Supporting Information

For the manuscript:

Carbohydrate Sensing using a Fluorescent Molecular Tweezer

Experimental

Materials

Nuclear magnetic resonance spectra were run in CDCl₃, CD₃OD, DMSO- d_6 or CDCl₃ (0.8 ml) and CD₃OD (2 drops). Where a Bruker AVANCE 300 was used ¹H spectra were recorded at 300.22 MHz and ¹H-¹³C at 75.50 MHz. Where a Bruker AVANCE 400 was used ¹H spectra were recorded at 400.13 MHz and ¹³C at 100.62 MHz. Chemical shifts (δ) are expressed in parts per million and are reported relative to the residual solvent peak or to tetramethylsilane as an internal standard in ¹H and ¹³C spectra. The multiplicities and general assignments of the spectroscopic data are denoted as: singlet (s), doublet (d), triplet, (t), quartet (q), quintet (quin), doublet of doublets (dd), doublet of doublets of doublets (ddd), doublet of doublets of doublets of doublets (dddd), doublet of triplets (dt), triplet of triplets (tt), unresolved multiplet (m), apparent (app), broad (br) and aryl (Ar). Where stated the ¹³C NMR spectra were subject to the polarisation that is nurtured during attached nucleus testing (PENDANT) technique, resulting in primary and tertiary carbon atoms having a different phase to secondary and quaternary carbon atoms.¹ The phases are reported as (+) positive phase and (-) negative phase. In certain instances the structural assignments reported for the ¹H NMR spectra were elucidated with the aid of correlated spectroscopy (COSY), heteronuclear multiple quantum coherence (HMOC) and heteronuclear multiple bond connectivity (HMBC) NMR experiments. Infrared spectra were obtained (following internal background calibration) in the range 600-4000 cm⁻¹ from KBr discs or CDCl₃ solutions using a Perkin-Elmer 1600 FT spectrometer. Characteristic absorption peaks are reported in wavenumbers (cm⁻¹). Mass spectra were recorded by the EPSRC National Mass Spectrometry Service Centre, Swansea. Electrospray ionisation measurements were performed in positive ionisation mode (ES^+). High resolution ES^+ measurements were conducted on either a Finnigan MAT95 high resolution double focussing mass spectrometer or a MAT900 high resolution double focussing mass spectrometer with tandem ion trap. Capillary

melting points were determined using a Büchi 535 melting point apparatus. The readings were taken from a mercury-in-glass thermometer and are reported uncorrected as the meniscus point (unless stated otherwise), rounded to the nearest 1 °C with a heating ramp rate of 0.5 °C minute⁻¹. Where the sample changed colour or evolved gas during or after the melt, thermal decomposition (dec) is noted.² Fluorescence measurements were performed on a Perkin-Elmer Luminescence Spectrophotometer LS 50B, utilising Starna Silica (quartz) cuvets with 10 mm path lengths, four faces polished. Data was collected via the Perkin-Elmer FL Winlab software package. All pH measurements taken during fluorescence experiments were recorded on a Hanna Instruments HI 9321 Microprocessor pH meter which was routinely calibrated using Fisher Chemicals standard buffer solutions (pH 4.0 phthalate, 7.0 - phosphate, and 10.0 - borate). All solvents used in fluorescence measurements were HPLC or Fluorescence grade and the water was deionised. All saccharides used in fluorescence measurements were certified as > 99% pure. Solvents and reagents were reagent grade unless stated otherwise and were purchased from Acros Organics, Avocado Research Chemicals Ltd. Fisher Scientific UK, Frontier Scientific Europe Ltd, Lancaster Synthesis Ltd and Sigma-Aldrich Company Ltd and were used without further purification, unless stated otherwise. Where dry solvents were required, dichloromethane was distilled from calcium hydride, whilst was distilled from sodium benzophenone ketyl, under a nitrogen atmosphere. Thin layer chromatography was performed using commercially available Merck or Macherev-Nagel aluminium backed plates coated with a 0.20 mm layer of silica gel 60 with fluorescent indicator UV_{254} . These plates were visualised using either ultraviolet light of 254 nm or 365 nm wavelength, or by staining the plates with vanillin or ninhydrin solution. Silica gel column chromatography was carried out using Davisil LC 60A silica gel (35-70 µm).

