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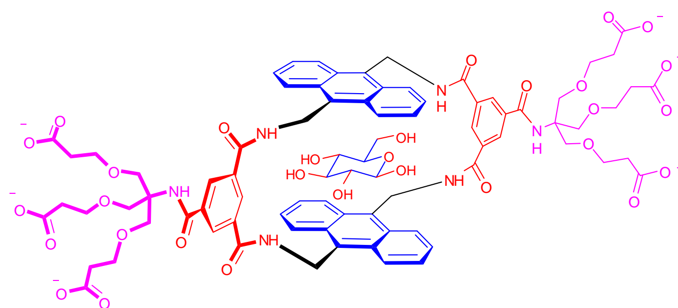
GRAPHICAL ABSTRACT

Selective carbohydrate binding is a difficult task, usually accomplished by proteins (lectins) or complex synthetic analogues. It has now been achieved by a remarkably simple structure, accessible in just 5 steps from commercially available materials. The new receptor is highly selective for all-equatorial carbohydrates, and may be used to sense glucose through changes in anthracene fluorescence.



A simple and accessible synthetic lectin for glucose recognition and sensing

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Binding carbohydrates from water is a difficult task, even for the natural carbohydrate-binding proteins known as lectins. The design of synthetic lectin mimics is correspondingly challenging, especially if good selectivities are required. In previous work we have shown that success is possible, but only for complex polycyclic architectures requiring lengthy and low-yielding syntheses; for example, one glucose-selective system was made in 21 steps and only 0.1% overall yield. Here we report the discovery of a simple monocyclic host which matches the earlier designs but is far more accessible, having been prepared in just 5 steps and 23% overall yield. The new synthetic lectin binds glucose with excellent selectivity vs. other common monosaccharides (for example, ~50:1 vs. galactose), and sufficient affinity for glucose sensing at the concentrations found in blood. It also features a built-in signalling system in the form of strong and guest-dependent fluorescence emission. The effectiveness and simplicity of this molecule suggests potential for development into new methodology for practical glucose monitoring.

Carbohydrate recognition in aqueous solution is a particular challenge for supramolecular chemistry[1,2]. Carbohydrates are hydrophilic species, and therefore difficult to extract from water. Being coated with hydroxyl groups they are also hydromimetic, blending easily into a background of water molecules. Distinguishing between substrate and solvent is thus a difficult problem for receptors, whether natural or artificial. Achieving selectivity is also non-trivial; while carbohydrate structures allow great scope for variation, differences are often subtle (for example, the configuration of a single asymmetric centre). Carbohydrate-binding proteins (lectins) show notably low affinities[3,4], and often quite modest selectivities.

Lectins are important tools for research and medicine (especially diagnostics)[5-9], so their limitations present opportunities for synthetic analogues. However, unsurprisingly, the design of synthetic lectins is not straightforward. Although progress has been made[8,10-18], affinities are mostly low and good selectivities are rare. Moreover, success usually comes at the cost of structural complexity. The octalactam **2** (Fig. 1a), reported previously by our group[18], provides a good example. This tricyclic system is able to surround a β-glucose unit **1**, providing polar and apolar surfaces which complement the all-equatorial substitution pattern of the substrate. Selectivity for glucose is excellent; for example, ratios of binding constants are 20:1 for glucose *vs.* galactose, and 60:1 for glucose *vs.* mannose. The affinity for glucose, at 60 M^{-1}, may seem low, but this is state-of-art for a synthetic system operating in water through non-covalent interactions. Furthermore, it is not too low to be useful. A key application for glucose-selective receptors is the monitoring of glucose in diabetics. As blood glucose levels are relatively high, affinities should be moderate to avoid saturation[18].

Although the properties of **2**, and related systems, are very promising, the elaborate structures impose a barrier to further development. The synthesis of **2** requires 20 steps, with an overall yield of just ~0.1%. Preparing substantial quantities is difficult, and further modification (e.g. to link the receptor to a surface) represents a major undertaking. We have therefore considered alternative approaches to the design of synthetic lectins, which might involve simpler structures requiring less synthetic effort. Herein we report a surprising success in the form of bis-anthracenyl system **5** (Fig. 1b) As discussed below, this new receptor is available in just 5 steps from commercially-available starting materials, a dramatic improvement on **2**. Despite its simplicity and accessibility, **5** matches the performance of **2** as a glucose-selective receptor while possessing improved signalling properties due to the anthracene fluorescence. Receptor **5** could point the way to genuinely practical blood glucose monitors, and also to a new strategy for improving the efficiency of synthetic lectin designs.

