# Anthracene-based open and macrocyclic receptors in the flurometric detection of urea†

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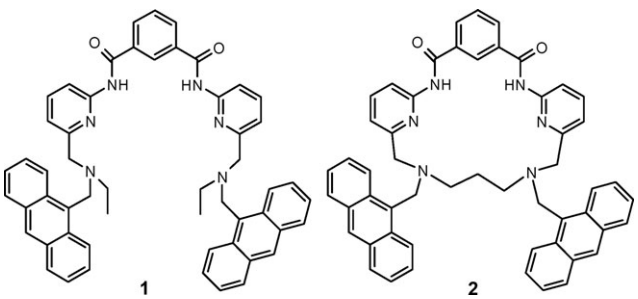
Anthracene-based open and macrocyclic receptors **1** and **2** have been designed and synthesized for the recognition of urea in less polar $CHCl_3$ and more polar $CH_3CN$ solvents. Receptor **1** binds urea strongly in $CHCl_3$ and exhibits a significant increase in the emission of the anthracene. Macrocyclic analogue **2** shows higher binding and good sensibility towards urea due to the macrocyclic effect. In $CH_3CN$, the binding constant values are lower in magnitude, but the trend is similar to that seen in $CHCl_3$. Both the receptors **1** and **2** are examples of PET sensors for the recognition of urea.



## Introduction

Molecular recognition between molecules is one of the most fundamental processes in both chemistry and biology. The study of synthetic molecules to detect biologically important species is important in the area of molecular recognition.[1,2] Considerable efforts have been made in last few decades to design molecules for the development of good receptors for specific tasks. It is worth mentioning that the recognition and detection of bioactive substrates like urea,[3] biotin,[4] carbohydrates,[5] carboxylic acids,[6] amino acids,[7] *etc*. have been considerably successful. Among these substrates of biological significance, urea is important because it is toxic, a pollutant and causes serious biological disorders.[8,9] The detection of this small molecule by a suitable synthetic receptor is therefore important. Fluorescent receptors with high sensitivity and selectivity in this aspect are mentionable. To the best of our knowledge, very few fluorescent receptors for urea recognition are known in the literature. Goswami *et al*. reported the fluorometric detection of urea by a macrocyclic receptor.[10] A 2,6-bis(2-benzimidazole)pyridine receptor, as developed by Chetia and Iyer, showed urea binding with a concomitant change in fluorescence.[11] Non-fluorescent urea receptors with a considerable binding ability are also known. Crown ether-based receptors, as developed by Pedersen,[3] and the carboxylic acid-containing crown ether receptor of Reinhoudt *et al*.[12] are known to bind urea. Reinhoudt *et al*. have also explored the concept of using an electrophilic center to bind urea in the cavity of a crown ether.[13] Bell and Liu reported the synthesis of naphthyridine-fused polyaza heterocycles, which are potentially effective for urea recognition.[14] Goswami and Mukherjee reported the solubilization and recognition of urea using a simple dinaphthyridine receptor.[15] Recently, we have shown that a pyridine amide-based macrocyclic receptor can form a strong inclusion complex with urea.[16]

Thus, the noteworthy progress made in this area inspired us to work on the recognition of urea, and accordingly, we have designed, synthesized and envisioned the sensing properties of acyclic **1** and macrocyclic receptor **2** for urea in both $CHCl_3$ and $CH_3CN$. Herein, we report the synthesis of **1** and **2**, and our findings on their abilities to recognize and sense urea using $^1H$ NMR, fluorescence and UV-vis spectroscopic methods.




## Results and discussion

Receptors **1** and **2** were synthesized according to Scheme 1 and Scheme 2, respectively. Initially, Schiff base **3** was obtained by condensing 9-anthraldehyde with ethylamine in $CH_3OH$. The reduction of **3** using $NaBH_4$ afforded amine **4**, which, on reaction with 2-*N*-(pivaloyl)-6-bromomethylpyridine in the presence of $K_2CO_3$ in dry acetone, gave product **5** in 75% yield. The cleavage of the amide linkage of **5** using 30% KOH in aqueous ethanol produced amine **6** in 78% yield. The reaction of amine **6** with isothaloyl diacid chloride in dry THF afforded open receptor **1** in 64% yield.

Similarly, diamine **10**, the precursor of macrocycle **2**, was obtained from 9-anthraldehyde by performing sequential reactions, as shown in Scheme 2. The high dilution reaction of diamine **10** with isothaloyl diacid chloride in the presence of $Et_3N$ in dry THF yielded macrocycle **2** in 12% yield.

In designed receptors **1** and **2**, the anthracene acts as a fluorescent probe that is connected to the binding sites *via* a $-CH_2-$ linkage to fulfil the characteristic feature of a PET

