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Marie-Paule Teulade-fichou[a]; Jean-Pierre Vigneron[a]; Jean-Marie Lehn[a]
[a] Laboratoire de Chimie des Interactions Moléculaires, Collège de France, Paris, France

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Molecular recognition of nucleosides and nucleotides by a water-soluble cyclo-bis-intercaland receptor based on acridine subunits

MARIE-PAULE TEULADE-FICHOU, JEAN-PIERRE VIGNERON and JEAN-MARIE LEHN*

Laboratoire de Chimie des Interactions Moléculaires, Collège de France, 11 Place Marcelin Berthelot UPR 285 CNRS, 75005 Paris, France

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The macrocyclic bisacridine receptor 1 prepared by diamine-dialdehyde condensation has been found to complex nucleotides strongly in water. The important changes of the fluorescence properties of 1 upon complexation revealed the remarkable ability of the receptor to discriminate between the two types of nucleobase: addition of purine nucleotides produced a strong quenching of the emission whereas a considerable enhancement of the fluorescence intensity was induced by pyrimidine nucleotides. By comparison with the binding properties of the acyclic monochromophoric reference compound 7 these opposite variations of the fluorescence have been interpreted in terms of electronic interaction (π/π stacking) and conformational changes of the receptor 1 (increase of the interchromophoric distance). The stability constants were determined by fluorescence measurements and high values ranging from 10^4 to 10^8 M^{-1} were obtained for the 1/1 complexes. The selectivities observed were shown to be dependent on both electrostatic and structural factors.

INTRODUCTION

The study of the selective recognition of nucleobases and their derivatives by synthetic hosts sheds light on biological processes and contributes to the design of artificial receptor molecules useful in analytical and medicinal fields. Since many natural positively charged compounds strongly bind the negatively charged phosphate groups of polynucleotides (biological polyamines, protamines, histones ...),[1] molecular recognition of nucleobases by linear or cyclic artificial polyammonium cationic receptors has received much attention.[2] In such cases strong electrostatic interactions lead to very high binding constants, up to 10^{11} M^{-1}.[2f] In order to achieve a base-selective recognition it is necessary to endow the receptor molecules with other binding sites capable of interacting with the nucleic bases or with the sugar moieties. Interactions with the former may be achieved by hydrogen bonding with sites capable of forming complementary hydrogen bonding patterns and/or by stacking with π-systems of the host compounds. So far a number of model complexation studies of nucleobases have been performed in organic solvents where the complementary hydrogen bonding is most efficient.[3] Although intracellular environment is not a mere aqueous solution, we have chosen to study molecular recognition of nucleotides in water[4] where they are most soluble and where electrostatic interactions and hydrophobic forces play a significant role.[5]

By analogy to the fixation of intercalators between the plateaux of base pairs in double-stranded polynucleotides and nucleic acids, our approach rests on the design of molecules containing two flat subunits of large area situated at a distance suitable for the intercalation of flat organic substrates. Of special interest as structural subunits are the planar heterocyclic dye molecules which possess photochemical and electrochemical properties and may interact with double stranded nucleic acids by intercalation between base pairs.[1,6] Incorporation of such groups into macrocyclic structures yields receptor molecules of cyclointercaland type that may bind flat substrate molecules and perform photo or electroinduced reactions on the bound substrate species.[7]

We have already reported the synthesis and some properties of macrocycles containing one or two intercaland groups derived from porphyrin,[7a], phenazine,[4a,b] acridine,[4,c,d,e] phenanthridine[4f] and naphthalene[4g]. The binding of planar species by other types of intercaland

*To whom correspondence should be addressed.

receptor molecules has also been studied recently [see for instance reference[4d]] and stacking effects have been shown to reinforce base-pairing[3,8] or electrostatic interactions.[2f,9,10]

We now report the synthesis of the molecular receptor **1** based on acridine subunits and its ability to bind, around biological pH, nucleosides and nucleotides in water.[11]

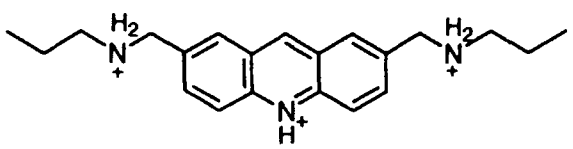
RESULTS AND DISCUSSION

Synthesis and properties of receptor 1

The macrocyclic receptor **1** was obtained according to the procedure previously described for the synthesis of a parent compound (Scheme 1).[4g] The 2 + 2 condensation between diethylenetriamine and 2,7-acridinedicarboxaldehyde **5** gave the tetraimine **6** which was reduced by $NaBH_4$; **1** was isolated as its octachlorhydrate **1**–$8H^+$ (69% yield for the two consecutive reactions). It is also possible to isolate the tetraimine **6** (94% yield, crude product; decomposition ~ 230°C).

