Tricyclic Octaurea "Temples" for the Recognition of Polar Molecules in Water

Claire S. Webster, Federica Balduzzi and Anthony P. Davis*

School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, UK.

Table of Contents

1. Synthesis and Characterisation of Receptors 5 and 6

Synthesis

Commercial reagents were purchased from Sigma-Aldrich, Alfa-Aesar, Fisher Scientific, Fluorochem or Acros Organics and were used without further purification unless otherwise specified. All air and moisture sensitive manipulations were carried out using standard vacuum line and Schlenk techniques. Solvents for air and moisture sensitive manipulations were obtained from an Anhydrous Engineering Solvent Purification System,[1] distilled and dried over activated molecular sieves, or purchased from Acros Organics.

Flash column chromatography was performed using silica gel 60 Å (Sigma Aldrich, particle size 35-70 micron) and a suitable eluent. Reverse phase flash chromatography was performed on a Biotage Isolera One or Biotage Selekt with Biotage SNAP Ultra C18 25 µm columns or Biotage Sfär C18 30 µm columns, typically with acetone/water eluents. CV ≡ column volume(s). Thin layer chromatography (TLC) was performed using aluminium backed TLC plates (Merck-Keiselgel 60 F_{254}) and visualised using UV fluorescence (254 or 365 nm) and/or developed using ninhydrin, potassium permanganate or bromocresol green.

HPLC analyses were performed using a Waters 600 Controller with a Waters 2998 Photodiode Array Detector. For analytical runs a XSELECT CSH C18 5 µm (4.6 × 150 mm) column was used and for preparative runs a XSELECT CSH Prep C18 5 µm OBD (19 × 250 mm) column was used with the solvent conditions stated.

LC-MS analyses were performed using a Waters autopurification system comprising of a SFO, 2767 autosampler and 2545 pump with a XSELECT CSH C18 3.5µm (4.6 × 100 mm) column at a 1.0 mL/min pump rate using the solvent conditions stated. Detection was carried out using a Waters 2998 diode array detector monitoring between 210 and 600 nm and a Waters SQD2 ESI mass spectrometer detecting in positive mode between 150 and 2100 m/z.

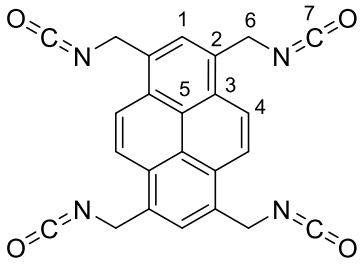
NMR spectra were recorded at 25°C (unless stated otherwise) on either a Varian VNMR 400 MHz, Bruker 400 MHz, Varian VNMRS 500 MHz, Bruker Avance III HD Cryo 500 MHz, Varian VNMRS Cryo 600 MHz or the Bruker Advance III HD Cryo 700 MHz spectrometers. All ^{1}H and ^{13}C NMR chemical shifts are reported relative to the ^{1}H (residual) and ^{13}C chemical shifts of the solvent as standard. Assignments of the recorded signals were made with assistance of COSY, HSQC, HMBC and NOESY experiments, where necessary.

UV-Vis spectra were recorded on an Agilent Cary 300 spectrometer. Fluorescence spectra were recorded on a Horiba FluoroMax Plus spectrometer. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer with an ATR accessory and frequencies reported in wavenumbers (cm^{-1}). ESI-HRMS (electrospray ionisation high resolution mass spectrometry) was performed on a Bruker Daltonics microTOF II.

Tetra-azide (**8**),[2] Fmoc protected *tert*-butyl ester G2[*] linker (**10**),[3] NDI **33**[4] NDI **34**[4] and NDI **35**[5] were prepared according to literature procedures. The detailed synthetic procedures and characterisation data for compound in the synthetic sequence towards receptors **5** and **6** are given in the following pages, accompanied by the relevant NMR spectra as evidence of purity and characterisation.

[*] "G2" is used throughout to denote "second-generation dendrimer".

1,3,6,8-tetrakis(isocyanatomethyl)pyrene (9)



An autoclave was charged with azide **8**[2] (44 mg, 0.095 mmol) and polymer supported triphenylphosphine (3 mmol/g, 254 mg, 0.757 mmol), before closing under a N_2 atmosphere. Anhydrous toluene was added and the vessel pressurised with CO_2 (20 bar) before heating to 50 °C. After 5 h, the reaction was cooled to rt and the autoclave vented. The reaction mixture was filtered and washed with toluene (3 x 20 mL). The solvent was removed under vacuum to give the title compound (38 mg, 95%) as a yellow solid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.35 (s, 4H, C4*H*), 8.15 (s, 2H, C1*H*), 5.26 (s, 8H, C6H_2); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 131.2 (C2), 128.3 (C3), 126.6 (C1), 125.8 (C5), 124.3 (C7), 123.4 (C4), 45.2 (C6); **ν_{max}/cm^{-1}** (solid) 2921 (m), 2852 (m), 2251 (s). *The compound was too unstable for a mass spectrum to be obtained.*

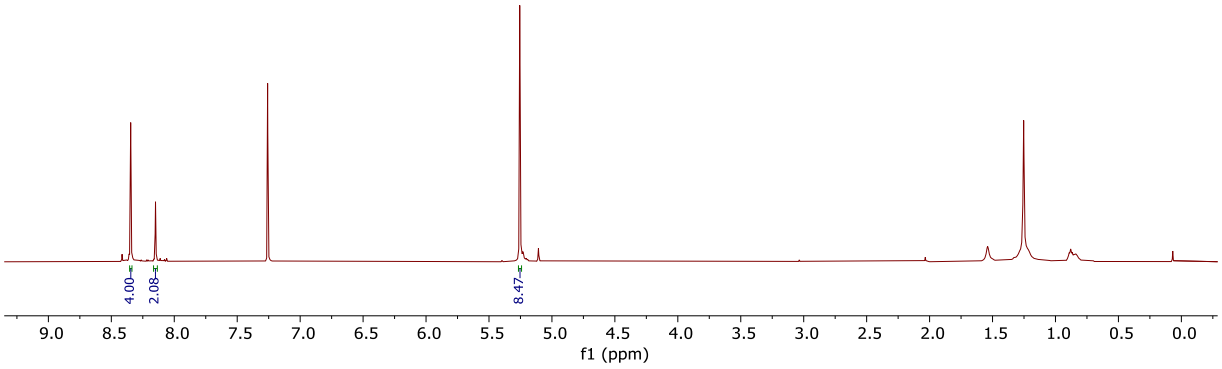
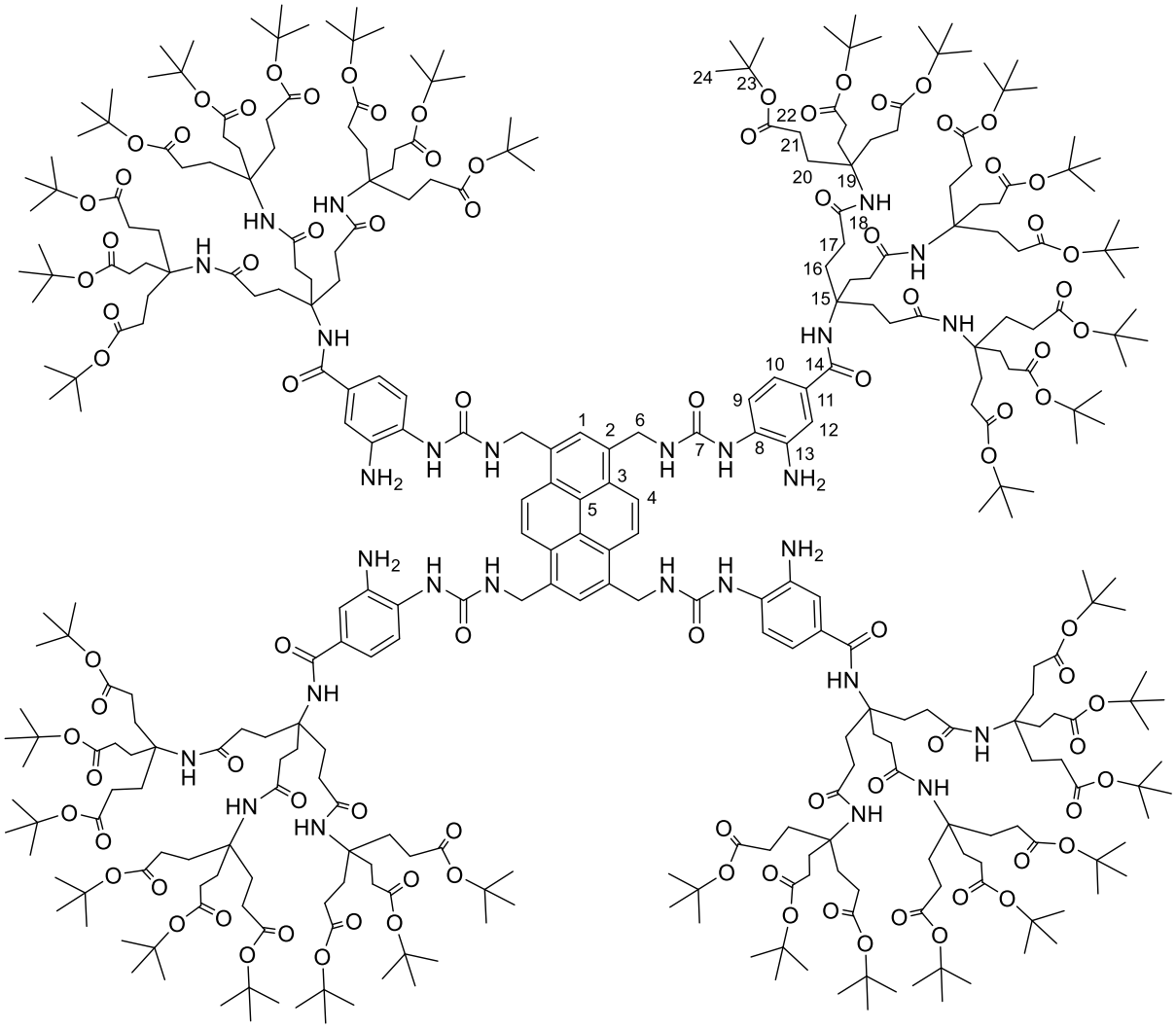


Figure S1. H NMR (500 MHz, $CDCl_3$, 298 K) spectrum of isocyanate **9**.

***Tert*-butyl protected pyrenyl half receptor (11)**



Under an N_2 atmosphere, isocyanate **9** (36 mg, 85 µmol) and amine **10**[3] (0.73 g, 0.41 mmol) were dissolved in anhydrous DCM (4 mL). Anhydrous pyridine (40 µL, 0.51 mmol) was added and the reaction mixture stirred for 16 h at 34 °C. The solvent was removed under vacuum and the crude product was then purified by reverse phase flash column chromatography (Biotage Sfär, C18 Bio 25 g) eluting with acetone/water (25:75 → 80:20 over 8 CV then 80:20→ 100% acetone over 4 CV) to give the Fmoc protected compound (0.50 g, 77%) as a yellow solid.

Fmoc protected half receptor (0.12 g, 15.7 µmol) was dissolved in anhydrous DCM (1 mL) under an N_2 atmosphere and cooled to 0 °C. DBU (20 µL, 0.125 mmol) was added and the reaction stirred for 1 h at 0 °C. On warming to rt, solvent was removed under vacuum. The crude residue was then purified by flash column chromatography (DCM 100% → DCM:MeOH 95:5) to give the title compound (97 mg, 92%) as a yellow solid. **^{1}H NMR** (500 MHz, CD_3OD) δ 8.46 (s, 4H, C4*H*), 8.18 (s, 2H, C1*H*), 7.41 (d, *J* = 8.5 Hz, 4H, C9*H*), 7.29 (t, *J* = 2.0 Hz, 4H, C12*H*), 7.20 (dd, *J* = 8.5, 2.0 Hz, 4H, C10*H*), 5.17 (s, 8H,

C6H_2), 2.28 – 2.20 (m, 24H, C17H_2), 2.20 – 2.15 (m, 72H, C21H_2), 2.12 – 2.05 (m, 24H, C16H_2), 1.97 – 1.88 (m, 72H, C20H_2), 1.42 (s, 324H, C24H_3); **^{13}C NMR** (126 MHz, CD_3OD) δ 175.5 (C18), 174.4 (C22), 170.0 (C14), 158.4 (C7), 141.9 (C13), 134.1 (C2), 133.1 (C11), 129.7 (C8), 129.4 (C3), 128.2 (C1), 126.9 (C5), 125.1 (C9), 124.1 (C4), 118.8 (C10), 117.3 (C12), 81.6 (C23), 59.4 (C15), 58.7 (C19), 43.1 (C6), 32.5 (C16), 32.2 (C17), 30.7 (C21), 30.5 (C20), 28.5 (C24); **NSI$^+$-HRMS** for $C_{356}H_{578}N_{28}O_{92}^{4+}$ $[M+4H]^{4+}$ calculated; 1680.5385, found; 1680.5364.

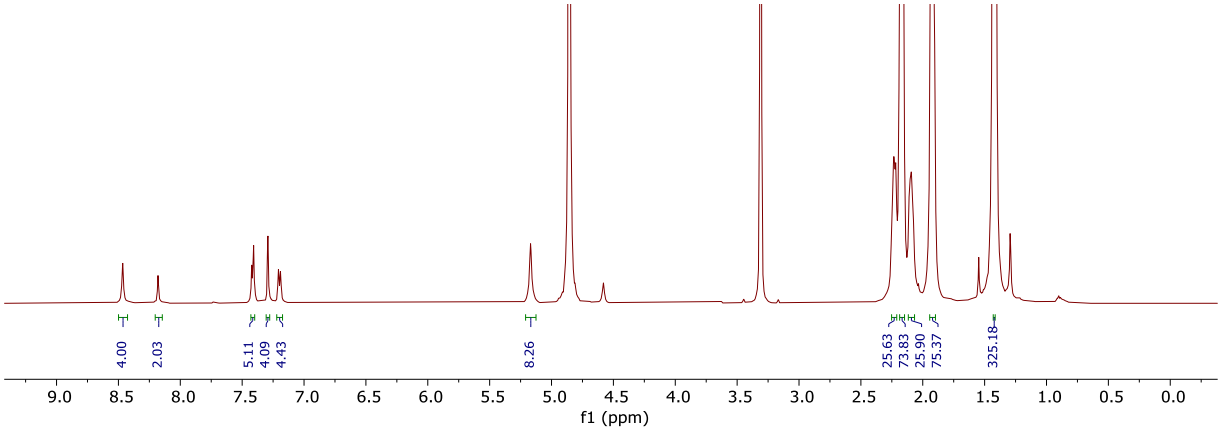
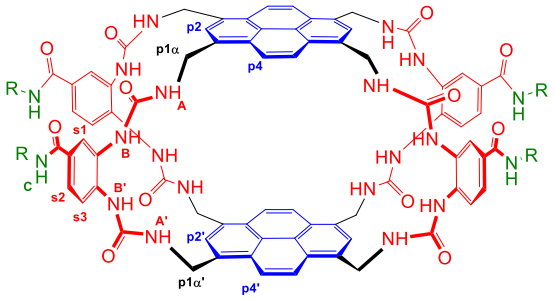
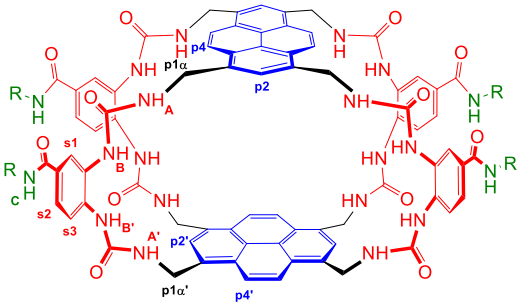


Figure S2. ^{1}H NMR (400 MHz, CD_3OD, 298 K) spectrum of half receptor **11**.

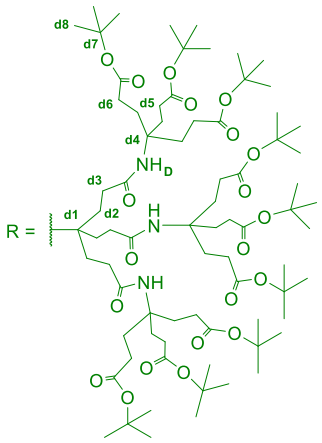
***Tert* butyl protected pyrene-pyrene macrocycles (12 & 13)**



12



13



Under an inert N_2 atmosphere, half-receptor **11** (0.13 g, 19.7 µmol) was dissolved in an anhydrous DMF/pyridine mixture (1:3, 5 mL:15 mL). A solution of isocyanate **9** (10 mg, 23.6 µmol) in anhydrous DCM (5 mL) was added at a rate of 0.3 mL/hr and the resulting solution stirred at 34 °C for 3 days. The reaction mixture was cooled to room temperature and the volatiles removed under vacuum, followed by co-evaporation with toluene (3 x 25 mL). The residue was purified by preparative HPLC eluting with acetone/water (70:30 → 100:0 over 40 min). The components eluting at 33 min and 34 min were collected, the volatiles evaporated and then the samples were freeze dried to yield **12** (36 mg, 26%, faster-eluting peak) and **13** (13 mg, 10%, slower-eluting peak) as pale-yellow solids.

Data for 12 (eluting at 33 min)

^{1}H NMR (700 MHz, $(CD_3)_2SO$, 313 K) δ 8.50 (s, 4H, p4), 8.33 (s, 4H, p4'), 8.10 (s, 4H, s1), 8.02 (d, J = 8.0 Hz, 4H, s3), 8.00 (s, 2H, p2), 7.97 (s, 2H, p2'), 7.76 (s, 4H, C), 7.73 (s, 4H, B'), 7.56 (d, J = 8.6 Hz, 4H, s2), 7.47 (s, 4H, B), 7.22 (s, 12H, D), 6.98 (s, 4H, A'), 6.85 (s, 4H, A), 5.19 (s, 4H, p1α), 5.15 (s, 4H, p1α'), 4.62 (s, 4H, p1α'), 4.53 (s, 4H, p1α), 2.19 – 2.03 (m, 96H, d3 & d6), 2.04 – 1.92 (m, 24H, d2), 1.87 – 1.74 (m, 72H, d5), 1.38 (s, 324H, d8); **^{13}C NMR** (176 MHz, $(CD_3)_2SO$, 313 K) δ 172.9 (d3CO), 172.5 (d6CO), 165.9 (NH_CCO), 135.5 (Ar C_q), 131.7 (p2), 131.2 (p2'), 130.7 (Ar C_q), 130.1 (Ar C_q), 129.5 (Ar C_q), 128.6 (Ar C_q), 124.5 (s1), 124.2 (p4), 124.0 (p4'), 123.5 (s2), 119.8 (s3), 79.9 (d7), 56.9 (d4), 41.6 (p1α), 41.2 (p1α'), 31.2 (d2), 30.9 (d3), 29.5 (d6), 29.3 (d5), 28.1 (d8); **NSI$^+$-HRMS** for $C_{380}H_{588}N_{32}O_{96}^{4+}$ $[M+4H]^{4+}$ calculated;

1786.0638, found; 1786.0631; t_R: (Acetone:water 70:30→ 100% acetone over 40 minutes, 16 mL/min flow rate) 38.8 mins (analytical), 33.9 mins (preparative). *^{13}C data assigned using HSQC & HMBC.*

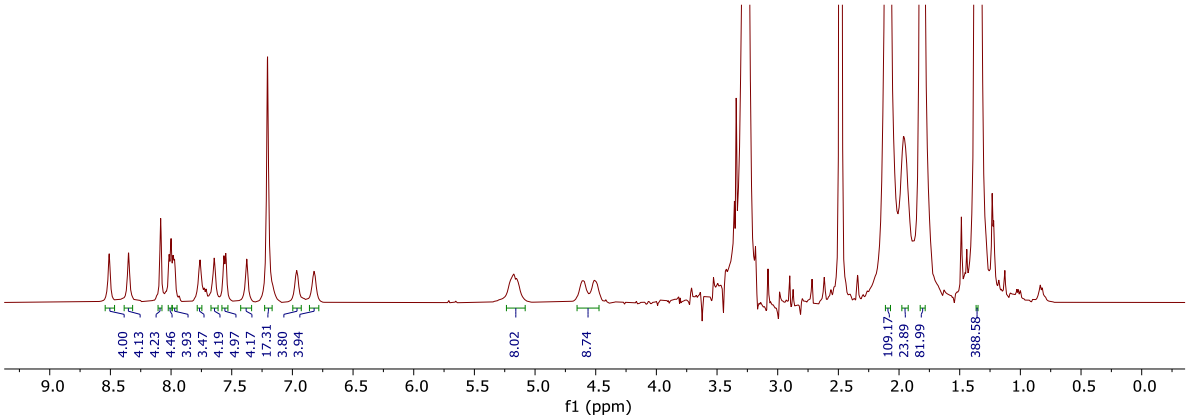


Figure S3. ^{1}H NMR (500 MHz, $(CD_3)_2SO$, 313 K) spectrum of eclipsed protected macrocycle **12**

Data for 13 (eluting at 34 min)

^{1}H NMR (700 MHz, $(CD_3)_2SO$, 313 K) δ 8.42 (s, 4H, p4), 8.25 (s, 4H, p4'), 8.15 (s, 4H, s1), 8.11 (d, J = 8.1 Hz, 4H, s3), 8.07 (s, 2H, p2), 8.02 (s, 2H, p2'), 7.74 (s, 8H, C & B'), 7.56 (d, J = 8.6 Hz, 4H, s2), 7.46 (s, 4H, B), 7.23 (s, 12H, D), 7.08 (s, 4H, A'), 6.96 (s, 4H, A), 5.24 – 5.08 (m, 8H, p1α & p1α'), 4.63 (s, 4H, p1α'), 4.57 (s, 4H, p1α), 2.18 – 2.07 (m, 96H, d3 & d6), 2.02 – 1.92 (m, 24H, d2), 1.89 – 1.77 (m, 72H, d5), 1.39 (s, 324H, d8); **^{13}C NMR** (176 MHz, $(CD_3)_2SO$, 313 K) δ 172.9 (d3CO), 172.6 (d6CO), 166.0 (NH_cCO), 135.5 (Ar C_q), 132.1 (p2), 131.6 (p2'), 130.9 (Ar C_q), 129.2 (Ar C_q), 128.6 (Ar C_q), 124.7 (s1), 124.0 (p4), 124.0 (p4'), 123.3 (s2), 119.3 (s3), 79.9 (d7), 56.9 (d4), 41.7 (p1α), 41.4 (p1α'), 31.2 (d2), 30.9 (d3), 29.5 (d5), 29.5 (d6), 28.1 (d8); **NSI$^+$-HRMS** for $C_{380}H_{588}N_{32}O_{96}^{4+}$ $[M+4H]^{4+}$ calculated; 1786.0638, found; 1786.0614; t_R: (Acetone:water 70:30→ 100% acetone over 40 minutes, 16 mL/min flow rate) 39.2 mins (analytical), 34.4 mins (preparative). *^{13}C data assigned using HSQC & HMBC.*

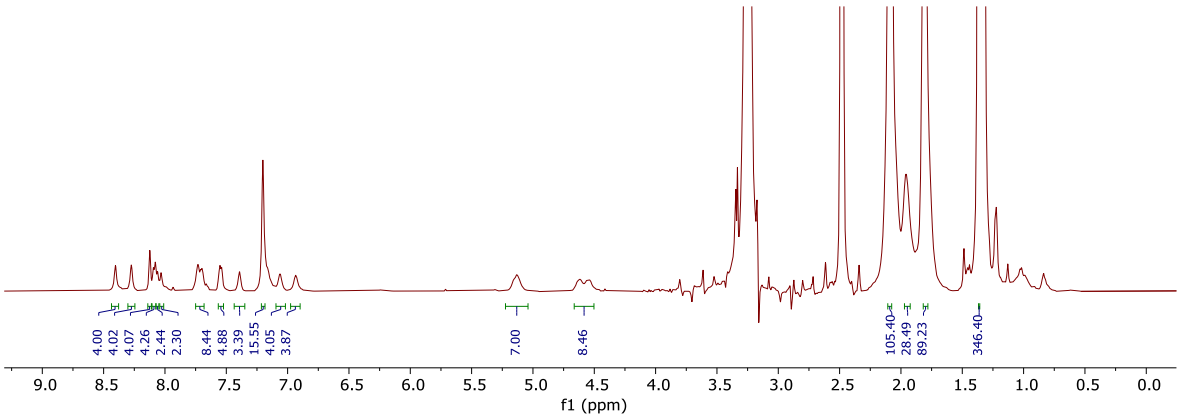
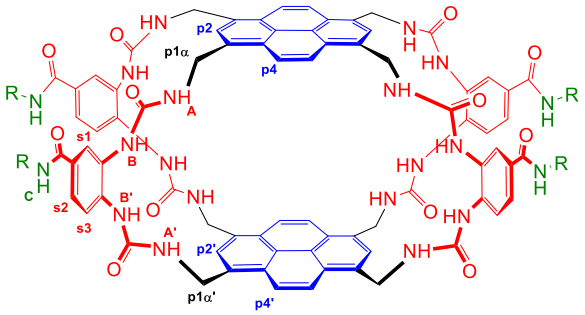
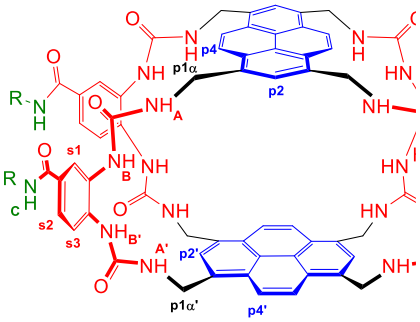


Figure S4. ^{1}H NMR (500 MHz, $(CD_3)_2SO$, 313 K) spectrum of staggered protected macrocycle **13**.

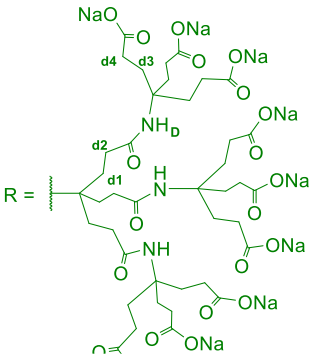
Deprotected pyrene-pyrene macrocycles (5 & 6)



5



6



Protected receptor **12** or **13** (10 mg, 1.40 µmol) was dissolved in DCM (4 mL) and the solution cooled to 0°C. TFA (2 mL) was added dropwise, and the solution warmed to rt before stirring for 24 h. The solvent was removed under a steady stream of N_2 before being suspended in water and freeze dried. The resulting solid was re-suspended in water and the pH adjusted to 7.4 by addition of 0.1 M NaOH. The solution was filtered (0.45 µM syringe filter) and freeze dried, affording the deprotected receptor (**5** or **6**) (7 mg, 85%) as a pale-yellow solid.

The ^{1}H and ^{13}C NMR spectra of the free receptors were preliminarily assigned using 2D methods at 85 °C due to signal broadening at room temperature. Some signals were still not observed at high temperatures, presumed to be due to signal broadening persisting. Additionally, prolonged experiments at this temperature led to partial degradation of the receptors which further hindered characterisation. The receptors were later fully characterised as host:NDI complexes.

