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Macrocyclic *versus* open-chain carbazole receptors for carboxylate binding†

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The anion recognition properties of six synthetic acyclic and macrocyclic carbazole-based receptors have been studied by ^{1}H-NMR as well as with COSMO-RS calculations towards acetate, benzoate, lactate, sorbate and formate. The receptors differed by the number and geometry of hydrogen-bond donor (HBD) sites, the nature and length of the linker(s) between the HBD sites and the cyclic or non-cyclic nature. The binding ability of the receptors is strongly influenced by the structure and steric variables of the receptors and anions. It was found that when urea was replaced with the flexible diglycolyl as the connecting linker between carbazole subunits, the carboxylate binding affinity of the receptor decreased significantly. The effects of the receptors' structure on anion binding have been investigated and several intriguing cases have been identified and analysed. The current findings shed light on carboxylate anion binding and contribute to the systematic synthesis of receptors with beneficial functional selectivity for carboxylate anions.

Introduction

The development of synthetic (*i.e.* not biological) hosts[1–4] to recognize and sense cations, anions, and neutral molecules has developed into a prominent area of research on the borderline of organic, physical and analytical chemistry. The fundamental role of anions in environmental and biological processes necessitates the development of receptor molecules capable of sensing anions.[5–8] On the other hand, using receptor molecules or sensors, it is more difficult to sense and recognize anions than cations.[9] For these reasons, significant efforts have been put into developing synthetic receptors for anion detection and sensing. As a result, numerous examples of anion receptors have been developed.[4,10–16]

In particular, carboxylate anions are of paramount interest as target species in molecular recognition due to their ubiquitous presence in nature and their many biological roles.[17] Amino acids, enzymes, antibodies, and metabolic intermediates, as well as other natural products, possess a variety of carboxylate functionalities that are responsible for their specific biochemical behaviour[18] Many anion-binding motifs have been explored.[8,19–25] Most commonly contain (thio)urea,[14,24] imidazolium cations,[26] guanidinium ions,[20,21] *etc.* as potential binders of carboxylates.[10] These binding motifs have been used in a myriad of receptors of different complexities.[4,5,24,27–30] For example, Liu *et al.* have reported the development of a cholic-acid-based fluorescent receptor, exploiting the thiourea motif that is capable of binding anions through hydrogen bonding (HB) interactions.[24,31] Moreno-Valle *et al.* successfully synthesized a new group of receptors containing the urea and benzamide groups.[5] It has been found that pyrrole with the more acidic NH has higher hydrogen-binding potential. As can be concluded from the pK_a values (in DMSO) of pyrrole (23.0), indole (20.9) and carbazole (19.9),[32] the acidity of the pyrrole ring can be increased not only by attaching electron-withdrawing groups but also by conjugation with the benzene ring.[33] The groups of Jurczak,[34] Sessler,[35] and Gale[36,37] and our group[38–40] have used the carbazole ring as an effective component of anion receptors. The carbazole ring is used not only because of its higher acidity but also for its rigidity. It has been demonstrated that carbazole's rigid skeleton enables the introduction of various functionalities, which makes it an ideal moiety for the design of anion receptors.[34–40]

As can be seen from the literature, the most common interaction in anion–receptor binding is hydrogen bonding (HB) between the HB donor groups of the receptor and the anionic centre(s) of the anion.[38] However, HB alone does not enable satisfactory selectivity.[38,41,42] Variations in the hydrocarbon moiety or additional functional groups have a strong impact on the properties of carboxylate anions.[41,42] In the case of

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simple carboxylates, there is a strong relation between binding affinity and anion basicity.[42,43] But again, this alone is insufficient for useful discrimination between different carboxylates.

In order to improve selectivity, additional interactions are necessary, which can be achieved by host shape manipulation so that guests of certain shapes/geometries would be preferred.[22,44,45] Available evidence suggests that the most promising candidates for high-affinity and selective receptors are macrocyclic and/or cage-like molecules because they provide possibilities for interacting with all parts of the anion. Moreover, in aqueous media, binding of the anion may enable displacement of the so-called high-energy water from the binding cavity, which greatly enhances binding.[46–48] In spite of the considerable efforts made to create anion receptors, only a limited number of macrocyclic or cage-like structures are in use. In addition, some of them, *e.g.* the bambusurils[49] developed by the Sindelar[49] group, while being highly efficient in binding some anions, are available in limited size/shape varieties of the binding cavity, limiting the possibilities of tuning selectivity.

This study is a continuation of our ongoing research[38,39,50,51] that aims at increasing the selectivity of synthetic receptors towards common carboxylate anions. We recently investigated[39] the possibility of differentiating between carboxylates using biscarbazolylurea based macrocycles of different sizes featuring aliphatic chains of different lengths as the linkers for closing the binding cavity. It was demonstrated that varying just the ring size alone is of limited use in achieving selectivity. In this study we have created a family of hitherto unknown carbazolyl-urea-based acyclic and macrocyclic anion receptor molecules with different HBD sites and different ring chemistry (Fig. 1) for carboxylate recognition. Their synthesis and binding affinities towards different carboxylates are described.

Results and discussion

Synthesis of receptors

The main starting material, CzU (Fig. 1), was prepared according to a previously published procedure.[39]

For the synthesis of receptors Cz–Gly–NeP and CzU–NeP (see Fig. 1 for structures), one of the amine groups of carbazole (Cz) was protected. Then, the acylation of the monoprotected Cz with *tert*-butylacetyl chloride and the subsequent deprotection of the boc-NH group with trifluoroacetic acid (96%) gave the desired monoamide intermediate *N*-(8-amino-3,6-di-*tert*-butyl-9*H*-carbazol-1-yl)-3,3-dimethylbutanamide. The coupling of this intermediate with *N*,*N*′-carbonyldiimidazole (CDI) afforded the bis(carbazolyl)urea CzU–NeP in 75% yield. The treatment of same intermediate with commercially available diglycolyl chloride afforded the target dicarbazole receptor Cz–Gly–NeP in 86% yield.

