Covalent capture of dynamic hydrogen-bonded assemblies

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Covalent linkage of the three calix[4]arene units in hydrogen-bonded assemblies $\mathbf{1}_3$•$(DEB)_6$ *via* a threefold ring closing metathesis (RCM) reaction quantitatively converts the dynamic assemblies into covalent systems (123-membered macrocycles) that can be easily characterized using MALDI-TOF MS and HPLC.

Dynamic combinatorial libraries have recently attracted a great deal of attention in the rapidly expanding field of combinatorial chemistry.[1] Such libraries have the potential to amplify the formation of the strongest binder as a result of a templating effect exerted by an added host or guest molecule.[2–7] Most literature examples involve dynamic systems based on the reversible formation of *covalent* bonds[2–5] or coordinative bonds.[6,7] Dynamic libraries based on the reversible formation of weak noncovalent interactions, like multiple hydrogen-bonding, have so far not received a great deal of attention, most likely because of severe characterization problems. Our group has reported the synthesis and characterization of a 4-membered dynamic library of hydrogen-bonded assemblies and recently we have shown that guest-templating effects are also applicable to these systems.[8,9] Increasing the structural diversity in these systems is easy and can be performed simply by mixing the appropriate number of individual components under thermodynamically controlled conditions. However, the serious lack of suitable characterization techniques currently limits the size of the libraries that can be made.

Here we describe the covalent capture of dynamic libraries of hydrogen-bonded assemblies $\mathbf{1}_3$•$(DEB)_6$, which converts these dynamic libraries into covalent analogues that can be readily characterized using conventional techniques like mass spectrometry and HPLC. We used the ring-closing metathesis (RCM) reaction,[10] because it is compatible with the hydrogen-bonded network in assembly $\mathbf{1}_3$•$(DEB)_6$. Moreover, it has been used previously for the cyclization of cyclic peptides,[11] catenane formation,[12,13] and post-modification of dendrimers.[14]

Reaction of assembly $\mathbf{1a}_3$•$(DEB)_6$ (R = H, n = 6),[15] carrying oct-7-enyl side chains, with Grubbs catalyst in CD_2Cl_2 resulted in the covalent linkage of the three calix[4]arene units **1a** (Fig. 1) *via* a threefold metathesis reaction giving the 123-membered macrocycle **2a** as the corresponding hydrogen-bonded assembly **2a**•$(DEB)_6$ in 96% yield (Fig. 2).† Monitoring the reaction by ^{1}H NMR spectroscopy (Fig. 3) clearly showed that the reaction occurs without destroying the assembly. The signals for the terminal vinylic protons at δ 5.8 and 4.9 gradually disappear during the reaction and a new signal at δ 5.47 for the internal vinylic protons in **2a**•$(DEB)_6$ is observed [Fig. 3(*b*)].‡ HPLC analysis of the reaction mixture (at different time intervals) showed that **1a** is rapidly consumed (> 95% after 36 min) upon addition of the Ru catalyst, initially giving intermediate products (linear dimer and trimer) that are slowly converted into the final product **2a**.§ The clean formation of assembly **2a**•$(DEB)_6$ (detected as **2a** after loss of DEB under the MS conditions) was confirmed by MALDI-TOF MS (observed m/z = 3100 for $[\mathbf{2a} + H]^+$ containing the most abundant natural isotopes; calc. for $C_{180}H_{240}N_{36}O_{12}$ = 3100; Fig. 4). Both HPLC and MALDI-TOF MS clearly showed that the cyclic monomer **3a** (calc. m/z for $C_{60}H_{80}N_{12}O_4$ = 1032) is not formed, which emphasizes the high degree of preorganization of the reactive double bonds within the assembly.

The clean formation of assembly **2a**•$(DEB)_6$ was only observed under conditions where assembly $\mathbf{1a}_3$•$(DEB)_6$ is present. Not a single trace of **2a** was formed when **1a** was reacted with the Ru catalyst either in the absence of DEB or in the presence of 4 equiv. of *N*-propyl-5,5-diethylbarbituric acid (PDB), a substitute for DEB that cannot form an assembly similar to $\mathbf{1a}_3$•$(DEB)_6$. Moreover, RCM reactions carried out at $[\mathbf{1a}_3$•$(DEB)_6]_{t=0}$ = 0.01 mM (250-fold dilution) gave the cyclic monomer **3a** as the major product due to extensive dissociation of the assembly at this concentration.