Eclipsed Receptor 5:

^{1}H NMR (500 MHz, D_2O, 358 K) δ 8.40 (s, 4H, ArH), 8.32 (s, 2H, ArH), 8.26 – 8.07 (m, 8H, ArH), 5.34 (s, 4H, p1α), 5.14 (s, 4H, p1α), 2.90 – 2.67 (m, 96H, d2 & d4), 2.67 – 2.57 (m, 24H, d1), 2.51 – 2.32 (m, 72H, d3). **^{13}C NMR** (126 MHz, D_2O, 358 K) δ 126.5 (Ar), 126.3 (Ar), 123.1 (Ar), 42.6 (p1α), 42.2 (p1α), 31.8 (d3), 31.6 (d2 & d4), 31.5 (d1); **ESI^{+}-LRMS** for $C_{236}H_{304}N_{32}O_{96}^{4+}$ $[M+4H]^{4+}$ calculated; 1281.0, found; 1281.7; t_R**:** (H_2O:MeCN 98:2→ 100% MeCN over 20 minutes, 1 mL/min flow rate) 5.30 min (analytical).

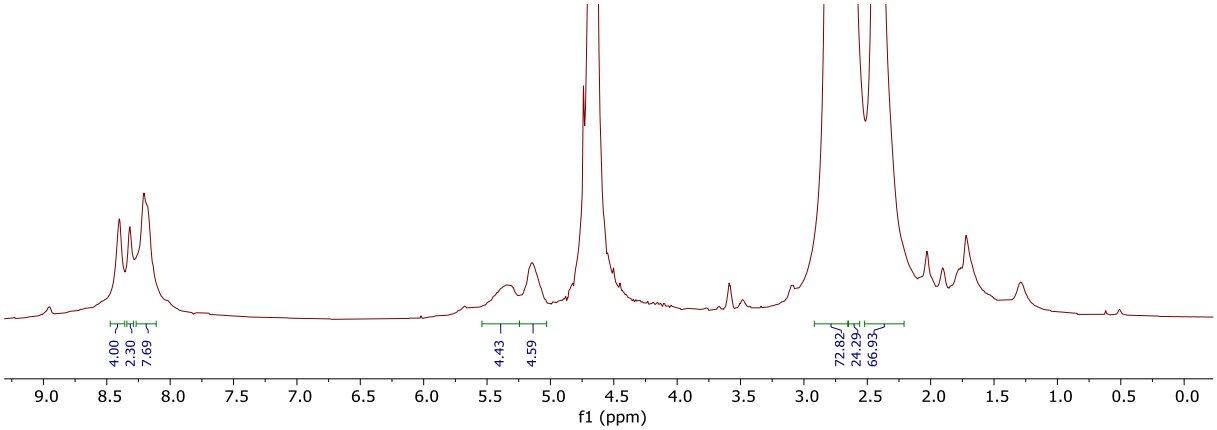


Figure S5. ^{1}H NMR (500 MHz, D_2O, 358 K) spectrum of eclipsed receptor **5**.

Staggered Receptor 6:

^{1}H NMR (500 MHz, D_2O, 358 K) δ 8.93 (s, 2H, p4), 8.27 (d, J = 8.5 Hz, 4H, s2/s3), 8.25 – 8.13 (m, 6H, s2/3 & p2), 8.09 (s, 4H, s1), 5.75 – 5.51 (m, 4H, p1α), 5.41 – 5.27 (m, 2H, p1α), 5.24 – 5.10 (m, 2H, p1α), 2.82 – 2.69 (m, 24H, d2), 2.70 – 2.54 (m, 96H, d1 & d4), 2.46 – 2.19 (m, 72H, d3); **^{13}C NMR** (126 MHz, D_2O, 358 K) δ 126.6 (p2), 126.5 (s1), 126.3 (s2/s3), 124.4 (s2/s3), 123.9 (p4), 43.3 (p1α), 42.7 (p1α), 42.4 (p1α), 41.8 (p1α), 32.4 (d1), 31.7 (d2), 31.7 (d3), 31.6 (d4); **ESI^{+}-LRMS** for $C_{236}H_{304}N_{32}O_{96}^{4+}$ $[M+4H]^{4+}$ calculated; 1281.0, found; 1281.7; t_R**:** (H_2O:MeCN 98:2→ 100% MeCN over 20 minutes, 1 mL/min flow rate) 5.66 mins (analytical).

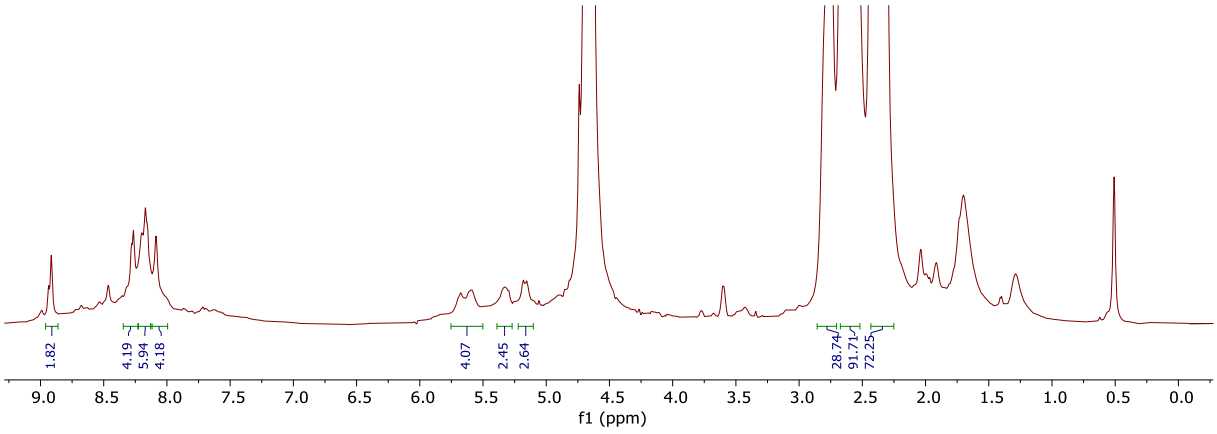


Figure S6. ^{1}H NMR (500 MHz, D_2O, 358 K) spectrum of staggered receptor **6**.

2. Properties

Eclipsed receptor 5

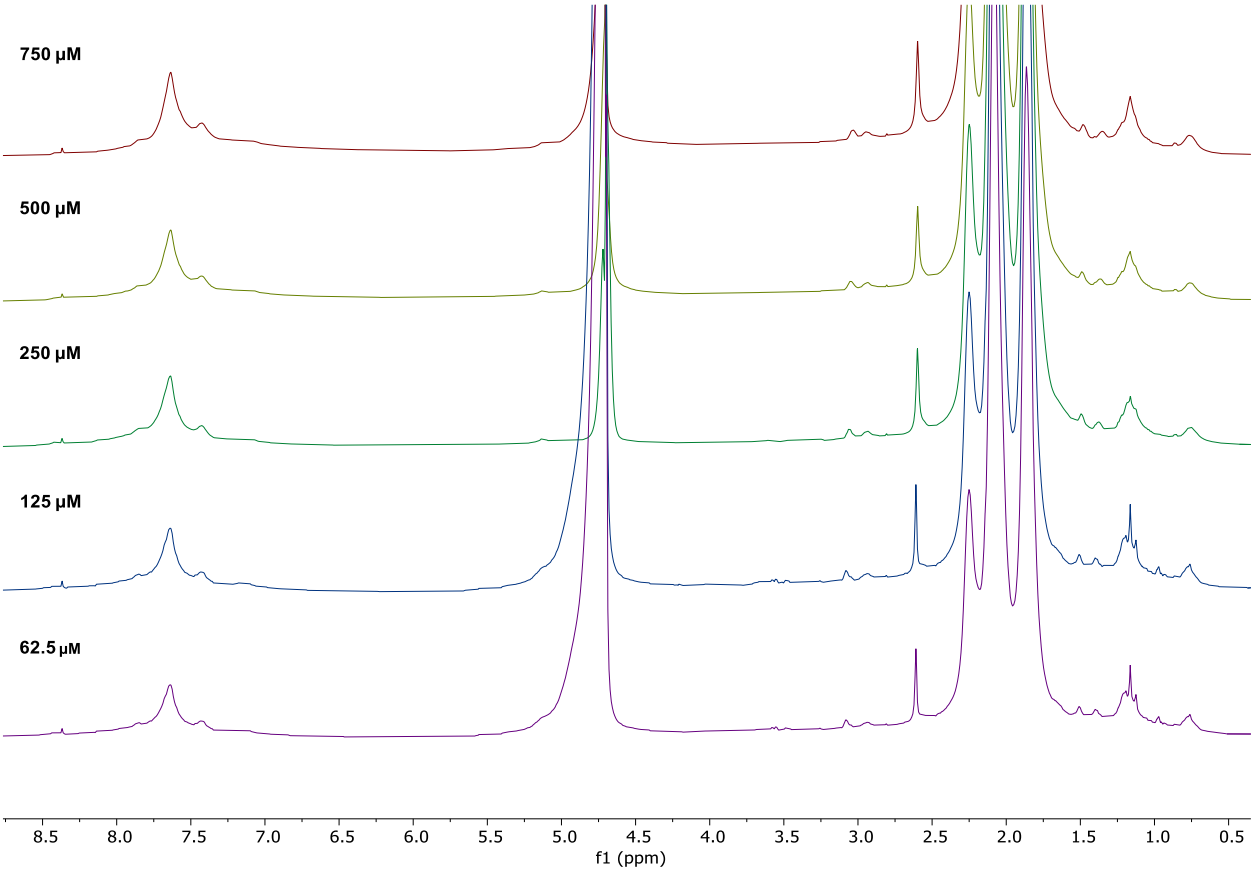


Figure S7. ^{1}H NMR (500 MHz, 298 K, D_2O) of receptor **5** at varying concentrations.

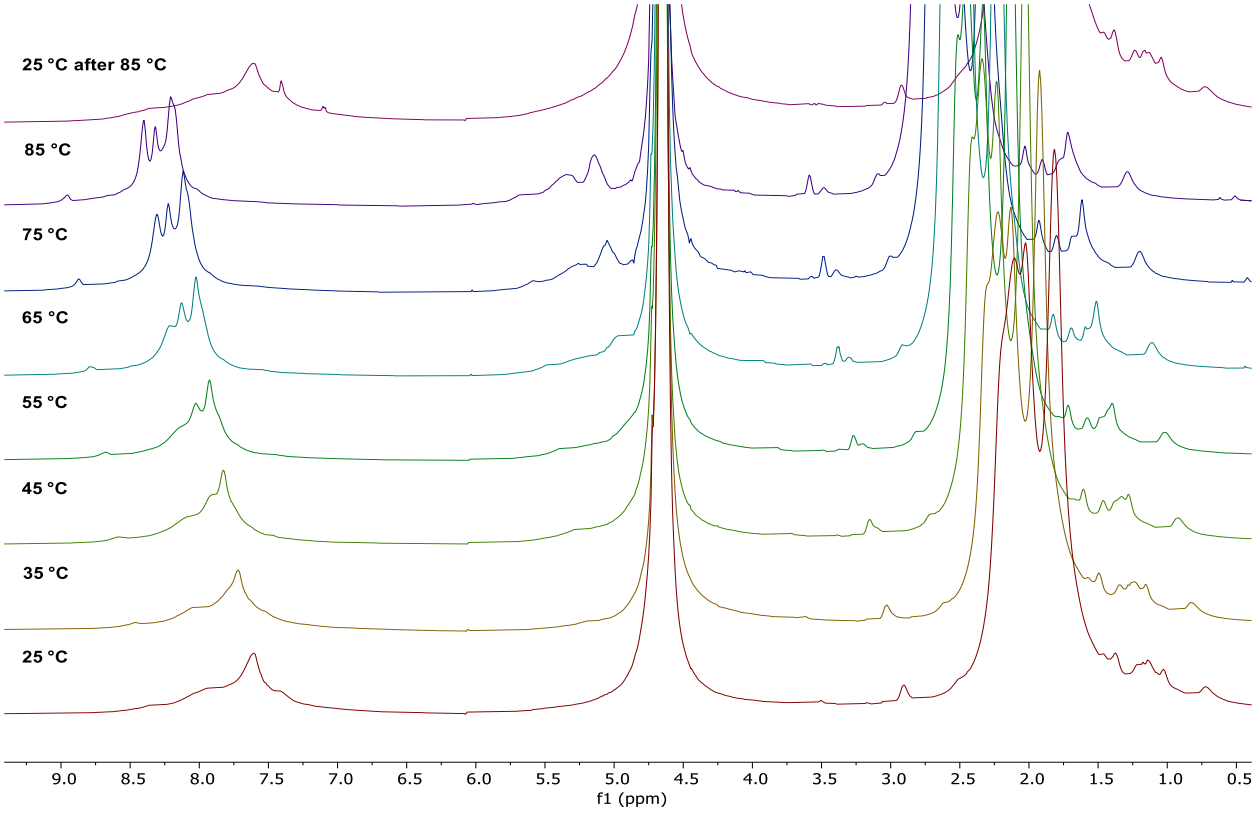


Figure S8. ^{1}H-NMR (500 MHz, D_2O) spectra of receptor **5** at a range of temperatures, 25 °C to 85 °C in 10 °C steps before returning to 25 °C. Signals at 7.09 ppm and 7.40 ppm that appear at 25 °C after heating, are the result of partial degradation.

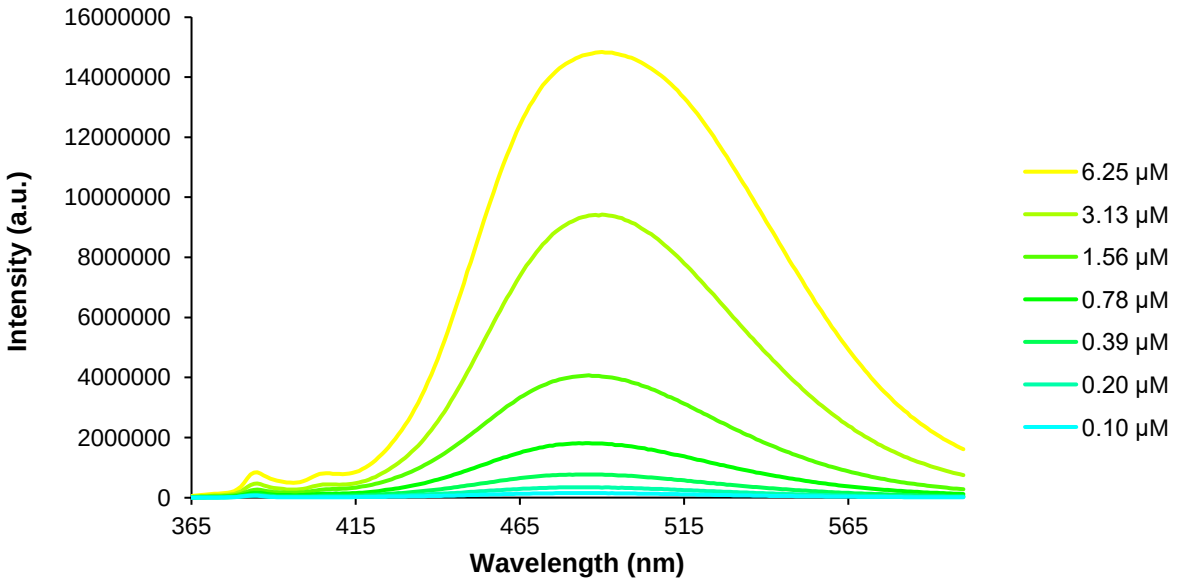


Figure S9. Fluorescence emission spectrum for receptor **5** at concentrations 6.25 to 0.10 µM in H_2O at 298 K, excited at 340 nm.

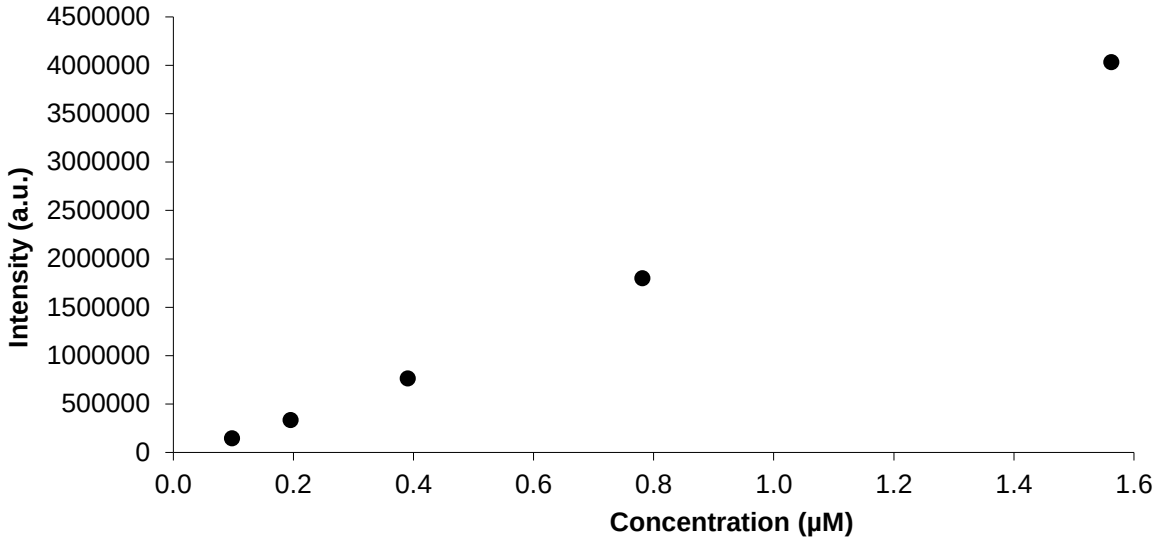


Figure S10. Fluorescence emission intensity at 490 nm for receptor **5**, plotted against increasing receptor concentration in H_2O at 298 K. A roughly linear relationship is observed below 1.56 μM.

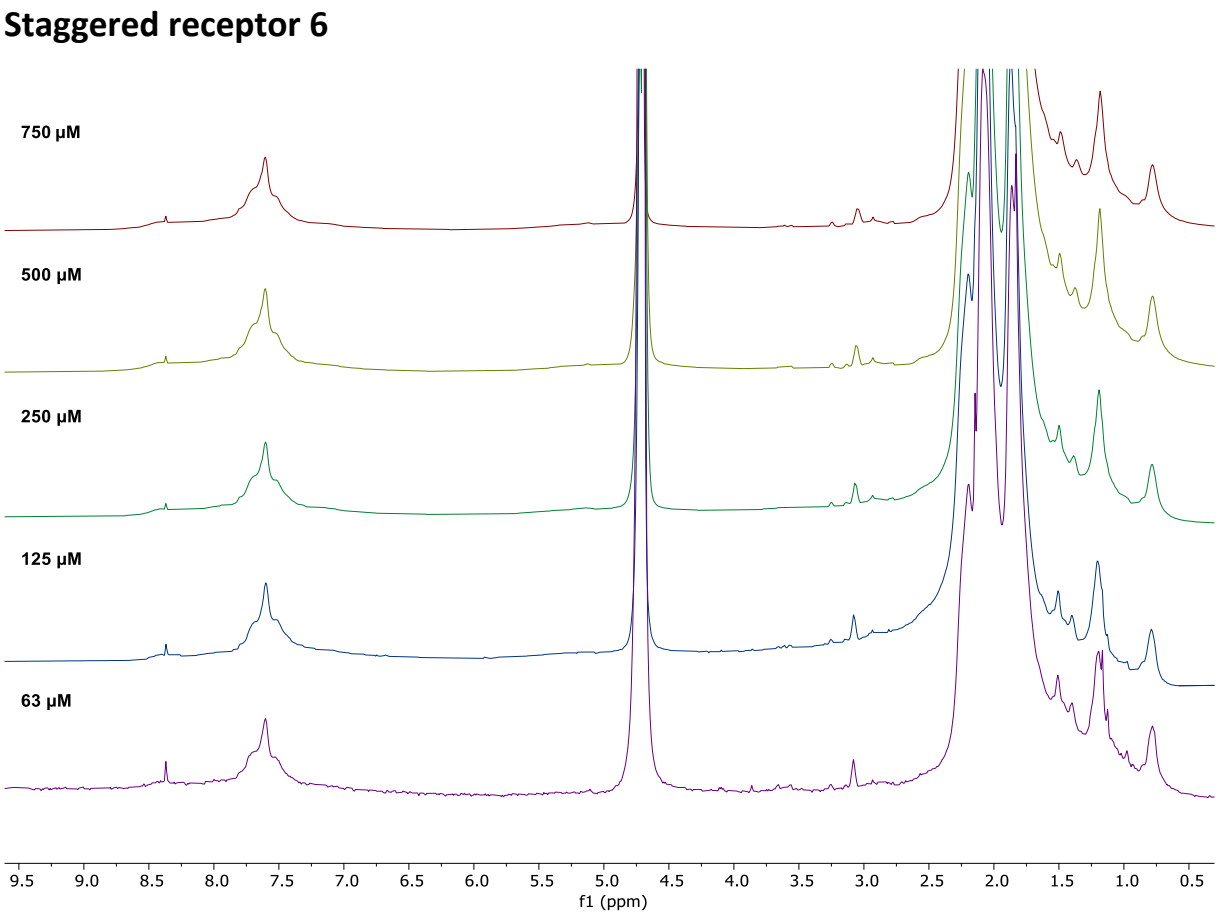


Figure S11. ^{1}H NMR (500 MHz, 298 K, D_2O) of receptor **6** at varying concentrations.

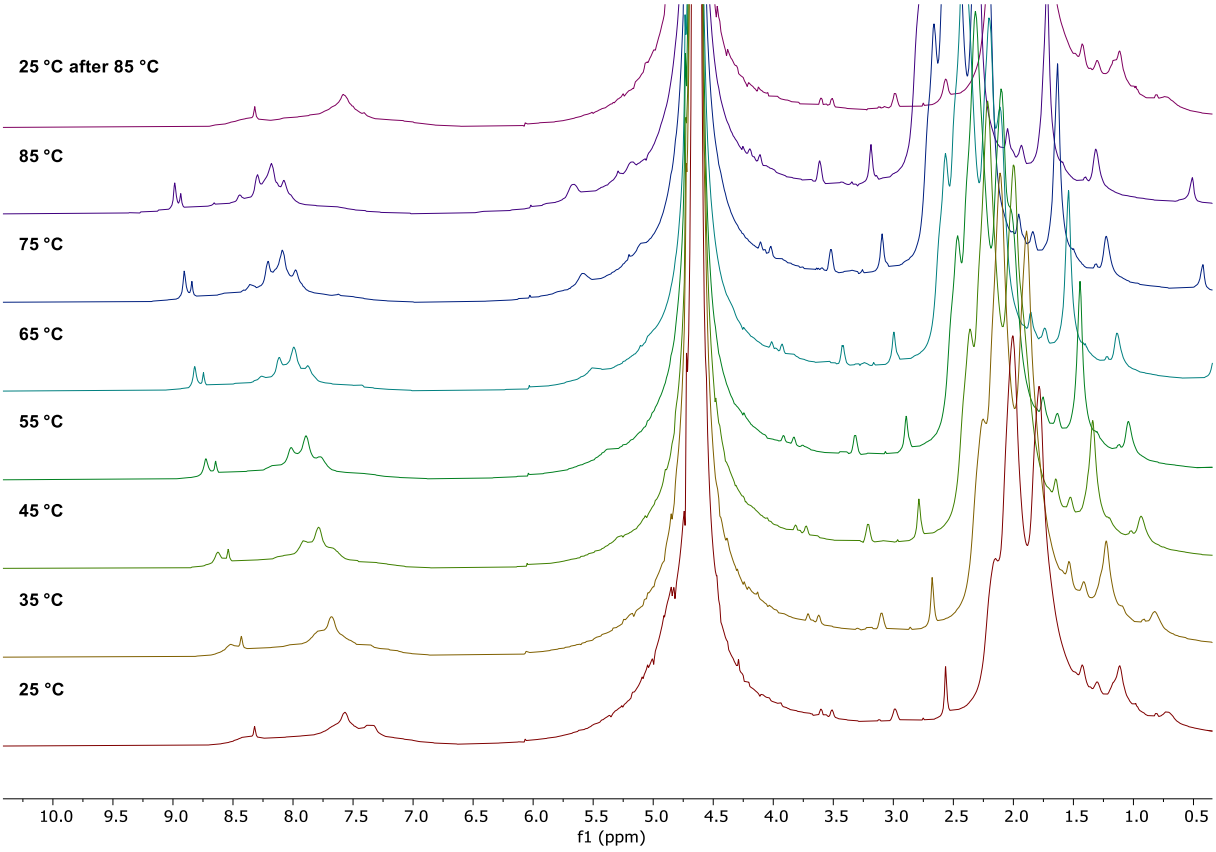


Figure S12. ^{1}H-NMR (500 MHz, D_2O) spectra of receptor **6** at a range of temperatures, 25 °C to 85 °C in 10 °C steps before returning to 25 °C.

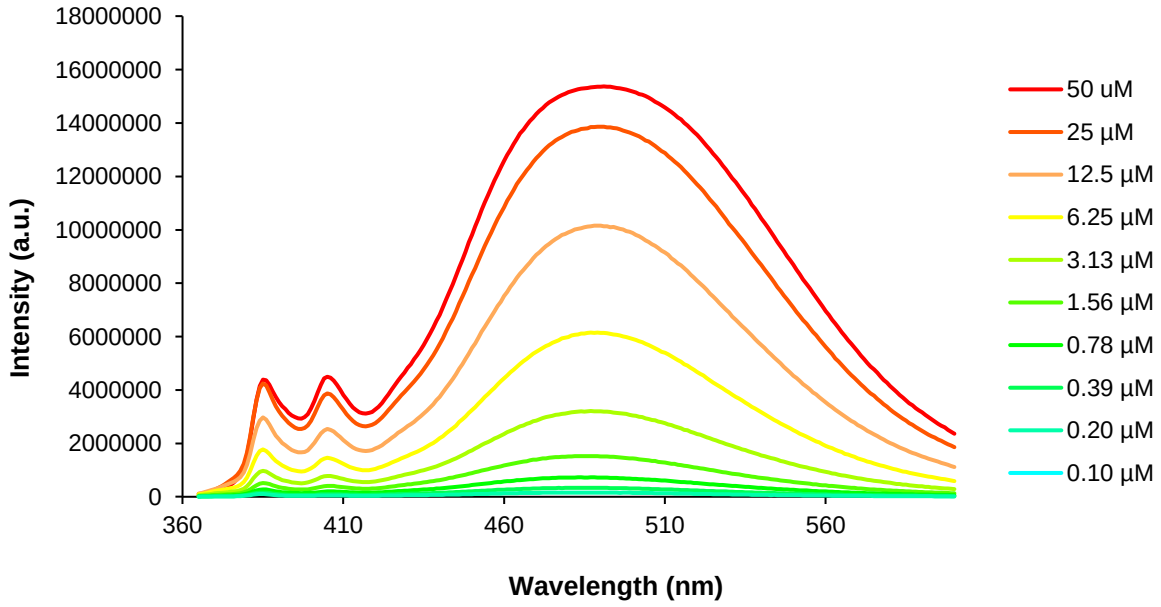


Figure S13. Fluorescence emission spectrum for receptor **6** at concentrations 50 to 0.10 µM in H_2O at 298 K, excited at 340 nm.

