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# Alternative Strategy for Adjusting the Association Specificity of Hydrogen-Bonded Duplexes

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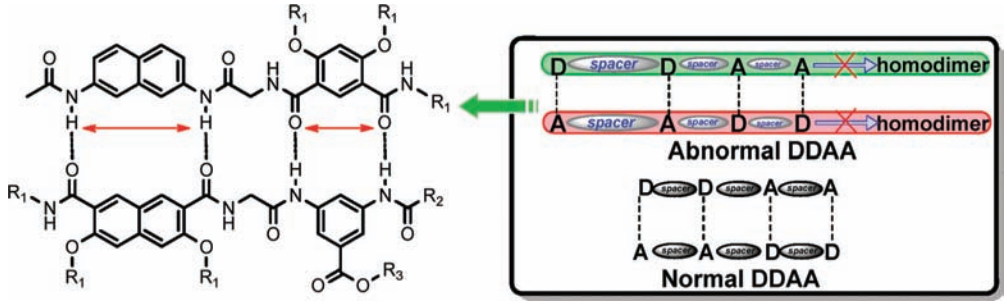
## ABSTRACT




**A strategy for creating new association specificity of hydrogen-bonded duplexes by varying the spacings between neighboring hydrogen bonds is described. Incorporation of naphthalene-based residues has provided oligoamide strands that pair into duplexes sharing the same H-bonding sequences (e.g., DDAA) but differing in the spacings between their intermolecular hydrogen bonds, leading to homo- or heteroduplexes. The ability to manipulate association-specificity as demonstrated by this work may be extended to other multiple hydrogen bonded systems, thereby further enhancing the diversity of multiple hydrogen-bonded association units for constructing supramolecular structures.**

Artificial duplexes with arrays of hydrogen bond donors (D) and acceptors (A) are of great importance for the design of various host−guest and self-assembling systems.[1] Many hydrogen-bonded complexes of different size, shape, and number of hydrogen bonds have been utilized as association modules.[2,3] Among known examples, the ureido-pyrimidinone derivatives (UPy)[4] and deazapterin (DeAP)[5] carrying the DDAA hydrogen-bonding sequence have been successfully applied for developing supramolecular polymers,[2a,d,6] and for constructing other molecular architectures[7] because of their high dimerization constant ($K_{dimer} \approx 10^7$ $M^{-1}$ in

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$CDCl_3$) and synthetic accessibility. Efforts on addressing the limit often associated with the simultaneous presence of interconverting tautomers in heterocycle-based systems led to the design of various multiply (number of hydrogen bonds >3) hydrogen-bonded homo- and heterodimers with enhanced strength, directionality, and specificity.[8] Recently, a self-complementary amidourea motif stabilized by four intermolecular hydrogen bonds was found to be highly stable in polar solvents of low polarity.[9]

Besides stability, specificity is the other major parameter that determines the success and usefulness of designed association modules.[10] Among known systems, few allow the adjustment (or programming) of association specificity. We have constructed oligoamide strands carrying multiple amide H and O atoms. These oligoamide strands associate into duplexes via multiple hydrogen-bonding interactions involving their amide H and O atoms. Our hydrogen-bonded duplexes[11] were found to be free of the tautomerism that typically accompanies heterocycle-based complexes. In addition, secondary electrostatic interaction,[12] a phenomenon associated with most hydrogen-bonded heterocycles, is absent in our oligoamide duplexes. As a result, the stability of a hydrogen-bonded duplex is readily predictable, being directly proportional to its number of interstrand hydrogen bonds. The tuning of hydrogen-bonding sequence-specificity has so far relied on varying the arrangement of hydrogen bond donors and acceptors. Herein we describe a new approach that adds to the diversity of association specificity while maintaining the same sequence of hydrogen donors and acceptors.

Instead of varying the arrangement of hydrogen bond donors and acceptors, it was reasoned that changing the spacings between interstrand hydrogen bonds in a duplex should lead to altered association specificity. Such a possibility was first explored by the design of two oligoamide strands **1** and **2** that contain napthalene residues with the spacing of 7.2 Å between two close H-bonds. The benzene residies, however, could only afford H-bonding sites with a distance of 4.9 Å between two neighboring H-bonds, which is obviously shorter than naphthalene units (Figure 1A).[13] Sharing the same (DDAA) hydrogen-bonding sequence that leads to the self-dimerization of the originally designed oligoamide (e.g., **6** in Figure 2) with the spacing of ca. 4.8 Å, neither **1** nor **2** could undergo self-dimerization because of the expanded spacing between the amide NH or carbonyl groups attached to the napthalene residue. Instead, strands **1** and **2** carry complementary hydrogen-bonding sequences, pairing of which leads to a heteroduplex **1·2**.

