Acyclic Cucurbit[n]uril-Type Receptors: Optimization of Electrostatic Interactions for Dicationic Guests

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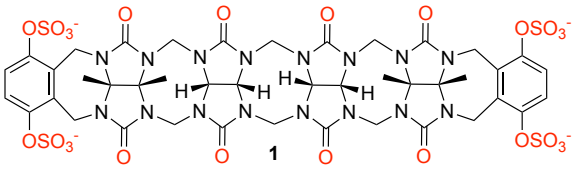
Supporting Information Placeholder

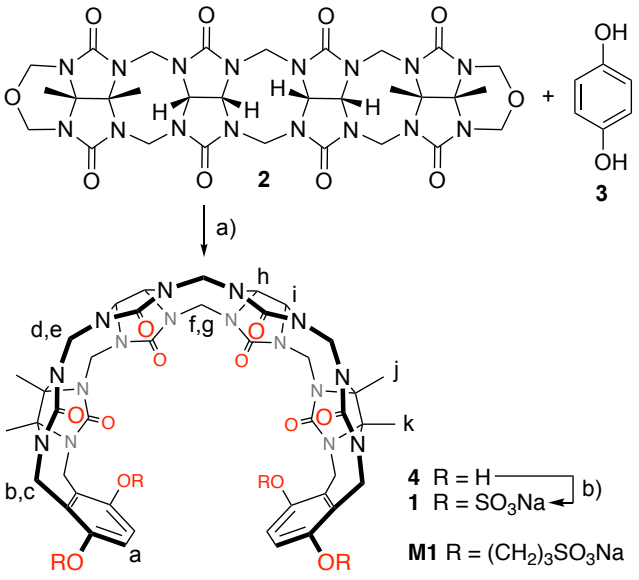
ABSTRACT: The synthesis of acyclic CB[n]-type host (**1**) is reported. By optimizing the placement of the sulfate groups nearby the electrostatically negative ureidyl C=O portals, the binding affinity of this class of receptors toward hydrophobic (di)ammonium guest molecules (**5** – **23**) is maximized. The x-ray crystal structures of **1**•**6a** and **1**•**6d** are reported.



Molecular container compounds and their use in basic science and real world applications is a focal point of modern supramolecular chemistry.[1-6] For example, cyclodextrins are used as solubilizing excipients for hydrophobic drugs, as the active ingredient in Febreeze™, and as a sequestration agent for neuromuscular blockers (NMBA, Sugammadex).[7-9] Cucurbit[n]urils (CB[n]) have become increasingly popular due to their high affinity toward hydrophobic (di)cations (K_a commonly $>10^6$ M^{-1}) and their stimuli responsive host•guest binding properties.[10] CB[n] hosts are thereby well suited as components of functional systems (e.g. sensing ensembles, drug delivery systems, and supramolecular materials).[10-14] Recently, we and others, have been exploring the synthesis and molecular recognition properties of acyclic CB[n]-type receptors (e.g. **M1**, Scheme 1) which retain the essential binding properties of macrocyclic CB[n] but are more easily functionalized.[12,15-17] For example, **M1** functions as a solubilizing excipient for insoluble drugs and a sequestration agent for NMBAs and drugs of abuse (e.g. methamphetamine and fentanyl).[18-19] These applications require hosts with maximal binding affinities to outcompete the cognate biological receptors.[20-22] Herein, we report the synthesis of **1** whose anionic sulfate groups are positioned at the ureidyl C=O portals to complement cationic guests.

