A Highly Stable, Six-Hydrogen-Bonded Molecular Duplex

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Abstract: This paper describes the design, synthesis, and characterization of a hydrogen-bonded molecular duplex (**3·4**). Two oligoamide molecular strands, **3** and **4**, with the complementary hydrogen-bonding sequences ADAADA and DADDAD, respectively, were found to form an extremely stable ($K_a = (1.3 \pm 0.7) \times 10^9$ M^{-1}) molecular duplex (**3·4**) in chloroform. Evidence from 1D and 2D ^{1}H NMR spectroscopy, isothermal titration calorimetry, and thin-layer chromatography confirmed the formation and the high stability of the duplex. The exceptional stability is explained by positive cooperativity among the numerous hydrogen-bonding and van der Waals interactions and the preorganization of the individual strands by intramolecular hydrogen bonds. This design has opened a new avenue to supramolecular recognition units with programmable specificities and stabilities.

Introduction

Currently there is intense interest in constructing supramolecular structures through the self-assembly of both biological and synthetic systems.[1] In Nature, the cooperative action of numerous noncovalent forces leads to highly specific molecular recognition events. As a result, nanoscale supramolecular structures are ubiquitous in Nature. On the other hand, the preparation of nanostructures with even the best present-day synthetic methods still presents one of the most daunting challenges. Based on noncovalent interactions, particularly hydrogen-bonding interactions, numerous artificial self-assembly systems have been developed.[1b] For example, Whitesides et al.[1c] described multicomponent structures based on the cyanuric acid−melamine motif. The groups of Zimmerman[2] and Meijer[3] reported heterocyclic complexes with arrays of hydrogen-bond donors (D) and acceptors (A). Hamilton et al.[4] developed complexes based on the 2-aminopyridinecarboxylic acid system. By combining multiple hydrogen bonds, Zimmerman,[2b] Lehn,[5] and Mascal[6] described the creation of cyclic self-assembling structures. In addition, Reinhoudt et al.[7] and de Mendoza et al.[8] have shown the assembly of calix[4]arene derivatives. Ghadiri et al.[9] designed dimeric structures with eight hydrogen bonds using modified cyclic peptides. Extremely stable dimers based on guanine were reported recently by Sessler et al.[10] Rebek et al.[11] designed concave molecules that assembled into structures reminiscent of tennis balls.

A most well-known and intensively studied example of self-assembly involves the formation of the DNA double helix.[12] The pairing of nucleic acid strands is perhaps the most elegant example of self-assembly in Nature. Nucleobase complementarity allows the specification of intermolecular association of nucleic acids, by "sticky-ended" association.[13] DNA is the molecule with the most readily predictable and programmable intermolecular interactions. The unfavorable entropy price paid for the association of two strands of DNA is compensated by numerous noncovalent interactions that act cooperatively. DNA assembly is characterized by sequence specificity and positive cooperativity. By taking advantage of the programmable sequence specificity of duplex DNA, Seeman has described an elegant strategy for the construction of a variety of DNA-based nanostructures.[14] Although duplex DNA is able to specify intermolecular associations, the conjugation of DNA molecules with other types of structural units, such as peptide and unnatural structures, is not always a trivial task synthetically. In addition, the requirement for using DNA in aqueous media is incompatible with many nonbiological applications. Therefore, if a diverse

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(1) For reviews, see: (a) Lindsey, J. S. *New J. Chem.* **1991**, *15*, 153−180. (b) Lawrence, D. S.; Jiang, T.; Levett, M.; *Chem. Rev.* **1995**, *95*, 2229. (c) 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. (d) Philip, D.; Stoddart, J. F. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1155. (e) Conn, M. M.; Rebek, J., Jr. *Chem. Rev.* **1997**, *97*, 1647. (f) de Mendoza, J. *Chem.−Eur. J.* **1998**, *4*, 1373. (g) Sijbesman, R. P.; Meijer, E. W. *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 24.

(2) (a) Corbin, P. S.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1998**, *120*, 9710−9711. (b) Kolotuchin, S. V.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1998**, *120*, 9092−9093.

(3) (a) Beijer, F. H.; Sijbesma, R. P.; Kooijman, H.; Spek, A. L.; Meijer, E. W. *J. Am. Chem. Soc.* **1998**, *120*, 6761−6769. (b) Beijer, F. H.; Kooijman, H.; Spek, A. L.; Sijbesma, R. P.; Meijer, E. W. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 75−78. (c) Folmer, B. J. B.; Sijbesma, R. P.; Kooijman, H.; Spek, A. L.; Meijer, E. W. *J. Am. Chem. Soc.* **1999**, *121*, 9001−9007.

(4) Yang, J.; Fan, E.; Geib, S. J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 5314−5415.

(5) Marsh, A.; Silvestri, M.; Lehn, J.-M. *Chem. Commun.* **1996**, 1527−1528.

(6) Mascal, M.; Hext, N. M.; Warmuth, R.; Moore, M. H.; Turkenburg, J. P. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2204−2206.

(7) Vreekamp, R. H.; van Duynhoven, J. P. M.; Hubert, M.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1215−1218.

(8) González, J. J.; Prados, P.; de Mendoza, J. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 525.

(9) (a) Ghadiri, M. R.; Kobayashi, K.; Granja, J. R.; Chadha, R. K.; McRee, D. E. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 93. (b) Kobayashi, K.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 95.

(10) Sessler, J. L.; Wang, R. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1726.

(11) (a) Szabo, T.; Hilmersson, G.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1998**, *120*, 6193−6194. (b) Rebek, J., Jr. *Acc. Chem. Res.* **1999**, *33*, 278−286.

(12) (a) Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1984. (b) Blackburn, G. M. In *Nucleic Acids in Chemistry and Biology*; Blackburn, G. M., Gait, M. J., Ed.; IRL Press: Oxford, U.K., 1990; p 17.

(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. *Acc. Chem. Res.* **1997**, *30*, 357−363.

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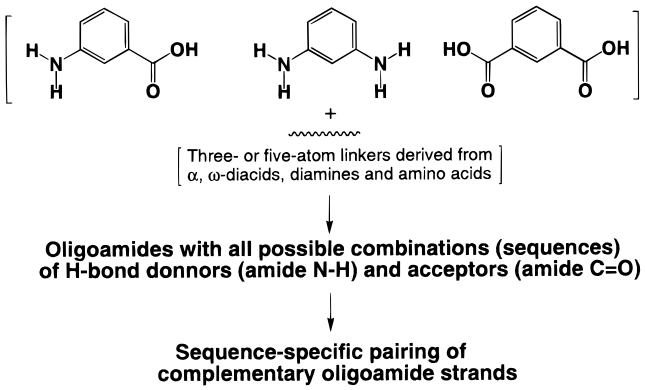


Figure 1. Oligoamides consisting of the three building blocks conjugated by proper linkers, leading to molecular strands with all possible combinations of hydrogen-bond donors and acceptors.

set of readily modifiable structural units leading to highly specific intermolecular interactions become available, the development of artificial nanostructures will be greatly facilitated.

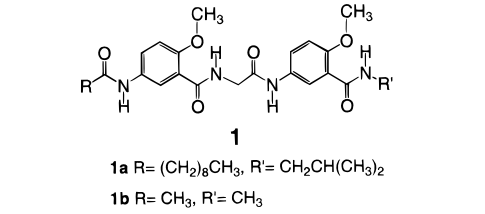
We are interested in developing unnatural molecular recognition units with programmable strength and specificity similar to those demonstrated by DNA. The underlying strategy involves the combination of the simplest hydrogen-bond donors and acceptors, i.e., amide O and H atoms. As shown in Figure 1, linking building blocks derived from 3-aminobenzoic acid, 1,3-benzenedicarboxylic acid (isophthalic acid), and 1,3-diaminobenzene (1,3-phenylenediamine) with proper three- or five-atom linkers should generate oligoamides with all possible combinations (sequences) of donors and acceptors. The number *N* of duplexes with *n* intermolecular hydrogen-bonding sites can be calculated on the basis of the following equations:

$$N = 2^{n-2} + 2^{(n-3)/2} \quad \text{when } n = \text{odd number} \qquad (1)$$

$$N = 2^{n-2} + 2^{(n-2)/2} \quad \text{when } n = \text{even number} \qquad (2)$$

When *n* is an odd number, only complementary (or hetero) duplexes are possible. With *n* being an even number, both complementary and self-complementary duplexes become possible. The first term in the second equation is the number of complementary duplexes and the second term is the number of self-complementary (or homo) duplexes. The number *N* increases very rapidly as the number of intermolecular hydrogen-bonding sites increases. For example, there should be six different quadruply hydrogen-bonded duplexes. For oligoamides that associate with six hydrogen bonds, the number of different duplexes jumps to 20.

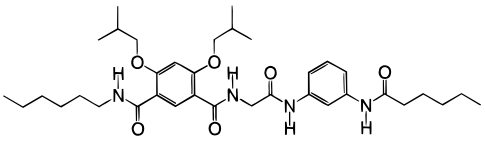
We recently described the design and characterization of oligoamides **1** and **2**, with the self-complementary hydrogen-bonding sequences DADA and DDAA, which formed stable duplexes via hydrogen-bonding interactions between the backbone amide O and H atoms.[15] Incorporating alkoxy groups into **1** and **2** led to the formation of the highly favorable S(6) type[16] intramolecular hydrogen-bonded rings that have been observed in numerous structures.[17] The S(6) systems preorganized the benzamide groups in a way that facilitated the dimerization of these molecules. The incorporation of intramolecular hydrogen bonds also helped to block unwanted hydrogen-bonding interactions that may have led to the formation of polymeric aggregates. The self-complementary dimers of **1** and **2** showed similar stabilities (10^4 M^{-1} < K_{dimer} < 10^5 M^{-1}) in chloroform. The stabilities of these hydrogen-bonded duplexes are thus sequence-independent. This is in sharp contrast to the case of previously reported hydrogen-bonded dimers, whose stability depended on the particular arrangement of hydrogen-bond donor and acceptor sites due to secondary electrostatic interactions.[18] In fact, the dimers of **1** and **2** represent the first examples of unnatural hydrogen bonded duplexes free of secondary interactions. This system inaugurated the design and systematic study of self-assembling duplexes presenting cooperativity and adjustable specificity.



