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An extremely stable, self-complementary hydrogen-bonded duplex†

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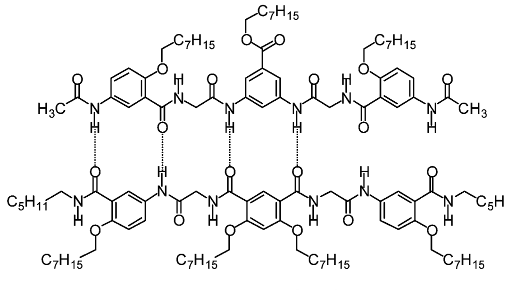
This paper describes the design, synthesis and characterization of a self-complementary six-H-bonded duplex with an association constant greater than 10^9 M^{-1} in $CHCl_3$.

Numerous unnatural self-assembly systems have been developed in recent years.[1–3] Most of these previously described systems are case-dependent, *i.e.*, the individual components carry the information that defines only the formation of the specific assembly. An alternative approach involves the design of highly specific and highly stable recognition units (modules) that are compatible with a variety of structural components. Such recognition modules or "molecular glues" then direct the assembly of these structural components. In this regard, hydrogen-bonded complexes based on rigid heterocycles with multiple H-bonding donor (D) and acceptor (A) sites have received the most attention in recent years.[4–10] Other complexes, most based on H-bonding interactions, have also been reported.[11–21] Highly stable, self-complementary H-bonded complexes are particularly attractive for developing supramolecular homopolymers of very high molecular weights.[22] In spite of the intriguing perspective, only a very small number of self-complementary H-bonded complexes with high stabilities are known. The best known examples involve two pairs of quadruply H-bonded, self-complementary complexes, both based on the AADD-DDAA array, and with association constants greater than 10^7 M^{-1}.[23,24]

We have described the design of molecular recognition units with programmable strength and specificity.[9,25–28] Based on building blocks derived from 1,3-disubstituted benzene rings linked by α-amino acid residues, oligoamide strands with a variety of combinations (sequences) of H-bond donors and acceptors were obtained. H-bonded duplexes were sequence-specifically formed from complementary or self-complementary strands. These duplexes are free of secondary electrostatic interactions and tautomerism, *i.e.*, the stability of a duplex depends only on the number of intermolecular hydrogen bonds it contains. For quadruply H-bonded duplexes, the association constants lie in the 10^4 M^{-1} range. Increasing the number of H-bonds led to a dramatic increase in the stability of the corresponding H-bonded duplex. For example, we reported recently that the six-H-bonded duplex **1·2**, consisting of two mutually complementary H-bonding sequences, was extremely stable. In chloroform, the association constant of **1·2** was so high ($\geq 10^9$ M^{-1}) that it could not be measured accurately by ^1H NMR or isothermal titration calorimetry.[26]

Given the sequence-independent stability and structural simplicity of our duplex system, the development of extremely stable, self-complementary H-bonded duplexes should be readily achievable. By simply increasing the number of intermolecular hydrogen bonds, a rapid increase in the diversity of duplexes is expected. For duplexes with six H-bonding sites, four self-complementary duplexes with unique H-bonding sequences are possible. We report here the design and characterization of our first six-H-bonded, self-complementary duplex that contains the AADADD-DDADAA array (Fig. 1).‡



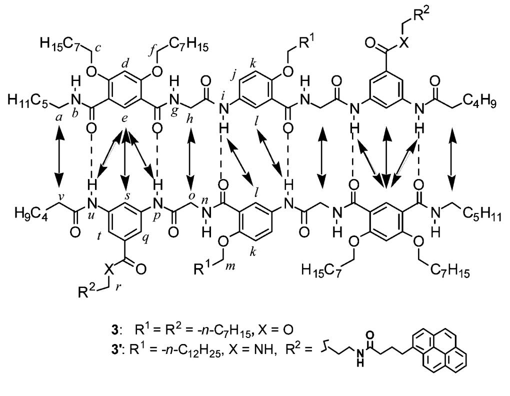
1

2

1•2 ($K_a \geq 10^9$ M^{-1})

At room temperature, ^1H NMR binding studies from 10 mM to 2 μM on **3·3** in $CDCl_3$ found no concentration-dependent change in the chemical shifts of its amide NH signals. Assuming a 10% dissociation of **3·3** at 2 μM, a lower limit of 4.5×10^7 M^{-1} for the association constant of duplex **3·3** was estimated. Based on the inaccurately determined K_a of duplex **1·2**, the association constant for **3·3** can only be higher than this conservatively estimated lower limit. Attempts were also made to measure the association constant of **3·3** by increasing temperatures. Unfortunately, no concentration-dependent changes in NH chemical shifts were observed at raised temperatures (up to 60 °C), which in turn indicated the high stability of **3·3**. It needs to be pointed out that no new peaks appeared as temperature or the concentration of **3·3** was varied, indicating that **3·3** was not influenced by tautomeric equilibrium.

