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Bis-urea anion receptors: influence of receptor structure and anion nature on binding affinity and stability†

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Complexation behavior of bis-urea receptors bearing nitro substituent at different proximities from urea binding site was investigated using isothermal titration calorimetry (ITC) and ^{1}H NMR (Nuclear Magnetic Resonance) titration experiments. Their binding interactions with fluoride (F^-), acetate (OAc^-), and dihydrogen phosphate ($H_2PO_4^-$) anions were examined to evaluate affinities, stoichiometries, and thermodynamic parameters. The nature of the anion and the position of the nitro substituent significantly influenced receptor binding ability. Receptors with *ortho*-nitro groups underwent decomposition upon interaction with F^-, forming 2-benzimidazolinone cyclic urea as evidence from relatively large positive enthalpy ($\Delta H°$ = 13.78 kJ mol^{-1}) and entropy ($\Delta TS°$ = 30.90 kJ mol^{-1}). Furthermore, X-ray diffraction analysis revealed that the cyclic urea engages in complexation with the fluoride anion. This degradation was suppressed in *meta*-substituted analog. Notably, *meta*-nitro receptor exhibited high binding affinity toward acetate ($\Delta H°$ = −27.73 kJ mol^{-1}), while $H_2PO_4^-$ binding for all receptors showed large entropic contributions, due to the geometry and size of the anion. These results offer insights into designing selective and stable anion receptors.

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Introduction

In recent years, significant attention has been given to the development of simple receptors capable of selectively recognizing biologically important anions such as fluoride, chloride, phosphate, and carboxylate.[1] These anion receptors can be either neutral or positively charged, with their interactions typically involving hydrogen bonding and/or electrostatic attraction. In some instances, deprotonation of the acidic hydrogen bond donor may also occur.[2] While neutral receptors exhibit lower intrinsic electrostatic affinity for guest anions compared to their cationic counterparts, they do not inherently require a counter-anion, potentially enhancing their selectivity.[3] However, it is important to recognize that neutral receptors may also bind the counter-cation of the anion, which can influence the receptor's overall selectivity.[4]

Among neutral anion receptors, those incorporating amide, (thio)urea, and pyrrole functional groups have demonstrated effectiveness due to their ability to form strong hydrogen bonds with anions.[5] Amides and ureas, due to their relatively straightforward synthesis, are among the most widely used hydrogen bond donors in anion-binding receptors.[6] However, the presence of a C=O hydrogen bond acceptor in these binding sites may sometimes lead to unwanted aggregation effects.[7] This limitation is mitigated when anion-binding sites feature pyrrole or imidazole groups, which explains their increasing use in recent years.[8] Typically, charge-neutral anion receptors incorporate multiple strong hydrogen bond donor groups, forming an effective anion-binding motif due to the converging NH binding sites.[9] Furthermore, additional functional groups such as amides and indole moieties, both excellent hydrogen bond donors, enhance receptor efficacy.[10]

The concept of preorganization has played a crucial role in the advancement of 'second-generation' anion receptors, making highly efficient anion-chelating agents.[11] For instance, certain anion hosts utilize a rigid cholesterol framework that maintains the receptor in a preorganized conformation optimized for anion binding.[12] Moreover, the introduction of electron-withdrawing substituents such as trifluoromethyl ($-CF_3$) and nitro ($-NO_2$) has been shown to increase the hydrogen bond acidity of urea and sulfonamide groups, further strengthening their ability to bind anions effectively.[13]

Bis-urea-based receptors have garnered significant attention in supramolecular chemistry due to their ability to form strong hydrogen bonds with various anions, leading to high binding affinities and selectivity.[14–18] Recent studies have demonstrated that incorporating flexible linkers, such as 1,2-phenoxyethane and 1,2-ethoxyethane moieties, into bis-urea derivatives enhances their solubility in common organic solvents, thereby facilitating effective anion recognition under more

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concentrated conditions.[19] Additionally, the design of tritopic bis-urea receptors, which integrate polyether bridges and chromogenic units, has been shown to improve the recognition of oxyanions and enable ion-pair recognition through cooperative mechanisms.[20,21] These advancements highlight the versatility and efficacy of bis-urea receptors in the selective binding of anionic species, making them valuable tools for applications in sensing, environmental monitoring, and catalysis.[22]

Anion recognition by synthetic receptors is accompanied by several difficulties because of the peculiar characteristic of anions.[23,24] The relatively large size of anions compares to cations require a much larger receptor. For example, the size of the smallest ionic radius of anions, fluoride ion (1.33 Å) is comparable to the radius of the potassium cation (1.38 Å). Chloride ion (1.81 Å) is larger than the cesium cation (1.70 Å). Another aspect of the design of anionic receptors is the different geometry of anions ranging from simple spherical, linear, trigonal plannar, tetrahedral to octahedral and often show multiple coordination geometries.

Recently, we reported the synthesis and binding properties of urea-functionalized receptors derived from constitutional isomers of tetra-bromo-functionalized pillar[5]arenes toward halide anions.[25] Building upon this, the present work describes the synthesis of bis-urea functionalized receptors incorporating nitro substituents at varying proximities to the urea group. The effect of receptor structure on selectivity and binding affinity toward fluoride, acetate, and dihydrogen phosphate is investigated using ^{1}H NMR titration, isothermal titration calorimetry (ITC), and X-ray diffraction analysis.

Results and discussion

Synthesis

A urea functional group, widely utilized in the synthesis of anion receptors, was employed to investigate the effect of proximal electron-withdrawing groups on binding affinity towards fluoride, acetate, and dihydrogen phosphate. Accordingly, *o*-phenylenediamine was reacted with nitrophenyl isocyanate derivatives, yielding three bis-urea anion receptors, as shown in Fig. 1.

