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Zwitterionic triple- and quadruple hydrogen-bonding motifs†

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Zwitterionic triple and quadruple hydrogen-bonding heterocyclic modules based on an easily accessible deazapterin scaffold are reported. The solid-state structure of 3H-bonding zwitterionic molecules revealed the formation of various hydrogen-bonded 1D chains composed of water, methanol, or a chloride anion, depending on the extent of protonation. The association of newly designed AAD and AADA (D = hydrogen-bond donor, A = hydrogen-bond acceptor) zwitterionic monomers was studied in solution to identify complementary partners and to evaluate binding strength. Both AAD and AADA motifs form 3H-bonded dimers after the addition of half an equivalent of acid, representing a negatively-charged analogue of the C–C$^+$ pair in naturally occurring DNA i-motifs. The protonation of the AADA motif with hydrochloric acid converts it into a non-H-bonding form where the urea moiety is complexed with the chloride anion. The exchange of chloride with non-nucleophilic anions unlocks the rotation of the urea group, resulting in the formation of the ADDA–DAAD heterocomplex with 2,7-diamido-1,8-naphthyridine.

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Introduction

Hydrogen-bonding (H-bonding) motifs serve as the cornerstone of supramolecular chemistry, enabling the formation of dynamic, complex and functional molecular systems through non-covalent interactions.[1] Since an individual H-bond is not sufficiently strong for precise positioning and holding molecular components in supramolecular assemblies, a collection of H-bond donors (D) and acceptors (A) forming a multipoint array is used instead.[2] Such arrays are typically represented by heterocyclic scaffolds featuring additional polarised N–H and/or O–H bonds. The five main natural nucleobases adenine, cytosine, guanine, thymine and uracil that mediate the self-assembly of DNA are perhaps the most iconic examples of H-bonding motifs.[3] To achieve the full complementarity between the motifs, the complete planarity of the array is often necessary. Planarisation through intramolecular H-bonding proved to be a very efficient way to pre-organise the array by constraining flexibility, which reduces the energy required to adopt the correct binding conformation. This is especially important for systems where part of the D and A sites is residing in peripheral substituents, such as urea or amide moieties.

Different rotamers represent different H-bonding arrays, each stabilised by internal H-bonding and arising from the rotation of these groups, with their proportion depending on the chemical environment or binding partner. Tautomerisation, where D and A sites are swapped by the relocation of the H-atom, is yet another process determining the identity of the H-bonding motif.[4] Such dynamic behaviour can be explored to design complex responsive supramolecular systems with interchangeable geometries.[5]

Among all H-bonding motifs, the arrays representing 3H-bonding are special, as they are easy to access synthetically, though they possess an odd number of H-bond donors and acceptors; the latter feature prevents their full self-complementarity, and only part of the A and D can engage in H-bonding, resulting in weaker assemblies. To cope with this limitation, we recently explored the tautomerisation phenomenon using the isocytosine heterocycle as a model. In so-called tautoleptic aggregation, the only possible 2H self-association is transformed into fully saturated 3H-bonding *via* tautomerization of one of the isocytosines binding partners (Fig. 1a).[4c] Since such dimers also create the complementary 2H-bonding interface

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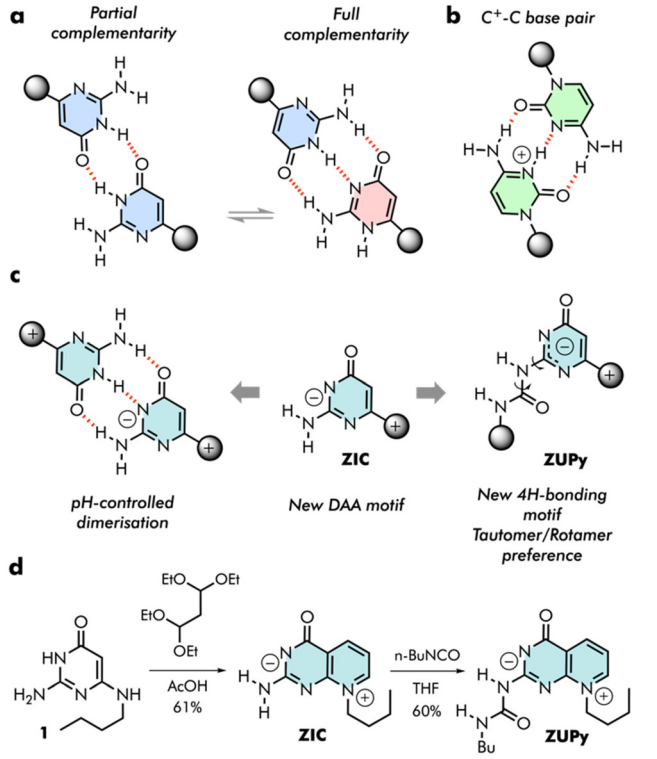


Fig. 1 Achieving full complementarity in 3H-bonding motifs through tautomeric equilibrium (a) or partial protonation (b); (c) possible design and applications of zwitterionic 3H- and 4H-bonding motifs; (d) synthesis of zwitterionic motifs.

perpendicular to 3H-bonding, it can be utilised in the assembly of supramolecular polymers with intriguing geometries.[4e] Another way to achieve maximum utility of all H-bonding sites involves ionisation of the 3H-bonding unit. Nature is taking advantage of this strategy in cytidine-rich i-motifs (intercalated-motif)[6] where hemi-protonation of cytidines at low pH leads to the formation of 3H-bonded C–C^+ dimers with stabilities (−169.7 kJ mol^{-1}) exceeding that of the canonical guanidine–cytidine pair (−96.6 kJ mol^{-1}).[7]

Herein, we describe our efforts in generalising the concept of ionised H-bonding motifs of multiple valencies by introducing zwitterionic analogues of isocytosine and of the most utilised 4H-bonding motif, Meijer's ureidopyrimidinone (Fig. 1c).[8] We reasoned that the isocytosine ring, possessing a negative charge delocalised over the lactam moiety (*i.e.*, the NH–CO fragment), would create a new DAA motif complementary to the protonated version of itself. This could open the possibilities for pH-responsive self-association or introducing a new module for DAA–AAD heterocomplexation.[9] The recent report on the incorporation of non-natural acidic nitro-substituted cytosine that forms a complementary pair under basic conditions into oligonucleotide structure further highlights the potential of such motifs.[10] Applying the same logic to the 4H-bonding unit would not only provide a new DDAA unit that is negatively charged and sensitive to pH, but it would also give us a model system to study the distribution of rotamers and tautomers and how that affects the self-assembly. The discovery of H-bonding arrays with high binding fidelity is essential in achieving the orthogonality required for the assembly of complex supramolecular networks.[5a,d,11,12] In this context, new charged motifs would open new perspectives to endow these systems with stimuli-responsive fidelity and consequently, dynamicity.

