ORGANIC LETTERS

2000
Vol. 2, No. 26
4121−4124

Self-Assembly of Polar Functionalities Using Noncovalent Platforms

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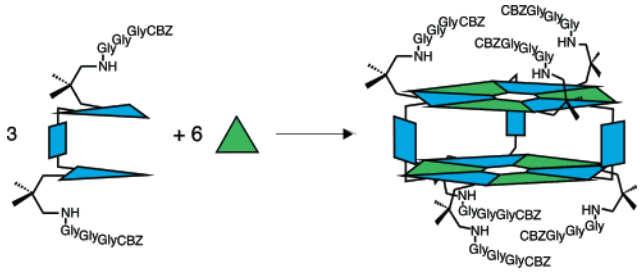
Received August 25, 2000

ABSTRACT



Small peptide fragments functionalized with dimelamine units spontaneously form well-defined assemblies. The hydrogen-bond donating and accepting sites in the peptide units are perfectly compatible with the hydrogen-bond assembly motif and slightly stabilize the assembly via additional hydrogen-bond formation.

The concave 3-D positioning of different amino acid residues around a central cavity via noncovalent interactions provides the basis for natural receptors to bind substrates and catalyze (bio)chemical transformations with exquisite regio- and stereoselectivity. Efforts to mimic such functional group arrays in covalent[1] or metal-coordinated systems[2] have recently led to the synthesis of artificial protein receptors mimicking the binding properties of natural antibodies.[3] However, the self-assembly of polar functionalities using noncovalent interactions has so far received very little attention, presumably due to incompatibility with the noncovalent scaffolds.[4] In this paper we show that hydrogen-bond-directed self-assembly can indeed be used to assemble polar functionalities, such as amines, amides, ureas, or small peptide fragments. These functionalities do not decrease the thermodynamic stability of the assemblies to a significant extent but in certain cases even lead to a slight increase in the stability.

In previous papers we reported on the noncovalent synthesis of double rosette assemblies $\mathbf{1}_3\cdot(\mathrm{DEB})_6$ via the cooperative formation of 36 hydrogen bonds, using the rosette motif introduced by Whitesides.[5] In view of our objective to generate functional group diversity in or around noncovalently assembled cavities,[6] we studied the assembly

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10.1021/ol000244o CCC: $19.00 © 2000 American Chemical Society
Published on Web 12/06/2000

of calix[4]arene dimelamines **2a**−**e** carrying amino (**2a**), ureido (**2b**), amido (**2c**), sulfonamido (**2d**), and phosphoramido (**2e**) functionalities that are connected via a 2,2-dimethylpropyl spacer to the melamine units (see Figure 1).

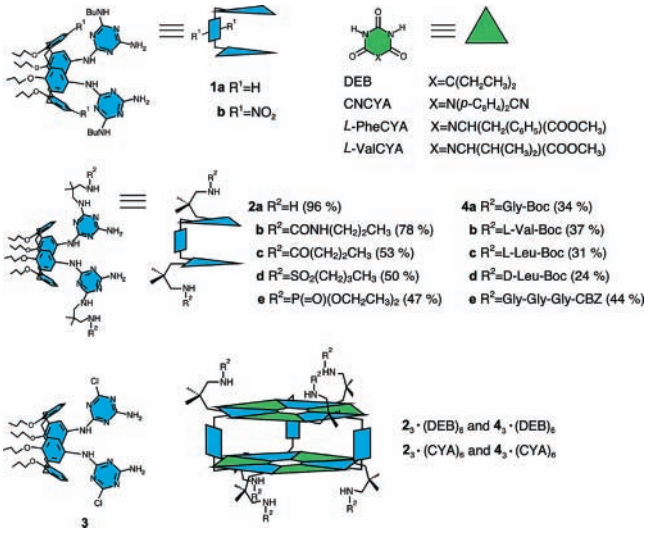

Figure 1. Molecular structures and schematic representations of the molecular components **1**−**4** and the corresponding hydrogen-bonded assemblies $\mathbf{2}_3\cdot(\mathrm{DEB})_6$ and $\mathbf{4}_3\cdot(\mathrm{DEB})_6$ or $\mathbf{2}_3\cdot(\mathrm{CYA})_6$ and $\mathbf{4}_3\cdot(\mathrm{CYA})_6$. Reaction conditions for the formation of product **2a**: compound **3**, 2,2-dimethyl-1,3-propanediamine (large excess), 90 °C overnight; product **2b**: compound **2a**, propyl isocyanate (50 equiv), 2 h, rt; products **2c**−**2e**: compound **2a**, corresponding acid chloride derivative (50 equiv), 1 h, rt; products **4**: compound **2a**, corresponding *N*-protected amino acid/peptide (2.2 equiv), EDC/HATU (2.2 equiv), DIPEA (4 equiv) 24−48 h, rt.