1

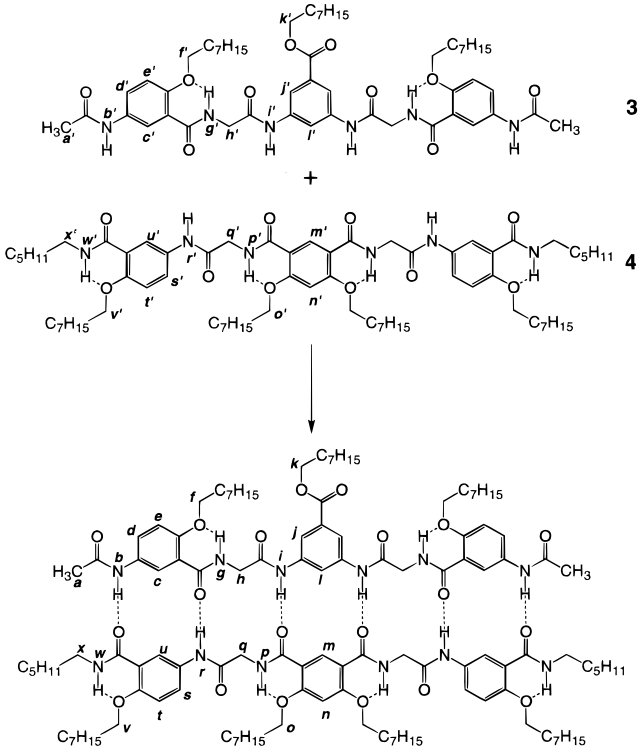
1a R= $(CH_2)_8CH_3$, R'= $CH_2CH(CH_3)_2$

1b R= CH_3, R'= CH_3



2

To develop the molecular system represented by **1** and **2** into a more general and predictable platform for designing molecular recognition units, oligoamides **3** and **4**, with the complementary hydrogen-bonding sequences DADDAD and ADAADA, were designed and synthesized. The self-assembly of **3** and **4** into a hydrogen-bonded duplex should address the following questions: (1) Instead of self-complementary arrays, can duplexes



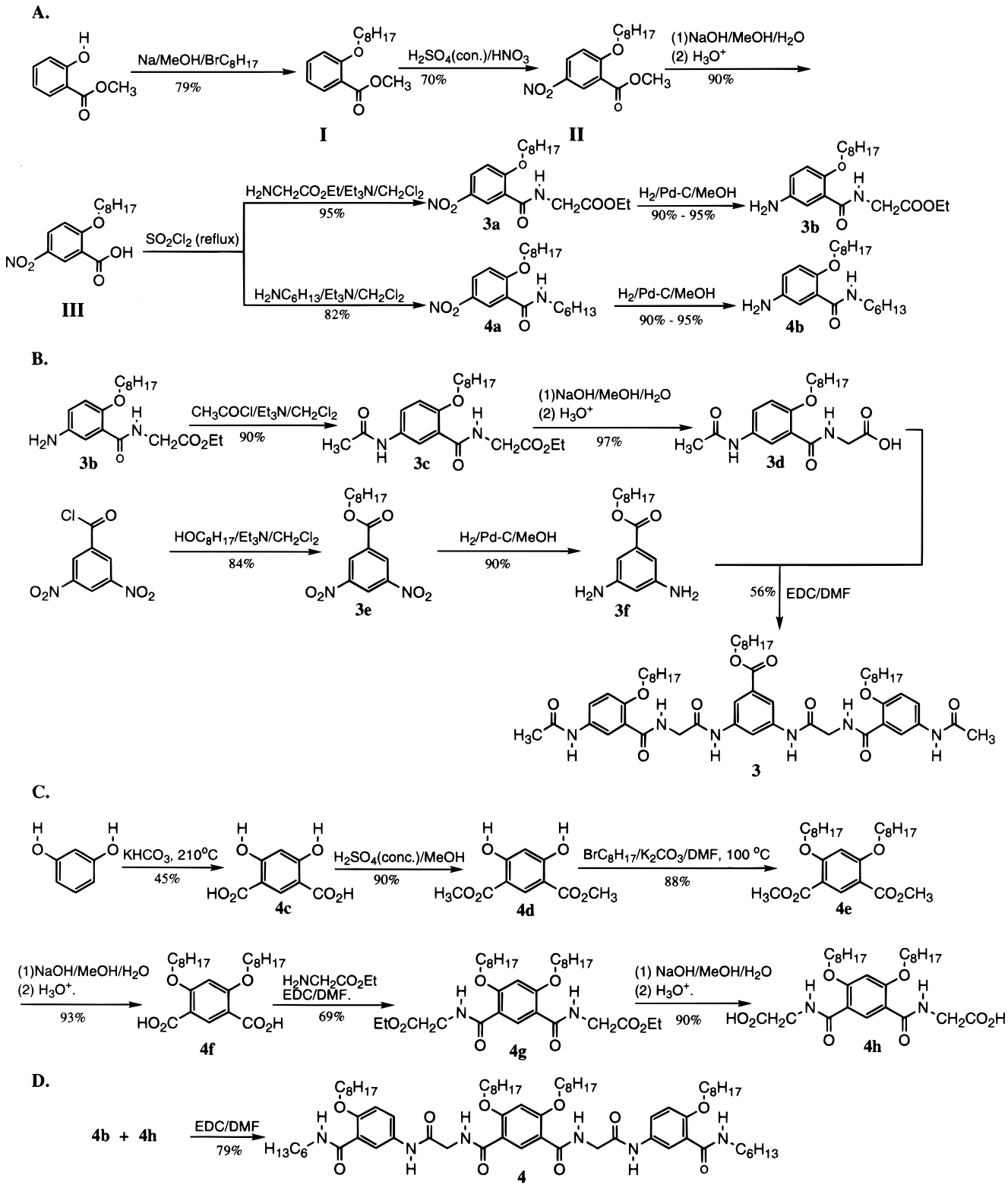
Duplex 3•4

(15) Gong, B.; Yan, Y.; Zeng, H.; Skrzypczak-Jankunn, E.; Kim, Y. W.; Zhu, J.; Ickes, H. *J. Am. Chem. Soc.* **1999**, *121*, 5607−5608.

(16) Berstein, J.; Davis, R. E.; Shimoni, L.; Chang, N.-L. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1555−1573.

(17) Etter, M. C. *Acc. Chem. Res.* **1990**, *23*, 120−126.

Scheme 1. Synthesis of **3** and **4**



containing two different strands of complementary hydrogen-bonding sequences form? (2) Will the backbones of longer strands stay in register to allow pairing of the duplexes? (3) In the formation of a longer duplex, is there any cooperativity among the building blocks and among the hydrogen-bonding sites? As illustrated, these two strands may form a molecular duplex held together by six hydrogen bonds. Given its increased number of hydrogen-bonding sites and the possibility of cooperative interactions among the hydrogen bonds, such a duplex is expected to be more stable than the homologous quadruply hydrogen-bonded duplexes. The long-term objective of this work is to develop hydrogen-bonded molecular duplexes whose formation is characterized by programmable sequence specificity and adjustable stability and which may be used as

(18) (a) Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810−2819. (b) Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008−2010. (c) Murray, T. J.; Zimmermann, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010−4011.