tert-Butyl 6-aminohexylcarbamate



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tert-Butyl phenyl carbonate (8.50 ml, 45.8 mmol) was added to a stirred solution of hexamethylene-1,6-diamine **3** (5.12 g, 44.0 mmol) in ethanol (50 ml) and heated at 78 °C overnight. The reaction mixture was allowed to cool before the solvent was evaporated and dichloromethane (100 ml) was added. The organic phase was extracted with an aqueous HCl solution (4 × 75 ml at $3 \le \text{pH} \le 7$). The aqueous phases were combined and the pH was increased to 14 by the addition of NaOH and the aqueous phase was extracted with dichloromethane (5 × 100 ml). These organic phases were recombined, dried over magnesium sulfate, filtered, and evaporated to give **4** as a peach suspension (4.25 g, 45%); v_{max} (CDCl₃) / cm⁻¹ 3456, 3018, 2980, 2932, 2858, 1708, 1508; δ_{H} (300 MHz, CDCl₃) 1.07 (2 H, s, N*H*₂), 1.23-1.30 (4 H, m, NH₂(CH₂)₂(C*H*₂)₂), 1.33-1.43 (13 H, m, CH₂C*H*₂(CH₂)₂C*H*₂CH₂, C(C*H*₃)₃), 2.61 (2 H, t, ³*J*_{HH} 4.5, NH₂CH₂), 3.03 (2 H, dt, ³*J*_{HH} 4.9, ³*J*_{HH} 4.9, *CH*₂NH), 4.77 (1 H, br s, N*H*Boc); δ_{C} PENDANT (75 MHz, CDCl₃) 26.8(+), 26.9(+), 28.6(-), 30.3(+), 33.9(+), 40.6(+), 42.3(+), 78.8(+), 156.3(+); *m*/z (ES⁺) 217.1908 ([M + H]⁺. C₁₁H₂₅N₂O₂ requires 217.1911).





Isophthaloyl dichloride (292 mg, 1.44 mmol) in dry dichloromethane (50 ml) was added dropwise to a solution of tert-butyl 6-aminohexylcarbamate 4 (651 mg, 3.01 mmol) and triethylamine (0.40 ml, 2.85 mmol) in dry dichloromethane (50 ml) at room temperature under nitrogen. The system was allowed to stir for 4 hours and the solvent was then evaporated. The resulting solid was dissolved in dichloromethane (75 ml) and washed with water (3×30 ml). The organic phase was evaporated to give **5** as a cream powder (780 mg, 96%); mp 119 °C (dec); v_{max} (KBr) / cm⁻¹ 3353, 2976, 2937, 2868, 1683, 1634, 1520; δ_H (300 MHz, CDCl₃) 1.34 (26 H, s, (CH₂)₂(CH₂)₂NHCOOC(CH₃)₃), 1.37-1.46 (4 H, m, CH₂CH₂NHCOOC), 1.54 (4 H, tt, ³*J*_{HH} 6.9, ³*J*_{HH} 6.9, CONHCH₂C*H*₂), 3.06 (4 H, t, ³*J*_{HH} 6.5, C*H*₂NHCOOC), 3.37 (4 H, dt, ³J_{HH} 6.3, ³J_{HH} 6.3, CONHCH₂), 4.62 (2 H, br s, NHCOOC), 6.82 (2 H, br s, CONH), 7.44 (1 H, app t, ³J_{HH} 7.7, ArH-5), 7.94 (2 H, app dd, ³J_{HH} 7.7, ⁴J_{HH} 1.7, ArH-4/6), 8.21 (1H, app t, ${}^{4}J_{\text{HH}}$ 1.5 ArH-2); δ_{C} PENDANT (75 MHz, CDCl₃) 26.0 (+), 26.3 (+), 28.8 (-), 29.6 (+), 30.4 (+), 39.9 (+), 40.3 (+), 79.6 (+), 125.3 (-), 129.3 (-), 130.7 (-), 135.1 (+), 156.8 (+), 167.1 (+); m/z (ES⁺) 563.3808 ([M + H]⁺. C₃₀H₅₁O₆N₄ requires 563.3803).