These compounds were synthesized starting from bis(chlorotriazine) **3** via reaction with an excess of 2,2-dimethyl-1,3-propanediamine (**2a**, >95%), followed by reaction with propyl isocyanate (**2b**, 78%), butyryl chloride (**2c**, 53%), butanesulfonyl chloride (**2d**, 50%), or diethyl phosphoryl chloride (**2e**, 47%) (for details, see Supporting Information).

Without exception the ^{1}H NMR spectra of these assemblies exhibit diagnostic signals at 14.1−14.0 (H_a), 13.3−13.2 (H_b), 8.6−8.4 (H_c), 7.8−7.6 (H_d), 6.7 (H_e), 6.9−7.1 (H_f), 7.1−7.0 (H_g), and 6.1−6.0 (H_h) (see Figure 2). The assemblies are formed in yields varying from 75 to 99% as determined by integration of the appropriate ^{1}H NMR signals.[7] Characterization of the corresponding assemblies $\mathbf{2}_3\cdot(\mathrm{CNCYA})_6$ by MALDI-TOF using Ag^+ labeling[8] also confirmed the successful formation of the hydrogen-bonded assemblies. The only exception is assembly $\mathbf{2a}_3\cdot(\mathrm{CNCYA})_6$, which is unstable due to proton transfer between the strongly acidic CNCYA ($pK_a \sim 4.7$) and the basic amino function.

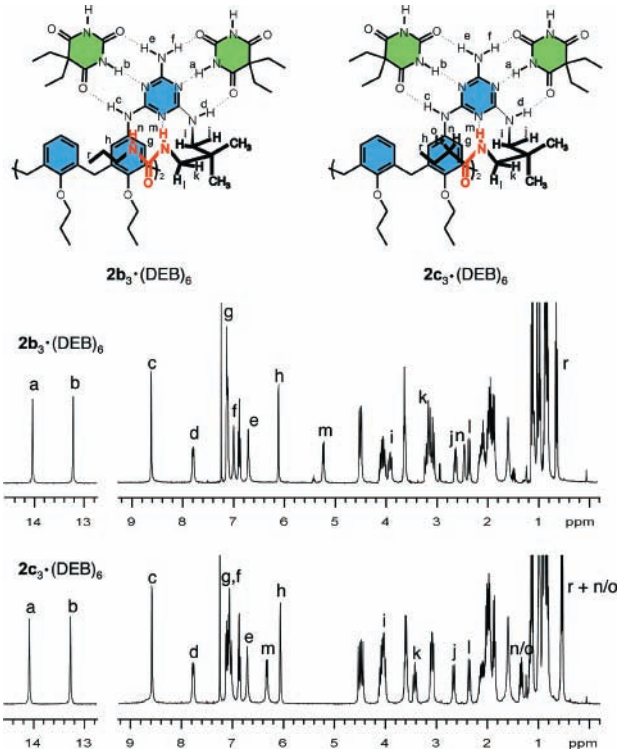

Figure 2. Characterization of assemblies $\mathbf{2b}_3\cdot(\mathrm{DEB})_6$ and $\mathbf{2c}_3\cdot(\mathrm{DEB})_6$ by ^{1}H NMR spectroscopy. Spectra were recorded at 400 MHz in $CDCl_3$ at 303 K.

Subsequently, we investigated the effect of amino (**2a**), ureido (**2b**), and amido (**2c**) functionalities on the thermodynamic stability of the corresponding hydrogen-bonded assemblies by ^{1}H NMR titrations with DMSO-d_6 (see Table 1).[9] We found that the stability of assemblies $\mathbf{2b}_3\cdot(\mathrm{DEB})_6$ and $\mathbf{2c}_3\cdot(\mathrm{DEB})_6$, carrying six ureido and six amido functionalities, respectively, is significantly higher ($\chi_{DMSO} = 14$ and 15) than that for the corresponding assembly $\mathbf{1}_3\cdot(\mathrm{DEB})_6$ ($\chi_{DMSO} = 10$). The stability of assembly $\mathbf{2a}_3\cdot(\mathrm{DEB})_6$ is equal to that of $\mathbf{1}_3\cdot(\mathrm{DEB})_6$, indicating that there is no effect from the amino substituents.