Results and discussion

To implement zwitterionic modules, we selected isocytosine derivatives fused with the pyridine ring. Deprotonation of the isocytosine would result in a negatively charged unit, whereas alkylation of the pyridine ring would introduce the positive charge. The synthesis of 3H-bonding zwitterionic isocytosine (**ZIC**) and the corresponding 4H-bonding ureidopyrimidinone (**ZUPy**) is outlined in Fig. 1d. The modified Bernetti synthesis[13] toward *N*5-deazapterins was developed based on 2-amino-6-(butylamino)pyrimidin-4-ol **1** and malonaldehyde bis(diethylacetal). A one-step condensation reaction in acetic acid afforded **ZIC** in good yield. Treatment of **ZIC** with butyl isocyanate in THF gave **ZUPy** in 60% yield. The *n*-butyl chain was sufficient to provide adequate solubility of the target compounds in a range of solvents, including chloroform.

To confirm the molecular structure of **ZIC**, the crystals were grown by slow evaporation of a methanol solution of **ZIC**. The inspection of the X-ray structure, however, revealed peculiar structural features (Fig. 2). Unexpectedly, the **ZIC** was found to co-crystallise as methanol solvates of both the neutral hydrate (**ZIC**·H_2O) and the HCl salt (**ZIC**·HCl) in a 1 : 1 ratio. The compounds formed a symmetric 3H-bonded dimer composed of zwitterionic **ZIC** and its protonated counterpart.

The crystal is nevertheless centrosymmetric, indicated by the averaged structure of the dimer with the central proton equally distributed on both **ZIC** molecules (Fig. 2a). The dimer observed represents the negative analogue of the C–C^+ pair found in i-motifs.

The H-bonded dimers form stacks with 3.2 Å distance between the aromatic rings, indicating π–π interactions. The outer hydrogen atom of the amino group not involved in the dimer serves as a donor for H-bonding with chloride anions and water molecules. Finally, the methanol molecule bridges the gap between the chloride and water with two additional H-bonds into a 1D $(\cdots CH_3OH\cdots H_2O\cdots CH_3OH\cdots Cl\cdots)_n$ chain. The non-polar part of the chain (CH_3 groups of CH_3OH) is further accommodated in a hydrophobic channel formed by the butyl substituents from two adjacent H-bonded stacks (Fig. 2b–d).

The presence of a protonated **ZIC** form in the solid state was surprising since no hydrochloric acid was used in the synthesis. A control experiment indicated that the most likely source of chloride was tap water used for workup and extraction. The high affinity of **ZIC** for chloride was demonstrated by quantitative analysis of the tap water treated sample, which showed 1 wt% of chloride (5.2 wt% corresponds to a pure

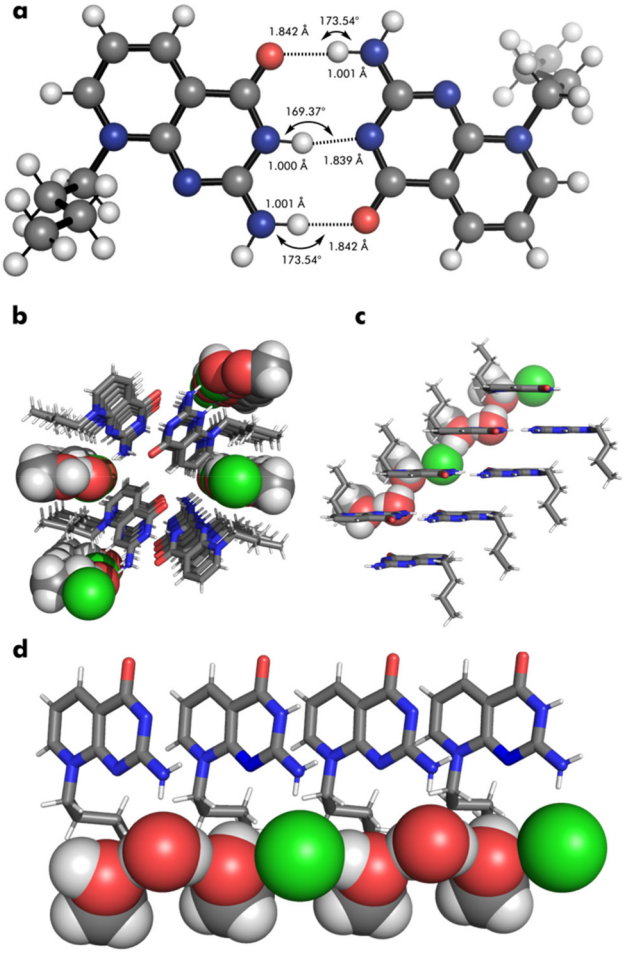


Fig. 2 (a) Structural parameters of **ZIC**-**ZIC** HCl 3H-bonded dimer; (b) top view of the channels; (c) side view of the stack of **ZIC**-**ZIC** HCl pair together with 1D $(-CH_3OH-H_2O-CH_3OH-Cl^-)_n$ chain; (d) close-up view of the 1D chain with H-bonded **ZIC** molecules.

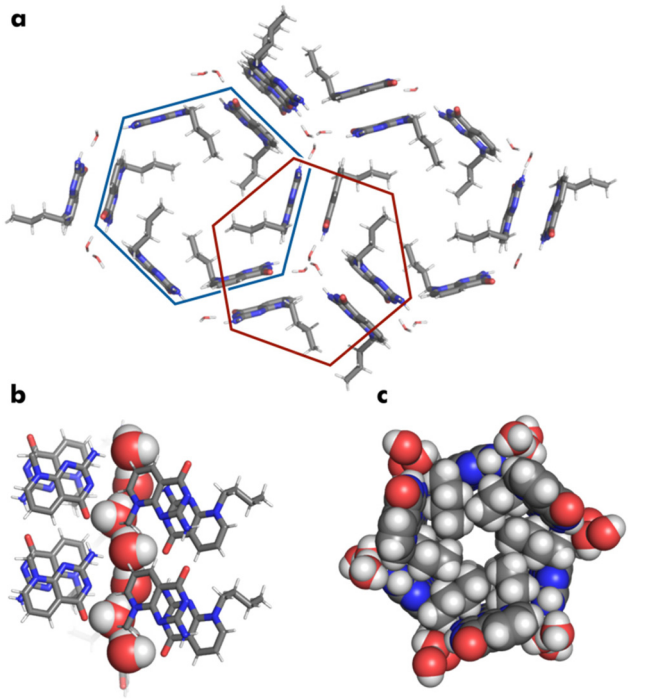


Fig. 3 (a) Top view of hydrophobic (blue hexagon) and hydrophilic (red hexagon) channels in **ZIC**; (b) side view 1D H_2O helix within hydrophilic channel; (c) top view space filling structure of hydrophobic channel.