The condensation reaction between biscarbazolylurea (CzU) and pyrrole-2-carboxaldehyde gave the orange solid CzU–Py as the desired product in 25% yield. The same condensation procedure was used for the synthesis of (CzU–DPM). At room temperature, the condensation of di-(acid chloride) (A and B)[52,53] with CzU afforded the macrocyclic receptors CzU–BA and CzU–BB in overall yields of 22% and 18%, respectively.

Binding studies

Binding studies were carried out using an NMR titration method that was developed previously by our group.[50,51] The measurements were carried out by using 1,3-dicarbazolylurea and 1,3-diindolylurea as reference compounds, with binding affinities of the anions available from a previous work.[38] When the receptors Cz–Gly–NeP and CzU–NeP were titrated with different carboxylate anions (Fig. 1) as tetra(*N*-butyl) ammonium (TBA) salts, all of the three NH peaks from the carbazole and amide groups became increasingly deshielded. The change in resonance frequency resulting from titrant addition was observed not only for NH protons, but also for protons of the neopentyl groups as well as the *tert*-butyl groups connected to the carbazole ring. The study of the *tert*-butyl protons gave valuable data of host orientation relative to the anion. The smaller but fairly observable chemical shift changes of *tert*-butyl protons, after adding aliquots of anions, suggest different conformational changes of the receptor for each anion.

Fig. 2 shows the changes of chemical shifts related to signals of *tert*-butyl groups attached to the carbazole moieties in Cz–Gly–NeP upon binding of different anions. It can be seen from Fig. 2 that in the case of the acetate anion, the *tert*-butyl signal moves upfield until one equivalent of CH_3COO^- is added, while by adding an excess of the acetate anion, the chemical shifts start increasing and the signal returns almost to its initial position. With other anions, except sorbate, the *tert*-butyl signals show steady upfield changes.

In the case of the *tert*-butyl signal of the carbazole ring, which appears at ~1.4 ppm, we noticed that for acetate and sorbate, its chemical shift is almost constant. When adding one equivalent of anions, the signal begins to split. By adding additional acetate or sorbate, respectively, one half of the 36 *tert*-butyl protons become more shielded while the other half become deshielded.

In the case of benzoate, all 36 protons shift to a higher frequency. The chemical shift of this signal is almost consistent with those of lactate and formate, even when more than one equivalent of anions is added.

For CzU–NeP, substantial changes in the NMR spectra were observed for the three NH protons, the protons of the neopentyl group and the carbazole protons at positions 2 and 7. All three NH protons show profound chemical shift changes in the ^{1}H-NMR spectra, and shift heavily downfield upon addition of the anion, indicative of hydrogen bonding with carboxylate anions. The signals of protons belonging to the *tert*-butyl groups, connected to carbazole ring as well as neopentyls, are almost fixed and are not differentiated by the association of the host (please refer to the ESI†).

The difference between CzU–NeP and Cz–Gly–NeP is the connecting group of two carbazole subunits. As can be found

