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Complexation Studies of Nucleotides by Tetrandrine Derivatives Bearing Anthraquinone and Acridine Groups

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Water soluble dicationic cyclophane-type macrocycles bearing two anthraquinone (1) or two acridine groups (2) have been prepared by chemical modification of S,S-(+)-tetrandrine. Complexation of nucleotide anions (mono-di- and triphosphates) and D/L phenylalanine by tetrandrine derivatives has been studied by UV-Visible and fluorescence titrations in water.

Association of nucleotides to semi-synthetic hosts 1 and 2 increases with increasing guest charge in the series $AMP^{2-} < ADP^{3-} < ATP^{4-}$. It was demonstrated for the 1- ATP and ADP complexes that association strength is increased in the absence of salt (NaCl). Besides, receptor 1 shows a slight preference for the complexation of UMP over nucleotides containing A (adenine), G (guanine), C (cytosine) or T (thymine) as bases. The major forces stabilizing the complexes result from ion pairing between the charged groups of host and guest and from stacking or hydrophobic interactions. Receptor 1 displayed chiral recognition towards D/L phenylalanine.

Keywords: Complexation; Nucleotides; Semisynthetic macrocycles; Tetrandrine

INTRODUCTION

Anions are important components of chemical and biological systems and for that reason different research groups have focused their investigations on anion detection and quantification [1–7]. Nucleotides are one of the most important class of anionic molecules and because of its many biological implications, nucleotide recognition in water is an important challenge in supramolecular chemistry. Within this context several classes of synthetic and natural cationic host molecules have been studied: for example synthetic systems such as open chain polyammonium receptors [8]; macrocyclic receptors such as azonia-crown and azoniacyclophanes [9–13]; sapphyrins and porphyrin derivatives [14–16]; polymers [17]; and natural systems such as peptides [18]. Among the synthetic host compounds of the previous list, a considerable number of them show large binding constants towards nucleotides in water. The main strategies considered in host design are the presence of multiple cationic sites for phosphate moieties and aromatic units to simultaneously stack with the nucleotide base. However, these systems have great disadvantages: the synthetic routes for obtaining them are generally sophisticated, sometimes they show poor water solubility and frequently the ionic state of these hosts is pH-dependent.

We demonstrated previously that the use of semisynthetic macrocycles may be a viable and easily accessible alternative to synthetic receptors [19–21]. Specifically, we reported a semisynthetic cyclophane type receptor, obtained by quaternization of nitrogen atoms of the bisisoquinoline alkaloid S,S-(+)-tetrandrine with benzyl groups (DBT, Scheme 1), who showed a moderate affinity towards phosphate-containing compounds adenosine mono-, di- and triphosphates, with an irregular dependence on the guest charge [19].

With this in mind, we expected that quaternization of both nitrogen atoms of the alkaloid with apolar and planar units of larger surfaces than those groups employed in DBT, i.e., anthraquinone or polycyclic arenes such as acridine, would transform it into a dicationic receptor with affinity for negative charges of phosphate-containing compounds such as nucleotides and with an extended cavity capable of significant hydrophobic and $\pi-\pi$ stacking interactions with aromatic moieties of nucleotides in aqueous medium.

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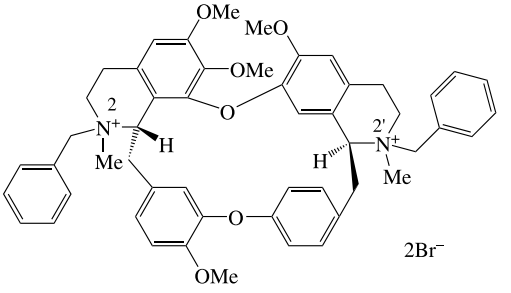
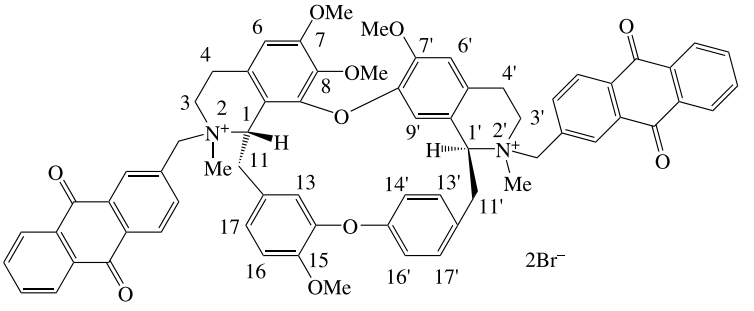


DBT



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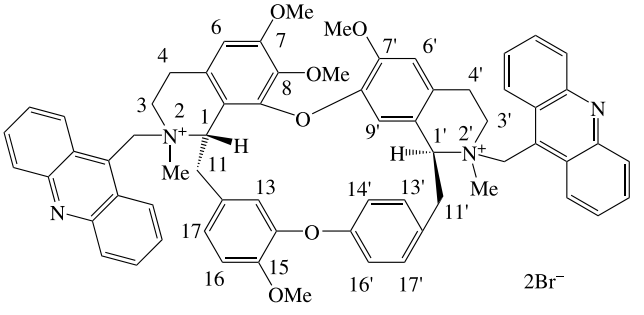


2

SCHEME 1 Chemical structures of N,N′-dibenzylated S,S-(+)-tetrandrine (DBT) and new tetrandrine derivatives with two anthraquinonylmethyl (**1**) or two acridinylmethyl (**2**) groups.

