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Self-assembly and recognition properties of a tetraanionic macrocyclic boronate ester in aqueous medium†

Mayte A. Martínez-Aguirre, Jorge M. del Campo, Sigfrido Escalante-Tovar and Anatoly K. Yatsimirsky*

Facultad de Química, Universidad Nacional Autónoma de México, 04510 México D.F., México. E-mail: anatoli@unam.mx.

Abstract

A tetraanionic [2+2] boronate ester macrocycle is self-assembled from a dicatechol 3,3,3′,3′-tetramethyl-1,1′-spirobiindane-5,5′,6,6′-tetraol and 1,4-benzenediboronic acid in the presence of 2 equivalents of NaOH in water containing 0-5 % vol. DMSO. No templating by potential guest molecules is required for the self-assembly with close to quantitative yield of the macrocycle. The macrocycle is stable and binds efficiently various cationic guests at pH 9. For alkali and R_4N^+ cations the association constants K_A are growing in the order Cs^+ < Me << Et << n-Pr with K_A = 180 M^{-1} for Cs^+ and >10^5 M^{-1} for Pr_4N^+ The inclusion of R_4N^+ cations is confirmed by 1D and 2D ^{1}H NMR and is further characterized by quantum mechanical calculations. The macrocycle binds efficiently some biologically important cationic guests (choline, acetylcholine, 1-methylnicotinamide) and discriminate Arg-OMe over Lys-OMe. It forms highly fluorescent complex with an isoquinoline alkaloid berberine, which can be used for optical sensing of tetraalkylammonium guests by displacement or ternary complex formation mechanism. These results extend previously limited to solid state or non-aqueous media applications of self-assembled boronate ester hosts for molecular recognition to practically more important aqueous solutions.

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† Electronic supplementary information (ESI) available: ^{11}B, ^{13}C, ^{1}H ROESY and NOESY NMR spectra of **3** and its complex with Pr_4N^+, NMR titration data, potentiometric titrations of mixtures of **1** and **2** in 50% DMSO, energies and Cartesian coordinates for calculated structures.

Introduction

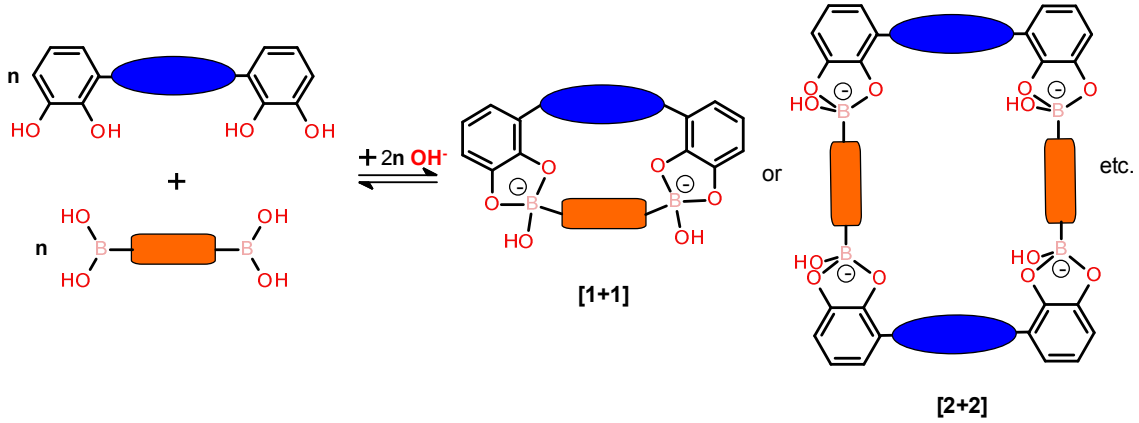
The reversible formation of boronate esters is widely employed in preparation of self-assembled supramolecular structures [1] such as covalent organic frameworks,[2] or discrete macrocycles,[3,4,5] cages and capsules.[6,7,8] Due to low stability of esters towards hydrolysis this area is limited to solid state or non-aqueous media, although in some occasion a macrocyclization e.g. via intramolecular boronic acid ester formation between fragments of phenylboronic acid and galactose attached to the opposite ends of a peptoid [9] or by crosslinking of bis(polyhydroxyalkyl)amines with boric acid [10] was proposed on basis of some indirect evidences (see also [11e]). Formation of more stable tetrahedral hydroxocomplexes of boronate esters in basic aqueous solutions [11] allows preparation of pH and/or sugar responsive polymers,[12] gels [13] and micelles,[14] which rely on multiple weak covalent interactions, but no discrete supramolecular structures requiring stronger and more preorganized interactions, were reported yet in water.

Formation of anionic tetrahedral boronate esters stabilized by acetate or methoxide anions bound to boron atom, which are structurally related to hydroxocomplexes, was employed in self-assembly of molecular capsules from tripodal boronic acid and catechole units in mixed methanol – acetonitrile solvent.[7] The important feature of these systems is that the self-assembly is driven by a guest (ammonium cation) encapsulation in the host cavity. Although the existence of empty capsules in solution was demonstrated spectroscopically, the equilibria of host-guest interactions were studied either by ability of guests to promote capsule formation from free components [7b] or by competition with a tetramethylammonim guest already bound to the capsule.[7a] No true association host-guest constants were determined and a limited set of tested simple guests demonstrated the order of affinities as $Et_4N^+ > Me_4N^+ >$ alkali cation.

Several spiroborate macrocyclic structures involving a dicatechol 3,3,3′,3′-tetramethyl-1,1′-spirobiindane-5,5′,6,6′-tetraol [15] or 2,2′,3,3′-tetrahydroxy-1,1′-binaphthyls [16] based on tetrahedral anionic borate ester links were assembled in the solid state. In all cases the anionic macrocycles were obtained with substituted ammonium counter-ions some of which were included in macrocycles as the guests, but no host-guest equilibria were reported.

An extension of boronate ester based self-assembling processes to aqueous solutions may have many useful applications in design of receptor molecules for recognition and sensing of

practically important analytes. Taking into consideration known factors favoring formation of more stable boronate diol esters in aqueous solutions [11] one may consider as potentially efficient self-assembling components 1,2-aryldiols and arylboronic acids in sufficiently basic medium to allow formation of tetrahedral hydroxocomplexes of boronate esters. In particular with dicatechols and diboronic acids the expected self-assembling process can produce in a favorable case a set of [n+n] macrocycles in accordance with Scheme 1.



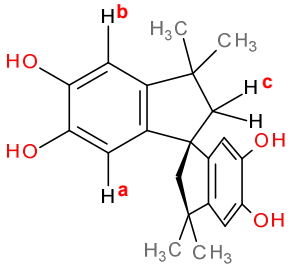
Scheme 1. Possible self-assembling process between dicatechols and diboronic acids in water.

