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Carboxylate Receptors

Towards the Discrimination of Carboxylates by Hydrogen-Bond Donor Anion Receptors

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Abstract: The binding constants ($\log K_{ass}$) of small synthetic receptor molecules based on indolocarbazole, carbazole, indole, urea and some others, as well as their combinations were measured for small carboxylate anions of different basicity, hydrophilicity and steric demands, that is, trimethylacetate, acetate, benzoate and lactate, in 0.5% H_2O/[D_6]DMSO by using the relative NMR-based measurement method. As a result, four separate binding affinity scales (ladders) including thirty-eight receptors were obtained with the scales anchored to indolocarbazole. The results indicate that the binding strength is largely, but not fully, determined by the strength of the primary hydrogen-bonding interaction. The latter in turn is largely determined by the basicity of the anion. The higher is the basicity of the anion the stronger in general is the binding, leading to the approximate order of increasing binding strength, lactate $<$ benzoate $<$ acetate $\leq$ trimethylacetate, which holds with all investigated receptors. Nevertheless, there are a number of occasions when the binding order changes with changing of the carboxylate anion, sometimes quite substantially. Principal component analysis (PCA) reveals that this is primarily connected to preferential binding of trimethylacetate, supposedly caused by an additional hydrophobic/solvophobic interaction. These findings enable making better predictions, which receptor framework or cavity is best suited for carboxylate anions in receptor design.

Introduction

Carboxylates are among the most important anions in nature and in technology.[1] Smaller carboxylates are important metabolites whereas carboxylic acids with long aliphatic chains are crucial in the formation of fats. Amino acids are the key components in the formation of peptides and proteins and many widely used anti-inflammatory drugs such as aspirin and ibuprofen are carboxylic acids.[2] Between pH 7 and 8, that is, under physiological conditions, carboxylic acids exist predominantly in their anionic form. For these reasons the synthesis of receptors capable of binding carboxylates in analytical applications[3] (e.g., sensors) or acting as anion transporters[4] in membranes is attracting intense current interest.

Carboxylate anions have a distinct geometry with equal CO bond lengths (1.26 Å in acetate)[5] and bond angles between the CO bonds (close to 120° in acetate)[5] and a distance between the oxygen atoms around 2.2 Å.[6] The negative charge of carboxylate ions is largely distributed between the two oxygen atoms making these ions strongly solvated in hydrogen-bond-donating solvents, especially in water.[7] Because carboxylic acids are quite strong acids in aqueous media[8] they are deprotonated quite readily, although the respective carboxylates are not protonated so easily. The geometry of carboxylates enables formation of hydrogen-bonded complexes with chelating receptors of suitable geometry in 1:1 stoichiometry. The alkyl or aryl moiety (often with substituents) of a carboxylate ion significantly modifies its properties (e.g., size, basicity and hydrophilicity) and forms the basis of differentiating between different carboxylates.

Numerous synthetic receptor molecules have been proposed for binding carboxylates. In 2005, Gale and co-workers reported the anion-binding ability of acyclic receptors containing *ortho*-phenylenediamine-based bis-urea units.[9,10] The four NH urea protons stabilise hydrogen-bond interactions with the negatively charged oxygen atoms of the carboxylate, producing a complex through four hydrogen bonds with functionalised systems offering additional amide hydrogen-bond donors.[9,10] Other similar structures have been reported, for example, receptors based on 1,8-diaminocarbazole[11] and 1,2-dia-

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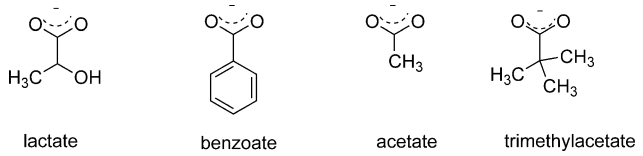
Supporting information (containing the relative binding measurement spectra (^{1}H NMR and UV/Vis spectrophotometric), additional compound characterisation data (^{1}H, ^{13}C and ^{15}N NMR spectra, IR and HR MS data) of the synthesised compounds) for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201405858.

minoanthraquinone.[12] In 2005, Beer and co-workers introduced indolo[2,3-*a*]carbazole as a useful binding fragment for anions, specifically for carboxylates.[13]

The ultimate goal of synthetic carboxylate receptor research would be developing receptor molecules that are strongly binding and can discriminate between carboxylates with different structures. Simple receptor molecules can be neither sufficiently sensitive nor sufficiently selective to differentiate between different carboxylates as a simple receptor molecule will interact first of all with the carboxylate centre and the binding will be influenced by the rest of the ion through its influence on the electron density of the carboxylate. Other interactions will be limited by the small size of the receptor. Such small molecules can, however, be regarded as building blocks of more complex receptors. Understanding the relationship between molecular structures and the binding behaviour towards different carboxylate anions is important for designing more complex receptors.

In this work, four different small carboxylate anions of different basicity, hydrophilicity and steric demand—acetate, trimethylacetate, benzoate and lactate (see Scheme 1)—were selected for an experimental study of their binding to thirty-eight different synthetic urea-, indole-, carbazole-, thiourea- and indolocarbazole-based receptors (see Scheme 2) with the aim of studying possible "embryonic" stereoselectivity of the receptors towards selected anions and to discuss it in terms of anion properties as well as structural parameters of the receptors (number and positions of NH groups, locations of other nearby groups, size of the binding cavity, etc).



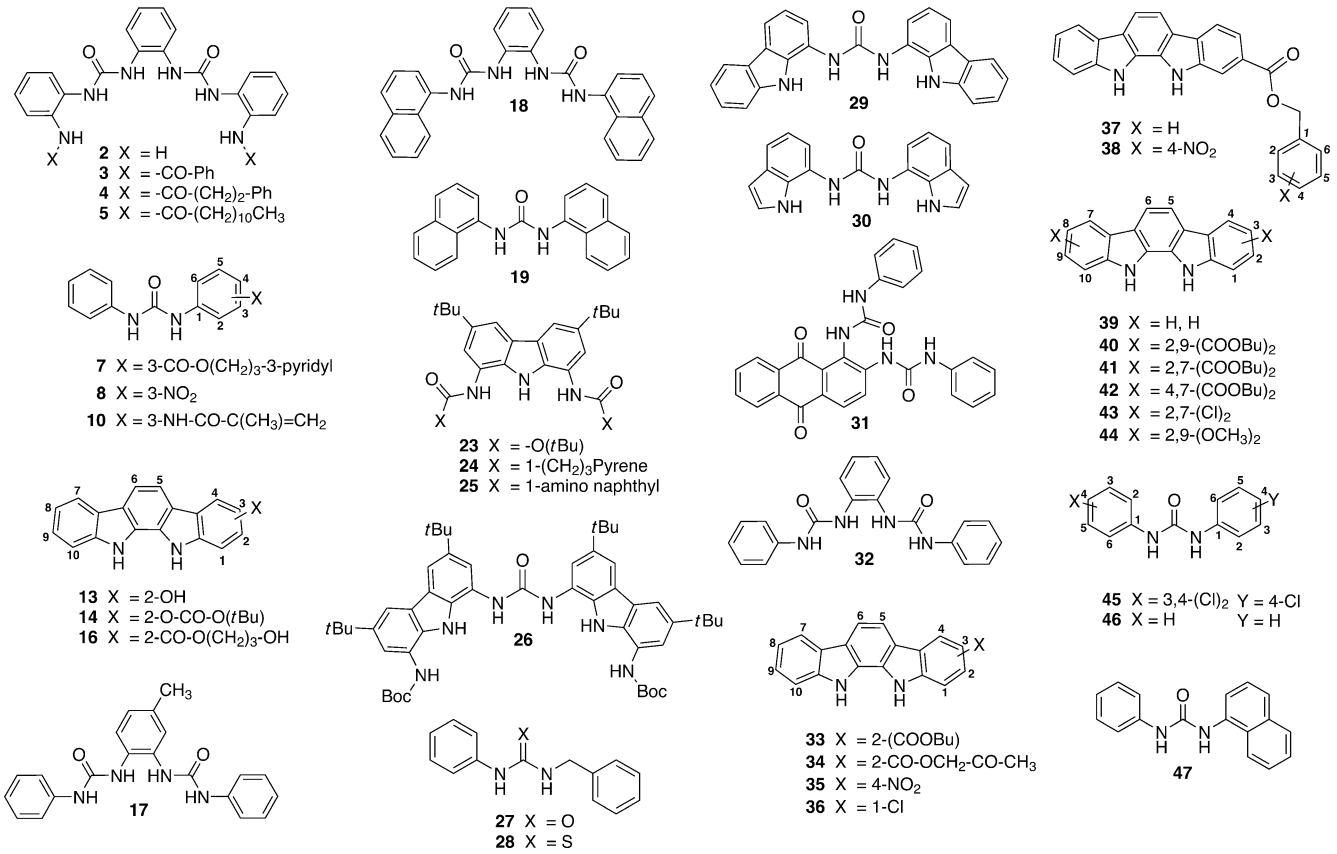
Scheme 1. Structures of the investigated anions.

The binding affinity of a receptor R towards an anion A^- to form a receptor–anion complex RA^- according to a 1:1 stoichiometry is quantified by the binding (association) constant K_{ass} according to the following equilibria [Eqs. (1) and (2)]; in Equation (2) *a* denotes the activities of the different species.

$$R + A^- \overset{K_{ass}}{\rightleftharpoons} RA^- \quad (1)$$

$$K_{ass} = \frac{a_{RA^-}}{a_R a_{A^-}} \quad (2)$$

The binding constant measurements are based on the relative measurement method recently introduced by our group and applied both for UV/Vis[14] and NMR spectroscopy[15] as techniques. The NMR version of this method enables the unprecedented ability to differentiate between very close binding constants, that is, $\log K_{ass}$ differences of 0.05 are detectable. An



Scheme 2. Structures of the molecular receptors for which the $\log K_{ass}$ values were determined.

additional advantage of the NMR measurement method is its ability to simultaneously measure the binding of several different receptors to the same anion. This relative NMR measurement method is also used in this work and its abilities have been better characterised than previously.

Results

^{1}H NMR-based relative binding measurements

The compounds **1**–**47** were synthesised by methods that were used by our groups previously[15] as described below. All the relative NMR measurements were performed under the fast exchange conditions. On binding of the carboxylate anions to form the receptor–anion complexes, complex formation is easily identifiable through the shifts of the NH protons in the NMR spectra (see the Supporting Information). The binding affinities of all four anions towards thirty-eight receptors were measured in $[D_6]$DMSO/H_2O (99.5:0.5 m/m) by using the relative measurement method as previously reported for the acetate anion.[15] The binding constants of the receptors are presented in Table 1 and in the binding scales containing all relative measurement results in the Supporting Information.

At low and moderate anion concentrations a 1:1 stoichiometry was found with all receptor–anion pairs. The $\log K_{ass}$ values were calculated from the NMR titration data according to the 1:1 binding model [Eq. (1)] in the case of all receptors. In the case of receptors **3**–**5** at higher anion concentration a 1:2 stoichiometry (two anions bound to one receptor) was observed as described in reference [10]. In the case of these receptors only titration points with a low anion concentration were taken into account where formation of the 1:2 complexes is

Table 1. Binding constants of the carboxylate anions in $[D_6]$DMSO/H_2O (99.5:0.5 m/m) at 25 °C.[a]

Receptor	Lactate anion			Benzoate anion			Acetate anion			Trimethylacetate anion		
	$\log K_{ass}$	u_c[b]	u_c[c]	$\log K_{ass}$	u_c[b]	u_c[c]	$\log K_{ass}$	u_c[b]	u_c[c]	$\log K_{ass}$	u_c[b]	u_c[c]
receptor **25**	3.38	0.02	0.05	3.88	0.01	0.05	4.67	0.01	0.09	4.88	0.01	0.06
1,3-dicarbazolylurea **29**	3.25	0.01	0.05	4.08	0.01	0.05	4.56	0.01	0.09	5.33	0.01	0.06
1,3-diindolylurea **30**	3.19	0.01	0.05	4.01	0.01	0.05	4.63	0.01	0.09	5.07	0.01	0.06
receptor **26**	3.17	0.01	0.05	3.96	0.03	0.06	4.94	0.01	0.09	4.95	0.01	0.06
receptor **45**	2.87	0.01	0.05	3.53	0.01	0.05	4.13	0.01	0.08	4.16	0.01	0.06
1-(3-NO_2-phenyl)-3-phenyl urea **8**	2.68	0.01	0.05	3.34	0.01	0.05	3.90	0.01	0.08	3.98	0.01	0.06
4-NO_2-indolocarbazole **35**	2.58	0.01	0.05	3.22	0.01	0.05	3.88	0.01	0.08	3.85	0.01	0.06
receptor **5**	2.55	0.01	0.05	3.27	0.01	0.05	3.88	0.01	0.09	4.08	0.01	0.06
4,7-$(BuOCO)_2$-indolocarbazole **42**	2.53	0.01	0.05	3.16	0.01	0.05	3.77	0.01	0.08	3.81	0.01	0.06
receptor **32**	2.53	0.01	0.05	3.11	0.01	0.05	3.70	0.01	0.08	4.03	0.01	0.06
2,7-$(BuOCO)_2$-indolocarbazole **41**	2.52	0.01	0.05	3.18	0.01	0.05	3.79	0.01	0.08	3.84	0.01	0.06
2,9-$(BuOCO)_2$-indolocarbazole **40**	2.51	0.01	0.05	3.17	0.01	0.05	3.82	0.01	0.08	3.82	0.01	0.06
2,7-Cl_2-indolocarbazole **43**	2.45	0.01	0.05	3.07	0.01	0.05	3.67	0.01	0.08	3.72	0.01	0.06
receptor **17**	2.44	0.01	0.05	2.99	0.01	0.05	3.64	0.01	0.09	3.96	0.01	0.06
receptor **4**	2.44	0.01	0.05	3.18	0.01	0.05	3.85	0.01	0.09	3.99	0.01	0.06
receptor **3**	2.43	0.02	0.05	2.89	0.01	0.05	3.62	0.01	0.09	3.75	0.01	0.06
receptor **7**	2.42	0.01	0.05	3.08	0.01	0.05	3.58	0.01	0.09	3.66	0.01	0.06
receptor **38**	2.39	0.01	0.05	2.91	0.01	0.05	3.59	0.01	0.08	3.58	0.01	0.06
receptor **34**	2.39	0.01	0.05	2.98	0.01	0.05	3.54	0.01	0.08	3.60	0.01	0.06
receptor **33**	2.39	0.01	0.05	2.95	0.01	0.05	3.58	0.01	0.08	3.59	0.01	0.06
receptor **2**	2.37	0.01	0.05	3.00	0.02	0.05	3.67	0.01	0.09	3.93	0.01	0.06
receptor **37**	2.35	0.01	0.05	2.93	0.01	0.05	3.55	0.01	0.08	3.54	0.01	0.06
receptor **18**	2.30	0.01	0.05	3.25	0.01	0.05	3.74	0.01	0.09	4.22	0.01	0.06
receptor **16**	2.29	0.01	0.05	2.94	0.01	0.05	3.56	0.01	0.09	3.55	0.01	0.06
1,3-diphenylurea **46**	2.27	0.01	0.05	2.82	0.01	0.05	3.33	0.01	0.08	3.39	0.01	0.06
receptor **10**	2.22	0.01	0.05	2.73	0.01	0.05	3.24	0.01	0.09	3.23	0.01	0.06
receptor **14**	2.18	0.01	0.05	2.76	0.01	0.05	3.36	0.01	0.09	3.36	0.01	0.06
indolocarbazole **39**	**2.14**	0.01	0.05	**2.70**	0.01	0.05	**3.27**	0.01	0.09	**3.28**	0.01	0.06
2,9-$(MeO)_2$-indolocarbazole **44**	2.13	0.01	0.05	2.63	0.01	0.05	3.26	0.01	0.08	3.14	0.01	0.06
receptor **24**	2.05	0.01	0.05	2.96	0.01	0.05	3.38	0.01	0.09	3.76	0.01	0.06
receptor **13**	1.96	0.01	0.05	2.63	0.01	0.05	3.16	0.01	0.09	3.21	0.01	0.06
receptor **31**	1.91	0.01	0.05	2.49	0.01	0.05	3.09	0.01	0.09	3.40	0.01	0.06
1-naphthalen-1-yl-3-phenyl-urea **47**	1.89	0.01	0.05	2.44	0.01	0.05	2.85	0.03	0.09	3.02	0.01	0.06
1-Cl-indolocarbazole **36**	1.85	0.01	0.05	2.48	0.01	0.05	2.89	0.01	0.08	3.01	0.01	0.06
1-benzyl-3-phenyl-thiourea **28**	1.74	0.01	0.05	2.31	0.01	0.05	2.80	0.01	0.09	2.90	0.01	0.06
receptor **23**	1.65	0.01	0.05	2.27	0.01	0.05	2.41	0.01	0.09	3.03	0.01	0.06
1-benzyl-3-phenyl-urea **27**	1.62	0.01	0.05	2.08	0.01	0.05	2.51	0.01	0.09	2.57	0.01	0.06
1,3-di-naphthalen-1-yl-urea **19**	1.62	0.01	0.05	2.11	0.01	0.05	2.45	0.01	0.09	2.72	0.01	0.06