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Bis(carbamate) **5** (5.41 g, 9.61 mmol) was dissolved in a solution of hydrochloric acid (10 ml, S.G. 1.16, 32%), ethyl acetate (100 ml) and methanol (10 ml). The reaction mixture was then stirred at room temperature for 30 minutes, after which the solvents were evaporated to yield **11** as a cream powder (4.20 g, 100%); mp 241 °C (dec); v_{max} (KBr) / cm⁻¹ 3324, 2967, 2921, 2862, 1634, 1601, 1582, 1535; $\delta_{\rm H}$ (300 MHz, MeOD) 1.43-1.51 (8 H, m, (CH₂)₂(CH₂)₂NH₂HCl), 1.62-1.75 (8 H, m, CH₂(CH₂)₂CH₂ CH₂NH₂HCl), 2.94 (4 H, t, ³*J*_{HH} 7.5, CH₂NH₂HCl), 3.42 (4 H, t, ³*J*_{HH} 7.2, CONHCH₂), 7.57 (1 H, app t, ³*J*_{HH} 7.8, ArH-5), 7.97 (2H, app dd, ³*J*_{HH} 7.8, ⁴*J*_{HH} 1.8, ArH-4/6), 8.31 (1H, app t, ⁴*J*_{HH} 1.6 ArH-2); $\delta_{\rm C}$ PENDANT (75 MHz, MeOD) 27.4 (+), 27.8 (+), 28.9 (+), 30.6 (+), 41.1 (+), 41.2 (+), 127.7 (-), 130.3 (-), 131.6 (-), 136.6 (+), 169.8 (+); *m*/z (ES⁺) 363.2755 ([M + H - 2HCl]⁺. C₂₀H₃₅O₂N₄ requires 363.2755).





Pyrene-1-carboxaldehyde (328 mg, 1.43 mmol) was added to a solution of ammonium hydrochloride salt 11 (271 mg, 0.622 mmol) and caesium carbonate (192 mg, 0.589 mmol) in dry tetrahydrofuran (100 ml) and methanol (100 ml). The reaction mixture was heated at reflux for 7 hours, after which it was cooled to 0 °C and sodium borohydride (165 mg, 4.36 mmol) added slowly. The reaction was stirred at room temperature for a further 2 hours before the careful addition of 1 M hydrochloric acid (10 ml). The solvents were evaporated, prior to the extraction of the intermediate in dichloromethane $(3 \times 100 \text{ ml})$ from 0.5 M sodium hydroxide (100 ml). Methanol (100 ml) was added to the combined organic fractions and this was stirred to afford a clear solution, before being dried over magnesium sulfate, filtered and evaporated. The residue was dissolved in a solution of hydrochloric acid (10 ml, S.G. 1.16, 32%), methanol (50 ml) and dichloromethane (50 ml), then stirred for 30 minutes at room temperature. The solution was evaporated and the resulting salt was dissolved in the minimum amount of boiling, dry dichloromethane and then heated under reflux for 1 hour before allowing the system to cool to room temperature. The resulting suspension was slurried over a glass sinter, filtered and washed with dry dichloromethane (250 ml) to yield 6 as a yellow powder (365 mg, 68%); mp 181 °C (onset dec); v_{max} (KBr) / cm⁻¹ 2931, 2782, 1634, 1540, 847; δ_{H} (300 MHz, DMSO)

1.26-1.41 (8H, br m, $(CH_2)_2(CH_2)_2$ NHCH₂), 1.48-1.59 (4H, br m, CH_2 CH₂NHCH₂), 1.67-1.79 (4H, br m, CONHCH₂CH₂), 3.04-3.14 (4H, br m, CH_2 NHCH₂Ar), 3.20-3.29 (4H, br m, CONHCH₂), 4.90 (4H, br s, NHCH₂Ar) 7.52 (1H, app t, ${}^{3}J_{HH}$ 7.7, isophthalamido ArH-5), 7.96 (2H, app dd, ${}^{3}J_{HH}$ 7.7, ${}^{4}J_{HH}$ 1.7, isophthalamido ArH-4/6), 8.07-8.56 (18H, m, pyrenyl ArH), 8.69 (1H, br app t, ${}^{4}J_{HH}$ 5.6, isophthalamido ArH-2), 9.28 (2H, br s, CONH); δ_{C} (75 MHz, DMSO) 25.7, 26.2, 26.3, 29.2, 47.3, 47.5, 123.6, 124.0, 124.2, 125.1, 126.0, 126.05, 126.10, 126.2, 126.5, 126.9, 127.6, 128.5, 128.6, 129.5, 129.7, 130.0, 130.5, 131.0, 131.7, 135.1, 166.1; *m/z* (ES⁺) 791.4327 ([M + H - 2HCI]⁺. C₅₄H₅₅O₂N₄ requires 791.4320).



