Organic & Biomolecular Chemistry

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Cite this: *Org. Biomol. Chem.*, 2011, **9**, 7457

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PAPER

A simple ionic triphenylene receptor for catecholamines, serotonin and D-glucosamine in buffered water†

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Received 6th July 2011, Accepted 2nd August 2011
DOI: 10.1039/c1ob06099f

The combination of hydrophobic effects and ionic pairing within a triphenylene-based receptor were exploited for the binding of biological phenylethylamines, serotonin and D-glucosamine in phosphate buffered water.

Introduction

Binding of ammoniums and biological ammoniums is a well-documented field of supramolecular chemistry, due to the ubiquity of these small compounds in molecular biology.[1] In this context, catecholamines and other amphiphilic phenylethylamines have been attractive targets for artificial receptors since the 80's. Boronic acid-based receptors of catecholamines were developed but still lack selectivity for the series of catechol derivatives and 1,2-diol compounds.[2] Besides, based upon classical supramolecular concepts, cavitands and macrocycles were employed as complementary hosts for biological ammoniums that bring to play solvophobic and electrostatic forces such as hydrogen bonds, ion–dipole interactions (especially π–cation) and ionic interactions. Generally, an appropriate combination of interactions is the key factor of a selective recognition, depending on the ammonium degree of substitution, its steric hindrance and/or the presence of extra binding sites within the guest. However, if the hydrophobic effect is the dominant force for the (host:guest) association, poor selectivity is observed for amphiphilic ammoniums against other aromatic species.[3] In contrast, when the process of binding is driven by electrostatic attraction, the values of association constants (K_{ass}) dramatically decrease when operating in water with ionic strength (NaCl, buffer).[4]

Indeed, a small number of receptors is efficient in salt buffered water at neutral pH, which is a more representative medium of physiological conditions than pure water.[5,6] To properly evaluate a host designed for biological amines and catecholamines, its properties should be investigated under suitable experimental conditions, towards a large series of guests. Moreover, a topical question deals with the necessity of designing elaborate receptors, through time and energy demanding synthesis. Thus, a novel challenge for supramolecular chemistry would be the design of cheap simple hosts to further allow the elaboration of analytical devices based on supramolecular hosts.[7] Then, the conception of supramolecular receptors is anticipated to move towards uncluttered structures of easy synthetic access.

Recently, we described the elementary synthesis of triphenylene **1**[8] and its binding ability towards acetylcholine (ACh, K_{ass} (1 : 1) = 94 M^{-1}) against other similar aliphatic ammoniums such as choline (Ch, K_{ass} = 0 M^{-1}) in phosphate buffered water at pH 7.1 (Chart 1).[9] This remarkable property in a competitive medium was not totally clarified. An investigation of the (host:ACh) complex monitored by infrared showed that ionic pairing occurs between the binding partners, which is assisted by a process of desolvatation, detected on the C=O group of ACh. Except for (metallo)porphyrin-based receptors,[1] such a combination of hydrophobic and ionic forces that assembles a non-concave host and a guest in water are scarce[10] and, in principle, could allow the complexation of various amphiphilic targets. Thus, we proposed to deeply investigate, and possibly generalize, the binding properties of the simple hydrosoluble polyaromatic host **1** towards biological amphiphilic ammoniums, through ionic pairing and suitable hydrophobic effects, under more biomimetic conditions, *i.e.*, buffered water at neutral pH (Chart 1). Additionally, a (host:guest) binding model would be proposed based on molecular modeling.

Thus, to evaluate the hydrophobic impact of the triphenylene core on the host binding properties, a series of model ammoniums **2a–c** with phenyl hydrophobic groups and catechol **2d** were submitted to (host:guest) titrations in the presence of **1** (Table 1). Then, classical targets for artificial receptors were selected as guests to assess the attractiveness of host **1** (Chart 1): catecholamines **3a–c**, (−)-ephedrine **3d**, tyramine **3e** and serotonin **3f**. α-Amino acids **4a–d**, and *C*-protected tryptophan **4e** complete the test. To extend the scope of the host properties, D-glucosamine **5a** was also investigated as a guest and compared to D-glucose **5b**, D-fructose **5c**, D-cellobiose **5d** and vitamin C **5e**.

