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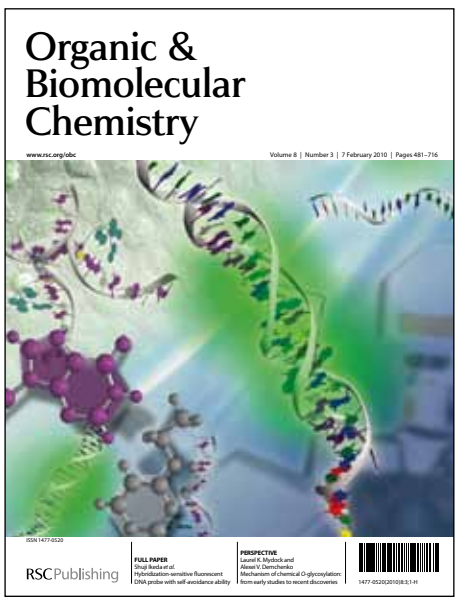

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ARTICLE TYPE

Design, synthesis and binding studies of a novel quadruple ADDA hydrogen-bond array

Maria L. Pellizzaro,[a] Simon A. Barrett,[a] Julie Fisher[a] and Andrew J. Wilson*[a,b]

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The design and synthesis of a novel ADDA hydrogen-bond array is described. The ureidodiimidazole motif (UDIM) **2** engages in interactions with complementary diamidonaphthyridine (DAN) **3** motifs with an association constant $K_a = 825 \pm 16\ M^{-1}$ in chloroform. ^{1}H NMR and molecular modelling studies were carried out in order to explain the unexpected behaviour of this new supramolecular motif. These revealed that a combination of effects including; an energetic bias for the folded conformer, subtle differences in shape complementarity between the two components and the potential for self-association of UDIM **2** disfavour higher affinity interactions between the two components.

Introduction

The design and synthesis of linear arrays possessing hydrogen bond donor and acceptor moieties represents an ongoing endeavour in supramolecular chemistry.[1] Such motifs enhance our fundamental understanding of co-operative non-covalent interactions and form the bases of higher order supramolecular assemblies.[2, 3] The strength with which linear arrays participate in molecular recognition with complementary partners is determined by a complex interplay of different factors that is not wholly understood. These factors include: the number and arrangement of donor and acceptor interactions, the tautomeric and conformational properties of the array and remote substituent effects.[1, 4, 5,6] Additional subtle effects occur as a consequence of proximal functional groups capable of weak interactions (e.g. CH···O hydrogen-bonds)[7] and small differences in shape complementarity that arise as a result of small differences in bond length within the covalent framework of the array (e.g. the C-O, C-N and C-C bond length all differ).[7] Although arrays employing large numbers (≥4) of hydrogen bond donor and acceptor moieties have been described,[8-15] linear arrays employing four hydrogen bonds have received considerable attention as targets for design and synthesis,[16-33] presumably because they represent an ideal balance between synthetic accessibility and predictable or controllable recognition properties. Our group previously described a series of conformer independent linear arrays that participate in molecular recognition through three hydrogen bonds.[15, 34-38] In the current manuscript we describe the logical extension of our work with *N*-alkyl/ arylureidoimidazoles (e.g. UIM **1**)[34, 36, 38] to the development of a quadruply hydrogen-bonded motif. Ureidodiimidazole (UDIM) **2** retains the conformer independent features of the *N*-acyl-2-aminoimidazole core (Figure 1) and presents an acceptor-donor-donor-acceptor (ADDA) arrangement of functional groups in the fully extended conformation. The diamidonaphthyridine (DAN) motif, which has been extensively studied[39, 40] as a DAAD array, was selected as a complementary partner to study the molecular recognition properties of this new supramolecular building block (in this work we used the novel derivative **3** with *n*-butyl chains). Our results reveal weaker than anticipated heterodimerisation resulting from a complex interplay of effects, including a variety of stable conformational states, subtle differences in shape complementarity, steric interactions and the potential for homodimerisation of UDIM **2**.