The 2,7-acridinedicarboxaldehyde **5** was prepared in the following way (Scheme 1). Ullmann condensation between 5-methyl 2-amino benzoic acid and *p*-bromotoluene yielded the intermediate **2** (62% yield) which was subjected to $POCl_3$ cyclization to give 9-chloro 2,7-dimethylacridine **3** (81% yield). Reductive dehalogenation of this compound (H_2, Ni Raney)[12] followed by $FeCl_3$ oxidation[13] of the acridane intermediate gave 2,7 dimethylacridine **4** (88% yield).[14]

The preparation of the dicarboxaldehyde **5**, via the 2,7-bisbromomethylacridine obtained by N-bromosuccinimide treatment being unsatisfactory, another approach was sought. The classical oxidation by selenium dioxide in refluxing dioxane[15] failed, but heating around 180°–200°C an equimolecular mixture of 2,7-dimethylacridine and selenium dioxide, in the presence of a large excess of naphthalene as solvent, gave 2,7-acridinedicarboxyaldehyde **5** (76% yield after recrystallisation in acetonitrile). These drastic conditions were successful in several cases where other methods failed.[16] An acyclic monochromophoric reference compound was required to understand and properly interpret the spectroscopic properties of a bischromophoric structure such as **1**. To this end we prepared the bisaminoacridine **7** from the condensation of the dicarboxaldehyde **5** with *n*-propylamine followed by reduction and protonation of the resulting diimine as described above for the macrocycle **1**.

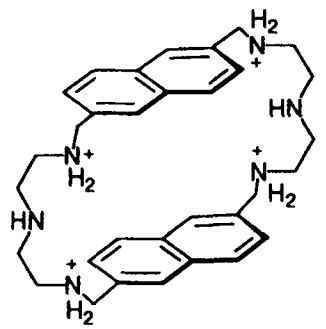


7 (anions : 3 Cl^-)

Properties of the macrocycle 1 in solution

The macrocycle **1** is a highly basic compound possessing eight protonation sites. Its pK_a values were determined in order to be able to select an appropriate pH for the measurement of the binding constants of a given protonated form with various substrates.

According to our previous work on the related bisnaphthalene macrocycle **8**[4g] the pK_a's of the triamine



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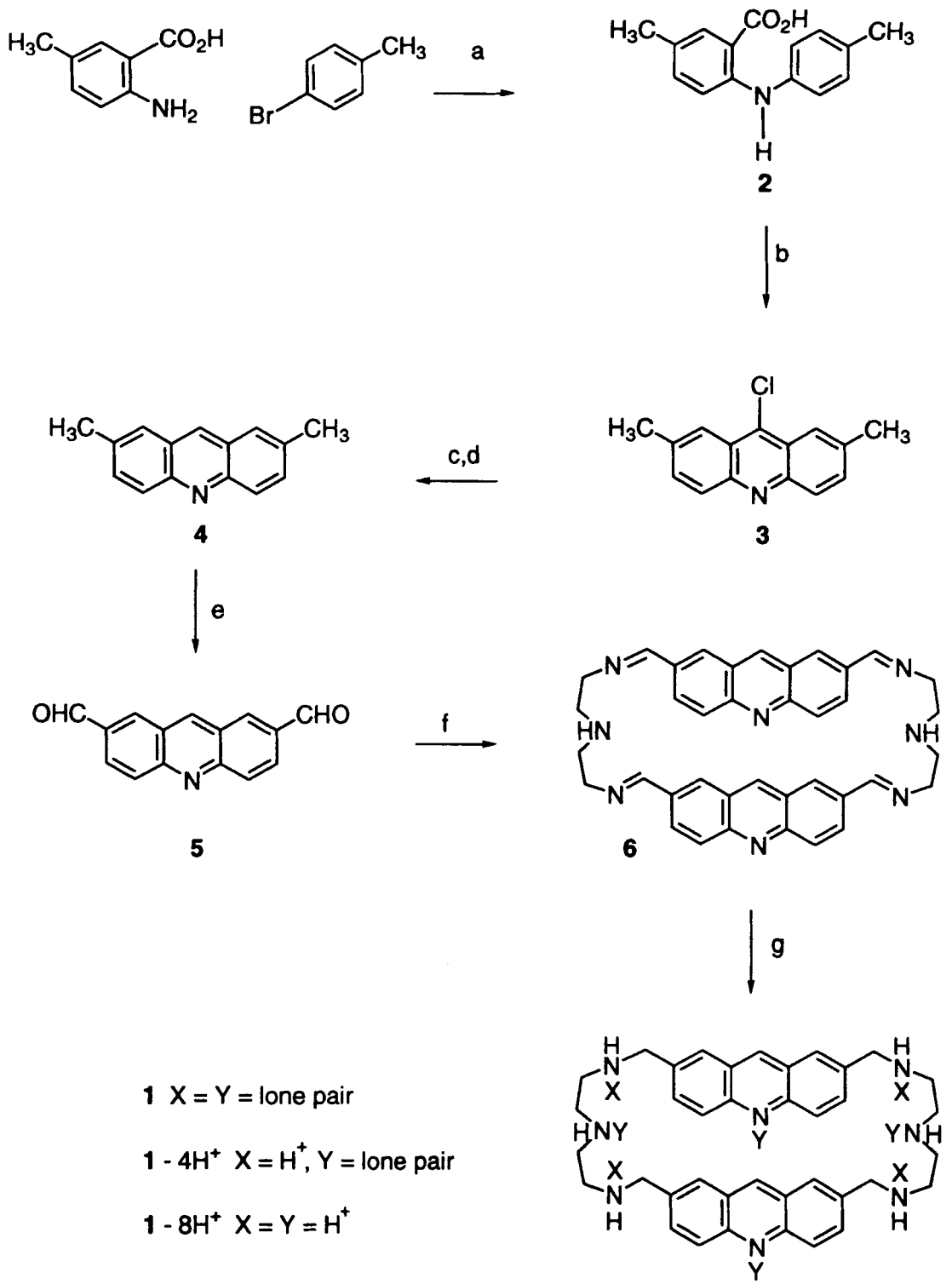
linkers may be estimated as follows: central NH: 2.5–3; lateral NHs: 7.4–10. Furthermore the pK_a's of the reference monomer compound **7** determined by pHmetric titration were found to be 3.65 (acridine), 8.80 and 9.80 (side NHs). The acridine pK_a value is low compared to the unsubstituted acridine (pK_a 5.6) due probably at least in part to the presence of protonated sites in the lateral groups. The incorporation of the acridine in a polycationic cyclic structure should lower the pK_a of the ring nitrogen and thus the pK_a values of the two acridines of **1** may be estimated to lie below 3.65. This is confirmed by the similarity in the pH dependence of the UV-VIS absorption spectra of **1** compared to that of **7**. The spectra are similar in shape and for both compounds the variation of the pH in the range 1.8 to 5 results in a continuous and important drop of the molar absorption coefficients of the π-π^* transition whereas no significant changes were observed for higher pH values [ε_1(360nm) = 34000 (pH 1.8), 14200(pH > 5) ; ε_7 (358nm) = 26200 (pH 1.8), 14000 (pH > 5)]. This decrease is representative of the acid-base equilibrium between the cationic and neutral forms of the acridine nuclei in both **1** and **7**, the molar absorption coefficient of the acridinium salts being higher than that of the deprotonated acridine. We can assume that at a fixed pH of 6 the acridines units will be in their neutral form and each linker in a diprotonated state, thus the overall charge on the macrocycle **1** is expected to be 4^+.

