis of hydrazo rotaxane[2H]-1



Scheme S1. i) 9-Aminomethylanthracene (**3**), Et_3N, $CHCl_3$, 25 ºC, 96h, 40%; ii) 1,8-bis(dimethylamino)naphthalene (proton sponge), $CHCl_3$, 60 ºC, 72h, 79%; iii) $N_2H_4{\cdot}H_2O$, EtOH, reflux, 5 h, 95%; iv) $Ph_2CHCH_2O(CH_2)_{11}NH_2$ (**5**), Et_3N, $CHCl_3$, 65 ºC, 48h, 58%; iii) 5-nitroisophthaloyl dichloride, *p*-xylylenediamine, Et_3N, $CHCl_3$, 25 ºC, 4h (x2), 41%.

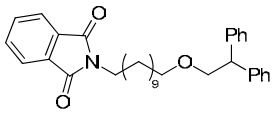
Phenyl 2-(anthracen-9-ylmethylcarbamoyl)hydrazinecarboxylate, 4



To a solution of 9-anthracenylmethylamine[1] (**3**) (1.81 g, 8.71 mmol) in chloroform (200 mL) was added diphenyl hydrazodicarboxylate[2] (**2**) (7.45 g, 26.13 mmol) followed by triethylamine (1.8 mL, 13.06 mmol). The reaction mixture was stirred for 96 h. at room temperature. During this time a white precipitated was formed in the reaction mixture which was filtered through a fritted glass filter. The resulting solution was concentrated *in vacuo* to one-half of its initial volume and sequentially washed with water (2 x 100 mL), 1 M NaOH solution (2 x 100 mL) and brine (2 x 100

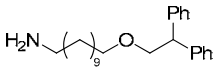
mL). The organic phase was dried with anhydrous $MgSO_4$, and concentrated to afford a crude material which was purified by column chromatography on silica gel eluting with a $CHCl_3$/MeOH (97/3) mixture as eluent to give the title product as a yellow solid (**4**, 1.24 g, 37%). M.p. 117-119 ºC; ^{1}H NMR (400 MHz, d_6-DMSO): δ = 9.37 (s, 1H, NH), 8.60 (s, 1H, H_m), 8.44 (d, J = 4.2 Hz, 2H, H_o), 8.13 (d, J = 6 Hz, 2H, H_n), 7.89 (s, 1H, NH); 7.74-7.08 (m, 9H, Ph+H_p+H_q), 6,9 (s, 1H, NH), 5.29 (d, J =10,4 Hz, 2H, H_a), HRMS (ESI) calcd for $C_{23}H_{20}N_3O_3$ $[M + H]^+$ 386.1426, found 386.1500.

_N_-(11-(2,2-diphenylethoxy)undecyl)phthalimide, S2



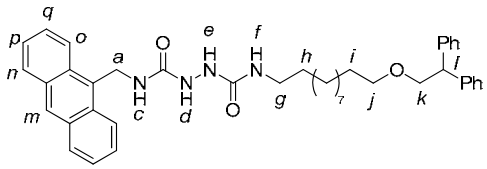
To a solution of 2,2-diphenylethanol (3.60 g, 18.16 mmol) in chloroform (30 mL) under nitrogen atmosphere was added 1,8-bis(dimethylamino)naphthalene (proton sponge, 10.37 g, 48.4 mmol). To the previous mixture was added dropwise a solution of the triflate[3] **S1** (5.43 g, 12.1 mmol) in chloroform (30 mL) and the resulting yellow solution was refluxed under nitrogen for 78 h. During this time the reaction turned dark orange/brown and a precipitate was formed. After cooling, the precipitate was removed by filtration and the filtrate was washed with 1M HCl solution (2 x 75 mL) and brine (1 x 90 mL), and dried with anhydrous $MgSO_4$. After removal of the solvent, the residue was purified by silica gel chromatography eluting with 1:9 AcOEt/hexanes to furnish compound **S2** as a colourless oil (4.75 g, 79%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.89-7.68 (m, 4H, Phth), 7.33-7.18 (m, 10H, Ph), 4.30 (t, J = 7.2 Hz, 1H, CH), 3.96 (d, J = 7.2 Hz, 2H, CHCH_2), 3.70 (dd, J = 7.2, 6.4 Hz, 2H, CH_2), 3.46 (t, J = 6.6 Hz, 2H, CH_2), 1.69 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 1.35-1.23 (m, 14H, alkyl chain); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.40 (CO), 142.40 (*q*), 133.76, 132.16 (*q*), 128.31, 128.27, 126.29, 123.08, 73.96, 71.16, 53.37, 50.99 (CH), 38.03, 29.45, 29.42 (x2), 29.30, 29.13, 28.55, 26.82, 26.02; HRMS (ESI) calcd for $C_{33}H_{40}NO_3$ $[M + H]^+$ 498.30082, found 498.29910.

11-(2,2-diphenylethoxy)undecylamine, 5



To a solution of the phthalimide **S2** (1.99 g, 3.99 mmol) in EtOH (75 mL) was added N_2H_4 H_2O (2 mL, 40.00 mmol) in one go. The mixture was stirred at reflux temperature for 4 h. After cooling to room temperature, the insoluble phthalhydrazide was separated by filtration, and then the filtrate was concentrated *in vacuo* to provide off-white solid. The solid was triturated twice with $CHCl_3$ (200 mL) and filtered. The filtrate was dried over anhydrous Na_2SO_4. After filtration the solution was concentrated under reduced pressure to afford the amine **5** as a colorless oil (1.39 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31-7.18 (m, 10H, Ph), 4.29 (t, *J* = 7.3 Hz, 1H, CH), 3.95 (d, *J* = 7.3 Hz, 2H, CHC*H*$_2$), 3.42 (t, *J* = 6.6 Hz, 2H, CH_2), 2.69 (dd, *J* = 7.4, 6.7 Hz, 2H, CH_2), 1.61 (br s, 2H, NH_2), 1.53 (m, 2H, CH_2), 1.45 (m, 2H, CH_2), 1.29-1.24 (m, 14H, alkyl chain); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 142.35 (*q*), 128.28, 128.26, 126.29, 73.93, 71.15, 50.95 (CH), 42.14, 33.65, 29.55, 29.50, 29.46, 29.43 (x2), 29.32, 26.83, 26.01; HRMS (ESI) calcd for $C_{25}H_{38}NO$ $[M + H]^+$ 368.29534, found 368.29410.