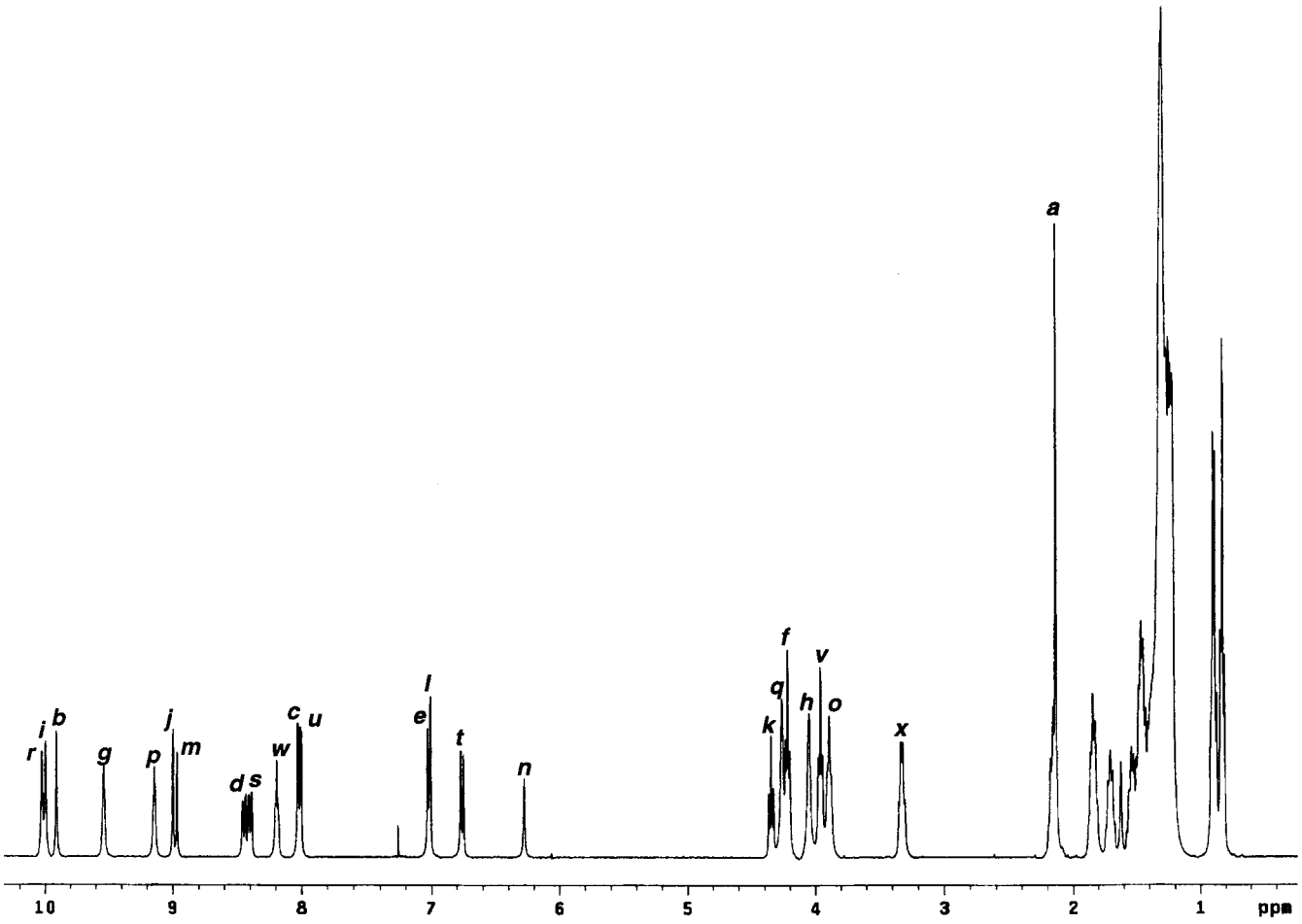


Figure 2. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of duplex **3·4**.

specific molecular recognition units (or "molecular glues") for constructing supramolecular structures.

Results and Discussion

Synthesis. Oligoamides **3** and **4**, which contain six hydrogen-bonding sites with the sequences DADDAD and ADAADA, respectively, were synthesized by iterative coupling steps using either acid chloride or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (Scheme 1). Methyl salicylate was first alkylated with 1-bromooctane; product **I** was then nitrated into ester **II**, which was converted into 5-nitro-2-(octyloxy)benzoic acid, **III**. Acid **III** was converted into the corresponding acid chloride, which was then treated with the corresponding amines to give ester **3a** or amide **4a**. The nitro compounds **3a** and **4a** were then reduced to the corresponding amines **3b** and **4b** by catalytic hydrogenation. Compound **3a** was acetylated into **3c**, which was hydrolyzed to give acid **3d**. Diamine **3f** was prepared from 3,5-dinitrobenzoyl chloride via the octyl benzoate ester **3e**. Coupling of **3d** and **3f** in DMF using EDC gave **3**.

Resorcinol was converted into 4,6-dihydroxy-1,3-benzenedicarboxylic acid, **4c**, by modifying a reported procedure.[19] The acid **4c** was esterified and alkylated into dimethyl 4,6-bis-(octyloxy)-1,3-benzenedicarboxylate, **4e**. This was followed by hydrolysis to give the acid **4f**. The coupling between **4f** and glycine ethyl ester using EDC in DMF gave **4g**. Diester **4g** was then hydrolyzed into the dicaid **4h**. Coupling between **4h** and **4b** using EDC in DMF led to **4**.

(19) Gore, T. S.; Inamdar, P. K.; Nagarkar, P. M. *Indian J. Chem.* **1974**, 946−947.

Preparation and Characterization of the Hydrogen-Bonded Duplex. Duplex **3·4** was prepared by mixing 1 equiv of **3** with 1 equiv of **4** in chloroform (1 mL). Although the solubility of **3** (<1 mM) or **4** (≤10 mM) in the same solvent was relatively low, the 1:1 mixture of **3** and **4** was highly soluble (≫100 mM) in chloroform. This observation suggested that duplex **3·4** indeed formed, which shielded the polar amide groups from the solvent, thus preventing the formation of polymeric aggregates.[20] Attempts to obtain X-ray-quality crystals of **3·4** have not been successful so far. Slow evaporation of solvents from solutions of complex **3·4** in chloroform and in chloroform/ethanol led to amorphous masses.

(1) ^{1}H NMR Spectroscopy. Figure 2 shows the 1D ^{1}H NMR spectrum of duplex **3·4**. The assignment of this spectrum to the structure of **3·4** was assisted by using COSY and NOESY spectra.

^{1}H NMR spectroscopy in chloroform-*d* ($CDCl_3$) revealed downfield shifts of the aniline NH signals of the 1:1 mixture of **3** and **4** (at 1 mM: H_b 9.93 ppm, H_i 10.09 ppm, and H_r 10.19 ppm) compared to the corresponding signals of either **3** (H_b <7.5 ppm and H_i <7.5 ppm; the signals overlap with other peaks) or **4** (H_r 10.04 ppm), suggesting the formation of duplex **3·4**.

Mixing **3** and **4** in stoichiometries other than 1:1 revealed separate sets of signals corresponding to both duplex **3·4** and the uncomplexed strands. Figure 3 shows the NMR spectrum of a 1:4 mixture of **3** and **4**. This spectrum is consistent with

(20) Whitesides et al. observed similar phenomena for the solubilities of hydrogen-bonded aggregates: Mathias, J. P.; Simanek, E. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1994**, *116*, 4326−4340.

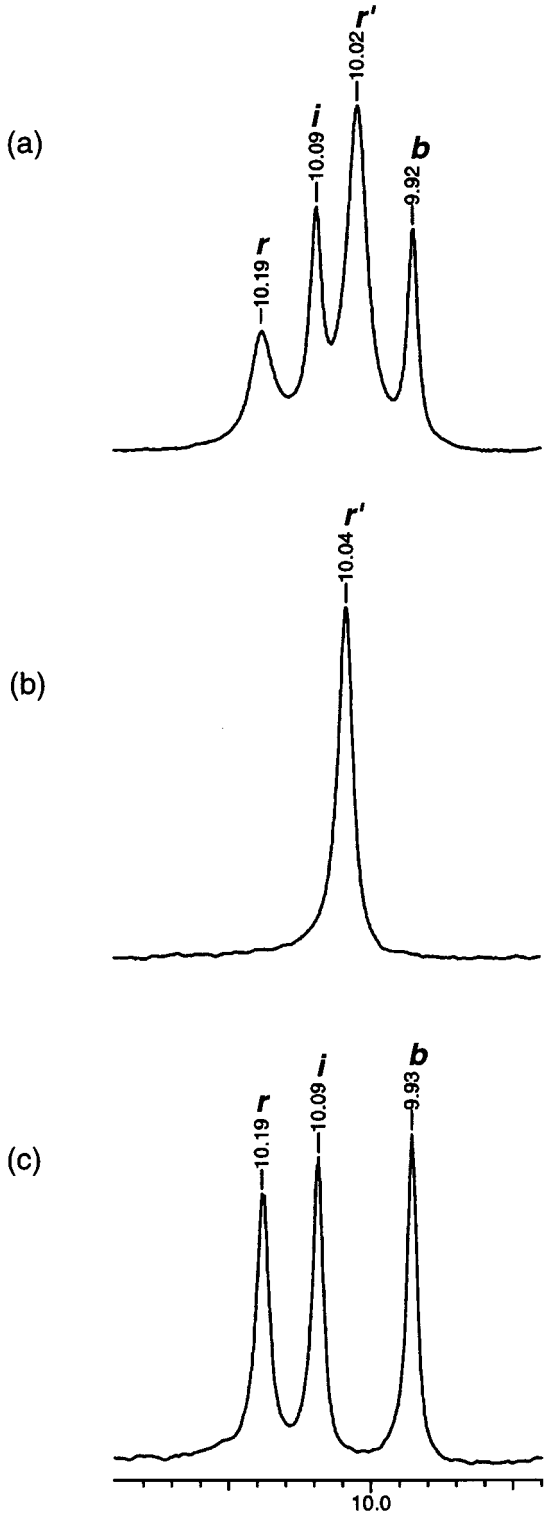


Figure 3. Aniline NH resonances of (a) a 1:4 mixture of **3** and **4**, (b) amide **4** at 1 mM, and (c) duplex **3·4** at 1 mM.

the existence of the three aniline signals corresponding to duplex **3·4** and one aniline signal corresponding to uncomplexed **4** (Figure 3). The observation of separate sets of resonances for duplex **3·4** and the uncomplexed **4** at this stoichiometry for **3**:**4** indicated that exchange between the assembled and the uncomplexed states was very slow on the time scale of NMR spectroscopy, which provided another piece of evidence for the very high stability of the duplex. Similar phenomena were observed by Whitesides et al.[20] for highly stable hydrogen-bonded aggregates with multiple components.

