Design and synthesis of preorganized tripodal fluororeceptors based on hydrogen bonding of thiourea groups for optical phosphate ion sensing

Shin-ichi Sasaki,[a] Daniel Citterio,[b] Satoru Ozawa [a] and Koji Suzuki *[a,b]

[a] *Department of Applied Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan. E-mail: suzuki@applc.keio.ac.jp; Fax: +81-45-566-1568*
[b] *Kanagawa Academy of Science and Technology (KAST), KSP West-614, 3-2-1 Sakato, Kawasaki 213-0012, Japan*

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Expecting a preorganization effect, tripodal anion host molecules were designed and synthesized. It was demonstrated that two kinds of new thiourea derivatives having a six-fold substituted benzene ring as a preorganized spacer were effective for recognition of the tetrahedral dihydrogen phosphate anion. A tripodal fluororeceptor **1** having a pyrene moiety adjacent to the thiourea binding site showed long-wavelength emission upon addition of guest anions in acetonitrile. On the other hand, a tripodal thiourea receptor **2** connected to anthracene groups *via* methylene units showed a decrease in fluorescence intensity upon addition of anions. In both cases, the degree of the change in emission intensity was in the order of $H_2PO_4^- > CH_3COO^- > Cl^- \gg ClO_4^-$, which is clearly different from that of reference compounds having only one binding site. Association constants (K_a) of these tripodal fluororeceptors also confirmed the dihydrogen phosphate selectivity over the more basic acetate anion in this homogeneous solution system. The characteristics of the fluororeceptors using the preorganization effect were shown to be promising for the development of chemical ion sensors with a specific anion selectivity.

Introduction

With application to chemical sensors in mind, the design and synthesis of new receptor molecules for the selective complexation of anions have been actively studied.[1,2] Among them, for the development of neutral anion receptors, the focus has been on urea or thiourea groups as anion binding sites, because the hydrogen bonding of these functional groups is directional in character and results in relatively strong complexes with biologically important anions such as acetate, phosphate, or chloride. So far, several urea or thiourea derivatives connected with a series of spacer units including cyclic structures have been synthesized, and their anion binding ability or selectivity has been studied.[3] Some of them were, for example, successfully applied to ion sensors as neutral ionophores for ion-selective electrodes (ISEs) to quantify chloride,[4] sulfate,[5] or acetate anions.[6] On the other hand, some thiourea derivatives were also used for optical anion sensing based on absorption spectra.[7] However, the number of studies on these chromoreceptors is relatively limited in spite of the possibility of their application to practically useful optical sensing systems such as optodes.[8] Furthermore, only a few thiourea derivatives having fluorescent units are known so far, and it is reported that the selectivity of these receptors for guest anions in homogeneous solution systems (acetonitrile) follows the order of anion basicity, apparently due to their simple structures.[7a,9] To our knowledge, no example of a "preorganization approach" to recognizing biologically important oxoanions has been examined for chromo- or fluororeceptors based on urea or thiourea hydrogen bonding. In this study, two kinds of preorganized tripodal fluororeceptors were designed and synthesized, and their binding selectivity and spectral characteristics toward several guest anions were examined. As a result, it was demonstrated that thiourea derivatives having a six-fold substituted benzene ring as a rigid spacer were effective for recognition of the tetrahedral dihydrogen phosphate anion.

