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Complexation of dicarboxylates and phosphates by a semisynthetic alkaloid-based cyclophane in water

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ABSTRACT: Dicationic *N,N′*-dibenzylated cyclophane-type derivative **1** of a bisisoquinoline macrocyclic alkaloid, *S,S*-(+)-tetrandrine, was prepared and characterized. In aqueous solution **1** undergoes dimerization, which was studied by the concentration dependence of its ^{1}H NMR signals. The salt effect on the dimerization constant was analyzed by using different versions of Debye–Hückel-type equations. ^{1}H NMR titration of **1** by 10 dicarboxylate anions of different structures revealed strong peak binding selectivity for succinate in the series of α,ω-dicarboxylates. Aromatic carboxylates have larger binding constants and for *o*-phthalate the formation of both 1:1 and 1:2 host–guest complexes is observed. Binding of nucleotides (AMP, ADP and ATP) to **1** is surprisingly insensitive to the guest charge, indicating a major contribution of stacking interactions of the nucleobase with **1**. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: complexation; dicarboxylates; phosphates; alkalord-based cyclophane

INTRODUCTION

Anions are important components of chemical and biological systems, and their recognition by synthetic receptors attracts considerable interest (for reviews, see Ref. 1). Often fairly selective anion complexation via hydrogen bonding to neutral receptors is efficient only in aprotic solvents.[2] In protic solvents, in particular in water, the main driving forces for anion complexation are ion pairing and ionic hydrogen bonding.[3–7] With anions possessing sufficiently large apolar moieties, additional hydrophobic[8] and/or stacking[9] interactions with the receptor apolar surface can also contribute significantly. Large binding constants and significant selectivity were reported for recognition of highly charged tricarboxylates[6,10,11] and phosphates[12,13] by polyprotonated azamacrocycles and cleft-type receptors containing multiple ammonium or guanidinium groups. Complexation of dicarboxylates in water is generally weak and of low selectivity.[3a,4] Ion pairing of dicarboxylates with open-chain diammonium cations was found to be essentially independent of the structure of both components with log $K = 2.1 \pm 0.2$ at zero ionic strength.[14] A recently reported cleft-type diamidinium receptor specially designed for dicarboxylate binding shows high binding constants of the order of 10^4 l mol^{-1}, but in non-aqueous media (methanol), and demonstrates only marginal selectivity.[15]

We demonstrated previously that the use of natural macrocyles may be a viable and easily accessible alternative to synthetic receptors.[16] Specifically, the cationic form of a bisisoquinoline alkaloid, *d*-(+)-tubocurarine, was found to be an efficient receptor for organic anions.[16a] The purpose of this work was to test the dicarboxylate recognition properties of a semisynthetic cyclophane-type receptor obtained by quaternization of nitrogen atoms of another bisisoquinoline alkaloid, *S,S*-(+)-tetrandrine.

The crystal structure of tetrandrine shows that it has the conformation of a triangle with a small cavity.[17] We expected that quaternization of both nitrogen atoms of the alkaloid with large apolar groups would transform it into a dicationic receptor with an extended cavity capable of significant hydrophobic interaction with apolar moieties of dicarboxylates. In addition, a rigid aromatic skeleton of the macrocycle may create increased binding se-

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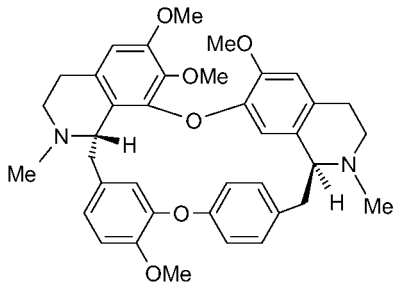
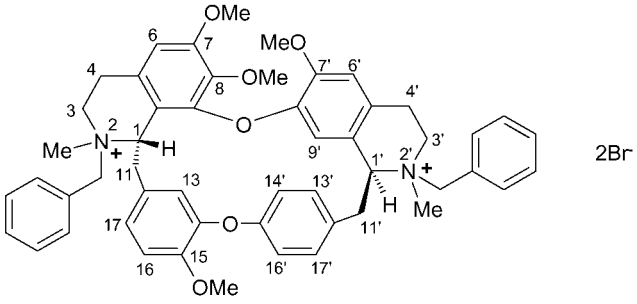
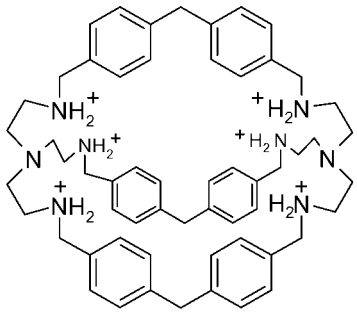
Contract/grant sponsor: National Institute of General Medical Sciences; *Contract/grant number:* GM6035201.
Contract/grant sponsor: Petroleum Research Fund; *Contract/grant number:* 33098-AC4.



S,S-(+)-tetrandrine



1



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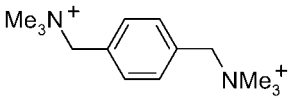

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Chart 1.

lectivity due to steric effects. With this in mind, we prepared a bisbenzylated derivative (**1**) of tetrandrine which indeed demonstrated significant complexation selectivity towards dicarboxylates. For comparison, the complexation of adenosine mono-, di- and triphosphates with **1** was studied.