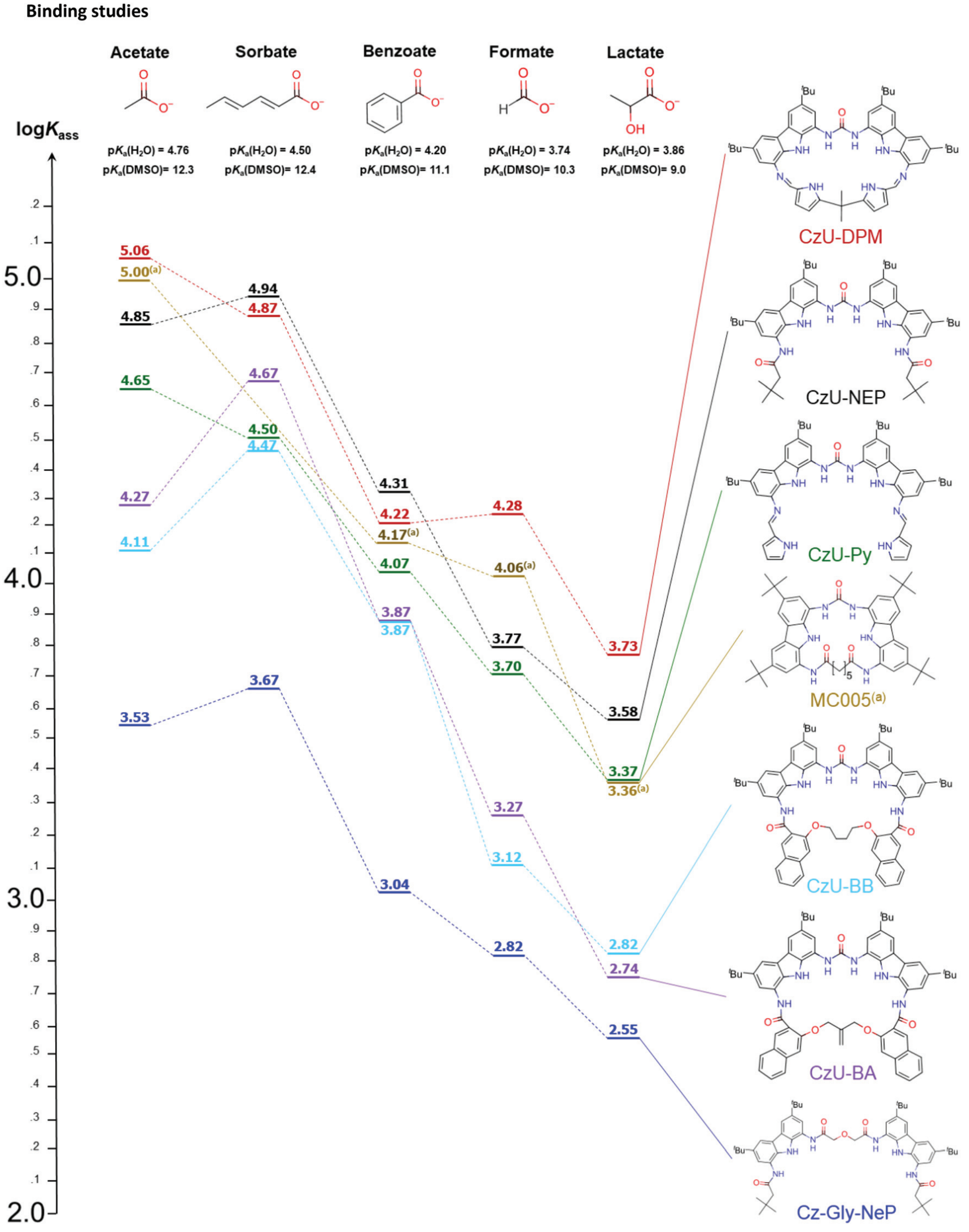

Fig. 1 Binding ladder with investigated synthetic receptors and analysed TBA-carboxylates (solvent: DMSO-d_6 + 0.5% H_2O). [a] Values from the literature[39] for comparison. Acidity values in water and DMSO are taken from ref. 38 and 54–56.

from Fig. 1, CzU–NeP shows a considerably higher binding affinity for all studied carboxylates compared to those of Cz–Gly–NeP. When its binding data is compared to previous results obtained with similar receptors,[38,39] it is clear that CzU–NeP has a higher affinity for carboxylate anions, in particular for sorbate and acetate. Fig. 1 shows that CzU–NeP has nearly the same affinity for anions as the two reference compounds. It was discovered that the affinity of CzU–NeP was the highest for benzoate, followed by formate and sorbate among the twenty-two receptors previously investigated.[38]

The binding affinity of CzU–BA and CzU–BB for different anions is in the same range. Both show the highest affinity for

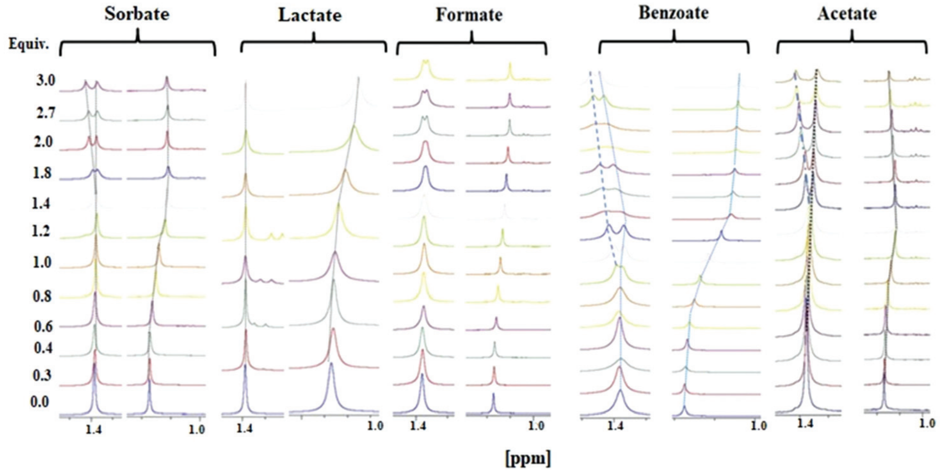

Fig. 2 The chemical shifts related to *t*-Bu protons of receptor Cz–Gly–NeP with increasing amounts of different carboxylate anions in DMSO-d_6 + 0.5% H_2O at 298 K.

sorbate. This similarity was expected as both receptors have similar hydrogen bond donor groups and cavity geometry. Both of these receptors are somewhat similar to MC005, except that the linkers connecting the –NH–CO– fragments are not aliphatic chains but are more sophisticated. Looking at the geometries of the receptors and their anion complexes, it turns out that such linkers lead to suboptimal space for accommodating the anions and in addition, the groups fragments in the linkers tend to form hydrogen bonds with the amide NH fragments thereby partially deactivating the latter in terms of anion binding.

Among the six synthetic receptors presented in this work, CzU–DPM has the highest affinity for most anions. When comparing to related synthetic receptors from previous works[38,39] it is evident that CzU–DPM is among the best binders for most carboxylates. The binding affinity of selected carboxylates loosely follows the basicity order for these anions as well. It is interesting to compare CzU–DPM and MC005[39] from our previous study. These macrocyclic receptors have nearly identical cavity sizes and hydrogen bonding numbers (Fig. 3 and Table 1).

The notable distinction between these two receptors is that MC005 has a methylene spacer of five units between two amides, whereas CzU–DPM has a dipyrromethane core. As seen in Fig. 1, CzU–DPM performs slightly better than MC005 for any given anion. The only anion with a significant difference is lactate. Looking at the most stable lactate complexes of MC005 and CzU–DPM (Fig. 4), it can be seen that while lactate seems to have one less HB interaction in the CzU–DPM complex, the overall geometry of the complex is sterically less strained. As the CzU–DPM structure allows lactate to interact with both the pyrrolo-, the biscarbazolyl- and the urea NH groups, the anion is oriented in such a way that the steric strain on the receptor is reduced. For MC005, the end of the lactate anion may be spatially hindered by the alkyl linker, which could cause suboptimal angles of HB interactions.

The binding affinities of the synthesized receptors are clearly related to the basicity of the anions. The affinity increases as the basicity of the anions increases (Fig. 5).

