Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron 63 (2007) 5460–5469
Tetrahedron

A branched, hydrogen-bonded heterodimer: a novel system for achieving high stability and specificity

Jason B. Bialecki,* Li-Hua Yuan and Bing Gong*

Department of Chemistry, University at Buffalo, State University of New York, Buffalo, NY 14260-3000, USA

Received 9 February 2007; revised 10 April 2007; accepted 12 April 2007
Available online 20 April 2007

Abstract—Presented is a description of the design, synthesis, and characterization of a novel, branched, six hydrogen-bonded heterodimer termed 'trident'. Two branched oligoamide molecules, **1** and **2**, with complementary hydrogen-bonding sequences 3(AD) and 3(DA), respectively, were found to form a very stable ($K_a \geq 9\times10^6$ M^{-1}) heterodimer in chloroform. Confirmation of the high stability of the heterodimer was obtained through 1D and 2D ^{1}H NMR spectroscopy, thin-layer chromatography (TLC), and vapor pressure osmometry (VPO). The stability of the trident occurs through the cooperative action of six programmed hydrogen-bonding interactions, which are facilitated by the pre-organization of the individual strands through intramolecular hydrogen bonding.

1. Introduction

Recently, there has been intense interest by chemists to construct self-assembling aggregates that will lead to supramolecular structures.[1,2] In nature supramolecular systems are made up of multi-component architectures that assemble through highly specific intermolecular interactions.[3] These highly specific interactions are from the cooperative action of numerous non-covalent forces such as Van der Waals interactions, steric effects, and, most prominently, hydrogen bonding. Nanoscale structures are commonplace throughout nature. However, the preparation of artificial nanostructures presents one of the most daunting challenges to modern chemists and biochemists alike. Based on these non-covalent forces, numerous artificial self-assembly systems have been developed.[4–11]

The most well known and most intensely studied example of self-assembly through molecular recognition is the DNA double helix.[12] The pairing of complementary nucleobases through 'sticky ended' association is the key driving force for the formation of the DNA double helix.[13] The formation of this secondary structure is entropically unfavorable. However, this energy loss is made up for by the cooperative action of many enthalpically favorable H-bond interactions between complementary nucleobases. This sequence-specificity between nucleobase pairs is so powerful that duplex DNA is the molecule with the most readily predictable and programmable intermolecular interactions.

Seeman[14] has taken advantage of the programmable sequence-specificity of DNA and has reported an elegant strategy for the construction of a variety of DNA-based nanostructures. Conjugating these DNA-based nanostructures to other structural units such as peptides and unnatural structures could implement specific intermolecular interactions but synthetically this would be a great challenge. Besides, DNA must be used in aqueous media. Many applications, particularly in materials science, are not compatible with aqueous media. Unnatural H-bonding units with high sequence-specificity should greatly facilitate the design of a variety of nanostructures. In fact, the design of unnatural H-bonding structural motifs that demonstrate DNA-like programmable sequence-specificity has been an area of intense interest. The development of a diverse set of molecular subunits as associating units for supramolecular assembly represents a continuing challenge.[15]

Meijer et al.[16] and Zimmerman and Corbin[17] have reported quadruply hydrogen-bonded heterocyclic complexes with arrays of H-bond donors (D) and acceptors (A). The dimerization between the reported heterocycles shows high specificity. However, these systems are often complicated by tautomerism, which is usually associated with nitrogen-based heterocycles.

Another feature previously reported and commonly observed in H-bonded complexes is secondary electrostatic interactions.[18,19] Such interactions can display either repulsive or attractive forces between adjacent H-bonding sites in

Keywords: Hydrogen-bonding; Heterodimer; Self-assembly; Oligoamide; NMR.
* Corresponding authors. Fax: +1 716 645 6963 (B.G.); fax: +1 201 216 8240 (J.B.B.); e-mail addresses: crotalus97@yahoo.com; bgong@buffalo.edu

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.04.043

host–guest complexes. These non-covalent forces can drastically change the binding strength in complexes with the same number of H-bonding sites. On the basis of differences in secondary electrostatic interactions, triply hydrogen-bonded complexes of DDA·AAD arrays showed roughly a 10-fold increase in stability over triply hydrogen-bonded complexes of DAD·ADA arrays.

A new class of H-bonded duplexes free of secondary interactions and tautomeric effects has been developed.[20] These H-bonded duplexes have unnatural backbones and are characterized by adjustable strength and programmable sequence-specificity. This is the first systematic design of unnatural molecular recognition systems with programmable strength and specificity similar to those demonstrated by DNA. These duplexes are synthesized by linking residues derived from 1,3-diaminobenzene, 1,3-benzenedicarboxylic acid, and 3-aminobenzoic acid with one or more amino acid linkers (usually glycine) to generate oligoamides with various numbers and sequences of H-bonding sites. Ether and ester side chains are incorporated into the molecular structure to adjust solubility. Intramolecular H-bonds, especially the S(6) type[21] and the unconventional aromatic H-bond (Fig. 1), are engineered into the oligoamide strands to pre-organize the molecules and facilitate binding. An example of this system is displayed in Figure 2. A duplex strand is designed to sequence-specifically pair with another strand of its complementary sequence. Depending on the H-bonding sequence, either homodimers or heterodimers will form in solution. The binding strength of duplexes greatly increases with an increase in the number of programmed intermolecular hydrogen bonds.[22] Duplexes demonstrate high specificity in their formations by including all possible arrays that are allowable by the amino acid linkers.

A six hydrogen-bonded molecular duplex (Fig. 2), which formed from a 1:1 ratio of oligoamides **A** and **B**, was reported[23] to be extremely stable in chloroform. For high stability to be achieved, each oligoamide strand must pre-organize into a ridged, flat structure prior to duplex formation.

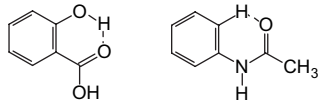


Figure 1. (a) S(6) type H-bonding in salicylic acid (15–20 kJ/mol); (b) unconventional aromatic H-bonding in *N*-phenylacetamide (2–4 kJ/mol).

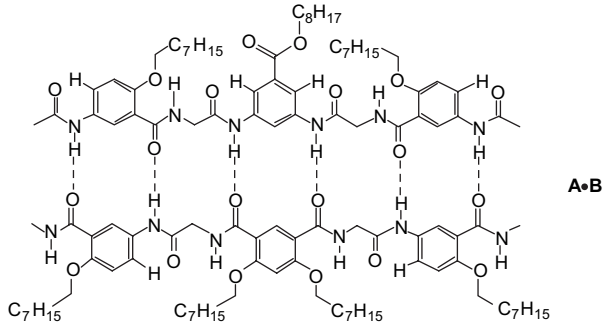


Figure 2. The six H-bonded heterodimer duplex **A**·**B** with a K_a of ~10^9 M^{-1} in $CHCl_3$.

This pre-organization is driven by intramolecular H-bonds such as the S(6) type, which occurs adjacent to the aromatic ring, and the five-membered type, which occurs within the glycine linker.