EXPERIMENTAL

S,S-(+)-Tetrandrine, carboxylic acids, adenosine mono-, di- and triphosphates, inorganic salts and components of buffer solutions were purchased from commercial suppliers and used without further purification. All solutions were prepared in purified water (Milli-Q Reagent Water System). NMR spectra were recorded on Varian UNITY INOVA 400 and 500 MHz spectrometers.

*N,N′-Dibenzyltetrandrine dibromide (**1**).* To a solution of *S,S*-(+)-tetrandrine (0.467 g, 0.735 mmol) in acetone (130 ml) a solution of benzyl bromide (0.38 g, 2.2 mmol) in acetone (7 ml) was added and the mixture was refluxed for 15 h. The solid precipitate was washed with acetone and recrystallized from acetone–water affording **1** (0.596 g, 84%). FAB-MS: *m/z* 402.3 (**1**−2Br$^-$, M^{2+}), 885.7 (**1**−Br$^-$, MBr$^+$). ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 2.57 (s, 3H); 2.76 (d, J = 16.5 Hz, 1H); 3.14 (dd, 1H); 3.19 (s, 3H); 3.241 (d, J = 6 Hz, 1H); 3.278 (d, J = 6 Hz, 1H); 3.34 (s, 3H); 3.42 (m, 1H); 3.43 (m, 1H); 3.467 (s, 3H); 3.52 (m, 1H); 3.56 (m, 1H); 3.72 (d, J = 7.5 Hz, 1H); 3.76 (s, 3H); 3.78 (s, 3H); 3.88 (m, 1H); 4.34 (m, 1H); 4.40 (m, 1H); 4.515 (m, 1H); 4.54 (m, 1H); 4.59 (dd, J = 5.75, 11.25 Hz, 1H); 4.644 (m, 1H); 4.939 (d, J = 9.5 Hz, 1H); 6.05 (s, 1H); 6.4 (d, J = 1.5, 1H); 6.56 (dd, J = 2, 8.5 Hz, 1H); 6.69 (dd, J = 3, 8.3 Hz, 1H); 6.8 (s, 1H); 6.91 (dd, J = 2, 8.5 Hz, 1H); 6.95 (d, J = 8.5 Hz, 1H); 7.1 (dd, J = 2, 8 Hz, 1H); 7.113 (s, 1H); 7.52 (m, 1H). Anal. calc. for $C_{52}H_{56}N_2O_6Br_2{\cdot}4H_2O$ (1036.92), C 60.23, H 6.22, N 2.70; found, C 60.18, H 6.1, N 2.69%. Assignment of ^{1}H NMR signals of **1** in DMSO-d_6 was done by using standard two-dimensional techniques (COSY, ROESY, TOCSY, HMBC and HMQC) (Table 1). Chemical shifts of **1** in water were poorly resolved and concentration dependent (see Results and discussion). Assignment of signals in water was made by comparison of spectra recorded at the lowest possible concentrations with that in DMSO-d_6. Benzylation of nitrogen atoms of tetrandrine leads to the formation of two new chiral centers and therefore a mixture to four stereoisomers can be produced. There are, however, several pieces of evidence in favor of the formation of a single isomer.

Table 1. Assignment of ^{1}H NMR signals of **1**[a]

Proton	δ in DMSO- d_6(ppm)	δ in D_2O (ppm)
2-N-CH_3	2.57	2.428
2-N-CH_2	4.515, 4.644	4.317, –
2′-N-CH_3	3.34	
2′-N-CH_2	4.54, 4.644	4.346, –
1	4.939	4.783
1′	4.59	4.467
3	3.88, 4.34	–, 4.16
3′	3.72, 4.40	3.63, –
4	3.278, 3.42	
4′	3.241, 3.43	
6	6.8	6.675
6′	7.113	7.053
9′	6.05	5.892
11	2.76, 3.52	2.636, –
11′	3.14, 3.56	3.017, 3.474
13	6.40	6.112
13′	6.56	6.54
14′	6.69	6.54
16	6.95	6.88
16′	6.91	6.74
17	7.10	6.824
17′	7.52	7.177
7-O-CH_3	3.76	3.674
7′-O-CH_3	3.78	3.7
8-O-CH_3	3.19	
15-O-CH_3	3.467	3.4

[a] Chemical shifts of aromatic protons of benzyl groups appear between 7.33 and 7.55 ppm.

First, the ^{1}H NMR spectrum shows a single set of signals for all hydrogens. Second, the reversed-phase HPLC analysis of **1** shows a single elution peak under different elution conditions (in a typical experiment a 40.3 mg ml^{-1} stock solution of **1** in DMSO was prepared, the injection volume was 50 µl, the chromatographic column (C_{18}) was 250 × 4.6 mm i.d., the mobile phase was 40% methanol –60% water, the flow-rate was 0.5 ml min^{-1}, UV detection was used and the capacity factor was $k' = 4.49$).