[a] All $\log K_{ass}$ values correspond to Equation (1). The binding constant values of the acetate anion in the table are on an average by 0.13 log units higher than the values previously published for the same receptors in reference [15], because a systematic mistake was found in the calculation of the anchor point $\log K_{ass}$ values in reference [15] and those values were recalculated. [b] Standard uncertainties for comparing $\log K_{ass}$ values within the same scale. [c] Standard uncertainties for comparing $\log K_{ass}$ values between different scales or with those from other research groups.

negligible. In all cases the binding affinity was measured by the chemical shift change induced by anion complexation upon addition of anionic guests in the form of tetrabutylammonium salts.[15]

The numbers of relative binding measurements carried out were 77, 89, 47 and 86 with lactate, benzoate, acetate and trimethylacetate, respectively. The consistency parameters of the resulting four binding scales are 0.01 log units, indicating excellent consistency. The $\log K_{ass}$ ranges of the receptors are the following: lactate anion 1.76, benzoate anion 1.99, acetate anion 2.53 and trimethylacetate anion 2.76 orders of magnitude. The scales are anchored to the absolute $\log K_{ass}$ values of binding of the respective anions to indolocarbazole **39** (see Table 2).

The binding constant values of the acetate anion in Table 1 are on an average by 0.13 log units higher than the values previously published for the same receptors in reference [15], because a systematic mistake was found in the calculation of the anchor point $\log K_{ass}$ values in reference [15] and those values were recalculated. However, all relative binding affinities remain the same. For compounds not included in this study the $\log K_{ass}$ values from reference [15] can be corrected by adding 0.13 log units.

With an NMR instrument of sufficient signal separation (700 MHz in our case) it is possible to simultaneously measure the $\Delta\log K_{ass}$ values between a number of receptors (towards the same anion) from the same set of solutions. In this work, we were able to successfully measure simultaneously $\Delta\log K_{ass}$ values between up to six receptors in one solution (see the Supporting Information). Figure 1 shows the ^{1}H NMR spectra of the measurement of $\Delta\log K_{ass}$ values between receptors **2**, **29**, **30** and **42** towards the benzoate anion. The differences of the chemical shifts of the NH protons of the free receptor and the receptor–anion complex are the following: receptor **2** ($\Delta\delta =$ 1.49 and 1.06 ppm), receptor **29** ($\Delta\delta = 0.95$ and 2.25 ppm), receptor **30** ($\Delta\delta = 0.99$ and 2.14 ppm) and receptor **42** ($\Delta\delta =$ 3.5 ppm). As expected from the structure, receptors **29** and **30** have significantly stronger binding affinities towards the benzoate anion than the remaining receptors, due to the carbazole and indole rings. Overall, the binding affinity order for the benzoate anion is **29** > **30** > **42** > **2**.

Comparison with literature data

The binding affinities of receptors **29** and **30** towards the acetate and benzoate anions in the same solvent have been published[16,17] and were found to be "above 10^4", which is in agreement with our data. The $\log K_{ass}$ value in the same solvent has also been published for receptor **3** with the acetate and benzoate anions and values of 3.78 and 4.00, respectively, were obtained.[10] The $\log K_{ass}$ value for the acetate anion agrees very well with our value, that is, 3.62. However, our

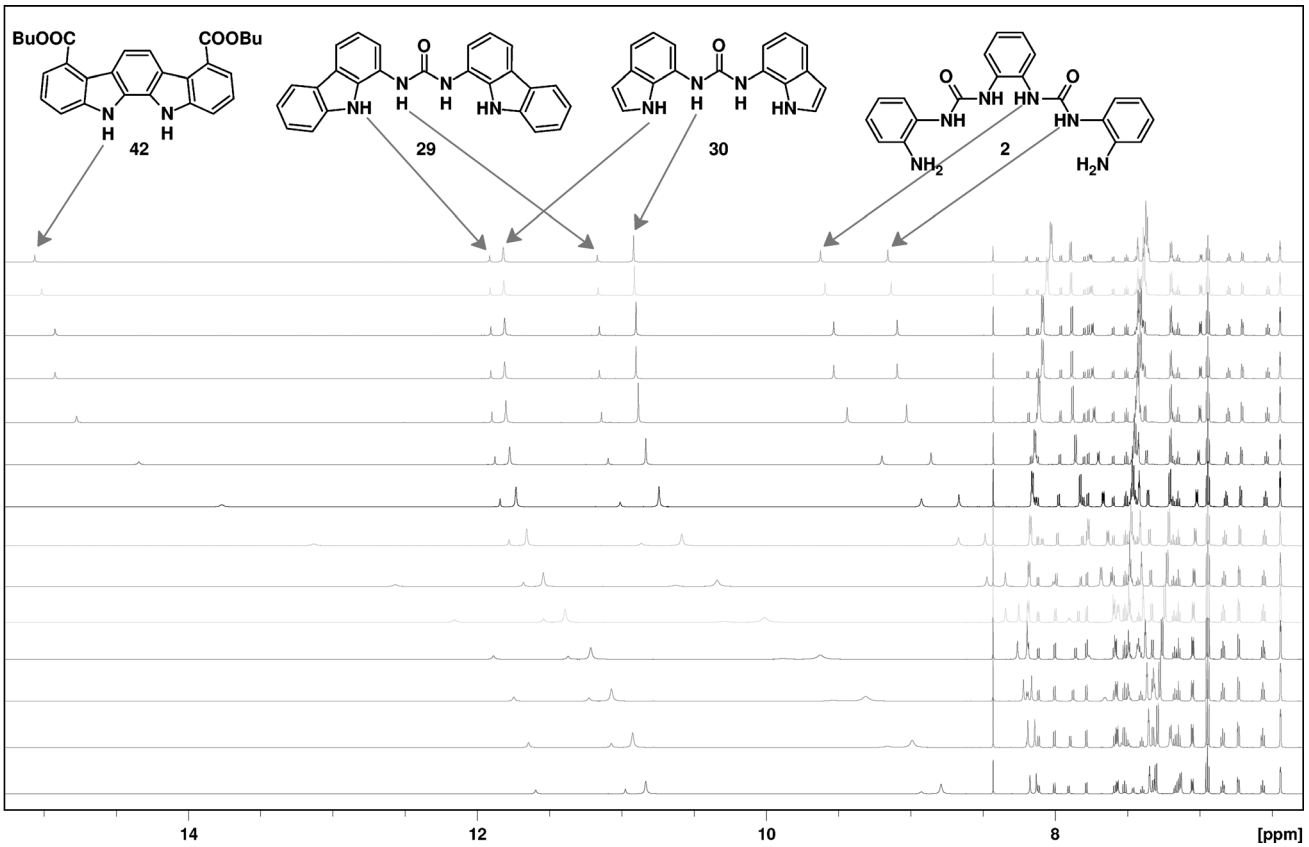


Figure 1. ^{1}H NMR spectra of the relative binding affinity measurement between receptors **2**, **29**, **30** and **42** in [D_6]DMSO/H_2O (99.5:0.5 m/m) with the benzoate anion. Titration proceeds from bottom to top. The bottom-most spectrum corresponds to a solution without titrant added.

value of 2.89 for the benzoate anion is by an order of magnitude lower than that published in reference [10]. To some extent, this discrepancy can be due to the different fitting model used in reference [10], but this cannot explain the difference of one order of magnitude. The measurement of log K_{ass} between the benzoate anion and receptor **3** was carried out separately by using the absolute UV/Vis method. The log K_{ass} value obtained was 3.05, which agrees with the value obtained from the relative measurement.

A separate ^{1}H NMR titration of receptors **29** and **30** with the trimethylacetate anion was carried out and found that in both cases there is a hydrogen-bonding interaction in the receptor–anion complex between the H-3 proton as demonstrated both by NMR data and COSMO-RS calculations (see Figures 2 as well as S271, S301 and S302 in the Supporting Information) and the carbonyl oxygen atoms of the receptor. According to Figure 2, the H-3 protons are deshielded during the addition of the trimethylacetate anion (almost $\Delta\delta = 0.80$ ppm deshielding effect). The effect is caused by 1) favourable orientation of the H-3 proton near the carbonyl oxygen atoms in the complex and 2) the increased negative partial charge on the carbonyl oxygen atoms in the receptor–anion complex. Similar, but weaker, effects were observed with other carboxylate anions. According to COSMO-RS calculations both free receptors are preferably in conformations where the indole/carbazole NH bonds are turned towards the carbonyl oxygen atoms (*anti–anti* conformation, see Figures S301 and S302 in the Supporting Information)

A similar hydrogen-bond interaction has been observed in reference [18] for the acetate and benzoate anions, where it was demonstrated that receptor **30** has three possible conformations (i.e., *syn–syn*, *syn–anti* and *anti–anti*). In the absence of anion, the receptor is in the *anti–anti* conformation and in the presence of an anionic guest the complex adopts the *syn–syn* conformation.

Receptor **29** enables naked-eye detection of carboxylates: the colour of a solution in [D_6]DMSO/H_2O (99.5:0.5 m/m) changes from colourless to light pink with all carboxylate anions. An especially strong colour is observed upon addition of the trimethylacetate anion.

In relative binding measurement experiments some minor aromatic proton interactions in receptors **7**, **8**, **10**, **18**, **19** and **47** with all the carboxylate anions were observed (see the Supporting Information), resulting in $\Delta\delta$ values being mostly in the range of 0.2–0.3 ppm.

Absolute binding measurements

The absolute log K_{ass} values with all investigated anions were measured with the unsubstituted indolocarbazole **39**. For each anion the log K_{ass} value was measured on at least two different days. The log K_{ass} value for receptor **39** with the lactate anion was obtained by using the UV/Vis method, the log K_{ass} value with the benzoate anion was obtained by using NMR, UV/Vis and fluorescence methods and the log K_{ass} values with the acetate and trimethylacetate anions were obtained by using the NMR and UV/Vis methods. Several independent datasets were obtained on each day and for each of the data sets three calculation procedures were applied (see Refs. [14] and [15] for details). The results of the measurements are presented in Table 2.

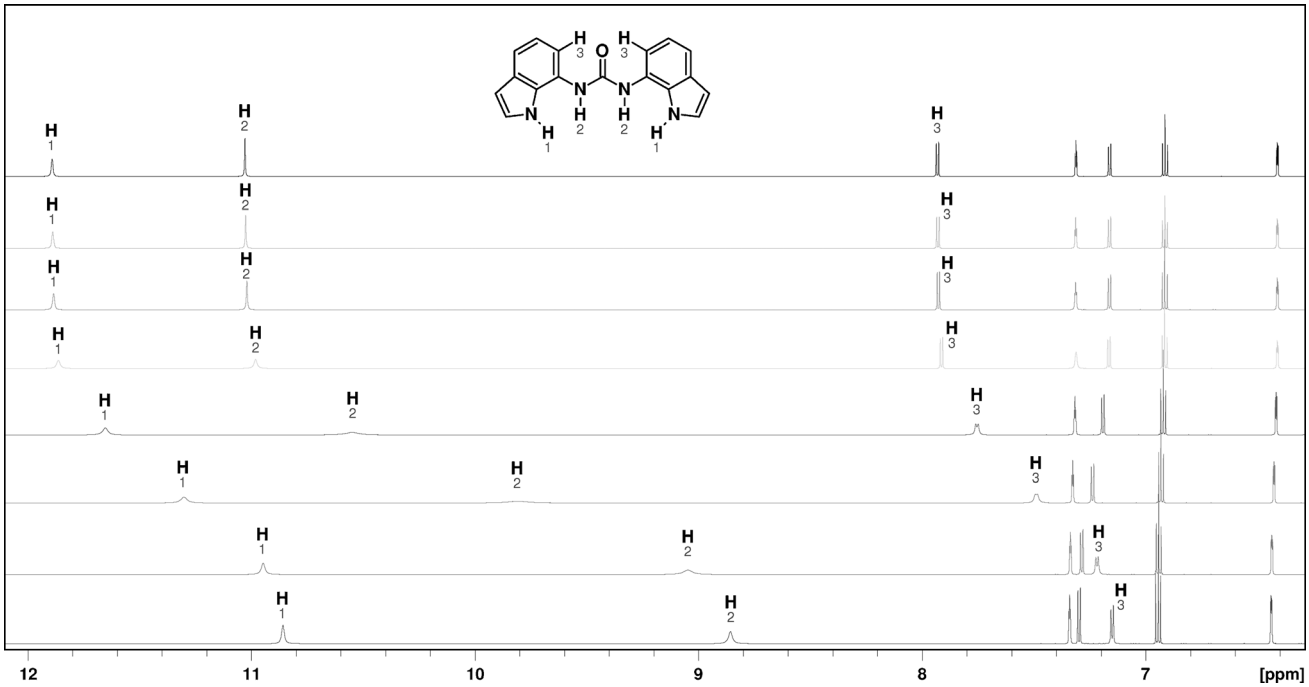


Figure 2. ^{1}H NMR spectra of the titration of receptor **30** in [D_6]DMSO/H_2O (99.5:0.5 m/m) with the trimethylacetate anion. Titration proceeds from bottom to top. The bottom-most spectrum corresponds to a solution without titrant added.