The synthesized compounds were fully characterized by NMR and HRMS (see Experimental section). Additionally,

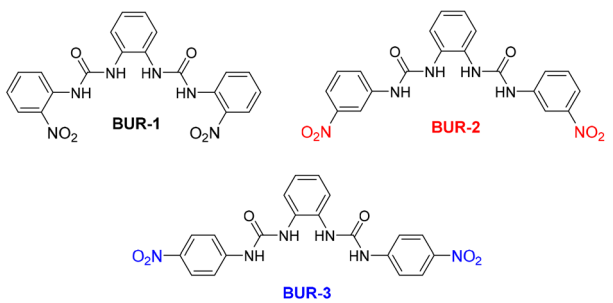

Fig. 1 Chemical structures of the synthesized bis-urea anion receptors bearing nitro substituents.

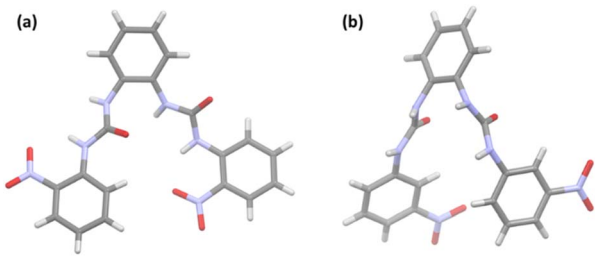

Fig. 2 X-Ray single-crystal structure of the synthesized receptors **BUR-1** (a), and **BUR-2** (b). Gray, C; red, O; light blue, N; hydrogen, white.

suitable single crystals for X-ray single crystal diffraction analysis for receptors **BUR-1** and **BUR-2** were grown by slow evaporation from a solution of THF (Fig. 2). Unfortunately, attempts to grow suitable single crystals for **BUR-3** were unsuccessful.

Binding studies

Isothermal titration calorimetry (ITC) has been effectively employed to study various reversible, noncovalent supramolecular interactions in solutions. This technique provides quantitative insights into the binding affinity and thermodynamic parameters governing the interactions between bis-urea receptors (**BUR-(1–3)**) and tetrabutylammonium anions, including tetrabutylammonium fluoride (**TBA-F**), tetrabutylammonium acetate (**TBA-OAc**), and tetrabutylammonium dihydrogen phosphate (**TBA-H_2PO_4**). A summary of the ITC measurements is presented in Table 1. In addition, the complexation behavior between the bis-urea functionalized receptors and tetrabutylammonium anion salts was examined using ^{1}H NMR titration experiments. This technique is highly effective for probing binding interactions at the molecular level, as it monitors the changes in proton chemical shifts that occur upon complex formation. Consequently, ^{1}H NMR titration provided a clear and detailed picture of the interactions between the bis-urea receptors and the tetrabutylammonium anion salts.

Complexation with tetrabutylammonium fluoride (TBAF)

ITC experiments were performed to investigate the interactions between the synthesized anion receptors and tetrabutylammonium fluoride (**TBAF**) in chloroform at 25 °C. All experimentally obtained binding molar ratios (n) were close to two, indicating a 1 : 2 stoichiometric ratio of complexation, with association constant (K) values listed in Table 1. The measured rate constant (k) for the *o*-nitro substituent receptor **BUR-1** (K_1 = 1.35 ± 0.05 × 10^4 M^{-1}) is primarily related to the deportation/decomposition rate, as evidence from thermodynamic data determined from ITC experiment (Table 1). The association constant K value of the *o*-nitro substituent receptor **BUR-2** (K_1 = 4.28 ± 0.03 × 10^4 M^{-1}) and **BUR-3** (K_1 = 3.90 ± 0.04 × 10^4 M^{-1}) are comparable. On the other hand, the ITC thermograms exhibited distinct variations in the interactions between the receptors and fluoride anions (F^-) (Fig. 3 and 4). Notably, the

Table 1 ITC thermodynamic parameters associated with the complexation between bis-urea anion receptors (BUR1–3) and various TBA-ions[a]

Receptor	Ion	$\Delta H^{\circ b}$ (kJ mol^{-1})	$-T\Delta S^{\circ b}$ (kJ mol^{-1})	K^b (M^{-1})	$n^{b,d}$	K^g (M^{-1})
BUR-1	F^-	13.78	−30.90	$1.35 \pm 0.05 \times 10^{4c}$	2.15	N.D.[h]
				$1.40 \pm 0.05 \times 10^{3c}$		N.D.[h]
	OAc^-	−9.83	−15.17	$1.10 \pm 0.02 \times 10^{4d}$	2.07	$1.42 \pm 0.15 \times 10^4$
				$2.59 \pm 0.02 \times 10^{3e}$		$1.11 \pm 0.09 \times 10^3$
	$H_2PO_4^-$	−3.75	−19.32	$3.83 \pm 0.04 \times 10^{4d}$	2.32	$1.92 \pm 0.15 \times 10^3$
				$1.10 \pm 0.05 \times 10^{3e}$		$7.48 \pm 0.10 \times 10^2$
BUR-2	F^-	−16.70	−0.43	$4.28 \pm 0.03 \times 10^{4d}$	2.06	$3.65 \pm 0.17 \times 10^4$
				$1.23 \pm 0.05 \times 10^{3\ e}$		$7.78 \pm 0.22 \times 10^2$
	OAc^-	−27.73	7.31	$3.78 \pm 0.05 \times 10^{4f}$	1.32	$4.66 \pm 0.45 \times 10^4$
	$H_2PO_4^-$	−7.18	−12.72	$3.07 \pm 0.02 \times 10^{3d}$	1.95	$1.60 \pm 0.11 \times 10^3$
				$1.82 \pm 0.05 \times 10^{2\ e}$		$6.63 \pm 0.15 \times 10^2$
BUR-3	F^-	−6.70	−17.45	$3.90 \pm 0.04 \times 10^{4d}$	2.10	N.D.[h]
				$3.22 \pm 0.05 \times 10^{3e}$		N.D.[h]
	OAc^-	−18.95	−3.27	$1.41 \pm 0.03 \times 10^{3f}$	0.95	$2.50 \pm 0.07 \times 10^3$
	$H_2PO_4^-$	−6.03	−15.16	$5.16 \pm 0.05 \times 10^{2f}$	1.07	$3.25 \pm 0.56 \times 10^3$