The presence of bromo or iodo substituents at position R in assembly $\mathbf{1}_3$•$(DEB)_6$ significantly decreases the yields of the corresponding cyclic trimers **2b** (21%) and **2c** (16%) (Fig. 2). When the covalent capture was carried out in CD_2Cl_2 significant amounts of the cyclic monomers **3b** (12%) and **3c** (28%) were formed.¶ In toluene-d_8 formation of the cyclic monomers **3b** and **3c** was not observed, but still the yields of the cyclic trimers **2b** (37%) and **2c** (10%) were low in comparison to that of **2a** under the same conditions (100%). These results clearly indicate that the bromo and iodo substituents at position R significantly reduce the thermodynamic stability of the

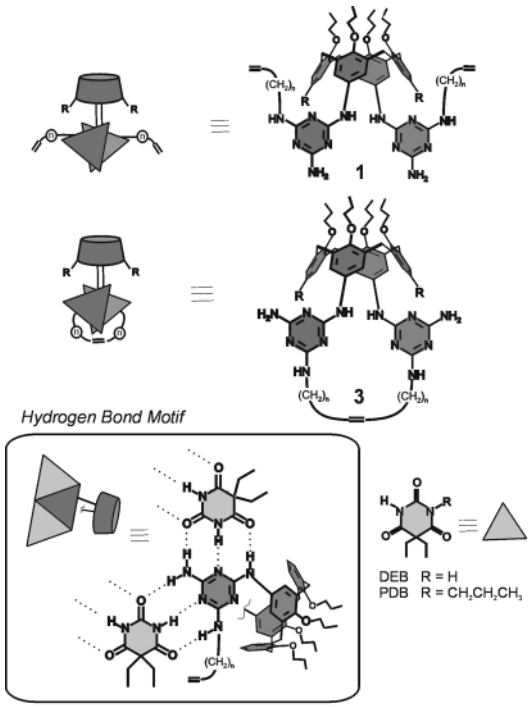

Fig. 1 Molecular structures and schematic representations of calix[4]arene dimelamine **1**, cyclic monomer **3** and barbituric acid derivatives DEB and PDB.

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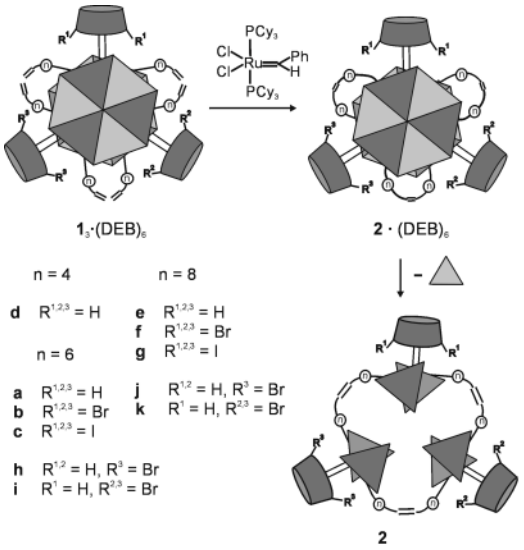

Fig. 2 Schematic representation of the covalent capture of hydrogen-bonded assemblies $\mathbf{1}_3$•$(DEB)_6$.

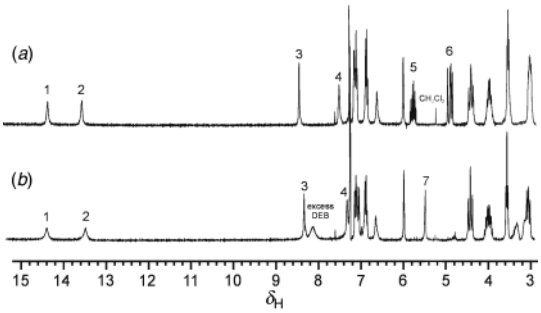

Fig. 3 ^{1}H NMR spectra of (*a*) assembly $\mathbf{1a}_3$•$(DEB)_6$ and (*b*) assembly **2a**•$(DEB)_6$ after reaction of assembly $\mathbf{1a}_3$•$(DEB)_6$ with Grubbs catalyst. Peak designations: NH_{barb} protons (1 and 2), NH_{Ar} protons (3), NH_{CH2} protons (4), terminal (5,6) and internal (7) vinylic protons. Spectra were recorded in $CDCl_3$ on a 300 MHz spectrometer.

