

Cite this: *Chem. Sci.*, 2025, 16, 1288

All publication charges for this article have been paid for by the Royal Society of Chemistry

Received 21st October 2024

Accepted 6th December 2024

DOI: 10.1039/d4sc07150f

rsc.li/chemical-science

Reversing selectivity of bambusuril macrocycles toward inorganic anions by installing spacious substituents on their portals†

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Two chiral bambusurils, which are diastereomers to each other, show remarkable differences in their binding affinity and selectivity toward inorganic anions as determined by isothermal titration calorimetry. These differences are explained by quantum-chemical calculations.

Introduction

Artificial molecules with the ability to act as receptors of inorganic anions are investigated in many fields, including anion sensing,^{1,2} organocatalysis,^{3,4} anion remediation in industrial wastewater,^{5–7} and others. Supramolecular chemists dealing with the host–guest chemistry of inorganic anions are intrigued by the design of anion receptors with optimal binding affinity and selectivity toward the selected analyte.^{3,7–9} This design is particularly challenging in the case of receptors for inorganic anions featuring relatively small differences in their sizes. Among supramolecular host molecules, macrocycles are especially appreciated for their ability to discriminate particular anions because they are more rigid compared to their acyclic analogues.¹⁰ Macrocycles are valued for their high affinity, which is achieved thanks to multiple binding motifs. Moreover, the selectivity of macrocycles toward a particular anion can be tuned by the number of their repeating units, as can be illustrated on calix[*n*]pyrroles or hemicucurbit[*n*]urils. While calix[4]pyrroles show 100 times higher selectivity for fluoride compared to other halides and phosphates, calix[6]pyrroles preferentially bind iodide.¹¹ Similarly, hemicucurbit[6]urils prefer to bind chloride over iodide, while hemicucurbit[8]urils form the most stable complexes with PF₆[−] and SbF₆[−].¹²

Bambus[*n*]urils are anion receptors consisting of *n* glycoluril units connected by *n* methylene bridges.^{13,14} Until now, only four (*n* = 4) and six (*n* = 6) membered bambusuril homologues

have been reported.^{15,16} As the four-membered bambusurils do not bind to any anion, the six-membered homologues form stable complexes with many inorganic anions. The typical complex in the solution consists of a single anion included within the center of the macrocycle cavity. It has been reported that the binding affinity of bambus[6]urils (BU[6]s) to anions can be modulated by installing different substituents on the nitrogen atoms of the BU[6] portals.^{17,18} However, independently of their substituents, BU[6]s have shown essentially the same selectivity for all inorganic anions ranging from small F[−] to large ClO₄[−] in the same solvent. This is mainly due to the flexibility of the BU[6] macrocyclic framework and its ability to adapt to the size of these anions. Thus, the selectivity of BU[6] is mostly dictated by anion solvation and correlates with the charge density of anions.^{13,19} Previously, we prepared enantiomerically pure bambusurils **BU1** and **BU2** (Fig. 1) and demonstrated their enantioselectivity in binding different chiral carboxylates.²⁰ Here, we demonstrate that diastereomers **BU1** and **BU2** bind the same inorganic anions with very different affinities and selectivities. To the best of our knowledge, this is the first example of any receptor of inorganic anions, in which the type and position of substituents attached to the receptor binding site impact the receptor selectivity. The binding differences were rationalized with the help of theoretical calculations.

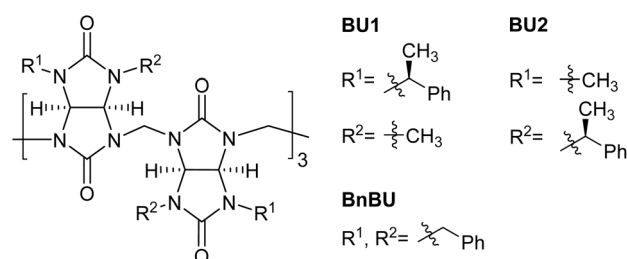


Fig. 1 Structures of bambusuril macrocycles investigated in this work.

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† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d4sc07150f>

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Results and discussion

The binding of **BU1** and **BU2** with a series of inorganic anions was investigated by isothermal titration calorimetry in chloroform. For all titrations, the experimental data fit well with the 1 : 1 binding model, which is consistent with the 1 : 1 host–guest stoichiometry previously described for the number of bambusuril–anion complexes. The reported association constants are apparent, as we did not consider the competition of TBA cations and bambusuril macrocycles for anions.