[a] Fixed concentration of ion-receptor (2 mM) and varying concentration tetra-*n*-butylammonium (TBA) ions (50 mM) in DMF at 25 °C. [b] Calculated from ITC measurement. [c] deportation/decomposition rate constant (k_1) and (k_2). [d] Experimental binding molar ratio. 1 : 2 binding constant (K_1). [e] and (K_2). [f] 1 : 1 binding constant (K_a). [g] Calculated from ^{1}H NMR titrations. [h] Not determined.

thermodynamic parameters obtained from these measurements were influenced by the structural differences of the receptors. Generally, the higher affinity toward F^- is evident from larger negative enthalpy change (ΔH°).

From Table 1, the **BUR-2** exhibits relatively large of negative enthalpy change ($\Delta H^\circ = -16.70$ kJ mol^{-1}) compared to **BUR-3** ($\Delta H^\circ = -6.70$ kJ mol^{-1}) indicates better binding toward fluorine anion. Whereas the positive enthalpy change (ΔH° = 13.78 kJ mol^{-1}) observed in the **BUR-1** complexation indicates that the system gains energy due to the abstraction of hydrogen atoms from the urea functional groups by fluoride ions (F^-). Furthermore, the large positive (+) entropy changes (ΔTS° = 30.90 kJ mol^{-1}) for the complexation of **BUR-1** with F^- suggests greater disorder within the system, which indicates receptor decomposition. This is clearly demonstrated by the overlaid thermograms obtained from ITC experiments involving the addition of fluoride to receptors **BUR-1** and **BUR-2**, as shown in Fig. 3. Receptor **BUR-3** initially binds to F^- but undergoes decomposition as the fluoride anion concentration increases, as illustrated in the overlaid thermograms in Fig. 4.

NMR studies

To gain a clearer understanding of the molecular–level interactions, the changes in proton chemical shifts upon complex formation were carefully monitored. To achieve this, ^{1}H NMR titration experiments were conducted between the tetrabutylammonium anions and the synthesized bis-urea receptors **BUR-(1–3)** in DMSO-d_6 at 298 K. Similar to the results obtained from the ITC measurement, ^{1}H NMR spectra obtained after the addition of the anions, is dependent on the nature of the anions and the structure of the receptors.

Fig. 5 and 6 shows the expanded ^{1}H NMR spectra of a mixture of **BUR-1** (2 mM) and various equivalents of the

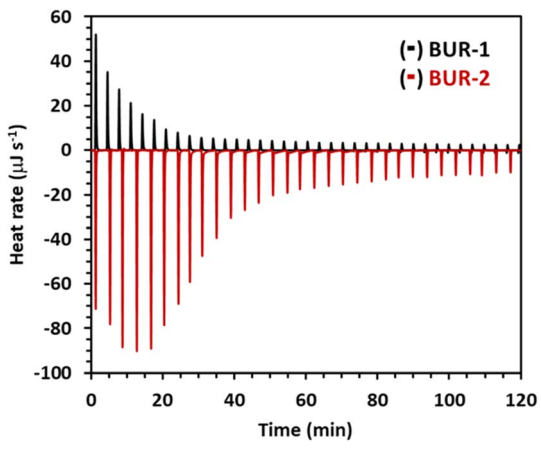

Fig. 3 Overlaid ITC raw heats for sequential injection of the fluorine anion (F^-) solution to 2.0 mM solutions of receptor **BUR-1** and **BUR-2** in DMF at 25 °C.

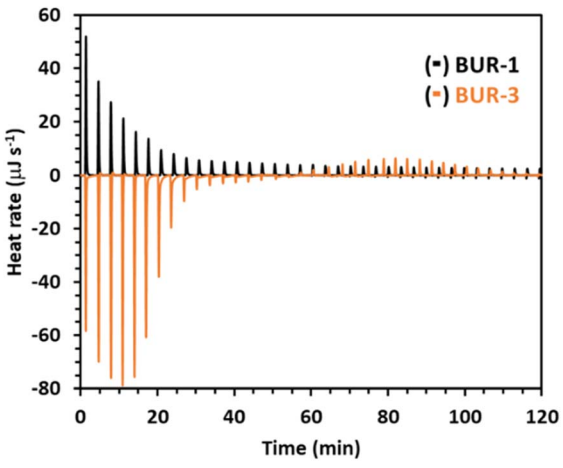

Fig. 4 Overlaid ITC raw heats for sequential injection of the fluorine anion (F^-) solution to 2.0 mM solutions of receptor **BUR-1** and **BUR-3** in DMF at 25 °C.