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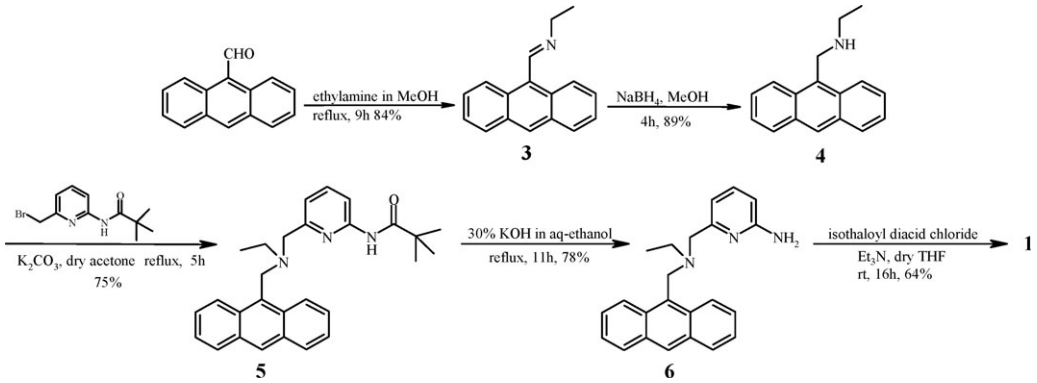
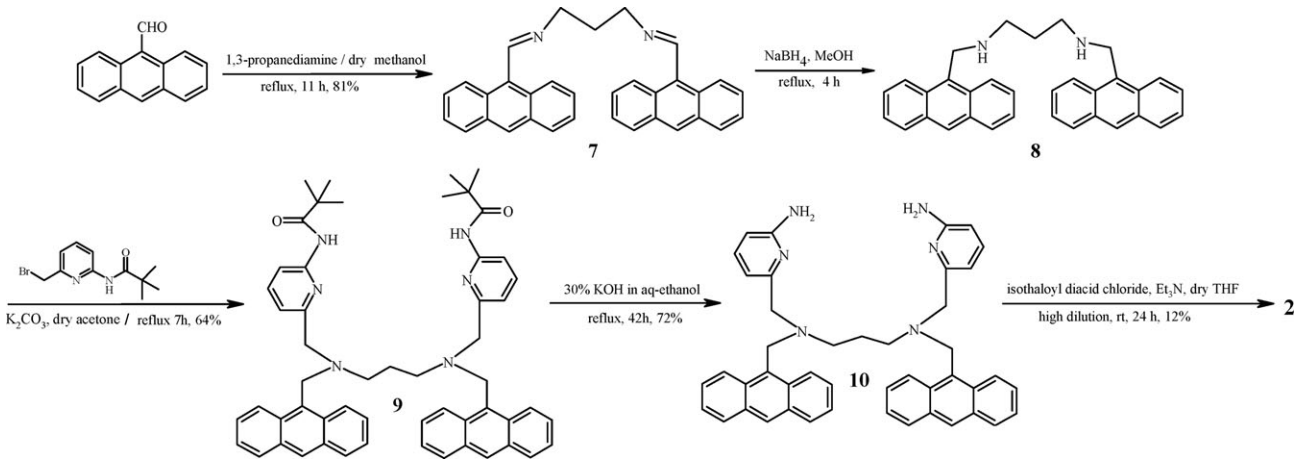
† Electronic supplementary information (ESI) available: Absorption and emission spectra of **1** and **2** in $CHCl_3$, methods for the Job plot, and methods for the determination of binding constant values. See DOI: 10.1039/b909536e




**Scheme 1** The synthesis of receptor **1**.




**Scheme 2** The synthesis of receptor **2**.



system.[17] In **1** and **2**, the amide NHs are well arranged in the cavities for complexation of the urea carbonyl oxygen. The other hydrogen bond acceptors, including the pyridine ring nitrogens, are also correctly oriented for effective complexation of the $-NH_2$ groups of urea.

The optimization of the geometries of **1** and **2** was performed at the AM1 level.[18] It is evident from Fig. 1 that the open cleft of **1** is able to form a strong complex with a urea molecule by utilizing all the hydrogen bond donors and acceptors together. In comparison, the disposition of the two anthracene moieties of macrocycle **2** creates partial steric crowding during the complexation of urea (Fig. 1(b)). Fig. 1(c) shows the mode of complexation of urea in the cavity, where the urea molecule is found to interact from outside the cavity.

To look into the binding properties of **1** and **2** in solution, $^1$H NMR spectra were recorded in dry $CDCl_3$. Urea powder was added to $CDCl_3$ solutions of **1** and **2**, followed by thorough sonication for about 10 min. The excess urea was removed by filtration, and the filtrate was used to record $^1$H NMR spectra. Significant downfield shifts of the amide protons ($\Delta\delta$ = 0.89) and the isophthaloyl peri proton ($\Delta\delta$ = 0.1) in **1** were observed in the presence of urea. In addition, a new peak at $\delta$ 4.95 for the urea protons was observed (Fig. 2). The integration ratio of the signals for the urea protons to the protons of receptor **1** revealed a 1 : 1 stoichiometry for the complex. Dilution of this complex in $CDCl_3$ did not cause any positional movement of the amide signals. This indicates the strong complexation of urea in the open cleft of **1**. A similar study was performed using thiourea. In the presence of thiourea, the amide protons of **1** underwent a minor downfield chemical shift ($\Delta\delta$ = 0.09), and a weak signal for the thiourea protons at $\delta$ 5.87 was observed (Fig. 2(c)). This indicates a weak interaction of receptor **1** with thiourea.

Simultaneously, similar experiments were performed with macrocyclic receptor **2**. Fig. 3, shows the changes in the $^1$H NMR spectrum of **2** upon the complexation of urea and thiourea.

Interestingly, here, the amide protons did not exhibit any observable change upon complexation with urea. However a signal at $\delta$ 4.58 for the $-NH_2$ groups of urea was seen (Fig. 3(b)). This finding is in accordance with the molecular modelling study (Fig. 1(c)), where the urea molecule interacts from outside of the macrocycle, possibly due to steric congestion. In connection with this, our previous report on urea binding by a pyridine-based macrocycle[16] also supports the present observation. The stoichiometry of the complex was 1 : 1, as established from the integration ratio in the $^1$H NMR spectrum. In contrast to this, the amide protons of macrocycle

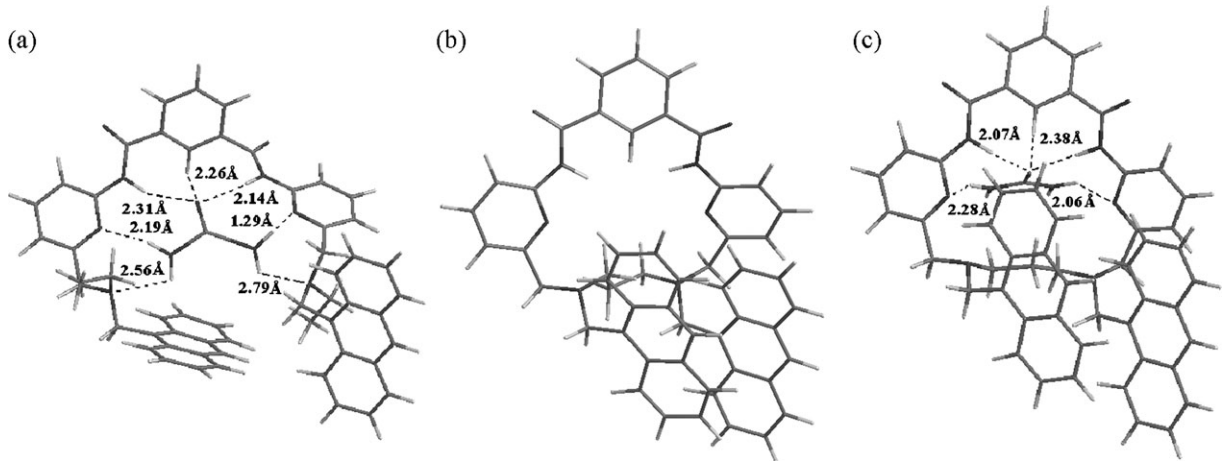



**Fig. 1** (a) AM1-optimized geometries of the **1**·**urea** complex (heat of formation = 391.17 kcal), (b) macrocycle **2** (heat of formation = 257.28 kcal) and (c) **2**·**urea** complex (heat of formation = 213.16 kcal).

