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Synthesis and crystal structures of fluorescent receptors for 9-butyladenine

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ABSTRACT

Two pyrene containing fluorescent receptors, 2-(1-pyrenyl)benzoic acid (FR-1) and 8-(1-pyrenyl)-1-naphthoic acid (FR-2), have been designed and synthesized to mimic a pyrene dinucleotide for molecular recognition of 9-butyladenine (9-BuA). The X-ray crystal structures of the receptors FR-1 and FR-2 along with the binding substrate 9-BuA have been determined. FR-1 has the carboxyl group in the same plane as the phenyl group whereas the pyrenyl group is perpendicular to the phenyl group. However, both carboxyl and pyrenyl groups in FR-2 are parallel to each other but perpendicular to the naphthyl group. The binding constant for FR-2 to 9-BuA was found to be 7896±2187 M^{-1}, which is 8.3-fold greater than that for FR-1 (953±129 M^{-1}). The results indicate that the complex of 9-BuA with FR-2 is more stable than that with FR-1 by 1.2 kcal/mol. In addition, the molecular recognition of 9-BuA with the receptors can also be observed using fluorescence spectroscopy.

1. Introduction

A recent innovation is the application of fluorescent hydrocarbons as DNA base analogues in biological chemistry and drug discovery.[1–5] Pyrene is approximately the same size as a DNA base pair and can therefore mimic an entire DNA base pair and selectively bind with a DNA abasic site during DNA replications promoted by DNA polymerases. As a result, pyrene nucleotide was applied as a molecular wedge, DNA base pusher, and enzyme activity rescuer and promoter during enzymatic DNA base flipping and DNA repair.[6,7] One of the key features of pyrene is its large surface area for π–π stacking interactions. Kool and co-workers reported that while pyrene was used as a dangling nucleotide base analogue, it contributed more stability to duplex DNA than any DNA base, such as adenine, cytosine, guanine or thymine.[8,9] Although the solution structures of pyrene-containing DNA have been determined, attempts to obtain crystal structures have been unsuccessful.[10]

Another approach to studying the interactions of pyrene dinucleotides is to synthesize molecular mimics and study their binding with a suitable DNA base analogue. Therefore, two fluorescent receptors, 2-(1-pyrenyl)benzoic acid (FR-1) and 8-(1-pyrenyl)-1-naphthoic acid (FR-2), were designed based on the structure of a pyrene dinucleotide using chemical mimicry (Scheme 1).[8,9] The rigid phenyl and naphthyl rings were used to mimic the DNA phosphate backbone, and carboxyl groups were used to resemble the DNA base thymine, which is a complementary base of adenine in a DNA base pair. Herein, we report the synthesis of these two receptors by Suzuki coupling and X-ray structures of the receptors and the DNA adenine analogue, 9-butyladenine (9-BuA).[11] Binding constants of the receptors with the 9-butyladenine have been determined using NMR spectroscopy. Molecular recognition of 9-BuA has also been carried out using fluorescence spectroscopy.

2. Results and discussion

2.1. Synthesis of FR-1, FR-2, and 9-BuA

A standard Suzuki coupling was applied in the synthesis of both receptors FR-1 and FR-2.[12] In the synthesis of FR-1 (Scheme 2), 2-bromoiodobenzene (**1**) was used as starting material because of the different reactivity for iodine and bromine during palladium catalyzed coupling reactions. Indeed, coupling product **2** was the only product isolated. Compound **2** was then converted to the carboxylic acid using *n*-butyllithium to generate a carbanion, which reacted with carbon dioxide to form lithium carboxylate. Following acidification, receptor FR-1 was obtained in moderate yield.[13] Receptor FR-2 was synthesized using a similar procedure with 1,8-dibromonaphthalene (**3**) as starting material (Scheme 3).

9-Butyladenine was synthesized as described in a preliminary report from adenine and freshly prepared 1-iodobutane (Scheme 4).[14] In the purification step, the product was recrystallized from toluene followed by aqueous ethanol solution (10%).[15]

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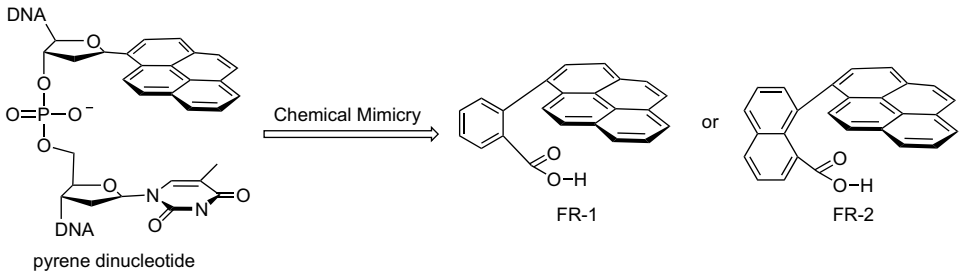
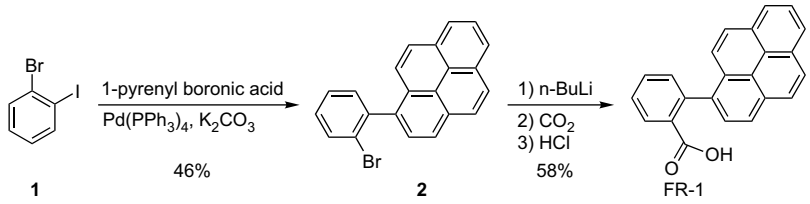
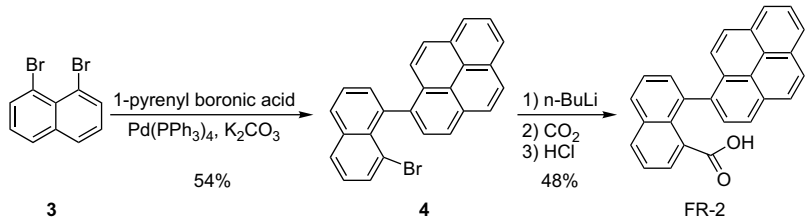


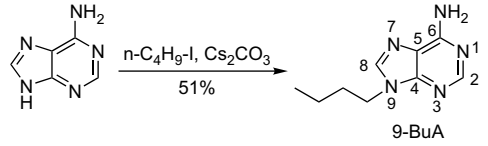
Scheme 1. Chemical mimicry of pyrene dinucleotide using receptors FR-1 and FR-2.