Results and discussion

Design and synthesis of fluororeceptors

To obtain new fluororeceptors with the aim of complexing guest anions selectively, consideration must be given to the shape of the target anion in the design of receptor molecules. In order to recognize the biologically important phosphate anion, a tripodal structure based on a six-fold substituted benzene ring as a preorganized spacer was selected.[10a] Anslyn *et al.* have recently reported several examples of anion receptors based on this unit.[10b–e] Moreover, since this unit has been successfully used in the development of ammonium ionophores,[11] it is expected that a preorganized tripodal receptor with suitable anion binding sites can complementarily recognize the tetrahedral phosphate anion as illustrated in Fig. 1 (a). A molecular modeling study was performed using a simple model compound, and the results indicated that the use of the hydrogen bonding of thiourea groups connected to the benzyl position of the spacer benzene ring is suitable for forming a complex with the tetrahedral dihydrogen phosphate anion. Pyrene and anthracene groups, which can be connected to the *N′* position of the thiourea groups, were chosen to detect the anion binding by emission spectra. Moreover, three butyl groups were introduced to the 2,4,6-positions of the spacer benzene ring, not only for the effect of preorganization[12] but also to increase the solubility of the receptor in organic solvents. Based on these considerations, the new fluororeceptors **1** and **2** were designed and synthesized with the corresponding monothioureas **3**[9a] and **4** as reference compounds of **1** and **2**, respectively (Fig. 1 (b)).

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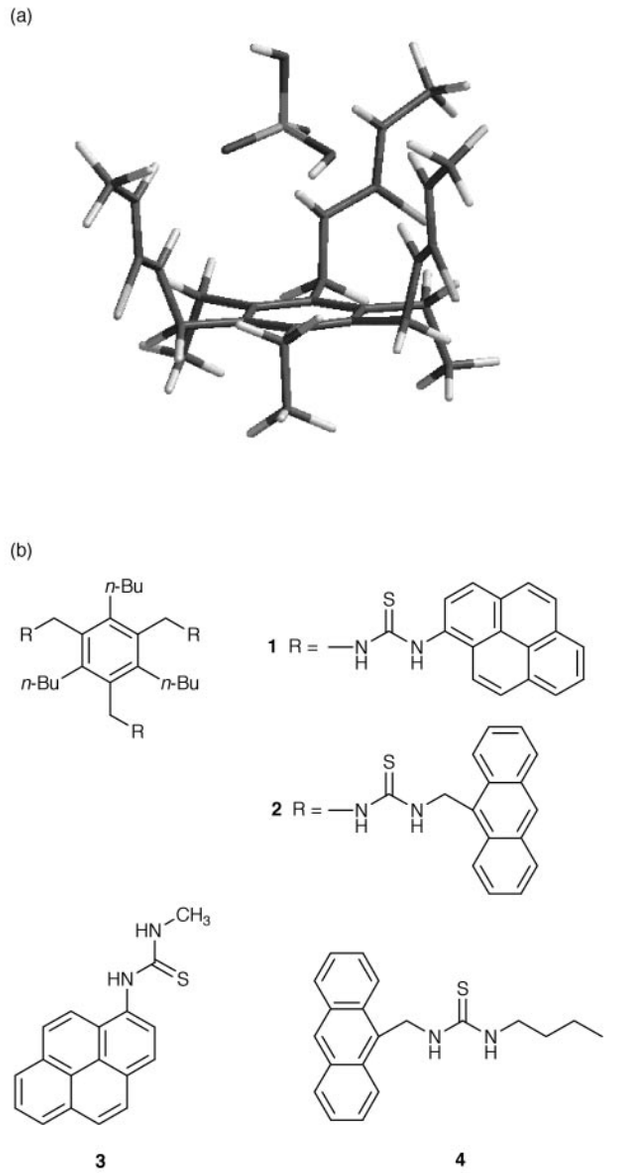
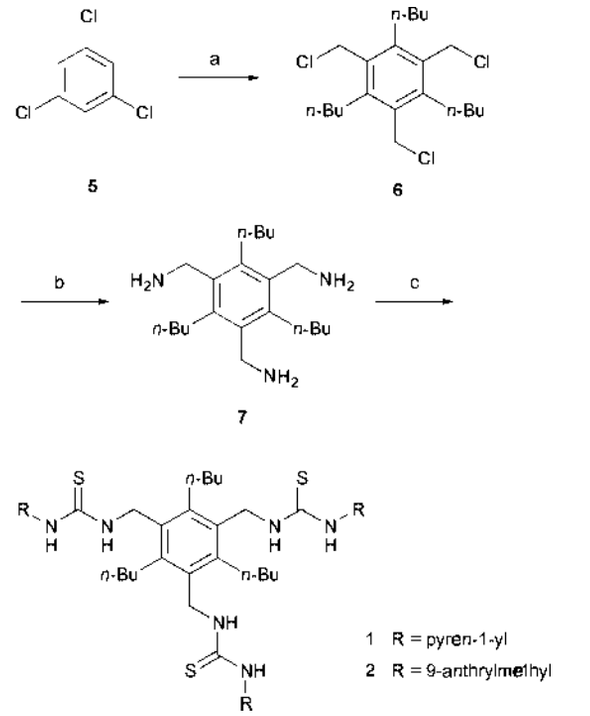


