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Acyclic cucurbituril featuring pendant cyclodextrins

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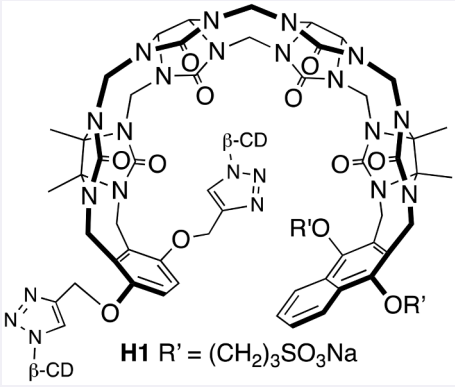
ABSTRACT

Acyclic cucurbit[n]uril–β-cyclodextrin chimeric host **H1** is presented. The goal of the study is to deepen the cavity of the receptor to allow β-CD complexation of moieties on the guest (especially fentanyl) that protrude from the cavity to enhance binding affinity and deliver new supramolecular antidotes for fentanyl intoxication. ^{1}H NMR spectroscopy was used to deduce the geometry of the complexes between **H1** and **H2** and the guest panel whereas isothermal titration calorimetry was used to determine the thermodynamic parameters of complexation. Hosts **H1** and **H2** retain the essential molecular recognition features of CB[n] receptors, but **H1** binds slightly stronger towards the guest panel than **H2**. Compared to tetraanionic **M1** and **M2**, dianionic **H1** and **H2** are less potent receptors which reflects the importance of electrostatic interactions in this series of hosts. The work highlights the challenges inherent in the optimisation of binding affinity of hosts as potential supramolecular antidotes.



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Introduction

The covalent synthesis and non-covalent self-assembly of molecular container compounds and studies of their molecular recognition properties has occupied a central space in the field of supramolecular chemistry for the past several decades [1]. For example, the design principles for the preparation of metal-organic cages and frameworks by reversible metal•ligand interactions have been delineated, their fundamental host•guest recognition properties studied, and a variety of applications have been demonstrated (e.g. supramolecular nanoreactors, components of sensing arrays, drug delivery vehicles, supramolecular metallo drugs, and materials for separation and sequestration) [2]. Within the realm of covalent molecular containers, a variety of classes of compounds (Figure 1) have been studied including cyclodextrins (CD), calixarenes, cyclophanes, cucurbiturils (CB[n]), resorcinarenes and related compounds, and most recently pillararenes. [1b,1f,3] These covalent host systems have been used for a variety of applications including the purification of precious metals, the construction of molecular machines, the preparation of (chiral) stationary phases, as transmembrane ion channels, as household deodorising products, as supramolecular antidotes, and as critical components of glucose sensors [4]. Most relevant to human health has been the *in vivo* use of HP-β-CD and Captisol^{TM} as solubilising excipients for hydrophobic insoluble pharmaceuticals [5] and Sugammadex as a reversal agent for the neuromuscular blockers rocuronium and vecuronium [6].

Our research group has been most interested in the synthesis and molecular recognition properties of the

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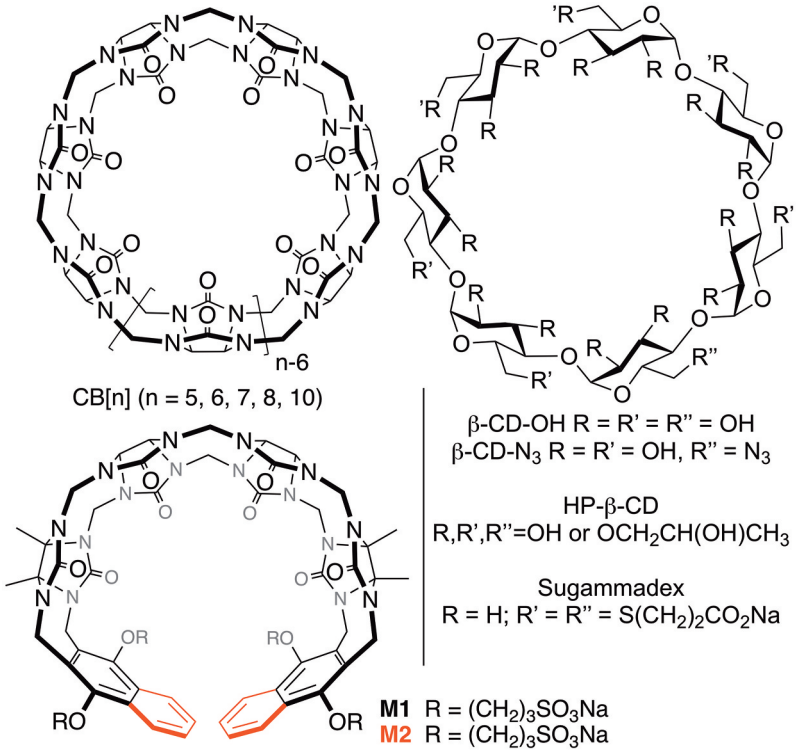


Figure 1. Structures of cyclodextrins and (acyclic) cucurbit[n]urils.