ZIC : **ZIC-HCl** dimer). The **ZIC** : **ZIC-HCl** having the lowest solubility most likely aids in it being preferentially crystallised out from solution.

Nevertheless, the pure zwitterionic form of **ZIC** could be obtained using distilled water, with single crystals suitable for SCXRD being grown from MeOH/ethyl acetate (Fig. 3). The crystals of the trigonal *R*3 space group displayed a porous structure with two channels of opposite polarity (Fig. 3a). The hydrophilic channel is formed by the exposed NH_2, C=N, and C=O groups in the polar edges of three isocytosine rings along the crystal *c*-axis held in place by additional head-to-tail π–π stacking with the aromatic system of another **ZIC** molecule. This channel is filled with water with the amino and carbonyl groups forming H-bonds with oxygen and hydrogen atoms of the water molecules, respectively. This results in a helical arrangement of water molecules within the chain, with three H_2O molecules completing the full turn (Fig. 3b). The π–π stacking of the aromatic rings around the polar channel leads to a hexagonal arrangement of **ZIC** molecules with non-polar alkyl chains filling the internal space. The so-formed hydrophobic pores have a diameter of around 1.5 Å (Fig. 3c).

Intrigued by the above intricate packing and inclusion patterns, we also sought to prepare the protonated analogue of **ZIC**. After treating the latter with a methanolic HCl solution and triturating with diethyl ether, **ZIC-HCl** was obtained in 80% yield, and with single crystals grown from methanol (Fig. 4). The crystal structure features π–π stacked **ZIC-HCl** molecules providing a polar environment for coordination of water molecules and chloride anions (Fig. 4b). Two pairs of centrosymmetrically related stacks accommodate two alternating $(Cl\cdots H_2O\cdots)_n$ 1D chains (Fig. 4a). The amino group forms bifurcated hydrogen bonds with the oxygen atom of the water molecule in one chain and the chloride anion in the other. Moreover, interaction with chloride ion is reinforced with an additional H-bond with the lactam N–H bond. A water molecule connects two neighbouring chloride anions *via* H-bonding. Two additional **ZIC** stacks facing the above chains with pyridinium rings provide additional interactions with the chloride ions. They are nested on the aromatic surface of these rings by electrostatic and, most likely, anion–π interactions (Fig. 4c).[14]

We further explored the potential of **ZIC** as a new DAA motif in heterocomplexation studies in solution. Since solid-state analysis convincingly demonstrated dimerisation of hemi-protonated **ZIC**, we selected isocytosine **IC** as the ADD counterpart (Fig. 5). The spectrum of **ZIC** in d_6-DMSO displays well-defined resonances for pyridinium aromatic protons and

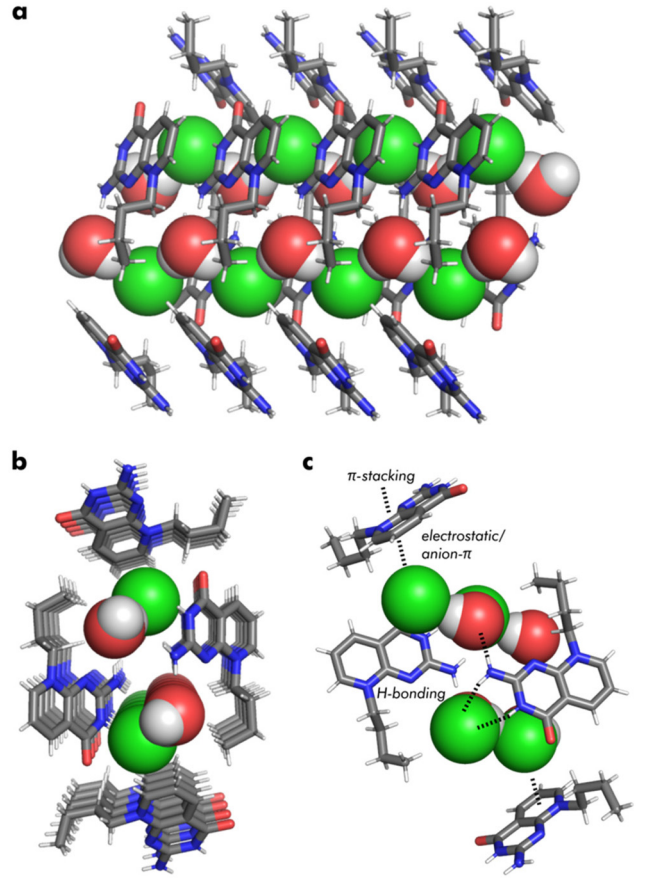


Fig. 4 Side view (a) and top view (b) of $(H_2O{-}Cl^-)_n$ 1D chains within the channels in **ZIC-HCl**; (c) non-covalent interactions stabilising $(H_2O{-}Cl^-)_n$ chains.

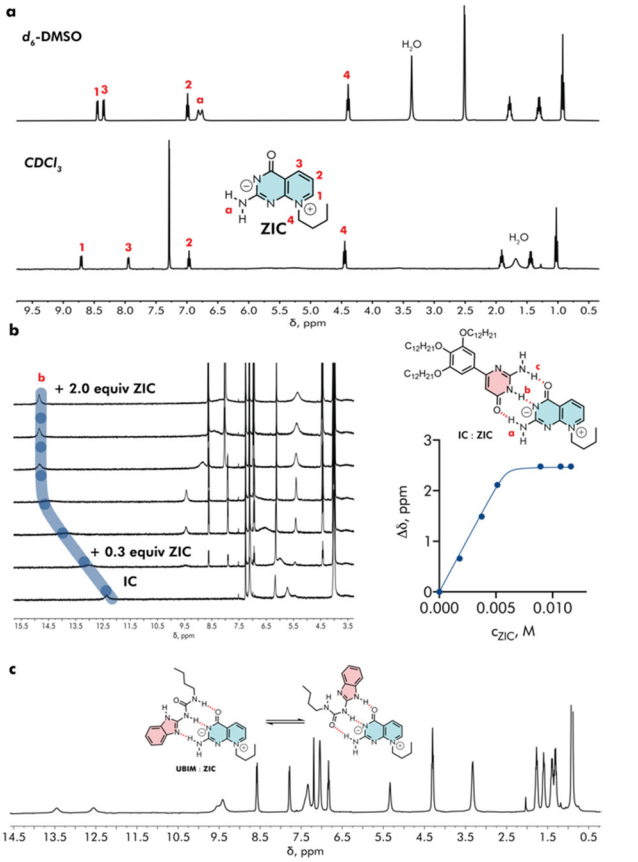


Fig. 5 (a) ^{1}H NMR spectra of **ZIC** in d_6-DMSO (top) and $CDCl_3$ (bottom); (b) ^{1}H NMR titration experiment of **IC : ZIC** complex; (c) ^{1}H NMR ($CDCl_3$) spectrum of heterocomplex **UBIM : ZIC**.

