Fluorescence '*Reading-out*' of the Molecular-recognition Process

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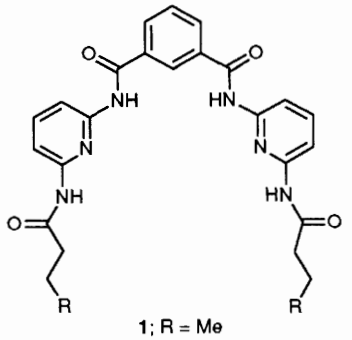
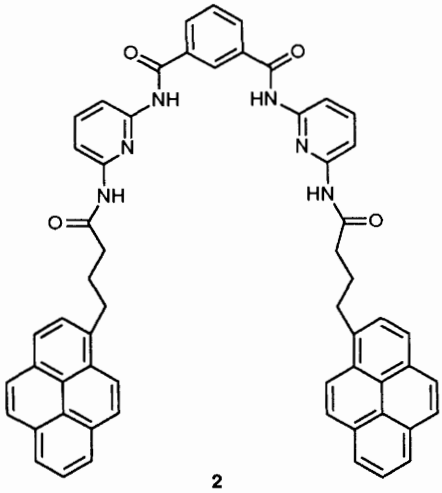
The binding of guest molecules sensitively influences the fluorescent behaviour of barbiturate-incorporated fluorescent pyrenes, making it possible to '*read-out*' the molecular-recognition process by a fluorescence spectroscopic technique.

The molecular design of artificial receptors that can precisely recognise guest molecules has been the focus of much recent attention.[1,2] In the papers reported so far hydrogen-bonding interactions play a central role.[1,2] This approach inevitably results in some limitation in the detection of the molecular-recognition process, ^{1}H NMR spectroscopy being the 'sole' effective method of characterization. We considered that if the molecular-recognition process can be 'read-out' more conveniently, we could apply the molecular-recognition unit as a useful 'transducer' of chemical to physical signals.



1; R = Me
1'; R = Et



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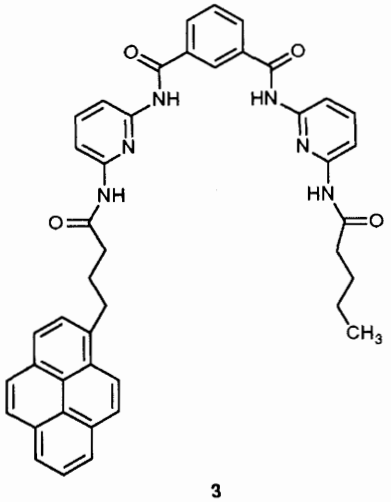

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We previously introduced two pyrene groups or a fluorophore and quencher pair onto the lower rim of a calix[4]arene *via* ester spacers serving as a metal-binding site.[2–4] We found that the fluorescence properties of these functionalized calix[4]arenes are sensitive to metal-binding events.[2,3] The results tempted us to design artificial receptors in which the molecular-recognition process can be 'read-out' by a fluorescence method. Two studies of fluorescence 'reading-out' of molecular recognition involving hydrogen-bonding interactions are known.[5,6] Hamilton *et al.*[1,7]† developed an efficient receptor **1** for barbiturates and have also performed fluorescence experiments with a related receptor containing a 2,7-dioxynaphthalene fluorophore.[8] We, therefore, synthesized a fluorescent receptor **2** with two pyrene groups, predicting that the binding of barbiturates would influence the ratio of monomer *vs.* excimer emission from these two pyrene rings. Compound **3** was used as a reference for **2**.

† Hamilton *et al.* found that in compound **1** weak fluorescence emission arising from the pyridine π-systems is observed, which is useful for the estimation of the association constants, see ref. 7.