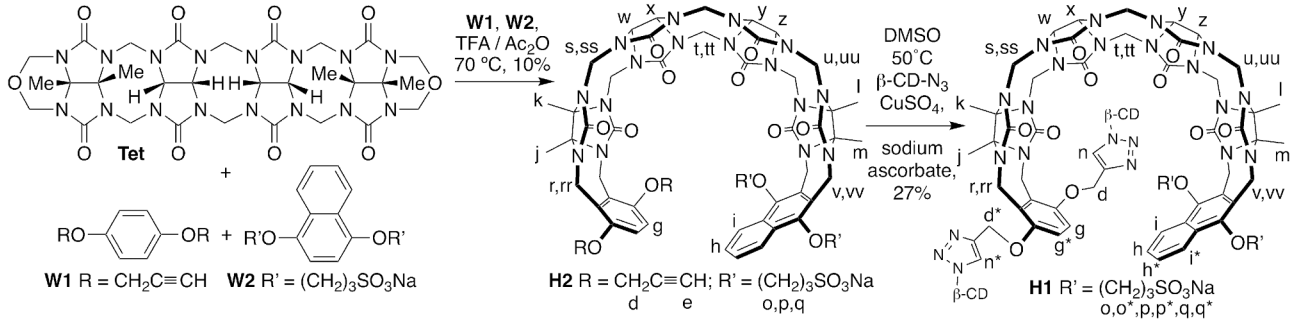
CB[n] family of macrocycles (Figure 1) [7]. CB[*n*] are composed of *n* glycoluril units connected by 2 *n* CH_2-groups which define a central hydrophobic cavity flanked by two electrostatically negative ureidyl carbonyl lined portals [8]. Accordingly, CB[n] hosts bind with unusually high affinity and selectivity to hydrophobic diammonium ions with K_a values up to 10^{17} M^{-1} in water. [3e,9] CB[n]•guest complexes are, therefore, highly responsive to electrochemical, photochemical, and chemical (e.g. pH or competing guest) stimuli [10]. Accordingly, macrocyclic CB[n] and their derivatives have been used in a variety of applications including as components of sensing arrays, molecular machines, separations materials, supramolecular materials, non-covalent inducers of protein dimerisation, as reversal agents, and for (targeted) drug delivery. [4a,10a,11] More recently, we and others, [10d,12] have explored the synthesis and molecular recognition properties of acyclic CB[n]-type receptors (e.g. **M1** and **M2**, Figure 1). Acyclic CB[n]-type receptors are composed of a central glycoluril oligomer, two aromatic sidewalls, and alkoxy chains terminated by sulphonate solubilising groups. Despite being acyclic, the polycyclic backbone of **M1, M2**, and analogues preorganises them into a C-shaped conformation that preserves the essential recognition properties of macrocyclic CB[n] but with more straightforward routes towards synthetic modification [12e]. In a series of papers, we have explored the influence of the glycoluril oligomer length (monomer – pentamer), the aromatic sidewall (e.g. benzene, naphthalene, anthracene, triptycene), the length of the alkylene linking group, and the nature of the ionic group (e.g. carboxylate, sulphonate, ammonium, sulphate) on the molecular recognition properties of acyclic CB[n]-type receptors [13]. By virtue of its high aqueous solubility, acyclic CB[n]-type receptor **M1** was shown to be particularly effective as a solubilising excipient for insoluble drugs for *in vivo* drug delivery [13a,14]. Conversely, the high affinity displayed by acyclic CB[n]-type receptors towards their guests enables **M1** and analogues to function as *in vivo* reversal agents for the neuromuscular blockers rocuronium and vecuronium which are commonly used by anaesthesiologists in the operating room as well as the anaesthetics ketamine and etomidate [15]. Most recently, the ability of **M1** and **M2** to function as *in vivo* sequestration agents for fentanyl and methamphetamine by complexation of their phenethyl ammonium ion moieties, respectively, have been demonstrated [16]. In this paper, we explore the replacement of the $O(CH_2)SO_3Na$ groups of acyclic CB[n]-type receptors with β-CD units to deepen the cavity of the receptor and complement regions of guests that protrude from the central acyclic CB[n] cavity as a means to enhance binding affinity towards fentanyl to create an improved supramolecular antidote.

Results and discussion

This results and discussion section is organised as follows. First, we describe the design and synthesis of acyclic cucurbituril–cyclodextrin chimeric host **H1** along with receptor **H2** as comparator. Next, we describe the selection of the guests used in this study along with qualitative investigations of their host•guest binding processes by ^{1}H NMR spectroscopy. Subsequently, we perform direct isothermal titration calorimetry (ITC) titrations to measure the thermodynamic parameters of host•guest binding. Finally, we discuss the results and offer some conclusions.