The self-assembling of the type shown in Scheme 1 in aqueous medium is significantly more difficult process than macrocyclization via formation of neutral boronic acid esters in the absence of water. Esters of the latter type are too unstable toward hydrolysis and addition of hydroxide anions is the only way to increase their stability to acceptable level. However, creation of negative charges on boronate links should destabilize the macrocycle due to mutual repulsion of similar charges. Moreover, formation of a hydroxoboronate ester group on one end of a diboronic acid will transform a weakly electron acceptor $B(OH)_2$ group into a strongly electron donor $B(OR)_2(OH)^-$ group, which by inductive effect will increase pK_a of the second boronic acid group and thus reduce its affinity to a diol.[11d] It is not surprising therefore that mentioned above molecular capsules assembled via anionic tetrahedral boronate esters need for their stability encapsulation of a cationic guest, which may compensate these unfavorable effects by neutralizing the negative charge of the macrocycle. In this paper we report a first example of self-assembling of a cyclophane-type tetraanionic macrocycle from a diboronic acid and a dicatechol in water sufficiently stable to measure the host-guest equilibrium constants by direct

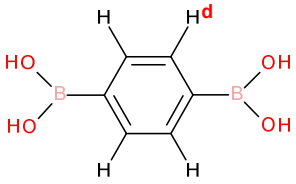
inclusion of a guest into an empty host macrocycle and explore its recognition properties with a wide range of guests including some important biological compounds like acetylcholine, derivatives of nicotinamide and amino acids.

Results and discussion

In preliminary experiments reactions of a series of commercially available dicatechols and diboronic acids were tested for their feasibility to the self-assembly in water by potentiometric and spectroscopic titrations. Initially the observed stability constants of complexes of some dicatechols with phenylboronic acid at optimum pH for each dicatechol were measured. For such dicatechols as gallein, pyrogallol red and ellagic acid the optimum stability constants were less than 10^3 M^{-1} which means that the yield of the complex in a millimolar range of concentrations of both components was less than 50%. On the other hand stability constants for two natural dicatechols nordihydroguaiaretic acid and 3,3,3′,3′-tetramethyl-1,1′-spirobiindane-5,5′,6,6′-tetraol (**1**) were above 10^4 M^{-1} for phenylboronic acid and these compounds were tested for possible macrocyclization with 1,4-benzenediboronic, 1,3-benzenediboronic and 4,4'-biphenyldiboronic acids. Analysis of ^{1}H NMR spectra of equimolar mixtures of dicatechols and diacids in basic media revealed formation of individual compounds in reactions of nordihydroguaiaretic acid with 1,3-benzenediboronic acid and of **1** with 1,4-benzenediboronic and 4,4'-biphenyldiboronic acids while in other cases apparently polymeric materials were formed. Among these more successful cases only the system **1** - 1,4-benzenediboronic acid (**2**) was sufficiently stable towards oxidative degradation and was chosen for further study.



1



2

The following procedure was employed for the self-assembly. To a mixture of **2** and NaOH in molar ratio 1:2 (typically 5 and 10 mM respectively) in water one equivalent of **1** in

DMSO (typically as 0.1 M solution) was added with stirring to the final content of DMSO less or equal to 5 % vol. All added **1** was dissolved completely and the ^{1}H NMR spectrum of the mixture (Fig. 1) indicated nearly quantitative formation of a single product with all signals of aromatic protons of **1** and **2** shifted up-field and signals of aliphatic protons of **1** practically unchanged. A single chemical shift of protons of phenylene group of the diboronic acid indicates the equivalence of boronate groups and therefore a symmetrical structure of the product.

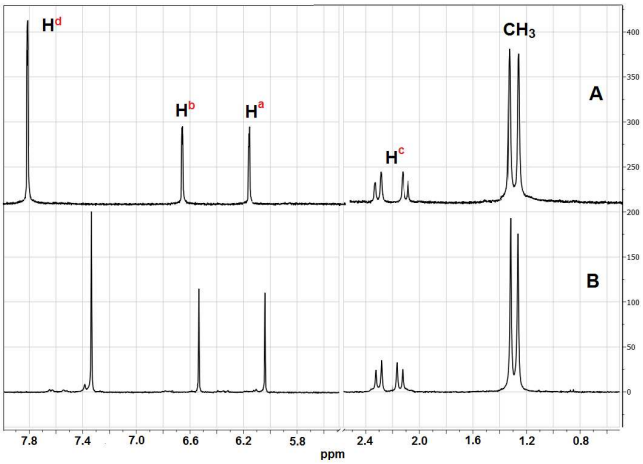


Figure 1. ^{1}H MMR spectra of the equimolar mixture of **1** and **2** without added base (A) and in the presence of 2 equivalents of NaOH (B) in 5% vol. DMSO-d_6/D_2O.

To further characterize the self-assembled product its ^{11}B, ^{13}C and ^{1}H ROESY NMR spectra were recorded (Figures 1S-3S, ESI). The ^{11}B spectrum shows a single peak at 9.17 ppm indicating the presence of equivalent tetrahedral boronate atoms and ^{13}C spectrum confirms the symmetrical structure of the product. All these results agree with formation of a [n+n] symmetrical product in line with Scheme 1. The ESI-MS spectrum obtained in the positive mode (Fig. 2) shows the presence of two intense peaks at m/z 1051.1 and 1033.1 with characteristic isotope distribution patterns for a species containing 4 boron atoms of the composition $C_{54}H_{52}O_{12}B_4Na_5^+$ and $C_{54}H_{50}O_{11}B_4Na_5^+$ respectively. They correspond to cationized by Na^+ neutral [2+2] boronate ester macrocycle (tetraanion plus four sodium counterions) and its dehydrated form respectively. Interestingly the ESI-MS spectrum obtained in the negative mode does not show the presence of corresponding boronate anionic species presumably because of their instability in the gas phase due to mutual repulsion of anionic boronate groups which must

be compensated by the counter ion binding.