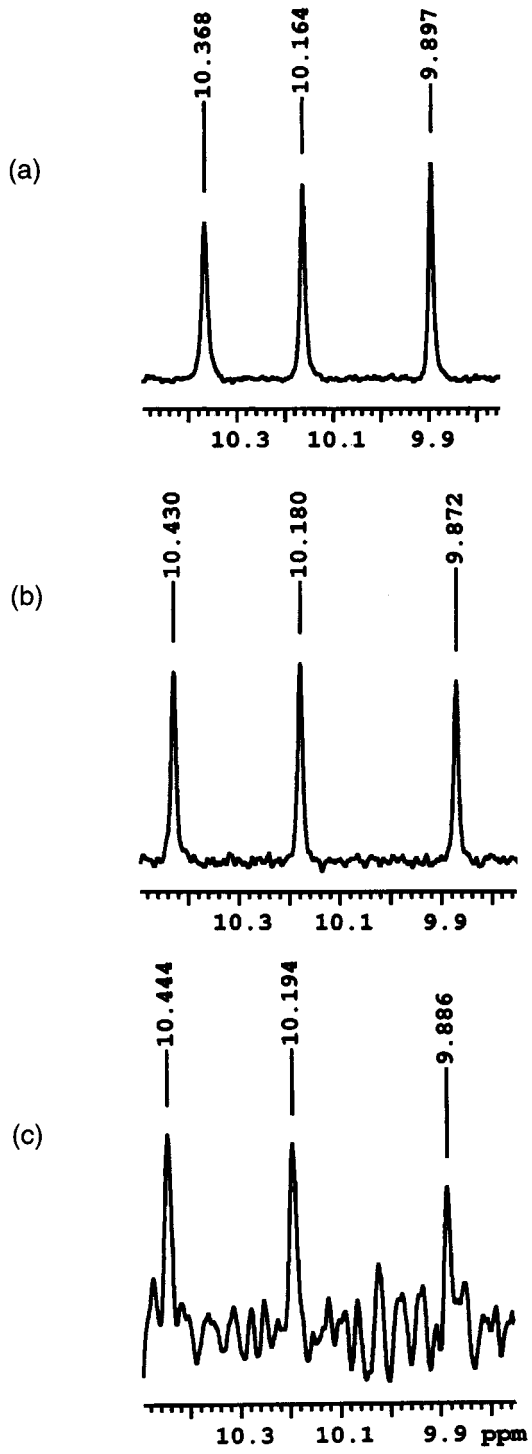


Figure 4. Aniline resonances of duplex **3·4** at (a) 100 μM, (b) 10 μM, and (c) 1 μM.

In an attempt to determine the association constant, ^{1}H NMR binding studies were carried out in $CDCl_3$ by diluting a solution of the 1:1 mixture of **3** and **4**. Within the sensitivity of detection of the NMR spectrometer (400 MHz), no significant upfield shifts of the aniline NH signals were observed across a broad concentration range (100 mM to 1 μM). Figure 4 shows the regions of the three aniline NH signals at 100, 10, and 1 μM. Detection of the NH signals at 1 μM was made possible by first determining the spin−lattice relaxation time T_1 of duplex **3·4**. The ^{1}H NMR spectrum (400 MHz) of **3·4** at 1 μM was recorded for a total of ~29 h (acquisition time = 0.251 s; delay time = 0.05 s; excitation pulse width = 25 ms; number

of acquisitions = 332 168). The short acquisition time was based on the spin−lattice relaxation time of **3·4** that was experimentally determined to be 0.6 s. On the basis of this short relaxation time, a short repetition time was adopted, which led to a large acquisition number within a reasonable period of time. Because the signal-to-noise ratio is proportional to the square root of the acquisition number, the large acquisition number allowed a spectrum with much improved sensitivity. The three aniline NH signals, which were involved in intermolecular hydrogen-bonding interactions, remained sharp and well above the background under these NMR conditions. Instead of moving upfield, one NH signal corresponding to H_r moved slightly (0.078 ppm) downfield upon dilution from 100 to 10 μM. This was probably due to the weakening of stacking interactions among the dimers. Since there was no detectable change in the chemical shifts and number of the aniline NH signals, assuming conservatively a 10% dissociation at 1 μM led to a lower limit of 9×10^7 M^{-1} for the association constant of duplex **3·4**.[3a]

Variable-temperature measurements of the amide NH signals of **4** were carried out to probe the possibility for this molecule to adopt folded conformations with intramolecular hydrogen bonds.[21] At 1 mM **4** and from 25 to 65 °C in $CDCl_3$, the temperature-dependent change of the aniline NH signal (H_r) of **4** was 1.4×10^{-2} ppm/K. The two glycine NH signals, which were involved in forming the S(6) intramolecular hydrogen bonds, showed much smaller changes in the same temperature range: 3.9×10^{-3} ppm/K (H_w) and 3.9×10^{-3} ppm/K (H_p). These results clearly indicated that the aniline NH group in **4** was not involved in an intramolecular hydrogen-bonding interaction, and **4** was thus very likely to adopt an extended, rather than folded, conformation in its uncomplexed form. Similar variable-temperature experiments could not be carried out on **3** due to overlap of its amide NH signals with other aromatic resonances in its ^{1}H NMR spectrum. However, given its structural similarity to **4**, particularly in its backbone rigidity, **3** is also very likely to adopt an extended conformation in solution.

The self-association of **4** was investigated by diluting its solution in $CDCl_3$ from 10 to 0.375 mM. Upfield shifts of its aniline NH signal from 10.11 to 9.72 ppm was observed. A dimerization constant of $(4.4 \pm 2.0) \times 10^4$ M^{-1} was obtained for **4**.[22] This value is surprisingly similar to those for the self-complementary quadruply hydrogen-bonded duplexes we reported recently.[15] One possibility is that the self-association of **4** involves four intermolecular hydrogen bonds. Examining the sequence of hydrogen-bond donors and acceptors in **4** leads to only one possible arrangement of a self-complementary dimer with four attractive hydrogen-bonding interactions and one repulsive interaction involving two amide O atoms (Figure 5a). The only repulsive interaction in this dimer could be alleviated by slightly twisting the backbone of the two molecules, which leads to a dimer with a stability similar to those of quadruply hydrogen-bonded **1** and **2**. Given the considerable degree of rotational freedom in **4**, such a twist of the backbone in **3** should be easily realized. The self-association of **3** could not be investigated because of the limited solubility of this compound in $CDCl_3$. However, given its hydrogen-bonding sequences, it

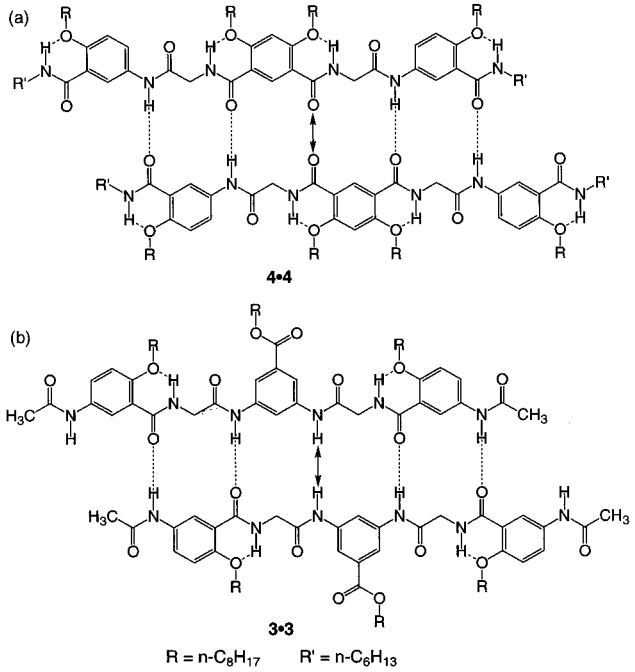
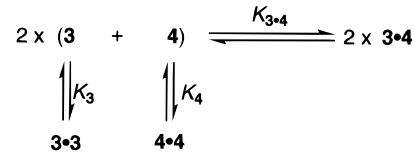


Figure 5. (a) Amide **4** possibly associating into dimer **4·4** with four attractive hydrogen bonds (dotted lines) and one repulsive interaction between two amide O atoms (double-headed arrow). (b) Amide **3** possibly forming dimer **3·3** with a stability similar to that **4·4**.

is reasonable to imagine that the self-association of **3** may also involve four intermolecular hydrogen bonds (Figure 5b).

Considering the equilibrium



the dimerization of **4** (and very likely that of **3**) should not interfere with the pairing of **3** and **4**. Despite its relatively large value, K_4 is still 5 orders of magnitude (see the ITC data below) smaller than $K_{3\cdot4}$. On the basis of the above equilibrium, if $K_3 \approx K_4$ and the initial (added) concentrations of **3** and **4** are the same, a simple calculation shows that at equilibrium only 0.001% of **4** (or **3**) will be the dimer **3·3** or **4·4**, while 99.999% of **4** and **3** will exist as **3·4**. It needs to be pointed out that this conclusion is consistent with the ^{1}H NMR observations, which indicated that diluting a solution of **3·4** to as low as 1 μM did not result in the appearance of any signals corresponding to uncomplexed **3** (Figure 4).

Unequivocal evidence for the formation of **3·4** in solution was provided by two-dimensional (2D) NMR (NOESY, $CDCl_3$, 400 MHz) studies (Figure 6). Numerous interstrand contacts were observed. For example, contacts between protons l and m, i and m, and h and q, as well as a and x, were particularly diagnostic of duplex formation. In addition, contacts between protons f ang g, o and p, and w and v were indicative of the intramolecular hydrogen bonds that act to preorganize both **3** and **4**. These 2D NMR data indicated that, in solution, **3** and **4** indeed aligned and associated in accord with the original design. Once again, the extraordinary stability of **3·4** is demonstrated by the fact that NOESY spectra recorded at 1 or 10 mM **3·4** showed the same intra- and interstrand contacts.

(2) Isothermal Titration Calorimetry (ITC). Dilution experiments showed that the stability constant of duplex **3·4**

(21) (a) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512−523. (b) Hamuro, Y.; Geib, J.; Hamilton, A. D. *J. Am. Chem. Soc.* **1996**, *118*, 7529−7541.

(22) Wilcox, C. S. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Durr, H., Eds.; VCH: New York, 1991. The dimerization constant was obtained by fitting the NMR data to a modified dimerization equation with the program Kaleidagraph on a Macintosh computer.

