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PAPER

Combined study of anion recognition by a carbazole-based neutral tripodal receptor in a competitive environment†

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Anion recognition studies have been carried out on a series of neutral synthetic receptors in which carbazole-2-carboxamide has been used as building block. Different ligands which include one to three carbazole units in their structure have been prepared. Binding experiments have been performed under competitive conditions in DMSO and DMSO–water solutions. The tripodal receptor offered a better host–guest association due to the synergistic effect of a well arranged set of hydrogen bonds. A selective response towards the biologically important pyrophosphate anion has been achieved. This selectivity is enhanced when studies are carried out with an increasing water content, which gets as high as 20% (v/v) in NMR experiments. The significance of this result lies in the use of a neutral receptor which exclusively interacts with the anionic guest through hydrogen bonding. The influence of multiple equilibria in the studied system has been analysed. Several techniques (^{1}H NMR, ^{31}P NMR, mass spectrometry, diffusion-NMR, ITC, absorption and emission spectroscopy) have been employed to get a better understanding of the different processes taking place in solution for the evaluated receptors.

Introduction

Anion recognition and sensing is becoming a well settled discipline in supramolecular chemistry.[1] The important roles that anions play in biological systems and the environment make them very interesting analytical targets.[2] In this regard, the detection of pyrophosphate[3] becomes specially relevant due to its presence as a product of DNA amplification in PCR (polymerase chain reaction) processes,[4] as well as its relation to different pathologies.[5] The design of new synthetic receptors for anionic species utilises a wide range of approaches depending on the driving force for the anion recognition. In the case of pyrophosphate sensing in a highly competitive environment, receptors based on metal coordination complexes, which interact with the anion through Coulombic attractions and strong dative covalent bonds, represent a very efficient approach.[3,6] Additionally, cationic receptors which combine the electrostatic interaction with hydrogen bonding are also quite common.[7] Conversely, it is very rare to find neutral receptors for pyrophosphate whose binding ability is exclusively based on much weaker interactions such as hydrogen bonds,[8] especially in those cases in which aqueous media are involved.[9]

In this context we have synthesised a new family of receptors which incorporate the carbazole unit with different topologies, ranging from a simple 2-carbamoylcarbazole, to a tripodal carbazole-2-carboxamide (Fig. 1). This binding motif defines a cavity where three different types of hydrogen bonding can be established from an amide NH function, a pyrrole-like NH and an aromatic CH. The structural arrangement highlights the utility of the carbazole system in anion supramolecular chemistry both as binding and signalling unit, which has not been extensively used in the design of synthetic receptors.[10] Although several anions were initially tested, our studies finally focused on fluoride, acetate, dihydrogenphosphate and pyrophosphate.[11] In this regard, a stronger interaction has been determined for the pyrophosphate anion, resulting in a highly selective complexation.

Results and discussion

The synthesis of receptors **1**, **2** and **3** was carried out as depicted in Scheme 1. Initially, 4-iodobenzoic acid was nitrated to obtain 4-iodo-3-nitrobenzoic acid, **4**. The corresponding ethyl ester **5** was then isolated by refluxing **4** in ethanol under acidic catalysis. A Suzuki–Miyaura cross-coupling between compound **5** and phenylboronic acid yielded the corresponding biphenyl derivative **6**. The carbazole unit was synthesised through a Cadogan reaction by refluxing **6** with triphenylphosphine in 1,2-

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†Electronic supplementary information (ESI) available: ^{1}H NMR spectra, ^{13}C NMR spectra, titration fitplots, ^{31}P-titration isotherms, mass spectrometry, NOESY spectra, diffusion NMR fitplots, emission spectra and X-ray diffraction data. CCDC 853238. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2ob06868k

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dichlorobenzene.[12] Hydrolysis of the ester function under basic conditions led to the isolation of the desired carbazole 2-carboxylic acid, **8**. The reaction of this acid with benzylamine, *m*-xylylenediamine or 2,4,6-triethyl-1,3,5-tri(aminomethyl) benzene,[13] in the presence of PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate), enabled the isolation of ligands **1**, **2** and **3**, respectively.

All the compounds were characterised by the usual techniques, *i.e.*, ^{1}H NMR, ^{13}C NMR and mass spectrometry. In the case of compound **3**, single crystals could be grown, by slow evaporation from a DMSO solution, suitable for X-ray diffraction analysis. In the crystal, the molecular structure of the receptor shows a conformation where the three bulky carbazole-2-carboxamide groups present different dispositions, with two of them pointing to one side of the plane defined by the benzene core and the third one pointing to the opposite side of that plane (Fig. 2). This unexpected conformation, might be due to a solid state packing pattern influenced by several intramolecular and intermolecular non-covalent interactions. In this regard two unusually short intramolecular C–H···H–C contacts (1.830 and 1.946 Å) between the amide *N*-methylene group and both methylene groups of the ethyl substituents on the benzene ring could be detected. Furthermore, all three carbazole-2-carboxamide units are not planar, but carboxamide–carbazole dihedral angles of 29.4(1), 31.9(3) and 15.5(3)° have been found. This fact could be due to the formation of receptor dimers through intermolecular NH(carboxamide)···O=C hydrogen bonds with two amide groups of one molecule acting as hydrogen bond donors to a O=C bifurcated acceptor of an inversely related molecule (Fig. 2).

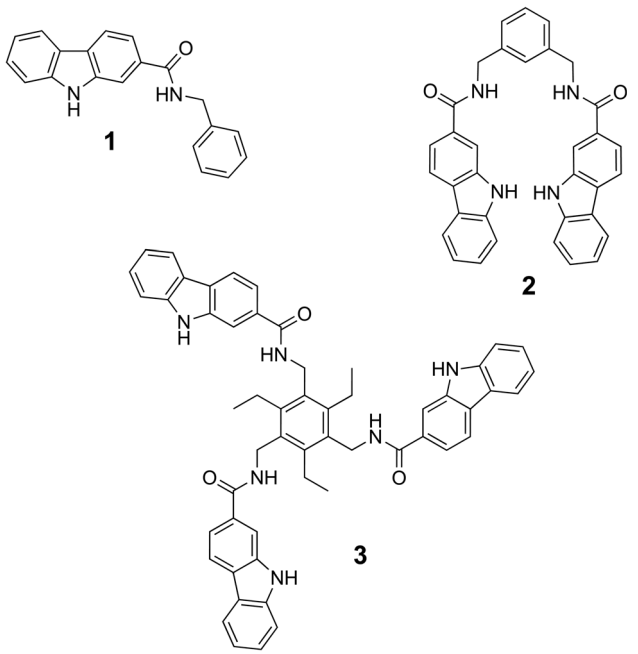
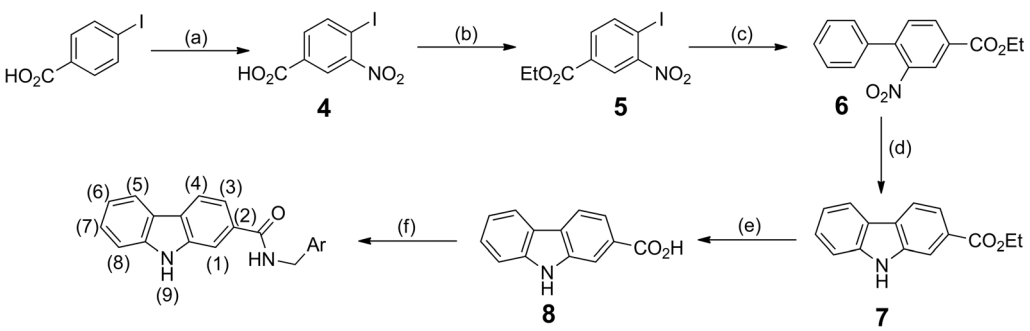


