Synthesis and assembly of monofunctionalized pyrogallolarene capsules monitored by fluorescence resonance energy transfer†

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Received (in Cambridge, UK) 17th August 2007, Accepted 12th September 2007
First published as an Advance Article on the web 25th September 2007
DOI: 10.1039/b712713h

Pyrogallol[4]arenes were monofunctionalized with fluorophores and fluorescence resonance energy transfer (FRET) was used to follow the self-assembly and exchange of the hexameric capsules at micromolar concentrations.

Pyrogallol[4]arenes **1** are shallow, bowl-shaped macrocycles (Fig. 1) that self-assemble into hexameric hydrogen bonded capsules $\mathbf{1_6}$ in the crystalline state,[1] in solution,[2] and even in the gas phase.[3] Despite the structural similarities between pyrogallolarenes and resorcin[4]arenes **2**, several differences in their respective hydrogen bonded assemblies $\mathbf{1_6}$ and $\mathbf{2_6}$ and their encapsulation preferences have been identified.[1b,2] While NMR methods are appropriate for monitoring the host/guest behavior at millimolar concentrations, assembly dynamics of hydrogen-bonded capsules are best followed at micro- to nanomolar concentrations.[4] We report here the application of fluorescence resonance energy transfer (FRET) to the formation of capsule $\mathbf{1_6}$ and its exchange of subunits.

Earlier we synthesized resorcinarenes labeled with donor and acceptor fluorophores and used these to probe the dynamic behavior of the capsules $\mathbf{2_6}$ through FRET studies.[4c] When a fluorescent donor and acceptor are present in the same hexameric assembly, FRET is observed on photoexcitation of the donor (Fig. 1). While a huge number of combinations and isomeric arrangements of donors and acceptors are possible on a single hexameric capsule (only one donor and one acceptor are shown, and they are placed nearest each other in Fig. 1), they are apparently all identical with respect to FRET behavior. A single, tight isosbestic point is seen, consistent with a two-state system: capsules bearing only one type of fluorophore and capsules bearing both fluorophores.

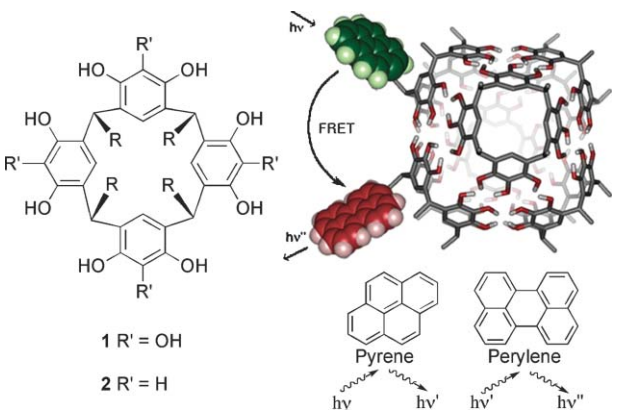

Fig. 1 Left: Structures of pyrogallolarenes **1** and resorcinarenes **2**. Right: Schematic representation of donor (**D**) and acceptor (**A**) labeled pyrogallolarenes brought within FRET distance in a hexameric assembly. (Pendant R groups and solvent molecules removed for clarity). Pyrene and perylene are the donor and acceptor fluorophores, respectively and only one of each is shown; in the experiments, each module of the hexamer bears a fluorophore.

Although resorcinarenes have previously been monofunctionalized and widely used,[5] the chemistry of pyrogallolarenes is relatively underdeveloped and, to our knowledge, no reports of monofunctionalized pyrogallolarenes exist in the literature. Fluorophores have been crystallized as guests *inside* pyrogallolarene hexamers,[6] but not attached to the outer surface. Efforts were initially directed towards the synthesis of a monohydroxyl footed pyrogallolarene, analogous to the resorcinarenes.[5e] Problems arose in finding a protection strategy that would reveal the alcohol for further functionalization of the lower rim while blocking the 12 phenolic positions of the hindered upper rim. An alternate approach, whereby the fluorophores were incorporated into the pyrogallolarene framework from the outset, proved successful. Pyrogallolarenes are synthesized by condensing alkyl aldehydes with pyrogallol in the presence of hot, concentrated hydrochloric acid. A simple alkyl linker between the fluorophore and aldehyde functions was used to withstand these relatively harsh reaction conditions.

Accordingly, pyrene butanol **3** was oxidized with Dess–Martin periodinane (DMP) and an acid catalyzed condensation of the resulting pyrene aldehyde **4**, dodecyl aldehyde, and pyrogallol afforded a mixture of pyrogallol[4]arenes with 0–2 appended pyrenes (Scheme 1). Pivalate protection of the hydroxyls allowed for isolation of the monopyrene functionalized pyrogallolarene **5**. For the acceptor, 3-bromoperylene[7] **7** was coupled with 4-pentyn-1-ol under Sonogashira conditions and the alcohol was then protected as the *tert*-butyldimethylsilyl (TBS) ether **8**. Hydrogenation of the alkyne, TBAF deprotection, and oxidation of the liberated alcohol using DMP yielded 3-(5-pentanal)perylene **9**. Condensation of aldehyde **9** with dodecyl aldehyde and pyrogallol provided a mixture of pyrogallolarenes.‡ Pivalate protection of the mixture as before allowed for purification and afforded monoperylene pyrogallolarene **10**. Facile deprotection of **5** and **10** with hydrazine yielded the requisite pyrene donor and perylene acceptor pyrogallolarenes **6** (**D**) and **11** (**A**) respectively.

