EurJOC
European Journal of Organic Chemistry

A Journal of
ChemPubSoc Europe

Accepted Article

Title: Exploring selectivity of 22 acyclic urea-, carbazole- and indolocarbazole-based receptors towards 11 monocarboxylates

Authors: Kerli Martin, Juuli Nõges, Kristjan Haav, Sandip A. Kadam, Astrid Pung, and Ivo Leito

To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201700931

Link to VoR: http://dx.doi.org/10.1002/ejoc.201700931

Supported by
ACES Asian Chemical Editorial Society

WILEY-VCH

Exploring selectivity of 22 acyclic urea-, carbazole- and indolocarbazole-based receptors towards 11 monocarboxylates

Kerli Martin, Juuli Nõges, Kristjan Haav, Sandip A. Kadam, Astrid Pung and Ivo Leito*

Abstract: Carboxylates are attractive target analytes in supramolecular analytical chemistry. 22 acyclic synthetic receptors having a different number and geometric arrangement of hydrogen bond donor (HBD) fragments and hydrophobic moieties have been applied to study experimentally selective binding of 11 carboxylate anions of widely differing basicity, hydrophobicity and steric demand, resulting in 242 accurately determined binding constants. It was found that besides the basicity of the anions structural and steric factors of anions and receptors influence the binding. Several interesting cases are pinpointed and analysed. The ability of selected receptors to discriminate between anions according to structural features (hydrophilicity, substitution at α-carbon, etc.) is demonstrated. The present results give insight into carboxylate anion binding and make an important step towards systematic development of receptors with useful selectivity patterns and thereby to the practical use of receptor series in sensor arrays for carboxylate fingerprinting in mixtures.

Introduction

Carboxylates are perhaps the most diverse group of anionic compounds ranging from some of the smallest anions like formate and acetate to large peptides. Variations in the carbon chain length or addition of functional groups can greatly alter their chemical properties and functionalities.[1],[2] In terms of the molecular recognition process, binding of a receptor only to the carboxyl group via hydrogen bonding[3] or ion-ion interactions is insufficient for achieving good selectivity. In the case of simple carboxylates, there is a strong relation between binding affinity and anion basicity.[4] This relation alone is by far 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 shape/geometry would be preferred.[5] The receptor should not only be able to differentiate between similar carboxylates but also other anionic species and be capable of overcoming solvent and counter-ion competition. Large variety in the properties of carboxylates (basicity, chirality, hydrophobicity/hydrophilicity, polarizability, *etc.*) creates diverse ways to design carboxylate receptors. A number of works demonstrate good affinity and moderate selectivity of synthetic receptors towards different carboxylates.[5,6] However, to the best of the knowledge of the authors of this paper, a comprehensive analysis of structural features of carboxylates related to their binding to synthetic receptors is lacking. Binding characterization is a major part in receptor design and synthesis and gives guidelines for designing receptors with better sensitivity and selectivity. Small studies with 1-2 receptors and anions in differing solvent media provide little possibility for making generalizations and make it difficult to do any meaningful comparison of binding data. Therefore, comparable binding measurements of a larger set of receptors and anions in one solvent medium are necessary.

The aim of this work is to quantitatively (via logK_{ass} values) characterize the binding of a series of monocarboxylate anions to a diverse series of synthetic multidentate HBD receptors. In total 11 carboxylates of different size, geometry, basicity and hydrophilicity/hydrophobicity were selected: formate, acetate, anions of propionic acid derivatives (ibuprofen, ketoprofen, naproxen, pivalic acid and lactic acid), hexanoate, sorbate, benzoate, and glucuronate (see Scheme 1).

Formate

pK_a(H$_2$O) = 3.74[7]
pK_{a_exp}(DMSO) = 10.3[8]
log$P_{o\text{-}w}$ = -0.54[9]
E_R = 0

Acetate

pK_a(H$_2$O) = 4.76[7]
pK_{a_exp}(DMSO) = 12.3[10]
log$P_{o\text{-}w}$ = -0.17[9]
E_R = 17

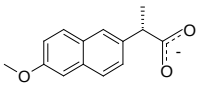
Pivalate

pK_a(H$_2$O) = 5.03[11]
pK_{a_exp}(DMSO) = 12.9[12]
log$P_{o\text{-}w}$ = 1.47[9]
E_R = 59

Lactate

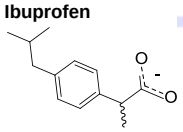
pK_a(H$_2$O) = 3.86[7]
pK_{a_calc}(DMSO) = 9.0
log$P_{o\text{-}w}$ = -0.72[9]
E_R = 48

Naproxen



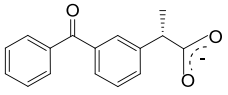
pK_a(H$_2$O) = 4.15[13]
pK_{a_calc}(DMSO) = 11.7
log$P_{o\text{-}w}$ = 3.34[9]
E_R = 66

Ibuprofen



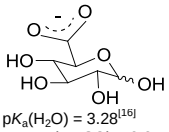
pK_a(H$_2$O) = 4.40[14]
pK_{a_calc}(DMSO) = 11.6
log$P_{o\text{-}w}$ = 4.5[9]
E_R = 66

Ketoprofen



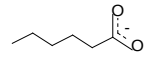
pK_a(H$_2$O) = 4.30[15]
pK_{a_calc}(DMSO) = 11.1
log$P_{o\text{-}w}$ = 3.12[9]
E_R = 66

Glucuronate



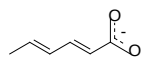
pK_a(H$_2$O) = 3.28[16]
pK_{a_calc}(DMSO) = 8.3
log$P_{o\text{-}w}$ = -2.57[9]
E_R = *57*

Hexanoate



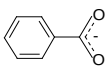
pK_a(H$_2$O) = 4.88[11]
pK_{a_calc}(DMSO) = 12.5
log$P_{o\text{-}w}$ = 1.92[9]
E_R = 36

Sorbate



pK_a(H$_2$O) = 4.50[17]
pK_{a_calc}(DMSO) = 12.4
log$P_{o\text{-}w}$ = 1.33[9]
E_R = *36*

Benzoate



pK_a(H$_2$O) = 4.20[7]
pK_{a_exp}(DMSO) = 11.1[10]
log$P_{o\text{-}w}$ = 1.87[9]
E_R = *45*

Scheme 1. Investigated anions X-COO$^-$ together with pK_a values of X-COOH in water (experimental) and DMSO (experimental, if available, otherwise computational, see the SI for details), log$P_{o\text{-}w}$ values (given for the respective neutral conjugate acids) and substituent repulsive energies E_R (kcal mol^{-1}) as estimators of the steric demand[18] of X. E_R values printed in italic have been estimated on the basis of structural analogy.