two broadened resonances for the NH_2 group. The splitting of the latter resonances is caused by DMSO complexation and restricted rotation of the NH_2 group. In comparison, the NH_2 resonances in $CDCl_3$ are not visible due to a fast exchange with water molecules (Fig. 5a). The addition of an equimolar amount of **IC** resulted in a new set of resonances indicating complexation. Unfortunately, due to the broadening of some resonances, the full assignment of all N–H signals was not possible even at 248 K (Fig. S10†). The low-temperature experiment also served as indirect evidence for hetero-complexation, as moderately soluble **ZIC** was retained in solution upon complex formation with "greasy" **IC**. Diffusion-ordered spectroscopy (DOSY) also corroborated the formation of complex evidenced by significant decrease in diffusion coefficient from $D = 7.7 \times 10^{-10}$ m^2 s^{-1} for free **ZIC** to $D = 3.7 \times 10^{-10}$ m^2 s^{-1} for **IC : ZIC** complex (Fig. S6 and S9†). The NMR titration experiment was performed and analysed using a 1 : 1 binding isotherm to give an association constant (K_{assoc}) of $(3.8 \pm 0.9) \times 10^4$ M^{-1} (Fig. 5b). This value is comparable with other DDA–AAD systems ($K_{assoc} = 10^4$–10^5 M^{-1}) reported in literature.[2]

As an alternative to isocytosine, in which only the H[3] tautomer serves as a DDA module, we synthesised ureidobenzimidazole (**UBIM**) with a conformer-independent DDA H-bonding array (Fig. 5c).[15] Mixing **ZIC** with **UBIM** in $CDCl_3$ in a 1 : 1 ratio (12 mM) resulted in complete dissolution of otherwise insoluble **UBIM**. The ^{1}H NMR spectrum displays one set of resonances, with all the N–H atoms being observed. Due to the low solubility of **UBIM**, the precise estimation of K_{assoc} was not possible; however, dilution of a 1 : 1 complex solution below 1.0 mM caused its full dissociation, providing a rough K_{assoc} estimate of $\sim 10^3$ M^{-1}. Thus, the positive effect of intramolecular H-bonding in **UBIM**, which preorganises the DDA array, is likely offset by a less optimal geometry caused by the five-membered imidazole ring.

The zwitterionic quadruple H-bonding unit **ZUPy** was investigated next. The ^{1}H NMR spectrum in $CDCl_3$ agreed well with the proposed structure, with two N–H resonances identified by proton exchange with CD_3OD and the ^{1}H-^{13}C HSQC experiment (Fig. 6a). Unfortunately, the localisation of negative charge could not be determined unambiguously in solution. On the other hand, crystallisation of **ZUPy** from solvents of different polarities, such as dichloromethane and methanol provided structures with ADAA motif (Fig. 6c). The **ZUPy** in crystals obtained from methanol acquires the rotamer in which an internal H-bond is formed between urea terminal nitrogen and N1 nitrogen of the isocytosine ring. The stacks of

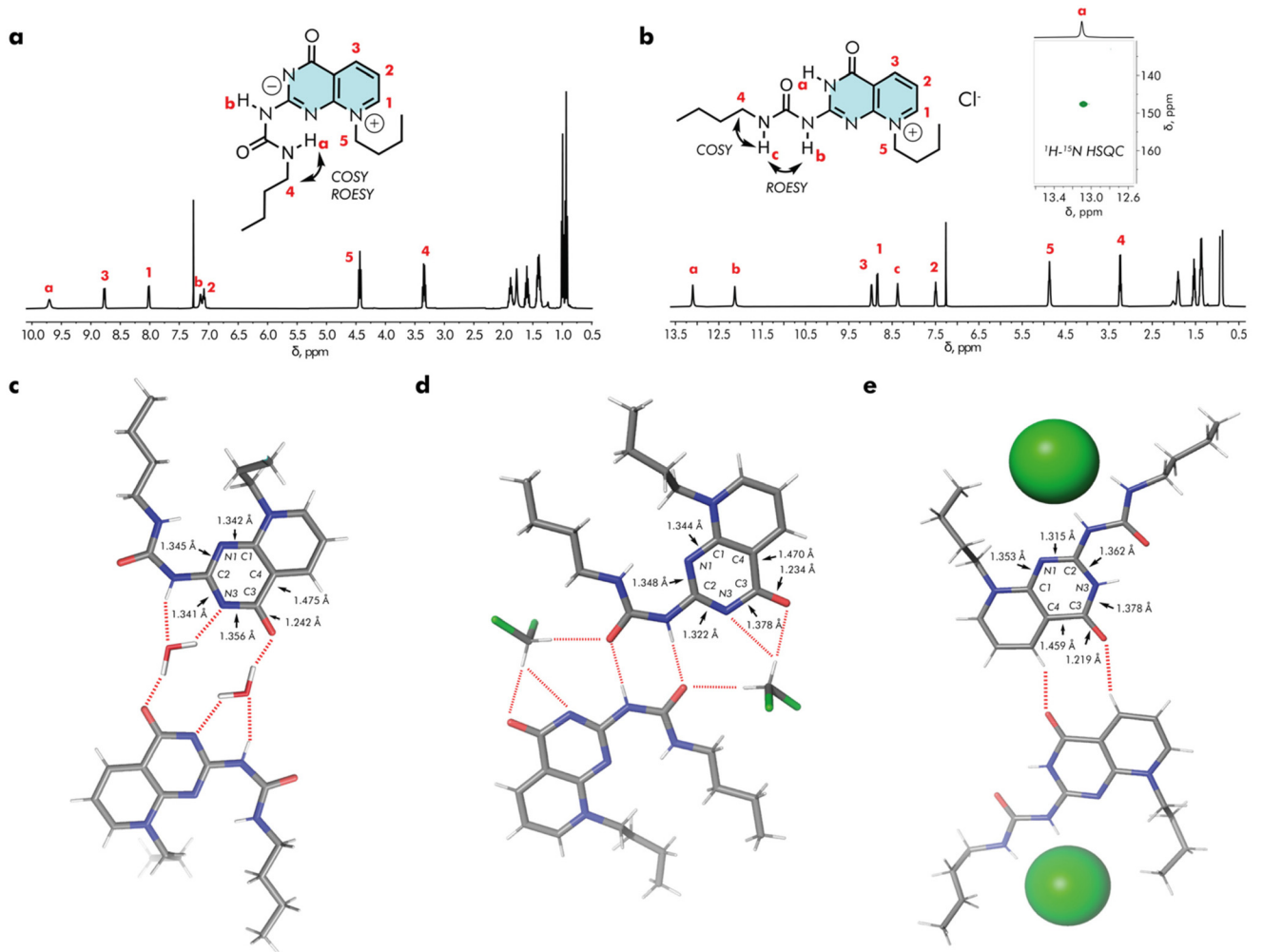


Fig. 6 (a) ^{1}H NMR spectrum of **ZUPy**; (b) ^{1}H NMR spectrum of **ZUPy-HCl** with part of ^{1}H–^{15}N HSQC spectrum shown; x-ray structures of **ZUPy** (H_2O) (c), **ZUPy** (CH_2Cl_2) (d) and **ZUPy-HCl** (e) with the lengths of selected bonds indicated.