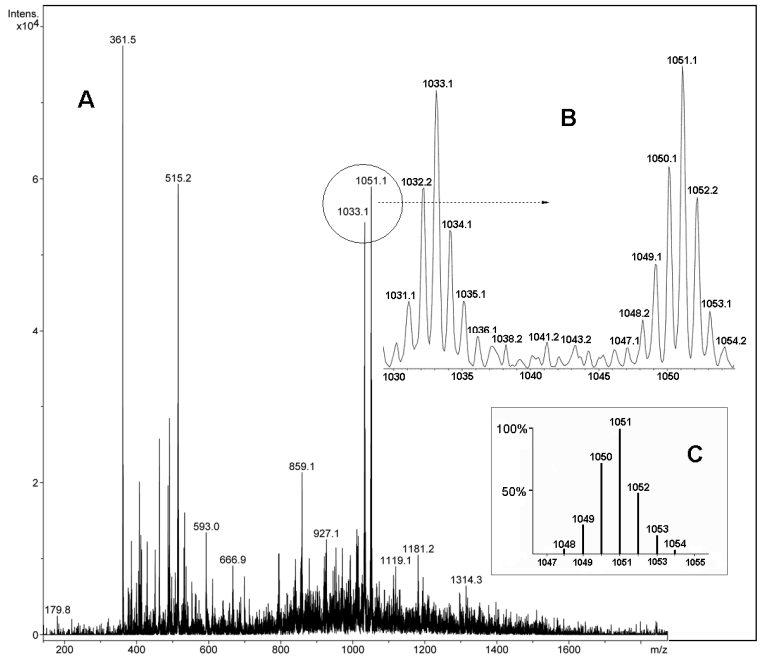
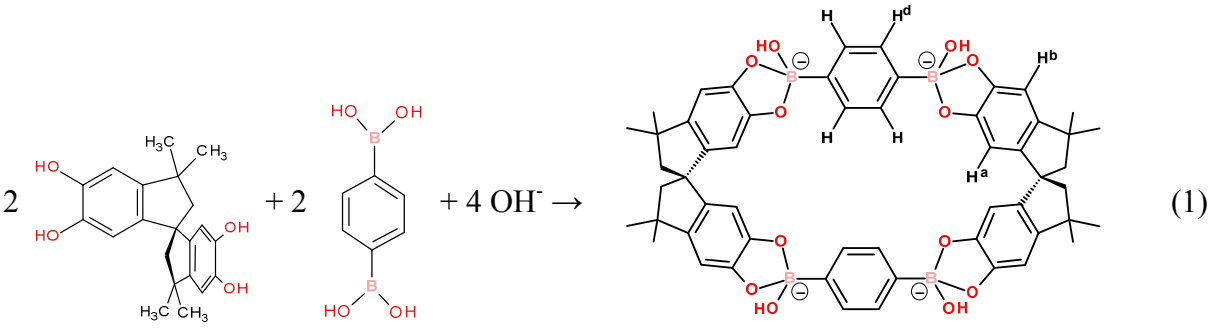


Figure 2. (A) ESI-MS spectrum in the positive mode of the equimolar mixture of **1** and **2** in the presence of 2 equivalents of NaOH; (B) expanded area between 1030 and 1050 m/z; (C) simulated isotope distribution pattern for $C_{54}H_{52}O_{12}B_4Na_5^+$.

Additional confirmation of the stoichiometry of the reaction between **1** and **2** in the presence of NaOH was obtained by potentiometric titration in 50% vol aqueous DMSO (higher proportion of the organic co-solvent was used because of insufficient solubility for this technique of **1** in water containing lower amount of DMSO). The results shown in Fig.4S (ESI) demonstrate formation of the final product of stoichiometry 2:2:4 (**1**:**2**:OH^-) with intermediate formation of the products of stoichiometry 1:1:1 and 2:2:3 corresponding to acyclic boronate esters (see Supporting Information for details). On basis of all these observations we conclude that the reaction between **1** and **2** proceeds with nearly quantitative formation of a tetraanionic [2+2] boronate ester macrocycle (**3**) in accordance with reaction (1).



3

(1)

The resulting solution has pH 9.0 and the compound is stable for at least one week when kept in solution at 6°C. A slow oxidation manifested in progressive coloring occurs during the storage. Decreasing in pH below ca. 8.5 and dilution of the compound below 1 mM induced the dissociation of the marcocycle, however. The macrocycle **3** can be prepared also in pure water by the following procedure. One equivalent of solid **1** is added to a mixture of one equivalent of **2** and two equivalents of NaOH in pure water and briefly heated at 60-70 °C with stirring under nitrogen until all **1** dissolves. A product with the same NMR spectrum as in Fig.1B is obtained, however, it is difficult to avoid significant degree of oxidation during the heating and for this reason all further studies were performed with samples containing 5% DMSO prepared by the first procedure.

The recognition properties of **3** were tested towards several types of cationic species by NMR titrations. Figure 3 illustrates typically observed spectral changes with Pr_4N^+ as a guest. Mixing of the guest with an excess of host induces strong up-field shifts in all signals of Pr_4N^+ as a result of its inclusion into the aromatic host (Fig. 3B). The aromatic signals of the host also move up-field while signals of methyl groups remain practically unchanged and signals of methylene protons appear as a singlet. Interestingly, the relative intensity of signals around 7.6 ppm corresponding to traces of the intermediate acyclic complex (see Supporting Information) decreases in the presence of the guest evidently due to the shift in the equilibrium towards **3** induced by complexation. NOESY and ROESY spectra of the Pr_4N^+/**3** mixtures (Figures 6S and 7S, ESI) contain the cross-peaks corresponding to contacts of the guest with both components of the macrocycle confirming the guest inclusion.

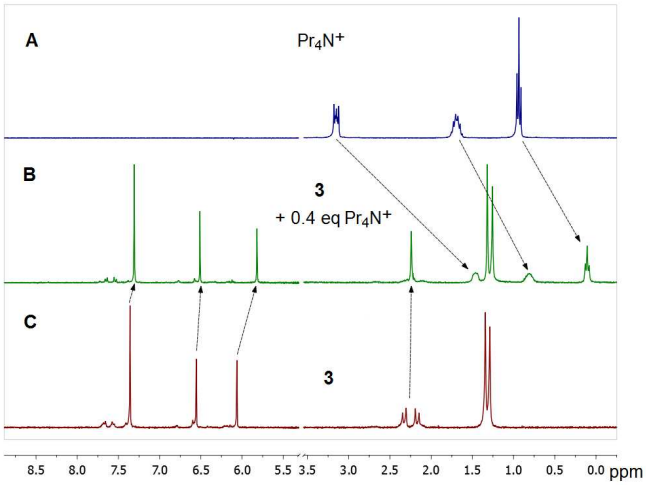


Figure 3. ^{1}H NMR spectra (5% vol. DMSO-d_6/D_2O) of 2.5 mM macrocycle **3** alone (C) and in the presence of 1 mM Pr_4NCl (B).

