2013

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Citation/Publisher Attribution
Radaram, B., Potvin, J., & Levine, M. Highly Efficient Non-Covalent Energy Transfer in All-Organic Macrocycles. *Chemical Communications* 49.74 (2013), 8259-8261.
Available at: http://dx.doi.org/10.1039/C3CC45128C

Journal Name

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Highly efficient non-covalent energy transfer in all-organic macrocycles

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

The use of aromatic organic macrocycles as supramolecular hosts for non-covalent energy transfer is reported herein. These macrocycles lead to stronger binding and more efficient energy transfer compared to commercially available γ-cyclodextrin. This energy transfer was particularly efficient for the highly toxic benzo[*a*]pyrene with a fluorescent BODIPY acceptor, with up to a 5-fold increase in the fluorophore emission observed.

The complexation of small molecules in organic macrocycles is a highly active area of research, with applications including supramolecular catalysis,[1] small-molecule detection,[2] and macrocycle-promoted energy transfer.[3] We have previously shown that γ-cyclodextrin, a well-known supramolecular host,[4] promotes efficient energy transfer from several polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) to fluorophore acceptors.[5] This energy transfer occurs with up to 35% efficiency, and has significant potential applications in developing array-based detection schemes.[6]

The use of aromatic macrocycles as supramolecular hosts can lead to even stronger binding of aromatic guests and higher energy transfer efficiencies, as these macrocycles can bind aromatic guests via π-π stacking[7] in addition to hydrophobic binding.[8] Four examples of such macrocycles were synthesized (Figure 1) (synthetic details provided in the ESI). Briefly, a double Williamson etherification reaction[9] followed by a double Suzuki reaction[10] rapidly assembled the linear precursors. The key macrocyclization reactions were accomplished via a double etherification reaction (for compound **1**) [11] or via a double Mitsunobu reaction (for compounds **2-4**). [12]

These macrocycles include three structures that are electronically-dissymmetric (**1-3**), with clearly defined electron-rich and electron-deficient components to the macrocycle, and one that is electronically symmetric (**4**). The electronically dissymmetric structures are designed to bind an electron-rich analyte near the electron-deficient component of the macrocycle, and an electron-deficient fluorophore near the electron-rich segment of the macrocycle, to form a stack of four aromatic components with alternating electronic character that will undergo efficient energy transfer. Whether such dissymmetry improves the binding and energy transfer efficiencies was tested by comparison to control macrocycle **4**, which lacks such dissymmetry.

Semi-empirical PM3-level calculations of the macrocycles indicate that all of them have internal dimensions analogous to that of γ-cyclodextrin (Table 1),[13] and sufficiently large to promote intra-cavity energy transfer.

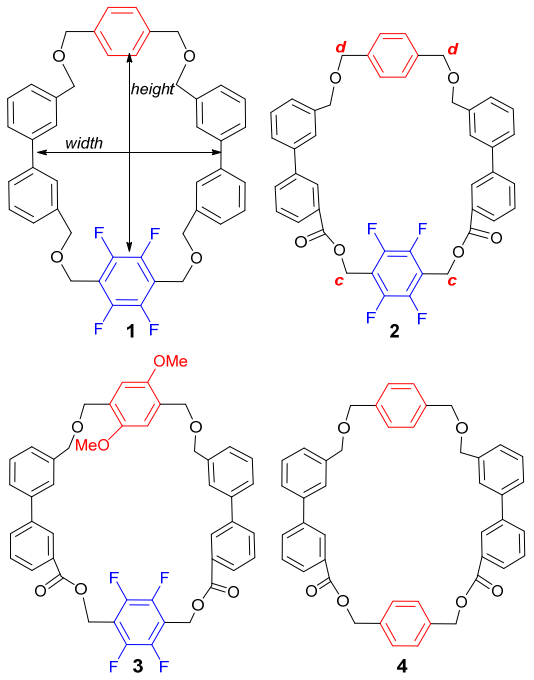

Figure 1: Structures of supramolecular hosts, with electron-rich segments highlighted in red, and electron-deficient segments in blue. Height and width dimensions are shown on macrocycle **1**, and the key protons involved in NMR studies are indicated by letters "c" and "d"

