ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA

Croat. Chem. Acta **2019**, *92*(2), 163–171
Published online: July 29, 2019
DOI: 10.5562/cca3507

HKD 1926

Acyclic Cucurbit[n]uril-Type Containers as Receptors for Neuromuscular Blocking Agents: Structure–Binding Affinity Relationships

David Shaya, Lyle Isaacs*

Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742, United States
* Corresponding author's e-mail address: LIsaacs@umd.edu

RECEIVED: March 25, 2019 * REVISED: May 1, 2019 * ACCEPTED: May 3, 2019

THIS PAPER IS DEDICATED TO PROF. KATA MLINARIĆ-MAJERSKI ON THE OCCASION OF HER 70TH BIRTHDAY

Abstract: Acyclic cucurbit[n]uril molecular containers **1** and **2**C3 have previously been shown to strongly bind to the neuromuscular blocking agents rocuronium, vecuronium, pancuronium, and cisatracurium *in vitro* by optical methods and to reverse neuromuscular block *in vivo* in rats. In this paper we study the *in vitro* binding of a panel of acyclic CB[n]-type receptors toward the four neuromuscular blocking agents and acetylcholine to develop structure-binding affinity relationships. The selected variants include those with different aromatic sidewalls (e.g. **1**Me_4 with dimethyl *o*-xylylene walls; **3** with 1,8-linked naphthalene walls), with different glycoluril oligomer lengths (e.g. **4** and **5** based on glycoluril trimer), and with different linker lengths between aromatic wall and SO_3^- solubilizing group (e.g. **2**C2–**2**C4). Based on the analysis of complexation induced changes in ^{1}H NMR chemical shift we conclude that the hydrophobic regions of the guests bind in the hydrophobic cavity of the hosts with the cationic moieties of the guest binding at the ureidyl C=O portals by ion-dipole and ion-ion interactions. The thermodynamic parameters of binding were determined by direct and competition isothermal titration calorimetry experiments. We find that hosts **4** and **5** based on glycoluril trimer form significantly weaker complexes with the streroidal NMBAs than with the analogues hosts based on glycoluril tetramer (**1** and **2**C3). Similarly, hosts **1**Me_4 and **3** with different length and height aromatic walls do not exhibit the extreme binding constants displayed by **2**C3 but rather behave similarly to **1**. Finally, we find that hosts **2**C2 and **2**C4 bind only slightly more weakly to the NMBAs than **2**C3, but retain the ability to discriminate against acetylcholine, and possess higher inherent water solubility than **2**C3. Host **2**C4, in particular, holds potential for future *in vivo* applications.

Keywords: cucurbituril, neuromuscular blocking agent, reversal agent, molecular container, drugs.

INTRODUCTION

THE administration of neuromuscular blocking agents (NMBAs) is an essential element of care given to over 400 million patients per year during surgical procedures in operating rooms, intensive care units, and emergency medicine departments.[1] Specifically, NMBAs are used during general anesthesia to block neuromuscular transmission which prevents movement during surgery and optimizes surgical conditions. The NMBAs exert their function by binding to the Acetyl choline receptor (AChR) at the neuromuscular junction.[1,2] The most widely used NMBAs in clinical practice are rocuronium (**roc**), vecuronium (**vec**), and cisatracurium (**cis**) (Figure 1).[1] Unfortunately, an estimated 20–50 % of patients that receive NMBAs experience postoperative respiratory disfunction that can lead to airway obstruction, hypoxia, and longer stays in the post-anesthesia care unit all of which increase the risk of morality and healthcare costs.[3] Accordingly, strategies to reverse neuromuscular block at the end of surgery are important in clinical practice to control costs and improve patient outcomes. Classical reversal strategies involve the administration of small molecules like neostigmine and edrophonium that bind to and inhibit the activity of Acetylcholine esterase which increases the concentration of acetyl choline (**ACh**) at the neuromuscular junction to more effectively displace the NMBA from the AChR.[4] An alternative strategy is to directly compete with the AChR for binding to the NMBA. This strategy was first implemented in the form of the γ-cyclodextrin derivative Sugammadex which displays

high affinity binding to rocuronium (K_a = 1.05 × 10^7 M^{-1}) in water *in vitro*.[5] *In vivo*, Sugammadex reverses neuromuscular block by sequestering rocuronium and vecuronium in the bloodstream, thereby depleting their concentration at the neuromuscular junction, and promoting the clearance of the Sugammadex•rocuronium complex in the urine.[6] Sugammadex has been having a major impact on the clinical practice of anesthesia in Europe since its approval in 2008, but was only approved by the US FDA in December 2015 after concerns about allergic reactions and hemorragic side effects had been addressed. Worldwide sales of Sugammadex under the tradename Bridion™ by Merck amounted to $ 704 million in 2017.[7] Accordingly, when we started our work in this area in 2010 we saw a need to develop new classes of molecular containers that could act as broad spectrum reversal agents (e.g. **roc**, **vec**, and **cis**).[8]