Previously, we studied the influence of the $(CH_2)_n$ linker length between the aromatic walls and the SO_3^- groups on their binding affinity toward guests but did not observe large differences for n = 2-4.[23] We reasoned that these alkylene linkers result in the anionic sulfonate groups being positioned away from the C=O portals of **M1** and reduce the electrostatic driving force toward guest complexation. Accordingly, we hypothesized that complete removal of the linker would position the OSO_3^- groups closer to the cation binding site at the C=O portals and thereby increase binding affinity toward cationic guests. Synthetically, we allowed tetramer (**2**)[24] to react with **3** in TFA at RT to deliver **4** in 99% yield. Reaction of **4** with pyridine•SO_3 at 90 °C delivered **1** (60%, 0.5 gram scale) after purification by gel permeation chromatography. Host **1** was characterized spectroscopically and its structure was confirmed by x-ray crystallography of its host•guest complexes (*vide infra*). For example, the ^{1}H NMR spectrum of **1** shows six resonances for the diastereotopic CH_2-groups of the glycoluril oligomer in the expected 4:4:4:4:2:2 ratio along with a singlet for the aromatic H-atoms, two Me resonances, and two glycoluril methine resonances which is consistent with the depicted C_{2v}-symmetric structure of **1**. In the ^{13}C NMR we observe all 14 resonances expected based on the depicted of the C_{2v}-symmetric structure of **1**. Finally, the electrospray ionization mass spectrum for **1** as its complex with **6d** exhibits a doubly charged ion ($[M+\mathbf{6d}\text{-}2Cl^-]^{2-}$), calcd. for $C_{54}H_{70}N_{18}O_{24}Na_4^{2-}$ 787.1642, found 787.1679. Host **1** exhibits high water solubility (>40 mM). Next, we performed dilution experiments to ensure that self-association of **1** does not impinge upon the planned binding constant measurements.[1]

Scheme 1. Synthesis of **1**. Conditions: a) TFA, 25 °C, N_2, 16 h; b) pyridine•SO_3, 90 °C, 18 h.



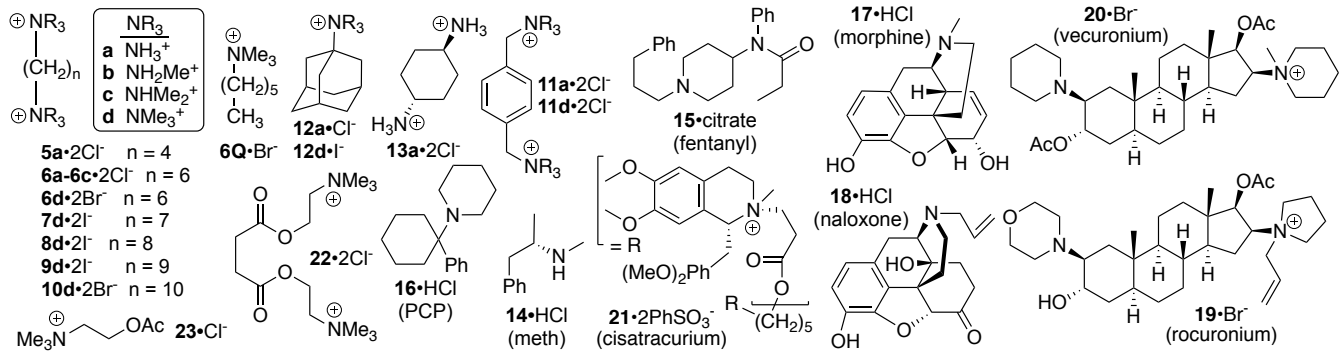


Figure 1. Structures of guests **5** – **23**.

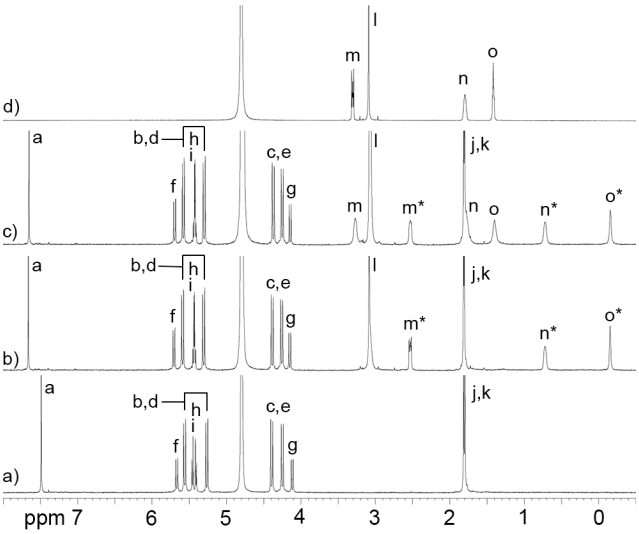


Figure 2. ^{1}H NMR spectra (D_2O, 600 MHz) recorded for: a) **1** (1 mM), b) **1** (1 mM) and **6d** (1 mM), c) **1** (1 mM) and **6d** (2 mM), and d) **6d** (1 mM). *Resonances for **1**•**6d**.