Scheme 2. Synthesis of receptor FR-1.



Scheme 3. Synthesis of receptor FR-2.



Scheme 4. Synthesis of 9-BuA.

2.2. Crystallographic study of receptors FR-1, FR-2, and 9-BuA

Crystallographic studies were performed to explore the structures of the receptors FR-1 and FR-2. Suitable crystals for crystallographic study were grown as follows. Both receptors FR-1 and FR-2 were dissolved in hot acetonitrile. After cooling to room temperature and slow evaporation, dark brown crystals formed on the surface of the containers. The solvent was then decanted, the crystals dried under vacuum, and carefully scraped from the containers.[15,16] Colorless crystals of 9-butyladenine were obtained by slow cooling of a saturated hot aqueous alcohol (10%) solution.[15]

The molecular structures of FR-1 and FR-2 as determined by crystallography are shown in Figs. 1 and 2. As expected, both receptors form dimers through hydrogen bond association of carboxyl groups (Figs. 3 and 4). The parameters for these hydrogen bonds are given in Table 1. The distance from the carbonyl oxygen to carboxyl proton of another molecule is 1.86 Å for both FR-1 and FR-2. Interestingly, the structure of FR-1 shows the carboxyl group in approximately the same plane as the phenyl group [O=C–C1(ph)–C2(ph) torsion angle=161.3(3)°] and the pyrenyl group perpendicular to the phenyl group [C2(py)–C1(py)–C2(ph)–C1(ph) torsion angle=−63.5(3)°].[17] In addition, pyrenyl groups are turned away from each other in the dimer (Fig. 3). The structure of FR-2 shows carboxyl [O=C–C1(ph)–C2(ph) torsion angle=−56.4(6)°] and pyrenyl [C2(py)–C1(py)–C8(naph)–C7(naph) torsion angle=−64.8(6)°] groups parallel to each other but perpendicular to the naphthyl group. The distance between the two pyrenyl groups is 6.79 Å (Fig. 4) indicating that these two pyrenyl groups should interact with each other through π–π interactions.[8,9] The closeness of the pyrenyl groups in the dimer provides evidence for the formation of pyrene excimer

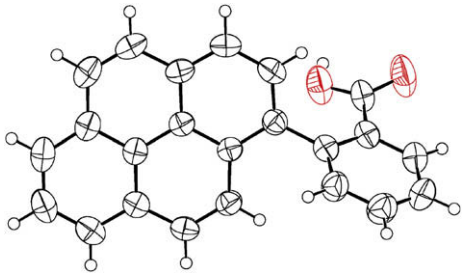


Figure 1. Thermal ellipsoid drawing of an FR-1 molecule.

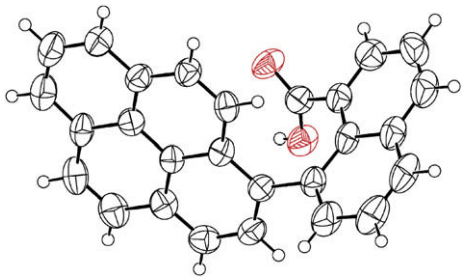


Figure 2. Thermal ellipsoid drawing of an FR-2 molecule.

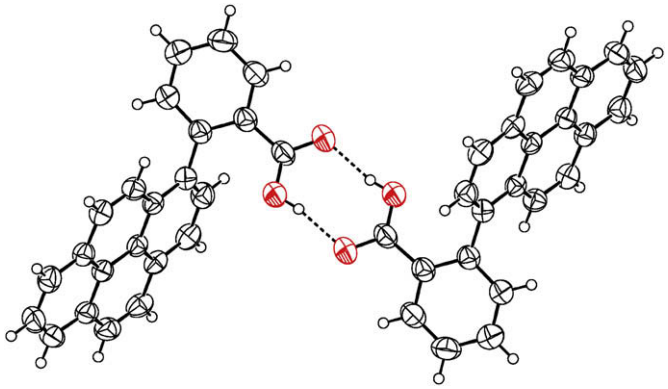


Figure 3. Thermal ellipsoid diagram of FR-1 dimer.

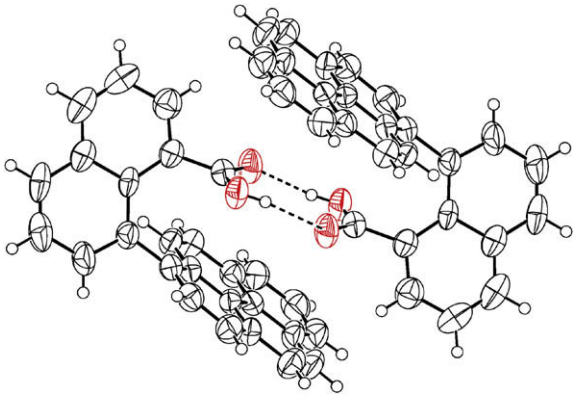


Figure 4. Thermal ellipsoid diagram of FR-2 dimer.

