Realia et naturalia

DISSERTATIONES
CHIMICAE
UNIVERSITATIS
TARTUENSIS
145

SANDIP ASHOKRAO KADAM

Anion receptors: synthesis and accurate binding measurements

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS
145

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS
145

SANDIP ASHOKRAO KADAM

Anion receptors: synthesis and accurate binding measurements

UNIVERSITY OF TARTU
PRESS
1632

Institute of Chemistry, Faculty of Science and Technology, University of Tartu.
Dissertation is accepted for the commencement of the Degree *of Doctor philosophiae* in Chemistry on June 18, 2015 by the Doctoral Committee of the Institute of Chemistry, University of Tartu.

Supervisor: Professor Ivo Leito (PhD)
Institute of Chemistry, University of Tartu, Estonia

Opponent: Professor Carsten Schmuck (Dr)
University of Duisburg-Essen, Germany

Commencement: August 25, 2015 at 10.00, Ravila 14a, room 1021

This work was supported by the grant No 9105 from the Estonian Science Foundation, the Estonian National Research and Development Infrastructure Development Programme of measure 2.3 'Promotion of development activities and innovation' (Regulation No. 34) funded by the Enterprise Estonia foundation and by the Institutional funding project IUT20-14 from the Estonian Ministry of Education and Science.

Publication of this dissertation is granted by University of Tartu.

ISSN 1406-0299
ISBN 978-9949-32-879-6 (print)
ISBN 978-9949-32-880-2 (pdf)

University of Tartu Press
www.tyk.ee

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following articles:

I. Haav, K.;[§] **Kadam, S. A**.;[§] Toom, L.; Gale, P. A.; Busschaert, N.; Wenzel, M.; Hiscock, J.; Kirby, I.; Haljasorg, T.; Lõkov, M.; Leito, I. Accurate method to quantify binding in supramolecular chemistry. *J. Org. Chem.* **2013**, *78*, 7796–7808. § equal contribution

II. **Kadam, S. A**.; Haav, K.; Toom, L.; Haljasorg, T.; Leito, I. NMR method for simultaneous host-guest binding constant measurement. *J. Org. Chem*., **2014**, *79*, 2501–2513.

III. **Kadam, S. A**.; Martin, K.; Haav, K.; Toom, L.; Mayeux, C.; Pung, A.; Gale, P.A.; Hiscock, J. R.; Brooks, S. J.; Kirby, I. L.; Busschaert, N.; Leito, I. Towards discrimination of carboxylates by hydrogen-bond donor anion receptors. *Chem. Eur. J.* **2015**, 21, 5145–5160.

Author's contribution

Paper I. Synthesized all the molecules and participated in manuscript preparation.

Paper II. Synthesized all the molecules, carried out all the NMR binding measurements and data analysis and was key participant in manuscript preparation.

Paper III. Synthesized all the molecules, carried out most of the NMR binding measurements and data analysis and was key participant in manuscript preparation.

ABBREVIATIONS

AcOH	Acetic acid
Ac_2O	Acetic anhydride
AcO^-	Acetate anion
$AlCl_3$	Aluminum trichloride
ACN	Acetonitrile
BzO^-	Benzoate anion
Bu	Butyl
Boc_2O	Di-tert-butyl-dicarbonate
δ	Chemical shift (ppm)
DMSO	Dimethyl sulfoxide
DMSO-d_6	Deuterated dimethyl sulfoxide
DMF	*N*,*N*-Dimethylformamide
DCM	Dichloromethane
DMAP	Dimethylaminopyridine
EtOH	Ethanol
EDC·HCl	1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride
HB	Hydrogen bond
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HOBt	Hydroxybenzotriazole
HNO_3	Nitric acid
$\log K_{ass}$	Logarithmic association constant
MeOH	Methanol
NMR	Nuclear magnetic resonance spectroscopy
NOE	Nuclear overhauser enhancement
N_2	Nitrogen
$N_2H_4 \cdot H_2O$	Hydrazine hydrate
pK_a	Negative logarithm of ionization constant
ppm	Parts per million
TLC	Thin layer chromatography
THF	Tetrahydrofuran
TBAOH	Tetrabutylammonium hydroxide
TBA	Tetrabutylammonium
TFA	Trifluoroacetic acid
UV-vis	UV-visible spectroscopy
λ	Wavelength (nm)

I. INTRODUCTION

The design, synthesis and study of molecular receptors for anions is an ever-expanding research area in supramolecular chemistry. Many receptor molecules incorporating functional groups such as urea, thiourea, indole, pyrrole, carbazole, indolocarbazole, etc have been successful in selectively complexing a range of anionic guests via hydrogen bonding. An important objective of supramolecular analytical chemistry is to design synthetic receptors that could bind particular analytes with both high sensitivity and selectivity. Selective anion receptors can be utilized in anion sensing, extraction or transport. These techniques can find direct application in determination of anions in real samples (by incorporating the receptors into a sensor) or increasing the efficiency of sample preparation procedures by using suitable receptors for selective extraction or transport.

The stability of a supermolecule is expressed by the binding constant (association constant) K_{ass} (or its logarithm $\log K_{ass}$) between species involved (often termed the host and guest). Binding constant is a key characteristic of a supramolecular assembly. When measured for different systems the $\log K_{ass}$ values enable obtaining useful information for predicting properties of new molecular assemblies. Developing a receptor molecule, which is specific to any single anion is not easy and probably impossible for some (simpler) anions. Therefore the focus of development has largely shifted to receptor arrays, where a number of receptors are grouped, each of them being sensitive to a number anions, but with different $\log K_{ass}$ values, enabling determining different anions after mathematical treatment of data. The requirement is however, that the involved receptors have *different* sensitivities towards the different anions.

