Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2018

Electronic Supplementary Information

Intramolecularly Enhanced Molecular Tweezers with Unusually Strong Binding for Aromatic Guests in Unfavorable Solvents

Xiaoyu Xing and Yan Zhao*

Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111, USA

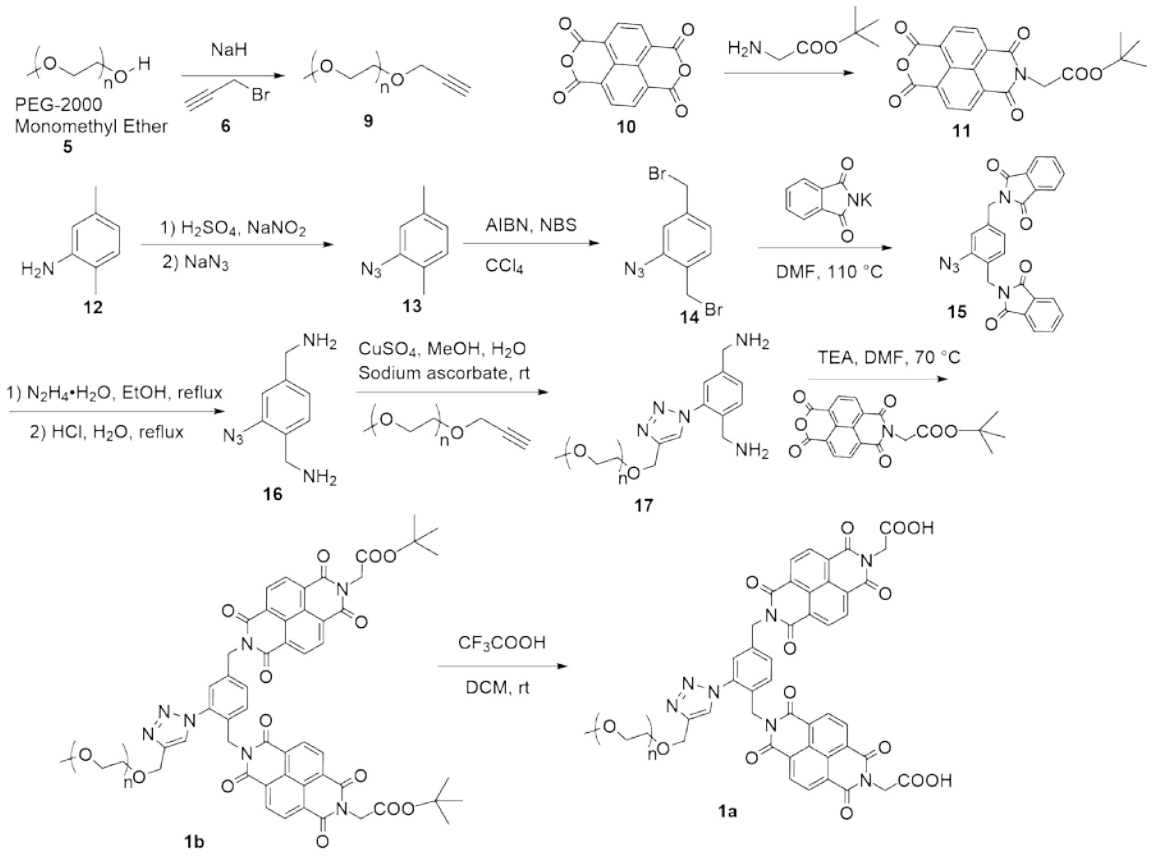
Contents

General Method 3
Scheme S1 4
Syntheses 4
Solvent Study UV-Vis Spectroscopy and Data Analysis Method 7
Titration by UV-Vis Spectroscopy and Data Analysis Method 8
Figure S1. 10
Figure S2. 11
Figure S3. 12
Figure S4. 13
Figure S5. 14
Figure S6. 15
Figure S7. 16
Figure S8. 17
Figure S9. 18
Figure S10. 19
Figure S11. 20
Figure S12. 21
Figure S13. 22
Figure S14. 23
Figure S15. 24
Figure S16. 25
Figure S17. 25
Figure S18. 26
Figure S19. 26
Table S1. 27
Table S2. 28
^{1}H and ^{13}C NMR spectra 29

General Method

For spectroscopic purpose, methanol, and dichloromethane (DCM) were of HPLC grade. All other reagents and solvents were of ACS-certified grade or higher, and were used as received from commercial suppliers. Routine ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400, on a Bruker AV II 600 or on a Varian VXR-400 spectrometer. Variable temperature ^{1}H NMR spectra, nuclear Overhauser effect spectroscopy (NOESY), and diffusion ordered spectroscopy (DOSY) were recorded on a Bruker AV II 600 spectrometer. UV-vis spectra were recorded at ambient temperature on a Cary 100 Bio UV-visible spectrophotometer. Dynamic light scattering (DLS) data were recorded at 25 °C using PDDLS/ CoolBatch 90T with PD2000DLS instrument.

Scheme S1



Syntheses

Syntheses of compounds **9**[1], **11**[2], **13**[3]and **14**[4]were previously reported.

Compound 15. Compound **14** (2.29 g, 7.52 mmol) and potassium phthalimide (3.06 g, 16.6 mmol) were dissolved in DMF (50 mL). After the reaction mixture was stirred at

[1] Zill, A. T.; Licha, K.; Haag, R.; Zimmerman, S. C. *New J. Chem.* **2012**, *36*, 419.
[2] Giichi, S; Moriyasu, M; Kuniyoshi, N; etc. Compounds Against Helicobacter Activity. Eur. Pat. WO 03095453, Nov. 2003.
[3] Sundberg, R. J.; Suter, S. R.; Brenner, M. *J. Am. Chem. Soc.* **1972**, *94*, 513.
[4] Boyle, M. M.; Gassensmith, J. J.; Whalley, A. C.; Forgan, R. S.; Smaldone, R. A.; Hartlieb, K. J.; Blackburn, A. K.; Sauvage, J.-P.; Stoddart, J. F. *Chem.–Eur. J.* **2012**, *18*, 10312.

110 °C overnight, TLC showed completion of the reaction. The mixture was cooled down to room temperature. The precipitate was filtered and washed with cold dichloromethane (3 × 15 mL) to give a white powder (2.36 g, 72%). The product was used in the next step without further purification.

Compound 16. Compound **15** (355 mg, 0.812 mmol) and hydrazine monohydrate (101 mg, 2.01 mmol) were combined in ethanol (30 mL). The reaction mixture was heated to reflux and stirred overnight. After TLC showed completion of the reaction, the mixture was concentrated by rotary evaporation. Aqueous hydrochloric acid (3M, 50 mL) was added and the reaction mixture was heated to reflux overnight. After the reaction mixture was cooled down to room temperature, sodium hydroxide pellets were added slowly until pH = 12. The mixture was extracted with dichloromethane (3 × 30 mL). The combined organic layer was washed with brine (30 mL) and dried with $MgSO_4$. The solvent was removed by rotary evaporation to give a green oil (131 mg, 91%). ^{1}H NMR (600 MHz, $CDCl_3$, δ) 7.24 (d, *J* = 7.7 Hz, 1H), 7.14 (s, 1H), 7.05 (d, *J* = 7.2 Hz, 1H), 3.89 (s, 2H), 3.76 (s, 2H). ^{13}C NMR (151 MHz, $CDCl_3$, δ) 143.9, 138.1, 133.2, 129.5, 123.8, 117.0, 46.1, 42.7. ESI-MS (*m/z*): $[M+H]^+$ cacld for $C_8H_{11}N_5$, 178.1087; found, 178.1090.

Compound 17. To a solution of **16** (91.8 mg, 0.518 mmol) in a1:1 water/methanol mixture (20 mL), copper sulfate hydrate (11.0 mg, 0.0441 mmol), sodium ascorbate (103 mg, 0.520 mmol), and **6** (1.06 g, 0.520 mmol) were added. After being stirred at room temperature for 12 h, the reaction mixture was concentrated by rotary evaporation. The resulting solution was diluted with water (30 mL) and extracted with dichloromethane (3 × 40 mL). The combined organic layer was washed with brine (30 mL). The dichloromethane solution was filtered through a layer of celite and the celite was washed with methanol (3 × 50 mL).

The organic solvents were removed by rotary evaporation to give a yellow-green solid, which was used in the next step without further purification.

Compound 1b. To a solution of **17** (424 mg, 0.191 mmol) and **11** (219 mg, 0.574 mmol) in DMF (20 mL), triethylamine (508 mg, 5.02 mmol) was added. After being stirred at 70 °C overnight, the reaction mixture was concentrated by rotary evaporation. The residue was purified by flash column chromatography over silica gel with 10:1 dichloromethane/methanol as the eluent to give a brown solid (132 mg, 27%). ^{1}H NMR (600 MHz, $CDCl_3$, δ) 8.75 (dd, *J* = 9.2, 4.8 Hz, 6H), 8.69 (d, *J* = 7.6 Hz, 2H), 8.08 (s, 1H), 7.65 (d, *J* = 8.3 Hz, 1H), 7.56 (s, 1H), 7.38 (d, *J* = 8.1 Hz, 1H), 5.39 (s, 2H), 5.27 (s, 2H), 4.84 (s, 4H), 4.80 (s, 2H), 3.80-3.52 (series of m, multiple H), 3.37 (s, 3H), 1.49 (s, 18H). ^{13}C NMR (151 MHz, $CDCl_3$, δ) 166.5, 166.4, 162.7, 162.5, 162.3, 162.3, 145.1, 137.1, 136.1, 132.2, 131.29, 131.27, 131.2, 128.7, 127.0, 126.85, 126.82, 126.7, 126.51, 126.46, 126.44, 126.38, 124.9, 82.7, 77.4, 71.9, 70.53, 70.47, 69.9, 64.6, 59.0, 55.9, 50.6, 43.0, 42.3, 39.7, 28.0. ESI-MS (*m/z*): $[M+3H]^{3+}$ multiple peaks centered at 978.7941 and separated by 14.6775 from the PEG chain.