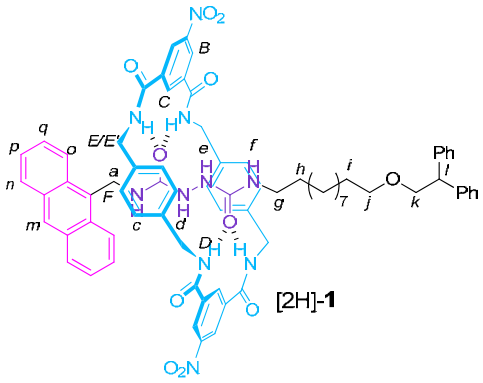
N^1-(9-anthracenylmethyl)-N^2-11-(2,2-diphenylethoxy)undecyl)-1,2-hydrazinedicarboxamide, [2H]-**6**



To a solution of the hydrazodicarboxylate **4** (2.5 g, 6.53 mmol) in chloroform (200 mL) was added the amine **5** (2.4 g, 6.53 mmol) followed by triethylamine (1.3 mL, 9.79 mmol). The reaction mixture was stirred for 48 hours at reflux temperature under nitrogen atmosphere. Then the resulting solution was sequentially washed with 1 M NaOH solution (2 x 150 mL) and brine (2 x 150 mL). The organic phase was dried with anhydrous $MgSO_4$, and concentrated to afford a crude material which was purified by column chromatography on silica gel eluting with a $CHCl_3$/MeOH (97/3) mixture as eluent to give [2H]-**6** as a yellow solid (2.49 g, 58%). M.p. 143-145 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.35 (s, 1H, CH_m), 8.20 (d, 2H, *J* = 8.6 Hz, CH_o), 7.92 (d, 2H, *J* = 8.3 Hz, CH_n), 7.52-7.35 (m, 4H, CH_p + CH_q), 7.34-7.10 (m, 10H, Ph), 7.12 – 6.94 (br s, 1H, NH_e), 6.73 (br s, 1H, NH_d), 5.95 (br s, 1H, NH_c), 5.65 (br s, 1H, NH_f), 5.22 (br s, 2H,

CH_a), 4.26 (t, 1H, J = 7.2 Hz, CH_l), 3.92 (d, 2H, J = 7.3 Hz, CH_k), 3.42 (t, 2H, J = 6.6 Hz, CH_j), 2.91 (t, 2H, J = 7.0 Hz, CH_g), 1.64-1.39 (m, 2H, CH_i), 1.29-0.94 (m, 18H, alkyl chain); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.76 (x2 CO), 142.56 (C_{Ar}), 131.57 (C_{Ar}), 130.46 (C_{Ar}), 129.30 (C_{Ar}), 128.48, 128.46, 128.31. 126.71, 126.49 (x2), 125.21, 123.90, 74.15 (CH_j), 71.37 (CH_k), 51.17 (CH_l), 40.09, 36.53, 29.87, 29.68, 29.65 (x2), 29.63, 29.54, 29.34, 26.82, 26.22 ppm; HRMS (ESI) calcd for $C_{42}H_{51}N_4O_3$ $[M + H]^+$ 659.39612, found 659.39720.

1,2-Hydrazodicarboxamide [2]rotaxane, [2H]-**1**



[2H]-**1**

The thread [2H]-**6** (0.58 g, 0.88 mmol) and Et_3N (3 mL, 21.12 mmol) in anhydrous $CHCl_3$ (300 mL) were stirred vigorously whilst solutions of *p*-xylylene diamine (0.96 g, 7.04 mmol) in anhydrous $CHCl_3$ (40 mL) and 5-nitroisophthaloyl dichloride[4] (1.75 g, 7.04 mmol) in anhydrous $CHCl_3$ (40 mL) were simultaneously added over a period of 4 h using motor-driven syringe pumps. After a further 4 h the resulting suspension was filtered through a Celite pad and then the solvent removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel eluting with a $CHCl_3$/MeOH (97/3) mixture as eluent to give the rotaxane [2H]-**1** as a bright yellow solid (0.46 g, 41%). M.p. decomp. > 190 ºC; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.75 (d, J = 1.3 Hz, 4H, CH_B), 8.51 (s, 2H, CH_C), 8.37 (s, 1H, CH_m), 8.27 (d, J = 8.2 Hz, 2H, CH_o), 8.06 (br s, 4H, NH_D) 7.95 (d, J = 8.4 Hz, 2H, CH_n), 7.42-7.41 (m, 4H, CH_q + CH_p), 7.35-7.20 (m, 10H, Ph), 7.18 (s, 8H, CH_F), 6.17 (br s, 1H, NH_c), 5.61 (br s, 1H, NH_d), 5.40 (s, 1H, NH_e), 5.18 (d, J = 4.8 Hz, 2H, CH_a), 5.07 (br s, 1H, NH_f), 4.50 (dd, J = 13.8, 5.2 Hz, 4H, CH_E), 4.2 (t, J =7.3 Hz 1H, CH_l), 4.26 (dd, J = 10.9, 4.1 Hz, 4H, $CH_{E'}$), 4.00 (d, J = 7.4 Hz, 2H, CH_k), 3.56 (t, J = 6.7 Hz, 2H, CH_j),

2.66 (m, 2H, CH_g), 1.50-1.48 (s, 4H, CH_i+CH_h), 1.13-0.93 (m, 14H, alkyl chain).[13]C NMR (100 MHz, $CDCl_3$): δ = 164.97 ($CO_{macrocycle}$), 158.12 (2 x CO), 148.29 (*q*), 142.34 (*q*), 136.98 (*q*), 136.00 (*q*), 131.73 (*q*), 131.51, 130.14 (*q*), 129.39, 128.93, 128.50, 128.40, 128.26, 126.55, 126.43, 125.31, 125.13, 124.04, 74.11, 71.38, 51.13 (CH), 44.83, 40.10, 36.51, 29.92, 29.52, 29.49, 29.43, 29.36, 29.22, 28.27, 26.79, 26.07 ppm; HRMS (ESI) calcd for $C_{74}H_{78}N_{10}O_{11}$ $[M + H]^+$ 1281.56950, found 1281.57452.