The rigidity and flatness of each oligoamide strand facilitate the alignment of the programmed donor/acceptor sites and maintain that they work cooperatively. It is possible that either strand (**A** or **B**) could self-dimerize, producing at best, a four hydrogen-bonded homodimer. However, due to the cooperative nature of the hydrogen bonds, the association constant of the six hydrogen-bonded duplex is five orders of magnitude stronger than that determined for the four H-bonded homodimers. The six H-bonded duplex (**A**·**B**) is so greatly favored that no homodimers (**A**·**A** or **B**·**B**) were detectable.[23]

The design of the new branched heterodimer 'trident' rests on the same design of the duplex, with the replacement of the rigid glycine linker with more flexible cyclohexane-based linkers. Figure 3 displays the structure and expected 3D arrangement for trident **1**·**2**. Although some rigidity will likely be lost due to the less rigid linker, the branched oligoamides should still pre-organize in such a way to facilitate binding. The benefits of the new linker system will allow for a third dimension of stability to be added and for the potential of designing an all donor/acceptor complementary heterodimer, which has not yet been obtained with this motif. Also, the design of these branched oligoamides will further discourage self-dimerization. Figure 4 shows that branched oligoamides **1** and **2** can at best self-dimerize to produce two H-bonded homodimers such as **1**·**1** or **2**·**2**. Two H-bonded homodimers similar to this have been reported to have very low association constants

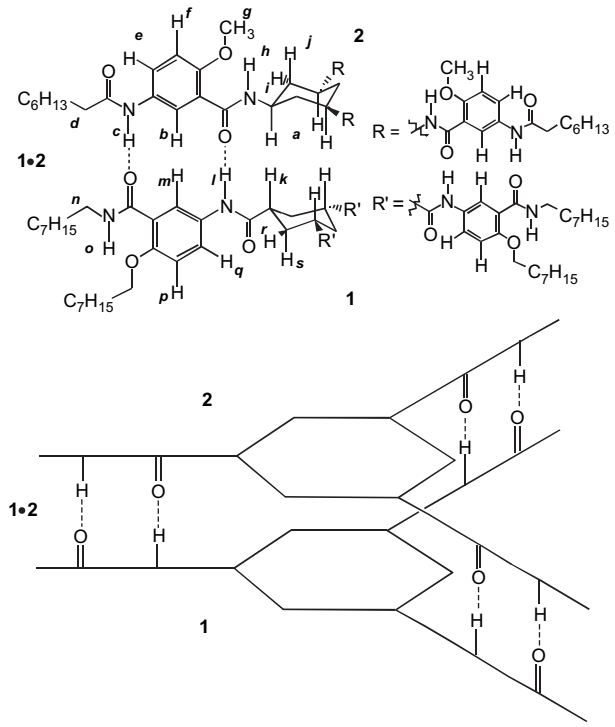


Figure 3. The chemical structure and spatial model of the six H-bonded trident **1**·**2**.

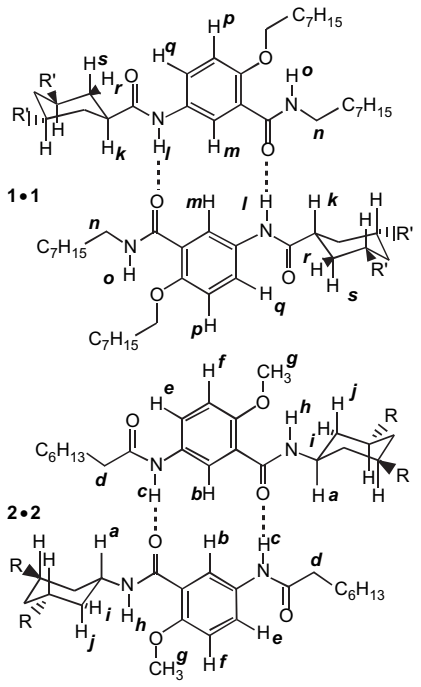


Figure 4. Likely configurations for homodimers **1·1** and **2·2**. Homodimers were undetectable via 2D NOESY studies. For **1·1** no inter-strand NOE contacts were observed between protons ***n*** and ***k***, and ***n*** and ***l***. For **2·2** no inter-strand NOE contacts were observed between protons ***a*** and ***c***, and ***a*** and ***d***.

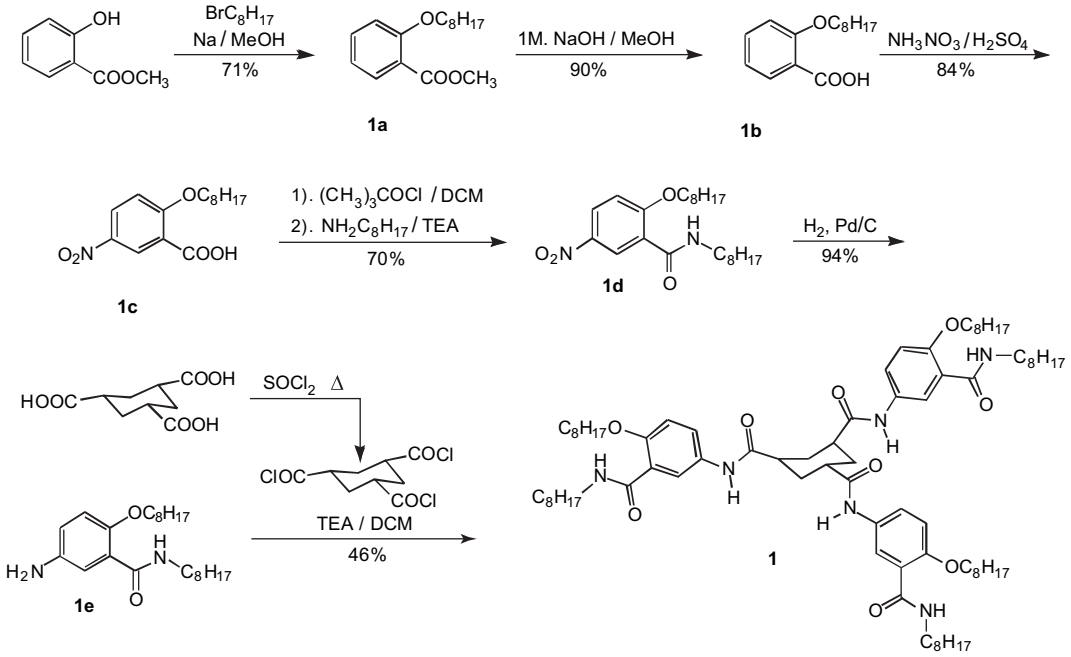
(~25 M^{-1}).[20] Therefore, we conclude that trident **1·2** will not be in equilibrium with homodimer formation(s). In fact, as mentioned in Figure 4 homodimers were undetectable through 2D NMR studies.

The linkers adopted for the synthesis of branched oligoamides **1** and **2** were *cis*,*cis*-1,3,5-cyclohexanetricarboxylic acid and *cis*,*cis*-1,3,5-triaminocyclohexane, respectively. The complementary sequences of **1** and **2** are 3(AD) and 3(DA). We asked if the branched complementary strands **1** and **2** would dimerize effectively and if they would bind more or less tightly than the six hydrogen-bonded duplex **A·B**.