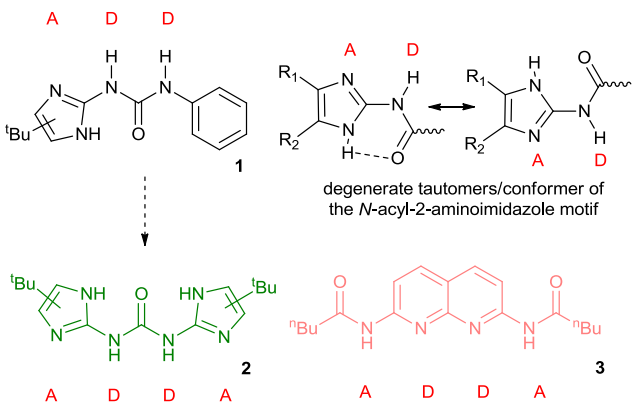


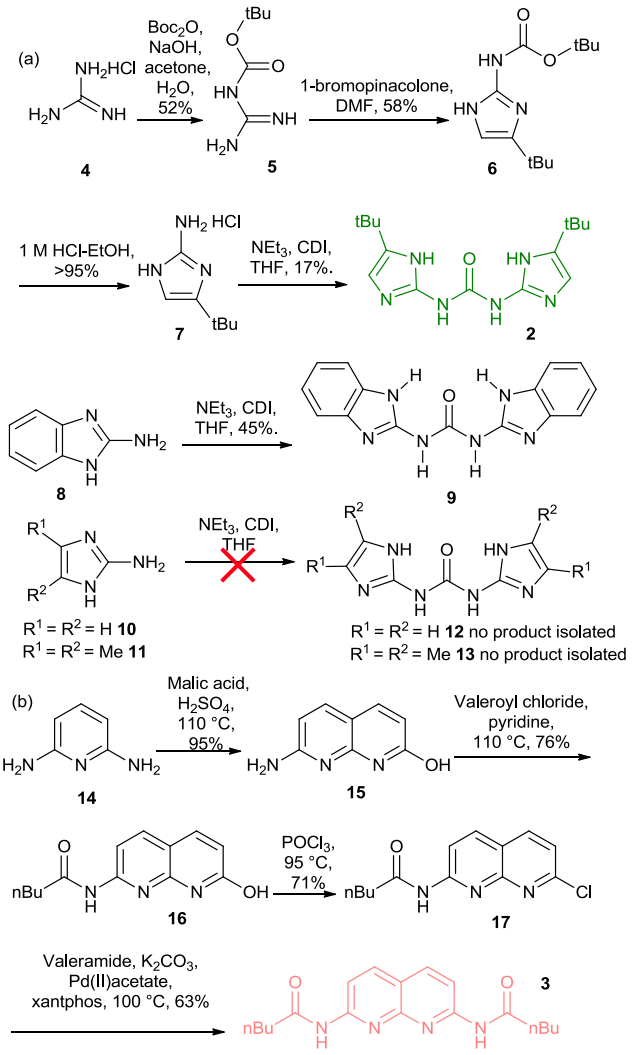
Figure 1 Structures of compounds **1-3** under study in this work together with an illustration depicting the degenerate conformational/ tautomeric properties of the *N*-acyl-2-aminoimidazole motif.

Results and Discussion

Synthesis

UDIM **2** was synthesised as illustrated in Scheme 1b by closely following the synthesis of UIM **1**.[34] Mono-protection of guanine hydrochloride **4** with a Boc-group to give **5**, followed by cyclisation using 1-bromopinacolone gave the protected aminoimidazole **6**. Deprotection using hydrochloric acid in ethanol gave the hydrochloride salt **7**, which was reacted with

1,1-carbonyldiimidazole (CDI) to give the final product, UDIM **2**. Because of the potential for UDIM **2** to adopt a range of conformational and tautomeric states, we attempted to synthesise more symmetrical derivatives. The product of the reaction between benzimidazole **8** and CDI gave the desired target **9** however this was insoluble in most solvents and we were only able to obtain a ^{1}H NMR spectrum. Aminoimidazole **10** and dimethlyaminoimidazole **11** both failed to yield the desired products **12** and **13** noting again that the reaction produced only insoluble precipitates. DAN **3** was synthesized following a slightly modified literature procedure as illustrated in Scheme 1b.[41]



Scheme 1 Synthesis of (a) UDIM **2** and (b) DAN **3**.

Conformational Analysis

UDIM **2** may exist in two different conformations as illustrated in Figure 2 which we refer to as *extended* (ex) or *folded* (fo). Similarly, two tautomers of each imidazole ring in UDIM **2** are possible, leading to seven plausible states. The ^{1}H NMR spectrum of UDIM **2** suggests that there is more than one conformer present in solution (Figure 3). VT ^{1}H NMR shows that at high temperatures (333K) the NH resonances begin to coalesce; indicating that inter-conversion between the different tautomers and conformers becomes more rapid on the NMR timescale. On cooling, the NMR spectrum is resolved further, however assignment of the individual conformers/tautomers was not possible by 2D NOESY NMR because of the presence of exchange peaks in the spectrum (see ESI). Two of the extended conformers are symmetrical whilst all the folded conformers are not; it is therefore tempting to suggest that a folded conformer predominates as there are four signals of comparable intensity in the low temperature spectrum, although this might equally suggest that two symmetrical extended conformers (e.g. **2-*ex*-i** and **2-*ex*-iii**) are present in comparable proportions.

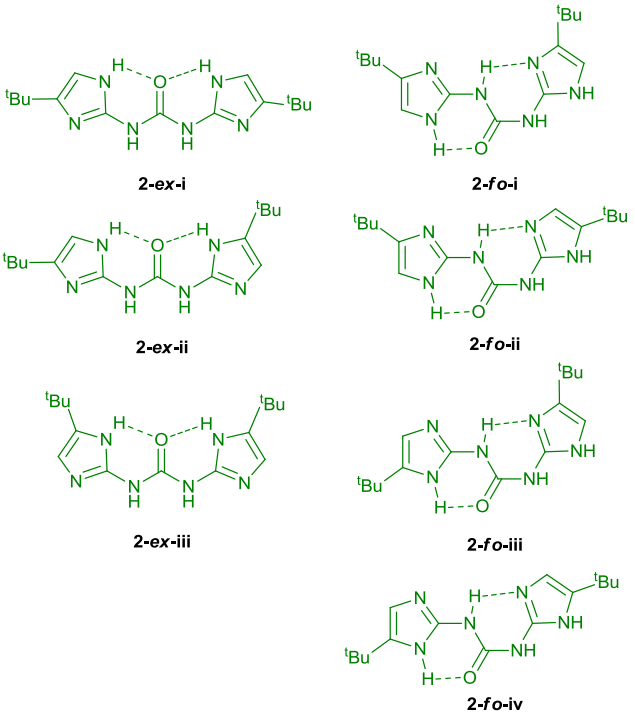


Figure 2 Plausible conformational states of UDIM **2**

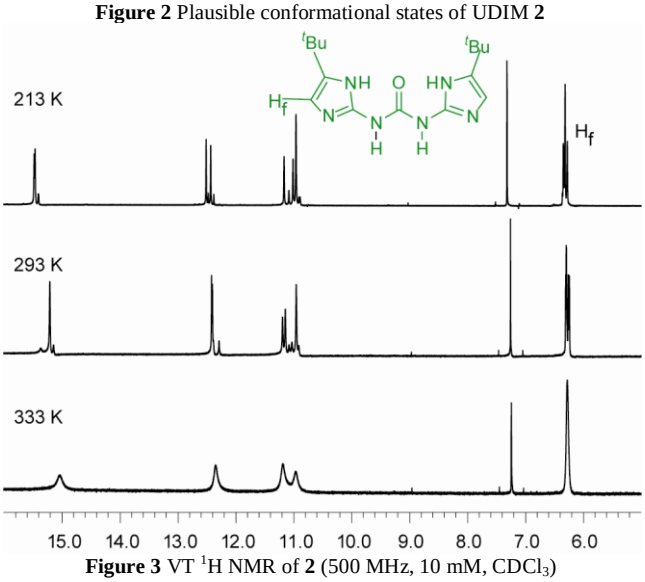


Figure 3 VT ^{1}H NMR of **2** (500 MHz, 10 mM, $CDCl_3$)