Compound 1a. To a solution of **1b** (29.6 mg, 0.0101 mmol) in dichloromethane (5 mL), trifluoroacetic acid (5 mL) was added. After being stirred at room temperature overnight, the reaction mixture was concentrated by rotary evaporation. The residue was purified by flash column chromatography over silica gel with 7:1 dichloromethane/methanol as the eluent to give a brown gel (21.1 mg, 74%). ^{1}H NMR (600 MHz, $CDCl_3$, δ) 8.71 (d, *J* = 14.5 Hz, 6H), 8.65 (d, *J* = 7.5 Hz, 2H), 8.06 (s, 1H), 7.67 (d, *J* = 8.3 Hz, 1H), 7.49 (s, 1H), 7.47 (s, 1H), 5.40 (s, 2H), 5.28 (s, 2H), 4.93 (s, 4H), 4.78 (s, 2H), 4.49 (t, *J* = 4.5 Hz, 3H), 3.87-3.36 (series of m, multiple H). ^{13}C NMR (151 MHz, $CDCl_3$, δ) 168.7, 162.6, 162.4,

162.0, 144.3, 137.1, 135.2, 131.0, 130.8, 130.60, 130.56, 129.4, 127.9, 126.7, 126.5, 126.4, 126.01, 125.99, 125.6, 125.5, 125.4, 125.3, 72.3, 71.2, 69.74, 69.71, 69.67, 69.6, 69.5, 69.1, 67.5, 67.4, 63.4, 60.2, 57.9, 42.7, 41.4. ESI-MS (*m/z*): $[M+3H]^{3+}$ multiple peaks centered at 970.7478 and separated by 14.6739 from the PEG chain.

Solvent Study UV-Vis Spectroscopy and Data Analysis Method

Stock solution of **1a** (4.0 mM) and **1b** (4.0 mM) in DCM were prepared. For the solvent titration, a typical procedure is as follows. An aliquot of the stock solution was added to 2.00 mL of the appropriate solvent in a quartz cuvette. Concentration of the receptors was15 μM. The sample was gently vortexed for 30 s before the absorbance spectrum was collected at 298 K.

Literature method[5] was followed in the two-state curve fitting for the absorbance data for hosts **1a**:

$$\textbf{Unfolded} \underset{}{\overset{Keq}{\rightleftharpoons}} \textbf{Folded}$$

According to the two-state model, at any given concentration of the denaturant (i.e., MeOH), only the folded and unfolded conformations are present and their fractions are represented by f_F and f_U. Fraction of the unfolded conformation can be calculated by:

$$K_{eq} = f_F/f_U$$

$$f_F = (I-I_U)/(I_F-I_U)$$

[5] Zhao, Y.; Zhong, Z. *J. Am. Chem. Soc.* **2005**, *127*, 17894.

$$f_U = 1 - f_F$$

I is the absorbance at a certain solvent composition. I_U is the absorbance at fully unfolded state, and I_F is that at fully folded state.

The equilibrium constant (K_{eq}) and the free energy (ΔG) for the folding reaction can be calculated using:

$$\Delta G = -RT \ln K_{eq} = -RT \ln(f_F/f_U) = -RT \ln[(1 - f_U)/f_U]$$

In the two-state model, the free energies are linearly related to the concentration of denaturant and are assumed to have the same relationship to the $E_T(30)$ values of the solvent:

$$\Delta G = \Delta G_0 + m\, E_T(30)$$

Then we can obtain equations:

$$f_F = 1/(1 + 1/\exp(-(\Delta G_0 + m\, E_T(30)/RT))$$

$$I = I_U + (I_F - I_U)/(1 + 1/\exp(-(\Delta G_0 + m\, E_T(30))/RT))$$

A nonlinear least-squares fitting of the experimental data to above equation affords the two-state folding-unfolding curves and f_U.

Titration by UV-Vis Spectroscopy and Data Analysis Method

Stock solution of **1a** (4.0 mM) and **1b** (4.0 mM) in DCM were prepared. Stock solutions of the guest (**2-4**) were prepared in the appropriate solvent mixture, in which titrations would be performed. For the titrations, a typical procedure is as follows. An aliquot of the host stock solution was added to 2.00 mL solvent in a quartz cuvette. Concentration of the

receptors varies from 15 μM to 30 μM based on signal strength. The sample was gently vortexed for 30 s before its UV-vis spectrum was recorded. Aliquots of the guest was added and the spectrum was recorded after each addition. The titration was continued until saturation was reached and the total volume of the guest solution added was kept below 100 μL. The binding constant was obtained by nonlinear least squares curving fitting of the absorbance values to the 1:1 binding isotherm.[6] Titrations were repeated at least twice. The average with standard deviation as uncertainty was reported as final value.

[6] Schneider, H. J.; Yatsimirsky, A. K. *Principles and methods in supramolecular chemistry*; New York: J. Wiley, 2000; pp 137-146.

Figure S1. Photographs of 2.5 mM (left) and 8 mM (right) pyrene **2** in $CDCl_3$ at 298 K.

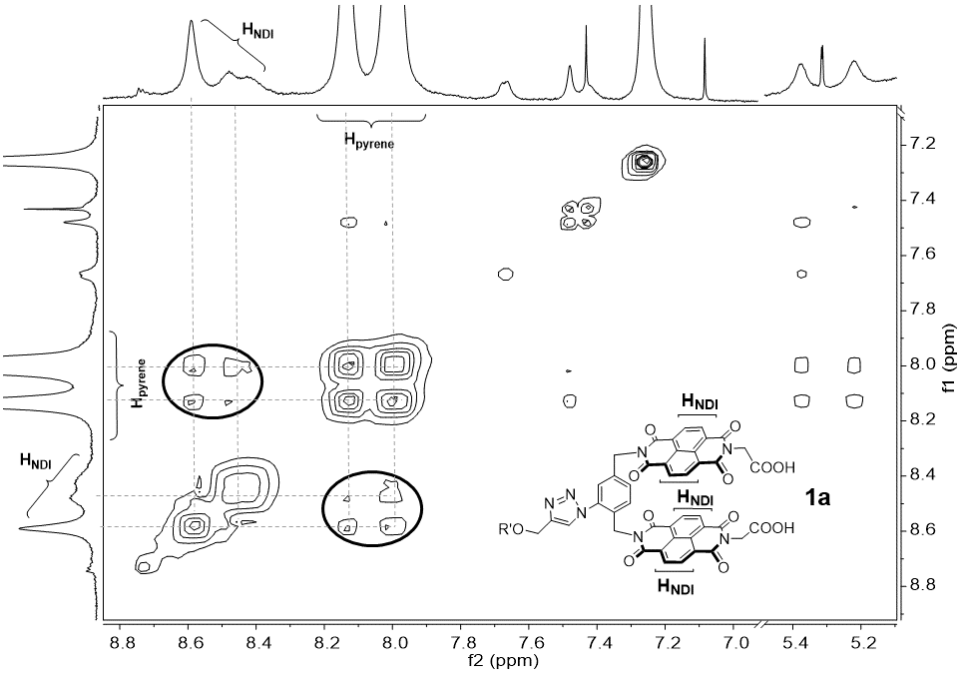


Figure S2. NOESY spectrum of 0.6 mM host **1a** with 2.5 mM pyrene in $CDCl_3$ at 253 K. Low temperature was used to enhance the binding and the cross-peaks. The NDI peaks were sharp at room temperature but became broad at 253 K, especially in the presence of pyrene (Figures S11–S12). The cross peaks in the circles were between the NDI protons of host **1a** and pyrene.

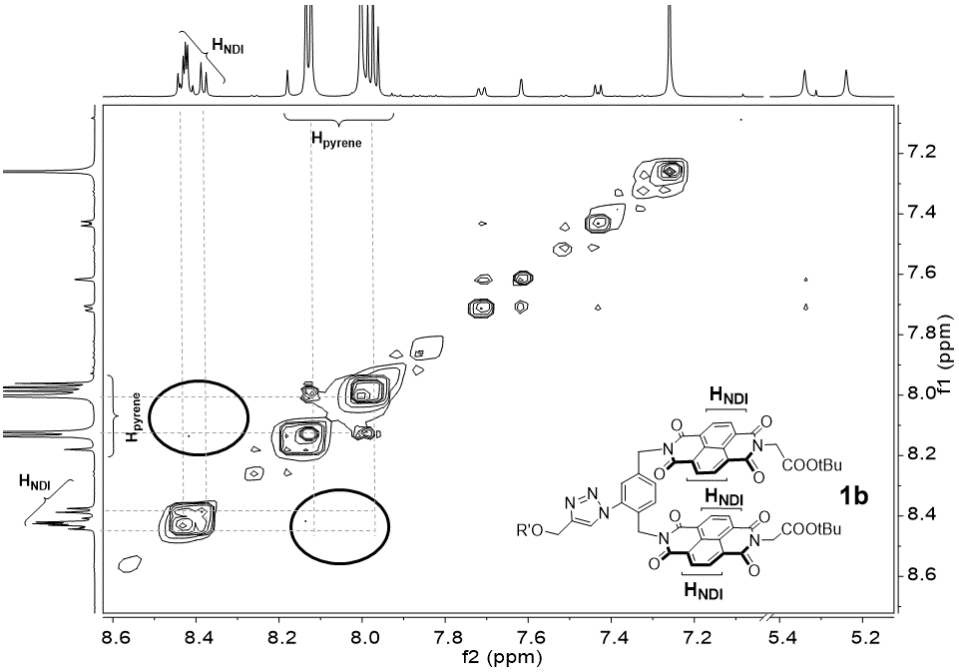


Figure S3. NOESY spectrum of 2 mM host **1b** with 8 mM pyrene in $CDCl_3$ at 253 K. Insignificant coupling was found between the NDI protons of host **1b** and pyrene.

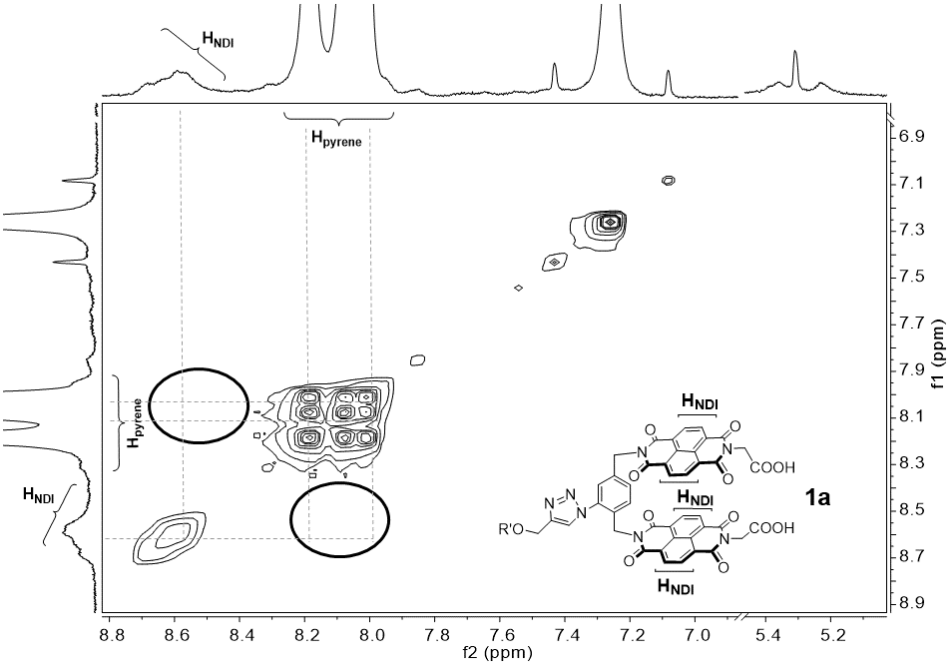


Figure S4. NOESY spectrum of 0.6 mM host **1a** with 2.5 mM pyrene and 11.4 mM ammonia in $CDCl_3$ at 253 K.