Among the noncovalent bonds, hydrophobic and π-stacking interactions are important ones for aromatic components. For example, intercalation of some kinds of arenes into base pairs in DNA and RNA helices are driven by π-stacking interaction. For that reason anthraquinone and acridine moieties are often incorporated in systems reported in literature [22–26]. Besides, taking into account the unique properties of anthraquinone and acridine units (chromophore, fluorophore or electrochemical) these make them useful as signaling units when incorporated in different structures [27,28].

In the present study we report on the binding properties of two new water soluble receptors derived from S,S-(+)-tetrandrine. These semi-synthetic receptors displayed substantial affinity for tetraanionic nucleotides in water solutions, while maintaining its chiral recognition.

RESULTS AND DISCUSSION

Synthesis of Tetrandrine Derivatives

Quaternization of nitrogen atoms of S,S-(+)-tetrandrine with 2-(bromomethyl)anthraquinone or 9-(bromomethyl)acridine gave respectively compounds **1** and **2** in good yields (85–90%). In both reactions two new chiral centers are produced at S,S-(+)-tetrandrine's nitrogens. In principle compounds **1** and **2** may be expected as a mixture of stereoisomers, however, in similar reactions with two previously reported tetrandrine derivatives [19–21], only one single steroisomeric product was afforded. Accordingly, the ^{1}H NMR spectra of macrocycles **1** and **2** show a single set of signals for all hydrogens (see Figs. 1S and 2S in the Supplementary Material), indicating the formation of a single stereoisomer in each case. In addition, due to the rigidity of the

cyclophane macrocycle one may expect that new introduced groups will appear only at positions occupied by the lone electron pairs of nitrogen atoms of the alkaloid.

Solution Behavior of Macrocycles

Self-association tendencies of **1** and **2** in water were studied by ^{1}H NMR. Qualitatively a significant self-association of both compounds is manifested in broadening of NMR signals in water, but too low solubility in this solvent did not allowed us to study the process by this technique quantitatively.

The high molar extinction coefficients of receptors prompted us to monitor the self-association process by UV–vis technique. Therefore, the relations of the absorbance upon the concentration were investigated in aqueous medium at pH 7.2. The absorbance of receptor **1** obeyed Beers Law and was directly proportional to concentration in the range 3.8×10^{-6}–1×10^{-4} M. The same behavior was observed for receptor **2** for concentrations in the range 3.8×10^{-6}–4.8×10^{-5} M. These experiments allowed us to obtaining the molar extinction coefficients for maximums of absorption spectra of both compounds (see Experimental section for values). Figs. 3S and 4S in the Supplementary Material contain absorption spectra of **1** and **2** at different concentrations.

On the other hand, only cyclophane **2** showed high quantum yields to being studied by fluorescence. The fluorescence spectra of **2** in water at pH 7.2 showed an emission band with a maximum at 440 nm by excitation at 350 nm in agreement with similar systems reported in literature [29]. Besides, it was found that emission obeys a linear relationship with the concentration in the range 1×10^{-6}–9×10^{-6} M. Figure 5S in the Supplementary Material contain emission spectra of **2** at different concentrations.