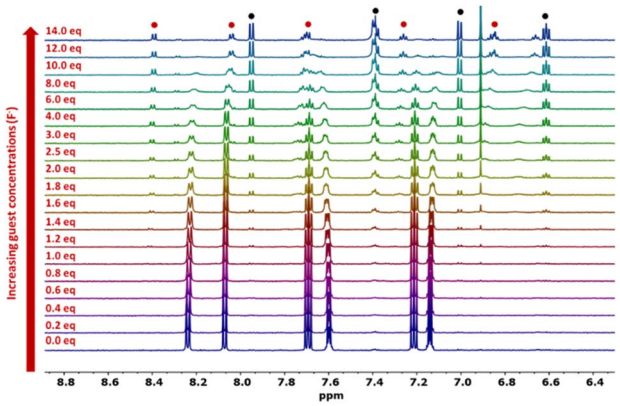

Fig. 5 Expanded ^{1}HNMR chemical shifts (600 MHz, DMSO-d_6) of aromatic protons (H's) measured upon incremental addition of the guest **TBAF** (0 → 14 eq) to a solution of **BUR-1** (2 mM) at 298 K. ^{1}H NMR Signals correspond to 1,3-dihydro-2*H*-benzimidazol-2-one (●) and to *o*-nitroaniline (●).

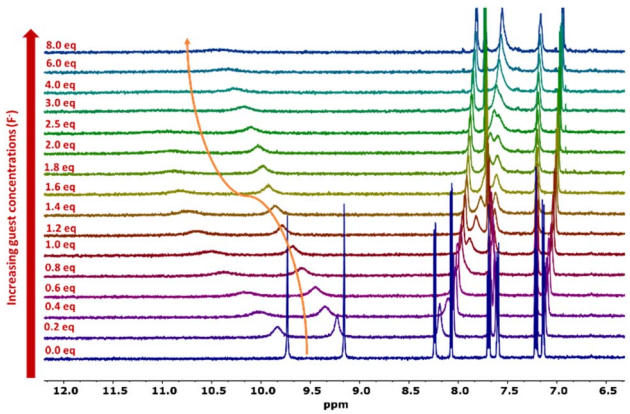

Fig. 6 ^{1}HNMR titration spectra (600 MHz, DMSO-d_6) upon incremental addition of the guest **TBAF** (0 → 14 eq) to a solution of **BUR-2** (2 mM) at 298 K.

tetrabutylammonium fluoride salt (**TBAF**). In the initial stage of the titration, no chemical shift was observed for the receptor proton signals. However, a new set of peaks began to emerge following the addition of one equivalent of the anion. Further Increase in concentration of F^- led to the appearance of nine additional peaks in the ^{1}H NMR spectrum. The number of these new signals exceeds those characteristics of the intact receptor, indicating that the receptor undergoes decomposition under these conditions.

Similar behavior was observed in the ^{1}H NMR titration experiment of **BUR-3** with fluorine anion, however the extent of the decomposition is significantly lower than **BUR-1** receptor (Fig. S5†). The overlaid ^{1}H NMR titration spectra show a gradual shift in the receptor proton signals, indicative of binding between the anion and the receptor. Concurrently, as the F^- concentration increases, a new set of peaks emerges, suggesting receptor decomposition. This behavioral pattern reflects the trends documented in the ITC experiment (Fig. 4). **BUR-2** exhibits only a gradual chemical shift of the receptor signals in

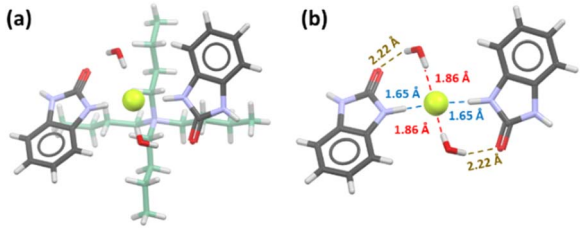

Fig. 7 Crystal structure of the complex formed between the 2-benzimidazolinone cyclic urea receptor and fluoride (F^-). (a) The structure shown in the presence of the tetrabutylammonium (TBA^+) counterion. (b) The structure shown with the tetrabutylammonium cation omitted for clarity.

their ^{1}H NMR titration spectra, indicating binding to the fluoride anion, as shown in Fig. 7. All ^{1}H NMR titration experiments show good consistency with the data acquired from isothermal titration calorimetry (ITC) measurements.

The stoichiometry of the complexation process was determined using the method of continuous variations, commonly known as Job's method. To investigate the interaction between the anionic receptor **BUR-2** and fluoride ions (F^-), a Job's plot was constructed by plotting the mole fraction of the receptor (χ) against the product of the observed chemical shift changes in the urea N–H protons at 9.16 ppm and the receptor mole fraction (χ). The resulting plot exhibited a maximum at a mole fraction of 0.33 (Fig. S6†). This stoichiometric inflection point in Job's plot is a characteristic of 1 : 2 host-to-guest stoichiometric ratio, indicating that each **BUR-2** receptor molecule binds to two fluoride anions in the complexation process. The data fitted well to a 1 : 2 binding isotherm, with the association constants determined to be $K_1 = (3.65 \pm 0.17) \times 10^4$ M^{-1} and $K_2 = 7.78 \pm 0.22 \times 10^2$ M^{-1}. These values are consistent with the association constants obtained from the ITC measurements (Table 1).