^{1}H NMR titrations. The diacids were converted into the respective dianions by adjusting the pH of their solutions in D_2O to be 2–3 units above second pK_a value by adding Na_2CO_3. Titrations were performed on Varian UNITY INOVA 400 and 500 MHz spectrometers by adding aliquots of the dianion stock solutions (typically 0.4 M) to a ca 4 mM solution of **1** in D_2O containing desired amount of background NaCl electrolyte. All titrations were repeated twice. The experimental data were fitted using non-linear least-squares regression with the Microcal Origin 3.5 program. At least 10 signals of different protons of **1** were used for the fitting and the binding constants obtained were averaged.

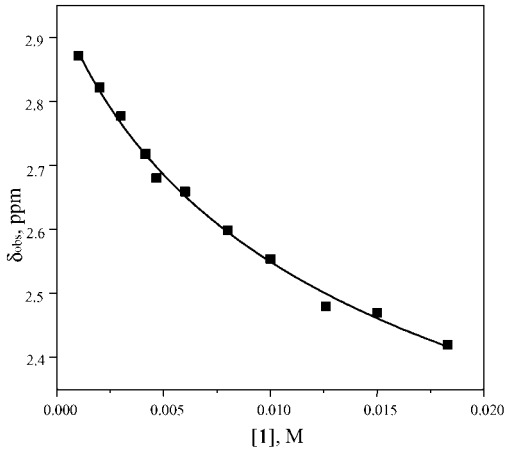


Figure 1. Concentration dependence of the observed chemical shift of H-11 of **1**. The solid line is the fitting curve in accordance with Eqn. (3)

RESULTS AND DISCUSSION

Self-association of 1

In preliminary experiments we observed a significant concentration dependence of the chemical shifts of **1** in water indicative of self-association of the dication. Figure 1 shows as an example the concentration profile of the observed chemical shift for the H-11 proton. Similar upfield shifts of the proton signals were observed on addition of NaCl to a solution of **1** in water (Table 2). Self-association of charged hydrophobic species in water is often observed, e.g. for charged porphyrins[18] and cyclophanes.[19] A positive salt effect on such aggregation

Table 2. Effect of NaCl on chemical shifts of 4.15 mM **1** in water (selected signals which undergo largest salt-induced shifts) and dimerization constants K_D at different NaCl concentrations

[NaCl] (M)	H-11	H-9′	H-13	H-13′	H-16′	K_D (l mol^{-1})
0	2.72	5.97	6.15	6.55	6.76	23 ± 8
0.05	2.64	5.88	6.1	6.53	6.72	34 ± 5
0.1	2.57	5.85	6.09	6.525	6.71	42 ± 6
0.15	2.53	5.82	6.07	6.52	6.69	49 ± 7
0.2	2.51	5.79	6.06	6.51	5.68	53 ± 8
0.3	2.48	5.77	6.05	6.505	6.67	63 ± 8

processes is also well documented and is attributed to decreased repulsion of similarly charged species at higher solution ionic strength.[20]

Quantitative analysis of this and other similar profiles for the chemical shifts of different protons shows that they are perfectly fitted to a simplest model involving dimerization of charged macrocycles according to the equation

$$2M^{2+} \rightleftharpoons M_2{}^{4+} \quad (1)$$

where M is the monomeric and M_2 is the dimeric species with the equilibrium dimerization constant K_D defined as

$$K_D = [M_2]/[M]^2 \quad (2)$$

The equation for observed chemical shift δ_{obs} on assumption of fast exchange between dimeric and monomeric forms has the form

$$\delta_{obs} = \delta_M + (\delta_D - \delta_M) \left\{1 + (1 - \sqrt{1 + 8K_D[H]_T})/4K_D[H]_T\right\} \quad (3)$$

where δ_M and δ_D are the chemical shifts of monomer and dimer, respectively, and $[H]_T$ is the total concentration of the host. Fitting of concentration profiles for different protons gives after averaging the value $K_D = 23 \pm 8$ l mol^{-1} in water without added electrolyte.

Since carboxylates and phosphates are charged species, titration with them should be performed in the presence of supporting electrolyte in order to minimize the possible increase in the degree of dimerization of **1** with increase in titrant concentration. In order to estimate the salt effect on K_D we calculated K_D values at different concentrations of NaCl added by Eqn. (3) using data from Table 2 and assuming that NaCl does not affect δ_M and δ_D values taken from fitting the concentration dependences. Averaged values of K_D calculated from salt-induced shifts for different protons are given in Table 2.

It has been shown that salt effects on host–guest equilibria can be treated satisfactorily in terms of a simple Debye–Hückel equation [Eqn. (4)] with the apparent charges of reacting species equal to or somewhat lower than the true charges:[7,21]

$$\log K = \log K^0 - 0.5(z_R{}^2 - z_P{}^2)\sqrt{I}/(1 + \sqrt{I}) \quad (4)$$

where I is the ionic strength, K^0 is the equilibrium constant at $I = 0$, $z_R{}^2$ is the sum of squares of charges of reactants and $z_P{}^2$ is the sum of squares of charges of products. The fitting of the results from Table 2 to Eqn. (4) shown in Fig. 2 (dashed line) gives $K_D{}^0 = 22$ l mol^{-1} and $z_R{}^2 - z_P{}^2 = -2.42$, which means that the dication of **1** behaves as a species with effective charge $z_M = +1.1$. However, the fitting to the Eqn. (4) shows an obvious