Design and synthesis of host H1

In a previous study we measured the binding of **M1** and **M2** towards a panel of drugs of abuse and observed tight binding ($K_a \approx 10^7$ M^{-1}) towards fentanyl in 20 mM phosphate buffered water; [16a] follow up *in vivo* experiments showed that **M1** is capable of modulating the physiological effects of fentanyl in Sprague Dawley rats. [16b,17] ^{1}H NMR investigations showed that **M1** and **M2** bound to the phenethyl ammonium ion binding epitope of fentanyl, whereas the pendant piperidine and amido groups are outside the cavity. Accordingly, as a means to improve binding affinity towards fentanyl and perhaps improve its function as an *in vivo* sequestration agent we decided to append β-cyclodextrin rings on the arms of the acyclic CB[n] receptor in the form of **H1** (Scheme 1) to complement the protruding functional groups of fentanyl. Molecular modelling (Supporting Information, Figure S44) of **H1**•fentanyl supports the molecular and supramolecular design elements. Synthetically, we allowed glycoluril tetramer (**Tet**) to react with a mixture of **W1** (2 equiv.) and **W2** (1 equiv.) in a 1:1 (v:v) mixture of CF_3CO_2H and Ac_2O as solvent at 70 °C to promote the envisioned double electrophilic aromatic substitution reactions [13c,18] which delivered **H2** in 10% yield. In this reaction we use an excess of **W1** with respect to **W2** to limit the quantity of **M2** formed and enhance the amount of the insoluble byproduct with two **W1** walls which allowed **H2** to be isolated by recrystallisation from H_2O/acetone mixtures [19]. Host **H2** features a mirror plane passing through its equator but is unsymmetrical from end-to-end due to the different aromatic sidewalls and accordingly is C_s-symmetric. Figure 2(a) shows the ^{1}H NMR spectrum recorded for **H2** in water. As expected based on C_s-symmetry, only 3 aromatic C-H resonances (H_g, H_h, H_i) are observed for **H2** at 8.08 (H_i), 7.47 (H_h), and 6.03 (H_g) ppm. The surprisingly upfield shifted resonance for H_g can be explained based on the conformation of **H2** in water which features edge-to-face π-π interactions between the tip of the benzene and face of the naphthalene sidewall which places H_g in the anisotropic shielding region of the naphthalene ring. Only two resonances (each integrating to 6 H) are observed for the four different Me groups of **H2** due to accidental overlap. On the basis of C_s-symmetry, a total of 35 ^{13}C NMR resonances would be expected for **H2**; experimentally, we observe 33 resonances (2 resonances missing due to overlap in the C=O and aromatic region). In the electrospray ionisation mass spectrum of **H2** we observe an ion at *m/z* = 1355 which can be assigned to the [**H2** – Na]$^-$ ion. Having firmly established the structure of **H2** we moved on to its transformation into **H1**. Scheme 1 shows the click reaction between **H2** and β-CD-N_3 which was carried out in DMSO at 50 °C in the presence of $CuSO_4$ and sodium ascorbate as catalyst system. Acyclic cucurbituril–cyclodextrin chimeric host **H1** was isolated in 27% yield after purification by silica gel chromatography eluting with acetonitrile–water mixtures. Because the β-CD units of **H1** are homochiral and enantiomerically pure, the mirror plane present in **H2** is no longer present in **H1**. Therefore, **H1** is C_1-symmetric and every proton and every carbon atom in the structure of **H1** ($C_{142}H_{198}N_{22}Na_2O_{86}S_2$; MW = 3699) is chemically different. The ^{1}H NMR



Scheme 1. Synthesis of **H1** featuring an acyclic CB[n] core with pendant β-cyclodextrins.

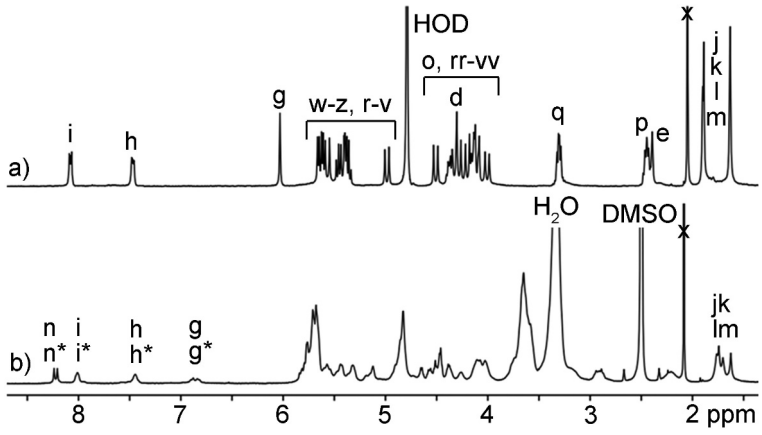


Figure 2. ^{1}H NMR spectra recorded for: a) **H2** (400 MHz, D_2O, RT), and b) **H1** (400 MHz, DMSO-d_6, RT).