Table 1 Cavity dimensions of compounds **1-4** in the energy-minimized conformations

Compound	Height	Width
1	9.1 Å	11.2 Å
2	5.0 Å	12.6 Å
3	5.7 Å	13.0 Å
4	9.7 Å	8.0 Å

Once synthesized, macrocycles **1-4** were used for two key applications: (a) as supramolecular hosts to bind aromatic PAHs; and (b) as hosts for non-covalent energy transfer from PAHs to fluorophore **7** (Figure 2).[14]

The binding of aromatic PAHs in macrocycles **1-4** was measured by adding concentrated solutions of the macrocycle and PAH in THF to an aqueous solution of phosphate buffered saline. The fluorescence emission spectrum of the PAH was measured in the presence of increasing amounts of the macrocycle. This experimental design resulted in a mostly aqueous solution, which maximized hydrophobic binding of the PAHs.

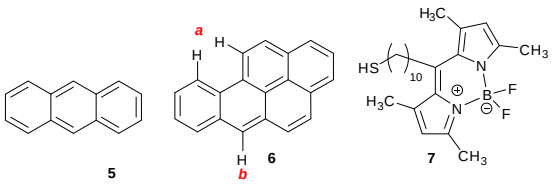

Figure 2: PAH energy donors (**5** and **6**) and fluorophore acceptor (**7**) used in macrocycle-promoted energy transfer

Among all macrocycles tested, macrocycle **2** was the most efficient supramolecular host for binding benzo[*a*]pyrene **6**, with other PAH-macrocycle combinations leading to negligible binding. This binding was quantified by measuring changes in the emission spectra of benzo[*a*]pyrene: the addition of 0.061 mM of macrocycle **2** to 0.029 mM solution of benzo[*a*]pyrene **6** resulted in a 4-fold increase in the benzo[*a*]pyrene emission (Figure 3a). The sharp increase in the excimer band around 500 nm with increasing amounts of the macrocycle strongly suggests a 1:2 host: guest complex, even in the presence of a ca. 2-fold excess of the supramolecular host. Fitting this data to a Benesi-Hildebrand equation for a 1:2 complex revealed an apparent binding constant of 5 x 10^9 M^{-2},[15] which is among the highest binding constants observed for this highly toxic analyte.[16] By comparison, the addition of macrocycle **2** to a solution of anthracene resulted in no significant changes in the anthracene emission beyond spectral broadening (Figure 3b).

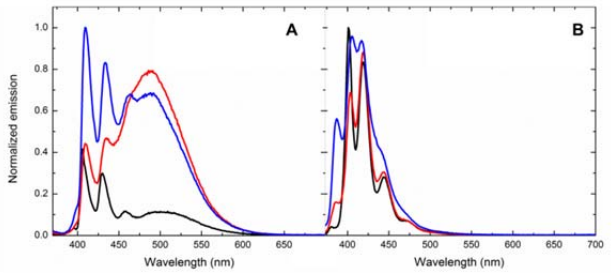

Figure 3: Analyte emission spectra in the presence of increasing amounts of macrocycle **2** for (a) benzo[*a*]pyrene and (b) anthracene. Black line: [**2**] = 0 mM; red line: [**2**] = 0.020 mM; blue line: [**2**] = 0.061 mM.

This binding was further confirmed by ^{1}H NMR titration studies.[17] The titration of benzo[*a*]pyrene into a solution of macrocycle **2** in $CDCl_3$ resulted in a shift of both the benzo[*a*]pyrene peaks and the macrocycle peaks (Table 2; Figure 4). The fact that macrocycle protons C and D shift noticeably indicates that benzo[*a*]pyrene associates with both sides of the macrocycle, although more with the electron-deficient side (as indicated by a larger shift in the C protons). The simultaneous shifts in the host and guest peaks suggest a close association between the host and the guest, and are consistent with the fluorescence data. ^{1}H NMR data also supports the formation of a 1:2 complex, as the NMR shifts of peaks A and B shifted substantially on going from 0 equivalents to 2 equivalents of benzo[*a*]pyrene, and only minimally between 2 equivalents and 4 equivalents of benzo[*a*]pyrene.