Table 1. Thermodynamic Stability Measurements of Assemblies $\mathbf{2}_3\cdot(\mathrm{DEB})_6$ and $\mathbf{2}_3\cdot(\mathrm{CYA})_6$

assembly	χ_{DMSO}[a]	assembly	χ_{THF}[a]
$\mathbf{1a}_3\cdot(\mathrm{DEB})_6$	10	$\mathbf{1a}_3\cdot(\text{L-PheCYA})_6$	34
$\mathbf{2a}_3\cdot(\mathrm{DEB})_6$	10	$\mathbf{2b}_3\cdot(\text{L-PheCYA})_6$	31
$\mathbf{2b}_3\cdot(\mathrm{DEB})_6$	14	$\mathbf{2c}_3\cdot(\text{L-PheCYA})_6$	35
$\mathbf{2c}_3\cdot(\mathrm{DEB})_6$	15	$\mathbf{2b}_3\cdot(\text{L-ValCYA})_6$	34
		$\mathbf{2c}_3\cdot(\text{L-ValCYA})_6$	34 (29)[b]

[a] χ = percentage of polar solvent at which 50% of the assembly is still intact. [b] Independently determined by THF ^{1}H NMR titration.

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(7) The percentage of assembly formed was determined by comparison of the $H^a + H^b$ integral with the ArCH_2Ar integral of the calix[4]arene part. As a standard we use assembly $\mathbf{1}_3\cdot(\mathrm{DEB})_6$, for which the integral ratio was arbitrarily set to 100%.

(8) (a) Jolliffe, K. A.; Crego Calama, M.; Fokkens, R.; Nibbering, N. M. M.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **1998**, *37*, 1247−1251. (b) Prins, L. J.; Jolliffe, K. A.; Hulst, R.; Timmerman, P.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2000**, *122*, 3617−3627.

Structural analysis of the assemblies $\mathbf{2b}_3\cdot(\mathrm{DEB})_6$ and $\mathbf{2c}_3\cdot(\mathrm{DEB})_6$ by ^{1}H NMR spectroscopy using 2D DQF,[10] TOCSY,[11] and NOESY[12] experiments clarifies the reasons for the improved stability. In both cases, the 2,2-dimethylpropyl side chain adopts a very rigid conformation as judged from the presence of strong NOE connectivities between H_d and H_j and between H_l and H_m, respectively, and the absence of NOE connectivities between H_d and H_i and between H_k and H_m, respectively. For assembly $\mathbf{2b}_3\cdot(\mathrm{DEB})_6$, the two urea proton signals resonate at very different chemical shifts in the ^{1}H NMR spectrum, i.e., at 5.2 (H_m) and 2.4 (H_n) ppm, and H_n shows a distinguished NOE cross-peak with one of the propoxy groups of the calix[4]arene unit. Therefore, we suggest that the side chain is folded back over the calix[4]arene aromatic rings, resulting in a high-field chemical shift for H_n because of an anisotropic shielding effect of one of the calix[4]arene aromatic rings. The folding back can be explained by the fact that the other urea proton (H_m) is involved in hydrogen bonding with one of the nitrogen atoms (N-1) of the triazine ring. This view is supported by the fact that the urea proton signals do not shift at all upon the addition of chloride (1−2 equiv) or carboxylate (1−2 equiv) anions, while free urea groups normally interact strongly with these anions, giving rise to 1−2 ppm downfield shifts.[13] Gas-phase molecular modeling calculations (Quanta 97, CHARMm 24.0) in which the observed NOE connectivities were used as distance constraints gave a structure in which the propylureido groups fold back over the calix[4]arene units.

Structural analysis of assembly $\mathbf{2c}_3\cdot\mathrm{DEB}_6$ showed similar results. The amide proton (H_m) resonate at 6.33 ppm, while the double triplets observed for the methylene protons ($NH_mC(O)CH_nH_o$) are strongly upfield shifted ($\delta = 1.4$ and 0.6 ppm, respectively) from their normal position between 2 and 3 ppm. These methylene protons also show a NOE cross-peak with one of the propoxy groups of the calix[4]arene unit which means that they occupy a well-defined position on top of the electron-rich aromatic ring of the calix[4]arene unit. Moreover, the $NHC(O)CH_2CH_2CH_3$ protons also experience upfield shifts ($\delta = 1.2$ and 0.54 ppm), albeit much smaller. Also in this case, gas-phase molecular modeling studies reproduce the hydrogen-bonding interaction between the amide proton and the triazine nitrogen (N-1) (see Figure 3).