Results and discussion

All experiments were conducted below the concentration of host self-aggregation (9 mM). During titrations monitored by

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† Electronic supplementary information (ESI) available: Titrations monitored by proton NMR and molecular modeling details. See DOI: 10.1039/c1ob06099f

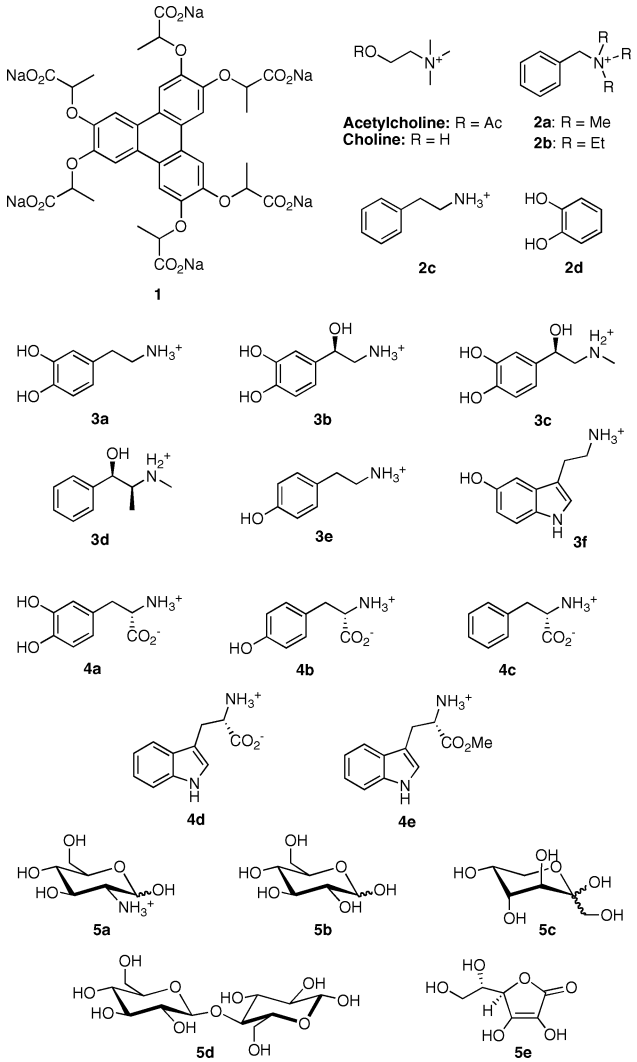

Chart 1 Molecular structures of receptor **1**, model ammoniums **2a–c**, catechol **2d** and biological guests (pH 7.1): acetylcholine, choline, catecholamines **3a–c**, (–)-ephedrine **3d**, tyramine **3e**, serotonin **3f**, related α-amino acids **4a–e**, carbohydrates **5a–d** and vitamin C **5e**.

^{1}H NMR, the chemical shifts of the host were unchanged. This observation indicates that π–π interactions are probably weak between host **1** and its guests, as tight complexes between triphenylene derivatives and aromatic targets usually induce significant upfield shifts on both partners.[11] In contrast, during these titrations, the signals of the guest were downshifted in the aliphatic and aromatic regions ($\Delta\delta$ = +0.1 to + 0.7 ppm) when complexation occurred (Table 1). Apparently, both aromatic and ionic moieties of the guests are involved in a recognition process of moderate strength.

All complexes were formed in a (1 : 1) (host : guest) stoichiometry. Concerning model ammoniums **2**, the observed binding constants were higher (K_{ass} = 150–350 M^{-1}) than the one determined for the less hydrophobic guest, ACh (K_{ass} = 94 M^{-1}), as expected by the proposed dual binding model. Benzyltrimethylammonium **2a** presented an association constant of 277 M^{-1} whereas benzyltriethylammonium **2b** showed a K_{ass} of 352 M^{-1}, probably due to its more hydrophobic structure. Confirming this tendency, the more hydrophilic primary ammonium **2c** interacted with **1** in a looser manner (K_{ass} = 149 M^{-1}) than **2a–b**. Interestingly, the latter value is relevant in buffered water for such a model of biological phenylethylamines.[6] Molecular modeling of the (**1**:**2c**) complex in water (MM level, Fig. 1a) showed that the guest is located above the hydrophobic surface of the host (distance between the aromatic planes is 3.50 Å), while ionic pairing occurs between the NH_3^+ group of the guest and two carboxylate groups of the host (d = 2.18 and 2.56 Å) positioned at the extremities of the triphenylene bay region, to optimize the combination of forces between host and guest. Besides, an aromatic guest without an ammonium moiety such as catechol **2d**, did not interacting with host **1**, probably due to the lack of ionic interaction. Receptor **1** can thus offer efficient binding when complementary guests are provided with both ammonium and hydrophobic moieties, depending on their hydrophobicity. This binding model was then further tested towards different hosts **3–5**.