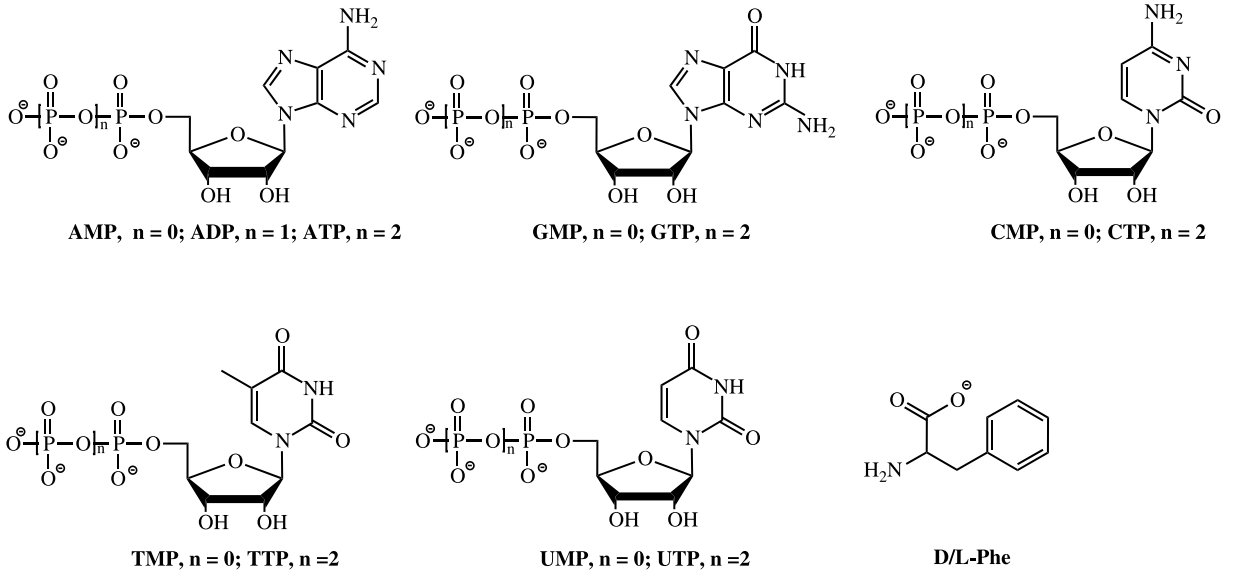
Complexation Studies

Dicationic cyclophanes **1** and **2**, bearing anthraquinone and acridine moieties were expected as multipoint recognition hosts capable of electrostatic and π-stacking interactions. Thus, **1** and **2** may recognize molecules holding both aromatic and anionic moieties such as nucleotides. Chemical structures of all guests used in this study are shown in Scheme 2. Also the anionic forms of D- and L-phenylalanine were included as guests to evaluate the chiral recognition ability of host **1**.

Complexation of Nucleotides

The complexation ability of **1** and **2** toward a set of nucleotides (Scheme 2) in aqueous solution was evaluated by titration experiments that were monitored by UV–vis spectroscopy. All nucleotides were used as sodium salts and studies were conducted at pH 7.2, in a phosphate buffer 0.032 M [23]. At this pH nucleotides are expected to be predominantly in their fully ionized form. Addition of nucleotides to both hosts induced hypochromism in their UV–vis absorption spectra as an indication of host–guest complexation. This hypochromic effect is frequently observed with similar complexes and can be attributed to π-stacking coordination [30,31]. Complex formation was observed and the absorptions recorded at 300 and 330 nm for **1** and at 350, 367 and 397 nm for **2** were plotted versus the guest concentration, at those wavelengths absorption of guest is negligible.

In the case of host **1** all data could be fitted to the 1:1 host–guest binding model since higher associations



SCHEME 2 Chemical structures of guests employed.

were not observed and for that reason were analyzed using the Eq. (1) [32]:

$$A_{obs} = (A_o + A_\infty K[G]_T)/(1 + K[G]_T) \qquad (1)$$

Where A_{obs} is the observed absorbance, A_o is the absorbance of free host, A_∞ is the maximum absorbance induced by the presence of a given guest, $[G]_T$ is the total concentration of the guest and K is the binding constant.

The 1:1 stoichiometry of the species formed between **1** and nucleotides was confirmed by Job's method of continuous variation [33]. As an example Fig. 1 shows the Job plot obtained for **1** in the presence of UMP. In this case a minimum at 0.5 is indicative of a 1:1 stoichiometry. This finding validates the use of the Eq. (1) for UV–vis complexation studies.

Typical titration plots for **1** and selected guests are shown in Fig. 2 and the average binding constants are collected in Table I. The following results were obtained:

Complexation strength increases with increasing guest charge: the association constant K increases in the series $AMP^{2-} < ADP^{3-} < ATP^{4-}$. Similar trends between guest charge and binding affinity had been previously observed in the complexation of these nucleotides with other cyclophanes. The increased affinity for nucleoside di- and triphosphates relative to monophosphates has been attributed in some instances to the electrostatic interactions displayed by the cationic host towards the multiply charged anionic guests [10,11,16,34].

Triphosphate nucleotides GTP, CTP, TTP and UTP associated with **1** at comparable levels to ATP. On the other hand, binding of **1** to monophosphate nucleotides (GMP, CMP, TMP and UMP) appeared much weaker than those to triphosphates. This fact also suggests that the electrostatic interactions (ion pairing) play a major role in host–guest association.

Complexation of receptor **1** to monophosphate nucleotides (AMP, GMP, CMP, TMP and UMP) showed no trend and it was found an unexpected higher association of **1** to UMP over the other

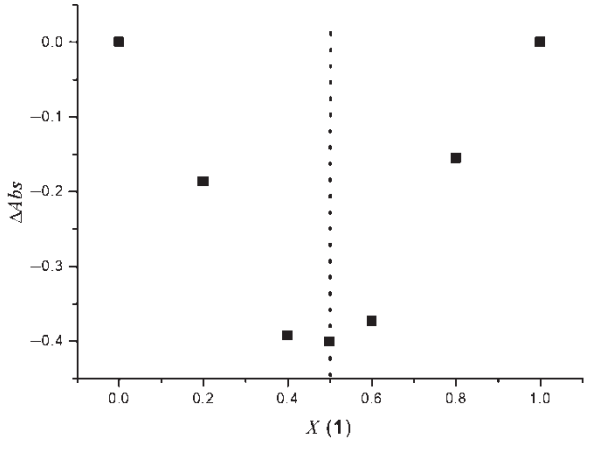


FIGURE 1 Job plot for the complex formed between **1** and UMP: ΔAbs of **1** (the difference in absorbance of complexed and free **1**) at 330 nm against its molar fraction, X (**1**). The experiment was carried out in phosphate buffer (pH 7.2) and 0.05 M NaCl at 25°C. The total concentration of **1** + UMP was kept constant at 5×10^{-5} M.