3. Additional ^{1}H NMR spectra of the hydrazinedicarboxamide, [2H]-6

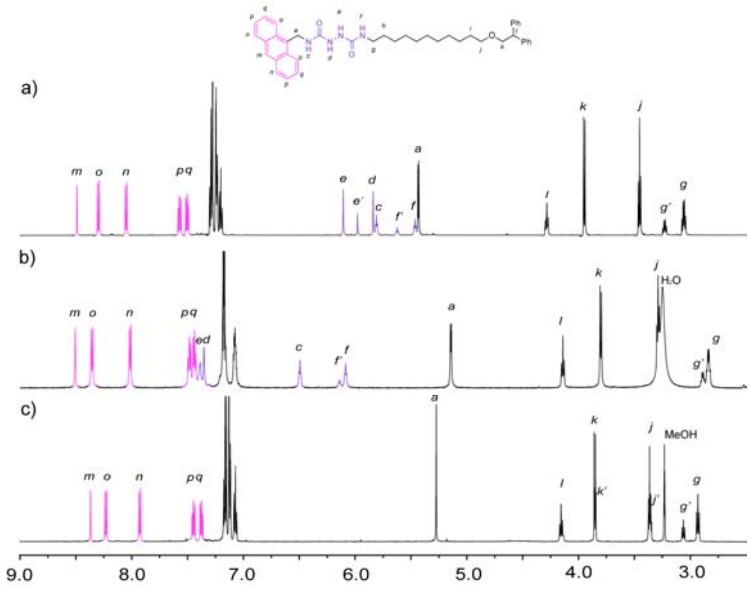


Figure S1. ^{1}H NMR spectra (600 MHz, 298 K) of the hydrazinedicarboxamide, [2H]-**6** in (a) $CDCl_3$, (b) $(CD_3)_2SO$, and c) CD_3OD.

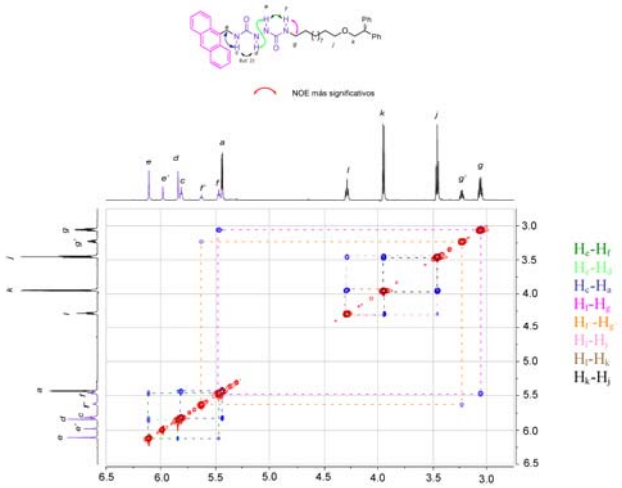


Figure S2. Selected region of 2D NOESY spectra (600 MHz, $CDCl_3$, 298 K) of the hydrazinedicarboxamide, [2H]-**6**.

4. Comparison of ^{1}H-^{15}N HSQC spectra of the thread [2H]-6 and the rotaxane [2H]-1

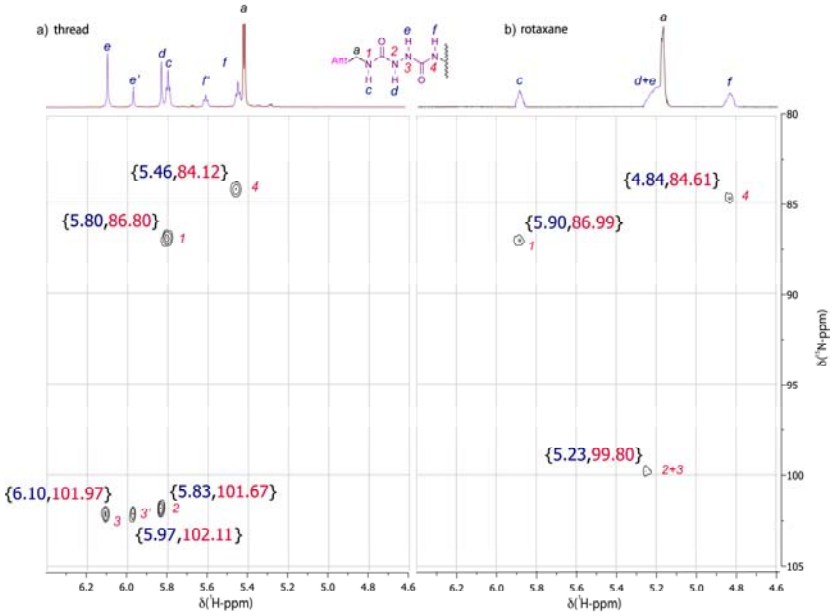
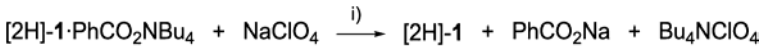


Figure S3. Selected region of the ^{1}H-^{15}N HSQC spectra (600 MHz, $CDCl_3$, 298 K) of (a) the hydrazinedicarboxamide [2H]-**6** and (b) the rotaxane [2H]-**1**.

Note: the chemical shift of corresponding nitrogen of the four equivalent amide groups of the macrocyclic tetralactame is 116.62 ppm, which is within the typical range for this type of functional group.

5. Recovering of [2]rotaxane, [2H]-1 from its complex with tetrabutyl ammonium benzoate

Method A. Ionic exchange

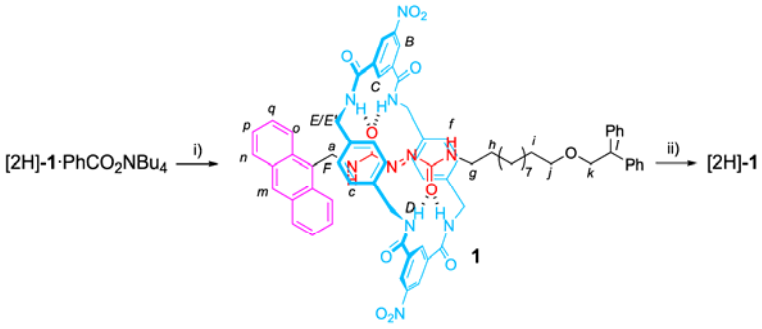


Scheme S2. i) $CDCl_3$, 25 ºC, 48h, quantitative

To a solution of the complex [2H]-**1**·$PhCO_2NBu_4$ in $CDCl_3$ (3 mL) was added a gentle excess of $NaClO_4$ (5 equiv). The resulting mixture was stirred was stirred for 48

hours at room temperature under nitrogen atmosphere. Afterwards the insoluble salts were separated by filtration, and then the filtrate was analyzed by ^{1}H NMR for obtaining an identical spectrum to that showed by an analytically pure sample of the rotaxane [2H]-**1.**

Method B. Oxidation/reduction protocol



Scheme S3. i) *N*-bromosuccinimide, pyridine, -30 ºC, CH_2Cl_2, 30 min, 99%; ii) N_2H_4, $CHCl_3$, 25 ºC, 30 min, 93%.

