

Electronic Supporting Information

Tuneable pseudorotaxane formation between a biotin-avidin bioconjugate and CBPQT⁴⁺.

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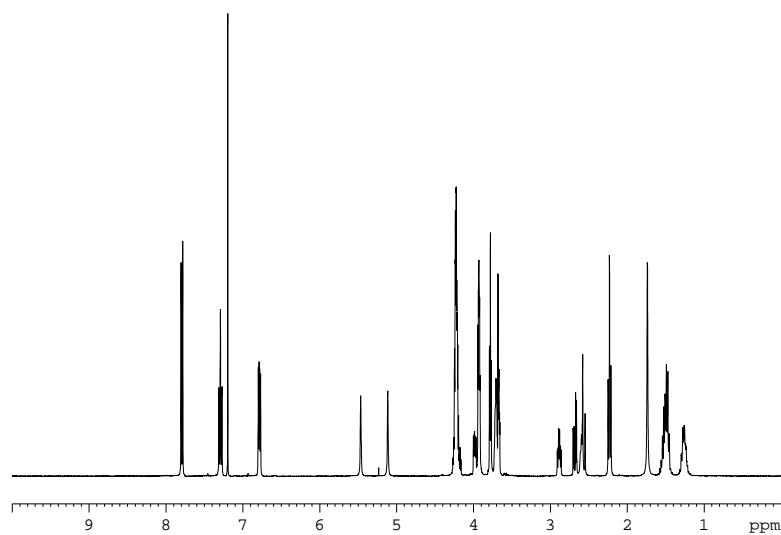
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1. Synthesis of compound 2

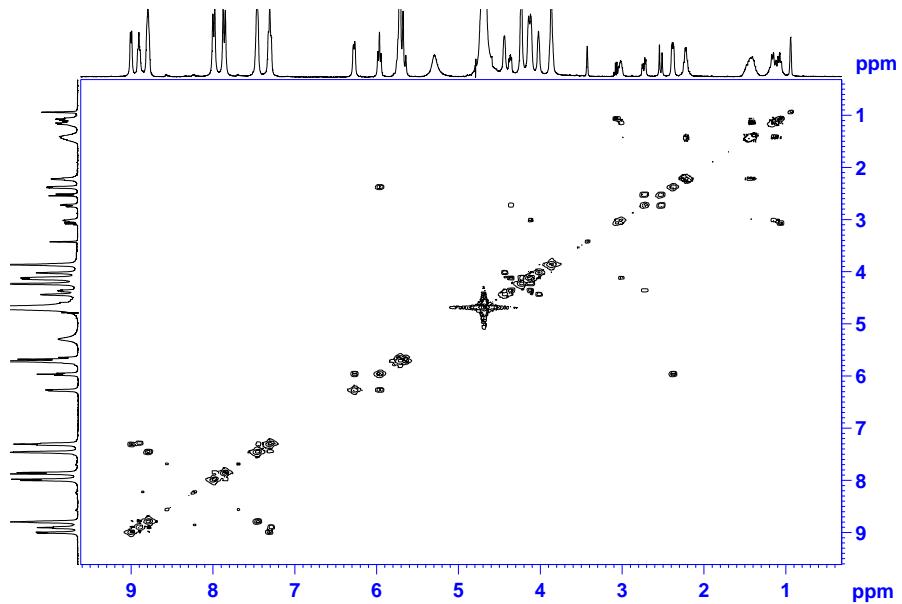
Biotin (125 mg, 0.5 mmol), pentafluorophenol (94 mg, 0.51 mmol), EDCI (0.5 mmol) DMAP (8 mg, 0.07 mmol) were dissolved in DMF (10 ml). The reaction was stirred at room temperature for 24 hrs. 1,5-Bis[2-(hydroxyethoxy)ethoxy]-naphthalene (200 mg, 0.6 mmol) was added and the reaction was stirred for a further 24 hrs. The solvent was removed under reduced pressure and the product was purified using silica gel column chromatography eluting with DCM/MeOH gradient elution to afford **2** as a yellow waxy solid (220 mg, 79% Yield).