Accordingly, we measured the ^{1}H NMR spectrum of **1** upon dilution from 40 to 1 mM. We did not observe significant changes in chemical shifts ($\Delta\delta$ <0.02 ppm) over this concentration range and therefore conclude that **1** is monomeric in water (Supporting Information).

Next, we performed qualitative host•guest binding studies of **1** with guests **5**-**13** (Figure 1, Supporting Information) as monitored by ^{1}H NMR. Figure 2 shows the ^{1}H NMR spectra recorded for **1**, **6d**, and 1:1 and 1:2 mixtures of **1**:**6d**. As expected the methylene resonances for guest **6d** (H_m, H_n, H_o) within the **1**•**6d** complex (Figure 2b) experience a sizable upfield shift upon complexation due to the anisotropic shielding effects of the aromatic walls and the glycoluril concavity.[15,25-26] At a 1:2 **1**:**6d** ratio, resonances are observed for both free **6d** and **1**•**6d** which indicates slow exchange on the ^{1}H NMR time scale which is usually observed only for tight host•guest complexes. Similar ^{1}H NMR measurements were made for the remainder of the guests (Supporting Information). We find that the narrow guests (e.g. **11a,d** and **6a-d**) display slow exchange kinetics whereas the bulkier guests **12a,d** display intermediate to fast exchange on the chemical shift timescale. We attribute this to their lower binding constants (*vide infra*) as a result of the expansion of the cavity of **1** required to accommodate the larger adamantane framework.

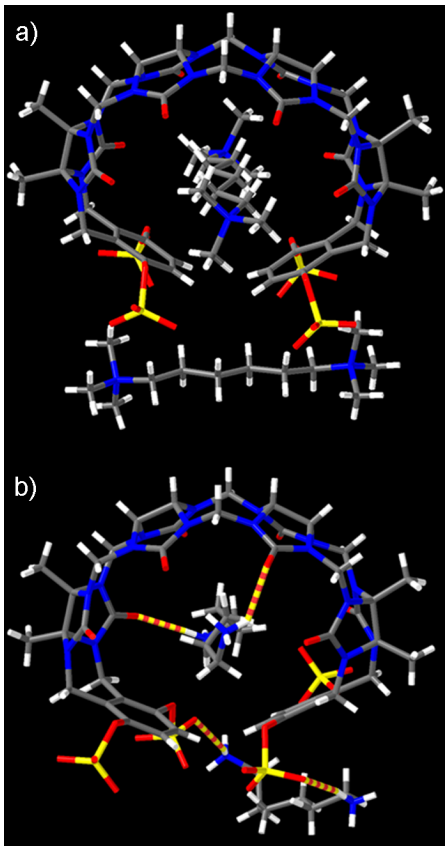


Figure 3. Renderings of the crystal structures of: a) **1**•**6d**, and b) **1**•**6a**. Color code: C, gray; H, white; N, blue; O, red; H-bonds red-yellow striped.

We obtained x-ray crystal structures of the **1**•**6d** (CCDC 2003520) and **1**•**6a** (CCDC 2003521) complexes (Figure 3; See Figure S54 for enlarged stereoviews). Figure 3a shows that **1**•**6d** adopts a geometry that optimizes Me_3N^+•••O=C electrostatic interactions at both portals and displays only small out-of-plane skewing of the terminal aromatic rings. The geometry of **1**•**6d** is reminiscent of CB[n]•guest complexes where the Me_3N^+•••O=C distances cluster in the 3.810-4.690 Å range to spread the positive charge to the carbonyl portals.[14] Interestingly, a second molecule of **6d** fits into a cleft created by the sidewalls and the outward pointing OSO_3^- groups to balance the overall 4- charge of **1**. These OSO_3^- groups also engage in electrostatic interactions with **6d** (Me_3N^+•••O-S distances: 3.808-4.722 Å). The crystal structure of **1**•**6a** (Figure 3b) also shows intracavity and extracavity molecules of **6a** but displays significant out-of-plane twisting of the aromatic termini. Interestingly, one of the four OSO_3- groups turns inward toward

the ammonium ion guest which establishes that this group can directly participate in the guest recognition process.