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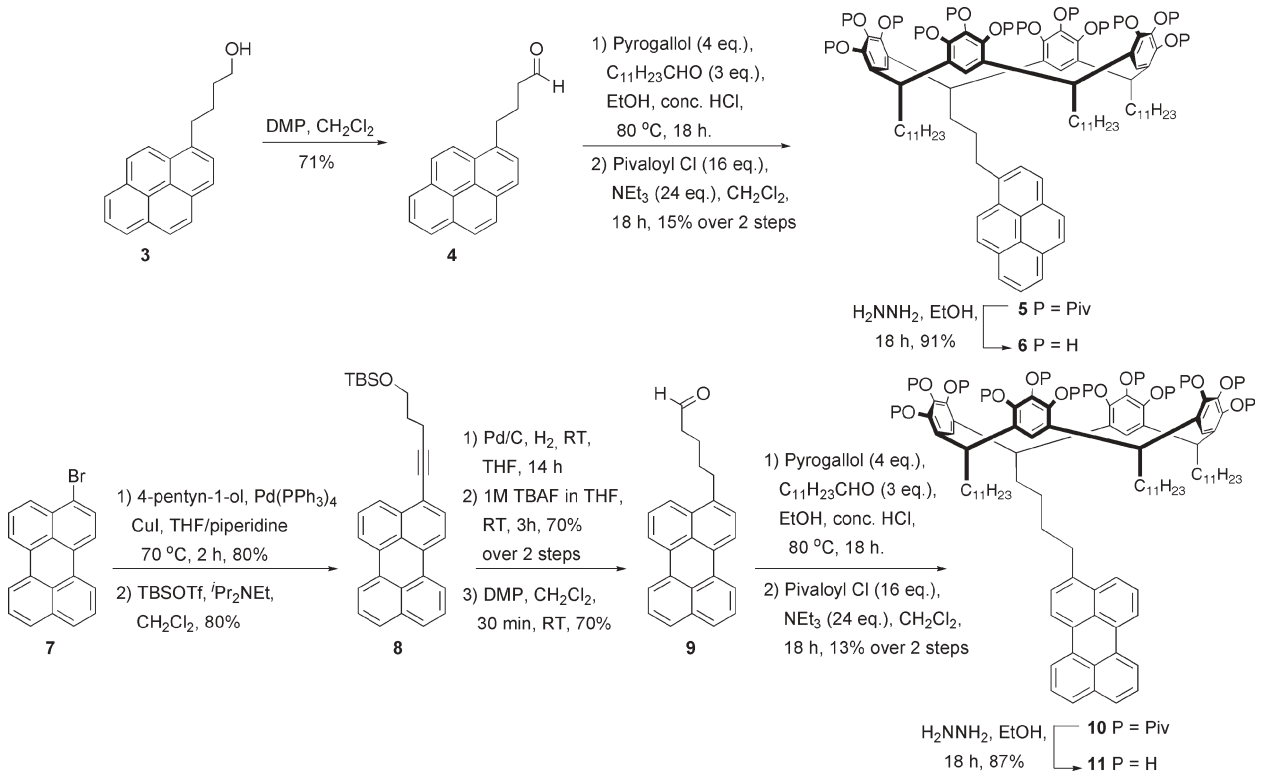
† Electronic supplementary information (ESI) available: Key experimental, absorption and emission spectra, details of kinetic experiments and methanol titrations. See DOI: 10.1039/b712713h



Scheme 1 Synthesis of pyrene donor and perylene acceptor labeled pyrogallolarenes **6** (**D**) and **11** (**A**).

The ^{1}H NMR spectrum of the perylene pyrogallolarene in d_6-acetone clearly reveals the reduced symmetry of the mono-functionalized macrocycle **11** *cf.* the unsubstituted pyrogallolarene **1** (Fig. 2). Additionally, ^{1}H NMR experiments with the labeled pyrogallolarenes in $CDCl_3$ showed clean formation of hexameric encapsulation complexes in the presence of known guest trihexylamine.

The absorption and emission spectra of the pyrene and perylene labeled pyrogallolarenes confirmed that the absorption of the acceptor overlaps significantly with the emission of the donor, allowing for non-radiative energy transfer between them when the donor is excited (see ESI†). The pivalate protected pyrene and perylene pyrogallolarenes **5** and **10** served as control compounds for our fluorescence experiments. They lack the hydrogen bonding sites needed for the hexamer to form, hence any FRET between the two compounds can only arise from the unassembled free dyes in solution. Dilution studies were thus performed with equimolar mixtures of **5** and **10**. At 25 μM there was a small amount of intermolecular FRET, however, upon 10-fold dilution to 2.5 μM, no FRET was observed. All subsequent experiments were performed at 1 μM where no intermolecular FRET is possible.