Binding Studies

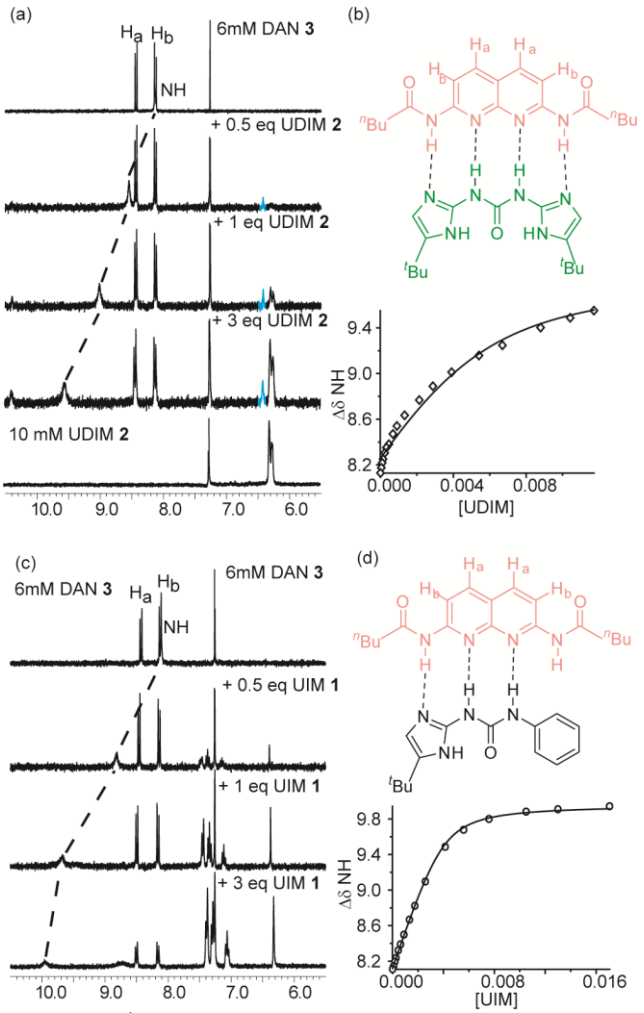
In order to study the interaction between UDIM **2** and DAN **3** in

deuterochloroform, a ^{1}H NMR spectrum of a 1:1 mixture was compared to that of the individual components. Significant shifts of the NH resonances of DAN **3** were observed, indicating that intermolecular hydrogen bonding occurs. In contrast resonances for UDIM **2**, exhibited only minimal complexation induced shifts (CIS), however an additional set of resonances were observed – this is most clearly seen for the imidazole CH resonance and will be discussed further below. It should be noted that there was no change in the NMR spectrum of this sample over a period of 24 hours. The result indicates that weak binding (see below) is not limited by slow interconversion between tautomeric and conformational states of UDIM **2**. The origin of this effect likely derives from either geometrical/steric incompatibility of **2-*ex*-i-iii** with DAN **3** or because **2** exhibits a strong preference for a conformational/ tautomeric state (i.e. **2-*fo*-i-iv)**) that is unproductive for binding to **3.** On the basis of the CIS for DAN **3**, ^{1}H NMR titrations were used to determine the association constant for the interaction. Aliquots of UDIM **2**, containing a small amount of DAN **3**, were added to a solution of DAN **3**. After each addition of UDIM **2** a ^{1}H NMR spectrum was recorded (Figure 4a) and the change in shift of the NH of DAN **3** was plotted against the concentration of UDIM **2**. Fitting the data to a 1:1 association model in HypNMR[42] gave a binding affinity of 825 ± 16 M^{-1} (Figure 4b). It should be noted that no attempt was made to account for self-association of either component in the analytical treatment of the data. Such an assumption is appropriate for DAN **3** which is known to only weakly self-associate, however it is not clear if the assumption is appropriate for UDIM **2**.

The additional set of resonances observed for UDIM **2** in the presence of DAN **3** is indicative of slow exchange between two or more species. In addition to a simple equilibrium between bound and unbound UDIM **2**, these might include two different conformations of UDIM **2** bound to DAN **3** and/or higher aggregates such as homodimers of UDIM **2**. A ^{1}H-^{1}H NOESY experiment was performed to provide additional structural information on the nature of interactions taking place, however it was difficult to assign correlations as through-space or exchange correlations (see ESI). A ^{1}H-^{1}H ROESY was therefore performed; the spectrum (Figure 5) exhibits intramolecular correlations only for the additional set of resonances that appear for UDIM **2** in the presence of DAN **3**. Such a result is consistent with the extended conformation of UDIM **2-*ex*-iii** being bound to DAN **3**

Formation of a complex in solution was also observed by diffusion ordered NMR spectroscopy (DOSY). DOSY is capable of measuring the size of assemblies that are present in solution and changes in the diffusion coefficients reflect changes in the size of these species.[43] All diffusion coefficients were measured on 3.6 mM samples at 20 °C in $CDCl_3$. The diffusion coefficients for DAN **3** and UDIM **2** in isolation were 10.61 and 9.33 $m^2s^{-1} \times 10^{-10}$ respectively. In the 1:1 mixture they both decreased (10.06 and 8.67 $m^2s^{-1} \times 10^{-10}$), indicating that they are within larger assemblies in solution, however the absence of coincident diffusion coefficients for both components indicates weak association between **2** and **3**. It is possible to calculate the association constant of the complex from the change in the diffusion coefficients (see ESI). The UDIM:DAN **2:3** complex was found to have an association constant of about 834 M^{-1}, in agreement with the ^{1}H NMR titrations (825 M^{-1}).