¹H NMR (CDCl₃) δ = 1.26 (2H, m), 1.50 (4H, m), 2.23 (2H, t) 2.56 (1H, d), 2.67 (1H, dd), 2.90 (1H, q), 3.68 (4H, m), 3.77 (2H, t), 3.92 (4H, m), 3.98 (1H, m), 4.22 (1H, m), 4.25 (6H, m), 4.65 (1H, s), 4.95 (1H, s), 6.80 (2H, t), 7.29 (2H, t), 7.80 (2H, d). ¹³C NMR (CDCl₃) δ = 24.65, 28.08, 28.18, 33.79, 40.43, 55.25, 60.03, 61.73, 61.81, 63.41, 67.94, 67.99, 66.47, 69.69, 69.76, 72.67, 105.76, 105.84, 114.57, 114.72, 125.17, 125.27, 126.73, 154.3, 162.56, 173.63. MS (FAB/NOBA) 563.1 (M + H)⁺. Mpt. 102-104 °C. C₂₈H₃₈N₂O₈S requires C, 59.77; H, 6.81; N, 4.98 %. Found C, 59.67; H, 6.79; N, 4.95.

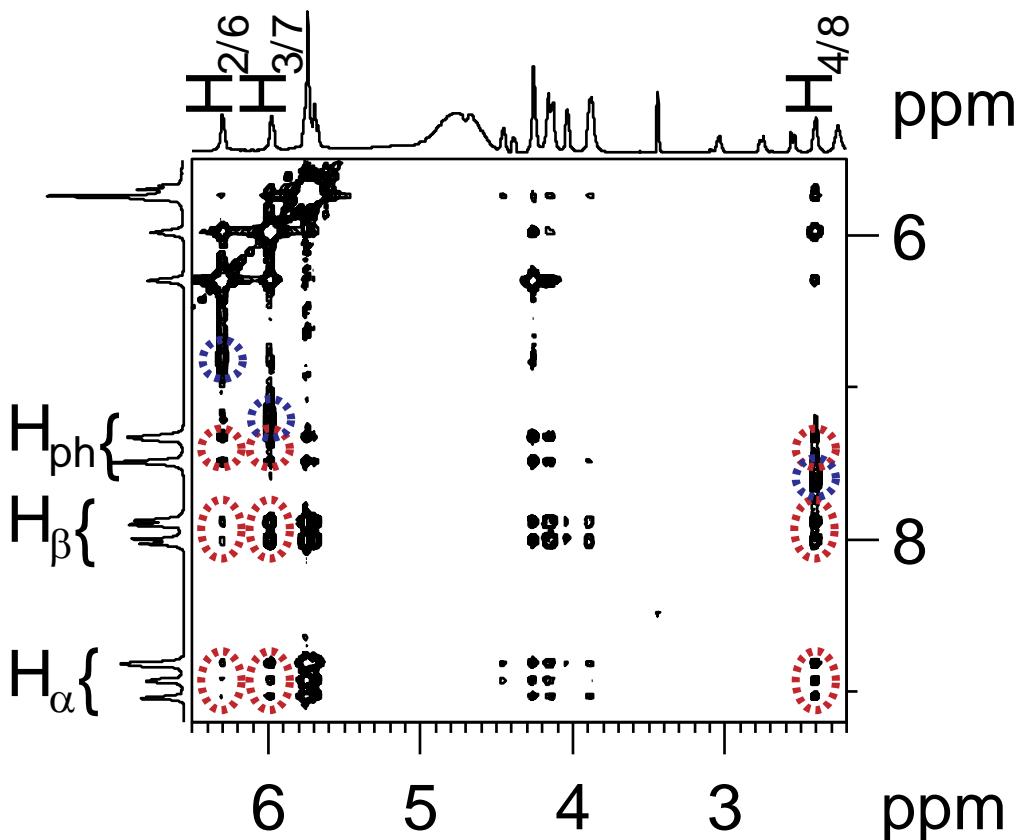


^1H NMR of compound **2** (CDCl_3 , 400 MHz)

2. NMR investigation of the complexation between **1 and **2****



2D COSY for the pseudorotaxane formed by **1** and **2** recorded in $\text{D}_2\text{O}/\text{EtOH}$ *d*-6 (70/30).