Results and Discussion

Receptor design and synthesis. Underlying this work was the notion that condensed aromatic units could play a useful role in the design of improved synthetic lectins. Contact between aromatic surfaces and

carbohydrate CH groups is often observed in lectin-saccharide complexes[19,20], and it is widely thought that CH-π interactions[21-23], allied to hydrophobic effects[24,25], make important contributions to binding. In our synthetic lectins to date (e.g. **2**), the aromatic surfaces have been provided by oligophenyl units. Though helpful synthetically,[11] the biphenyl bond tends to twist due to steric interference between ortho hydrogens, and this can disturb the interactions between rigidly positioned axial CH groups and the aromatic surfaces (Fig. 1d, left). By contrast, a condensed aromatic unit can make ideal contact with an array of axial CH groups (Fig. 1d, right). Moreover, the carbohydrate can slide across the surface without significant loss of binding energy, so that (a) other interactions can be maximised and (b) some freedom of movement can be retained within the complex (hence less entropy loss on binding). The optical properties of condensed aromatics should also be advantageous. These units tend to be strongly absorbing and fluorescent, with potential for modulation on binding and thus for application in sensing.

As a first test of this concept, the monocycle **5** seemed an interesting prospect. As shown in Fig. 1b, it should be accessible in just two steps (cyclisation and deprotection) from diamine **3** and the spacer component **4**[15]. An organic-soluble analogue had been prepared by the group of B. D. Smith via a similar cyclisation (although not applied to carbohydrate binding)[26]. Calculations suggested that **5** could adopt a range of conformations with different angles between the aromatic surfaces, but that all low-energy structures would feature a cleft or cavity (see, for example, Fig. 1c). It was not clear that this simple and rather flexible architecture would favour any particular saccharide, or indeed that it would show any notable carbohydrate-binding properties. However the enclosed, amphiphilic nature of the cavity did seem generally suitable for carbohydrate recognition. We were also aware that an acyclic receptor based on a single anthracene unit had shown evidence of binding to complex carbohydrate derivatives[27].

Macrocycle **5** was prepared in ~23% yield via the projected route (Fig. 1b). Diamine component **3**[28] is available commercially, but was also conveniently synthesised by bis-bromomethylation of anthracene followed by treatment with hexamethylenetetramine[29]. Diester **4** was prepared via a 3-step procedure which is well-established in our group. For comparison purposes we also synthesised the asymmetrical system **9** (Fig. 2), in which a single anthracene is paired with a smaller *p*-xylyl unit. Although a longer sequence was required for **9**, the process was straightforward. Aside from delivering this useful control, the route in Fig. 2

should be adaptable to a variety of analogues of **5**. It should thus be possible to tune the binding and/or optical properties of the receptor, for example by placing substituents in one anthracene unit.

Binding and structural studies. Macrocycle **5** dissolved in D_2O at concentrations up to ~4 mM giving clean, if slightly broadened, ^{1}H NMR spectra. Minor signal movements were observed on dilution to ~1 mM but no further effect was observed below this concentration, implying that the system is monomeric at 1 mM or less. Binding to carbohydrate substrates was studied initially by ^{1}H NMR titrations at 298 K. Addition of some carbohydrates to **5** caused substantial changes to the spectrum of the receptor, especially to the chemical shifts of isophthaloyl protons E and F (see Fig. 3 for key). For example, addition of glucose caused a downfield movement of the signal due to internally-directed protons E, with $\Delta\delta$ tending towards ~0.8 p.p.m (Fig. 4a). The signal due to externally-directed protons F also shifted downfield, by ~0.15 p.p.m. Small movements of the signals due to anthracene protons A and B were observed, while the spectrum sharpened considerably during the titration. The movements of protons E and F gave excellent fits to a 1:1 binding model (see Fig. 4b and Supplementary Information), yielding values for the association constant K_a of 58 and 54 M^{-1} respectively (average = 56 M^{-1}). Methyl β-D-glucoside **10** (Fig. 3) produced similar effects, analysed to give $K_a = 96$ M^{-1}. In this case a Job plot[30] was performed, providing further support for 1:1 stoichiometry (see Supplementary Fig. S71 online).