Figure 4 ^{1}H NMR based binding studies of UIM **1** and UDIM **2** towards



DAN **3** (a) ^{1}H NMR spectra for the titration of UDIM **2** into DAN **3** (300 MHz, $CDCl_3$, new UDIM CH resonance shown in light blue) (b) proposed structure of the complex and the binding isotherm for the titration shown in (a), (c) ^{1}H NMR spectra for the titration of UIM **1** into DAN **3** (300 MHz, $CDCl_3$) (d) proposed structure of the complex and the binding isotherm for the titration shown in (c).

The comparatively low binding constant determined and observation of a distinct set of resonances attributable to UDIM:DAN **2:3** prompted further study of the self-association of UDIM **2**. In particular, the major resonances for the NH protons are observed at low field (11-15 ppm) which is indicative of hydrogen-bonding however of the plausible conformations, only 2 NH's can be hydrogen-bonded at any one time, suggesting the possibility of intermolecular self-association. Dilution studies in chloroform resulted in no change in the chemical shifts of UDIM **2**. Similarly for the VT experiment illustrated in Figure 3, larger chemical shift changes than those observed would be anticipated for intermolecular hydrogen-bonds indicating either no interaction or a very strong interaction. We therefore also performed a DMSO titration to disrupt intermolecular hydrogen bonding; as the concentration of DMSO-d_6 increases in a 10 mM solution of UDIM **2** in $CDCl_3$, the NH resonances broaden and

then converge to two peaks which may support an intermolecular mode of association but could also arise due to a change in the position of conformational/ tautomeric equilibrium. We also performed a dilution study at 5% DMSO-d_6 in $CDCl_3$, however no change in the chemical shift of the key resonances was observed. Overall, these data are ambiguous in defining the extent of UDIM **2** self-association. However, in tandem with the diffusion co-efficient determined by DOSY, we tentatively suggest that UDIM **2** is largely monomeric under the conditions of the experiment and that its self-association behaviour is not a major characteristic that defines the intermolecular association behaviour with DAN **3**.

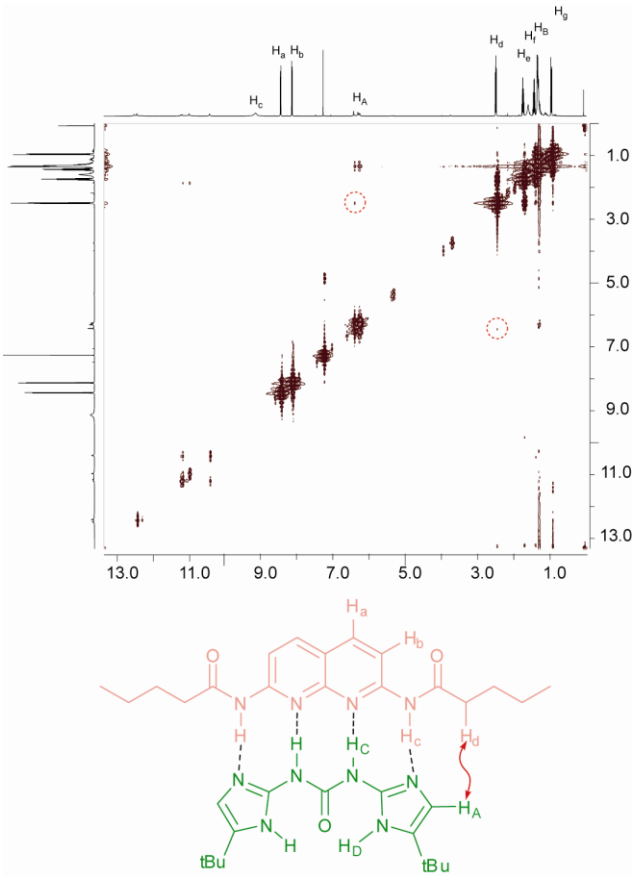


Figure 5 Phase sensitive ^{1}H-^{1}H ROESY spectrum of 1:1 mix of components UDIM **2** and DAN **3** at 10 mM concentration (with respect to each component) in $CDCl_3$, 500 MHz.

We found the weak nature of the interaction between UDIM **2** and DAN **3** surprising; although the VT NMR experiments suggest UDIM **2** adopts a variety of conformations and tautomers, we still anticipated that when presented with the complementary DAN array **3** the conformer **2-*ex*-i-iii** would be adopted in order to maximize hydrogen bonding interactions within the entire system. This property has been observed for other systems e.g. the ureidopyrimidine motif (UPy) despite forming a stable homodimer,[44] reconfigures in the presence of DAN derivatives to form stable heterodimers ($K_a > 10^5$ M^{-1}).[22, 45] Similarly, the *N, N'*-di-4-triazolylurea motif recently described by Hisamatsu and co-workers (DTU) also forms stable heterodimers with DAN derivatives ($K_a > 10^5$ M^{-1}).[29] In both instances the high affinity interaction may be (at least partially) reconciled by the fact that formation of a heterodimer maximizes the non-covalent interactions of the system as a whole, however this is not always the case; DAN derivatives have been shown to form weak complexes ($K_a \sim 10^3$ M^{-1})[19, 21] with dipyridylurea (DPU) derivatives which preferentially adopt a folded conformation and self-associate weakly.[10] It is noteworthy that multiple resonances for the UDIM **2** imidazole protons are still observed in the presence of DAN **3** consistent with there being no change in the distribution of conformers at equilibrium. It is possible that this derives from an excessive barrier to interconversion between tautomers and conformers; as stated previously, we believe this to be unlikely as our ^{1}H-^{1}H NOESY data revealed correlations indicative of exchange, whilst for UPy[16] and ureidodiguanidinium DDDD$^+$ motifs[31] such transitions are kinetically rapid on the NMR timescale.