To investigate the receptors decomposition, three experiments were conducted in which each receptor was stirred with two equivalents of **TBAF** in DMF at room temperature. The receptor **BUR-2** was recovered intact following column separation, consistent with the stability observed in both ^{1}H NMR titration and ITC experiments. In contrast, the **BUR-3** receptor exhibited significant decomposition under the experimental conditions, while the **BUR-1** receptor completely disappeared from the final reaction mixture, indicating extensive degradation. The two major spots observed in the TLC analysis of the reaction mixture of **BUR-1** with **TBAF** were successfully isolated by column chromatography (Scheme 1). Analysis of their ^{1}H NMR, ^{13}C NMR and HRMS data identified the compounds as *o*-

Scheme 1 Reaction of bis-urea receptor **BUR-1** with tetra-*n*-butylammonium (**TBAF**).

nitroaniline and 2-benzimidazolidinone (Fig. S8 and S9†). This cyclic urea is formed from the reaction of **TBAF** with receptor **BUR-3** with a slower rate as clearly dementated by both ^{1}H NMR and ITC experiments. The data consistent with previously reported use of **TBAF** in the formation is cyclic urea.[26] The presence of strong electron-withdrawing groups at the *ortho* and *para* positions relative to the urea enhances the N–H acidity, which triggers receptor decomposition and leads to the formation of the cyclic urea compound. The closer proximity of the nitro group in the *ortho*-nitro derivative **BUR-1** significantly increases the urea N–H acidity through both inductive and resonance effects, compared to the *para*-nitro derivative **BUR-3**, where the nitro group is further away. Previously, the *para*-nitro derivative **BUR-3** was reported to undergo decomposition and the formation of 2-benzimidazolidinone when treated with1,8-diazabicycloundec-7-ene (DBU).[18] In contrast, receptor **BUR-2** (with nitro groups at the *meta* positions) does not show a comparable increase in N–H acidity, as the meta position limits the resonance contribution.

Further confirmation of the formation of the 2-benzimidazolidinone when suitable single crystals for X-ray diffraction analysis were successfully obtained from the reaction mixture of **BUR-1** with tetrabutylammonium fluoride (**TBAF**). In the resulting crystal structure in Fig. 7, the N–H protons from two adjacent cyclic urea molecules formed strong and directional hydrogen bonds (N–H···F, 1.646 Å) with the fluoride anion (F^-), effectively coordinating the anion within the cyclic-urea receptor. These primary urea–fluoride interactions were further stabilized by the incorporation of water molecules into the crystal lattice (O–H···F, 1.86 Å). The water molecules played a critical role in consolidating the binding environment by forming an extensive hydrogen bonding network, bridging the fluoride anion to nearby carbonyl (C=O···H, 2.22 Å) of urea receptors and further enhancing the overall stability of the complex ass shown in Fig. 7b. This cooperative binding, involving both the urea moieties and the lattice water molecules, highlights the importance of secondary interactions in strengthening anion recognition in the solid state. Efficient supramolecular interactions are present in the crystal network of BIN·**TBAF**. The N–H and the carbonyl moieties of urea functional group in 2-benzimidazolinone are engaged in supramolecular interactions with its neighboring counterparts by O–H···O=C (1.95 Å) (Fig. S10†). As a result, supramolecular networks were successfully assembled in the solid state, as illustrated in Fig. S11 and S12 in the ESI.†

Complexation with tetrabutylammonium acetate (TBAOAc)

Acetate, compared to fluoride, is a larger, more polarizable anion with lower charge density, which makes it a softer base and better suited for hydrogen bonding interactions with urea, while fluoride, being smaller and more highly charged, tends to form stronger and more localized hydrogen bonds. The interaction between urea and acetate primarily driven by hydrogen bonding. Urea and acetate interact provides insights into molecular recognition processes and the behavior of similar systems in both biological and synthetic environments.

ITC studies

Isothermal titration calorimetry (ITC) experiments were carried out to investigate the binding interactions between the three synthesized receptors and tetrabutylammonium acetate (**TBAOAc**) in dimethylformamide (DMF) at 298 K. The resulting thermodynamic data are summarized in Table 1. The complexation behaviors of **BUR-2** and **BUR-3** were found to follow a clear 1 : 1 stoichiometry, as indicated by the binding molar ratios (n), which were consistently close to unity. In contrast, **BUR-1** receptor shows 1 : 2 ratio of complexation as indicated by the binding molar ration (n) which is close to two. The association constant K_a value of the nitro-substituent receptor **BUR-1** ($K_1 = 1.10 \pm 0.05 \times 10^4$ M^{-1}) and **BUP-2** ($K_1 = 3.78 \pm 0.03 \times 10^4$ M^{-1}) are comparable, whereas the **BUR-3** ($K_a = 3.90 \pm 1.41 \times 10^3$ M^{-1}) is one order of magnitude lower. The **BUR-2** exhibits significantly large of negative enthalpy change ($\Delta H° = -27.73$ kJ mol^{-1}) which indicates excellent binding ability toward acetate anion followed by **BUR-3** ($\Delta H° = -18.95$ kJ mol^{-1}), and **BUR-1** ($\Delta H° = -9.83$ kJ mol^{-1}). The complexation of **BUR-1** with acetate anion (OAc^-) exhibit large positive (+) entropy changes ($\Delta TS° = 19.32$ kJ mol^{-1}) compared to the complexation with **BUR-2** ($\Delta TS° = -7.31$ kJ mol^{-1}) and **BUR-3** ($\Delta TS° = 3.27$ kJ mol^{-1}) receptors which indicates receptor decomposition.