Our group has a long-standing interest in the synthesis and molecular recognition properties of macrocyclic cucurbit[n]uril (CB[n]) molecular containers (Figure 1).[9] CB[n] are pumpkin shaped hosts that comprise *n* glycoluril units connected by *2n* methylene brides that feature a hydrophobic cavity guarded by two symmetry equivalent electrostatically negative ureidyl C=O portals.[10] Guest compounds that feature a hydrophobic region flanked by two ammonium moieties can form extraordinarily tight complexes (K_a up to 10^{17} M^{-1}) with CB[n] hosts.[11] Accordingly, we and others, have considered the use of CB[n]-type receptors as potential reversal agents for NMBAs.[8a,12] Unfortunately, the CB[n] that are large enough to encapsulate the steroidal nucleus of **roc** and **vec** (CB[8] and CB[10]) display low μM solubility in water[10b,13] which greatly limits their potential *in vivo* applications. CB[7] which does have good water solubility (20 mM)[10b] was shown by Macartney to bind to the ammonium end groups of steroidal NMBAs rather than engulf the steroid ring system.[12b] Over the years, our group has sought to understand the mechanism of CB[n] formation and use that knowledge to prepare new CB[n] type receptors with enhanced solubility and clickable functional groups.[9b,14] For example, we prepared acyclic CB[n]-type receptors (e.g. **1** and **2**, Figure 2) that comprise a central glycoluril tetramer, two aromatic sidewalls, and four sodium sulfonate groups to greatly improve aqueous solubility.[15] Related receptors that lack the SO_3^- solubilizing groups have also been studied by Sindelar and co-workers.[16] Initially, we studied the ability of **1** and **2** to function as solubilizing agents for insoluble drugs and carbon nanotubes and as components of sensing arrays for pharmaceutical agents.[15,17] As part of follow-up studies of the use of **1** and **2** to function as solubilizing excipients, we had cause to prepare numerous structural variants containing different numbers of glycoluril rings (1, 2, 3, 4),[17d] different aromatic walls,[17b] and different solubilizing group linker lengths[18] including compounds **1**–**5** shown in Figure 2. In 2012, we reported that **1** and **2** bind to **roc**, **vec**, and **cis** *in vitro* with μM to nM binding affinity.[8a] *In vivo* experiments (rat) showed that **1** or **2** reverse the effects of **roc**, **vec**, and **cis** in a dose dependent manner and restores the train-of-four (TOF) ratio faster than placebo and neostigmine and comparable to or faster than Sugammadex.[8b,8c] As part of our efforts to further develop **2** as a broad spectrum reversal agent for NMBA reversal we studied the binding of **2** toward a panel of 27 drugs commonly used during or after surgery by experiment and simulation to assess the potential for displacement interactions that could lead to undesired recurarization.[8d] Most recently, we have investigated the ability of **1** and **2** to act as *in vivo* sequestration agents for drugs of abuse (e.g. methamphetamine).[19] In a complementary line of inquiry, the group of Ruibing Wang has pursued the use of macrocyclic cucurbiturils as reversal agents.[20] In this paper, we measure the binding affinity of a panel of previously prepared acyclic CB[n] type receptors (**1**–**5**) toward the panel of NMBAs (**roc**, **vec**, **pan**, **cis**) and acetylcholine (**ACh**) to more fully define the structure–binding affinity relationships.

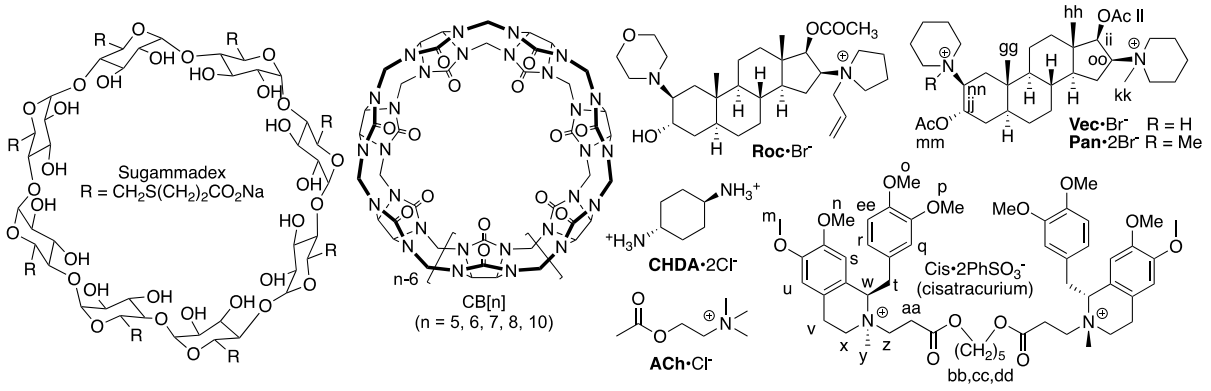

Figure 1. Chemical structures of Sugammadex, CB[n], and guests.