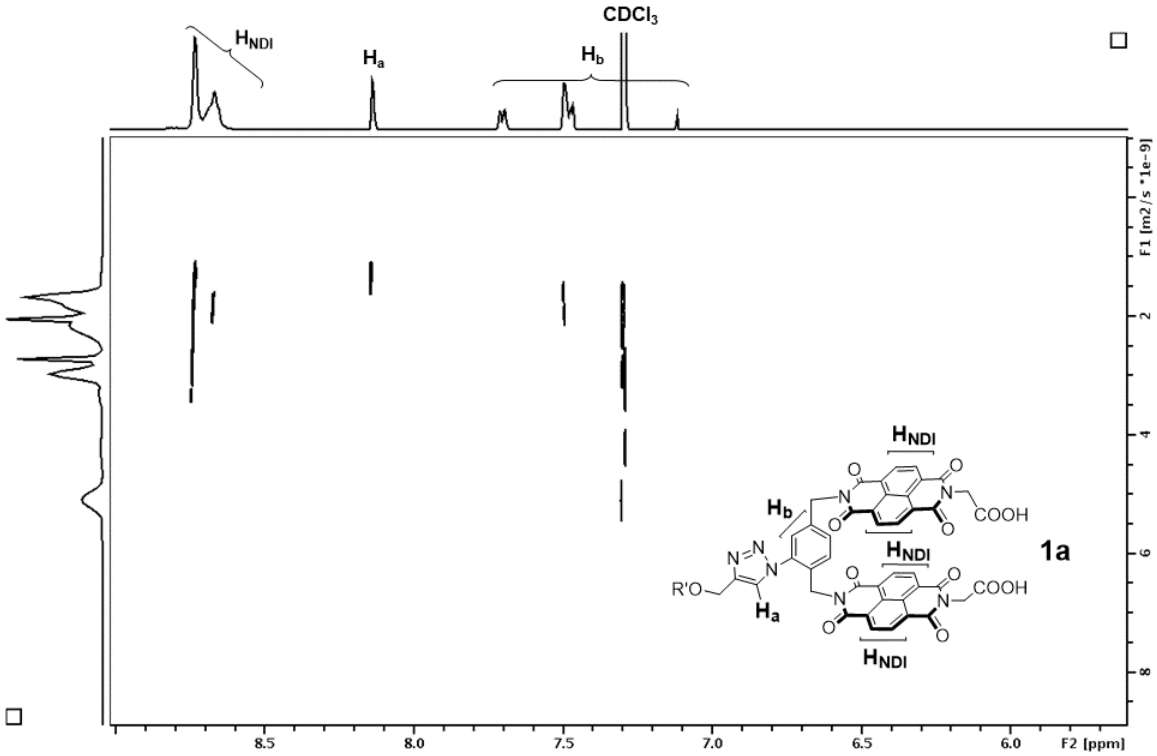


Figure S5. DOSY spectrum of 0.6 mM host **1a** in $CDCl_3$ at 253 K with diffusion coefficients of 2.1×10^{-9} m^2/s at 8.85-8.56 ppm (protons on NDI moieties), 2.0×10^{-9} m^2/s at 8.20-8.07 ppm.

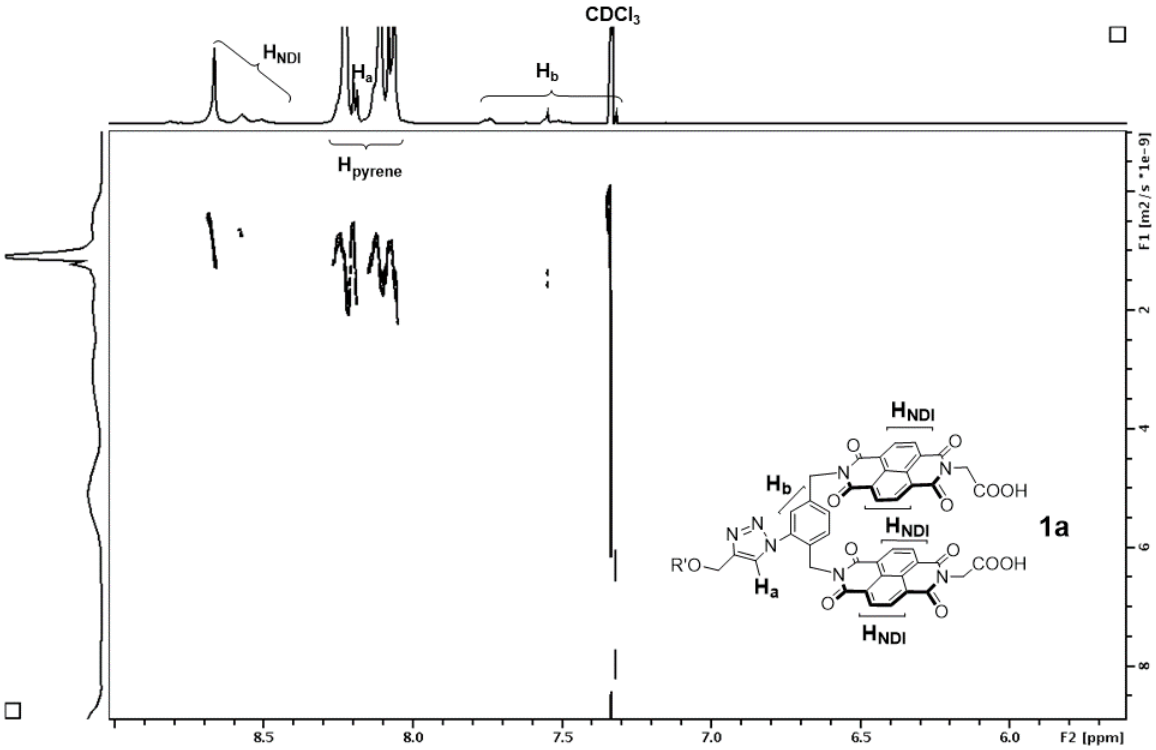


Figure S6. DOSY spectrum of 0.6 mM host **1a** with 2.5 mM pyrene in $CDCl_3$ at 253 K with diffusion coefficients of 7.0×10^{-10} m^2/s at 8.73-8.62 ppm and 7.6×10^{-10} m^2/s at 8.62-8.45 ppm (protons on NDI moieties), 1.1×10^{-9} m^2/s at 8.32-7.99 ppm (protons on pyrene).

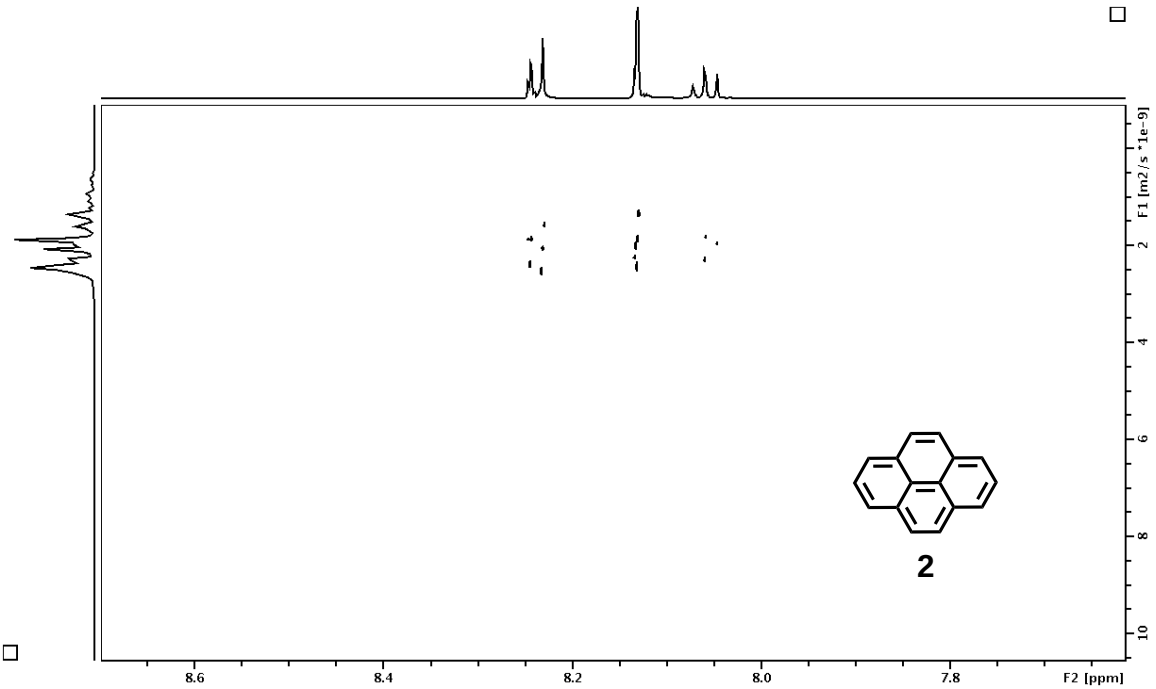


Figure S7. DOSY spectrum of 2.5 mM pyrene **2** in $CDCl_3$ at 253 K with diffusion coefficients of 2.0×10^{-9} m^2/s at 8.26-8.23 ppm, 1.9×10^{-9} m^2/s at 8.15-8.12 ppm, and 1.8×10^{-9} m^2/s at 8.08-8.04 ppm.