Table 2. Results of absolute log K_{ass} value measurements with indolocarbazole **39** in [D_6]DMSO/H_2O (99.5:0.5 m/m).

Anion	Method	Absolute log K_{ass} [a]	S[b]	N[b]	Assigned log K_{ass} [c]	CI (95%)[c]
lactate anion	UV/Vis	2.14	0.07	5	2.14	0.09
benzoate anion	UV/Vis	2.71	0.01	2	2.7	0.08
	NMR	2.79	0.01	2		
	fluorescence	2.66	0.02	2		
acetate anion	UV/Vis	3.33	0.02	5	3.27	0.13
	NMR	3.17	0.25	4		
trimethylacetate anion	UV/Vis	3.35	0.02	3	3.28	0.13
	NMR	3.16	0.06	2		

[a] Average log K_{ass} values from the different techniques. Acetate anion values were taken from reference [15], the remaining values are from this work. [b] Standard deviation of values from independent experiments and numbers of independent experiments (on different days). [c] Assigned log K_{ass} values and 95% confidence intervals of the assigned values.

Discussion

Selectivity toward carboxylate anions

Table 1 and Figure 3 indicate that the binding affinity in broad terms follows the order of anion basicity, that is, trimethylacetate $\geq$ acetate $>$ benzoate $>$ lactate. The aqueous pK_a values of the respective acids are 5.01, 4.76, 4.20 and 3.86, respectively[19] (the higher the pK_a value, the higher the basicity). Nevertheless, there are numerous smaller differences between the binding orders, which were revealed thanks to the high precision of the NMR-based relative binding measurement method.

The most pronounced binding order changes are observed between the acetate and trimethylacetate anions. With most indolocarbazoles and receptor **26** the log K_{ass} values for the acetate and trimethylacetate anions are similar. At the same time, with most urea- or amidocarbazole-based receptors the trimethylacetate anion binds more strongly than the other anions. The strongest carboxylate binders out of the studied receptors are receptors **25**, **26**, **29** and **30** (different anions having different binding orders). Their strong binding is caused by numerous suitably located hydrogen-bond donor sites leading to tetradentate binding and a suitable size of the pocket for fitting the anion, so that a nearly planar structure with little steric strain is formed, as is indicated by the computational geometries (see Figures S299–S302 in the Supporting Information). In Figure 3, receptor **25** has a relatively high binding affinity towards the lactate anion and an intermediate affinity towards the acetate anion but in the case of the benzoate and trimethylacetate anions it has relatively the lowest binding affinity of the other three receptors. Out of these four receptors, compound **26** has the highest binding affinity towards the acetate anion and the lowest affinity towards the lactate anion and an intermediate affinity towards the benzoate and trimethylacetate anions. Receptor **29** is the strongest binder of the benzoate and trimethylacetate anions but is the weakest one of the four towards the acetate anion. Compound **29** has a very high binding affinity difference between the trimethylacetate and acetate anions (Δlog K_{ass} 0.77), whereas the same difference in the case of receptor **26** is only 0.01. Receptor **30** is similar to compound **29**, but binds all anions except acetate weaker than receptor **29**.

Based on the binding data and the computational geometries of the receptors and the complexes the reason for these differences (especially those between receptors **29** and **26**) seems to be that compound **29** forms a suitable cavity for accommodating the trimethylacetate anion and partial shielding of its hydrophobic moiety from the polar solvent without introducing significant steric strain (see Figure S301 in the Supporting Information). The cavity formed by receptor **26** (Figure S300 in the Supporting Information) is smaller and is crowded by substituents. This hinders binding of the trimethylacetate anion and introduces a significant steric strain (Figure S300 in the Supporting Information), whereas the acetate anion fits better with receptor **26** because of its small size (Figure S300 in the Supporting Information).

The preferential binding of trimethylacetate with respect to acetate was also observed with receptors **18**, **23**, **24**, **31** and **32** and somewhat weaker with compound **3**–**5**. The receptors **23** and **24** from the family of the 1,8-disubstituted carbazoles, but also receptors **18**, **4** and **5** have bulky and hydrophobic substituents, which can be oriented around the large hydrophobic moieties of the trimethylacetate anion and also the benzoate anion in such a way that hydrophobic/solvophobic interactions can take place (but at the same time no steric hindrance is introduced), differently from acetate and lactate, which lack such hydrophobic moieties. The data of receptors **23** and **24** support this: they bind relatively stronger to the trimethylacetate (log K_{ass} 3.03 and 3.76, respectively) and benzoate anions (log K_{ass} 2.27 and 2.96, respectively) and relatively weaker to the acetate (log K_{ass} 2.41 and 3.38, respectively) and lactate (log K_{ass} 1.65 and 2.05, respectively) anions (Figure 3). The remaining receptors seem to bind preferentially the trimethylacetate anion but not to the benzoate anion.

Figure 3 also implies that some indolocarbazole-based receptors (i.e., compounds **35** and **44**) might bind the acetate anion stronger than the trimethylacetate anion. However, the uncertainties of the anchor compound log K_{ass} values are of the same order of magnitude as the differences, so it is impossible to claim this.

The phenyl- and/or naphthyl-substituted urea-based receptors **46**, **47** and **19** bind carboxylate ions distinctly weaker than the related indole- or carbazole-substituted receptors **26**, **29** and **30** and the more naphthyl groups are present the weaker is the binding. Naphthyl rings are more electronegative than phenyl rings and the positive polarisation of the NH hydrogen atoms increases on sequential replacement of phenyl rings by naphthyl rings. Nevertheless, the binding affinity towards all four anions decreases on this replacement. With the addition of each naphthyl ring the binding affinity decreases by around 0.2–0.5 log units. Examining the computational results sheds light on these results. Binding of the anions to receptor **46** does not introduce significant steric strain (see Figure 305 in

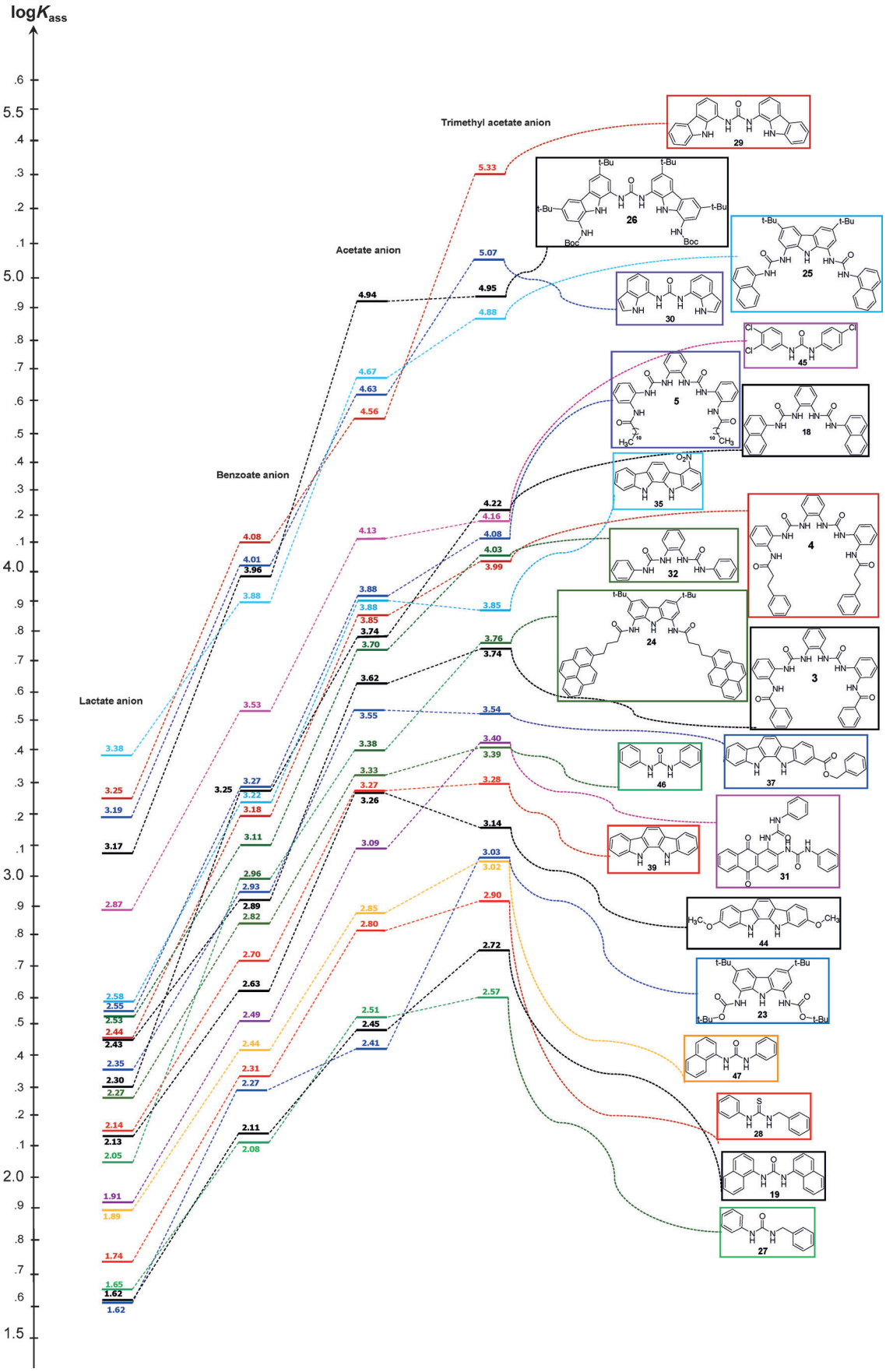


Figure 3. Trends of the binding constant changes with the trimethylacetate, acetate, benzoate and lactate anion and different families of receptors in [D_6]DMSO/H_2O (99.5:0.5 m/m).

the Supporting Information) but is only bidentate, thus it forms a distinctly weaker complex than in the cases of compounds **26**, **29** and **30**. Each of the naphthyl rings when introduced to receptor **46**, makes the binding less favourable. In contradistinction to the indolyl or carbazolyl groups, the naphthyl groups are unable to contribute additional hydrogen bonds (which would not be completely uncommon even for CH groups)[20,21] but are pushed out of the urea plane by the anions, thus creating a significant steric strain (see Figures S295 and S296 in the Supporting Information). The steric effect is caused first of all by the oxygen atoms of the carboxyl group. This explains the lack of sensitivity towards the anion size.

A different picture is observed for the receptor pair **32** and **18**. Here, receptor **18**, containing naphthyl rings, shows higher binding affinity towards the benzoate, acetate and trimethylacetate anions and lower binding affinity towards the lactate anion. The computations (Figures S303 and S295 in the Supporting Information) show that these receptors are significantly strained before anion binding and that the anions are bound in such a way as not to cause additional disturbance of the naphthyl rings.

The above discussion of binding orders of the anions to the receptors was done in terms of $\log K_{ass}$ values, molecular structures (of the receptors and receptor–anion complexes) as well as solvation effects. The binding orders are defined in terms of $\log K_{ass}$ values, relating them to free energy changes (ΔG_{ass}) on binding. This way of data analysis does not enable separating the ΔG_{ass} values into enthalpy (ΔH_{ass}) and entropy ($T\Delta S_{ass}$) components. Although in the majority of cases the enthalpy term governs the binding processes, it is increasingly realised that sometimes the entropy term can play a major (even decisive) role.[22–24] This is true first of all in structured solvents especially in water. Our experimental protocol does not enable the determination of enthalpy and entropy contributions without a very large additional experimental effort but for getting the information about general trends between binding and molecular structures of receptors and anions the ΔG_{ass} value (actually log K_{ass}) is sufficient and it is not inevitable to determine the ΔH_{ass} and $T\Delta S_{ass}$ terms, especially considering that in our case the content of water in the solvent is only 0.5%. The general conclusions on the binding ability of different receptors towards carboxylate anions presented below are based only on the log K_{ass} data:

1) The main factors determining the binding efficiency are the number of hydrogen-bond donor sites, their donicity, their mutual position and the possible steric crowding around the binding sites. In many cases the hydrogen-bond donicity and the steric crowding are mutually competitive. Not only is it important to have multiple hydrogen-bond donor sites but it is also crucial whether they are suitably positioned so that all of them can produce hydrogen bonds with the anion of interest.
2) A tetradentate system of four suitably located NH centres seems (out of the studied systems) to be the most successful combination for binding carboxylate anions, especially the one found in bis-indolyl or bis-carbazolyl ureas, for example, in receptors **26**, **29** and **30**.
3) The *ortho*-phenylenediamine-bis-urea receptors, although also featuring four or six hydrogen-bond donor fragments form weaker complexes, because of an unsuitable spatial arrangement of the NH groups (see for example, Figures S291–294 in the Supporting Information).
4) Carbazole-based tridentate receptors are inferior to the tetradentate systems and lead to asymmetrical binding of the anions, which is not optimal from the point of view of forming hydrogen bonds (see Figures S297–299 in the Supporting Information).
5) Bidentate diphenylurea and indolocarbazole centres have similar efficiency in binding carboxylate anions. This is caused by a trade-off between two factors, firstly, diphenylurea has a lower hydrogen-bond donicity because of a lower acidity of the NH protons (pK_a values in DMSO for indole, carbazole and urea are 20.95, 19.9, 26.95, respectively,[25] the acidity of indolocarbazole is expected to be in the range of indole and carbazole), but the bond angle in hydrogen-bond formation is more suitable and the respective urea is able to produce an eight-membered ring with two almost parallel individual hydrogen bonds. Secondly, the NH sites in the indolocarbazole are less suitably spatially oriented but have higher hydrogen-bond donicity due to higher acidity of the NH protons.