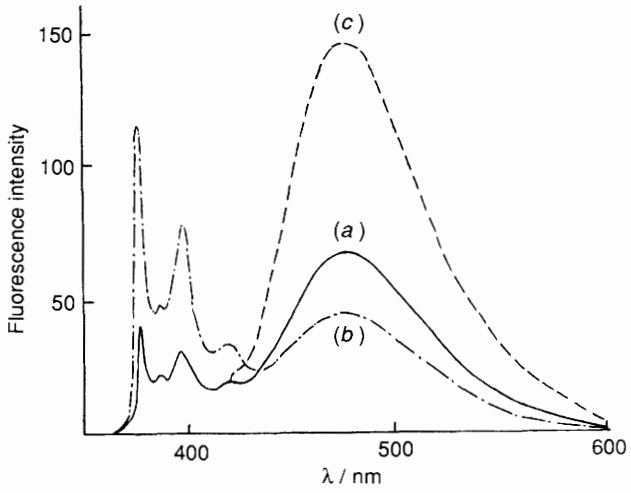


Fig. 1 Fluorescence spectra of **2** (2.00×10^{-6} mol dm^{-3}) in $CHCl_3$–cyclohexane (2:8 v/v). (*a*) **2**, (*b*) **2** + barbital (5.00×10^{-5} mol dm^{-3}), (*c*) **2** + ethyleneurea (5.00×10^{-3} mol dm^{-3}). Excitation 345 nm, temperature 25 °C.

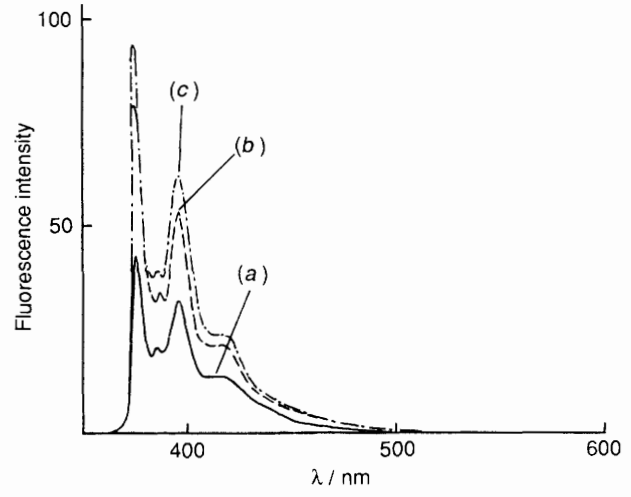


Fig. 2 Fluorescence spectra of **3** (2.00×10^{-6} mol dm^{-3}) in $CHCl_3$–cyclohexane (2:8 v/v). (*a*) **3**, (*b*) **3** + barbital (5.00×10^{-5} mol dm^{-3}), (*c*) **3** + ethyleneurea (5.00×10^{-3} mol dm^{-3}). Excitation 345 nm, temperature 25 °C.

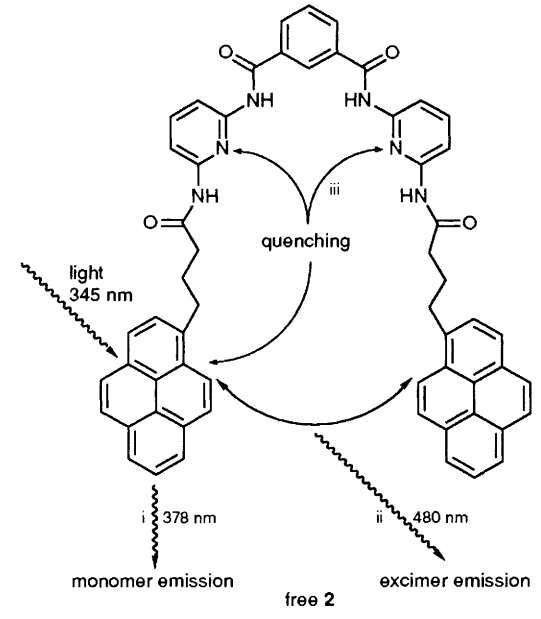
Compound **2** (m.p. 266 °C) was synthesized by the treatment of 1,3-bis{[(6-aminopyridin-2-yl)amino]carbonyl}benzene **9**[7] and 4-(pyren-1-yl)butanoyl chloride in the presence of triethylamine in THF (tetrahydrofuran). Compound **3** (m.p. 166 °C) was synthesized from **9** *via* two steps: the 1-butyl moiety was first introduced by the reaction of **9** and 1-pentanoyl chloride in the presence of triethylamine in THF and then the 4-(pyren-1-yl)butyl moiety was introduced in a manner similar to that described for **2**. The structures and the purities of **2** and **3** were ascertained by IR, ^{1}H NMR spectroscopy and elemental analysis.