Computational study of receptor–anion complexation

For further characterization of the receptor–anion complexation, the conformer stability of receptors CzU–NeP, Cz–Gly–NeP, CzU–Py, CzU–DPM, CzU–BA and CzU–BB and their 1 : 1 anion complexes in the studied solvent mixture (DMSO–0.5% H_2O) was investigated using DFT computational approach for geometry optimization and applying the COSMO-RS[57–59] computational method to investigate the conformer stability in solution, where DMSO with 0.5% H_2O was used as the solvent.

The DFT calculations were carried out using the program Turbomole with these computational parameters used for geometry optimization: Becke–Pedrew functional, TZVP basis set, wave function convergence criteria: max difference 10^{-6} Hartree, geometry convergence: max gradient $|dE/dxyz|$ 10^{-3} Hartree Bohr^{-1}.

The computationally predicted most stable conformers for every receptor and its combination with every anion are presented in the ESI.† The geometries were investigated to characterize the receptor cavity size in the complex *via* the distances between the carbazole NH atoms and the amide NH atoms in the receptor geometries. The receptor cavity size parameters are presented in Table 1, together with information on the number of HB interactions in the preferred receptor–anion complex geometries.

According to the computational investigation, in the CzU–NeP complex, acetate, formate and sorbate anions interact with 4 binding groups (one urea NH, two biscarbazolyl NH, and one amide NH). Lactate has HB interactions with all binding groups, but under suboptimal bond angles and distances. In the benzoate complex, the anion binds to one side of the cavity and there is an intramolecular hydrogen bond (IMHB) interaction in the receptor. In the case of CzU–Py, all

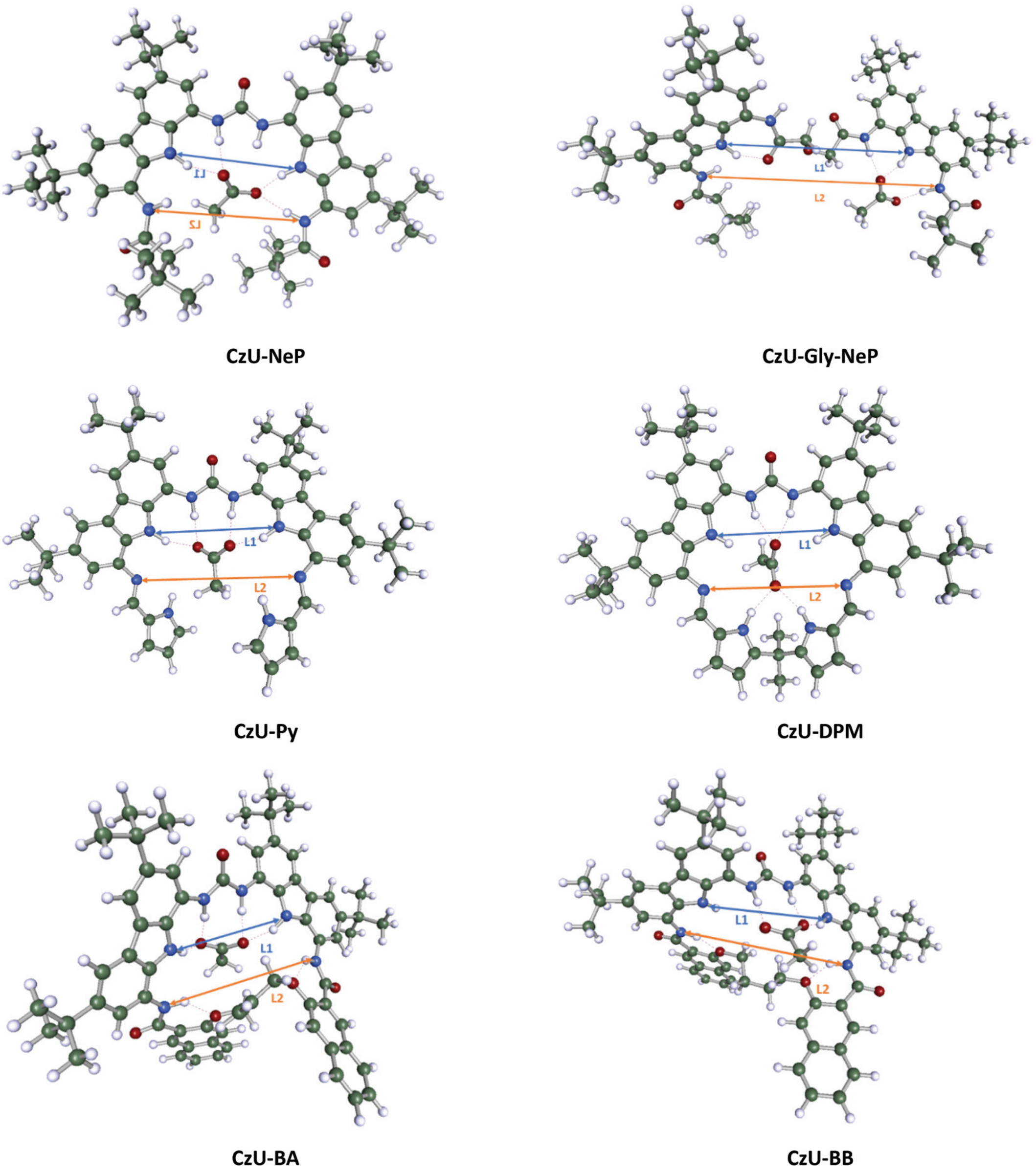

Fig. 3 Cavity size parameters L1 and L2.