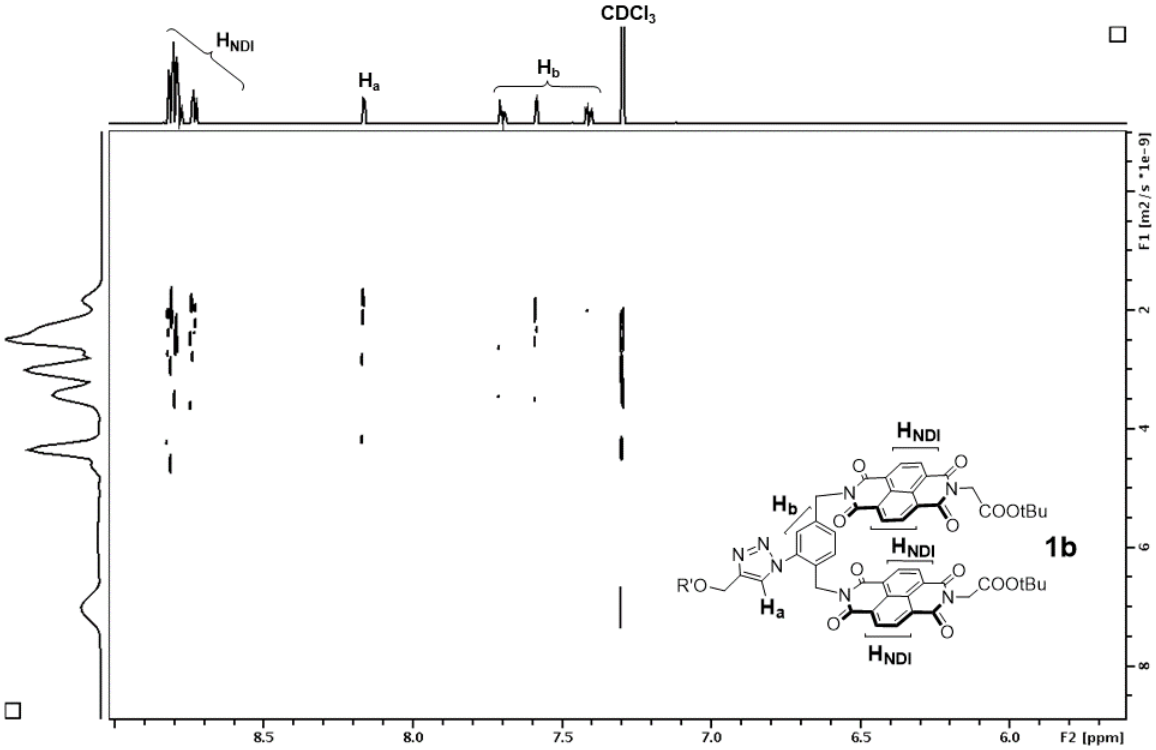


Figure S8. DOSY spectrum of 2 mM host **1b** in $CDCl_3$ at 253 K with diffusion coefficients of 3.4×10^{-9} m^2/s at 8.85-8.76 ppm, 3.6×10^{-9} m^2/s at 8.76-8.71 ppm (protons on NDI moieties), 3.1×10^{-9} m^2/s at 8.19-8.14 ppm.

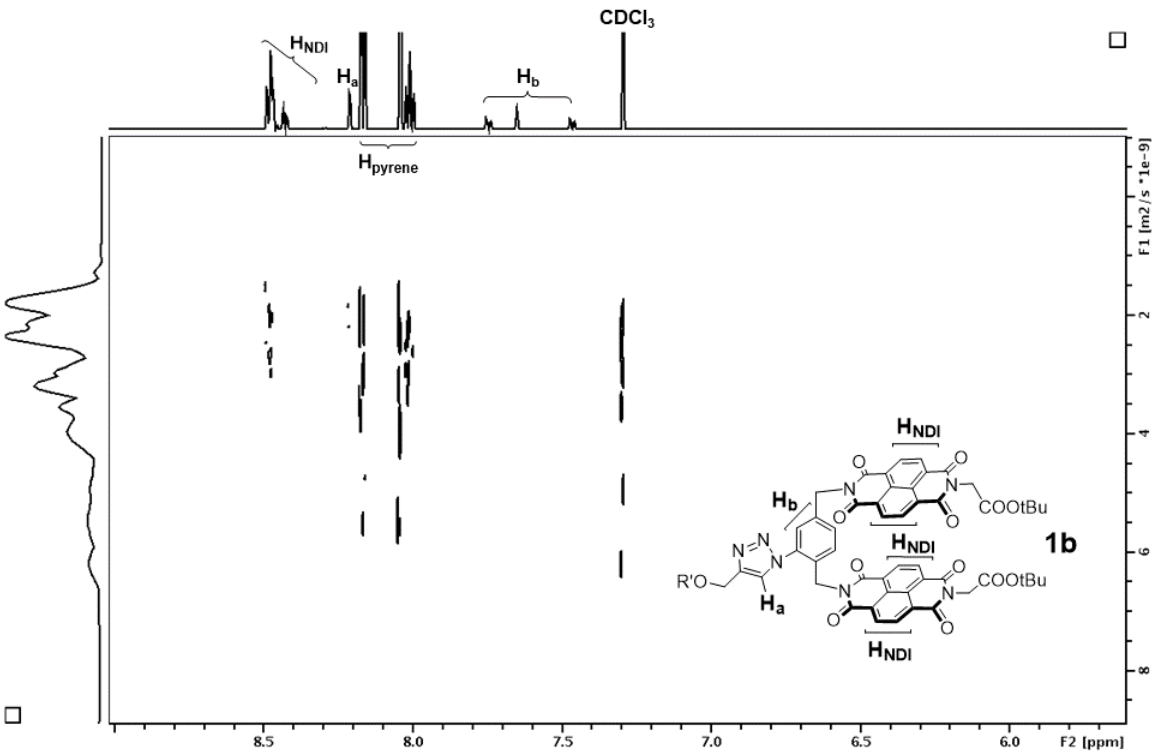


Figure S9. DOSY spectrum of 2 mM host **1b** with 8 mM pyrene in $CDCl_3$ at 253 K with diffusion coefficients of 2.8×10^{-9} m^2/s at 8.51-8.45 ppm and 3.6×10^{-9} m^2/s at 8.45-8.41 ppm (protons on NDI moieties).

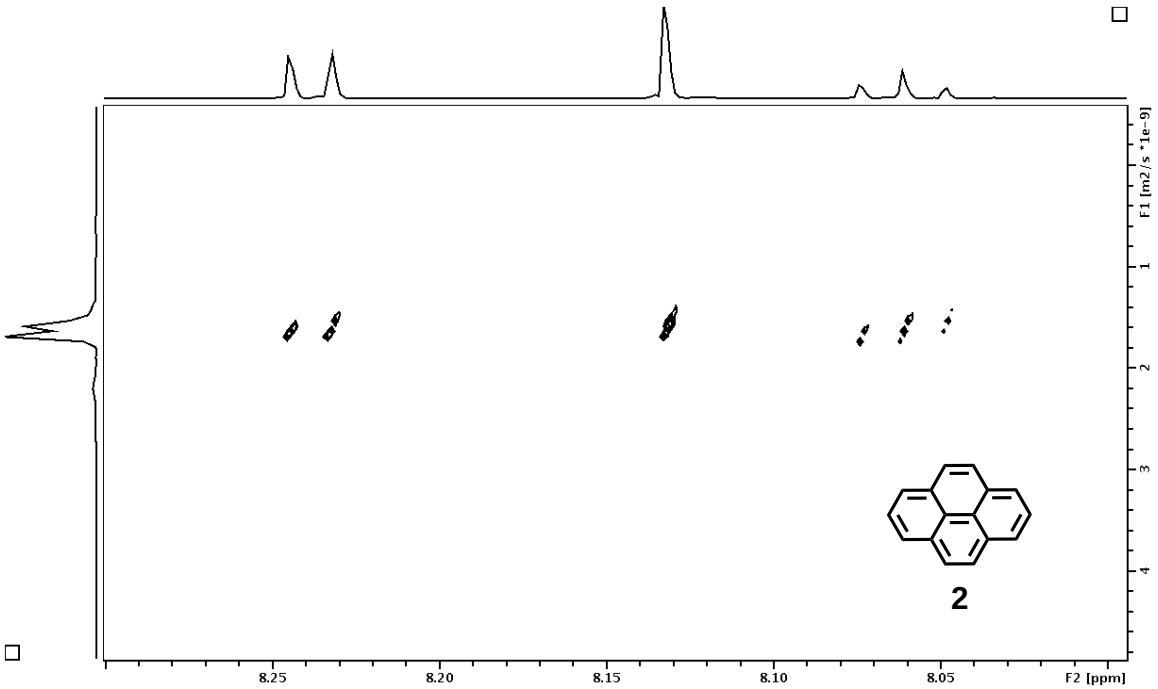


Figure S10. DOSY spectrum of 8 mM pyrene **2** in $CDCl_3$ at 253 K with diffusion coefficients of 1.7×10^{-9} m^2/s at 8.26-8.12 ppm, and 1.5×10^{-9} m^2/s at 8.08-8.04 ppm.

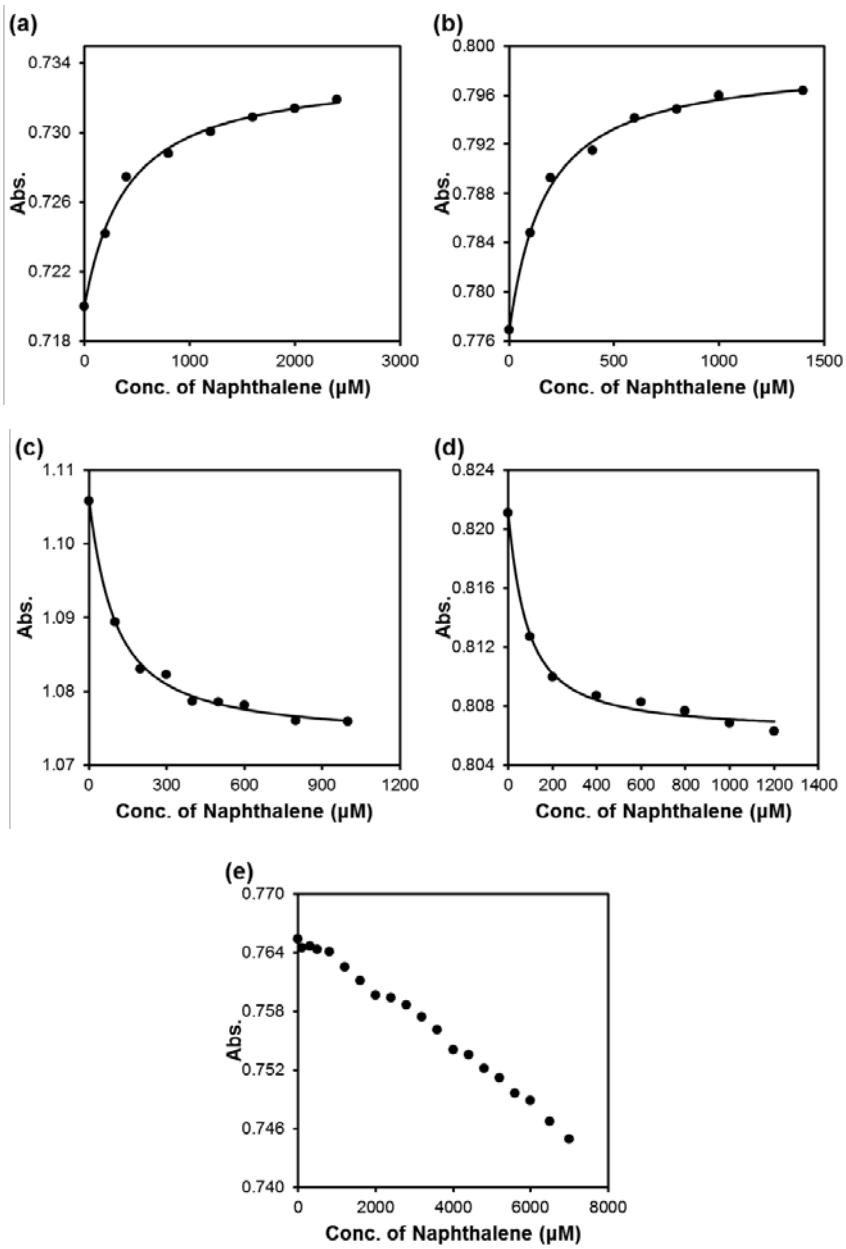


Figure S11. UV-vis titration curves of compound **1a** by naphthalene in (a) 1:4 DCM/MeOH (v/v), [**1a**] = 15 μM, (b) 4:1 DCM/MeOH (v/v), [**1a**] = 15 μM, (c) DCM, [**1a**] = 30 μM, (d) 1:4 Hexane/DCM (v/v), [**1a**] = 30 μM and (e) 3:2 Hexane/DCM (v/v), [**1a**] = 30 μM. The UV absorbance at 382 nm was monitored and the smooth curve was from nonlinear least squares curving fitting to a 1:1 binding isotherm.