Fig. 1 (a) Proposed structure of the complex of 1,3,5-triethyl-2,4,6-tris[(*N*′-methylthioureido)methyl]benzene, a simple model compound of tripodal receptors **1** and **2**, with the dihydrogen phosphate anion. The structure was optimized by the AM1 semiempirical method. (b) Chemical structures of fluororeceptors.

The synthesis leading to receptors **1** and **2** is outlined in Scheme 1. The coupling reaction of 1,3,5-trichlorobenzene **5** with *n*-butylmagnesium bromide using a $NiCl_2$(DPPP) catalyst, followed by chloromethylation using chloromethyl methyl ether catalyzed by conc. H_2SO_4 gave six-fold substituted benzene **6** in 35% yield. The trichloride **6** was converted to triamine **7** by the Gabriel reaction in 64% yield. The thiourea derivatives **1** and **2** were prepared by the reaction of triamine **7** with the corresponding isothiocyanates in 16 and 50% yield, respectively.

Spectroscopic properties of the fluororeceptors

The variation in the absorption spectra of receptor **1** in acetonitrile upon addition of acetate anions is shown in Fig. 2. The spectra reveal clear isosbestic points at 310 and 346 nm, and an association constant for a 1 : 1 complex could be calculated by monitoring the absorption change at 370 nm. Essentially the same spectral change was observed upon addition of dihydrogen phosphate anions. On the other hand, Fig. 3 shows the emission spectra of receptor **1** in the presence of excess anions excited at 310 nm. A weak broad band at around 500 nm in the absence of anions is ascribed to the intramolecular inter-



Scheme 1 *Reagents and conditions*: (a) (i) *n*-BuMgBr, $NiCl_2$(DPPP), ether; (ii) chloromethyl methyl ether, conc. H_2SO_4, 35%; (b) (i) phthalimidopotassium, DMF; (ii) $H_2NNH_2{\cdot}H_2O$, EtOH; (iii) 6 M HCl, EtOH, 64%; (c) 1-(isothiocyanato)pyrene or 9-(isothiocyanatomethyl)-anthracene, $CHCl_3$, (**1**: 16%; **2**: 50%).

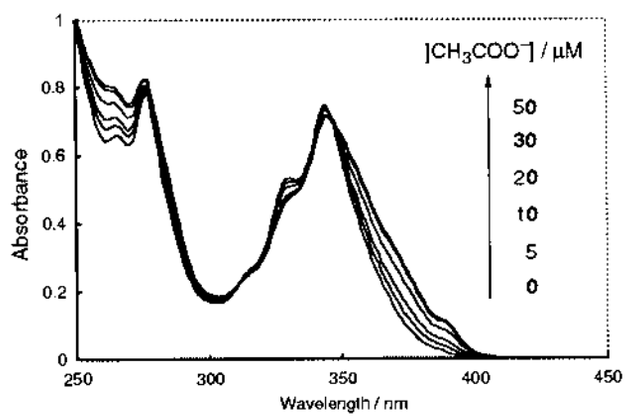


Fig. 2 UV-VIS absorption spectra of receptor **1** with increasing acetate anion concentration in acetonitrile. [**1**] = 1.0×10^{-5} M. Anion was used as tetrabutylammonium salt.