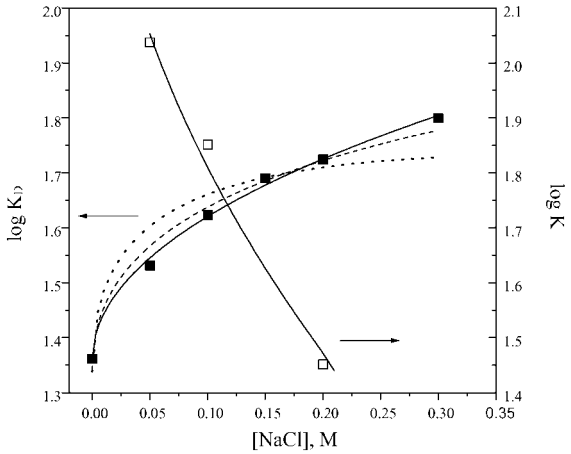


Figure 2. Salt effects on the dimerization constant of **1** (K_D, left axis, solid squares) and binding constant of terephthalate anion to **1** (K, right axis, open squares). Solid lines are the fitting curves in accordance with Eqn. (6) and parameters given in the text; dashed and dotted lines are the fitting curves to Eqns (4) and (5), respectively

systematic deviation from the experimental points. In a search for a better fit we tested also an empirical Debye–Hückel type equation [Eqn. (5)] successfully employed for a large number of ionic association equilibria with both organic and inorganic ions:[14,22]

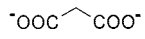
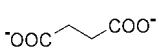
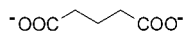
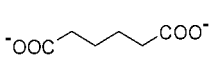
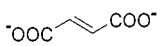
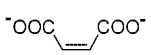
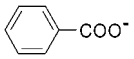
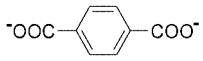
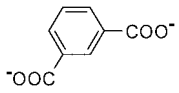
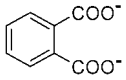
$$\log K = \log K^0 - (z_R{}^2 - z_P{}^2)\sqrt{I}/(2 + 3\sqrt{I}) + CI + DI\sqrt{I} \quad (5)$$

where $C = 0.11p^* + 0.20$ $(z_R{}^2 - z_P{}^2)$, $D = -0.075$ $(z_R{}^2 - z_P{}^2)$ and p^* is the difference in number of moles of reactants and products. The fitting to Eqn. (5) shown in Fig. 2 by a dotted line gives $K_D{}^0 = 22$ l mol^{-1} and $z_R{}^2 - z_P{}^2 = -3.5$ ($z_M = +1.3$), but the quality of the fitting is even worse than that with Eqn. (4). Bearing in mind the purely empirical nature of the fitting of these results to a Debye–Hückel-type equation derived for small, spherical ions rather than for large organic ions possessing highly dispersed charges, we tried also to use as an adjustable parameter the size parameter B in the original Debye–Hückel equation:

$$\log K = \log K^0 - 0.5(z_R{}^2 - z_P{}^2)\sqrt{I}/(1 + B\sqrt{I}) \quad (6)$$

The fitting to Eqn. (6) is shown in Fig. 2 by a solid line. It is considerably better and gives $K_D{}^0 = 23$ l mol^{-1}, $z_R{}^2 - z_P{}^2 = -1.68$ ($z_M = +0.92$) and $B = 0.074$. Remarkably, the results of the application of all three equations agree reasonably well with each other in spite of the obvious difference in fitting quality. The equilibrium constant at zero ionic strength is virtually the same for all three fitting equations and the effective monomer charge varies in a narrow range from 0.9 to 1.3. Most probably

Table 3. Binding constants of anions to and complexation-induced changes in selected chemical shifts of **1** at saturation at 25 °C and $I = 0.05$ in water[a]

Anion	K, (l mol^{-1})	Changes in chemical shifts at saturation				
		H-11	H-11′	H-9′	H-13	H-13′
	<10					
	58.0 ± 5.3	−0.033	−0.025	−0.032	−0.015	–[c]
	<10					
	–[b]					
	43.2 ± 4.2	−0.062	−0.040	−0.061	−0.042	–[c]
	46.7 ± 4.1	−0.024	−0.013	–[c]	<−0.008	–[c]
	11.6 ± 3.3	−0.38	−0.21	−0.33	−0.43	−0.26
	110 ± 4	−0.123	−0.115	−0.106	−0.107	−0.105
	48.9 ± 3.6	−0.284	−0.236	−0.226	−0.22	−0.205
	$K_1 = 135 \pm 12$ $K_3 = 12.4 \pm 1.5$	−0.017 −0.290	−0.05 −0.06	−0.033 −0.25	−0.01 −0.16	−0.01 −0.13
AMP[2]	$K_1 = 48 \pm 7$	−0.081	−0.02	−0.014	−0.134	−0.079
	$K_2 = 55 \pm 10$	−0.246	−0.14	−0.084	−0.201	−0.221
ADP[3]	39.6 ± 3.6	−0.244	−0.214	−0.11	−0.298	–[c]
ATP[4]	110.2 ± 8.5	−0.211	−0.155	−0.108	−0.264	–[c]

[a] Errors in K are the standard deviations from mean values calculated by the averaging of values obtained by fitting of experimental points for 10 NMR signals measured in duplicate; errors in changes in chemical shifts at saturation obtained as the second fitting parameter were typically within ±10%).
[b] No interaction detected.
[c] Not determined because of signal overlapping.

the estimated low effective charge of the dication reflects a high degree of charge delocalization in **1**.