Two-dimensional (2D) NMR (NOESY, 400 MHz) experiments revealed numerous inter-strand NOEs at both high (20 mM) and low (1 mM) concentrations.[29] As indicated in Fig. 1, these NOEs include those between protons *a′* and *v′*, *e′* and *s′*, *h′* and *o′*, as well as *i′* and *l′*. The observed NOEs are fully consistent with the formation of duplex **3·3**. The fact that



3 (3')

3 (3')

Fig. 1 The design of the self-complementary duplexes **3·3** and **3′·3′**. Cross-strand NOEs from the NOESY spectrum of **3·3** are shown by arrows. The H atoms are labelled for the convenience of discussion (see text).

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† Electronic supplementary information (ESI) available: 2D ^1H NMR spectra; details of mass spectrometry and fluorescence experiments; fluorescence emission spectra; synthetic procedures. See http://www.rsc.org/suppdata/cc/b3/b301791e/

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NOESY spectra recorded at 1 mM or 20 mM of **3·3** showed the same inter-strand contacts again indicates that there was no isomerization of **3** as concentration was varied.

The formation of duplex **3·3** was also confirmed by mass spectrometry (ESI). Fig. 2 shows the spectrum corresponding to the “molecular ion” [**3** + **3** + Cl]$^{-1}$ ($C_{140}H_{220}ClN_{12}O_{22}$). The experimentally measured isotopic distribution shows excellent agreement with that of the calculated one. Interestingly, the mass spectrum also indicates the existence of 20% of the doubly charged ion [**3** + **3** + Cl]$_2^{2-}$.

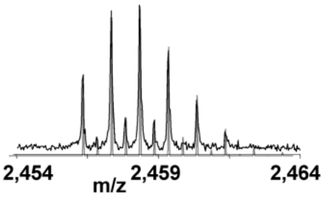

Fig. 2 Isotopic distributions of the [**3·3** + Cl]$^{-1}$ ion as determined by ESI-FTICRMS from chloroform–Ph_4PCl solution (black). The experimental result matches very well with that from calculation (grey).

The stability of duplex **3·3** was then compared with that of **1·2** by assaying with straight-phase thin-layer chromatography (TLC).[26] The polarities of duplexes **1·2** and **3·3** should be similar if **3·3**, instead of single strand **3**, was present in solution. As shown in Fig. 3, the R_f value of duplex **3·3** was found to be the same as that of **1·2** (0.92). This result confirmed that, similar to **1·2**, duplex **3·3** had a very high stability. Interestingly, in contrast to the tailing observed for duplex **1·2**, the absence of any tailing for **3·3** on the TLC plate indicated that **3·3** did not dissociate under the TLC conditions (SiO_2 plate, 10% DMF in $CHCl_3$) and was therefore even more stable than **1·2**.

The dimerization constant of pyrene-labeled **3′·3′** was determined by a fluorescence method. The excimer species of pyrene in solution is well known, showing an emission band that is well separated from that of the monomer.[30] Using a method reported previously,[24] the dimerization constant of pyrene-labeled strand **3’** was determined. Concentration-dependent changes in the intensities of the pyrene excimer band at 509 nm appeared in the range of 10^{-12} M to 10^{-10} M in chloroform. A dimerization constant of $(6.8 \pm 4.1) \times 10^9$ M^{-1} (triplicate runs) was obtained by nonlinear regression analysis[24,31] of the fluorescence data. This very large association constant confirms the above-discussed results from NMR dilution and TLC experiments.

In summary, self-complementary **3·3** was found to be an extremely stable duplex. This duplex should find immediate application in the design of supramolecular homopolymers with very high molecular weights. More importantly, the similar high stability of **3·3** and **1·2** demonstrates the generality of our system for designing duplexes with extremely high stability and fidelity. This fully established these duplexes as reliable, extendable molecular recognition units with systematically tunable stabilities. The other three pairs of six-H-bonded, self-complementary duplexes can be similarly designed and prepared. The ether, acyl and amide side chains of the duplexes provide numerous handles for attaching a variety of structural units such as natural and unnatural oligomer or polymer chains. These self-complementary duplexes (homoduplexes), along with their mutually complementary counterparts (heteroduplexes) with six or more H-bonds, should provide a set of readily available associating modules with extremely high stability and orthogonal specificity for the directed assembly of sophisticated supramolecular structures.

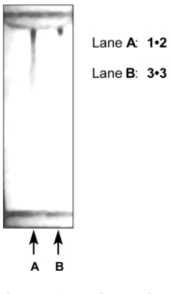

Fig. 3 TLC of duplexes **1·2** (lane A) and **3·3** (lane B). TLC conditions: SiO_2 plate/10% DMF in $CHCl_3$. The developed TLC plate was visualized under short wavelength UV light.

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Notes and references

‡ Compounds **3** and **3’** were synthesized based on similar procedures for preparing **1** and **2**.[26,27] Both **3** and **3’** give satisfactory analytical data. Fluorescence binding experiments were carried out using a Perkin-Elmer LS 55 Luminescence Spectrometer. The dimerization constants were obtained by fitting the fluorescence data into a modified dimerization equation with the program Kaleidagraph on a Macintosh computer.

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