The titration experiments were performed by adding aliquots of concentrated solutions of chloride salts of the guests in D_2O to **3** prepared in situ in 5% vol. DMSO/D_2O. Guests did not shift initial pH 9.0 of typically 2.5 mM solutions of **3**. As an example of titration experiment Fig. 4 shows a complete set of NMR spectra for titration with acetylcholine. Titration plots for a series of [R_4N]Cl salts are shown in Fig. 5. Plots for other guests and complete sets of NMR spectra for titrations with several other representative guests are given in ESI, Figures 8S-10S.

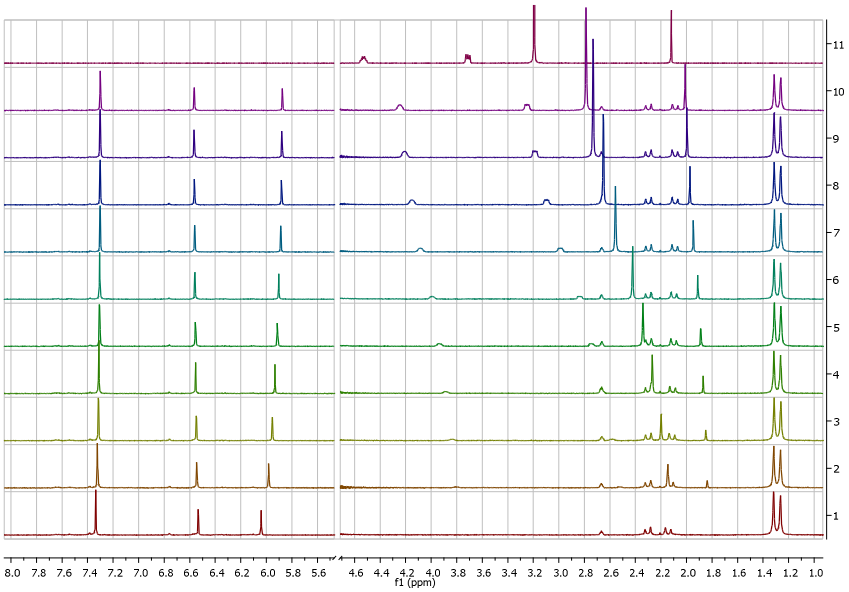


Figure 4. ^{1}H NMR titration of 2.5 mM **3** by acetylcholine. Concentrations of acetylcholine: (1) –

0; (2) – 1.0 mM; (3) – 1.5 mM; (4) – 2.0 mM; (5) – 2.5 mM; (6) – 3.0 mM; (7) – 4.0 mM; (8) – 5.0 mM; (9) – 6.0 mM; (10) – 7.0 mM; (11) – free acetylcholine.

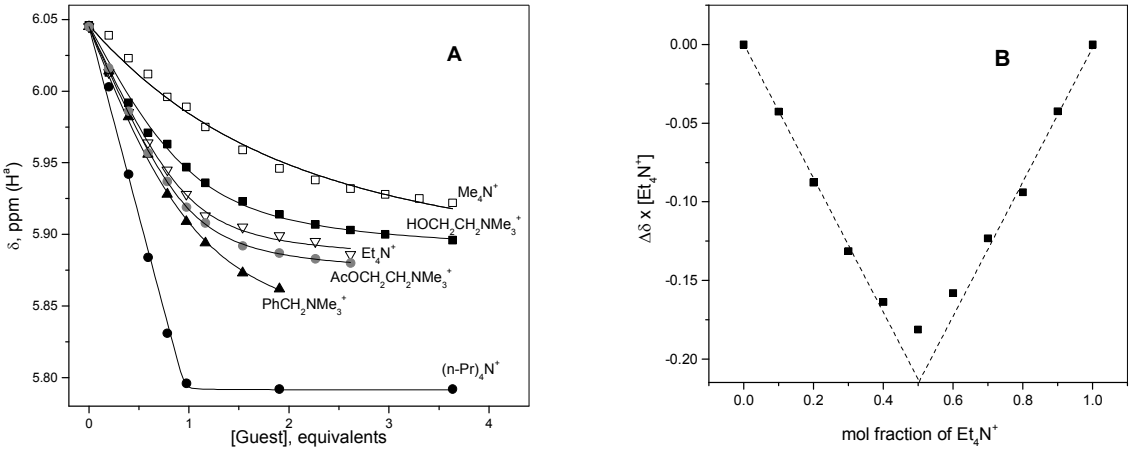


Figure 5. (A) Titration plots of 2.5 mM **3** prepared by mixing of 5 mM **1** with 5 mM **2** and 10 mM NaOH in D_2O containing 5% vol DMSO-d_6 by tetraalkylammonium guests. The guest concentration is given as number of equivalents to total **3**. (B) The Job plot for Et_4N^+ as a guest (signals of guest methyl groups).

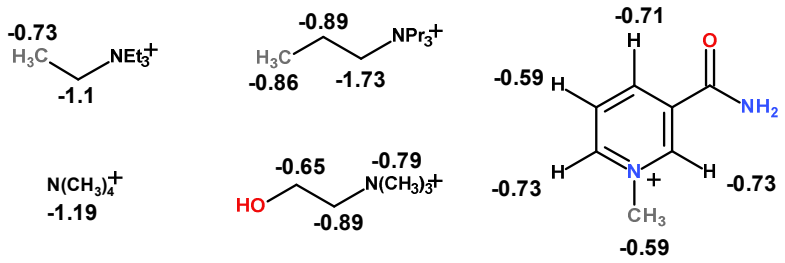
The profile for most tightly bound Pr_4N^+ clearly shows the saturation break at the molar ratio 1:1. The Job plot (Fig. 5B) with Et_4N^+ indicates the same stoichiometry. Titration plots were fitted to the equation (2) where [H] and [G] are total concentrations of host and guest, K_A is the association constant and $\Delta\delta = (\delta_{HG}-\delta_H)$ is the complexation induced shift in the signal.[17]

$$\delta_{obs} = \delta_H + 0.5\ (\Delta\delta/[H])\ ([H]+[G]+K_A^{-1}-(([H]+[G]+K_A^{-1})^2-4[H][G])^{0.5}) \qquad (2)$$

The calculated K_A and $\Delta\delta$ are summarized in Table 1 and Scheme 2 shows the complexation induced shifts in selected guests.

Table 1. Association constants (K_A with standard errors) and complexation induced shifts (Δδ) for macrocycle **3** at pH 9.0.