spectrum recorded for **H1** in water is broadened, but the spectrum recorded in DMSO-d_6 (Figure 2(b)) is sufficiently sharp to analyse essential features. For example, two singlets are observed at 8.24 and 8.21 ppm which are assigned to the two different triazolyl protons (H_n and H_{n*}) along with two resonances for H_i/H_{i*} and H_h/H_{h*} centred at 8.01 and 7.44 ppm. Protons H_g and H_{g*} appear as a pair of coupled doublets at 6.88 and 6.84 ppm as expected. The downfield shift of the H_g and H_{g*} resonances in DMSO-d_6 (Figure 2(b)) relative to that observed for H_g (6.03, Figure 2(a)) of **H1** in D_2O is due to differences in cavity solvation where DMSO acts as a guest that changes the orientation of the tips of the aromatic sidewalls. Finally, four methyl resonances are observed (j, k, l, m) at 1.76, 1.74, 1.70, 1.63 ppm as expected which reflects the absence of left-to-right symmetry. The electrospray ionisation mass spectrum shows an ion at *m/z* = 1826 which can be ascribed to the [**H1** – 2Na]$^{2-}$ ion. Having established the structures of **H1** and **H2** we moved on to an examination of their host•guest recognition properties.

Qualitative Investigation of the Host Guest properties of H1 and H2. After completing the synthesis and charaterization of the **H1** and **H2** hosts, we decided to qualitatively investigate their host•guest recognition properties by ^{1}H NMR spectroscopy. We selected guests **G1** – **G8** and fentanyl as the members of our guest panel (Figure 3). Guests **G1** – **G4** were selected because they are commonly investigated with macrocyclic and acyclic CB[n]-type receptors, [9a,20] and differ in the width/length of the hydrophobic moiety between the cationic N-atoms. Compounds **G5** – **G8** are derivatives of **G3** which contain a common central hexanediammonium ion moiety which constitutes an excellent binding domain for acyclic CB[n] hosts and two pendant $(CH_2)_n$ Ph groups (n = 1, 2, 3, 4) that are intended to protrude from the acyclic CB[n] cavity and allow complexation by the pendant β-cyclodextrin rings of **H1**. Compounds **G5** – **G7** are known in the literature [21] but the synthetic routes and characterisation data were not reported for **G6** and **G7**, whereas **G8** is unknown. Compounds **G5 – G8** were prepared by the alkylation of N,N,N′,N′-tetramethyl hexanediamine with the appropriate alkyl halide in hot DMF to give **G5 – G8** in 50–56% yield. Finally, we selected fentanyl as a member of the guest panel because it represents a biologically relevant target that can benefit from the presence of the pendant β-CD cavities of **H1**.

To qualitatively assess the host-guest recognition properties of **H1** and **H2** towards the guest panel,

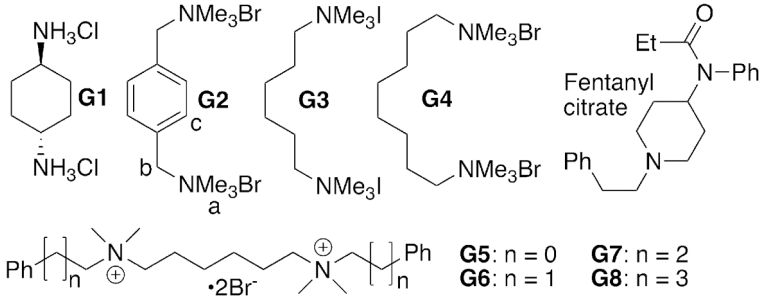


Figure 3. Chemical structures of guests **G1** – **G8** and fentanyl that we investigated in this study.

we initially collected ^{1}H NMR spectra of **H1** or **H2** in the presence of 1 equiv. and 2 equiv. of guests **G1** – **G8**. Figure 4 shows the ^{1}H NMR spectra recorded for mixtures of host **H1** and guest **G2**. The ^{1}H NMR spectrum of **H1** in D_2O is heavily broadened and spin-spin splitting cannot be observed; nonetheless some assignments can be made based on chemical shift and intensity. Interestingly, at a 1:1 **H1:G2** ratio (Figure 4(c)) the spectrum sharpens dramatically indicating a well defined **H1•G2** complex. Upon formation of the **H1•G2** complex, host resonances H_g and H_{g*} shift downfield as the edge-to-face π-π interactions between the benzene and naphthalene sidewalls are disrupted upon host•guest complexation [19]. Conversely, the four different host Me groups (j, k, l, m) become well resolved upon formation of the **H1•G2** complex. Interestingly, upon formation of the **H1•G2** complex, guest protons H_c shift upfield to 6.18 ppm and appear as a pair of coupled doublets which is due to the fact that the top and the bottom of **H1** are different. The upfield shifting is consistent with the binding of the *p*-phenylene unit of **G2** inside the anisotropic shielding region of **H1**. Similarly, two upfield shifted NMe_3 resonances (a′ and a″) are observed upon formation of **H1•G2**. When an excess of guest **G2** is present (Figure 4(d)), separate sharp resonances are observed for **H1•G2** and uncomplexed **G2** which indicates that the guest exchange process is slow on the chemical shift timescale. Slow kinetics of guest exchange are generally observed for tighter host•guest complexes which encouraged us to measure the thermodynamics of host•guest complexation by isothermal titration calorimetry as described below. Related ^{1}H NMR spectra were acquired for the remainder of the **H1**•guest and **H2**•guest complexes (Supporting Information). In these complexes, we generally observe upfield shifting of the resonances corresponding to the central hydrophobic domain of guests **G1 – G8** as they bind inside the anisotropic shielding region of the acyclic CB[n] cavity. Precipitation is observed when preparing mixtures of **H1** with **G5** – **G8** at 2.5 mM concentrations; sufficient concentrations of **H1•G5** – **H1•G8** remain in solution and ^{1}H NMR spectra were obtained (Supporting Information). This result could mean that the pendant aryl rings do not bind inside the intended β-CD cavity and instead cause intermolecular aggregation. However, we also observed precipitation when preparing host•guest complexes of **H2** with **G5** – **G8** which instead suggests that the complexation of dianionic hosts **H1** or **H2** with dicationic guests **G5** – **G8** results in the overall neutral zwitterionic complexes which might be expected to display lower aqueous solubility. Slow to intermediate kinetics of guest exchange on the chemical shift timescale are typically observed for **H1** and **H2**.