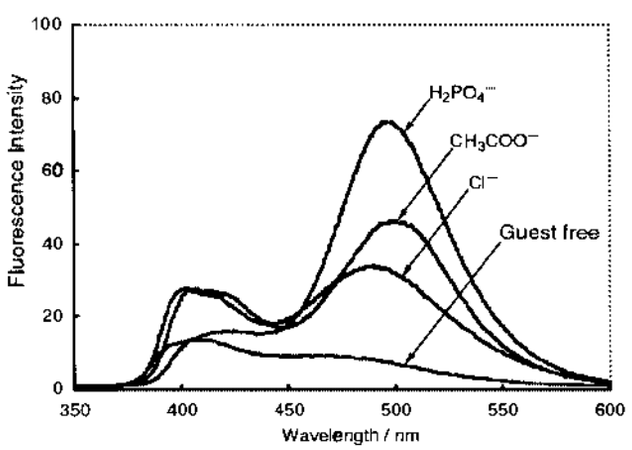


Fig. 3 Fluorescence spectra of receptor **1** with several guest anions in acetonitrile. [**1**] = 2.0×10^{-6} M. [Anion] = 4.0×10^{-4} M. Excitation wavelength: 310 nm. All anions were used as their tetrabutylammonium salts.

Table 1 Association constants (M^{-1}) of receptors **1**–**4** with dihydrogen phosphate and acetate anions in acetonitrile

Anion[a]	**1**[b]	**2**[c]	**3**[d]	**4**[c]
$H_2PO_4^-$	3.7×10^5	1.9×10^4	8.7×10^2	6.7×10^2
CH_3COO^-	1.9×10^5	1.4×10^4	6.2×10^3	2.9×10^3

[a] Counter-ion: tetrabutylammonium. [b] Association constants were determined by UV-VIS titration. [c] Association constants were calculated by nonlinear curve-fitting plots based on fluorescence spectra. [d] Data from ref. 7*a*.

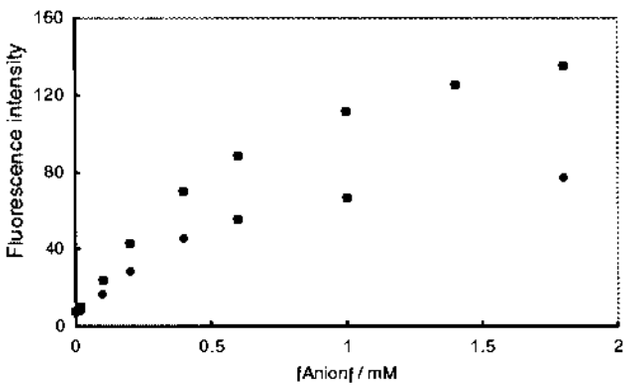


Fig. 4 Relation between the fluorescence intensity of receptor **1** at 500 nm and the anion concentration of (■) dihydrogen phosphate and (●) acetate. Excitation wavelength: 310 nm. [**1**] = 2.0×10^{-6} M in acetonitrile.

action of the pyrene rings, because such an emission was not observed for receptor **3**. Although no spectral change occurred upon addition of ClO_4^-, a long-wavelength emission at around 500 nm was observed upon addition of other guest anions as reported in the literature.[9a] However, it should be noted that the fluorescence intensity of receptor **1** increased in the order $H_2PO_4^- > CH_3COO^- > Cl^-$, which is different from the basicity of the guest anions. As shown in Fig. 4, a selectivity for dihydrogen phosphate over acetate was observed when the fluorescence intensity at 500 nm was plotted against the anion concentration. On the contrary, reference compound **3** shows acetate selectivity under the same conditions.