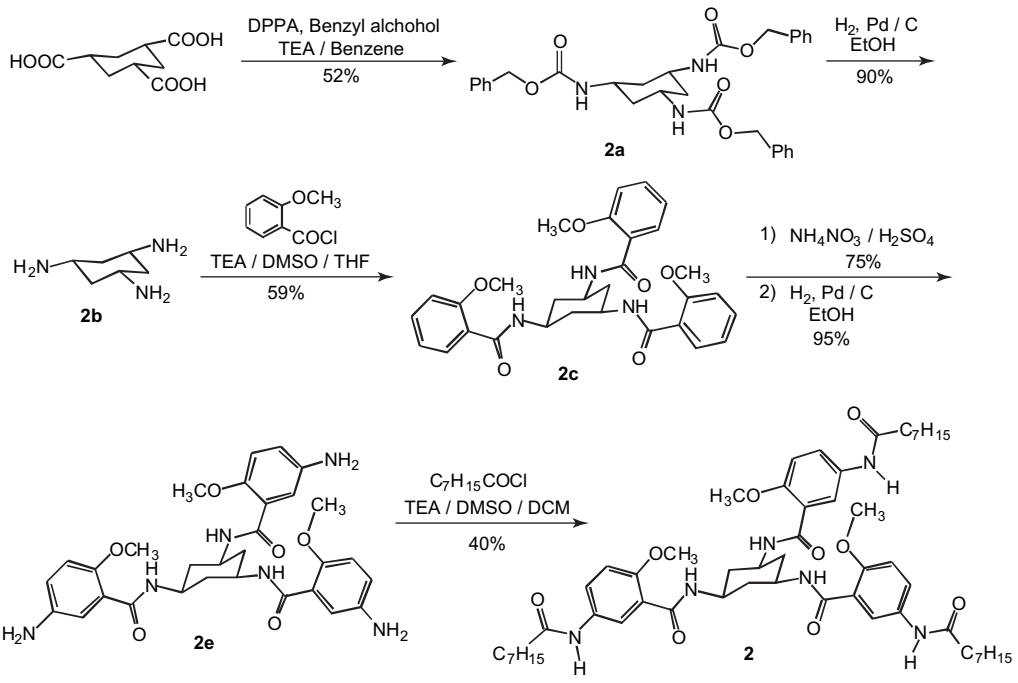
2. Results and discussion

2.1. Synthesis

Oligoamides **1** and **2** were synthesized by replicate coupling steps by conversion of the corresponding carboxylic acid to an acid chloride and coupling it to the appropriate amine. Synthesis of oligoamide **1** (Scheme 1) began with the alkylation of methyl salicylate with 1-bromooctane to afford product **1a**, which was then hydrolyzed to the acid **1b** and then nitrated with ammonium nitrate and sulfuric acid to yield the 5-nitro acid **1c**. The 5-nitro acid **1c** was converted into the corresponding acid chloride using trimethylacetyl chloride, and then treated with octylamine to yield the nitrated amide **1d**, which was then catalytically hydrogenated to produce amine **1e**. Amine **1e** of 3 equiv was coupled to the triacid chloride of *cis*,*cis*-1,3,5-cyclohexanetricarboxylic acid to produce oligoamide **1**. Oligoamide **2** (Scheme 2) was produced through a different approach. First a Curtius rearrangement was performed on *cis*,*cis*-1,3,5-cyclohexanetricarboxylic acid to yield the tricarbamate **2a**, which was then deprotected to *cis*,*cis*-1,3,5-triaminocyclohexane **2b** via catalytic hydrogenation. The triamine **2b** was then treated with 3 equiv of anisoyl chloride to produce the triamide **2c**, which was then nitrated with ammonium nitrate and sulfuric acid to give the tris(5-nitro) compound **2d** in surprisingly good yield. The tris(5-nitro) compound **2d** was catalytically hydrogenated to produce triamine **2e**, which was then finally treated with octanoyl chloride to yield oligoamide **2**.



Scheme 1. Synthesis of branched oligoamide **1**.



Scheme 2. Synthesis of branched oligoamide **2**.

2.2. Characterization of the hydrogen-bonded heterodimer

Trident **1**·**2** was prepared by mixing a 1:1 molar equivalent of strand **1** and strand **2** in $CDCl_3$ (1 mL). Strand **2** had a poor solubility of <5 mM in $CDCl_3$ while strand **1** was quite soluble (~50 mM). A 1:1 mixture of single strands **1** and **2** both became very soluble (>>50 mM) in $CDCl_3$, which was similar to the results previously reported for duplex **A**·**B**.[23] This observation indicated that the heterodimer formed. The formation of the heterodimer **1**·**2** shields the polar amide groups of each strand from the non-polar solvent and thus allows the solubility to drastically increase. Efforts to obtain dimeric crystals for X-ray crystallography were unsuccessful. Thus far, crystal structures have only been reported for homodimer duplexes.[20]

2.3. ^{1}H NMR spectroscopy

^{1}H NMR spectra of **1**, **2**, and **1**·**2** in $CDCl_3$ are shown in Figure 5. All signals were first assigned and then confirmed by assigning NOE intramolecular contacts obtained from 2D NMR (NOESY) (data not shown). Figure 5 reveals significant downfield shifts of the aniline NH signals. At 2 mM, H_I in **1** appeared at 9.01 ppm, the same proton in **1**·**2** at 24 °C appeared at 9.71 ppm. At 2 mM, H_c in **2** appeared at 7.38 ppm, whereas H_c in **1**·**2** at 24 °C appeared at 9.80 ppm. It is worth noting that the signal of H_I is shifted significantly downfield before the mixing of **1** and **2**. This suggests that **1** may not acquire a ridged conformation prior to dimerization and thus may be forming small aggregates. A closer look at the structure of **1** reveals that the stabilizing S(6) intramolecular H-bonds are on the outer portions of the branched molecule. The interior alignment of the molecule may not be completely achieved because it relies on the weak interactions of the unconventional aromatic H-bonds. However, the pre-organization of **2** appears to be much better as reflected by the upfield shift of H_c prior to the mixing of **1** and **2**. Here the S(6) intramolecular H-bonds are toward the center of the molecule where structural rigidity is most important.

To determine the association constant for **1**·**2** ^{1}H NMR binding studies were carried out in $CDCl_3$, by diluting a 1:1 mixture of **1**·**2** from 10 to 0.01 mM. However, no significant changes in the chemical shifts of aniline NH signals upon dilution (H_I moved 0.012 ppm upfield, H_c moved 0.004 ppm upfield) were observed. Assuming a 10% dissociation of **1**·**2** at 0.01 mM,[16] the association constant was conservatively estimated leading to a lower limit of $\geq 9\times10^6$ M^{-1} for the trident **1**·**2**. Our findings are comparable to other association constants that have been reported for duplexes with up to six hydrogen bonds.[22]

^{1}H NMR binding studies were also carried out in 5% DMSO-d_6/$CDCl_3$ and 10% DMSO-d_6/$CDCl_3$ for **1**·**2**. Table 1 shows a list of the various association constants determined for trident **1**·**2**. The trident is substantially weakened by the addition of small amounts of DMSO suggesting that it would not exist in polar solvents such as methanol or water. This trend, however, is consistent for molecular duplexes.

The 2D NMR (NOESY, $CDCl_3$, 500 MHz) studies on **1**·**2** provided the most conclusive evidence for formation of the trident in solution. The formation of the trident should lead to the observation of seven NOE inter-strand contacts as represented in Figure 6. The 2D NMR studies were performed at variable temperatures. The first study was performed at room temperature (24 °C), which led to the following results. In Figure 7 the NOESY spectra of **1**·**2** at 24 °C shows cross-strand NOE contacts between protons ***a*** and ***k***, ***a*** and ***l***, ***c*** and ***n***, and ***d*** and ***n***. Figure 8 shows the

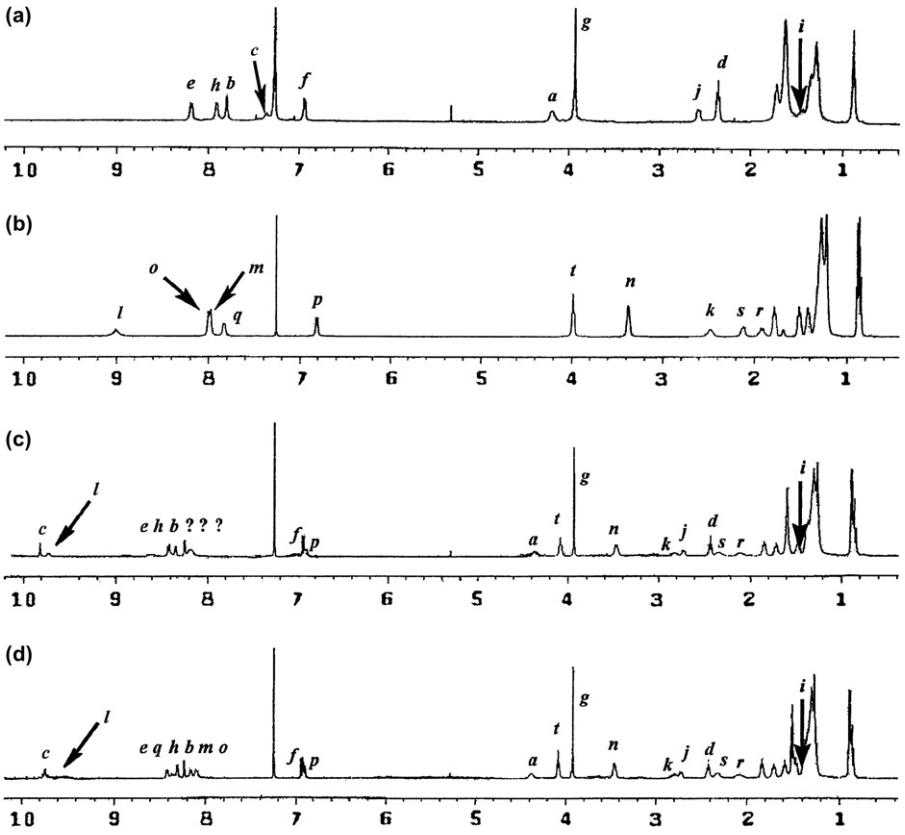


Figure 5. ^{1}H NMR (500 MHz, $CDCl_3$) spectra: (a) 2 mM of **2** at 24 °C; (b) 2 mM of **1** at 24 °C; (c) 2 mM of **1**·**2** at 24 °C; (d) 2 mM of **1**·**2** at 45 °C.