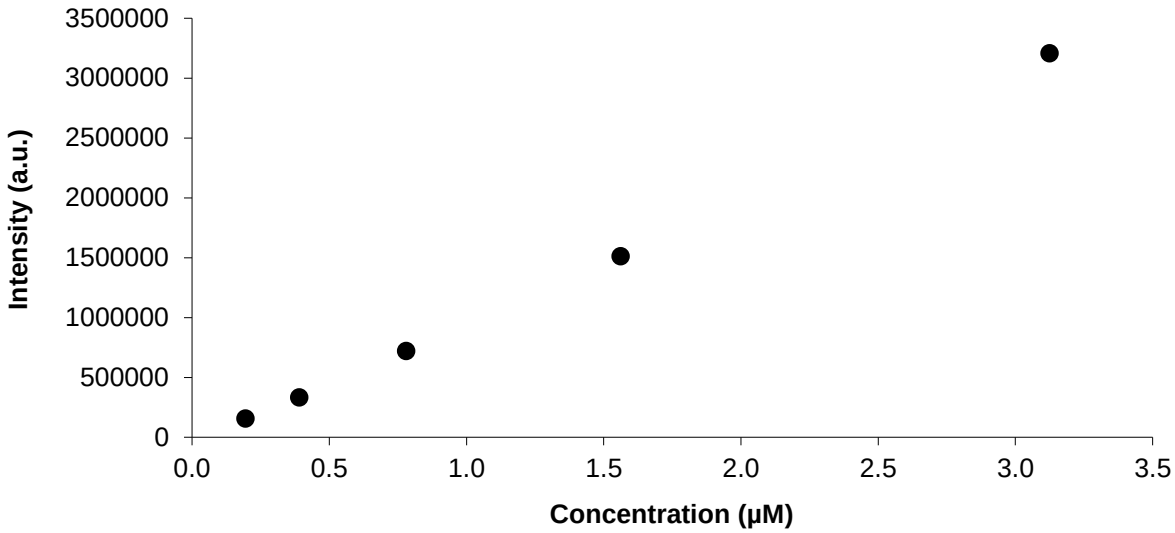


Figure S14. Fluorescence emission intensity at 490 nm for receptor **6**, plotted against increasing receptor concentration in H_2O at 298 K. A roughly linear relationship is observed below 3.13 μM.

3. Binding studies

3.1 Methods

^{1}H NMR titrations were performed on a Varian VNMRS Cryo 600 MHz or Bruker Advance III HD Cryo 500 MHz spectrometer. A stock solution of receptor in D_2O with 10 mM phosphate buffer (pH 7.4) was prepared. A stock solution of the guest was prepared by dissolving the desired guest in the aforementioned receptor stock solution. Where the guest was a reducing sugar, the solution was left to equilibrate overnight before use. Aliquots of this solution were then added to an NMR tube containing a known concentration of receptor solution. The receptor concentration was therefore held constant whilst the guest concentration was increased. The NMR tube was shaken after each addition, manually centrifuged and the ^{1}H-NMR spectra acquired at 298 K.

Fluorescence titrations were performed on a Horiba FluoroMax Plus spectrometer at 25 °C, maintained using the thermostat feature. A stock solution of receptor in HPLC grade H_2O with 10 mM phosphate buffer (pH 7.4) was prepared. A stock solution of the guest was prepared by dissolving the desired guest in the aforementioned receptor stock solution. Where the guest was a reducing sugar, the solution was left to equilibrate overnight before use. In order to maintain a constant volume over the course of the titration, a known volume was removed from a four-sided Hellma 111-QS quartz cuvette (3 mL, 10 mm path length) containing a known concentration of receptor solution. This volume was then replaced using the receptor:guest stock solution. After the addition, the contents of the cuvette were stirred with micro stirrer bar for 2 mins. The solution allowed to settle for a further 1 min before the spectrum was collected twice ensuring the intensity was stable. Fluorescence emission intensity was recorded in arbitrary units (a.u.) and emission wavelength in nanometres (nm).

Calculation of binding constants (K_a) were performed using a specifically written non-linear least squares curve-fitting programme using a 1:1 binding model implemented within Microsoft Excel.

3.2 Binding data and analyses

3.2.1 Eclipsed receptor 5

Hypoxanthine

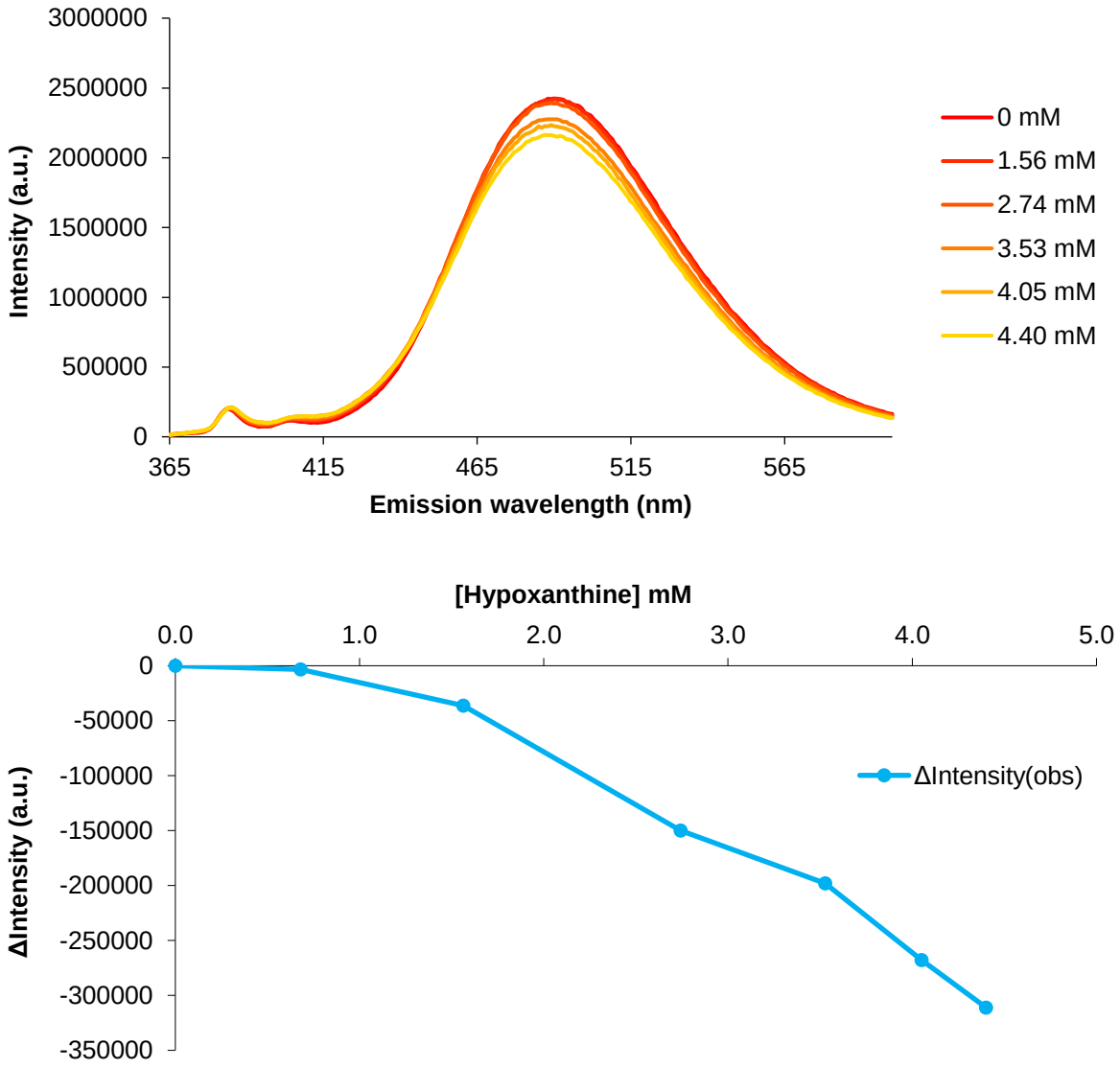


Figure S15. Fluorescence emission for receptor **5** (350 nM) titrated with a combined solution of hypoxanthine **24** (5 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The insolubility of the guest in water meant only points in the low millimolar range could be obtained. Therefore, it was not possible to obtain a binding constant. The experiment was performed twice with similar results.

Adenine

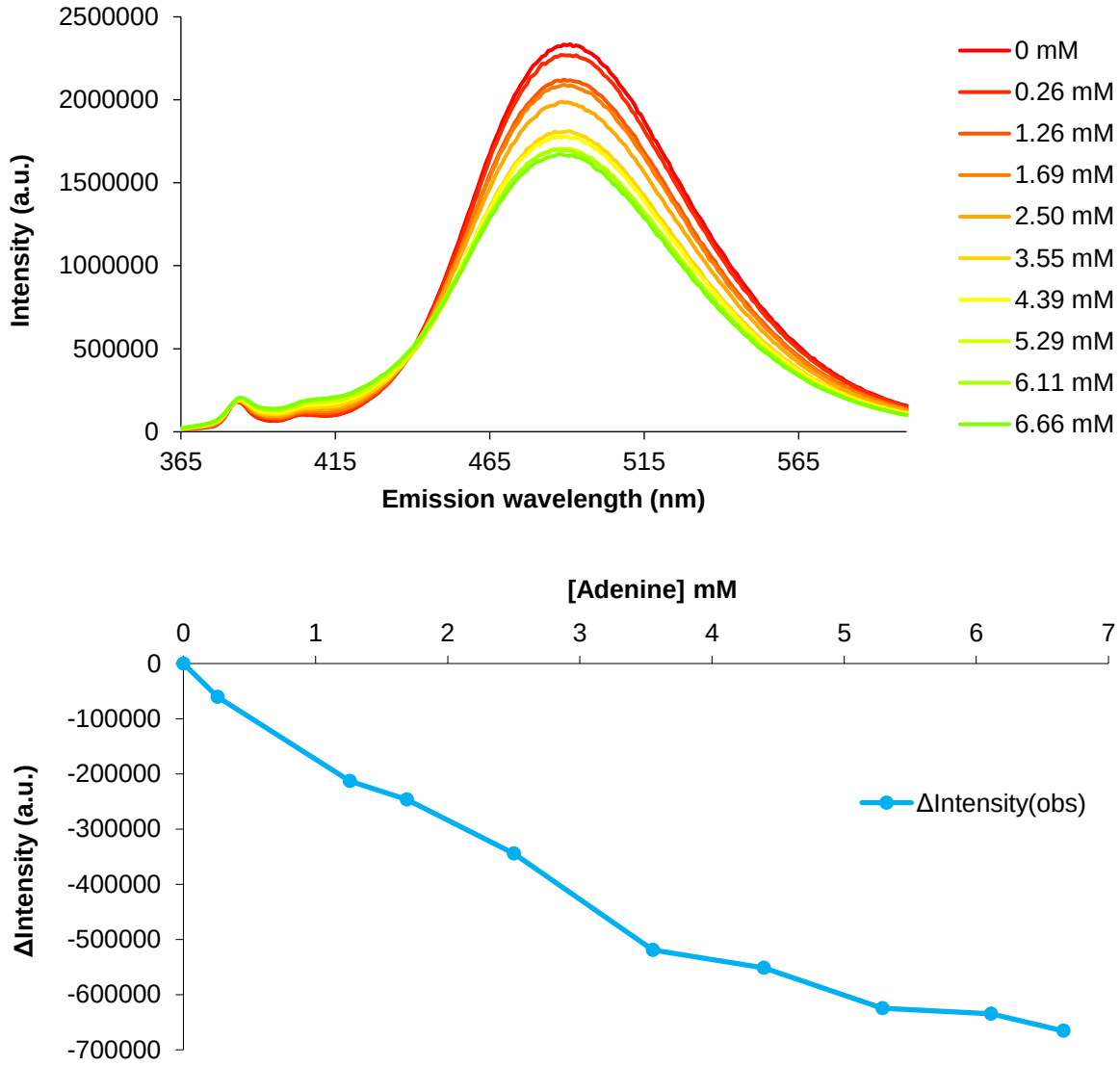


Figure S16. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (350 nM) titrated with a combined solution of adenine **25** (8 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The insolubility of the guest in water meant only points in the low millimolar range could be obtained. Therefore, it was not possible to obtain a binding constant. The experiment was performed twice with similar results.

Thymine

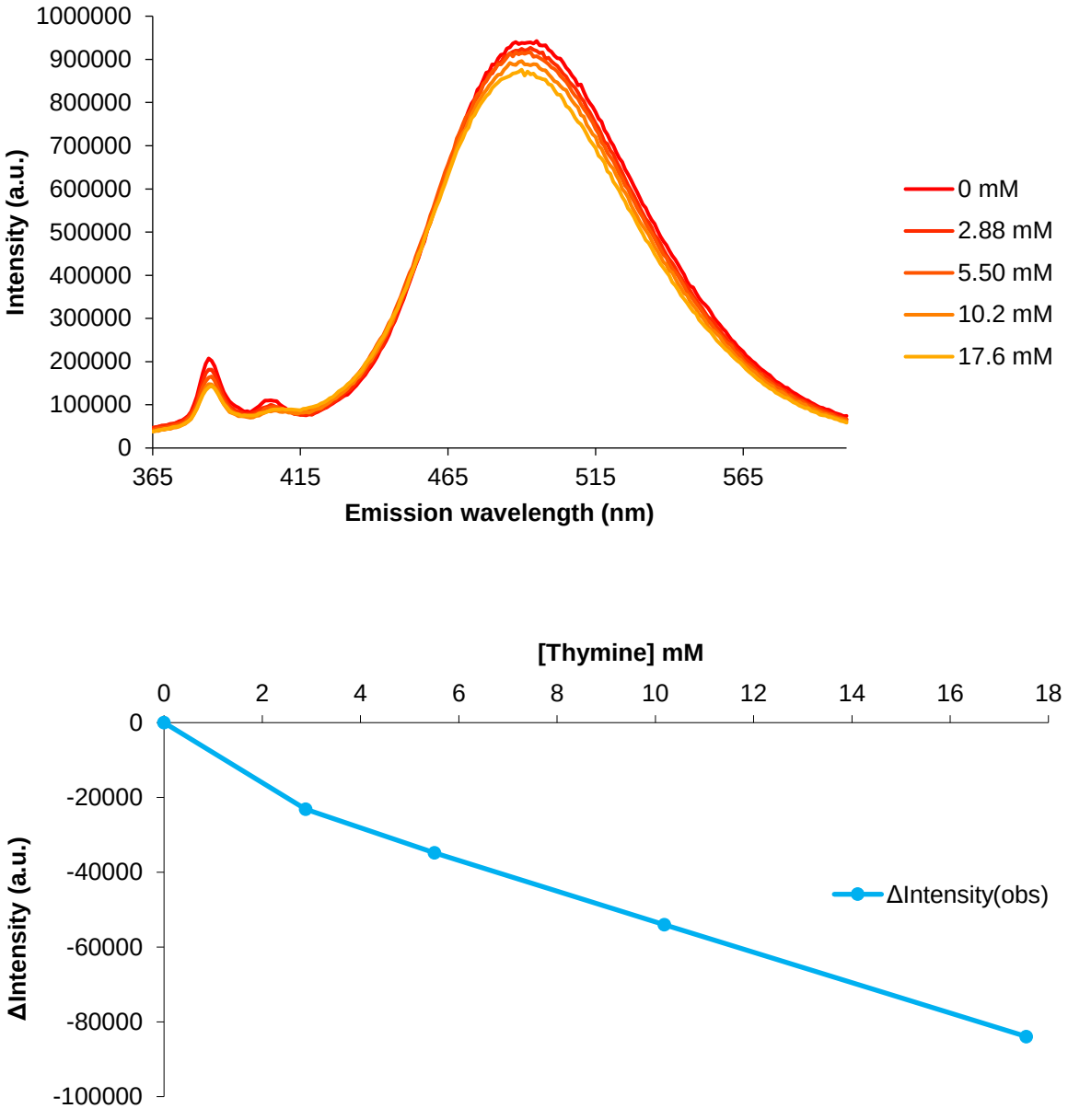


Figure S17. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor 5 (350 nM) titrated with a combined solution of thymine **26** (27 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The insolubility of the guest in water meant only points in the low millimolar range could be obtained. Therefore, it was not possible to obtain a binding constant. The experiment was performed once.

L-Tryptophan

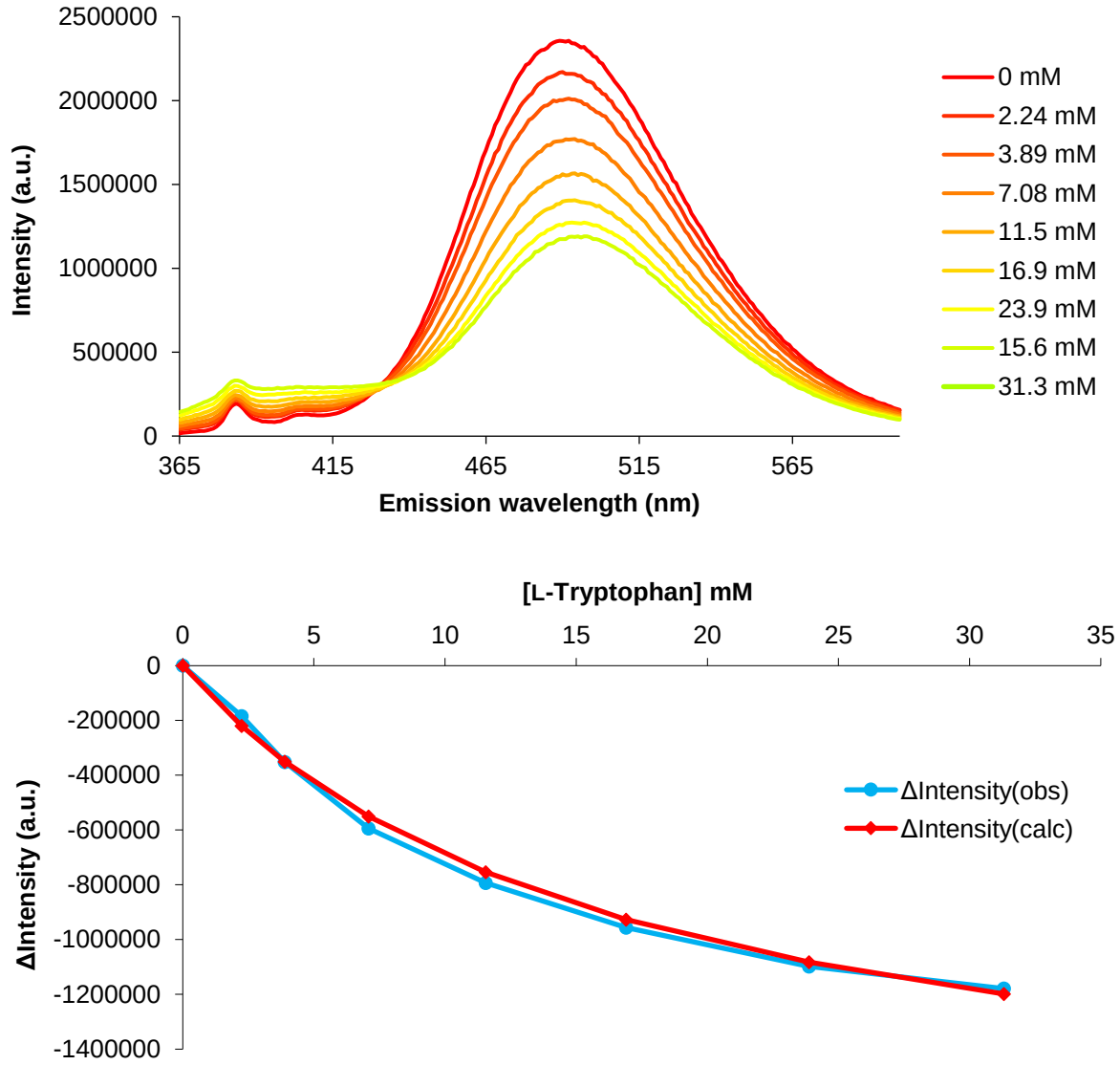


Figure S18. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (350 nM) titrated with a combined solution of L-tryptophan **27** (52 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 61 ± 5 M^{-1}. The experiment was performed once.

Indole

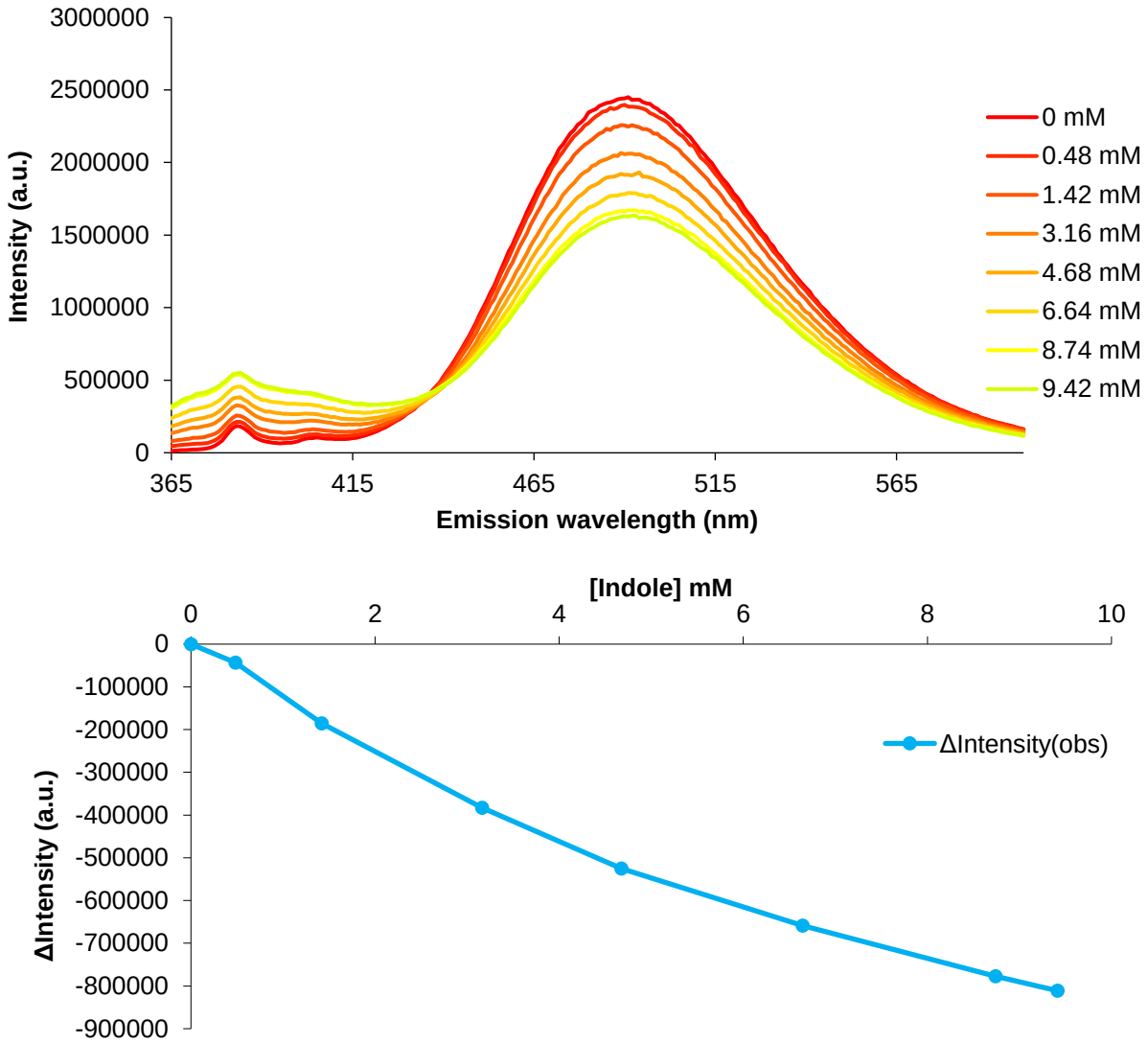


Figure S19. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (350 nM) titrated with a combined solution of indole **28** (15 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The insolubility of the guest in water meant only points in the low millimolar range could be obtained. Therefore, it was not possible to obtain a binding constant. The experiment was performed once.

Paracetamol

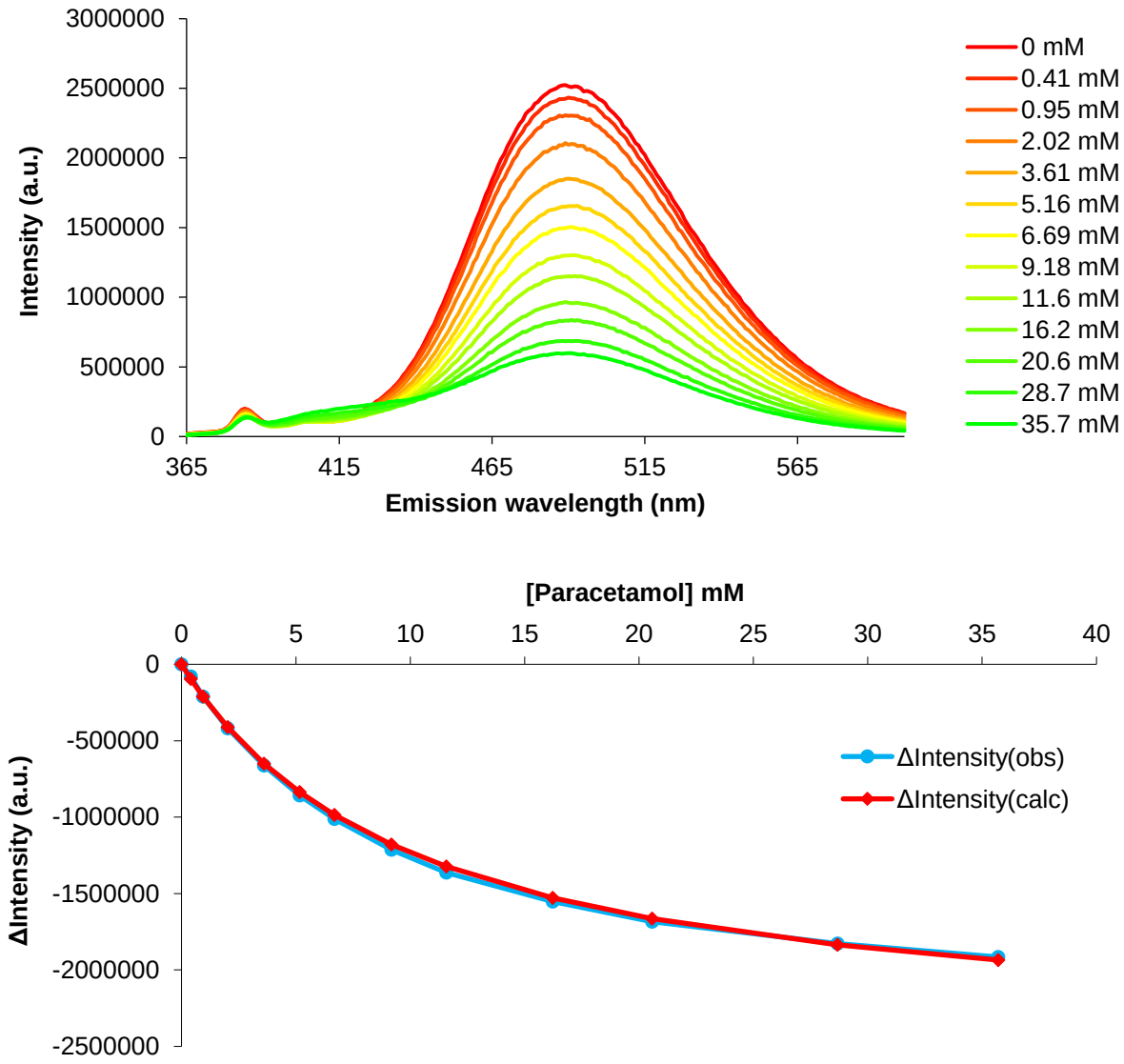


Figure S20. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (350 nM) titrated with a combined solution of paracetamol **29** (81 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 98 ± 5 M^{-1}. The experiment was performed once.