The stability of the functionalized assemblies was also studied by CD spectroscopy via the addition of THF (see Table 1). For these measurements, we make use of the fact that double rosette assemblies with chiral cyanurates, such as L-ValCYA and L-PheCYA, display very intense and characteristic CD spectra, which are not observed for the individual components.[5a,14] For assemblies $\mathbf{2}_3\cdot$(L-PheCYA)$_6$ and $\mathbf{2}_3\cdot$(L-ValCYA)$_6$ (see Figure 4) with ureido (**2b**) and amido (**2c**) functionalities, we found that the thermodynamic stability is essentially equal to that of $\mathbf{1a}_3\cdot$(L-PheCYA)$_6$. The small difference in relative stability of assemblies $\mathbf{2}_3\cdot$(L-PheCYA)$_6$ and $\mathbf{2}_3\cdot(\mathrm{DEB})_6$ is most likely due to steric interac-

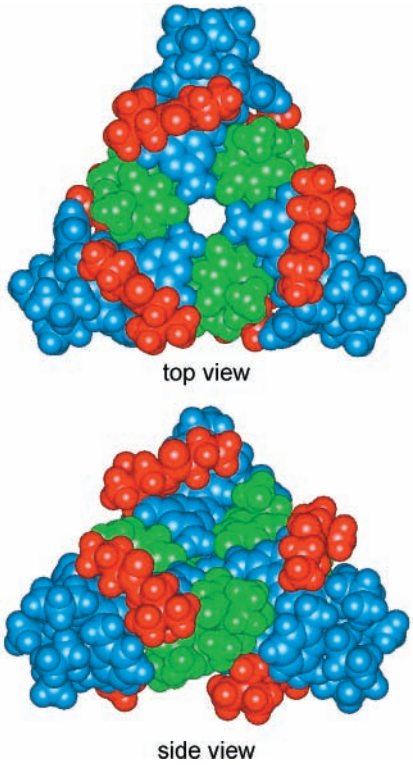

Figure 3. Gas-phase-minimized structure of assembly $\mathbf{2c}_3\cdot(\mathrm{DEB})_6$ using the observed NOE connectivities as distance constraints. The blue parts represent the calix[4]arene dimelamine components, the green parts the barbiturate fragments, and the red parts the amido side chains.

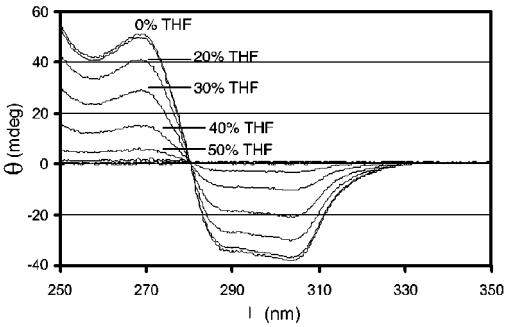

Figure 4. Determination of the thermodynamic stability of assembly $\mathbf{2c}_3\cdot$(L-ValCYA)$_6$ using CD spectroscopy. Spectra were recorded in CH_2Cl_2/THF mixtures (1 mM) at 298 K.

(9) (a) Mammen, M.; Simanek, E. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 12614−12623. (b) The stability of the hydrogen bond assemblies was measured by titration of a chloroform-d_1 solution of the assembly with DMSO-d_6 using 1,4-dinitrobenzene ($\delta = 8.3$−8.4 ppm) as an internal standard. The χ_{DMSO} value represents the % of DMSO corresponding to formation of only 50% of the assembly.

(10) Rance, M.; Sørensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wütrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479.

(11) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355−360.

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tions between the more bulky side chains of L-ValCYA or L-PheCYA and the 2,2-dimethylpropyl spacer in **2b** and **2c**, which counterbalances the previously observed stabilizing effect. Assembly $\mathbf{2a}_3\cdot(\text{L-PheCYA})_6$ is unstable, even in neat chloroform, due to proton transfer between the strongly acidic L-PheCYA ($pK_a \sim 4.7$) and the basic amino function.