Principal component analysis of the obtained data

Principal component analysis (PCA) was performed on the binding constant data to further assess the differences between the studied receptors in their selectivity patterns towards the selected carboxylate anions, that is, the possibility of finding receptors able to differentiate between the carboxylate anions. This kind of multivariate analysis of data from a number of receptors is the basis of achieving selectivity by receptor arrays,[25] where none of the individual receptors by themselves are selective enough.

The plot of scores of different receptors according to the PC1 and PC2 is presented in Figure 4. PC1 describes 97% of the variance. The axes of the binding constants of all four anions are quite well aligned with PC1, so PC1 shows the general binding affinity of the receptors towards the carboxylate anions. The receptors having the highest binding affinities are positioned to the left-hand side of the plot. PC2 describes 2% of the variance. Looking at the axes of the binding constants of the anions indicates that PC2 characterises the selectivity between large hydrophobic and small hydrophilic ions. Receptors that have a (relatively) lower affinity towards the acetate and lactate anions and a relatively higher affinity towards the trimethylacetate and benzoate anions are positioned in the upper part of the plot. The plot also reveals that the selected receptors are almost incapable of differentiating between the acetate and lactate anions.

Four groups of receptors emerge on the plot: 1) receptors **25**, **26**, **29** and **30**, which are the strongest binders, 2) recep-

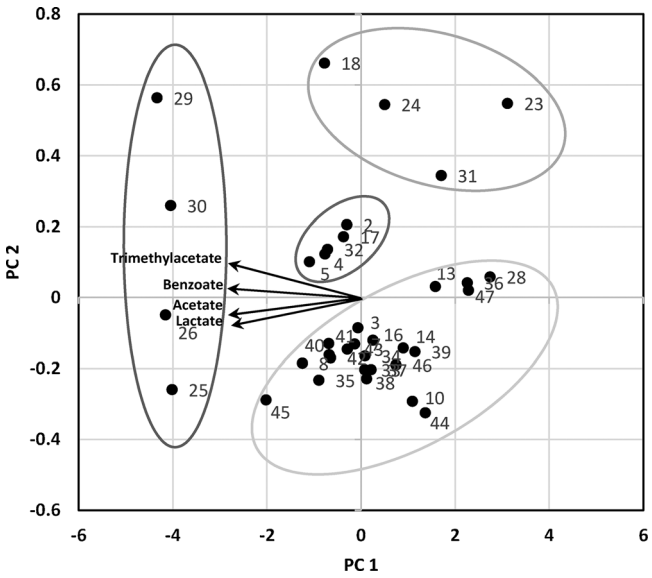


Figure 4. PCA plot of the binding constant data. Scores of PC2 versus scores of PC1.

tors **18**, **23**, **24** and **31**, which have relatively the strongest affinity towards the trimethylacetate anion, supposedly made possible by hydrophobic/solvophobic interaction, 3) receptors **2**, **4**, **5**, **17** and **32**, which are all based on combining two urea moieties through an 1,2-phenylene fragment and can also possibly have some solvophobic interaction and 4) all other receptors, which seem to bind anions mostly by hydrogen bonding, without significant involvement of other interactions. Examining the PCA plot together with the structural features can be of help in picking molecular fragments for designing new receptors.

Conclusions

We have reported a series of anion receptors from different compound families, including indolocarbazole, carbazole, indole, urea, thiourea and amide moieties. The binding affinities (expressed as $\log K_{ass}$ values) of receptors described in this work were measured with the trimethylacetate, acetate, benzoate and lactate anions.

It is evident that binding of the carboxylate anions to the investigated receptors is primarily determined by the basicity of the anion and the binding affinity follows the approximate order: lactate < benzoate < acetate ≤ trimethylacetate. The reason for this is that the binding is predominantly determined by hydrogen-bonding interactions. Tetradentate receptors with planar structures seem to be one of the most suitable. The carboxylate anions also have planar structures and can form two bifurcated hydrogen bonds with tetradentate receptors. However, other important factors to consider are the size and the geometry of the anion and the receptors as well as hydrophobic interactions and steric demands. The addition of functional groups can introduce additional interactions between the receptor and the anion. PCA analysis indicates that the studied receptors can only weakly differentiate between small carboxylate anions. The results of this work help in predicting, which binding moieties are most suitable to be used for the construction of synthetic receptors for carboxylate anions.

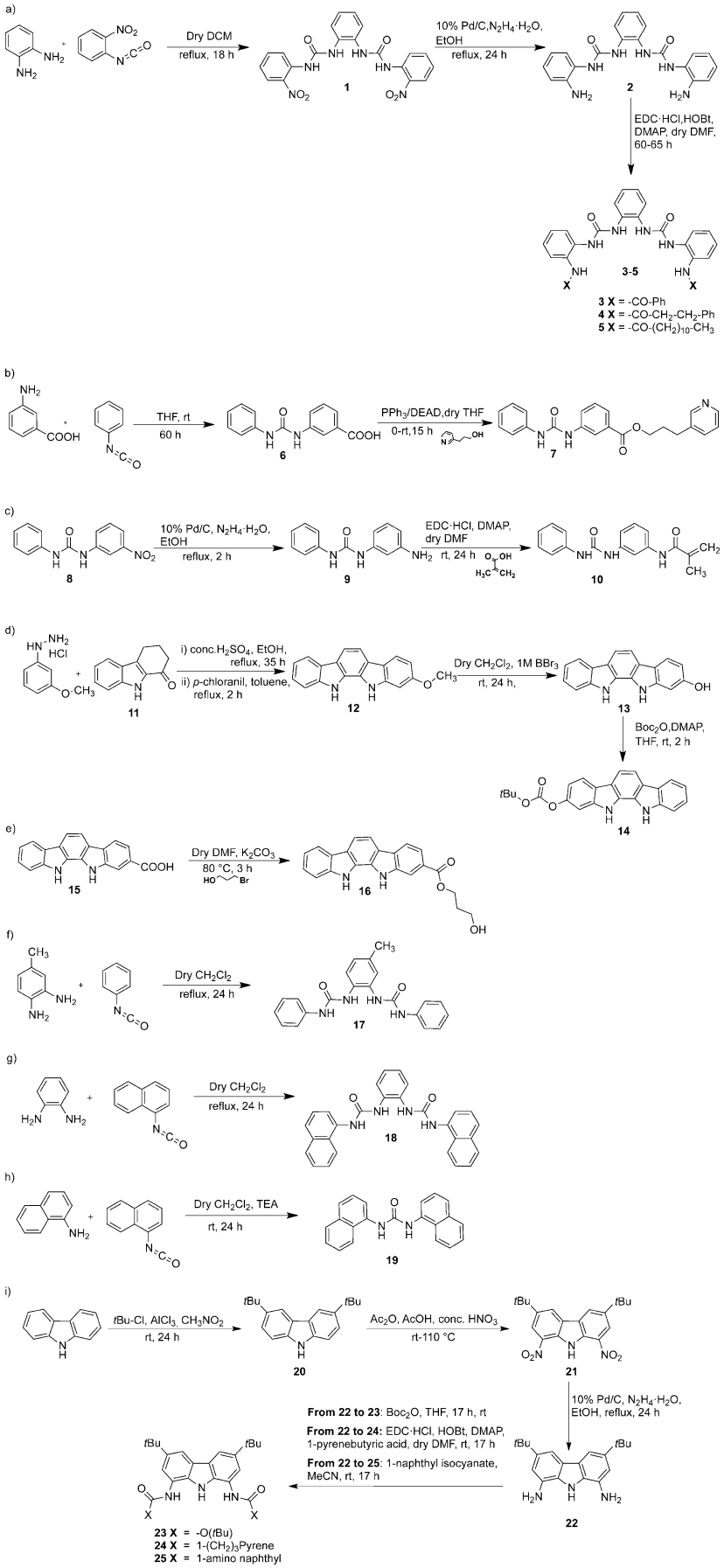
Experimental Section

Instruments and methods: NMR measurements were carried out on a 200 MHz NMR Bruker Avance II 200 NMR spectrometer or a 700 MHz NMR Bruker Avance II 700 NMR spectrometer. UV/Vis spectrophotometric measurements were carried out by using a Thermo Nicolet Evolution 300 spectrophotometer and fluorescence spectrofluorometric measurements were carried out by using a Horiba FluoroMax-4 spectrofluorometer. All receptor molecules were characterised on a 400 MHz Bruker Avance II 400 NMR spectrometer of a 700 MHz NMR Bruker Avance II 700 NMR spectrometer. The water content of the DMSO solvent was checked with a Mettler Toledo DL 32 titrator. Melting points were determined in open capillaries and are uncorrected. COSMO-RS calculations were done by using COSMOthermX version C30_1401 parametrisation BP_TZVP_C30_1401, solvent DMSO with 0.5% water.[26] We used only the geometries from the COSMO-RS calculations for discussions because it has been demonstrated that the ability of COSMO-RS to reproduce experimental energetics of hydrogen-bond formation is insufficient.[26] ATR-IR spectra were recorded on a Thermo Electron Nicolet 6700 FTIR device by using a micro-ATR accessory with a diamond crystal.[27] The spectrometer had a DTGS CsI detector and a CsI beamsplitter. About 128 scans were recorded over the range of $\tilde{\nu}$ = 400–4000 cm^{-1}. High-resolution mass spectra were obtained on a Varian (now Agilent) 930 FT-ICR mass spectrometer equipped with a 7 T superconducting magnet and an IonSpec Omega data system, by using electrospray ionisation (ESI) or matrix-assisted laser desorption/ionisation (MALDI).[28] In the case of the ESI source the ionisation chamber temperature was 40 °C, the spray needle voltage was set to −3000 V, the shield voltage was set to −300 V, the nebulising gas (N_2) pressure was set to 15 psi, the drying gas (N_2) pressure was set to 10 psi at 200 °C. The ion optic was optimised for every compound infused. In the case of the MALDI source positive ions were generated from a target obtained by evaporation under vacuum of a DMSO/*i*PrOH solution of the studied compound/2,5-dihydroxybenzoic mixture deposited as a thin layer on a MALDI target plate. The samples for the HR-MS were dissolved in DMSO/*i*PrOH solution (10:90 μL) leading to a stock solution with a concentration of 20 mg mL^{-1}. An aliquot (1 μL) was dissolved a) in *i*PrOH (1 mL) and infused (ESI) with a flow rate of 3 μL min^{-1} or b) in a 0.1 mol L^{-1} solution of 2,5-dihydroxybenzoic acid in *i*PrOH (for details see the Supporting Information). Purification of the compounds was performed by column chromatography on silica gel (pore size 60 Å, 230–400 mesh). Analytical thin-layer chromatography (TLC) was conducted on TLC plates (silica gel 60 with fluorescent UV_{254} marker on aluminium sheets).

Solvents and chemicals: The solvent for the binding measurements (DMSO with 0.5% of water) was prepared by using anhydrous DMSO 99.9% (for UV/Vis and fluorescence measurements) or [D_6]DMSO 99.8% (for NMR measurements) and water from a MilliQ Advantage A10 system. The final water content of the solvent was checked with a Karl–Fischer titration and was mostly between 0.48 and 0.52% and always between 0.45 and 0.55%. Titrant solutions were prepared from tetrabutylammonium acetate and tetrabutylammonium benzoate (99% Sigma–Aldrich). The solvent for the synthesis, THF (Romil, 99.9%, water content less than 5 ppm, according to Karl–Fisher titration), was additionally dried by means of

continuous circulation through a column filled with alumina and was delivered inside a glovebox. Dichloromethane and DMF were dried as described in reference [29].

Synthesis of the different receptor families: The *ortho*-phenylenediamine-urea-type receptors **1**–**5** have been prepared from *ortho*-phenylenediamine coupled with 2-nitrophenylisocyanate to form compound **1**, which was reduced with 10% Pd/C and hydrazine hydrate in ethanol to form compound **2** (Schemes 3a–i). Compound **2** was coupled with lauric acid, benzoic acid or 3-phenylpropanoic acid by using *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimde (EDC·HCl), 4-dimethylaminopyridine (DMAP) and 1-hydroxybenzotriazole (HOBt) in dry DMF to form compounds **3**–**5**. Compound **7** was prepared from 3-amino-benzoic acid by reacting with phenyl isocyanate to get compound **6**, which in turn was coupled with substituted pyridine by using diethyl azodicarboxylate (DEAD)/PPh_3 to get compound **7**. Compound **10** was prepared from compound **8** (obtained as described in reference [15]) reduced with 10% Pd/C and the hydrazine hydrate **9** and coupled with methacrylic acid to form compound **10**. The substituted indolocarbazole **13** was prepared from compound **11** (obtained as described in reference [14]), which was transferred to compound **12** and then demethylated in BBr_3 (1 M in dry DCM). Compound **14** was prepared by reacting compound **13** with *tert*-butoxycarbonyl (Boc) anhydride. Compound **16** was prepared from compound **15** (prepared as described in reference [15]) coupled with 3-bromopropan-1-ol by using K_2CO_3 in dry DMF.[15] Compounds **17**–**19** were prepared from 4-methylbenzene-1,2-diamine, *ortho*-phenylenediamine and 1-aminonaphthalene with phenylisocyanate and 1-naphthyl isocyanate in dichloromethane. Compounds **20**–**22** were prepared by following published methods.[30,31] Compound **23** was prepared by Boc-protection of compound **22**. Compound **24** was prepared from compound



Scheme 3. Synthesis of different substituted indolocarbazole-, urea- and carbazole-based receptors.