Caffeine

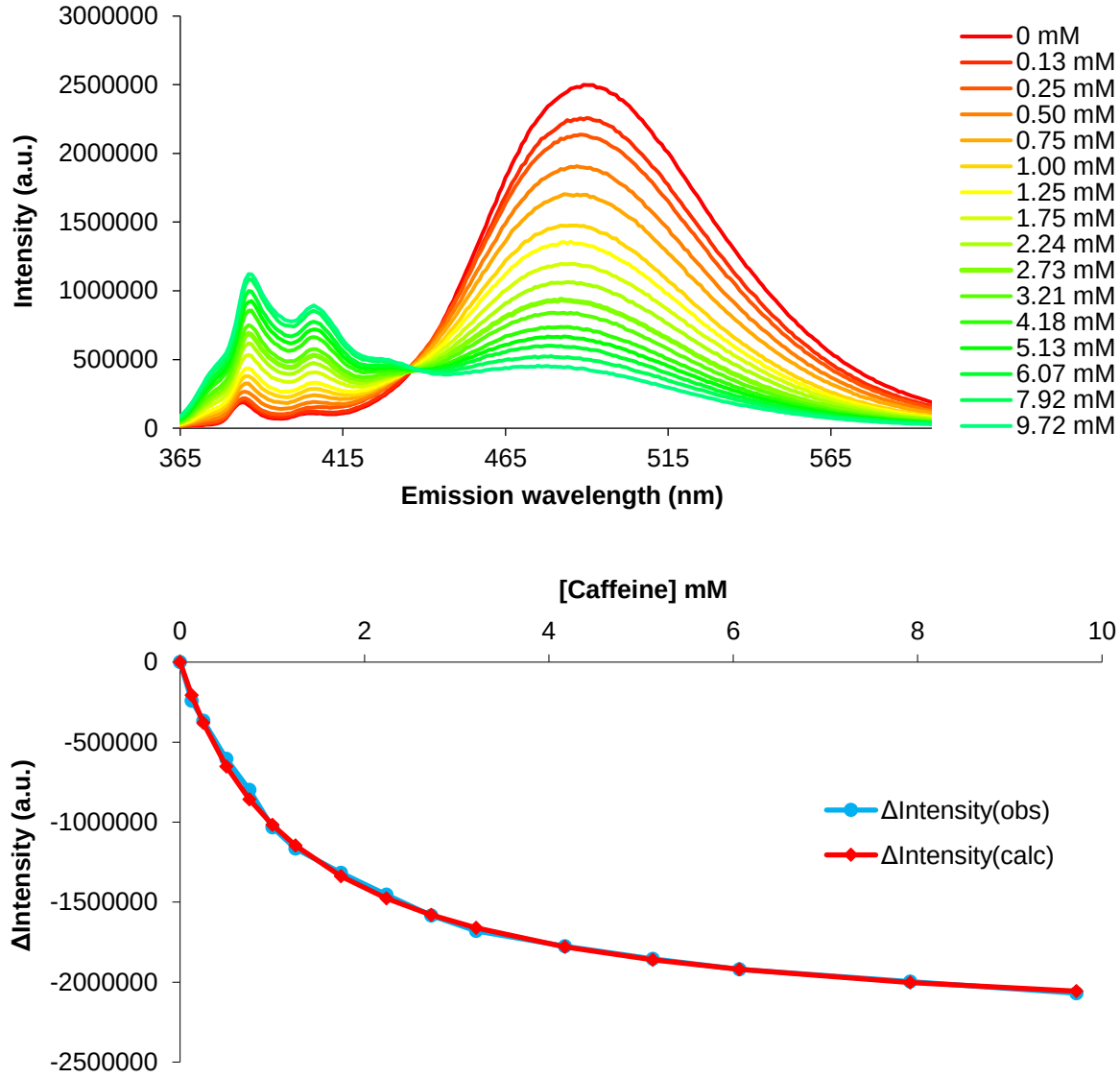


Figure S21. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (350 nM) titrated with a combined solution of caffeine **30** (76 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 774 ± 41 M^{-1}. The experiment was performed three times with similar results.

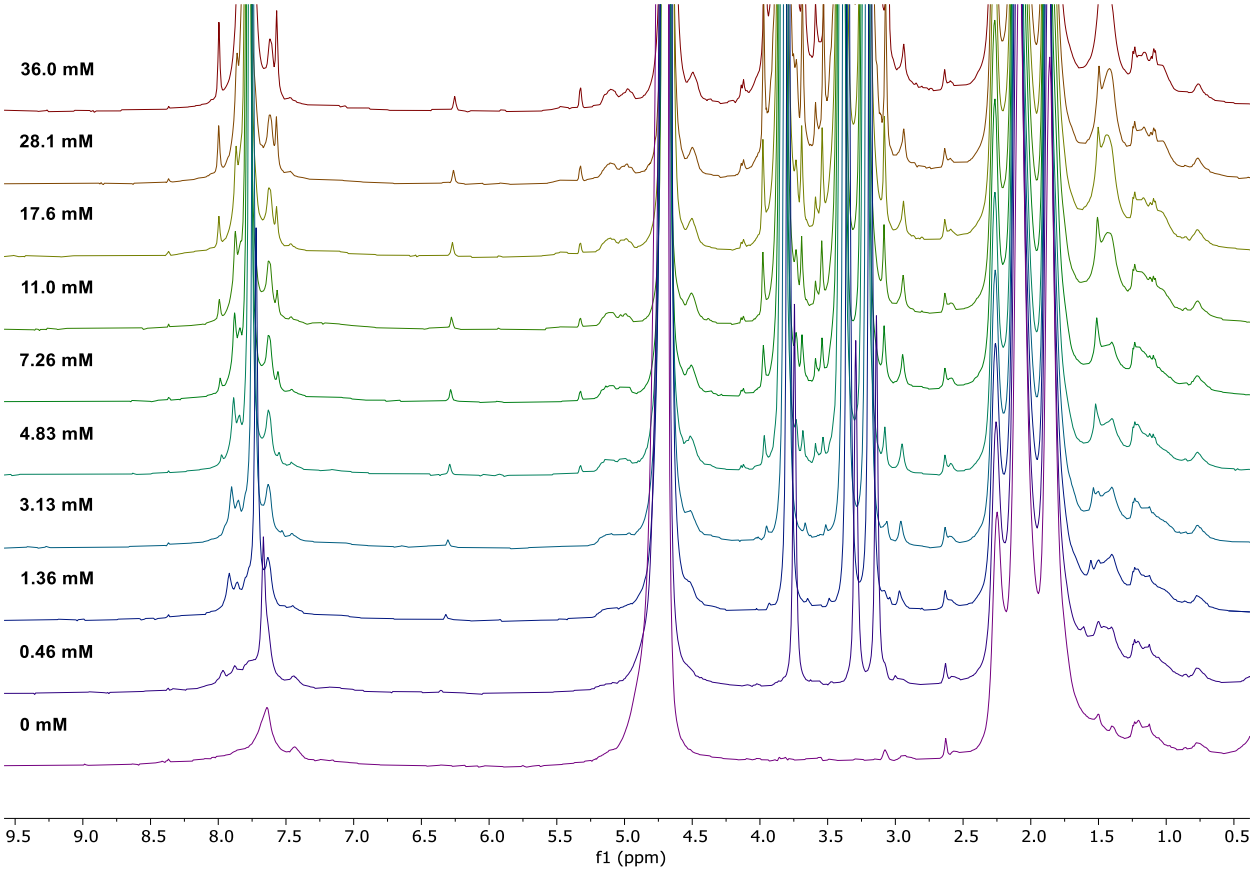


Figure S22. ^{1}H NMR (500 MHz, D_2O with 10 mM phosphate buffer (pH 7.4), 298 K) spectra for receptor **5** (150 µM) titrated with a combined solution of caffeine **30** (92 mM) and receptor **5** (150 µM). Spectral changes imply binding; however this could not be quantified from this study.

Theobromine

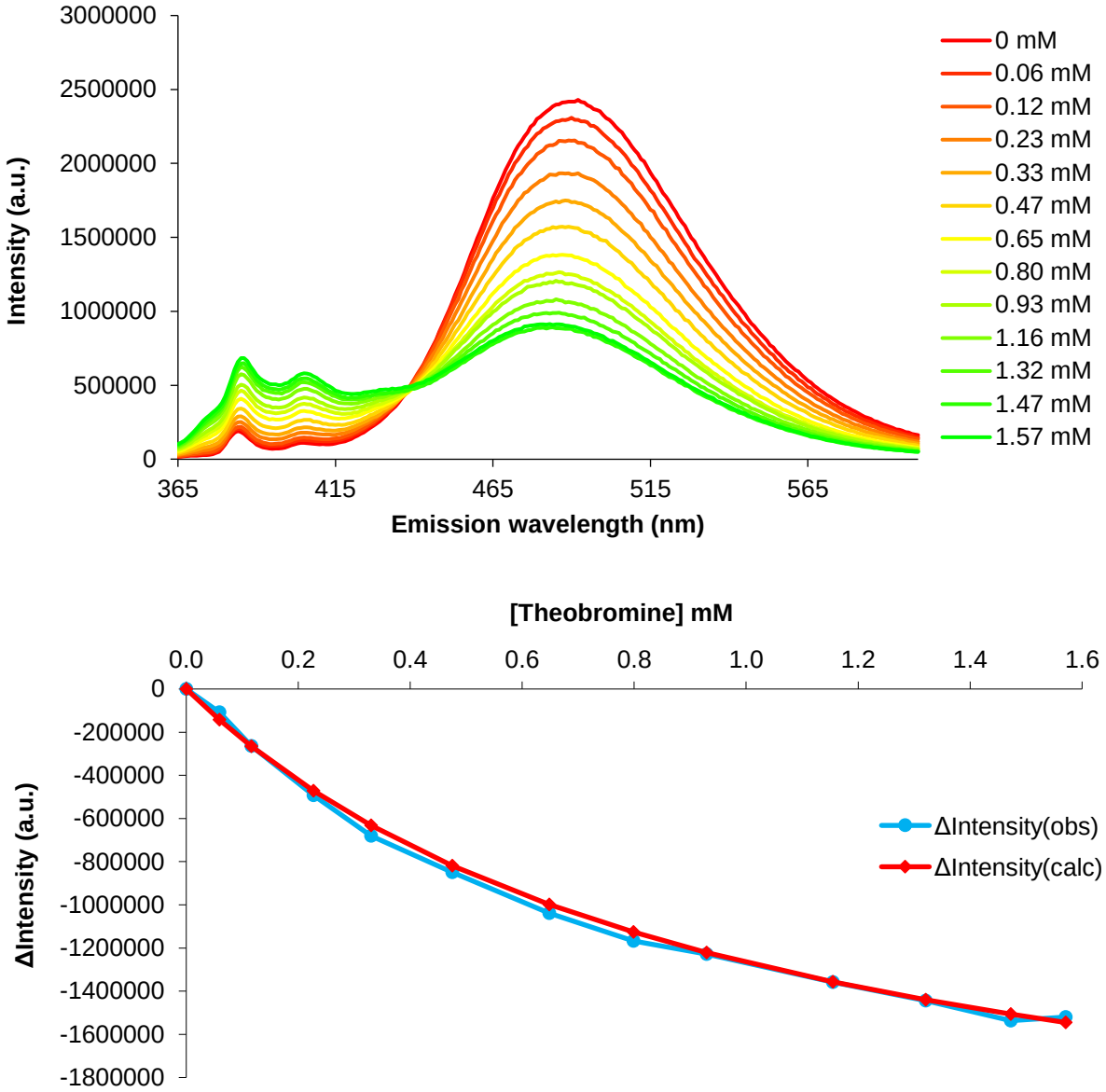


Figure S23. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (350 nM) titrated with a combined solution of theobromine **31** (1.8 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 1017 ± 82 M^{-1}. Due to guest insolubility in water, only points in the low millimolar range could be obtained and hence, saturation of the receptor was not reached. The experiment was performed three times with similar results.

Theophylline

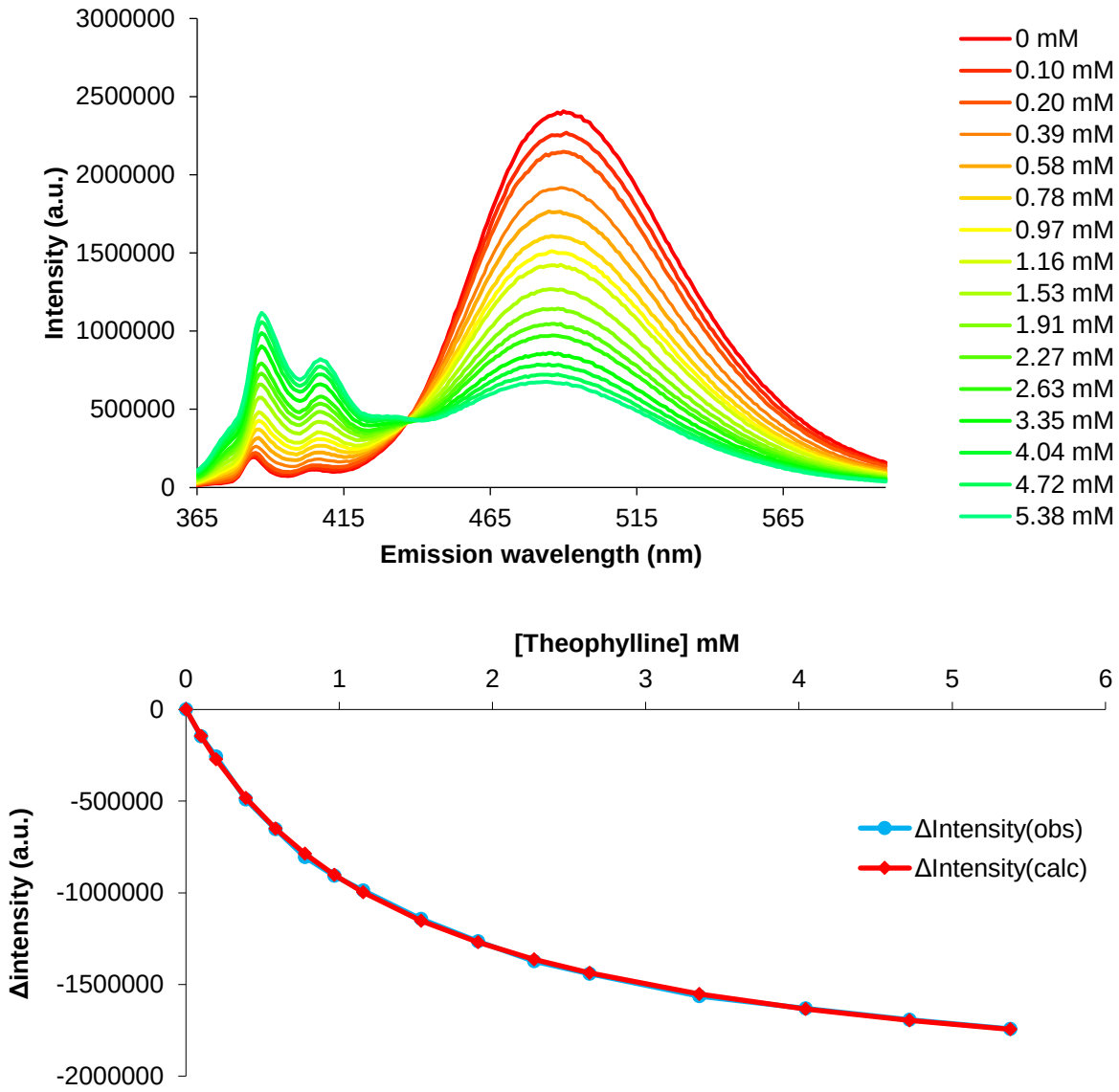


Figure S24. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (350 nM) titrated with a combined solution of theophylline **32** (29 mM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 720 ± 13 M^{-1}. The experiment was performed three times with similar results.

Bis(dimethylamino) NDI **33**

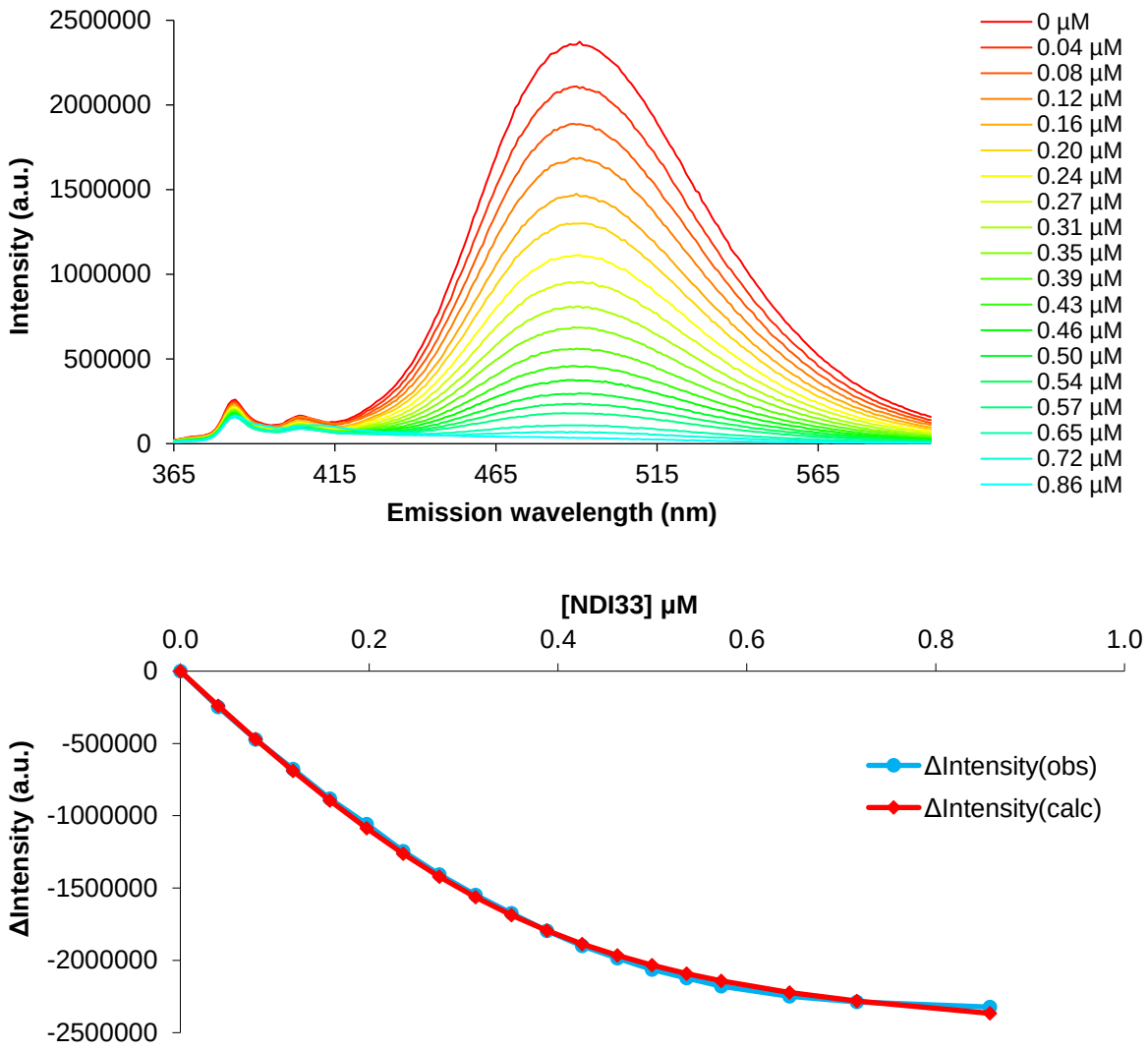


Figure S25. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (370 nM) titrated with a combined solution of NDI **33** (6 µM) and receptor **5** (370 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 1.5 x 10^7 M^{-1} ± 1.55%. The experiment was performed three times with similar results.

Bis(trimethylammonium) NDI **34**

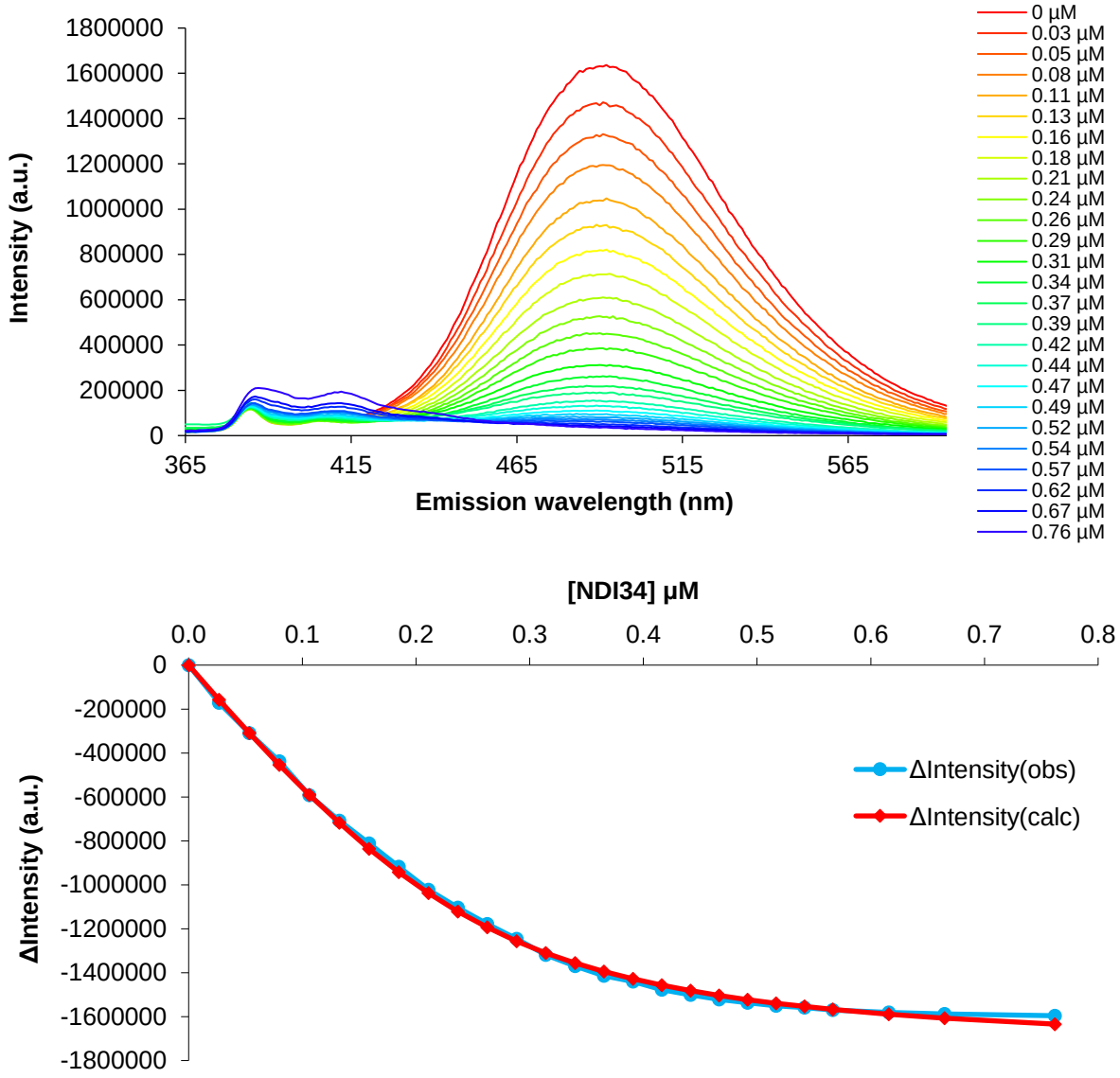


Figure S26. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (250 nM) titrated with a combined solution of NDI **34** (8 µM) and receptor **5** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 2.2 x 10^7 M^{-1} ± 2.42%. The experiment was performed three times with similar results.

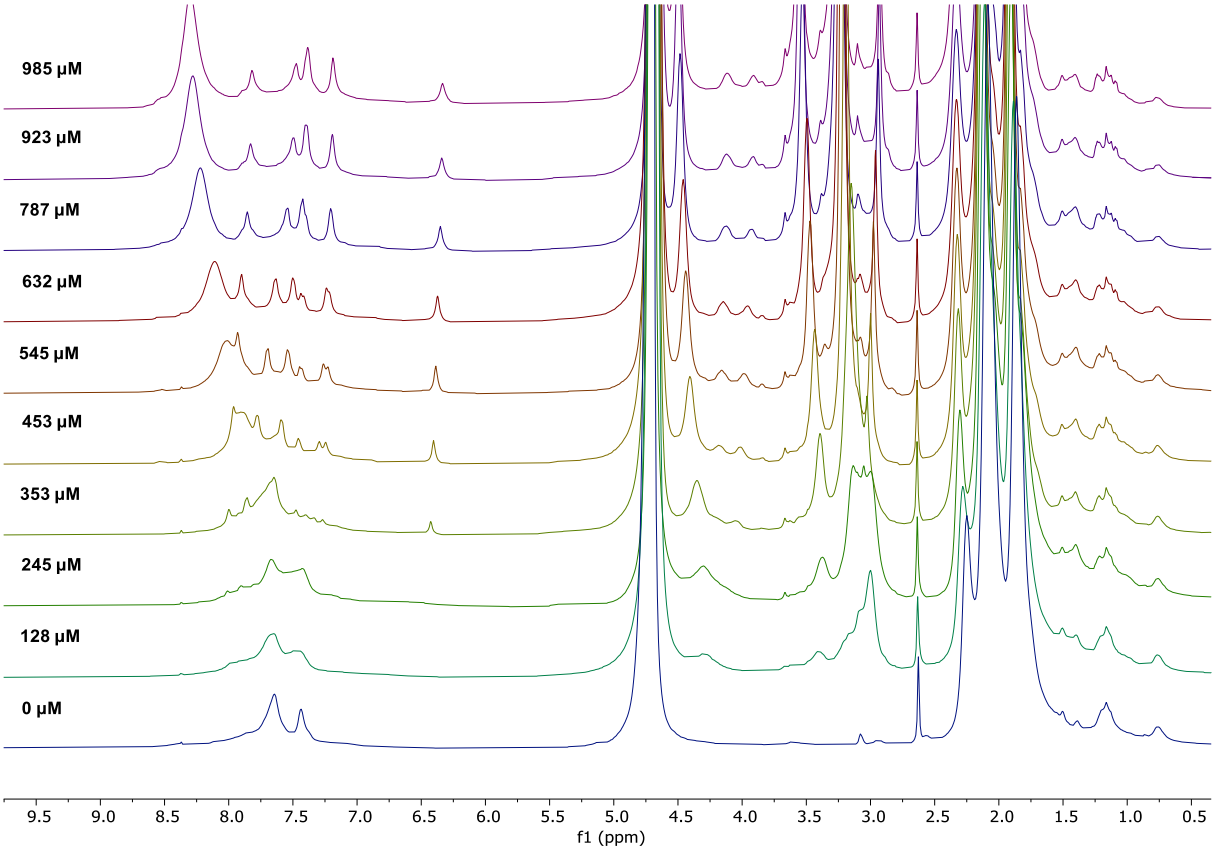


Figure S27. ^{1}H NMR (500 MHz, D_2O with 10 mM phosphate buffer (pH 7.4), 298 K) spectra for receptor **5** (163 µM) titrated with a combined solution of NDI **34** (3 mM) and receptor **5** (163 µM). Spectra imply binding; however, this could not be quantified from this study.