Fig. 1 Structures of carbazole-2-carboxamide receptors.

Initially, anion binding studies were carried out in DMSO-d_6 without any drying treatment.[14] As expected, ligand **1** displayed weaker responses than receptors **2** and **3**. In general, it is worth highlighting that no response was detected for chloride, bromide, hydrogensulfate, nitrate, azide and cyanide anions; carboxylate anions such as acetate and benzoate offered a very weak response in the NMR spectra; the more relevant results were obtained for F^-, $H_2PO_4^-$ and $HP_2O_7^{3-}$.

Studies on compound **1** were performed as a useful set of experiments to evaluate the validity of the carbazole-2-carboxamide binding structure. Thus, when **1** was titrated with fluoride anion the carbazole NH proton shifted downfield, corresponding to a hydrogen bond to the anion, and disappeared after the addition of 1 equivalent of anion. However, no interaction was initially detected for the peaks corresponding to the CH on position 1 of the carbazole and the amide NH, which was displaced to lower chemical shifts from one equivalent of added anion onwards (Fig. 3). The small size of the fluoride anion would explain the absence of bonding to this proton and the sufficiency of the interaction with the carbazole NH. Besides, the observed upfield shift should be due to a shielding effect provoked by the deprotonation of the carbazole by fluoride anion, as has been argued by some other authors.[15,16] Similar upfield shift could also be observed in the ^{1}H NMR spectrum for other protons on the carbazole unit. The deprotonation by fluoride anion could be confirmed by the detection of the HF_2^- triplet at 16.2 ppm. This acid–base interaction was further supported by the titration of **1** with TBAOH, which displays a similar chemical shift pattern for most of the monitored nuclei (see the ESI†).[17]

The fluoride titrations of **2** and **3** (Fig. 3) showed a different evolution. In both cases the carbazole NH disappeared after



Scheme 1 Synthesis of carbazole-2-carboxamides: (a) HNO_3, H_2SO_4; (b) EtOH, H_2SO_4; (c) $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME; (d) PPh_3, *o*-DCB; (e) NaOH, H_2O, EtOH; (f) PyBOP, Et_3N, CH_2Cl_2, benzylamine (**1**), *m*-xylylenediamine (**2**) or 2,4,6-triethyl-1,3,5-tri(aminomethyl)benzene (**3**).

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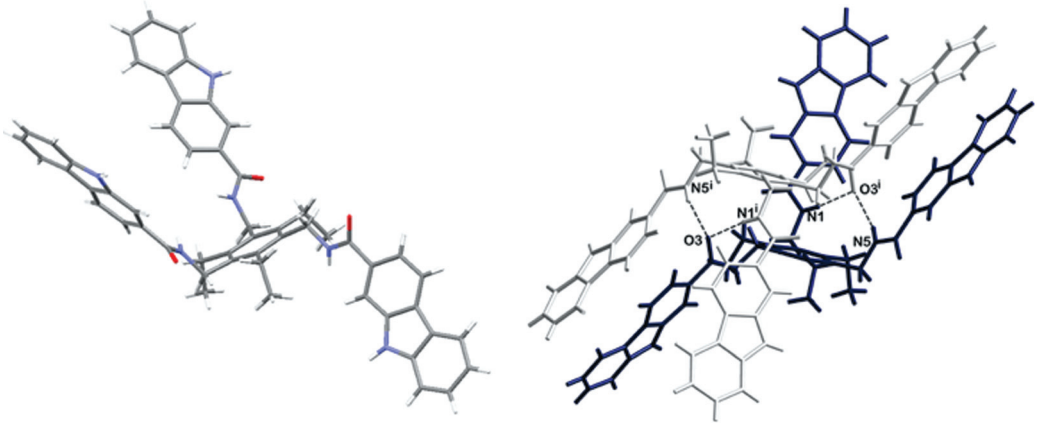

Fig. 2 X-ray structure of receptor **3** (left) and its dimer (right). Solvent molecules have been omitted for the sake of clarity.

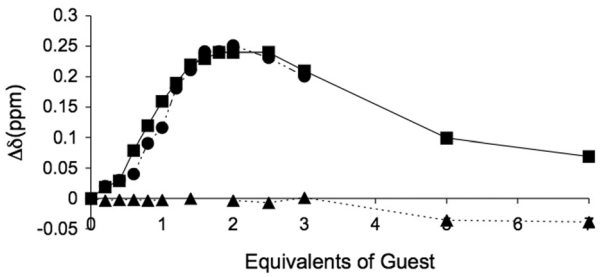

Fig. 3 Evolution of the chemical shifts of the carbazole CH(1) proton of **1** (▲), **2** (■) and **3** (●), 2×10^{-3} M in DMSO-d_6, upon titration with TBAF.