In the practical sense, on the basis of above results we chose for further studies the concentration of added NaCl as 0.05 M and that of **1** as ca 0.004 M. Under these conditions ca 80% of **1** should remain in the monomeric form on variation of the titrant concentration from 0 to ca 0.04 M, provided that the 'ionic strength' created by the titrant is equivalent to that created by NaCl. Another problem in the titration of a self-associating host is the unknown degree of self-association of the host–guest complex. If it is higher than for the free host, the observed binding constant will be larger than its true value. The positive salt effect on the dimerization of **1** indicates that in principle neutral host–guest complexes may have larger dimerization constants than the dicationic host. Such a complexation-induced increase in the dimerization constant should affect the shape of the titration curve, but within the limits of experimental errors the deviation may be difficult to detect. On the other hand, the contribution of this effect evidently should be larger for higher host concentrations. Therefore, titrations with several guests (isomeric phthalate anions) were performed at different concentrations of **1** in the range 2–8 mM in the presence of 0.05 M NaCl. The observed binding constants were found to be independent of the host concentration, indicating a small if any contribution of possible complexation-induced increases in the dimerization constant.

Anion complexation

The anionic guests used in this study are listed in Table 3. In all cases, addition of a guest anion induced upfield shifts of proton signals of **1** and the plots of observed

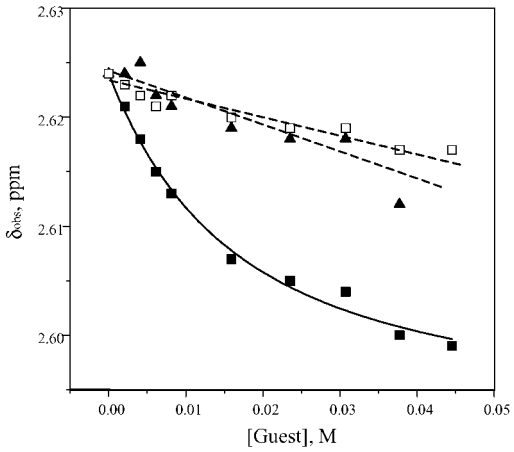


Figure 3. Titration plots for **1** and malonate (triangles), succinate (solid squares) and glutamate (open squares) anions (chemical shifts for H-11). Solid lines are the fitting curves in accordance with Eqn. (7)

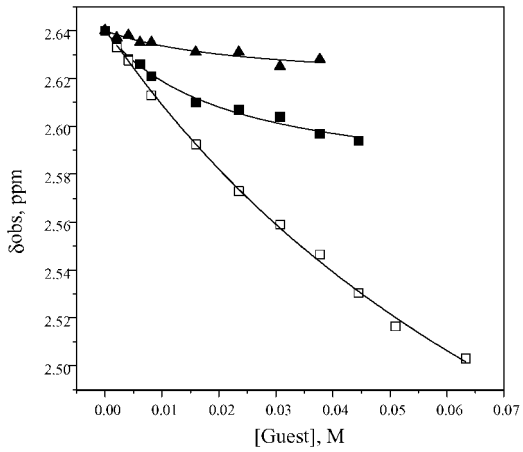


Figure 4. Titration plots for **1** and maleate (triangles), fumarate (solid squares) and benzoate (open squares) anions (chemical shifts for H-11). Solid lines are the fitting curves in accordance with Eqn. (7)

chemical shifts vs guest concentration followed Eqn. (7) derived for a 1:1 complexation scheme:

$$\delta_{obs} = \delta_H + 0.5\Delta\delta([H]_T + [G]_T + 1/K - \sqrt{([H]_T + [G]_T + 1/K)^2 - 4[H]_T[G]_T})/[H]_T \quad (7)$$

where δ_H is the chemical shift of **1**, $\Delta\delta$ is the difference in chemical shifts of complexed and free **1** (complexation-induced shift at saturation, CIS), $[H]_T$ and $[G]_T$ are the total concentrations of the host **1** and the guest and K is the binding constant. Also, a simplified equation [Eqn. (8)] which assumes the free guest concentration to be approximately equal to its total concentration was used when appropriate:

$$\delta_{obs} = \delta_H + \Delta\delta K[G]_T/(1 + K[G]_T) \quad (8)$$

The 1:1 stoichiometry was confirmed by the construction of Job plots for terephthalate as the guest using chemical shifts of different protons.

Additions of anions of aliphatic monocarboxylic acids did not induce any detectable changes in the ^{1}H NMR spectra of **1**, but dicarboxylates showed such changes, as is illustrated in Fig. 3. Only the results for succinate show sufficient curvature to allow an estimation of K and CIS values. The respective binding constants and selected (largest) CIS values for some protons are given in Table 3. With malonate and glutarate rather dispersed linear dependences of chemical shifts on guest concentrations are observed. In order to obtain at least an upper estimate of the binding constants for these anions, the results were fitted to Eqn. (8) assuming that the CIS values for these anions are similar to those for succinate. Such estimates show that the K values for both anions are lower than 10 l mol^{-1}. Additions of adipic acid did not induce noticeable spectral changes.