NDI **35**

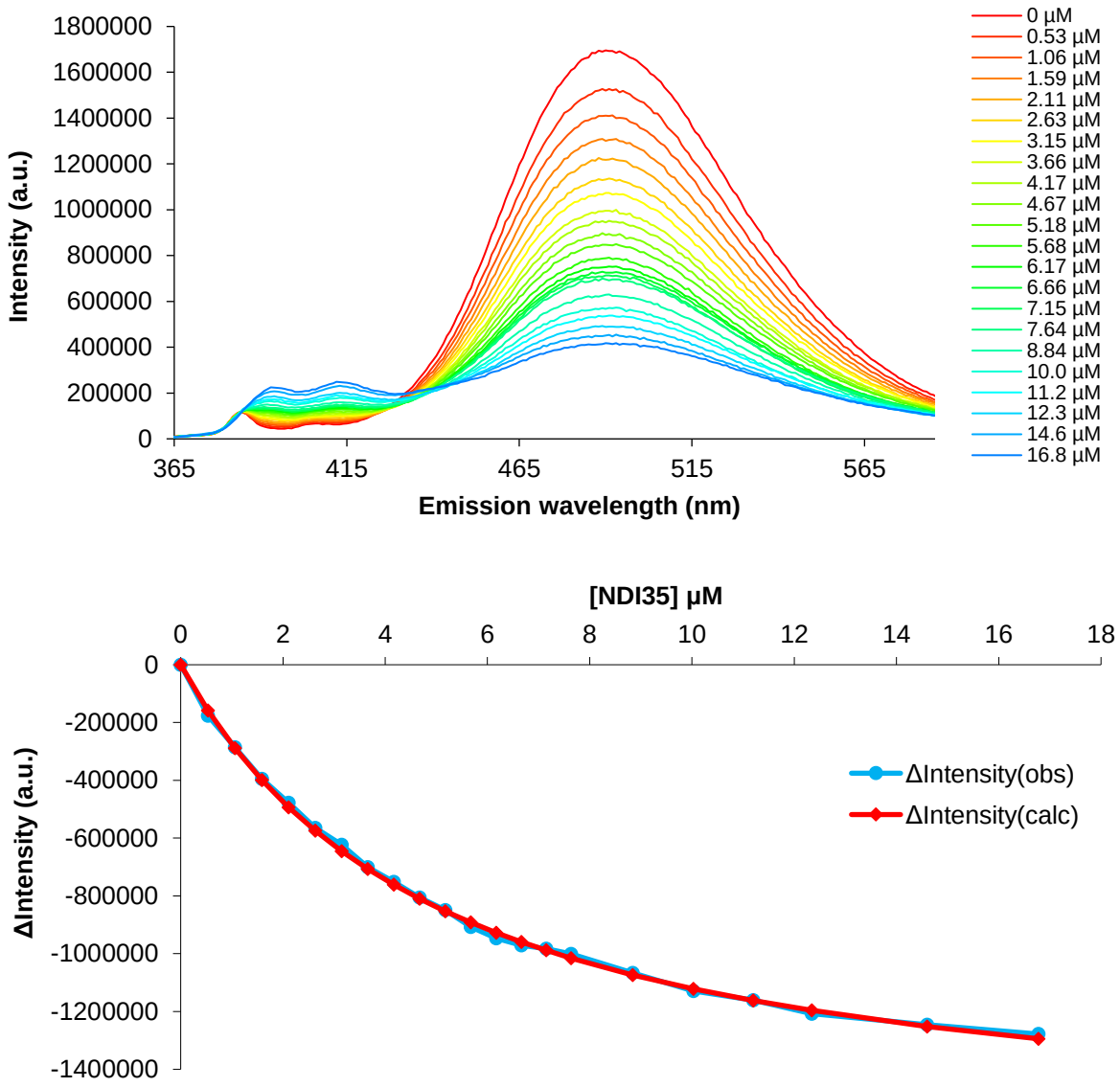


Figure S28. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **5** (340 nM) titrated with a combined solution of NDI **35** (80 µM) and receptor 5 (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 2.1 x 10^5 M^{-1} ± 3.01 %. The experiment was performed three times with similar results.

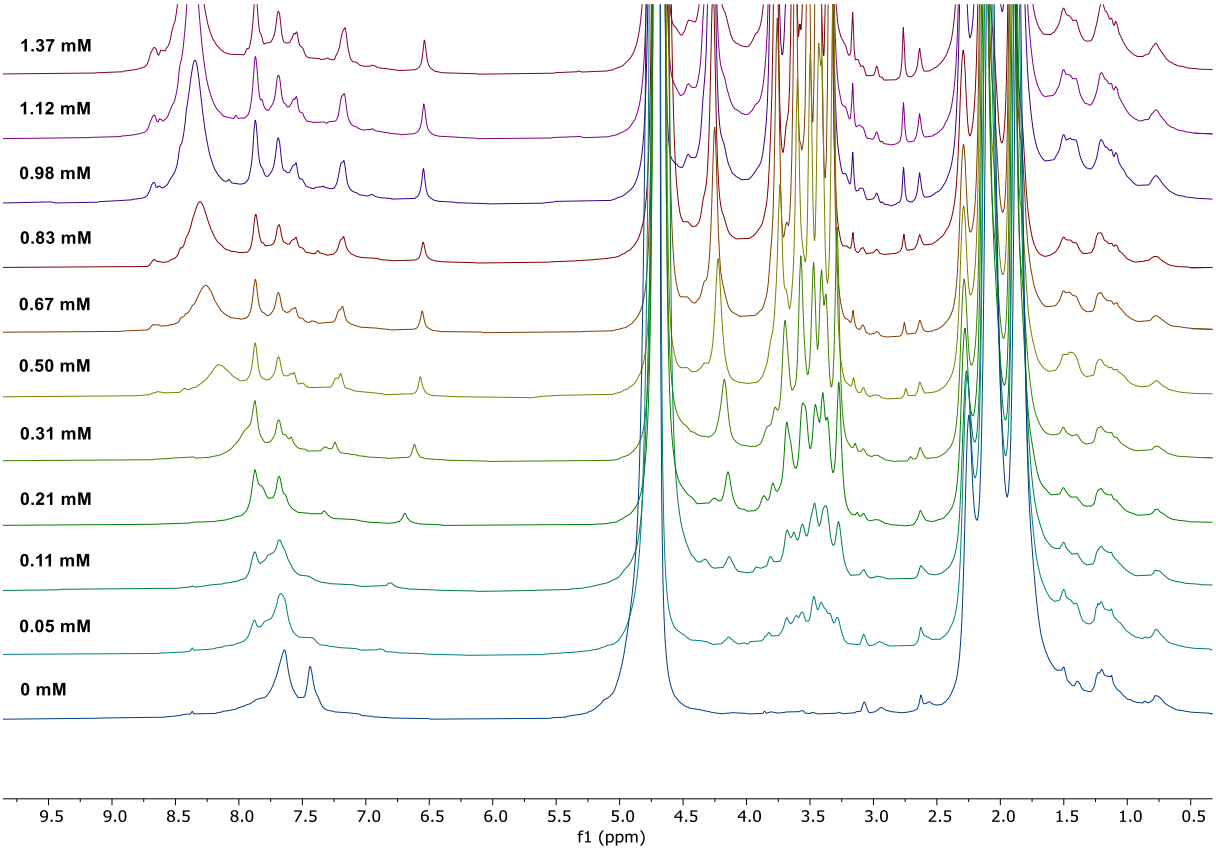


Figure S29. ^{1}H NMR (500 MHz, D_2O with 10 mM phosphate buffer (pH 7.4), 298 K) spectra for receptor **5** (160 µM) titrated with a combined solution of NDI **35** (5 mM) and receptor **5** (160 µM). Spectra imply binding; however, this could not be quantified from this study.

3.2.2 Staggered receptor 6

Hypoxanthine

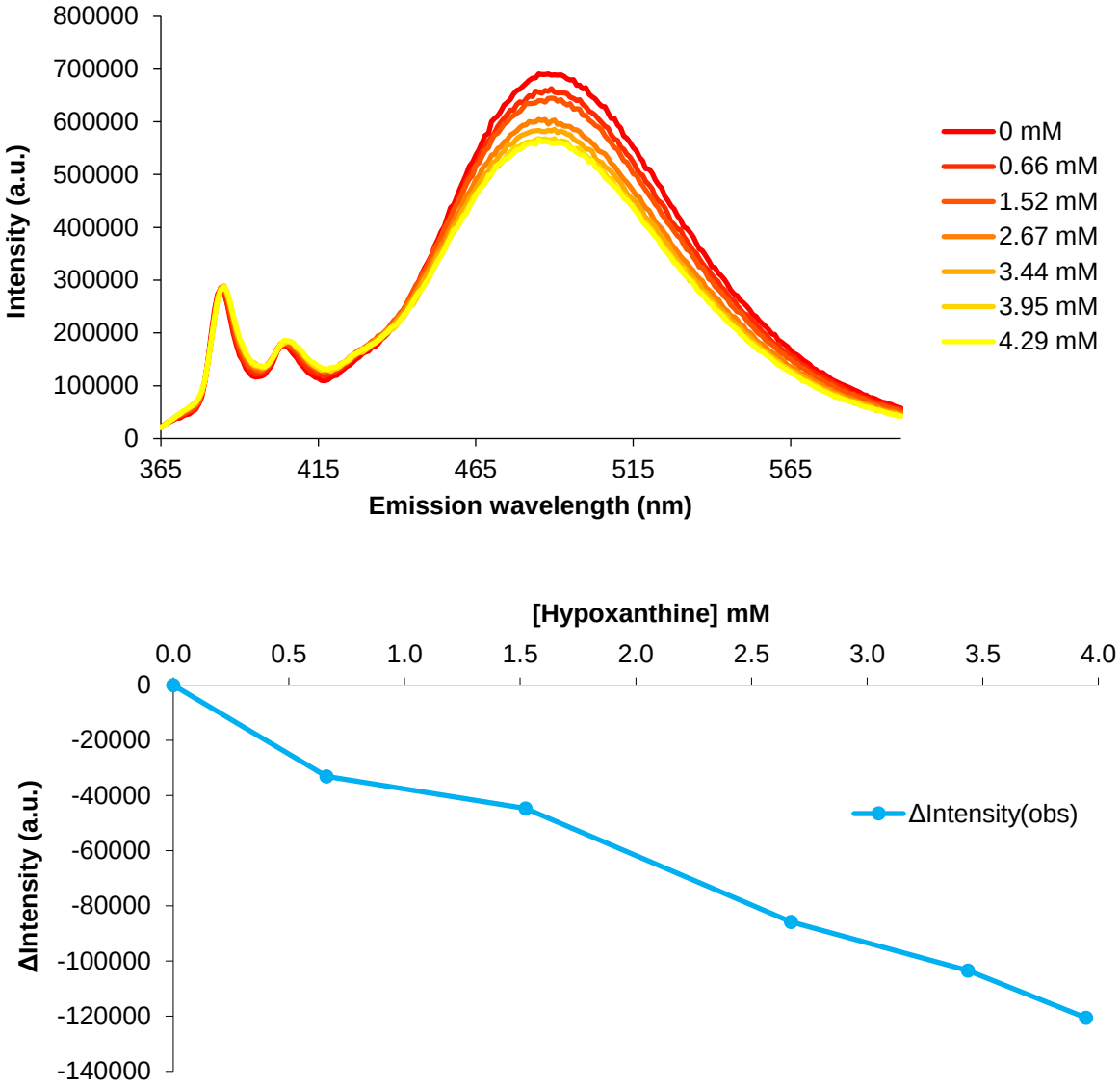


Figure S30. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of hypoxanthine **24** (5 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The insolubility of the guest in water meant only points in the low millimolar range could be obtained. Therefore, it was not possible to obtain a binding constant. The experiment was performed once.

Adenine

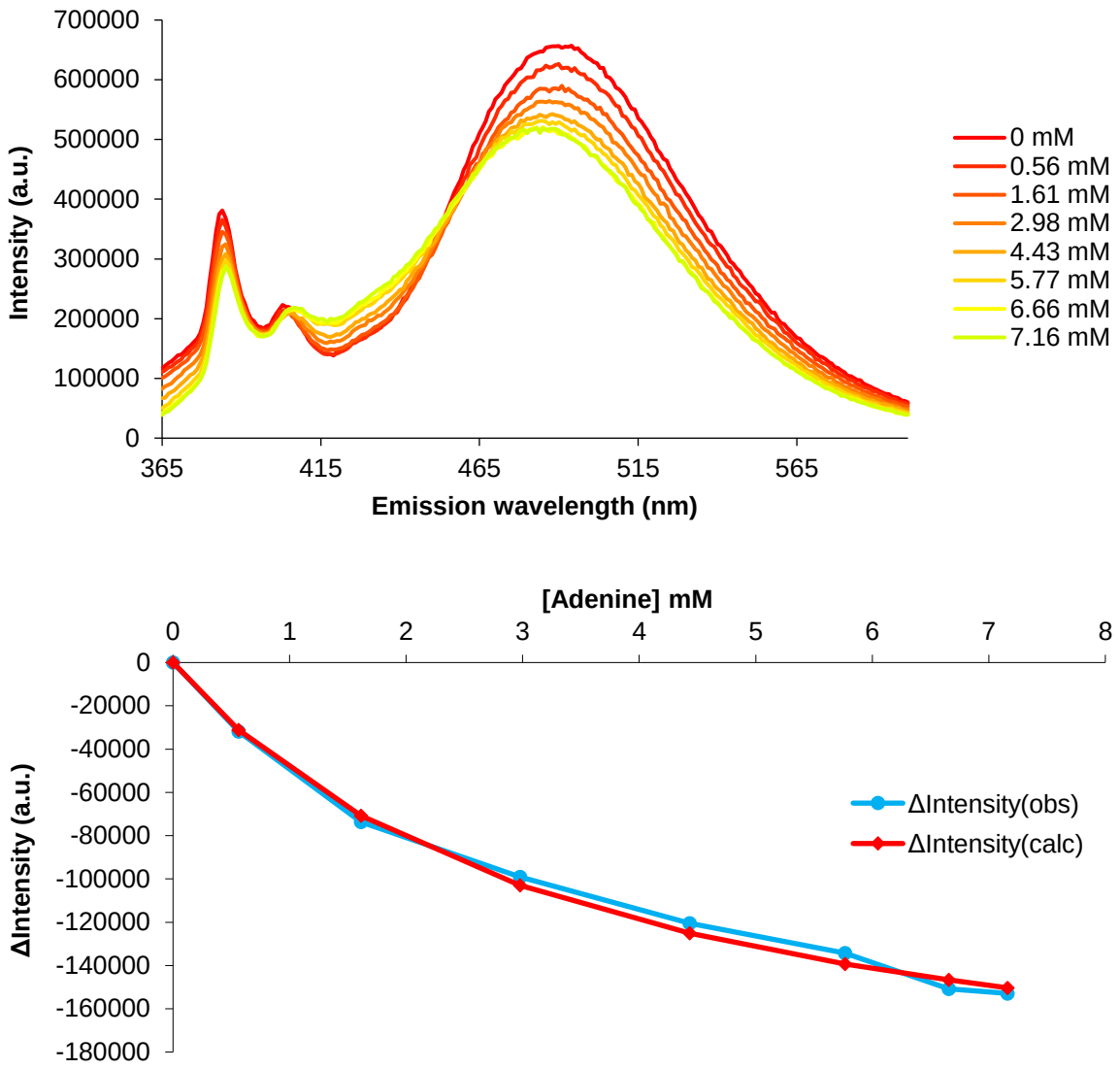


Figure S31. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of adenine **25** (8.4 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 288 ± 10 M^{-1}. The experiment was performed once.

Thymine

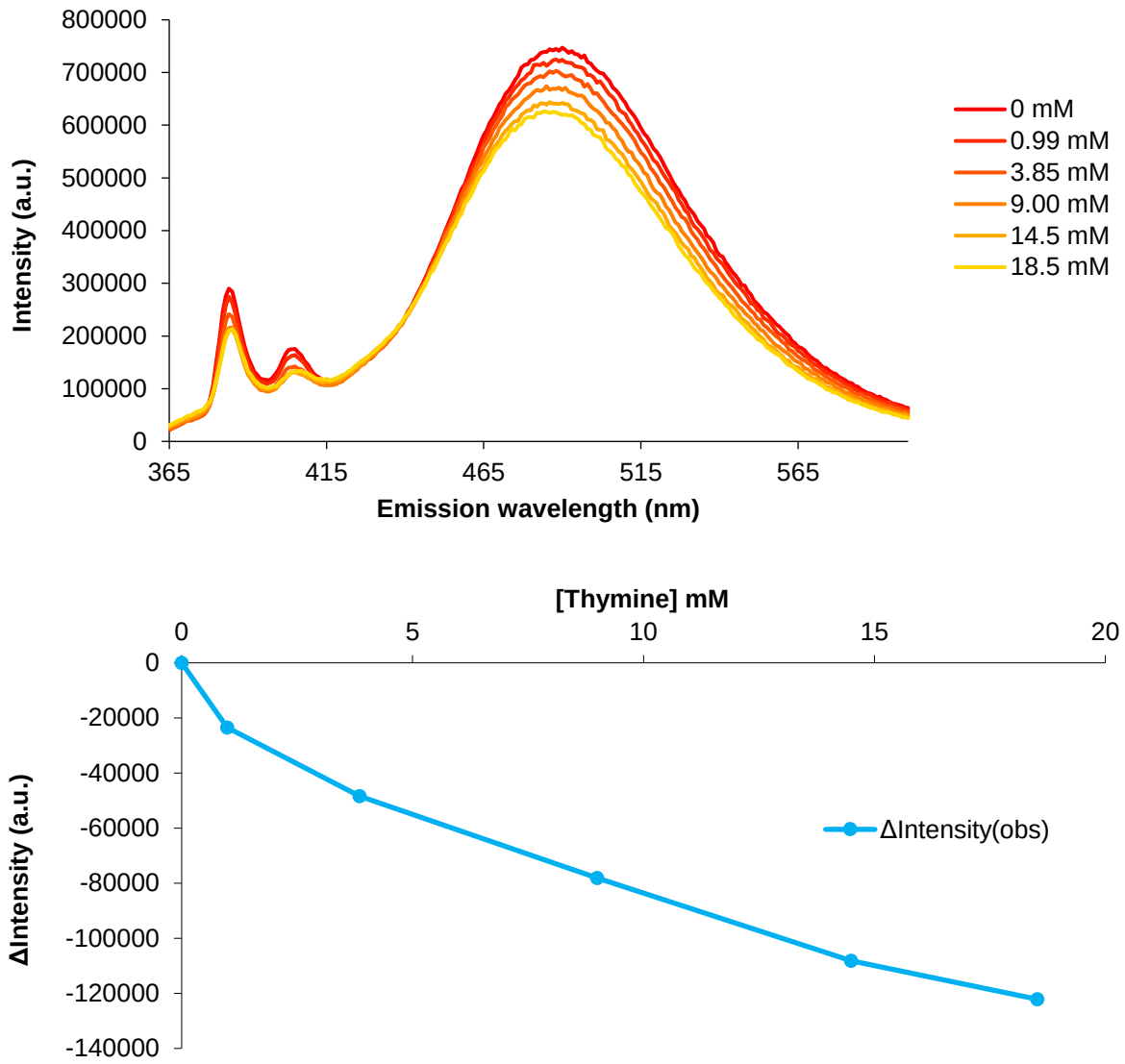


Figure S32. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of thymine **26** (30 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The insolubility of the guest in water meant only points in the low millimolar range could be obtained. Therefore, it was not possible to obtain a binding constant. The experiment was performed once.

L-tryptophan

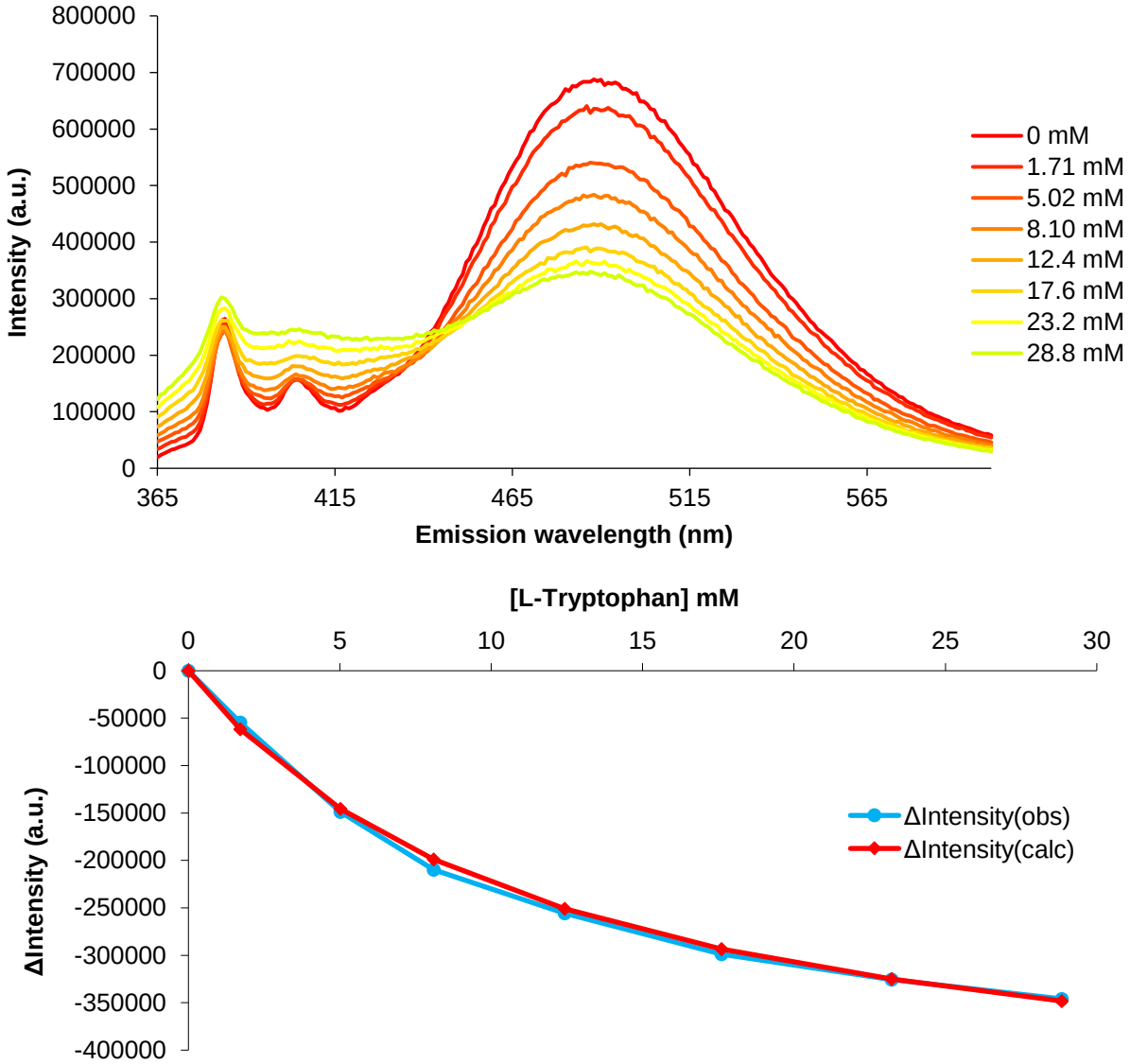


Figure S33. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of L-tryptophan **27** (51 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 84 ± 4 M^{-1}. The experiment was performed once.

Indole

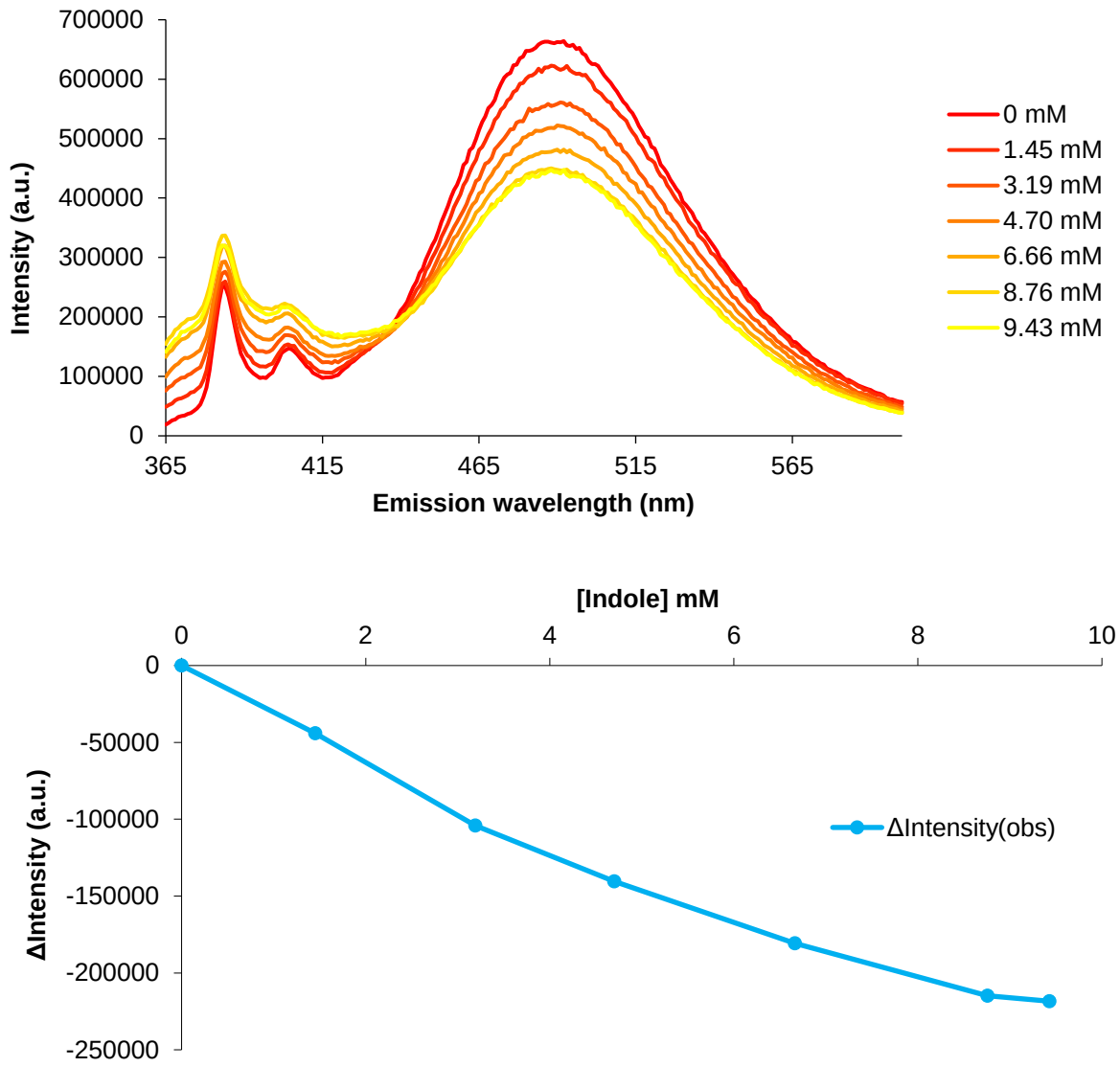


Figure S34. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of indole **28** (14.5 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The insolubility of the guest in water meant only points in the low millimolar range could be obtained. Therefore, it was not possible to obtain a binding constant. The experiment was performed once.