Phenylethylamines **3a–f** were complexed by host **1** with a (1 : 1) binding constant of ~200 M^{-1}, a significant value in such a medium of high ionic strength. However, as observed in the literature,[4–6] the proposed binding principle cannot distinguish between such

Table 1 Binding constants (K_{ass}, M^{-1}) between receptor **1** and ammonium guests **2–5** in buffered water and selected maximum binding-induced chemical shift variations ($\Delta\delta$, ppm) observed for guest protons[a,b]

Guest[c]	K_{ass} (1 : 1)[d]	$\Delta\delta_{max}$ H_{aliph}[e]	$\Delta\delta_{max}$ H_{arom}[f]
Acetylcholine ACh	94	+0.18	—
BnN^+Me_3 **2a**	277	+0.41	+0.30
BnN^+Et_3 **2b**	352	+0.79	+0.44
Phenylethylammonium **2c**	149	+0.09	+0.09
Catechol **2d**	< 1	0.00	0.00
Dopamine **3a**	194	+0.53	+0.55
L-Noradrenaline **3b**	186	+0.15	+0.12
L-Adrenaline **3c**	204	+0.11	+0.14
(–)-Ephedrine **3d**	183	+0.08	+0.08
Tyramine **3e**	237	+0.25	+0.26
Serotonin **3f**	201	+0.69	+0.71
L-DOPA **4a**, L-tyrosine **4b**, L-phenylalanine **4c** and L-tryptophan**4d**	< 1	0.00	0.00
L-Tryptophan methyl ester **4e**	160	+0.34	+0.20
D-Glucosamine **5a**	87	+0.11	—
D-Glucose **5b**, D-fructose **5c**, D-cellobiose **5d** and vitamin C **5e**	< 1	0.00	0.00

[a] D_2O, Na_2HPO_4 100 mM, pH 7.1, 298 K. [b] Proton NMR monitoring. [c] Chloride as a counterion. [d] Estimated errors ±15%. [e] Experimental $\Delta\delta_{max}$ concerning aliphatic protons. [f] Experimental $\Delta\delta_{max}$ concerning aromatic protons.

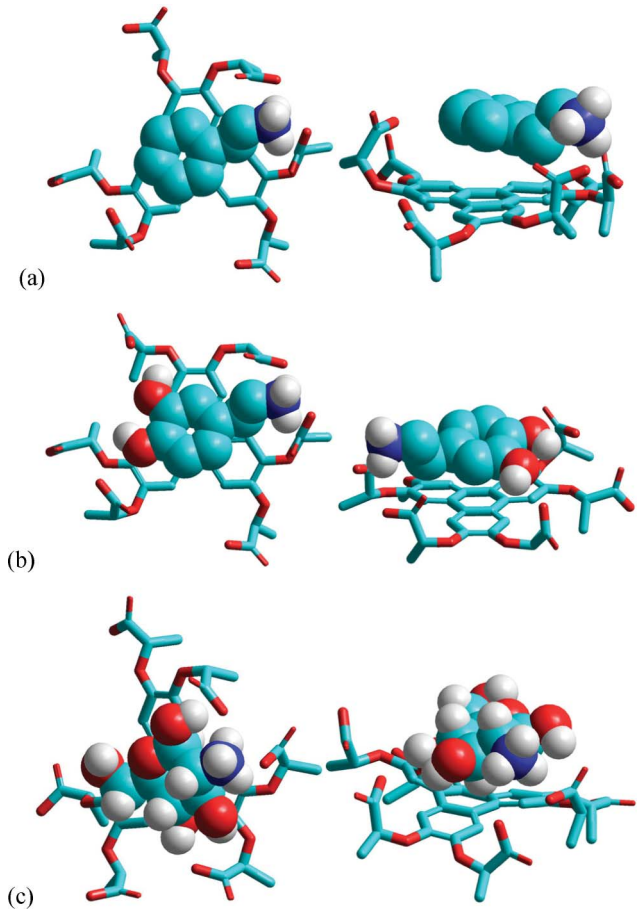

Fig. 1 Energy-minimized structure of the complexes (HyperChem 8.0, AMBER Force field) between receptor (*R*,*R*,*S*,*R*,*S*,*S*)-**1** (stick) and: (a) ammonium **2c** (CPK), (b) dopamine **3a** (CPK), or (c) D-glucosamine **5a** (CPK) in water, top (left) and side (right) views. Water molecules and host's protons were omitted for clarity.