Oligoamide strands **1**−**5** and **7** were synthesized in the presence of EDCI and HOBt by standard amide coupling chemistry.[13,14] These strands could either self-dimerize (i.e., **3** and **7**) into a homoduplex or pair (i.e., **1** and **2**, or **4** and **5**) into a heteroduplex.

Oligoamide strand **6**, with its DDAA array, was found to dimerize in chloroform with a binding constant of $6.5 \times 10^4$ $M^{-1}$.[11a] In contrast, $^1$H NMR dilution experiments revealed a dimerization constant of $\sim$33 $M^{-1}$ for **1** in chloroform, confirming the weak self-association expected of this strand. However, a $^1$H NMR titration experiment using **1** upon addition of **2** from 1:0 to 1:1.8 at 1.8 mM revealed a

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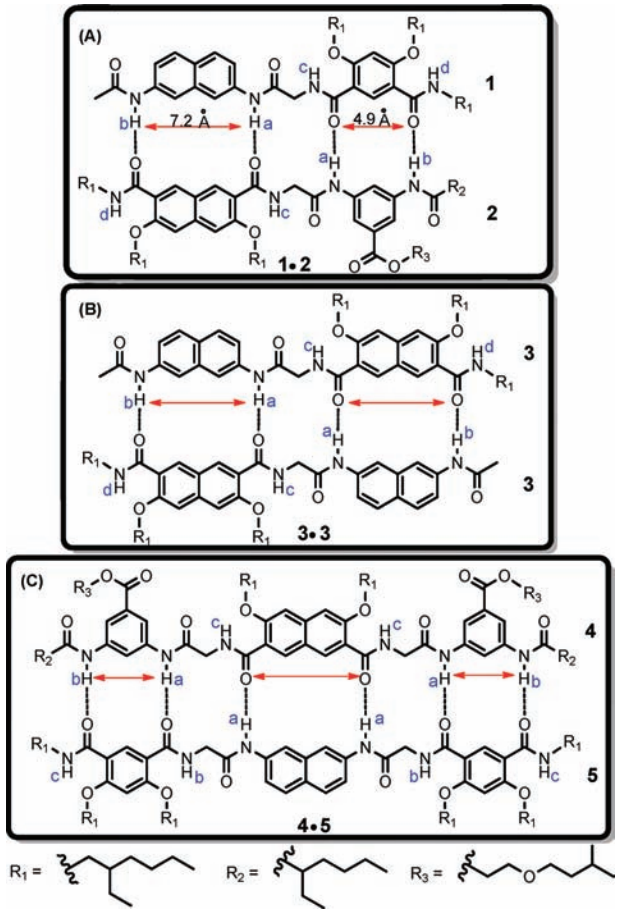


**Figure 1.** Oligoamide strands containing naphthalene residues and sharing the same (DDAA) sequences but assemble into heteroduplexes **1**·**2** and **4**·**5** or homoduplex **3**·**3**.

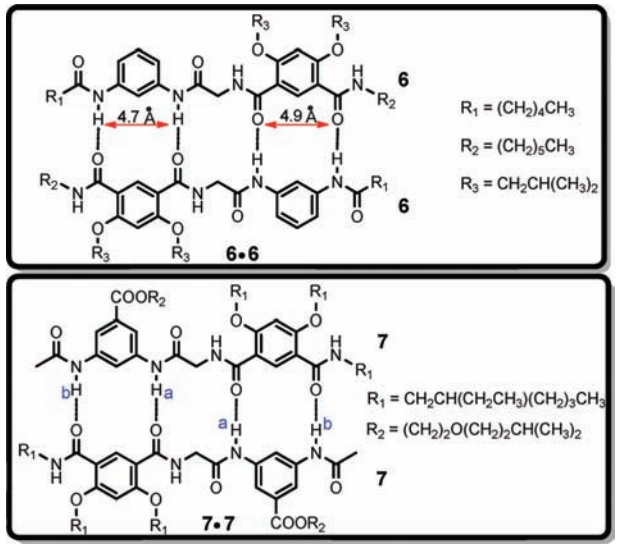


**Figure 2.** Self-complementary oligoamide dimer structures of **6**·**6**[11a] and **7**·**7** with the DDAA arrays.

**Table 1.** Binding Constants ($M^{-1}$) from $^1$H NMR Dilution and Titration Experiments[a]

| compound | $K_{dim}$ ($M^{-1}$) | compound | $K_a$ ($M^{-1}$) |
|---|---|---|---|
| **1**·**1** | $(3.3 \pm 0.3) \times 10$ | **1**·**2** | $(1.9 \pm 0.6) \times 10^4$ |
| **2**·**2** | $(1.7 \pm 0.2) \times 10$ | **1**·**7** | $(9.6 \pm 0.8) \times 10^2$ |
| **3**·**3** | $(1.6 \pm 0.3) \times 10^3$ | **2**·**7** | $(6.8 \pm 0.7) \times 10^2$ |
| **4**·**4** | $(4.2 \pm 0.3) \times 10$ | **4**·**5**[b] | $(2.4 \pm 0.7) \times 10^5$ |
| **5**·**5** | $(5.2 \pm 0.4) \times 10$ | | |