When **BU1** and **BU2** were compared, striking differences in their binding affinities of more than two orders of magnitude were observed (Table 1). For example, the binding selectivity of **BU2** over **BU1** for F^- , Cl^- , Br^- , and I^- is 592, 1273, 891, and 750. The highest selectivity of **BU2** over **BU1** of 5822 was observed for NO_3^- . We also compared the binding characteristics of **BU1** and **BU2** to those of the previously studied **BnBU** (Table 1 and Fig. 2).¹⁹ This macrocycle bears 12 benzyl substituents on its portals. We compared the binding affinities of all three bambusurils according to the volume of bound anions. The stability of complexes with **BU1** and **BU2** increases from F^- to I^- , while it decreases for anions with large diameters, such as ClO_4^- , ReO_4^- , and PF_6^- . In contrast, the binding affinity of **BnBU** increases from F^- up to ClO_4^- and decreases for the larger anions. This clearly illustrates that the cavity of **BnBU** is more flexible and can even adapt to larger ClO_4^- , while the **BU1** and **BU2** cavities are best suited for smaller I^- and bind ClO_4^- less efficiently.

The absolute values of association constants of **BnBU** complexes are comparable with those of **BU2** but significantly higher than those of **BU1** (Table 1). Further comparison of **BnBU** with **BU2** revealed that all halides are bound by **BU2** more strongly than by **BnBU**, but the reverse is observed for ClO_4^- and large anions. This clearly shows the preferential binding of **BU2** to smaller spherical anions.

Another crucial difference between the investigated bambusurils is in their selectivity. Specifically, **BnBU** preferentially binds ClO_4^- over Cl^- with selectivity exceeding 1600. In contrast, the selectivity for ClO_4^- over Cl^- in the cases of **BU1**

and **BU2** is reduced to 206 and 4. Moreover, **BnBU** and **BU1** bind ReO_4^- more than 8 and 50 times more strongly than Cl^- , while **BU2** preferentially binds Cl^- over ReO_4^- with a selectivity of 14. The reverse selectivity among the investigated bambusurils was observed also for the NO_3^-/Br^- anion pair (Table 1). Inspired by the reviewers' comments, we decided to test if the reverse selectivity will be recorded in a solvent different from chloroform. We determined the association constants for the complexes of **BU1** and TBA salts of Br^- ($2.6 \times 10^7 M^{-1}$) and NO_3^- ($2.9 \times 10^6 M^{-1}$) in acetonitrile (Table S3†). The results showed that **BU1** preferentially binds Br^- over NO_3^- in both chloroform and acetonitrile with selectivities of 22 and 9, while **BnBU** preferentially binds NO_3^- over Br^- with a selectivity of 3 in chloroform.

These results clearly show that replacing benzyl with (*S*)-1-phenylethyl substituents in the bambusuril structure leads to changes in binding affinity and, consequently, to reverse selectivity toward anions. This contrasts with previous studies,^{17,21} in which the selectivity toward anions remained the same for differently substituted bambusurils.

We also decided to test whether the observed differences in anion binding could be rationalized by the ability of bambusuril to act as a heterotopic ion-pair receptor interacting simultaneously with the anion and cation of the salt. We conducted an ITC titration of **BU1** with tetraethylammonium (TEA) chloride in chloroform. We determined that K_a for the **BU1**· Cl^- complex is about 4.6 times lower when TEA instead of TBA salt was used. This reflects the higher affinity of TEA towards Cl^- compared to TBA and supports that the bambusuril does not act as a heterotopic ion-pair receptor.

We also performed 1H NMR titration of the **BU2** solution using TBACl in chloroform (Fig. S24†). The results showed that TBA proton signals experience an upfield chemical shift in the presence of **BU2**. This is explained by shielding TBA^+ from the chloride anion encapsulated inside the macrocycle, excluding the possibility of **BU2** cooperatively interacting with the cation. The binding of chloride within the **BU2** cavity is slow on the NMR time scale, in contrast to TBA^+ , which exhibits fast exchange upon interaction with the macrocycle. This indicates that the TBACl ion pair dissociates upon interacting with the macrocycle. The DOSY spectra of an equimolar solution of TBACl and **BU2** in chloroform showed that the diffusion coefficient for TBA^+ is higher compared to that of the macrocycle but lower than that of TBA^+ measured in the absence of the macrocycle (Fig. S25 and S26†). This indicates that TBA^+ and the **BU2**· Cl^- complex interact to some extent. We performed dilution experiments of an equimolar mixture of **BU2** and TBACl in chloroform to determine the ion-pair association constant (K_{ip}) between the **BU2**· Cl^- complex and TBA^+ to be $1.2 \times 10^5 M^{-1}$ (Fig. S27†). Although we do not know the value of K_{ip} of TBACl in chloroform, we expect it to be about the same order of magnitude as the published K_{ip} value of $9 \times 10^9 M^{-1}$ for TEACl in the same solvent.²² The found K_{ip} value of $1.2 \times 10^5 M^{-1}$ for the **BU2**· Cl^- complex and TBA^+ is several orders of magnitude lower than that for TEACl, showing that the macrocycle dramatically weakened the ion pairing. Moreover, the ITC experiments performed in acetonitrile and chloroform showed similar