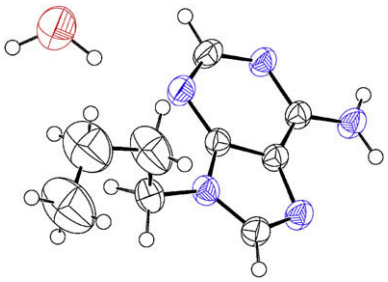


Figure 5. Thermal ellipsoid drawing of a 9-BuA molecule.

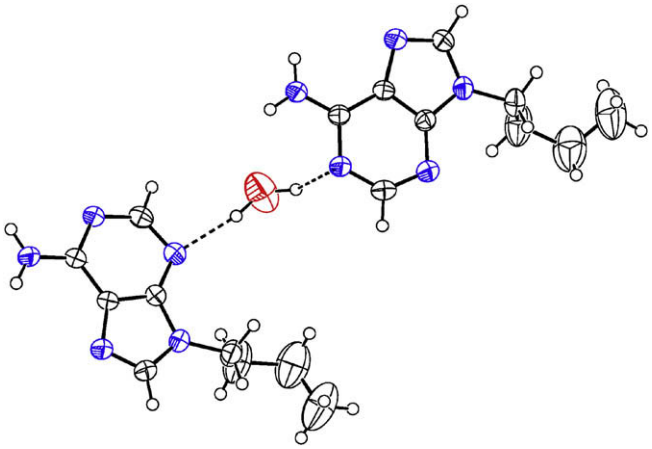


Figure 6. Thermal ellipsoid diagram of 9-BuA dimer.

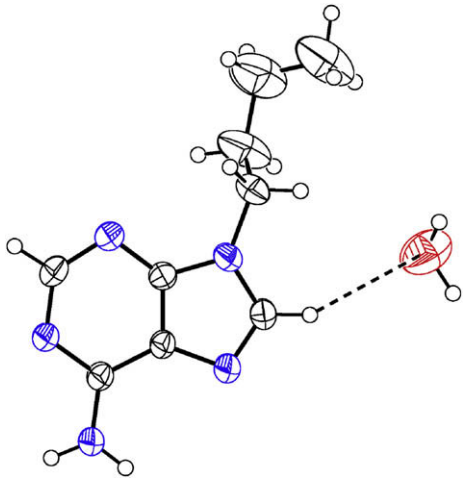


Figure 7. Close contact between C8-H of 9-BuA to a water molecule.

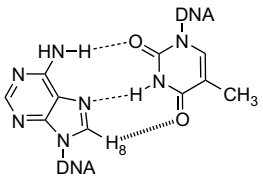
Table 1
Hydrogen bonding parameters for FR-1, FR-2, and 9-BuA

Compound	D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (°)
FR-1	O–H···O=C[a]	0.83	1.86	2.684(3)	169
FR-2	O–H···O=C[b]	0.82	1.86	2.672(3)	172
9-BuA	N–H···7-N[c]	0.87	2.18	3.050(4)	177
	N–H···1-N[d]	0.87	2.09	2.928(4)	161
	O–H···3-N	1.01(5)	1.94(5)	2.913(4)	163(5)
	O–H···1-N[e]	1.01(6)	2.16(7)	3.064(5)	148(5)

[a] $-x, 2-y, 1-z$.
[b] $2-x, 2-y, -z$.
[c] $-x, 1/2+y, -1/2-z$.
[d] $-x, -1/2+y, -1/2-z$.
[e] $x, 3/2-y, 1/2+z$.

for FR-2 with the maximum emission at 472 nm in chloroform.[18,19] However, the pyrenyl groups in the FR-1 dimer do not overlap explaining why no pyrene excimer emission for FR-1 is observed. Although the structures of the two dimers are very different, the dimerization constants, 824±36 and 960±10 M^{-1}, respectively, were very similar (Figs. S1 and S2).

The X-ray structure of 9-butyladenine was also determined (Fig. 5). In the crystal, a water molecule is present, which forms hydrogen bonds with N3 of one 9-butyladenine and N1 of another (Fig. 6). Furthermore, a close contact of 2.69 Å is observed between H8 of 9-BuA and the oxygen of a water molecule [C–H: 0.94 Å; C···O (x, $1+y$, z): 3.537(5) Å; C–H···O: 150°] (Fig. 7). This observation suggests that H8 may be involved in a DNA base pair between adenine and thymine, and provides evidence of the *spectator H-bond* (Scheme 5).[20–22]



Scheme 5. Possible involvement of adenine H8 in hydrogen bonding with oxygen of thymine in a DNA base pair.

More importantly, the 9-butyladenine molecules form hydrogen bonded chains. The adenine moiety forms both Watson–Crick and Hoogsteen hydrogen bonding with two other molecules (Fig. 8).[23] The distances from hydrogen atoms of the amino group to nitrogen atoms of the purine moiety are short (Table 1) indicating that strong hydrogen bonding is the key interaction between adenine molecules.[15]

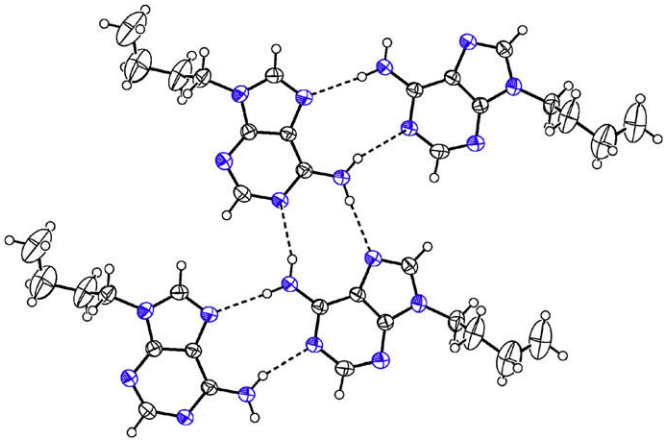
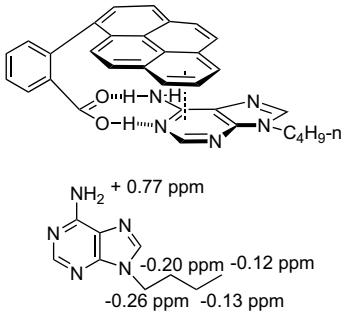