[*] K. Martin, J. Nõges, K. Haav, S. A. Kadam, A. Pung, Prof. I. Leito
Institute of Chemistry
University of Tartu
Ravila 14a, 50411 Tartu, Estonia
E-mail: ivo.leito@ut.ee
WWW: http://analytical.chem.ut.ee/

Supporting information for this article is given via a link at the end of the document.

As Scheme 1 displays, this selection includes anions X-COO$^-$ with different basicities (ranging from highly basic pivalate and hexanoate to glucuronate and lactate having low basicity), hydrophilicities/hydrophobicities (ranging from highly hydrophobic anion of ibuprofen to highly hydrophilic glucuronate and formate), size (ranging from the smallest carboxylates formate and acetate to large anions of naproxen and glucuronic acid) and steric demand of the substituent X (ranging from non-existent in the case of formate to serious in the case of pivalate and the propionic acid derivatives). The counterion for all anions is tetrabutylammonium (TBA). Binding of these anions to 22 urea-, carbazole- and indolocarbazole-based receptors (see Scheme 2) was measured in DMSO-d_6 with 0.5 % of water. Polydentate systems where several NH centers can form hydrogen bonds with carboxylate center are among the most suitable building blocks for selecting receptors for carboxylate anions.[19,20] For this reason tetra- to hexadentate receptors dominate the selection. Some bi- and tridentate receptors are included for comparison. While these receptors are designed to recognize monocarboxylates, the results described herein are also relevant to research related to synthesis of ditopic receptors (e.g. for dicarboxylates)[21,22], design of macrocyclic anion sensors[23], and the like.

Results and Discussion

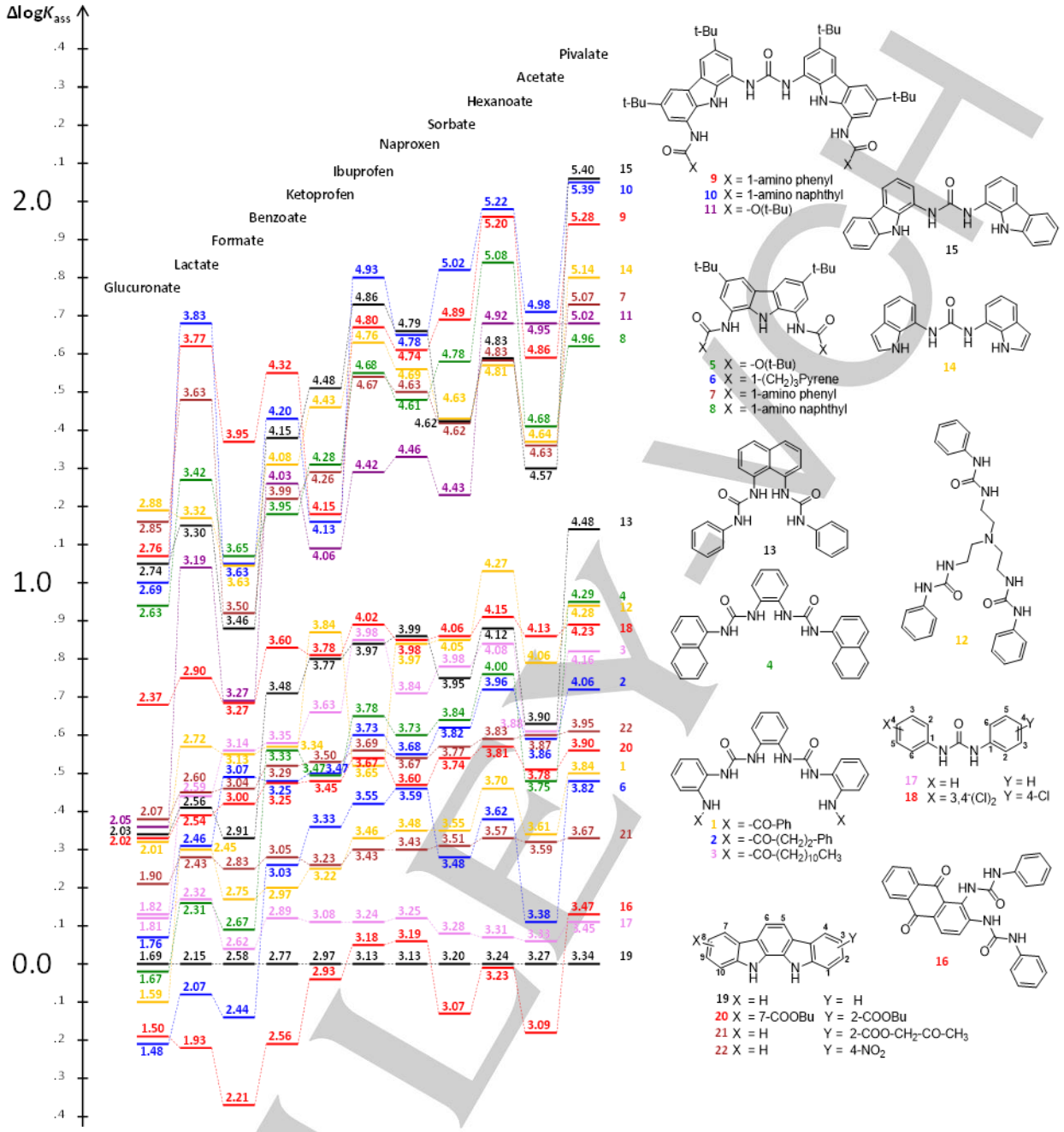
Binding affinities were determined using ^{1}HNMR-based relative binding affinity measurement method.[24] 11 binding affinity scales – one for every anion – were constructed (SI pages S162 to S172). Absolute logK_{ass} values were obtained by anchoring scales to absolute logK_{ass} values of **15**, **19**, **21** and **22** according to the procedure described in ref. [25]. In total 242 logK_{ass} values were determined (see Table 1). The summary of experimental results is presented in Scheme 2. The anion-receptor complex structures of receptors **8**, **10**, **11** and **15** have been computed for all 11 anions (complexes between **8**, **11**, **15** and lactate, benzoate, acetate, and pivalate are available from ref. [4]). From practical reasoning receptors showing high binding affinity towards anions were selected for computations.