The comparison of the absorption spectra of **1** and **7** also provides information on the relative position of the two acridines in the receptor **1**. An hypochromism of the π-π^* transition is observed in **1** with respect to the monomer **7** ($\varepsilon_1 < 2\varepsilon_7$) and is more pronounced as the pH increases. This effect is characteristic of bischromophoric systems[17] and is an indication of some weak interaction between the two rings. Its increase with the



a) Cu / CuI , K_2CO_3 n-pentanol , 150 °C, 5h ; b) $POCl_3$,reflux, 3h ; c) H_2/ Raney Ni,Ethanol
d) $FeCl_3$; e) SeO_2,naphtalene 200 °C, 30 min ; f) diethylenetriamine, CH_2Cl_2/MeOH ,15h
g) $NaBH_4$, MeOH 0° C, 3h; HCl/ethanol

Scheme 1

pH is an agreement with a decrease of the interchromophoric distance: at acidic pH the receptor **1** experiences maximum electrostatic repulsion due to complete protonation and the two acridines should be parted from each other; at pH > 5 the deprotonation of the central NHs on the linkers and of the heterocyclic nitrogens brings the two aromatic rings closer.

The low fluorescence quantum yield of the macrocycle **1** relative to that of the monochromophoric compound **7** ($\phi_1/\phi_7 = 0.16$) is in accordance with a certain degree of interaction between the two acridines. However the emission spectrum of **1** (λ_{em} = 430nm) is similar in shape to that of **7** (λ_{em} = 427nm) and exhibits no intramolecular excimer fluorescence (Figure 1). The absence of excimer fluorescence indicates that the two acridines are isolated and have low probability of reaching close proximity within the excited monomer lifetime. In addition the ^{1}H NMR spectra show no significant upfield shift of the aromatic signals between **1** and **7**, and argue against an intramolecular stacking of the two chromophores.

All these observations are consistent with the existence at pH 6 of the tetraprotonated form **1**-4H$^+$ of the macrocycle; the two neutral acridine units may be held in a more or less parallel orientation by the bis cationic polyammonium linkers as schematically shown in Figure 2 but there is no definite proof that this is the case. Such a semi-closed conformation should be appropriate to accommodate anionic planar aromatic guests as revealed by examination of CPK molecular models.

Interaction of receptor 1 with nucleosides and nucleotides

Due to the remarkable fluorescent properties of the acridine ring a convenient method to monitor the complexation between **1** and the nucleotide substrates is provided by fluorescence spectroscopy.