NMR studies

^{1}H NMR titration experiments were carried out between tetrabutylammonium acetate (**TBAOAc**) and the synthesized bis-urea receptors **BUR-(1–3)** in DMSO-d_6 at 298 K. ^{1}H NMR spectra were recorded for a mixture of **BUR-1** (2 mM) and increasing equivalents of tetrabutylammonium acetate to monitor the interaction between the receptor and the acetate anions (Fig. 8). During the initial stages of the titration, gradual downfield and upfield shifts were observed for the proton signals of **BUR-1**, indicating the formation of a receptor-acetate complex as the acetate concentration increased. However, at higher acetate concentrations, a new set of peaks began to emerge, indicating the

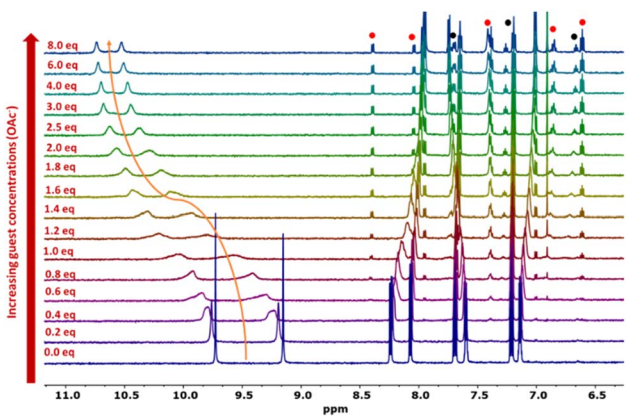

Fig. 8 ^{1}HNMR titration spectra (600 MHz, DMSO-d_6) measured upon incremental addition of the guest **TBAOAc** (0 → 8 eq) to a solution of **BUR-1** (2 mM) at 298 K. ^{1}H NMR Signals correspond to 1,3-dihydro-2*H*-benzimidazol-2-one (●) and to *o*-nitroaniline (●).

decomposition of receptor **BUR-1**. These newly emerging peaks were identical to those previously detected in titration experiments between **BUR-1** and fluoride anions, suggesting a similar decomposition pathway. The extent of receptor decomposition in the presence of acetate anions was found to be lower compared to fluoride, which can be attributed to the lower basicity of acetate relative to fluoride anions. The extent of decomposition observed during the ^{1}H NMR titration is significantly lower for **BUR-3** compared to **BUR-1.** This reduced decomposition is due to the positioning of the nitro groups at the *para* position relative to the urea functional groups in **BUR-3**. This behavior is consistent with the trends previously observed during the titration of fluoride anions, where **BUR-3** also exhibited greater structural stability compared to **BUR-1**. In contrast, **BUR-2** exhibited no detectable decomposition during the ^{1}H NMR titration experiments with tetrabutylammonium acetate (**TBA-OAc**), as shown in Fig. 9. As previously observed, enhanced stability is attributed to the positioning of the nitro groups at the *meta* positions relative to the urea functional groups. The absence of decomposition in **BUR-2** is consistent with their behavior in previous titrations with fluoride anions (Fig. 6).

The stoichiometry of the complexation is determined by using the method of continuous variations for **BUR-2**, and **BUR3** with acetate anions. The resulting plot exhibited a maximum at a mole fraction of 0.5 which is characteristic of a 1 : 1 host-to-guest stoichiometric ratio (Fig. S20–S22†). The binding data for the receptor fit well to a 1 : 1 binding isotherm, with the association constants determined to be $K_a = (4.66 \pm 0.45) \times 10^4$ M^{-1} (**BUR-2**), and $K_a = (2.50 \pm 0.07) \times 10^3$ M^{-1} (**BUR-3**) for the acetate binding event. For **BUR-1**, a Job's plot exhibited a maximum at a mole fraction of 0.33, which is characteristic of a 1 : 2 host-to-guest stoichiometric ratio. The association constants determined to be $K_1 = (1.42 \pm 0.15) \times 10^4$ M^{-1} for the first binding event, and $K_2 = (1.11 \pm 0.09) \times 10^3$ M^{-1} for the second acetate binding event, based on the chemical shift changes of the urea N–H protons (Fig. S18 and S19†). These values are in good agreement with the association constants obtained from isothermal titration calorimetry (ITC) measurements (Table 1). From the complexation experiments, the receptor **BUR-2** exhibits excellent binding affinity for acetate anion.

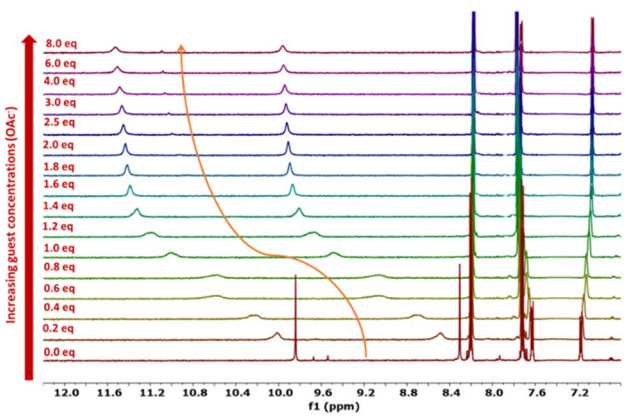

Fig. 9 ^{1}HNMR titration spectra (600 MHz, DMSO-d_6) measured upon incremental addition of the guest **TBAOAc** (0 → 8 eq) to a **BUR-2** (2 mM) at 298 K.

