A Modular Phosphorylated Glycoluril-Derived Molecular Tweezer for Potent Binding of Aliphatic Diamines

Michael Heilmann[a] and Konrad Tiefenbacher*[a,b]

Abstract: A molecular tweezer based on a glycoluril-derived framework bearing four phosphate groups was synthesized and shown to be capable of binding organic amines in aqueous solution. We report the K_a values for 30 complexes of this molecular tweezer and amine guests, determined via ^{1}H NMR titrations. Both the hydrophobic cavity and the phosphate groups contribute to the binding. Bulkier molecules and molecules bearing negatively charged groups like carboxylates in amino acids bind less tightly due to a steric clash and coulombic repulsion. The narrow cavity and the strong ionic interactions of the phosphate groups with ammonium guests favor binding of aliphatic diamines. These binding properties clearly distinguish this system from structurally related molecular clips and tweezers.

Selectively binding biologically relevant molecules is a topic that has grown in attention over the past decades.[1] Over that same time period, our understanding of molecular recognition in complex systems, both biological and supramolecular ones has grown substantially.[2] Several examples of supramolecular hosts capable of binding drugs or druglike molecules[3] and protein surfaces[4] have been developed. Hosts can form strong interactions with several different classes of guest molecules both in organic solvents[5] and water,[6] demonstrating the vast potential of tailor-made artificial structures. One class of host molecules are molecular clips/tweezers, which feature two aromatic panels held in place via a rigid linkage unit.[7] A common feature in many water-soluble molecular clips and tweezers is the concept of attaching polar groups like phosphates, sulfonates, carboxylates etc. to facilitate solubilization of the hydrophobic core structure.[8] One intensively investigated example was developed by Klärner, Schrader and co-workers: molecular tweezer **1** (Figure 1) that comprises an electron rich hydrophobic cavity with two phosphate moieties attached to the framework. They were able to show that **1** is an excellent binder of lysine not only in solution, but also on the surface of particular proteins by which it can act as an inhibitor for protein-protein interactions.[4b, 7a, 8c, 9] Besides this tweezer and its close relatives,[10] a variety of different frameworks for molecular tweezers and clips have been investigated, for instance by the groups of Zimmerman,[11] Nolte[6a] and Isaacs.[8a, 8b, 12] In particular, Isaacs and co-workers investigated a class of acyclic congeners of cucurbit[*n*]urils (CB[*n*]) that share in common that they incorporate varying numbers of glycoluril units. By changing this number, they have developed tweezers that accommodate large aromatic dyes,[8b, 13] but also ones that mimic CB[*6*], like **2** (Figure 1) with a smaller cavity that mainly accommodates aliphatic amines.[8a] However, binding of an organic ammonium ion inside **2** is mostly dependent on cation-dipole interactions with the glycoluril carbonyl moieties; therefore the observed binding constants were lower than in systems like **1** that bind guests through strong ionic interactions.

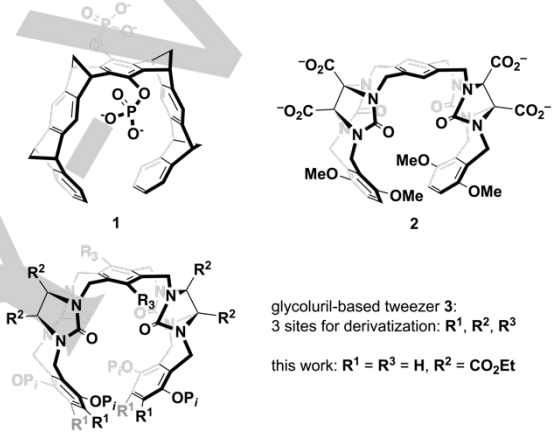

Figure 1. Chemical structures of the similarly sized phosphate-bearing tweezer **1**, glycoluril-derived tweezer **2** and the general framework for phosphorylated glycoluril-derived tweezer **3**.[8a, 8c] OP_i: OPO_3H_2.

Due to our interest in the application of supramolecular containers for catalysis,[14] we became interested in tweezers **1** and **2** since we envisioned that long term they may offer the potential to bind and derivatize lysine residues on protein surfaces selectively. For instance, in a hypothetical lysine-binding system a suitably placed base and electrophile on the tweezer backbone would facilitate derivatization of the primary amine. We chose tweezer **2** as starting point, as the presence of four hydroxyl groups should enable a more facile attachment of groups than the less functionalized tweezer **1** while retaining a similarly sized cavity. As a first step towards our long-term goal, we decided to investigate the attachment of phosphate groups (inspired by tweezer **1**) onto tweezer **2**, as they should increase affinity to amine guests, and report our results in this communication.