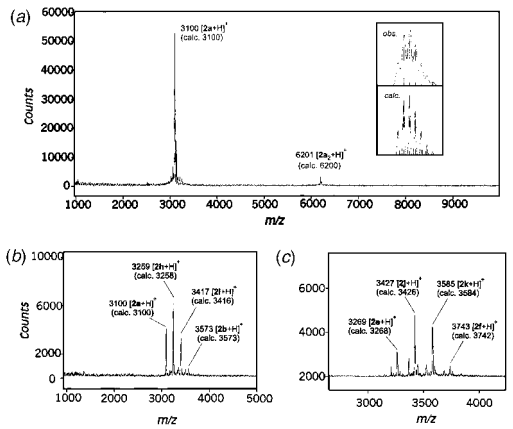

Fig. 4 MALDI-TOF mass spectra of the crude reaction mixtures of the covalent capture of (*a*) assembly $\mathbf{1a}_3$•$(DEB)_6$ (observed and calculated isotopic patterns for $[\mathbf{2a}+H]^+$ are given as inserts); (*b*) dynamic library $\mathbf{1a}_{3-x}\mathbf{1b}_x$•$(DEB)_6$ (x = 0–3, R = H, Br, n = 6); (*c*) dynamic library $\mathbf{1e}_{3-x}\mathbf{1f}_x$•$(DEB)_6$ (x = 0–3, R = H, Br, n = 8).

assemblies[15] and strongly hinder the reaction of the two alkene units, leading to competitive polymerization of the assemblies and/or intermediates.

Substitution of the oct-7-enyl chains in **1a** for hex-5-enyl chains, as in **1d**, completely inhibits the covalent capture of the corresponding assembly $\mathbf{1d}_3$•$(DEB)_6$ and formation of cyclic trimer **2d** does not occur to a significant extent either in CD_2Cl_2 or in toluene-d_8. The effect of an increase in the alkenyl chain length is much less pronounced. Covalent capture of assembly $\mathbf{1e}_3$•$(DEB)_6$, carrying dec-9-enyl side chains, in toluene-d_8 gave assembly **2e**•$(DEB)_6$ in 71% yield. The introduction of bromo substituents at positions R in these assemblies seems to decrease the yield of the corresponing assembly **2**•$(DEB)_6$ to a much smaller extent. For example, covalent capture of assembly $\mathbf{1f}_3$•$(DEB)_6$ (R = Br, n = 8) in toluene-d_8 gave assembly **2f**•$(DEB)_6$ in 61% yield.

Covalent capture of the 4-component dynamic libraries[8] $\mathbf{1a}_{3-x}\mathbf{1b}_x$•$(DEB)_6$ (x = 0–3, R = H, Br, n = 6), and $\mathbf{1e}_{3-x}\mathbf{1f}_x$•$(DEB)_6$ (x = 0–3, R = H, Br, n = 8) under standard RCM conditions in toluene-d_8 clearly showed the formation of all four possible trimers **2a**, **2b**, **2h** and **2i** (Fig. 4, relative ratio 20:43:30:7 determined by HPLC) and **2e**, **2f**, **2j** and **2k** (relative ratio 10:38:40:12 determined by HPLC), respectively. The product distribution for the library with oct-7-enyl side chains is slightly different from statistical (*i.e.* 12:38:38:12), due the lower stability of the Br-containing assemblies.

In conclusion we can state that the covalent capture of hydrogen-bonded assemblies $\mathbf{1}_3$•$(DEB)_6$ occurs with high efficiency and provides a new tool for the characterization of dynamic libraries using conventional techniques like HPLC and mass spectrometry.

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Notes and refences

† Reaction conditions for RCM reactions: (*a*) CD_2Cl_2, room temp., 40 h; $[\mathbf{1}]_{t=0}$ = 9.0 mM; $[DEB]/[\mathbf{1}]_{t=0}$ = 2.5; $[Ru\ cat]_{t=0}$ = 20 mol%, additional 10 mol% at t = 18 h. (*b*) toluene-d_8, room temp., 48 h; $[\mathbf{1}]_{t=0}$ = 6.0 mM; $[DEB]/[\mathbf{2}]_{t=0}$ = 2.5; $[Ru\ cat]_{t=0}$ = 20 mol%, additional 10 mol% at t = 18 and 24 h. Reactions were quenched by extensive bubbling with oxygen.

‡ A mixture of *cis* (*c*) and *trans* (*t*) isomers is most probably formed (the HPLC analysis of assembly **3a**•$(DEB)_6$ shows two shoulders), but the different isomers (*ccc*, *cct*, *ctt*, *ttt*) were not resolved even in a 600 MHz ^{1}H NMR spectrum.

§ HPLC analysis was performed on crude reaction mixtures (no intermediate workup). Eluent: 95% CH_2Cl_2–4.75% MeOH–0.25% NH_3 (25% in H_2O); Column: Resolve Silica 90 Å 5 μm 3.9 × 150 mm. Yields were determined using DEB as an internal standard.

¶ In all reactions that show the formation of cyclic monomer **3** by MS and HPLC, precipitation of significant amounts of the monomer was observed, resulting in overall yields being much lower than 100%.

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