Figure S1. ¹H NMR spectrum, recorded at 300 MHz, of **6**, before conversion to a salt, in CDCl₃.

 N^2 , N^3 -bis(6-(N^1 , N^4 -(2-boronobenzyl)- N^1 , N^4 -((pyren-1- yl)methyl)amino)hexyl)iso-phthalamide



2

Potassium carbonate (494 mg, 3.57 mmol) was added to a solution of ammonium hydrochloride salt 6 (157 mg, 0.181 mmol) in tetrahydrofuran (100 ml) and acetonitrile (100 ml). The solution was stirred for 10 minutes before 2-(2-(bromomethyl)phenyl)-5,5-dimethyl-1,3,2-dioxaborinane³ (175 mg, 0.618 mmol) was added and the system heated under reflux for 2 days. The solvent was evaporated and the residue obtained was dissolved in water (50 ml) and hydrochloric acid (1 M) was used to adjust the pH to \sim 7. Dichloromethane (3 × 50 ml) was used to extract the product and the organic phases were combined, dried over magnesium sulphate, filtered and evaporated. The resulting oil was purified by flash chromatography (eluent methanol / dichloromethane 3 : 97 to 90 : 10) to yield 2 as a vellow powder (27.2 mg, 14%). δ_{H} (400 MHz, CDCl₃ with a few drops of CD₃OD) 1.00 (4 H, tt, ${}^{3}J_{\text{HH}}$ 7.1, ${}^{3}J_{\text{HH}}$ 7.1, $CH_{2}(\alpha)$), 1.10 (4 H, tt, ${}^{3}J_{\text{HH}}$ 7.4, ${}^{3}J_{\text{HH}}$ 7.4, $CH_{2}(\beta)$), 1.31 (4 H, tt, ${}^{3}J_{\text{HH}}$ 7.3, ³*J*_{HH} 7.3, *CH*₂(γ)), 1.47 (4 H, tt, ³*J*_{HH} 7.7, ³*J*_{HH} 7.7, *CH*₂(δ)), 2.46 (4 H, t, ³*J*_{HH} 7.9, $CH_2CH_2N(\zeta)$), 3.16 (4 H, t, ${}^{3}J_{HH}$ 7.2, $CONHCH_2(\eta)$), 3.82 (4 H, s, $NHCH_2Ar(\theta)$), 4.16 (4 H, s, NHC H_2 Ar(1)), 7.22-7.38 (9H, m, phenyl ArH(v) and isophthalamido ArH-5(λ)), 7.75 (2 H, app dd, ${}^{3}J_{HH}$ 7.7, ${}^{4}J_{HH}$ 1.7, isophthalamido ArH-4/6(μ)), 7.87-

8.08 (19 H, m, pyrenyl Ar*H*(ξ) and isophthalamido Ar*H*-2(o)); $\delta_{\rm C}$ PENDANT (100 MHz, CDCl₃ with a few drops of CD₃OD) 24.8 (-, CH₂(δ)), 26.4 (-, CH₂(β)), 26.9 (-, CH₂(α)), 29.1(-, CH₂(γ)), 39.8 (-, CH₂(η)), 39.9 (-, CH₂(η)), 53.1 (-, CH₂(ζ)), 54.3 (-, CH₂(ι)), 62.0 (-, CH₂(θ)), 123.1 (+), 124.5 (+), 124.6 (-), 124.8 (-), 125.0 (+), 125.16 (+), 125.23 (+), 125.8 (+), 127.29 (+), 127.34 (+), 127.4 (+), 128.5 (+), 128.7 (+), 129.7 (+), 129.8 (-), 130.0 (+), 130.4 (-), 130.6 (-), 130.8 (-), 131.1 (+), 134.8 (-), 136.0 (+), 141.6 (-), 166.77 (-, CO), 166.84 (-, CO); *m/z* (ES⁺ in CD₃OD/CDCl₃) 1063.5132 ([M - 4H + 4D + H]⁺. C₆₈H₆₅D₄O₆N₄B₂ requires 1063.5669 (mass is -50.5 ppm, exact mass calculated by Bruker Daltonics IsotopePattern).



Figure S2. ¹H NMR spectrum, recorded at 300 MHz, of **2** in CDCl₃ with a few drops of MeOD.