Data for the other substrates were analysed to give the binding constants listed in Table 1. Values for tricyclic system **2** are recorded for comparison. Given the relative simplicity of **5**, one might expect reduced performance. Remarkably, however, the two systems behave quite similarly, the main difference being that **5** is the *more* selective for glucose vs. other monosaccharides. Thus, both **2** and **5** prefer the all-equatorial carbohydrate moieties, binding well to glucose (for which the K_a values are almost identical), methyl β-D-glucoside and, to a lesser extent, 2-deoxyglucose, *N*-acetylglucosamine and xylose. Receptor **5** also binds fairly strongly to anionic glucuronic acid derivatives. Selectivity against other monosaccharides is generally good for both systems, but **5** is appreciably superior. Aside from methyl α-D-glucoside, all “non-target” monosaccharides were bound by **5** with $K_a \leq 1$ M^{-1}. With disaccharides, **5** seems to bind significantly to any system containing β-glucosyl (cellobiose, maltose, lactose). Here there is a qualitative difference from **2**, which binds cellobiose well but shows no affinity for maltose.

Complex formation could also be studied by fluorescence spectroscopy. On excitation with UV light at 394 nm, **5** emitted in the blue-violet region, peaking at 423 nm, with a quantum yield of 2.4% (20 μM aqueous solution). Addition of glucose caused the emission intensity to increase by factors of up to 2.5 (Fig. 4c). Analysis of the changes gave, again, an excellent fit to a 1:1 binding curve. Binding constants obtained by this method were in good agreement with those measured by NMR titrations (see Table 1). Moreover these fluorescence characteristics are promising for practical glucose sensing, especially when compared to the biphenyl-based synthetic lectins. The excitation wavelength is only just outside the visible region, thus relatively safe and obtainable with inexpensive UV LEDs. In contrast, the biphenyl-based systems require light at ~280 nm for excitation, and produce far weaker emission which does not always change on binding.

Binding of **5** to glucose and methyl β-D-glucoside was studied by a third technique, Isothermal Titration Calorimetry (ITC). Measured affinities were again consistent with those determined by NMR titrations (Table 1), and revealed that complexation was enthalpy-driven with significant negative entropies (e.g. for glucose, ΔH = -3.8 kcal mol^{-1}, $T\Delta S$ = -1.4 kcal mol^{-1}). This contrasts with the oligophenyl-based synthetic lectins, where binding entropies are positive (e.g. for **2**+**10**, ΔH = -0.6, $T\Delta S$ = 2.3 kcal mol^{-1})[16-18]. However, negative binding entropies are common for natural lectins[3]. The difference between **2** and **5** may reflect a greater conformational freedom in the latter, which is presumably lost on binding. The observation of negative ΔS does not preclude a role for hydrophobic interactions[30]. Indeed, with fewer polar spacers, it seems likely that **5** is less dependent on polar interactions than tricyclic cages such as **2**, and thus more reliant on the displacement of high-energy water. This is supported by experiments in less polar media, where H-bonding must dominate. Thus the organic soluble (t-butyl protected) precursor of **5** bound octyl β-D-glucoside in chloroform with K_a = 3200 M^{-1}. The corresponding value for a biphenyl-based system was ~100 times higher[31].

The role of non-polar interactions was highlighted by studies on control macrocycle **9**. This compound possesses the same polar groups as **5**, but provides less apolar surface for hydrophobic/CH-π interactions. Addition of some carbohydrates (e.g. glucose, xylose) to **9** yielded minor changes in the ^{1}H NMR spectrum of the macrocycle. However signal movements were almost linear with concentration, implying K_a too small to measure.

The 3D structure of **5** and its complex with methyl β-D-glucoside **10** was studied by 2D nOe spectroscopy (NOESY) at 600 MHz (^{1}H) (for details see Supplementary Information). In the case of **5** itself, the main issue is the orientation of the annular amide groups, which can be positioned such that either NH or CO point inward. Strong NOESY cross- peaks between NH protons *D* and spacer CH *E* (see Fig. 3 for labelling), and the absence of connections *D-F*, indicated that inward-directed NH groups are preferred (see Supplementary Figs. S62-S64 online). The data thus support the calculated structure shown in Fig. 1c. To study the complex **5**.**10**, an excess of **10** was added such that ~90% of **5** was in the bound state. Again, the intramolecular NOESY signals showed a strong preference for the "NH-in" arrangement (see Supplementary Figs. S66 and S67 online). A large number of intermolecular cross-peaks were observed at long nOe mixing times (see Supplementary Fig. S68 online), but at short mixing times the connections *D-4* and *D-5* stood out strongly, followed by $D\text{-}6_R$. The data are best accommodated by structures in which the substrate CH_2OH passes through the tetralactam ring so that H4 and H5 can come into contact with two diametrically opposite protons *D*. One such structure is shown in Fig. 5. This substrate positioning allows the formation of 4 intermolecular NH···O bonds to four substrate oxygens, as well as 6 CH-π contacts. Interestingly, the distance between the aromatic surfaces in this structure is smaller than previously determined for a biphenyl-based synthetic lectin.[17] This suggests a tight fit, which may contribute to the negative entropy of binding. The loss of receptor mobility, and the change from angled to parallel anthracene units, may account for the change in fluorescence. The structure in Fig. 5 can help to explain the selectivities. An axial OH group, as in galactose or mannose, would clearly disrupt this structure, while the loss of CH_2OH from the substrate (to give xylose) removes both polar and apolar binding interactions. On the other hand, several of the better substrates (glucuronides, cellobiose, maltose, lactose) do not seem compatible with this binding geometry, so other modes of interaction could be possible.