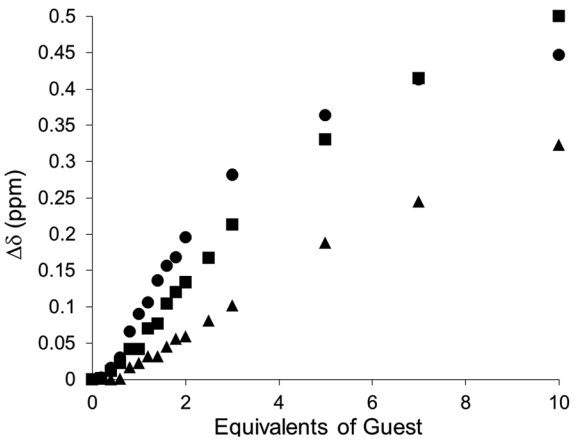

Fig. 4 Evolution of the chemical shifts of the amide NH proton of **1** (▲), **2** (■) and **3** (●), 2×10^{-3} M in DMSO-d_6, upon titration with $TBAH_2PO_4$.

adding the first aliquot of anion. Nevertheless the peak corresponding to CH(1) experienced a downfield shift which reverted to an upfield shift after a certain anion concentration (2–3 equivalents).[18] This behaviour could be related to an initial binding process favoured by the assistance of multiple branches in **2** and **3**, followed by the deprotonation of the carbazole unit.[16] A similar titration profile was also detected for the titration of **2** and **3** with TBAOH (see the ESI†), which would confirm the influence of an acid–base equilibrium during the titration with fluoride anion.

When compound **1** was titrated with the $H_2PO_4^-$ anion a weak binding could be determined. The evolution of the ^{1}H NMR spectra revealed that carbazole NH, CH(1) and amide proton shifted downfield due to hydrogen bonding to the anionic guest (Fig. 4), which contrasts with the result previously discussed for the fluoride anion. The larger size of the $H_2PO_4^-$ anion causes the involvement of all the available hydrogen bond donor functions in the structure of the receptors. A similar titration profile was confirmed for both receptors **2** and **3**, with a stronger binding observed for the tripodal ligand, which has more anchoring points and a better geometrical correspondence with the tetrahedral anionic guest. Regarding the rest of the carbazole signals, they did not experience any significant displacement during the titration experiment.

Experimental data could be fitted to a 1 : 1 model by nonlinear regression. The determined binding constants are summarised in Table 1.[19]

The anion binding equilibrium was also followed by ^{31}P NMR titration for the more stable complex **3**·$H_2PO_4^-$ (see the ESI†). In this case, aliquots of the receptor were added into a solution of the $H_2PO_4^-$ anion, whose chemical shift decreased upon complexation. Reasonably good agreement was found between the binding constants determined by ^{1}H NMR (K = 440 M^{-1}) and ^{31}P NMR (K = 670 M^{-1}) when fitted to a 1 : 1 stoichiometry.

Titration experiments carried out with the $HP_2O_7^{3-}$ anion evidenced a remarkably high affinity towards the carbazole-2-carboxamide unit (Fig. 5). Once again, the studies performed for compound **1** showed an early vanishing of the carbazole NH. The amide NH experienced a downfield shift before disappearing in the proximity of one equivalent of added anion. Interestingly, the peak corresponding to CH(1) did not initially displace much, but between half an equivalent and one equivalent of $HP_2O_7^{3-}$ it moved to a higher chemical shift, describing a sigmoidal titration profile. This result is indicative of CH(1) hydrogen bonding the anion. In agreement with this evolution we could postulate that, due to the size of the $HP_2O_7^{3-}$ anion, this may induce the formation of a 2 : 1 host : guest complex, when working at substoichiometric concentration. Then, as the

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Table 1 Binding constants for a 1 : 1 host : guest model in DMSO-d_6

	1	2	3
AcO^-	55	65	165
$H_2PO_4^-$	14	20	440
$HP_2O_7^{3-}$	—[a]	>10 000	—[a]

[Host] = 2×10^{-3} M, T = 25 °C. Nonlinear regression analysis of the CH(1) signal gave errors <10% in all cases.[a] Experimental data could not be fitted.

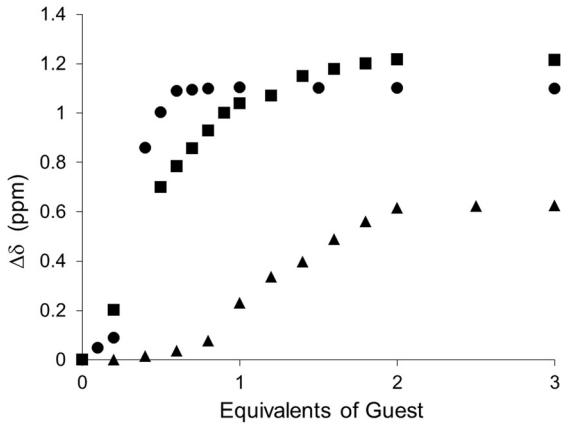

Fig. 5 Evolution of the chemical shifts of the carbazole CH(1) proton of **1** (▲), **2** (■) and **3** (●), 2×10^{-3} M in DMSO-d_6, upon titration with $TBA_3HP_2O_7$.

concentration of the anion increases, the complex rearranges to a 1 : 1 stoichiometry, which would demand more participation from CH(1) proton.

As far as receptor **2** is concerned, the signals of the carbazole and amide NHs disappeared after the addition of 0.2 equivalents of $HP_2O_7^{3-}$ anion. Nevertheless, the latter appeared again after the addition of 0.6 equivalents of anion, at much higher chemical shift, as did the CH(1) proton. Accordingly, the CH(2) proton on the *m*-xylylene spacer also shifts downfield, which confirms its contribution to the anion binding as well as the optimum conformation of the receptor. In general, all the peaks in the ^{1}H NMR spectrum broadened at the initial stage of the titration but sharpened as the titration progressed as a consequence of the displacement of the equilibrium towards the more stable anionic complex. The titration profile could be fitted to a 1 : 1 model. The calculated binding constant shows an extraordinary enhancement of three orders of magnitude for the complexation of $HP_2O_7^{3-}$ anion with respect to the other tested anions, which reinforces the suitability of the carbazole-2-carboxamide binding unit.

In this regard, when receptor **3** was titrated with a solution of $HP_2O_7^{3-}$ anion a different behaviour could be detected (Fig. 6). During the initial part of the titration all the signals broadened and some of them were difficult to follow. Nevertheless, for those detectable signals defining the binding cavity, namely CH(1) and amide NHs, two set of peaks could be detected between 0.3 and 0.7 equivalents. During the course of the titration, one of these groups of signals faded out as the other set of peaks got better defined. With this particular receptor, even the

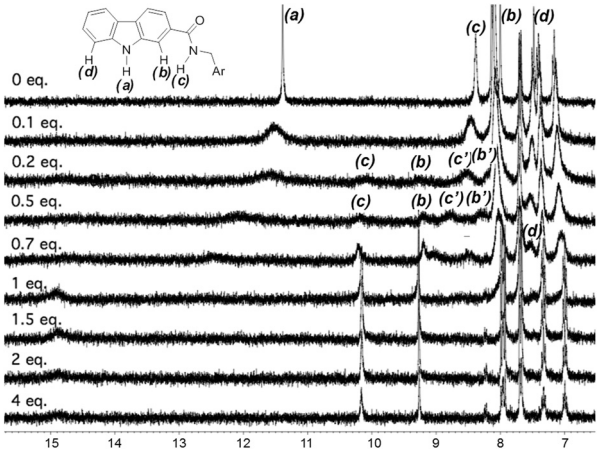

Fig. 6 Evolution of the ^{1}H NMR spectra of receptor **3** upon titration with $TBA_3HP_2O_7$. 1 : 1 complex signals (a, b, c, d). 2 : 1 (H : G) complex signals (b′, c′).

carbazole NH could be observed for some of the intermediate points of the titration experiment. Additionally, it could be noticed that even proton CH(8) on the carbazole unit experienced a downfield shift caused by hydrogen bonding to the large $HP_2O_7^{3-}$ anion.