22 coupled with 1-pyrenebutyric acid by using EDC·HCl, HOBt and DMAP and compound **25** was prepared from compound **22** coupled with 1-naphthyl isocyanate in acetonitrile. The following receptors were synthesised as described in literature: **26**,[31] **27**,[32] **28**,[33] **29**,[16] **30**,[17] **31**[12] and **32**–**47**.[14,15]

Preparation of compound 1: *ortho*-Phenylenediamine (0.20 g, 1.85 mmol) was dissolved in dry dichloromethane (60 mL) then 2-nitrophenylisocyanate (0.67 g, 4.07 mmol) was added drop-wise through a syringe. During the addition a precipitate was formed. The reaction mixture was stirred at reflux temperature for 18 h. The reaction was monitored by TLC. After completion of the reaction the mixture was cooled to room temperature, filtered and the obtained solid was washed with diethyl ether (50 mL) to get compound **1** (0.70 g, 1.60 mmol, 87.5%) as a light-yellow solid. M.p. 220.7 °C; $R_f = 0.78$ (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): $\delta = 9.74$ (brs, 2H), 9.17 (brs, 2H), 8.24 (ddd, $^3J(H,H) = 8.5$, $^4J(H,H) = 1.3$, $^5J(H,H) = 0.4$ Hz, 2H), 8.07 (ddd, $^3J(H,H) = 8.4$, $^4J(H,H) = 1.6$, $^5J(H,H) = 0.4$ Hz, 2H), 7.69 (ddd, $^3J(H,H) = 8.5$, $^3J(H,H) = 7.2$, $^4J(H,H) = 1.6$ Hz, 2H), 7.60 (AA′ of AA′XX′, 2H), 7.21 (ddd, $^3J(H,H) = 8.4$, $^3J(H,H) = 7.2$, $^4J(H,H) = 1.3$ Hz, 2H), 7.14 ppm (XX′ of AA′XX′, 2H); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): $\delta = 152.6$, 138.1, 134.9, 134.7, 130.9, 125.4, 124.6, 124.5, 123.0, 122.5 ppm; IR (ATR-FTIRS): $\tilde{\nu} = 3276$, 1650, 1492, 1338, 1278, 732 cm^{-1}; MALDI FTICR: (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{20}H_{16}N_6O_6+Na]^+$: 459.10235 $[M+Na]^+$; found: 459.10268.

Preparation of compound 2: 10% Pd/C (0.04 g) and hydrazine monohydrate (1.0 mL) were added to a stirring suspension of compound **1** (0.55 g, 1.26 mmol) in ethanol (100 mL) and the reaction mixture was heated to reflux for 24 h under nitrogen atmosphere. The mixture was then allowed to cool to room temperature and the resulting product (white precipitate) and Pd/C was removed by using filtration. The product was re-dissolved in DMF (30 mL) and filtered to remove Pd/C. The DMF solution was quenched with H_2O (100 mL) to obtain the crude compound **2** as a white precipitate. The product was removed by using filtration and washed with H_2O (50 mL) followed by methanol (3×10 mL) to afford compound **2** (0.40 g, 1.06 mmol, 84.4%) as a white solid. M.p. 317.2 °C; $R_f = 0.45$ (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): $\delta = 8.15$ (brs, 2H; 1,2-(N*H*)$_2$-C$_6$H$_4$), 8.11 (brs, 2H; 1′-N*H*-C$_6$H$_4$), 7.56 (AA′ of AA′XX′, 2H; CH-3,6$_{C6H4}$), 7.32 (ddm, $^3J(H,H) = 7.9$, $^4J(H,H) = 1.5$ Hz, 2H; CH-6′$_{C6H4}$), 7.05 (XX′ of AA′XX′, 2H; CH-4,5$_{C6H4}$), 6.84 (ddd, $^3J(H,H) = 7.9$, $^3J(H,H) = 7.2$, $^4J(H,H) = 1.5$ Hz, 2H; CH-4′$_{C6H4}$), 6.73 (ddd, $^3J(H,H) = 7.9$, $^4J(H,H) = 1.5$, $^5J(H,H) = 0.3$ Hz, 2H; CH-3′$_{C6H4}$), 6.56 (ddd, $^3J(H,H) = 7.9$, $^3J(H,H) = 7.2$, $^4J(H,H) = 1.5$ Hz, 2H; CH-5′$_{C6H4}$), 4.81 ppm (brs, 4H; NH$_2$); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): $\delta = 153.9$ (NH-CO-NH), 141.1 (C-2′$_{C6H4}$), 131.5 (C-1,2$_{C6H4}$), 124.58 (CH-4′$_{C6H4}$), 124.57 (C-1′$_{C6H4}$), 124.1 (CH-6′$_{C6H4}$), 123.9 (CH-3,6$_{C6H4}$), 123.7 (CH-4,5$_{C6H4}$), 116.7 (CH-5′$_{C6H4}$), 115.8 ppm (CH-3′$_{C6H4}$); IR (ATR-FTIRS): $\tilde{\nu} = 3388$, 3225, 1692, 1558, 1504, 742 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{20}H_{20}N_6O_2+Na]^+$: 399.15399 $[M+Na]^+$; found: 399.15375.

Preparation of compound 3: Compound **2** (0.10 g, 0.26 mmol), EDC·HCl (0.15 g, 0.79 mmol), DMAP (0.09 g, 0.73 mmol), HOBt (0.10 g, 0.74 mmol) and benzoic acid (0.10 g, 0.82 mmol) were dissolved in dry DMF (5 mL). The reaction mixture was stirred at room temperature for 60 h. The reaction was monitored by TLC. After completion of the reaction (determined by disappearance of the starting material and intermediate from TLC) the mixture was quenched with water (100 mL). The formed precipitate was filtered, washed with methanol (50 mL) and dichloromethane (40 mL) to give compound **3** (0.10 g, 0.17 mmol, 63.9%) as an off-white solid. M.p. 190.3 °C; $R_f = 0.46$ (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): $\delta = 10.05$ (brs, 2H; NH-amide), 8.60 (brs, 2H; 1,2-(N*H*)$_2$-C$_6$H$_4$), 8.43 (brs, 2H; 2′-N*H*-C$_6$H$_4$), 8.00 (m, 4H; CH-2,6$_{C6H5}$), 7.79 (ddm, $^3J(H,H) = 8.2$, $^4J(H,H) = 1.5$ Hz, 2H; CH-3′$_{C6H4}$), 7.58 (m, 2H; CH-4$_{C6H5}$), 7.54 (AA′ of AA′XX′, 2H; CH-3,6$_{C6H4}$), 7.51 (m, 4H; CH-3,5$_{C6H5}$), 7.41 (ddm, $^3J(H,H) = 7.9$, $^4J(H,H) = 1.6$ Hz, 2H; CH-6′$_{C6H4}$), 7.20 (ddd, $^3J(H,H) = 8.2$, $^3J(H,H) = 7.4$, $^4J(H,H) = 1.6$ Hz, 2H; CH-4′$_{C6H4}$), 7.09 (ddd, $^3J(H,H) = 7.9$, $^3J(H,H) = 7.4$, $^4J(H,H) = 1.5$ Hz, 2H; CH-5′$_{C6H4}$), 7.04 ppm (XX′ of AA′XX′, 2H; CH-4,5$_{C6H4}$); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): $\delta = 165.6$ (CO-amide), 153.8 (NH-CO-NH), 134.2 (C-1$_{C6H5}$), 134.1 (C-2′$_{C6H4}$), 131.8 (CH-4$_{C6H5}$), 131.1 (C-1,2$_{C6H4}$), 128.48 (C-1′$_{C6H4}$), 128.45 (CH-3,5$_{C6H5}$), 127.8 (CH-2,6$_{C6H5}$), 127.1 (CH-6′$_{C6H4}$), 126.2 (CH-4′$_{C6H4}$), 124.12 (CH-3,6$_{C6H4}$), 124.06 (CH-4,5$_{C6H4}$), 123.0 (CH-5′$_{C6H4}$), 122.4 ppm (CH-3′$_{C6H4}$); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): $\delta = 120.95$ (NH-amide), 102.86 (2′-NH-C$_6$H$_4$), 100.71 ppm (1,2-(NH)$_2$-C$_6$H$_4$); IR (ATR-FTIRS): $\tilde{\nu} = 3276$, 3250, 3060, 3026, 1650, 1595, 1507, 1291, 748, 696 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{34}H_{28}N_6O_4+Na]^+$: 607.20643 $[M+Na]^+$; found: 607.20567.

Preparation of compound 4: Compound **2** (0.14 g, 0.37 mmol), EDC·HCl (0.21 g, 1.12 mmol), DMAP (0.14 g, 1.12 mmol), HOBt (0.15 g, 1.12 mmol) and 3-phenylpropionic acid (0.17 g, 1.12 mmol) were dissolved in dry DMF (5 mL). The reaction mixture was stirred at room temperature for 60 h. The reaction was monitored by TLC. After completion of the reaction (determined by disappearance of the starting material and intermediate from TLC) the mixture was quenched with water (100 mL). The formed precipitate was filtered, washed with methanol (40 mL), dichloromethane (30 mL) and diethyl ether (30 mL) to give compound **4** (0.15 g, 0.23 mmol, 63.2%) as an off-white solid. M.p. 188.2–189.4 °C; $R_f = 0.47$ (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): $\delta = 9.55$ (brs, 2H; NH-amide), 8.55 (brs, 2H; 1,2-(N*H*)$_2$-C$_6$H$_4$), 8.25 (brs, 2H; 2′-N*H*-C$_6$H$_4$), 7.73 (ddm, $^3J(H,H) = 8.2$, $^4J(H,H) = 1.4$ Hz, 2H; CH-3′$_{C6H4}$), 7.57 (AA′ of AA′XX′, 2H; CH-3,6$_{C6H4}$), 7.27 (m, 4H; CH-3,5$_{C6H5}$), 7.23 (m, 2H; CH-6′$_{C6H4}$), 7.23 (m, 4H; CH-2,6$_{C6H5}$), 7.18 (m, 2H; CH-4$_{C6H5}$), 7.11 (ddd, $^3J(H,H) = 8.2$, $^3J(H,H) = 7.4$, $^4J(H,H) = 1.6$ Hz, 2H; CH-4′$_{C6H4}$), 7.09 (XX′ of AA′XX′, 2H; CH-4,5$_{C6H4}$), 7.01 (ddd, $^3J(H,H) = 7.9$, $^3J(H,H) = 7.4$, $^4J(H,H) = 1.4$ Hz, 2H; CH-5′$_{C6H4}$), 2.90 (m, 4H; C*H*$_2$-3), 2.64 ppm (m, 4H; CH$_2$-2); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): $\delta = 171.3$ (CO-CH$_2$), 153.6 (NH-CO-NH), 141.1 (C-1$_{C6H5}$), 133.2 (C-2′$_{C6H4}$), 131.3 (C-1,2$_{C6H4}$), 128.9 (C-1′$_{C6H4}$), 128.4 (CH-3,5$_{C6H5}$), 128.3 (CH-2,6$_{C6H5}$), 126.0 (CH-4$_{C6H5}$), 125.9 (CH-6′$_{C6H4}$), 125.6 (CH-4′$_{C6H4}$), 124.3 (CH-3,6$_{C6H4}$), 124.2 (CH-4,5$_{C6H4}$), 123.2 (CH-3′$_{C6H4}$), 123.1 (CH-5′$_{C6H4}$), 37.5 (CH$_2$-2), 31.0 ppm (CH$_2$-3); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): $\delta = 126.98$ (NH-amide), 102.30 (2′-NH-C$_6$H$_4$), 100.78 ppm (1,2-(NH)$_2$-C$_6$H$_4$); IR (ATR-FTIRS): $\tilde{\nu} = 3250$, 3020, 1650, 1522, 1507, 1291, 748, 696 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{38}H_{36}N_6O_4+Na]^+$: 663.26902 $[M+Na]^+$; found: 663.26961.

Preparation of compound 5: Compound **2** (0.10 g, 0.26 mmol), EDC·HCl (0.13 g, 0.66 mmol), triethylamine (TEA) (0.13 g, 0.66 mmol), HOBt (0.09 g, 0.66 mmol) and lauric acid (0.13 g, 0.66 mmol) were dissolved in dry DMF (5 mL). The reaction mixture was stirred at room temperature for 65 h. The reaction was monitored by TLC. After completion of the reaction (determined by disappearance of the starting material and intermediate from TLC) the mixture was quenched with water (100 mL). The formed precipitate was filtered and washed with methanol (60 mL) and dichloromethane (50 mL) to give compound **5** (0.14 g, 0.19 mmol, 72.7%) as a white solid. M.p. 179.6–180.8 °C; $R_f = 0.53$ (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): $\delta = 9.48$ (brs, 2H; NH-amide), 8.53 (brs, 2H; 1,2-(N*H*)$_2$-C$_6$H$_4$), 8.23 (brs, 2H; 2′-N*H*-C$_6$H$_4$), 7.70 (ddm, $^3J(H,H) = 8.2$, $^4J(H,H) = 1.4$ Hz, 2H; CH-3′$_{C6H4}$), 7.56 (AA′ of AA′XX′, 2H; CH-3,6$_{C6H4}$), 7.29 (ddm, $^3J(H,H) = 7.9$, $^4J(H,H) = 1.6$ Hz, 2H; CH-6′$_{C6H4}$), 7.13 (ddd, $^3J(H,H) = 8.2$,

^3J(H,H) = 7.4, ^4J(H,H) = 1.6 Hz, 2H; CH-4′$_{C6H4}$), 7.07 (XX′ of AA′XX′, 2H; CH-4,5$_{C6H4}$), 7.03 (ddd, ^3J(H,H) = 7.9, ^3J(H,H) = 7.4, ^4J(H,H) = 1.4 Hz, 2H; CH-5′$_{C6H4}$), 2.31 (t, ^3J(H,H) = 7.5 Hz, 4H; CH_2-2), 1.57 (m, 4H; CH_2-3), 1.15–1.35 (m, 32H; CH_2-4-11), 0.84 ppm (vt, 6H; CH_3); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): δ = 172.1 (CO-CH_2), 153.7 (NH-CO-NH), 133.0 (C-2′$_{C6H4}$), 131.3 (C-1,2$_{C6H4}$), 129.2 (C-1′$_{C6H4}$), 125.8 (CH-6′$_{C6H4}$), 125.5 (CH-4′$_{C6H4}$), 124.2 (CH-3,6$_{C6H4}$), 124.1 (CH-4,5$_{C6H4}$), 123.4 (CH-3′$_{C6H4}$), 123.2 (CH-5′$_{C6H4}$), 36.0 (CH_2-2), 31.4 (CH_2-10), 25.2 (CH_2-3), 29.13, 29.10, 29.06, 28.9, 28.82, 28.79 (CH_2-4-CH_2-9), 22.2 (CH_2-11), 14.0 ppm (CH_3); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 126.97 (NH-amide), 102.30 (2′-NH-C_6H_4), 100.66 ppm (1,2-(NH)$_2$-C_6H_4); IR (ATR-FTIRS): $\tilde{\nu}$ = 3281, 2919, 2851, 1646, 1519, 1452, 748 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{44}H_{64}N_6O_4+Na]^+$: 763.48812 [*M*+Na]$^+$; found: 763.48786.