Table 1. ^{1}H NMR dilution studies of trident **1**·**2** in $CDCl_3$ and in DMSO-d_6/$CDCl_3$

Concn range	$CDCl_3$	5% DMSO-d_6/$CDCl_3$	10% DMSO-d_6/$CDCl_3$
	0.01–10.0 mM	0.05–5.0 mM	0.05–10.0 mM
Unit	K_a (M^{-1})	K_a (M^{-1})	K_a (M^{-1})
H_l	$\geq 9\times10^6$	5.0×10^4	1.1×10^3
H_c	$\geq 9\times10^6$	1.1×10^4	1.2×10^3

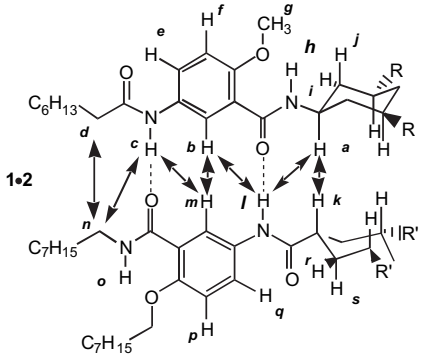


Figure 6. The seven proposed and observed inter-strand NOE contacts for trident **1**·**2**.

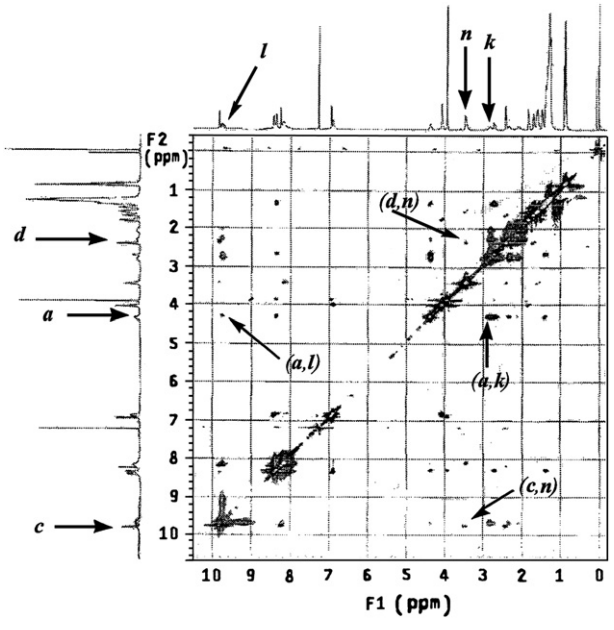


Figure 7. NOESY spectra of **1**·**2** (2 mM in $CDCl_3$ at 24 °C, 500 MHz, mixing time 0.5 s) showing cross-strand contacts between protons ***a*** and ***k***, ***a*** and ***l***, ***c*** and ***n***, and ***d*** and ***n***.

cross-strand NOE contact between protons ***b*** and ***l***. The NOESY spectra of **1**·**2** at 24 °C gives substantial evidence for the formation of the trident dimer, but two predicted cross-strand NOE contacts between protons, ***c*** and ***m***, and ***b*** and ***m*** could not be observed because of signal overlap.

Because of this signal overlap, variable temperature ^{1}H NMR (500 MHz) experiments of **1**·**2** were performed in $CDCl_3$ from −10 to −2 °C at 10 mM and again from 24 to 50 °C at 2 mM. Figure 9 shows the partial 1D spectra corresponding to the aromatic region of **1**·**2** obtained from NMR

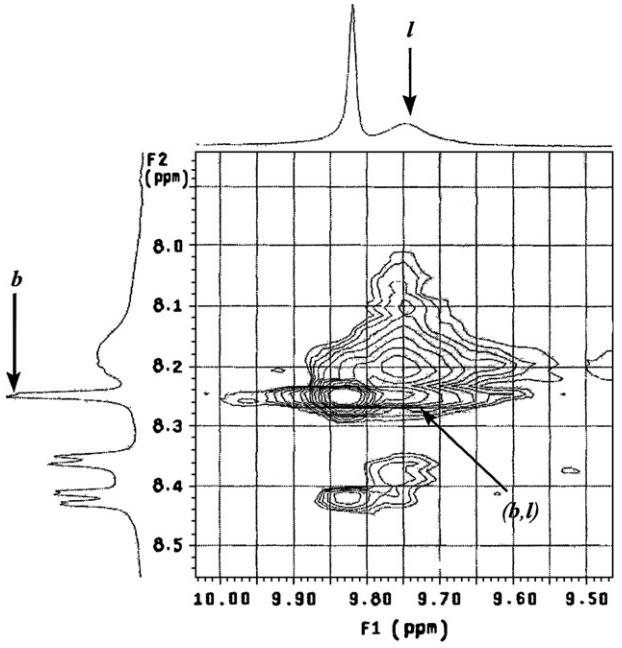


Figure 8. NOESY spectra of **1·2** (2 mM in $CDCl_3$ at 24 °C, 500 MHz, mixing time 0.5 s) showing cross-strand contacts between protons ***b*** and ***l***.

variable temperature experiments. As temperature increased, the resolution of the 1D NMR spectra improved. At 45 °C, sufficient resolution of all signals was obtained in the 1D spectrum.

At 45 °C, some of the newly resolved signals in the aromatic region looked unfamiliar and had to be assigned using their observed intramolecular NOE contacts. All 10 signals in the aromatic region were found to have at least one NOE contact with a previously confirmed ^{1}H signal.

Results of the 2D NMR (NOESY, $CDCl_3$, 500 MHz, 45 °C, mixing time 0.5 s) showed cross-strand NOE contacts between protons ***m*** and ***c*** (Fig. 10), and protons ***b*** and ***m*** (Fig. 11) as well as the five previous cross-strand NOE contacts that were observed at 24 °C. In total, all seven cross-strand NOE contacts that were proposed in Figure 6 for trident **1·2** were observed.

Another interesting feature that was observed in the ^{1}H NMR variable temperature study was the dramatic change in broadness of signal H_l. H_l was unexpectedly broad at room temperature and continued to broaden with increased temperature (Fig. 7). When temperatures were lowered H_l would sharpen. The explanation for this observation is the interconversion of the chair conformation of the cyclohexane rings. The most stabile conformation of the cyclohexane ring is the chair conformation. However, it is known that even at room temperature the cyclohexane ring undergoes rapid interconversion between chair conformations (ring-flip). When temperature increases, this process becomes more rapid. This conformational change would affect the interior H-bonds, i.e., the three H-bonds that are close to the cyclohexane ring, more than it does to the other three exterior H-bonds. This conformational flexibility would oscillate the interior NH groups and carbonyl O atoms, which would cause the corresponding NMR signal to broaden. We would then expect the K_a value based on