Although it is not common to resort to CH bonds as hydrogen bond donors, it is evident that a correct orientation of these groups, as is the case for the carbazole system, can render useful and thermodynamically favourable interactions which will contribute to gain efficiency in the molecular recognition processes.

The graphical display of these data (Fig. 5) led us to an interpretation based on the coexistence of two possible complexes with 2 : 1 and 1 : 1 host : guest stoichiometries. Nevertheless, the sigmoidal titration isotherm abruptly reaches the saturation zone and thwarts any possibility of calculating a binding constant. The fact that a mixture of species can be detected, along with the apparent strength of the anion binding, makes the elucidation of the system difficult. However, a qualitative analysis of the titration profile unequivocally correlates to a stronger complexation than the other studied receptors. As expected, the increasing number of carbazole units enhances the $HP_2O_7^{3-}$ anion recognition due to a better size correspondence between the receptor and the guest, as well as the higher charge of this anionic guest.

In order to get further evidence of the processes taking place in solution, the system (**3** + $HP_2O_7^{3-}$) was studied in more detail by isothermal titration calorimetry (Fig. 7).

The plot of the thermodynamic data displays a complex pattern, which cannot be fitted to any binding model but reinforces our previous observation, which denotes the occurrence of multiple equilibria. The initial part of the experiment shows an endothermic drop[20] which is followed by two exothermic processes. This titration profile presents two inflection points at 0.5 and 1 guest–host molar ratio, respectively, which would confirm the evolution from a 2 : 1 to a 1 : 1 host : guest complex.

Additionally, NOESY experiments performed in the absence and the presence of $HP_2O_7^{3-}$ anion unveiled the induced fit effect of the anion (see the ESI†). At this point, it is worth mentioning that the NMR spectra of the free receptor **3** showed a

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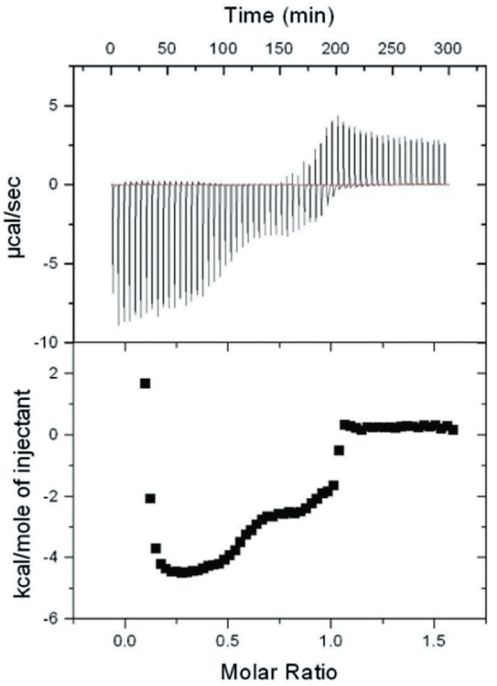

Fig. 7 ITC enthalpogram of receptor **3**, 2×10^{-3} M in DMSO, upon addition of $TBA_3HP_2O_7$. The heats of dilution were subtracted from the raw data after a blank experiment.

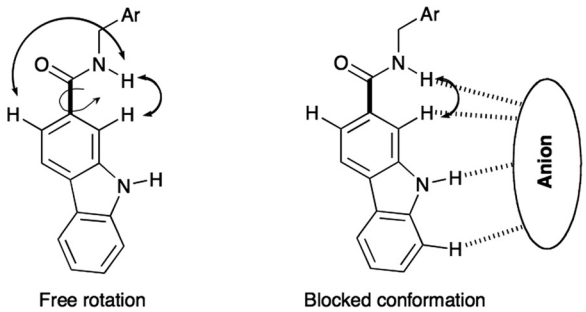

Fig. 8 NOEs detected in receptor **3** for the amide NH proton in the absence (left) and the presence (right) of $HP_2O_7^{3-}$ anion.

single set of signals, which proves the magnetic equivalence of all the appended carbazole units in solution at room temperature. This differs from what was observed in the X-ray analysis of **3**. Interestingly, 2D-NOE spectra of **3** showed how the amide NH proton presented a NOE with the protons H(1) and H(3) on the carbazole. This confirms the rotation of the carbazole unit around the C(2)–CO sigma bond. When one equivalent of $HP_2O_7^{3-}$ anion was added the most significant change corresponded to the amide NH showing a NOE with proton H(1) of the carbazole only (Fig. 8).

Thus, we could conclude that upon $HP_2O_7^{3-}$ complexation the anion sitting in the cavity of the receptor blocks its conformational freedom and makes the carbazole units point inwards, which improves the binding ability of the tripodal receptor **3**.

A ^{31}P NMR titration was carried out, where a solution of receptor **3** was added over a solution of $TBA_3HP_2O_7$. Only one phosphorus signal could be followed during the experiment. The

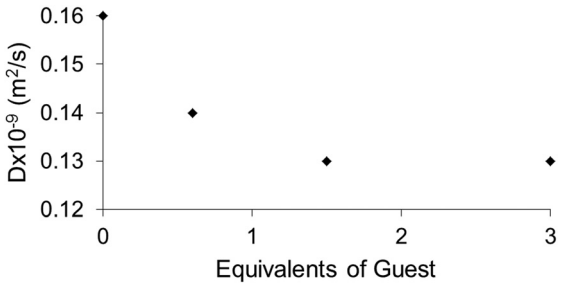

Fig. 9 Evolution of the diffusion coefficient with increasing $HP_2O_7^{3-}$ anion concentration.