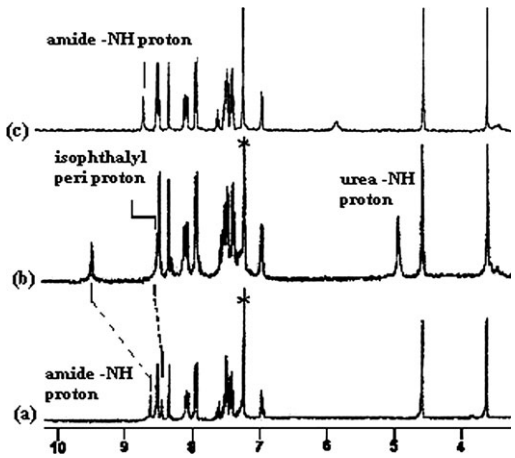



**Fig. 2** (a) Partial $^1$H NMR spectra of receptor **1** ($c = 2.58 \times 10^{-3}$ M), (b) a 1 : 1 complex of receptor **1** with urea and (c) a 1 : 1 complex of receptor **1** with thiourea.

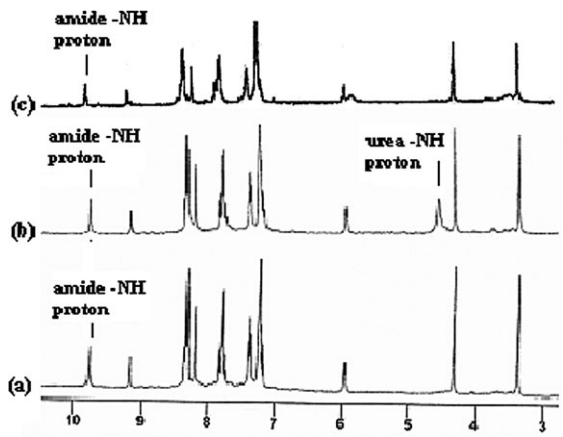



**Fig. 3** (a) Partial $^1$H NMR spectra of receptor **2** ($c = 2.69 \times 10^{-3}$ M), (b) a 1 : 1 complex of receptor **2** with urea and (c) a 1 : 1 complex of receptor **2** with thiourea.

**2** were found to be almost non-interacting with thiourea, as evidenced from the negligible shift of the amide protons ($\Delta\delta = 0.01$), although the thiourea protons appeared at $\delta$ 5.88. These $^1$H NMR experiments thus corroborate that receptors **1** and **2** effectively interact with urea rather than thiourea in $CDCl_3$.

To understand the sensing and binding potencies of **1** and **2** for urea and thiourea, UV and fluorescence titrations were performed by careful dilution of the 1 : 1 complexes of urea and thiourea with the receptors in $CHCl_3$. Fig. 4 shows the change in absorbance of the anthracene moiety with **1**·**urea** complex concentration; the linear change in absorbance at 368 nm with complex concentration indicates a 1 : 1 stoichiometry of the complex of **1** with urea (see inset of Fig. 4). A similar finding was also observed for thiourea with receptor **1**.†

Likewise, the complexation-induced change in absorbance of **2** in the presence of urea and thiourea was recorded in $CHCl_3$. Fig. 5 represents the change in absorption of the complex of **2** with urea upon dilution with $CHCl_3$. Here also, the change in absorbance at 370 nm with complex concentration was found to be linear, which confirms the 1 : 1 stoichiometry of the complex.

To evaluate the sensing properties of receptor **1**, fluorescence titrations were performed in the presence and absence of urea

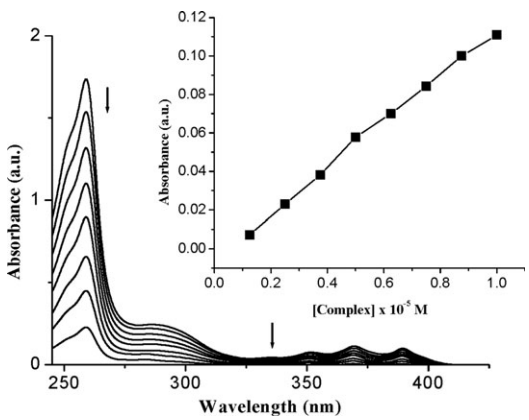



**Fig. 4** UV spectra of the **1**·**urea** complex ($c = 1 \times 10^{-5}$ M) and its change in absorbance upon dilution. Inset: a plot of absorbance *vs.* concentration of the 1 : 1 complex of urea with **1**.

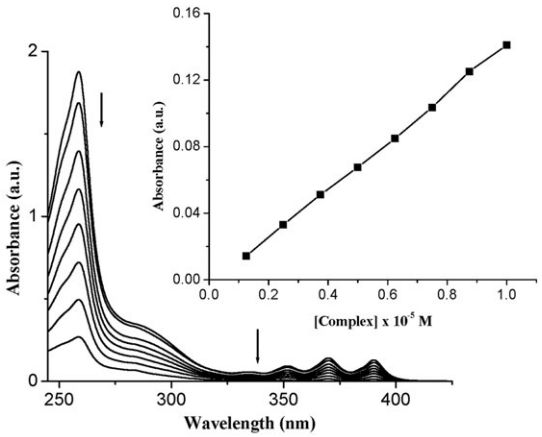



**Fig. 5** UV spectra of the **2**·**urea** complex ($c = 1 \times 10^{-5}$ M) and its change in absorbance upon dilution. Inset: a plot of absorbance *vs.* concentration of the 1 : 1 complex of urea with **2**.