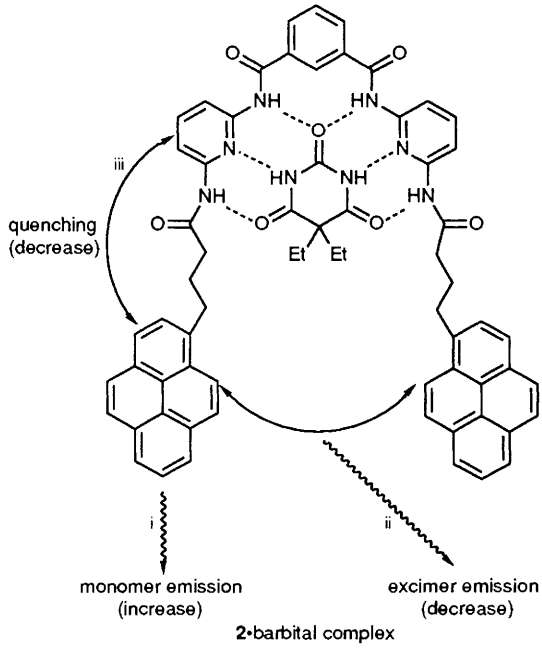
As shown in Figs. 1 and 2 compound **3** gives only monomer emission (378 and 398 nm) whereas compound **2** gives both monomer and excimer emission (480 nm). In $CHCl_3$–cyclohexane (2:8 v/v) the addition of barbital induced an decrease in excimer emission and an increase in monomer

Table 1 Fluorescence change induced by added barbital (25 °C)[a]

Solvent	Compound **2**		Compound **3**
	$\Delta I_m/I_m^\circ$	$\Delta I_{ex}/I_{ex}^\circ$	$\Delta I_m/I_m^\circ$
$CHCl_3$	0.44	−0.04	0.58
CH_2Cl_2	0.25	0.09	—
Benzene	0.48	−0.06	—
$CHCl_3$–c-C_6H_{12} (4 : 6 v/v)	1.52	0.01	0.77
$CHCl_3$–c-C_6H_{12} (2 : 8 v/v)	1.67	−0.31	1.00
EtOEt	0.23	−0.15	—
THF	0.01	0.00	—
Dioxane	0.00	0.00	—
MeCN	0.00	0.00	—

[a] [**2** or **3**] = 2.00×10^{-6}, [barbital] = 1.00×10^{-3} mol dm^{-3}, excitation 345 nm, $\Delta I_m = I_m - I_m^\circ$ (I_m: fluorescence intensity in the absence of barbital) at 378 nm, $\Delta I_{ex} = I_{ex} - I_{ex}^\circ$ (I_{ex}: fluorescence intensity in the absence of barbital) at 480 nm.





Scheme 1

Table 2 Fluorescence change induced by various guest molecules (25 °C)[a]

Guest	**2** in $CHCl_3$		**2** in $CHCl_3$–c-C_6H_{12} (2 : 8 v/v)		**3** in $CHCl_3$	**3** in $CHCl_3$–c-C_6H_{12} (2 : 8 v/v)
	$\Delta I_m/I_m^\circ$	$\Delta I_{ex}/I_{ex}^\circ$	$\Delta I_m/I_m^\circ$	$\Delta I_{ex}/I_{ex}^\circ$	$\Delta I_m/I_m^\circ$	$\Delta I_m/I_m^\circ$
4	0.44	−0.04	1.67	−0.31	0.58	1.00
5	0.10	1.15	0.32[c]	0.65[c]	—	0.54[c]
6	0.99[c]	0.49[c]	—	—	—	—
7	0.12	2.48	0.10	1.23	1.37	1.47
8	[b]	[b]	0.87	0.09	—	—

[a] [**2** or **3**] = 2.00×10^{-6} mol dm^{-3}, [guest] = at the concentration where I_m and I_{ex} are saturated, $\Delta I_m = I_m - I_m^\circ$ (I_m: fluorescence intensity in the absence of guest) at 378 nm, $\Delta I_{ex} = I_{ex} - I_{ex}^\circ$ (I_{ex}: fluorescence intensity in the absence of guest) at 480 nm. [b] Fluorescence was quenched by **8**, which made the determination of $\Delta I_m/I_m^\circ$ and $\Delta I_{ex}/I_{ex}^\circ$ difficult. [c] Because of the limited solubility of the guest molecule the saturated I_m and I_{ex} values could not be obtained. The values listed herein were estimated from the analysis of plots of [guest] *vs.* $\Delta I_m/I_m^\circ$ and $\Delta I_{ex}/I_{ex}^\circ$ by the Benesi–Hildebrand expression.