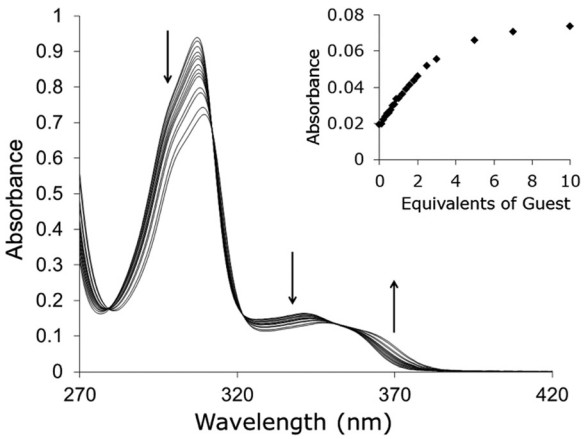

Fig. 10 Absorption spectra of receptor **3**, 2×10^{-5} M in DMSO, upon titration with $HP_2O_7^{3-}$ anion. Inset: titration profile at 370 nm.

titration profile clearly describes a sigmoidal curve, which again should be interpreted as a result of a multiple equilibria system, and is sharply differentiated from the result previously obtained for the ^{31}P NMR titration with $H_2PO_4^-$ anion, which fitted well to a 1 : 1 binding model (see the ESI†).

When the addition of aliquots of $HP_2O_7^{3-}$ anion to a solution of **3** was monitored by mass spectrometry, a peak corresponding to a 1 : 1 complex was detected (see the ESI†).[21]

Furthermore, diffusion NMR studies were performed to gain a better understanding of the speciation of the system. The diffusion coefficient of **3** (0.16×10^{-9} m^2 s^{-1}) was calculated by following the attenuation of the carbazole signals. A decrease in this coefficient was determined after the addition of aliquots of the $HP_2O_7^{3-}$ anion, as a result of the receptor **3** swelling up to accommodate the anionic guest (Fig. 9). The evolution of the experiment showed a drop in the value of the diffusion coefficient which did not cease until the addition of one equivalent of anion. This result, in agreement with those obtained previously from the different techniques discussed above, indicates that, within the concentration range of 10^{-3} M, the species in equilibrium evolve towards the formation of a 1 : 1 complex.[22]

The presence of carbazole as part of the tripodal receptor allows the analysis of the anion complexation by following the changes by absorption and emission spectroscopy.[23] The absorption spectrum of **3** resembled that of the carbazole unit in DMSO solution, showing an intense band at 307 nm and a weaker band at 341 nm with a shoulder at 358 nm (Fig. 10).

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The addition of anion caused a general decrease in the absorbance accompanied by a red shift of the bands. These changes in the spectra were evident for the $HP_2O_7^{3-}$ anion. However, the changes induced by $H_2PO_4^-$, F^- or AcO^- anions were irrelevant. Therefore, the dilution of the host enhanced the selectivity of **3** towards $HP_2O_7^{3-}$ anion. Under these diluted conditions the possible 2 : 1 host : guest complex does not seem to manifest itself since its concentration is negligible. Moreover, the presence of four clearly resolved isosbestic points at 280, 312, 322 and 351 nm supports the prevalence of a 1 : 1 host : guest equilibrium. Accordingly, the titration profile could be fitted to a 1 : 1 model with a binding constant value $K = 1.8 \times 10^4$ M^{-1}.[24]

The inherently higher sensitivity of emission spectroscopy represents an important advantage of this technique within the area of molecular recognition. Consequently, fluorescence titrations were performed for receptor **3** with the above mentioned anions. As occurred in previous experiments only the pyrophosphate anion offered a good response, with the rest of the tested anions causing marginal changes in the emission spectrum (Fig. 11) (see the ESI†).

The fluorescence spectrum has a band at 389 nm with a subtle shoulder at 380 nm. A quenching in the emission intensity could be clearly observed for $HP_2O_7^{3-}$ (Fig. 12) which, in agreement with the UV-vis results, fitted well to a 1 : 1 binding model (K = 1.5 × 10^4 M^{-1}). Curiously, an incipient growth of the fluorescence could be detected in the range of 450–550 nm which is responsible for an analytically useful colour change in the fluorescence of the receptor.

This made us question the possibility of having a deprotonation process interfering with the anion complexation equilibrium, perhaps facilitated by the diluted conditions of the experiment.[25,26] Thus, control experiments were then carried out by titrating TBAOH into a solution of **3**. The effect of the addition of a strong base was a fluorescence quenching during the initial stage of the titration followed by the complete disappearance of the original spectrum and the emergence of the emission from a new species. This new spectrum has an emission maximum at 493 nm which differs from the spectrum resulting from the presence of $HP_2O_7^{3-}$ anion in large excess, whose emission maximum is at 463 nm (Fig. 13). Therefore, this rules out the possibility of interpreting the response towards $HP_2O_7^{3-}$ as a mere deprotonation process and indicates that the recognition of the anion is the dominant process taking place in solution.[27]

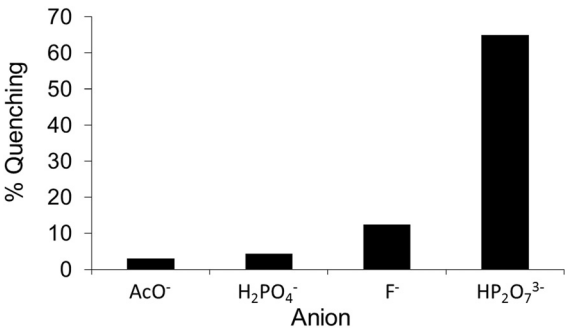

Fig. 11 Ratio of emission quenching of receptor **3**, 2 × 10^{-5} M in DMSO, in the presence of 10 equivalents of different anions.

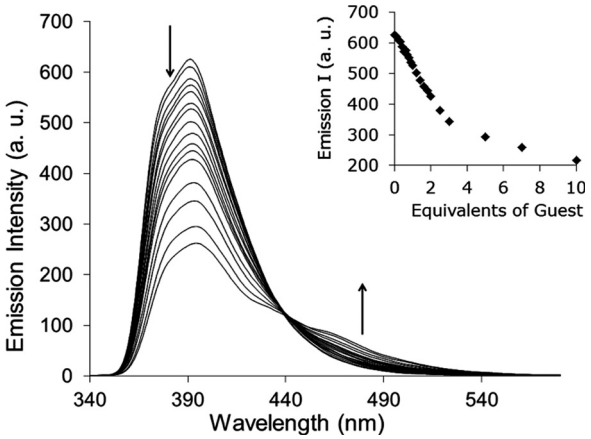

Fig. 12 Emission spectra of receptor **3**, 2 × 10^{-5} M in DMSO, upon titration with $HP_2O_7^{3-}$ anion. λ_{exc} = 300 nm. Inset: titration profile at 391 nm.