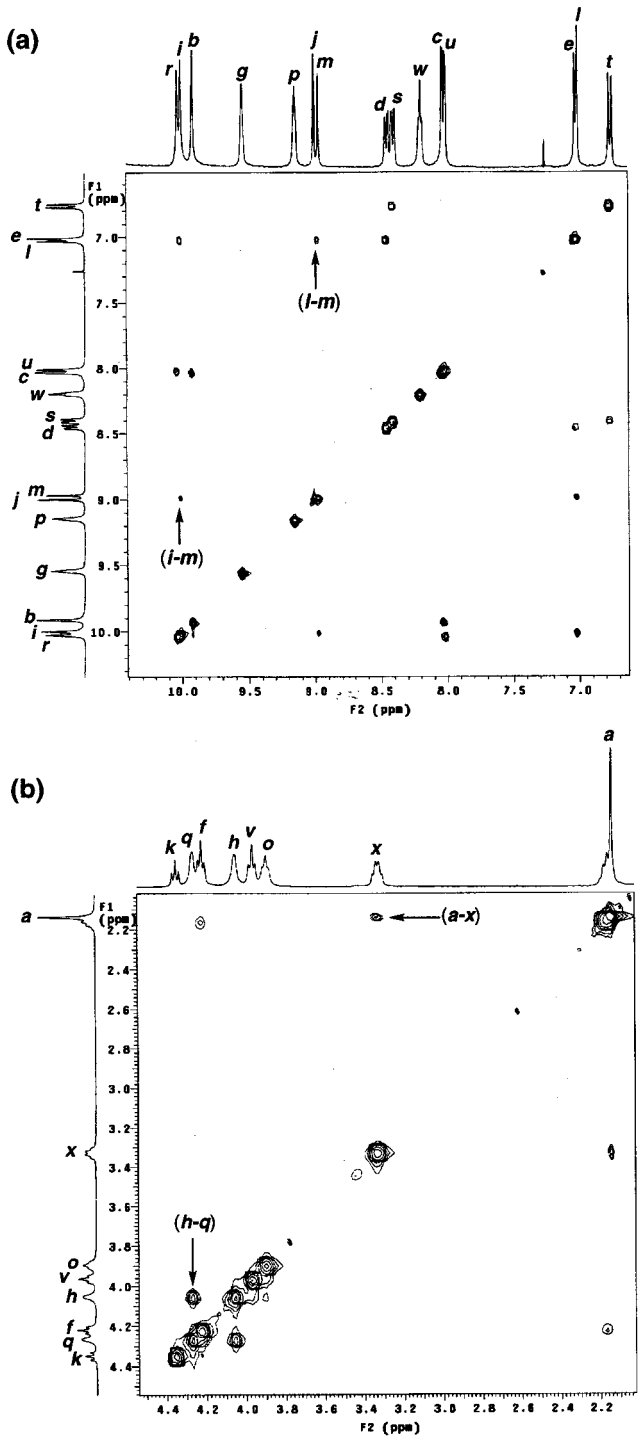


Figure 6. NOESY spectra of **3·4** (8 mM) in $CDCl_3$ (mixing time 0.5 s): (a) cross-strand contacts between protons l and m and protons i and m; (b) cross-strand contacts between protons a and x and protons h and q. NOESY spectra of **3·4** recorded at concentrations of 1 and 100 mM are similar to the ones shown here.

was far above the detection limit of the NMR method. Titrations using isothermal titration microcalorimetry can directly measure $\Delta H°$ and the association constant over a large range of K_a values, so this method was employed to acquire thermodynamic profiles for complexation. We first carried out titration of **4** into **3** in pure $CHCl_3$. By titration of a solution of 0.1 mM **3** with 0.8 mM **4** in chloroform, an association constant of $(1.3 \pm 0.7) \times 10^9$ M^{-1} was obtained. Due to the very high stability of **3·4**, the concentrations of **3** and **4** had to be decreased significantly to the micromolar range to obtain a reasonable binding isotherm.

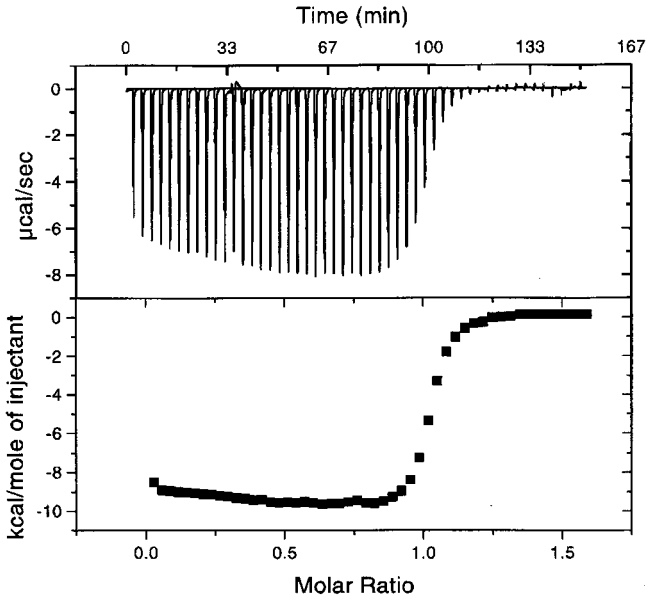


Figure 7. Calorimetry binding isotherm for the titration of **3** with **4** in 5% DMSO/chloroform.

Since the experiment was carried out at micromolar concentrations, the measurements were carried out near the detection limit of the calorimeter. When even lower concentrations of **3** and **4** were tested, the resulting data led to association constants with greater (>10%) error. In all cases, analysis of the resulting binding isotherm in $CHCl_3$ showed that the binding constant was in the range of 10^9 M^{-1}.

More accurate data were obtained by carrying out the ITC titration in 5% DMSO/$CHCl_3$. Titration of **4** into **3** produced the thermogram shown in Figure 7. Integration of the heat data plotted vs the mole ratio of host and guest produced the isotherm also shown in Figure 7. An association constant of $(3.5 \pm 1.3) \times 10^6$ M^{-1} was obtained, along with $\Delta G = -8.9 \pm 0.2$ kcal/mol and $\Delta H = -9.7 \pm 0.2$ kcal/mol. The excellent fit obtained for the thermogram, in combination with the NMR data, supports our belief that the values reported here accurately describe the thermodynamic equilibrium.

Given the relatively small entropic change ($-T\Delta S \approx 0.8$ kcal/mol), the ITC data showed that the formation of **3·4** was driven almost entirely by enthalpy. This result implies that **3** and **4** adopt preorganized, extended conformations, which may have provided optimal orientations for pairing the two strands. This is consistent with the results of the variable-temperature measurements, which indicated that **4** was more likely to adopt an extended, rather than folded, conformation in solution. To account for the thermodynamic data from the ITC experiment, a very likely, and yet simplified, scenario can be proposed: dimer **4·4** (and perhaps **3·3**) dissociates into single-stranded **4**, which then combines with single-stranded **3** to form the highly stable **3·4**. This process involves mainly enthalpic, rather than entropic, changes, during which the existence of any uncomplexed, folded molecules is very unlikely.

(3) Thin-Layer Chromatography (TLC). The stability of **3·4** was further confirmed by straight-phase thin-layer chromatography (TLC) analysis (silica gel plate, 10% DMF in chloroform). As shown in Figure 8, the presence of duplex **3·4** is clearly demonstrated by the different R_f values of **3** (R_f = 0.00), **4** (R_f = 0.10), and **3·4** (R_f = 0.96). The fact that **3·4** shows a tailing on the TLC plate indicates its partial dissociation under the rather polar analytical conditions. That **3·4** could be detected under the rather harsh straight-phase TLC conditions

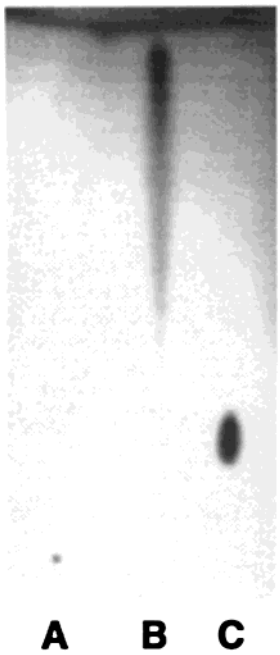


Figure 8. Thin-layer chromatography of **3** (lane A), **3** + **4** (1:1, lane B), and **4** (lane C). TLC condition: silica gel plate/10% DMF in $CHCl_3$. The spot corresponding to **3** (lane A) is weak due to the low solubility of **3**. The developed TLC plate was detected under short-wavelength UV light.

is very surprising and is a direct confirmation of the extremely high stability shown by this duplex.[23]

Positive Cooperativity in the Self-Assembling Process. Comparing the stabilities of doubly ($K_a = 25\ M^{-1}$, -0.9 kcal/mol for each H bond) and quadruply (dimers of **1** and **2**, $10^4\ M^{-1} < K_a < 10^5\ M^{-1}$, -1.4 to -1.7 kcal/mol^{-1} for each H bond) hydrogen-bonded duplexes[15] with that of **3·4** ($1 \times 10^9\ M^{-1}$, 2.0 kcal/mol for each H bond) indicates that the increase in stabilities is not just due to the additive effect from increased numbers of hydrogen bonds. Instead, these data clearly demonstrate that the self-assembly of **3·4** is highly cooperative: after the initial association of the two strands, which may involve one or, at most, two hydrogen bonds and is entropically unfavorable, the subsequent hydrogen-bond formation during the growth of the duplex is enhanced by multiple, enthalpically favorable interactions. This type of cooperativity is one of the most prominent features of the self-assembly of DNA and lies at the heart of many self-assembling and self-organizing systems.[1a] In addition to hydrogen-bonding interactions, van der Waals contacts between the methylene hydrogens of the glycine linkers may also contribute to the stability of the duplexes. Such contacts, as clearly shown by the NOESY spectra of **3·4**, may play a dominant role in the future design of duplexes that will be stable in aqueous media.