To a solution of the complex [2H]-**1**·$PhCO_2NBu_4$ in dichloromethane (5 mL) were added pyridine (1.1 equiv) and *N*-bromosuccinimide (1 equiv) at -30º C. The resulting orange solution was stirred at room temperature for 30 min. Then the reaction mixture was diluted with dichloromethane (10 mL) and washed with water (2 x15 mL) and brine (2 x 20 mL). The organic phase was dried with anhydrous $MgSO_4$, and concentrated *in vacuo* to quantitatively yield the azo rotaxane **1** as an orange solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.78 (d, *J* = 0.9 Hz, 4H, CH_B), 8.53 (s, 1H, CH_m), 8.37 (s, 2H, CH_C), 8.24 (d, *J* = 8.9 Hz, 2H, CH_o), 8.08 (d, *J* = 8.4 Hz, 2H, CH_n), 7.49 (t, *J* = 6 Hz, 2H, CH_p), 7.33 (t, *J* = 3 Hz, 2H, CH_q), 7.28–7.11 (m, 14H, 10Ph + 4CH_D), 6.58 (s, 8H, CH_F), 5.44 (d, *J* = 5.6 Hz, 2H, CH_a), 5.34 (t, *J* = 5.7 Hz, 2H, NH_f+NH_c), 4.31 (dd, *J* = 14.3, 5.5 Hz, 4H, CH_E), 4.26 (t, *J* = 7.5 Hz, 1H, CH_l) 4.07 (dd, *J* = 14.3, 4.9 Hz, 4H, $CH_{E'}$), 3.93 (d, *J* = 7.3 Hz, 2H, CH_k), 3.41 (t, *J* = 6.6 Hz, 2H, CH_j), 3.22 (dd, *J* = 13.5, 6.7 Hz, 2H, CH_g), 2.22 (t, *J* = 9 Hz, 2H, CH_i), 2.03-1.97 (m, 2H, CH_h), 1.45-1.10 (m, 14H, alkyl chain). To a degassed solution of this solid in chloroform (5 mL) was added hydrazine monohydrate (1.5 equiv) in one go. The orange solution was transformed to a colourless solution in less than 15 min. The reaction mixture was dried with a high vacuum pump to afford a colorless solid which was analyzed by ^{1}H NMR for obtaining an indistinguishable spectrum to that showed by an analytically pure sample of the

rotaxane [2H]-**1**. The ^{1}H NMR spectra ($CDCl_3$, 300 MHz, 298 K) of rotaxane **1** and rotaxane [2H]-**1** are shown in Figure S4.

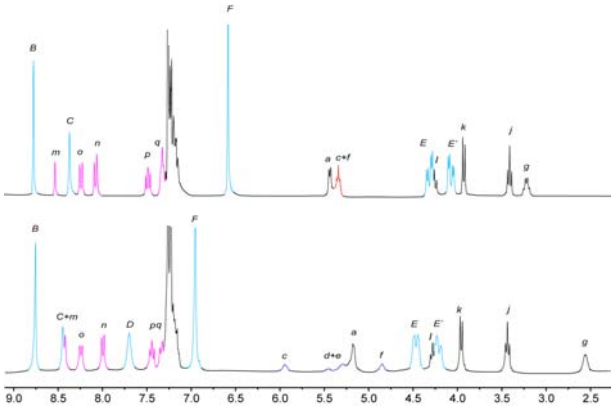


Figure S4. ^{1}H NMR spectra (300 MHz, $CDCl_3$, 298 K) of (a) azodicarboxamide rotaxane **1**, (b) hydrazodicarboxamide rotaxane [2H]-**1**. The assignments correspond to the lettering shown in Scheme S1 and S3.

6. Continuous variation method using the hydrazo[2]rotaxane [2H]-1 and tetrabutyl ammonium benzoate

A 1 mM solution of the rotaxane [2H]-**1** and other solution of tetrabutylammonium benzoate of identical concentration were prepared. Then a set of solutions of [2H]-**1** were prepared in 10 NMR tubes (5 mm) by mixing different volumes of both solutions so that the volume of all final solutions was 0.5 mL, being the molar fraction of rotaxane [2H]-**1**: 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1. Afterwards, their corresponding ^{1}H NMR spectrum (298K, 400 MHz) was recorded and the proton chemical shift was plotted against the molar fraction of rotaxane (Figure 5b) to estimate the stoichiometry of the complex.

7. Titration of the hydrazo[2]rotaxane [2H]-1 with tetrabutyl ammonium benzoate by fluorescence spectroscopy

Due to the inherent limitations of the NMR method for the accurate determination of association constants higher than 10^4 M^{-1} fluorescence titration was carried out. Fluorescence experiments were conducted on a Cary Eclipse spectrophotometer, at 298 K, in chloroform. The sample volume was 3 mL. The titration between the rotaxane and

the ammonium salt in chloroform was carried out by adding small aliquots of a more concentrated solution of benzoate. The concentration of the fluorescent rotaxane (20×10^{-6} M) was maintained constant all along the titration. The association constants were calculated representing the intensity of fluorescence emission (at λ_{exc} = 365 nm) *versus* concentration of tetrabutylammonium benzoate in the program SPECFIT/32 using a 1:1 binding model. The variation in the emission spectra of [2H]-**1** in chloroform with increasing concentrations of BzO^- (λ_{exc} = 365 nm) is displayed in Figure 5a. In the Figure **S5** is showed the speciation distribution of the concentrations of the hydrazodicarboxamide rotaxane [2H]-**1** (blue) and the complex [2H]-**1**·$PhCO_2NBu_4$ (red).