Finally, a number of further experiments were performed to test the potential of **5** for glucose monitoring *in vivo*. Lactate and mannitol are carbohydrate-like molecules which can be present in blood, and which often bind to boron-based receptors[32,33]. Neither produced any response when added to **5**. Binding to glucose was also studied at physiological temperature (310 K). The affinity measured by NMR titration was 33 M^{-1}, lower than at room temperature (as expected). However this is still potentially useful, implying receptor occupancy of 6 - 25% across the physiological range of 2 - 10 mM glucose.

Photobleaching of **5** was found to be relatively slow. Under continuous UV irradiation in a fluorescence spectrometer, emission decayed by <10% in 5 hours (see Supplementary Fig. S61 online). In a practical device this would translate to a long lifetime as the receptor would be subjected only to short pulses of light every few seconds.

Conclusions

Recent research on synthetic lectins suggests that, for some applications, they have real potential as alternatives to their natural counterparts. For example, as previously noted[18], tricyclic octalactam **2** is considerably more glucose-selective than the lectins normally used to bind this substrate (e.g. Concanavalin A, Lens Culinaris Agglutinin, Pisum Sativum Agglutinin). In the present work, we have shown that similar performance (in some respects better) can be obtained with a far simpler and highly accessible structure. Moreover, this new receptor **5** possesses fluorophore units with convenient absorption and emission characteristics, which respond to binding and provide a built-in signalling system for glucose sensing. The mode of binding is such that substituents added to the anthracene chromophores are unlikely to cause large effects, implying that tuning the chromophores to further optimise the optical properties should have only minor effects on binding. This system therefore presents an excellent starting point for a new approach to practical blood glucose monitoring. While alternatives employing boron-based receptors or natural lectins are more mature,[34,35] they are not without problems and may not prove optimal. Our work also highlights the potential of condensed aromatic units as hydrophobic surfaces in synthetic lectins. The success of such a simple system based on this strategy bodes well for future, more sophisticated designs employing a similar approach.

Methods

Synthesis of 5. A solution of **4**[15] (1.6 g, 1.55 mmol) in anhydrous THF (45 mL) was added dropwise over 30 h to a solution of **3** (367 mg, 1.55 mmol) and di-isopropylethylamine (5 mL) in anhydrous THF (1 L) under nitrogen. After a further 24 h stirring, the solvent was removed under reduced pressure. The residue was dissolved in DCM (100 mL) and washed with saturated aqueous NH_4Cl (100 mL), water (100 mL) and brine (100 mL). The organic solution was dried over Na_2SO_4 and evaporated in *vacuo*. The residue was taken up in DMSO (12 mL), filtered and purified by preparative HPLC to yield the O-t-butyl-protected analogue of

macrocycle **5** (370 mg, 0.21 mmol, 27%). This material was dissolved in DCM (20 mL) and cooled in ice. Trifluoroacetic acid (TFA) (5 mL) was added drop-wise and the mixture was allowed to warm to room temperature then stirred for 3 h. The solvent was removed in *vacuo*, and the residue was suspended in water (5 mL). NaOH aq. (0.5 M, ca. 8 equiv.) was added dropwise until the suspended material dissolved to form a clear solution. The solution was freeze-dried and further purified by preparative HPLC to give receptor **5** (150 mg, 85%). For further details and characterisation data, and details of the synthesis of **9**, see Supplementary Information pages S2-S15 online.

^{1}H NMR titrations. Titrations were performed on a Varian 500 MHz spectrometer. Solutions of reducing carbohydrates were prepared in D_2O and kept overnight at room temperature before the titration experiments, in order to ensure equilibration of anomers. In a typical titration, aliquots of carbohydrate solution were added to receptor solution (DSS as internal standard) and the ^{1}H NMR spectra were recorded. Variations in chemical shifts were entered into a specifically written non-linear least squares curve-fitting program implemented within Excel. Assuming 1:1 stoichiometry, the programme calculates K_a and the limiting change in chemical shift $\Delta\delta$. The assumption is supported by the generally good fits between observed and calculated data. For further details, including spectra and fitting curves, see Supplementary Information page S16 and Figs. S8-S55 online.