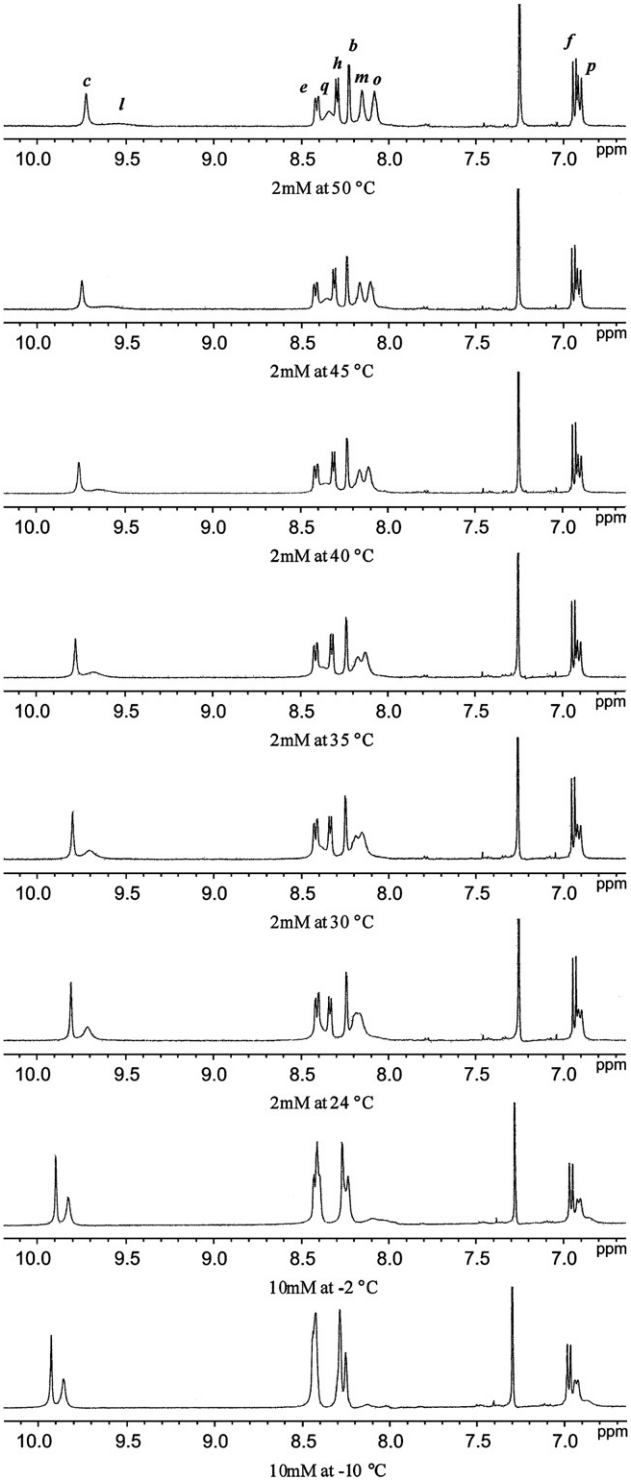


Figure 9. ^{1}H NMR (500 MHz, $CDCl_3$) spectra of the aromatic region of **1·2** at variable temperatures. All signals in the aromatic region were resolved at 45 °C.

the concentration-dependent changes of H_l to be lower than that obtained from H_c. In fact the difference is about five-fold in 5% DMSO-d_6/$CDCl_3$ (Table 1).

2.4. TLC studies

The dimerization of **1·2** was further confirmed by straight-phase thin-layer chromatography (TLC) analysis (silica

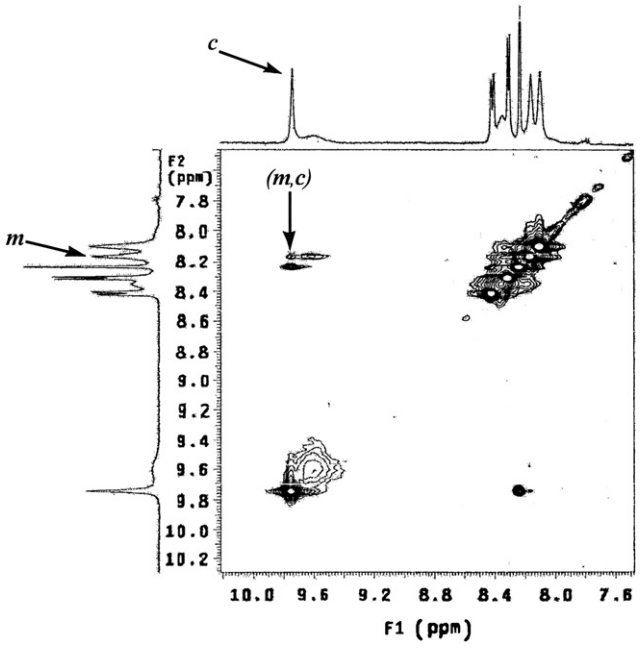


Figure 10. NOESY spectra of **1·2** (2 mM in $CDCl_3$ at 45 °C, 500 MHz, mixing time 0.5 s) showing cross-strand contacts between protons ***m*** and ***c***.

gel, 3:1 DCM/acetone). As shown in Figure 12, the presence of trident **1·2** is demonstrated by the different R_f values of **1** (R_f=0.12), **2** (R_f=0.11), and **1·2** (R_f=0.46). Dimerization of **1·2** substantially increases the R_f value by shielding the polar amide groups from the solid phase. The reason that **1·2** displays tailing on the TLC plate indicates that it is partially dissociated under the relatively polar assaying conditions. The fact that **1·2** could be detected under such polar conditions is a direct confirmation of the very high stability shown by this trident.[24] Trident **1·2** had to be developed using slightly less polar solvent conditions than for duplex **A·B**

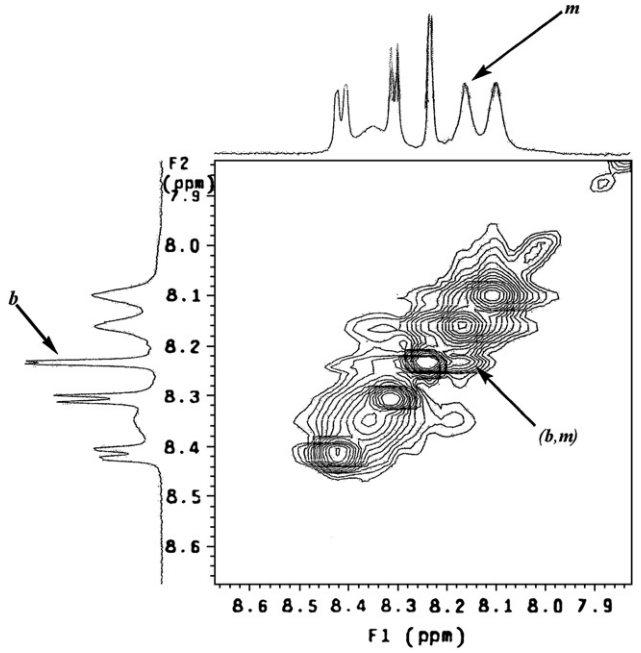


Figure 11. NOESY spectra of **1·2** (2 mM in $CDCl_3$ at 45 °C, 500 MHz, mixing time 0.5 s) showing cross-strand contacts between protons ***b*** and ***m***.

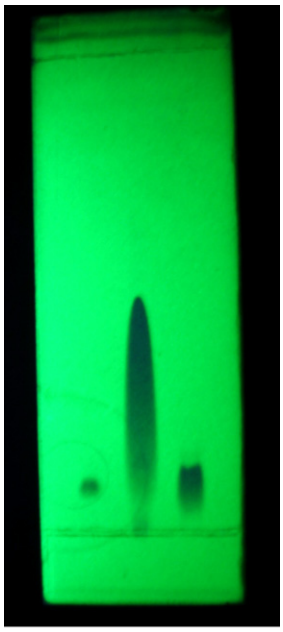


Figure 12. Thin layer chromatography (TLC) of **2**, **1·2**, and **1** from left to right. TLC developing conditions were 25% acetone in DCM. The TLC plate was viewed under short wavelength UV light.

(10% DMF/$CHCl_3$).[23] This is evidence that **1·2** has a lower binding constant than duplex **A·B**.

2.5. VPO studies

Using polystyrene as a molecular weight standard, vapor pressure osmometry (VPO) studies of a 1:1 mixture of **1** and **2** at ambient temperature in $CHCl_3$ were performed over the concn range of 2–20 mM. The average value obtained over this concn range was a molecular weight (MW) of 2260.[25] This value is consistent with the calculated MW of **1+2** (2247 g/mol), which suggests the formation of a tightly bound dimer between **1·2**.