ZUPy in the crystal are connected *via* two molecules of water that are H-bonded to the carbonyl oxygen of one **ZUPy** molecule and to the N=C and N–H groups of the other molecule (Fig. 6c). On the other hand, the crystal structure of **ZUPy** crystallised from dichloromethane features 2H-bonding between urea moieties, while dichloromethane molecules are connected *via* C–H H-bonds to the carbonyl groups of the urea and isocytosine, as well as the N3 atom. To gain an insight into the distribution of the negative charge, a control sample of **ZUPy-HCl** was prepared by protonation, and its structure determined by SCXRD (Fig. 6b and e). Interestingly, **ZUPy-HCl** crystallises as the H[3] tautomer, stabilised by an intramolecular H-bond between the urea carbonyl group and the N3–H bond. The selection of this rotamer is perhaps determined by the bifurcated H-bonds formed between the urea moiety and the chloride counterion. Finally, a pair of C–H···O=C H-bonds are established between two molecules. The same form of **ZUPy-HCl** is also observed in $CDCl_3$, as confirmed by the ^{15}N shift δ = 147 ppm, characteristic of the H[3] tautomer and ROESY cross-peaks between urea N–H bonds (Fig. 6b).[5a,b]

Analysis of the bond lengths in **ZUPy** crystals obtained from methanol suggested the delocalisation of the negative charge over both isocytosine nitrogen atoms and, to some degree, the cytosine carbonyl group. Specifically, the N1–C2 bond (1.345(2) Å) was significantly longer than the N1–C2 double bond in the **ZUPy-HCl** reference (1.315(2) Å). Likewise, the C2–N3 (1.341(2) Å) bond was also longer than expected for a C=N double bond (Fig. 6c and e). Contrary, shortening of the N3–C3 bond (1.356 (1) Å *vs.* 1.378(2) Å in **ZUPy-HCl**) and lengthening of the C=O bond (1.242(2) Å *vs.* 1.219(2) Å in **ZUPy-HCl**) point toward partial delocalisation of the electron density on the carbonyl group. Interestingly, the distribution of the electron density in dichloromethane solvate was found to be different. The relatively short C2–N3 (1.322(3) Å) and long C3–N3 (1.378(4) Å) bonds clearly indicate the localisation of the negative charge on the N1 atom.

To probe the heterocomplexation properties of the newly obtained AADA module, the complementary unit pyridylurea amide **PyAU** was selected (Fig. 7). As was shown in previous studies on structurally related compounds, heterocyclic ureas

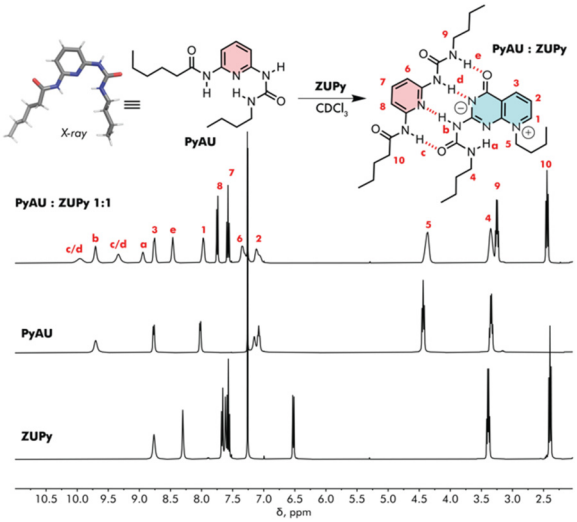


Fig. 7 ^{1}H NMR spectra of **PyAU** : **ZUPy** complex and monomers.

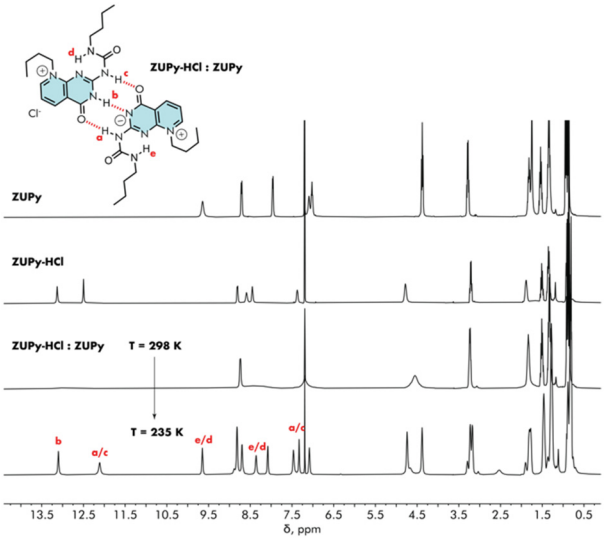


Fig. 8 ^{1}H NMR spectra of **ZUPy** : **ZUPy-HCl** complex and monomers.

in solution prefer a folded form stabilised by intramolecular H-bonds.[16] We have confirmed this *via* the SCXRD structure of **PyAU** (Fig. 7). Upon exposure to the AADA motif, unfolding of **PyAU** and heterocomplex formation are anticipated. Mixing **ZUPy** and **PyAU** in a 1 : 1 ratio in $CDCl_3$ (61 mM) resulted in a new single set of resonances consistent with the AADA-DDAD heterodimer. Despite the formation of quadruple H-bonds, the complex was moderately stable, and dilution of the solution to 1.35 mM resulted in full dissociation. The results provided an approximate estimate of K_{assoc} of 10^3 M^{-1}. Compared to a few structurally unrelated AADA motifs reported in literature, the low association constant (<900 M^{-1}) seems to be a rule rather than an exception for this system.[17] The increase of the H-bonding acceptor strength by introducing the negative charge on the nitrogen atom thus provides little improvement in binding strength.

The pH sensitivity of **ZUPy** was further employed in preparing a complex between **ZUPy** and **ZUPy-HCl** by the addition of half an equivalent of HCl. The emergence of the new set of broad resonances and the absence of the resonances of individual components suggested the formation of a heterodimer (Fig. 8). To aid the characterisation, the sample was cooled to 235 K. The sharp resonances obtained allowed the assignment of all H-bond donors to N–H groups using the ^{1}H–^{15}N HSQC spectrum, ruling out the presence of the enolic form. The chemical exchange between monomers, unfortunately, prevented full characterisation (Fig. S27 and S28†). Nevertheless, the spectral data was consistent with the formation of a 3H-bonded complex utilising the H[3] tautomer with urea groups forming intramolecular H-bonds to N1. The **ZUPy·ZUPy-HCl** structure therefore mimics the behaviour of the **ZIC·ZIC-HCl** pair, with the added advantage of more convenient functionalisation of the monomer *via* a urea handle.