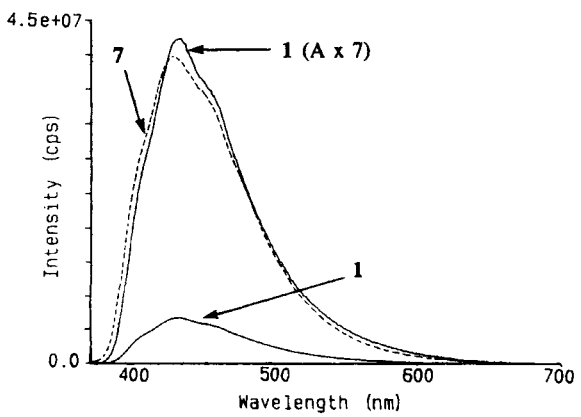
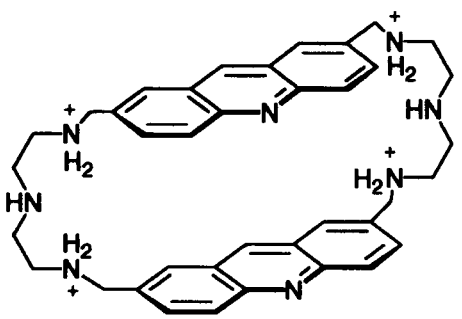


Figure 1 Corrected fluorescence emission spectra of **1**(——) (5 × 10^{-6}mol dm^{-3}) (λ_{em}: 430nm; λ_{exc}: 360nm) and **7** (- - -) (5.3 × 10^{-6}mol dm^{-3}) (λ_{em}: 427nm, λ_{exc}: 358nm), in cacodylate buffer 10mM, pH 6, 25°C, (A = peak area).



1

Figure 2 Representation of the tetraprotonated form **1**-4H$^+$ of the receptor **1** at pH 6.

The measurements were performed at pH 6 in cacodylate buffer 10mM without additional salt, the concentration of NaCl in the buffer being sufficiently high to maintain a constant ionic strength. Complexation is expected to induce higher effective acidity of the anionic substrate and thus mono, di and trinucleotides are expected to be mainly in their fully ionized form (second pK_a of the phosphate group: 6.3). For all the series tested there was no clear isobestic point in absorption so the excitation wavelength was fixed at 365nm where no variation of the absorption occurred. The titrations were carried out by gradual addition of the different substrates to a solution of the receptor **1**.

The complexation of nucleotides displays a remarkable behaviour with two types of response which reach a limiting value at high (> 1eq.) proportion of substrate: strong quenching of the fluorescence intensity (70%–80%) with the purine derivatives, considerable enhancement of the emission (70%–130%) with pyrimidine compounds (typical experiments are shown in Figures 3a & 4a). These strong variations of the intensity are accompanied by only small shifts of the maximum wavelength [λ_{em} (**1**) = 430 nm] (Table 1).

It is well known that the fluorescent properties of the acridines are modified upon interaction with nucleotides, generally a strong quenching is observed with the purine derivatives while the pyrimidines induce moderate variations[18]. These effects are commonly attributed to an overlap between the acridine ring and the nucleobase indicating the formation of a molecular complex. A similar behaviour is observed for the reference monomer compound **7**: a strong quenching is induced by AMP ($\Phi/\Phi 0$ = 0.16) (Figure 5a) whereas no significant variation of the emission is produced by UMP (Figure 5b). Similarly the spectral changes of receptor **1** produced by the binding of the purine nucleotides reflect the occurrence of a ring/ring interaction between the nucleobase and the aromatic subunits of the macrocycle. However, besides the

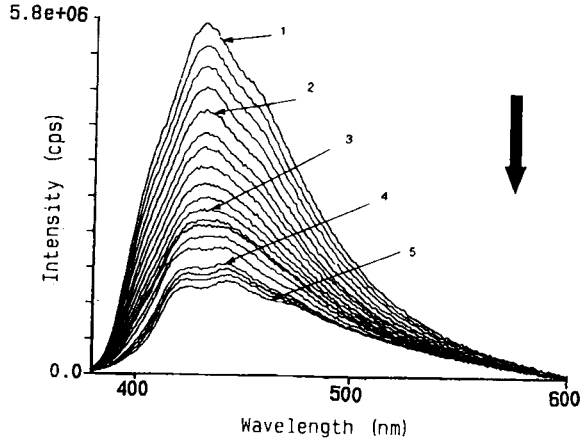


Figure 3a Fluorimetric titration of **1** (5×10^{-6}mol dm^{-3}) with AMP in cacodylate buffer 10mM, (λ_{exc}: 365nm); (AMP)/(**1**): 1,0; 2,0.5; 3,1; 4,2.5; 5,5.8.