For simple receptor structures – e.g. indolocarbazole (**19**) and diphenylurea (**17**) – the binding affinity (logK_{ass}) of selected carboxylates approximately follows the basicity order (see Scheme 1). This is because HB is the primary binding interaction in these complexes and the higher the pK_a of the acid the higher is generally the hydrogen bond acceptor ability (HBA) of the anion.

Table 1. Experimentally[a] determined binding constants (logK_{ass})[b] towards selected carboxylates in DMSO-d_6:H_2O (99.5%:0.5% m/m).

Rec	G	L	F	B	K	I	N	S	H	A	P
1	1.59	2.45[c]	2.75	2.97[c]	3.22	3.46	3.48	3.55	3.70	3.61[c]	3.84[c]
2	1.76	2.46[c]	3.07	3.25[c]	3.47	3.73	3.68	3.82	3.96	3.86[c]	4.06[c]
3	1.81	2.59[c]	3.14	3.35[c]	3.63	3.98	3.84	3.98	4.08	3.88[c]	4.16[c]
4	1.67	2.31[c]	2.67	3.33[c]	3.47	3.78	3.73	3.84	4.00	3.75[c]	4.
5	0.84	1.70[c]	1.44	2.34[c]	2.36	2.55	2.56	2.44	2.63	2.41[c]	3.
6	1.48	2.07[c]	2.44	3.03[c]	3.33	3.55	3.59	3.48	3.62	3.38[c]	3.
7	2.85	3.63	3.50	3.99	4.26	4.67	4.63	4.62	4.83	4.63	5
8	2.63	3.42[c]	3.65	3.95[c]	4.28	4.68	4.61	4.78	5.08	4.68[c]	4.
9	2.76	3.77	**3.95**	**4.32**	4.15	4.80	4.74	4.89	5.20	4.86	5
10	2.69	**3.83**	3.63	4.20	4.13	**4.93**	4.78	**5.02**	**5.22**	**4.98**	5
11	2.05	3.19[c]	3.27	4.03[c]	4.06	4.42	4.46	4.43	4.92	4.95[c]	5.
12	2.01	2.72	3.13	3.34	3.84	3.65	3.97	4.05	4.27	4.06	4
13	2.03	2.56	2.91	3.48	3.77	3.97	3.99	3.95	4.12	3.90	4
14	**2.88**	3.32[c]	3.63	4.08[c]	4.43	4.76	4.69	4.63	4.81	4.64[c]	5.
15	2.74	3.30[c]	3.46	4.15[c]	**4.48**	4.86	**4.79**	4.62	4.83	4.57[c]	**5.**
16	1.50	1.93[c]	2.21	2.56[c]	2.93	3.18	3.19	3.07	3.23	3.09[c]	3.
17	1.82	2.32[c]	2.62	2.89[c]	3.08	3.24	3.25	3.28	3.31	3.33[c]	3.
18	2.37	2.90[c]	3.27	3.60[c]	3.78	4.02	3.98	4.06	4.15	4.13[c]	4.
19	1.69	2.15[c]	2.58	2.77[c]	2.97	3.13	3.13	3.20	3.24	3.27[c]	3.
20	2.02	2.54[c]	3.00	3.25[c]	3.45	3.67	3.60	3.74	3.81	3.78[c]	3.
21	1.90	2.43[c]	2.83	3.05[c]	3.23	3.43	3.43	3.51	3.57	3.59[c]	3.
22	2.07	2.60[c]	3.04	3.29[c]	3.50	3.69	3.67	3.77	3.83	3.87[c]	3.

[a] logK_{ass} values were measured in DMSO-d_6:H_2O (99.5%:0.5% m/m) at 25 °C. [b] See the Scales (in the SI) for uncertainty ranges. Rec = Receptor (Host); G = Glucuronate; L = Lactate; F = Formate; B = Benzoate; K = Ketoprofen; I = Ibuprofen; N = Naproxen; S = Sorbate; H = Hexanoate; A = Acetate; P = Pivalate. [c] logK_{ass} reported in ref. [4], some of them slightly changed due to additional measurements. As there are two anomers of glucuronate, α- and β-form, binding constants for glucuronate are apparent binding constants. **Bold** type is used to indicate the strongest binder for the particular anion. Underline indicates (unexpected) binding affinities that are discussed in the text.



Scheme 2. Binding-structure relationship plot comparing the binding affinity changes of all anions under study. Receptor **5** with lowest logK_{ass} values has been omitted for clarity and depicted in the SI.

Principal component analysis (PCA) was performed (using $\Delta\log K_{ass}$ values relative to indolocarbazole, **19**) to elucidate the trends of binding selectivity of carboxylates to different receptors. The results (Figure 1) show that most of the investigated carboxylates can be discriminated by the used receptors to a statistically significant extent.

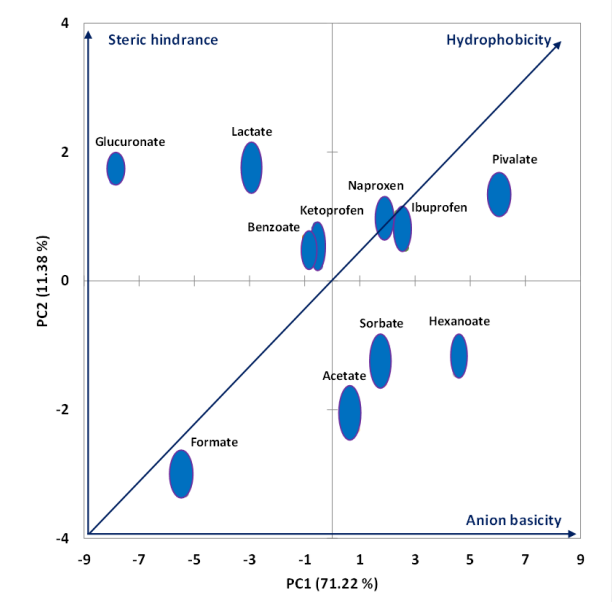


Figure 1. PCA plot of binding constant data (relative to indolocarbazole (19)). The dot size and shape take into account the uncertainties of the experimental $\log K_{ass}$ values.