[a] Measured by a dilution and titration experiment in $CDCl_3$. Errors represent the standard error of the data fit to the calculated isotherm. [b] Measured by a titration experiment in $CDCl_3$−5% DMSO-$d_6$.

substantial downfield change for **1**-H$^a$ ($\Delta\delta = 1.10$ ppm) and **1**-H$^b$ ($\Delta\delta = 2.15$ ppm), indicating that protons H$^a$ and H$^b$ of **1** are involved in intermolecular hydrogen bonding, whereas protons **1**-H$^c$ ($\Delta\delta = 0.58$ ppm) and **1**-H$^d$ ($\Delta\delta = 0.23$ ppm), which are intramolecularly hydrogen-bonded, experienced only a minor change. By fitting the concentration-dependent change of the chemical shifts of proton H$^a$ of **1** to a 1:1 binding motif,[15] an association constant of $1.9 \times 10^4$ $M^{-1}$ was obtained (Table 1).[13] Two-dimensional NOE spectroscopy (2D-NOESY) in $CDCl_3$ provided the evidence for formation of heteroduplex **1**·**2**.[13] Cross-strand NOEs between **1**-H$^a$ and **2**-H$^f$, **1**-H$^b$ and **2**-H$^e$, **1**-H$^l$ and **2**-H$^a$, **1**-H$^l$ and **2**-H$^j$, and **1**-H$^f$ and **2**-H$^f$ in a 1:1 mixture of **1** and **2** (10 mM each) were observed. Another piece of evidence confirming the formation of hydrogen-bonded **1**·**2** came from mass spectrometry (ESI), which showed a peak (*m*/*z* 1789.23) of high intensity that points to the presence of the species [**1**·**2** + H]$^+$.[13] Furthermore, ESI result clearly shows the absence of both **1**·**1** dimer and **2**·**2** homodimers, demonstrating the fidelity of heterodimer formation.

The DDAA sequences of **1** and **2**, although they bear nominal similarity in sequence order to benzene-based oligoamide strands such as **6** and **7**,[11a] only led to the formation of a heteroduplex. Such an altered association specificity is further demonstrated by a competition experiment that involved adding stand **7** from 0 to 2 equiv to a mixture of **1** and **2** (2.0 mM each) in 1:1 molar ratio. The chemical shifts of both protons H$^a$ and H$^b$ of **1** and **2** showed insignificant changes ($\Delta\delta = 0-0.09$ ppm) in the presence of **7** (Figure 3a)[13] as compared to a change of 0.24 or 0.19 ppm in the control experiments by adding **7** to **1** or to **2**. This observation clearly indicates that duplex **1**·**2** experiences only a very small change despite the presence of an oligoamide strand having also borne a DDAA sequence.

Besides expanding diversity of association specificity, the availability of naphthalene-derived building blocks allows many new duplexes to be constructed. For example, with two naphthalene residues in its structure, strand **3** is self-complementary and was found to dimerize into hydrogen-bonded duplex **3**·**3** with a $K_{dimer}$ of $1.6 \times 10^3$ $M^{-1}$ obtained by standard nonlinear least-squares regression analysis of the concentration-depdendent chemical shift changes of proton **3**-H$^b$.[13] 2D-NOESY experiment (10 mM in $CDCl_3$) and ESI-

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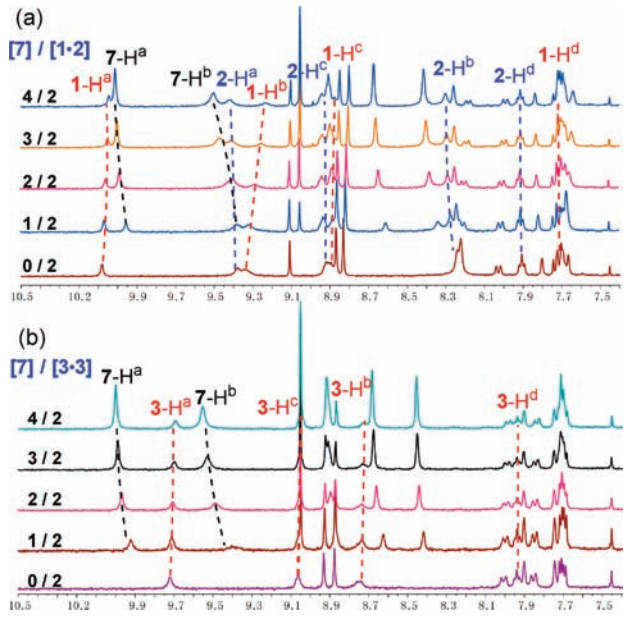


**Figure 3.** $^1$H NMR titration of strand **7** from 0 to 2 equiv into (a) duplex **1·2** (2.0 mM) and (b) duplex **3·3** (2.0 mM) in $CDCl_3$ at 298 K.