With the knowledge that the amido functionalities in **2c** significantly stabilize the corresponding hydrogen-bonded assembly, we subsequently investigated assemblies of the calix[4]arene dimelamines **4** that are functionalized with the Boc-protected amino acids Gly (**4a**), L-Val (**4b**), and L- (**4c**) or D-Leu (**4d**). Dimelamines **4** were synthesized starting from **2a** using standard peptide coupling conditions (EDC, HATU, for details see Supporting Information). ^{1}H NMR spectra show that assembly $\mathbf{4a}_3\cdot(\text{DEB})_6$ is formed in quantitative yield in chloroform. The very close similarity between the ^{1}H NMR spectra of $\mathbf{4a}_3\cdot(\text{DEB})_6$ and $\mathbf{2c}_3\cdot(\text{DEB})_6$ suggests that both assemblies have very similar structures. Moreover, the corresponding chiral assembly $\mathbf{4a}_3\cdot(\text{L-PheCYA})_6$ displays a CD virtually identical to that of assembly $\mathbf{2c}_3\cdot(\text{L-PheCYA})_6$. For dimelamine **4b**, with the bulky isopropyl side chain, formation of the assembly with DEB in $CDCl_3$ occurs only partially (~40%). The decrease in stability is clearly caused by steric hindrance from the isopropyl group. A further increase in the size of the side chain, as for **4c** (L-Leu), totally inhibits formation of the hydrogen-bonded assembly in chloroform. However, in a less polar solvent such as toluene, the assembly is formed quantitatively (>95%). These results clearly show that the formation and stability of assemblies $\mathbf{4}_3\cdot(\text{DEB})_6$ are extremely sensitive to steric effects but seem to be only marginally affected by the presence of the amide or carbamate functionalities.

The assemblies $\mathbf{4b}_3\cdot(\text{DEB})_6$ and $\mathbf{4c}_3\cdot(\text{DEB})_6$ display a highly characteristic CD for calix[4]arene double rosette assemblies, which originates from diastereoselective formation of the *P*-isomer.[5a] Despite the fact that the chiral centers in the side chains are six atoms away from the core of the assembly, the induction of the *P*-helicity is virtually complete (>95%) as judged from the single set of signals for the $H_{a,b}$ protons in the NMR spectrum. This fact further emphasizes the very close proximity of the chiral side chains to the assembly.

We finally investigated by ^{1}H NMR and CD spectroscopy the formation of assemblies $\mathbf{4e}_3\cdot(\text{DEB})_6$ and $\mathbf{4e}_3\cdot(\text{L-PheCYA})_6$ (R = Gly_3CBZ), both assemblies carrying a total of *18 amide and 6 carbamate* functionalities. Assembly $\mathbf{4e}_3\cdot(\text{DEB})_6$ is formed in 80% in chloroform, and the ^{1}H NMR spectrum clearly shows the characteristic signals for assembly formation. The spectrum is slightly broadened, most likely as the result of nonspecific aggregation. Assembly $\mathbf{4e}_3\cdot(\text{L-PheCYA})_6$ displays the characteristic CD, which proves its formation. The CD intensity has dropped to 75% in comparison to that of $\mathbf{4a}_3\cdot(\text{L-PheCYA})_6$, which corresponds well with the stability as determined by NMR, assuming that the molar CD absorptivities of both assemblies are comparable.

In this paper we have for the first time shown that the formation of noncovalent hydrogen assemblies can be used for the self-assembly of polar hydrogen bond donating and accepting functionalities in solution. Our results clearly show that the stability of the assemblies is comparable to those of nonfunctionalized assemblies. Furthermore, it has been shown that bulky side chains cause steric hindrance, which strongly decreases the thermodynamic stability of the assemblies. Future work will concentrate on noncovalent combinatorial libraries of these peptide-functionalized assemblies[15] in which structural diversity can be generated by random variation of the oligopeptide sequences.

Acknowledgment. We thank Mr. R. Fokkens and Prof. N. M. M. Nibbering for the MALDI-TOF measurements, The Technology Foundation for financial support (project number TST 4624, J.M.C.A.K), the EC for the Marie Curie Research Training Grant to Dr. M. Crego-Calama (No. ERBFMBICT 961445) as a part of the TMR Program, and FWO Vlaanderen for financial support (I.L.).

Supporting Information Available: Experimental procedures, characterization for assemblies $\mathbf{2}\cdot(\text{DEB})_6$, and MALDI-TOF data for $\mathbf{2}_3\cdot(\text{CNCYA})_6$. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) In the absence of any other source of chirality, the double rosette assembly exists as a racemic mixture of the *M*- and *P*-enantiomers. An assembly of 3 equiv calix[4]arene dimelamines with 6 equiv of L- or D-PheCYA, respectively, gives quantitatively either the *M*- or the *P*-enantiomer, both with a highly characteristic CD. The maximum of the CD signals is measured as a function of the volume fraction THF in CH_2Cl_2. In analogy to the DMSO titrations, the χ_{THF} value represents the % of THF corresponding to formation of only 50% of the assembly.

(15) Crego Calama, M.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **2000**, *39*, 755−758.