The fluorescence intensity of receptor **2** decreased upon addition of guest anions as shown in Fig. 5 (a). The anthracene moiety of **2** is connected to the thiourea group *via* one methylene unit; therefore, the emission quenching is assumed to be due to a photoinduced electron transfer (PET) process, as described for a similar case based on a pyrene fluorophore.[9b] Regarding the degree of intensity changes, receptor **2** showed quenching in the order $H_2PO_4^- > CH_3COO^- > Cl^- \gg ClO_4^-$. In contrast, as shown in Fig. 5 (b), the strongest quenching was observed when acetate anions were added to a solution of reference compound **4**. Therefore, it can be concluded that the anion selectivity between these two oxoanions is caused by the tripodal structure of receptors **1** and **2**, because the selectivity of the reference compounds **3** and **4** having only one anion binding site follows the order of anion basicity.

Association constants of fluororeceptors

Table 1 summarizes the association constants (K_a) of receptors **1**–**4** with dihydrogen phosphate and acetate anions in acetonitrile. The binding constants of the tripodal receptors **1** and **2** are larger than those of reference compounds **3** and **4**, apparently due to the increased number of binding sites. The K_a values of receptor **1** are large compared to those of **2**, which can be explained by the enhanced acidity of the thiourea groups of receptor **1** caused by the direct connection of aromatic pyrene rings. For the anion selectivity, it should be pointed out that the K_a values of tripodal receptors **1** and **2** for dihydrogen

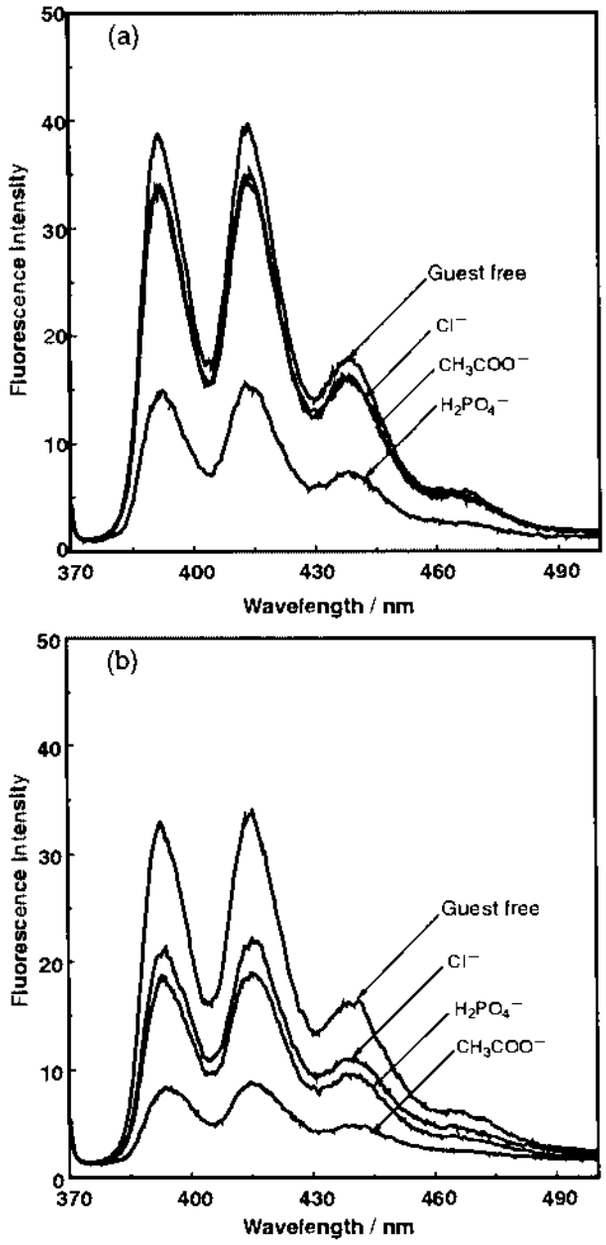


Fig. 5 Fluorescence spectra of (a) receptor **2** and (b) receptor **4** with several guest anions in acetonitrile. [Receptor] = 1.0×10^{-5} M. [Anion] = 2.0×10^{-3} M. Excitation wavelength: 366 nm. All guest anions were used as their tetrabutylammonium salts.

phosphate are larger than for acetate, whereas the K_a values of the simple thioureas **3** and **4** show selectivity for acetate over dihydrogen phosphate. These results are in accordance with the selectivity observed in the emission spectral change. Thus, it was shown that the preorganization effect of the tripodal receptors increases the dihydrogen phosphate selectivity by about one order of magnitude compared to the model receptors with only one binding site.