Given the high binding constants typically observed for host•guest complexes (acyclic) CB[n]-type receptors,[10,15] we elected to use isothermal titration calorimetry (ITC) to measure the K_a values between host **1** and guests **5**–**23**. For the weaker binding complexes ($K_a \leq 10^7$ M^{-1}), we performed the direct titration of host **1** in the ITC cell with a solution of guest in the syringe and fitted the data to a 1:1 binding model implemented by the PEAQ ITC software to obtain K_a and ΔH values (kcal mol^{-1}). Table 1 reports the thermodynamic data for **1•5**, **1•6Q**, **1•12a**, **1•13a**, **1•14**-**1•18**, and **1•21**-**1•23** obtained by direct ITC titrations (Supporting Information). Complexes with K_a values that exceed 10^7 M^{-1} cannot be measured accurately by direct titrations, so we turned to ITC competitive titrations.[27-28] In competitive titrations, a solution of host and an excess of a weak guest of known ΔH and K_a is titrated with a solution of a tighter binding guest. Fitting of the heat released during the displacement process is analyzed by a competitive binding model in the PEAQ ITC data analysis software which delivers ΔH and K_a for the tighter complex. Figure 4a shows the thermogram recorded when a mixture of **1** and **13** was titrated with **6d**; Figure 4b shows the fitting of the integrated heats to a competitive binding model to determine $K_a = 6.79\times10^9$ M^{-1} and ΔH = -12.1 kcal mol^{-1}. Table 1 reports K_a and ΔH values for the remaining **1**•guest complexes obtained in an analogous manner (Supporting Information).

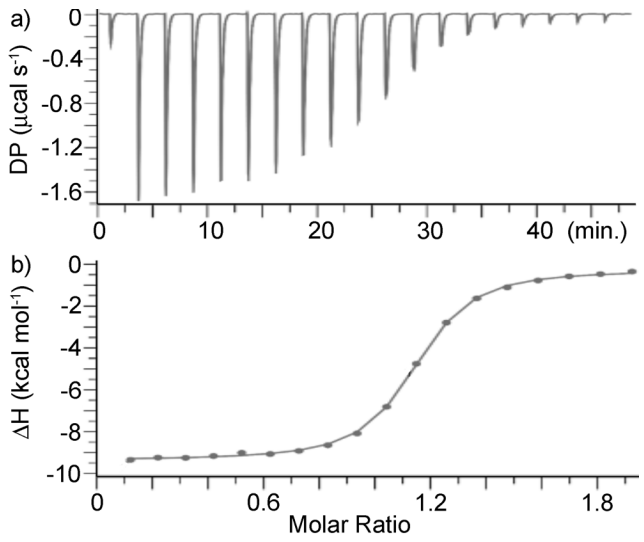


Figure 4. a) ITC titration of **1** (100 µM) and **13** (2 mM) in the cell with a solution of **6d** (1.0 mM) in the syringe, and b) data fitting to a competition binding model to extract $K_a = 6.79\times10^9$ M^{-1} and ΔH = -12.1 kcal mol^{-1}.

Table 1. Binding constants measured by ITC for 1•guest complexes and comparative literature values for M1. Conditions: 20 mM sodium phosphate buffered H_2O, pH 7.4, 25 °C.