Conclusions

The assembly of **3·4** indicates that (1) in addition to self-complementary duplexes, highly stable duplexes containing two different strands can also be designed, (2) the backbones of **3·4** do stay in register to allow the formation of all the hydrogen bonds and thus the pairing of the duplexes, and (3) the self-assembly of **3·4** is highly cooperative, which results in a very stable duplex.[24] These results, along with those for **1** and **2**, have paved the way for the design of longer duplexes. The development of a wide variety of extremely tight-binding duplexes with high sequence specificities can be envisioned. In addition, this class of molecules offers several advantages, including ease of synthesis based on highly efficient amide (peptide) chemistry and readily available building blocks. Structural modification can be conveniently carried out on this system. As a result, duplexes compatible with a variety of environmental conditions should be easily generated. Highly specific, tight-binding duplexes should find numerous applications in chemistry, biochemistry, and materials science.

Experimental Section

General Methods. All chemicals were purchased from Aldrich, Fluka, and Sigma and were used as received unless otherwise noted. The organic phases from all liquid extractions were dried over Na_2SO_4 unless specified otherwise. All products were detected as single spots by thin-layer chromatography (precoated 0.25 mm silica plates from Aldrich). All samples were purified either by recrystallization or by flash column chromatography and dried completely under high vacuum before being characterized by ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and elemental analysis. All ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXR 400 spectrometer (400 MHz). NMR chemical shifts are reported in ppm relative to TMS. For the ^{1}H NMR dilution experiments, $CDCl_3$ (99.8% D) and DMSO-d_6 (99.8% D) were purchased from Carmbridge Isotope Laboratory and used without further purification. T_1 measurements were carried out with degassed samples (freeze−thaw cycles with nitrogen). NOE measurements were performed with the steady-state NOEDIF protocol on degassed samples.

Methyl 2-(Octyloxy)benzoate (I). Sodium metal (2.76 g, 120 mmol; washed with clean petroleum ether prior to use) was added in small pieces to anhydrous MeOH (120 mL) in a well-ventilated hood. The resulting solution was heated under reflux. Methyl salicylate (15.2 g, 100 mmol) was then introduced, followed by 1-bromooctane (23.2 g, 120 mmol). The reaction was allowed to proceed for 2 days under reflux. The solid was then filtered off, and the solvent was removed under reduced pressure. Excess 1-bromooctane was removed in vacuo, and the residue was dissolved in ethyl acetate. The solution was washed with diluted NaOH solution and was dried over Na_2SO_4. Evaporation of the solvent gave pure liquid product **I** (25.1 g, 79%). ^{1}H NMR ($CDCl_3$): δ 7.77 (q, J = 1.2, 7.6, 1H), 7.43 (m, 1H), 6.95 (m, 2H), 4.02 (t, J = 6.6, 2H), 3.88 (s, 3H), 1.83 (m, 2H), 1.48 (m, 2H), 1.34−1.28 (m, 8H), 0.88 (t, J = 6.6, 2H). (All J values are reported in hertz.) ^{13}C NMR ($CDCl_3$): δ 167.01, 158.66, 133.17, 131.51, 120.79, 120.01, 113.40, 69.10, 51.75, 31.80, 29.29, 29.22, 25.95, 22.62, 14.01. Anal. Calcd for $C_{16}H_{24}O_3$: C, 72.69; H, 9.15. Found: C, 72.63; H, 9.25.

Methyl 2-(Octyloxy)-5-nitrobenzoate (II). Compound **I** (26.4 g, 100 mmol) was added dropwise to concentrated H_2SO_4 (60 mL) cooled in an ice−water bath, to which a mixture of 70% HNO_3 (7.7 mL, d 1.41 g/mL, 120 mmol) and concentrated H_2SO_4 (32.3 mL) was added dropwise over a period of 20 min at 0 °C. After being stirred for 1.5 h, the reaction mixture was poured into cracked ice (1 kg) and the mixture was left overnight while the temperature was kept at about 0 °C. The precipitate was dissolved in ether (600 mL), and the solution was washed with 1 N NaOH (2 × 30 mL). Drying over Na_2SO_4 and evaporation of the solvent gave the crude product as a solid, which was then recrystallized from MeOH, the give the pure product as a white solid (21.66 g, 70%). ^{1}H NMR ($CDCl_3$): δ 8.70 (d, J = 3.2, 1H), 8.33 (q, J = 3.2, 9.2, 1H), 7.03 (d, J = 9.2, 1H), 4.14 (t, J = 6.4, 2H), 3.92 (s, 3H), 1.88 (m, 2H), 1.50 (m, 2H), 1.38−1.29 (m, 8H), 0.88 (t, J = 6.8, 3H). ^{13}C NMR ($CDCl_3$): δ 164.69, 163.22, 140.54, 128.69, 127.79, 120.84, 112.72, 69.921, 52.35, 31.77, 29.19, 28.88, 25.81, 22.63, 14.03. Anal. Calcd for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.20; H, 7.75; N, 4.78.

(23) Previously only reverse-phase TLC had been used for the characterization of hydrogen-bonded complexes: Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 6409.

(24) There were reports on hydrogen-bonded complexes with very high stabilities: (a) Bell, T. W.; Hou, Z.; Zimmerman, S. C.; Thiessen, P. A. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2163. (b) Sessler, J. L.; Wang, R. *J. Org. Chem.* **1998**, *63*, 4079.

2-(Octyloxy)-5-nitrobenzoic Acid (III). The ester **II** (3.09 g, 10 mmol) was dissolved in hot MeOH (40 mL), to which 1 N NaOH (20 mL, 20 mmol) was added. The mixture was heated under relux for 30−40 min, and more water (100 mL) was added. The aqueous layer was neutralized by addition of concentrated HCl to pH 3.0. The precipitated crude product was collected, and recrystallization from MeOH gave a white solid (2.66 g, 90%). ^{1}H NMR ($CDCl_3$): δ 10.62 (br, 1H), 9.04 (d, J = 2.8, 1H), 8.43 (q, J = 2.8, 9.2, 1H), 4.34 (t, J = 6.6, 2H), 1.97 (m, 2H), 1.51 (m, 2H), 1.41−1.29 (m, 8H), 0.89 (t, J = 6.8, 3H). ^{13}C NMR ($CDCl_3$): δ 129.87, 129.63, 129.55, 118.79, 113.09, 71.41, 71.33, 31.67, 29.09, 20.04, 28.75, 25.75, 22.58, 14.01. Anal. Calcd for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 60.89; H, 7.21; N, 4.79.

***N*-((Ethoxycarbonyl)methyl)-2-(octyloxy)-5-nitrobenzamide (3a).** The acid **III** (2.95 g, 10 mmol) was dissolved in 10 mL of thionyl chloride, and the mixture was heated under reflux with a calcium chloride drying tube for 2 h. The solvent was removed in vacuo, and anhydrous ether (2 × 30 mL) was added and then removed. The crude acid chloride was directly used for the next step without further purification.

To a solution of glycine ethyl ester hydrochloride (10 mmol) and 2.02 g (20 mmol) of triethylamine in CH_2Cl_2 was added a solution of the above prepared acid chloride in 40 mL of methylene chloride over a period of 5 min at 0 °C. The ice−water bath was removed, and the reaction mixture was allowed to warm to room temperature. After 6 h, the solvent was evaporated, the residue was dissolved in ethyl acetate, and the solution was washed alternatively with diluted HCl and NaOH solutions. Drying over Na_2SO_4 and evaporation of solvent gave the crude product, which was recrystallized from MeOH, to give the product as a white solid (3.61 g, 95%). ^{1}H NMR ($CDCl_3$): δ 9.10 (d, J = 2.8, 1H), 8.44 (s, 1H), 8.32 (q, J = 3.4, 9.2, 1H), 7.08 (d, J = 9.2, 1H), 2.26 (m, 6H), 2.01 (m, 2H), 1.508 (m, 2H), 1.41−1.29 (m, 13H), 0.88 (t, J = 6.8, 3H). ^{13}C NMR ($CDCl_3$): δ 169.70, 162.91, 161.55, 141.72, 128.56, 128.21, 121.70, 112.54, 70.70, 61.57, 42.26, 31.75, 29.19, 29.14, 28.81, 26.07, 22.61, 14.18, 14.05. Anal. Calcd for $C_{19}H_{28}N_2O_6$: C, 59.98; H, 7.42; N, 7.37. Found: C, 59.93; H, 7.34; N, 7.47.

***N*-Hexyl-2-(octyloxy)-5-nitrobenzamide (4a).** This compound was synthesized by the same procedure used for preparing **3a**. Yield: 3.10 g, 82%. ^{1}H NMR ($CDCl_3$): δ 9.10 (d, J = 2.8, 1H), 9.29 (q, J = 2.8, 8.8, 1H), 7.78 (s, 1H), 7.05 (d, J = 9.2, 1H), 4.23 (t, J = 6.6, 2H), 3.47 (m, 2H), 1.93 (m, 2H), 1.61 (m, 2H), 1.51 (m, 2H), 1.41−1.30 (m, 14H), 0.90 (m, 6H). ^{13}C NMR ($CDCl_3$): δ 162.90, 161.13, 141.91, 128.52, 127.70, 122.84, 112.41, 70.32, 40.11, 31.75, 31.54, 29.49, 29.27, 29.19, 29.10, 26.83, 26.17, 22.60, 22.59, 14.01, 13.99. Anal. Calcd for $C_{21}H_{34}N_2O_4$: C, 66.65; H, 9.06; N, 7.40. Found: C, 66.53; H, 9.22; N, 7.48.