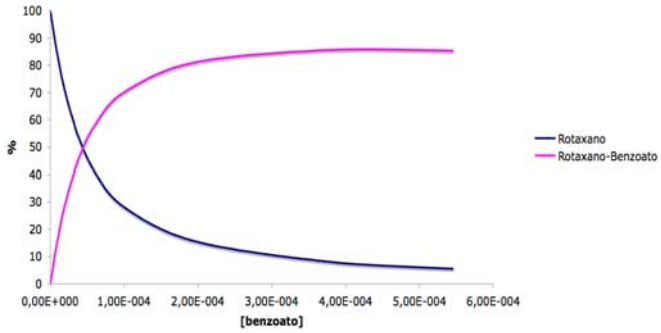
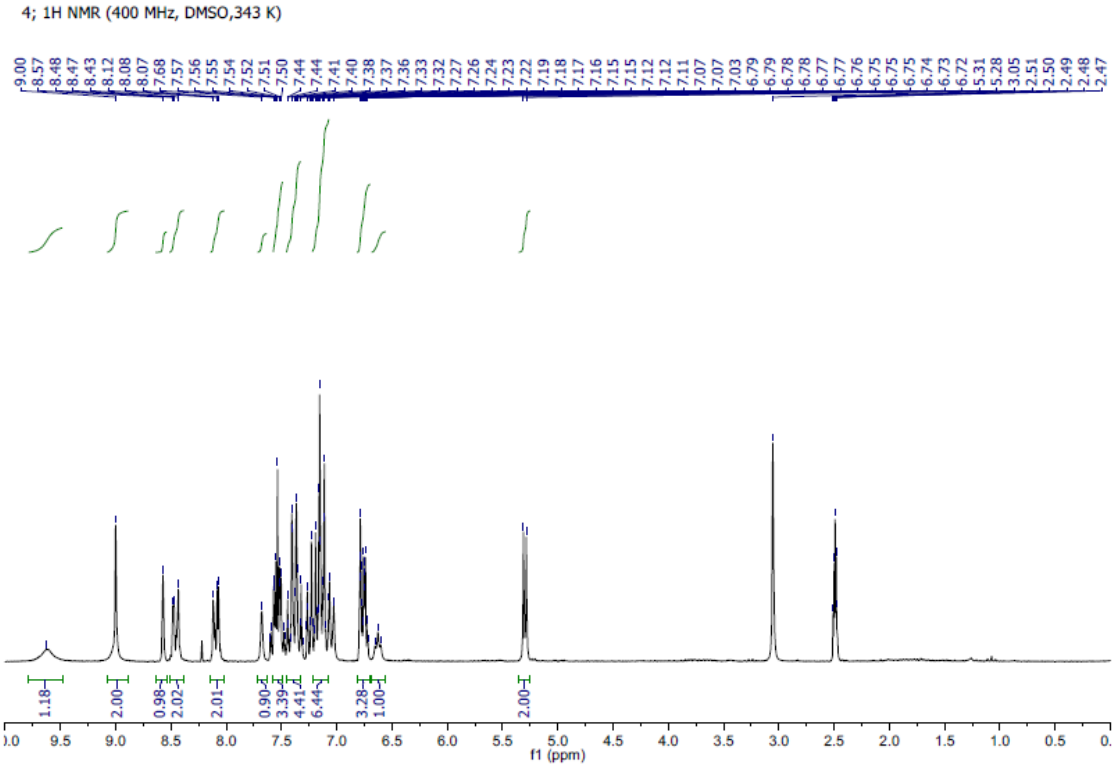
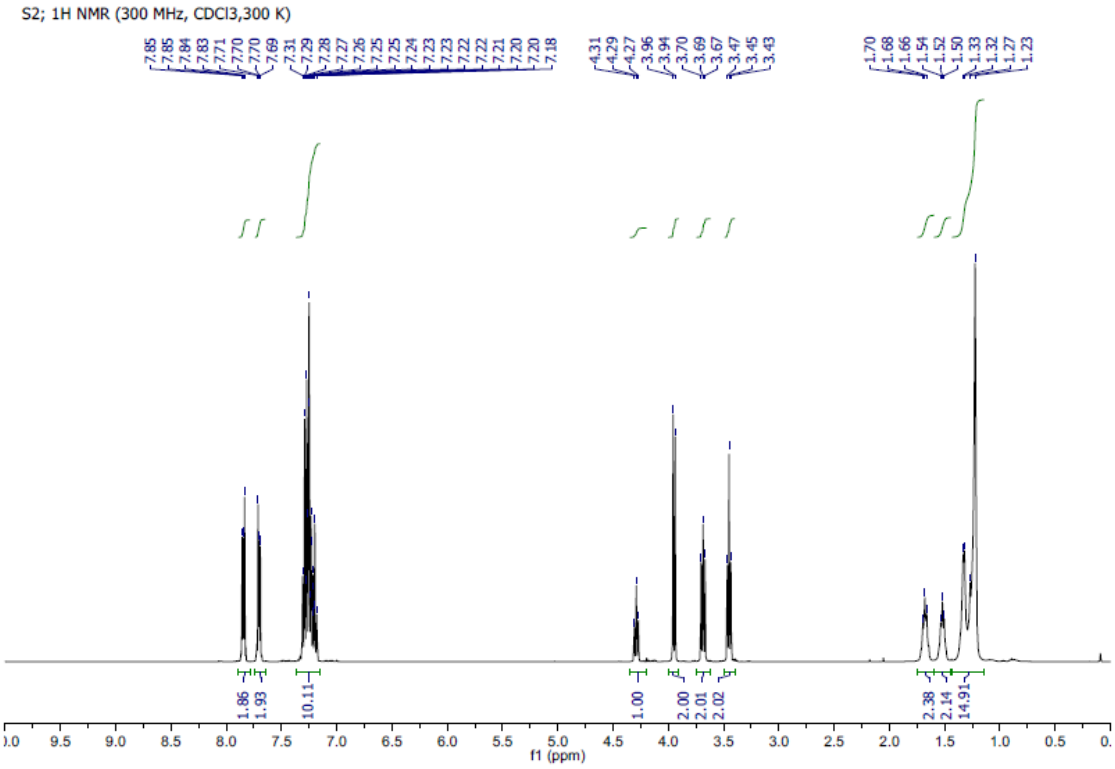
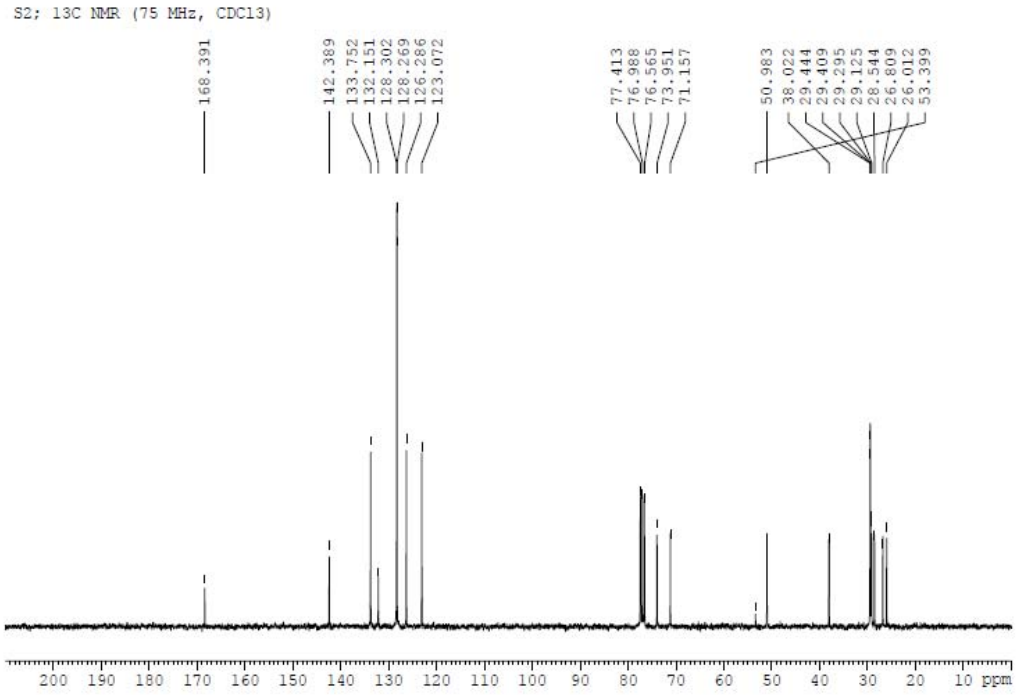