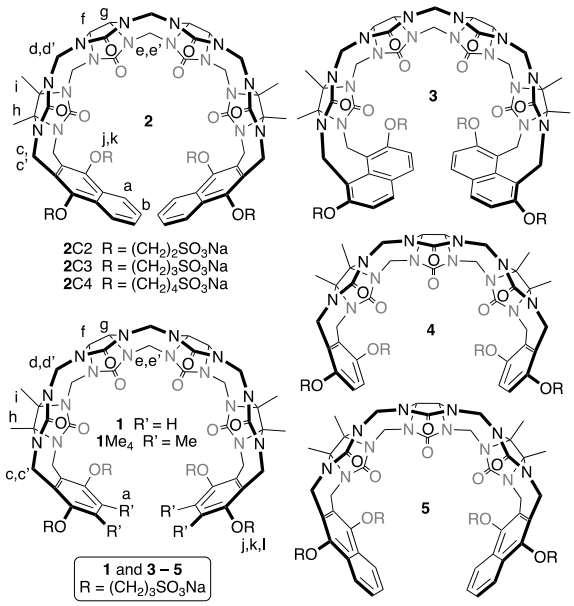

Figure 2. Chemical structures of acyclic CB[n]-type receptors used in this study.

EXPERIMENTAL

General

Hosts **1**–**5** were prepared by the literature procedures.[15,17a,17b,17d] The neuromuscular blocking agents (**roc**, **vec**, **pan**, and **cis**) and **ACh** were purchased from commercial suppliers and used without further purification. ^{1}H NMR spectra were measured on commercial NMR spectrometers operating at 400 MHz. ITC data was collected on a Malvern Microcal PEAQ-ITC instrument with a 200 µL cell volume. We used an injection syringe of 40 µL capacity. In each case, the host and guest solutions were prepared in a 20 mM NaH_2PO_4 buffer (pH 7.4). The sample cell was filled to capacity (200 µL) with the host solution and the guest solution was titrated (first injection = 0.4 µL, subsequent 18 injections = 2 µL) into the cell. Competition (displacement) titrations were performed for container•drug complexes with binding constants exceeding K_a = 5 × 10^6 M^{-1} using **CHDA** as a weaker binding ligand included with the host in the ITC cell. Data was fitted, as appropriate, with either the single set of sites model or the competitive binding model within the MicroCal PEAQ-ITC analysis software.

RESULTS AND DISCUSSION

This results and discussion section is organized as follows. First, we describe the rationale for our selection of the eight different hosts used in this study. Next, we present the results of ^{1}H NMR experiments that provide qualitative information on the geometry of the host•guest complexes. Subsequently, we present the results of isothermal titration calorimetry experiments used to measure the thermodynamic parameters for the various host•guest complexes. Finally, we discuss the trends in the K_a values as a function of host structure.

Selection of Hosts 1–5

As described above, our investigations into the use of acyclic CB[n]-type compounds as reversal agents have focussed on lead compounds **1** and **2** due to their high binding affinity and their excellent biocompatibility in vitro and in vivo (e.g. high maximum tolerated dose, no hERG activity, no genotoxicity).[15,18,21] In addition to **1** and **2**, we also selected compounds **1**Me_4 and **3** that feature dimethyl o-xylylene and 2,7-dialkoxynaphthalene sidewalls. These structural changes influence the depth (e.g. **3** is deeper), the size (e.g. **1**Me_4 is intermediate in size between **1** and **2**), and the nature (e.g. the aliphatic Me groups of **1** become part of the walls that define the molecular recognition surface) of the cavity. Finally, we selected compounds **4** and **5** that differ from **1** and **2** in that they are based on a central glycoluril trimer but possess common aromatic sidewalls.[17d] The cavities of hosts **4** and **5** are smaller than those of **1** and **2** and may be more complementary to the narrower diammonium region of cis.[17d] Finally, we selected compounds **2**C2 and **2**C4 which differ in the length of the alkylene linker between the aromatic sidewall and the solubilizing sulfonate groups.[18] Previously, **2**C2 (68 mM) and **2**C4 (196 mM) have been shown to have higher aqueous solubility than **2** (14 mM) which would be advantageous for formulation if the binding constants of **2**C2 or **2**C4 were comparable to those of 2 toward the NMBAs.

1H NMR Investigations of the Host-Guest Complexes

Initially, we performed qualitative ^{1}H NMR investigations of the interactions between containers **1**–**5** and guests (**roc**, **vec**, **pan**, **cis**, **ACh**, and **CHDA**) to glean information about complex geometry and stoichiometry. It is well known that the ureidyl C=O region of macrocyclic CB[n] and acyclic CB[n]-type receptors constitute ammonium ion binding regions due to ion-dipole interactions.[10a,22] Conversely, the cavity of macrocyclic CB[n] and acyclic CB[n]-type receptors constitutes a hydrophobic binding region; the cavity is also a magnetic shielding region by virtue of the π-systems of the glycoluril units and the aromatic sidewalls.[9b,23] In accord with these expectations, the ^{1}H NMR spectra of complexes between hosts **1**–**5** and guests **ACh** and **CHDA** (Supporting Information) feature significant upfield shifting for the methylene protons of **ACh** and **CHDA** which indicates they are located in the cavity of the host. Somewhat surprisingly, the NMe_3 protons of **ACh** also undergo an upfield shifting which probably reflects an out-