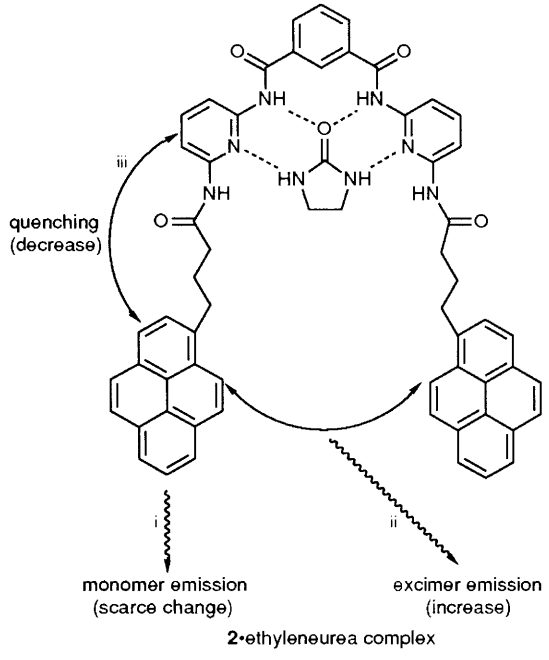
emission (Table 1).‡ On the other hand, hydrogen-bond acceptor solvents such as THF, dioxane and MeCN, scarcely influenced the fluorescence intensities. The results indicate that barbital is bound to **2** through hydrogen-bonding interactions and the bound barbital suppresses the interaction of two pyrene rings in the excited state.

In $CHCl_3$ and CH_2Cl_2, in contrast, the I_m (monomer emission intensity at 378 nm) is increased upon addition of barbital while the I_{ex} (excimer emission intensity at 480 nm) is scarcely decreased. This implies that in these solvents the I_m increase is not caused by the suppression of the interaction of two pyrene rings. We noticed that a similar I_m increase is observed for **3**, which has only one pyrene ring. The above observations are rationalized in terms of intramolecular fluorescence quenching of the pyrene excited state by the pyridine π-systems: the quenching ability of the pyridine π-systems is suppressed by the formation of hydrogen bonds between the guest molecule and the pyridine nitrogens, leading to the increase in the monomer emission. Hence, the photochemical processes possible in **2** are depicted as follows (Scheme 1): the excited pyrene can follow three different routes, (*i*) monomer emission, (*ii*) excimer emission and (*iii*) quenching by the intramolecular pyridines. One can therefore conclude that the bound guest molecule suppresses either (*ii*) by the steric effect or (*iii*) by the formation of the hydrogen bond or both. Fages *et al.* have reported a case of steric suppression of excimer emission.[9] Barbital as a guest suppresses only (*iii*) in solvents such as $CHCl_3$ and CH_2Cl_2 but both (*ii*) and (*iii*) in $CHCl_3$–cyclohexane (2 : 8 v/v). Probably, two pyrene rings tend to associate in apolar solvents such as $CHCl_3$–cyclohexane (2 : 8 v/v). As a result, the steric effect (*ii*) leading to the decrease in excimer emission is particularly marked in this solvent.

Interestingly, we found that $\Delta I_m/I_m^\circ$ and $\Delta I_{ex}/I_{ex}^\circ$ used as parameters for the molecular recognition vary sensitively with the structure of guest molecules. In contrast to barbital **4**, which features the $\Delta I_m/I_m^\circ$ increase and the $\Delta I_{ex}/I_{ex}^\circ$ decrease, 5,5-dimethylhydantoin **5**, 5,5-diphenylhydantoin **6** and ethyleneurea **7** caused the increase in both $\Delta I_m/I_m^\circ$ and $\Delta I_{ex}/I_{ex}^\circ$ (Table 2). The increase in $\Delta I_m/I_m^\circ$ is readily

‡ The absorption and fluorescence spectra of **2** in pure cyclohexane were quite different from those in other solvents, suggesting the intermolecular aggregation of **2**.