Paracetamol

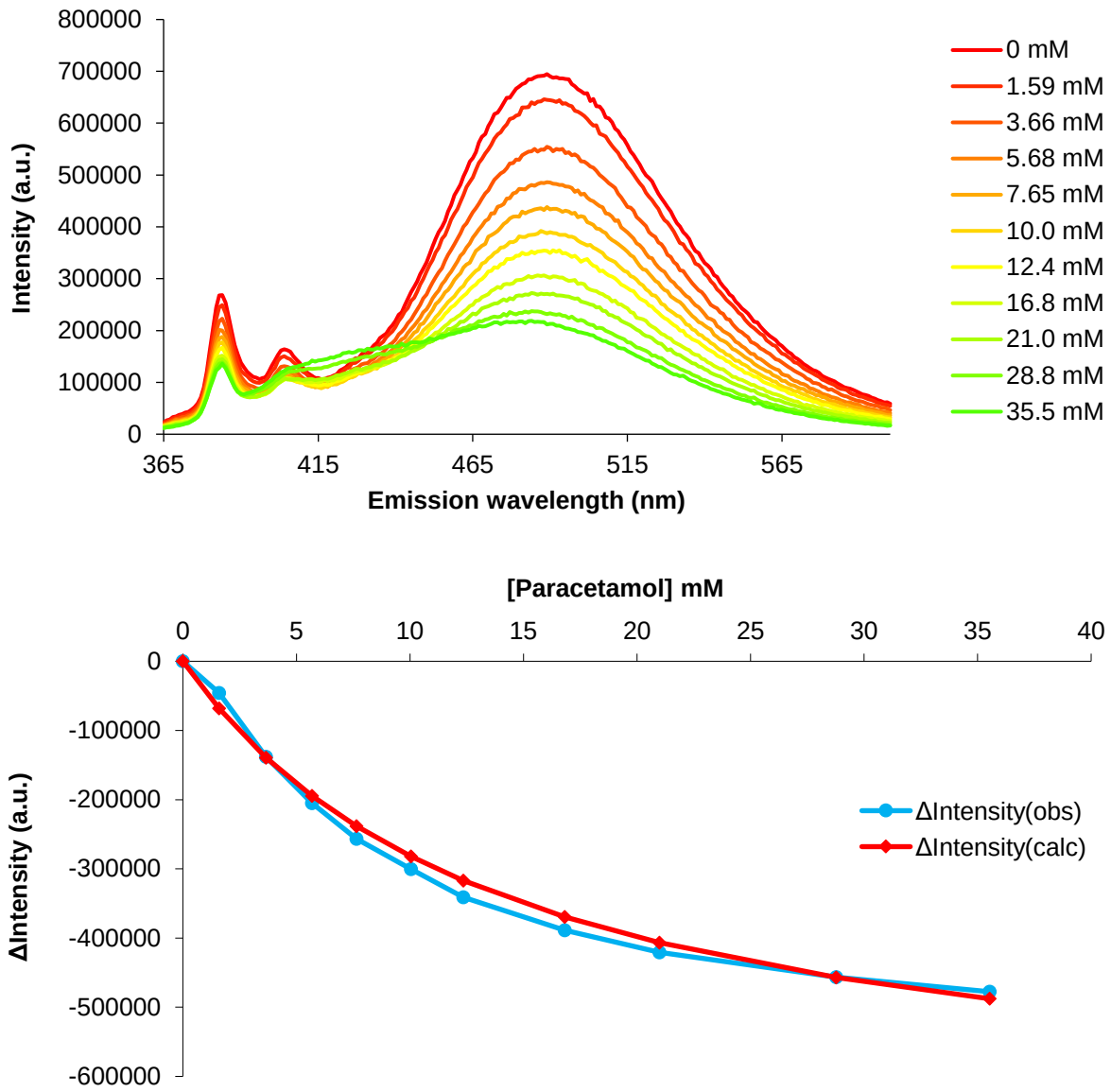


Figure S35. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of paracetamol **29** (79 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 70 ± 8 M^{-1}. The experiment was performed once.

Caffeine

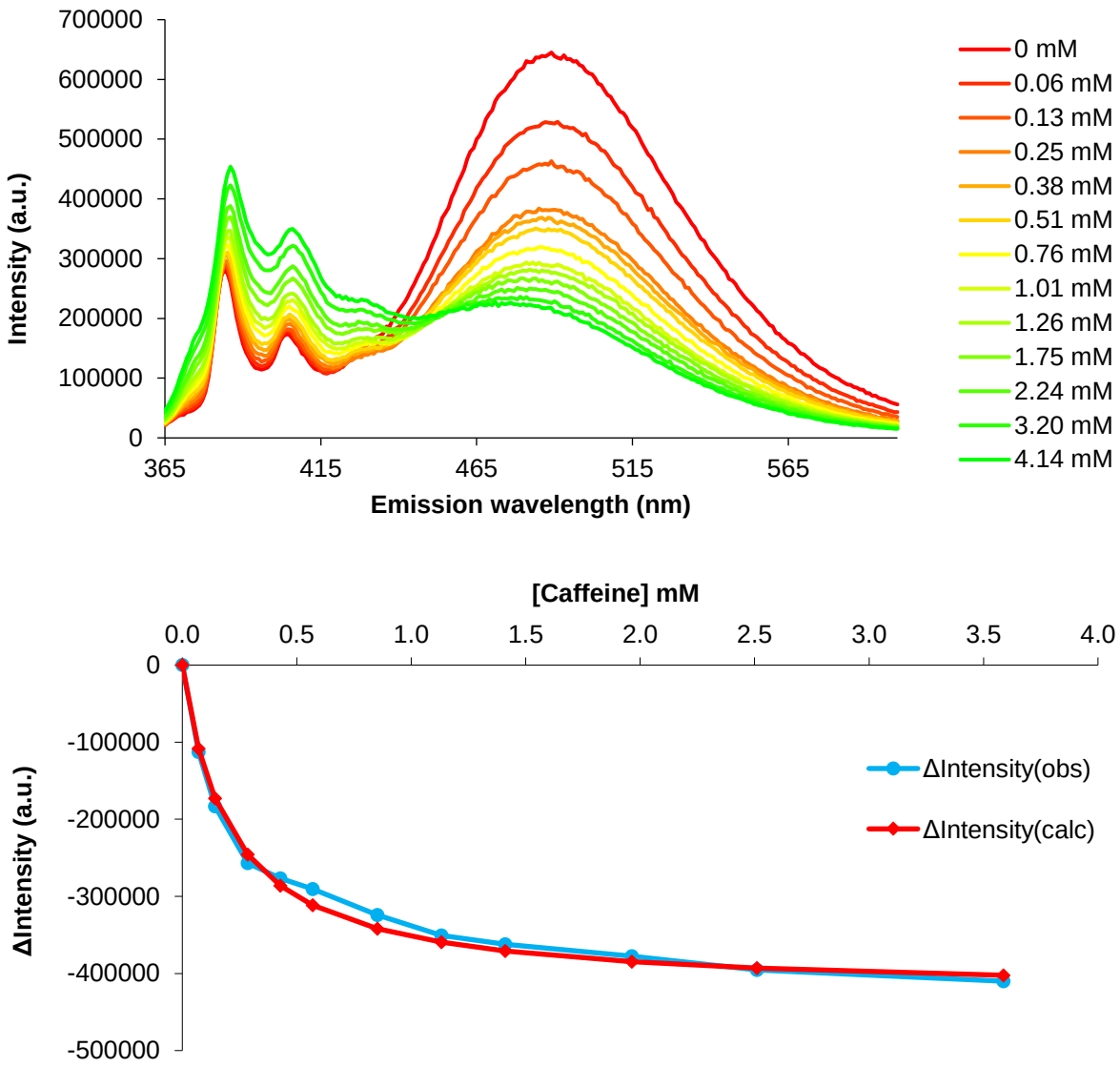


Figure S36. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of caffeine **30** (43 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 4790 ± 204 M^{-1}. The experiment was performed three times with similar results.

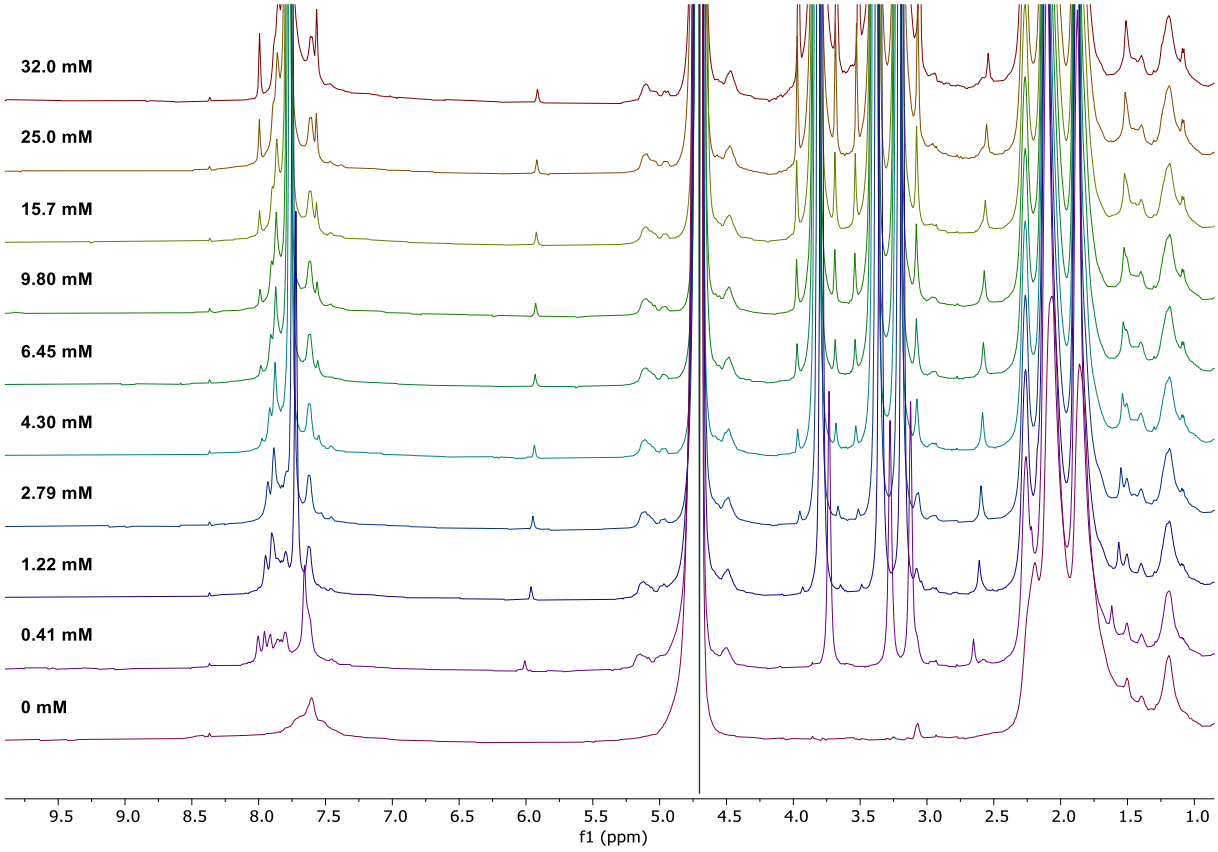


Figure S37. ^{1}H NMR (500 MHz, D_2O with 10 mM phosphate buffer (pH 7.4), 298 K) spectra for receptor **6** (150 µM) titrated with a combined solution of caffeine **30** (82 mM) and receptor **6** (150 µM). Spectral changes imply binding; however, this could not be quantified from this study.

Theobromine

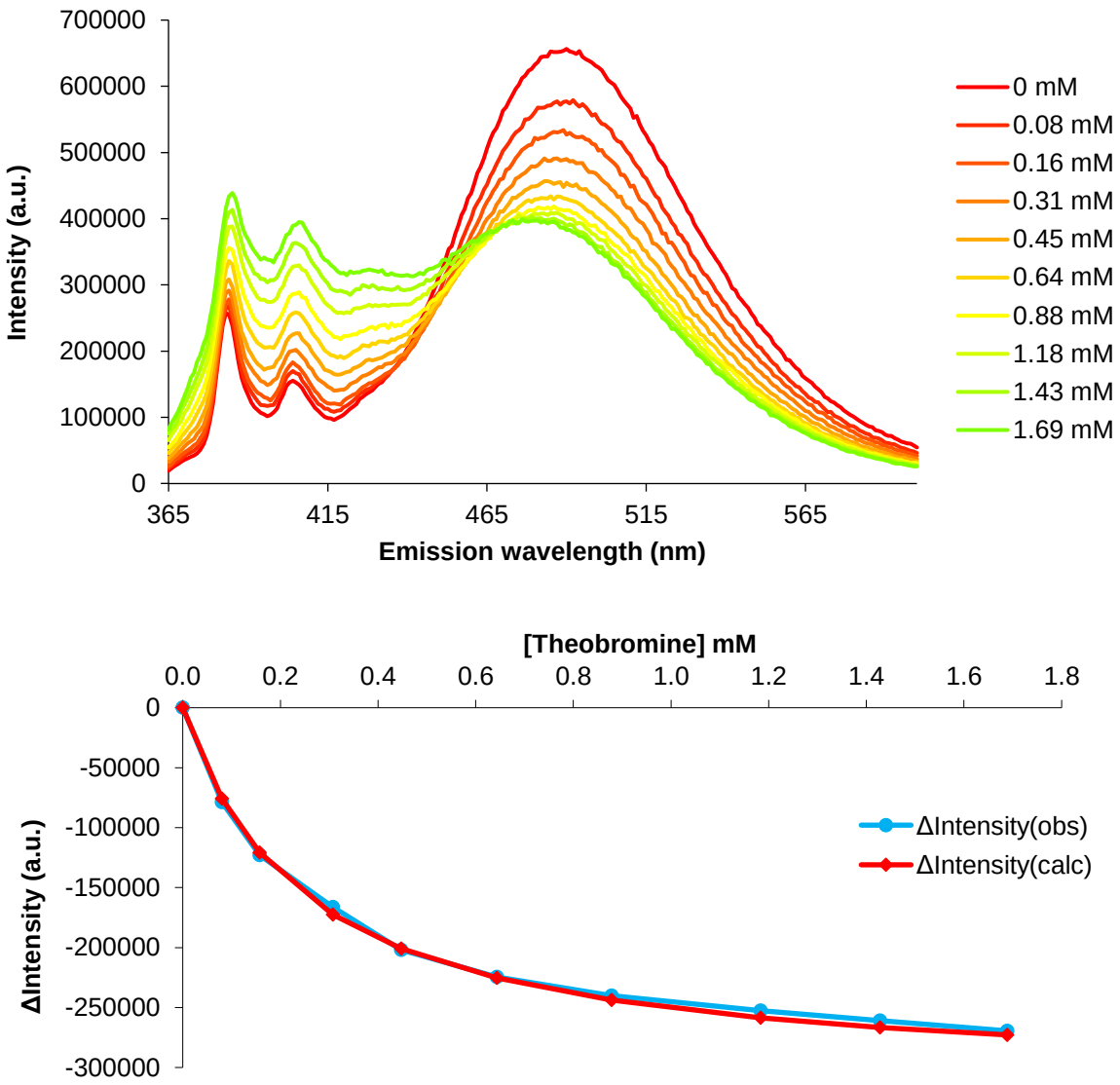


Figure S38. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of theobromine **31** (2.4 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 3987 ± 111 M^{-1}. The experiment was performed three times with similar results.

Theophylline

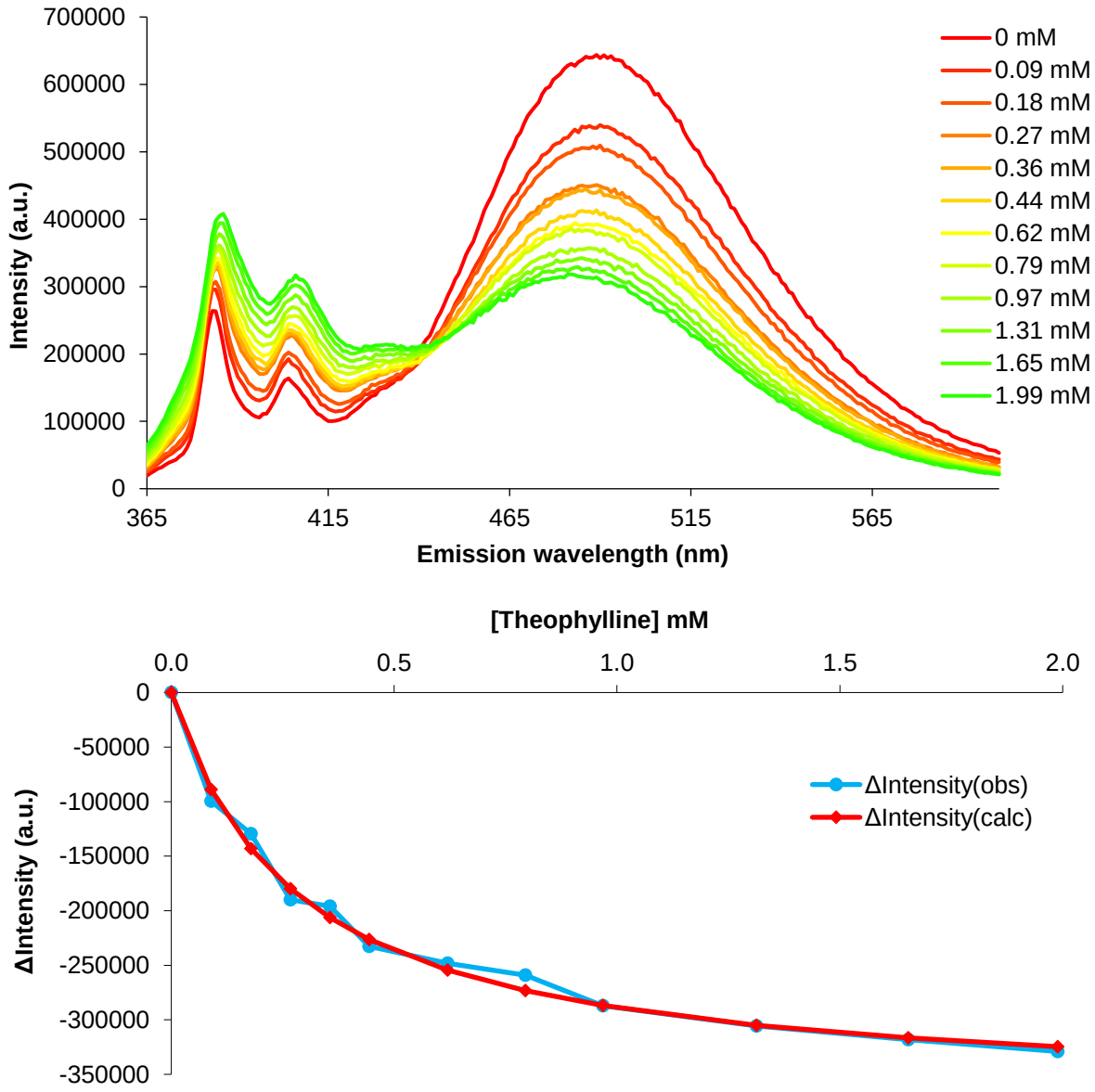


Figure S39. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (350 nM) titrated with a combined solution of theophylline **32** (27 mM) and receptor **6** (350 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 3526 ± 203 M^{-1}. The experiment was performed three times with similar results.

Bis(dimethylamino) NDI **33**

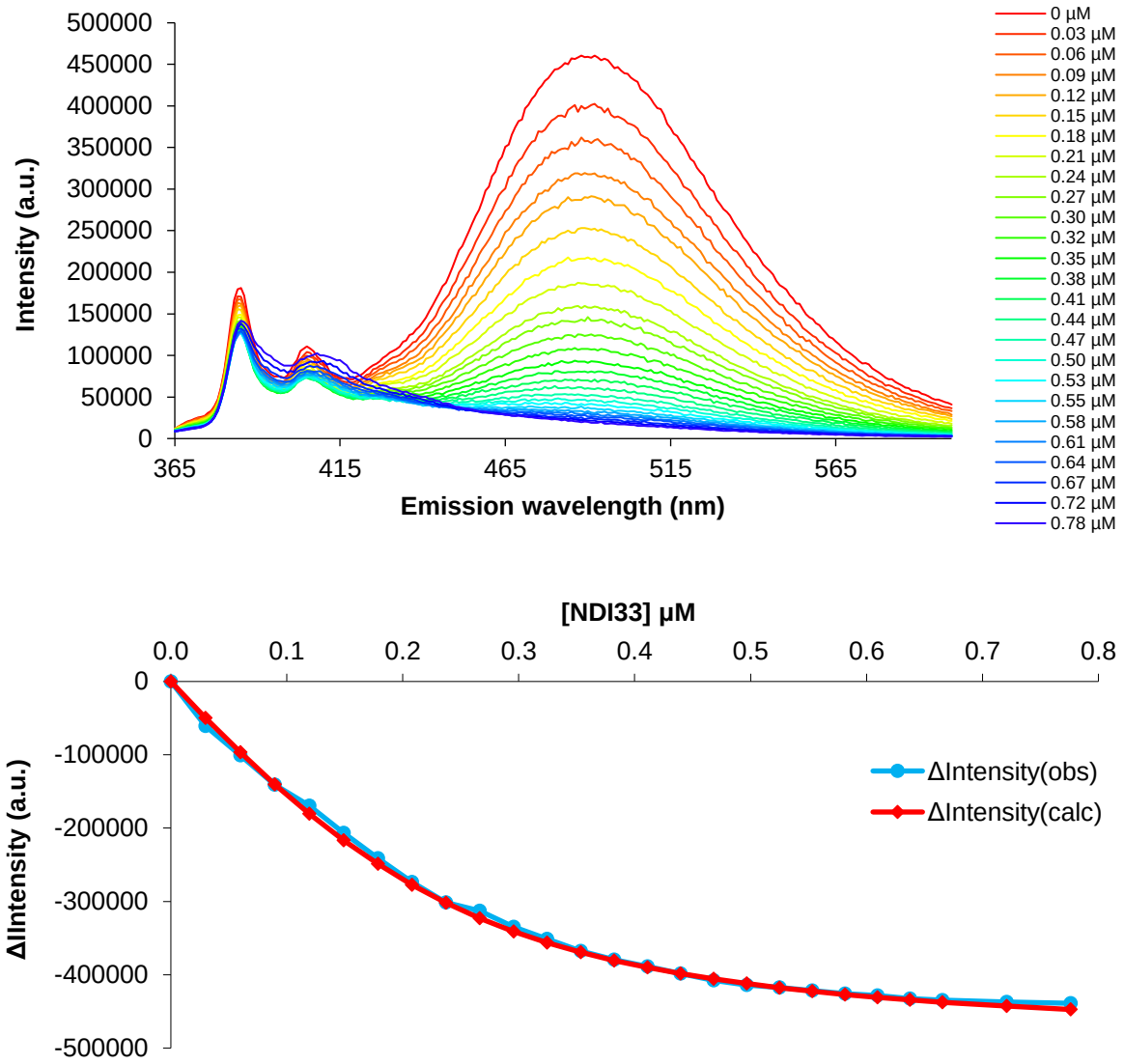


Figure S40. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (230 nM) titrated with a combined solution of NDI **33** (9 µM) and receptor **6** (230 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 1.5 x 10^7 M^{-1} ± 4.98%. The experiment was performed three times with similar results.

Bis(trimethylammonium) NDI **34**

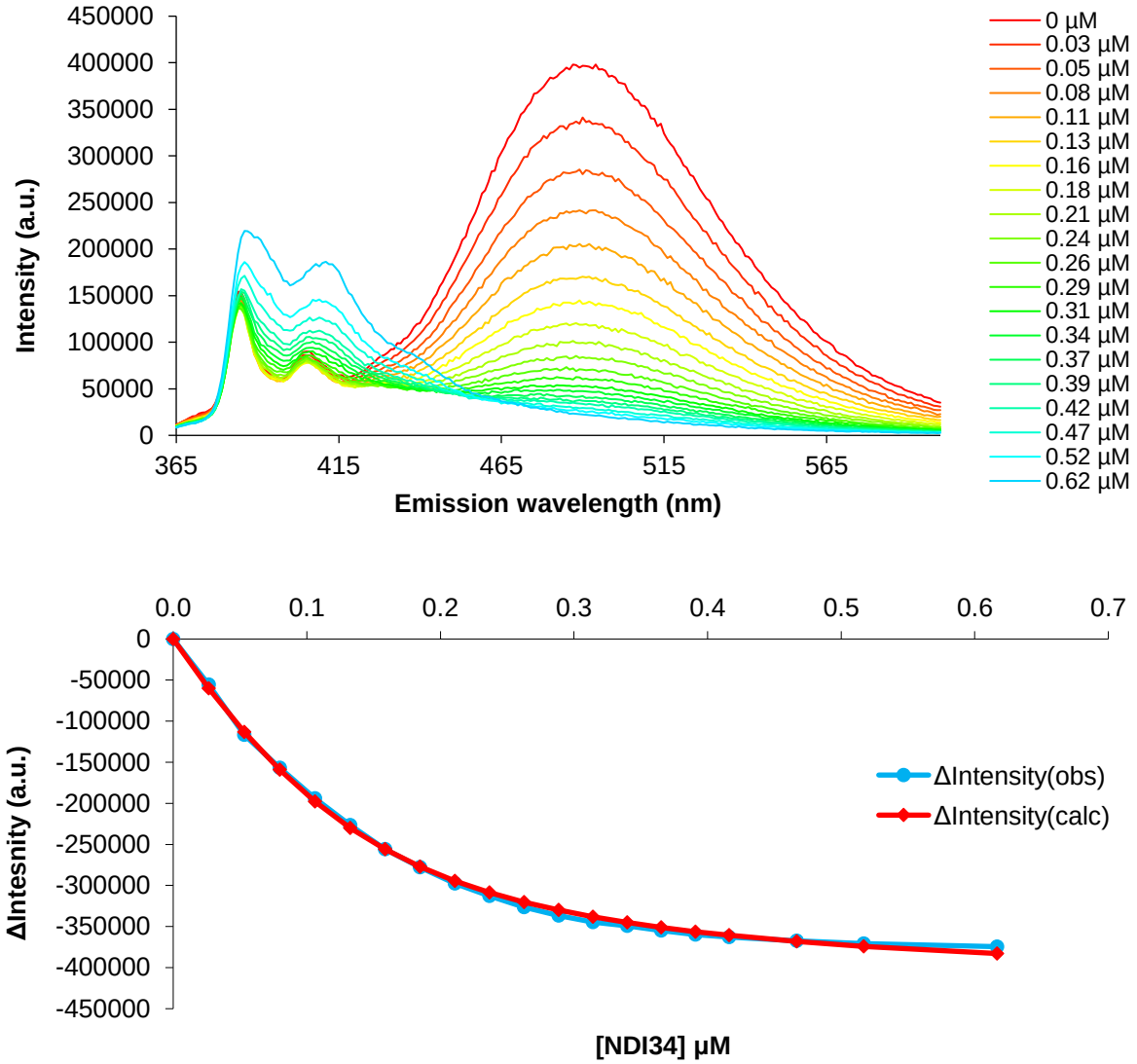


Figure S41. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (125 nM) titrated with a combined solution of NDI **34** (8 µM) and receptor **6** (125 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 1.8 x 10^7 M^{-1} ± 2.26%. The experiment was performed three times with similar results.