Preparation of compound 6: 3-Aminobenzoic acid (0.50 g, 3.64 mmol) was dissolved in dry THF (60 mL) then phenyl isocyanate (0.43 g, 3.64 mmol) was added drop-wise. The reaction mixture was stirred at room temperature for 60 h. The reaction was monitored by TLC (disappearance of the starting material from TLC). The formed white precipitate was filtered and washed with dichloromethane (70 mL) and diethyl ether (60 mL) to obtain compound **6** (0.80 g, 0.31 mmol, 85.6%) as a white solid. M.p. 153-154 °C; R_f = 0.25 (10% methanol in dichloromethane); ^{1}H NMR (400.1 MHz, [D$_6$]DMSO, +25 °C): δ = 12.90 (brs, 1H; COOH), 8.88 (brs, 1H; C_6H_4-N*H*), 8.68 (brs, 1H; C_6H_5-N*H*), 8.12 (ddd, ^4J(H,H) = 2.4, ^4J(H,H) = 1.6, ^5J(H,H) = 0.5 Hz, 1H; CH-2), 7.63 (ddd, ^3J(H,H) = 8.1, ^4J(H,H) = 2.4, ^4J(H,H) = 1.1 Hz, 1H; CH-4), 7.55 (ddd, ^3J(H,H) = 7.7, ^4J(H,H) = 1.6, ^4J(H,H) = 1.1 Hz, 1H; CH-6), 7.46 (m, 2H; CH-2′,6′), 7.40 (ddd, ^3J(H,H) = 8.1, ^3J(H,H) = 7.7, ^5J(H,H) = 0.5 Hz, 1H; CH-5), 7.28 (m, 2H; CH-3′,5′), 6.98 ppm (m, 1H; CH-4′); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, +25 °C): δ = 167.2 (COOH), 152.5 (NH-CO-NH), 140.0 (C-3), 139.5 (C-1′), 131.3 (C-1), 128.9 (CH-5), 128.7 (CH-3′,5′), 122.6 (CH-6), 122.3 (CH-4), 121.9 (CH-4′), 118.8 (CH-2), 118.3 ppm (CH-2′,6′); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 108.98 (C_6H_5-NH), 108.74 ppm (C_6H_4-NH); IR (ATR-FTIRS): $\tilde{\nu}$ = 3288, 2632, 2566, 1685, 1592, 1564, 1305 cm^{-1}; ESI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{14}H_{12}N_2O_3-H]^-$: 255.07752 [*M*−H]$^-$; found: 255.07735.

Preparation of compound 7: Compound **6** (0.31 g, 1.21 mmol) and 3-(pyridin-3-yl)propan-1-ol (0.22 g, 1.60 mmol) were dissolved in dry THF (100 mL). Then PPh_3 (0.60 g, 2.29 mmol) was added. The reaction mixture was stirred at 0 °C for 15 min. The catalyst DEAD (40% assay in toluene) (0.32 g, 1.83 mmol) was added drop-wise. The reaction mixture was stirred at 0 °C for 1 h then stirred for 15 h at room temperature. After disappearance of the starting material (monitored by TLC) the reaction mixture was concentrated under reduced pressure. The formed oily gel was diluted with ethyl acetate (50 mL) and washed with water (100 mL). The organic layer was concentrated under reduced pressure. The product was purified by column (230–400 mesh silica) chromatography eluting with 1% methanol in dichloromethane to give compound **7** (0.33 g, 0.88 mmol, 72.7%) as a white solid. M.p. 140.5 °C; R_f = 0.45 (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): δ = 8.94 (brs, 1H; NH-Bn), 8.71 (brs, 1H; Ph-NH), 8.49 (dm, ^4J(H,H) = 2.3 Hz, 1H; CH-2$_{Pyr}$), 8.41 (ddm, ^3J(H,H) = 4.7, ^4J(H,H) = 1.7 Hz, 1H; CH-6$_{Pyr}$), 8.17 (ddd, ^4J(H,H) = 2.3, ^4J(H,H) = 1.6, ^5J(H,H) = 0.4 Hz, 1H; CH-2$_{Bn}$), 7.70 (ddd, ^3J(H,H) = 7.8, ^4J(H,H) = 2.3, ^4J(H,H) = 1.7 Hz, 1H; CH-4$_{Pyr}$), 7.67 (ddd, ^3J(H,H) = 8.1, ^4J(H,H) = 2.3, ^4J(H,H) = 1.1 Hz, 1H; CH-4$_{Bn}$), 7.55 (ddd, ^3J(H,H) = 7.6, ^4J(H,H) = 1.6, ^4J(H,H) = 1.1 Hz, 1H; CH-6$_{Bn}$), 7.47 (m, 2H; CH-2,6$_{Ph}$), 7.43 (ddd, ^3J(H,H) = 8.1, ^3J(H,H) = 7.6, ^5J(H,H) = 0.4 Hz, 1H; CH-5$_{Bn}$), 7.32 (ddd, ^3J(H,H) = 7.8, ^3J(H,H) = 4.7, ^5J(H,H) = 0.9 Hz, 1H; CH-5$_{Pyr}$), 7.29 (m, 2H; C*H*-3,5$_{Ph}$), 6.98 (m, 1H; CH-4$_{Ph}$), 4.27 (t, ^3J(H,H) = 6.4 Hz, 2H; CH_2-1), 2.77 (m, 2H; CH_2-3), 2.05 ppm (m, 2H; CH_2-2); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): δ = 165.7 (COO), 152.5 (NH-CO-NH), 149.7 (CH-2$_{Pyr}$), 147.4 (CH-6$_{Pyr}$), 140.2 (C-3$_{Bn}$), 139.5 (C-1$_{Ph}$), 136.6 (C-3$_{Pyr}$), 135.9 (CH-4$_{Pyr}$), 130.3 (C-1$_{Bn}$), 129.2 (CH-5$_{Bn}$), 128.8 (CH-3,5$_{Ph}$), 123.5 (CH-5$_{Pyr}$), 122.8 (CH-4$_{Bn}$), 122.5 (CH-6$_{Bn}$), 122.1 (CH-4$_{Ph}$), 118.6 (CH-2$_{Bn}$), 118.4 (CH-2,6$_{Ph}$), 63.9 (CH_2-1), 29.5 (CH_2-2), 28.7 ppm (CH_2-3); ^{15}N NMR (70.9 MHz, [D$_6$]DMSO, +20 °C): δ = 317.3 (N-1$_{Pyr}$), 108.86 (NH-Ph), 108.67 ppm (NH-Bn); IR (ATR-FTIRS): $\tilde{\nu}$ = 3275, 1719, 1592, 1562, 1462, 1294, 1082 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{22}H_{21}N_3O_3+Na]^+$: 398.14751 [*M*+Na]$^+$; found: 398.14810.

Preparation of compound 9: Compound **8**[15] (0.18 g, 0.70 mmol), and 10% Pd/C (0.030 g) were suspended in EtOH (40 mL) then hydrazine monohydrate (0.5 mL) was added drop-wise. The reaction mixture was stirred at reflux temperature for 2 h under nitrogen atmosphere. After disappearance of the starting material (monitored by TLC) the mixture was allowed to cool to room temperature and Pd/C was removed by using filtration. The filtrate was concentrated under reduced pressure to get compound **9** as (0.15 g, 0.66 mmol, 94.4%) a white solid. M.p. 188.2 °C; R_f = 0.51 (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): δ = 8.55 (brs, 1H; NH), 8.37 (brs, 1H; NH), 7.43 (m, 2H; CH-2,6$_{Ph}$), 7.26 (m, 2H; CH-3,5$_{Ph}$), 6.94 (m, 1H; CH-4$_{Ph}$), 6.88 (ddd, ^3J(H,H) = 8.0, ^3J(H,H) = 7.9, ^5J(H,H) = 0.3 Hz, 1H; CH-5$_{C6H4}$), 6.77 (ddd, ^4J(H,H) = 2.2, ^4J(H,H) = 2.1, ^5J(H,H) = 0.3 Hz, 1H; CH-2$_{C6H4}$), 6.55 (ddd, ^3J(H,H) = 8.0, ^4J(H,H) = 2.1, ^4J(H,H) = 1.0 Hz, 1H; CH-4$_{C6H4}$ or CH-6$_{C6H4}$), 6.18 (ddd, ^3J(H,H) = 7.9, ^4J(H,H) = 2.2, ^4J(H,H) = 1.0 Hz, 1H; CH-4$_{C6H4}$ or CH-6$_{C6H4}$), 5.04 ppm (brs, 2H; NH_2); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): δ = 152.4, 149.2, 140.3, 139.9, 129.1, 128.8, 121.6, 118.0, 108.1, 106.1, 103.7 ppm; IR (ATR-FTIRS): $\tilde{\nu}$ = 3322, 3288, 1650, 1545, 1492, 1441, 1312, 1222 cm^{-1}; ESI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{13}H_{13}N_3O_1-H]^-$: 226.09859 [*M*−H]$^-$; found: 226.09845.

Preparation of compound 10: Compound **9** (0.16 g, 0.70 mmol), EDC·HCl (0.24 g, 1.25 mmol) and DMAP (0.17 g, 1.39 mmol) were dissolved in dry DMF (5 mL) then 2-methylacrylic acid (0.08 g, 0.93 mmol) was added drop-wise. The reaction mixture was stirred at room temperature for 24 h. After disappearance of the starting material (monitored by TLC) the reaction mixture was quenched with water (100 mL). The formed precipitate was extracted in ethyl acetate (50 mL). The organic layer was washed with water (50 mL) and dried over $MgSO_4$, filtered and concentrated under reduced pressure. The obtained product was washed with diethyl ether (50 mL) to get compound **10** (0.15 g, 0.51 mmol, 72.6%) as an off-white solid. M.p. 210.4 °C; R_f = 0.58 (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): δ = 9.78 (brs, 1H; amide-NH), 8.70 (brs, 1H; NH-C_6H_4), 8.62 (brs, 1H; Ph-NH), 7.85 (ddd, ^4J(H,H) = 2.0, ^4J(H,H) = 1.5, ^5J(H,H) = 1.0 Hz, 1H; CH-2$_{C6H4}$), 7.44 (m, 2H; CH-2,6$_{Ph}$), 7.27 (m, 2H; CH-3,5$_{Ph}$), 7.26 (m, 1H; CH-6$_{C6H4}$), 7.19 (m, 2H; CH-4$_{C6H4}$ and CH-5$_{C6H4}$), 6.96 (m, 1H; CH-4$_{Ph}$), 5.79 (dq, ^2J(H,H) = 0.9, ^{4}J(H,H) = 0.9 Hz, 1H; =CH_Z), 5.50 (dq, ^4J(H,H) = 1.6, ^2J(H,H) = 0.9 Hz, 1H; =CH_E), 1.94 ppm (dd, ^4J(H,H) = 1.6, ^4J(H,H) = 0.9 Hz, 3H; CH_3); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): δ = 167.0 (amide-CO), 152.5 (NH-CO-NH), 140.5 (*C*=CH_2), 139.9 (C-3$_{C6H4}$), 139.8 (C-1$_{Ph}$), 139.5 (C-1$_{C6H4}$), 128.91 (CH-3,5$_{Ph}$), 128.86 (CH-5$_{C6H4}$), 121.9 (CH-4$_{Ph}$), 120.0 (=CH_2), 118.2 (CH-2,6$_{Ph}$), 114.0 (CH-6$_{C6H4}$), 113.5 (CH-4$_{C6H4}$), 110.2 (CH-2$_{C6H4}$), 18.9 ppm (CH_3); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 128.27 (amide-NH), 109.02 (NH-C_6H_4), 108.71 ppm (NH-Ph); IR (ATR-FTIRS): $\tilde{\nu}$ = 3301, 1643, 1597, 1535, 1487, 1314, 1296, 693, 632 cm^{-1}; ESI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{17}H_{17}N_3O_2-H]^-$: 294.12480 [*M*−H]$^-$; found: 294.12516.

Preparation of compound 13: Compound **12**[14] (0.31 g, 1.08 mmol) was dissolved in dry dichloromethane (20 mL) and the solution was cooled for 30 min at 0 °C. Then a 1 M solution of BBr_3 (4 mL) was added drop-wise. The reaction mixture was stirred at 0 °C for 15 min and then 24 h at room temperature. After disappearance of the starting material (monitored by TLC) the reaction mixture was cooled to 0 °C, quenched by adding methanol (5 mL) drop-wise and concentrated under reduced pressure. Thereafter the following treatment was carried out two times (for removing boric acid in the form of its volatile trimethyl ester): 5% solution of methanol in dichloromethane (150 mL) was added to the concentrated mixture and the mixture was concentrated again under reduced pressure. A solid product was obtained and washed with diethyl ether (30 mL) to give compound **13** (0.21 g, 0.77 mmol, 71.4%) as an off-white solid. M.p. decomposed above 350 °C; R_f = 0.12 (30% ethyl acetate in hexane); ^{1}H NMR (400.1 MHz, [D$_6$]DMSO, +25 °C): δ = 10.89 (brs, 1H; NH-11), 10.73 (brs, 1H; NH-12), 9.33 (brs, 1H; OH), 8.10 (dddd, ^3J(H,H) = 7.8, ^4J(H,H) = 1.2, ^5J(H,H) = 0.8, ^5J(H,H) = 0.6 Hz, 1H; CH-7), 7.89 (ddd, ^3J(H,H) = 8.5, ^5J(H,H) = 0.6, ^5J(H,H) = 0.5 Hz, 1H; CH-4), 7.82 (dd, ^3J(H,H) = 8.2, ^5J(H,H) = 0.4 Hz, 1H; CH-6), 7.75 (dd, ^3J(H,H) = 8.2, ^5J(H,H) = 0.4 Hz, 1H; CH-5), 7.64 (ddd, ^3J(H,H) = 8.1, ^4J(H,H) = 1.0, ^5J(H,H) = 0.8 Hz, 1H; CH-10), 7.35 (ddd, ^3J(H,H) = 8.1, ^3J(H,H) = 7.0, ^4J(H,H) = 1.2 Hz, 1H; CH-9), 7.17 (ddd, ^3J(H,H) = 7.8, ^3J(H,H) = 7.0, ^4J(H,H) = 1.0 Hz, 1H; CH-8), 7.01 (dd, ^4J(H,H) = 2.2, ^5J(H,H) = 0.5 Hz, 1H; CH-1), 6.69 ppm (dd, ^3J(H,H) = 8.5, ^4J(H,H) = 2.2 Hz, 1H; CH-3); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, +25 °C): δ = 155.7 (C-2), 140.6 (C-12′), 138.9 (C-10′), 125.7 (C-11′), 124.9 (C-11″), 124.1 (CH-9), 123.9 (C-7′), 120.7 (C-5′), 120.2 (CH-4), 119.4 (CH-7), 119.0 (C-6′), 118.7 (CH-8), 116.7 (C-4′), 111.34 (CH-10), 111.25 (CH-6), 110.8 (CH-5), 108.7 (CH-3), 97.1 ppm (CH-1); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 112.27 (NH-12), 112.21 ppm (NH-11); IR (ATR-FTIRS): $\tilde{\nu}$ = 3402, 3050, 1625, 1324, 1151, 740 cm^{-1}; ESI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for [$C_{18}H_{12}N_2O_1$−H]$^-$: 271.08769 [*M*−H]$^-$; found: 271.08761.