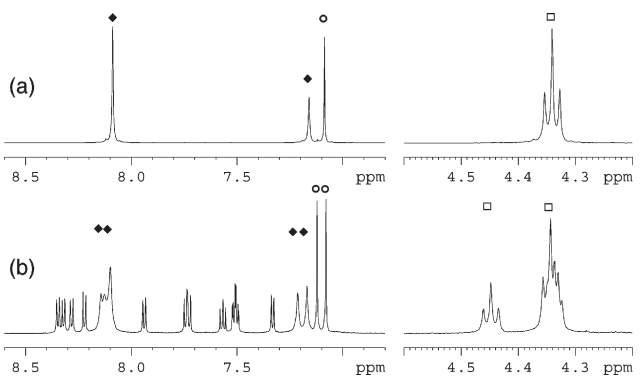


Fig. 2 Expansion of the ^{1}H NMR spectra of (a) pyrogallolarene **1** (R = $C_{11}H_{23}$) and (b) perylene pyrogallolarene **11** (600 MHz, d_6-acetone, 300 K). The reduced symmetry of pyrogallolarene **11** *cf.* **1** is clearly seen by the multiple methine (□), phenolic (◆), and aromatic pyrogallolarene (○) proton environments. The remaining signals shown in (b) arise from the perylene protons.

To probe the dynamic behavior of the pyrogallolarene hexamers, dilute solutions (1 μM) of **6** (**D**) and **11** (**A**) labeled pyrogallolarenes in dichloromethane were mixed at room temperature and the fluorescence emission of the solution was monitored over time. As the species equilibrated, increasing FRET was observed: the donor pyrene fluorescence intensity decreased as the acceptor perylene fluorescence increased (Fig. 3). This gradual development of FRET indicates the exchange of pyrogallolarene monomers in solution and formation of pyrogallolarene hexamers containing both **D** and **A** species. Once equilibrated, titration of the solution with methanol, a solvent which disrupts the hydrogen bonds of the capsule, resulted in dissociation of the pyrogallolarene hexamers to the constituent monomers and the disappearance of the FRET signal (see ESI†).

These pyrogallolarene exchange experiments revealed several differences compared to our earlier study of the resorcinarene hexameric capsules.[4c] First, the pyrogallolarene monomers exchange at a much slower rate than the resorcinarene monomers at the same concentration, with a half-life for the system to reach equilibrium of *ca.* 60 min (*cf.* 10 min for the resorcinarenes). Second, pyrogallolarene hexamer assembly occurred in both water-saturated and dry dichloromethane, whereas the resorcinarenes

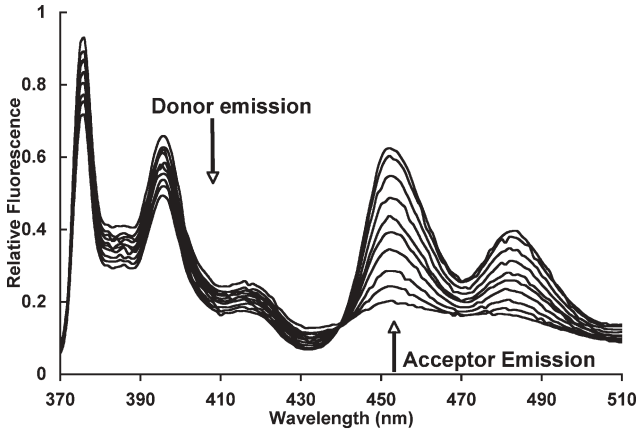


Fig. 3 Development of FRET with time upon mixing of **6** (**D**) and **11** (**A**) solutions at 1 μM in CH_2Cl_2. (Times from 0 to 6.5 h). λ_{exc} = 346 nm.

required the water-saturated solvent for hexamer formation. Third, the pyrogallolarene hexamers were found to be more stable and necessitated the addition of a higher concentration of methanol for the complete disassembly of the hexameric capsule.

In summary, the first synthesis of monofunctionalized pyrogallolarenes has been achieved and used to attach donor and acceptor fluorophores. The self-assembly of pyrogallolarene hexamers was studied by FRET and differences in the dynamic behavior of pyrogallolarenes and resorcinarenes were identified. The interactions of the resorcinarene and pyrogallolarene hexamers can now be determined and will be reported in due course.

We are grateful to the Skaggs Institute and the NIH (GM 50174) for financial support. ESB and TJD are Skaggs postdoctoral and predoctoral fellows, respectively.

Notes and references

‡ It appears important to have a long enough chain length between the aldehyde and the fluorophore to facilitate pyrogallolarene formation; initial attempts to use perylene propanal in place of the perylene pentanal were unsuccessful.

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