Table 1 Receptor cavity size parameters L1, L2 and the number of HB interactions in receptor–anion complexes. Computational geometries from DFT calculations, COSMO-RS method applied to determine the most stable geometries. Solvent: DMSO, 0.5% H_2O

Receptor Complex	L1 (Å) (carbazolyl NH distance)					L2 (Å) (amide NH distance)					No. of HB interactions in the complex[b]				
	For	Ac	Lac	Bz	Sor	For	Ac	Lac	Bz	Sor	For	Ac	Lac	Bz	Sor
CzU–NEP	6.7	6.7	6.7	7.1	6.6	8.2	8.2	8.4	9.6	7.9	5 (0)	5 (0)	5 (0)	4 (1)	5 (0)
Cz–Gly–NEP	11.3	11.2	9.4	11.5	11.0	14.7	14.5	12.6	14.9	14.5	3 (1)	3 (1)	4[a] (2)	3 (1)	3 (1)
CzU–Py	7.1	7.1	7.2	7.0	6.9	9.0	9.2	9.6	8.7	8.6	4 (0)	4 (0)	5 (0)	4 (0)	4 (0)
CzU–DPM	6.5	6.5	6.5	6.5	6.5	7.4	7.4	7.4	7.5	7.4	5 (0)	5 (0)	5 (0)	5 (0)	5 (0)
CzU–BA	6.8	6.8	6.8	6.9	7.2	8.6	8.6	8.6	8.6	9.8	4 (2)	4 (2)	5[a] (2)	4 (2)	3 (1)
CzU–BB	7.2	6.8	6.8	7.3	7.3	10.1	9.8	9.8	10.1	10.1	3 (2)	3 (3)	4[a] (3)	3 (1)	3 (2)
MC005[39]	6.5	6.5	6.5	6.5	6.5	7.6	7.6	7.7	7.6	7.6	5 (0)	5 (0)	6[a] (0)	5 (0)	5 (0)

[a] One HB interaction is a HB in the lactate anion. [b] Conditions for counting HB interactions: max distance between atoms, 2.5 Å; and max angle, 120°. First number: HB interactions between anion and receptor, number in brackets: IMHB interactions in the receptor.

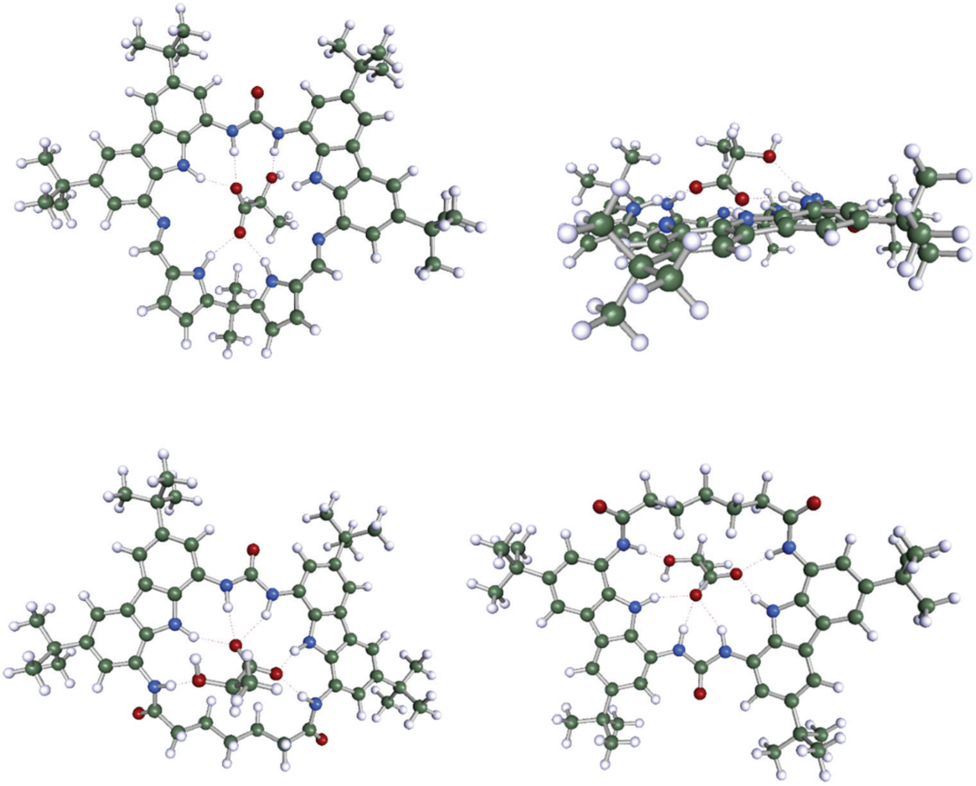

Fig. 4 The most stable conformers of CzU–DPM (above) and MC005 (below) complexed with a lactate anion.