of-plane geometrical helical distortion of the hosts which allows the NMe_3^+ group to engage in cation-π interactions with the aromatic sidewalls of the hosts. Figure 3a-c show the ^{1}H NMR spectra recorded for **cis**, **2**C2, and an equimolar mixture of **cis** and **2**C2. Upon formation of the **2**C2•**cis** complex we observe a large upfield change in chemical shift for H_{cc} and H_{dd} which means that host resides in large part on the central $(CH_2)_5$ linker between the benzyl isoquinoline units as expected. Conversely, we observe a significant downfield change in chemical shift of H_a and H_b on the naphthalene sidewalls of **2**C2 upon complex formation. We attribute this change to the presence of intramolecular edge-to-face π-π interactions between the naphthalene walls of uncomplexed **2**C2 which are disrupted upon complex formation.[17b] Related downfield changes in chemical shift of the protons on the aromatic sidewalls are also seen for hosts **1**, **2**, and **3**.[17b] For other host-guest combinations, multiple resonances are observed for the aromatic walls upon complexation. For example, upon formation of the **3**•**pan** complex (Figure S54) we observe that the two symmetry equivalent aromatic protons (H_a and H_b) of C_{2v}-**3** split into 8 resonances which reflects the overall C_1-symmetry of

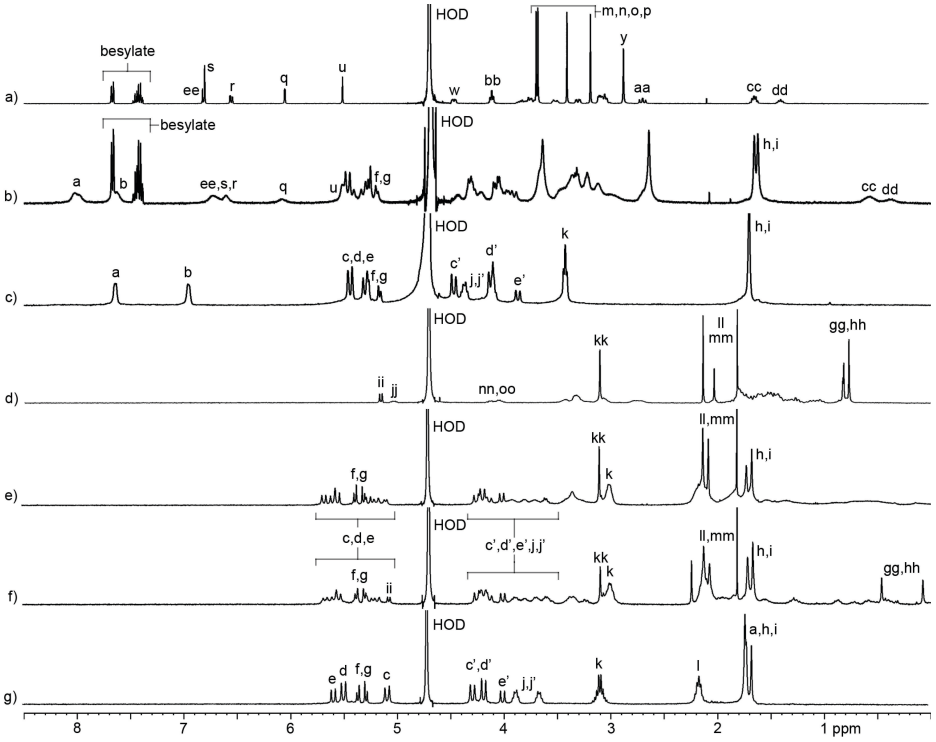

Figure 3. ^{1}H NMR spectra recorded (400 MHz, RT, D_2O) for solutions containing: a) **cis** (1 mM), b) **cis** (1 mM) and **2**C2 (1 mM), c) **2**C2 (1 mM), d) **vec** (1 mM), e) **1**Me_4 (1 mM) and **vec** (2 mM), f) **1**Me_4 (1 mM) and **vec** (2 mM), and g) **1**Me_4 (1 mM).

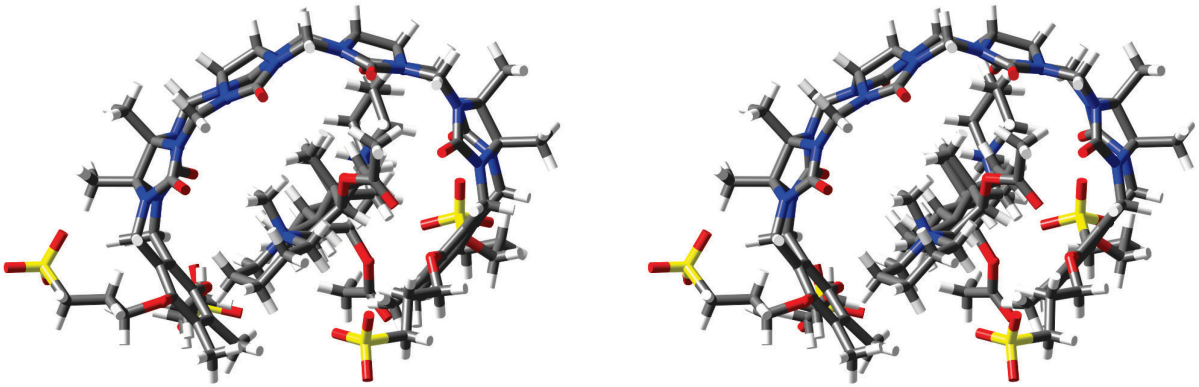

Figure 4. Cross-eyed stereoview of the MMFF minimized geometry of **1**Me_4•**vec**. Color code: C, grey; H, white; N, blue; O, red; S, yellow.