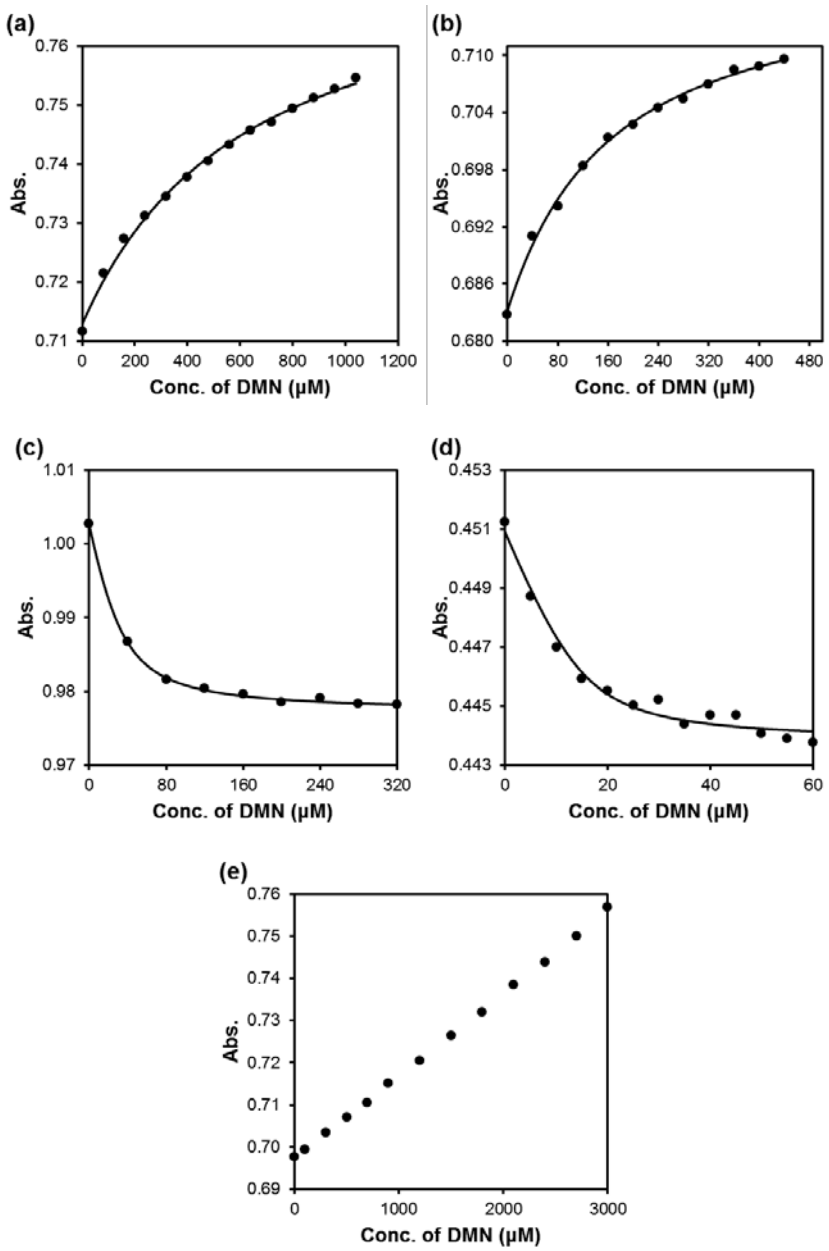


Figure S12. UV-vis titration curves of compound **1a** by 1,5-dimethoxynaphthalene in (a) 1:4 DCM/MeOH (v/v), [**1a**] = 15 μM, (b) 4:1 DCM/MeOH (v/v), [**1a**] = 15 μM, (c) DCM, [**1a**] = 30 μM, (d) 1:4 Hexane/DCM (v/v), [**1a**] = 15 μM and (e) 3:2 Hexane/DCM (v/v), [**1a**] = 30 μM. The UV absorbance at 382 nm was monitored and the smooth curve was from nonlinear least squares curving fitting to a 1:1 binding isotherm.

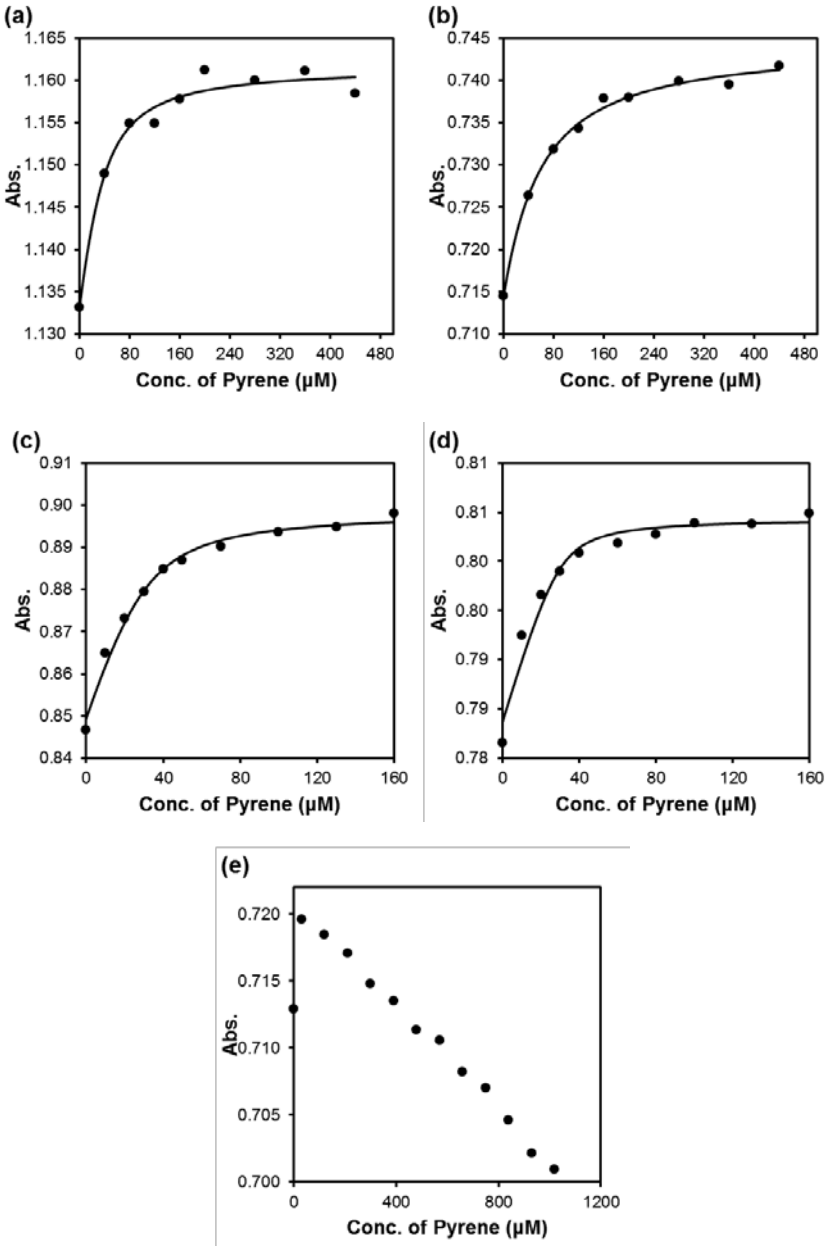


Figure S13. UV-vis titration curves of compound **1a** by pyrene in (a) 1:4 DCM/MeOH (v/v), [**1a**] = 30 μM, (b) 4:1 DCM/MeOH (v/v), [**1a**] = 15 μM, (c) DCM, [**1a**] = 30 μM, (d) 1:4 Hexane/DCM (v/v), [**1a**] = 30 μM and (e) 3:2 Hexane/DCM (v/v), [**1a**] = 30 μM. The UV absorbance at 382 nm was monitored and the smooth curve was from nonlinear least squares curving fitting to a 1:1 binding isotherm.

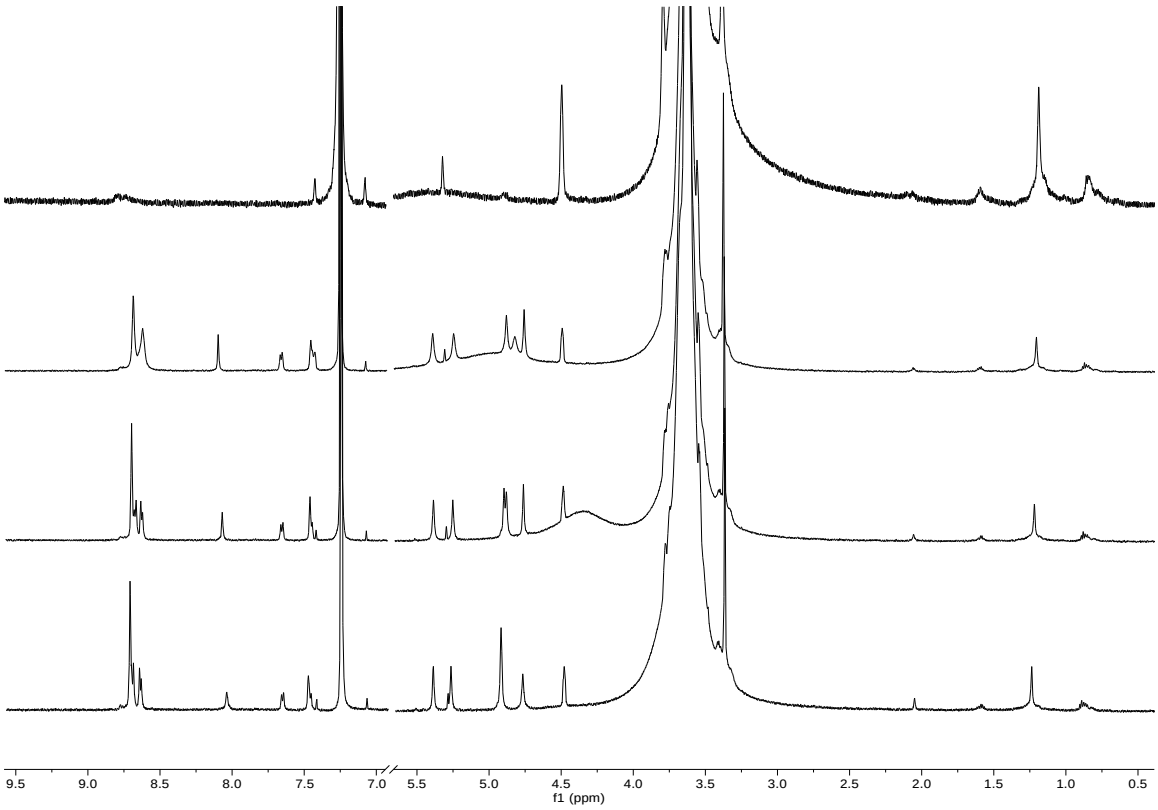


Figure S14. NMR spectra of 2 mM host **1a** in $CDCl_3$ at different temperatures. Temperature from bottom to top was 298 K, 273 K, 253 K, and 233 K.