Fluorescence titrations. Titrations were carried out at 298 K on a PerkinElmer LS45 spectrometer in PBS buffer solution (pH 7.1, 100 mM). The carbohydrate stock solutions were prepared by dissolving the carbohydrates in buffer containing the receptor at the concentration to be used in the titration (thus avoiding dilution of the receptor during the experiment). The solutions were kept overnight at room temperature before the titration experiments, in order to ensure equilibration of anomers. The wavelength to be used for fluorescence excitation was determined by measurement of the UV-visible spectrum of receptor **5** in the presence of carbohydrates. 394 nm was chosen, because at this wavelength the absorption of receptor was almost independent of carbohydrate concentration. In a typical titration, aliquots of carbohydrate-receptor solution were added to receptor solution (2.5 mL) in a quartz cuvette (3 mL, 10 mm pathway). The solution was stirred for 2 min and left to stand for another 1 min before the emission spectrum was recorded. Binding constants were calculated using non-linear curve fitting assuming 1:1 binding stoichiometry, employing both

Kaleidagraph and a customized Excel spreadsheet. Errors were estimated at < 5%. For examples of spectra and fitting curves see Supplementary Figs. S56-S58 online.

Isothermal titration microcalorimetry (ITC) experiments. ITC experiments were performed on a VP-ITC (Microcal, Inc., Northampton, MA) at 298 K. Stock solutions of carbohydrates were made up in pure HPLC grade water and allowed to equilibrate overnight. Receptor solutions were made up in pure water. All the solutions were degassed and thermostated using the ThermoVac accessory before titration. The sample cell volume was 1.4226 mL. Each titration experiment included 25-35 successive injections. For output traces and data analyses see Supplementary Figs. S59 and S60 online.

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Author contributions.

The experimental work was performed mainly by C.K., with a contribution from H.D. towards the synthesis of **9**. C.K., A.P.D. and M.P.C. planned the work and analysed the results. The paper was jointly written by C.K. and A.P.D. with assistance from the other authors.

Additional information

The authors declare no competing financial interests. Supplementary information and chemical compound information accompany this paper at www.nature.com/naturechemistry. Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/. Correspondence and requests for materials should be addressed to A.P.D.

Table 1. Association constants K_a to receptors **5** and (for comparison) **2** in aqueous solution.

	Substrate	K_a (M^{-1})	
		5	**2**‡
All-equatorial monosaccharides	D-glucose	56 (58*, 55†)	60
	methyl β-D-glucoside **10**	96 (121*, 101†)	130
	2-deoxy-D-glucose	36	29
	N-acetyl-D-glucosamine	10	7
	D-xylose	9	17
	D-glucuronic acid, sodium salt	24	
	methyl β-D-glucuronide, sodium salt	87	
Other monosaccharides	methyl α-D-glucoside	6	15
	D-galactose	1	3
	D-mannose	1	~1
	D-fructose	~0	
	L-rhamnose	~0	~0
	L-fucose	~0	3
	D-arabinose	1	4
	D-lyxose	~0	~1
	D-ribose	~0	6
Disaccharides and miscellaneous substrates	D-cellobiose	28	71
	D-maltose	35	~0
	D-lactose	16 (9*)	8
	D-sucrose	~0	
	D-trehalose	~0	
	mannitol	~0	
	sodium lactate	~0	

Association constants measured by ^{1}H NMR titration in D_2O at 298 K. For structures of substrates see Supplementary Fig. S7 online. Values denoted ~0 were too small for analysis. Errors were estimated at ≤ 10% for most cases where $K_a \geq 10$ M^{-1}. For further details, see Supplementary Information. *Measured by fluorescence titration in phosphate buffer solution (pH 7.1, 0.1 M) at 298 K. †Measured by ITC titration in water at 298 K. ‡Data from ref 18.