Table 1 Apparent association constants (K_a , M^{-1}) of BUs with various anions in $CHCl_3$ at 298.15 K determined by ITC

| Anions | BU1 | BU2 | BnBU ^a |
|--------------|-------------------|----------------------|--------------------------|
| $CH_3CO_2^-$ | 1.9×10^4 | 2.0×10^6 | 5.6×10^5 |
| $MeSO_3^-$ | 8.2×10^4 | 9.4×10^5 | 7.3×10^5 |
| NO_3^- | 5.1×10^5 | 3.0×10^9 | 1.8×10^9 |
| ReO_4^- | 1.7×10^7 | 3.0×10^7 | 1.1×10^8 |
| ClO_4^- | 6.8×10^7 | 1.7×10^9 | 2.1×10^{10} |
| PF_6^- | 1.2×10^7 | 4.1×10^8 | 8.7×10^8 |
| F^- | 1.3×10^5 | 7.7×10^7 | 1.9×10^6 |
| Cl^- | 3.3×10^5 | 4.2×10^8 | 1.3×10^7 |
| Br^- | 1.1×10^7 | 9.8×10^9 | 6.7×10^8 |
| I^- | 1.6×10^8 | 1.2×10^{11} | 1.6×10^{10} |

^a Published previously, see ref. 19. Standard deviations were calculated from two or three independent experiments and are lower than 17%, except for the **BU2**· ReO_4^- complex, for which it is 30%.



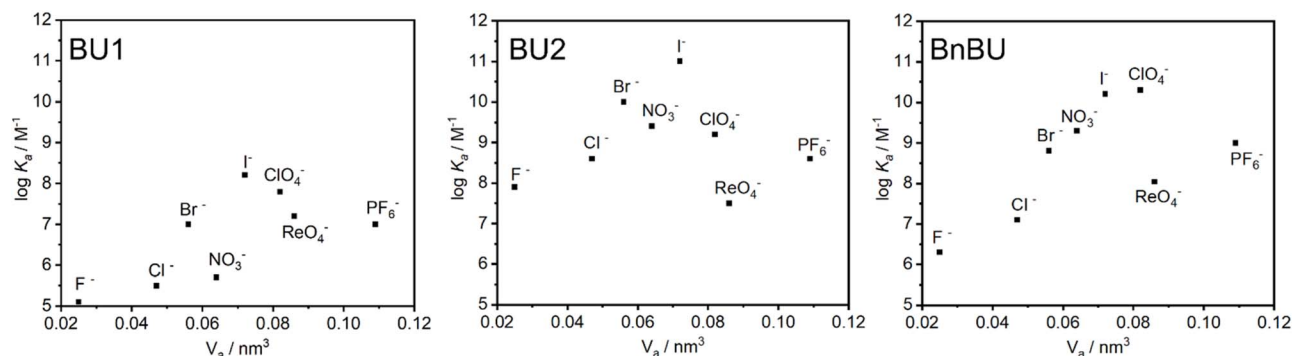


Fig. 2 Dependence of apparent association constants on anions size.

preferences of **BU1** for Br^- over NO_3^- . As ion-pairing in acetonitrile is weak (K_{ip} for TBACl is 5 M^{-1}),²² we believe that this experiment further supports the insignificance of cations in the observed preferential anion binding.

The observed differences in binding affinities between **BnBU**, **BU1**, and **BU2** are reflected in their enthalpy–entropy compensation (Fig. 3). All complexes are strongly exothermically favorable, but most of them are counterbalanced by an entropic cost. The enthalpy–entropy compensation is most significant for **BU2** halide complexes and the least for the complexes of **BU1**.

We performed a computational study to better understand bambusuril binding affinity and selectivity differences. Quantum-chemical calculations were performed for the macrocycles with empty cavities and their complexes with halides, all in implicit chloroform solvent. Bambusurils **BU1** and **BU2** are diastereomers to each other. They differ only in the placement of the 1-phenylethyl and methyl groups, which are attached to the nitrogen atoms of the glycoluril building blocks in opposite positions (see R^1 and R^2 in Fig. 1). In addition to these distinct arrangements, the 1-phenylethyl groups can rotate along the N–C bond, resulting in different conformers of the macrocycles. Here, we tested two boundary conformations

(Fig. 4), which differ by the orientation of the methyl and phenyl groups of the 1-phenylethyl substituent relative to glycoluril five-membered rings.