Guest	K_a [M^{-1}]; ΔH (kcal mol^{-1}) Host **1**	Host **M1**[e]
5	1.68×10^6; -6.76 ±0.020[a]	–
6a	3.70×10^8; -8.60±0.021[b]	5.05×10^7; -6.23±0.014
6b	5.26×10^8; -9.82±0.038[c]	9.43×10^7; -7.15±0.025
6c	5.74×10^8; -10.5±0.028[c]	4.81×10^7; -7.66±0.073
6d	6.71×10^9; -12.1±0.042[c]	8.93×10^7; -9.35±0.021
6Q	7.57×10^6; -9.68±0.063[a]	1.24×10^6; -5.67±0.033
7d	6.06×10^9; -12.2±0.041[c]	–
8d	1.75×10^9; -10.5±0.032[c]	–
9d	7.57×10^8; -10.2±0.030[c]	–
10d	5.43×10^8; -10.3±0.088[c]	–
11a	9.71×10^8; -9.69±0.014[b]	1.67×10^8; -8.09±0.018
11d	1.05×10^9; -12.0±0.030[b]	1.78×10^8; -11.4±0.022
12a	9.90×10^5; -4.45±0.021[a]	9.62×10^5; -6.55±0.029
12d	6.66×10^6; -7.36±0.030[d]	1.70×10^7; -9.09±0.027
13a	3.41×10^6; -2.92±0.019[a]	1.95×10^6; -5.70±0.027
14	3.02×10^6; -9.28±0.058[a]	7.5×10^6
15	3.64×10^6; -12.2±0.076[a]	1.1×10^7
16	1.89×10^5; -6.18±0.069[a]	4.7×10^4
17	7.69×10^5; -8.03±0.07[a]	5.3×10^5
18	4.85×10^6; -5.90±0.205[a]	–
19	6.29×10^8; -12.9±0.056[b]	8.4×10^6
20	1.00×10^9; -9.62±0.036[c]	5.8×10^6
21	5.32×10^5; -15.4±0.174[a]	9.7×10^5
22	2.41×10^4; -5.26±0.372[a]	–
23	2.31×10^5; -8.54±0.063[a]	2.4×10^4

– not reported. a) Direct titration. Competive ITC using: b) **5** as competitor, c) **13a** as competitor, d) **6d** as competitor, e) Literature values.[18,29-31]

The binding constant data reported in Table 1 allows us to draw some conclusions about the molecular recognition preferences of host **1** in comparison to **M1**. As expected, we find that the **1**•guest complexes are uniformly driven by favorable enthalpic (ΔH) contributions. In the CB[n] series of hosts these favorable enthalpy values are attributed to the presence of high energy host intracavity water molecules that are released upon guest binding.[32-33] Host **1** displays high affinity toward hexanediammonium ion guests **6a** – **6d** with K_d values in the single digit nM to sub-nM range. Host **1** prefers the quaternary ammonium ion guest **6d** by ≈10-fold over the primary – tertiary ammonium ions **6a** – **6c**. In selected contexts, related preferences have been seen for CB[7][34] where they are attributed to the more efficient spreading of positive charge to the entire ureidyl C=O portal. Host **1** binds quaternary monoammonium ion guest **6Q** 890-fold weaker than the corresponding quaternary diammonium **6d**; this ≈10^3 M^{-1} difference in affinity is also noted for CB[n]-type receptors.[10] Importantly, we find that **1** binds to guests **6a** – **6c** 5.6 – 11.9-fold stronger than **M1**, but 75-fold stronger than **M1** toward bis(quaternary) guest **6d**. Similar preferences are observed for dicationic guests **11a** and **11d** but not for monocationic guests **12a** and **12d** which suggests that the defined separation between OSO_3^- groups in **1** makes it especially complementary to diammonium ion guests. Host **1** also binds with high affinity (single digit nM to sub nM K_d values) toward the longer alkanediammonium ions **7d** – **10d** although **6d** is the tightest binder in this series which reflects the ability of acyclic CB[n] to flex their cavity to accommodate larger guests and optimize binding affinity. Related preferences have previously been seen for **M1** and related receptors toward primary alkane diammonium ion guests.[24]

We have previously studied the use of **M1** and a naphthalene walled analogue known as **M2** as *in vivo* sequestration agents for drugs of abuse (e.g. methamphetamine (**14**)).[18] Accordingly, we decided to measure the binding affinities of some compounds (**14**

– **18**) relevant to counteracting the effects of drugs of abuse. We find that host **1** binds less tightly than **M1** toward **14** and **15**. In contrast, host **1** binds somewhat tighter to PCP (**16**) and morphine (**17**) than **M1** does, but the single digit μM dissociation constants are unlikely to render **1** an efficient *in vivo* sequestration agent for **16** and **17**. Accordingly, **1** is not an improved lead compound for the sequestration of drugs of abuse (**14** – **17**). This is perhaps not surprising given that **1** has a distinct preference for bis(quaternary) diammonium ions whereas **14** – **17** are secondary and tertiary ammonium ions.