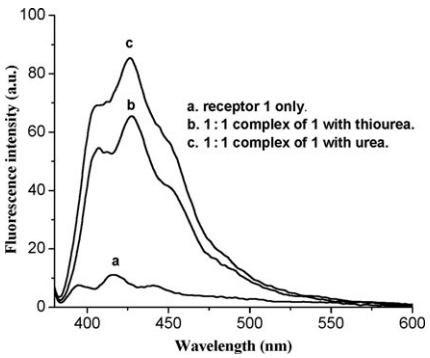



**Fig. 6** The change in emission of receptor **1** ($c = 1 \times 10^{-5}$ M) and its 1 : 1 complexes with urea and thiourea in $CHCl_3$.

and thiourea. It is evident from Fig. 6 that the emission at 416 nm in $CHCl_3$ is markedly increased, or 'switched on', with a red shift of 7 nm, in presence of an equivalent amount of urea and thiourea. During complexation, no peak at a higher wavelength was observed due to an excimer between the pendant anthracenes. The change in emission of the anthracene moiety upon dilution of the 1 : 1 complex of **1** with urea by $CHCl_3$ (Fig. 7) is almost linear in nature, and is shown in the inset of Fig. 7. The increase in emission of **1** upon complexation is explained by inhibition of the photoinduced electron transfer (PET) from the binding site to the excited anthracene moiety.

Under similar experimental conditions, the sensitivity and selectivity of receptor **2** was evaluated in $CHCl_3$ at an excitation wavelength of 368 nm. In the presence of an equivalent amount of urea, the emission of the anthracene moiety at 425 nm was increased (Fig. 8), without showing any extra peaks at higher wavelengths corresponding to excimer or exciplex formation. The increase in the emission of the anthracene moiety of **2**, with a red shift of 5 nm, in presence of urea we also attribute to the inhibition of the PET process from the binding site to the excited anthracene moiety, as for receptor **1**. The change in emission of the anthracene moiety at 425 nm for the 1 : 1 complex of **2** with urea is presented in the inset of Fig. 8, showing an almost linear response with

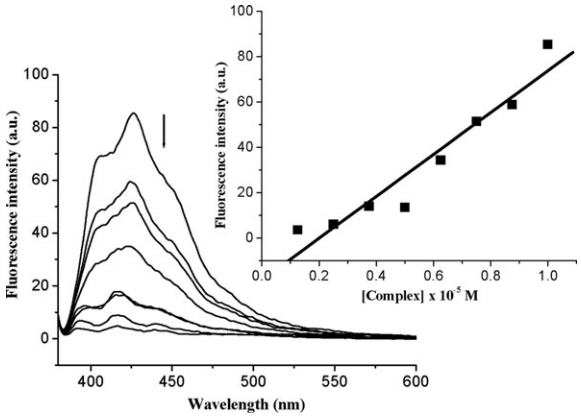



**Fig. 7** The change in emission of receptor **1** ($c = 1 \times 10^{-5}$ M) in $CHCl_3$ in the presence of stoichiometric amounts of urea, and dilution spectra of the 1 : 1 complex ($\lambda_{ex} = 368$ nm). Inset: plot of fluorescence intensity *vs.* concentration of the 1 : 1 complex of urea with **1**.

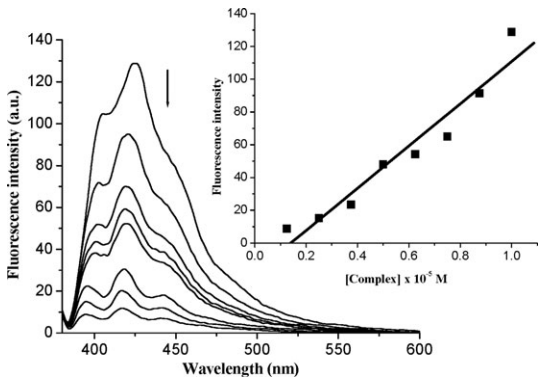



**Fig. 8** The change in emission of receptor **2** ($c = 1 \times 10^{-5}$ M) in $CHCl_3$ in the presence of stoichiometric amounts of urea, and dilution spectra of the 1 : 1 complex ($\lambda_{ex} = 368$ nm). Inset: plot of fluorescence intensity *vs.* concentration of the 1 : 1 complex of urea with **2**.

complex concentration. In the presence of thiourea, the increase in the emission of **2** was relatively less than that observed with urea.†

Thus, the PET-based fluorescence behaviors of receptors **1** and **2** in presence of urea are potentially applicable to detect urea in less polar solvents. Both open and macrocyclic receptors **1** and **2**, in this regard, can act as good sensors for urea.

Binding constant values were determined by the dilution method[10] from a fluorescence titration in pure dry $CHCl_3$ (Table 1). It is evident from Table 1 that macrocyclic receptor **2** shows a stronger binding with urea than open receptor **1**. Acyclic receptor **1** also shows a marked selectivity for urea over thiourea. On the other hand, receptor **2** exhibits about a

**Table 1** Binding constant values ($K_a/M^{-1}$) in $CHCl_3$ by the fluorescence method

| Guest | Receptor **1** | Receptor **2** |
|---|---|---|
| Urea | $1.14 \times 10^5$ | $1.97 \times 10^6$ |
| Thiourea | $2.71 \times 10^4$ | $1.35 \times 10^6$ |

10 times higher binding constant value for urea due to the macrocyclic effect. Surprisingly, the macrocycle shows a poor selectivity in binding between urea and thiourea. We suggest that receptor **2** interacts with urea and thiourea outside the cavity of the macrocycle, as supported by our $^1$H NMR study described above.

We further investigated the hydrogen bonding interactions of **1** and **2** with urea and thiourea in the polar solvent $CH_3CN$ by the direct titration method. The recognition properties of **1** and **2** were evaluated by analyzing the changes in both fluorescence and absorption spectra. In $CH_3CN$, the emission of **1** ($c = 1 \times 10^{-5}$ M) appeared as triplet, with $\lambda_{max} = 415$ nm, when excited at 368 nm. Upon adding urea, the fluorescence intensity of **1** at 415 nm increased gradually without producing any other physical change (Fig. 9(a)). No such significant response was observed for thiourea (Fig. 9(b)). Thus, the increase in the emission of **1** upon increasing the concentration of urea is attributed to the strong complexation of urea, for which the PET process occurring in between the binding site and excited state of anthracene of **1** is deactivated. Due to the bigger size of thiourea, the open cleft of **1** is less capable of strong binding, as well as inhibiting the PET process effectively.