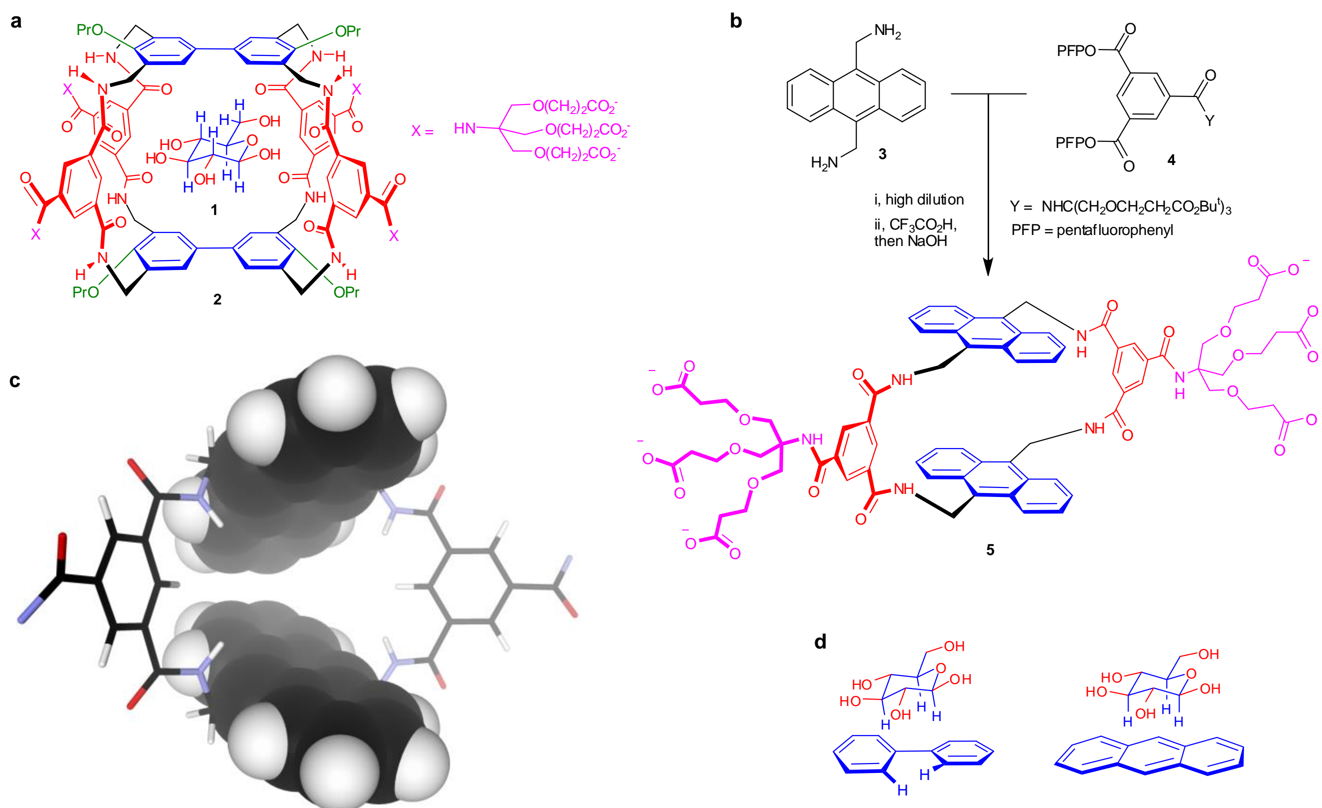

Figure 1 | Synthetic lectins - old and new designs. a, Previously reported[18] biphenyl-based system **2**, shown binding β-D-glucose **1**. Complex formation is thought to be driven by hydrophobic/CH-π interactions between substrate CH and biphenyl surfaces (blue), and by polar interactions between substrate OH groups and receptor isophthalamides (red). The propoxy groups are required for optimal glucose-selectivity. **b,** Synthesis and structure of the monocyclic synthetic lectin **5** described in this paper. **c,** The ground state conformation of **5** as predicted by Monte Carlo Molecular Mechanics calculations. The water-solubilising side chains are removed for clarity, and the anthracene units are shown in space-filling mode. **d,** Comparison of the interactions of (left) biphenyl and (right) condensed aromatic units with β-D-glucose **1**.