It was possible that the protonated version of the H[3] tautomer of **ZUPy** might demonstrate the ADDA motif in one of the rotamers. We therefore attempted the complexation of **ZUPy-HCl** with the commonly utilised DAAD naphthyridine diamide module-**DAN**.[18] Surprisingly, no complex formation was observed in $CDCl_3$ (Fig. S30†). We reasoned that energetically favourable complexation of Cl^- anions by the urea group prevents its rotation to establish an intermolecular H-bond. To test this assumption, the chloride anion in **ZUPy-HCl** was exchanged with $[N(SO_2CF_3)_2]^-$ (NTf_2^-) using $AgNTf_2$, which resulted in the formation of the desired complex (Fig. 9). Adding 1.5 equivalents of cetyltrimethylammonium chloride (CTAC) to the above solution disrupted the complex and resulted in a spectral pattern identical to Cl^- coordinated monomeric **ZUPy-HCl**.

It should be noted that in addition to its response upon changing the counterion, **ZUPy-HCl** also displays perfect ortho-

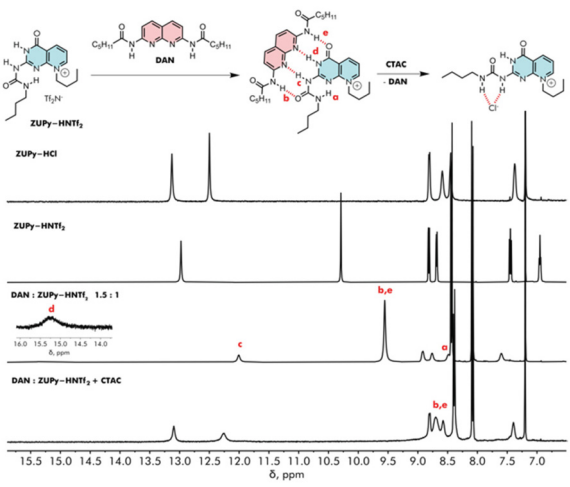
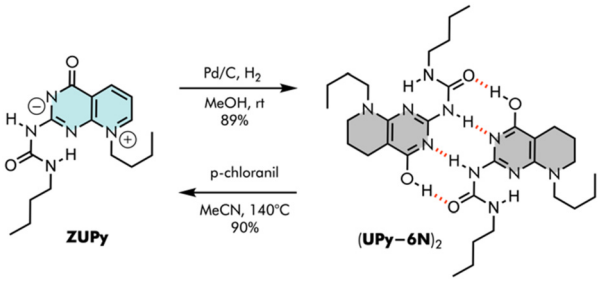


Fig. 9 Chloride anion modulated heterocomplexation of **DAN** and **ZUPy-HNTf$_2$** (c = 11.4 mM).



Scheme 1 Redox-switching between monomeric and 4H-bonded systems.

gonality to classical Meijer's UPys, as revealed by narcissistic self-sorting of their mixture (Fig. S29†).

Given the well-known fact that 6-amino-substituted ureidopyrimidinones exist in the 4H-bonding ADAD enol form[18b] and considering that the alkylated pyridine ring is activated for reduction, we devised a scheme where the H-bonding monomer would change its binding properties as a result of a chemical transformation (Scheme 1). Treating the **ZUPy** with dihydrogen in the presence of a Pd/C catalyst, converted **ZUPy** into **UPy-6N** in good yield. By performing dilution and temperature titrations we confirmed that **UPy-6N** forms very stable dimers in the enolic form (Fig. S34 and S35†). To recover **ZUPy**, **UPy-6N** was treated with chloranil. This redox-responsive H-bonding module has great potential and might find even broader applications in smart materials, especially when coupled with milder photoredox processes.

Conclusions

The development of zwitterionic isocytosine and ureidopyrimidinone derivatives introduces a versatile and responsive class of building blocks for both solid- and solution-phase supramolecular chemistry. Through the strategic use of tautomerism, rotamer interconversion, acid–base chemistry and anion complexation, these systems demonstrate dynamic behaviour and high fidelity in molecular recognition. Although the presence of a negative charge on a H-bond donor does not provide a large improvement in binding strength, new motifs described extend the collection of available AAD and AADA modules.

By partially protonating a single component 3H-bonding system of a zwitterionic isocytosine derivative, full complementarity was attained, providing an example of an anionic counterpart of the naturally occurring C–C$^+$ pair. Further applications of this motif for selective binding of DNA/RNA guanine or sensing guanosine phosphates can be envisioned based on H-bonding complementarity assisted by ionic interactions. Formation of porous structures with well-defined 1D chains of water, anions, or both, provides a convenient platform to study ion conductivity[19] and water transport mechanisms[20] in a confined space relevant to material and life sciences, and showcases the rich supramolecular chemistry in the solid phase displayed by the **ZIC** motif.

Experimental section

Synthesis of ZIC

To a suspension of **1** (0.90 g, 4.9 mmol) in deionized water (10 mL) and glacial acetic acid (5.0 mL) was added 1,1,3,3-tetraethoxypropane (1.2 mL, 4.9 mmol). The mixture was heated at 100 °C for 4 hours under a nitrogen atmosphere, cooled to room temperature and the solvent was removed *in vacuo*. The residue was purified by flash chromatography (DCM/MeOH = 50/1) to give **ZIC** as a light-yellow solid (0.66 g, 61%).

^{1}H NMR (400 MHz, d_6-DMSO) δ 8.44 (dd, J = 6.5, 1.8 Hz, 1H), 8.34 (dd, J = 7.4, 1.7 Hz, 1H), 6.98 (t, J = 6.8 Hz, 1H), 6.80 (s, 1H), 6.75 (s, 1H), 4.38 (t, J = 7.3 Hz, 2H), 1.77 (p, J = 7.5 Hz, 2H), 1.30 (h, J = 7.4 Hz, 2H), 0.91 (t, J = 7.3 Hz, 3H).

^{13}C NMR (101 MHz, d_6-DMSO) δ 167.57, 164.02, 156.41, 145.19, 140.89, 117.10, 113.03, 51.58, 30.70, 19.17, 13.56.

HRMS (ESI) calcd for ([M + H]$^+$): $C_{11}H_{15}N_4O$ 219.1246; found: 219.1252.