In the closed form, the aromatic parts of 1-phenylethyl groups are aligned with the axial axis of the macrocycle, seemingly “closing” the cavity.

However, a short MD simulation reveals that the cavity is not effectively sealed but rather extended due to the tumbling of the phenyl groups. The cavity is closed upon halide binding. In contrast, the aromatic parts head out of the cavity in the open form, exposing the cavity to the solvent, similar to non-derivatized bambusurils.²³ In all cases, the calculations showed that **BU1** prefers the closed form, while **BU2** prefers the open form (Fig. 4). The magnitude of the preference is comparable to the binding affinities, and it does not depend on the type of anion occupying their cavity (Table S5†). The calculation agrees with the previously determined crystal structure of the **BU2** complex with Br^- ,²⁰ in which the macrocycle adapts the open-form conformation. The stable conformations, the closed for **BU1** and the open for **BU2**, while completely different, share the same structural feature. In both cases, the methine group of

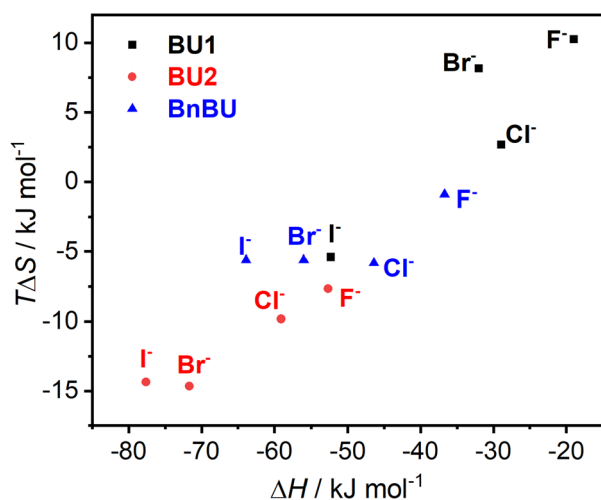


Fig. 3 Enthalpy–entropy compensation plot of the BU complexes with halides.

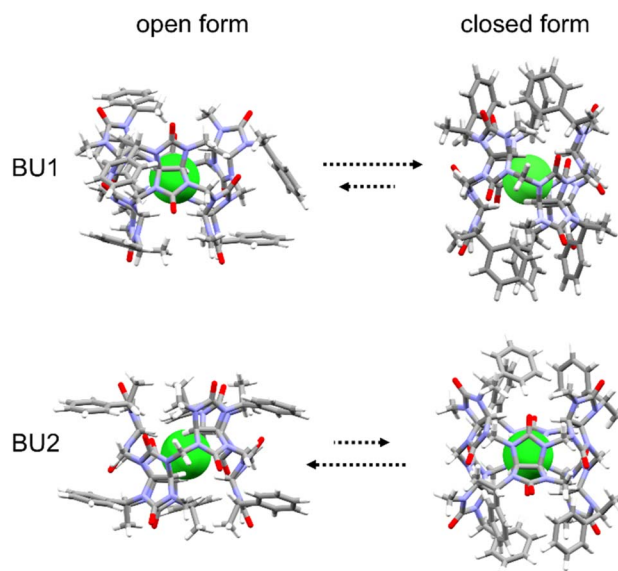


Fig. 4 Two boundary conformations for **BU1** and **BU2**.



Table 2 Experimental (ΔG_b) and calculated (ΔE_b) binding affinities for **BU1** and **BU2** halide complexes. The binding affinity difference ($\Delta\Delta G_b$ and $\Delta\Delta E_b$) between **BU2** and **BU1** and its decomposition into the interaction $\Delta\Delta E_i$, the deformation $\Delta\Delta E_d$, and the solvation $\Delta\Delta E_s$ contributions. All energies are in kJ mol⁻¹