Conclusions

We have shown that thiourea derivatives having a six-fold substituted benzene ring as a rigid spacer are useful for recognizing the tetrahedral dihydrogen phosphate anion with selectivity over the acetate anion. The preorganization effect of the tripodal structure is found to be effective in reversing the selectivity pattern of these two oxoanions in contrast to the anion basicity. These receptors are expected to be useful, for example, as components of optode membranes for the detection of the biologically important phosphate anion.[8] Thus, it is

demonstrated that the characteristics of the well preorganized fluororeceptors are promising for the development of chemical sensors for a specific anion.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-GSX 270 spectrometer in $CDCl_3$ or DMSO-d_6. Coupling constants are given in Hz, and all chemical shifts are reported relative to an internal standard of tetramethylsilane or using the residual solvent peak as a standard. Abbreviations Ar, An, and Py represent aromatic, anthryl, and pyrenyl, respectively. Melting points were determined on a BÜCHI B-545 apparatus and are uncorrected. All moisture-sensitive reactions were carried out under an atmosphere of nitrogen. 1,3,5-Trichlorobenzene, chloromethyl methyl ether, 1-bromobutane, hydrazine monohydrate, 9-(chloromethyl)anthracene, and 1-aminopyrene were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). [1,3-Bis(diphenylphosphino)propane]nickel(II) chloride [$NiCl_2$-(DPPP)] and phthalimidopotassium were purchased from Kanto Chemical Co. (Tokyo, Japan). Solvents were dried (drying agent in parentheses) and distilled prior to use: ether ($LiAlH_4$), THF (sodium ketyl benzophenone), DMF (CaH_2).

Preparation of fluororeceptors

1,3,5-Tributyl-2,4,6-tris(chloromethyl)benzene 6. To a stirred suspension of 1,3,5-trichlorobenzene (25.0 g, 0.138 mol) and $NiCl_2$(DPPP) (753 mg, 1.4 mmol) in ether (150 mL) was added a solution of 1-butylmagnesium bromide prepared from 1-bromobutane (113 g, 0.825 mol) and magnesium (20.1 g, 0.827 mol) in ether (200 mL) over a period of 20 min at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 40 min; during that period, an exothermic reaction started. The mixture was then refluxed for 8 h, cooled in an ice-bath, and hydrolyzed with 10% aqueous HCl. The organic layer was separated and the aqueous layer was extracted twice with ether. The combined organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to leave a brown oil (31.4 g).

To a solution of the above crude 1,3,5-tributylbenzene in chloromethyl methyl ether (200 mL) was added concentrated sulfuric acid (10 mL), and the mixture was refluxed for 18 h. During that period, chloromethyl methyl ether (100 mL) and concentrated sulfuric acid (5 mL) were added twice. The reaction mixture was poured into ice–water and extracted three times with ethyl acetate. The combined extract was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The product was dissolved in CH_2Cl_2 and hexane was added. The resulting precipitate was separated and dried to give the trichloride **6** (17.5 g, 35%) as a white solid. Mp 129–130 °C; δ_H ($CDCl_3$) 1.04 (9H, t, J = 7.1, CH_3), 1.51–1.62 (12H, m, CH_2CH_2CH$_3$), 2.86 (6H, m, ArCH_2CH$_2$), 4.70 (6H, s, ArCH_2Cl).