accounted for by the suppressed quenching efficiency of the pyridine rings. How then can we explain the increase in $\Delta I_{ex}/I_{ex}°$? It is known that barbital forms six neat hydrogen bonds with four NH protons and two pyridine nitrogens in **1** (as shown in Scheme 1 for **2**).[1,7] Particularly, the hydrogen bonds with the terminal NH protons would efficiently suppress the molecular motion of two pyrene rings. In the complex with **7**, on the other hand, two pyrene rings are fixed to a *syn* conformation but the freedom for the pyrene–pyrene interaction still remains because of the absence of the hydrogen bonds with the terminal NH protons. This fixation mode leads to the increase in $\Delta I_{ex}/I_{ex}°$. The $\Delta I_{ex}/I_{ex}°$ increase is in the order **4**<**6**<**5**<**7**. This is exactly in line with the number of hydrogen bonds between **2** and these guest molecules (**6**<**5** is attributed to the steric effect of two phenyl rings in **6**).



Scheme 2

These binding modes are more clearly demonstrated by use of ^{1}H NMR spectroscopy. In $CDCl_3$ at 25 °C in the presence of **2** (5.00×10^{-3} mol dm^{-3}) the NH protons in **4** (5.00×10^{-3} mol dm^{-3}) shift to lower magnetic field (δ 7.98 → 12.42; Δδ 4.44) owing to the formation of hydrogen bonds, whereas the ethyl protons shift to higher magnetic field (CH_3 δ 0.89 → 0.30, CH_2 δ 2.04 → 1.30; Δδ −0.59 and −0.74, respectively). The result substantiates the idea that when the ureido moiety is bound to **2**, the two ethyl groups interfere with the stacking interaction of the pyrene rings. Although **2** in the presence of **4** showed sufficient solubility in $CDCl_3$ for the measurement of ^{1}H NMR spectra, it was not so soluble in the presence of other guest molecules. We therefore used the more soluble **1′** instead of **2**. In the **1′**·**4** complex ([**1′**] = [**4**] = 5.00×10^{-2} mol dm^{-3}) both the terminal NH protons in **1′** and the NH protons in **4** largely shift to lower magnetic field (δ 8.41 → 9.58; Δδ 1.17 for **1′** and δ 7.98 → 11.60; Δδ 3.62 for **4**). In the **1′**·**5** and **1′**·**6** complexes the CONHCO proton in **5** or **6** shifts largely to lower magnetic field (δ 8.20 → 11.29; Δδ 3.09 ppm for **5** and Δ 7.38 → 9.25; Δδ 1.87 ppm for **6**) whereas the $CONHCR_2$ proton shifts to a smaller extent (δ 5.87 → 6.65; Δδ 0.78 for **5** and δ 5.92 → 6.68; Δδ 0.76 for **6**). The terminal

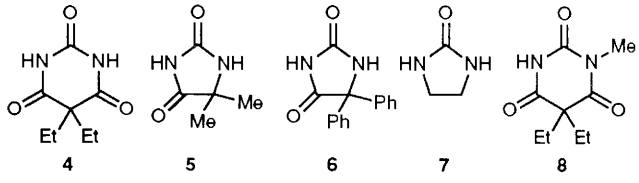
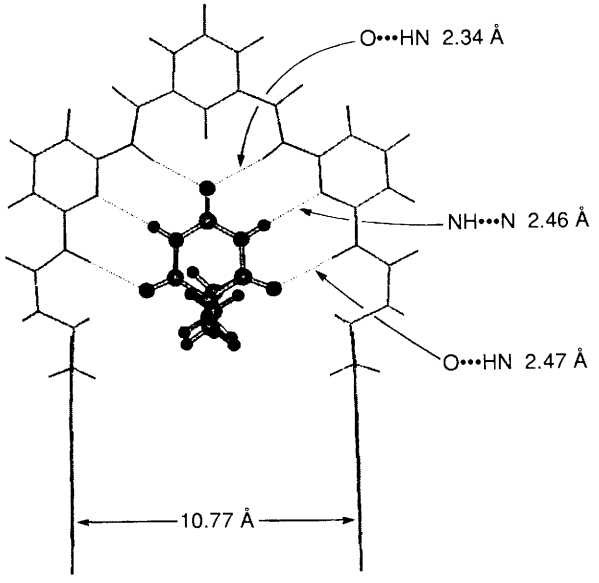
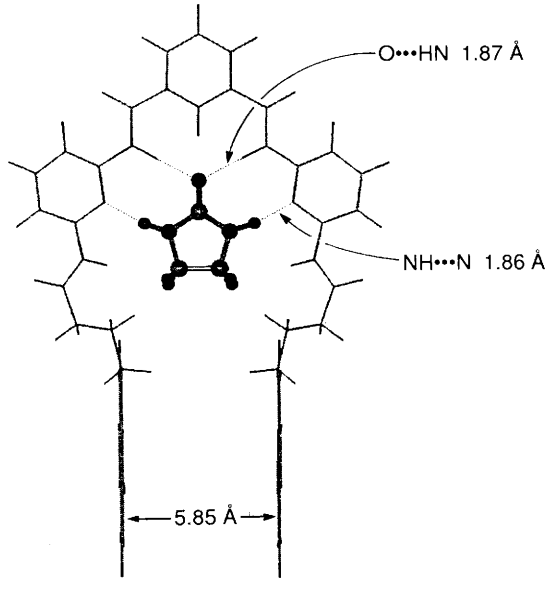