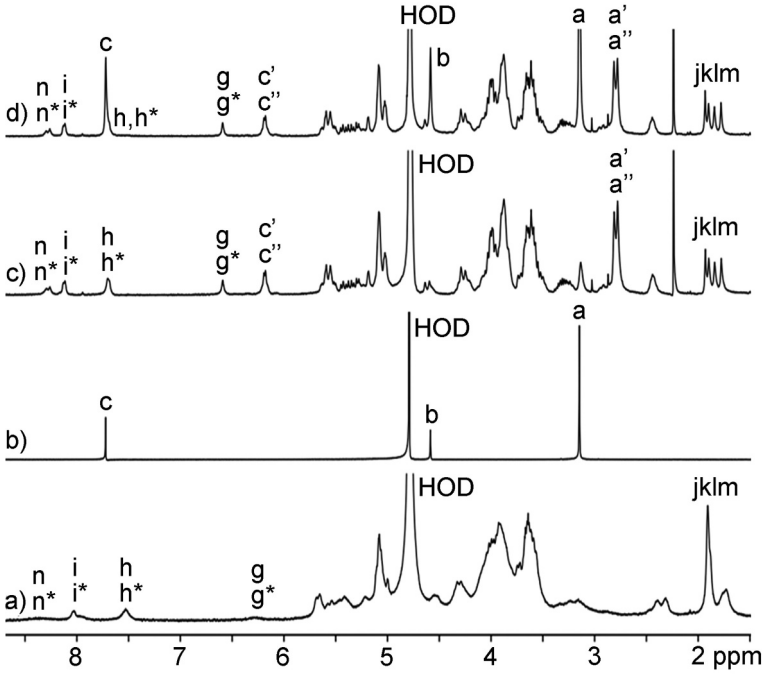


Figure 4. ^{1}H NMR spectra recorded (400 MHz, D_2O, RT) for: a) **H1** (2.5 mM), b) **G2** (2.5 mM), c) a mixture of **H1** (2.5 mM) and **G2** (2.5 mM), and d) a mixture of **H1** (2.5 mM) and **G2** (5.0 mM).

Determination of the thermodynamic parameters for host•guest complexes by isothermal titration microcalorimetry

After having demonstrated binding of guests **G1 – G8** into the cavity of **H1** and **H2** by analysis of the complexation induced changes in chemical shift, we decided to measure the thermodynamic parameters for formation of the complexes. Host•guest complexes of macrocyclic and acyclic CB[n] are often too strong to measure by ^{1}H NMR or even UV/Vis titrations, so we turned to ITC which can be conducted at low μM concentrations and deliver K_a values up to ≈ 10^7 M^{-1} reliably by direct ITC titrations. Figure 5(a) shows the thermogram recorded during the titration of a solution of **H1** (50 μM) in the ITC cell with a solution of fentanyl (500 μM) in the ITC injection syringe. In this experiment we used [**H1**] = 50 μM so as to lower the c-value (c = K_a × [Host]) into the range required for reliable measurements [22]. Figure 5(b) shows the a plot of △H versus the **H1**:fentanyl molar ratio with was fitted with the PEAQ ITC analysis software