1,3,5-Tris(aminomethyl)-2,4,6-tributylbenzene 7. Phthalimidopotassium (2.22 g, 12 mmol) was added to a solution of **6** (1.18 g, 3.0 mmol) in DMF (10 mL). The reaction mixture was stirred at 60 °C for 3 h and then cooled to room temperature. The mixture was poured into 0.5 M aqueous NaOH and extracted three times with $CHCl_3$. The combined extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to leave an oily solid. To the residue was added CH_2Cl_2 (3 mL) followed by hexane (20 mL). The resulting solid was filtered, washed with ether, and dried to afford 1,3,5-tributyl-2,4,6-tris(phthalimidomethyl)benzene (1.39 g) as a white solid. δ_H ($CDCl_3$) 0.63 (9H, t, J = 7.0, CH_3), 1.16–1.64 (12H, m, CH_2CH_2CH$_3$), 2.98–3.04 (6H, m, ArCH_2CH$_2$), 4.92 (6H, s, ArCH_2N), 7.66–7.82 (12H, m, *Ar*).

To a refluxing suspension of the above imide in EtOH (20 mL) was added hydrazine monohydrate (1.0 g, 20 mmol), and the mixture was refluxed again for 30 min. After cooling, 6 M aqueous HCl was added to acidify the mixture and it was refluxed for 30 min. The mixture was poured into aqueous NaOH and extracted three times with $CHCl_3$. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to leave triamine **7** (640 mg, 64% for two steps) as a white solid. The product was used for the following reaction without further purification. δ_H ($CDCl_3$) 0.98 (9H, t, J = 7.0, CH_3), 1.36 (6H, s, NH_2), 1.45–1.51 (12H, m, CH_2CH_2CH$_3$), 2.71–2.77 (6H, m, ArCH_2CH$_2$), 3.84 (6H, s, ArCH_2NH$_2$).

9-(Isothiocyanatomethyl)anthracene. 9-(Aminomethyl)anthracene[13] was prepared from 9-(chloromethyl)anthracene by the Gabriel reaction as described for the preparation of triamine **7**. To a solution of amine (638 mg, 3.1 mmol) in THF (10 mL) was added NaOH (246 mg, 6.2 mmol) dissolved in a small amount of water, and then CS_2 (2.35 g, 30.8 mmol) was slowly added. The mixture was stirred for 1 h at room temperature. 30% H_2O_2 was added dropwise with cooling in an ice-bath, and the mixture was acidified with 10% HCl. The mixture was diluted with water and extracted three times with $CHCl_3$. The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by chromatography on silica gel (hexane–EtOAc, 9 : 1) to give the desired isothiocyanate (506 mg, 66%) as a yellow solid. δ_H ($CDCl_3$) 5.59 (2H, s, AnCH_2NCS), 7.50–7.55 (2H, m, *An*), 7.60–7.66 (2H, m, *An*), 8.06 (2H, dd, J = 0.7, 8.1, *An*), 8.23 (2H, d, J = 8.4, *An*), 8.53 (1H, s, *An*).

1-(Isothiocyanato)pyrene[14]. This compound was prepared from 1-aminopyrene by the same procedure.

1,3,5-Tributyl-2,4,6-tris[(*N*′-(1-pyrenyl)thioureido)methyl]-benzene 1. Into a nitrogen-purged flask a solution of triamine **7** (25 mg, 0.075 mmol) in $CHCl_3$ (5 mL) was added followed by a solution of 1-(isothiocyanato)pyrene (78 mg, 0.30 mmol) in $CHCl_3$ (5 mL). The mixture was refluxed for 6 h and concentrated to remove the solvent. The product was purified by recrystallization from 1,1,2,2-tetrachloroethane to afford receptor **1** (13 mg, 16%) as colorless needles. Mp 180–182 °C; δ_H (DMSO-d_6) 1.00 (9H, t, J = 6.4, CH_3), 1.57 (12H, br, CH_2CH_2CH$_3$), 2.86 (6H, br, ArCH_2CH$_2$), 4.87 (6H, br, ArCH_2NH), 7.74 (3H, br, CH$_2$N*H*), 8.04–8.33 (27H, m, *Py*), 9.86 (3H, br, PyN*H*); δ_C (DMSO-d_6) 13.9 (CH$_3$), 23.1, 29.8, 34.4 and 43.0 (CH$_2$), 121.9, 122.0, 123.8, 124.4, 124.6, 125.0, 125.3, 125.8, 126.4, 126.8, 127.1, 127.3, 128.8, 130.4, 130.7, 132.3, 133.0 and 142.9 (ArC and PyC), 182.0 (S=C) (Found: C, 77.64; H, 5.94; N, 7.47; S, 8.61. Calc. for $C_{72}H_{66}N_6S_3$: C, 77.80; H, 5.98; N, 7.56; S, 8.65%). Receptors **2** and **4** were prepared similarly.

