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Understanding the binding properties of phosphorylated glycoluril-derived molecular tweezers and selective nanomolar binding of natural polyamines in aqueous solution†

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A modular synthetic platform for the construction of flexible glycoluril-derived molecular tweezers was developed. The binding properties of four exemplary supramolecular hosts obtained *via* this approach towards 16 organic amines were investigated by means of ^{1}H NMR titration. In this work, we compare the K_a values obtained this way with those of three structurally related molecular tweezers and provide a computational approach towards an explanation of the observed behavior of those novel hosts. The results showcase that certain structural modifications lead to very potent and selective binders of natural polyamines, with observed binding of spermine below 10 nM.

Introduction

Over the past decades, the growing understanding of intermolecular processes has set the foundation for studying molecular recognition, both in biological and artificial systems, in an increasingly exact fashion.[1] Numerous synthetic supramolecular host structures with different sizes, shapes and functionalization patterns have been shown to interact with a multitude of classes of guest molecules.[2] Among those are biologically highly relevant targets, including proteins, drugs or drug-like molecules or biogenic small molecules.[3] One class of such constructs called molecular tweezers is defined as U-shaped molecules with aromatic panels that are connected with rigid linkers.[4] Several different frameworks for such tweezers have been shown suitable to accommodate a guest molecule within the pre-organized cavity.[2b,4h,k,5] Importantly, by deliberate decoration of a hydrophobic core structure with solubilizing groups like phosphates, phosphonates, or carboxylates, such tweezers can be adapted to bind organic guests in aqueous solution.[6] For instance, the phosphate derivative of tweezer **1** (Fig. 1A, R = O$^-$) has been shown to be remarkably selective for lysine and arginine derivatives and was even successfully employed to tightly bind these moieties in oligopeptides and on protein surfaces.[7] However, its structural design limits the possibilities of facile derivatization. On the other hand, the glycoluril building block is a well-established subunit for an entire family of acyclic tweezers of varying size, shape and flexibility that have been utilized as hosts for a broad range of viable guest molecules.[3d] One example is host **2**, which is very similar to **1** in size and shape of its cavity, but has a higher level of flexibility due to the lack of rigid bicyclic linkers.[6b,9] Inspired by these contributions and motivated by our group's interest in catalysis inside supramolecular containers and within molecular tweezers,[10] we sought to develop a modular and derivatization-friendly platform for the synthesis of molecular tweezers. Ultimately, we envision this to enable the selective binding and modification of biologically relevant targets. We recently showed that the conformationally flexible phosphorylated host **3** binds very tightly to numerous sterically undemanding diamine guests.[8] Guided by these results, we have designed a streamlined synthetic procedure for tweezers of the general structure **4** with the goal of understanding host–guest interactions of these hosts in more detail. Here, we report a comparative study of the binding properties of four derivatives of the general structure **4** and the previously described tweezers **1**, **2** and **3**.

Results and discussion

For this study, we chose to synthesize and investigate the following derivatives (Scheme 1): benzo-tipped tweezer **4a** which