The Zimmerman group have shown that motifs capable of interaction through only three hydrogen bonds can form stronger interactions in comparison to arrays presenting four hydrogen bonding groups,[46] although it should be noted that this is functional group dependent.[47] We therefore also performed a ^{1}H NMR titration on the UIM:DAN, **1**:**3** complex (Figure 4c). CIS were observed and these were in fact significantly larger than those observed for UDIM:DAN **2**:**3**. The binding affinity for the UIM:DAN **1**:**3** interaction – 2140 ± 42 M^{-1} (Figure 4d) – is stronger than the UDIM:DAN **2**:**3** complex and is presumably weaker than triply hydrogen-bonded complexes (made by **1**)[38] because the remaining H-bond donor on DAN **3** contributes an additional unfavourable secondary interaction. Taken with the ^{1}H NMR observations this suggests that both the extend conformer **2-*ex*-i-iii** and folded conformer **2-fo-i-iv** of UDIM should be capable of interaction with DAN **3**.

Molecular Modelling

We performed modelling studies to provide additional insight on the experimental observations. Gaussian 03[48] was used to perform density functional theory (DFT) calculations, which can predict the geometry of complexes that interact *via* intermolecular hydrogen bonds. Gas phase calculations were run using the hybrid B3LYP functional. Due to the size of the molecules, it was necessary to use a mixed basis set C, 6-31G; N/O, 6-31G*; H, 6-31++G** as we did in earlier work.[38] This approach allows polarisation and diffusion functionals to be added to heteroatoms involved in hydrogen bonding, whilst atoms not directly involved in the formation of hydrogen bonds (i.e. carbon), can be calculated using a smaller basis set, reducing the computational requirements. The structures and minimum energies of the individual molecules were calculated first. For UDIM **2** we first performed calculations on the version without the ^{t}Bu groups present (compound **12**) to minimize computational time. The folded conformer **12-*fo*** is more stable than the extended conformer **12-*ex*** by 3.6 kJ mol^{-1} (Figure 6a). We also preformed the calculations for all conformers and tautomers of **2** – the energies of which were all within 5 kJ mol^{-1} (see ESI). We next calculated the structure and energy of DAN **3** in the binding conformation (again the butyl chains were shortened to methyl groups (compound **18**)); alternative amide rotamers were not considered because, given our earlier observations on amidonaphthyridine derivatives,[38] these are disfavoured due to electrostatic repulsion with the nitrogen atoms in the aromatic

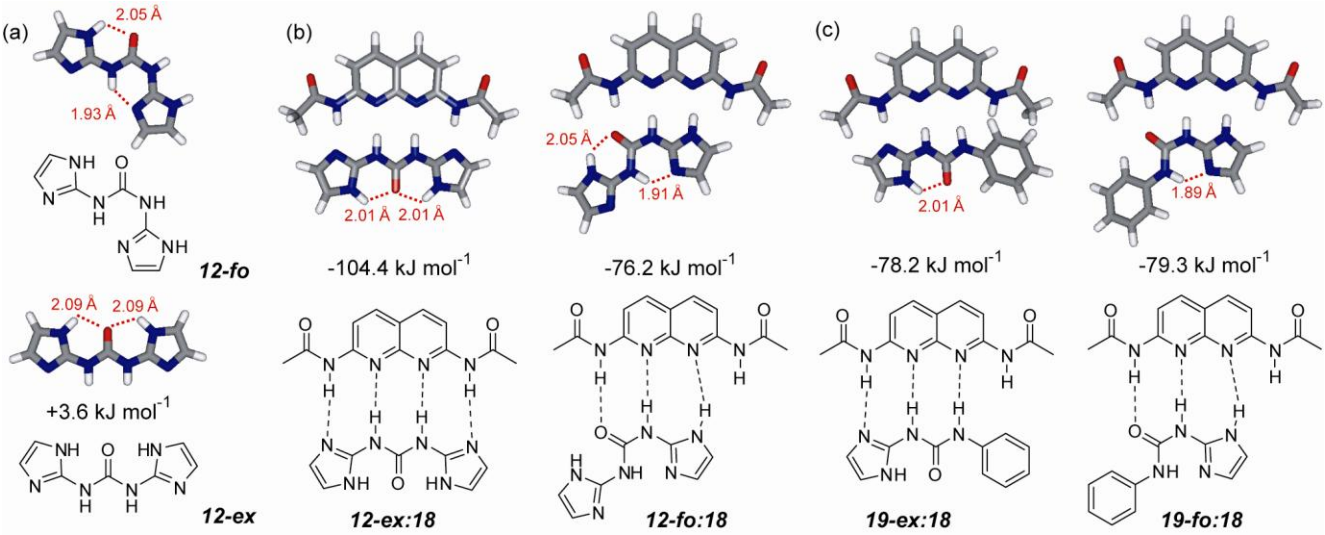


Figure 6 Optimized geometries and calculated energies of monomers and complexes used in this work as calculated using DFT (B3LYP, C, 6-31G; N/O, 6-31G*; H, 6-31++G**), (a) UDIM in folded and extended conformations, (b) UDIM **12** bound to DAN **18** in folded and extended conformations, (c) UIM **19** bound to DAN **18** in both conformations.