The existence of a sharp optimum in the series of guests $^-OOC(CH_2)_nCOO^-$ might indicate the best complementarity of guest negative charges and ammonium charges of **1** at $n = 2$, but the distance between the N-2 and N-2′ atoms in tetrandrine is 9.75 Å,[17] whereas the distance between carboxylate oxygens of succinate is only ca 6 Å. Quaternization of nitrogens in **1** may change the distance, but most probably it will be even larger because of repulsion of ammonium cations. Therefore, better charge complementarity should be expected for guests with larger n and the optimum for succinate may be due to the best complementarity of its hydrocarbon chain to the host shape. Probably its methylenes are included in the small and rigid host cavity, which allows van der Waals contacts of both carboxylates with ammonium sites, but owing to steric restrictions larger chains cannot be accommodated inside the cavity.

Typical titration plots for fumarate and maleate are shown in Fig. 4. These anions have binding constants similar to that for succinate (Table 3). Inspection of CIS values (Table 3) for all three anions shows that although they differ considerably in absolute magnitude, in all cases complexation affects the chemical shifts of protons situated around both ammonium groups of **1** and no guest shows a preferable location around one of the host nitrogens. It is worth noting that CIS values with all anions studied show a non-specific pattern, being of similar magnitude for very distant protons (Table 3). Therefore, analysis of the complexation-induced shifts of different signals is unfortunately of limited help for the identification of the location of the bound guest in this case. It seems that the association of anions with **1** leads to the formation of a series of rapidly equilibrating, on the NMR time-scale, isomeric complexes with each guest

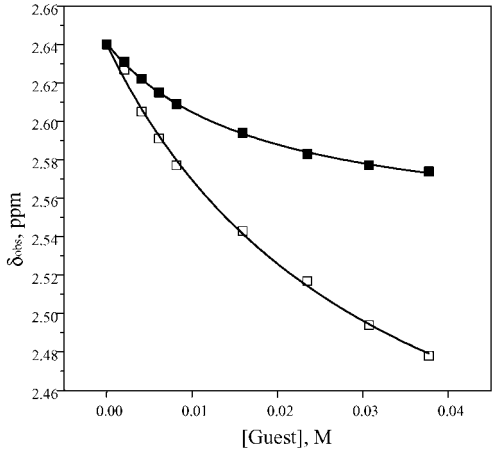


Figure 5. Titration plots for **1** and terephthalate (solid squares) and isophthalate (open squares) anions (chemical shifts for H-11). Solid lines are the fitting curves in accordance with Eqn. (7)

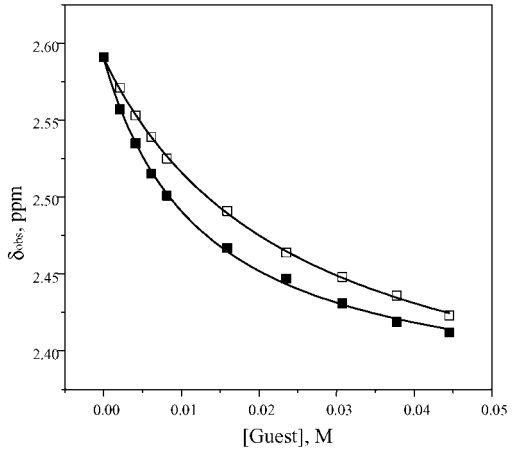


Figure 7. Titration plots for **1** and ATP (solid squares) and ADP (open squares) anions (chemical shifts for H-11). Solids lines are the fitting curves in accordance with Eqn. (7)

and the observed changes in chemical shifts are the averaged values. Hence one should rely more on structure–affinity relationships in determining the mode of anion binding to **1**.

With aromatic carboxylates one may expect an additional contribution from stacking or hydrophobic interactions. Indeed, benzoate anion, in contrast to aliphatic monocarboxylates, interacts with **1** although with a small binding constant, (Table 3, Fig 4). As discussed above for dicarboxylates, benzoate induces changes in the chemical shifts of protons situated around both ammonium groups (Table 3). Since the monoanion certainly cannot form a salt bridge with both ammonium groups of **1**, the observation of considerable CIS values for distant protons can be explained only by assuming that benzoate participates in a fast equilibrium between two or more isomeric complexes in which the carboxylate group is in a contact with either the N-2 or N-2′ site, in agreement with the assumption made above for dicarboxylates.

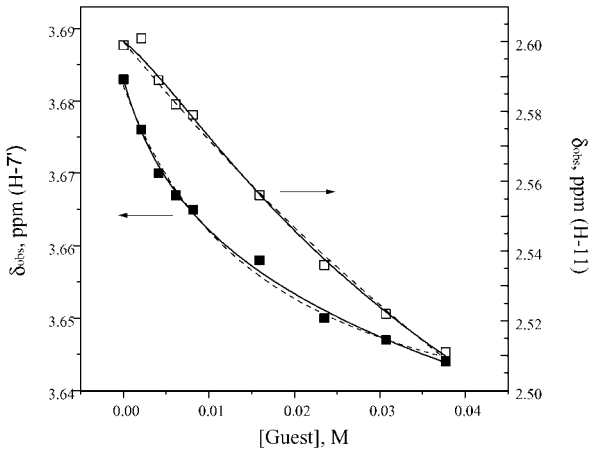


Figure 6. Titration plots for **1** and phthalate anions (chemical shifts for H-7′ and H-11). Solid lines are the fitting curves in accordance with Eqn. (9) and dashed lines in accordance with Eqn. (7)