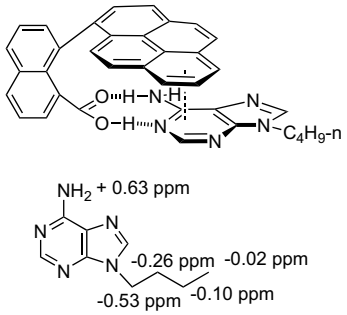
Figure 8. Thermal ellipsoid diagram of showing four molecules of 9-BuA chain.

2.3. Molecular recognition of 9-BuA with receptors FR-1 and FR-2

Both of the receptors FR-1 and FR-2 were applied in the molecular recognition of a substrate 9-BuA as studied by titrations using NMR spectroscopy. First, the variation in proton chemical shifts of the amino and alkyl groups of 9-BuA (1.00 mM) was determined based on NMR experiments in the presence or absence of the same molar amount of a receptor (1.00 mM) (Scheme 6). In the presence of the receptor FR-1, the proton chemical shift of the amino group moved downfield by 0.77 ppm, suggesting that the amino group was hydrogen bonding with the carboxyl group of the receptor. All proton chemical shifts of the butyl group moved upfield. For example, the chemical shift of the N–CH_2 protons moved upfield by 0.26 ppm indicating that the butyl group was in a shielded area and there were π–π stacking interactions between the adeninyl group in 9-BuA and the pyrenyl group of the receptor FR-1. With receptor FR-2, the chemical shift of the amino group protons of 9-BuA moved downfield by 0.63 ppm suggesting that the 9-BuA also formed hydrogen bonding with the carboxyl group of FR-2 (Scheme 7). The chemical shift of the N–CH_2 protons in 9-BuA moved upfield by 0.53 ppm, indicating that the 9-BuA is involved in stronger π–π stacking interactions with the pyrenyl group in receptor FR-2 than in FR-1.[24]



Scheme 6. Molecular recognition of 9-BuA with receptor FR-1 and chemical shift changes for 9-BuA after binding.



Scheme 7. Molecular recognition of 9-BuA with receptor FR-2 and chemical shift changes for 9-BuA after binding.

Job's plots, generated to identify the binding stoichiometry between 9-BuA and the receptors, showed 1:1 binding association for both receptors FR-1 and FR-2 (Figs. S3 and S4).[25] The binding constant for the complex between FR-1 and 9-BuA was determined to be 953±129 M^{-1} using a ^1H NMR titration experiment with deuteriochloroform ($CDCl_3$) as solvent (Fig. S5). Using a similar method, the binding constant for FR-2 and 9-BuA was determined to be greater than 10^4, which is the upper limit for reliability of this experiment.[26,27] Therefore, the binding constant for the FR-2:9-BuA complex was estimated to be 7896±2187 M^{-1} using a ^1H NMR competitive titration experiment (Table S1).[26,27] The latter value is 8.3-fold greater than the former one, suggesting that receptor FR-2 is a better receptor than FR-1 for 9-BuA. The complex FR-2:9-BuA is more stable than FR-1:9-BuA by 1.2 kcal/mol, indicating that the former has stronger π–π stacking interactions and hydrogen bonding. The free energy difference between the two complexes was calculated using Eq. 1.

$$\Delta\Delta G = \Delta G_{\mathrm{FR-2:9-BuA}} - \Delta G_{\mathrm{FR-1:9-BuA}} = -RT \ln K_{\mathrm{b(FR-2:9-BuA)}}/K_{\mathrm{b(FR-1:9-BuA)}} \quad (1)$$

where $\Delta\Delta G$ is the free energy difference between complexes and $\Delta G_{\mathrm{FR\text{-}2:9\text{-}BuA}}$ and $\Delta G_{\mathrm{FR\text{-}1:9\text{-}BuA}}$ are the free energies for the complexes of 9-BuA with receptors FR-2 and FR-1. $K_{\mathrm{b(FR\text{-}2:9\text{-}BuA)}}$ and $K_{\mathrm{b(FR\text{-}1:9\text{-}BuA)}}$ are the binding constants for receptors FR-2 and FR-1 with 9-BuA.

An important question to resolve is the contribution to the intermolecular interaction that results from π–π stacking interactions of the pyrenyl groups in the receptors with 9-BuA. Rao and co-workers reported a binding constant of 150 M^{-1} for complex formation between 9-BuA and an aromatic acid receptor, benzoic acid.[28] The relative values of the binding constants of receptors FR-1 and FR-2 to that of benzoic acid are 6.4 and 53 (Table 2). Thus, the energy stabilizations from π–π stacking interactions were estimated to be 1.1 and 2.3 kcal/mol for the receptors FR-1 and FR-2, respectively, using a treatment as in Eq. 1.