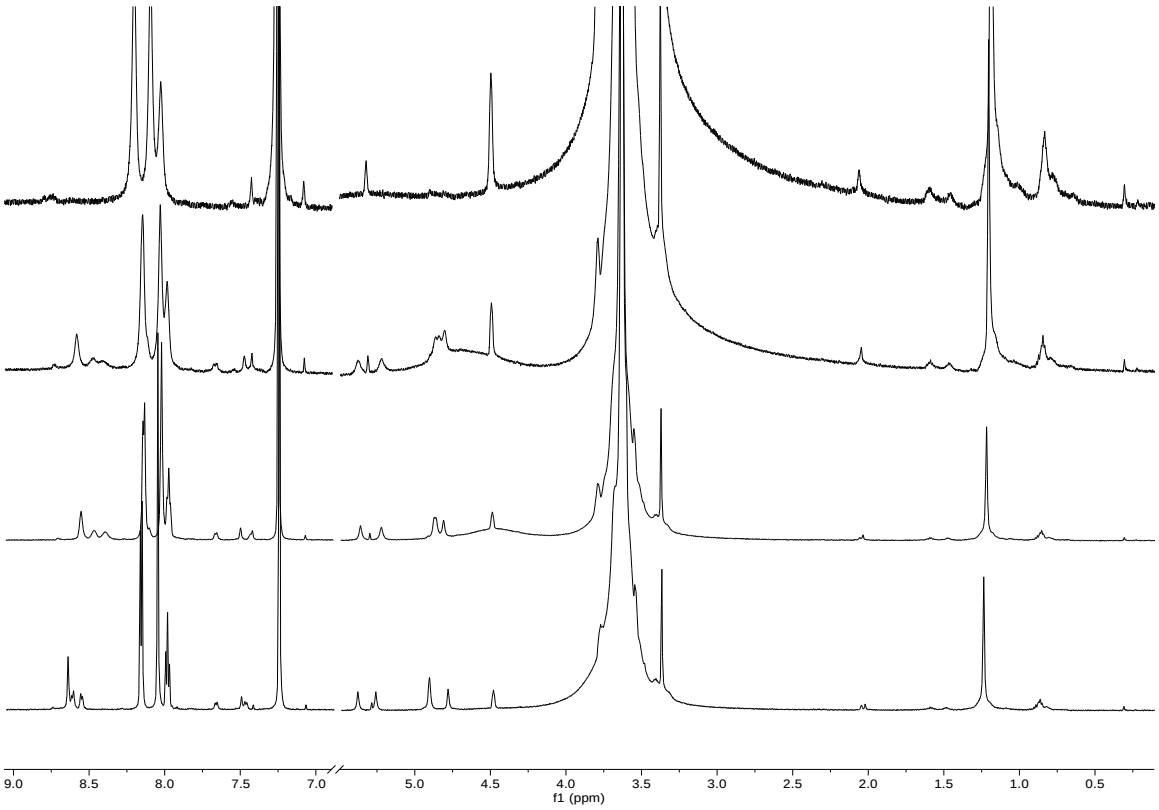


Figure S15. NMR spectra of 2 mM host **1a** with 3 mM pyrene in $CDCl_3$ at different temperatures. Temperature from bottom to top was 298 K, 273 K, 253 K, and 233 K.

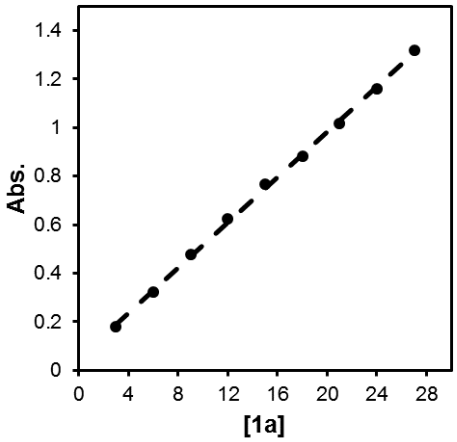


Figure S16. UV-vis absorbance as a function of [**1a**] in μM with linear trend line of R^2=0.9991.

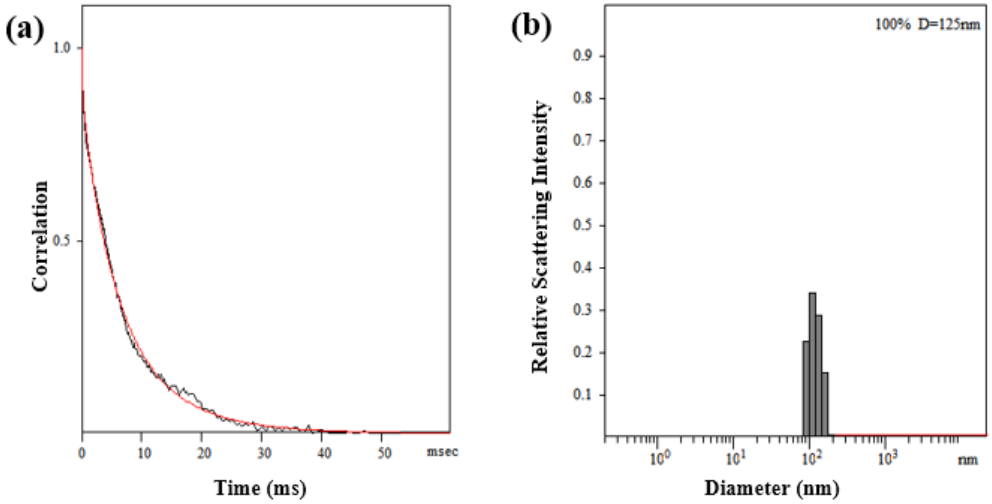


Figure S17. Representative dynamic light scattering (a) correlation curve and (b) distribution of the hydrodynamic diameter of **1a** with pyrene in DCM, [**1a**] = 100 μM, [pyrene] = 500 μM. The diameter average from 50 raw data is 121 nm.

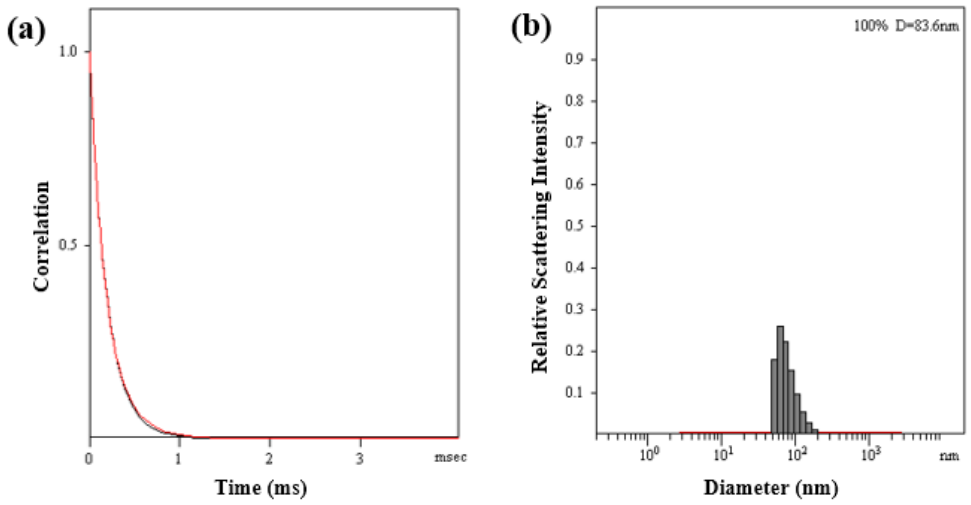


Figure S18. Representative dynamic light scattering (a) correlation curve and (b) distribution of the hydrodynamic diameter of **1a** with pyrene in hexane/DCM 3/2, [**1a**] = 100 μM, [pyrene] = 500 μM. The diameter average from 50 raw data is 79.1 nm.

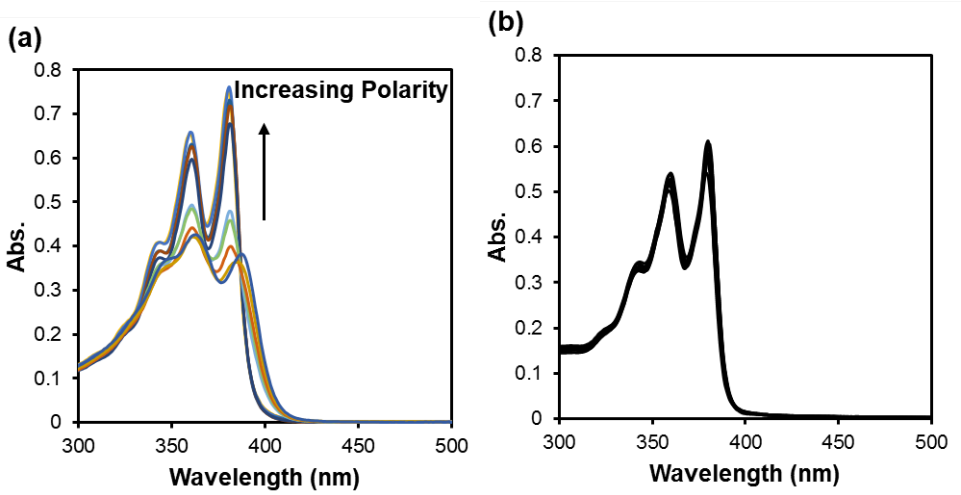


Figure S19. (a) UV-Vis spectra of compound **1a** in DCM/methanol mixtures and hexane/DCM mixtures. [**1a**] = 15 μM. (b) UV-vis spectra of compound **1b** in DCM/methanol mixtures and hexane/DCM mixtures. [**1b**] = 30 μM.