Initially, we devised our synthetic route towards the final product analogously to the synthesis of **2**, followed by deprotection and phosphorylation.[8a] However, after a considerable amount of experimentation, we were not able to achieve demethylation of the tetraethyl ester of **2** and under most reaction conditions observed either no reaction or decomposition

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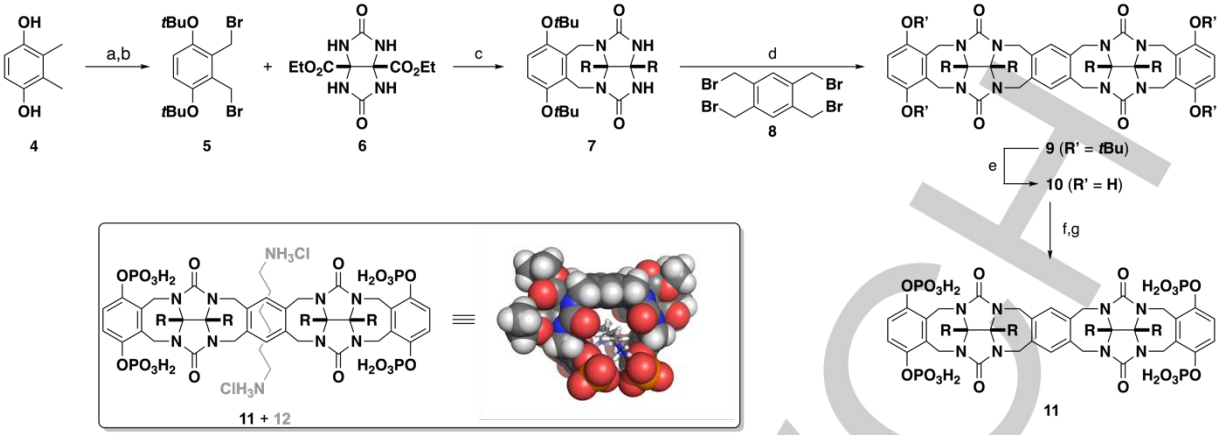


Scheme 1. Synthesis of the tetraphosphate Tweezer **11**. **R** = **CO_2Et**. a) 20% $Mg(ClO_4)_2$, Boc_2O, 40 °C, 16 h, 70%. b) AIBN, NBS, CCl_4, 95 °C, 16 h, 93%. c) **5**, **6**, KO*t*Bu, DMSO, rt, 3 h, 48%. d) KO*t*Bu, **7**, 29%. e) TfOH, HFIP, rt, 48 h, 94%. f) diethyl phosphite, CCl_4, NEt_3, MeCN, rt, 16 h, 61%. g) TMSBr, MeCN, rt, 16 h then H_2O, *quant.*. The structure of **11** binding hexanediamine dihydrochloride (**12)** has been optimized based on the PM6 semi-empirical method. Boc: *tert*-butyloxycarbonyl, AIBN: azobis-*iso*-butyronitrile, NBS: *N*-bromosuccinimide, HFIP: hexafluoro-*iso*-propanol, TMS: trimethylsilyl.