| | BU1 | BU2 | BU2–BU1 | BU1 | BU2 | BU2–BU1 | | | |
|-----------------|--------------|--------------|--------------------|--------------|--------------|--------------------|--------------------|--------------------|--------------------|
| Anion | ΔG_b | ΔG_b | $\Delta\Delta G_b$ | ΔE_b | ΔE_b | $\Delta\Delta E_b$ | $\Delta\Delta E_i$ | $\Delta\Delta E_d$ | $\Delta\Delta E_s$ |
| F [−] | −29.7 | −43.5 | −13.7 | −45.4 | −65.1 | −19.7 | −31.4 | 8.0 | 3.7 |
| Cl [−] | −31.6 | −48.5 | −16.8 | −85.1 | −103.9 | −18.8 | −26.3 | 1.6 | 5.9 |
| Br [−] | −41.4 | −51.6 | −10.2 | −95.7 | −106.3 | −10.5 | −19.6 | 3.0 | 6.0 |
| I [−] | −47.5 | −61.9 | −14.4 | −108.0 | −113.7 | −5.7 | −10.5 | 3.4 | 1.5 |

the 1-phenylethyl group interacts with carbonyl oxygens at the central belt through hydrogen bonding. This seems to be a key interaction already observed in the other BU derivatives, such as **BnBU** or dodecamethylbambusuril.^{19,20}

Next, we calculated the binding affinities of halide anions to **BU1** and **BU2**. In agreement with the experiment, we found that **BU2** exhibits binding affinities higher than **BU1** (Table 2). Compared to experimental values, the calculated values are overestimated because they do not include entropy contributions. Better agreement can be observed for the calculated ($\Delta\Delta E_b$) and experimental ($\Delta\Delta G_b$) binding energy differences between **BU2** and **BU1**. In addition, to trace the origin of this preference, we decomposed the obtained binding affinities ($\Delta\Delta E_b$) into (i) the interaction of the halide anion with the bambusuril cavity ($\Delta\Delta E_i$), (ii) macrocycle deformation upon halide anion binding ($\Delta\Delta E_d$), and (iii) solvation contributions ($\Delta\Delta E_s$). Their values clearly reveal that the binding difference between **BU1** and **BU2** is predominantly the result of interaction factors, as solvation and deformation effects do not substantially differ for different anions.

We tried to decipher possible structural factors responsible for the observed binding preference. The differences in the preferred conformation of **BU1** and **BU2** are clearly caused by the placement of 1-phenylethyl groups and impact the cavity arrangement. The **BU1** cavity is screw-like distorted, as indicated by the different lengths of C–H \cdots X[−], by about 0.3 Å for each of the two methine protons of glycoluril units (Table S6†). In contrast, all C–H \cdots X[−] hydrogen bonds in **BU2** are equalized with the same length. A similar situation can be found in the experimental structure of **BnBU** complexed with chloride,¹⁹ where all C–H \cdots Cl[−] hydrogen bonds are equalized with the same length of 3.13 Å. This result confirms that the structure of the **BU2** macrocycle is more flexible compared to **BU1** and better adapts to the anion size. On the other hand, the spacious arrangement of the 1-phenylethyl units in **BU1** induces distortion of the macrocycle cavity, resulting in a significantly lower anion binding affinity of this macrocycle compared to **BU2** and **BnBU**.^{22–31}

Conclusions

In conclusion, we showed that the position of methyl and 1-phenylethyl groups on the glycoluril building blocks of diastereomeric bambusurils **BU1** and **BU2** induces significant differences in their anion binding. **BU1** binds inorganic anions up to

three orders of magnitude less strongly than **BU2**. Quantum-chemical calculations revealed that these binding differences are due to distortion of the **BU1** cavity, which is forced by the arrangement of spacious 1-phenylethyl groups, while the more relaxed arrangement is proposed for the **BU2** diastereomer. Moreover, both **BU1** and **BU2** and previously reported **BnBU** feature very different or even reverse selectivity for pairs of inorganic anions. This is a unique achievement in bambusuril chemistry, as, until today, different substituents on the macrocycle portals resulted only in the modulation of their binding affinity, while selectivity among anions remained similar. Thus, our results show that bulky substituents could be used to rigidify the structures of any flexible macrocyclic receptor to tune its binding selectivity toward guest molecules.

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

V. S. conceived the project. J. S. synthesized the macrocyclic compounds. C. R. and S. G. conducted ITC measurements. S. G. performed NMR experiments. P.K. carried out the computational studies. V. S. and P. K. drafted the manuscript. All authors contributed to the writing of the ESI† and to the revision of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the Czech Science Foundation (23-05271S). We thank the RECETOX Research Infrastructure (LM2023069) financed by the Ministry of Education, Youth and Sports. This project was supported by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 857560 (CETOCOEN Excellence). This publication reflects only the author's view and the European Commission is not responsible for any use that may be made of the information it contains. Computational resources were



provided by the e-INFRA CZ project (ID: 90254), supported by the Ministry of Education, Youth and Sports of the Czech Republic.

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