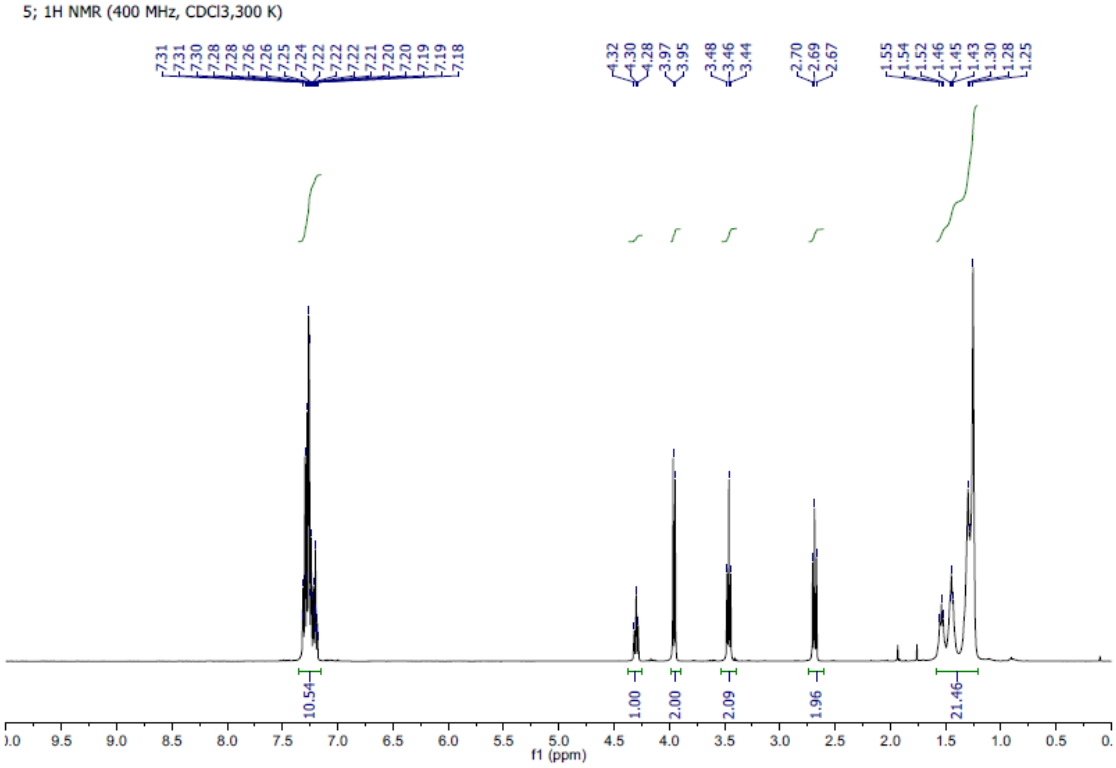
Figure S5. Speciation distribution diagram for the binding of BzO^- to [2H]-**1**.

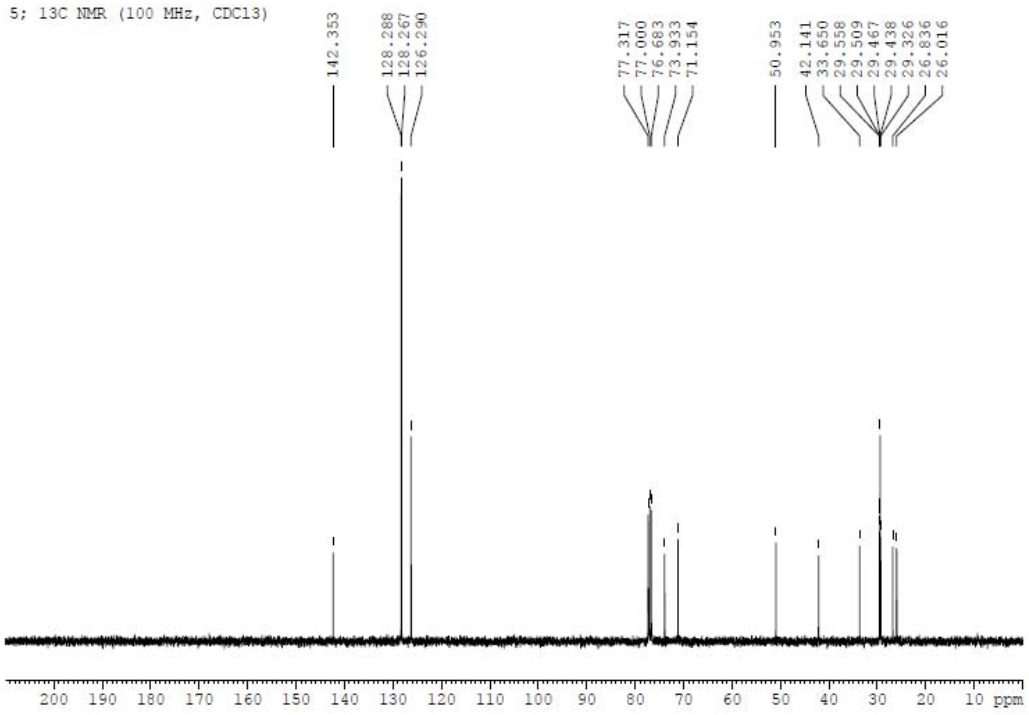
8. ^{1}H and ^{13}C NMR spectra of synthesized compounds