Table 2
The energy stabilizations from π–π stacking interactions besides hydrogen bonding in the molecular recognition of 9-BuA with the receptors FR-1 and FR-2 in $CDCl_3$ (296 K)

Receptor	K_b (M^{-1})	$K_{b(receptor)}/K_{b(benzoic\ acid)}$	$\Delta\Delta G$ (kcal/mol)
Benzoic acid	150[a]	—	—
FR-1	953	6.4	−1.1
FR-2	7896	53	−2.3

[a] Literature data.[28]

Moreover, the structures of the complexes of 9-BuA with the receptors FR-1 and FR-2, individually were calculated using Spartan'06 software at the RM1 level (Figs. 9 and 10).[29] The distances and bond angles in the hydrogen bonds were then analyzed (Table S2). The complex structures show both receptors formed π–π stacking interactions with the binding substrate 9-BuA. However, hydrogen bonding in the complex FR-2:9-BuA was stronger than that in FR-1:9-BuA based on hydrogen bonding parameters. The average calculated hydrogen bond length, 1.771 Å in the complex FR-2:9-BuA was shorter than the average value of 1.813 Å in FR-1:9-BuA, and the average calculated hydrogen bond angle of 169° in the complex FR-2:9-BuA was closer to 180° than that in FR-1:9-BuA (157°). In terms of π–π stacking interactions, the adeninyl ring of 9-BuA was approximately parallel to the pyrenyl group with a distance of 5.37 Å for the complex FR-2:9-BuA. For the complex FR-1:9-BuA, the π–π stacking interactions were weaker because the adeninyl ring of 9-BuA was much less parallel to the pyrenyl group and there was a slightly greater average distance of 5.68 Å (Figs. S6 and S7). The calculated structures explain why protons in the butyl group of the complex FR-2:9-BuA were shielded more significantly than those in the FR-1:9-BuA complex. Therefore, theoretical calculations agree with the experimental results in demonstrating that FR-2 was a better mimic for the pyrene dinucleotide than FR-1. The results also indicate that both π–π stacking interactions and hydrogen bonding were important in the stabilization of DNA duplex and should work in concert for maximum stabilization.

2.4. Pyrene fluorescence spectroscopic study of FR-1 and FR-2 in the molecular recognition of 9-BuA

The fluorescence spectra of receptors FR-1 and FR-2 in chloroform were obtained. FR-1 has a maximum emission peak at 441 nm, while for FR-2 the maximum emission peak occurs at 472 nm. The shift to higher wavelength for FR-2 can be explained because the emission for this receptor is from an excimer facilitated in the dimer through intermolecular interactions.[18,19]

Molecular recognition studies of 9-BuA by receptors FR-1 and FR-2 were performed using fluorescence spectroscopy.[30] The experiments were carried out with chloroform as solvent at room temperature (296 K). Pyrene fluorescence changes were monitored during titration of the receptors FR-1 and FR-2 with 9-BuA (Schemes 6 and 7). It is interesting to note that the molecular recognition of 9-BuA by FR-1 resulted in a decrease of pyrene fluorescence intensity at 441 nm (Fig. 11) while the binding of the FR-2 receptor by 9-BuA resulted in an increase of pyrene fluorescence at the same wavelength (Fig. 12). For FR-2, there was a blue shift of the maximum pyrene fluorescence peak from 472 to 441 nm with increasing 9-BuA concentration. Remarkably, during the binding of the receptor FR-2 by 9-BuA, a visual color change of the fluorescence emission from greenish blue of receptor FR-2 to dark blue of the complex FR-2:9-BuA (Fig. 13) was observed. The blue shift and emission color change during molecular recognition of 9-BuA by FR-2 are consistent with the diminishing of FR-2's excimer emission at 472 nm and recovery of the maximum

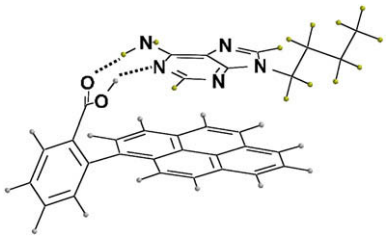


Figure 9. Calculated structure of the complex FR-1:9-BuA.

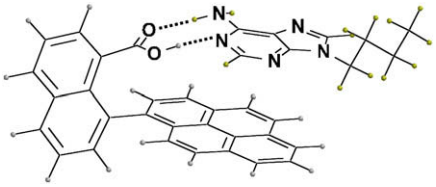


Figure 10. Calculated structure of the complex FR-2:9-BuA.

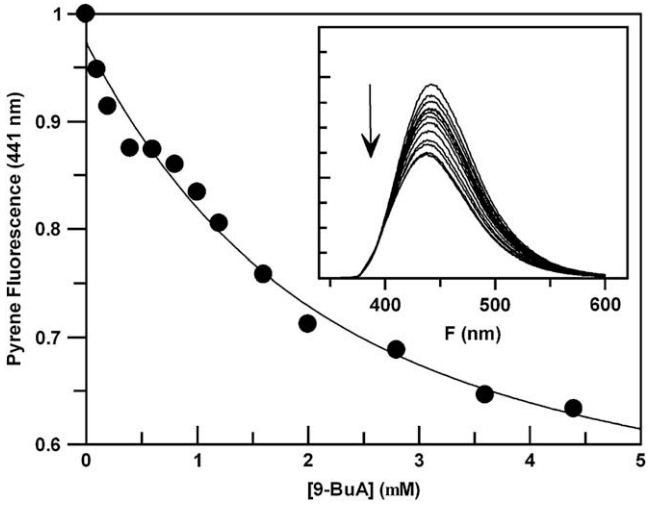


Figure 11. Titration of FR-1 with 9-BuA monitored by fluorescence.