instead of the desired transformation. Since the originally devised route *via* **2** was not feasible, we started by exploring alternative protecting groups. After the investigation of several acyl, phosphoryl and silyl groups, we only identified *tert*-butyl protection as well-suited for our purpose. Therefore, we started our synthesis from 2,3-dimethylhydroquinone (**4**, Scheme 1) and were able to obtain dibenzyl bromide **5** in two steps.[15] Subsequent alkylation of glycoluril **6** with one equivalent of **5**[8a] provided **7**, two equivalents of which were linked via alkylation with tetrabromodurene (**8**) to give the *tert*-butyl protected framework of the tweezer **9**. It is noteworthy that, contrary to the reported synthesis of its methyl analogon, only one diastereomer was obtained under optimized conditions. In the C-shaped diastereomer **9** formed, all R-groups are positioned on the same side of the molecule (see Supporting Information for details).[8a] After several attempts to remove the *tert*-butyl groups under Lewis- or Brønsted-acidic conditions,[15-16] including triflic acid in trifluoroethanol,[17] only led to decomposition of **9** without productive formation of **10**, we found that using triflic acid in hexafluoro-*iso*-propanol (HFIP) gave rise to **10** in a yield of 94%. With **10** in hand we were able to obtain the desired tetraphosphate tweezer **11** in a sequence of Atherton-Todd phosphorylation and deprotection of the formed tetrakis(diethyl)phosphate. When we first subjected **11** to the aliphatic monoamine guest decylammonium tetrafluoroborate (**13**, Table 1), we found that it is a comparably moderate binder (K_a = 353 M^{-1}, 70 mM phosphate buffer in D_2O, pD = 7.2) for monoamines, but we also realized that it is binding aliphatic diamines very strongly. **11** neatly accommodates hexane-1,6-diammonium guest **12** inside its cavity (K_a = 1.33•10^5 M^{-1}, 70 mM phosphate buffer in D_2O, pD = 7.2, Table 1). Additionally, isothermal titration calorimetry of **11** and **12** indicates a strongly enthalpy-driven binding (see Supporting Information). Figure 2 displays the ^{1}H NMR spectra of **11**, **12** and their equimolar mixture, indicating strong perturbations of chemical shifts in both host and guest.

After having obtained these initial results, we were looking into the binding properties of **11** in more details. Since structurally comparable systems have been reported to dimerize in solution, we first determined that **11** only undergoes weak dimerization (K_s = 5.07 M^{-1}), which is in good agreement especially with other glycoluril-based tweezers.[6b, 7a, 8b] Subsequently, the guest scope of **11** was explored. Table 1 summarizes the binding constants K_a determined for the interaction of **11** with 29 different guests in phosphate buffered D_2O (pD = 7.2). Values were determined *via* ^{1}H NMR experiments using non-linear regression[18] and span over a wide range (10^0 M^{-1} – 10^7 M^{-1}). Given the structure of **11**,

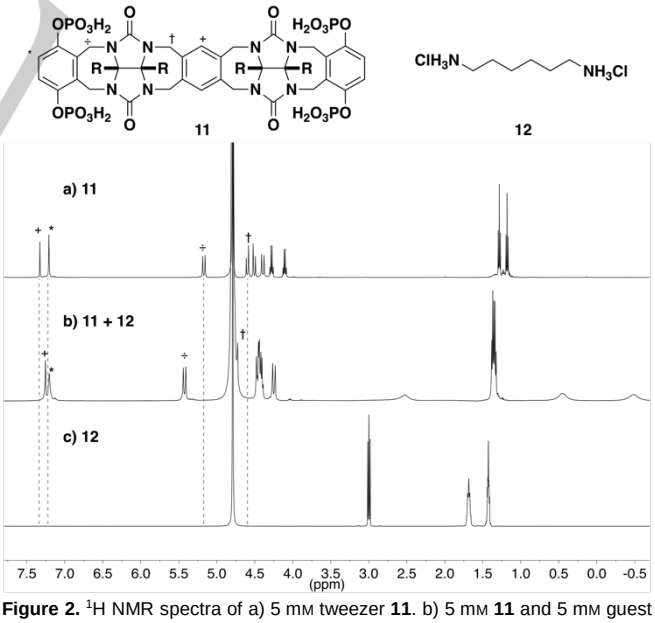


Figure 2. ^{1}H NMR spectra of a) 5 mM tweezer **11**. b) 5 mM **11** and 5 mM guest hexanediammonium chloride (**12**). c) 5 mM **12**. All spectra were recorded in 70 mM phosphate buffer (pD = 7.2).

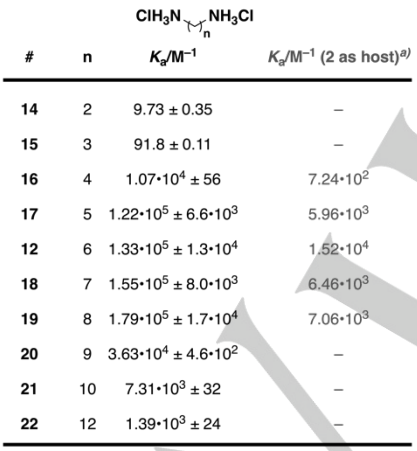
we expected it to bind aliphatic diamines strongly dependent on the length of their methylene linkers. In fact, while di- and trimethylene linked diamine species **14** and **15** were only very