Under identical conditions, receptor **2** also showed a similar behavior. The change in emission of **2** in the presence of 70 equivalents of urea and thiourea is shown in Fig. 10(a). While the emission of the anthracene moiety in **2** increased in the presence of urea, it decreased in presence of thiourea. The change in emission of **2** upon gradually adding urea is represented in Fig. 10(b).

Furthermore, in the presence of urea, the intensity of absorption of the anthracene moiety in receptor **1** decreased gradually. Fig. 11(a) shows the change in absorption of receptor **1** in the presence of urea. In the presence of thiourea, the intensity of absorption of the anthracene moiety in receptor **1** decreased, and an isosbestic point at 275 nm (Fig. 11(b)) was observed. This indicates the presence of two species (receptor and receptor–thiourea complex) existing in equilibrium.

A UV-vis titration of macrocyclic receptor **2** was carried out in the presence of urea and thiourea. Fig. S3 and Fig. S4 in the ESI show the change in absorbance of receptor **2** in the presence of urea and thiourea, respectively.†

The 1 : 1 stoichiometry of the urea complexes of both **1** and **2** was determined by a UV Job plot (Fig. 12).[19]

The binding constants for receptors **1** and **2** with urea in $CH_3CN$ were determined by both fluorescence and UV methods[20] (Table 2).

As can be seen from Table 2, receptor **2** binds urea and thiourea more effectively than the receptor **1**. It is notable that in $CH_3CN$, binding constant values are lower in magnitude than those obtained in $CHCl_3$. This is due to the more polar character of $CH_3CN$, in which the hydrogen bonding interactions between the receptor and the urea/thiourea molecule are significantly reduced.

## Conclusion

We have designed and synthesized charge-neutral, anthracene-based, both open and macrocyclic receptors as fluorescent chemosensors for the detection and sensing of the biologically-important substrate urea in the less polar solvent $CHCl_3$, as well as in the more polar solvent $CH_3CN$. The binding constant values determined are variously moderate and appreciable. The receptors act as good PET sensors by fulfilling the criteria of a PET system. The intrinsic fluorescence of the anthracene moiety in the receptors effectively senses the presence of notoriously insoluble urea in $CHCl_3$ solution by exhibiting a significant increase in emission upon complexation. The same is true in the more polar solvent $CH_3CN$. Both receptors effectively bind urea in $CHCl_3$. $CH_3CN$, being more polar than $CHCl_3$, reduces hydrogen bonding interactions between the receptors and the urea/thiourea molecules. This is evident from the lower binding constant values of the receptors for urea and thiourea. To the best of our knowledge, such anthracene-based PET systems for the detection of urea are unknown in the literature.

## Experimental

### Syntheses

***N*1-[(*E*)-1-(9-Anthryl)methylidene]-1-ethanamine (3).** To an MeOH solution of 9-anthraldehyde (1 g, 4.85 mmol) at room temperature was slowly added ethylamine (0.28 mL, 4.97 mmol). After stirring at room temperature for 30 min, the resulting solution was refluxed for 9 h. After cooling, the precipitated yellow solid was filtered and washed with ether. The crude yellow product was recrystallised from ethanol to yield almost pure Schiff base **3** (0.95 g) in 84% yield (mp 62 °C).

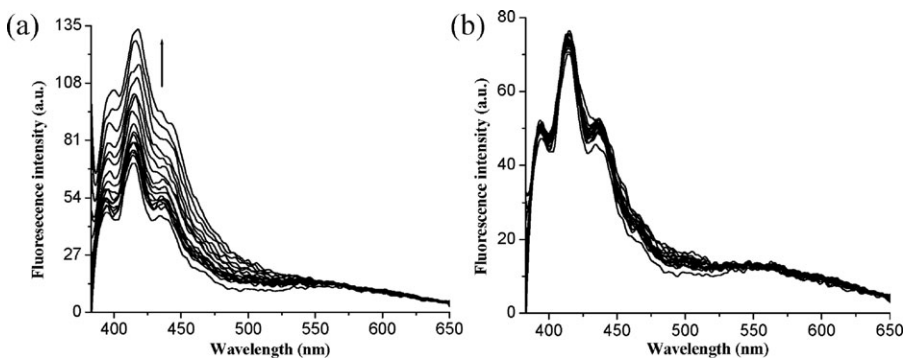



**Fig. 9** The fluorescence change of receptor **1** ($c = 1 \times 10^{-5}$ M) in presence of (a) urea and (b) thiourea in $CH_3CN$.

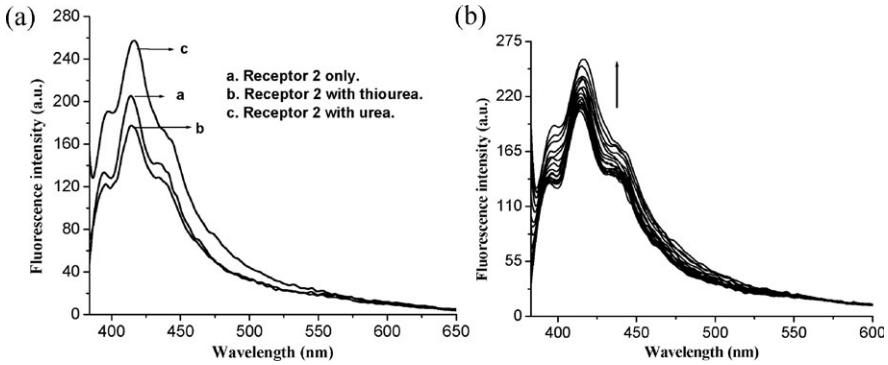



**Fig. 10** (a) A comparison of the fluorescence change of receptor **2** upon adding of 70 equivalents of guests ($c = 1 \times 10^{-5}$ M) and (b) the fluorescence change of receptor **2** ($c = 1 \times 10^{-5}$ M) in presence of urea in $CH_3CN$.