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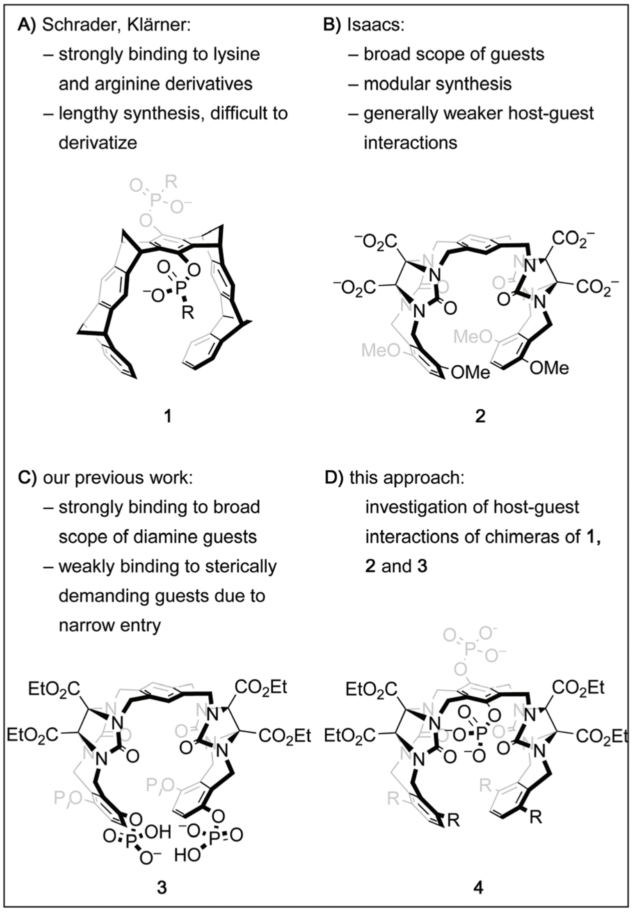
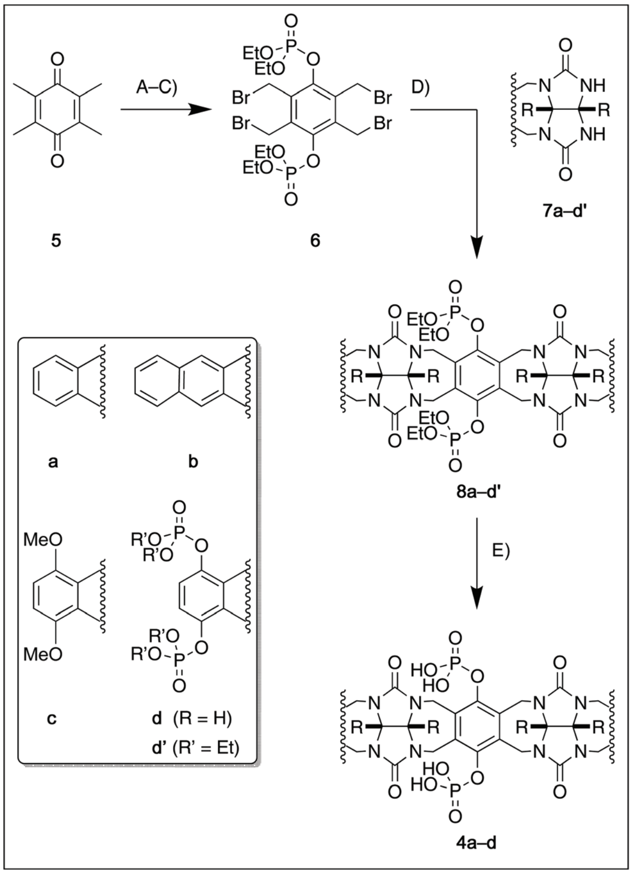


Fig. 1 Chemical structures of molecular tweezer 1[6a] and the similarly sized tweezer 2.[6b] Tweezer 3[8] shares the framework with 2 but the phosphate groups attached to it provide unique binding properties. Tweezers of the general structure 4 were synthesized with the goal of having access to a broad range of chimeras *via* a modular platform.



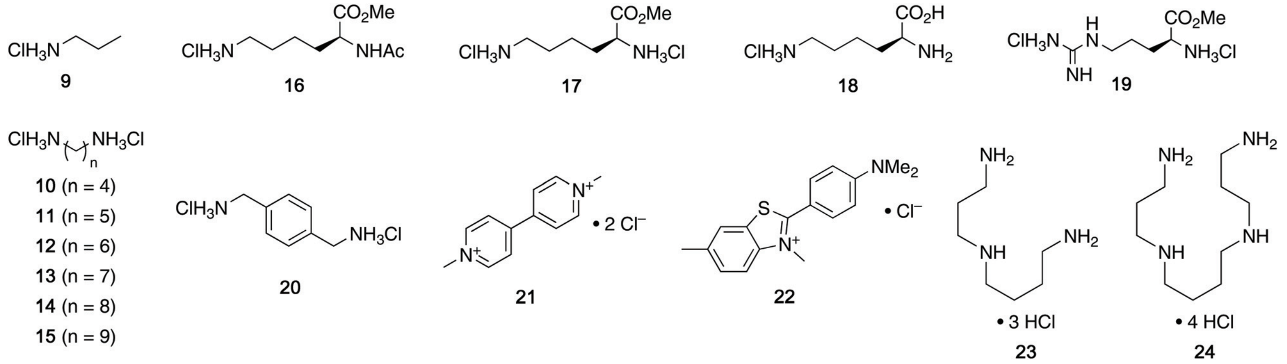
Scheme 1 Synthesis of tweezers **4a–d**. **R = CO_2Et**. (A) Zn, AcOH, 130 °C, 60 min. (B) NaH, $(EtO)_2P(O)Cl$, DMF, 0 °C, 18 h, 64% (2 steps). (C) NBS, AIBN, CCl_4, 95 °C, 16 h, 87%. (D) **6**, **7** (2.0 equiv.), Cs_2CO_3, TMAI, 35–58% (dr = 1.3/1 … 1.9/1). (E) TMSBr, 0 °C, 24 h then EtOH, rt, 60 min, 82–88%. NBS: *N*-Bromosuccinimide, AIBN: azobis-iso-butyronitrile, TMAI: tetramethylammonium iodide, TMS: trimethylsilyl.