the **3•pan** complex due to enantiomerically pure **pan** guest and slow guest exchange kinetics. For the **5•vec** and **5•pan** complexes (Figures S57 and S60) we observe splitting of the two aromatic resonances of C_{2v}-**5** (H_a and H_b) into four resonances (rather than eight) because fast guest exchange kinetics averages the two possible orientations (e.g. up or down) of **vec** or **pan** within the cavity of **5**. Related increases in the number of resonances are observed for the CH_2-groups of the glycoluril backbone of C_{2v}-**5** (e.g. two doublets from 5.0–5.5 ppm; Figure S57) upon formation of the **5•vec** complex (e.g. four doublets from 5.0 – 5.6 ppm). Figure 3d-g show the ^{1}H NMR spectra recorded for **vec**, **1**Me_4, and 1 : 1 and 1 : 2 mixtures of **1**Me_4 and **vec**. As expected, at a 1 : 1 ratio of **1**Me_4:**vec**, we observe significant upfield shifts for the axial steroidal CH_3-groups (H_{gg}, H_{hh}) which indicates that the steroidal nucleus of **vec** is bound inside the cavity of **1**Me_4 which allows the pendant ammonium groups to engage in ion-ion and ion-dipole interactions with the C=O portals and SO_3^- solubilizing groups of the host. At a 1 : 2 ratio of **1**Me_4:**vec** the resonances for the axial steroidal Me groups are broadened into the baseline which indicates that exchange processes with intermediate kinetics occur on the ^{1}H NMR timescale. Figure 4 shows a cross-eyed stereoview of an MMFF minimized geometry of the **1**Me_4•**vec** complex which illustrates the three dimensional arrangement of **vec** inside the cavity of the host. Interestingly, the axial Me-groups point into the concavity shaped by the glycoluril tetramer whereas the axial steroidal C-H bonds on the α-face are oriented toward the aromatic walls of the host. Overall, our analysis of the changes in ^{1}H NMR chemical shifts upon complexation are consistent with the complexation of the hydrophobic portions of the guests within the hydrophobic cavity of the acyclic CB[n]-type receptors with electrostatic interactions at the portals as expected based on the literature precedents.[9b,22]

Determination of the Thermodynamics of Host•Guest Complexes by Isothermal Titration Calorimetry

After having observed clear cavity binding of the NMBA guests within the cavity of hosts **1**–**5** by ^{1}H NMR spectroscopy, we set out to determine the strength and thermodynamic parameters of the complexes. Given that the tight binding previously observed with **2**[8a] and the complexity and broadening observed in the ^{1}H NMR of many of the complexes would complicate K_a determination by ^{1}H NMR spectroscopy we decided to use ITC which would deliver the full thermodynamic parameters. Table 1 presents the K_a, Δ_rG, Δ_rH, and $T\Delta_rS$ values for the various host•guest complexes. For the weaker complexes (e.g. $K_a \leq 5 \times 10^6$ M^{-1}) we were able to perform direct titrations of host with guest. For example, Figure 5a shows the thermogram obtained when a solution of **2**C4 (70 μM) in the cell was titrated with a solution of **CHDA** (1 mM) in the syringe. Fitting of the data to a 1 : 1 binding model implemented within the PEAQ-ITC analysis software allowed a determination of K_a = 1.00×10^6 M^{-1} and Δ_rH = –3.59 kcal mol^{-1}; complexation is