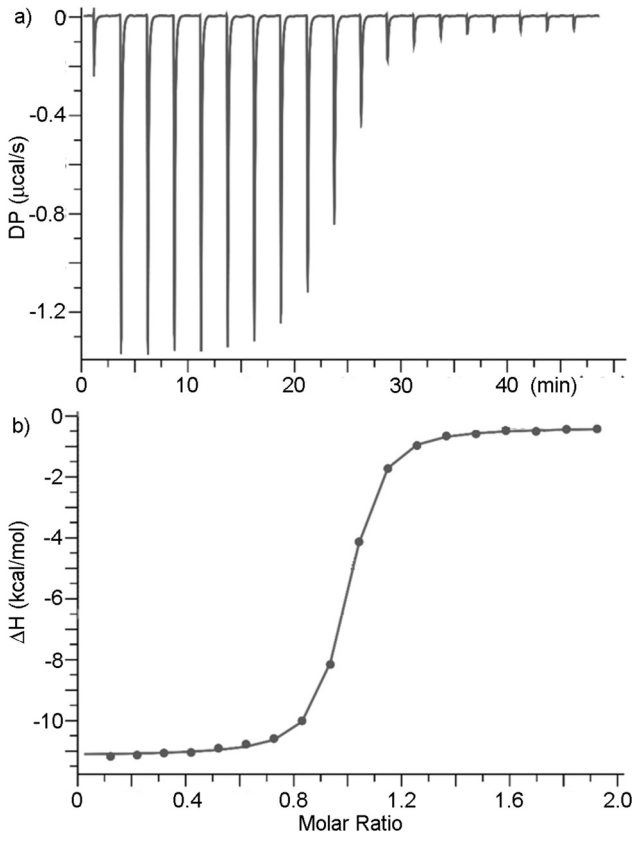


Figure 5. a) Thermogram obtained during the titration of a solution of **H1** (50 μM) in the cell with a solution of fentanyl (500 μM) in the syringe at 298 K in 20 mM sodium phosphate buffered water at pH 7.4. b) Plot of △H (kcal mol^{-1}) versus host: guest molar ratio which was fitted to a single set of sites model to extract K_a = (5.30 ± 0.23) × 10^6 M^{-1} and △H = −10.8 ± 0.04 kcal mol^{-1}.

to a single set of sites binding model with K_a = (5.30 ± 0.23) × 10^6 M^{-1} and △H = −10.8 ± 0.04 kcal mol^{-1}. Related direct ITC titrations were performed for the remainder of the host•guest complexes of **H1** and **H2** with guests **G1** – **G8** and fentanyl and the values of K_a and △H are presented in Table 1. Table 1 also presents the thermodynamic parameters for the complexes between hosts **M1** and **M2** with guests **G1** – **G3** and fentanyl drawn from the literature. [13d,16a,20b,23] The K_a values for **H1** and **H2** fall in the relatively narrow range of 1.72 × 10^5 M^{-1} – 1.78 × 10^7 M^{-1}. Interestingly, **H1** is generally (8 out of 9 cases) a more potent than **H2** towards a specific guest ranging from a low of 0.78-fold (for **G6**) to a high of 40-fold (for **G4**). Clearly the presence of the pendant β-CD rings increase the binding affinity of **H1** relative to **H2**. However, the reasons for this increase remain unclear because one would expect higher K_a values only for guests **G5** – **G8** and fentanyl which contain pendant aryl rings that protrude from the acyclic CB[n] cavity but not for guests **G1** – **G4** which does not agree with the experimental results. The potential influence of the inclusion of the hydrophilic sulphonate groups of **H1** in the hydrophobic cavities of the adjacent β-CD units on binding thermodynamics is considered unlikely. As expected based on precedent from macrocyclic and acyclic CB[n]-type receptors, the complexation enthalpies (△H) are uniformly negative values which reflect the fact that the uncomplexed receptors encapsulate H_2O molecules that do not have a full complement of H-bonds which leads to enthalpic gains upon host•guest complexation [24]. The △H values for the complexes of **H1** with a specific guest are uniformly larger negative values than the corresponding complex of **H2**. One can also perform a comparison between the complexation abilities of **H1** and **H2** with the previously studied **M1** and **M2**. [13d,20b,23] Of course, **H1** and **H2** possess one phenyl wall and one naphthalene wall whereas **M1** possesses two phenyl walls and **M2** possesses two naphthalene walls. As such, the comparisons should be made with caution. Table 1 shows that **M1** and **M2** bind slightly more weakly (≈ 0.6-fold) than **H1** towards cyclohexanediammonium ion **G1** which is not surprising given that **G1** is never a tight binder to acyclic CB[n] because the 4 C-atom spacing between N-atoms is not optimal to complement the distance between ureidyl carbonyl portals of the host. For guests **G2** and **G3**, however, host **H1** is inferior to hosts **M1** and **M2** (**G2: M1** 10-fold and **M2** 142-fold; **G3: M1** 29-fold and **M2** 151-fold). This is not surprising given that **M1** and **M2** are two of our tightest binding hosts (13a,15a,16) and that **H1** and **H2** are dianionic hosts whereas **M1** and **M2** are tetraanionic hosts. The electrostatic component of the binding of macrocyclic and acyclic CB[n]-type receptors

Table 1. Thermodynamic parameters (K_a (M^{-1}); △H (kcal mol^{-1})) measured for the host•guest complexes by isothermal titration calorimetry. Conditions: 20 mM sodium phosphate buffered water, pH 7.40, 298 K.