***N*-((Ethoxycarbonyl)methyl)-5-amino-2-(octyloxy)benzamide (3b).** Compound **3a** was reduced by catalytic hydrogenation in methanol at room temperature, using Pd−C (10%) as the catalyst. Removal of catalyst and solvent gave the crude product, which was used for the next step without isolation and further purification. Typical yield: 90−95%. ^{1}H NMR ($CDCl_3$): δ 8.74 (s, 1H), 7.56 (d, J = 2.8, 1H), 6.79 (m, 2H), 4.24 (q, J = 6.6, 13.2, 4H), 4.05 (t, J = 6.6, 2H), 3.54 (s, 2H), 1.90 (m, 2H), 1.46 (m, 2H), 1.35−1.28 (m, 13H), 0.88 (t, J = 6.8, 3H). ^{13}C NMR ($CDCl_3$): δ 169.97, 165.36, 150.66, 140.04, 121.40, 119.70, 118.48, 114.02, 69.94, 61.26, 42.07, 31.78, 29.29, 29.21, 26.17, 22.63, 14.19, 14.06. Anal. Calcd for $C_{19}H_{30}N_2O_4$: C, 65.11; H, 8.63; N, 8.00. Found: C, 64.82; H, 8.71; N, 7.97.

***N*-Hexyl-5-amino-2-(octyloxy)benzamide (4b).** This compound was prepared by the same procedure for used preparing **3b**. ^{1}H NMR ($CDCl_3$): δ 8.154 (s, 1H), 7.57 (d, J = 3.2, 1H), 6.76 (m, 2H), 4.01 (t, J = 6.4, 2H), 3.53 (s, 2H), 3.44 (q, J = 6.8, 12.8, 2H), 1.82 (m, 2H), 1.59 (m, 2H), 1.46 (m, 2H), 1.43−1.29 (m, 14H), 0.89 (t, J = 6.4, 6H). ^{13}C NMR ($CDCl_3$): δ 165.21, 150.17, 140.34, 122.42, 118.97, 118.51, 113.95, 69.72, 39.77, 31.80, 31.60, 29.56, 29.51, 29.38, 29.26, 26.89, 26.30, 22.63, 22.61, 14.06. Anal. Calcd for $C_{21}H_{36}N_2O_2$: C, 72.38; H, 10.41; N, 8.04. Found: C, 72.38; H, 10.40; N, 8.21.

***N*-((Ethoxycarbonyl)methyl)-5-acetamido-2-(octyloxy)benzamide (3c).** To a solution of **3b** (3.52 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) in 60 mL of methylene chloride was added dropwise acetyl chloride (0.79 g, 10 mmol) over 5 min at 0 °C, after which the ice−water bath was removed. After 6 h, the solvent was evaporated, the residue was dissolved in ethyl acetate, and the solution was washed with diluted HCl and NaOH solutions alternatively. Drying and evaporation of the solvent gave the crude product, which was recrystallized from MeOH, giving the pure product as a white solid (3.56 g, 90%). ^{1}H NMR ($CDCl_3$): δ 8.70 (s, 1H), 8.13 (q, J = 2.4, 8.8, 1H), 7.83 (d, J = 2.4, 1H), 7.38 (s, 1H), 6.96 (d, J = 8.8, 1H), 4.25 (q, J = 8.8, 14.0, 4H), 4.13 (t, J = 6.6, 2H), 2.17 (s, 3H), 1.93 (m, 2H), 1.48 (m, 2H), 1.37−1.29 (m, 12H), 0.88 (t, J = 6.4, 3H). ^{13}C NMR ($CDCl_3$): δ 169.76, 168.49, 164.96, 153.89, 131.80, 125.59, 123.24, 120.64, 113.06, 69.74, 61.41, 42.16, 31.78, 29.27, 29.20, 29.07, 26.14, 24.35, 22.63, 14.19, 14.06. Anal. Calcd for $C_{21}H_{32}N_2O_5$: C, 64.26; H, 8.22; N, 7.14. Found: C, 64.51; H, 8.45; N, 7.16.

***N*-(Carboxymethyl)-5-acetamido-2-(octyloxy)benzamide (3d).** Hydrolysis of **3c** based on the procedures described for **1c** gave the crude product, which was crystallized from MeOH, to give the pure product as a white solid (97%). ^{1}H NMR (DMSO): δ 12.80 (br, 1H), 9.95 (s, 1H), 8.52 (t, J = 5.0, 1H), 8.0 (s, 1H), 7.78 (d, J = 8.8, 1H), 7.10 (d, J = 8.4, 1H), 4.08 (t, J = 6.2, 2H), 4.01 (d, J = 3.6, 2H), 2.0 (s, 3), 1.80 (m, 2H), 1.40−1.24 (m, 10H), 0.843 (t, J = 6.2, 3H). ^{13}C NMR (DMSO): δ 171.07, 167.99, 164.23, 152.49, 132.64, 123.59, 121.71, 121.19, 113.57, 69.16, 41.55, 31.20, 28.70, 28.62, 28.43, 25.53, 23.78, 22.07, 13.96. Anal. Calcd for $C_{19}H_{28}NO_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.62; H, 7.97; N, 7.68.

Octyl 3,5-Dinitrobenzoate (3e). To a solution of 3,5-dinitrobenzoyl chloride (18.5 g, 80 mmol) and triethylamine (8.1 g, 80 mmol) in 250 mL of methylene chloride was added dropwise 1-octanol in 40 mL of methylene chloride over 5 min at 0 °C. The ice−water bath was removed. After 6 h, the solvent was evaporated and the residue was dissolved in ethyl acetate and washed with diluted HCl and NaOH solutions alternatively. The ethyl acetate solution was dried over Na_2SO_4. Evaporation of the solvent and recrystallization of the crude product from MeOH gave the product as a white solid (25.9 g, 84%).^{1}H NMR ($CDCl_3$): δ 9.22 (t, J = 2.0, 1H), 9.16 (d, J = 2.0, 2H), 4.48 (t, J = 2.0, 2H), 1.83 (m, 2H), 1.47−1.29 (m, 10H), 0.88 (t, J = 6.6, 3H). ^{13}C NMR ($CDCl_3$): δ 162.23, 148.43, 133.93, 129.04, 121.92, 66.83, 31.43, 28.84, 28.25, 25.58, 22.29, 13.71. Anal. Calcd for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.22; N, 8.64. Found: C, 55.45; H, 6.34; N, 8.70.

Octyl 3,5-Diaminobenzoate (3f). Hydrogenation of **3e** based on procedures similar to those described for **1e** gave crude **3f** (90%), which was used directly for the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 6.78 (d, J = 1.6, 2H), 6.18 (s, 1H), 4.25 (t, J = 6.6, 2H), 3.67 (s, 4H), 1.73 (m, 2H), 1.42−1.28 (m, 10H), 0.88 (t, J = 6.0, 3H). ^{13}C NMR ($CDCl_3$): δ 166.91, 147.47, 132.56, 106.94, 105.55, 64.97, 31.80, 29.25, 29.19, 28.76, 26.05, 22.63, 14.05. Anal. Calcd for $C_{15}H_{24}N_2O_2$: C, 68.15; H, 9.15; N, 10.60. Found: C, 68.14; H, 9.14; N, 10.67.

Octyl 3,5-Bis[((((((5-acetamido-2-(octyloxy)phenyl)carbonyl)amino)methyl)carbonyl)amino]benzoate (3). To a solution of acid **3d** (3.64 g, 10 mmol), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (1.92 g, 10 mmol), and 1-hydoxybenzotriazole (1.35 g, 10 mmol) in 30 mL of dimethylformamide (DMF) was added the diamine **3f** (1.32 g, 5 mmol) in 10 mL of DMF. The reaction was allowed to proceed overnight. Distilled water (50 mL) was then added to the reaction mixture. The precipitated solid was filtered off and stirred with anhydrous MeOH under reflux for 2 h. The solution was cooled to room temperature and filtered, giving the crude product. Recrystallization from DMF/$CHCl_3$ gave the pure product as a white solid (2.6 g, 56%). ^{1}H NMR (DMSO): δ 10.36 (s, 2H), 9.92 (s, 2H), 8.66 (t, J = 4.8, 2H), 8.10 (s, 1H), 8.05 (d, J = 2.8, 2H), 8.02 (d, J = 1.2, 2H), 7.79 (q, J = 2.8 Hz, 9.2, 2H), 7.12 (d, J = 9.2, 2H), 4.24 (t, J = 6.6, 2H), 4.19 (d, J = 2.4, 4H), 4.11 (t, J = 6.4, 4H), 2.00 (s, 6H), 1.83 (m, 4H), 1.69 (m, 2H), 1.41−1.14 (m, 26H), 0.82 (t, J = 6.6, 3H), 0.741 (t, J = 6.6, 6H). ^{13}C NMR (DMSO): δ 167.76, 167.35, 165.29, 164.14, 152.46, 139.25, 132.57, 130.66, 123.50, 121.72, 121.27, 114.75, 113.93, 113.50, 69.24, 64.57, 43.44, 31.01, 28.57, 28.48, 28.44, 28.38, 28.05, 25.58, 25.25, 23.59, 21.84, 21.82, 13.66, 13.62. Anal. Calcd for $C_{53}H_{76}N_6O_{10}$: C, 66.50; H, 8.00; N, 8.78. Found: C, 66.12; H, 8.12; N, 8.79.

