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Selective recognition of biogenic amine hydrochlorides by heteroditopic dihomooxacalix[4]arenes†

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Ion-pair recognition/complexation of monoamine neurotransmitter and trace amine hydrochlorides takes place—within outstanding efficiency and selectivity—within the π-rich cavity and the pocket formed at the lower rim by the two ureido moieties of tailor-made dihomooxacalixarene receptors 1 and 2. The solid-state structure of 1 is also reported.

The so-called ‘monoamine neurotransmitters’ (e.g. dopamine, histamine, norepinephrine, and serotonin) and ‘trace amines’ (e.g. 2-phenylethylamine and tyramine) are structurally related species that share an arylethylamine moiety as a common structural feature. The ability to selectively detect such target molecules is of enormous significance in medical and clinical research/analysis as these molecule play a key role in the physiological nervous system’s signaling processes, as well as in several psychiatric disorders and neurodegenerative pathologies (e.g., schizophrenia and Parkinson’s disease).1

In recent years, a number of different artificial receptors, based on calixarenes,2 homooxacalix[3]arenes,3 hemicycrophanes4 and other molecular frameworks2 have been prepared, but selective recognition nevertheless remains an open challenge. We have a long-standing interest in the fine-tuning of macrocyclic compounds of different type/size as artificial receptors5 and now, urged by the very recent findings that 18-membered p-tert-butyldihomooxacalix[4]arenes 3b,c can efficiently bind linear alkylammonium substrates, providing a ‘superweak’ counterion is used,7 we wish to report our latest results on the ion-binding properties and selectivities of new heteroditopic dihomooxacalix[4]arenes 1 and 2 towards n-butylammonium halides and a number of biogenic amine hydrochlorides (see Fig. 3). Based on our earlier findings on 3b,c –where the mandatory presence of a very loose anion (such as tetras[3,5-bis(trifluoromethyl)phenyl]-borate8) was required for alkylammonium cation endo-cavity complexation to occur—two of the lower rim alkyl/benzyl moieties of these receptors were replaced9 with ureido pendant groups with the specific aim of obtaining ditopic host molecules.10

Derivatives 1 and 2,9 owing to their additional ureido anionic recognition sites,11 were then expected to simultaneously bind the two ionic counterparts of alkylammonium salts and, as a result, minimize the detrimental ion-pairing effect12 typically encountered in the recognition/binding process of a charged guest by a neutral host.

To verify this hypothesis, 1H NMR titration experiments were carried out by adding increasing amounts of n-butylammonium chloride, bromide and iodide (0.5, 1.0 and 2.0 equiv.) to a 1.0 mM solution of receptors 1 and 2 in CDCl3 to preliminary assess slow ν fast mode of complexation on the NMR timescale. Doubling of all the receptor resonances together with a new set of signals compatible with the presence of a guest molecule bound to the host were observed right from the addition of the first salt aliquot. n-Butylammonium cation inclusion, inside the homooxacalixarene cavity, is unequivocally substantiated by the appearance of the alkyl-chain resonances in the –1.4 to 0.0 ppm region of the 1H NMR spectrum.3a,7,13 Owing to the chiral environment—arising from the asymmetric substitution pattern present at the lower rim of the hosts 1 and 2— the pairs of enantiotopic hydrogen atoms belonging to the α- and β-CH2 groups of the included guest display chemically non-equivalent signals once the alkylammonium ion is firmly anchored within the dihomooxacalixarene cavity.† On the other hand, simultaneous halide binding to the ureido moieties is underlined by the downfield shift observed for all the resonances assigned to the NHs (Fig. 1 and Fig. S4; ESI†). Collectively, these observations are
fully consistent with a slow complexation mode on the NMR timescale. Further addition of salt aliquots caused the disappearance of the free hosts’ resonances.