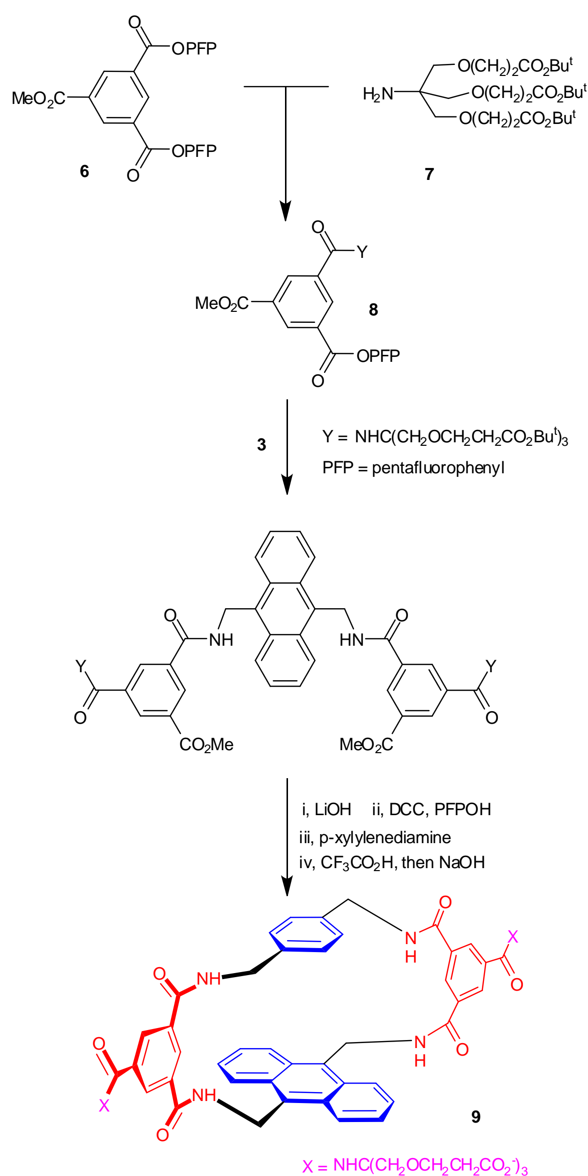

Figure 2 | Synthetic route to control macrocycle 9. Adaptation of this stepwise procedure should grant access to further analogues of **5** with dissimilar hydrophobic units.

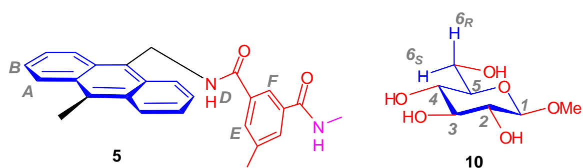

Figure 3 | Labelling systems for NMR binding and structural studies.