Distribution of anions across the PCA graph indicates differentiation of the anions by basicity, hydrophobicity, and steric hindrance. PC1 mainly describes anion basicity. A large spread of anion basicity (even though damped to some extent by the use of relative binding affinities) causes this effect to dominate the binding. To some extent hydrophobicity also contributes to PC1. The hydrophobic anions are a group in the upper right corner and hydrophilic anions (glucuronate, formate, acetate, and lactate) position themselves in the lower and left sides of the plot. As anion basicity strongly dominates binding, hydrophilic lactate is positioned not far from hydrophobic benzoate and ketoprofen. PC2 expresses mainly the steric hindrance of the anionic center. Formate is the least sterically hindered anion, followed by acetate, sorbate, and hexanoate where the steric hindrance is low. On the other hand, in anions where substituents are close to carboxylate group they hinder binding (e.g. in glucuronate or pivalate). Although glucuronate is significantly larger than lactate or pivalate, the effect of steric hindrance is of similar magnitude. When anions have similar basicity then these secondary effects might determine the binding order. Ketoprofen, naproxen, and ibuprofen form a group of structurally related (all can be considered derivatives of 2-phenylpropanoate) large hydrophobic anions and are expectedly close to each other. Also, benzoate is linked to this group. These four anions are distributed in the graph in the order of their hydrophobicities, not basicities.

The differences between receptors in terms of anion binding are visualized in Scheme 2. In general, the stronger binders are strong (in relative terms) towards all anions and vice versa. Closer examination reveals, however, numerous cases where in a group of two receptors (R1 and R2) and two anions (A1 and A2) R1 binds A1 stronger than A2, while R2 binds A2 stronger than R1 (so-called “selectivity reversals”). Let us examine some of them in the "upper group" of the scheme (receptors **7-11** and **14-15**), as there receptors, being the best binders, are the most interesting in practical terms. An interesting case is the behavior of the three carboxylates with aliphatic chain: acetate, hexanoate, pivalate. The three anions have similar basicity and very similar binding constants with receptors operating solely on the basis of HB (e.g. **17-19**). Thus the differences in binding with the same receptors are caused by other effects. As a big picture, acetate is bound weaker by most of the receptors in the upper group. This is most probably caused by the solvophobic effect contribution: all these receptors have some hydrocarbon moieties that according to the structure of the complexes can interact with the alkyl chains of anions. Among the three anions this effect is the weakest in the case of acetate, which possesses by far the smallest alkyl chain. If one examines specific receptors then striking contrast is observed with receptor **15** binding the three carboxylates: **15** is the weakest binder of acetate in the upper group and at the same the strongest binder of pivalate of all studied receptors. Examining the geometries of the complexes reveals the reason: the "half-pocket" of **15** exactly accommodates pivalate, allowing solvophobic effects between its *t*-Bu group and the outer aromatic rings of the carbazole fragments. At the same time, for acetate the half-pocket is too big and the anion will have no interactions with the aromatic rings. The situation with hexanoate is intermediate – it has interaction with one of the aromatic rings. Another interesting receptor is **11**, which binds the three carboxylates with almost equal affinity, meaning that it is among the best binders of acetate and second worst in the upper group for pivalate. Examining the geometry of the complex reveals that pivalate with its *t*-Bu group will not fit into the pocket, while acetate has just the right size. It is interesting that out of the upper group **11** is by far the weakest binder of formate. The structure reveals that – similarly to acetate and **15** – formate is too small for the binding pocket of **11**.

Receptors **9** and **10** are for the majority of anions the most potent binders in this work. Noticeable differences (0.65 and 0.80 $\log K_{ass}$ units, respectively) in binding is between ibuprofen and ketoprofen are evident with receptors **9** and **10**: in the upper group **9** and **10** are among the strongest binders of ibuprofen and among weakest with ketoprofen. At the same time, indolocarbazole (**19**) that has only 2 HBD centers, and where binding is expected to be dependent only on anion basicity, binds ibuprofen only by 0.16 $\log K_{ass}$ units stronger. Both anions are 2-phenylpropionate derivatives, the steric surroundings of the carboxylate group are almost identical and the anions differ only by the substituents in the aromatic ring: 4-isobutyl vs 3-benzoyl, respectively. The aqueous pK_a values of the conjugate

acids of the anions differ by only 0.1 pK_a units. The computational geometries (SI S192-S193) suggest that carboxylate groups of ibuprofen and ketoprofen are bound by the same number of NH groups and the substituents have small effect on the structure of the complexes. In this situation the large binding differences of **9** and **10** with these anions are surprising.

Receptors **9** and **10** are the most sophisticated ones where besides HBs with the carboxylate group an appreciable solvophobic effect can be observed between the hydrophobic residues of the anions and the receptors. The difference between these interactions between the highly hydrophobic isobutyl group and markedly more polar benzoyl group might be the main reason for the large difference in binding affinities. This finding demonstrates that minor (and distant from the anionic center) structural effects in anions can have large impact on binding affinity.

It is also interesting that ibuprofen is bound stronger by all selected receptors except for **12** that binds ketoprofen by 0.18 logK_{ass} units stronger, which in our interpretation might be caused by an additional HB between the ketoprofen's benzoyl group and one of the urea fragments of **12**.

As a rule, receptors with 4 or more HBD groups dominate in terms of binding affinity. Out of the bidentate HBD molecules 3,4,4'-Cl_3-diphenylurea (**18)** is on an average the strongest binder, because of the inductive effect of the three chloro substituents, enhancing both the acidity and HBD ability of **18**. It has been found, however, that contrary to intuitive expectation, the correlation between HBD ability and acidity of a molecule is not strong.[26] Combinations of carbazole (or indole) and urea (**7-11**, **14** and **15**) have the highest binding affinities. There is an optimal number of HBD groups that should be implemented into receptor framework. Computational modeling suggests that as a maximum, 5-6 NH groups can come to close proximity of a carboxylate group to form HBs in a co-operative manner. Furthermore, it is important that the atoms N-H···O are aligned as closely on one line as possible. **9** and **10** possess 8 NH groups in total and indeed, together they are the strongest binders for 7 anions out of 11. For instance, according to the computational criteria specified in the SI (Computational structures section), receptor **10** forms five hydrogen bonds with ibuprofen, two HBs with one carboxylate group's oxygen and three with another oxygen atom. The lowest N-H···O angle is 148°.