Guest	K_A, M^{-1}	Δδ (ppm)	
		H^a	H^d
Cs^+	180±10	-0.014	0.026
Me_4N^+	300±60	-0.19	-0.033
Et_4N^+	(3.8±0.7)×10^3	-0.16	-0.027
Pr_4N^+	>10^5	-0.25	-0.027
Bu_4N^+	Precipitate		
Me_4GuanH^+	(1.1±0.2)×10^3	-0.059	-0.035
Choline	(1.9±0.2)×10^3	-0.16	-0.027
Acetylcholine	(4.1±0.3)×10^3	-0.17	-0.036
$PhCH_2NMe_3^+$	(1.5±0.3)×10^3	-0.21	-0.15
1-Methylnicotinamide	(2.0±0.2)×10^3	-0.003	-0.16
Nicotinamide	No interaction		
n-$BuNH_3^+$	220±30	-0.032	0.006
1,4-Diaminobutane	(5.5±0.9)×10^3	-0.055	0.014
Lys-OMe	No interaction		
Arg-OMe	240±20	-0.050	-0.017

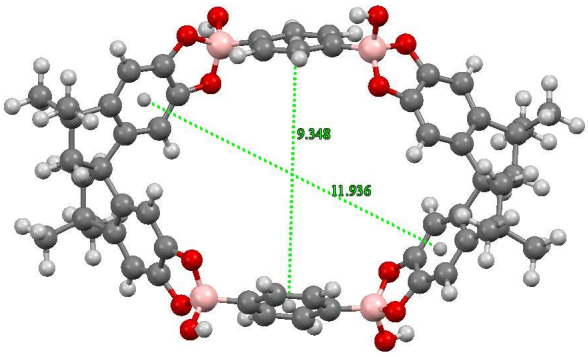


Scheme 2. Complexation induced shifts (ppm) in selected guests.

The macrocycle **3** binds R_4N^+ guests with high affinity close to that reported for best receptors for these cations operating in water,[18] such as cucurbiturils,[19] sulfonatocalixarenes [20] and resorcinarene cavitands,[21] but the selectivity is different. While the reported macrocyclic receptors either do not discriminate R_4N^+ guests with different length of the radical R [19a,20] or prefer smaller guests,[21b,c] **3** shows a strong preference to larger cations. With R = n-Bu the precipitation occurs already after addition of ca. 0.2 equivalent of the guest indicating possibly even stronger interaction.

In order to get a deeper insight into the nature of host-guest complexation with the series

of tetraalkylammonium cations high level quantum mechanical calculations were performed for **3** and its complexes. Figure 6 shows the calculated structures of the macrocycle and its complexes with Me_4N^+ and Pr_4N^+ cations. In agreement with NMR results the macrocycle has a symmetrical structure with ellipse shape cavity with a minor axis of approximately 6 Å estimated as the distance between nearly coplanar phenylene rings of 1,4-benzenediboronic acid fragments (Figure 6A) taking into account the van der Waals radius of carbon. This approximately corresponds to the cavity size of β-cyclodextrin [22] or curcurbit[6]uril.[23] The R_4N^+ cations fit the cavity with N atom of the guest occupying the center of the cavity (see top and side views of the Pr_4N^+ complex in Fig. 6 C and D). The center of the guest cation is positioned closer to the H^a than to H^b atom of the spirobiindane fragment (e.g. 5.695 and 7.248 Å respectively for Me_4N^+, Figure 6B), which explains stronger complexation induced shift in the position of H^a signal (see Figures 3 and 4). The distance is shorter in the Pr_4N^+ complex than in the Me_4N^+ complex (cf. Figures 6B and 6C), which explains larger shift in the former complex (Table 1). Also the first methylene group of Pr_4N^+ forms a shorter contact with the aromatic ring of the spirobiindane fragment than the methyl group of Me_4N^+ guest (cf. Figures 6B and 6C) in accordance with larger shift induced in the former case (Scheme 2).



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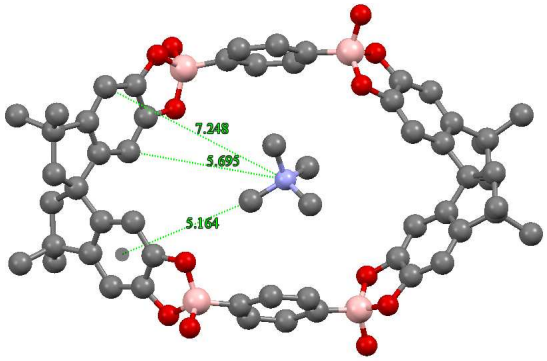

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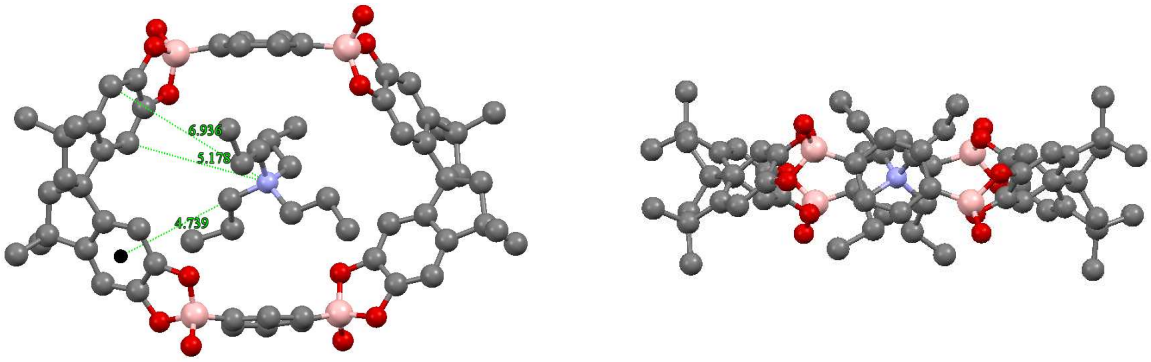

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Figure 6. (A) Minimized structure of Na_4**3** (sodium cations ommitted); (B) top view of the structure of the inclusion complex of **3** with Me_4N^+; (C) top view of the structure of the inclusion complex of **3** with Pr_4N^+; (D) side view of the structure of the inclusion complex of **3** with Pr_4N^+.

The calculated gas phase complex formation energies (Table 2S ESI) are negative, but predict the opposite to the experimentally observed trend $Me_4N^+ > Et_4N^+ > Pr_4N^+$ reflecting only electrostatic contribution to the binding. The observed selectivity $Cs^+ < Me_4N^+ < Et_4N^+ < Pr_4N^+$ can be rationalized in terms of significant contribution of hydrophobic interactions into the binding free energy. In agreement with this K_A for n-$BuNH_3^+$ is close to that for Me_4N^+ possessing similar charge and number of aliphatic carbon atoms. Also $PhCH_2NMe_3^+$ and Et_4N^+ possesing similar hydrophobicity (one phenyl group is equivalent to four methylenes in terms of π hydrophobicity scale [24]) have close to each other values of K_A. On the other hand choline and acetylcholine obviously possess higher affinities than expected on basis of only hydrophobic effect possibly because of better fit of these guests to the macrocycle cavity. Also tetramethylguanidinium cation (Me_4GuanH^+ in Table 1) forms significantly more stable complex than tetramethylammonium cation indicating a better fit of a planar guanidinium group.