3. Conclusion

Experimental results conclude that the designed complementary strands **1** and **2** were successful in forming the highly stable dimer **1·2** in chloroform. Although considerable flexibility exists in the structures of **1** and **2**—the six separate points of free rotation (N(H)–$C_{cyclohexane}$ and C(=O)–$C_{cyclohexane}$ bonds) and the ring-flip of the cyclohexane linkers—these two molecules were still able to undergo molecular recognition of one another and bind at all six programmed hydrogen-bonding sites. Evidence from TLC and ^{1}H NMR studies suggests that branched dimer **1·2** does not bind as tightly as the six H-bonded duplex **A·B** mentioned above. The varying factor between these two is the unit linker. The binding strength of the duplex and trident relies on two aspects. First, the number of programmed H-bonding donor/acceptor sites and second, the rigidity and pre-organization of each strand. Overall, we conclude that glycine works better than cyclohexanetricarboxylic acid to pre-organize and rigidify the strands and therefore is the main reason why duplex **A·B** binds more tightly than trident

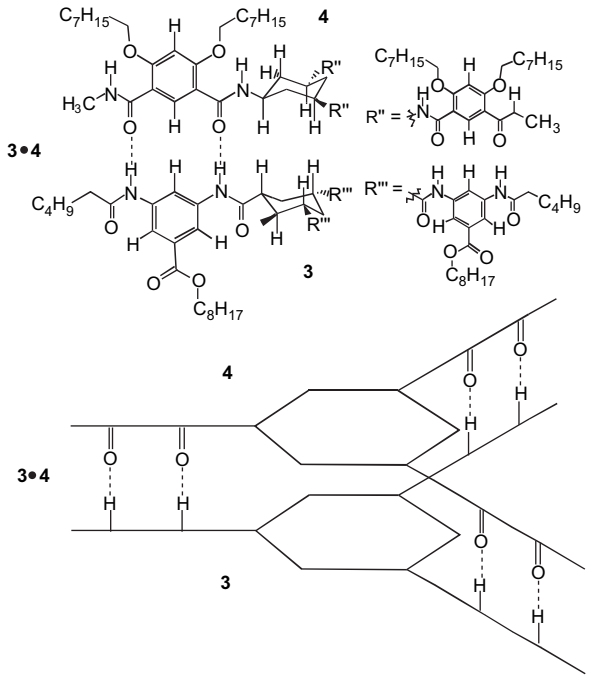


Figure 13. The chemical structure and proposed spatial model of six H-bonded trident **3·4**.

1·2. At this point it is difficult to deduce whether the added dimension of the trident helps to significantly increase the binding strength.

Although the cyclohexane linkers do not enforce as much rigidity as glycine they do incorporate enough rigidity to prevent the individual strands from folding prior to dimerization. Ideas for future studies are the synthesis of a pair of all donor/acceptor strands, which should yield a novel, six H-bonded heterodimer as shown in Figure 13. Also it would be interesting to incorporate adamantane (*cis*,*cis*-1,3,5-adamantanetricarboxylic acid and *cis*,*cis*-1,3,5-triaminoadamantane) linkers emplace of the cyclohexane (*cis*,*cis*-1,3,5-cyclohexanetricarboxylic acid and *cis*,*cis*-1,3,5-triaminocyclohexane) linkers to gauge the effects the cyclohexane ring-flip has on the binding strength of the trident. The success of the **1·2** trident may potentially lead to a whole new avenue of molecular design and alternative strategies for generating new sequence-specificity.

4. Experimental

4.1. General

All chemicals were purchased from Aldrich or Fluka unless specified. All products were detected as one spot on thin-layer chromatographic analysis (pre-coated 0.25 mm silica plates purchased from Fischer), and further characterized by ^{1}H NMR, ^{13}C NMR, and elemental analysis or mass spectral (MS) analysis. All elementary analyses were performed by Atlantic Microlab, Inc. High-resolution mass spectra were measured on a Waters-Micromass Q-TOF API-US spectrometer equipped with a nanoelectrospray ion source. Signals were acquired in the W-mode operation. Ion series generated by water clusters charged by the ammonium ion were used as reference mass peaks. All ^{1}H NMR spectra were recorded from a Varian VXR 400 spectrometer (400 MHz). ^{1}H NMR data are reported in parts per million downfield from tetramethylsilane (TMS). All coupling constants (J=values) are reported in hertz (Hz). All ^{13}C NMR spectra are decoupled and were recorded on a Varian VXR 400 spectrometer (100.6 MHz). Chemical shifts are reported relative to the central peak of $CDCl_3$ at 77.0 ppm or the central peak of DMSO-d_6 at 39.5 ppm. $CDCl_3$ (99.8% D) and DMSO-d_6 (99.8% D) were purchased from Cambridge Isotope Laboratories.

4.2. Synthesis

4.2.1. Methyl 2-octyloxybenzoate (1a). A mixture of methyl salicylate (3.61 g, 16 mmol), K_2CO_3 (4.1 g, 30 mmol), octyl bromide (6.80 g, 35 mmol), and DMF (40 mL) was heated at 90 °C for 24 h. The solution was diluted with water (125 mL) and extracted with EtOAc (3×20 mL). The combined organic extracts were dried over $MgSO_4$, and evaporated under reduced pressure to afford the product (3.0 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (q, 1H, J=8.8, 2.4), 7.54 (q, 1H, J=8.8, 2.4), 6.94 (t, 1H, J=8.8), 6.87 (t, 1H, J=8.8), 4.24 (d, 2H, J=6.8), 3.87 (s, 3H), 2.06 (m, 2H), 1.58 (m, 2H), 1.37 (m, 8H), 0.89 (t, 3H, J=6.8). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.0, 157.3, 135.8, 133.9, 123.3, 117.0, 111.6, 70.1, 58.2, 31.8, 29.0, 28.7, 25.7, 22.5, 19.1. Anal. Calcd for $C_{16}H_{24}O_3$: C, 72.69; H, 9.15. Found: C, 72.63; H, 9.25.

4.2.2. 2-Octyloxybenzoic acid (1b). The ester **1a** (3.09 g, 10 mmol) was dissolved in hot MeOH (40 mL), to which 1 M NaOH (20 mL, 20 mmol) was added. The mixture was refluxed for 2 h and diluted with water (100 mL). The aqueous solution was acidified with concd HCl (2 mL) to precipitate the crude product, which was collected and recrystallized from MeOH to yield a pure white solid (2.66 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.38 (s, 1H), 8.09 (q, 1H, J=9.0, 2.8), 7.53 (q, 1H, J=9.0, 2.8), 6.92 (t, 1H, J=9.0), 6.87 (t, 1H, J=9.0), 4.31 (d, 2H, J=6.8), 1.98 (m, 2H), 1.59 (m, 2H), 1.36 (m, 8H), 0.89 (t, 3H, J=6.8). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 166.7, 157.8, 134.9, 134.0, 123.0, 117.6, 113.1, 69.9, 31.7, 29.0, 28.6, 25.7, 22.6, 14.1. Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 71.73; H, 8.98.

4.2.3. 2-Octyloxy-5-nitrobenzoic acid (1c). The product was prepared according to the literature procedure.[26] The acid **1b** (2.01 g, 8.00 mmol) was dissolved in concd H_2SO_4 (30 mL) and cooled to 0 °C in an ice bath. NH_4NO_3 (0.71 g, 8.81 mmol) was added in small portions over a period of 40 min. The solution was poured into cold water (125 mL). A white precipitate formed was collected, washed with water, and dried to give **1c** as a white solid (1.98 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.63 (s, 1H), 9.06 (q, 1H, J=9.2, 2.8), 8.45 (d, 1H, J=9.2), 7.19 (d, 1H, J=9.2), 4.38 (t, 2H, J=6.8), 2.00 (m, 2H), 1.54 (m, 2H), 1.33 (m, 8H), 0.91 (t, 3H, J=6.8). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.4, 162.9, 140.0, 128.4, 126.30, 121.6, 113.3, 70.1, 31.8, 29.2, 28.9, 25.8, 22.6, 14.0. Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 60.88; H, 7.21; N, 4.79.