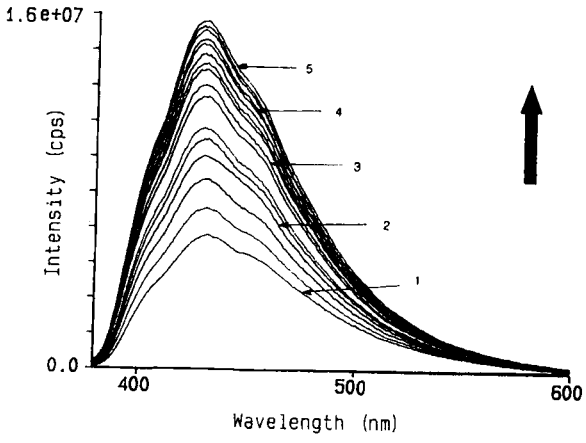


Figure 4a Fluorimetric titration of **1** (5×10^{-6}mol dm^{-3}) with CMP in cacodylate buffer 10mM, (λ_{exc}: 365nm); (CMP)/(**1**): 1,0; 2,2.9; 3,9.8; 4,21.8; 5,49.8.

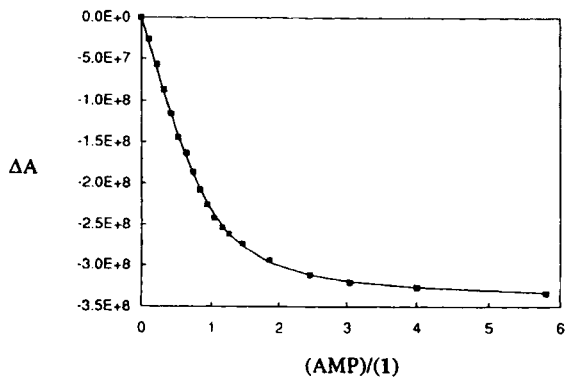


Figure 3b Titration curve of **1** with AMP / (A = peak area).

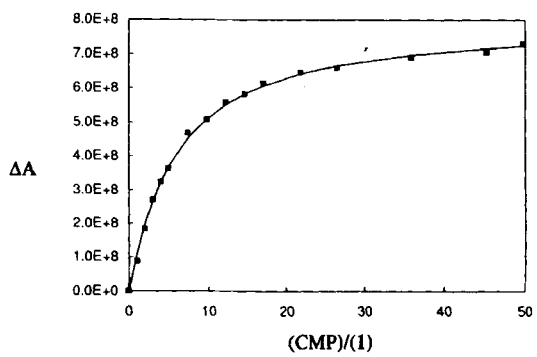


Figure 4b Titration curve of **1** with CMP/(A = peak area).

electronic π/π interaction a structural effect resulting from the increase of the interchromophoric distance between the two acridines can be expected. Indeed this is clearly seen in the case of pyrimidines: an increase of the fluorescence is obtained and the spectrum of the complex (λ_{em} = 428nm) tends to resemble that of the monochromophoric compound **7** (λ_{em} = 427 nm); the substrate keeping apart the two acridines moieties decreases their mutual interaction and to the extent that the probable π/π stacking doesn't induce any spectral changes, this enhancement should be indicative of the opening of the cavity of the macrocycle. In the case of purines the extinction of the fluorescence probably hinders the detection of the conformational modification and is only representative of the electronic interaction. Thus the opposite variations of the emissions observed in both series suggest a partial or complete insertion of the nucleobase between the two acridines.

The titration experiments have been analyzed by a non-linear least-square curve fitting procedure. In every case the best statistical fit has been obtained for the formation of complexes of 1/1 stoichiometry as illustrated

Table 1 Maximum emission wavelength, relative fluorescence quantum yields (Φ/Φ_o) and stability constants (log K_s) calculated for the complexes of receptor **1** with nucleosides and nucleotides.

Substrates[a]	$\lambda em^{(nm)}$	Φ/Φ_o[b]	log K_s[c]
A	423;444	0.19	3.5
AMP^{2-}	424;443	0.19	6.1
$cAMP^{1-}$	421;445	0.28	4.0
ADP^{3-}	424;442	0.21	7.2
ATP^{4-}	423;444	0.32	8.4
G	—	—	—[d]
GMP^{2-}	445;450	0.21	4.9
GTP^{4-}	440;447	0.22	7.2
C	429	1.13	< 2
CMP^{2-}	429	2.42	4.6
U	430	1.17	< 2
UMP^{2-}	427	1.76	3.8
UDP^{3-}	428	1.9	4.2
UTP^{4-}	427	3.0	5.5

[a] A : adenosine; G : guanosine; C : cytidine; U : uridine; AMP^{2-}, GMP^{2-}, CMP^{2-}, UMP^{2-} : monophosphates of A, G, C, U respectively; ADP^{3-}, UDP^{3-} : diphosphates of A and U; ATP^{4-}, GTP^{4-}, UMP^{4-}: triphosphates of A, G, U respectively. All the anionic substrates were used as sodium salts;
[b] the Φ/Φ_0 values were obtained from the ratio of the emission peaks areas of receptor **1** without substrate and with substrate at saturation;
[c] Standard deviation ± 0.2;
[d] not measurable due to precipitation.