closely resembles **1**; naphtho-tipped tweezer **4b** which is related to **4a** but features a larger cavity due to increased angular distortion resulting from repulsion of the larger tips; dimethoxybenzo-tipped tweezer **4c** which shares features of both **1** and **2**; and hexaphosphorylated tweezer **4d** which serves as a chimera of **3** and **4a**. The synthetic strategy of tweezers **4a–d** is based on the convergent alkylation of the respective glycoluril derivatives **7a–d′** with tetrabromide **6** (Scheme 1). Tetrabromide **6** is accessible from commercially available duroquinone (**5**) in three steps. After a zinc mediated reduction[11] and phosphorylation employing sodium hydride and diethyl chlorophosphate, fourfold benzylic bromination delivered **6** in good yield (55% over three steps). While the preparation of the partially substituted glycoluril derivatives **7a–d′** was easily accomplished by alkylation of the parent unsubstituted glycoluril with the corresponding *o*-xylylene dibromides (see ESI† for details), the direct construction of the final frameworks **8a–d′** proved to be a highly challenging transformation. Numerous attempts of employing different inorganic and organic bases in a variety of solvents led to virtually no conversion at room temperature, likely due to the steric congestion of electrophile **6**, and resulted in decomposition without formation of **8** when heated. The use of differently protected derivatives of **6**, including several alkyl, acetal, or acyl protecting groups, did not resolve the issue. Attempts to use different leaving groups instead of bromides as in **6**, including readily accessible chlorides and acetates, were not successful either.[12] Gratifyingly, trace product formation was observed when we subjected **6** and **7a** to the weak inorganic base potassium carbonate and sodium iodide at 40 °C. Further optimization showed that using catalytic amounts of tetramethylammonium iodide and cesium carbonate smoothly gave the desired compounds in good yields. Unlike in our previously presented synthesis of **3**[8] but also observed in the synthesis of **2**,[6b] mixtures of the “C-shaped” **8a–d′** and their undesired “S-shaped” diastereomers were obtained that necessitated separation *via* HPLC (see ESI†). Subsequent deprotection of the obtained diastereopure diethylphosphates by addition of bromotrimethylsilane followed by ethanol furnished the desired phosphate tweezers **4a–d** in excellent yields.

With the desired tweezers in hand, we started investigating their binding properties by means of ^{1}H NMR titration in buffered D_2O (70 mM phosphate buffer, pD = 7.2), and determining the equilibrium constants *via* non-linear regression.[13] We found that **4a** and **4d** undergo weak dimerization (K_{dim} = 32.7 M^{-1} and 35.0 M^{-1}, respectively), while this process is considerably more pronounced in **4b** (773 M^{-1}) and **4c** (624 M^{-1}), with the orders of magnitude in good agreement with comparable systems.[6a,7a,9b] Job plots indicated that these tweezers bind their guests in a 1 : 1 fashion (see ESI†). Next, the guest scope was explored in detail. Table 1 summarizes the binding constants (K_a) of complexes of 16 guests and the four tweezers **4a–d** as well as available literature values of tweezers **1–3** for comparison. Similar to previous findings with **2** and **3**, **4a** and **4c** bind short aliphatic monoamine compounds like propylammonium chloride (**9**) relatively weakly, and **4b** did not show significant uptake of such short-chained diammonium guests. The stronger affinity of **4d** to **9** is in agreement with the stronger ionic interactions facilitated by the six phosphate moieties in **4d** as compared to two in **1** and **4a-c**. Interestingly, the binding properties of tweezers **3** and **4a–d** did not change substantially when we investigated the same equilibrium in unbuffered D_2O, although we observed diminished solubility of **4a–d**, likely due to stronger self-aggregation in this solvent.[6b] As expected, we were not able to observe any binding in control titration experiments of the exemplary S-shaped deprotected construct *dia*-**4c**; in agreement with the exclusive accommodation of the aliphatic chains of the guests in the tweezers' C-shaped cavity.