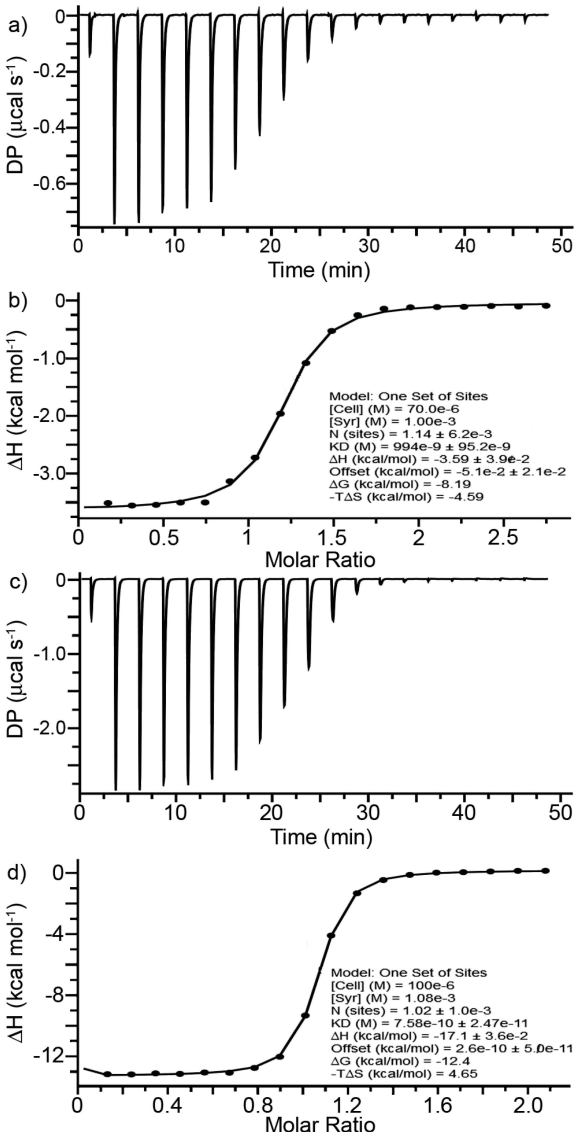

Figure 5. Thermograms recorded (298.0 K, 20 mM sodium phosphate buffered H_2O, pH 7.4) during the titration of: a) a solution of **2**C4 (70 μM) with a solution of **CHDA** (1 mM) in the syringe, and c) a solution of **2**C4 (100 μM) and **CHDA** (500 μM) in the cell with a solution of **Roc** (1.08 mM) in the syringe. The data was fitted using the PEAQ-ITC analysis software to: b) a one-set-of-sites binding model to extract the thermodynamic parameters for **2**C4•**CHDA**, and d) to a competitive binding model using the thermodynamic parameters for **2**C4•**CHDA** as inputs to determine the thermodynamic parameters for **2**C4•**Roc**.

also entropically favourable with $-T\Delta_rS$ = –4.59 kcal mol^{-1}. For complexes with higher K_a values, direct titrations are not reliable because the c-values are larger than 1000.[24] Accordingly, to measure $K_a > 10^7$ M^{-1} we performed competitive ITC titrations. In competitive ITC titrations, a solution of host and weak binding guest (K_a and Δ_rH previously determined) in the cell is typically titrated with a solution of the tighter binding guest.[25] As the guest exchange process occurs, the difference in Δ_rH between the two competing complexes is evolved so it is important to select the competitor wisely. We have found that **CHDA** is a suitable competitor in many cases. Figure 5c shows the thermogram obtained during the titration of a solution of 2C4 (70 μM) and **CHDA** (0.5 mM) with a solution of **roc** (1.08 mM) in the syringe. Figure 5d shows the fitting of the data to a competitive binding model using the known thermodynamic parameters for the **2**C4•**CHDA** complex (Figure 5b) as inputs which allowed us to determine K_a = 1.32 × 10^9 M^{-1} and Δ_rH = –17.1 kcal mol^{-1} for the **2**C4•**roc** complex. The thermodynamic parameters for the remaining host•guest complexes (Table 1) were measured by analogous direct or competitive ITC titrations as documented in the Supporting Information.

Table 1. Thermodynamic parameters (K_a (M^{-1}), Δ_rH, Δ_rG, and $-T\Delta_rS$ (kcal mol^{-1}) and determined for the host•guest complexes by ITC. [a] competition ITC titration using **CHDA** as competitor.

K_a / 10^6 / M^{-1}; Δ_rH / kcal mol^{-1}; Δ_rG / kcal mol^{-1}; $-T\Delta_rS$ / kcal mol^{-1}						
	CHDA	**ACh**	**roc**	**vec**	**pan**	**cis**
1 K_a	2.30	0.0229	3.51	4.2	0.758	0.132
Δ_rH	–5.69	–6.87	–8.64	–5.56	–4.35	–16.4
Δ_rG	–8.68	–5.95	–8.93	–9.05	–8.02	–6.99
$-T\Delta_rS$	–2.99	0.917	–0.291	–3.49	–3.67	9.40
1Me$_4$ K_a	0.730	0.0302	2.51	5.41	1.41	0.0914
Δ_rH	–6.91	–6.31	–14.9	–6.68	–5.46	–10.1
Δ_rG	–8.00	–6.41	–8.73	–9.19	–8.39	–6.77
$-T\Delta_rS$	–1.09	–1.17	6.21	–2.51	–2.93	3.30
2C2 K_a	2.00	0.158	1090[a]	862[a]	148[a]	0.13
Δ_rH	–6.64	–8.32	–16.1	–9.29	–8.69	–13.2
Δ_rG	–8.60	–7.09	–12.3	–12.2	–11.1	–6.83
$-T\Delta_rS$	–1.96	1.23	3.74	–2.90	–2.46	6.35
2C3 K_a	2.49	0.179	5780[a]	4020[a]	800[a]	0.128
Δ_rH	–5.05	–7.27	–13.5	–9.33	–9.31	–13.3
Δ_rG	–8.73	–7.17	–13.3	–13.1	–12.1	–6.97
$-T\Delta_rS$	–3.68	0.102	0.201	–3.77	–2.84	6.34
2C4 K_a	1.00	0.0588	1320[a]	231[a]	200[a]	0.194
Δ_rH	–3.59	–4.57	–17.1	–6.26	–6.11	–10.9
Δ_rG	–8.19	–6.51	–12.4	–11.4	–11.3	–7.21
$-T\Delta_rS$	–4.59	–1.94	4.65	–5.15	–5.21	3.64
3 K_a	0.140	n.b.	0.847	0.971	0.195	0.0971
Δ_rH	-4.79		–9.05	–4.15	–4.68	–8.30
Δ_rG	-7.02		–8.09	–8.17	–7.22	–6.80
$-T\Delta_rS$	-2.24		0.967	–4.02	–2.53	1.49
4 K_a	n.d.	0.000704	0.0214	0.0348	0.0382	0.0225
Δ_rH		–4.99	–6.08	–3.67	–4.01	–6.69
Δ_rG		–3.88	–5.91	–6.20	–6.25	–5.94
$-T\Delta_rS$		1.11	0.173	–2.53	–2.24	0.754
5 K_a	n.d.	0.0015	1.31	0.98	0.588	0.0562
Δ_rH		–3.78	–7.21	–4.89	–3.63	–7.06
Δ_rG		–4.33	–8.35	–8.18	–7.87	–6.48
$-T\Delta_rS$		–0.555	–1.14	–3.28	–4.24	0.582