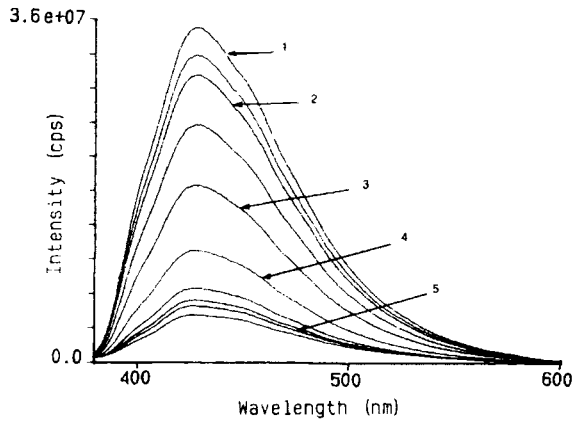


Figure 5a Fluorimetric titration of **7** (5.3×10^{-6}mol dm^{-3}) with AMP in cacodylate buffer 10mM, (λ_{exc}: 365nm); (AMP)/(**7**): 1,0; 2, 73,9; 3,498; 4,1137; 5, 2792.

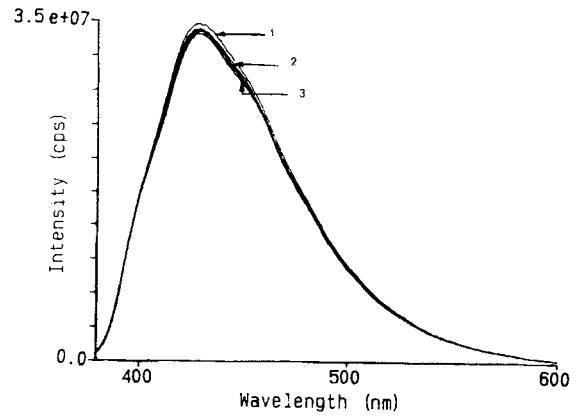


Figure 5b Fluorimetric titration of **7** (5.3×10^{-6}mol dm^{-3}) with UMP in cacodylate buffer 10mM, (λ_{exc}: 365nm); (AMP)/(**7**): 1,0; 2, 252; 3, 3849.

in Figures 3b & 4b. The values of the binding constants are summarized in Table 1.

The comparison between electroneutral nucleosides and negatively charged nucleosides phosphates shows clearly the influence of the number of charges carried by the substrates on the association constant. The affinity is enhanced by a factor 10^2 to 10^3 from a nucleoside to a nucleoside monophosphate and then by one order of magnitude for every additional phosphate group (nucleoside di- and triphosphates). These results point to the predominant stabilizing role played by the electrostatic attraction on the receptor/substrate association. This effect is non-specific and thus was observed to be the same independently of the nucleobase attached to the ribose.

The hydrophobic effects are illustrated by the preferential complexation of the purines; the equilibrium constant for AMP is 190 times higher than that for UMP. This selectivity, frequently observed when stacking interactions are involved, is commonly attributed to the larger size of the purines that allows a greater contact area with aromatic complexing units. This observation confirms the probable inclusion of the nucleobases between the two acridines moieties.

The ability of receptor **1** to discriminate between the two types of nucleobase is remarkable both quantitatively and qualitatively (opposite variations of the fluorescence). Furthermore receptor **1** exhibits a higher affinity for adenine than for guanine derivatives, the association constant for AMP being 14 times that for GMP. A similar result has been recently reported in the literature[2i] and has been ascribed to the higher polarizability of the adenine which stabilizes the complex in terms of van der Waals forces (induced dipole). Both features may be of interest for the use of **1** as a site selective marker of nucleic acids.

The increase of the electrostatic interaction doesn't induce a decrease of the selectivity between purines and pyrimidines. Indeed the ratio of the constants for the di- and trinucleotides is much larger (K_{ATP}/K_{UTP} = 780; K_{ADP}/K_{UDP} = 1100) than for the corresponding mononucleotides (K_{AMP}/K_{UMP} = 190). Similarly the selectivity adenine > guanine is conserved (K_{ATP}/K_{GTP} = 16). Thus despite the predominance of the electrostatic interactions, the structural factors (van der Waals, hydrophobic) still play a significant role in the binding of the di and trinucleotides by the receptor **1**.

The comparison of the present work with the binding properties of the related bisnaphtalene macrocycle **8**[4g] confirms the considerable influence of the van der Waals and solvophobic effects on the complexation. The replacement of the bicyclic naphtalene by the larger tricyclic acridine unit produces a large increase of the binding constants specially for the purine derivatives as shown in Table 2 (an increase of 2 to 3 orders of magnitude is observed for AMP, GMP and ATP).

Finally the drop of affinity measured for the cyclic mononucleotide cAMP may be attributed to the decrease of the total charge in the substrate ($cAMP^{1-}/AMP^{2-}$) and possibly to a lesser structural adjustment due to the rigidity of the cyclic phosphate residue.