We next investigated the dependency of aliphatic diamine guest binding on the length of their methylene linkers. As expected, we found moderate-length guests to be optimal, the size of which suit the tweezers' cavities most closely (C_7-diammonium chloride **13** for **4a** and **4c**, C_8-diammonium chloride **14** for the larger host **4b**). However, the binding of diammonium guests in **4a–c** is weak compared to the derivatives

Table 1 Guest molecules investigated and binding constants K_a (M^{-1}) of their complexes with molecular tweezers **4a–d**, determined *via* ^{1}H NMR titration, and comparison to related systems. Titrations performed at 100 μM host in 70 mM phosphate buffer (pD 7.2). The precision of the measurements is discussed in detail in the ESI;† the error for 95% confidence is generally below ±5% (see ESI† for exact values)



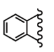
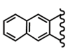
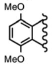
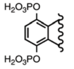
9 **16** **17** **18** **19**

10 (n = 4)
11 (n = 5)
12 (n = 6)
13 (n = 7)
14 (n = 8)
15 (n = 9)

20 **21** **22**

• 3 HCl
23

• 4 HCl
24

Guest	**4a** (K_a/M^{-1}) 	**4b** (K_a/M^{-1}) 	**4c** (K_a/M^{-1}) 	**4d** (K_a/M^{-1}) 	Related tweezers Schrader **1** (K_a/M^{-1})[c]	Isaacs **2** (K_a/M^{-1})[e]	Our prev. work **3** (K_a/M^{-1})[f]
9	138	<10	88.7	3310		105	345
9[a]	208	<10	275	4260	890[d]		295
10	35.8	<10	<10	26 100		724	10 700
11	247	83.5	377	287 000		5960	122 000
12	569	354	1170	148 000		15 200	133 000
13	838	870	2580	49 600		6460	155 000
14	517	1200	1900	14 300		7060	179 000
15	240	831	861	3150			36 300
16	<10	<10	<10	28.3	58 800		<10
17	88.0	<10	23.0	1430			4220
18	32.1	<10	<10	30.8	47 600		57.1
19	14.3	<10.0	14.8	1260			253
20	26.2	103	99.2	18 800		496	33 800
21	144	1750	130.4	27 100		2060	21 600
22	147	585	175	<10			54.0
23	200	45	94.4	2 370 000[b]			388 000
24	815	<10	325	148 000 000[b]			7 920 000

[a] Titration performed in unbuffered D_2O. [b] Determined *via* competitive displacement titration at 100 μM **4d** and 10 mM or 200 mM **21** as a competitor. [c] Values for the phosphate derivative of tweezer **1** as determined by Klärner & Schrader.[6a] [d] Value for the methylphosphonate derivative of tweezer **1** as determined by Klärner & Schrader.[14] [e] Values for tweezer **2** as determined by Isaacs.[6b] [f] Values for tweezer **3** as determined in our previous work.[8]

4d and **3**.[8] Interestingly, the values for **10–14@4c** and **10–14@2** suggest that the phosphates present in **4c** but missing in **2** do not increase but even decrease binding.[6b] This observation was surprising as the added phosphates were expected to increase binding substantially due to strong ion–ion interactions. We interpret these results as an indication that the central phosphate moieties destabilize the desired tweezer conformation, potentially favouring more open conformations. The conformational flexibility of the related host **2** was described by Isaacs and co-workers previously.[6b] While **4d** proved to be a much stronger binder than **4a–c**, a similar observation was made: the additional phosphate moieties in **4d** compared to **3** appeared to offer a limited contribution to the binding of diamine guests **10–15**, although a stronger preference for shorter methylene linkers and reduced affinity for longer-chained guests was observed (maximum with C_5-diammonium chloride **11** for **4d** and maximum with C_8-diammonium chloride **14** for **3**).[8]

We were also interested in the formation of complexes of the novel tweezers and basic amino acid derivatives. Very little interaction was observed between tweezer **4a** and the investigated lysine derivatives Ac-Lys-OMe (**16**, $K_a < 10\ M^{-1}$), H-Lys-OMe (**17**, 88.0 M^{-1}) and H-Lys-OH (**18**, 32.1 M^{-1}) as well as arginine derivative H-Arg-OMe (**19**, 14.3 M^{-1}). These results were surprising, since the size and shape of the cavity of **4a** is very similar to that of **1**, which binds lysine derivatives very tightly,[6a] but are in good agreement with the previous observation of comparably poor binding in **3**. It is important to note that **4a** has a much more flexible framework than **1** and that it can populate different non-active conformations besides the desired "tweezer-like" conformation by means of rotation around the methylene bridges (*vide infra*). Similar to **4a**, interactions between amino acid-derived guests **16–19** and hosts **4b** and **4c** are weak. The binding properties of **4d** towards these guests are very similar to those of **3**, which is in good agreement with the finding that the central phosphate moieties do not contribute to binding.