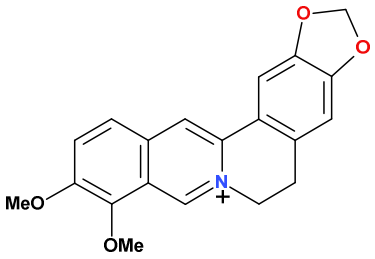
Besides hydrophobic interactions, the principal driving forces for inclusion in **3** are charge-charge and possibly cation-π interactions. This is evident from the absence of interaction with neutral nicotinamide in comparison with strong binding of cationic 1-methylnicotinamide and strongly increased K_A value for dication of 1,4-diaminobutane as compared to monocationic n-$BuNH_3^+$ (Table 1). Additions of polyamines (spermidine, spermine) to **3** induced precipitation even at low guest concentrations. A contribution from cation-π interactions is highly probable, however these interactions are expected to induce down-field shifts in NMR signals of aromatic

protons [25] and we observe mostly the up-field shifts (Table 1), which may be attributed to changes in hydration due to contacts with hydrophobic guests.

The inclusion of cationic guests into the macrocycle cavity is evidenced by observation of complexation induced shifts of all protons of guest cations (Scheme 2). The observed negative shifts agree with well-known through-space shielding effect of aromatic rings on C-H protons. [26]

Due to observed increased affinity of **3** to guanidinium as compared to ammonium cations (see above) we attempted using the boronate macrocycle for discrimination of lysine and arginine derivatives. Table 1 shows the results for binding of methyl esters of both amino acids, which demonstrate the expected higher affinity to the arginine derivative.

Important group of cationic biological compounds constitute isoquinoline alkaloids among which perhaps the most extensively studied is berberine.[27] Recently inclusion of berberine in different macrocyclic hosts, such as cucurbiturils,[28] cyclodextrins[29] and calixarenes[30] was studied with a purpose of improvement of fluorescent properties of the alkaloid for analytical applications.[31]



Berberine

In aqueous solution berberine has very low fluorescence, but inclusion in the hydrophobic cavity of a macrocyclic host induces a 10 to 100 fold increase in the fluorescence intensity. Similar effect was observed with **3**: addition of 1 mM **3** to the berberine aqueous solution induced a 10 fold increase in fluorescence (Figure 7, green and red lines correspond to the fluorescence of free and bound berberine respectively). The calculated structure of the berberine complex with **3** (Figure 8) demonstrates that indeed a significant portion of the guest can fit to the macrocycle cavity.

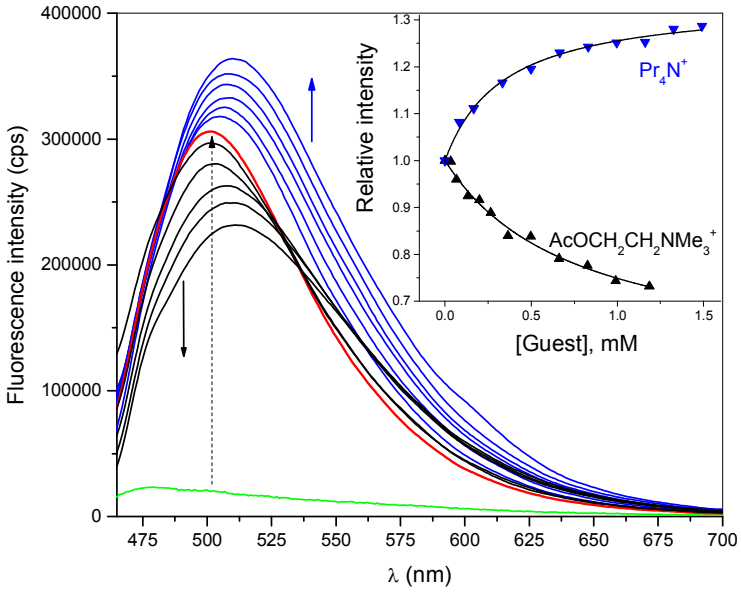


Figure 7. Fluorescence spectra (excitation wavelength 400 nm) of 3 μM berberine in water (green line), after addition of 1 mM **3** (red line) and after subsequent additions of acetylcholine (black lines) or Pr_4N^+ (blue lines). Arrows show the trends on additions of increased amounts of cationic guests. Inset: relative fluorescence intensity as a function of concentrations of added guests to berberine in the presence of 1 mM **3**.

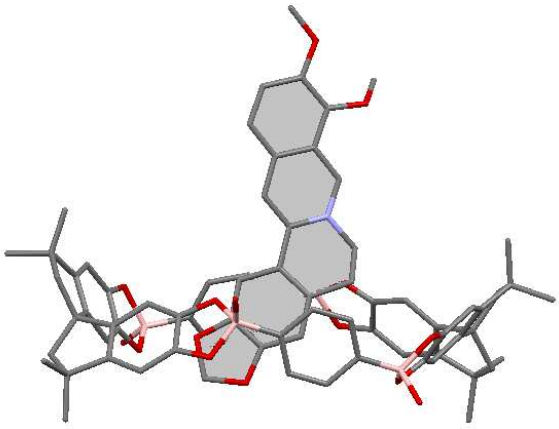


Figure 8. Minimized structure of the inclusion complex of **3** with berberine.

We attempted to use this effect for possible optical sensing of other cationic guests like tetraalkylammonium cations by the indicator displacement assay. Additions of acetylcholine (Figure 7, black lines) induced the expected decrease in fluorescence and the titration plot shown

in the inset of the figure demonstrates a possibility to detect ca. 0.1 mM of the added guest. The displacement of berberine was confirmed also by ^{1}H NMR results which showed that after addition of 4 mM acetylcholine to a 1:1 mixture of berberine and **3** the aromatic signals of macrocycle coincided with those for the aceylcholine-**3** complex. Surprisingly, additions of Pr_4N^+ cation to the berberine-**3** complex instead of the expected fluorescence quenching due to displacement of berberine from the host cavity, induced an increase in fluorescence intensity accompanied by a red shift of the emission maximum (Figure 7, blue lines). A possible reason for such behavior is that more hydrophobic Pr_4N^+ cation can form a ternary complex with **3** and berberine providing less polar environment around the alkaloid favorable for its fuorescence.[28] These results demonstrate possibility of using **3** in a complex with a fluorescent indicator molecule for optical sensing of cationic guests.