Expectedly their binding affinities towards those anions differ on an average only by 0.15 logK_{ass} units from the next receptors (by 0.3 logK_{ass} units, as maximum). Thus, their binding affinity is well comparable to the remaining urea-carbazole combined receptors. Receptors **7**–**11** and **15** are the strongest binders with different carboxylates. In spite of their similar core structures, receptors **9**-**11** show different binding patterns. Pivalate, hexanoate, and acetate are bound by **11** with similar affinity, while **9** and **10** prefer pivalate and hexanoate against acetate. As for the remaining anions, they are bound stronger by **9** and **10** than by **11**. Some anions have additional HBA centers, most notably lactate and glucuronate. Out of the computationally investigated receptors **7**, **8**, **10** and **11** form additional hydrogen bond with lactate's 2-OH group as evidenced by computations (the additional HB is present in the dominant conformers). Such additional HBs are probably the reason that lactate binds stronger with **7**, **8**, **9** and **10** than could be expected from its low basicity. The strong binding of lactate with **9** and **10** is noteworthy. The computational geometry of the complex with **10** reveals that even two HBs are formed between the urea fragment attached to naphthyl and lactate's OH. Ibuprofen and naproxen (as well as acetate and sorbate) are bound to **8**, **9** and **10** with similar affinity patterns. However, the affinity pattern of these receptors towards ketoprofen is different, despite very similar vicinity of the carboxylate group in the three anions.

Glucuronate has, similarly to lactate, OH groups that can behave as additional HBA (or HBD) centers to have additional interactions with a receptor. Still, lactate is bound considerably stronger than glucuronate. Lactate is bound ~ 0.75 logK_{ass} units stronger to receptors **1**-**8** and **12**, ~ 1 logK_{ass} units stronger to **9**, **10** and **11** and around 0.5 logK_{ass} units stronger to receptors **13**-**22**. Glucuronate is more hydrophilic and a weaker base. The first factor causes stronger solvation by solvent molecules and the second factor causes weaker interaction between receptor's NH groups and glucuronate's carboxylate group. 1,3-bis(carbazolyl) urea based receptors **9**, **10** and **11** form a binding 'pocket' of suitable size for lactate but the spatial fit for glucuronate is not optimal. Lactate's OH group might be less accessible to solvent molecules in the complexed state than glucuronate's OH groups.

Conclusions

Differential binding of 11 carboxylate anions of widely differing basicity, hydrophobicity and steric demand with 22 neutral synthetic receptors was demonstrated. The results of PCA reveal that carboxylates with dissimilar structures can be distinguished using the differential sensing paradigm. Additionally, this work provides experimentally determined 242 binding constants that can be directly compared with each other. Several cases of interesting relations between structure and binding are pinpointed and analysed. Lengthening of the carbon chain (acetate vs hexanoate) appears to play modest role in differentiating between the anions. Anion basicity is a key factor in determining binding and additional influence is exerted by other groups in the anions, by steric factors and by subtle structural effects. It is demonstrated that the relative binding affinity of acetate and pivalate is to a large extent governed by the suitability of the size of the binding site dimensions to support solvophobic effect (stronger with pivalate than acetate). Large binding differences were found between the very similar anions of naproxen and ibuprofen – anions with similar structure and basicity and identical surroundings of the carboxylate group. The presented results give insight into carboxylate anion binding and contribute towards systematic development of receptors with useful selectivity patterns and thereby to the practical use of receptor series in sensor arrays for carboxylate fingerprinting in mixtures.

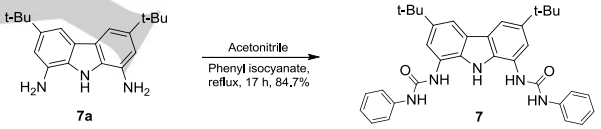
Experimental Section

Instruments. For compound characterization of ^{1}H and ^{13}C NMR spectra were recorded at 700.1 MHz (^{1}H), 176.1 MHz (^{13}C) and NMR-based relative binding affinity measurements were carried out at 700.1 MHz on a Bruker AVANCE III 700 instrument. UV-Vis spectrophotometric measurements were carried out using Thermo Nicolet Evolution 300 spectrophotometer and fluorescence spectrofluorometric measurements were carried out using Horiba FluoroMax-4 spectrofluorometer. All the NMR, UV-Vis, and fluorescence measurements carried out at 25 °C. ATR-IR spectra were recorded on a Thermo Electron Nicolet 6700 FT-IR device using a Smart Orbit micro-ATR accessory with diamond crystal. The spectrometer had a DTGS detector and CsI beamsplitter. 128 scans were recorded over the range of 400–4000 cm^{-1}. For HRMS-analysis the samples were first dissolved in MeCN/H_2O (80/20) with a concentration of ~1 mg/ml and then diluted in MeCN/H_2O (80/20) for ESI-measurements. The concentration was chosen so that an appropriate signal was achieved in LRMS part of the machine. The final analyte concentration of infused solutions remained in range 0.5-2 µg/ml. High-resolution ESI-ICR spectra were obtained on a hybrid Varian 910-FT-ICR-MS system, which is coupled to Varian-J320 triple-quadrupole MS. Ionization parameters were as follows: spray chamber temperature, 40 °C; spray needle voltage, -4500 V; nebulizing gas (N_2) pressure, 30 psi; API-drying gas (N_2), 15 psi at 250 °C; shield voltage, 600 V; and capillary voltage 32 V. Mass range of ions, selected by quadrupole, were guided into the FT-ICR analyzer cell. Ion guide parameters and FT-ICR ion guide and excitation parameters were optimized for mass range (m/z = 100–1000). Ion collection time varied from 300 to 1000 ms; FT-ICR analyzer cell parameters were: DAC rate: 8000 kHz for m/z range of 100–800 direct (broadband); ADC rate: 4 MHz; transient length: 1024 K; 262.144 ms or 2048 K; 524.288 ms. For the calibration of the mass axis, samples were spiked with the in-house prepared internal calibration mixture containing perfluorinated Brønsted superacids. Ions used for calibration were: $C_{12}F_{10}NO_4S_2^-$ (m/z = 475.91145), $C_8F_{17}NO_2SH^-$ (m/z = 497.94620), $C_8F_{18}NO_4S_2^-$ (m/z = 579.89868), $C_{12}F_{26}NO_4S_2^-$ (m/z = 779.88591)[27]. The concentration of the calibrants in the infused solutions remained within 0.5–1.0 µM.

Solvents and Chemicals. The solvent for binding measurements, DMSO with 0.5% of water (m/m), was prepared using anhydrous DMSO 99.9% (for UV-Vis and fluorescence measurements) or DMSO-d_6 99.8% (for NMR measurements) and water from MilliQ Advantage A10 system. The water content of the DMSO solvent was checked with Mettler Toledo DL 32 titrator. Titrant solutions for binding measurements were prepared from respective tetrabutylammonium salts.