weakly interacting with **11**, we observed longer-chained species up to dodecanediammonium chloride (**22**) to bind tightly to **11**, with a maximum of K_a = 1.79•10^5 M^{-1} in C_8-diammonium chloride **19**. This length of a methylene linker for the best-binding guest is notably higher than in several different related systems like cucurbiturils and other glycoluril-derived tweezers,[8a, 8b, 19] unless these systems were modified in a similar way of attaching additional charged groups at their periphery.[20] Furthermore, the affinity of **11** to these aliphatic diamines is considerably higher than of **2**,[8a] all of which results indicate that the phosphate groups of **11** are involved in binding the ammonium moieties of guests, possibly in addition to the glycoluril moieties.

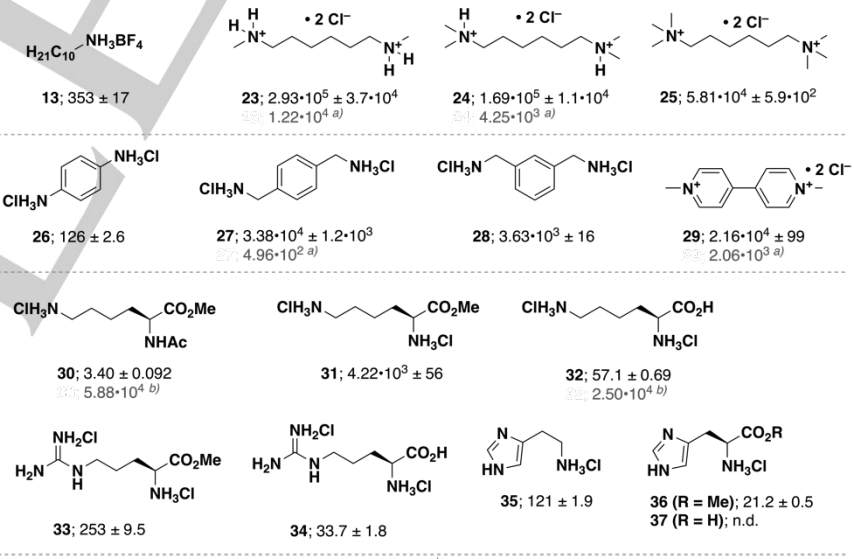
In order to gain more insight into the binding mode, we also investigated the binding properties of **11** with di-, tetra- and hexamethylated hexanediamine species **23**, **24** and **25**. Since the binding constants of **12**@**11** (K_a = 1.33•10^5 M^{-1}), **23**@**11** (2.93•10^5 M^{-1}), **24**@**11** (1.69•10^5 M^{-1}) and **25**@**11** (5.81•10^4 M^{-1}) are all within a margin of a factor of 5, a dominating contribution of hydrogen bonding with the glycoluril moieties in **11** can be excluded. This is also supported by the binding of both **23** and **24** being reported to be weaker to the strictly hydrogen-bonding tweezer **2** than to **11**, and no binding constant being reported for **25**@**2**. The binding appears dominated by ionic interactions with the phosphate groups. The lower binding of **25** compared to the other guests might be attributed to increased steric bulk of the several groups have reported molecular tweezers that are strongly binding aromatic guests, mostly rationalized by π-π interactions,[6b, 8b] we were interested to study the binding behaviour of **11** with several aromatic guests. It came to no surprise that the short phenylene linked guest **26** was binding to **11** rather weakly (K_a = 126 M^{-1}), whereas both *p*-xylylene linked **27** (3.38•10^4 M^{-1}) and *m*-xylylene linked **28** (3.63•10^3 M^{-1}) were interacting with **11** more strongly. However, it is noteworthy that the binding constants of **27**@**11** and **28**@**11** vary by an order of magnitude, although the calculated N–N distances (7.01 Å and 6.88 Å, respectively) only differ marginally. The difference in binding may be attributed to **27** fitting more neatly into the cavity of **11**, whereas **28** already induces increased steric strain. Considering that aromatic guests are viable guests and the binding may be attributed to ion-pairing, the high binding constant of **11** and methyl viologen dichloride (**29**) (K_a = 2.16•10^4 M^{-1}) and the fact that **11** is binding those small aromatic guests more tightly than **2** was consistent with our expectations.