ring.[38] Calculations on the dimers were run using counterpoise in order to overcome the basis set superposition error, using the basis set outlined above. The calculations were limited to **12**:**18** due to the size of **2**. Energies are counterpoise corrected and given for the global minimum. The large energies relative to those obtained by experiment derive from the fact that the calculations were performed in the gas phase and no solvation energies are incorporated. For this reason, caution must be exercised in interpreting the results however the qualitative observations are still useful. The UDIM:DAN complex when optimized using UDIM in the extended conformation **12-*ex*:18** has a binding affinity of -104 kJ mol^{-1} and in the folded conformation **12-*fo*:18** an energy of -76 kJ mol^{-1} (Figure 6b). For comparison the energies of both conformers of UIM (with the tBu groups removed **19)** bound to DAN **18** were also calculated and found to be -78 and -79 kJ mol^{-1} respectively (Figure 6c). These results suggest that (a) the difference in energy between the folded and extended states of UDIM **2/12** is quite small and (b) the extended conformation of UDIM **12** (and most likely **2**) should be favourably recognized by DAN derivatives with reasonable affinity, which conflicts with the experimental observations. There are a number of explanations for this discrepancy – (i) solvation energies affect the solution stability of the two conformations of UDIM **2/12** to the extent that the difference in stability is much larger than calculated (ii) solvation energies offset the difference in experimental binding energies (**3**:**2-*ex*** and **3**:**2-*fo***) such that they do not match with those calculated (**18**:**12-*ex*** and **18**:**12-*fo***) (iii) the calculated energies do not account for additional factors. In the latter case, one such factor may be the bond lengths within the covalent framework. Because aromatic C-N bonds are shorter than aromatic C-C bonds, the DAN **18** motif is slightly curved, not linear.[46] Similarly, the intramolecular hydrogen bonds for UDIM **12-*fo*** undergo minimal change upon complexation with DAN **18** to form three hydrogen-bonds, whereas in UDIM 1**2-*ex*** they become shorter upon complex formation with DAN **18**, implying significant distortion of the covalent framework of UDIM **12** is necessary in order to form the complex. For triply hydrogen-bonded complexes based upon UIM **19**, these distortions are not observed. Thus in order to interact through the desired ADDA-DAAD array both DAN **3/18** and UDIM **2/12** must adopt distorted and unfavorable geometries. A further consideration is that the *t*-butyl groups in conformation **2-*ex*-i-ii** might sterically impede interaction with **3,** however we do not attribute the weak binding affinity solely to this feature as our data clearly indicate interconversion between the different conformations and tautomers is possible; if strong hydrogen bonds formed this would drive the equilibrium in favour of conformations capable of making these interactions.

Conclusions

In conclusion, we have described the design and synthesis of a novel hydrogen-bonding motif that has the potential to present a quadruple ADDA array. Lower than anticipated binding affinities towards a complementary partner were observed that may be attributed to a number of factors. This may include an energetic bias for the folded conformer alongside subtle differences in shape complementarity and steric interactions that hinder the desired interaction. The results highlight the fact that the conformational and tautomeric states adopted by hydrogen-bonding motifs are difficult to predict and control. In addition to the UPy[16] and ureidodiguanidinium[31] examples referred to previously, where maximizing non-covalent interactions seems to drive formation of a heterocomplex, the ureidodiguanidinium which is capable of presenting a DDDD^{+} and engages in high affinity interactions with AAAA arrays (described recently by the Leigh group)[31] is a similar motif to UDIM **2**. On the other hand, our observations follow the trend observed with DPUs, where a strong preference for the folded conformation dominates molecular recognition behaviour. Although undesired states can be designed out as in the case of ureidoguanosine (UG), where the energy of the self-complementary conformer is much higher than the conformer that presents the desired ADDA array

(facilitating high affinity and fidelity interaction with DAN derivatives), this remains difficult to do predictably.[49] Our future work in this area will focus on this objective.

Experimental

General Considerations

All reagents were purchased from Aldrich or Alfa-Aesar and used without further purification unless otherwise stated. Where anhydrous solvents were required, THF was freshly distilled from sodium benzophenone ketyl radical, CH_2Cl_2 was freshly distilled from calcium hydride and $CHCl_3$ was freshly distilled from calcium chloride under a nitrogen atmosphere. Anhydrous DMF was obtained "sure-sealed" from Sigma-Aldrich. Triethylamine was distilled from calcium hydride and stored, under nitrogen, over potassium hydroxide pellets. All non-aqueous reactions were carried out under a nitrogen atmosphere. Analytical thin layer chromatography (TLC) was conducted using Merck Kieslegel 0.25 mm silica gel pre-coated aluminium plates with fluorescent indicator active at UV_{254}. Purification by column chromatography was carried out using Merck Kieselgel 60 silica gel. NMR spectra were obtained using Bruker DRX500 or Bruker DPX300 spectrometers operating at 500.13 MHz or 300.13 MHz for ^1H spectra and 125.76 MHz or 75.47 MHz for ^{13}C spectra as stated. Proton spectra are referenced to TMS at 0.00 ppm, and carbon spectra to $CDCl_3$ at 77.4 ppm, unless otherwise stated. Melting points were determined using a Griffin D5 variable temperature apparatus and are uncorrected. IR spectra were obtained using Perkin-Elmer FTIR spectrometer. Microanalysis was carried out on a Carlo Erba Elemental Analyser MOD 1106 instrument. High Resolution Mass Spectra (HRMS) were recorded on a Micromass GCT Premier using electron impact ionisation (EI) or a Bruker Daltonics micrOTOF using electro spray ionisation (ESI).

Synthetic Procedures

2-*tert*-Butoxyamido-4-*tert*-butylimidazole and UIM **1**, were synthesized as described previously by our group.[36]