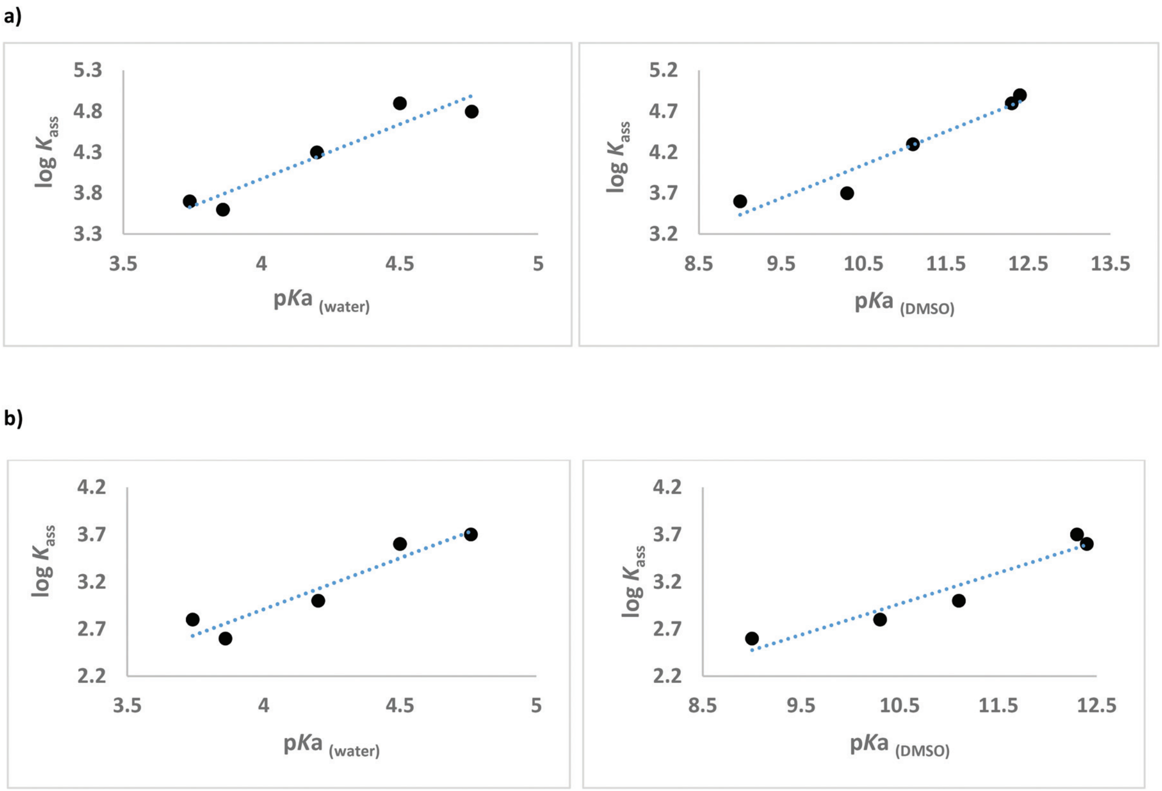

Fig. 5 The correlation between anion affinities of (a) CzU–NeP and (b) Cz–Gly–NeP and the basicity of the studied carboxylate anions in H_2O and DMSO.

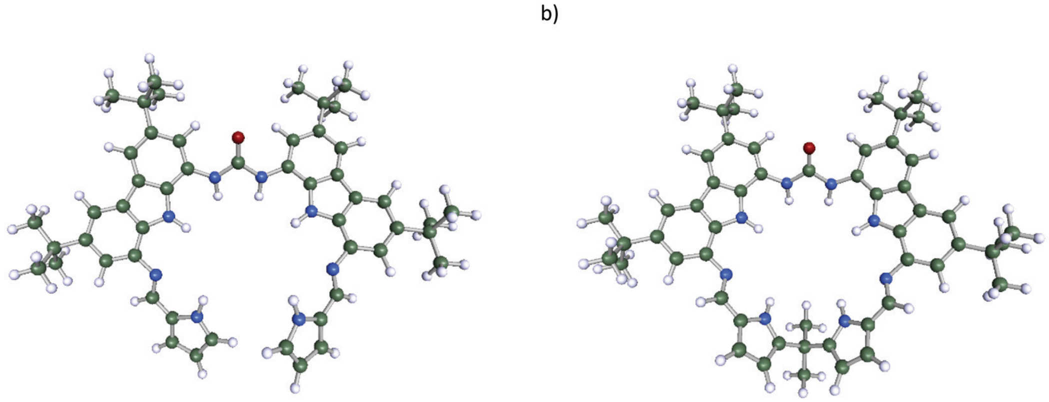

Fig. 6 The E-out-in-E-out-in isomer of (a) CzU–Py and (b) CzU–DPM.

anions have 4 HB interactions with the urea and biscarbazolylurea NH bonds. In the case of lactate, an additional interaction occurs with one of the pyrrole NH bonds. In the case of Cz–Gly–NeP, anions can interact with the binding groups on one side of the receptor (3 NH groups in total) and three IMHB interaction(s) exist in the receptor, as well as in the receptor–anion complexes. In comparison to CzU–NeP, Cz–Gly–NeP has unused binding groups in the receptor, which are unable to interact with the anion and this may explain the correspondingly lower binding affinity.

The CzU–DPM anion complexes are similar in structure to CzU–Py. This is not unexpected, as the anion-binding groups are the same. However, because of the significantly different conformational flexibility, the binding mechanisms differ. In the complexes of CzU–Py, the anions interact with the urea and biscarbazolyl NH bonds (4 total interactions), except lactate, which also interacts with one pyrrole NH. In the CzU–DPM complexes, the anions generally interact in addition with the urea NH and pyrrole NH binding groups. Experimental investigation showed that CzU–DPM has a higher binding affinity, which may be explained by the larger number of hydrogen bonds enabled by the cyclic orientation of the NH groups in CzU–DPM being more suitable for targeting carboxylate anions – in a closed cyclic structure, the geometry is more optimal for carboxylate binding and allows stronger HB interactions. Nevertheless, the ring size of CzU–DPM is too small for accommodating most of the investigated anions, which leads to them being essentially “on the receptor surface”. The exception is formate, which resides inside the ring. This may be the reason why CZU–DPM binds formate by close to 0.6 log units stronger than CZU–Py – the largest difference for any anion.

In the complexes with CzU–BA, all anions, except sorbate, have similar preferred complex geometries – the anion interacts with 4 HBs (urea and biscarbazolyl NH bonds) and the receptor has 2 IMHB interactions. The sorbate anion is larger and so only interacts with 3 NH groups (2 urea NHs, one biscarbazolyl NH), and the receptor has one IMHB interaction in the complex. For CzU–BB, the receptor structure is more rigid and the binding groups are not oriented in the same plane, so anions can mostly interact with only 3 HB interactions (urea NHs and one biscarbazolyl NH). 1–3 IMHB interactions also exist in the complex. From the study of computational conformer stability, it can be seen that all geometries of CzU–BA and CzU–BB have steric strain, the carboxylate anions do not fit well to interact with the binding groups and the receptor prefers to keep some IMHB interactions also inside the receptor–anion complexes.