4.2.4. *N*-Octyl-2-octyloxy-5-nitrobenzamide (1d). The acid **1c** (3.21 g, 10.9 mmol) was dissolved in DCM

(25 mL), then TEA (2.12 g, 21.0 mmol) and trimethylacetyl chloride (1.33 g, 11.0 mmol) were added, and the solution was stirred for 1 h at room temperature. A second solution of octylamine (1.70 g, 13.2 mmol) and TEA (2.12 g, 21.0 mmol) in DCM (25 mL) was placed into an ice bath to which the first solution was added. After stirring overnight, the solution was washed with 2 M HCl (200 mL), dried over $MgSO_4$, and the solvent was removed. The residue was recrystallized from MeOH to afford pure **1d** (3.17 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (d, 2H, J=2.8), 8.49 (q, 2H, J=9.2, 2.8), 7.95 (d, 2H), 7.25 (d, 2H, J=9.2), 4.22 (t, 2H, J=6.6), 3.68 (q, 2H, J=6.6), 1.83 (t, 2H, J=6.6), 1.59 (t, 2H, J=6.6), 1.47 (m, 20H), 0.94 (m, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 163.4, 161.7, 142.3, 128.9, 128.2, 123.2, 113.0, 70.8, 40.6, 32.3, 32.3, 23.0, 29.8, 29.8, 29.8, 29.7, 29.6, 27.7, 26.7, 23.1, 23.1, 14.6. Anal. Calcd for $C_{23}H_{38}N_2O_4$: C, 67.95; H, 9.42; N, 6.89. Found: C, 67.80; H, 9.44; N, 6.81.

4.2.5. *N*-Octyl-5-amino-2-octyloxybenzamide (1e). Compound **1d** (1.21 g, 2.91 mmol), 10% Pd/C (0.11 g), and MeOH (25 mL) were stirred at room temperature under 1 atm of hydrogen for 4 h. The solvent was filtered and evaporated to afford the aryl amine (1.03 g, 94%). This amine was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.74 (s, 1H), 6.98 (s, 1H), 6.85 (s, 1H), 4.06 (t, 2H, J=6.4), 3.46 (q, 2H, J=6.4), 1.85 (t, 2H, J=6.4), 1.61 (t, 2H, J=6.4), 1.49 (t, 2H, J=6.4), 1.32 (m, 18H), 0.90 (m, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.8, 150.7, 140.9, 122.8, 119.5, 119.0, 114.3, 70.1, 40.3, 32.3, 32.2, 29.8, 29.7, 29.6, 29.6, 29.5, 29.5, 27.7, 26.6, 23.1, 23.0, 14.4. Anal. Calcd for $C_{23}H_{40}N_2O_2$: C, 73.36; H, 10.71; N, 7.44. Found: C, 73.19; H, 10.85; N, 7.36.

4.2.6. Oligoamide (1). *cis*,*cis*-1,3,5-Cyclohexanetricarboxylic acid (0.12 g, 0.54 mmol) was dissolved in thionyl chloride (4 mL) and refluxed for 3 h. The solvent was removed under reduced pressure to yield a residue, which was dissolved in DCM (10 mL) and added drop wise to a solution of amine **1e** (0.64 g, 1.70 mmol), DCM (15 mL), and TEA (0.35 g, 3.4 mmol) and stirred overnight. The reaction mixture was washed with 2 M HCl (20 mL), dried over $MgSO_4$, and evaporated to yield an amber solid, which was recrystallized twice from MeOH and washed with EtOAc to afford pure **5** as a white solid (0.34 g, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 3H), 8.01 (t, 3H, J=6.0), 7.97 (s, 3H), 7.87 (d, 3H, J=9.0), 6.83 (d, 3H, J=9.0), 4.00 (t, 2H, J=6.0), 3.39 (q, 2H, J=6.0), 2.45 (s, 3H), 2.13 (d, 3H, J=12.2), 1.91 (q, 3H, J=12.2), 1.79 (m, 6H), 1.52 (m, 6H), 1.42 (m, 6H), 1.27 (m, 48H), 0.88 (m, 18H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 174.0, 165.2, 153.7, 132.0, 126.7, 124.8, 121.6, 113.0, 69.5, 44.3, 40.3, 32.1, 32.1, 29.7, 29.7, 29.7, 29.6, 27.5, 26.5, 22.9, 14.3, 14.3. Anal. Calcd for $C_{78}H_{126}N_6O_9$: C, 72.50; H, 9.85; N, 6.51. Found: C, 72.26; H, 9.90; N, 6.26. ESIHRMS Calcd for $C_{78}H_{127}N_6O_9$ $[M+H]^+$: 1291.9659. Found: 1291.9673.

4.2.7. *N*,*N*′,*N*″-Tris(benzylcarbamyl) *cis*,*cis*-1,3,5-triaminocyclohexane (2a). The product was prepared according to a literature procedure.[27] Under nitrogen, *cis*,*cis*-1,3,5-cyclohexanetricarboxylic acid (8.0 g, 37 mmol) was added to a solution of benzene (400 mL), TEA (15.6 mL, 112 mmol), and DPPA (30.85 g, 112 mmol). The mixture was vigorously stirred and heated at 80 °C until the solids dissolved (~2 h). Benzyl alcohol (13.34 g, 123.5 mmol) was added and the solution was refluxed for 18 h during which a precipitate formed. After cooling to ambient temperature, the product was collected under vacuum filtration, washed with a minimal amount of cold benzene, and dried to yield **6a** (10.2 g, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.34 (m, 15H), 5.01 (s, 6H), 3.38 (m, 2H), 1.89 (d, 2H, J=12.0), 1.06 (q, 2H, J=12.0). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 146.8, 128.7, 120.4, 119.9, 119.3, 113.7, 111.4, 56.7, 38.2, 37.1. ESIHRMS Calcd for $C_{30}H_{34}N_3O_6$ $[M+H]^+$: 532.2442. Found: 532.2432.

4.2.8. *cis*,*cis*-1,3,5-Triaminocyclohexane (2b). A mixture of carbamate **2a** (0.4 g, 0.76 mmol), 10% Pd/C (0.30 g), and 95% EtOH (100 mL) was heated and shaken at 35 °C under 5 atm of hydrogen for 12 h. The Pd/C was filtered off and the solvent was removed under reduced pressure to give **2b** as a white solid (0.09 g, 90%). ^{1}H NMR (400 MHz, D_2O) δ 3.26 (t, 3H, J=11.6), 2.28 (d, 3H, J=11.6), 1.44 (q, 3H, J=11.6). ^{13}C NMR (100.6 MHz, D_2O) δ 45.5, 32.5. ESIHRMS Calcd for $C_6H_{16}N_3$ $[M+H]^+$: 130.1339. Found: 130.1338.

4.2.9. *N*,*N*′,*N*″-Tris(anisoyl) *cis*,*cis*-1,3,5-triaminocyclohexane (2c). Compound **2b** (0.07 g, 0.5 mmol) was dissolved in dry DMSO (5 mL) and TEA (0.5 mL). An ice cold solution of anisoyl chloride (0.31 g, 1.8 mmol) in dry THF (10 mL) was added. The reaction mixture was stirred overnight. Distilled water (50 mL) was added to the reaction mixture and a precipitate formed within minutes. The solid was collected and washed with distilled water to yield **2c** as a white solid (0.17 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, 3H, J=8.0), 7.88 (d, 3H, J=6.8), 7.43 (t, 3H, J=8.0), 7.07 (t, 3H, J=8.0), 6.95 (d, 3H, J=8.0), 4.15 (m, 3H), 3.94 (s, 9H), 2.58 (d, 3H, J=12.0), 1.59 (q, 3H, J=12.0). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 164.7, 157.6, 133.0, 132.5, 121.8, 121.6, 111.6, 56.2, 46.7, 38.3. ESIHRMS Calcd for $C_{30}H_{34}N_3O_6$ $[M+H]^+$: 532.2442. Found: 532.2431.

4.2.10. *N*,*N*′,*N*″-Tris(2-methoxy-5-nitrobenzoyl) *cis*,*cis*-1,3,5-triaminocyclohexane (2d). Compound **2c** (0.17 g, 0.32 mmol) was dissolved into concd H_2SO_4 (6 mL) and placed in an ice bath. Solid NH_4NO_3 (0.086 g, 1.1 mmol) was added in small portions to the mixture over a period of 30 min. After the addition was completed the mixture was stirred for 15 min and poured into cold water (100 mL). The white precipitate that formed was collected, washed with water, and dried to give **2d** as a pale gray solid (0.16 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (d, 3H, J=2.8), 8.33 (q, 3H, J=9.2, 2.8), 7.81 (d, 3H, J=7.2), 7.17 (d, 3H, J=9.2), 4.18 (m, 3H), 4.10 (s, 9H), 2.52 (d, 3H, J=12.0), 1.59 (q, 3H, J=12.0). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 163.1, 162.2, 141.3, 127.9, 126.6, 124.9, 113.0, 57.5, 46.4, 38.8. ESIHRMS Calcd for $C_{30}H_{31}N_6O_{12}$ $[M+H]^+$: 667.1994. Found: 667.2002.