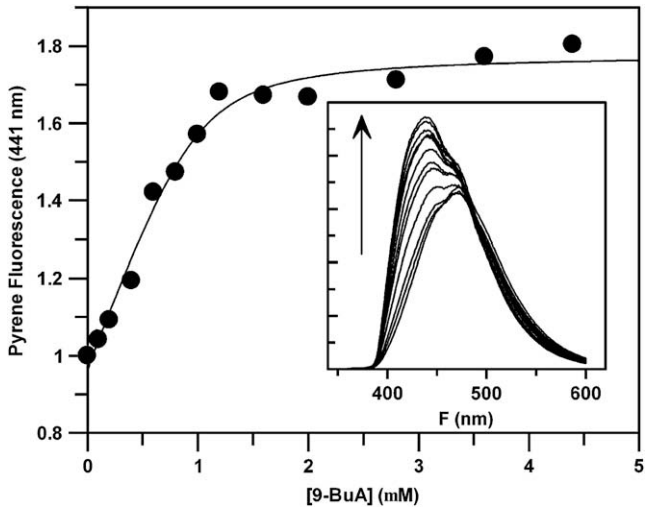


Figure 12. Titration of FR-2 with 9-BuA monitored by fluorescence.

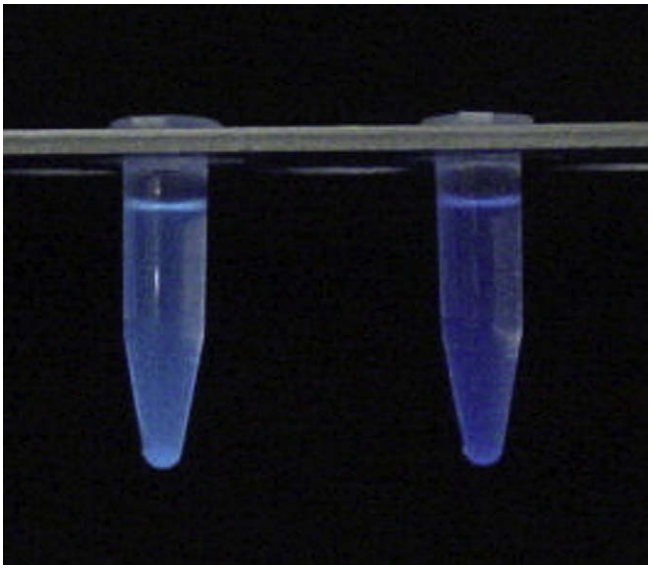


Figure 13. FR-2's fluorescence change from greenish blue (left, FR-2, 0.343 mM) to deep blue (right, complex of FR-2 with 9-BuA, FR-2, 0.343 mM, 9-BuA, 3.43 mM).

emission at 441 nm of the complex FR-2:9-BuA.[19] The observed changes in pyrene fluorescence behavior are indicative of the binding of 9-BuA by the receptors.[19]

3. Conclusion

In order to mimic pyrene dinucleotide, the receptors FR-1 and FR-2 were designed and synthesized. Their binding substrate 9-butyladenine was also synthesized. The three compounds were crystallized and their crystal structures determined. Both receptors FR-1 and FR-2 form dimers through hydrogen bonding between carboxyl groups of two molecules. In the FR-1 dimer, the carboxyl group is in the same plane as the phenyl group while the carboxyl group is vertical to the naphthyl group in the FR-2 dimer. In the FR-1 dimer, pyrenyl moieties are too far separated to interact. On the other hand, the pyrenyl groups in the FR-2 dimer are parallel with a distance of 6.79 Å. The crystal structure of 9-butyladenine shows that it forms an extensive hydrogen bonding network.

The molecular recognition of 9-butyladenine with receptors FR-1 and FR-2 established that the binding constant for complex formation of 9-BuA with FR-2 is 8.3-fold greater than with FR-1. The binding of 9-butyladenine by the receptor FR-2 is more energetically favorable than by FR-1 by 1.2 kcal/mol, suggesting π–π stacking and hydrogen bonding interactions are stronger in the complexation of 9-butyladenine with FR-2 than FR-1. These results indicate that the receptor FR-2 is a better mimic for pyrene dinucleotide. The energy stabilization from π–π stacking is estimated to be 2.3 kcal/mol in the complex FR-2:9-BuA. Fluorescence spectroscopic studies indicate that the binding of 9-BuA by FR-1 results in a decrease of fluorescence intensity. In contrast, molecular recognition of 9-BuA by receptor FR-2 results in a blue shift in emission wavelength and an increase of the fluorescence intensity.

4. Experimental

4.1. General

All chemicals were purchased from Sigma–Aldrich and used without further purification. Flash chromatography was performed with silica gel (70–230 mesh from Sorbent Technologies) and monitored by thin layer chromatography (TLC) with silica gel plates (Merck, Kieselgel 60 F_{254}). The melting points were determined using a MEL-TEMP apparatus. IR spectra were recorded on a Genesis II FT-IR™ spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Varian instrument. The spectra were recorded in deuteriochloroform ($CDCl_3$) or hexadeuteriodimethylsulfoxide (DMSO-d_6). Chemical shifts of protons are given in parts per million with TMS as internal standard. The chemical shifts of carbons are reported in parts per million with solvent peaks used as internal standards. Dilution and titration experiments with NMR were carried out at 296 K. ESI spectra were recorded on an API 150EX. The complex structures of 9-BuA with the receptors FR-1 and FR-2 were calculated with Spartan'06 from Wavefunction, Inc.[29]

X-ray structures were determined using a Rigaku R-AXIS SPIDER system. Structure solution was accomplished with *SIR-92*[31] while structure refinement was performed using *SHELX-97*.[32] Thermal ellipsoid drawings of the molecular structures were generated with *PLATON*.[33]

Fluorescence spectra were measured on a FluoroMax-3 from Horiba Jobin Yvon. The general setting was increment 1, integration 0.5, slit widths 3 (excitation) and 3 (emission) for both excitation and emission fluorescence spectra. All fluorescence spectra were recorded at 296 K and maintained using a circulating water bath. The fluorescence cuvette used was made of quartz with a volume of 4 mL. The final volume of solutions for all the measurements was 2 mL. The excitation wavelength was 350 nm.