CONCLUSION

The present results demonstrate the respective contributions of electrostatic and hydrophobic interactions in the complexation of nucleotides with a macrocyclic polyca-

Table 2 Comparison of the stability constants (log K_S) calculated for the complexes of the bisacridine **1** and bisnaphthalene **8** receptors with nucleotides

Substrate	*Bisacridine 1*	*Bisnaphthalene 8*
AMP^{2-}	6.1	4.1
ATP^{4-}	8.4	5.2
GMP^{2-}	5	4.6
CMP^{2-}	4.6	3.6
UMP^{2-}	3.8	3.8

tionic structure such as the bisacridine receptor **1**. The increase of the binding constants produced by the replacement of naphthalene by acridine subunits is in favor of the stacking of the substrate between the two aromatics dyes of the host molecule and the probable formation of inclusion complexes.

The large fluorescence enhancements induced by the binding of pyrimidines could be of importance for the development of fluorescent sensors for detecting this type of substrate. Furthermore the efficient complexation of purines nucleotides and specially adenine derivatives, if transposable to biological systems, might find useful application to the specific recognition of purines sequences in polynucleotides.

Finally the photochemical properties of the compound **1** and particularly its ability to perform light-induced DNA cleavage are under investigation.

EXPERIMENTAL

All commercially available chemicals employed were reagent grade and used without further purification. Melting points were determined on an Electrothermal digital melting-point apparatus. Proton NMR spectra were recorded on a Brucker AC 200 spectrometer. The microanalyses and the mass spectra were performed at the Service Central de Microanalyse du CNRS, Lyon and Service regional de Microanalyse de l'Université Pierre et Marie Curie (Paris).

4,4'-Dimethyldiphenylamine-2-carboxylic acid (2)

A mixture of 2-amino-5-methyl benzoic acid (33.2 g, 0.22 mol), *p*-bromotoluene (34.2 g, 0.2 mole), potassium carbonate (27.6 g, 0.2 mole), copper iodide (0.6 g) and copper powder (0.6 g) in dry *n*-pentanol (100 ml) was refluxed (150°C) under vigorous stirring for 5 h. The hot mixture was filtered through a pad of celite and the celite was thoroughly washed with methanol. The biphasic filtrate was acidified to pH 4.5–5 and the deposited solid was filtered off after cooling for 1 h in the fridge. This crude product was recrystallized from ethanol to give the title compound **2** (26.5 g): yellow crystals, mp 192°C. A second crop was obtained by evaporating the dark filtrate to dryness and dissolving the residue in CH_2Cl_2 (11). This solution was filtered through a short column of silica (5 cm height) and evaporated to dryness. The crude residue was recrystallised from ethanol to yield **2** (3.7 g, mp 192°C, total yield: 62%).^{1}H-NMR(DMSO d^6) 2.10 (s,3H), 2.39 (s,3H), 7.12 (m,6H), 7.70 (s,1H); Anal. Calcd for $C_{15}H_{15}NO_2$: C, 74.66; H,6.27; N,5.81. Found: C,74.76; H, 6.27; N,5.65.

9-Chloro-2,7-dimethylacridine (3)

A solution of **2** (26.4 g, 0.109 mole) in $POCl_3$ (80 ml) was refluxed for 3h. After evaporation to dryness the residue was cooled in an ice-bath and a 15% NH_4OH solution (120 ml) was added. The suspension was extracted with CH_2Cl_2 and the organic phase was washed with water, dried over Na_2SO_4 and evaporated to dryness to give crude **3** which was recrystallized from ethanol yielding pure **3** (21.1 g, 80%, in three crops) : yellow crystals, mp 156–158°C; ^{1}H-NMR (DMSO d^6) 2.58 (s,6H), 7.71 (d,2H, J = 8.75Hz), 8.07 (d,2H, J = 8.80Hz), 8.09 (s,2H); Anal. Calcd for $C_{15}H_{42}ClN$: C,74.53; H,5.00; N,5.79. found : C,74.34; H,5.00; N,5.66.

2,7-Dimethylacridine (4)

A solution of **3** (4.83 g, 0.02 mole) and pellets of 85% KOH (1.32 g, 0.02 mole) in C_2H_5OH (300 ml) was hydrogenated at ordinary pressure and temperature, in the presence of Raney nickel (about one teaspoon). When the theoretical quantity of hydrogen was absorbed, the catalyst was filtered off; a solution of $FeCl_3$, $6H_2O$ (10.8 g, 0.04 mole) in C_2H_5OH (50 ml) was then added to the hot filtrate (around 60°C). After cooling, a 15% NH_4OH solution (50 ml) was added; the precipitate was removed by filtration over celite and the clear filtrate was evaporated. Water was added and the desired product was extracted with CH_2Cl_2; the organic phase was dried over Na_2SO_4 evaporated and the crude product recrystallised from EtOH to give **4** (3.6 g, 88%) as yellow crystals, mp 180°C Litt. [14a] 171°). ^{1}H-NMR ($CDCl_3$) 2.58 (s,6H), 7.61 (d,2H, J = 9.0Hz), 7.73 (s,2H), 8.12 (d, 2H, J = 9.0 Hz), 8.56 (s, 1H).