	Host H1		Host H2		Host M1 (16a,23)		Host M2 (13d,16a,20b)	
Guest	K_a	△H	K_a	△H	K_a	△H	K_a	△H
G1	$(3.02 \pm 0.30) \times 10^6$	−7.25 ± 0.070	$(5.26 \pm 0.36) \times 10^5$	−4.35 ± 0.059	$(1.95 \pm 0.09) \times 10^6$	−5.70 ± 0.027	$(1.77 \pm 0.09) \times 10^6$	−5.54 ± 0.03
G2	$(1.78 \pm 0.18) \times 10^7$	−9.74 ± 0.073	$(9.20 \pm 0.54) \times 10^6$	−7.88 ± 0.035	$(1.78 \pm 0.07) \times 10^8$	−11.4 ± 0.022	$(2.53 \pm 0.12) \times 10^9$	−14.3 ± 0.04
G3	$(3.02 \pm 0.30) \times 10^6$	−7.71 ± 0.050	$(1.46 \pm 0.07) \times 10^6$	−5.74 ± 0.036	$(8.93 \pm 0.33) \times 10^7$	−9.35 ± 0.021	$(4.59 \pm 0.09) \times 10^8$	−10.6 ± 0.15
G4	$(6.99 \pm 0.42) \times 10^6$	−7.06 ± 0.034	$(1.72 \pm 0.28) \times 10^5$	−3.80 ± 0.031	n.d.	n.d.	n.d.	n.d.
G5	$(4.15 \pm 0.25) \times 10^6$	−20.00 ± 0.260	$(1.67 \pm 0.29) \times 10^5$	−6.87 ± 0.340	n.d.	n.d.	n.d.	n.d.
G6	$(2.36 \pm 0.25) \times 10^5$	−9.30 ± 0.270	$(3.01 \pm 0.52) \times 10^5$	−5.62 ± 0.240	n.d.	n.d.	n.d.	n.d.
G7	$(2.84 \pm 0.38) \times 10^6$	−8.90 ± 0.120	$(3.14 \pm 0.25) \times 10^5$	−3.88 ± 0.077	n.d.	n.d.	n.d.	n.d.
G8	$(3.84 \pm 0.82) \times 10^6$	−10.10 ± 0.200	$(2.92 \pm 0.18) \times 10^6$	−4.21 ± 0.027	n.d.	n.d.	n.d.	n.d.
Fentanyl	$(5.30 \pm 0.23) \times 10^6$	−10.80 ± 0.040	$(6.81 \pm 0.28) \times 10^5$	−5.16 ± 0.038	$1.1 \pm 0.04 \times 10^7$	−20.9 ± 0.06	$(7.6 \pm 0.5) \times 10^6$	−20.2 ± 0.07

n.d. = not determined

is known to make a significant contribution towards the overall binding free energy of the host•guest complexes.

Conclusions

In summary, we have reported the design and synthesis of acyclic CB[n]-type receptor **H1** which contains two pendant β-cyclodextrin rings via click reaction of β-CD-N_3 with **H2** as a potential route to deepen the cavity of the host and complement pendant groups on the guest (especially fentanyl) that protrude from the cavity of the acyclic CB[n] receptors. The molecular recognition properties of **H1** and **H2** towards a panel of diammonium ion guests (**G1** – **G8**) and fentanyl were investigated by means of ^{1}H NMR spectroscopy and isothermal titration calorimetry. We find that **H1** and **H2** retain the essential molecular recognition features of macrocyclic and acyclic CB[n]-type receptors (e.g. the ability to bind to diammonium ions in water with single digit μM to 100 nM dissociation constants). Acyclic CB[n]-cyclodextrin chimeric host **H1** binds slightly stronger towards the guest panel than **H2** but the origin of the tighter binding cannot be ascribed to the occupation of the β-CD cavities by the pendant functional groups on the guest. Dianionic hosts **H1** and **H2** are less potent receptors than tetraanionic hosts **M1** and **M2** which reflects the importance of electrostatic (ion-ion and ion-dipole) interactions on the strength of acyclic CB[n]•guest complexes. In conclusion, we find that the incorporation of β-CD rings into **H1** slightly enhances guest binding relative to propargylated **H2**. However, the removal of the two anionic sulphonate groups that synthetically enabled β-CD attachment by click reaction more than offset the expected gains in binding affinity relative to **M1** and **M2**. The work highlights the challenges inherent in the optimisation of binding affinity of hosts as potential supramolecular antidotes.[4a,4j]

Experimental Details

Compounds **Tet, W1, W2, M1, M2** and β-CD-N_3 were prepared according to literature procedures [14,25]. NMR spectra were measured on 400 MHz, 500 MHz, and 600 MHz spectrometers (400, 500, 600 MHz for ^{1}H NMR; 100, 126 MHz for ^{13}C NMR) at room temperature in the stated deuterated solvents unless otherwise stated. Low resolution mass spectrometry was performed using a JEOL AccuTOF electrospray instrument.