Preparation of compound 14: Compound **13** (0.10 g, 0.36 mmol) was dissolved in THF (5 mL) then DMAP (0.022 g, 0.18 mmol) was added, the reaction mixture was stirred at 0 °C, then di-*tert*-butyl dicarbonate (0.16 g, 0.74 mmol) was added drop-wise. The reaction mixture was stirred for 2 h and then concentrated under reduced pressure. The crude product was purified by column chromatography (230–400 mesh silica) eluting with 3% ethyl acetate in hexane to get compound **14** (0.07 g, 0.18 mmol, 50.5%) as a brown solid. M.p. 340.6 °C; R_f = 0.40 (30% ethyl acetate in hexane); ^{1}H NMR (400.1 MHz, [D$_6$]DMSO, +25 °C): δ = 11.12 (brs, 1H; NH-11), 11.10 (brs, 1H; NH-12), 8.15 (dddd, ^3J(H,H) = 7.8, ^4J(H,H) = 1.2, ^5J(H,H) = 0.8, ^5J(H,H) = 0.6 Hz, 1H; CH-7), 8.14 (ddd, ^3J(H,H) = 8.4, ^5J(H,H) = 0.6, ^5J(H,H) = 0.5 Hz, 1H; CH-4), 7.93 (dd, ^3J(H,H) = 8.3, ^5J(H,H) = 0.5 Hz, 1H; CH-6), 7.90 (dd, ^3J(H,H) = 8.3, ^5J(H,H) = 0.5 Hz, 1H; CH-5), 7.68 (ddd, ^3J(H,H) = 8.1, ^4J(H,H) = 1.0, ^5J(H,H) = 0.8 Hz, 1H; CH-10), 7.55 (dd, ^4J(H,H) = 2.2, ^5J(H,H) = 0.5 Hz, 1H; CH-1), 7.39 (ddd, ^3J(H,H) = 8.1, ^3J(H,H) = 7.1, ^4J(H,H) = 1.2 Hz, 1H; CH-9), 7.20 (ddd, ^3J(H,H) = 7.8, ^3J(H,H) = 7.1, ^4J(H,H) = 1.0 Hz, 1H; CH-8), 7.01 (dd, ^3J(H,H) = 8.4, ^4J(H,H) = 2.2 Hz, 1H; CH-3), 1.54 ppm (s, 9H; CMe_3); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, +25 °C): δ = 151.8 (C=O), 148.2 (C-2), 139.0 (C-12′), 138.9 (C-10′), 126.1 (C-11″), 125.5 (C-11′), 124.5 (CH-9), 123.6 (C-7′), 121.6 (C-4′), 120.04 (C-6′), 119.97 (CH-4), 119.68 (CH-7), 119.65 (C-5′), 118.9 (CH-8), 112.8 (CH-3), 111.9 (CH-6), 111.5 (CH-5), 111.4 (CH-10), 104.5 (CH-1), 82.9 (CMe_3), 27.3 ppm (CMe_3); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 113.29 (NH-11), 114.22 ppm (NH-12); IR (ATR-FTIRS): $\tilde{\nu}$ = 3403, 2925, 1762, 1274, 1250, 1137, 744 cm^{-1}; ESI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for [$C_{23}H_{20}N_2O_3$−H]$^-$: 371.14012 [*M*−H]$^-$; found: 371.13983.

Preparation of compound 16: Compound **15**[15] (0.10 g, 0.33 mmol) and K_2CO_3 (0.09 g, 0.66 mmol) were dissolved in dry DMF (3 mL) then 3-bromopropan-1-ol (0.055 g, 0.40 mmol) was added drop-wise. The reaction mixture was stirred for 3 h at 80 °C under N_2 atmosphere. After disappearance of the starting material (monitored by TLC) the reaction mixture was cooled to room temperature and quenched with water (75 mL). The product was extracted with ethyl acetate (3×20 mL). The ethyl acetate solution was washed with water (50 mL) and a saturated aqueous solution of NaCl (25 mL) and thereafter concentrated under reduced pressure. The obtained yellow solid was washed with ethyl acetate (10 mL) and diethyl ether (25 mL) to get the pure compound **16** (0.08 g, 0.22 mmol, 67.1%) as yellow solid. M.p. decomposed above 350 °C; R_f = 0.53 (30% ethyl acetate in hexane); ^{1}H NMR (400.1 MHz, [D$_6$]DMSO, +25 °C): δ = 11.33 (brs, 1H; NH-11), 11.31 (brs, 1H; NH-12), 8.37 (dd, ^4J(H,H) = 1.5, ^5J(H,H) = 0.7 Hz, 1H; CH-1), 8.26 (ddd, ^3J(H,H) = 8.2, ^5J(H,H) = 0.7, ^5J(H,H) = 0.6 Hz, 1H; CH-4), 8.18 (dddd, ^3J(H,H) = 7.8, ^4J(H,H) = 1.2, ^5J(H,H) = 0.8, ^5J(H,H) = 0.6 Hz, 1H; CH-7), 7.97 (brs, 2H; CH-5 and CH-6), 7.83 (dd, ^3J(H,H) = 8.2, ^4J(H,H) = 1.5 Hz, 1H; CH-3), 7.70 (ddd, ^3J(H,H) = 8.1, ^4J(H,H) = 1.0, ^5J(H,H) = 0.8 Hz, 1H; CH-10), 7.42 (ddd, ^3J(H,H) = 8.1, ^3J(H,H) = 7.1, ^4J(H,H) = 1.2 Hz, 1H; CH-9), 7.22 (ddd, ^3J(H,H) = 7.8, ^3J(H,H) = 7.1, ^4J(H,H) = 1.0 Hz, 1H; CH-8), 4.40 (t, ^3J(H,H) = 6.5 Hz, 2H; OCH_2), 3.63 (t, ^3J(H,H) = 6.2 Hz, 2H; CH_2OH), 1.95 ppm (tt, ^3J(H,H) = 6.5, ^3J(H,H) = 6.2 Hz, 2H; $CH_2CH_2CH_2$); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, +25 °C): δ = 166.6 (COO), 139.2 (C-10′), 138.3 (C-12′), 127.5 (C-4′), 127.5 (C-11″), 125.5 (C-2), 125.4 (C-11′), 124.9 (CH-9), 123.6 (C-7′), 121.0 (C-6′), 119.9 (CH-7), 119.7 (CH-3), 119.5 (CH-4), 119.4 (C-5′), 119.1 (CH-8), 113.2 (CH-1), 112.4 (CH-5 or CH-6), 112.1 (CH-5 or CH-6), 111.7 (CH-10), 61.9 (OCH_2), 57.4 (CH_2OH), 31.8 ppm ($CH_2CH_2CH_2$); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 114.99 (NH-12), 114.32 ppm (NH-11); IR (ATR-FTIRS): $\tilde{\nu}$ = 3351, 2960, 2888, 1672, 1613, 1240, 1045, 754 cm^{-1}; ESI FTICR (solvent ≈0.01% DMSO)*i*PrOH): *m/z* calcd for [$C_{22}H_{18}N_2O_3$−H]$^-$: 357.12446 [*M*−H]$^-$; found: 357.12514.

Preparation of compound 17: 4-Methylbenzene-1,2-diamine (0.09 g, 0.75 mmol) was dissolved in dry dichloromethane (25 mL) then phenylisocyanate (0.21 g, 1.71 mmol) was added drop-wise. The reaction mixture was heated to reflux under N_2 atmosphere for 24 h. After disappearance of the starting material (monitored by TLC) the formed white precipitate was filtered and washed with diethyl ether (30 mL) to obtain the pure compound **17** (0.25 g, 0.69 mmol, 93.2%) as a white solid. M.p. 241.3 °C; R_f = 0.60 (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): δ = 9.09 (brs, 1H; 2-NHCON*H*), 8.98 (brs, 1H; 1-NHCON*H*), 8.01 (brs, 1H; 2-NH), 7.94 (brs, 1H; 1-NH), 7.48 (dm, ^4J(H,H) = 2.1 Hz, 1H; CH-3$_{C6H3}$), 7.47 (m, 2H; CH-2,6$_{Ph-2}$), 7.46 (m, 2H; CH-2,6$_{Ph-1}$), 7.40 (dm, ^3J(H,H) = 8.1 Hz, 1H; CH-6$_{C6H3}$), 7.27 (m, 2H; CH-3,5$_{Ph-2}$), 7.26 (m, 2H; CH-3,5$_{Ph-1}$), 6.96 (m, 1H; CH-4$_{Ph-2}$), 6.95 (m, 1H; CH-4$_{Ph-1}$), 6.89 (ddq, ^3J(H,H) = 8.1, ^4J(H,H) = 2.1, ^4J(H,H) = 0.8 Hz, 1H; CH-5$_{C6H3}$), 2.27 ppm (dm, ^4J(H,H) = 0.8 Hz, 3H; CH_3); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): δ = 153.4 (1-NH-CO), 153.1 (2-NH-CO), 140.0 (C-1$_{Ph-1}$), 139.9 (C-1$_{Ph-2}$), 133.4 (C-2$_{C6H3}$), 131.7 (C-1$_{C6H3}$), 128.83 (CH-3,5$_{Ph-2}$), 128.81 (CH-3,5$_{Ph-1}$), 128.2 (C-4$_{C6H3}$), 124.5 (CH-6$_{C6H3}$), 124.4 (CH-5$_{C6H3}$), 124.0 (CH-3$_{C6H3}$), 121.8 (CH-4$_{Ph-2}$), 121.7 (CH-4$_{Ph-1}$), 108.13 (CH-2,6$_{Ph-2}$), 108.11 (CH-2,6$_{Ph-1}$), 20.7 ppm (CH_3); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +20 °C): δ = 108.64 (2-NHCO*N*H), 108.24 (1-NHCO*N*H), 100.06 (2-NH), 98.22 ppm (1-NH); IR (ATR-FTIRS): $\tilde{\nu}$ = 3275, 3055, 1629, 1599, 1565, 1540, 1310, 1211, 689 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for [$C_{21}H_{20}N_4O_2$+Na]$^+$: 383.14785 [*M*+Na]$^+$; found: 383.14772.

Preparation of compound 18: *ortho*-Phenylenediamine (0.10 g, 0.92 mmol) was dissolved in dry dichloromethane (50 mL) then 1-naphthyl isocyanate (0.37 g, 2.22 mmol) was added drop-wise. The

reaction mixture was heated to reflux under N_2 atmosphere for 24 h. After disappearance of the starting material (monitored by TLC) the formed white precipitate was filtered and washed with dichloromethane to obtain the pure compound **18** (0.35 g, 0.78 mmol, 78.3%) as a white solid. M.p. 273.5 °C; R_f = 0.58 (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): δ = 9.13 (br s, 2H; NH-Naph), 8.58 (br s, 2H; NH-Ph), 8.21 (dm, ^3J(H,H) = 8.5 Hz, 2H; CH-8$_{Naph}$), 8.04 (dd, ^3J(H,H) = 7.6, ^4J(H,H) = 1.1 Hz, 2H; CH-2$_{Naph}$), 7.93 (dm, ^3J(H,H) = 8.1 Hz, 2H; CH-5$_{Naph}$), 7.69 (AA′ of AA′XX′, 2H; CH-3,6$_{Ph}$), 7.64 (m, 2H; CH-4$_{Naph}$), 7.59 (ddd, ^3J(H,H) = 8.5, ^3J(H,H) = 6.8, ^4J(H,H) = 1.4 Hz, 2H; CH-7$_{Naph}$), 7.54 (ddd, ^3J(H,H) = 8.1, ^3J(H,H) = 6.8, ^4J(H,H) = 1.2 Hz, 2H; CH-6$_{Naph}$), 7.48 (dd, ^3J(H,H) = 8.2, ^3J(H,H) = 7.6 Hz, 2H; CH-3$_{Naph}$), 7.12 ppm (XX′ of AA′XX′, 2H; CH-4,5$_{Ph}$); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): δ = 131.3 (C-1,2$_{Ph}$), 153.7 (CO), 134.5 (C-1$_{Naph}$), 133.8 (C-4a$_{Naph}$), 128.5 (CH-5$_{Naph}$), 125.983 (CH-6$_{Naph}$), 125.976 (CH-3$_{Naph}$), 125.9 (C-8a$_{Naph}$), 125.7 (CH-7$_{Naph}$), 124.00 (CH-3,6$_{Ph}$), 123.97 (CH-4,5$_{Ph}$), 123.0 (CH-4$_{Naph}$), 121.6 (CH-8$_{Naph}$), 117.5 ppm (CH-2$_{Naph}$); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 101.94 (NH-Naph), 100.25 ppm (NH-Ph); IR (ATR-FTIRS): $\tilde{\nu}$ = 3261, 3046, 1644, 1555, 1499, 1398, 1274, 1245, 784 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for [$C_{28}H_{22}N_4O_2$+Na]$^+$: 469.16350 [*M*+Na]$^+$; found: 469.16354.