Complexation with tetrabutylammonium dihydrogen phosphate ($TBAH_2PO_4$)

ITC studies. The experimentally determined binding molar ratios ("*n*") for **BUR-1** and **BUR-2**, the binding molar ratios are close to two, indicating a 1 : 2 host-to-guest stoichiometry (Table 1) while **BUR-3**, the binding molar ratio is close to unity, consistent with a 1 : 1 host-to-guest complexation ratio. The thermodynamic parameters, including the binding constants (K), enthalpy changes (ΔH), and entropy contributions ($T\Delta S$), demonstrate a clear dependence on the structure of receptors (Fig. S24†). Fron ITC, the higher affinity toward $H_2PO_4^-$ was evident from larger negative enthalpy changes of **BUR-3** ($\Delta H° = -18.95$ kJ mol^{-1}) compared to **BUR-2** ($\Delta H° = -7.18$ kJ mol^{-1}) and **BUR-1** ($\Delta H° = -3.75$ kJ mol^{-1}). The larger dihydrogen phosphate anion ($H_2PO_4^-$), with its tetrahedral geometry, exhibits a strong dependence on the position of the substituents on the receptors. Due to its bulkier size and more complex spatial arrangement compared to smaller anions such as fluoride or acetate, effective binding of $H_2PO_4^-$ requires a well-defined binding site that can accommodate its tetrahedral geometry. Thus, all these for receptors indicate an increase in the positive entropy indicating high order in complexation due to the large size of hydrogen phosphate anion (Table 1).

NMR studies. Similarly, the noncovalent interactions of the obtained anionic receptors were investigated with tetrabutylammonium dihydrogen phosphate (**TBA·H2PO4**$^-$) using a ^{1}H NMR titration method. ^{1}H NMR spectra of mixtures of **BUR-(1–3)** (2 mM) and varying concentrations of **TBA·H2PO4**$^-$ in DMSO-d_6 at 25 °C confirmed host–guest complexation, as evidenced by an up-field shift of resonances for the urea protons and aromatic protons (H's) of the receptors (Fig. S25–S27†). At higher guest concentrations, the receptor aromatic proton signals reached a saturation point, where no further chemical shift changes ($\Delta\delta$) were observed, indicating complete complexation between the receptor and dihydrogen phosphate anion with no indication of receptor decomposition due to the weaker basicity of dihydrogen phosphate compared to acetate and fluorine anions. For receptors **BUR-1** and **BUR-2**, Job's plot between the mole fraction of the receptor (χ) and the observed chemical shift changes of aromatic protons on the receptors in ^{1}H NMR multiplied by the guest mole fraction (χ) show maxima at a mole fraction of 0.33, which indicate a 1 : 2 host-to-guest stoichiometric ratios of complexation. The data fitted to a 1 : 2 binding isotherm and the association constant K_1 were determined to be $1.92 \pm 0.15 \times 10^3$ M^{-1} ($K_2 = 7.48 \pm 0.10 \times 10^2$ M^{-1}) and $1.60 \pm 0.11 \times 10^3$ M^{-1} ($K_2 = 6.63 \pm 0.15 \times 10^2$ M^{-1}) for **BUR-1** and **BUR-2** respectively. In agreement with ITC measurements, stoichiometry of complexation of host–guest for **BUR-3** showed maxima at a mole fraction of 0.5, which favors the formation of a 1 : 1 host-to-guest stoichiometric ratio of complexation with calculated association constant K_a of 3.25 ±

0.56 $\times 10^3$ M^{-1}. The larger size of the dihydrogen phosphate anion, and weaker basicity decreased the binding affinity of the synthesized receptors compared to acetate anions.

Experimental

Materials and methods

NMR spectroscopy was conducted using a Bruker Avance II 600 MHz (Germany) spectrometer and using a Bruker DPX Avance 400 MHz (Germany) spectrometer. Single-crystal data analysis was carried out utilizing R-AXIS RAPID II (Rigaku, Japan) diffractometer. All ITC studies were performed using affinity ITC (TA Instruments, USA) and the data were analyzed using NanoAnalyze software (version 3.10.0). Electron impact ionization (EI) mass spectrometry was performed using Thermo Scientific DFS High Resolution GC/MS (Germany) mass spectrometer. Dimethylformamide (DMF), chloroform, and dichloromethane were distilled prior to use. All other reagents and solvents were of reagent grade and used without further purification.

^{1}H NMR titration

A 0.5 mL sample of **BUR-(1–3)** solution was prepared at a concentration of 2.0 mM in DMSO-d_6. A guest solution (2 mL) was prepared at a concentration of 0.1 M in DMSO-d_6. All titration experiments were conducted in NMR tubes at 298 K, and ^{1}H-NMR spectra were recorded upon the successive addition of aliquots of the stock solution of the appropriate guests *via* a microsyringe. The ^{1}H-NMR spectral changes were fitted to a 1 : 1 or 1 : 2 binding isotherm by nonlinear least-squares treatment using Microsoft Excel to determine the association constant, *K*.[27]

Preparation of single crystals for X-ray diffraction

Single crystals of the receptors reported in this study were grown using solvent evaporation method. Crystal data collection was performed using Rigaku Rapid II diffractometer with Cu-Kα radiation at room temperature. The data collected from the Rigaku system were analyzed using the "Crystalclear" software package. The structures were solved using direct techniques with the crystallographic software program "CrystalStructure," and refined using SHELXL-2019/2. The crystallographic data for the structures reported in this paper have been deposited at the Cambridge Crystallographic Data Centre as a supplementary publication (CCDC 2418794, 2418801 and 2418802).

ITC measurements

For the ITC host–guest complexation experiments, the receptor **BUR-(1–3)** dissolved in DMF (300 μL, 2 mM) was added to the reaction cell, while DMF was placed in the reference cell. The tetrabutylammonium anions were prepared at a concentration of 50 mM. The titration was carried out with 32 injections of 2 μL each, with a time interval of 240 s between injections. All titrations were performed at 298 K.