In a separate line of inquiry, we have shown that **M1** and **M2** act as *in vivo* reversal agents for NMBAs **19**-**21**.[29,35-37] Accordingly, we measured the binding constants of **1** toward a panel of compounds relevant to its potential use as an *in vivo* reversal agent. Table 1 shows that **1** possesses higher binding affinity toward **19** (75-fold) and **20** (172-fold) than **M1**. Importantly, **1** binds >2700-fold tighter to **19** or **20** than to **23**. Acetylcholine is also present in the neuromuscular junction and must not be sequestered. The affinity of **1**•**19** ($6.29\times10^8\ M^{-1}$) and **1**•**20** ($1.00\times10^9\ M^{-1}$) are comparable to those of **M2**•**19** ($3.4\times10^9\ M^{-1}$) and **M2**•**20** ($1.6\times10^9\ M^{-1}$) which function very well *in vivo*.[29] Host **1**, however, possesses superior solubility (>40 mM) compared to **M2** (18 mM) which might prove advantageous for formulation purposes.

In summary, we have presented the synthesis of host **1** with OSO_3^- groups directly connected to the aromatic walls. Host **1** has excellent solubility (40 mM), does not self-associate, and binds to quaternary diammonium ions tighter than **M1** which features propylene linkers. The x-ray crystal structures of **1**•**6a** and **1**•**6d** show cavity inclusion of the diammonium guest and an external diammonium ion that balances the overall charge of tetraanionic **1**. In conclusion, we find that the OSO_3^- groups do not merely function as solubilizing groups, but rather their close proximity to the C=O portals of **1** delivers enhanced binding affinity toward quaternary diammonium ions including important NMBAs **19** and **20**. Host **1** should be considered alongside **M1** and **M2** as *in vivo* reversal agents for neuromuscular blockers.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on ACS publication website at DOI: xxxxxxx.

Experimental procedures, characterization data, ^{1}H and ^{13}C NMR spectra for new compounds, ^{1}H NMR spectra for **1**•guest complexes, and data from ITC experiments (PDF); crystallographic information files for **1**•**6a** and **1**•**6d** (CIF).

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Author Contributions

†X.L. and S.Z.N. contributed equally. X.L. and S.Z.N. performed experiments and analyzed data. P.Y.Z. collected x-ray diffraction data and solved the crystal structures. L.I., X.L., and S.Z.N. wrote the paper. All authors have given approval to the final version of the manuscript. L.I. supervised the entire project.

Notes

L.I. is an inventor on patents held by the University of Maryland on the use of acyclic CB[n]-type receptors in biomedical applications.

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References.

1) Diederich, F., Complexation of neutral molecules by cyclophane hosts. *Angew. Chem., Intl. Ed. Engl.* **1988**, *27,* 362-386.

2) Ogoshi, T.; Yamagishi, T.-A.; Nakamoto, Y., Pillar-shaped macrocyclic hosts pillar[n]arenes: New key players for supramolecular chemistry. *Chem. Rev.* **2016**, *116,* 7937-8002.

3) Gutsche, C. D., Calixarenes. *Acc. Chem. Res.* **1983**, *16,* 161-170.

4) Cram, D. J., The design of molecular hosts, guests, and their complexes (nobel lecture). *Angew. Chem. Int. Ed.* **1988**, *27,* 1009-1020.

5) Rebek, J., Molecular behavior in small spaces. *Acc. Chem. Res.* **2009**, *42,* 1660-1668.

6) Harris, K.; Fujita, D.; Fujita, M., Giant hollow mnl2n spherical complexes: Structure, functionalisation and applications. *Chem. Commun.* **2013**, *49,* 6703-6712.

7) Bom, A.; Bradley, M.; Cameron, K.; Clark, J. K.; Van Egmond, J.; Feilden, H.; MacLean, E. J.; Muir, A. W.; Palin, R.; Rees, D. C.; Zhang, M.-Q., A novel concept of reversing neuromuscular block: Chemical encapsulation of rocuronium bromide by a cyclodextrin-based synthetic host. *Angew. Chem., Int. Ed.* **2002**, *41,* 265-270.