Table S1. Averaged hydrodynamic diameters for **1a** with pyrene in DCM and 3:2 hexane/DCM from dynamic light scattering at 298 K. [**1a**] = 100 μM. Viscosity and refractive index of solvent mixture was approximately calculated from linear composition of volume fraction of individual solvent.

Entry	Solvent	[Pyrene] (μM)	Diameter[a] (nm)
1	**DCM**	0	119 ± 23
2		100	118 ± 21
3		200	134 ± 57
4		300	124 ± 27
5		400	129 ± 41
6		500	121 ± 38
7	**3:2 Hexane/DCM**	0	85.6 ± 3.6
8		100	85.3 ± 3.4
9		200	81.6 ± 2.8
10		300	75.1 ± 4.4
11		400	84.0 ± 3.7
12		500	79.1 ± 4.6

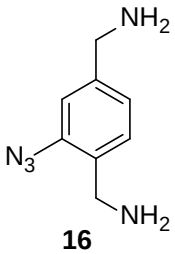
[a] Individual hydrodynamic diameter was generated from dynamic light scattering. Average was calculated from nearly 50 raw data, standard deviation of which was used as uncertainty.

Table S2. Binding constants of hosts **1a** and **1b** with guests in different solvents at 298K. All binding constants were averages of two titrations with standard error as uncertainty.

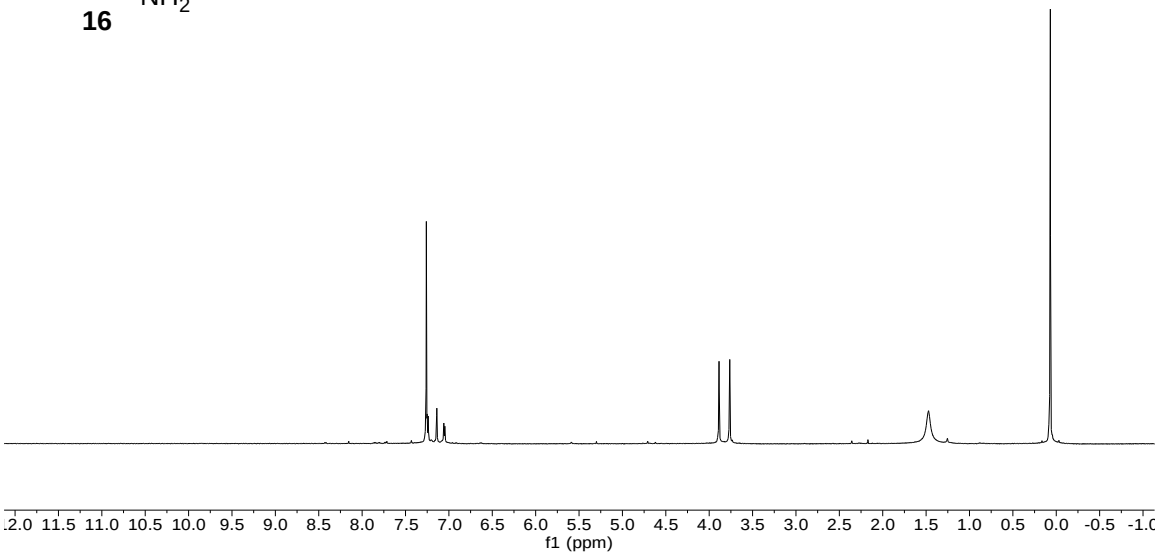
Entry	Host	Guest	Solvent(s)	K_a (M^{-1})
1[a]	**1a**	Naphthalene	Hex/DCM 3/2 (v/v)	-
2	**1a**	Naphthalene	Hex/DCM 1/4 (v/v)	$(2.2 \pm 0.1) \times 10^4$
3	**1a**	Naphthalene	DCM	$(1.0\pm 0.3) \times 10^4$
4	**1a**	Naphthalene	DCM/MeOH 4/1 (v/v)	$(7.1 \pm 1.4) \times 10^3$
5	**1a**	Naphthalene	DCM/MeOH 1/4 (v/v)	$(2.57\pm 0.04 \times 10^3$
6[a]	**1a**	DMN	Hex/DCM 3/2 (v/v)	-
7	**1a**	DMN	Hex/DCM 1/4 (v/v)	$(3.2 \pm 1.1) \times 10^5$
8	**1a**	DMN	DCM	$(7.4 \pm 0.8) \times 10^4$
9	**1a**	DMN	DCM/MeOH 4/1 (v/v)	$(7.8 \pm 3.4) \times 10^3$
10	**1a**	DMN	DCM/MeOH 1/4 (v/v)	$(1.3 \pm 0.8) \times 10^3$
11[a]	**1a**	Pyrene	Hex/DCM 3/2 (v/v)	-
12	**1a**	Pyrene	Hex/DCM 1/4 (v/v)	$(3.4 \pm 1.0) \times 10^5$
13	**1a**	Pyrene	DCM	$(2.4 \pm 1.4) \times 10^5$
14	**1a**	Pyrene	DCM/MeOH 4/1 (v/v)	$(2.8 \pm 1.1) \times 10^4$
15	**1a**	Pyrene	DCM/MeOH 1/4 (v/v)	$(4.0 \pm 1.6) \times 10^4$
16[a]	**1b**	DMN	Hex/DCM 1/4 (v/v)	-
17[a]	**1b**	DMN	DCM	-
18[a]	**1b**	DMN	DCM/MeOH 4/1 (v/v)	-
19[a]	**1b**	DMN	DCM/MeOH 1/4 (v/v)	-
20[a][b]	**1a**	DMN	Hex/DCM 1/4 (v/v)	-
21[a][b]	**1a**	DMN	DCM	-
22[a][b]	**1a**	DMN	DCM/MeOH 4/1 (v/v)	-
23[a][b]	**1a**	DMN	DCM/MeOH 1/4 (v/v)	-
24[a][c]	**1a**	DMN	Hex/DCM 1/4 (v/v)	-
25[a][c]	**1a**	DMN	DCM	-
26[a][c]	**1a**	DMN	DCM/MeOH 4/1 (v/v)	-
27[a][c]	**1a**	DMN	DCM/MeOH 1/4 (v/v)	-

[a] Saturation could not be reached even with high concentrations of guest. Binding was weak. [b]The binding titration were conducted after saturation with DIPEA (20-40 equiv. to **1a**). [c] The binding titration were conducted after saturation with NH_3 (10-20 equiv. to **1a**). (DMN: 1,5-dimethyl naphthalene)