In addition to their ability to bind PAHs, macrocycles **1-4** were also investigated for their ability to promote energy transfer from analytes **5** and **6** to highly fluorescent BODIPY **7**.[18] The success of this proximity-induced energy transfer depends significantly on whether the PAH donors and fluorophore acceptors bind in the macrocycle interior. Because macrocycle **2** binds benzo[a]pyrene with high affinities, the likelihood of its success as a host for supramolecular energy transfer was increased.

Table 2 ^{1}H NMR chemical shifts for **2:6** complex

NMR proton	Initial (ppm)	Final (ppm)	Change in ppm
Peak A	9.005	9.021	+0.016
Peak B	8.471	8.482	+0.021
Peak C	5.542	5.465	-0.077
Peak D	4.571	4.549	-0.022

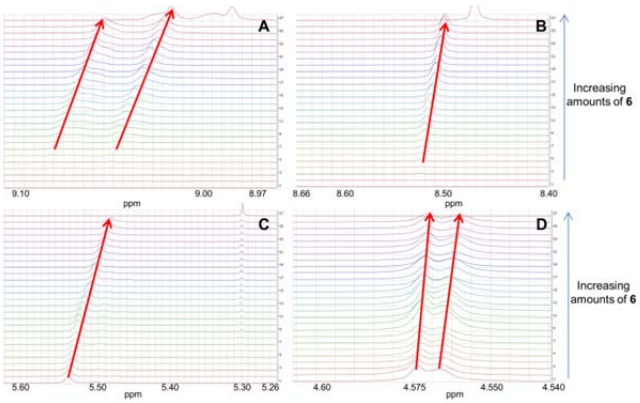

Figure 4: ^{1}H NMR chemical shifts for **2**: **6** complex. (a) Protons A; (b) Protons B; (c) Protons C; (d) Protons D. The designation of protons A-D are shown in Figures 1 and 2.

The efficiency of such energy transfer was quantified in two ways:

(a) by measuring the decrease in the donor emission from adding an energy acceptor, according to Equation 1:

$$\text{Donor decrease} = F_{DA}/F_D \quad (1)$$

where F_{DA} and F_D are the integrated emission of the donor in the presence and absence of acceptors;[19]

and (b) by measuring the increase in the acceptor emission from adding the energy donor, according to Equation 2:

$$\text{Fluorophore increase} = I_{DA}/I_A \quad (2)$$

where I_{DA} is the integrated emission of the fluorophore from analyte excitation, and I_A is the integrated emission of the fluorophore (from excitation at the same wavelength) in the absence of the analyte.

The results of macrocycle-promoted energy transfer are summarized in Table 3. These experiments were conducted under mostly aqueous conditions to maximize the favourable hydrophobic binding and π-π stacking between the aromatic PAH donor, aromatic fluorophore acceptor, and aromatic macrocycle.

Table 3 Results of macrocycle-promoted energy transfer between compound **6** and compound **7**

Host	Fluorophore Increase	Donor Decrease
Macrocycle **1**	3.3	0.90
Macrocycle **2**	5.3	0.57
Macrocycle **3**	2.4	0.80
Macrocycle **4**	3.6	0.72

a 360 nm excitation in all cases; fluorophore increase calculated according to Equation 2 and donor decrease calculated according to Equation 1. Control experiments in the absence of a macrocycle showed no significant energy transfer.

The results clearly indicate that macrocycle **2** was the most efficient host for non-covalent energy transfer, as measured both

by the increase in fluorophore emission more than 5-fold and by the decrease in donor emission to 57% of its initial value (Figure 5a and 5b). The minimal amount of excimer emission observed in these spectra strongly suggests that fluorophore **7** displaces one molecule of benzo[*a*]pyrene from the macrocycle's interior.