8) Stella, V. J.; Rajewski, R. A., Cyclodextrins: Their future in drug formulation and delivery. *Pharm. Res.* **1997**, *14,* 556-567.

9) https://www.febreze.com/en-us/safety/science-and-chemicals-in-febreze (accessed March 9).

10) Shetty, D.; Khedkar, J. K.; Park, K. M.; Kim, K., Can we beat the biotin–avidin pair?: Cucurbit[7]uril-based ultrahigh affinity host–guest complexes and their applications. *Chem. Soc. Rev.* **2015**, *44,* 8747-8761.

11) Dsouza, R. N.; Hennig, A.; Nau, W. M., Supramolecular tandem enzyme assays. *Chem. Eur. J.* **2012**, *18,* 3444-3459.

12) Liu, J.; Lan, Y.; Yu, Z.; Tan, C. S. Y.; Parker, R. M.; Abell, C.; Scherman, O. A., Cucurbit[n]uril-based microcapsules self-assembled within microfluidic droplets: A versatile approach for supramolecular architectures and materials. *Acc. Chem. Res.* **2017**, *50,* 208-217.

13) Walker, S.; Oun, R.; McInnes, F. J.; Wheate, N. J., The potential of cucurbit[n]urils in drug delivery. *Isr. J. Chem.* **2011**, *51,* 616-624.

14) Yin, H.; Wang, R., Applications of cucurbit[n]urils (n=7 or 8) in pharmaceutical sciences and complexation of biomolecules. *Isr. J. Chem.* **2017**, *58,* 188-198.

15) Ganapati, S.; Isaacs, L., Acyclic cucurbit[n]uril-type receptors: Preparation, molecular recognition properties and biological applications. *Isr. J. Chem.* **2018**, *58,* 250-263.

16) Bauer, D.; Andrae, B.; Gass, P.; Trenz, D.; Becker, S.; Kubik, S., Functionalisable acyclic cucurbiturils. *Org. Chem. Front.* **2019**, *6,* 1555-1560.

17) Mao, D.; Liang, Y.; Liu, Y.; Zhou, X.; Ma, J.; Jiang, B.; Liu, J.; Ma, D., Acid-labile acyclic cucurbit[n]uril molecular containers for controlled release. *Angew. Chem. Int. Ed.* **2017**, *41,* 12614-12618.

18) Ganapati, S.; Grabitz, S. D.; Murkli, S.; Scheffenbichler, F.; Rudolph, M. I.; Zavalij, P. Y.; Eikermann, M.; Isaacs, L., Molecular containers bind drugs of abuse in vitro and reverse the hyperlocomotive effect of methamphetamine in rats. *ChemBioChem* **2017**, *18,* 1583-1588.

19) Thevathasan, T.; Grabitz, S. D.; Santer, P.; Rostin, P.; Akeju, O.; Boghosian, J. D.; Gill, M.; Isaacs, L.; Cotton, J. F.; Eikermann, M., Calabadion 1 selectively reverses respiratory and central nervous system effects of fentanyl in a rat model. *Br. J. Anaesth.* **2020**, *124*, DOI: 10.1016/j.bja.2020.02.019.

20) Zhang, X.; Xu, X.; Li, S.; Li, L.; Zhang, J.; Wang, R., A synthetic receptor as a specific antidote for paraquat poisoning. *Theranostics* **2019**, *9,* 633.

21) Zhang, X.; Cheng, Q.; Li, L.; Shangguan, L.; Li, C.; Li, S.; Huang, F.; Zhang, J.; Wang, R., Supramolecular therapeutics to treat the side effects induced by a depolarizing neuromuscular blocking agent. *Theranostics* **2019**, *9,* 3107-3121.

22) Huang, Q.; Cheng, Q.; Zhang, X.; Yin, H.; Wang, L.-H.; Wang, R., Alleviation of polycation-induced blood coagulation by the formation of polypseudorotaxanes with macrocyclic cucurbit[7]uril. *ACS Appl. Bio Mater.* **2018**, *1,* 544-548.