Receptor Molecules. Receptors **1-6, 8, 11, 14-16** are the same as used in ref. [4], **17-19, 22** are from ref. [25], and **20, 21** from ref. [24]. Synthesis procedures of compounds **9** and **10** are described in ref [28]. Literature approaches were used for the synthesis of **12**[29] and **13**[30].

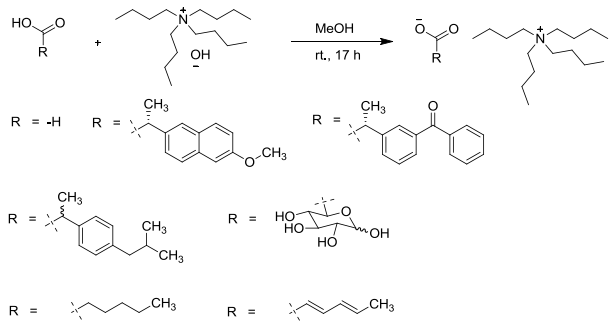
Preparation of Compound 7. Compound **7a** (0.10 g, 0.32 mmol), prepared as in ref. [4], was dissolved in acetonitrile (20 mL) then phenyl isocyanate was added drop-wise (0.081 g, 0.67 mmol). The reaction mixture was stirred at reflux temperature under N_2 atmosphere for 17 h. After the disappearance of the starting material (monitored by TLC) formed white precipitate was cooled and filtered. The white solid washed with diethyl ether to obtain the pure compound **7** (0.15 g, 0.27 mmol, 84.7%) as a white solid**.**



(1)

Data for **7**: Mp: 349.5-352.9 °C. R_f = 0.57 (10% methanol in DCM). ^{1}H NMR (700.1 MHz, DMSO-d_6, +20 °C) δ: 10.01 (bs, 1H, NH); 8.83 (bs, 2H NH); 8.77 (bs, 2H, NH); 7.91 (d, *J*=2.1 Hz, 2H); 7.54-7.51 (m, 4H); 7.42 (d, *J*=2.1 Hz, 2H); 7.29-7.26 (m, 4H); 6.99-6.96 (m, 2H); 1.41 (s, 18H, CH_3). ^{13}C NMR (176.0 MHz, DMSO-d_6, +20 °C) δ: 153.5 (CO); 142.3; 140.3; 131.6; 129.2; 125.2; 123.5; 122.3; 118.9; 116.4; 112.3; 34.9 (C-CH_3); 32.3 (C-CH_3). IR (ATR-FT-IRS) ν̃: 3297, 3042, 2950, 2899, 1647, 1597, 1557, 1497, 1226, 859, 746, 692, 648, 503 cm^{-1}. MALDI FT-ICR (*m/z*): solvent ~ 0.01% DMSO:isopropanol, $[M+Na]^+$ calcd for $[C_{42}H_{41}N_5O_2+Na]^+$ 570.28449, found 570.28461.

Preparation of tetrabutylammonium carboxylate salts. Commercially available TBA salts of acetate and benzoate were used. TBA salts of pivalate (trimethylacetate) and lactate are previously described in ref. [4]. TBA salts of formate, (S)-(+)-naproxen, ibuprofen (as a racemate), (S)-(+)-ketoprofen, D-glucuronate, hexanoate and sorbate were prepared by adding 1 equiv. 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 glove box under argon atmosphere.



(2)

Data for TBA formate: ^{1}H NMR (700.1 MHz, DMSO-d_6, +20 °C) δ: 8.57 (s, 1H); 3.20-3.17 (m, s8H); 1.59-1.54 (m, 8H); 1.32-1.28 (m, 8H); 0.92 (t, *J* = 7.0 Hz, 12H). ^{13}C NMR (176.0 MHz, DMSO-d_6, +20 °C) δ: 165.2; 57.9; 23.6; 19.7; 14.0. IR (ATR-FT-IRS) ν̃: 2959, 2873, 2590, 1597, 1491, 1456, 1332, 882, 587 cm^{-1}.

Data for TBA naproxen: ^{1}H NMR (700.1 MHz, DMSO-d_6, +20 °C) δ: 7.69 (d, *J* = 8.4 Hz, 1H); 7.62 (d, *J* = 8.4 Hz, 1H); 7.58 (s, 1H); 7.45 (dd, *J* = 7.0, 2.1 Hz, 1H); 7.21 (d, *J* = 2.1 Hz, 1H); 7.07 (dd, J = 7.0, 2.1 Hz, 1H); 3.85 (s, 3H); 3.30-3.29 (m, 1H); 3.16-3.14 (m, 8H); 1.57-1.52 (m, 8H); 1.32-1.27 (m, 11H); 0.93 (t, *J* = 7.0 Hz, 12H). ^{13}C NMR (176.0 MHz, DMSO-d_6, +20 °C) δ: 174.3; 155.7; 141.7; 131.8; 128.2; 128.0; 127.4; 124.9; 124.1; 117.3; 105.0; 56.9; 54.5; 49.1; 48.0; 22.5; 19.8; 18.7; 13.0. IR (ATR-FT-IRS) ν̃: 3187, 2959, 2874, 1589, 1485, 1375, 1339, 1212, 1032, 810, 746, 476 cm^{-1}. ESI FT-ICR MS (*m/z*): solvent MeCN/H_2O (80/20), [M]$^-$ calcd for $[C_{14}H_{13}O_3]^-$ 229.08702, found 229.08717.

Data for TBA ibuprofen: ^{1}H NMR (700.1 MHz, DMSO-d_6, +20 °C) δ: 7.14 (d, *J* = 7.7 Hz, 2H); 6.94 (d, *J* = 7.7 Hz, 2H); 3.19-3.14 (m, 8H); 3.14-3.12 (m, 1H); 2.36 (d, *J* = 7.0 Hz, 2H); 1.81-1.75 (m, 1H); 1.59-1.54 (m, 8H); 1.33-1.28 (m, 8H); 1.18 (d, *J* = 7.0 Hz, 3H); 0.93 (t, *J* = 7.0 Hz, 12H); 0.86 (d, *J* = 7.7 Hz, 6H). ^{13}C NMR (176.0 MHz, DMSO-d_6, +20 °C) δ: 175.6; 144.5; 137.5; 128.3; 127.6; 57.9; 49.7; 48.9; 44.9; 30.2; 23.6; 22.7; 20.9; 19.7; 14.0. IR (ATR-FT-IRS) ν̃: 3187, 2959, 2874, 1589, 1485, 1375, 1339, 1212, 1032, 810, 746, 476 cm^{-1}. ESI FT-ICR MS (*m/z*): solvent MeCN/H_2O (80/20), [M]$^-$ calcd for $[C_{13}H_{17}O_2]^-$ 205.12340, found 205.12340.