1,3-Bis(5-tert-butyl-1H-imidazol-2-yl)urea 2

2-*tert*-Butoxyamido-4-*tert*-butylimidazole **6** (1.36 g, 5.68 mmol) was dissolved in 1M HCl-EtOH (100 mL) before being stirred at reflux for 17 hr. It was then evaporated to dryness *in vacuo* to give the aminoimidazole hydrochloride as a yellow oil. Triethylamine (1.98 mL, 14.20 mmol) was added to a solution of the aminoimidazole hydrochloride (1.00 g, 5.68 mmol) in THF (100 mL) and the reaction mixture was stirred at room temperature for 2 hr. It was then heated to reflux and 1,1-carbonyldiimidazole (0.55 g, 3.41 mmol) was added. The reaction mixture was then heated at reflux for a further 20 hr before being cooled to room temperature and filtered. The filtrate was evaporated *in vacuo* and the resultant solid was purified by column chromatography (gradient elution: 0:1-1:19 MeOH-CH_2Cl_2) followed by trituration (MeOH) to give *1,3-bis(5-tert-butyl-1H-imidazol-2-yl)urea* **2** (150 mg, 17%) as a colourless solid; m.p. decomposes > 280 °C (Found: C, 59.1; H, 7.95; N, 27.7; $C_{15}H_{24}N_6O$ requires C, 59.2; H, 7.95; N, 27.6%); R_f 0.45 (1:9 MeOH-CH_2Cl_2); δ_H (300 MHz, $CDCl_3$); 15.20 (1H, s, NH), 12.40 (1H, s, NH), 11.16 (1H, s, NH), 10.96 (1H, s, NH), 6.30 and 6.26 (2H, 2 × s, 2 × ArCH), 1.33 (18H, s, ^{t}Bu); δ_C (125 MHz, $CDCl_3$); 162.9, 162.8 (× 2), 162.7 (× 2), 149.9, 149.1 (× 2), 149.0, 148.1, 141.3 (× 2), 140.1 (× 2), 136.7, 134.7, 111.8, 110.4 (× 2), 103.2, 30.9 (× 3), 30.8, 30.2 (× 2), 29.8 (× 2), 29.7; ESI-HRMS found *m/z* 305.2087 $[M + H]^+$ $C_{15}H_{25}N_6O$ requires 305.2084.

1,3-Di(1*H*-benzo[*d*]imidazol-2-yl)urea 9

2-Aminobenzimidazole (100 mg, 0.75 mmol) was added to a solution of triethylamine (262 μL, 1.88 mmol) in THF (10 mL). The reaction mixture was then heated to reflux and 1,1-carbonyl diimidazole (73 mg, 0.45 mmol) was added. The reaction mixture was then heated at reflux for a further 20 hr, before being cooled to 0 °C and filtered. The resultant solid was washed (Et_2O) to give *1,3-di(1*H*-benzo[*d*]imidazol-2-yl)urea* **8** (51 mg, 45%) as a colourless solid; m.p. > 350 °C; R_f 0.00 (1:9 MeOH–EtOAc); δ_H (300 MHz, DMSO-d_6); 12.15 (2H, s, 2 × NH), 7.47-7.44 (4H, m, 4 × ArCH), 7.14-7.11 (4H, m, 4 × ArCH); Molecule was too insoluble to obtain δ_C; ν_{max}/cm^{-1} (neat); 3369-2681 (br), 1633, 1593, 1564, 1274, 752; ESI-HRMS found *m/z* 293.1142 $[M + H]^+$, $C_{15}H_{13}N_6O$ requires 293.1145.

7-Amino-1,8-naphthyridin-2-ol 15

Concentrated sulphuric acid (10 mL) was added dropwise to a ground mixture of 2,6-diaminopyridine **14** (2.2 g, 20.0 mmol) and malic acid (3.0 g, 22 mmol) cooled to 0 °C. The reaction mixture was then heated to 110 °C for 3 hr before being cooled to 0 °C. Ammonium hydroxide solution was then added dropwise to pH 9 and the reaction mixture was filtered and washed with water and Et_2O to give *7-amino-1,8-naphthyridin-2-ol* **15** (3.04 g, 95%) as an orange solid; m.p. decomposes > 350 °C [Lit. > 350 °C][50]; R_f 0.00 (EtOAc); δ_H (300 MHz, DMSO-d_6); 11.91 (1H, s, OH), 7.66 (1H, d, J = 9.5 Hz, ArCH), 7.65 (1H, d, J = 8.4 Hz, ArCH), 7.03 (2H, s, NH_2), 6.36 (1H, d, J = 8.4 Hz, ArCH), 6.12 (1H, d, J = 9.5 Hz, ArCH); δ_C (75 MHz, DMSO-d_6); 164.1, 160.9, 150.7, 140.1, 137.7, 115.2, 105.6, 105.4; ESI found *m/z* 162.1 $[M + H]^+$, $C_8H_8N_3O$ requires 161.2.

***N*-(7-Hydroxy-1,8-naphthyridin-2-yl)pentanamide 16**

Valeroyl chloride (3.70 mL, 31.7 mmol) was added dropwise to a solution of 7-amino-1,8-naphthyridin-2-ol **15** (3.00 g, 18.6 mmol) in pyridine (20 mL) and heated to 110 °C for 20 hr before the reaction mixture was allowed to cool to room temperature. The pyridine was evaporated and then co-evaporated with toluene and the resultant black residue was dissolved in hot $CHCl_3$ (20 mL) and hexane (200 mL) was added. The solid was filtered and triturated (MeOH) to give *N-(7-hydroxy-1,8-naphthyridin-2-yl)pentanamide* **16** (3.47 g, 76%) as a pale yellow powder; m.p. 316–319 °C; R_f 0.39 (1:19 MeOH–CH_2Cl_2); δ_H (300 MHz, DMSO-d_6); 11.84 (1H, s, OH), 10.48 (1H, s, NH), 8.04 (1H, d, J = 8.5 Hz, ArCH), 7.94 (1H, d, J = 8.5 Hz, ArCH), 7.84 (1H, d, J = 9.4 Hz, ArCH), 6.42 (1H, d, J = 9.4 Hz, ArCH), 2.45 (2H, t, J = 7.4 Hz, CH_2), 1.55 (2H, m, CH_2), 1.32 (2H, m, CH_2), 0.89 (3H, t, J = 7.3 Hz, CH_3); ESI-HRMS found *m/z* 246.1239 $[M + H]^+$, $C_{13}H_{16}N_3O_2$ requires 246.1237.