23) Sigwalt, D.; Moncelet, D.; Falcinelli, S.; Mandadapu, V.; Zavalij, P. Y.; Day, A.; Briken, V.; Isaacs, L., Acyclic cucurbit[n]uril-type molecular containers: Influence of linker length on their function as solubilizing agents. *ChemMedChem* **2016**, *11,* 980-989.

24) Ma, D.; Zavalij, P. Y.; Isaacs, L., Acyclic cucurbit[n]uril congeners are high affinity hosts. *J. Org. Chem.* **2010**, *75,* 4786-4795.

25) Masson, E.; Ling, X.; Joseph, R.; Kyeremeh-Mensah, L.; Lu, X., Cucurbituril chemistry: A tale of supramolecular success. *RSC Adv.* **2012**, *2,* 1213-1247.

26) Mock, W. L.; Shih, N.-Y., Structure and selectivity in host-guest complexes of cucurbituril. *J. Org. Chem.* **1986**, *51,* 4440-4446.

27) Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L.-N., Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Anal. Biochem.* **1989**, *179,* 131-137.

28) Broecker, J.; Vargas, C.; Keller, S., Revisiting the optimal c value for isothermal titration calorimetry. *Anal. Biochem.* **2011**, *418,* 307-309.

29) Ma, D.; Zhang, B.; Hoffmann, U.; Sundrup, M. G.; Eikermann, M.; Isaacs, L., Acyclic cucurbit[n]uril-type molecular containers bind neuromuscular blocking agents in vitro and reverse neuromuscular block in vivo. *Angew. Chem. Int. Ed.* **2012**, *51,* 11358-11362.

30) Xue, W.; Zavalij, P. Y.; Isaacs, L., Triazole functionalized acyclic cucurbit[n]uril-type receptors: Host-guest recognition properties. *Org. Biomol. Chem.* **2019**, *17,* 5561-5569.

31) Xue, W.; Zavalij, P. Y.; Isaacs, L., Acyclic cucurbit[n]uril type receptors: Secondary versus tertiary amide arms. *Supramol. Chem.* **2019**, *31,* 685-694.

32) Biedermann, F.; Uzunova, V. D.; Scherman, O. A.; Nau, W. M.; De Simone, A., Release of high-energy water as an essential driving force for the high-affinity binding of cucurbit[n]urils. *J. Am. Chem. Soc.* **2012**, *134,* 15318-15323.

33) Biedermann, F.; Nau, W. M.; Schneider, H.-J., The hydrophobic effect revisited-studies with supramolecular complexes imply high-energy water as a noncovalent driving force. *Angew. Chem. Int. Ed.* **2014**, *53,* 11158-11171.

34) Cao, L.; Sekutor, M.; Zavalij, P. Y.; Mlinaric-Majerski, K.; Glaser, R.; Isaacs, L., Cucurbit[7]uril.Guest pair with an attomolar dissociation constant. *Angew. Chem. Int. Ed.* **2014**, *53,* 988-993.

35) Hoffmann, U.; Grosse-Sundrup, M.; Eikermann-Haerter, K.; Zaremba, S.; Ayata, C.; Zhang, B.; Ma, D.; Isaacs, L.; Eikermann, M., Calabadion: A new agent to reverse the effects of benzylisoquinoline and steroidal neuromuscular-blocking agents. *Anesthesiology* **2013**, *119,* 317-325.

36) Haerter, F.; Simons, J. C. P.; Foerster, U.; Moreno Duarte, I.; Diaz-Gil, D.; Ganapati, S.; Eikermann-Haerter, K.; Ayata, C.; Zhang, B.; Blobner, M.; Isaacs, L.; Eikermann, M., Comparative effectiveness of calabadion and sugammadex to reverse non-depolarizing neuromuscular-blocking agents. *Anesthesiology* **2015**, *123,* 1337-1349.

37) Ganapati, S.; Zavalij, P. Y.; Eikermann, M.; Isaacs, L., In vitro selectivity of an acyclic cucurbit[n]uril molecular container towards neuromuscular blocking agents relative to commonly used drugs. *Org. Biomol. Chem.* **2016**, *14,* 1277-1287.