4.2.11. *N*,*N*′,*N*″-Tris(5-amino-2-methoxybenzoyl) *cis*,*cis*-1,3,5-triaminocyclohexane (2e). Compound **2d** (0.11 g, 0.17 mmol) was dissolved in DMF (0.1 mL) and EtOH (0.25 mL), mixed with 10% Pd/C (0.02 g), and reduced under 4 atm of hydrogen at 35 °C for 3 h. The Pd/C was

removed by filtration and the solvent was removed under a high-pressure vacuum to afford (0.90 g, 95%) amine **2e**, which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (d, 3H, J=7.6), 7.09 (d, 3H, J=2.8), 6.88 (d, 3H, J=9.0), 6.74 (d, 3H, J=2.8, 9.0), 3.97 (m, 3H), 3.77 (s, 9H), 2.11 (d, 3H, J=12.0), 1.36 (q, 3H, J=12.0). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 165.7, 150.8, 140.8, 122.8, 119.6, 118.9, 114.5, 57.1, 46.3, 38.9. ESIHRMS Calcd for $C_{30}H_{37}N_6O_6$ $[M+H]^+$: 577.2769. Found: 577.2775.

4.2.12. Oligoamide (2). Amine **2e** (0.16 g, 0.27 mmol) was dissolved in dry DMSO (3 mL), then DCM (7 mL) and TEA (0.32 g, 3.2 mmol) were added to the solution. Octanoyl chloride (0.26 g, 1.6 mmol) in DCM (10 mL) was added to the solution. The mixture was stirred overnight. Water (50 mL) was added to the solution and the bottom layer was extracted with DCM. The solvent was removed under high-pressure vacuum to afford crude **2**. The crude product was purified by column chromatography (silica gel) using 1:20 MeOH/DCM as the eluant (0.11 g, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (q, 3H, J=9.2, 2.0), 7.90 (d, 3H, J=7.2), 7.78 (d, 3H, J=2.0), 7.33 (s, 3H), 6.93 (d, 3H, J=9.2), 4.16 (d, 3H, J=7.2), 3.92 (s, 9H), 2.56 (d, 3H, J=11.6), 2.34 (d, 6H, J=7.2), 1.71 (m, 6H), 1.44 (q, 3H, J=11.6), 1.28 (m, 24H), 0.88 (t, 9H, J=7.2). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 172.6, 164.4, 153.7, 133.4, 125.0, 123.5, 121.9, 112.4, 56.7, 46.1, 39.1, 37.5, 32.1, 29.6, 29.4, 26.1, 23.0, 14.5. Anal. Calcd for $C_{54}H_{78}N_6O_9$: C, 67.88; H, 8.25; N, 8.80. Found: C, 67.67; H, 8.13; N, 8.71. ESIHRMS Calcd for $C_{54}H_{79}N_6O_9$ $[M+H]^+$: 955.5903. Found: 955.5922.

Acknowledgements

We thank Athula B. Attygalle (Stevens Institute of Technology) for granting access to his Micromass/Waters Q-TOF-API-US mass spectrometer. This project was supported by the National Institute of Health (NIH).

References and notes

1. Philip, D.; Stoddart, J. F. *Angew. Chem., Int. Ed.* **1996**, *35*, 1154–1196.
2. Whitesides, G. M.; Simanek, E. E.; Mathias, J. P.; Seto, C. T.; Chin, D. N.; Mammen, M.; Gordon, D. M. *Acc. Chem. Res.* **1995**, *28*, 37–44.
3. Lawrence, D. S.; Jiang, T.; Levett, M. *Chem. Rev.* **1995**, *95*, 2229–2260.
4. Kolotuchin, S. V.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1998**, *120*, 9092–9093.
5. Klok, H. A.; Joliffe, K. A.; Schauer, C. L.; Prins, L. J.; Spatz, J. P.; Moller, M.; Timmerman, P.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1999**, *121*, 7154–7155.
6. Seebach, D.; Matthews, J. L.; Meden, A.; Wessels, T.; Baerlocher, C.; McCusker, L. B. *Helv. Chim. Acta* **1997**, *80*, 173–182.
7. Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, *366*, 324–327.
8. (a) Castellano, R. K.; Rebek, J. *J. Am. Chem. Soc.* **1998**, *120*, 3657–3663; (b) Castellano, R. K.; Clark, R.; Craig, S. L.; Nuckolls, C.; Rebek, J. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12418–12421; (c) Castellano, R. K.; Nuckolls, C.; Eichhorn, S. H.; Wood, M. R.; Lovinger, A. J.; Rebek, J. *Angew. Chem., Int. Ed.* **1999**, *38*, 2603–2606.
9. (a) Szabo, T.; Hilmersson, G.; Rebek, J. *J. Am. Chem. Soc.* **1998**, *120*, 6193–6194; (b) Rebek, J. J. *Acc. Chem. Res.* **1999**, *33*, 278–286.
10. Boucher, E.; Simard, M.; Wuest, J. D. *J. Org. Chem.* **1995**, *60*, 1408–1412.
11. Sanford, A. R.; Yuan, L.; Gong, B. *Eur. J. Biochem.* **2004**, *271*, 1416–1425.
12. (a) Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, NY, 1984; (b) Blackburn, G. M. *Nucleic Acids in Chemistry and Biology*; Gait, M. J., Ed.; IRL: Oxford, 1990.
13. Cohen, S. N.; Chang, A. C. Y.; Boyer, H. W.; Helling, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3240–3244.
14. Seeman, N. C. *Acc. Chem. Res.* **1997**, *30*, 357–363.
15. Zimmerman, S. C.; Corbin, P. S. *Struct. Bonding (Berlin)* **2000**, *96*, 63–94.
16. Beijer, F. H.; Sijbesma, R. P.; Kooijman, H.; Spek, A. L.; Meijer, E. W. *J. Am. Chem. Soc.* **1998**, *120*, 6761–6769.
17. Corbin, P. S.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1998**, *120*, 9710–9711.
18. Murray, T. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010–4011.
19. (a) Jorgenson, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008–2010; (b) Pranata, J.; Wierschke, S. G.; Jorgenson, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810–2819.
20. (a) Gong, B.; Yan, Y.; Zeng, H.; Skrzypczak-Jankunn, E.; Kim, Y. W.; Zhu, J.; Ickes, H. *J. Am. Chem. Soc.* **1999**, *121*, 5607–5608; (b) Gou, H.; Li, S.; Xie, D.; Gong, B. *Chem. Phys. Lett.* **2005**, *410*, 264–268.
21. (a) Berstein, J.; Davis, R. E.; Shimoni, L.; Chang, N. L. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1555–1573; (b) Etter, M. C. *Acc. Chem. Res.* **1990**, *23*, 120–126.
22. (a) Zeng, H.; Ickes, H.; Flowers, R. A.; Gong, B. *J. Org. Chem.* **2001**, *66*, 3574–3583; (b) Zeng, H.; Yang, X.; Brown, A. L.; Martinovic, S.; Smith, R. D.; Gong, B. *Chem. Commun.* **2003**, 1556–1557.
23. Zeng, H.; Miller, R. S.; Flowers, R. A.; Gong, B. *J. Am. Chem. Soc.* **2000**, *122*, 2635–2644.
24. Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *120*, 6409–6411.
25. The values of three separate determinations were MW=2260, 2280, 2250.
26. De Paulis, T.; Janowski, A.; Kessler, R. M.; Clanton, J. A.; Smith, H. E. *J. Med. Chem.* **1988**, *31*, 2027–2033.
27. Planalp, R. P.; Brechbiel, M. W. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 807–810.