Experimental section

General experimental methods. Commercially available solvents and reagents were used without further purification. ^{1}H NMR spectra were recorded at 300 MHz or 400 MHz on Varian Unity Inova or Varian VNMRS NMR spectrometers respectively, ^{13}C NMR proton decoupled spectra were recorded at 75.4 MHz on Varian Unity Inova NMR spectrometer and ^{11}B NMR spectra were recorded at 128.3 MHz with $Et_2O{\cdot}BF_3$ in $CDCl_3$ as the external standard on a Varian VNMRS NMR spectrometer, data was processed with MestReNova version 6.0.2-5475.

NMR titrations. The NMR titration experiments were performed by adding aliquots of concentrated solutions of chloride salts of the guests in D_2O to **3** prepared in situ in 5% vol. DMSO/D_2O. Analysis of ^{1}H NMR titration data by using equation (2) was performed by non-linear least-squares fitting with Microcal Origin 8.5 program for signals of all protons underwent significant changes and the calculated association constants given in Table 1 are the mean values.

Potentiometry. Potentiometric titrations in 50% vol. DMSO/water containing 0.05 M NaCl as background electrolyte were performed in a 25-mL thermostatted cell kept under nitrogen at 25°C. Measurements of pH were carried out using an Orion model 710-A research digital pH meter while the titrant (NaOH) solution was added to the system in small increments. The electrode was calibrated in terms of p[H^+] by titrations of 1–3 mM HCl. From these data, the

autoprotolysis constant pK_W = 15.9±0.1 was determined in good agreement with published values in this medium. The program HYPERQUAD 2003, version 3.0.51 was used to calculate all equilibrium constants.

Mass spectra. ESI-MS experiments were performed on a Bruker Esquire 6000 mass spectrometer operated in the positive mode with electrospray ionization source. Samples were run by direct infusion from a syringe pump. Data were collected and analyzed on Bruker Daltonics 3.2 software.

Calculation method. Structural optimization was performed using the restricted PBE [32] exchange–correlation functional in combination with the 6-311++G** basis set for all atoms using the TeraChem (1.5 version) software package [33] employing Cartesian coordinate optimization. For the empty macrocycle **3** and its inclusion complexes the negative formal charges were compensated by adding Sodium cations, thus a net (+1) charge was requested for the inclusion system and a zero net charge for the empty macrocycle.

In order to assess the fact that the interaction between the two fragments is not an artifact, a single point potential energy evaluation was performed by putting the tetraalkylammonium cation far away from the macrocycle center with the nitrogen atom of the guest at 10 Å with respect to the top position. The calculated interaction energy resulted in a favorable inclusion process for this level of theory. For the case of the berberine complex a 6-311G** basis set and PBE0 [34] hybrid exchange–correlation functional was employed; for this case we have tested just the inclusion model and during the optimization process it remained inside the macrocycle. Calculated absolute energies for all complexes are given in Table S2 (ESI).

Conclusions

In conclusion, the results of this study demonstrate that formation of anionic tetrahedral hydroxocomplexes of boronate esters can provide sufficient driving force for the self-assembly of discrete macrocyclic structures in aqueous medium. In contrast to previously reported self-assembled macrocyclic systems based on anionic complexes of boronate esters, which need a stabilizing (templating) effect of a cationic guest for their formation [7] (see Introduction), a nearly quantitative formation of tetraanionic **3** occurs in the absence of any guest cation. A possible reason for this is that the self-assembly of **3** takes place in aqueous medium which strongly

reduces destabilizing effect of mutual repulsion of negative charges of the macrocycle. The macrocycle **3** shows promising recognition properties towards biologically important guests. It binds alkylammonium, alkylpyridinium and tetraalkylammonium cations including choline and acetylcholine with high affinity and selectivity governed by charge and hydrophobicity of the guest, discriminates ammonium and guanidinium cations and strongly enhances the fluorescence of an isoquinoline alkaloid berberine. The berberine–**3** complex can be used for optical sensing of cationic guests. The macrocycle **3** suffers however from rather narrow range of stability: dilution below 1 mM and decrease in pH below 9 induced dissociation of the macrocycle. Further progress in this area can be achieved by using both catechol and boronic acid components with lower pK_a values, which will provide an increased thermodynamic stability of the self-assembled compound and a shift in optimum pH of its formation to lower values ideally to a "physiological" range around 7.

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References

1 For recent reviews on the use of boronic acids in self-assembly, see: (a) N. Fujita, S. Shinkai, and T. D. James, *Chem. Asian J*., 2008, **3**, 1076; (b) K. Severin, *Dalton Trans*., 2009, 5254; (c) R. Nishiyabu, Y. Kubo, T. D. James and J. S. Fossey, *Chem. Commun*., 2011, **47**, 1124; (d) E. Sheepwash, B. Icli and K. Severin, *Chimia*, 2012, **66**, 212; (e) S. D. Bull, M. G. Davidson, J. M. H. Van Den Elsen, J. S. Fossey, A. T. A. Jenkins, Y.-B. Jiang, Y. Kubo, F. Marken, K. Sakurai, J. Zhao, and T. D. James, *Acc. Chem. Res*., 2013, **46**, 312.

2 (a) A. P. Côté, A. I. Benin, N. W. Ockwig, M. O'Keeffe, A. J. Matzger and O. M. Yaghi, *Science*, 2005, **310**, 1166; (b) E. Sheepwash, V. Krampl, R. Scopelliti, O. Sereda, A. Neels and K. Severin, *Angew. Chem. Int. Ed.*, 2011, **50**, 3034.

3 (a) Y. Kikuchi, H. Takahagi, K. Ono and N. Iwasawa, *Chem. Asian J*., 2014, **9**, 1001; (b)

N. Iwasawa and H. Takahagi, *J. Am. Chem. Soc*., 2007, **129**, 7754; (c) H. Takahagi and N. Iwasawa, *Chem. Eur. J.,* 2010, **16**, 13680; (d) S. Ito, K. Ono and N. Iwasawa, *J. Am. Chem. Soc.*, 2012, **134**, 13962.

4 M. K. Smith, N. E. Powers-Riggs and B. H. Northrop, *Chem. Commun.,* 2013, **49**, 6167.

5 H. Danjo, N. Mitani, Y. Muraki, M. Kawahata, I. Azumaya, K. Yamaguchi and T. Miyazawa, *Chem. Asian J.*, 2012, **7**, 1529.