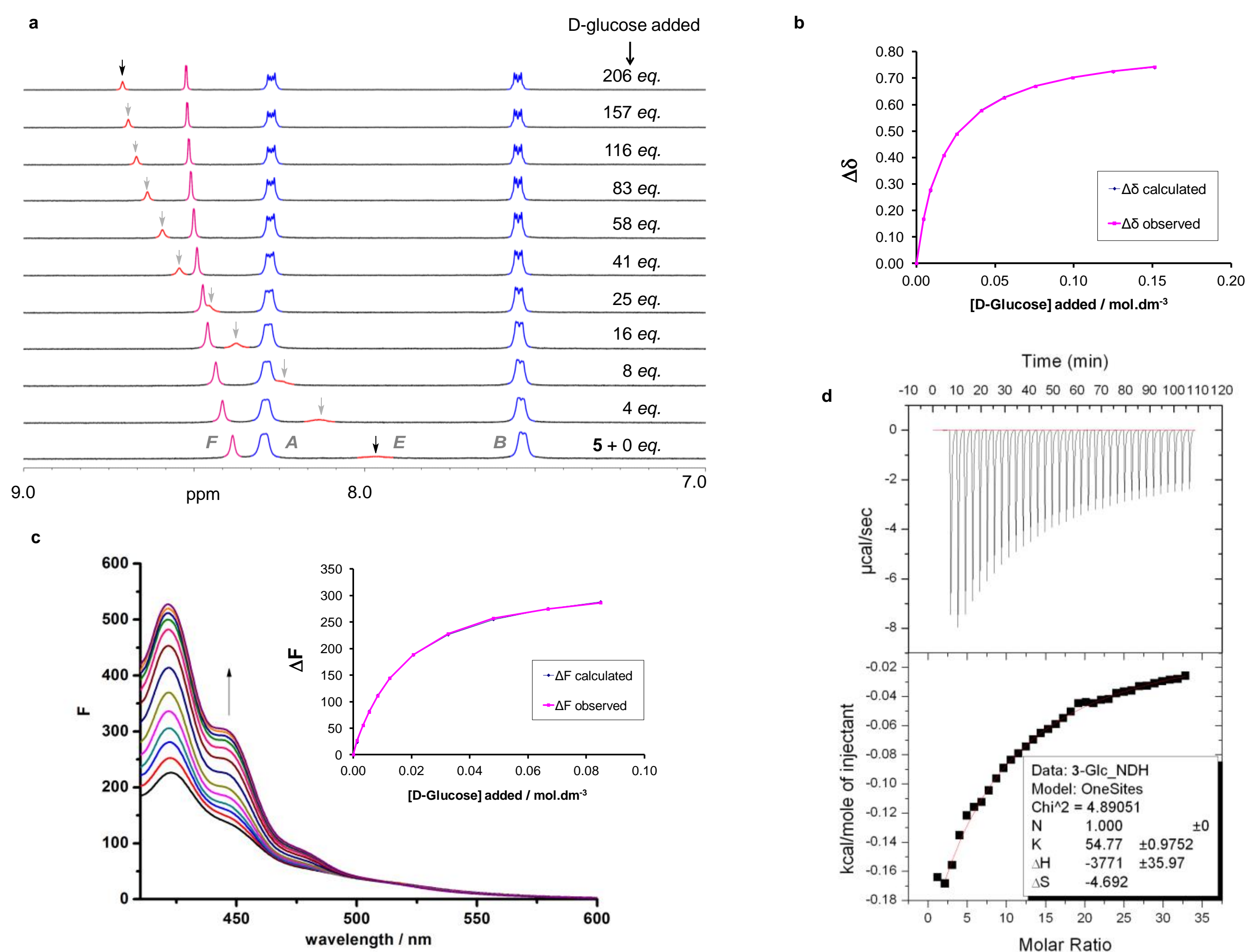

Figure 4 | Data from binding studies on 5 + glucose. a, Partial ^{1}H NMR spectra from the titration of **5** (1.1 mM) with D-glucose (0 to 200 mM). For the key to peak assignments see Fig. 3. **b,** Observed and calculated binding curves for NMR proton *E*. **c,** Fluorescence titration of **5** (18.8 μM) with the addition of glucose in phosphate buffer solution (pH 7.1, 0.1 M) at 298 K. Cell pathway: 10 mm, excitation wavelength: 394 nm. Inset: binding data (423 nm) and fitting curve, K_a = 58 M^{-1}. **d,** Output from ITC experiment, K_a = 55 M^{-1}.

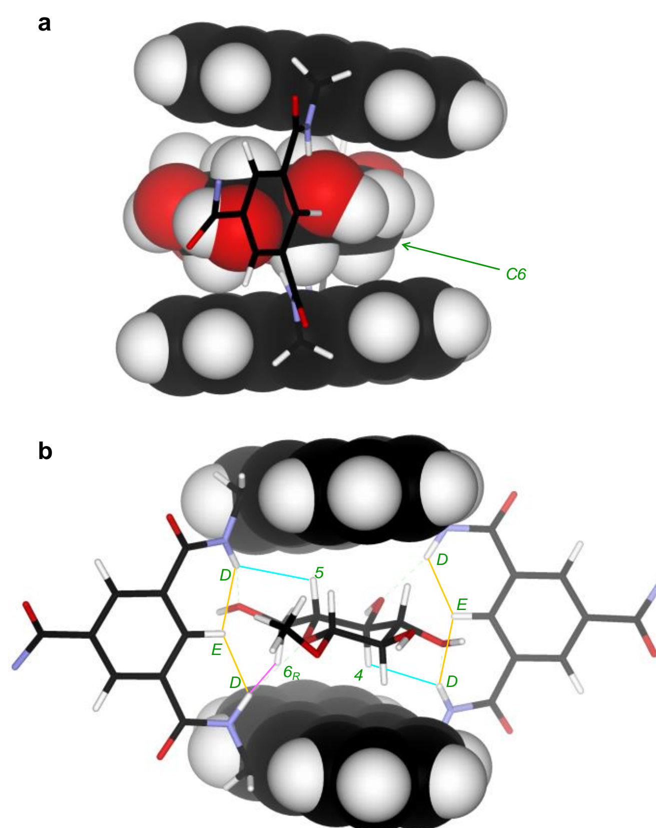

Figure 5 | NMR-based structure for the complex of 5 with methyl β-D-glucoside 10. For details of modelling see Supplementary Information. **a,** View of the complex roughly parallel to the tetralactam ring. The anthracene units and the substrate are shown in space-filling mode, while the solubilising side-chains are removed for clarity. **b,** View roughly perpendicular to the tetralactam ring. The shortest intermolecular distances according to NOESY (*D-4* and *D-5*) are shown as cyan lines, the longer $D\text{-}6_R$ distance is magenta, and the intramolecular *D-E* contacts are gold. The four NH···O hydrogen bonds appear as green dotted lines.