Fluorescence Measurements

Buffer and Solvents

The fluorescence titrations of **2** with different saccharides were carried out in a pH 8.21 aqueous methanolic buffer. The buffer was prepared according to the literature method of Perrin and Dempsey⁴ and contained: 52.1wt% HPLC grade methanol in deionised water with KCl (10.0 mM), KH₂PO₄ (2.75 mM), and Na₂HPO₄ (2.75 mM). When not in use the buffer was stored in the dark at 4 °C, with the solution being allowed time to return to room temperature before each use.

Fluorescence Titrations

In each instance the required sensor was weighed out in a small volumetric flask. The sample was then made up to the required volume with HPLC grade methanol to generate a stock solution of known molarity. 50 μ L of the stock solution were then transferred *via* microsyringe to a beaker containing a known volume of the stirred pH 8.21 aqueous methanolic buffer (the buffer was transferred by pipette). This method therefore generated a solution of known molarity (with a typical volume of 50 ml or 100 ml).

Fluorescence spectra were recorded as increasing amounts of solid saccharide (D-glucose, D-galactose, D-fructose and D-mannose) were added to the stirred sensor solutions. Typically the fluorescence response was evaluated over a saccharide concentration range from 0 to 0.1 mol dm^{-3} .

Data Analysis

Data was collected *via* the Perkin-Elmer FL Winlab software package on a PC running a Microsoft Windows. The observed stability constants (K_{obs}) with coefficient of determination (r^2) were calculated by the fitting of emission intensity at a single wavelength *versus* saccharide concentration using custom written non-linear (Levenberg-Marquardt algorithm) curve fitting.⁵ Fitting at two wavelengths was done using Scientist (MicroMath).

Spectra



pH Titrations

Figure S3. Fluorescence intensity versus pH profile of **2** (0.1 μ M, $\lambda_{ex} = 342$ nm, $\lambda_{em} = 377$ nm) displaying PET (•)alone and with (•)D-fructose, in an aqueous methanolic solution (52.1 wt% methanol).



Figure S4 The fluorescence emission spectrum of **2** 0.1 μ M, $\lambda_{ex} = 342$ nm) with increasing amounts of D-galactose **9** (0.00 to 0.10 M) in a pH 8.21 aqueous methanolic buffer solution [52.1 wt% methanol (KCl (10.0 mM), KH₂PO₄ (2.75 mM) and Na₂HPO₄ (2.75 mM)].



Figure S5 The fluorescence emission spectrum of **2** 0.1 μ M, $\lambda_{ex} = 342$ nm) with increasing amounts of D-mannose **10** (0.00 to 0.10 M) in a pH 8.21 aqueous methanolic buffer solution [52.1 wt% methanol (KCl (10.0 mM), KH₂PO₄ (2.75 mM) and Na₂HPO₄ (2.75 mM)].



Figure S6. Relative fluorescence intensity versus carbohydrate concentration profile of **2** (0.1 μ M, $\lambda_{ex} = 342$ nm, $\lambda_{em} = 377$ nm) displaying PET with (\blacktriangle) D-glucose, (\bullet) D-fructose, (\bullet) D-galactose, (\bullet) D-mannose in a pH 8.21 aqueous methanolic buffer solution [52.1 wt% methanol (KCl (10.0 mM), KH₂PO₄ (2.75 mM) and Na₂HPO₄ (2.75 mM)].



Figure S7. Relative fluorescence intensity versus carbohydrate concentration profile of **2** (0.1 μ M, $\lambda_{ex} = 342$ nm, $\lambda_{em} = 470$ nm) displaying excimer emission with (\blacktriangle) Dglucose, (•) D-fructose, (•) D-galactose, (•) D-mannose in a pH 8.21 aqueous methanolic buffer solution [52.1 wt% methanol (KCl (10.0 mM), KH₂PO₄ (2.75 mM) and Na₂HPO₄ (2.75 mM)].

References

- 1. J. Homer and M. C. Perry, J. Chem. Soc., Perkin Trans. 2, 1995, 533-536.
- 2. A. Gehenot, R. C. Rao, G. Maire and M. Gachon, *Int. J. Pharm.*, 1988, **45**, 13-17.
- 3. D. Stones, S. Manku, X. S. Lu and D. G. Hall, *Chem. Eur. J.*, 2004, **10**, 92-100.
- 4. D. D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control*, Chapman & Hall, London, 1974.
- 5. C. R. Cooper and T. D. James, J. Chem. Soc., Perkin Trans. 1, 2000, 963-969.