6 (a) N. Nishimura and K. Kobayashi, *J. Org. Chem*., 2010, **75**, 6079; b) N. Nishimura, K. Yoza and K. Kobayashi, *J. Am. Chem. Soc*., 2010, **132**, 777; (c) N. Nishimura and K. Kobayashi, *Angew. Chem. Int. Ed*., 2008, **47**, 6255; (d) H. Takahagi, S. Fujibe and N. Iwasawa, *Chem. Eur. J*., 2009, **15**, 13327.

7 (a) K. Kataoka, S. Okuyama, T. Minami, T. D. James and Y. Kubo, *Chem. Commun*., 2009, 1682; (b) K. Kataoka, T. D. James and Y. Kubo, *J. Am. Chem. Soc*., 2007, **129**, 15126.

8 (a) B. Icli, E. Sheepwash, T. Riis-Johannessen, K. Schenk, Y. Filinchuk, R. Scopelliti and K. Severin, *Chem. Sci.*, 2011, **2**, 1719; (b) J. Tçnnemann, R. Scopelliti, K. O. Zhurov, L. Menin, S. Dehnen and K. Severin, *Chem. Eur. J.*, 2012, **18**, 9939.

9 S. Chirayil and K. J. Luebke, *Tetrahedron Letters* 2012, **53**, 726.

10 J. van Haveren, H. Lammers, J. A. Peters, J. G. Batelaan and H. van Bekkum, *Carbohydr. Res*., 1993, **243**, 259.

11 (a) J. P. Lorand and J. O.Edwards, *J. Org. Chem*., 1959, **24**, 769; (b) J. Yan, G. Springsteen, S. Deeter and B. Wang, *Tetrahedron*, 2004, **60**, 11205; (c) G. Springsteen, S. Deeter and B. Wang, *Tetrahedron*, 2002, **58**, 5291; (d) M. A. Martínez-Aguirre, R. Villamil-Ramos, J. A. Guerrero-Alvarez and A. K. Yatsimirsky, *J. Org. Chem.*, 2013, **78**, 4674; (e) J. A. Peters, *Coord. Chem. Rev*. 2014, **268**, 1.

12 J. N. Cambre and B. S. Sumerlin, *Polymer*, 2011, **52**, 4631.

13 (a) M. Nakahata, S. Mori, Y. Takashima, A. Hashidzume, H. Yamaguchi and A. Harada, *ACS Macro Lett.*, 2014, **3**, 337; (b) T. Yang, R. Ji, X.-X. Deng, F.-S. Du andZ.-C. Li, *Soft Matter* 2014, **10**, 2671.

14 Y. Li, W. Xiao, K. Xiao, L. Berti, J. Luo, H. P. Tseng, G. Fung and K. S. Lam, *Angew. Chem. Int. Ed.*, 2012, **51**, 2864.

15 (a) B. F. Abrahams, B. A. Boughton, H. Choy, O. Clarke, M. J. Grannas, D. J. Price and

R. Robson, *Inorg. Chem*., 2008, **47**, 9797; (b) B. F. Abrahams, D. J. Price and R. Robson, *Angew. Chem., Int. Ed.*, 2006, **45**, 806.

16 H. Danjo, K. Hirata, S. Yoshigai, I. Azumaya and K. Yamaguchi, *J. Am. Chem. Soc.*, 2009, **131**, 1638.

17 H.-J.Schneider and A. K. Yatsimirsky, *Principles and methods in supramolecular chemistry*; John Wiley and Sons, Chichester, 2000; p 144.

18 A. Späth and B. König, *Beilstein J. Org. Chem.*, 2010**, 6***,* 1.

19 (a) A. D. St-Jacques, I. W. Wyman and D. H. Macartney, *Chem. Commun.*, 2008, 4936; (b) I. W. Wyman and D. H. Macartney, *Org. Biomol. Chem.*, 2010, **8**, 253.

20 (a) C. Bonal, Y. Israëli, J.-P. Morel and N. Morel-Desrosiers, *J. Chem. Soc*., *Perkin Trans. 2*, 2001, 1075; (b) D.-S. Guo, K. Wang and Y. Liu, *J. Incl. Phenom. Macrocycl. Chem.*, 2008, **62**, 1; (c) G. Arena, S. Gentile, F. G. Gulino, D. Sciotto and C. Sgarlata, *Tetrahedron Lett.*, 2004, **45**, 7091

21 (a) F. Hof, L. Trembleau, E. C. Ullrich and J. Rebek Jr., *Angew. Chem. Int. Ed.*, 2003, **42**, 3150; (b) P. Ballester and M. A. Sarmentero, *Org. Lett*., 2006, **8**, 3477; (c) H. J. Schneider, D. Güttes and U. Schneider, *J. Am. Chem. Soc.*, 1988, **110**, 6449.

22 K. A.Connors, *Chem. Rev.* 1997, **97**, 1325.

23 E. Masson, X. Ling, R. Joseph, R., L. Kyeremeh-Mensah and X. Lu, X. *RSC Advances*, 2012, **2**, 1213.

24 (a) C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology;* Wiley, New York, 1979. (b) A. Leo, C. Hansch and D. Elkins, D. *Chem. Rev.* 1971, **71**, 575.

25 A. Hassan, T. C. Dinadayalane, S. J. Grabowski and J. Leszczynski, *Phys. Chem. Chem. Phys.,* 2013, **15**, 20839.

26 N. H. Martin, R. M. Floyd, H. L. Woodcock,.S. Huffman and C.-K. Lee, *J. Mol. Graphics Modell.* 2008, **26**, 1125.

27 Md. M. Islam, A. Basu, M. Hossain, G. Sureshkumar, S. Hotha and G. S. Kumar, *DNA Cell Biol.* 2011, **30**, 123.

28 M. Megyesi, L. Biczok and I. Jablonkai, *J. Phys. Chem. C* 2008**, 112***,* 3410.

29 J.-S. Yu, F.-D. Wei, W. Gao and C.-C. Zhao, *Spectrochim. Acta Part A*, 2002, **58**, 249.

30 M. Megyesi and L. Biczok, *Chem. Phys. Lett.* 2006, **424**, 71.

31 (a) H.-J. Schneider, P. Agrawal and A. K. Yatsimirsky, *Chem. Soc. Rev.* 2013, **42**, 6777. (b) A. K. Yatsimirsky, *Natural Prod. Comm.* 2012, **7**, 369.

32 J. P. Perdew, K. Burke and M. Ernzerhof, *Phys. Rev. Lett.* 1996, **77**, 3865.

33 I. S. Ufimtsev and T.J. Martinez, *J. Chem. Theory Comput.* 2009, **5**, 2619.

34 (a) M. Ernzerhof and G. E. Scuseria, *J. Chem. Phys.* 1999, **110**, 5029. (b) C. Adamo and V. Barone, *J. Chem. Phys.* 1999, **110**, 6158.