Synthesis of ZUPy

To a suspension of **ZIC** (0.68 g, 3.1 mmol) in dry tetrahydrofuran (20 mL) was added butyl isocyanate (3.5 mL, 31 mmol). The mixture was heated at 100 °C overnight under a nitrogen atmosphere. After cooling down to room temperature, the solvent was removed *in vacuo*. The residue was purified by flash chromatography (DCM/MeOH = 20/1) to give **ZUPy** as a yellow solid (0.60 g, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (s, 1H), 8.77 (d, J = 7.4 Hz, 1H), 8.02 (d, J = 6.3 Hz, 1H), 7.15 (s, 1H), 7.08 (t, J = 6.9 Hz, 1H), 4.44 (t, J = 7.5 Hz, 2H), 3.34 (q, J = 6.8 Hz, 2H), 1.89 (q, J = 7.6 Hz, 2H), 1.60 (p, J = 7.4 Hz, 2H), 1.40 (hd, J = 7.3, 2.5 Hz, 4H), 0.99 (t, J = 7.3 Hz, 3H), 0.93 (t, J = 7.3 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.17, 162.03, 156.40, 154.36, 143.51, 143.20, 119.07, 114.28, 53.64, 39.94, 32.12, 31.42, 20.30, 19.87, 13.94, 13.66, 0.08.

HRMS (ESI) calcd for ([M + H]$^+$): $C_{16}H_{24}N_5O_2$ 318.1930; found: 318.1935.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article (NMR spectra and crystallographic data) have been included as part of the ESI.†

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References

1 (*a*) L. J. Prins, D. N. Reinhoudt and P. Timmerman, Noncovalent synthesis using hydrogen bonding, *Angew. Chem., Int. Ed.*, 2001, **40**, 2382–2426; (*b*) A. J. Wilson, Non-covalent polymer assembly using arrays of hydrogen-bonds, *Soft Matter*, 2007, **3**, 409–425; (*c*) Y. Liu, L. Wang, L. Zhao, Y. Zhang, Z.-T. Li and F. Huang, Multiple hydrogen bonding driven supramolecular architectures and their biomedical applications, *Chem. Soc. Rev.*, 2024, **53**, 1592–1623; (*d*) Z.-T. Li and L.-Z. Wu, *Hydrogen Bonded Supramolecular Materials*, Springer, 2015; (*e*) S. J. Grabowski, Hydrogen Bonding—New Insights, in *Challenges and Advances in Computational Chemistry and Physics*, ed. J. Leszczynski, Springer-Verlag, New York, 2006.

2 S. C. Zimmerman and P. S. Corbin, in *Structure and Bonding*, Springer, Berlin, Heidelberg, 2000, pp. 63–94.

3 S. S. Sivakova and S. J. Rowan, Nucleobases as supramolecular motifs, *Chem. Soc. Rev.*, 2005, **34**, 9–21.

4 (*a*) F. H. Beijer, R. P. Sijbesma, H. Kooijman, A. L. Spek and E. W. Meijer, Strong dimerization of ureidopyrimidones via quadruple hydrogen bonding, *J. Am. Chem. Soc.*, 1998, **120**, 6761–6769; (*b*) S. H. M. Söntjens, R. P. Sijbesma, M. H. P. van Genderen and E. W. Meijer, Stability and lifetime of quadruply hydrogen bonded 2-ureido-4[1H]-pyrimidinone dimers, *J. Am. Chem. Soc.*, 2000, **122**, 7487–7493; (*c*) E. Orentas, C. Wallentin, K. Bergquist, M. Lund, E. Butkus and K. Wärnmark, Topology selection and tautoleptic aggregation: Formation of an enantiomerically pure supramolecular belt over a helix, *Angew. Chem., Int. Ed.*, 2011, **50**, 2071–2074; (*d*) D. Račkauskaitė, K.-E. Bergquist, Q. Shi, A. Sundin, E. Butkus, K. Wärnmark and E. Orentas, A remarkably complex supramolecular hydrogen-bonded decameric capsule formed from an enantiopure C_2-symmetric monomer by solvent-responsive aggregation, *J. Am. Chem. Soc.*, 2015, **137**, 10536–10546; (*e*) A. Neniškis, D. Račkauskaitė, Q. Shi, A. J. Robertson, A. Marsh, A. Ulčinas, R. Valiokas, S. P. Brown, K. Wärnmark and E. Orentas, A tautoleptic approach to chiral hydrogen–bonded supramolecular tubular polymers with large cavity, *Chem. – Eur. J.*, 2018, **24**, 14028–14033.

5 (*a*) A. Jozeliūnaitė, T. Javorskis, V. Vaitkevičius, V. Klimavičius and E. Orentas, Fully supramolecular chiral hydrogen-bonded molecular tweezer, *J. Am. Chem. Soc.*, 2022, **144**, 8231–8241; (*b*) A. Jozeliūnaitė, A. Neniškis, A. Bertran, A. M. Bowen, M. Di Valentin, S. Raišys, P. Baronas, K. Kazlauskas, L. Vilčiauskas and E. Orentas, Fullerene complexation in a hydrogen-bonded porphyrin receptor via induced-fit: Cooperative action of tautomerization and C–H···π interactions, *J. Am. Chem. Soc.*, 2023, **145**, 455–464; (*c*) Q. Shi, X. Zhou, W. Yuan, X. Su, A. Neniškis, X. Wei, L. Taujenis, G. Snarskis, J. S. Ward, K. Rissanen, J. De Mendoza and E. Orentas, Selective formation of S_4- and T-symmetric supramolecular tetrahedral cages and helicates in polar media assembled via cooperative action of coordination and hydrogen bonds, *J. Am. Chem. Soc.*, 2020, **142**, 3658–3670; (*d*) Q. Shi, T. Javorskis, K.-E. Bergquist, A. Ulčinas, G. Niaura, I. Matulaitienė, E. Orentas and K. Wärnmark, Stimuli-controlled self-assembly of diverse tubular aggregates from one single small monomer, *Nat. Commun.*, 2017, **8**, 14943.

6 (*a*) K. Gehring, J.-L. Leroy and M. Guéron, A tetrameric DNA structure with protonated cytosine-cytosine base pairs, *Nature*, 1993, **363**, 561–565; (*b*) S. Benabou, A. Aviñó, R. Eritja, C. González and R. Gargallo, Fundamental aspects of the nucleic acid i-motif structures, *RSC Adv.*, 2014, **4**, 26956–26980.

7 B. Yang, R. R. Wu and M. T. Rodgers, Base-pairing energies of proton-bound dimers and proton affinities of 1-methyl-5-halocytosines: Implications for the effects of halogenation on the stability of the DNA i-Motif, *J. Am. Soc. Mass Spectrom.*, 2015, **26**, 1469–1482.