n.d.: not determined because competition experiments were not necessary.
n.b.: no binding detected.

DISCUSSION OF THE THERMODYNAMIC PARAMETERS

There are a number of interesting trends observed in the thermodynamic parameters of binding that are worthy of discussion. First, and most relevant toward the proposed use of **2**C3 as an *in vivo* reversal agent for steroidal neuromuscular blocking agents is that **2**C3 displays nanomolar affinity toward **roc**, **vec**, and **pan** and displays 4–20-fold higher affinity than either **2**C2 or **2**C4. Figure 6 presents the thermodynamic data in bar chart form to allow straightforward comparisons. Host **2**C3 has previously been found to be a more efficient solubilizing agent for insoluble drugs which can be traced to its higher binding constants than **2**C2 and **2**C4 in accord with present results.[18] Hosts **1**, **1**Me$_4$, and **3**–**5** display lower affinity (2 × 10^4 – 5 × 10^6 M^{-1}) toward **roc**, **vec**, and **pan**. Amongst these three steroidal NMBA's **pan** usually forms the weakest complex which is surprising given that **pan** contains two permanent quaternary ammonium groups which would be expected to enhance binding.[11d] Host **2**C3 binds **roc** 32000-fold stronger than **ACh** which is important because the sequestration of **ACh** from the neuromuscular junction is undesirable. In this regard, it is worth noting that **2**C4 (**2**C2) is 22000-fold (6900-fold) selective for **roc** over **ACh** while maintaining sub nM affinity toward **roc**. Host **2**C4 has higher inherent solubility (196 mM) than **2**C3 (14 mM) and **2**C2 (68 mM) which suggests that **2**C4 may be more easily formulated for potential *in vivo* use.[18]

Part of the impetus to perform this structure–binding affinity study was to determine whether superior acyclic CB[n]-type receptors could be discovered for the benylisoquinoline NMBA **cis** which is the third most popular NMBA in clinical use. We hoped that the narrow $(CH_2)_5$ chain of the guest would thread more efficiently through

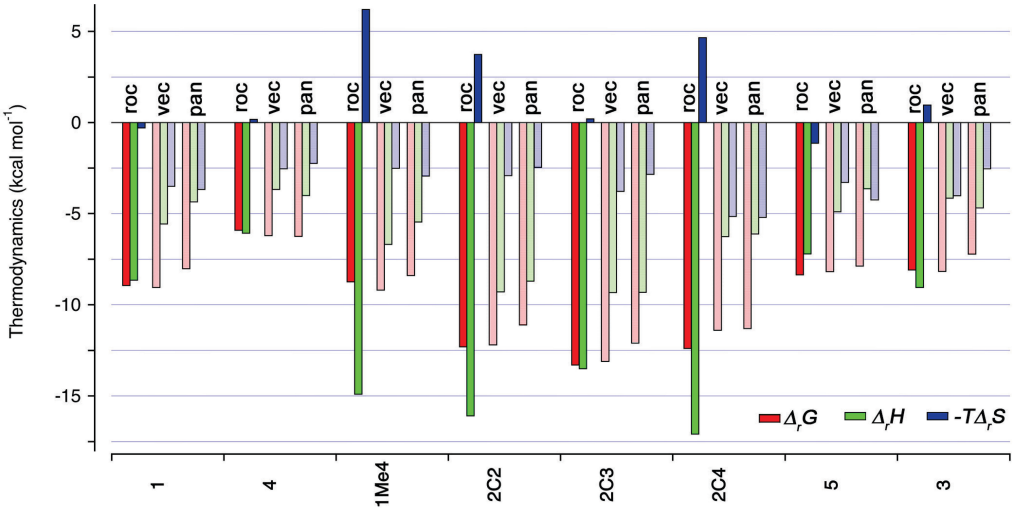

Figure 6. Bar chart depicting the thermodynamic parameters of binding ($\Delta_r G$, red bars; $\Delta_r H$, green bars; $-T\Delta_r S$, blue bars) in kcal mol^{-1} for complexes of **roc**, **vec**, and **pan** toward hosts **1**, **4**, **1**Me$_4$, **2**C2, **2**C3, **2**C4, **5**, and **3**.

the narrower hosts **4** and **5** created from glycoluril trimer or host **3** with taller sidewalls. Experimentally, we found that **1**–**3** bound **cis** with K_a values in the 10^5 M^{-1} range and that **4** and **5** do so even more weakly with K_a values from 22500–56200 M^{-1}. Host **2**C4 displays highest affinity toward **cis** and possesses excellent aqueous solubility. Interestingly, the host•guest complexes with **cis** are all entirely driven by favourable enthalpic changes and feature entropically unfavourable contributions to $\Delta_r G$. We believe that the conformational restriction of the long oligomethylene chain of **cis** needed to optimize binding is responsible for this thermodynamic signature.