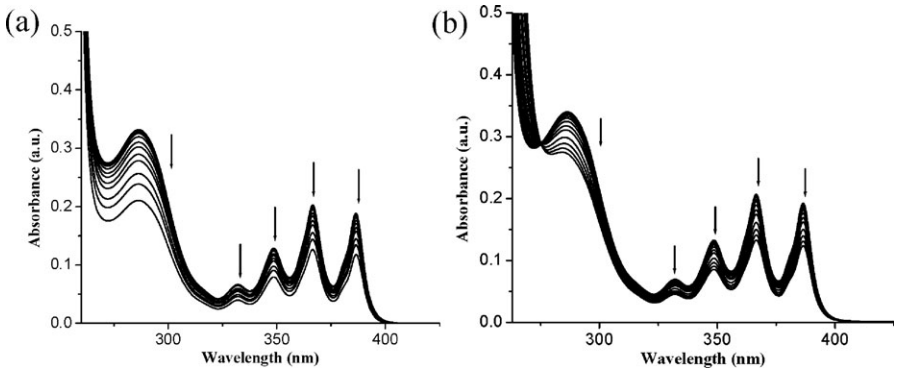



**Fig. 11** The change in absorption of receptor **1** ($c = 1 \times 10^{-5}$ M) in the presence of (a) urea and (b) thiourea in $CH_3CN$.

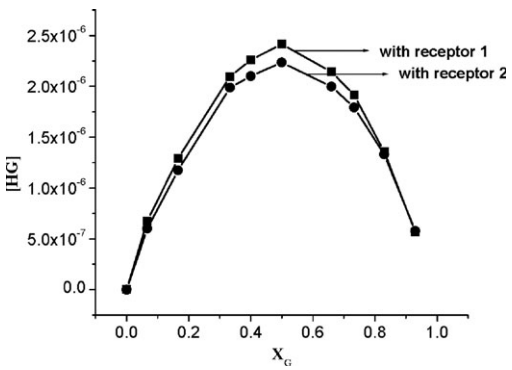



**Fig. 12** UV-vis Job plot of receptors **1** and **2** with urea; [host] + [guest] = $1 \times 10^{-5}$ M.

**Table 2** Binding constant ($K_a/M^{-1}$) values in $CH_3CN$

| | Fluorescence method | | UV method | |
|---|---|---|---|---|
| Guest | Receptor **1** | Receptor **2** | Receptor **1** | Receptor **2** |
| Urea | $3.05 \times 10^3$ | $9.27 \times 10^3$ | $2.56 \times 10^3$ | $5.07 \times 10^3$ |
| Thiourea | [a] | [a] | $1.27 \times 10^3$ | $2.69 \times 10^3$ |

[a] It was difficult to obtain an accurate value due to the minor changes in fluorescence.

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 9.43 (s, 1H), 8.46 (d, $J = 8$ Hz, 3H), 8.01 (d, $J = 8$ Hz, 2H), 7.75–7.44 (m, 4H), 3.98 (q, $J = 8$ Hz, 2H) and 1.52 (t, $J = 4$ Hz, 3H). FT-IR (KBr): 3040, 2967, 2842, 1636 and 1515 cm$^{-1}$.

### *N*1-(6-{[(9-Anthrylmethyl)(ethyl)amino]methyl}-2-pyridyl)-2,2-dimethylpropanamide (5)

Schiff base **3** (0.8 g, 3.43 mmol) was dissolved in MeOH (20 mL), to which a slight excess of solid $NaBH_4$ (0.26 g, 6.84 mmol) was added slowly to the methanolic solution; the resulting solution was refluxed with stirring for 4 h. After cooling the reaction mixture, 2 M HCl was added to destroy the excess sodium borohydride. Once the effervescence had stopped, 2 M NaOH was added until pH 9 was obtained. The yellow solution was then extracted into $CHCl_3$, washed three times with water, separated and dried over anhydrous $Na_2SO_4$. Removal of solvent under reduced pressure afforded crude amine **4** (0.72 g, yield 89%). Crude amine **4** (0.6 g, 2.55 mmol) (without further purification) and 2-*N*-(pivaloyl)-6-bromomethylpyridine (0.69 g, 2.55 mmol) were dissolved in dry acetone (30 mL) containing $K_2CO_3$ (0.7 g, 5.07 mmol), and the mixture refluxed for 5 h. The solvent was removed under reduced pressure and the resulting mass extracted into $CHCl_3$. After removal of the solvent, the crude product was purified by column chromatography (ethyl acetate: petroleum



ether 60–80 = 1 : 9), providing **5** (0.81 g, yield 75%) as a gummy product.

$^1$H NMR (200 MHz, $CDCl_3$) $\delta$: 8.53 (d, $J$ = 8 Hz, 2H), 8.37 (s, 1H), 7.99–7.92 (m, 4H including NH), 7.55–7.40 (m, 5H), 6.87 (d, $J$ = 8 Hz, 1H), 4.67 (s, 2H), 3.66 (s, 2H), 2.76 (q, $J$ = 6 Hz, 2H) and 1.35–1.20 (m, 12H). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 176.9, 158.8, 150.3, 138.3, 134.0, 131.3, 130.0, 129.0, 127.5, 127.1, 125.5, 125.0, 124.7, 118.7, 111.5, 59.1, 50.4, 48.9, 27.4 and 12.0. FT-IR (KBr): 3433, 3054, 2967, 3932, 2871, 1682 and 1578 cm$^{-1}$. UV ($CHCl_3$; $c$ = 5.05 × 10$^{-5}$ M; $\lambda_{max}$/nm): 258, 283, 350, 368 and 388. MS (FAB): $m/z$ = 426 [M + H]$^+$, 396, 248 and 191.

### 6-[(9-Anthrylmethyl)(ethyl)amino]methyl-2-pyridinamine (6)

Compound **5** (0.5 g, 1.176 mmol) was dissolved in a 30% KOH aqueous ethanol (1 : 4) solution (50 mL) and the reaction mixture refluxed for 11 h. The solvent was removed under vacuum and extracted with $CH_2Cl_2$ (3 × 60 mL). The organic layer was separated, washed with water and dried over anhydrous $Na_2SO_4$. The solvent was then removed by rotary evaporation. Finally, the crude product was purified by column chromatography using 20% ethyl acetate in petroleum ether 60–80 as the eluent to give brownish solid **6** (0.32 g, yield 78%) (mp: 136–138 °C).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.55 (d, $J$ = 8 Hz, 2H), 8.36 (s, 1H), 7.96 (d, $J$ = 8 Hz, 2H), 7.50–7.41 (m, 4H), 7.26 (1H, obscure by the $CDCl_3$ peak), 6.67 (d, $J$ = 8 Hz, 1H), 6.25 (d, $J$ = 8 Hz, 1H), 4.58 (s, 2H), 4.29 (s, 2H, $NH_2$), 3.60 (s, 2H), 2.67 (q, $J$ = 8 Hz, 2H) and 1.19 (t, $J$ = 8 Hz, 3H). FT-IR (KBr): 3438, 3303, 1627, 1595, 1575, 1465 and 1444 cm$^{-1}$. EI-MS: $m/z$ = 342.1 [M + H]$^+$ and 191.1.