We were then looking into the possibility of using **11** to bind derivatives of basic amino acids. For derivatives of both lysine and arginine, it is obvious that the methyl esters are binding more tightly to **11** than the free carboxylic acids (K_a = 4.22•10^3 M^{-1} and 57.1 M^{-1} for lysines **31** and **32**, respectively; and K_a = 253 M^{-1} and 33.7 M^{-1} for arginines **33** and **34**, respectively). This strongly reduced affinity of the free carboxylic acids most likely stems from repulsion of the deprotonated carboxylate of the guest (at pD = 7.2) and the phosphates of **11**. We were surprised to observe a binding constant for arginine methyl ester (**33**) lower by an order of magnitude than for lysine methyl ester (**31**); this quaternary ammonium salt that may render proper placement in the cavity less favorable (see Supporting Information). Since

Table 1. Binding constants K_a of **11** and the corresponding guests (M^{-1}), determined via ^{1}H NMR titration. Titrations performed at 5 mM **11** or 100 µM **11**. n.d.: K_a could not be determined by means of NMR titration. *a)* Values for **2** as host.[8a] *b)* Values for **1** as host.[21] *c)* titration performed at 10 µM **11**. *d)* determined *via* competitive displacement titration at 100 µM **11** and 10 mM **29** as a competitor.



#	n	K_a/M^{-1}	K_a/M^{-1} (2 as host)[a]
14	2	9.73 ± 0.35	–
15	3	91.8 ± 0.11	–
16	4	1.07•10^4 ± 56	7.24•10^2
17	5	1.22•10^5 ± 6.6•10^3	5.96•10^3
12	6	1.33•10^5 ± 1.3•10^4	1.52•10^4
18	7	1.55•10^5 ± 8.0•10^3	6.46•10^3
19	8	1.79•10^5 ± 1.7•10^4	7.06•10^3
20	9	3.63•10^4 ± 4.6•10^2	–
21	10	7.31•10^3 ± 32	–
22	12	1.39•10^3 ± 24	–



13; 353 ± 17

23; 2.93•10^5 ± 3.7•10^4 (2a: 1.22•10^4 a)

24; 1.69•10^5 ± 1.1•10^4 (2a: 4.25•10^3 a)

25; 5.81•10^4 ± 5.9•10^2

26; 126 ± 2.6

27; 3.38•10^4 ± 1.2•10^3 (2a: 4.96•10^2 a)

28; 3.63•10^3 ± 16

29; 2.16•10^4 ± 99 (2a: 2.06•10^3 a)

30; 3.40 ± 0.092 (2a: 5.88•10^4 b)

31; 4.22•10^3 ± 56

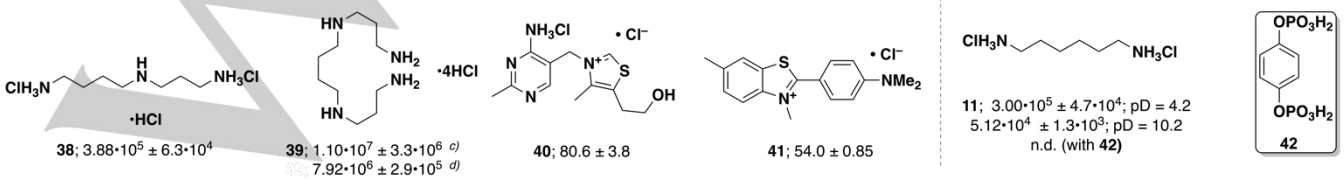
32; 57.1 ± 0.69 (2a: 2.50•10^4 b)

33; 253 ± 9.5

34; 33.7 ± 1.8

35; 121 ± 1.9

36 (R = Me); 21.2 ± 0.5
37 (R = H); n.d.



38; 3.88•10^5 ± 6.3•10^4

39; 1.10•10^7 ± 3.3•10^6 c) (2a: 7.92•10^6 ± 2.9•10^5 d)

40; 80.6 ± 3.8

41; 54.0 ± 0.85

11; 3.00•10^5 ± 4.7•10^4; pD = 4.2
5.12•10^4 ± 1.3•10^3; pD = 10.2
n.d. (with **42**)