1,3,5-Tris[(*N*′-(9-anthrylmethyl)thioureido)methyl]-2,4,6-tributylbenzene 2. This compound was obtained as a yellow solid after trituration with ether in 50% yield. Mp 253–255 °C; δ_H (DMSO-d_6) 0.80 (9H, t, J = 7.0, CH_3), 1.34–1.39 (12H, m, CH_2CH_2CH$_3$), 2.50 (ArCH_2CH$_2$ protons overlapped the residual solvent peak), 4.56 (6H, br s, ArCH_2NH), 5.58 (6H, d, J = 4.0, AnCH_2NH), 6.92 (3H, br s, ArCH$_2$N*H*), 7.49–7.56 (15H, m, *An*), 8.09–8.12 (6H, m, *An*), 8.32–8.35 (6H, m, *An*), 8.61 (3H, s, AnCH$_2$N*H*); δ_C (DMSO-d_6) 13.7 (CH$_3$), 22.8, 29.4, 34.2 and 42.3 (CH$_2$), 124.2, 125.2, 126.4, 127.5, 128.9, 129.8, 131.0, 132.3, and 142.0 (ArC and AnC), 181.6 (S=C) (Found: C, 76.52; H, 6.58; N, 7.63; S, 8.91. Calc. for $C_{69}H_{72}N_6S_3$: C, 76.63; H, 6.71; N, 7.77; S, 8.89%).

9-[(*N*′-butylthioureido)methyl]anthracene 4. This compound was obtained as a yellow solid after column chromatography

(silica gel, $CHCl_3$) in 90% yield. Mp 186–188 °C; δ_H ($CDCl_3$) 0.84 (3H, t, J = 7.2, CH_3), 1.22–1.35 (2H, m, $CH_2CH_2CH_3$), 1.48 (2H, quintet, J = 7.2, $CH_2CH_2CH_2$), 3.14 (2H, br, $NHCH_2CH_2$), 5.55 (1H, br, $NHCH_2CH_2$), 5.66 (2H, br, $AnCH_2NH$), 5.89 (1H, br, $NHCH_2An$), 7.47–7.60 (4H, m, *An*), 8.04 (2H, d, J = 7.7, *An*), 8.78 (2H, d, J = 8.1, *An*), 8.49 (1H, s, *An*); δ_C ($CDCl_3$) 13.6 (CH_3), 19.9, 30.7, 41.9 and 43.6 (CH_2), 123.8, 125.2, 126.8, 127.5, 128.4, 129.1, 130.5 and 131.4 (AnC), 181.4 (S=C) (Found: C, 74.25; H, 6.79; N, 8.59; S, 9.95. Calc. for $C_{20}H_{22}N_2S$: C, 74.49; H, 6.88; N, 8.69; S, 9.94%).

Fluorescence and absorbance measurements

Absorption and emission spectra in the UV-VIS region were recorded on Hitachi U-2001 and Hitachi F-4500 spectrophotometers, respectively, using a quartz cell. All guest anions were purchased as tetrabutylammonium salts and purified according to a literature procedure.[15] Acetonitrile for fluorometry was purchased from Kanto Chemical Co. and used as received. Association constants were calculated by titration experiments:[16] to a solution of a receptor in acetonitrile was added a solution of guest anion containing an equal concentration of the receptor, and the spectral changes were monitored as a function of anion concentration.

Acknowledgements

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