Comparison between structurally related hosts toward common guests is also worthwhile. For example, hosts **1** and **2**C3 are homologues of **4** and **5** containing one more glycoluril ring. This additional glycoluril ring was expected to increase the size (volume) of the host cavity and thereby influence binding affinity. Experimentally, we find that **1** is a significantly better host than **4** toward **roc** (164-fold), **vec** (121-fold), **pan** (20-fold), **cis** (6-fold), and **ACh** (32-fold). Similarly, we find that **2**C3 is a significantly better host than **5** toward **roc** (4400-fold), **vec** (4100-fold), **pan** (1360-fold), **cis** (2.3-fold), and **ACh** (119-fold). We believe the superior affinity and selectivity displayed by **1** and **2**C3 reflects the larger cavity size / volume which probably contains a larger number of high energy waters[23] that provide an enhanced driving force for complexation. Selectivity is lowest for the narrow **cis** guest which probably reflects a better complementarity between the narrow host with the narrower guest. A related comparison can be made between the behaviour of hosts **2**C3 and **3** which are isomers of one another. Host **2**C3 with its 2,3-linked naphthalene sidewalls possesses a wider cavity, but host **3** with its 1,8-linked naphthalene sidewalls possesses a narrower but deeper cavity. The SO_3^- solubilizing groups of **3** are also farther from the cavity and ureidyl C=O portals of the host. Experimentally, we find that **2**C3 is a better host than **3** for **roc** (6800-fold), **vec** (4100-fold), **pan** (4100-fold), and **cis** (1.3). Once again, the selectivity is largest for the larger steroidal guests that can properly fill the larger cavity of **2**C3 without requiring conformational changes of the host and lowest for **cis** whose $(CH_2)_5$ chain is more complementary to the narrower cavity of **3**. Finally, one can consider the binding trends for hosts **1**, **1**Me$_4$, and **2**C3 which differ systematically in the length of their aromatic sidewalls. For the smaller guests **CHDA**, **ACh**, and **cis** only small differences (1.4–7.8-fold) are observed in the binding constants toward **1**, **1**Me$_4$, and **2**C3 whereas for the larger steroidal guests **roc**, **vec**, and **pan** the differences are much larger (434–1055-fold). Our interpretation is that the cavity of **2**C3 is particularly poorly solvated (e.g. high energy waters) and is able to adjust its conformation (e.g. helical twist) to fully complement the larger steroidal guest in ways that are not possible for small hosts and not needed for the smaller guests. As found in previous studies,[17b,17d,18] hosts **2**C2–**2**C4 are the most potent hosts known to date in the acyclic CB[n] series.

CONCLUSION

In summary, we have studied the interaction of a series of acyclic cucurbit[n]uril-type receptors (**1**–**5**) toward four NMBAs, **ACh**, and **CHDA** to further define the structure–binding affinity relationships relevant for their development as *in vivo* reversal agents. Based on the result of

^{1}H NMR experiments, we conclude that the NMBAs bind with their hydrophobic residues located in the hydrophobic cavity of **1**–**5** which places their cationic moieties at the ureidyl C=O portals of the hosts. Isothermal titration calorimetry was used to measure the thermodynamic parameters for binding between hosts **1**–**5** and the NMBAs and **ACh**. We find that host **2**C3 and its relatives **2**C2 and **2**C4 display nanomolar affinity toward the steroidal NMBAs and maintain a high selectivity against **ACh** as needed for the proposed *in vivo* use. Hosts **2**C4 and **2**C2 with their higher aqueous solubility compared to **2**C3 are viable alternative reversal agents for steroidal NMBAs. All eight hosts displayed affinities toward **cis** in the $2 \times 10^4 - 2 \times 10^5$ M^{-1} range according to ITC. No binding improvement relative to compound **1** (K_a = 1.32 × 10^5 M^{-1} toward cis) which was previously used to reverse **cis** *in vivo* in rats[8b] was found. Finally, hosts **4** and **5** which are based on glycoluril trimer units rather than tetramer units (e.g. **1** and **2**) display 20–4400-fold weaker binding to the steroidal NMBAs which we attribute to the smaller host cavity and a small number of high energy waters[23] which are known to provide a driving force in CB[n] complexation. Amongst the acyclic CB[n] tested, we conclude that **2**C3 and its analogues **2**C4 and **2**C2 remain the most promising agents for further development as *in vivo* reversal agents for neuromuscular blocking agents.

Disclosure statement. The University of Maryland holds patents on the use of acyclic CB[n]-type receptors in biomedical applications where L. I. is named as an inventor.

Acknowledgment. We thank the National Science Foundation (CHE-1404911 and CHE-1807486 to L.I.) and the National Institutes of Health (CA-168365 to L.I.) for financial support. D.S. thanks the University of Maryland for a Howard Hughes Medical Institute undergraduate research fellowship.

Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca3507.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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