Preparation of compound 19: 1-Aminonaphthalene (0.15 g, 1.05 mmol) was dissolved in dry dichloromethane (20 mL) then TEA (0.1 mL) was added drop-wise and thereafter 1-naphthyl isocyanate (0.23 g, 1.36 mmol) was added drop-wise. The reaction mixture was stirred under N_2 atmosphere for 24 h at room temperature. After disappearance of the starting material (monitored by TLC) the formed precipitate was filtered and washed with diethyl ether (20 mL) to obtain the pure compound **19** (0.25 g, 0.80 mmol, 76.2%) as a white solid. M.p. 287.6 °C; R_f = 0.83 (10% methanol in dichloromethane); ^{1}H NMR (400.1 MHz, [D$_6$]DMSO, +25 °C): δ = 9.17 (br s, 2H; NH), 8.25 (dm, ^3J(H,H) = 8.5 Hz, 2H; CH-8), 8.08 (dd, ^3J(H,H) = 7.6, ^4J(H,H) = 1.2 Hz, 2H; CH-2), 7.96 (dddd, ^3J(H,H) = 8.1, ^4J(H,H) = 1.3, ^4J(H,H) = 0.7, ^5J(H,H) = 0.6 Hz, 2H; CH-5), 7.66 (dm, ^3J(H,H) = 8.1 Hz, 2H; CH-4), 7.64 (ddd, ^3J(H,H) = 8.5, ^3J(H,H) = 6.8, ^4J(H,H) = 1.3 Hz, 2H; CH-7), 7.57 (ddd, ^3J(H,H) = 8.1, ^3J(H,H) = 6.8, ^4J(H,H) = 1.2 Hz, 2H; CH-6), 7.50 ppm (dd, ^3J(H,H) = 8.1, ^3J(H,H) = 7.6 Hz, 2H; CH-3); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, +25 °C): δ = 153.3 (CO), 134.4 (C-1), 133.7 (C-4a), 128.4 (CH-5), 125.92 (C-8a), 125.90 (CH-6), 125.87 (CH-3), 125.7 (CH-7), 122.9 (CH-4), 121.4 (CH-8), 117.5 ppm (CH-2); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 102.17 ppm (NH); IR (ATR-FTIRS): $\tilde{\nu}$ = 3272, 3051, 1637, 1549, 1501, 1245, 1212, 1211, 783 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for [$C_{21}H_{16}N_2O_1$+Na]$^+$: 335.11548 [*M*+Na]$^+$; found: 335.11572.

Preparation of compound 23: Di-*tert*-butyl dicarbonate (0.30 g, 1.37 mmol) was added drop-wise to a solution of compound **22** (0.21 g, 0.68 mmol) in dichloromethane (70 mL) at 0 °C. The mixture was stirred 17 h at room temperature. After disappearance of the starting material (monitored by TLC) water (35 mL) was added, the mixture was extracted with dichloromethane (2×30 mL), dried with anhydrous Na_2SO_4, filtered, and evaporated to give a light-pink solid. This solid was purified by column chromatography (silica 230-400 mesh) eluting with 5% ethyl acetate in hexane to get compound **23** (0.22 g, 0.43 mmol, 63.0%) as a light-brown solid. M.p. 222.7 °C; R_f = 0.78 (30% ethyl acetate in hexane); ^{1}H NMR (400.1 MHz, [D$_6$]DMSO, +25 °C): δ = 10.32 (br s, 1H; NH-9), 9.11 (br s, 2H; NH-CO), 7.85 (d, ^4J(H,H) = 1.8 Hz, 2H; CH-4,5), 7.60 (br s, 2H; CH-2,7), 1.52 (s, 18H; OC(CH$_3$)$_3$), 1.38 ppm (s, 18H; CC(CH$_3$)$_3$); ^{13}C NMR (100.6 MHz, [D$_6$]DMSO, +25 °C): δ = 153.4 (CO), 141.5 (C-3,6), 130.4 (C-8a,9a), 123.9 (C-4a,4b), 122.5 (C-1,8), 116.0 (CH-2,7), 111.7 (CH-4,5), 79.1 (OCMe$_3$), 34.4 (CCMe$_3$), 31.9 (CC(CH$_3$)$_3$), 28.2 ppm (OC(CH$_3$)$_3$); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 109.77 (NH-9), 99.61 ppm (NH-CO); IR (ATR-FTIRS): $\tilde{\nu}$ = 3362, 3327, 2959, 1728, 1687, 1597, 1489, 1364, 1225, 1156 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for [$C_{30}H_{43}N_3O_4$+Na]$^+$: 532.31458 [*M*+Na]$^+$; found: 532.31419.

Preparation of compound 24: Compound **22** (0.10 g, 0.32 mmol) was dissolved in dry DMF (3 mL). The reaction mixture was stirred at room temperature, then EDC·HCl (0.15 g, 0.80 mmol), DMAP (0.04 g, 0.32 mmol), HOBt (0.09 g, 0.70 mmol) and 1-pyrenebutyric acid (0.20 g, 0.70 mmol) were added. The reaction mixture was stirred for 17 h at room temperature. After disappearance of the starting material (monitored by TLC) the reaction mixture was quenched with water (50 mL). The formed white precipitate was filtered, washed with water (50 mL), methanol (70 mL) and diethyl ether (30 mL) to give the pure compound **24** (0.17 g, 0.20 mmol, 61.9%) as a white solid. M.p. 311.3 °C; R_f = 0.88 (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): δ = 10.07 (br s, 2H; NH-amide), 9.87 (br s, 1H; NH-9$_{carb}$), 8.28 (d, ^3J(H,H) = 9.3 Hz, 2H; CH-10$_{pyr}$), 8.23 (dd, ^3J(H,H) = 7.7, ^4J(H,H) = 1.2 Hz, 2H; CH-6$_{pyr}$), 8.20 (dd, ^3J(H,H) = 7.9, ^4J(H,H) = 1.2 Hz, 2H; CH-8$_{pyr}$), 8.09 (d, ^3J(H,H) = 7.8 Hz, 2H; CH-3$_{pyr}$), 8.08 (d, ^3J(H,H) = 9.0 Hz, 2H; CH-5$_{pyr}$), 8.06 (d, ^3J(H,H) = 9.3 Hz, 2H; CH-9$_{pyr}$), 8.05 (d, ^3J(H,H) = 9.0 Hz, 2H; CH-4$_{pyr}$), 8.02 (dd, ^3J(H,H) = 7.9, ^3J(H,H) = 7.7 Hz, 2H; CH-7$_{pyr}$), 7.99 (d, ^4J(H,H) = 1.8 Hz, 2H; CH-4,5$_{carb}$), 7.81 (d, ^3J(H,H) = 7.8 Hz, 2H; CH-2$_{pyr}$), 7.45 (d, ^4J(H,H) = 1.8 Hz, 2H; CH-2,7$_{carb}$), 3.28 (m, 4H; CH$_2$-4), 2.50 (m, 4H; CH$_2$-2), 2.08 (m, 4H; CH$_2$-3), 1.39 ppm (s, 18H; CH$_3$); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): δ = 171.1 (CO), 141.7 (C-3,6$_{carb}$), 136.5 (C-1$_{pyr}$), 131.3 (C-8a,9a$_{carb}$), 130.9 (C-5a$_{pyr}$), 130.4 (C-8a$_{pyr}$), 129.3 (C-3a$_{pyr}$), 128.2 (C-10a$_{pyr}$), 127.51 (CH-2$_{pyr}$), 127.46 (CH-4$_{pyr}$), 127.2 (CH-9$_{pyr}$), 126.5 (CH-5$_{pyr}$), 126.2 (CH-7$_{pyr}$), 125.0 (CH-6$_{pyr}$), 124.9 (CH-3$_{pyr}$), 124.8 (CH-8$_{pyr}$), 124.7 (C-4a,4b$_{carb}$), 124.25 (C-10b$_{pyr}$), 124.17 (C-10c$_{pyr}$), 123.5 (CH-10$_{pyr}$), 122.5 (C-1,8$_{carb}$), 117.0 (CH-2,7$_{carb}$), 113.0 (CH-4,5$_{carb}$), 35.4 (CH$_2$-2), 34.5 (CMe$_3$), 32.1 (CH$_2$-4), 31.9 (CH$_3$), 27.3 ppm (CH$_2$-3); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 129.45 (NH-CO), 114.05 ppm (NH-9); IR (ATR-FTIRS): $\tilde{\nu}$ = 3273, 2955, 2865, 1652, 1548, 1235, 839 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for [$C_{60}H_{55}N_3O_2$+Na]$^+$: 872.41865; [*M*+Na]$^+$; found: 872.41702.

Preparation of compound 25: Compound **22** (0.10 g, 0.32 mmol) was dissolved in acetonitrile (25 mL) then 1-naphthyl isocyanate (0.14 g, 0.81 mmol) was added drop-wise. The reaction mixture was stirred at room temperature under N_2 atmosphere for 17 h. After disappearance of the starting material (monitored by TLC) the formed white precipitate was filtered and washed with diethyl ether (30 mL) to obtain the pure compound **25** (0.15 g, 0.23 mmol, 71.8%) as a white solid. M.p. 246.4-247.2 °C; R_f = 0.64 (10% methanol in dichloromethane); ^{1}H NMR (700.1 MHz, [D$_6$]DMSO, +20 °C): δ = 10.10 (br s, 1H; NH-9), 9.21 (br s, 2H; NH-C-1$_{carb}$), 8.96 (br s, 2H; NH-C-1$_{Naph}$), 8.21 (dm, ^3J(H,H) = 8.5 Hz, 2H; CH-8$_{Naph}$), 8.13 (dd, ^3J(H,H) = 7.6, ^4J(H,H) = 1.1 Hz, 2H; CH-2$_{Naph}$), 7.96 (d, ^4J(H,H) = 1.8 Hz, 2H; CH-4,5$_{carb}$), 7.95 (dm, ^3J(H,H) = 8.1 Hz, 2H; CH-5$_{Naph}$), 7.65 (dm, ^3J(H,H) = 8.2 Hz, 2H; CH-4$_{Naph}$), 7.59 (ddd, ^3J(H,H) = 8.5, ^3J(H,H) = 6.7, ^4J(H,H) = 1.4 Hz, 2H; CH-7$_{Naph}$), 7.54 (ddd, ^3J(H,H) = 8.1, ^3J(H,H) = 6.7, ^4J(H,H) = 1.2 Hz, 2H; CH-6$_{Naph}$), 7.48 (d, ^4J(H,H) = 1.8 Hz, 2H; CH-2,7$_{carb}$), 7.42 (dd, ^3J(H,H) = 8.2, ^3J(H,H) = 7.6 Hz, 2H; CH-3$_{Naph}$), 1.43 ppm (s, 18H; CH$_3$); ^{13}C NMR (176.0 MHz, [D$_6$]DMSO, +20 °C): δ = 153.5 (CO), 142.1 (C-3,6$_{carb}$), 134.4 (C-1$_{Naph}$), 133.8 (C-4a$_{Naph}$), 131.1 (C-8a,9a$_{carb}$), 128.5 (CH-5$_{Naph}$), 126.03 (C-8a$_{Naph}$), 125.97 (CH-6$_{Naph}$), 125.93 (CH-3$_{Naph}$), 125.8 (CH-7$_{Naph}$), 124.9 (C-4a,4b$_{carb}$), 123.2 (C-1,8$_{carb}$), 123.0 (CH-4$_{Naph}$), 121.4 (CH-8$_{Naph}$), 117.7 (CH-2$_{Naph}$), 115.8 (CH-2,7$_{carb}$), 112.0 (CH-4,5$_{carb}$), 34.5 (CMe$_3$), 31.9 ppm (CH$_3$); ^{15}N NMR (40.6 MHz, [D$_6$]DMSO, +25 °C): δ = 111.76 (NH-9), 103.14 (NH-C-1$_{carb}$), 101.14 ppm (NH-C-1$_{Naph}$); IR (ATR-FTIRS): $\tilde{\nu}$ = 3317, 3260, 2951,

1645, 1562, 1542, 1495, 1273, 1234, 782, 764 cm^{-1}; MALDI FTICR (solvent ≈0.01% DMSO/*i*PrOH): *m/z* calcd for $[C_{42}H_{41}N_5O_2+Na]^+$: 670.31524 $[M+Na]^+$; found: 670.31553.

Tetrabutylammonium carboxylate salt preparation: Tetrabutylammonium trimethylacetate and tetrabutylammonium lactate salts were prepared by adding one equivalent of tetrabutylammonium hydroxide in methanol to a solution of the corresponding acid (1 equiv) in methanol. The mixture was stirred at room temperature for 24 h, evaporated to dryness under reduced pressure and then dried under high vacuum at room temperature overnight. The salts are stored in a glovebox under argon atmosphere.

Measurement of the relative and absolute binding constants: The relative and absolute binding constant measurements were carried out by using the above-described two NMR instruments (200 or 700 MHz), UV/Vis and fluorescence instrument. All solutions were prepared in [D_6]DMSO or in DMSO with 0.5% water (m/m). The measurement and data treatment procedures of the NMR and UV/Vis measurements have been described previously.[14,15]

Fluorescence titration measurements were carried out in 1 cm quartz cells by using an excitation wavelength of $\lambda = 350$ nm and recording the emission spectra between $\lambda = 365$ and 500 nm. Titrations were performed by acquiring the changes in the fluorescence intensity at the peak of the emission spectrum at $\lambda = 386$ nm with a data recording interval of 0.1 nm. The slit width for excitation and emission monochromators were 1 and 5 nm respectively.

The working conditions and solvents used were the same as in the UV/Vis measurements of the absolute binding constants. For indolocarbazole **39** the $\log K_{ass}$ value measurements the concentrations of the stock solutions of the receptors were in the range of 0.00016–0.0016 M. The working concentration of indolocarbazole during the measurements (in the spectrofluorometer cell) was approximately 4×10^{-6} M. The concentration of TBA benzoate in the concentrated titrant solution was approximately 0.14 M and in diluted titrant solutions approximately 0.01 M. During titration the spectrofluorometric cell was weighed before and after each addition of titrant (in order to take volume correction into account). Over the course of titration 17–19 spectra were recorded. The spectrum of the free receptor was obtained before the first addition of titrant. The spectrum of the indolocarbazole–benzoate complex was obtained by adding a large amount of titrant. From the weighing data exact amounts of titrant added were found. The calculations of the $\log K_{ass}$ values were carried out the same way as in the case of UV/Vis measurements.

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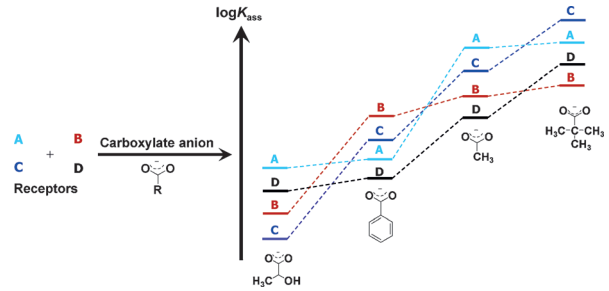

Can we discriminate? Comprehensive binding studies of four carboxylate anions with a number of anion receptors (see figure) reveals that the binding strength is largely determined by the basicity of the anion (leading to the general binding order lactate < benzoate < acetate ≤ trimethylacetate) and modulated by additional effects, such as the steric fit between the anion and the receptor as well as the hydrophilicity/hydrophobicity of the anion.

Carboxylate Receptors

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Towards the Discrimination of Carboxylates by Hydrogen-Bond Donor Anion Receptors