42

reduced binding efficacy may be explained the increased steric bulk of the guanidinium moiety of **33** compared to the smaller ammonium residue of **31**. When *N*-acetylated lysine methyl ester (**30**) was titrated with **11**, we observed very weak binding (K_a = 3.40 M^{-1}). This demonstrates the strong influence of charge, but also sterics of the guest on the interactions between host and guest, since the more accessible cavity of tweezer **1** has been reported to allow for strong interaction with both **30** and **32** (see Supporting Information). The low binding constant of weakly basic histamine hydrochloride (**35**) and **11** (K_a = 121 M^{-1}) and histidine methyl ester (**36**) and **11** (K_a = 21.2 M^{-1}) can be rationalized with the imidazole moiety predominantly not being protonated in the phosphate buffer we used, rendering the material a monoammonium species, which have already been shown to bind to **11** only moderately. We did not observe binding of histidine (**37)**. Taking into account that additional steric bulk of a carboxylate impairs the binding of lysine derivative **31** compared to its parent diamine **17** and that histamine is a weak binder, these results are in agreement with our expectations. We finally looked into the possibility to employ **11** to bind biologically relevant molecules. We therefore investigated the binding of both spermidine (**38**) and spermine (**39**) and found both guests to bind very tightly to the host system (for spermidine: $K_a = 3.88 \cdot 10^5$ M^{-1}; for spermine: $K_a = 1.10 \cdot 10^7$ M^{-1} determined *via* direct titration and $K_a = 7.92 \cdot 10^6$ M^{-1} determined *via* competitive displacement titration).[22] Similarly, the weak binding of thiamine (**40**) and thioflavin T (**41**) to **11** (K_a = 80.6 M^{-1} and 54.0 M^{-1}, respectively) met our expectations and may be attributed to the inability of the host-guest complex to form π-π interactions as well as the considerable steric hindrance of the guest molecules. Both, a more open access to the cavity and the feasibility of π-π interactions have repeatedly been reported to be key in high interactions of such more complex aromatic guests with other molecular tweezers.[8b, 23] Finally, we determined that **11** is also capable of binding **12** at different pD values with only minor changes in affinity ($K_a = 3.00\ 10^5$ M^{-1} at pD = 4.2 and $K_a = 5.12 \cdot 10^4$ M^{-1} at pD = 10.2), leaving host-guest interaction roughly unchanged as long as the charge of host and guest do not change dramatically. We also attempted to obtain a binding constant of **12** with phenylenediphosphoric acid (**42**), but were not able to observe any perturbation in chemical shifts over a large span of concentration. This result suggests that there is no interaction between **12** and **42** in buffered aqueous solution and therefore provides strong evidence for the observed interactions of **11** with the investigated guests to be driven by the existence of a cavity in **11** to accommodate a guest molecule.

In summary, we have developed a modular and derivatizable synthesis of the glycoluril-derived molecular tweezer **11** bearing four phosphate groups, which is capable of strongly binding aliphatic diamines in aqueous solution. The binding properties of **11** are unique compared to the phosphorylated tweezer **1** and the glycoluril-derived **2** in that it is a particularly strong binder of a large span of aliphatic and other sterically undemanding diamines and derivatives thereof. Binding is determined by two main factors: (1) The strong ionic interactions of protonated ammonium species with the deprotonated phosphate groups of the tweezer lead to a stronger enthalpy-driven binding than in **2**, in which hydrogen-bonding dictates the binding properties. (2) Due to the phosphate residues being located at the tips of the tweezer, the entry into the cavity of **11** is narrow compared to **1** and does not offer enough space for sterically more demanding guests. Our results suggest that **11** represents a system that is complementary to previously reported molecular tweezers. The highly modular synthesis of **11** allows for fast and easy derivatization, a process that is currently ongoing in our lab.

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Keywords: host-guest complexes • amines • molecular recognition • molecular tweezers • supramolecular chemistry

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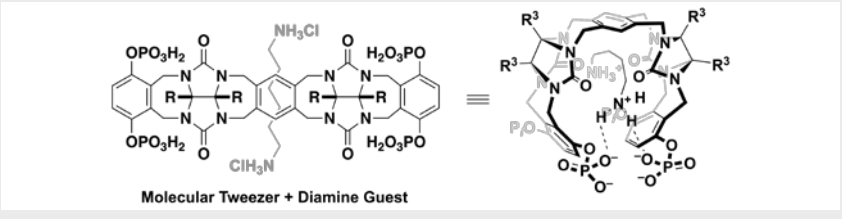

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We report the synthesis of a tetraphosphorylated glycoluril-derived molecular tweezer and its binding constants with several organic amine guests. It binds aliphatic diamines very tightly due to strong ionic interactions and its hydrophobic cavity.