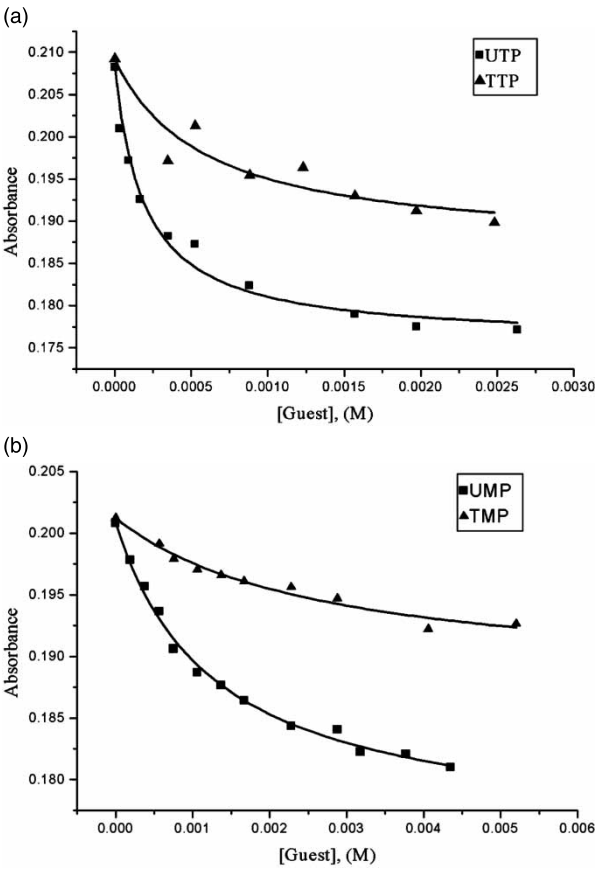


FIGURE 2 Plots of the absorbance at 330 nm of **1** (1.75×10^{-5} M) with respect to the concentration of added guest, in phosphate buffer at pH 7.2 and 0.05 M NaCl. Solid lines are the fitting curves in accordance with Eq. (1).

TABLE I Binding constants (K) and the change for Gibbs energy (ΔG) for complexes of nucleotides and phenylalanine with **1** and **2** at 25°C in buffer pH 7.2 and 0.05 M NaCl

Host	Guest	K, M^{-1}	Log K	ΔG^0 (kJ/mol)
1	AMP^{2-}	477 ± 128	2.68	−15.3
	ADP^{3-}	850 ± 241	2.93	−16.7
	ATP^{4-}	5405 ± 758	3.73	−21.3
	GMP^{2-}	–†		
	GTP^{4-}	–‡		
	CMP^{2-}	411 ± 160	2.61	−14.9
	CTP^{4-}	–‡		
	TMP^{2-}	373 ± 68	2.57	−14.7
	TTP^{4-}	3225 ± 756	3.51	−20.0
	UMP^{2-}	783 ± 61	2.89	−16.5
	UTP^{4-}	4721 ± 568	3.67	−21.0
	L-Phe^{1-}	167 ± 27	2.22	−13.0
	D-Phe^{1-}	23 ± 9	1.36	−8.0
2	AMP^{2-}	77 ± 5	1.88	−10.8
	ADP^{3-}	110 ± 15	2.00	−11.7
	ATP^{4-}	$K_{11} = 7191$	3.85	−22
		$K_{12} = 165$	2.21	−12.7

† Only estimated an order of $10^2 M^{-1}$. ‡ Only estimated an order of $10^3 M^{-1}$.

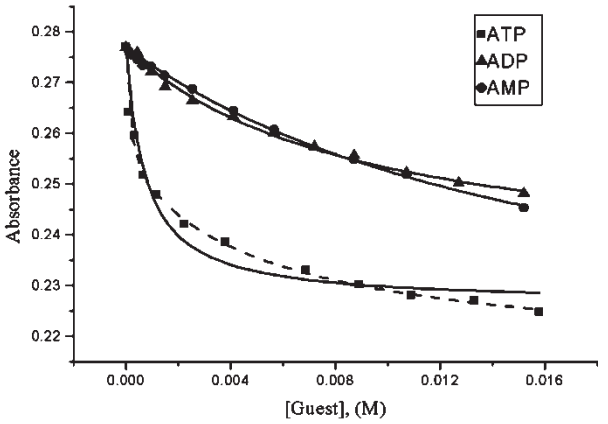


FIGURE 3 Plots of the absorbance at 367 nm of **2** (1.75×10^{-5} M) with respect to the concentration of added guest in phosphate buffer at pH 7.2 and 0.05 M NaCl. Solid lines are the fitting curves in accordance with Eq. (1) and dashed lines in accordance with Eq. (2).

monophosphate nucleotides, although differences in binding free energy are not very large (Table I). This result is very interesting considering that adenine has demonstrated the strongest stacking ability in model systems [10,11,34,35].

On the other hand, in the case of compound **2** were evaluated AMP, ADP and ATP as guests. Typical titration plots for all guests are shown in Fig. 3, and the binding constants K are collected in Table I. It is worth noting that in the case of ATP the experimental titration points do show clear deviations from the fitting curve corresponding to a 1:1 binding isotherm. For that reason, the experimental data were analyzed by using Eq. (2) [32]:

$$A_{\text{obs}} = \{(A_{\text{o}} + A_{11}K_{11}[\text{G}]_T + A_{12}K_{11}K_{12}[\text{G}]_T^2)/ (1 + K_{11}[\text{G}]_T + K_{11}K_{12}[\text{G}]_T^2)\} \quad (2)$$

Where A_{obs} is the observed absorbance, A_{o}, A_{11}, A_{12} are the absorbances of the free host and its 1:1 and 1:2 respective complexes, K_{11} and K_{12} are the corresponding stepwise formation constants, and $[\text{G}]_T$ is the total concentration of the guest. Thus, both complexes were considered: the 1:1 and 1:2 host–guest ratios. In addition, the 1:2 stoichiometry was confirmed by Job's method.