Data for TBA ketoprofen: ^{1}H NMR (700.1 MHz, DMSO-d_6, +20 °C) δ: 7.73-7.72 (m, 1H); 7.68-7.66 (m, 1H); 7.56-7.53 (m, 5H); 7.46-7.44 (m, 1H); 7.38-7.36 (m, 1H); 3.26-3.23 (m, 1H); 3.32-3.16 (m, 8H); 1.58-1.54 (m, 8H); 1.33-1.27 (m, 8H); 1.24 (d, *J* = 3.5 Hz, 3H); 0.92 (t, *J* = 7.0 Hz, 12H). ^{13}C NMR (176.0 MHz, DMSO-d_6, +20 °C) δ: 196.2; 174.1; 147.3; 137.5; 136.1; 132.4; 132.1; 129.6; 128.9; 128.5; 127.5; 126.4; 57.5; 49.6; 23.1; 20.2; 19.2; 13.4. IR (ATR-FT-IRS) ν̃: 2959, 2934, 2873, 2804, 1652, 1593, 1372, 1280, 1048, 864, 722, 704, 642 cm^{-1}. ESI FT-ICR MS (*m/z*): solvent MeCN/H_2O (80/20), [M]$^-$ calcd for $[C_{16}H_{13}O_3]^-$ 253.08702, found 253.08722.

Data for TBA glucuronate: ^{1}H NMR (700.1 MHz, DMSO-d_6, +20 °C) δ: 3.72-3.71 (m, 1H); 3.57-3.53 (m, 2H); 3.49-3.46 (m, 1H); 3.40-3.38 (m, 1H); 3.32-3.31(m, 1H); 3.17-3.15 (m, 8H); 1.59-1.54 (m, 8H); 1.33-1.28 (m, 8H); 0.93 (t, *J* = 7.7 Hz, 12H). ^{13}C NMR (176.0 MHz, DMSO-d_6, +20 °C) δ: 175.4; 72.8; 72.4; 72.1; 71.5; 64.2; 58.0; 23.5; 19.7; 14.0. IR (ATR-FT-IRS) ν̃: 3413, 3263, 2961, 2935, 2875, 1612, 1495, 1356, 1093, 1032, 881, 545. ESI FT-ICR MS (*m/z*): solvent MeCN/H_2O (80/20), [M]$^-$ calcd for $[C_6H_9O_7]^-$ 193.03538, found 193.03545.

Data for TBA hexanoate: ^{1}H NMR (700.1 MHz, DMSO-d_6, +20 °C) δ: 3.19-3.16 (m, 8H); 1.72-1.70 (m, 2H); 1.59-1.55 (m, 8H); 1.37-1.32 (m, 2H); 1.30-1.28 (m, 8H); 1.24-1.28 (m, 2H); 1.16-1.15 (m, 2H); 0.92 (t, *J* = 7.0 Hz, 12H); 0.83 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (176.0 MHz, DMSO-d_6, +20 °C) δ: 174.6; 57.5; 48.5; 32.0; 26.6; 23.1; 22.3; 19.2; 14.1; 13.5. IR (ATR-FT-IRS) ν̃: 2958, 2932, 2873, 1576, 1463, 1373, 883, 738 cm^{-1}. ESI FT-ICR MS (*m/z*): solvent MeCN/H_2O (80/20), [M]$^-$ calcd for $[C_6H_{11}O_2]^-$ 115.07645, found 115.07647.

Data for TBA sorbate: ^{1}H NMR (700.1 MHz, DMSO-d_6, +20 °C) δ: 6.58-6.54 (m, 1H); 6.07-6.03 (m, 1H); 5.74-5.70 (m, 1H); 5.61-5.59 (m, 1H); 3.19-3.15 (m, 8H); 1.72-1.71 (m, 3H); 1.59-1.54 (m, 8H); 1.33-1.28 (m, 8H); 0.92 (t, *J* = 7.0 Hz, 12H). ^{13}C NMR (176.0 MHz, DMSO-d_6, +20 °C) δ: 169.6; 134.9; 133.8; 132.1; 130.3; 58.0; 23.6; 19.7; 18.5; 13.9. IR (ATR-FT-IRS) ν̃: 3169, 2959, 2936, 2874, 1568, 1354, 996, 882, 740, 705, 570 cm^{-1}. ESI FT-ICR MS (*m/z*): solvent MeCN/H_2O (80/20), [M]$^-$ calcd for $[C_6H_7O_2]^-$ 111.04515, found 111.04522.

Measurement of the relative and absolute binding constants. The binding constant measurements were carried out in DMSO-d_6 or in DMSO with 0.5 % water (m/m) using the previously described[24,25] NMR and UV-Vis methodologies. For NMR relative binding measurements, the concentrations of TBA salt in the concentrated solution were approximately 0.63-2.20 M and in diluted solutions approximately 0.25-0.75 M, depending on the degree of solubility and anion basicity. The initial concentrations of receptors were around 0.006-0.015 M. For UV-Vis absolute binding measurements, the concentrations of receptors were in the following ranges: receptor **15** ($3.0\cdot10^{-5}$ M), receptor **19** ($3.0\cdot10^{-5}$ M), receptor **21** ($8.7\cdot10^{-5}$ M), and receptor **22** ($8.3\cdot10^{-5}$ M). For fluorescence absolute binding measurements, the concentration of receptor **15** was around $1.2\cdot10^{-6}$ M, and the concentration of receptor **19** was approximately $8.1\cdot10^{-6}$ M for formate and around $1.4\cdot10^{-5}$ M for ibuprofen. ^{1}H NMR measurements for absolute logK_{ass} determination were performed only with receptor **22** which concentration was around $7.0\cdot10^{-6}$ M.

Acknowledgements

This work was supported by the grant No 9105 from the Estonian Science Foundation, by the Estonian National R & D infrastructure development program of Measure 2.3 'Promotion of development activities and innovation' (Regulation No. 34) funded by the Enterprise Estonia Foundation, by the EU through the European Regional Development Fund (TK141 "Advanced materials and high-technology devices for energy recuperation systems") and by the Institutional funding project IUT20-14 from the Estonian Ministry of Education and Research. We thank the group of Philip Gale for supplying compounds **14** and **15**.