Synthesis of bis-urea-based receptor BUR-(1–3)

BUR-1: *o*-phenylenediamine (216 mg, 2 mmol) was dissolved in dichloromethane (CH_2Cl_2), followed by the addition of 2-nitrophenyl isocyanate (656 mg, 4 mmol). The reaction mixture was stirred at room temperature for 24 hours. Upon completion, a brownish-yellow precipitate formed, which was collected by filtration and washed several times with CH_2Cl_2 (80 mL). The crude product was dried under vacuum to yield **BUR-1** as a brownish-yellow solid; yield 869 mg (>99%). HRMS (EI): *m*/*z* calculated for $C_{20}H_{16}N_6O_6$ $[M]^+$: 436.1145, found: 436.1126. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.74 (s, 2H, 2NH), δ 9.16 (s, 2H, 2NH), δ 8.25–8.24 (d, 2H, Ar–H), δ 8.08–8.06 (d, 2H, Ar–H), δ 7.71–7.68 (t, 2H, Ar–H), δ 7.61–7.60 (dd, 2H, pheneylene-H), δ 7.23–7.20 (t, 2H, Ar–H), δ 7.16–7.14 (dd, 2H, pheneylene-H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 152.6, 138.9, 134.8, 134.6, 130.9, 125.3, 124.5, 122.9, 122.4.

BUR-2: *o*-phenylenediamine (216 mg, 2 mmol) was dissolved in dichloromethane (CH_2Cl_2), followed by the addition of 3-nitrophenyl isocyanate (656 mg, 4 mmol). The reaction mixture was stirred at room temperature for 24 hours. Upon completion, a brownish-yellow precipitate formed, which was collected by filtration and washed several times with CH_2Cl_2 (80 mL). The crude product was dried under vacuum to yield **BUR-2** as a brownish-yellow solid; yield 724 mg (83%). HRMS (EI): *m*/*z* calculated for $C_{20}H_{16}N_6O_6$ $[M]^+$: 436.1145, found: 436.1126. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.64 (s, 2H, 2NH), δ 8.57–8.56 (s, 2H, 2NH), δ 8.22 (s, 2H, Ar–H), δ 7.82–7.81 (d, 2H, Ar–H), δ 7.75–7.73 (d, 2H, Ar–H), δ 7.63–7.61 (dd, 2H, pheneylene-H), δ 7.57–7.55 (t, 2H, Ar–H), δ 7.17–7.15 (dd, 2H, pheneylene-H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 153.1, 148.1, 141.2, 131.2, 130.1, 124.6, 124.5, 124.2, 116.2, 112.1.

BUR-3: *o*-phenylenediamine[18] (216 mg, 2 mmol) was dissolved in dichloromethane (CH_2Cl_2), followed by the addition of 4-nitrophenyl isocyanate (656 mg, 4 mmol). The reaction mixture was stirred at room temperature for 24 hours. Upon completion, a brownish-yellow precipitate formed, which was collected by filtration and washed several times with CH_2Cl_2 (80 mL). The crude product was dried under vacuum to yield **BUR-3** as a brownish-yellow solid; yield 833 mg (96%). HRMS (EI): *m*/*z* calculated for $C_{20}H_{16}N_6O_6$ $[M]^+$: 436.1145, found: 436.1126. ^{1}H NMR (600 MHz, DMSO-d_6): δ 9.83 (s, 2H, 2NH), δ 8.30 (s, 2H, 2NH), δ 8.22–8.17 (dq, 4H, Ar–H), δ 7.73–7.67 (dq, 4H, Ar–H), δ 7.63–7.61 (dd, 2H, pheneylene H), δ 7.18–7.15 (dd, 2H, pheneylene-H).^{13}C NMR (150 MHz, DMSO-d_6): δ 152.6, 138.1, 134.8, 134.6, 130.9, 125.3, 124.6, 124.5, 122.9, 122.40.

Conclusion

In summary, the complexation behavior of the bis-urea receptors **BUR-(1–3)** was investigated using isothermal titration calorimetry (ITC) in combination with ^{1}H NMR titration experiments to explore their interactions with fluoride (F^-), acetate (OAc^-), and dihydrogen phosphate ($H_2PO_4^-$) anions. This combined approach provided a comprehensive understanding of the binding affinities, stoichiometries, and thermodynamic driving forces governing anion recognition in these systems.

The data obtained from the ITC measurements and ^{1}H NMR titration experiments are in excellent agreement. The complexation between the receptors and anions is dependent on the structure of the receptor and the nature of the anion. Extensive receptor decomposition was observed when bis-urea receptor with nitro groups *ortho* to the N–H was interacted with relatively high basic fluorine anion, F^-. The decomposition event was clearly revealed in both ITC and NMR experiments. The product of the decomposition was identified as a cyclic urea, namely 2-benzimidazolinone, which was fully characterized by spectroscopic techniques, and single-crystal X-ray analysis. The extent of decomposition reduces when the nitro group in the receptor is in the *para* position and eliminated when the nitro substituent is in the *meta* position. All the synthesized bis-urea receptors exhibit good binding with acetate anions, with excellent binding properties observed for the *meta*-nitro-based receptor **BUR-2**. The binding affinity for the dihydrogen phosphate anion is affected by the size and the geometry of the anion, as evidenced by the large entropy change observed for all receptors. The combination of ^{1}H NMR titration, ITC-derived thermodynamic profiles, and stoichiometry determination *via* Job's plots offers a comprehensive and quantitative understanding of how these receptors bind and discriminate between different anions. This study provides foundation for the design of future anion receptors with enhanced selectivity, stability, and tailored binding properties for applications in environmental sensing, biological recognition, and separation science.

Data availability

The data supporting this article have been included as part of the ESI,† and crystallographic data for [4 crystals] has been deposited at the [CCDC 2418794; 2418801 and 2418802] under and can be obtained from [URL of data record, **https://www.ccdc.cam.ac.uk/**].

Conflicts of interest

There are no conflicts to declare.

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