A simple inspection of data collected in Table I show that association of nucleotides to semi-synthetic host **2** increases with increasing guest charge in agreement with results found with its analogue **1**.

The complexation of ATP by **2** was also investigated by fluorimetric titrations. Studies were performed at pH 7.2, in a phosphate buffer (1×10^{-4} M) with 1 mM NaCl. For the fluorescence measurements the excitation wavelength was fixed at 350 nm and the emission was monitored at 437 nm. The addition of the nucleotide induced a progressive increase of the emission fluorescence spectra of **2** (a typical

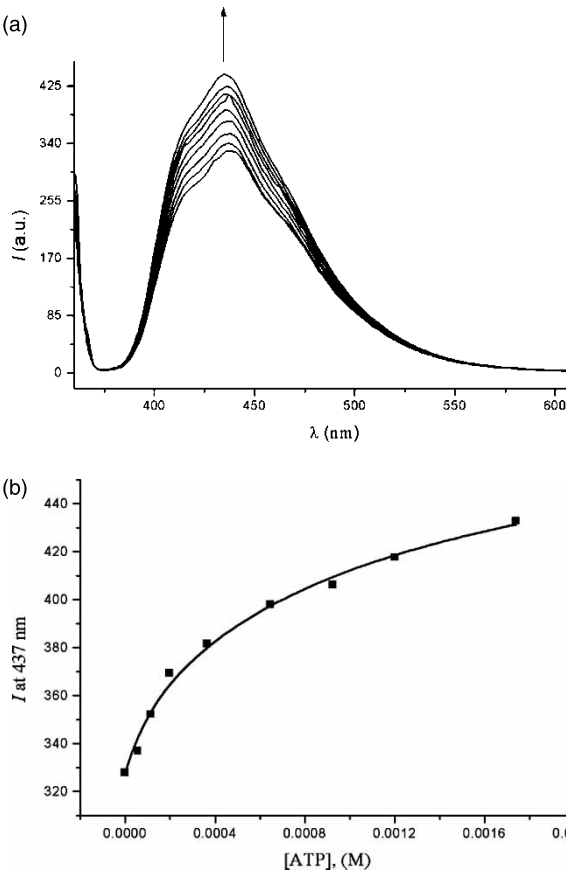


FIGURE 4 (a) Fluorimetric titration of **2** (1.3×10^{-6} M) with ATP (5.7×10^{-5}–1.7×10^{-3} M) in phosphate buffer (1×10^{-4} M) at pH 7.2 and 1 mM NaCl. (b) Plot of the intensity of emission at 437 nm of **2** ($\lambda_{\text{exc}} = 350$ nm) with respect to the concentration of added ATP. The solid line shows the fitted curve.

experiment is shown in Fig. 4). It is well known that upon binding to acridines nucleobases induce a modification of the fluorescence of these dyes, with a quenching or an enhancement that depends on the acridine derivative. This effect is attributed to an overlap between the acridine and the nucleobase rings [34]. The intensity emissions of **2** versus the ATP concentration were plotted and analyzed using a modified Eq. (2) suitable for fluorescence studies. The order of association constants K_{11} and K_{12} found by this study were in agreement with those for UV vis ($K_{11} = 7015.6$ and $K_{12} = 616.6$).

The effects of varying buffer type and salt (NaCl) concentrations on AMP, ADP and ATP binding by **1** were the subject of further studies. A UV–vis titration of **1** with ATP was performed in a non-phosphate buffer solution Trizma base (0.032 M at pH 7.2 with 0.05 M NaCl). The results were in good agreement with those from the study in the phosphate buffer obtaining $K = 5306\,\text{M}^{-1}$ for the **1**-ATP complex. This further rules out any inhibition or competitive binding due to the phosphate buffer.

Furthermore, it was found that the absence of inert salt increases the binding constant. Experiments

of the ATP, ADP and AMP binding by host **1** were carried out in phosphate buffer at pH 7.2 without NaCl. The absence of salt significantly altered the association constants of the **1**-ATP and **1**-ADP complexes which was reflected in the increased values by ca. one order, $K_{\mathbf{1}-ATP} = 63249\,M^{-1}$ and $K_{\mathbf{1}-ADP} = 2617\,M^{-1}$. On the other hand, the **1**-AMP complex showed an unexpected insensitivity to the absence of NaCl, resulting in an association constant of same order than obtained in the presence of 0.05 NaCl (see Table I). The fact that association constants decrease significantly when the ionic strength grows, have been demonstrated previously with many systems [8,13,17,19]. Besides, another aspect that deserves some comment is the association strength observed for **1**-ATP complex in buffered solution with no salt added: taking into account purely electrostatic binding between dication **1** and the ATP tetraanion its K value is comparable with those found for complexes of ATP and diammonium dications of different structures [36]. In contrast to these results, we can cite for example the work of Kejík *et al.* [16]. In this work they reported a tetracationic porphyrin alkaloid conjugated in an attempt to give the host sufficient positive charge to neutralize the ATP and at the same time establish aromatic π−π stacking interactions with the guest. However, the calculated value of association constant for complex between mentioned tetracationic host and ATP was 64,000 M^{-1} in a solvent mixture of 50:50 (v/v) methanol-HEPES buffer.