4.2. 1-(2-Bromophenyl)pyrene (2)

To a three-neck round bottom flask were added potassium carbonate (0.46 g, 3.3 mmol) and water (2.2 mL). The mixture was purged with nitrogen for 10 min. To the resulting mixture were added 2-bromoiodobenzene (0.283 g, 1.0 mmol), 1-pyrenyl boronic acid (0.246 g, 1.0 mmol), and 1,2-dimethoxyethane (5 mL) under nitrogen with stirring. After 2 min, the catalyst $Pd(PPh_3)_4$ (37 mg, 0.035 mmol, 3.5% molar amount) was added. After 5 min, the resulting mixture was heated at reflux in an oil bath at 120 °C for 14 h. After cooling to room temperature, water (5 mL) was added to the reaction and it was extracted with dichloromethane (3×5 mL). After evaporation of the solvents *in vacuo*, the residue was purified by column chromatography using silica gel with hexane as eluent, affording intermediate **2**, 1-(2-bromophenyl)pyrene (0.190 g white crystals, 47%), after removal of solvent. Mp 129–130 °C. IR (KBr, cm^{-1}): 513, 629, 650, 682, 721, 752, 847, 1005, 1023, 1047, 1115, 1175, 1192, 1243, 1422, 1434, 1455, 1466, 1558, 1601, 2854, 3039. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.37 (m, 1H), 7.48 (m, 2H), 7.75 (d, J=9.2 Hz, 1H), 7.83 (d, J=8.8 Hz, 1H), 7.92 (d, J=7.6 Hz, 1H), 8.02 (m, 2H), 8.12 (s, 2H), 8.22 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 124.3, 124.7, 124.7, 124.8, 125.1, 125.3, 125.4, 126.2, 127.4, 127.5, 127.5, 127.8, 128.8, 129.4, 131.0, 131.2, 131.5, 132.4, 132.9, 136.5, 141.8. HR-ESI-TOF calcd for $C_{22}H_{14}Br^{+}$ (M+H) 357.027, found 357.028.

4.3. 2-(1-Pyrenyl)benzoic acid (FR-1)

To a one-neck round bottom flask were added **2** (0.122 g, 0.34 mmol) and THF (2 mL). The mixture was cooled to −78 °C. To the chilled solution was added *n*-butyllithium (0.136 mL of 2.5 M in hexane, 0.34 mmol) with stirring. After 5 min, dry ice (0.03 g, 0.7 mmol) was added. The reaction mixture was then warmed to room temperature gradually. The solvent was removed *in vacuo*, the residue dissolved in sodium carbonate (0.5 M, 40 mL), and extracted with diethyl ether (2×20 mL) to remove impurities. The resultant aqueous solution was acidified with hydrochloric acid (6 N) to pH=2. The final product FR-1 was obtained by filtration, washing with water, and drying to constant weight (0.064 g, white solid, 58%). Mp 244–245 °C. UV–vis ($CHCl_3$, nm) λ_{max} 269, 279, 331, 346. IR (KBr, cm^{-1}): 495, 563, 632, 651, 683, 725, 753, 763, 848, 932,

1071, 1133, 1264, 1306, 1403, 1482, 1483, 1604, 1680, 1697, 2816, 2862, 3038. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 7.50 (d, J=7.6 Hz, 1H), 7.71 (m, 3H), 7.91 (m, 1H), 8.10 (m, 3H), 8.22 (s, 2H), 8.26 (d, J=7.6 Hz, 1H), 8.32 (d, J=7.2 Hz, 2H), 12.48 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ 124.3, 124.6, 125.0, 125.2, 125.5, 125.8, 126.9, 127.6, 127.8, 128.00, 128.4, 128.7, 130.4, 130.5, 130.9, 131.5, 132.0, 132.7, 133.3, 137.9, 141.1, 168.8. ESI-MS calcd for $C_{23}H_{14}O_2Na^+$ (M+Na), 345.3; found, 345.1. HR-ESI-TOF calcd for $C_{23}H_{15}O_2^+$ (M+H) 323.107, found 323.107.

4.4. 1-(8-Bromo-1-naphthyl)pyrene (4)

To a three-neck round bottom flask were added potassium carbonate (0.46 g, 3.3 mmol) and water (2.2 mL). The mixture was purged with nitrogen for 10 min. To the resulting mixture were added 1,8-dibromonaphthalene (0.286 g, 1.0 mmol), 1-pyrenyl boronic acid (0.246 g, 1.0 mmol), and 1,2-dimethoxyethane (5 mL) under nitrogen with stirring. After 2 min, the catalyst $Pd(PPh_3)_4$ (37 mg, 0.035 mmol, 3.5% molar amount) was added and the mixture stirred for 5 min. The resulting mixture was then heated to reflux in an oil bath at 120 °C for 14 h. After cooling to room temperature, water (5 mL) was added to the mixture and extracted with dichloromethane (3×5 mL). The residue from evaporation of the solvents *in vacuo* was purified by column chromatography using silica gel with 1–3% ethyl acetate (volume) in hexane as eluent, affording intermediate **4**, 1-(8-bromo-1-naphthyl)pyrene (0.219 g white solid, 54%), after removal of solvent. Mp 154–156 °C. IR (KBr, cm^{-1}): 493, 541, 721, 766, 821, 844, 1119, 1181, 1197, 1244, 1320, 1363, 1441, 1493, 1561, 2921, 3047. ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.33 (t, J=7.6 Hz, 1H), 7.60 (m, 3H), 7.73 (d, J=7.2 Hz), 7.90–8.23 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 120.3, 124.0, 124.5, 124.9, 125.0, 125.2, 125.6, 125.9, 126.0, 126.4, 127.4, 127.7, 128.7, 129.3, 129.6, 130.8, 130.9, 131.1, 131.6, 132.4, 133.9, 136.1, 138.3, 138.7. HR-ESI-TOF calcd for $C_{26}H_{16}Br^+$ (M+H) 407.043, found 407.042.