Table 3 Association constants (K/dm^3 mol^{-1}) for the 1 : 1 complexes formed between **2** and guest molecules (25 °C)

	Solvent	
Guest	$CHCl_3$	$CHCl_3$–c-C_6H_{12} (2 : 8 v/v)
4	4.1×10^4	2.5×10^6
5	1.9×10^2	1.4×10^4
6	1.4×10^2	—
7	41.9	1.1×10^3
8	—	1.0×10^3



(*a*)
2 • barbital complex



(*b*)
2 • ethyleneurea complex

Fig. 3 Energy-minimum structures for (*a*) **2**·barbital complex and (*b*) **2**·ethyleneurea complex estimated by a semiempirical molecular orbital calculation[8]

NH protons in these complexes shift to a lower magnetic field by less than 0.2 ppm. The results indicate that in these complexes one of two pyrenes can fluctuate more easily because of the absence of the hydrogen bond with one terminal NH proton. In the **1′·7** complex the two terminal NH protons in **1′** are expected to be free from the formation of the hydrogen bond. They shift to a lower magnetic field by less than 0.1 ppm. The increased mobility in the chain terminals leads to the increase in the excimer emission.

The above-mentioned emission mechanism is also supported by computational studies.§ When host **2** and guest **4** form six neat hydrogen bonds, the distance between the two pyrene rings is estimated to be 10.77 Å [Fig. 3(*a*)]. This distance is too large to give excimer emission.[10] On the other hand, when host **2** and guest **7** form four hydrogen bonds, the distance is estimated to be 5.85 Å [Fig. 3(*b*)]. This distance lies exactly in the range favourable for excimer emission.[10] The difference in the distance is crucially governed by the terminal NH (in **2**)···O=C (in **4**) hydrogen bonds. The cavity in host **2** is significantly enlarged by these hydrogen bonds.

The association constants for a molecular-recognition process have so far been estimated by ^{1}H NMR spectroscopy.[1] In the present system, on the other hand, they are readily estimated by fluorescence spectroscopy. The plots of [guest] *vs.* ΔI_m (for **4**, **6** and **8**) or ΔI_{ex} (for **5** and **7**) satisfied the equilibrium-shift method for the formation of a 1 : 1 complex. From the analysis we determined the association constants (K) (Table 3). The K value for the **2·4** complex in $CHCl_3$ (4.1×10^4 dm^3 mol^{-1}) is almost comparable with that for the **1·4** complex in $CDCl_3$ estimated by ^{1}H NMR spectroscopy (2.08×10^4 dm^3 mol^{-1}).[1]

We believe that the novel concept developed herein could lead to a new, promising host–guest-type sensory system.

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§ The semiempirical molecular orbital calculation was conducted by using MOPAC version 6.0 PM3 Hamiltonian. The graphic software used here for Fig. 3 is MOL-GRAPH/MA version 2.8. Since amide nitrogens tend to adopt the sp^2 orbital, we assumed that the atoms involved in the formation of hydrogen bonds all exist in the same plane and the two pyrene rings adopt a face-to-face orientation which is perpendicular to the hydrogen bond plane.

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