### *N*1,*N*3-Di(6-[(9-anthrylmethyl)(ethyl)amino]methyl-2-pyridyl)isophthalamide (1)

To a stirred solution of **6** (0.2 g, 0.586 mmol) and $Et_3N$ (0.2 mL, 1.44 mmol) in dry THF (10 mL) was added isophthaloyl diacid chloride (0.06 g, 0.295 mmol). The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 16 h. The solvent was removed under vacuum and extracted with $CH_2Cl_2$ (3 × 30 mL). The organic layer was washed with 20% aqueous $NaHCO_3$ solution, separated, dried over anhydrous $Na_2SO_4$ and the solvent removed under reduced pressure. Finally, the crude product was purified by column chromatography using 20% ethyl acetate in petroleum ether 60–80 as the eluent to give **1** (0.153 g, yield 64%) as a white solid (mp: 102–104 °C).

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$: 8.57 (s, 2H), 8.52 (d, $J$ = 9 Hz, 4H), 8.45 (s, 1H), 8.34 (s, 2H), 8.09–8.05 (m, 4H), 7.94 (d, $J$ = 8.50 Hz, 4H), 7.60 (t, $J$ = 10 Hz, 1H), 7.52–7.48 (m, 6H), 7.42 (t, $J$ = 7.65 Hz, 4H), 6.97 (d, $J$ = 7.50 Hz, 2H), 4.61 (s, 4H), 3.66 (s, 4H), 2.74 (q, $J$ = 7 Hz, 4H) and 1.23 (t, $J$ = 2 Hz, 6H). $^{13}$C (100 MHz, $CDCl_3$) $\delta$: 164.2, 159.2, 149.9, 138.4, 134.8, 131.4, 131.3, 130.7, 130.1, 129.3, 128.9, 127.5, 125.7, 125.5, 125.0, 124.7, 119.3, 111.7, 59.1, 50.5, 49.1 and 12.1. FT-IR (KBr): 3325, 2929, 2849, 1679, 1599, 1524 and 1453 cm$^{-1}$. UV ($CHCl_3$; $c$ = 1.725 × 10$^{-5}$ M; $\lambda_{max}$/nm): 258, 283, 350, 368 and 388. EI-MS: $m/z$ = 813.3 [M + H]$^+$, 637.2 and 623.3.

### *N*1,*N*3-Di[(*E*)-1-(9-anthryl)methylidene]-1,3-propanediamine (7)

To a solution of 9-anthraldehyde (1 g, 4.85 mmol) in dry MeOH (40 mL) was added 1,3-propanediamine (0.2 mL, 2.4 mmol), and the reaction mixture was stirred under reflux for 11 h. The solution was cooled and stirred at 0 °C for 2 h to give a yellow precipitate. The precipitate was filtered off and washed with MeOH several times, and finally dried under vacuum to give foam-like yellow solid **7** (0.87 g, yield: 81%) (mp: 128–130 °C).

$^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 9.55 (s, 2H), 8.56 (d, $J$ = 9 Hz, 4H), 8.50 (s, 2H), 8.04–8.01 (m, 4H), 7.52–7.41 (m, 8H), 4.21 (t, $J$ = 6.6 Hz, 4H) and 2.57–2.53 (m, 2H). FT-IR (KBr): 3047, 3024, 2866, 2841, 1637, 1517 and 1442 cm$^{-1}$. EI-MS: $m/z$ = 451.1 [M + H]$^+$ and 473.1 [M + Na]$^+$.

### *N*1-6-[((9-Anthrylmethyl)-3-[(9-anthrylmethyl)(6-[(2,2-dimethylpropanoyl)amino]-2 pyridylmethyl)amino]-propylamino)methyl]-2-pyridyl-2,2-dimethylpropanamide (9)

To a stirred solution of Schiff base **7** (0.8 g, 1.77 mmol) in dry MeOH (30 mL) was added $NaBH_4$ (0.335 g, 8.81 mmol) portion-wise at 0 °C; the reaction mixture was then refluxed for 4 h. The solvent was removed under vacuum and the crude mass extracted with $CH_2Cl_2$ (3 × 60 mL). The organic layer was washed with water and dried over anhydrous $Na_2SO_4$. The solvent was removed and the crude solid product remaining was pure enough to be used in the next step.

Compound **8** (0.7 g, 1.54 mmol) was dissolved in dry acetone (25 mL) containing $K_2CO_3$ (1.05 g, 7.61 mmol), and to this solution was added 2-*N*-pivaloyl-6-bromomethyl-pyridine (0.85 g, 3.14 mmol). The reaction mixture was refluxed for 7 h under a nitrogen atmosphere. The reaction's progress was monitored by TLC. After completion of the reaction, the $K_2CO_3$ was filtered off and the filtrate concentrated. The thick gummy mixture was dissolved in $CH_2Cl_2$. The organic layer was washed with water and dried over anhydrous $Na_2SO_4$. After removal of the solvent under vacuum, the crude product was obtained and purified by column chromatography using 15% ethyl acetate in petroleum ether 60–80 as the eluent to give light yellow solid **9** (0.82 g, yield: 64%) (mp: 86–90 °C).