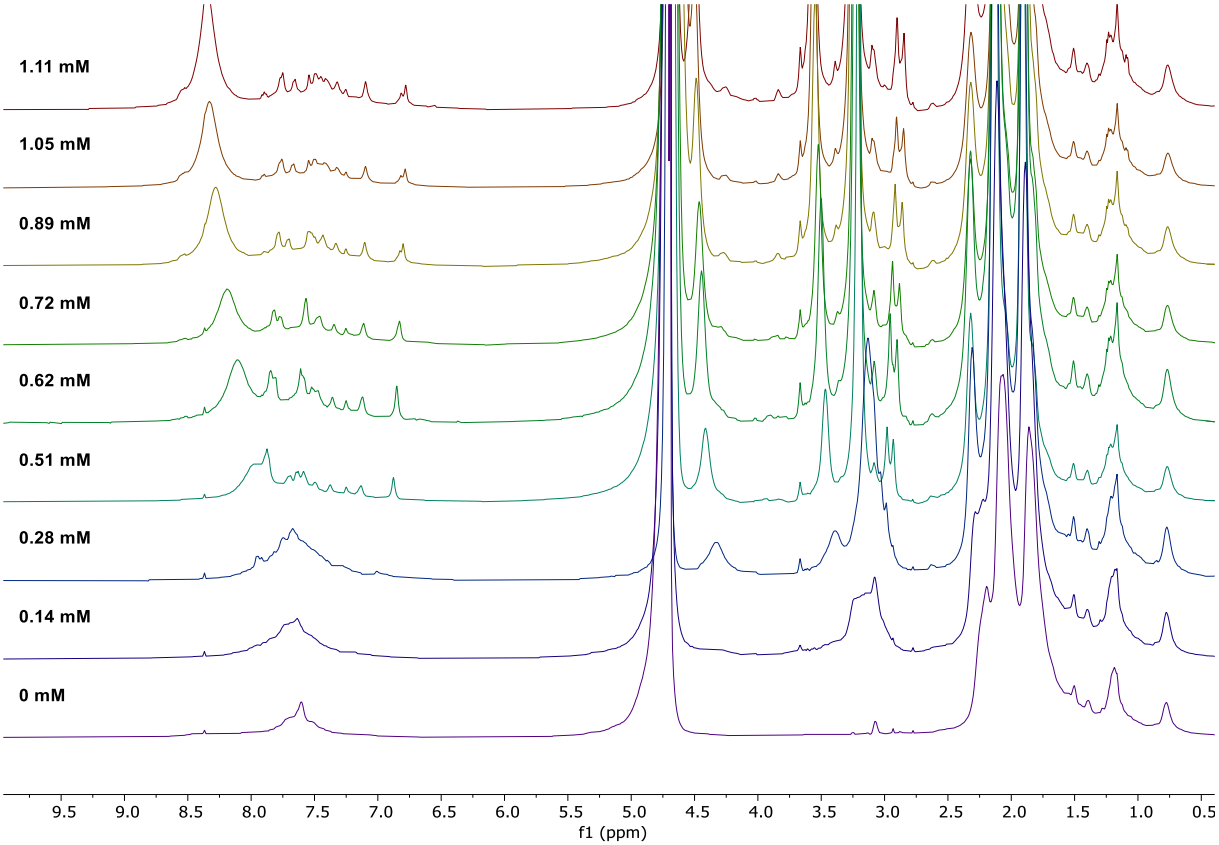


Figure S42. ^{1}H NMR (500 MHz, D_2O with 10 mM phosphate buffer (pH 7.4), 298 K) spectra for receptor **6** (159 µM) titrated with a combined solution of NDI **34** (3.4 mM) and receptor **6** (159 µM). Spectra imply binding; however, this could not be quantified from this study.

NDI **35**

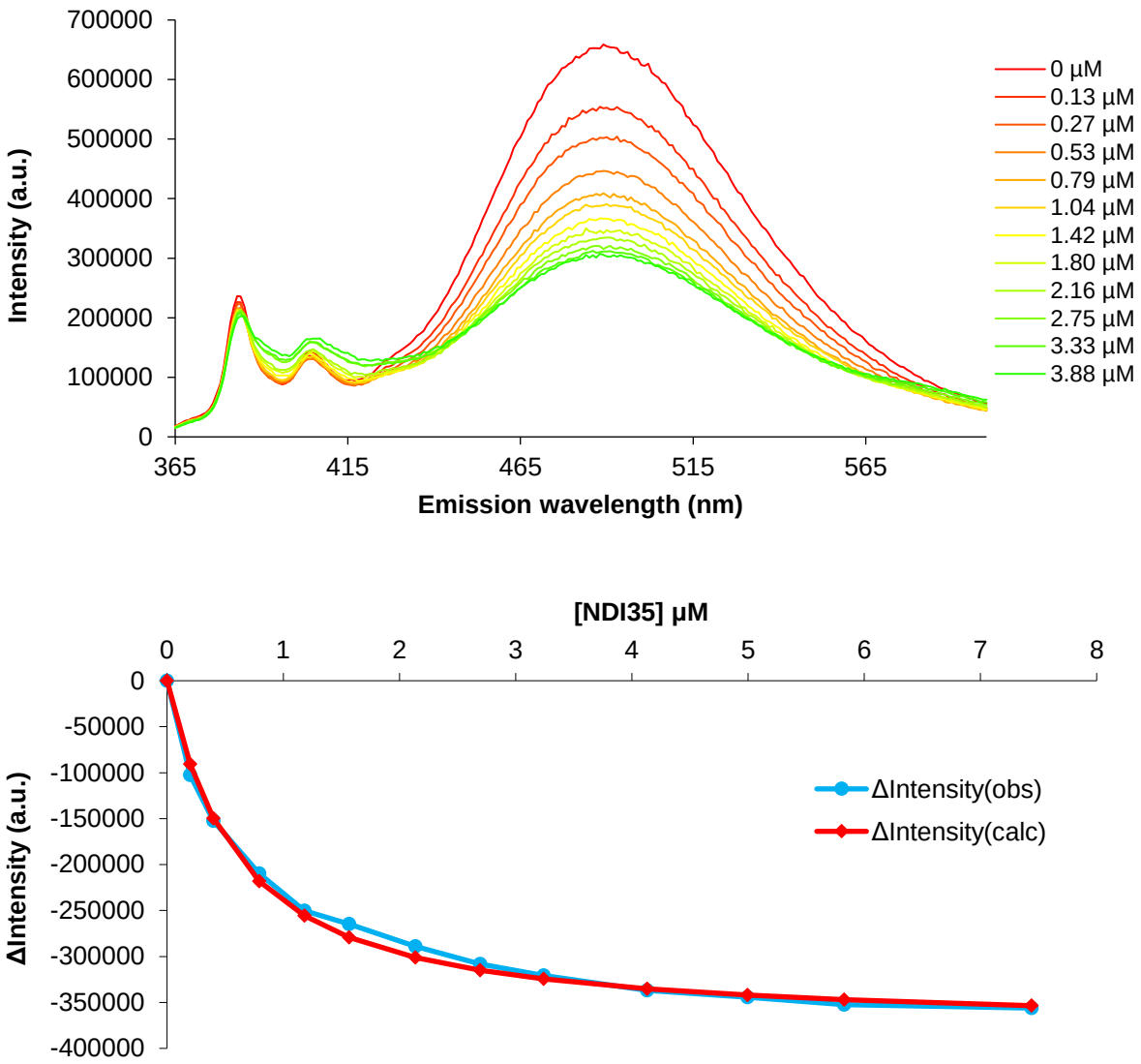


Figure S43. Fluorescence emission spectra (top) and binding analysis curve (bottom) for receptor **6** (150 nM) titrated with a combined solution of NDI **35** (30 µM) and receptor **6** (150 nM) in 10 mM phosphate buffer (pH 7.4) at 298 K. Excitation wavelength: 340 nm. Changes in emission intensity (ΔIntensity, a.u.) at emission wavelength 490 nm were plotted against increasing guest concentration (mM). The calculated values for ΔIntensity are overlaid with the observed values giving K_a = 1.9 x 10^6 M^{-1} ± 4.73 %. The experiment was performed three times with similar results.

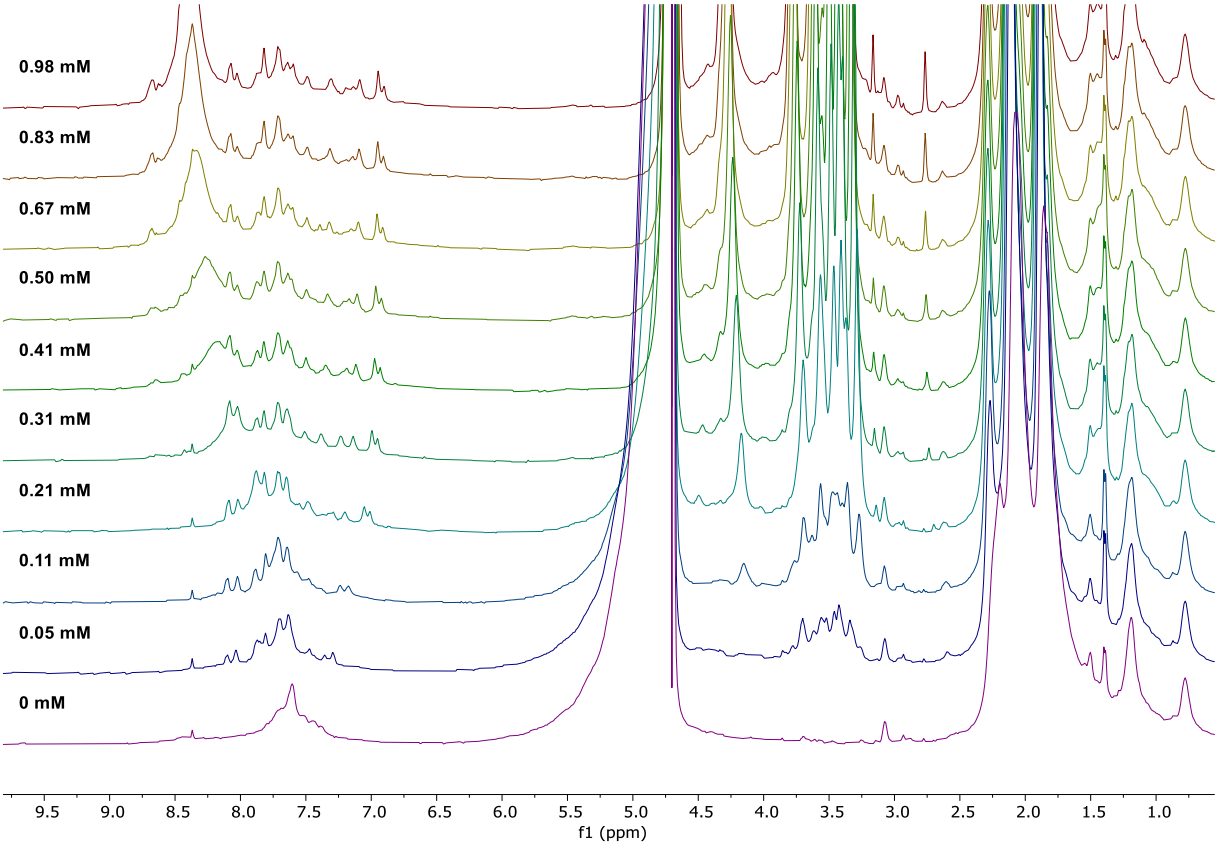


Figure S44. ^{1}H NMR (500 MHz, D_2O with 10 mM phosphate buffer (pH 7.4), 298 K) spectra for receptor **6** (155 µM) titrated with a combined solution of NDI **35** (5 mM) and receptor **6** (155 µM). Spectra imply binding; however, this could not be quantified from this study.

4. Characterisation of receptor-NDI complexes

Eclipsed Receptor 5-NDI 35

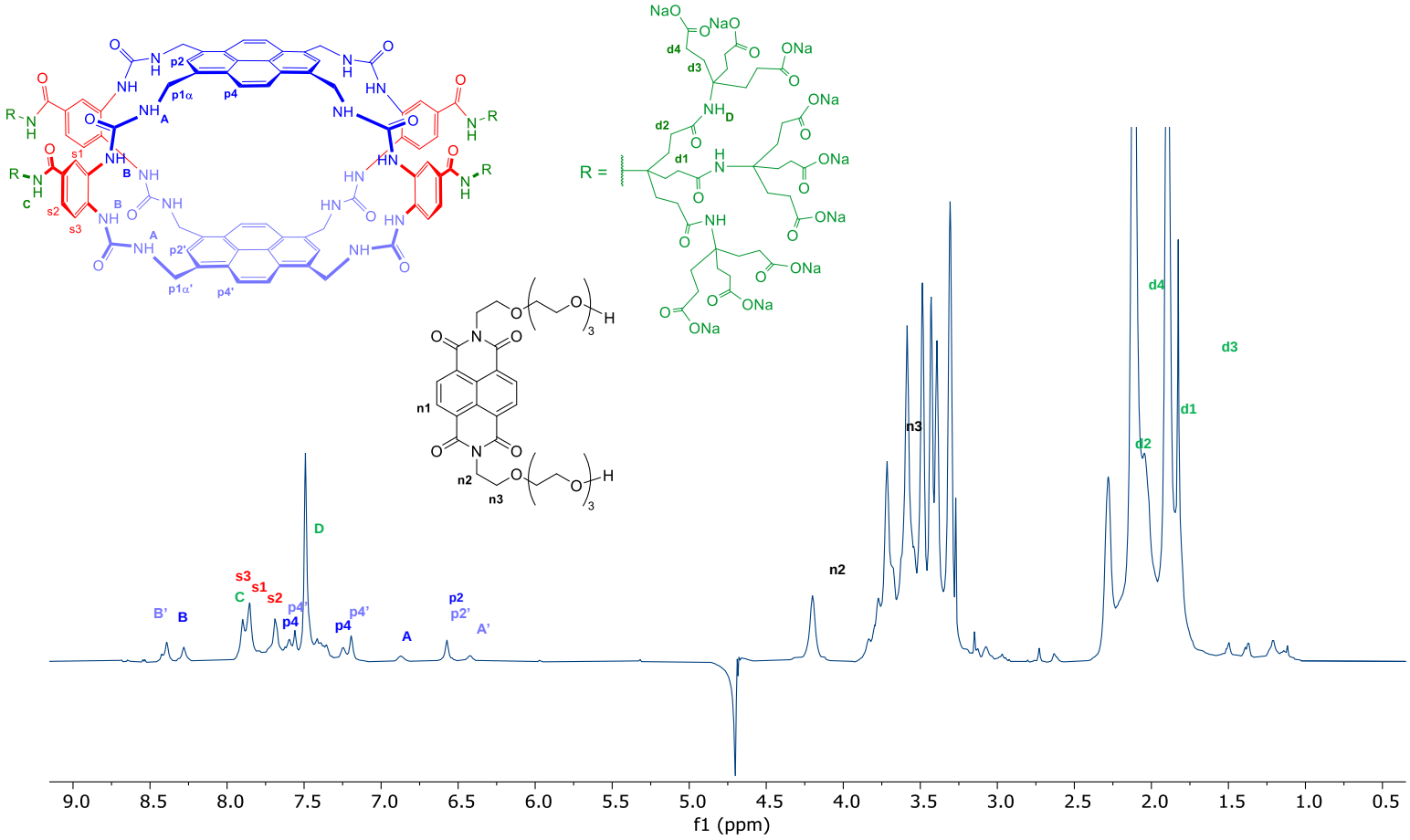


Figure S45. ^{1}H NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of eclipsed receptor **5** (250 µM) complexed with NDI **35** (750 µM). p1α signals are not seen due to PRESAT sequence used.

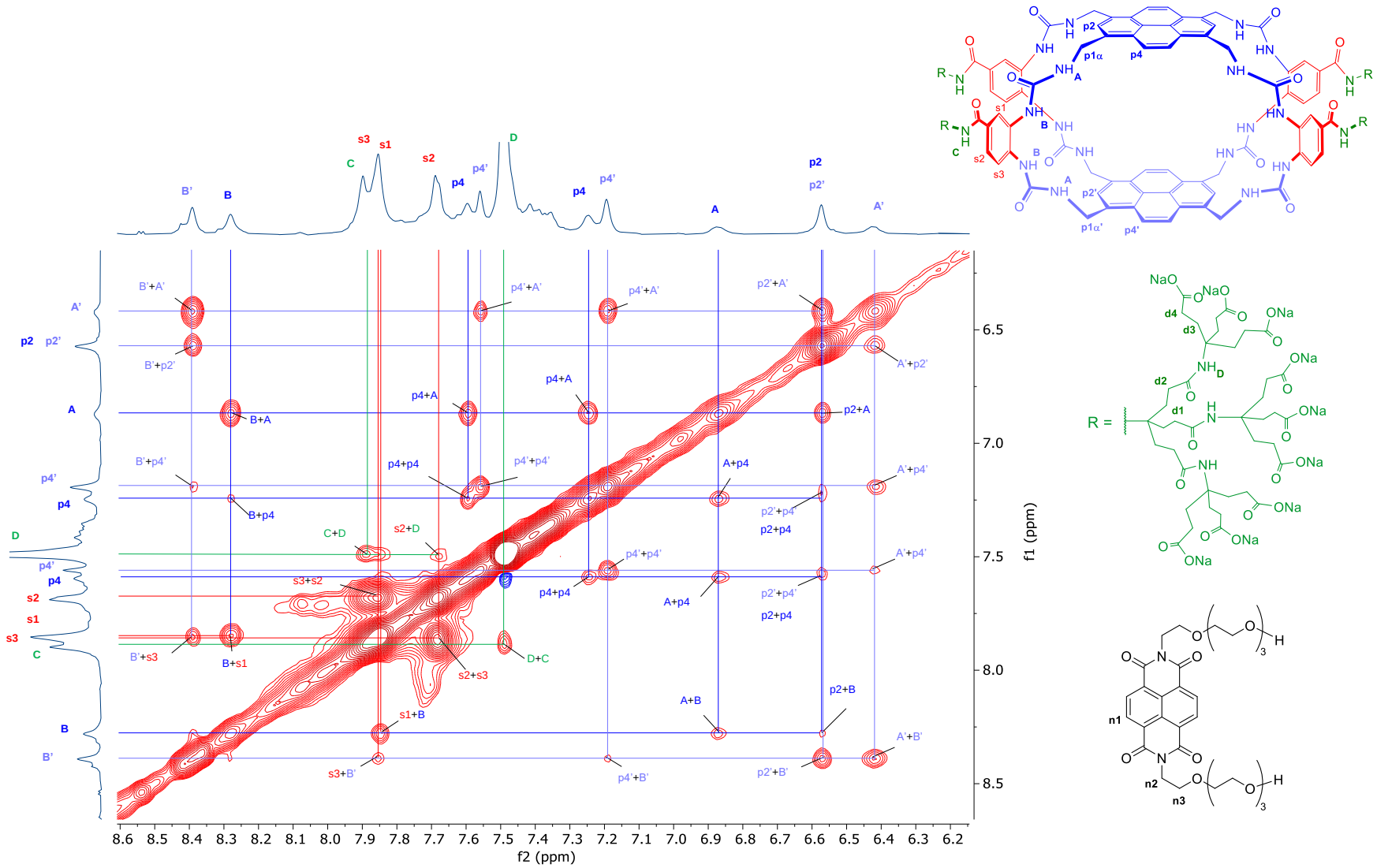


Figure S46. Partial ^{1}H-^{1}H NOESY NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of eclipsed receptor **5** (250 µM) complexed with NDI **35** (750 µM). Mixing time: 300 ms.

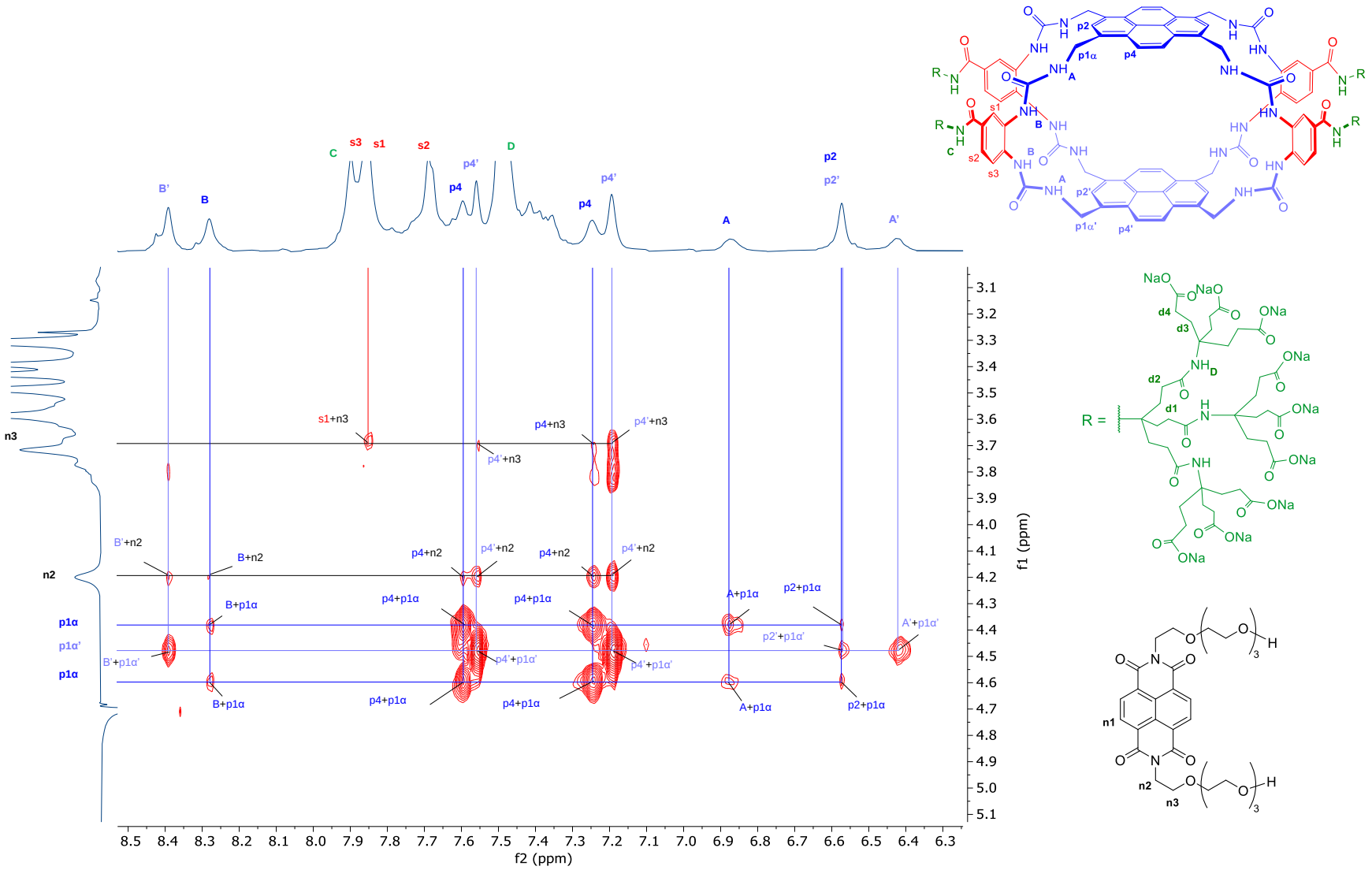


Figure S47. Partial ^{1}H-^{1}H NOESY NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of eclipsed receptor **5** (250 µM) complexed with NDI **35** (750 µM). Mixing time: 300 ms.