Partial 2D 400 ms NOESY spectrum of **1.2** recorded in $\text{D}_2\text{O}/\text{EtOH}$ *d*-6 (70/30) at 298K and 600 MHz. The nOe crosspeaks between the cyclophane and bound naphthalene moiety are highlighted as are the broad exchange peaks observed between the free and bound naphthalene shifts.

3. ITC Data.

Isothermal titration calorimetry (ITC) experiments were performed at 25 °C using a MicroCal VP-ITC titration microcalorimeter (MicroCal, Northampton, MA) with a sample cell volume of 1.4ml, following standard procedures.^{a,b} A 250 μl injection syringe was used with stirring at 310 rpm.

Samples were dissolved in deionised water/ethanol mix (70/30) and the solutions were degassed gently under vacuum before use. Each titration comprised an initial 1 μl pre-injection followed by 10 μl injections. A solution of **2** (0.569mM) was injected into avidin (0.053mM) until saturation. The syringe was then filled with a solution of **1** (0.959mM) and the titration continued.

Control experiments were performed with injections of **1** into a solution of avidin and also with injections of **1** or **2** into water/ethanol alone. These were used to correct titration data for heats of dilution prior to data analysis using a simple single-site binding model

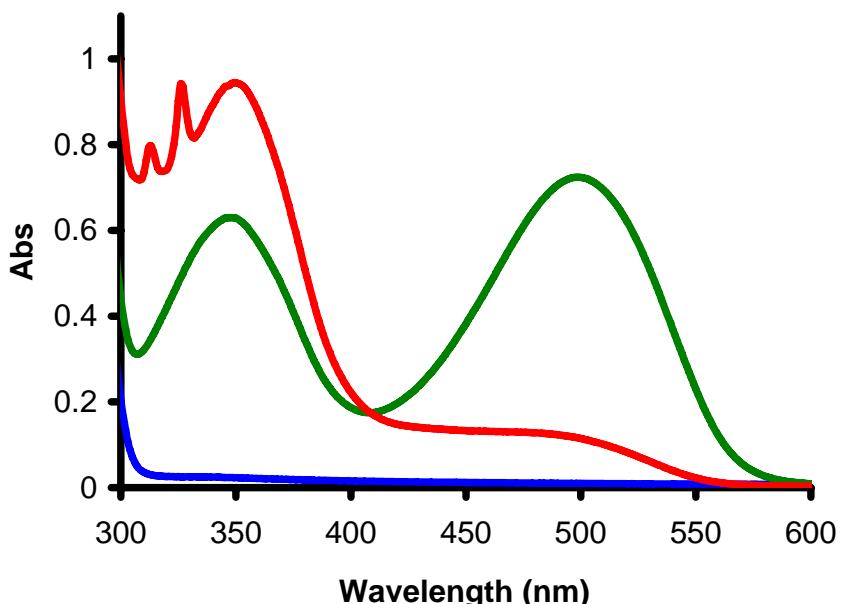
with non-linear regression (MicroCal Origin) to give apparent binding stoichiometry (N), association constant (K_a) and enthalpy of binding (ΔH). 1 cal = 4.184 J.

[a] T. Wiseman, S. Williston, J. F. Brandts, L. N. Lin, Rapid Measurement of Binding Constants and Heats of Binding Using a New Titration Calorimeter, *Anal. Biochem.* (1989) 179, 131-137.

[b] A. Cooper, C. M. Johnson, Isothermal Titration Microcalorimetry in C. Jones, B. Mulloy and A. H. Thomas (Eds.), *Microscopy, Optical Spectroscopy, and Macroscopic Techniques*. Humana Press, Totowa, NJ, (1994) p 137-150.

4. UV-vis Data.

UV-vis spectra were recorded using a Perkin-Elmer Lamda 25 spectrometer using optically matched 1cm cuvettes. The solvent used in all experiments was water:ethanol (70:30).



UV-vis spectra recorded in $\text{H}_2\text{O}/\text{EtOH}$ (70/30) showing: avidin (12×10^{-6} M, Blue line); and upon addition of HABA (5.25 mmol, green line); and upon the addition of **2** (5.25 mmol, red line)