$^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 8.40 (m, 4H), 8.34 (s, 2H), 7.96–7.93 (m, 6H), 7.82 (s, 2H), 7.44–7.37 (m, 10H), 6.80 (d, $J$ = 7.5 Hz, 2H), 4.48 (s, 4H), 3.56 (s, 4H), 2.52 (t, $J$ = 7.2 Hz, 4H), 1.87 (m, 2H) and 1.24 (s, 18H). $^{13}$C (75 MHz, $CDCl_3$) $\delta$: 176.9, 158.6, 150.3, 138.2, 134.0, 131.3, 129.9, 128.9, 127.5, 125.4, 124.9, 124.7, 118.8, 111.5, 59.9, 53.6, 51.2, 39.6, 27.4 and 24.6. FT-IR (KBr): 3433, 2962, 1687, 1598, 1577, 1517 and 1452. EI-MS: $m/z$ = 835.3 [M + H]$^+$ and 857.2 [M + Na]$^+$.

### Synthesis of macrocycle 2

Compound **9** (0.75 g, 0.897 mmol) was dissolved in 30% aqueous ethanolic KOH (50 mL), and the reaction mixture was refluxed for 42 h. After completion of the reaction, the volume of solvent was reduced and extracted with $CH_2Cl_2$

(60 × 3 mL). The organic layer was separated and dried over anhydrous $Na_2SO_4$, and the solvent removed under vacuum. Finally, the crude product was purified by column chromatography using 3% MeOH in $CHCl_3$ as the eluent to give brownish solid **10** (0.43 g, 72%) (mp: 92–94 °C). This material was used directly in the next step.

Compound **10** (0.4 g, 0.6 mmol) was dissolved in dry THF (25 mL) containing $Et_3N$ (0.1 mL, 0.72 mmol) in a dropping funnel. Isophthaloyldiacid chloride (0.061 g, 0.3 mmol) was dissolved in dry THF (25 mL) in another dropping funnel. The two dropping funnels were positioned over a two-necked round-bottomed flask containing dry THF (25 mL). The solutions from the dropping funnels were added dropwise to dry THF solvent (25 mL) over 8 h under a nitrogen atmosphere. The reaction mixture was then stirred for 36 h at room temperature. The solvent was removed under vacuum and extracted with $CH_2Cl_2$ (3 × 60 mL). The organic layer was washed with a 20% aqueous $NaHCO_3$ solution, separated and dried over $Na_2SO_4$, and the solvent removed using a rotary evaporator. Finally, the crude product was purified by column chromatography using 10% ethyl acetate in petroleum ether 60–80 as the eluent to give white solid **2** (0.057 g, yield: 12%) (mp: 152–154 °C).

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$: 9.78 (s, 2H), 9.15 (s, 1H), 8.36–8.32 (m, 6H), 8.21 (s, 2H), 7.86 (d, $J$ = 8 Hz, 2H), 7.81 (d, $J$ = 8.5 Hz, 4H), 7.71 (t, $J$ = 7.5 Hz, 1H), 7.40 (t, $J$ = 8 Hz, 4H), 7.27–7.24 (m, 4H), 7.20 (t, $J$ = 7.5 Hz, 2H), 5.97 (d, $J$ = 7.5 Hz, 2H), 4.35 (s, 4H), 3.40 (s, 4H), 2.24 (t, $J$ = 6.5 Hz, 4H) and 1.89 (m, 2H). $^{13}$C (100 MHz, $CDCl_3$) $\delta$: 163.8, 156.3, 150.8, 137.9, 134.2, 134.1, 131.9, 131.2, 131.0, 130.1, 129.6, 128.6, 127.5, 125.4, 125.2, 124.6, 118.7, 111.1, 61.7, 54.7, 50.8 and 28.2. FT-IR (KBr): 3423, 1683, 1604, 1575, 1539 and 1454 cm$^{-1}$. UV ($CHCl_3$; $c$ = 4.15 × 10$^{-5}$ M; $\lambda_{max}$/nm): 288, 350, 370 and 390. EI-MS: $m/z$ = 797.3 $[M + H]^+$, 701.5, 679.5 and 605.3.

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## References

1 H. Dugas, *Bio-organic Chemistry: A Chemical Approach To Enzyme Action*, Springer-Verlag, Inc., New York, 1996.
2 J. M. Lehn, *Supramolecular Chemistry: Concepts and Perspectives*, Wiley-VCH, Weinheim, 1995.
3 C. J. Pedersen, *J. Org. Chem.*, 1971, **36**, 1690.
4 S. Goswami and S. Dey, *J. Org. Chem.*, 2006, **71**, 7280 and references cited therein.
5 M. Mazic, M. Kuschel and W. Sicking, *Org. Lett.*, 2006, **8**, 855 and references cited therein.
6 S. Goswami, K. Ghosh and S. Dasgupta, *J. Org. Chem.*, 2000, **65**, 1907.
7 H. Wang, W.-H. Chan and A. W. M. Lee, *Org. Biomol. Chem.*, 2006, **6**, 929.
8 J. Cooke, *Nature*, 1962, **194**, 1262.
9 J. G. Morris and E. Payne, *J. Agric. Sci.*, 1970, **74**, 259.
10 S. Goswami, R. Mukherjee and J. Ray, *Org. Lett.*, 2005, **7**, 1283.
11 B. Chetia and P. K. Iyer, *Tetrahedron Lett.*, 2006, **47**, 8115.
12 C. J. van Staveren, V. M. L. J. Aarts, P. D. J. Grootenhuis, W. J. H. Droppers, J. van Eerden, S. Harkema and D. Reinhoudt, *J. Am. Chem. Soc.*, 1988, **110**, 8134 and references cited therein.
13 C. J. van Staveren, J. van Eerden, F. C. J. M. Veggel, S. Herkema and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 1988, **110**, 4994.
14 T. W. Bell and J. Liu, *J. Am. Chem. Soc.*, 1988, **110**, 3673.
15 S. Goswami and R. Mukherjee, *Tetrahedron Lett.*, 1997, **38**, 1619.
16 K. Ghosh, S. Adhikari and R. Frohlich, *Tetrahedron Lett.*, 2008, **49**, 5063.
17 (*a*) A. A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *Nature*, 1993, **364**, 42; (*b*) B. A. P. de Silva and K. R. A. S. Sandanayake, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 1173.
18 Calculations were performed using *Chem 3D version 7.0*, CambridgeSoft, Cambridge, MA, USA.
19 P. Job, *Ann. Chim.*, 1928, **9**, 113.
20 P. T. Chou, G. R. Wu, C. Y. Wei, C. C. Cheng, C. P. Chang and F. T. Hung, *J. Phys. Chem. B*, 2000, **104**, 7818.

 