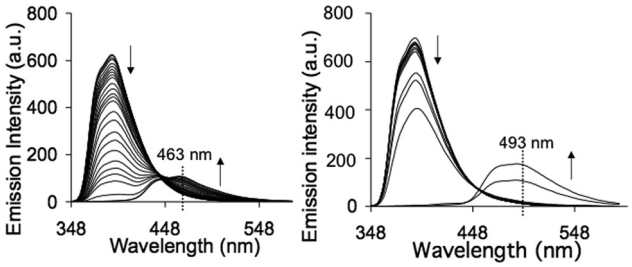

Fig. 13 Emission spectra of receptor **3**, 2 × 10^{-5} M in DMSO, upon titration with (left) $HP_2O_7^{3-}$ (0–50 equivalents) and (right) OH^- (0–20 equivalents).

After having checked the selectivity of **3** towards $HP_2O_7^{3-}$ anion in DMSO solution we performed experiments under more competitive conditions. Initially, the addition of five equivalents of dihydrogenphosphate, acetate and benzoate to a solution of **3** in DMSO did not produce important changes in the ^1H NMR spectrum as has been previously discussed. Upon titration of this mixture with $HP_2O_7^{3-}$ anion a noticeable modification of the chemical shifts was recorded (see the ESI†). The resulting titration curve was interpreted as a piece of evidence of the selective recognition of $HP_2O_7^{3-}$ anion.

Additionally, an evaluation of the increase in the water content of a solution of the tripodal host was performed (Fig. 14). As the concentration of water goes from 0.1 to 20% a perfectly readable response is obtained upon titration with $HP_2O_7^{3-}$ anion. The abrupt change, previously observed for the titration isotherm, became more rounded as the percentage of water increased. Simultaneously, the initial slope decreases its angle and the isotherm tends to reach a plateau at a higher guest–host ratio. All these changes indicate that upon increasing the amount of highly competitive water it is more difficult to displace the complexation equilibrium towards the formation of the anionic complex. However, even in such a competitive medium (DMSO–water, 20% v/v), the experimental data still reveal a binding process.

Similar studies were performed by fluorescence spectroscopy (Fig. 15). An appreciable emission quenching was still detected

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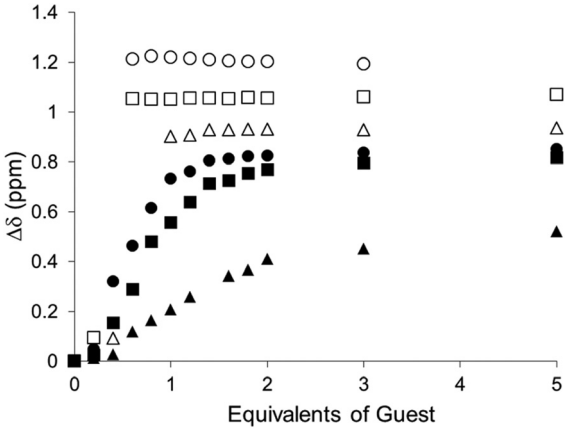

Fig. 14 Evolution of the chemical shifts of the carbazole CH(1) proton of **3** upon titration with $TBA_3HP_2O_7$ in DMSO-d_6 with 0.1% (○), 1% (□), 5% (△), 10% (●), 15% (■) and 20% (▲) water content (v/v).

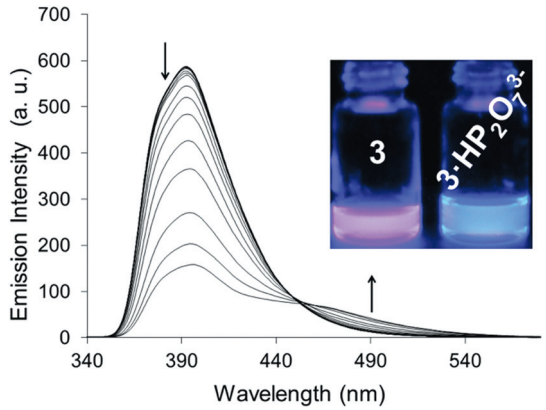

Fig. 15 Emission spectra of receptor **3**, 2×10^{-5} M in DMSO–water 5%, upon titration with $HP_2O_7^{3-}$ anion. λ_{exc} = 300 nm. Inset: fluorescence colour change upon complexation of $HP_2O_7^{3-}$ anion.

for the titration of **3** with $HP_2O_7^{3-}$ anion in DMSO–water 5% (v/v).

The higher dilution of the experiment increases the competitiveness of water which weakens the interaction with the anion to obtain a 1 : 1 complex (K = 145 M^{-1}). Nevertheless, the change in the colour of the fluorescence, from the pink free receptor to the blue anionic complex, makes the response of **3** a good analytical signal towards pyrophosphate anion in aqueous environment.

Conclusions

In summary, we have synthesised a new family of carbazole-2-carboxamide derivatives. Receptors bearing two and three carbazole units display a high selectivity towards $HP_2O_7^{3-}$ anion in DMSO solution. Receptor **3** shows a multiple equilibria behaviour under more concentrated conditions (10^{-3} M) which has been demonstrated by ^{1}H NMR, ^{31}P NMR and ITC experiments. The binding model simplifies to a 1 : 1 model and enhances its selectivity when diluted (10^{-5} M) as has been demonstrated by absorption and emission spectroscopy. The selective response for $HP_2O_7^{3-}$ anion is exclusive of receptor **3** when studies are performed under more competitive conditions such as the presence of other interfering anions or the study in aqueous environment. To conclude, the tripodal receptor **3** represents one of the few neutral receptors with selectivity towards $HP_2O_7^{3-}$ anion.