When we investigated (hetero-)aromatic guests, we found that the association constants for the complexes of **4d** with *p*-xylylenediammonium chloride (**20**, $K_a = 1.88 \times 10^4\ M^{-1}$) and methyl viologen chloride (**21**, $2.71 \times 10^4\ M^{-1}$) are similar to the values for **20@3** and **21@3** ($3.88 \times 10^4\ M^{-1}$ and $2.16 \times 10^4\ M^{-1}$, respectively). Additionally, both **4d** and **3** show no or very weak interaction with the sterically more demanding Thioflavin T (**22**). This further showcases that the installation of additional phosphate moieties at the central aromatic unit does not generally increase binding. Given this observation, we were not surprised to see relatively weak binding of **20** and **21** with **4a–c**. Interestingly, especially **20** and **21**, both comparably large aromatic cations, showed stronger binding to naphtho-tipped host **4b** ($K_a = 1.75 \times 10^3\ M^{-1}$ and $5.85 \times 10^2\ M^{-1}$, respectively) which displays larger aromatic surfaces than **4a** and **4c**.

We did, however, observe significantly stronger binding of the natural polyamines spermidine (**23**) and spermine (**24**) by the hexaphosphorylated host **4d** ($K_a = 2.37 \times 10^6\ M^{-1}$ for **23@4d** and $1.48 \times 10^8\ M^{-1}$ for **24@4**) than the tetraphosphorylated host **3** whereas **4a–c** interact only weakly with those guests. This particularly strong interaction is consistent with our observation that the additional phosphate moieties in **4d** compared to **3** were most advantageous for binding of diamines with relatively short aliphatic linkers. Given that the polyamine metabolism and homeostasis is strongly regulated in humans and elevated levels are linked to excessive cell proliferation and also neurodegenerative diseases, these are viewed as potential handle for future treatments.[15] Therefore, the nanomolar binding of these guests (422 nM for spermidine and 6.76 nM for spermine) in aqueous buffer is remarkable. Furthermore, while comparable affinities to spermidine and spermine have been reported for synthetic supramolecular hosts before,[9a,16] the level of selectivity for spermidine (**23**) and especially spermine (**24**) over their parent biosynthetic precursor putrescine (**10**) is substantially higher for **4d** compared to previously reported hosts.

In order to better understand the guest binding capabilities of the new hosts **4a–d**, we performed MD simulations of the conformational space of hosts **2** and **4c** in water (Fig. 2B and C, for detailed methodology see ESI†). The conformational changes in molecular tweezers are similar to those encountered in the folding process of small proteins.[17] However, the symmetric structure of the tweezers as well as their well-defined local conformations allows a simpler yet more efficient definition of their overall conformational state. In this case, ten different conformers are possible with regard to the four pairs of methylene bridges in these hosts, defining the correlation between the units that are linked by the methylene bridges either as *syn* (*s*, when the substituent at the glycoluril backbone and the aromatic unit are oriented towards each other) or *anti* (*a*, when they are oriented away from each other).[18] We found that while in both **2** and **4c** the *s,a,a,s* conformation is the most favored one, the free energy difference between this state and the "active" *a,a,a,a* conformation is lower in **2** (≈−9 kJ mol^{-1}) than in **4c** (≈−12 kJ mol^{-1}). This indicates that the active tweezer conformation is more accessible in solution for tweezer **2** than for **4c**. Furthermore, the energy barriers between the different conformations are much higher in **4c** than in **2**, indicating that interconversion between different conformers happens at lower rates in **4c** than in **2**. As a result, and in agreement with our observations in the binding studies with hosts **4a–d**, installation of phosphate groups at the central aromatic unit apparently destabilizes the desired tweezer conformation in favor of more open conformations that do not bind guest molecules efficiently. These results clarify the surprising finding that central phosphate groups do not generally increase binding although additional ion–ion interactions with cationic guests would become available. The binding of spermidine (**23**) and particularly spermine (**24**) to the hexaphosphate tweezer **4d** is the exception identified. We assume that the increased ion–ion interactions between the triply/quadruply charged guest and the hexaphosphate tweezer **4d** are overriding other factors in these two cases.