HRMS ([**3·3** + H]$^+$, *m*/*z*: 1647.75) provided the evidence for formation of homoduplex **3·3**.[13] When strand **3** (2.0 mM) was titrated with **7** from 0 to 2 equiv., the chemical shifts for the amide protons **3**-H$^a$ and **3**-H$^b$ were found to undergo negligible change ($\Delta\delta \approx 0.03$ ppm), which exhibits that the presence of strand **7** inflicted insignificant impact on the binding affinity of **3·3** (Figure 3b) and thus led to the high association specificity of duplex **3·3**. It is noteworthy that enlarged distance between the near two H-bonding sites caused a decrease in binding ability ($1.6 \times 10^3$ M$^{-1}$) compared to normal benzene-based oligoamide duplexes such as **6** ($\sim 10^4$ M$^{-1}$).

While shorter naphthalene-derived duplexes with matched sequence demonstrated appreciable affinity, does extension to the longer ones lead to the increased binding capability? Thus, strands **4** and **5** were prepared and examined for their hydrogen bond-mediated association. With heterocomplementary H-bonding sequences DDAADD and AADDAA, strands **4** and **5** are supposed to pair via six interstrand hydrogen bonds. 2D-NOESY experiment (10 mM in $CDCl_3$) provided the evidence for the formation of heteroduplex **4·5**.[13] Cross-strand NOEs between **4**-H$^d$ and **5**-H$^a$, **4**-H$^i$ and **5**-H$^a$, **4**-H$^a$ and **5**-H$^i$, and **4**-H$^a$ and **5**-H$^g$ were observed in a 1:1 mixture of **4** and **5** (5.0 mM each). In the ESI-HRMS spectra, an ion peak corresponding to duplex of **4·5** (*m*/*z*: 2640.62 for [**4·5** + H]$^+$) was observed. $^1$H NMR titration experiment of **5** with **4** from 0 to 2.0 equiv in $CDCl_3$−5% DMSO-$d_6$ revealed the downfield shift for amide protons **5**-H$^a$ and an upfield shift for protons **4**-H$^a$ and **4**-H$^b$, indicating the specific pairing of the two strands via hydrogen bonding. Since the six-hydrogen-bond benzene-based heteroduplex demonstrated an extremely high binding affinity with $K_{dimer}$ of $\sim 10^9$ M$^{-1}$ in chloroform, the association constant of **4·5** having the same number of H-bonding sites was expected to be beyond the limit of detection by $^1$H NMR technique. Thus, using $^1$H NMR titration in a solvent containing $CDCl_3$−5% DMSO-$d_6$, the $K_a$ of **4·5** was determined to be $2.4 \times 10^5$ M$^{-1}$, which is about 10-fold lower than that of six-hydrogen-bond benzene-derived duplexes ($\sim 10^6$ M$^{-1}$),[11b] strongly suggestive of the spacing effect of H-bonding sites in tuning both binding affinity as well as specificity by incorporating naphthalene residues.

When two different molecular components such as **1** and **2** associate via multiple hydrogen bonding interactions, the self-association of each individual component is usually inevitable, which compromises the stability of the target complex. Examining the self-dimerization of naphthalene-based oligoamide strands **1**, **2**, **4**, and **5** using $^1$H NMR dilution experiments indicates that the self-association of these strands was very weak, with $K_{dimer}$ values of 33, 17, 42, and 52 M$^{-1}$, respectively.[13] Therefore, as compared to our previously developed oligoamide strands, many of which undergo appreciable extent of self-association,[11b] the self-association of oligoamide strands containing naphthalene residues was significantly reduced.

In conclusion, by incorporating naphthalene-based residues, we have demonstrated an approach for creating new association specificity for hydrogen-bonded duplexes. Depending on their specific benzene or naphthalene residues, the designed duplexes may share the same hydrogen-bonding sequences but may form either homo- or heteroduplexes. Manipulating association specificity by varying the spacings between interstrand hydrogen bonds, as demonstrated by this work, may be extended to other multiple hydrogen bonded systems. Detailed studies on these aspects are currently being investigated and will be reported in due course.

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**Supporting Information Available:** Synthesis, analytical data, 2D NMR and ESI mass spectra, and the assemblies of the duplexes. This material is available free of charge via the Internet at http://pubs.acs.org.

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