Complexation of 1 with Free Amino Acids

The chiral recognition ability of compound **1** was evaluated also by UV-titrations. These studies were conducted in a bicarbonate buffer at pH 10. At this pH, amino acids are expected to be predominantly in their anionic form. Figure 5 illustrates the observed difference in titration curves for both enantiomers. Analysis of these plots with Eq. (1) allowed us to calculate binding association constants (Table I). The results showed that binding of phenylalanine is enantioselective and the binding enantioselectivity factor can be estimated as $K(L)/K(D) = 7$. This preference of **1** by the L enantiomer is in qualitative agreement with the enantioselectivity found for its analog DBT [20].

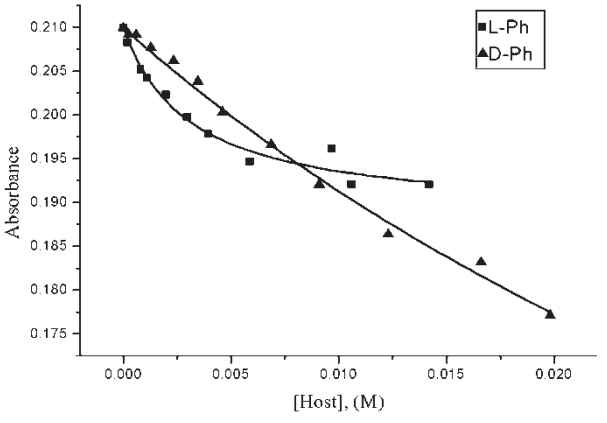


FIGURE 5 Plots of the absorbance at 330 nm of **1** (1.75×10^{-5} M) with respect to the concentration of added guest, in phosphate buffer at pH 7.2 and 0.05 M NaCl. Solid lines are the fitting curves in accordance with Eq. (1).

Molecular Modeling

In order to rationalize the binding mode of **1** and **2**, molecular modeling of the host structures and of the **1**-ATP and **2**-ATP complex was performed. The likely structures of **1** and **2** were determined by molecular mechanics simulations starting from the previously reported structure of their bisbenzylated analog [20]. Figure 6S shows the simulated structural conformation of hosts according to which one group attached to the N(2′) atom is directed out of the macrocycle cavity and the other group attached to the N(2) atom closes the cavity from the right side. This makes more probable entrance of guests from the left side; which is in agreement with previously reported DBT [20].

The complexation of ATP by **1** and **2** was investigated by computer modeling at semiempirical level. Results of energy minimizations were in agreement with the experimentally observed formation of stable complexes.

The estimated structure of the **1**-ATP (1:1) complex calculated by a semiempirical method is presented in Fig. 6. At least three oxygen atoms of phosphate moieties interact via ion pairing with N(2) ammonium centre by distances between 5.3 Å and 5.6 Å, sufficiently short for significant interaction. In addition, simulated complex shows interaction of the adenine base of ATP and the anthraquinone moiety attached via methyl group to the N(2′) atom of tetrandrine, which probably contributes to the complex stability by π-stacking or hydrophobic interactions.

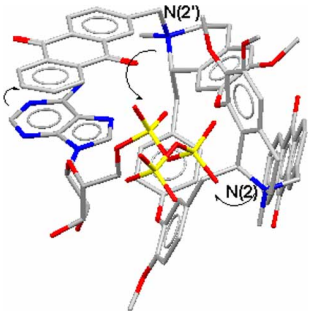


FIGURE 6 Simulated structure of **1**-ATP complex (1:1, H-G). Hydrogens omitted.

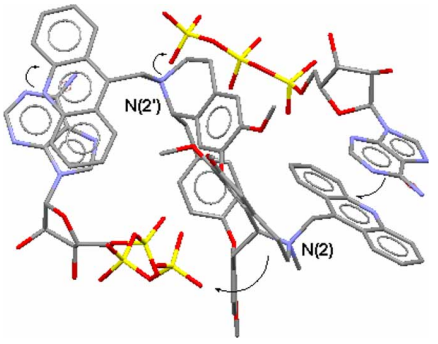


FIGURE 7 Simulated structure of **2**-ATP complex (1:2, H–G). Hydrogens omitted.

On the other hand, the estimated structure of the **2**-ATP (1:2) complex calculated by a semiempirical method is presented in Fig. 7. In this case, taking into account experimental results the simulated complex showed more than expected ion pairs between oxygen atoms of phosphate moieties of both ATP molecules and N(2) or N(2′) ammonium centers: at least four ionic pairs for each nitrogen with distances between 4.4 Å and 6.5 Å. This fact is reasonable considering that molecular modeling was made *in vacuo*. In addition, both anthraquinone moieties apparently contribute to the complex stability by interacting with each one adenine nucleobase.