All host-guest ternary systems under investigation (1.0 mM, 1:1 host/guest salt solutions in CDCl$_3$) displayed very high percentages of complexation ($\geq 95\%$, corresponding to $K_{\text{ass}} > 10^9$ M$^{-2}$), preventing us from reliably calculating the pertinent association constants by $^1$H NMR analysis. The complexation induced shifts (CISs) of the receptor NH$_\text{Ph}$ and the guest $\alpha$- to $\gamma$-CH$_2$ resonances (acting as anion and cation binding probes, respectively), observed upon dihomooxacalix[4]arene/n-BuNH$_2$·HX binding, indicate that halide ions interact with the ureido binding pocket of 1 and 2 in a similar fashion, while the alkylammonium chain, irrespective of its counterion, is accommodated at the same depth inside the macrocycle cavity. Accordingly, the CIS values of the latter probe-resonances do not vary in the presence of different anions, while those of the NH$_\text{Ph}$s decrease with an anion dependent pattern ($I^-$ < Br$^-$ < Cl$^-$) which may suggest a correlation with the halogen electronegativity scale (Table S4; ESI†). The solid-state structure of receptor 1 (Figs 2, S1, S2 and Tables S1–S3; ESI†) provides the means to explain the marked efficiency in the recognition of n-butylammonium halides. Similarly to what has been observed with calix[5]arenes, dihomooxacalix[4]arene 1 possesses a highly preorganized aromatic cavity, ready to accommodate the incoming butylammonium cation. In addition, the proclivity of the ureido moieties to act as anion-binding sites is highlighted by the fact that, even in the absence of guest molecules, they interact with each other, self-assembling into an infinite one-dimensional H-bonded array.

Fig. 2 The solid state structure of dihomooxacalix[4]arene 1, showing the presence of molecules 1 and 11 (depicted in the figure in grey and violet, respectively with the pertinent ring labeling) in the asymmetric unit. Molecules 1 and 11 are seen to adopt similar cone-in conformations with their phenylureido moieties being involved in an infinite array of intra- and intermolecular N-H···O hydrogen bonds.

Having assessed the heteroditopic nature of hosts 1 and 2, we then turned our attention to monoamine neurotransmitters and trace amines (as the corresponding hydrochlorides) 4–10 (Fig. 3).

Fig. 3 Structures of the monoamine neurotransmitters and trace monoamines (tested as the corresponding hydrochlorides): 2-phenylethylamine (Pea·HCl, 4), tyramine (Tyrm·HCl, 5), dopamine (Dopa·HCl, 6), serotonin (Sert·HCl, 7), histamine (Hist·2HCl, 8), norepinephrine (Nore·HCl, 9) and γ-aminobutyric acid (Gaba·HCl, 10).

$^1$H NMR selectivity screening experiments were carried out in a CDCl$_3$/CD$_3$OD solvent mixture (10:1, v/v) to ensure adequate solubility of both host and guest. Preliminary tests, carried out by adding 1 equiv. of ammonium guests 4–7 and 10 to a 1.0 mM solution of the host at 298 K caused a severe broadening of all $^1$H NMR signals, indicating a strong host-guest interaction, but gave no clues as to the mode and/or the stoichiometry, nor to the extent of binding taking place in solution. For a slow –on the NMR timescale– host-guest complexation/decomplexation regime to be fully established, lowering of the temperature to 233 K was necessary and, as a result, analysis of the complexation process became feasible (see Figs 4, S5 and S6 in the case of 1 and Tyrm·HCl; ESI†).
In conclusion, our studies demonstrate that dihomooxacalix[4]arenes 1 and 2 behave as heteroditopic receptors efficiently binding organic ammonium halides in organic media. Their preorganized n-rich cavities mimic the hydrophobic binding pockets of the G protein-coupled receptors, displaying high association constants and a good degree of selectivity towards 2-phenylethylamine, tyramine, dopamine and serotonin neurotransmitters. These features make receptor 1 and 2 promising candidates for the development of sensing devices capable of monitoring the level of biogenic amines in biological fluids.

**Experimental**

**General**

Dihomooxacalix[4]arenes 1 and 2 were available from previous studies. Amine hydrochlorides [4–10] were purchased from Sigma-Aldrich and were used as received.