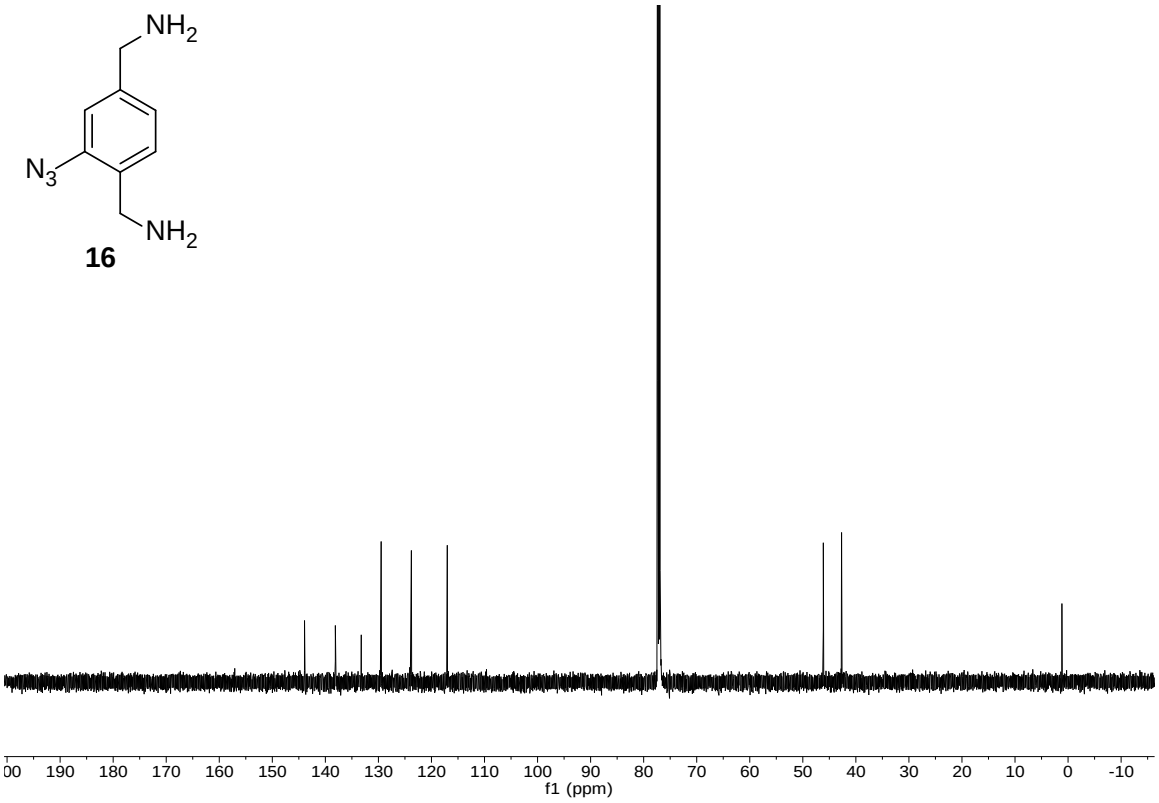
^{1}H and ^{13}C NMR spectra

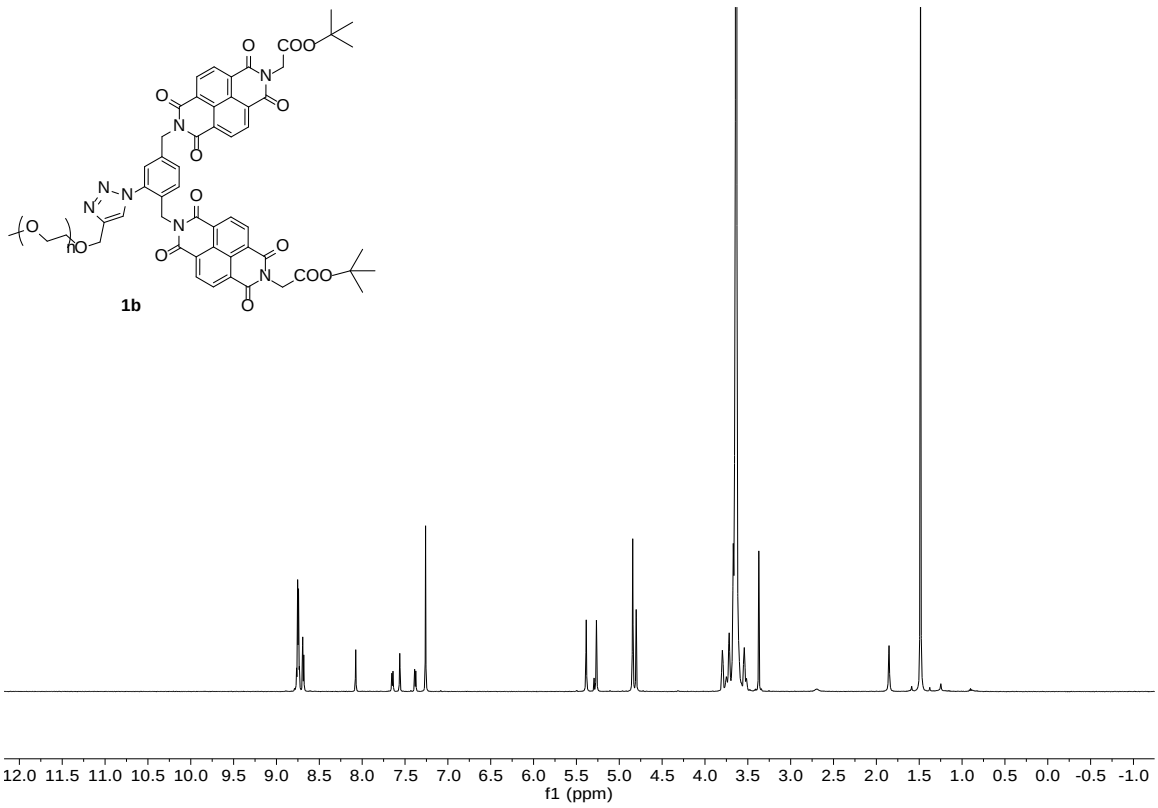


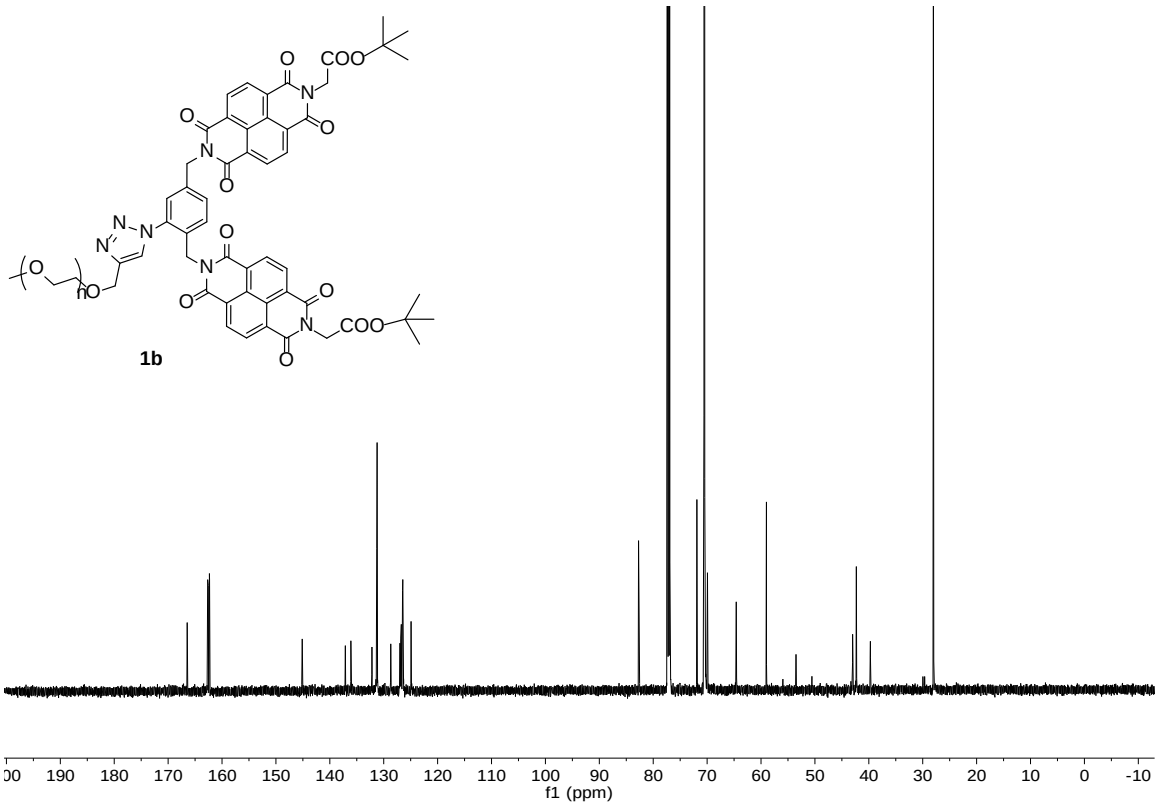
16

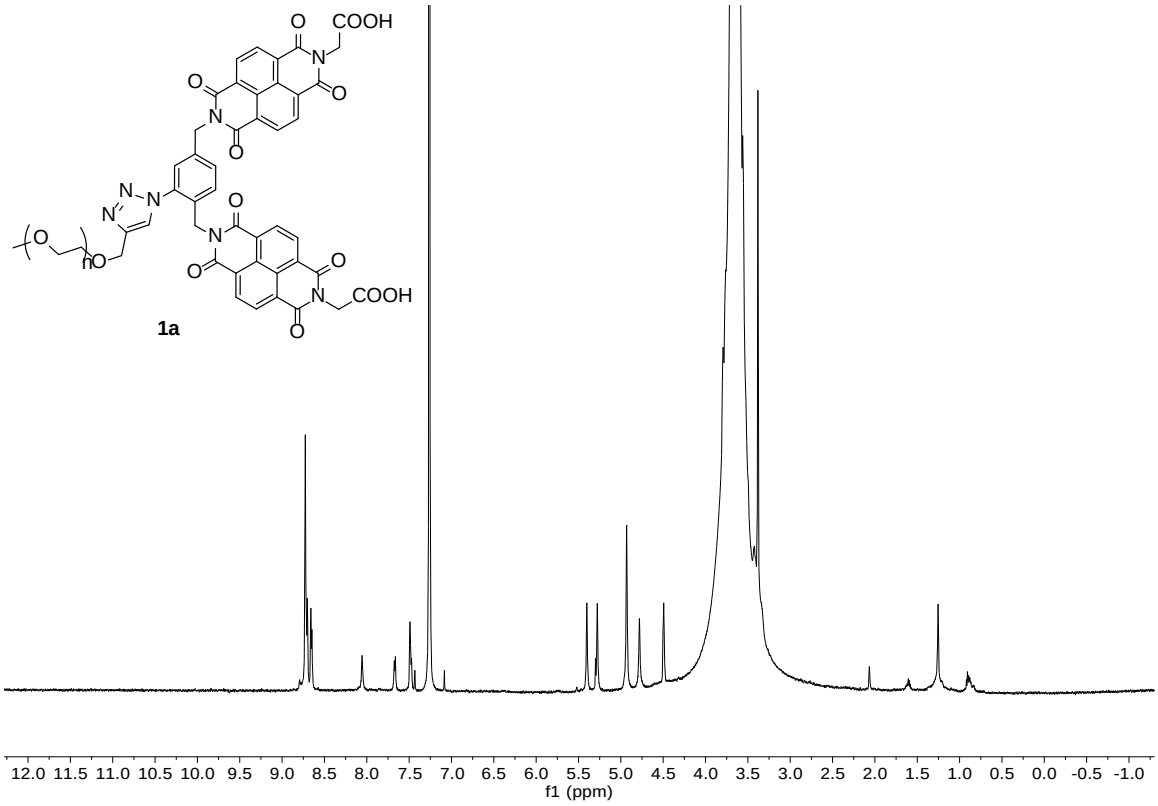


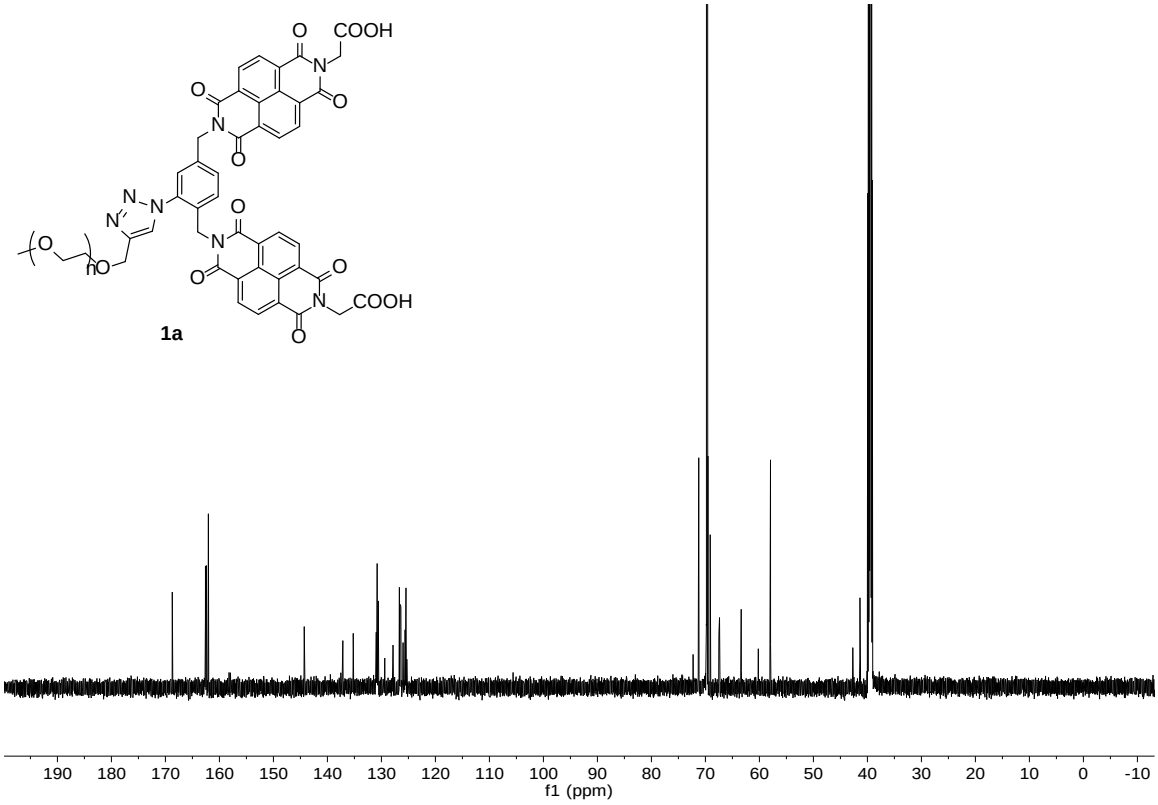
16




1b
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)


1b
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


1a
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)


1a
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)