8 (*a*) R. P. Sijbesma, F. H. Beijer, L. Brunsveld, B. J. B. Folmer, J. H. K. K. Hirschberg, R. F. M. Lange, J. K. L. Lowe and E. W. Meijer, Reversible polymers formed from self-complementary monomers using quadruple hydrogen bonding, *Science*, 1997, **278**, 1601–1604; (*b*) R. P. Sijbesma and E. W. Meijer, Quadruple hydrogen bonded systems, *Chem. Commun.*, 2003, **39**, 5–16; (*c*) Q. Chen, X. Su, E. Orentas and Q. Shi, Supramolecular crowns: A new class of cyclic hydrogen-bonded cavitands, *Org. Chem. Front.*, 2019, **6**, 611–617; (*d*) Q. Shi, K.-E. Bergquist, R. Huo, J. Li, M. Lund, R. Vácha, A. Sundin, E. Butkus, E. Orentas and K. Wärnmark, Composition- and size-controlled cyclic self-assembly by solvent- and C_{60}-responsive self-sorting, *J. Am. Chem. Soc.*, 2013, **135**, 15263–15268.

9 H. M. Coubrough, B. Balonova, C. M. Pask, B. A. Blight and A. J. Wilson, A pH–switchable triple hydrogen–bonding motif, *ChemistryOpen*, 2020, **9**, 40–44.

10 B. Wang, J. R. Rocca, S. Hoshika, C. Chen, Z. Yang, R. Esmaeeli, J. Wang, X. Pan, J. Lu, K. K. Wang, Y. C. Cao, W. Tan and S. A. Benner, A folding motif formed with an expanded genetic alphabet, *Nat. Chem.*, 2024, **16**, 1715–1722.

11 E. M. Todd, J. R. Quinn, T. Park and S. C. Zimmerman, Fidelity in the supramolecular assembly of triply and quadruply hydrogen–bonded complexes, *Isr. J. Chem.*, 2005, **45**, 381–389.

12 H. M. Coubrough, S. C. C. Van Der Lubbe, K. Hetherington, A. Minard, C. Pask, M. J. Howard, C. F. Guerra and A. J. Wilson, Supramolecular self–sorting networks using hydrogen–bonding motifs, *Chem. – Eur. J.*, 2018, **25**, 785–795.

13 R. Bernetti, F. Mancini and C. C. Price, Pyrido[2,3-d]pyrimidines from malonaldehydes, *J. Org. Chem.*, 1962, **27**, 2863–2865.

14 E. Bitega, R. Patil, M. Zeller and S. V. Rosokha, Charge-assisted anion–π interaction and hydrogen bonding involving alkylpyridinium cations, *ACS Omega*, 2024, **9**, 43058–43067.

15 M. L. Pellizzaro, A. M. McGhee, L. C. Renton, M. G. Nix, J. Fisher, W. B. Turnbull and A. J. Wilson, Conformer–independent ureidoimidazole motifs—Tools to probe conformational and tautomeric effects on the molecular recognition of triply hydrogen–bonded heterodimers, *Chem. – Eur. J.*, 2011, **17**, 14508–14517.

16 (*a*) L. V. Sudha and D. N. Sathyanarayana, ^{1}H and ^{13}C NMR spectra of some unsymmetric N,N′-dipyridyl ureas: Spectral assignments and molecular conformation, *J. Chem. Soc., Perkin Trans. 2*, 1997, 157–162; (*b*) P. S. Corbin and S. C. Zimmerman, Complexation-induced unfolding of heterocyclic ureas: A hydrogen-bonded, sheetlike heterodimer, *J. Am. Chem. Soc.*, 2000, **122**, 3779–3780; (*c*) M. Bolte, C. Kühl and U. Lüning, *N,N′*-Bis(6-methyl-2-pyridyl)-urea, *Acta Crystallogr., Sect. E:Struct. Rep. Online*, 2001, **57**, 502–504; (*d*) B. Ośmiałowski, K. Mroczyńska, E. Kolehmainen, M. Kowalska, A. Valkonen, M. Pietrzak and K. Rissanen, Association of *N*-(Pyridin-2-yl), *N*′-substituted ureas with 2-Amino-1,8-naphthyridines and benzoates: NMR and quantum chemical studies of the substituent effect on complexation, *J. Org. Chem.*, 2013, **78**, 7582–7593.

17 (*a*) G. B. W. L. Ligthart, D. Guo, A. L. Spek, H. Kooijman, H. Zuilhof and R. P. Sijbesma, Ureidobenzotriazine multiple H-bonding arrays: The importance of geometrical details on the stability of H-bonds, *J. Org. Chem.*, 2007, **73**, 111–117; (*b*) J. Taubitz and U. Lüning, The AAAA·DDDD hydrogen bond dimer. Synthesis of a soluble sulfurane as AAAA domain and generation of a DDDD counterpart, *Aust. J. Chem.*, 2009, **62**, 1550–1555; (*c*) J. Taubitz and U. Lüning, On the importance of the nature of hydrogen bond donors in multiple hydrogen bond systems, *Eur. J. Org. Chem.*, 2008, 5922–5927; (*d*) S. Brammer, U. Lüning and C. Kühl, A new quadruply bound heterodimer DDAD·AADA and investigations into the association process, *Eur. J. Org. Chem.*, 2002, 4054–4062; (*e*) S. H. Söntjens, J. T. Meijer, H. Kooijman, A. L. Spek, M. H. van Genderen, R. P. Sijbesma and E. W. Meijer, A multiple hydrogen-bond scaffold based on dipyrimidin-2-ylamine, *Org. Lett.*, 2001, **3**, 3887–3890.

18 (*a*) T. Park, E. M. Todd, S. Nakashima and S. C. Zimmerman, A quadruply hydrogen bonded heterocomplex displaying high-fidelity recognition, *J. Am. Chem. Soc.*, 2005, **127**, 18133–18142; (*b*) T. F. A. de Greef, G. B. W. L. Ligthart, M. Lutz, A. L. Spek, E. W. Meijer and R. P. Sijbesma, The mechanism of Ureido-Pyrimidinone: 2,7-Diamido-Naphthyridine complexation and the presence of kinetically controlled pathways in multicomponent hydrogen-bonded systems, *J. Am. Chem. Soc.*, 2008, **130**, 5479–5486.

19 Y.-R. Liu, Y.-Y. Chen, H.-Y. Zhao and G. Li, Exploration of single-crystal proton conduction in ordered networks, *Coord. Chem. Rev.*, 2023, **499**, 215516.

20 (*a*) P. Agre, Aquaporin water channels (Nobel Lecture), *Angew. Chem., Int. Ed.*, 2004, **43**, 4278–4290; (*b*) M. S. Kaucher, M. Peterca, A. E. Dulcey, A. J. Kim, S. A. Vinogradov, D. A. Hammer, P. A. Heiney and V. Percec, Selective transport of water mediated by porous dendritic dipeptides, *J. Am. Chem. Soc.*, 2007, **129**, 11698–11699.