Typical titration plots for terephthalate and isophthalate are shown in Fig. 5. Terephthalate has the larger binding constant, but the CIS values for the less tightly bound isophthalate are larger (Table 3). The situation with the *ortho*-isomer is more complicated. Fitting of the titration plots for *o*-phthalate monitored by the chemical shifts of different protons was satisfactory, but the binding constants were very much different for different monitored protons, ranging from ca 5 to ca 100 $\mathrm{l\,mol^{-1}}$. As an example, Fig. 6 shows the titration plots for *o*-phthalate monitored by the chemical shifts of H-7′ and H-11. Their fitting to Eqn. (7) shown by dashed lines gives $K = 87$ and 5.4 $\mathrm{l\,mol^{-1}}$, respectively. Such behavior indicates a more complex reaction scheme for this anion. Fitting these results to the equation

$$\delta_{\mathrm{obs}} = (\delta_{\mathrm{H}} + \delta_{\mathrm{HG}} K_1[\mathrm{G}] + \delta_{\mathrm{HG_2}} K_1 K_2[\mathrm{G}]^2)/(1 + K_1[\mathrm{G}] + K_1 K_2[\mathrm{G}]^2) \quad (9)$$

where δ_{H}, δ_{HG} and δ_{HG2} are chemical shifts of free host, 1:1 complex and 1:2 complex, respectively, and K_1 and K_2 are stepwise formation constants of these complexes, allowed us to obtain signal-independent binding constants $K_1 = 135$ and $K_2 = 12.4$ $\mathrm{l\,mol^{-1}}$ with major CIS values accompanying the formation of the second complex.

The association constants for terephthalate were determined at different concentrations of added NaCl (Fig. 2). Evidently the salt effect on the association equilibrium is much stronger than that on the dimerization of **1**. Fitting the results for terephthalate to Eqn. (6) gives $\log K^0 = 2.65$ and $z_{\mathrm{R}}{}^2 - z_{\mathrm{P}}{}^2 = 5.74$ with $B = 0.074$. Assuming that terephthalate behaves as a 'normal'

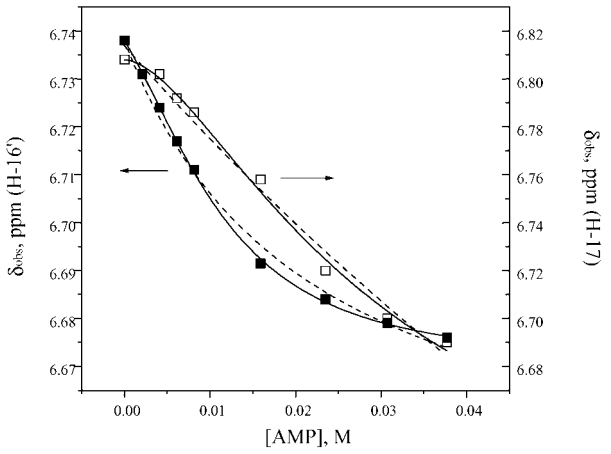


Figure 8. Titration plots for **1** and AMP anions (chemical shifts for H-17 and H-16′). Solid lines are the fitting curves in accordance with Eqn. (9) and dashed lines in accordance with Eqn. (7)

dianion with an effective charge of −2, one obtains from these parameters an effective charge of **1** of +1.36, in agreement with estimates from the salt effect on dimerization (see above).

Titration plots for ADP and ATP are illustrated in Fig. 7 and the respective binding constants are given in Table 3. In the case of AMP as in the case of *o*-phthalate, fitting to Eqn. (7) gives binding constants strongly dependent on the signal used for the fitting. As an example, the titration curves for two different protons are shown in Fig. 8. Again, fitting to Eqn. (9) allowed us to obtain signal-independent binding constants for the first and second complexes (Table 3). It is worth noting that in the case of AMP the experimental titration points do show clear deviations from the fitting curve corresponding to a 1:1 binding isotherm.

Large CIS values for nucleotides similar to those for aromatic carboxylates (Table 3) indicate possible contributions of stacking interactions for both types of guests. In the series of nucleotides the negative charge increases from 2 to 4 on going from AMP to ATP but the binding constants show little and irregular dependence on the guest charge: K decreases on going from AMP to ADP and then increases, but only by factor of ca 3, for ATP. The situation resembles that recently reported for nucleotide binding by a cationic porphyrin dimer.[9] Purely electrostatic binding of ATP to diammonium dications of different structures has K values of the order of 10^5 $\mathrm{l\,mol^{-1}}$ at $I = 0$,[23] much higher than the $K = 110\ \mathrm{l\,mol^{-1}}$ found for ATP and **1**. The association constants of ATP with ammonium monocations are of the order of 10^2–10^3 $\mathrm{l\,mol^{-1}}$ at $I = 0$.[24] Taking into account the fact of the formation of both 1:1 and 1:2 complexes between **1** and AMP, in which the dianion apparently is bound to only one of the host ammonium groups, we assume that ADP and ATP also are bound to only one of the host ammonium groups but the formation of 1:2 complexes for these anions is unfavorable because of mutual repulsion of highly charged guests. On the other hand, the values of K_1 and K_2 for AMP are too large for purely electrostatic binding of a dianion to a monocation. Typical values of association constants for such ionic pairs are ca 10 $\mathrm{l\,mol^{-1}}$ at $I = 0$.[14] The large binding constants for AMP therefore indicate a contribution from stacking interactions, which probably is less significant for nucleotides possessing higher negative charges.