4.5. 8-(1-Pyrenyl)-1-naphthoic acid (FR-2)

To a one-neck round bottom flask were added **4** (0.144 g, 0.35 mmol) and THF (2 mL). The mixture was cooled to −78 °C. To the resulting cold solution was added *n*-butyllithium (0.22 mL of 1.6 M in hexane, 0.35 mmol) with stirring. After 5 min, dry ice (0.031 g, 0.70 mmol) was added. The reaction mixture was then warmed to room temperature gradually. The solvent was removed *in vacuo*, the residue dissolved in sodium carbonate (0.5 M, 2×20 mL), and extracted with diethyl ether (2×20 mL) to remove impurities. The resulted aqueous solution was acidified with hydrochloric acid (6 N) to pH=2. The final product FR-2 was obtained by filtration, washing with water, and drying to constant weight (0.063 g off-white solid, 48%). Mp >260 °C, decomposed. UV–vis ($CHCl_3$, nm) λ_{max} 270, 281, 349. IR (KBr, cm^{-1}): 496, 639, 682, 722, 773, 846, 1155, 1183, 1210, 1293, 1413, 1460, 1503, 1692, 3039. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ 7.57–8.32 (m, 15H), 11.76 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ 124.7, 124.8, 125.4, 125.6, 125.7, 126.2, 126.4, 126.7, 127.6, 127.7, 128.1, 128.4, 129.0, 129.1, 129.2, 129.5, 130.7, 131.2, 131.5, 131.6, 132.6, 133.7, 135.0, 137.9, 138.2, 170.2. ESI-MS calcd for $C_{27}H_{16}O_2Na^+$ (M+Na), 395.4; found, 395.1. HR-ESI-TOF calcd for $C_{27}H_{17}O_2^+$ (M+H) 373.122, found 373.122.

4.6. Synthesis of 9-BuA

Adenine (302 mg, 2.25 mmol) was dissolved in hot DMF (20 mL). To the warm solution were added freshly prepared 1-iodobutane (205 mg, 1.12 mmol) and cesium carbonate (1.94 g, 3.36 mmol). The resulting mixture was stirred at 50 °C overnight. The DMF solvent was removed *in vacuo*. The residue was boiled with toluene (50 mL) for 5 min and the hot solution filtered. After evaporation of most of the solvent, the precipitate was collected by filtration. The crude product was recrystallized from toluene (5 mL) and then 10% ethanol in water (5 mL) to give pure product (110 mg, 51%). Mp 136–138 °C (lit.[34] 116 °C). UV–vis ($CHCl_3$, nm) λ_{max} 261. IR (KBr, cm^{-1}): 1029, 1071, 1491, 1596, 1676, 3286 (br, NH_2). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 0.96 (t, 3H, J=7.6 Hz, CH_3), 1.36 (m, 2H, CH_2), 1.87 (m, 2H, CH_2), 4.20 (t, 2H, J=7.2 Hz, CH_2), 5.71 (bs, 2H, NH_2), 7.79 (s, 1H, Ar-H), 8.37 (s, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm) δ 13.8, 20.1, 32.3, 43.9, 119.9, 140.7, 150.4, 153.2, 155.6. Mass (ESI) cal. for $C_9H_{14}N_5^+$ (M+H), 192.2; found, 192.4, and $C_9H_{13}N_5Na^+$ (M+Na), 214.2; found 212.4.

4.7. Determination of the crystal structures of receptors FR-1, FR-2, and 9-BuA by X-ray diffraction

Crystallographic data (excluding structure factors) for structures FR-1, FR-2, and 9-BuA in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 696757, 696758, and 697622, respectively. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk). Abbreviated crystallographic data are given below.

FR-1. $C_{23}H_{14}O_2$, MW=322.345, monoclinic, $P2_1/c$, a=9.2500 Å, b=7.0290 Å, c=25.8570 Å, α=90.00°, β=101.11°, γ=90.00°, V=1649.7 (13) Å^3, Z=4, T=223 K, μ=0.082 mm^{-1}, ρ_{calcd}=1.394 Mg m^{-3}, GOF on F^2=1.100, R=0.0602, R_w=0.1465 [$I>2\sigma(I)$].

FR-2. $C_{27}H_{16}O_2$, MW=372.405, monoclinic, $P2_1/n$, a=12.742(3) Å, b=11.796(3) Å, c=13.476(3) Å, α=90°, β=114.070(5)°, γ=90°, V=1849.3(8), Z=4, T=293 K, μ=0.083 mm^{-1}, ρ_{calcd}=1.338 Mg m^{-3}, GOF on F^2=1.091, R=0.0609, R_w=0.1309 [$I>2\sigma(I)$].

9-BuA. $C_{19}H_{15}N_5O$ (9-BuA hydrate), MW=209.26, monoclinic, $P2_1/c$, a=10.981(4) Å, b=8.240(3) Å, c=12.733(5) Å, α=90°, β=109.131(9)°, γ=90°, V=1088.5(7), Z=4, T=223 K, μ=0.089 mm^{-1}, ρ_{calcd}=1.277 Mg m^{-3}, GOF on F^2=1.097, R=0.0619, R_w=0.1789 [$I>2\sigma(I)$].

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Supplementary data

Dimerization constant determination, Job's plots, binding constant determination, structural information (hydrogen bonding and π–π stacking interactions) from calculations using Spartan'06, calculated complex structures using the Spartan'06, spectral data (^{1}H NMR and ^{13}C NMR) of the two receptors, their synthesis intermediates and 9-butyladenine are provided. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.10.041.

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