Experimental

General remarks

Reagents used as starting materials were commercially available and were used without further purification. Solvents were dried following the usual protocols (THF, Et_2O and toluene were distilled from sodium wire with benzophenone indicator; CH_3CN and CH_2Cl_2 were distilled from $CaCl_2$; EtOH and MeOH were distilled from magnesium and stored with molecular sieves). Unless stated otherwise, all reactions were carried out under nitrogen atmosphere. Column chromatography was run with silica gel 60 A CC 70–200 μm as stationary phase and using HPLC grade solvents. Melting points were measured in a Reichert instrument and are not corrected. ^{1}H NMR, ^{13}C NMR, NOE and NOESY experiments were recorded on either a Bruker AV300 instrument, or a Bruker AV400 instrument. Chemical shifts are referred to the residual peak of the solvent. In the experimental data "bp" stands for broad peak and "Cq" for quaternary carbon atom. Mass spectrometry was recorded on a HPLC-MS TOF 6220 instrument. Absorption spectra were recorded on a Cary 500 UV-vis-NIR spectrophotometer. Emission spectra were recorded on a Cary Eclipse spectrophotometer. Isothermal titration calorimetry experiments were run in a Microcal VP-ITC microcalorimeter and data were analysed using Origin software. X-ray structure determination was measured at 120(2) K using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) and ω scan mode on a Oxford Diffraction SuperNova diffractometer. 33 146 reflections were collected of which 10 672 were independent. The structure was solved by direct methods and all non-hydrogen atoms refined anisotropically on F^2 (SHELXS-97 and SHELXL-97, G.M. Sheldrick, University of Göttingen, 1997). Pulse gradient spin echo (PGSE) diffusion measurements were performed on a Bruker AV600 MHz spectrometer equipped with a HR Z-gradient BBO probe and using the standard *ledbpgp2s* pulse program from Bruker Topspin software (using a stimulated echo and longitudinal eddy current delay, with bipolar gradient pulses and two spoiling gradients). Sine shaped gradients were used and the measurements were recorded with 16k time domain data points in t_2 dimension and 16 t_1 increments, 24 transients for each t_1 increment and a relaxation delay of 5 s. The gradient length (δ = 2*P30) was 2.5 ms with diffusion time Δ (D20) = 70 ms. The D-values were determined following the integral decay of the carbazole signals.

4-Iodo-3-nitrobenzoic acid (4)

To a round bottomed flask containing 4-iodobenzoic acid (6 g, 24.19 mmol) was sequentially added HNO_3 65% (50 mL) and H_2SO_4 96% (50 mL). Orange vapours immediately evolved. The reaction was stirred at room temperature for 5 h. Afterwards, the crude was poured onto crushed ice to obtain a yellow precipitate. The solid was filtered, washed with water (3 × 20 mL) and dried

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in air to isolate the expected pure product (7 g, 98%). M. p. 214–215 °C; ^{1}H NMR (300 MHz, DMSO-d_6); δ (ppm) 8.31 (s, 1H), 8.25 (d, 1H, J = 8.1 Hz), 7.85 (d, 1H, J = 8.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6); δ (ppm) 165.1 (C═O), 153.2 (C_q), 141.8 (CH), 133.3 (CH), 132.0 (C_q), 125.0 (CH), 94.0 (C_q).

Ethyl 4-iodo-3-nitrobenzoate (5)

4-Iodo-3-nitrobenzoic acid, **4** (7.0 g, 23.9 mmol), was dissolved in ethanol (150 mL) and 1 mL of concentrated sulfuric acid was added. The solution was refluxed overnight. Then the reaction was allowed to cool down and the solvent was removed by rotary evaporation. The crude was dissolved in ethyl acetate (80 mL) and washed with a saturated solution of $NaHCO_3$ (3 × 50 mL). The organic extracts were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. A yellow solid was obtained which corresponds to the analytically pure ester (6.7 g, 87%). M.p. 88–89 °C; ^{1}H NMR (200 MHz, $CDCl_3$); δ (ppm) 8.44 (d, 1H, J = 1.8 Hz), 8.14 (d, 1H, J = 8.2 Hz), 7.88 (dd, 1H, J_1 = 2 Hz, J_2 = 8.2 Hz), 4.42 (q, 2H, J = 7.2 Hz), 1.41 (t, 3H, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$); δ (ppm): 164.1 (C═O), 153.0 (C_q), 142.3 (CH), 133.4 (CH), 132.0 (C_q), 126.0 (CH), 92.0 (C_q), 62.1 (CH_2), 14.2 (CH_3).

Ethyl 4-phenyl-3-nitrobenzoate (6)

A solution of ethyl 4-iodo-3-nitrobenzoate (1.00 g, 4.120 mmol) in dimethoxyethane (40 mL) was prepared in a 100 mL round bottomed flask equipped with a reflux condenser. This solution was deoxygenated by bubbling dry nitrogen for 15 minutes. $Pd(PPh_3)_4$ (0.11 g, 0.095 mmol), phenylboronic acid (0.57 g, 4.670 mmol) and aqueous Na_2CO_3 2 M were sequentially added. The mixture was refluxed for 18 h. After this time the reaction was allowed to cool to room temperature and was quenched by adding 50 mL of HCl (10%). Then the mixture was extracted with CH_2Cl_2 (4 × 50 mL). The organic extracts were combined, dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to obtain a yellow oil. This crude was purified by column chromatography (CH_2Cl_2 : n-hexane, 1 : 1) to isolate the desired product as a yellow solid (0.335 g, 83%). M. p. 63–64 °C; ^{1}H NMR (300 MHz, $CDCl_3$); δ (ppm) 8.28 (d, 1H, J = 0.6 Hz), 8.26 (dt, 1H, J_1 = 0.6 Hz, J_2 = 7.9 Hz), 7.54 (d, 1H, J = 8.1 Hz), 7.46–7.44 (m, 3H), 7.35–7.32 (m, 2H), 4.45 (q, 2H, J = 6.9 Hz), 1.44 (t, 3H, J = 7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$); δ (ppm) 164.4 (C═O), 149.2 (C_q), 140.2 (C_q), 136.4 (C_q), 132.7 (CH), 132.1 (CH), 130.7 (C_q), 128.8 (CH), 127.7 (CH), 125.1 (CH), 61.8 (CH_2), 14.2 (CH_3); HR-MS *m/z*: Calcd ($C_{15}H_{15}NO_5$, [M + 18]): 289.0950, Found: 289.1185.

Ethyl carbazole-2-carboxylate (7)

(0.323 g, 91%). M.p. 182–183 °C; ^{1}H NMR (200 MHz, $CDCl_3$); δ (ppm) 8.27 (s, 1H, br), 8.18–8.09 (m, 3H), 7.95 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.2 Hz), 7.49–7.47 (m, 2H), 7.34–7.23 (m, 1H, overlapped with solvent), 4.44 (q, 2H, J = 7.2 Hz), 1.45 (t, 3H, J = 7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$); δ (ppm) 167.3 (C═O), 140.7 (C_q), 138.8 (C_q), 127.6 (C_q), 127.2 (CH), 127.0 (C_q), 122.5 (C_q), 121.1 (CH), 120.6 (CH), 119.9 (2 × CH), 112.4 (CH), 110.9 (CH), 61.0 (CH_2), 14.4 (CH_3); HR-MS *m/z*: Calcd ($C_{15}H_{14}NO_2$, [M + H]): 240.1024, Found: 240.1021.