***N*-(7-Chloro-1,8-naphthyridin-2-yl)pentanamide 17**

N-(7-Hydroxy-1,8-naphthyridin-2-yl)pentanamide **16** (500 mg, 2.04 mmol) was dissolved in $POCl_3$ (10 mL) and heated to 95 °C for 3 hr. The reaction mixture was then cooled and $POCl_3$ was evaporated *in vacuo*. The residue was poured onto iced water (25 mL) and neutralised with concentrated ammonium hydroxide solution under vigorous stirring. The reaction mixture was extracted into CH_2Cl_2 (3 × 20 mL) and then the organic phase

was washed with saturated aqueous sodium bicarbonate (3 × 20 mL), water (3 × 20 mL) and saturated aqueous sodium chloride (20 mL). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo* before being crystallised (1:1 MeOH/MeCN–H_2O) to give *N-(7-chloro-1,8-naphthyridin-2-yl)pentanamide* **17** (380 mg, 71%) as yellow plates; m.p. 159–162 °C; R_f 0.60 (EtOAc); δ_H (300 MHz, $CDCl_3$); 8.57 (1H, d, J = 8.7 Hz, ArCH), 8.35 (1H, s, NH), 8.19 (1H, d, J = 8.7 Hz, ArCH), 8.07 (1H, d, J = 8.3 Hz, ArCH), 7.40 (1H, d, J = 8.3 Hz, ArCH), 2.48 (2H, t, J = 7.7 Hz, CH_2), 1.75 (2H, m, CH_2), 1.44 (2H, m, CH_2), 0.96 (3H, t, J = 7.4 Hz, CH_3); δ_C (75 MHz, $CDCl_3$); 172.7, 154.5, 154.4, 139.1, 138.8, 122.0, 119.2, 115.5, 37.7, 27.3, 22.3, 13.8;; ESI-HRMS found m/z 264.0896 $[M + H]^+$, $C_{13}H_{15}ClN_3O$ requires 264.0898. Found m/z 286.0713 $[M + Na]^+$, $C_{13}H_{14}ClN_3NaO$ requires 286.0718.

***N,N'*-(1,8-Naphthyridine-2,7-diyl)dipentanamide 3**

2-Pentanoylamino-7-chloro-1,8-naphthyridine **17** (100 mg, 0.38 mmol), valeramide (46 mg, 0.46 mmol), potassium carbonate (73 mg, 0.53 mmol), palladium (II) acetate (4 mg, 0.02 mmol) and xantphos (18 mg, 0.04 mmol) were suspended in 1,4-dioxane (10 mL) in a schlenck tube. The reaction mixture was heated to 100 °C for 23hr before being cooled to room temperature and filtered through celite. The solvent was evaporated *in vacuo* and the crude product was purified by column chromatography (gradient elution: 0:1-1:19 MeOH–CH_2Cl_2) followed by crystallisation (MeOH–H_2O) to give *N,N'-(1,8-naphthyridine-2,7-diyl)dipentanamide* **3** (79 mg, 63%) as colourless needles; m.p. 214–216 °C [Lit. 216–217 °C][10] (Found: C, 65.6; H, 7.45; N, 17.0; $C_{18}H_{24}N_4O_2$ requires C, 65.8; H, 7.37; N, 17.1%); R_f 0.66 (1:9 MeOH–CH_2Cl_2); δ_H (300 MHz, $CDCl_3$); 8.44 (2H, d, J = 8.8 Hz, 2 × ArCH), 8.35 (2H, s, 2 × NH), 8.12 (2H, d, J = 8.8 Hz, 2 × ArCH), 2.46 (4H, t, J = 7.5 Hz, 2 × CH_2), 1.73 (4H, m, 2 × CH_2), 1.42 (4H, m, 2 × CH_2), 0.95 (6H, t, J = 7.3 Hz, 2 × CH_3); δ_C (75 MHz, $CDCl_3$); 172.3, 153.9, 153.7, 139.0, 118.4, 113.4, 37.8, 27.3, 22.3, 13.8; ESI-HRMS found m/z 329.1968 $[M + H]^+$, $C_{18}H_{25}N_4O_2$ requires 329.1972.

DOSY experiments

DOSY NMR measurements were made on a Varian Inova 500 MHz spectrometer. All experiments were conducted at 20 °C on $CDCl_3$ solutions, and used a 5 mm ID probe. The bipolar pulse pair simulated echo (BPPSTE) sequence[51] was employed operating in ONESHOT mode.[52] Additional parameters: number of different gradient strengths, 20; gradient stabilisation delay 0.002 s; gradient length 0.002 s; diffusion delay 0.03 s; relaxation delay 2.5 s (following measurement of T1); acquisition time 2 s; kappa (unbalancing factor, 0.2). Data were processed using a 3 Hz line broadening and exponential multiplication. Data were zero-filled once. Spectra were phased and baseline corrected prior to production of the pseudo 2D DOSY plots.

Molecular Modelling

Calculations were run using B3LYP/C, 6-31G; N/O, 6-31G*; H, 6-31++G** level of theory in Gaussian03.

^{1}H NMR titrations

Titrations were performed as described previously.[36]

NOESY Data Aquisition

^{1}H-^{1}H NOESY data (256 increments, 1024 data points) was collected on the individual components and their 1:1 mixtures at 10 mmol concentrations in $CDCl_3$. Spectra were recorded on a Bruker Avance500 instrument operating at 300 K at a frequency of 500 MHz. A pulse sequence of 8.2 µs pulse, 2.0 s delay, 16.4 µs pulse, 1.2 s delay was used (note: spectra have been symmetrised for presentation purposes).

ROESY Data Aquisition

Phase sensitive ^{1}H-^{1}H ROESY experiments were performed on a 1:1 mixture of components UDIM **2** and DAN **3** at 10 mM concentration with respect to each component in $CDCl_3$ solvent. The sample was analysed immediately after preparation to avoid uptake of residual atmospheric H_2O. Spectra were recorded (104 increments, 572 data points) on a Bruker Avance DRX500 instrument at 253 K at a frequency of 500 MHz. A mixing time of 350 ms was applied using a constant wave spin lock mixing method. A pulse sequence repetition of 2.2 secs was used (note: spectra have been symmetrised for presentation purposes).

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Notes and references

[a] *School of Chemistry, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, United Kingdom, Fax: +44 (0)113 3436565; Tel: +44 (0)113 3431409; E-mail: A.J.Wilson@leeds.ac.uk*

[b] *Astbury Centre for Structural Molecular Biology, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, United Kingdom*

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