Keywords: Anion Receptors • Carboxylate • Binding Affinity • Hydrogen Bond • Discrimination

References

[1] S. Pagola, P. W. Stephens, D. S. Bohle, A. D. Kosar, S. K. Madsen, *Nature* **2000**, *404*, 307–310.
[2] C. N. R. Rao, S. Natarajan, R. Vaidhyanathan, *Angew. Chem. Int. Ed.* **2004**, *43*, 1466–1496.
[3] P. D. Beer, P. A. Gale, *Angew. Chem. Int. Ed.* **2001**, *40*, 486–516.

[4] S. A. Kadam, K. Martin, K. Haav, L. Toom, C. Mayeux, A. Pung, P. A. Gale, J. R. Hiscock, S. J. Brooks, I. L. Kirby, et al., *Chem. – Eur. J.* **2015**, *21*, 5145–5160.
[5] F. Ulatowski, J. Jurczak, *Asian J. Org. Chem.* **2016**, DOI 10.1002/ajoc.201600093.
[6] Y. Liu, T. Minami, R. Nishiyabu, Z. Wang, P. Anzenbacher, *J. Am. Chem. Soc.* **2013**, *135*, 7705–7712.
[7] R. J. Prankerd, *Profiles of Drug Substances, Excipients and Related Methodology*, Elsevier, London, **2007**.
[8] S. M. Petrov, Y. I. Umanskii, I. F. Mullin, A. N. Tertichnyi, Z. F. Khim, *Russ. J. Phys. Chem.* **1973**, *47*, 363, 647.
[9] C. Hansch, A. Leo, D. Hoekman, *Exploring QSAR Hydrophobic, Electronic, and Steric Constants*, American Chemical Society, Washington, DC, **1995**.
[10] F. G. Bordwell, *Acc. Chem. Res.* **1988**, *21*, 456–463.
[11] D. D. Perrin, B. Dempsey, E. P. Serjeant, *pKa Prediction for Organic Acids and Bases*, Chapman And Hall, **1981**.
[12] I. A. Koppel, J. B. Koppel, V. O. Pihl, *Org. React.* **1988**, *25 (1)*, 77–101.
[13] X. Li, M. A. Cooper, *Anal. Chem.* **2012**, *84*, 2609–2613.
[14] M. Meloun, S. Bordovská, L. Galla, *J. Pharm. Biomed. Anal.* **2007**, *45*, 552–564.
[15] A. Fini, P. De Maria, A. Guarnieri, L. Varoli, *J. Pharm. Sci.* **1987**, *76*, 48–52.
[16] R. Kohn, P. Kovac, *Chem Zvesti* **1978**, *32*, 478–485.
[17] E. Lück, M. Jager, N. Raczek, in *Ullmanns Encycl. Ind. Chem.* (Ed.: Wiley-VCH Verlag GmbH & Co. KGaA), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, **2000**.
[18] D. P. White, J. C. Anthony, A. O. Oyefeso, *J. Org. Chem.* **1999**, *64*, 7707–7716.
[19] N. H. Evans, P. D. Beer, *Angew. Chem. Int. Ed.* **2014**, *53*, 11716–11754.
[20] V. Blažek Bregović, N. Basarić, K. Mlinarić-Majerski, *Coord. Chem. Rev.* **2015**, *295*, 80–124.
[21] S.-Y. Liu, Y.-B. He, J.-L. Wu, L.-H. Wei, H.-J. Qin, L.-Z. Meng, L. Hu, *Org. Biomol. Chem.* **2004**, *2*, 1582–1586.
[22] C. L. Beck, A. H. Winter, *J. Org. Chem.* **2014**, *79*, 3152–3158.
[23] P. Piątek, J. Jurczak, *Chem Commun* **2002**, 2450–2451.
[24] S. A. Kadam, K. Haav, L. Toom, T. Haljasorg, I. Leito, *J. Org. Chem.* **2014**, *79*, 2501–2513.
[25] K. Haav, S. A. Kadam, L. Toom, P. A. Gale, N. Busschaert, M. Wenzel, J. R. Hiscock, I. L. Kirby, T. Haljasorg, M. Lõkov, et al., *J. Org. Chem.* **2013**, *78*, 7796–7808.
[26] S. Tshepelevitsh, A. Trummal, K. Haav, K. Martin, I. Leito, *J. Phys. Chem. A* **2017**, *121*, 357–369.
[27] A. Teearu, S. Vahur, T. Rodima, K. Herodes, W. Bonrath, T. Netscher, S. Tshepelevitsh, A. Trummal, M. Lõkov, I. Leito, *J. Mass Spectrom.* **2017**, DOI 10.1002/jms.3943.
[28] S. A. Kadam, K. Haav, L. Toom, A. Pung, C. Mayeux, I. Leito, *Eur. J. Org. Chem.* **2017**, DOI 10.1002/ejoc.201601583.
[29] N. Busschaert, P. A. Gale, C. J. E. Haynes, M. E. Light, S. J. Moore, C. C. Tong, J. T. Davis, W. A. Harrell, Jr., *Chem. Commun.* **2010**, *46*, 6252.
[30] J. Y. Lee, E. J. Cho, S. Mukamel, K. C. Nam, *J. Org. Chem.* **2004**, *69*, 943–950.

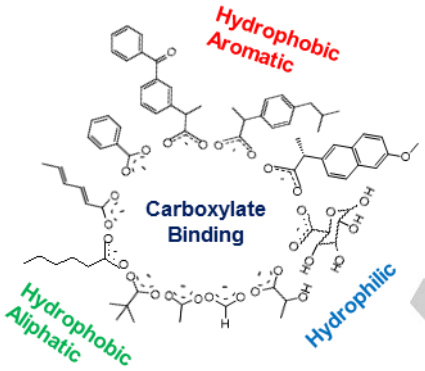
10.1002/ejoc.201700931

WILEY-VCH

Entry for the Table of Contents

FULL PAPER

242 accurately determined binding constants between 11 carboxylates and 22 synthetic receptors are used to reveal the main structural factors of anions and receptors governing the binding and demonstrate that the receptors selected for this study enable discriminating between anions according to structural features (hydrophilicity, substitution at α-carbon, etc).



Carboxylates Binding

*K. Martin, J. Nõges, K. Haav, S. A. Kadam, A. Pung, I. Leito**

Page No. – Page No.

Exploring selectivity of 22 acyclic urea-, carbazole- and indolocarbazole-based receptors towards 11 monocarboxylates