**1H NMR Complexation Experiments**

All spectra were recorded at 500 MHz at either 298 or 233 K. Percentages of the host-guest ternary complex formation (required for the calculation of the corresponding association constants, \( K_{ass} \)) were determined by direct \(^1\)H NMR integration analysis of the ‘free’ and ‘complexed’ resonances of the guest and/or the host, present at equilibrium, in an equimolar host-guest solution. A set of three different equimolar sample solutions was examined for each \( K_{ass} \) determination. In the case of dihomooxacalix[4]arenes 1 and 2 with \( n\)-BuNH\(_4\)\(\cdot\)X (X = Cl\(_2\), Br\(_2\) and I\(_2\)), where percentages of complexation were found to be ≥ 95%, \( K_{ass} \) values were estimated to be >10\(^8\) M\(^{-2}\). All samples were prepared by mixing together aliquots of stock solutions of host (600 mL) and guest (60 mL) to obtain a final equimolar host-guest solution (1.0 mM). The following stock solutions were used: [1–2] = 1.1 mM in CDCl\(_3\) or CDCl\(_3\)/CD\(_2\)OD (10:1, v/v), [\( n\)-BuNH\(_4\)\(\cdot\)X] = 11.0 mM in CDCl\(_3\) and [4–10] = 11.0 mM in CDCl\(_3\)/CD\(_2\)OD (10:1, v/v). For the assessment of the NH resonance complexation induced shifts a CDCl\(_3\)/CH\(_2\)OH (10:1, v/v) solution of [1] = [Tyrm·HCl] = 1.0 mM was used.

**X-ray crystallography**

Colourless very small single crystals (typical size 0.05 × 0.08 × 0.1 mm) suitable for X-ray analysis by synchrotron radiation were obtained from slow diffusion of CH\(_2\)OH into a CHCl\(_3\) solution of dihomooxacalix[4]arene 1 at room temperature. Data collection was carried out at the X-ray diffraction beamline of the Elettra Synchrotron, Trieste, Italy, employing the rotating-crystal method with the cryo-cooling technique. The crystal dipped in Paratone, as cryoprotectant, was mounted in a loop and flash frozen to 100 K under a stream of nitrogen. Diffraction data were indexed and integrated using MOSFLM\(^{18}\) and scaled with SCALA.\(^{19,20}\)

The structure was solved by direct methods using SIR2011.\(^{21}\) Non-hydrogen atoms were refined by full-matrix least-square methods on \( F^2 \) using SHELXL-13\(^{22}\) with the contribution of H atoms placed at the geometrically calculated positions and refined using the riding model. The crystallographic model is affected by disorder and a detailed description is provided in

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the ESI†. Crystallographic data and refinement details are reported in Table S1, see the ESI†.

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**Notes and references**

†Electronic supplementary information (ESI) available: additional 1H NMR spectra and crystal data for I. CCDC reference number 1009330. See DOI: 10.1039/x0xx00000x

‡Signal assignment follows from COSY experiments (see Fig. S3).

§The asymmetric unit of the triclinic cell contains two crystallographically independent receptor molecules (I and II, as seen in Fig. 2) and a methanol solvent molecule. See the ESI† for a full description.


9. A four-step synthesis of derivatives 1 and 2, –from the parent p-tert-butyldihomooxacalix[4]arene 3a– has just been described. 1 and 2, in agreement with their inherently chiral nature, were found to display distinctive 1H and 13C NMR signals (e.g. the ureido NH resonances; see trace a of Fig. 1 and Fig. 4S) fully compatible with the asymmetric substitution pattern present at the lower rim of their dihomoaxacalix[4]arene framework. See: (a) P. M. Marcos, F. A. Teixeira, M. A. P. Segurado, J. R. Ascenso, R. J. Bernardino, G. Brancatelli and S. Geremia, Tetrahedron, 2014, 70, 6497–6505; (b) P. M. Marcos, F. A. Teixeira, M. A. P. Segurado, J. R. Ascenso, R. J. Bernardino, S. Michel and V. Hubscher-Bruder, J. Org. Chem., 2014, 79, 742–751.


16. Binding of the chloride counterion to the ureido moieties of the host was confirmed, in the case of I and Tyr·HCl, by a parallel complexation experiment carried out in a 1:1 CDCl3/CH3OH solvent mixture at 233 K. In contrast with the case where CD3OD was used as co-solvent, under these conditions, exchange between the ureido and the CH3OH hydrogen atoms does not cause any signal loss and, as a result, CISs of the NH resonances –promoted by anion binding– can be fully appreciated in the 1H NMR spectrum (see Fig. S6). See:
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