Host H2

Compound **Tet** (1.00 g, 1.28 mmol) was first dissolved in a mixture of CF_3CO_2H and Ac_2O (v:v = 1:1, 30 mL) and heated at 70 °C for 5 min. Compounds **W1** (0.47 g, 2.56 mmol) and **W2** (0.57 g, 1.28 mmol) were added and the reaction mixture was stirred and heated at 70 °C for another 12 h. The reaction mixture was poured into acetone (40 mL) to give a yellow precipitate. The precipitate was collected by centrifugation and then dried under high vacuum overnight. The crude solid was recrystallised from water (15 mL) and acetone (30 mL) to yield **H2** as a white solid (0.18 g, 0.13 mmol, 10%). M.p. > 310 °C. IR (ATR, cm^{-1}): 1722s, 1457s, 1223s, 1179s, 1081 m, 795 m. ESI-MS: *m/z* 1355.59, calcd. for [**H2**-Na]$^-$: 1355.36. ^{1}H NMR (400 MHz, D_2O): δ 8.09 (ABq, 2H), 7.47 (ABq, 2H), 6.03 (s, 2H), 5.64 (d, *J* = 15.7 Hz, 2H), 5.62 (d, *J* = 15.3 Hz, 2H), 5.56 (d, *J* = 16.4 Hz, 2H), 5.47 (d, *J* = 9.0 Hz, 1H), 5.42 (d, *J* = 15.5 Hz, 2H), 5.39 (d, *J* = 9.0 Hz, 1H), 5.35 (d, *J* = 9.0 Hz, 1H), 4.99 (d, *J* = 16.1 Hz, 2H), 4.51 (d, J = 16.4, 2H), 4.45–4.35 (m, 2H), 4.30 (s, 2H), 4.28 (d, J = 16.0, 2H), 4.20 (d, J = 15.8, 2H), 4.20–4.10 (m, 2H), 4.14 (d, J = 15.4, 2H), 4.01 (d, J = 16.0, 2H), 3.31 (m, 4 H), 2.45 (m, 4 H), 2.39 (s, 2H), 1.89 (s, 6 H), 1.63 (s, 6 H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 155.4, 154.3, 154.1, 149.4, 148.3, 128.3, 127.9, 126.6, 122.8, 114.6, 79.9, 77.8, 77.6, 76.4, 76.3, 74.3, 70.5, 67.1, 67.0, 57.6, 52.7, 48.7, 48.5, 48.0, 36.0, 34.4, 30.7, 30.7, 26.5, 25.2, 25.1, 16.9, 16.0, 15.9, 15.7.

Host H1

Compound **H2** (0.020 g, 0.015 mmol) and β-CD-N_3 (0.036 g, 0.032 mmol) were dissolved in DMSO (3 mL) and stirred for 5 min. Subsequently, $CuSO_4$•$5H_2O$ (9.0 mg, 0.036 mmol) and sodium ascorbate (0.014 g, 0.072 mmol) were added to the solution and the reaction mixture was stirred and heated at 50 °C for 24 h. The reaction mixture was poured into acetone (20 mL) to give a yellow precipitate which was isolated by filtration and then dried under high vacuum. The crude solid was purified by column chromatography (SiO_2, CH_3CN/H_2O, 2:3) to give **H1** (15.0 mg, 27%) as a white solid. M.p. > 310 °C. IR (ATR, cm^{-1}): 3358 m, 1727 m, 1658 m, 1462 m, 1026s, 797 w. ESI-MS: *m/z* 1826, calcd. for [**H1**-2Na]$^{2-}$1826. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (s, 1H), 8.20 (s, 1H), 8.01 (m, 2H), 7.44 (m, 2H), 6.88 (d, *J* = 7.7 Hz, 1H), 6.84 (d, *J*= 6.7 Hz, 1H), 5.90–5.10 (m, 43 H), 5.00–4.75 (br m, 17 H), 4.75–3.90 (m, 30 H), 3.90–3.40 (m, 40 H), 3.05–2.80 (m, 6 H), 2.30–2.10 (br, 4 H), 1.76 (s, 3 H), 1.74 (s, 3 H), 1.70 (s, 3 H), 1.63 (s, 3 H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 156.1, 155.0, 154.8, 154.7, 150.7, 150.4, 148.8, 148.6, 143.4, 143.3, 128.7, 128.7, 128.3, 128.0, 127.2 (br), 126.9, 126.4, 123.2 (br), 114.8 (br), 102.7, 102.5 (br), 102.1, 101.9, 83.6, 82.4, 82.1, 81.9, 81.6, 78.1, 77.9, 76.9, 76.8, 74.5 (br), 73.5 (br), 72.9, 72.7, 72.5, 70.9, 70.6, 70.1, 66.8, 63.7, 63.4, 60.4 (br), 59.8, 53.0 (br), 50.5, 48.9, 48.4, 40.9, 36.4 (br), 34.9 (br), 31.1, 26.8, 17.5, 16.5, 16.1.

Disclosure statement

L.I. is an inventor on patents held by the University of Maryland on the biomedical application of acyclic cucurbiturils.

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