relatively similar structures. Molecular modeling of the complex between dopamine **3a** and **1** in water (Fig. 1b) shows that the guest can sit above the polyaromatic surface of the host (interplane distance = 3.50–3.70 Å), while the ammonium group of the guest interacts with two carboxylate substituents of the host (d = 2.20 and 2.63 Å). Secondary electrostatic interactions (ion–dipole) are seen between each hydroxyl group of the guest and a carboxylate substituent of **1** (d = 2.47 and 2.96 Å). Interestingly, these secondary interactions within the (**1**:**3a**) complex are possible due to the multivalency and the adaptability of the ionic receptor. Thus, the reported results for guests **3** are corroborating that a combination of ionic pairing and hydrophobic effects participate in the binding of amphiphilic ammoniums. Host **1** can then be considered as an attractive receptor for amphiphilic (aromatic) ammoniums in a competitive medium, with a low synthetic cost.

Concerning the selectivity of **1**, no complexation was detected with α-amino acids **4a–d** whereas *C*-protected tryptophan **4e** is recognized, showing a K_{ass} (1 : 1) of 160 M^{-1}. The latter constant is roughly in the same range as the ones measured for guests **3a–f**. Thus, anionic receptor **1** showed selectivity for amphiphilic ammoniums *versus* their corresponding α-amino acids, as predicted from the two-point binding model[12] necessary for the efficient complexation of unprotected amino acids.

In the series of carbohydrates **5** and vitamin C **5e**, receptor **1** only showed affinity towards D-glucosamine **5a**, with an association constant of 87 M^{-1} in a (1 : 1) stoichiometry, thus emphasizing the proposed dual binding principle. This value is close to the one observed with ACh, another aliphatic ammonium. To the best of our knowledge, this is the first study concerning the recognition of carbohydrates by supramolecular receptors conducted in phosphate buffered water.[13] As presented in Fig. 1c, two forces are in action within the (**1**:**5a**) complex: (i) the hydrophobic effects that favourably associate the CH bonds of the carbohydrate with the triphenylene surface (distance C–Hguest/$C_{aromatic}$ host = 2.64–2.99 Å) and, (ii) ionic pairing between the NH_3^+ group and two CO_2^- substituents of **1** (d = 2.67 and 2.82 Å). Moreover, the hydroxyl groups on position 1 and 4 of guest **5a** interact with one oxygen atom of each CO_2^- group involved in ionic pairing (d = 2.35 and 2.39 Å, respectively).

Conclusions

A simple polyaromatic structure revealed attractive binding properties towards amphiphilic ammoniums in phosphate buffered water (pH 7.1). In the presence of receptor **1**, catecholamines and serotonin formed (1 : 1) complexes with binding constants of ca. 200 M^{-1}, whereas D-glucosamine showed a K_{ass} of 90 M^{-1}. Based on ionic pairing combined with hydrophobic effects, the strength of the (host:ammonium) associations depends on the lipophilicity/hydrophilicity of the guest. Such binding of primary ammoniums is noticeable under the reported experimental conditions[4–6] and opens up prospects for further incorporation into functional molecular systems in buffered water. Additionally, in the context of greener host synthesis, polyaromatic receptor **1** allows for complexation with simple molecular tools with straightforward synthetic access.

Experimental part

General procedure for titrations

All solutions were freshly prepared and deuterated water was buffered with Na_2HPO_4 (100 mM). Proton NMR signal monitoring was conducted on guests **2–5**. A solution (250 µL) of host **1** (2 mM) was introduced into each NMR tube (12 to 15 experiments per titration). Increasing aliquots of guest stock solution (~50 mM) were added and the total volume (500 µL) was adjusted with buffered D_2O. Each titration was performed twice at least. The titration data ($\Delta\delta$ *versus* guest concentration) were fitted using the nonlinear curve-fitting procedure. HypNMR2006 program was employed using (1 : 1) binding models.[14] Job plots[15] were used to determine the stoichiometry of the host:guest complexes.

Molecular modeling

The molecular structures of the host and guests were submitted to a geometry optimization at the semi-empirical level (AM1). The resulting structures were subjected to energy minimization in a (1 : 1) (host : guest) stoichiometry, within a periodic box of water molecules (cube edge = 20.73 Å; 280 water molecules), using the molecular mechanics method (AMBER force field) as implemented in the software package HyperChem release 8. Calculations were conducted on three differents stereoisomers

of compound **1**: all-(*R*), all-(*S*) and (*R*,*R*,*S*,*R*,*S*,*S*), and their corresponding complexes. As the results were similar, the details are reported for the latter diastereoisomer (see Fig. 1).

Acknowledgements

This work was supported by the French Ministry of Research (C. G. Fellowship) and the Centre National de la Recherche Française.

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