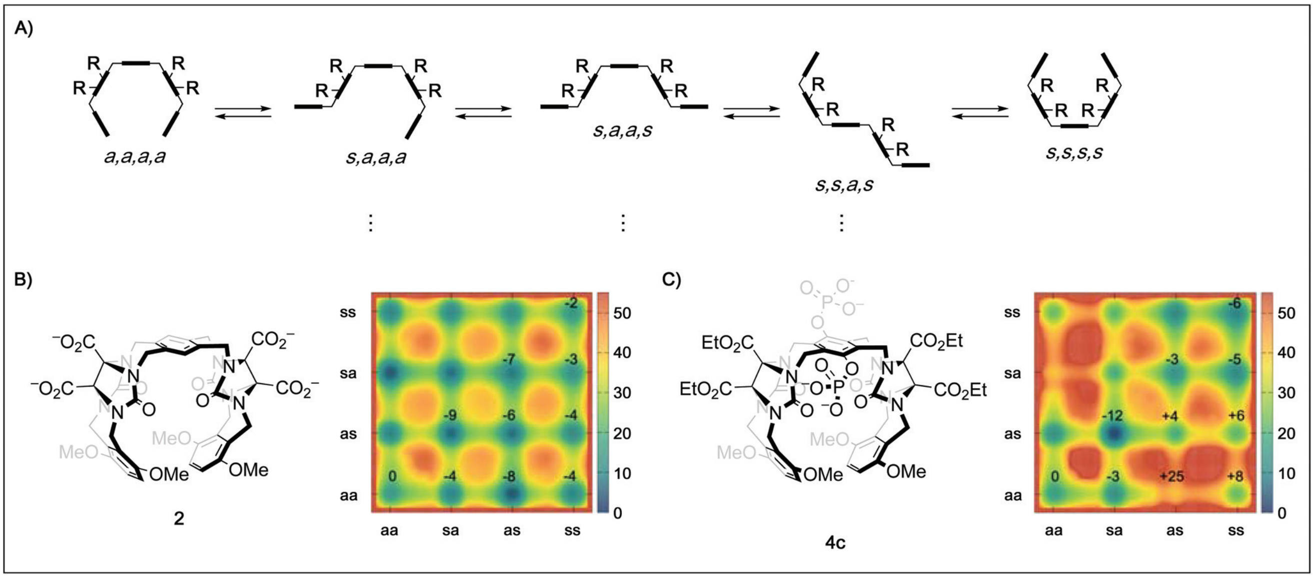


Fig. 2 (A) Schematic depiction of five of the ten possible conformers of a tweezer with the general framework of **2**, **3**, and **4** by rotation around the four pairs of methylene bridges using the nomenclature defined by Nolte.[18] (B) Metadynamics estimation of the free energy surface (kJ mol^{-1}) for the conformers of **2** in water reveal a global minimum for the *s,a,a,s* state. (C) Metadynamics estimation of the free energy surface (kJ mol^{-1}) for the conformers of **4c** in water also reveal a global minimum for the *s,a,a,s* state, but a much less favored "active" *a,a,a,a* state as well as higher energy barriers between the different conformations. Values given in the surface plots are relative to the *a,a,a,a* conformer.

Conclusions

In summary, we have developed a modular synthetic platform for phosphorylated molecular tweezers based on glycoluril linker units of the general structure **4** and were able to showcase the synthetic versatility by synthesizing the four derivatives **4a–d** with diverse tips. The comparative study of those and related tweezers shows that the installation of phosphate groups at the central aromatic unit of the tweezers did not generally improve binding of ammonium guests. Comparison of the binding properties of **4a** and **1**, **4c** and **2** as well as **4d** and **3** together with MD simulations of the conformational space of **4c** and **2** suggest that these central phosphate groups substantially reduce the stability of the active tweezer conformation and result in more open conformations in which the hosts cannot interact with guest efficiently in most cases. Nevertheless, in the case of host **4d**, the central phosphate groups appear to strongly contribute to the binding of natural polyamines in particular, resulting in nanomolar affinity (422 nM for spermidine and 6.76 nM for spermine) in aqueous solution. Together, these results grant an understanding of the impact of substituents on the binding properties depending on their location in the framework of this class of molecular tweezers. This combination of insights will guide efforts towards further generations of optimized flexible molecular tweezers. The short and highly modular synthetic route will enable the facile construction of future customized tweezer hosts.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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