4,6-Dihydroxy-1,3-benzenedicarboxylic Acid (4c). Resorcinol (2.2 g, 20 mmol) was mixed with anhydrous potassium bicarbonate (4.0 g, 40 mmol) in a Carius tube at 210 °C for 3 h. The mixture was then treated with water (100 mL), and the aqueous mixture was extracted with ether. The crude product was obtained upon acidification of the aqueous layer. Recrystallization from 2000 mL of boiling water gave pure **4c** (1.78 g, 45%). ^{1}H NMR (DMSO): δ 11.87 (br, 2H), 8.28 (s, 1H), 6.40 (s, 1H). ^{13}C NMR (DMSO): δ 171.02, 166.46, 134.31, 105.96, 103.16. Anal. Calcd for $C_8H_6O_6$: C, 48.50; H, 3.05. Found: C, 48.46; H, 3.09.

Dimethyl 4,6-Dihydroxy-1,3-benzenedicarboxylate (4d). A solution of **4c** (5.94 g, 30 mmol) and concentrated H_2SO_4 (~7.5 mL) in 120 mL of MeOH was heated under reflux for 2 days. The product was obtained upon cooling and was filtered off and washed with a small amount of MeOH, to give pure **4d** (6.11 g, 90%). ^{1}H NMR ($CDCl_3$): δ 11.25 (s, 2H), 8.40 (s, 1H), 6.48 (s, 1H), 3.94 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 169.73, 167.15, 134.16, 105.78, 104.20, 52.24. Anal. Calcd for $C_{10}H_{10}O_6$: C, 53.10; H, 4.46. Found: C, 52.93; H, 4.64.

Dimethyl 4,6-Bis(octyloxy)-1,3-benzenedicarboxylate (4e). A mixture of **4d** (6.79 g, 30 mmol), K_2CO_3 (24.86 g, 180 mmol), and 1-bromooctane (23.2 g, 120 mmol) in 150 mL of DMF containing 30 mL of methanol was heated at 100 °C for 2 days. The solid was filtered off, and the solvent was removed in vacuo at 130 °C. The residue was dissoloved in ethyl acetate, and the solution was washed with diluted HCl and NaOH solutions alternatively. Evaporation of the solvent gave pure **4e** (11.9 g, 88%). ^{1}H NMR ($CDCl_3$): δ 8.458 (s, 1H), 6.423 (s, H), 4.057 (t, J = 6.4, 4H), 3.85 (s, 6H), 1.87 (m, 2H), 1.51 (m, 4H), 1.35−1.28 (m, 16H), 0.88 (t, J = 6.8, 3H). ^{13}C NMR ($CDCl_3$): δ 165.43, 163.63, 137.01, 112.05, 97.70, 69.28, 51.62, 31.80, 29.28, 29.21, 29.06, 25.53, 22.64, 14.02. Anal. Calcd for $C_{26}H_{42}O_6$: C, 69.30; H, 9.40. Found: C, 69.46; H, 9.68.

4,6-Bis(octyloxy)-1,3-benzenedicarboxylic Acid (4f). Hydrolysis of **4e** based on procedures similar to those described for **1c** gave the product **4f**, which was recrystallized from MeOH, to give pure **4f** (93%). ^{1}H NMR (DMSO): δ 12.34 (br, 2H), 8.16 (s, 1H), 6.64 (s, 1H), 4.09 (t, J = 6.4, 4H), 1.70 (m, 4H), 1.42−1.24 (m, 20H), 0.84 (t, J = 6.4, 4H). ^{13}C NMR (DMSO): δ 165.84, 162.63, 135.92, 111.86, 98.24, 68.51, 31.16, 28.62, 28.57, 29.40, 25.28, 22.04, 13.90. Anal. Calcd for $C_{24}H_{38}O_6$: C, 68.22; H, 9.06. Found: C, 68.12; H, 9.21.

***N,N′*-Bis((ethoxycarbonyl)methyl)-4,6-bis(octyloxy)-1,3-benzenedicarboxamide (4g).** To a solution of **4f** (1.27 g, 3 mmol), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (1.15 g, 6 mmol), and 1-hydroxybenzotriazole (0.81 g, 6 mmol) in 40 mL of DMF were added glycine ether ester hydrochloride (0.98 g, 7 mmol) and triethylamine (0.7 g, 7 mmol) in 20 mL of DMF. The reaction mixture was stirred for 6 h, and the solvent was removed in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed with diluted HCl and NaOH solutions alternatively. The ethyl acetate solution was dried over Na_2SO_4. Evaporation of the solvent gave pure **4g** as a white solid (1.07 g, 69%). ^{1}H NMR ($CDCl_3$): δ 9.01 (s, 1H), 8.26 (t, J = 4.4, 2H), 6.44 (s, 1H), 4.23 (m, 8H), 4.14 (t, J = 6.6, 4H), 1.96 (m, 4H), 1.49 (m, 4H), 1.39−1.27 (m, 22H), 0.87 (t, J = 6.8, 6H). ^{13}C NMR ($CDCl_3$): δ 170.13, 164.18, 160.68, 137.33, 114.58, 96.44, 69.86, 61.20, 42.07, 31.75, 29.24, 29.14, 28.98, 26.14, 22.58, 14.15, 13.97. Anal. Calcd for $C_{32}H_{52}N_2O_8$: C, 64.84; H, 8.84; N, 4.73. Found: C, 64.85; H, 9.12; N, 4.81.

***N,N′*-Bis(carboxymethyl)-4,6-bis(octyloxy)-1,3-benzenedicarboxamide (4h).** Hydrolysis of **4g** based on procedures similar to those described for **1c** gave **4h**. Recrystallization of the crude product from MeOH gave pure **4h** as a white solid (90%). ^{1}H NMR (DMSO): δ 12.76 (br, 2H), 8.53 (s, 1H), 8.29 (t, J = 5.2, 2H), 6.79 (s, 1H), 4.25 (t, J = 6.4, 4H), 4.02 (d, J = 5.2, 4H), 1.85 (m, 4H), 1.43−1.20 (m, 20H), 0.85 (t, J = 6.8, 6H). ^{13}C NMR (DMSO): δ 170.88, 163.52, 160.14, 134.87, 113.87, 97.88, 69.44, 41.39, 31.02, 28.49, 28.41, 28.11, 25.33, 21.88, 13.73. Anal. Calcd for $C_{28}H_{44}N_2O_8$: C, 62.66; H, 8.26; N, 5.22. Found: C, 62.79; H, 8.56; N, 5.09.

***N,N′*-Bis[(((3′-((hexylamino)carbonyl)-4-(octyloxy)phenyl)amino)carbonyl)methyl]-4,6-bis(octyloxy)-1,3-benzenedicarboxamide (4).** To a solution of **4b** (2.48 g, 3.56 mmol) in 30 mL of DMF was added a solution of the diacid **4h** (1.91 g, 3.56 mmol), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (1.36 g, 3.5 mmol), and 1-hydroxybenzotriazole (0.96 g, 3.56 mmol) in 40 mL of DMF. The reaction was allowed to proceed for 6 h at room temperature, and the precipitated product was collected by filtration. Recrystallization from DMF/$CHCl_3$ gave pure **4** (3.37 g, 79%). ^{1}H NMR ($CDCl_3$): δ 10.12 (s, 2H), 8.96 (s, 1H), 8.57 (s, 2H), 8.29 (d, J = 8.8, 2H), 8.18−8.14 (m, 4H), 6.94 (d, J = 9.2, 2H), 6.47 (s, 1H), 4.62 (s, 4H), 4.159 (t, J = 6.6, 4H), 4.10 (t, J = 6.6, 4H), 3.49 (q, J = 7.2, 12.8, 4H), 2.03 (m, 4H), 1.86 (m, 4H), 1.61−1.25 (m, 56H), 0.91−0.83 (m, 18H). ^{13}C NMR ($CDCl_3$): δ 167.83, 165.01, 164.52, 160.54, 153.22, 136.44, 132.89, 124.95, 123,51, 121.85, 115.33, 112.97, 96.51, 69.91, 69.58, 44.69, 40.13, 31.92, 31.81, 31.62, 29.52, 29.47, 29.41, 29.38, 29.26, 29.24, 29.06, 26.86, 26.30, 22.65, 22.61, 22.60, 14.07, 13.99, 13.97. Anal. Calcd for $C_{70}H_{112}N_6O_{10}$: C, 70.20; H, 9.43; N, 7.02. Found: C, 70.10; H, 9.62; N, 7.24.

Binding Studies. The binding parameters were determined by titrating a solution of **3** with that of **4** in an Omega isothermal titration calorimeter (MicroCal, Northampton, MA). In chloroform, 0.1 mM **3** and 0.8 mM **4** were used. Lower concentrations of host and guest were used in pure chloroform due to the very high binding affinities. In 5% DMSO/$CHCl_3$, a solution of 0.5 mM **3** was titrated with a 4 mM stock solution of **4**. The cell was thermostated to ±0.1 °C using a circulating bath. All of the experiments were performed at 25 °C. The enthalpy of binding between **3** and **4** was determined from heats of multiple single injections. Injection volumes were 5 mL, with 3 min of equilibration time allowed between injections. The heat of dilution of both host and guest into solvent was determined, and the host−guest titration heat was adjusted by this contribution.

The binding constants, K, and the number of binding sites, n, were extracted from the calorimetric data by employing the Origin data analysis software supplied with the Omega titration calorimeter. A complete description of the data analysis has been published by Brandts and co-workers.[25]

NMR dilution experiments were performed on a Varian VXR 400 spectrometer at 25 °C in $CHCl_3$. The solution of a sample was diluted, and the chemical shifts of the NH protons were followed in the ^{1}H NMR spectra and analyzed with an equation described previously.[22]

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