CONCLUSIONS

The herein reported studies on the complexation of nucleotides and D/L phenylalanine by **1** and **2** semi-synthetic dicationic water soluble compounds, allow us to derive some general conclusions. First, results obtained with hosts on nucleotide complexation corroborate our hypothesis about introduction of anthraquinone and acridine moieties on tetrandrine improve interaction ability of these macrocycles with respect to previously reported DBT.

On the other hand, the fact that compounds **1** and **2** preferred triphosphate nucleotides rather than di- or monophosphate ones showed the role of electrostatic interactions in complexation. In addition a negative salt effect on these complexes also suggested that the electrostatic interactions (ion pairing) play a major role in host–guest association. However, we also observed significant contributions of stacking or hydrophobic interactions in complexation.

Among the results obtained in this paper, it is remarkable that observed association strength of cyclophanes **1** and **2** towards nucleotides demonstrates that these hosts are comparable to the artificial receptors reported so far. Besides, host **1** displayed chiral recognition properties in the presence of phenylalanine.

EXPERIMENTAL

General Procedures and Materials

All reagents for syntheses and molecular recognition studies were purchased from commercial suppliers and used without further purification. All solutions were prepared in purified water (Barnsted Nanopure Diamond Lab Water). UV/Vis and fluorescence spectra were recorded on a Perkin Elmer Lambda 2 and Perkin Elmer LS50B, respectively. A one-centimetre quartz cuvette cell holder was used. In all studies the cell holder was thermostatted at 25°C, through circulating water. NMR spectra were recorded on 400 MHz spectrometers Bruker AVANCE 400 and Varian UNITY INOVA. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane and J values are given in hertz. Mass spectra were recorded on a high resolution Jeol MStation 700 spectrometer in FAB technique. Spectrophotometric titrations were performed on 10^{-6}–10^{-5} M solutions of **1** or **2** on phosphate or bicarbonate buffer, depending on the guest used, and 0.05 M NaCl. When titration experiments were monitored by UV–vis spectroscopy it was employed a concentration of the receptor of 1.75×10^{-5} M and for the fluorescence experiments, only performed with **2**, a receptor concentration of 1.3×10^{-6} M. Typically aliquots of a fresh phosphate or carboxylate standard solutions of the anion were added to **1** or **2** solutions. The pH was checked at the beginning and at the end of the titration and was found constant in all cases. Guests were converted into the respective anions by adjusting the pH of their solutions in water by addition of Na_2CO_3. All spectrometric titration curves were fitted with the Microcal Origin 5.0 program. For the determination of the pK_a values of the compound **2** spectrophotometric pH-titration was performed with **2** (3×10^{-5} M) in equimolar (0.032 M) mixture of acetate, phosphate and borate buffers and 0.05 M NaCl.

Molecular Modeling

Molecular mechanics simulations were performed with Hypercube's hyperchem package, using the mm + force field as implemented in the 6.03 version of the program. The likely structures of **1** and **2** were determined by MM simulation starting from the known tetrandrine crystal structure followed by minimization *in vacuo* of the potential energy using a combination of conjugated gradient and Newton–Raphson algorithms using a 0.4184 kJ mol^{-1} convergence criterion in the minimization procedure. In all cases the macrocycle structures of **1** and **2** resembled the reported X-ray structure [37] very accurately.

Gaussian program package and the PM3 semiempirical method [38] were used for the minimization

of the binding energy and complex geometry of **1**-ATP and **2**-ATP. Optimized structure of the guest was placed at appropriate position maximally close to receptor, which undergo a favorable geometric arrangement with interactional complementarity for the development of electrostatic and π-stacking or hydrophobic interactions.

Synthesis of 1 ($C_{68}H_{60}N_2O_{10}Br_2$)

A solution of 2-(bromomethyl)anthraquinone (0.366 g, 1.18 mmol) in $CHCl_3$ (30 ml) was added to a solution of tetrandrine (0.300 g, 0.47 mmol) in $CHCl_3$ (80 mL), the mixture was refluxed and stirred for 24 h then the mixture was stirred for 48 h at room temperature. The solvent was evaporated and the solid product was dissolved in hot water. The small amount of solid precipitate was filtered off and the filtrated fraction was removed on a rotary evaporator with vacuum (0.535 g; yield: 90%). The compound was isolated as a yellow solid. Mp: 193–198°C. Anal. Calc. for $C_{68}H_{60}N_2O_{10}Br_2{\cdot}6H_2O$ (1333.1) C, 61.24; H, 5.44; N, 2.1; found C, 61.26; H, 5.44; N, 2.1%. UV absorptions $\lambda_{max}(H_2O/nm$ 256 ($\varepsilon/dm^3\,mol^{-1}\,cm^{-1}$ 96184), 330 (9883) and λ_{max}(MeOH)/nm 254 ($\varepsilon/dm^3\,mol^{-1}\,cm^{-1}$ 99540), 324 (9920). ^{1}H NMR (400 MHz; DMSO-d_6, Me_4Si): Partial assignment of signals was done by using COSY two-dimensional technique and by comparison with the spectra of tetrandrine and previously obtained *N,N′*-dibenzylated *S,S*-(+)-tetrandrine [19] in the same solvent: signals for N—CH_3 and O—CH_3 were found at $\delta_H = 2.67$ (3H, s), 3.27 (3H, s), 3.51 (3H, s), 3.74 (3H, s) and 3.81 (3H, s) while signals for N—CH_2 at $\delta_H = 4.47$ (1H, m), 4.77 (1H, d), 4.94 (1H, d) and 5.02 (1H, d) ppm. Signals for the 10 aromatic protons of cyclophane structure are at $\delta_H = 6.20$ (1H, s, H-9′), 6.51 (1H, s, H-13), 6.65 (2H, m, H13′ and H-14), 6.8 (1H, s, H-6), 6.69 (2H, m, H-16′ and H-16), 7.12 (1H, d, $J = 7.6$ Hz, H-17), 7.19 (1H, s, H-6′) and 7.55 (1H, d, $J = 7.5$ Hz, H-17′) ppm. Finally in the range 7.80–8.50 ppm the spectrum showed signals for hydrogens of the two anthraquinone units. IR ν_{max}/cm^{-1}: 3401.44b (C—H, conj.), 2938.86b (CH, aliphatic), 1676.46s (CO) y 1591.59s (C=C). MS *m/z* (FAB +) 532.61 ([**1**-2Br^-]–2 Br^-, $\mathbf{1}^{2+}$); 1145.12 ([**1**-2Br^-]–Br^-, $\mathbf{1}^+$).