Interestingly, macrocycle **4** was substantially less efficient than macrocycle **2** at promoting supramolecular energy transfer between benzo[*a*]pyrene **6** and BODIPY **7** (Figure 5c and 5d). The only difference between the two hosts is the replacement of the perfluorophenyl ring in macrocycle **2** with a phenyl ring in macrocycle **4**, which effectively removes the electronic dissymmetry from the structure. This direct comparison indicates that electronic dissymmetry provides a direct benefit for supramolecular energy transfer efficiencies.

Macrocycle **2** was also substantially more efficient at promoting such energy transfer compared to γ-cyclodextrin.[5] Using γ-cyclodextrin as a supramolecular host resulted predominantly in the formation of a benzo[*a*]pyrene excimer, with only weak energy transfer observed. This excimer effectively obscured the fluorophore emission peak, rendering such a system ineffectual for benzo[*a*]pyrene-based energy transfer and detection. In contrast, using macrocycle **2** resulted in a strong BODIPY peak and minimal benzo[*a*]pyrene excimer emission under identical experimental conditions. The ability to use benzo[*a*]pyrene in such energy transfer schemes (and detection schemes based on such energy transfer) is particularly relevant, due to the high toxicity and known carcinogenicity of benzo[*a*]pyrene.[20] Control experiments with macrocycle **2** and BODIPY **7** indicated that no energy transfer occurred from the very weakly fluorescent macrocycle to the BODIPY fluorophore.

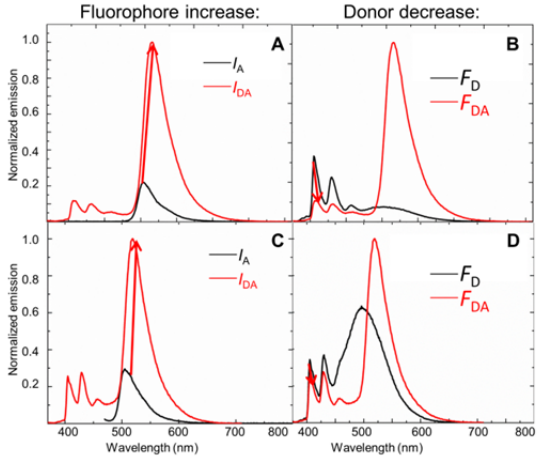

Figure 5: Comparison of the energy transfer in macrocycle **2** (5a and 5b) and macrocycle **4** (5c and 5d).

The reasons why macrocycle **2** is substantially more efficient than macrocycles **1**, **3**, and **4** at binding PAHs and promoting energy transfer are currently under investigation, but the following conclusions can already be drawn: (a) The electronic dissymmetry in macrocycle **2** led to better energy transfer efficiencies than electronically symmetric macrocycle **4**; (b) the ester linkages in macrocycle **2** led to better energy transfer efficiencies than the ether linkages of macrocycle **1**; and (c) the presence of the methoxy groups in macrocycle **3** led to less efficient energy transfer than macrocycle **2**, possibly due to the increased steric bulk.

In summary, reported herein is the use of aromatic organic macrocycles as supramolecular hosts for PAH binding and non-covalent energy transfer. One of the new macrocycles, compound **2**, is substantially more efficient than known macromolecules at binding benzo[*a*]pyrene and promoting energy transfer from this toxin to a fluorophore. More generally, the ability to modify the supramolecular host for this energy transfer via synthetic organic chemistry provides optimal flexibility in tuning and optimizing such non-covalent energy transfer. The scope of macrocycle-promoted energy transfer and its use in array-based detection scheme is currently under investigation, and results will be reported in due course.

Notes and references

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† This research was funded in part by a grant from the Gulf of Mexico Research Initiative (GOMRI).

† Electronic Supplementary Information (ESI) available: Syntheses of macrocycles **1-4**, fluorophore **7**, ^{1}H NMR titration details, fluorescence experimental details, copies of spectra for all new compounds. See DOI: 10.1039/b000000x/

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