Eclipsed Receptor 5-NDI 34

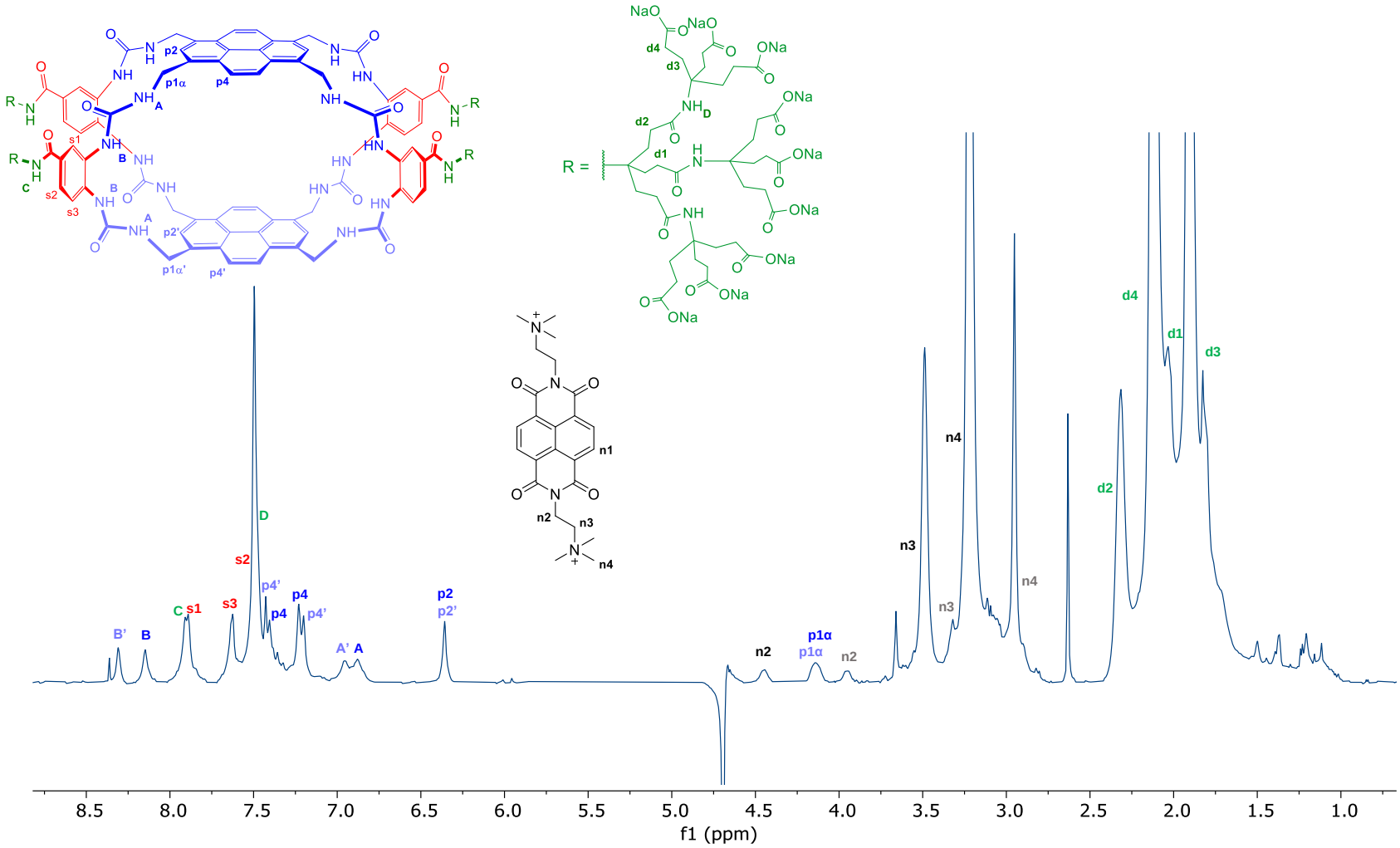


Figure S48. ^{1}H NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of eclipsed receptor **5** (250 μM) complexed with NDI **34** (750 μM). p1α signals are not seen due to PRESAT sequence used. Two sets of NDI signals are observed (black and grey labelling), both of which interact with the receptor, two hypothetical NDI structures are shown.

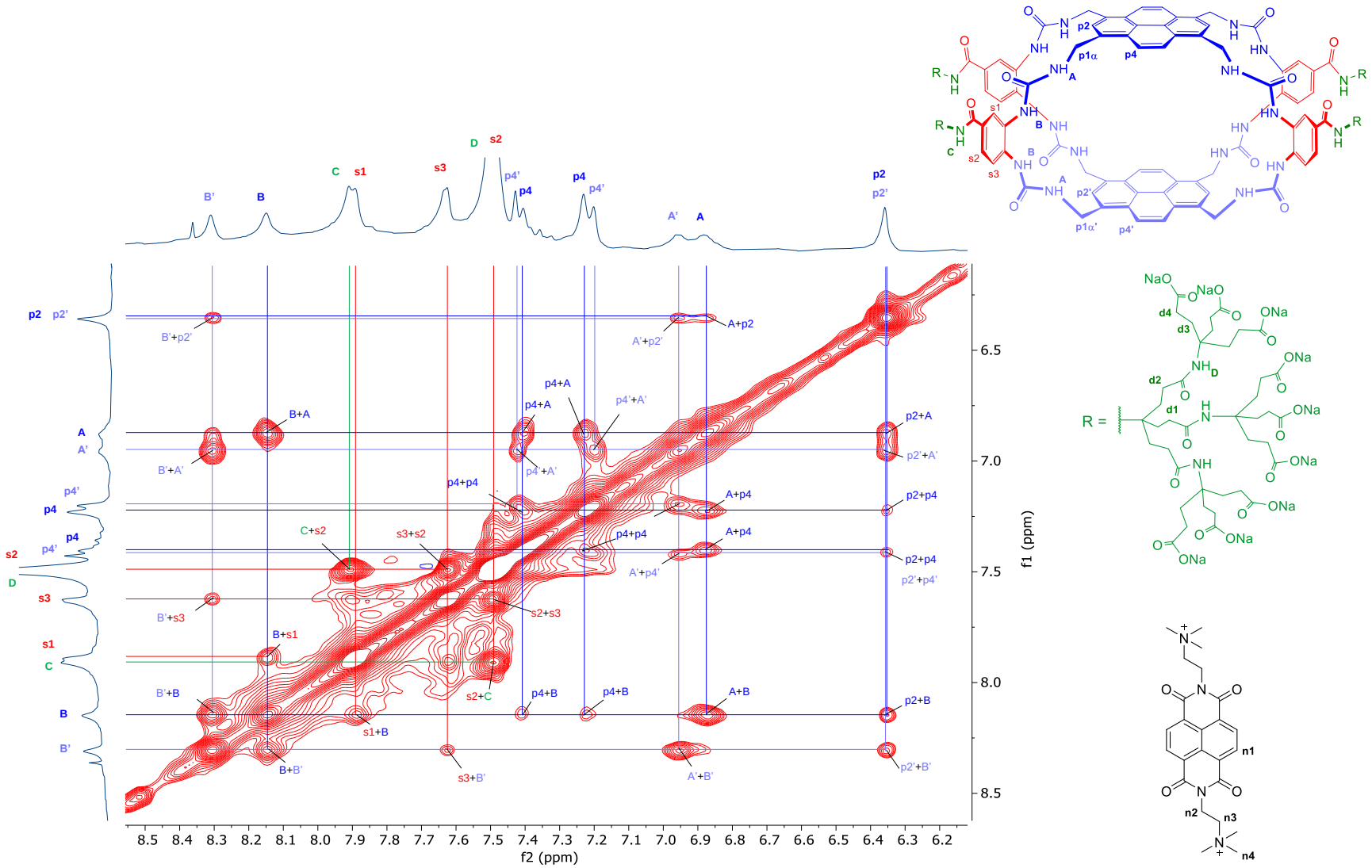


Figure S49. Partial ^{1}H-^{1}H NOESY NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of eclipsed receptor **5** (250 µM) complexed with NDI **34** (750 µM). Mixing time: 300 ms.

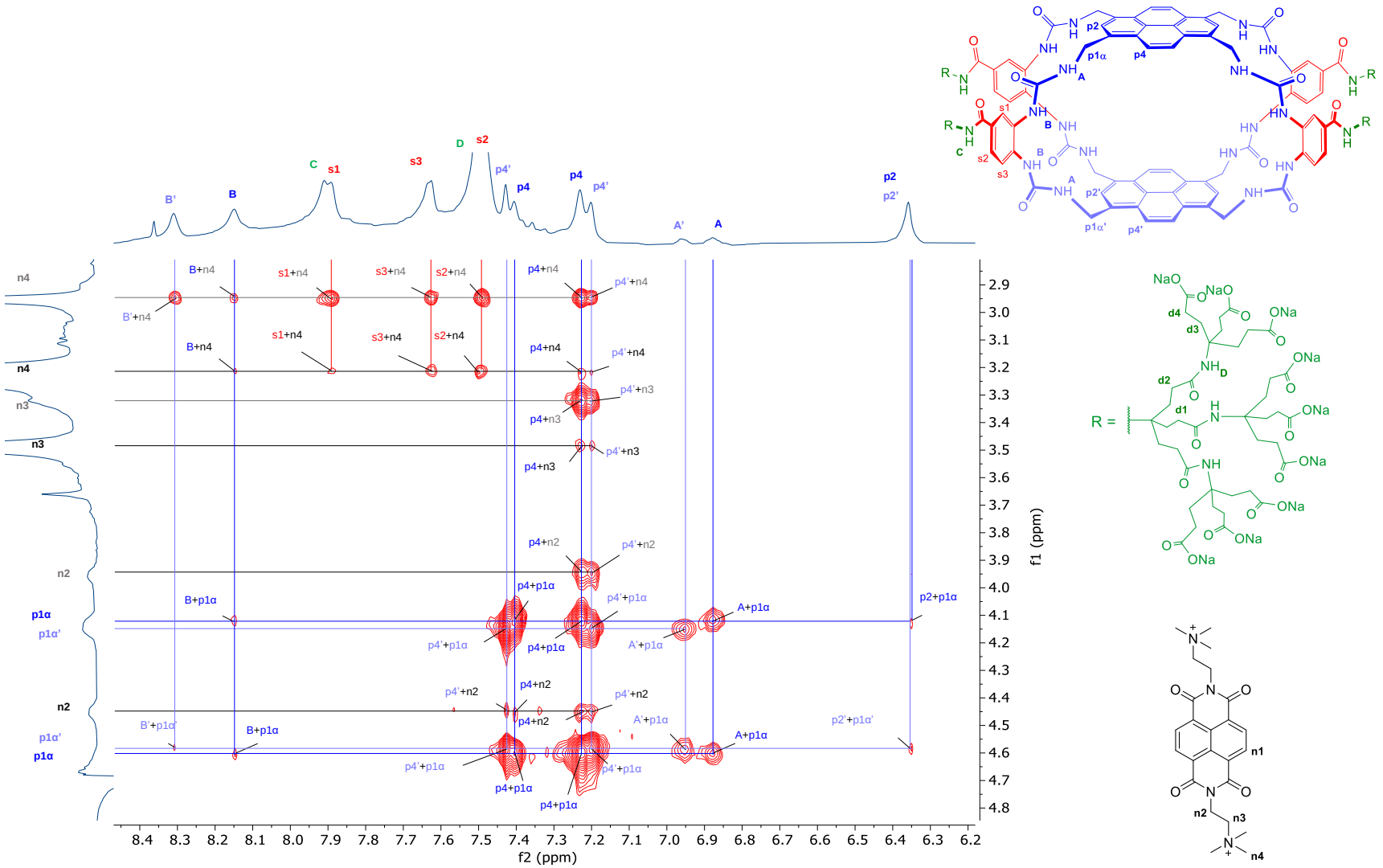


Figure S50. Partial ^{1}H-^{1}H NOESY NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of eclipsed receptor **5** (250 µM) complexed with NDI **34** (750 µM). Mixing time: 300 ms.

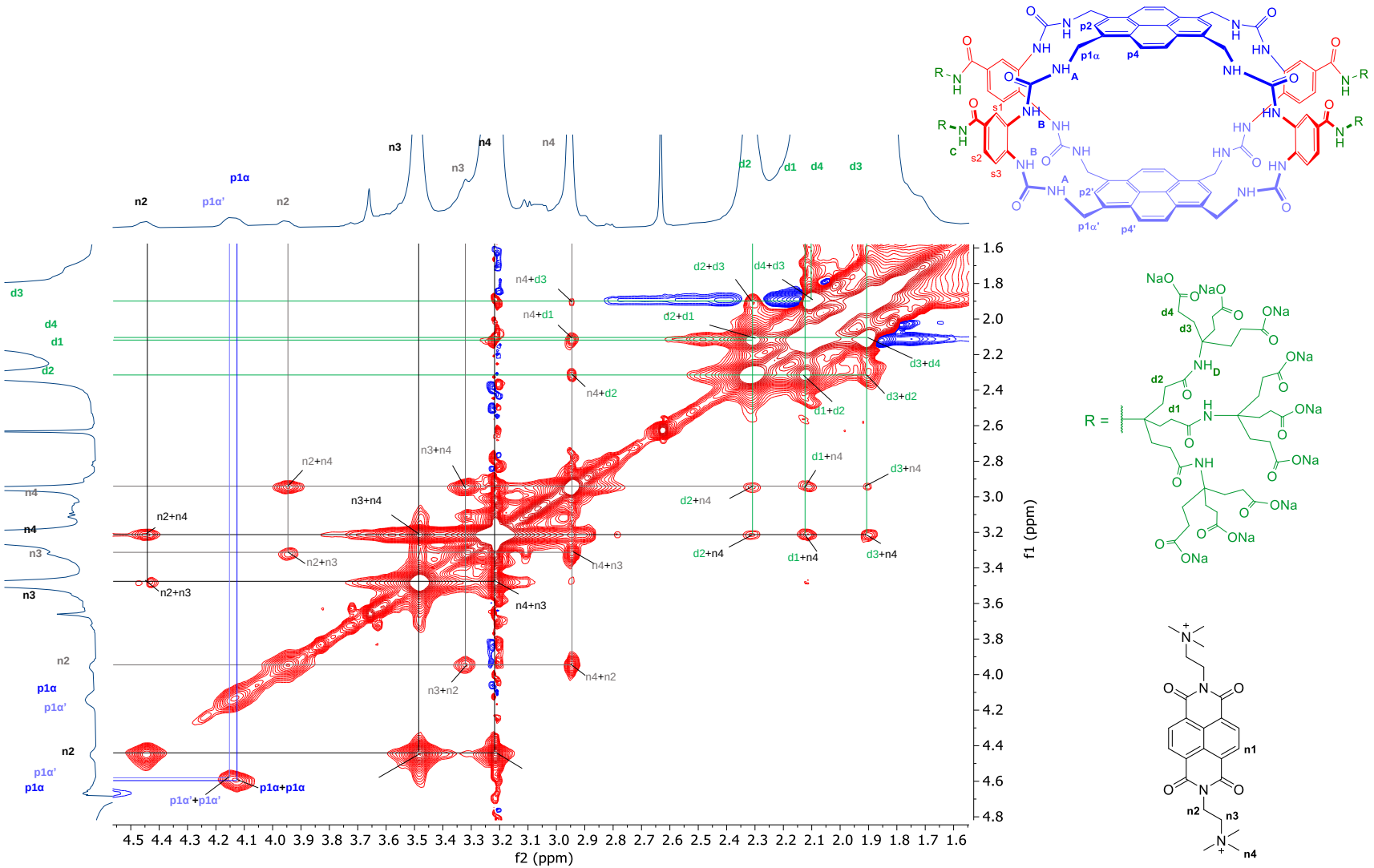


Figure S51. Partial ^{1}H-^{1}H NOESY NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of eclipsed receptor **5** (250 µM) complexed with NDI **34** (750 µM). Mixing time: 300 ms.

Staggered Receptor 6-NDI 35

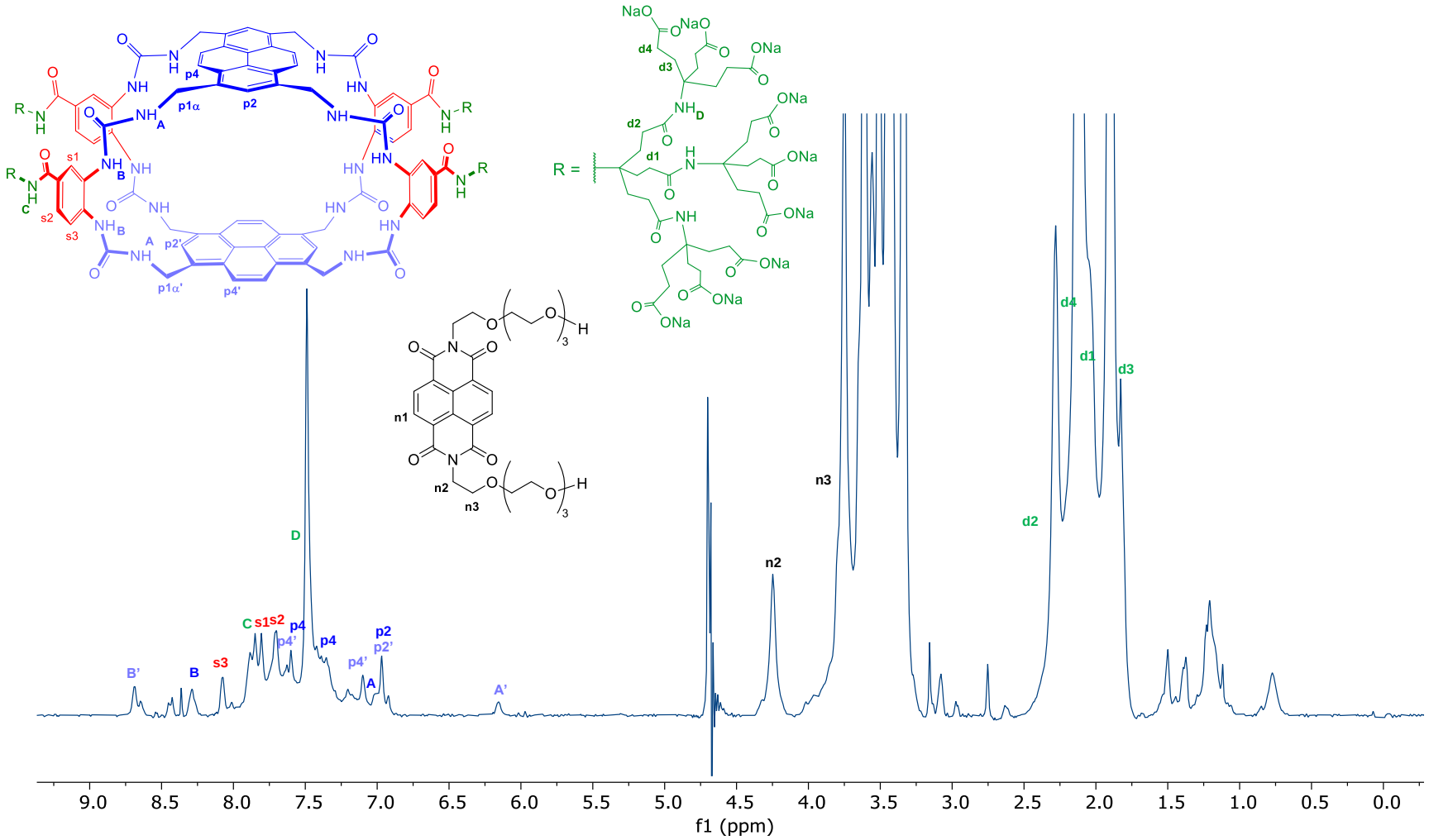


Figure S52. ^{1}H NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of staggered receptor **6** (250 µM) complexed with NDI **35** (750 µM). p1α signals are not seen due to PRESAT sequence used. Extra signals are observed for the other complex where the NDI guest enters the cavity in a different orientation.

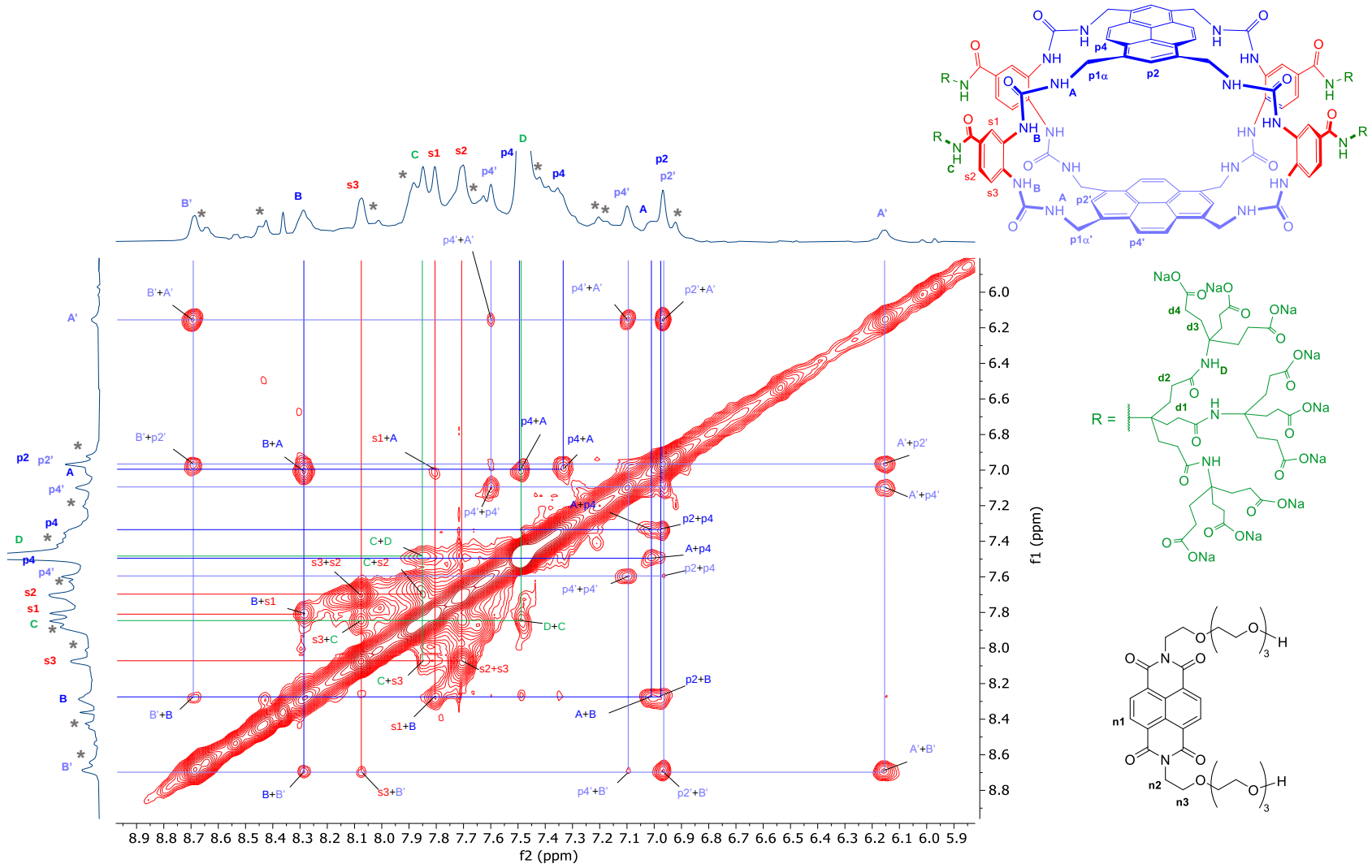


Figure S53. Partial ^{1}H-^{1}H NOESY NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of staggered receptor **6** (250 µM) complexed with NDI **35** (1 mM). Mixing time: 300 ms.

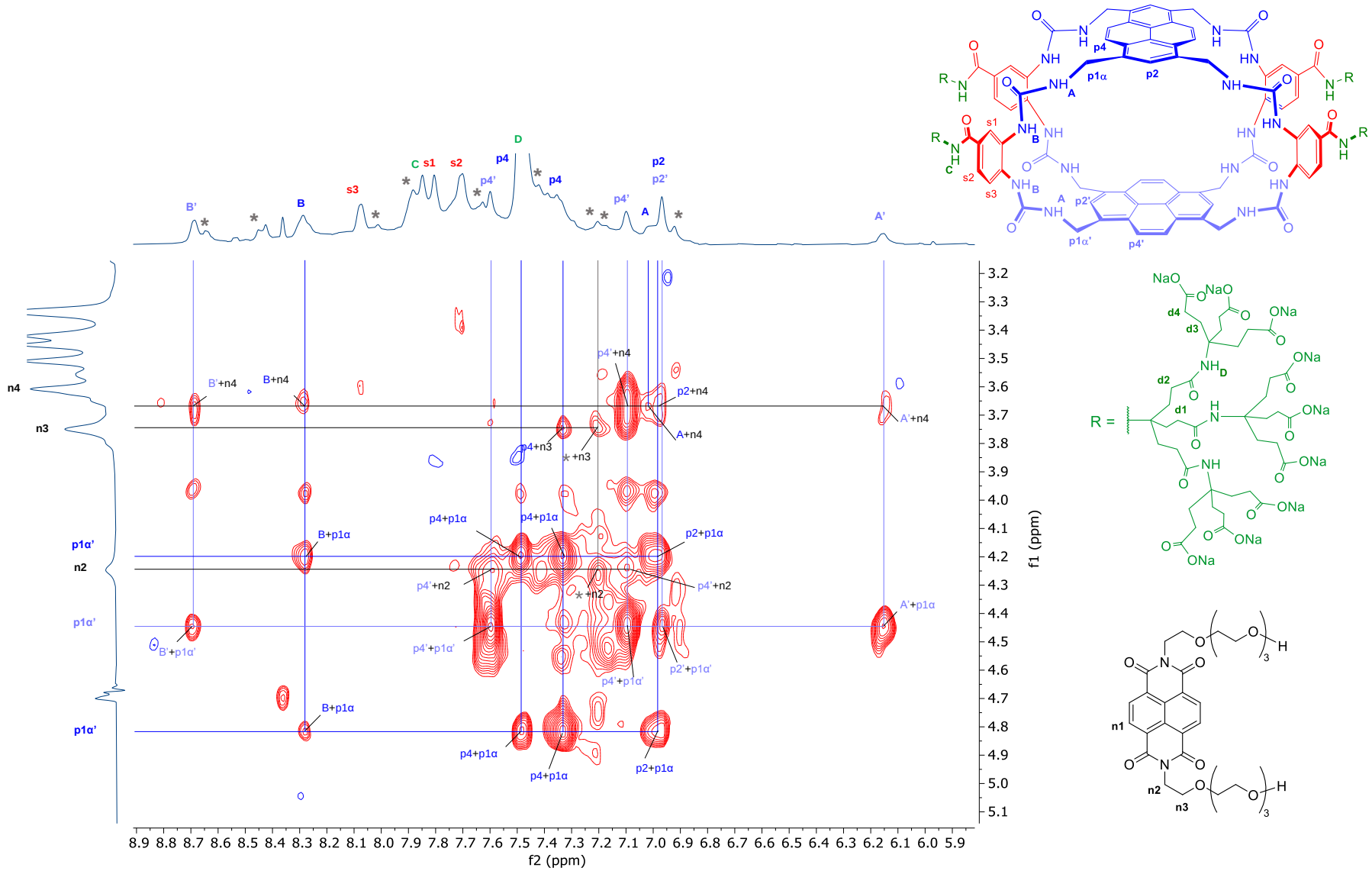


Figure S54. Partial ^{1}H-^{1}H NOESY NMR (700 MHz, 1:9 D_2O:H_2O, 298 K) spectrum of staggered receptor **6** (250 µM) complexed with NDI **35** (1 mM). Mixing time: 300 ms.

References

1. A. B. Pangborn, M. A. Giardello, R. H. Grubbs, R. K. Rosen and F. J. Timmers, Safe and convenient procedure for solvent purification, *Organometallics*, 1996, **15**, 1518-1520.
2. P. Rios, T. S. Carter, T. J. Mooibroek, M. P. Crump, M. Lisbjerg, M. Pittelkow, N. T. Supekar, G.-J. Boons and A. P. Davis, Synthetic receptors for the high-affinity recognition of O-GlcNAc derivatives, *Angew. Chem., Int. Ed.*, 2016, **55**, 3387-3392.
3. R. A. Tromans, T. S. Carter, L. Chabanne, M. P. Crump, H. Li, J. V. Matlock, M. G. Orchard and A. P. Davis, A biomimetic receptor for glucose, *Nature Chem.*, 2019, **11**, 52-56.
4. T. Türel and S. Valiyaveettil, A Naphthalene Diimide Based Macrocycle Containing Quaternary Ammonium Groups: An Electron-Deficient Host for Aromatic Carboxylate Derivatives, *ChemPlusChem*, 2020, **85**, 1430-1437.
5. A. H. G. David, P. García-Cerezo, A. G. Campaña, F. Santoyo-González and V. Blanco, [2]Rotaxane End-Capping Synthesis by Click Michael-Type Addition to the Vinyl Sulfonyl Group, *Chem. Eur. J.*, 2019, **25**, 6170-6179.