Synthesis of 2 ($C_{66}H_{62}N_4O_6Br_2$)

9-(bromomethyl)acridine (0.162 g, 0.579 mmol) and tetrandrine (0.147 g, 0.232 mmol) were reacted by following same procedure as that for the synthesis of compound **1**. The solid precipitate isolated was washed with acetone and dried with vacuum to give 0.2355 g (85% yield) of an orange solid. Mp: 140–145°C. Anal. Calc. for $C_{66}H_{62}N_4O_6Br_2{\cdot}5H_2O$ (1257.15) C, 63.05; H, 5.77; N, 4.45; found C, 62.65; H, 5.88; N, 4.69%. UV absorptions. $\lambda_{max}(H_2O$, pH = 7.2)/nm 350sh ($\varepsilon/dm^3\,mol^{-1}\,cm^{-1}$ 12032), 367 (17458), 397 (7749) and λ_{max}(DMSO)/nm 350sh ($\varepsilon/dm^3\,mol^{-1}$-$cm^{-1}$ 10797), 367 (15993), 397 (8139). ^{1}H NMR (DMSO-d_6, Me_4Si, δ_H/ppm): Partial assignment of signals was done in a similar way as its analog **1**: signals for N—CH_3 and O—CH_3 were found at $\delta_H = 2.23$ (3H, s), 3.03 (3H, s), 3.12 (3H, s), 3.5 (3H, s), 3.75 (3H, s) and 3.86 (3H, s) ppm while signals for N—CH_2 at $\delta_H = 4.34$ (1H, s), 4.92 (1H, m), 5.62 (1H, d, $J = 14.3$ Hz) and 5.76 (1H, d, $J = 13.7$ Hz) ppm. Signals for the 10 aromatic protons of cyclophane structure are at $\delta_H = 6.19$ (1H, s, H-9′), 6.35 (1H, s, H-13), 6.40 (1H, d, $J = 6.2$, H-13′), 6.51 (1H, s, H-6), 6.79 (2H, m, H-14′ and H-16), 6.95 (1H, d, $J = 6.9$, H-17), 7.09 (1H, d, $J = 7.2$, H-16′), 7.18 (1H, s, H-6′) and 7.29 (1H, d, $J = 7.2$, H-17′) ppm. Finally signals for hydrogens of both acridine units are in the range 7.60–9.0 ppm.

Determination of PKa values of 2

The pKa values of both acridine groups were determined by spectrophotometric titration of **2** in aqueous medium in the pH range 2–10 (see Fig. 7S). The absorption spectrum of **2** shows three maximums at 367 ($\varepsilon = 17458\,dm^3\,mol^{-1}\,cm^{-1}$ at pH 7.2), 397 ($\varepsilon = 7749\,dm^3\,mol^{-1}\,cm^{-1}$ at pH 7.2) and 415 nm and one shoulder at 350 ($\varepsilon = 12032\,dm^3\,mol^{-1}\,cm^{-1}$ at pH 7.2). For the maximum at 415 nm the absorbance increases in more acidic media and decreases significantly on increasing pH; lost of the orange colour of solution is observed as a consequence of the decrement of the acridinium ion species. A typical titration curve is illustrated in Fig. 7S. Titration results at four mentioned wavelengths were fitted to the Eq. (3) where A_{obs} is the absorbance at a given wavelength and ε_{HH}, ε_H and ε are the molar absorptivities of diprotonated, monoprotonated and unprotonated forms of **2**, respectively. The calculated pKa values were averaged affording a pK_a of 3.16 ± 0.01 for both acridine groups in agreement with similar systems reported in literature [39,40].

$$A_{obs} = (\varepsilon_{HH}[H^+]^2/K_{a1}K_{a2} + \varepsilon_H[H^+]/K_{a2} + \varepsilon) \times [\mathbf{2}]/(1 + [H^+]/K_{a2} + [H^+]^2/K_{a1}K_{a2}) \quad (3)$$

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