CzU–Py can, in theory, exist in many stereoisomeric forms due to the possible isomerization around the imine double bond and due to the rotation of two pyrrole rings. To gain additional insight into the geometry of the receptor CzU–Py, a computational investigation of conformationally relevant structures was performed. The most stable stereoisomer of the free CzU–Py receptor is the E-out-in-E-out-in isomer (Fig. 6). In this structure, both imine double bonds are oriented away from the cavity centre, thereby exposing the lone electron pair on the nitrogen to the inside of the cavity. Furthermore, both pyrrole hydrogens are oriented towards the centre of the molecule and therefore contribute to the effective stabilization of the anions. The energetically most stable geometry of CzU–Py is also the most suitable structure for successful anion binding. The macrocyclic receptor CzU–DPM showed similar results. The E-out-in-E-out-in isomer was also discovered to be the most stable conformer in this receptor.

Conclusion

Six previously unpublished anion binding receptor molecules based on the bis-carbazolylurea backbone were designed and synthesized. The design of these receptors incorporated chemical characteristics such as ether bridges or imine functionalities, which are less common in anion binding pockets. The binding properties of the receptor molecules were investigated towards five carboxylate anions. The obtained binding constants measured in DMSO-d_6 : H_2O (99.5% : 0.5%) spanned an average of 1.3 $\log K_{\text{ass}}$ units with the champion receptor reaching a log K_{ass} value above 5 towards acetate. The consistency parameters for all ladders showed good agreement of results between different measurements. For all receptors, the general trend showed increasing affinity with increasing anion basicity, which agrees with previous works. To further interpret the observed binding effects, all synthesized receptor molecules were further studied computationally, using DFT calculations and the COSMO-RS model, providing insight into anion–receptor complexation.

Experimental section

Instruments

Please refer to the ESI† for detailed descriptions of instruments and methods used in this study.

Synthesis

CzU–NeP. CDI (0.14 mmol, 22 mg) and *N*-(8-amino-3,6-di-*tert*-butyl-9*H*-carbazole-10yl)-3,3dimethylbutanamide (0.16 mmol, 64 mg) were dissolved in dry DMF (5 mL) under an argon atmosphere. The mixture was stirred overnight at 60 °C. Then the reaction was quenched with brine, affording a pale pink precipitate. The desired pure compound was isolated in 75% (63 mg) yield by filtration *in vacuo*.

^{1}H NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 9.96 (bs, 2H); 9.92 (s, 2H); 8.95 (bs, 2H); 7.96 (s, 2H);7.93 (s, 2H);7.64 (s, 2H); 7.51 (s, 2H); 2.21 (s, 4H); 1.42 (s, 18H); 1.39 (s, 18H); 0.98 (s, 18H).

^{13}C NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 170.15; 153.94; 141.83; 141.52; 130.75; 124.48; 124.41; 123.21; 122.61; 116.27; 112.57; 111.87; 49.25; 34.49; 34.46; 31.93; 31.89; 30.79; 29.67.

HR MS (ESI): *m*/*z* calcd [$C_{53}H_{72}N_6O_3$ + H] – 841.57387 found 841.57336.

Cz–Gly–NeP. (8-Amino-3,6-di-*tert*-butyl-9*H*-carbazole-10yl)-3,3dimethylbutanamide[60] (0.19 mmol, 78 mg) was dissolved in dry DCM (7 mL) and thereafter triethylamine (0.29 mmol, 40 μL) was added. Diglycolyl chloride (0.10 mmol, 11.4 μL) was slowly added at 0 °C to the mixture under an argon atmosphere. The reaction mixture was stirred at room temperature overnight. The next day the reaction was quenched by the addition of 20 ml aqueous solution of NaCl and extracted with DCM. After drying using a rotary evaporator, the crude product (grey solid) was obtained. Recrystallization was done in diethyl ether/petroleum ether and the pure product was obtained as a pale white solid in 86% (77 mg) yield.

^{1}H-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 10.22 (s, 2H); 9.86 (s, 2H); 9.78 (s, 2H); 8.06 (s, 2H); 7.98 (s, 2H); 7.63 (s, 2H); 7.42 (s, 2H); 4.44 (s, 4H); 2.29 (s, 4H); 1.39 (s, 36H); 1.08 (s, 18H).

^{13}C-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 170.02; 167.72; 141.82; 141.75; 132.50; 130.80; 124.75; 124.47; 122.69; 120.95; 118.98; 116.44; 114.18; 112.74; 70.55; 49.20; 34.47; 34.46; 31.86; 31.84; 30.85; 29.73.

HR MS (ESI): *m*/*z* calcd for [$C_{56}H_{776}N_6O_5$ + H] – 913.59500 found 913.59450.

CzU–Py. CzU (0.05 mmol, 34 mg) was dissolved in dry acetonitrile. Pyrrole-2-carboxaldehyde (0.22 mmol, 21 mg) was added dropwise to the solution at 0 °C and acidified using two drops of acetic acid. The ice bath was removed and the reaction mixture was left to stir at 90 °C for two days. After the disappearance of the starting material (monitored by TLC), the reaction was quenched with brine. The solvent was evaporated and washed with DCM, dried over $MgSO_4$, followed by purification using column chromatography (hexane/ethyl acetate). The desired product was obtained as an orange solid in 50% yield (20 mg).

^{1}H-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 11.69 (s, 2H); 10.31 (s, 2H); 8.88 (s, 2H); 8.59 (s, 2H); 7.97 (d, J = 1.4 Hz, 2H); 7.90 (d, J = 1.4 Hz, 2H); 7.69 (d, J = 1.1 Hz, 2H); 7.21 (d, J = 1.6 Hz, 2H); 7.03 (d, J = 1.4 Hz, 2H); 6.78 (m, 2H); 6.22 (m, 2H); 1.44 (s, 18H); 1.41 (s, 18H).