2,7-Acridinedicarboxaldehyde (5)

A mixture of 2,7-dimethylacridine **4** (1.55 g, 7.5 mmol), SeO_2 (1.875 g, 15 mmol + 10% excess) and naphthalene (7.5 g) was slowly heated to 200°C. Around 180°C–190°C a sudden seething took place and stopped after few min. After 30 min at 200°C the mixture was cooled to room temperature and the residue was dissolved in CH_2Cl_2/MeOH 1:1 (300 ml). The insoluble selenium residues were filtered off and the filtrate was evaporated to dryness. The residue was thoroughly washed with hexane in order to remove naphthalene and the crude product was recrystallized from CH_3CN to give **5** (1.34 g, 76%) as yellow crystals: m.p. 260°C. ^{1}H-NMR ($CDCl_3$) 8.34 (s,4H), 8.60 (s,2H), 9.17 (s,1H), 10.26 (s,2H). Anal. Calcd for $C_{15}H_9NO_2$: C,76.58; H,3.86; N,5.96. found: C,76.42; H,3.80; N,5.84.

2,5,8,21,24,27-Hexaaza[9,9](2,7)acridinophane (1)

A solution of diethylenetriamine (260 mg, 2.5 mmol) in CH_2Cl_2/MeOH 1:1 (20 ml) was added dropwise, at room temp and under N_2, to a well-stirred solution of 2,7-acridinedicarboxaldehyde **5** (5,85 mg, 2.5 mmol) in CH_2Cl_2/MeOH 1:1 (200 ml). The mixture was stirred at room temperature for 15h and then slowly evaporated to dryness without heating. The residue was washed with

diethylether and redissolved in CH_2Cl_2/MeOH 1:2 (150 ml). $NaBH_4$ (285 mg, 7.5 mmol) was added to this solution cooled at 0°C. After stirring for 3h the solvents were evaporated, the residue dissolved in water (40 ml) and extracted by CH_2Cl_2/MeOH 9:1. The organic phase was dried and evaporated. The green residue was recrystallized twice in 1N HCl/EtOH mixture to yield **1-8H⁺** (660 mg, 58%) as yellow crystals. ^1H NMR (D_2O, pH 2.5) 3.48 (s,16H), 4.57 (s,8H), 8.24 (q, 8H), 8.54 (s,4H), 9.84 (s,2H). Anal. calcd For $C_{38}H_{52}N_8Cl_8$, $4H_20$ (**1**-8HCl, $4H_2O$) C,46.72; H,6.19; N,11.47. Found: C,46.76; H,6.91; N,11.47.

2,7(Di-*n*propylaminomethyl) acridine (7)

2,7-acridinedicarboxaldehyde **5** (80mg, 0.34 mmol) was dissolved in a 1/1 mixture of CH_2Cl_2/CH_3OH (100ml) and added dropwise to *n*-propylamine (1g, 16.9ml) in CH_3OH (10ml). The mixture was stirred overnight then the solvent was evaporated and a pale yellow powder was collected (150mg) and dried under vacuum. It was dissolved in CH_3OH and cooled with an ice bath. $NaBH_4$ (30mg, 0.79mmol.) was added and stirring continued for 30 min. at 0°C and 1h at r.t.. Evaporation of the solvent left an oily residue which was poured into water (5ml) and then extracted with CH_2Cl_2/CH_3OH 40:1 (40ml). The organic phases were washed with water (3 × 5ml) and dried over $MgSO_4$. The evaporation gave a brown oily residue (95mg) which was dissolved in CH_3OH (10ml) and treated with a methanolic solution (2ml) saturated with gaseous HCl. The stirring was continued for 30 min. and then the solvent evaporated. A yellow green powder was collected and reprecipitated in CH_3OH to give compound **7-3H⁺** (82mg, 56%) ^1H NMR (D_2O, pH = 2.4) 0.87 (t,6H), 1.65 (m,4H), 3.03 (t,4H), 4.47(s,4H), 8.24 (q,4H), 8.46 (s,2H), 9.78 (s,2H). Anal. Calcd.for $C_{21}H_{30}N_3Cl_3$, $1CH_3OH$, $0.5H_2O$ (**7**-3HCl, $1CH_3OH$, $0.5H_2O$): C,55.99; H,7.47; N,8.90. Found: C,55.89; H,7.22; N,8.93.

Fluorescence titrations and analysis of the data:

The fluorescence spectra (λ_{exc} = 365nm) were recorded on a Fluoromax (Spex) spectrophotometer at ca 25°C under moderate irradiation (bandpass = 2nm) to avoid photochemical degradation of **1**. The titrations experiments were performed as follows: to an aqueous solution of **1** (5×10^{-6}mol dm^{-3}) in cacodylate buffer (10mM) were added aliquots of a mixture of the substrate diluted in an aqueous solution of **1** (5×10^{-6}mol dm^{-3}) in cacodylate buffer (10mM). In this manner the concentration of the receptor **1** was kept constant while the concentration of the substrate varied. The data were analysed by using a non-linear least-squares curve fitting procedure.

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