Trends in the complexation of dicarboxylates by **1** can be considered together with extensive literature data on ion pairing with these guests. Since any discussion of ionic equilibria requires corrections for salt effects, we estimated the association constants for dicarboxylates at $I = 0$ by using Eqn. (6) with parameters found for terephthalate. Logarithms of the thus corrected binding constants together with literature values for dicarboxylate binding to different cations are given in Table 4. As mentioned in the Introduction, purely electrostatic binding of dicarboxylates is rather non-specific. Dications possessing very different charge distributions, such

Table 4. Logarithms of association constants of dicarboxylates with **1** and other cationic species in water at $I = 0$

	$\mathbf{1}^{2+}$	Ca^{2+}[a]	$H_3N(CH_2)_3NH_3^{2+}$[a]	$H_3N(CH_2)_6NH_3^{2+}$[b]	$H_2N(CH_2)_6NH_3^{+}$[b]	$\mathbf{3}^{2+}$[c]	$\mathbf{2}^{6+}$[d]
Malonate	≤1	2.5		2.0	0.9		
Succinate	2.4	2.0	2.11	2.0	1.1		3.15
Glutarate	≤1	1.78	1.86	1.55[a,f]			3.36
Adipate	–[e]	2.2				2.61	3.41
Maleate	2.3	2.43					3.38
Fumarate	2.2	2.0					3.61
o-Phthalate	2.73	2.47	2.23	1.97[a,f]			
Isophthalate	2.3	2.0				2.74	
Terephthalate	2.65	2.05	2.15	2.27[a,f]		2.85	4.40

[a] Ref. 24.
[b] Ref. 14.
[c] Ref. 7.
[d] Ref. 4.
[e] No binding.
[f] *N*,*N*′-Permethylated diamine.

as spherical Ca^{2+} or diprotonated diamines with different spacers between ammonium groups, all bind dicarboxylates with $\log K \approx 2$. As an example of recognition by polyprotonated macrocycles, data for the macrobicyclic anion receptor **2**[4] are included, which show, of course, larger binding constants but practically no discrimination between aliphatic dicarboxylates. Complexation with the aromatic dication **3**[7] is stronger owing to some contribution from stacking and/or hydrophobic interactions, but also non-selective.

The most interesting feature of **1** in comparison with other dications is the strong peak selectivity for succinate in the series of α,ω-dicarboxylates. The similar binding constant for fumarate probably reflects similar conformations of this anion and succinate, but equally strong binding of maleate seems surprising. Comparison of CIS values (Table 3) shows that for maleate they are substantially smaller than for succinate and fumarate. It is possible, therefore, that in contrast to succinate and fumarate, maleate is bound to only one of the ammonium centers of **1** with its hydrocarbon moiety located outside the macrocycle cavity. Indirect evidence in favor of this assumption is the observation of formation of 1:1 and 1:2 complexes with *o*-phthalate, which possesses an arrangement of carboxylate groups similar to maleate and apparently forms complexes with each ammonium center of **1**. In the case of maleate, formation of the second complex can be easily overlooked because of the very small complexation-induced spectral shifts. Ion pairing of dicarboxylates with monocations is usually weak, as illustrated in Table 4 for malonate and succinate complexes with monoprotonated diaminohexane, which have association constants of only $10\ l\ mol^{-1}$. However, as can be seen from the data for Ca^{2+} (Table 4), maleate and *o*-phthalate form more stable ion pairs than other dicarboxylates owing to the higher charge density created by proximate carboxylate groups.

Binding constants for isomeric phthalates are in the range of values expected for ion pairing between dicarboxylates and dianions (Table 4), and the complexation is rather non-specific. An interesting feature is the ability of *o*-phthalate to form both 1:1 and 1:2 complexes, apparently due to the above-mentioned effect of higher negative charge density which allows sufficiently strong binding to a single ammonium center.

CONCLUSIONS

The use of natural macrocycles for the development of new receptors is promising in several respects. First, in this way one avoids the usually difficult step of macrocyclization inevitable in the preparation of synthetic receptors. Second, natural compounds often provide interesting three-dimensional arrangements of functional groups, which potentially may create high binding selectivity. Third, natural compounds are often chiral and are of a special interest for potential chiral recognition. The results obtained in this paper with bisbenzylated tetrandrine showed never before reported high peak selectivity of complexation of α,ω-dicarboxylates. We also observed significant contributions of stacking or hydrophobic interactions in the complexation of benzoate anion and nucleotides. These results create bases for further applications of **1** for more important chiral recognition of anions, e.g. *N*-acylated amino acids. Preliminary measurements with enantiomeric anionic derivatives of phenylalanine showed that **1** does have a significant capacity for chiral discrimination.

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