Carbazole-2-carboxylic acid (8)

Ethyl carbazole-2-carboxylate (0.50 g, 2.1 mmol) was placed in a round bottomed flask with 50 mL of ethanol. A solution of NaOH (0.72 g, 31.3 mmol) in water (10 mL) was added to obtain a clear solution whose colour changed from yellow to orange. The reaction was refluxed for 16 h. During this time a precipitate formed. The reaction was quenched by the addition of HCl 15% (150 mL) and stirred for 1 h. A pale yellow precipitate formed. This solid was filtered, washed with water (3 × 15 mL) and dried in the air to isolate the pure product (0.44 g, 99%). M.p. >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6); δ (ppm) 12.78 (s, 1H, br), 11.50 (s, 1H), 8.20–8.17 (m, 2H), 8.08 (s, 1H), 7.75 (d, 1H, J = 8 Hz), 7.467–7.431 (m, 2H), 7.19 (t, 1H, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO-d_6); δ (ppm): 168.0 (C═O), 140.9 (C_q), 139.0 (C_q), 127.5 (C_q), 126.8 (CH), 125.8 (C_q), 121.6 (C_q), 121.0 (CH), 119.9 (CH), 119.4 (CH), 119.0 (CH), 112.4 (CH), 111.3 (CH); HR-MS *m/z*: Calcd ($C_{13}H_{10}NO_2$, [M + 1]): 212.0711, Found: 212.0697.

N-Benzyl carbazole-2-carboxamide (1)

To a slurry of carbazole-2-carboxylic acid (0.20 g, 1 mmol) and PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) (1.56 g, 3 mmol) in CH_2Cl_2 (35 mL), triethylamine (0.4 mL, 3 mmol) was added to get a clear solution. This mixture was stirred at room temperature for 2 h. Then a solution of benzylamine (0.4 mL, 3 mmol) in CH_2Cl_2 (10 mL) was added dropwise and the reaction was stirred overnight. A white precipitate was formed during the course of the reaction. This was filtered, washed with water (3 × 15 mL) and dried in the air to obtain the pure product (0.18 g, 60%). M.p. 210–211 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 11.46 (s, 1H), 9.09 (t, 1H, J = 5.8 Hz), 8.19–8.14 (m, 2H), 8.03 (d, 1H, J = 0.8 Hz), 7.71 (dd, 1H, J_1 = 1.4 Hz, J_2 = 8.2 Hz), 7.54–7.10 (m, 8H), 4.52 (d, 2H, J = 6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ (ppm) 166.9 (C═O), 140.8 (C_q), 140.0 (C_q), 139.2 (C_q), 131.6 (C_q), 128.5 (CH), 128.3 (2 × CH), 127.2 (CH), 126.7 (CH), 126.4 (CH), 124.7 (C_q), 121.8 (C_q), 120.8 (CH), 119.8 (CH), 118.9 (CH), 117.7 (CH), 111.2 (CH), 110.5 (CH), 42.8 (CH_2); HR-MS-ES *m/z* Calcd ($C_{20}H_{17}N_2O$, [M + 1]): 301.1341, Found: 301.1342.

N,*N*′-(1,3-Phenylenebis(methylene))bis(carbazole-2-carboxamide) (2)

This compound was synthesised in a 40% yield using the same methodology as described for **1**. M.p. >300 °C; ^{1}H NMR (600 MHz, DMSO-d_6); δ (ppm) 11.43 (s, 2H), 9.11 (m, 2H), 8.10–8.07 (m, 4H), 8.02 (s, 2H), 7.68 (dd, 2H, J_1 = 1.2 Hz, J_2 = 8.4 Hz), 7.51 (d, 2H, J = 8.4 Hz), 7.42 (td, 2H, J_1 = 0.6 Hz, J_2 = 7.2 Hz), 7.36 (s, 1H), 7.316–7.291 (m, 1H), 7.24 (d, 2H, J = 7.8 Hz), 7.17 (t, 2H, J = 7.2 Hz), 4.52 (d, 4H, J = 5.4 Hz); ^{13}C NMR (50 MHz, DMSO-d_6); δ (ppm) 166.8 (C═O), 140.8 (C_q), 140.0 (C_q), 139.2 (C_q), 131.6 (C_q), 128.3 (CH), 126.4 (CH),

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126.0 (CH), 125.7 (CH), 124.6 (C_q), 121.8 (C_q), 120.8 (CH), 119.7 (CH), 118.9 (CH), 117.6 (CH), 111.2 (CH), 110.4 (CH), 42.7 (CH_2); HR-MS *m/z*: Calcd ($C_{34}H_{27}N_4O_2$, [M + 1]): 523.2134, Found: 523.2190.

N,N′,N″-(2,4,6-Triethylbenzene-1,3,5-triyl)tris(methylene)-tris-(carbazole-2-carboxamide) (3)

This compound was synthesised in a 12% yield using the same methodology as described for **1**. M.p. >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6); δ (ppm) 11.38 (s, 3H), 8.37 (bt, 3H), 8.14–8.11 (m, 6H), 8.00 (s, 3H), 7.69 (dd, 3H, J_1 = 1.6 Hz, J_2 = 8.6 Hz), 7.49 (d, 3H, J = 8 Hz), 7.41 (td, 3H, J_1 = 1.2 Hz, J_2 = 8 Hz), 7.16 (t, 3H, J = 7 Hz), 4.64 (d, 6H, J = 3.6 Hz), 2.94 (q, 6H, J = 7.2 Hz), 1.19 (t, 9H, J = 6.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6); δ (ppm) 166.8 (C═O), 143.8 (C_q), 140.7 (C_q), 139.1 (C_q), 132.2 (C_q), 131.6 (C_q), 126.4 (CH), 124.5 (C_q), 121.8 (C_q), 120.8 (CH), 119.6 (CH), 118.8 (CH), 117.9 (CH), 111.2 (CH), 110.6 (CH), 38.1 (CH_2), 22.8 (CH_2), 16.3 (CH_3);. HR-MS *m/z*: Calcd ($C_{54}H_{49}N_6O_3$, [M + 1]): 829.3866, Found: 829.3858.

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20 The initial endothermic evolution in the calorimetry experiment might be due to a preorganisation of the tripodal receptor to accommodate the anion afterwards.

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22 Although a 2 : 1 host : guest complex can be part of the multiple equilibrium, its ephemeral contribution (Fig. 6) only permits the diffusion coefficient to be measured by following the attenuation of signals corresponding to the 1 : 1 complex. This would explain the continuous decrease of the diffusion coefficient value.

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