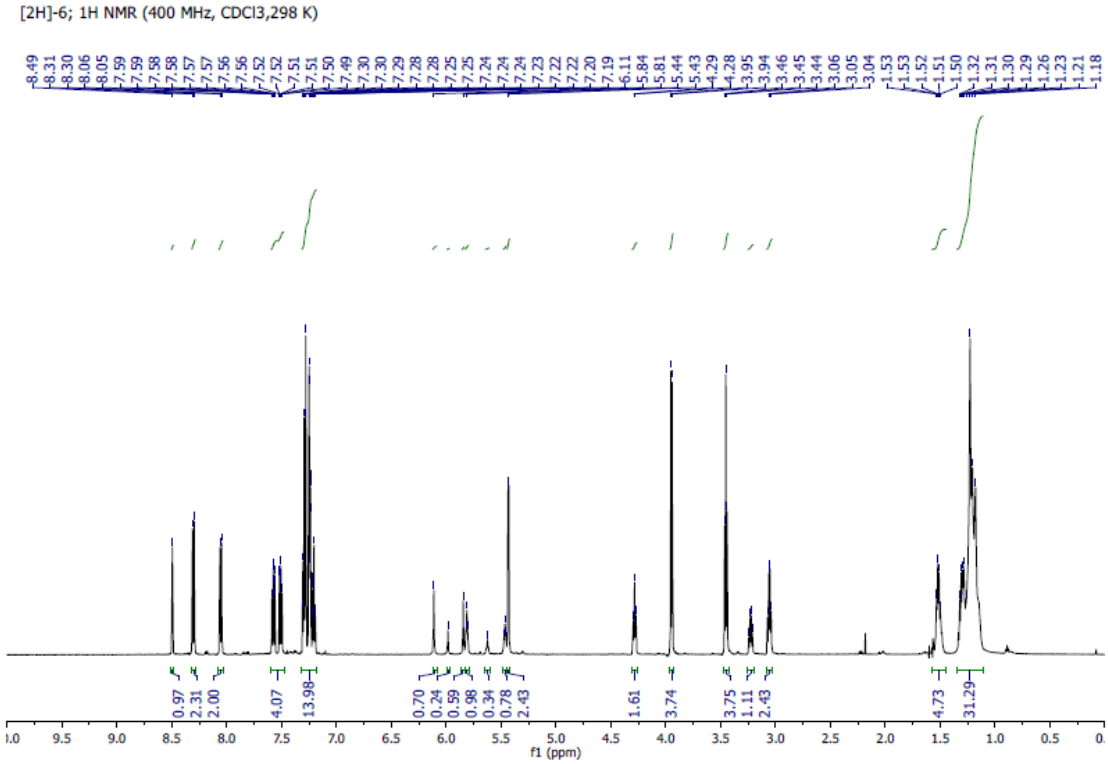


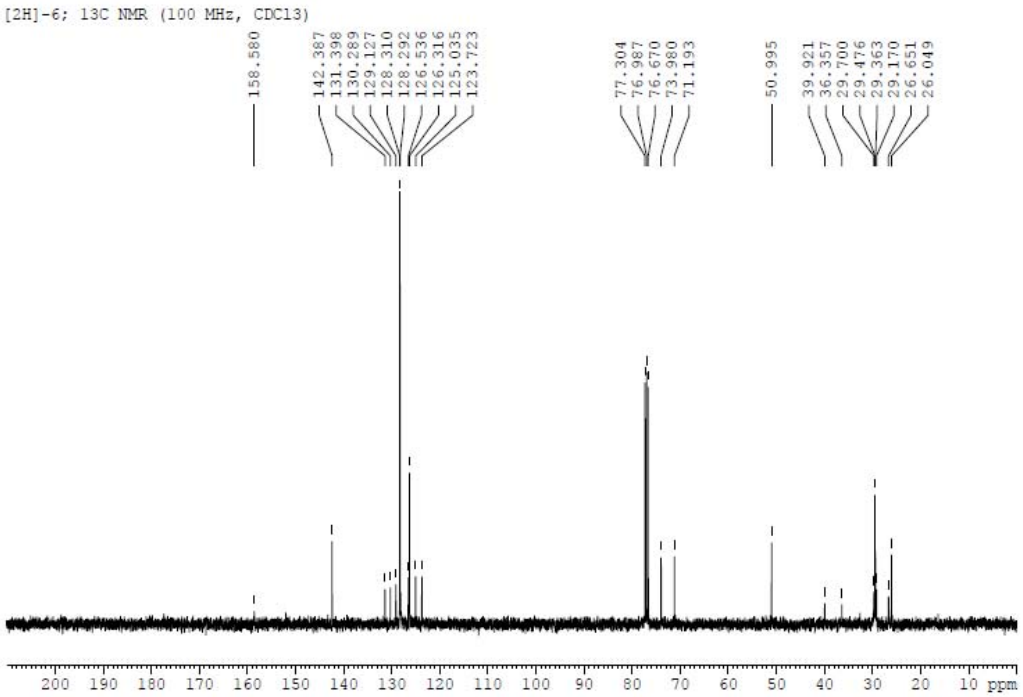


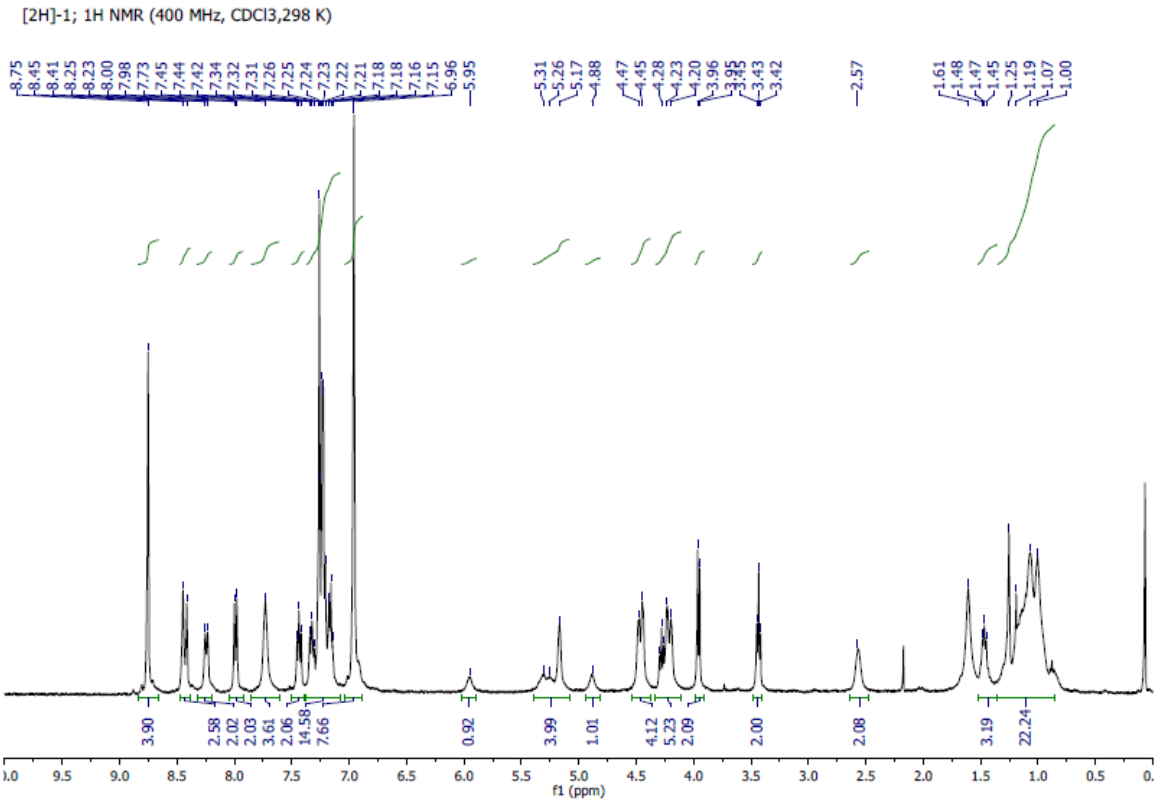

S2; 13C NMR (75 MHz, CDCl3)
168.391
142.389
133.752
132.151
128.302
128.269
126.286
123.072
77.413
76.988
76.565
73.951
71.157
50.983
38.022
29.444
29.409
29.295
29.125
28.544
26.809
26.012
53.399
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