^{13}C-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 150.18; 142.34; 141.74; 136.36; 132.85; 131.01; 130.35; 124.23; 123.71; 123.56; 123.30; 115.53; 115.48; 113.14; 111.47; 109.86; 45.71; 34.65; 34.50; 31.93; 31.91.

HR MS (ESI): *m*/*z* calcd for [$C_{51}H_{59}N_8O$]$^{+1}$ – 799.48063 found 799.47982.

CzU–DPM. CzU (0.09 mmol, 59 mg) was dissolved in dry acetonitrile and dipyrromethane (0.09 mmol, 20 mg), dissolved in 1 ml dry THF, was added dropwise at 0 °C. Then a few drops of HCl was added, the ice bath was removed and the reaction mixture was left to stir at room temperature for two days. After the disappearance of the starting material (monitored by TLC), the reaction was quenched with brine. The solvent was evaporated using a rotary evaporator and the residue was washed with DCM, dried over $MgSO_4$ and purified using column chromatography (hexane/ethyl acetate). The desired product was obtained as a yellow solid in 18% yield (14 mg).

^{1}H-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 11.28 (s, 2H); 9.65 (s, 2H); 8.73 (s, 2H); 8.39 (s, 2H); 8.08 (s, 2H); 7.94 (d, J = 1.5 Hz, 2H); 7.84 (d, J = 1.5 Hz, 2H); 7.02 (d, J = 1.6 Hz, 2H); 6.78 (d, J = 3.6 Hz, 2H); 6.27 (d, J = 3.6 Hz, 2H); 1.82 (s, 6H); 1.44 (s, 18H); 1.43 (s, 18H).

^{13}C-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 153.08; 152.78; 145.49; 143.27; 142.71; 138.85; 132.22; 130.90; 130.01; 124.35; 124.30; 124.18; 118.54; 114.12; 113.14; 113.05; 111.06; 107.30; 35.66; 35.12; 35.09; 32.45; 32.37; 28.57.

HR MS (ESI): *m*/*z* calcd for [$C_{54}H_{63}N_8O$]$^{+1}$ – 839.51194 found 839.51147.

CzU–BA. To a 25 mL round-bottom flask equipped with a magnetic stirrer, CzU (0.08 mmol, 49 mg) was added. The flask was closed with a septum and purged with argon. Thereafter, dried DCM (10 mL) and triethylamine (0.3 mmol, 42 μL) were added with a syringe. After cooling the reaction mixture to 0 °C, acid chloride A (0.08 mmol, 39 mg) was slowly added dropwise. Then the mixture was stirred for two days at room temperature. After the disappearance of the starting material (monitored by TLC), the mixture was quenched by adding one drop of brine and the precipitated by-product was filtered off. The filtrate was washed with an aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2. After purification with column chromatography (hexane/ethyl acetate), the desired compound was obtained as a light-yellow solid in 22% yield (18 mg).

^{1}H-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 10.56 (s, 2H); 10.08 (s, 2H); 8.78 (s, 2H); 8.24 (s, 2H); 7.99 (s, 2H); 7.96 (s, 2H); 7.93 (s, 2H); 7.91 (d, J = 8.1 Hz, 2H); 7.60 (d, J = 8.1 Hz, 2H); 7.48 (s, 2H); 7.44 (t, J = 7.2 Hz, 2H); 7.38 (m, 4H); 5.52 (s, 2H); 5.03 (s, 4H); 1.43 (s, 18H); 1.39 (s, 18H).

^{13}C-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 177.82; 164.87; 152.77; 141.86; 141.65; 139.04; 134.55; 132.37; 130.55; 129.49; 128.15; 127.66; 127.60; 126.45; 124.66; 124.36; 124.31; 123.09; 122.54; 118.20; 116.45; 112.28; 107.84; 68.61; 68.25; 34.51; 34.43; 31.87; 31.84; 27.40; 21.73.

HR MS (ESI): m/z calcd for $[C_{67}H_{68}N_6O_5 + H]^-$ 1037.53240 found 1037.53168.

CzU–BB. Same as CzU–BA, except that in this case we used acid chloride B instead of A.

^{1}H-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 10.42 (s, 2H); 10.26 (s, 2H); 8.84 (s, 2H); 8.33 (s, 2H); 8.27 (s, 2H); 8.01 (s, 2H); 7.98 (s, 2H); 7.91 (d, J = 8.3 Hz, 2H); 7.76 (d, J = 7.8 Hz, 2H); 7.46 (t, J = 7.7 Hz, 2H); 7.36 (t, J = 7.1 Hz, 2H); 7.34 (s, 2H); 7.29 (s, 2H); 3.96 (s, 4H); 2.01 (s, 4H); 1.46 (s, 18H); 1.37 (s, 18H).

^{13}C-NMR (700.1 MHz, DMSO-d$_6$, +20 °C): δ: 164.58; 162.37; 153.37; 141.88; 141.59; 134.92; 131.54; 129.89; 129.81; 128.24; 127.60; 127.50; 127.22; 126.48; 124.67; 124.25; 124.19; 122.93; 122.77; 116.98; 115.78; 112.53; 112.34; 107.15; 67.91; 45.73; 35.82; 34.64; 34.40; 30.81; 25.58.

HR MS (ESI): m/z calcd for $[C_{67}H_{70}N_6O_5 + H] -$ 1039.54805 found 1039.54805.

Binding measurements

The binding of anions in solution was studied using our previously published relative ^{1}H-NMR titration method.[50] The consistency standard deviation s[56] was 0.01 for acetate, sorbate, and benzoate and 0.04 for formate and lactate. Please see the ESI† for further details.

Conflicts of interest

The authors declare no conflict of interest.

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