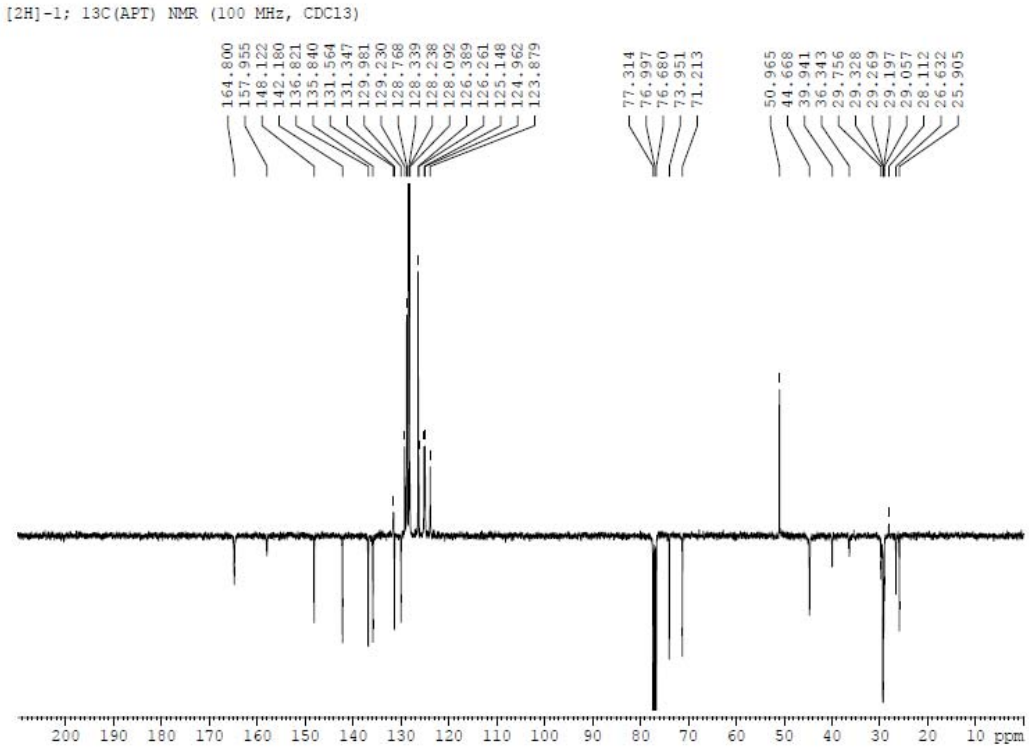

5; 1H NMR (400 MHz, CDCl3,300 K)
7.31
7.31
7.30
7.28
7.28
7.26
7.26
7.25
7.24
7.22
7.22
7.22
7.21
7.20
7.20
7.19
7.19
7.18
4.32
4.30
4.28
3.97
3.95
3.48
3.46
3.44
2.70
2.69
2.67
1.55
1.54
1.52
1.46
1.45
1.43
1.30
1.28
1.25
10.54
1.00
2.00
2.09
1.96
21.46
f1 (ppm)











9. References

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