In order to design receptor molecules with different sensitivities towards different anions it is very useful to have extensive, accurate and comparable (i.e. obtained in the same solvent) data on the binding affinity of different simpler receptors/building blocks with different anions, which would enable to see binding affinity trends depending on anion and receptor structure. Such data have until recently been unavailable, in part because of absence of sufficiently accurate measurement methods.

The general objective of this work was to synthesize a number of different simple anion receptors, which could be regarded as building blocks for more complex receptors, and accurately measure their binding affinity towards different simple carboxylate anions. The specific aims of the thesis are the following:

- Synthesize different families of simple receptors based on the core structures of indolocarbazole, urea, thiourea, carbazole, indole (and their combinations).
- Develop a simple, robust, fast and accurate method for measuring the binding affinity using relative measurement by NMR.
- Measure the binding affinities of the receptor molecules towards lactate, benzoate, acetate and trimethylacetate anions.
- Study the structural and solvent effects on binding affinity.

2. LITERATURE OVERVIEW

2.1. Host-Guest Supramolecular chemistry

Supramolecular chemistry has been defined by Nobel laureate Jean-Marie Lehn as "*the chemistry of molecular assemblies and of the intermolecular bond*"[1] It involves the non-covalent interactions between different species and formation of supramolecular structures (supermolecules).[2] One of the first seminal contributions to supramolecular chemistry was made in 1967 by Pedersen who reported on a series of macrocyclic polyethers capable of binding potassium ions.[3–5] Later, Cram developed the concept of molecular recognition, self-organization, self-replication, self-assembly, transport and catalyst in supramolecular chemistry.[6–8] Recently supramolecular chemistry has involved into an interdisciplinary field with connections in biology, chemistry and physics.[2]

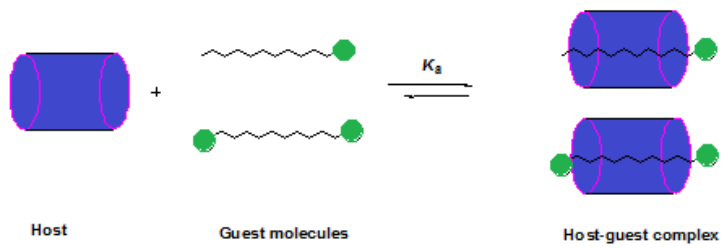


Figure 1. Host-Guest complex

Non-covalent interactions play a significant role in supramolecular chemistry. The energies of non-covalent interactions are usually in the range of 4 to 120 kJ/mol. This is weaker than covalent bonds, which are typically in close proximity to 350 kJ/mol for single bond.[9] In the following section different types of intermolecular forces, which are often used in the formation host-guest complex chemistry, are described.

Non-covalent interactions differ from covalent bonds in that they do not involve sharing of electrons but involve a somewhat more "remote" interaction between molecules or within molecules. Non-covalent interactions are responsible for binding the components of supermolecules in supramolecular chemistry. Non-covalent interactions can be classified into the following categories: hydrogen bond interaction, electrostatic interaction, Van der Waal forces, π-π interactions, hydrophobic interaction. It is important to stress that this is one of the commonly used classifications and is not fully rigorous. For example, hydrogen bonding involves charge-charge interaction: π-π interaction is a kind of Van der Waals interaction, etc.

2.1.1. Hydrogen bond interaction

HB interactions occur between hydrogen bond donor and acceptor sites. The donor site X-H has a positively polarized hydrogen atom. A HB donor molecule (or a cation) can contain numerous donor sites. The acceptor site carries a (partial) negative charge and has a lone electron pair or a π bond.[11,12] A HB acceptor molecule (or anion) can have numerous acceptor sites. The same molecule can simultaneously contain both HB donor and HB acceptor sites.

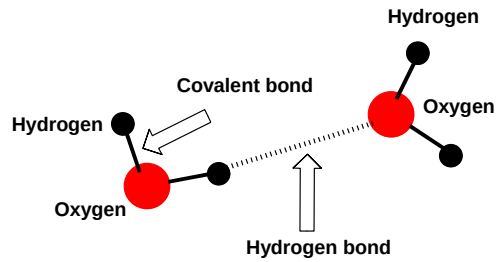


Figure 2. Non covalent interaction between two atoms

The strength of HB can vary significantly. Strong HB can be almost as strong as weak covalent bonds. Weak hydrogen bonds have similar strength to Van der Waals interactions.[11,12] Hydrogen bond energies usually are between 0.5 and 40 kJ/mol. The debate about the relative importance of electrostatic interaction and overlapping of orbitals (i.e. chemical bonding) in HB is still ongoing,[10] but usually HB is nowadays considered as being more an electrostatic interaction than a chemical bond. The most HB acceptors (important electron pair donors) are the nitrogen atoms in amines and heterocycles as well as oxygen atoms in alcohols, ethers and carbonyl compounds. Strong hydrogen bonds are formed between the following fragments: N-H···O, O-H···O, F-H···O etc. The main factors influencing the strength of a HB interaction in a system R-H···A, as well as the distance between R–H and A are the partial charges on H and A, as well as the steric factors (accessibility) and the bond angle between R, H and A. The higher are the partial charges and the better the R-H and A can access each other the stronger is the HB. HBs are directional interactions and will be stronger when the R-H···A angle is closer to 180°. In contrast, HBs of moderate strength will be slightly bent with bond angles between approximately 130 and 180°, while weak hydrogen bonds can be between 90 and 150°.[11,12] HB is probably the most important non-covalent interaction in binding hosts and guests in supramolecular chemistry.

2.1.2. Van der Waals interactions

Van der Waals interactions involve dipole-dipole and dipole-induced dipole interactions as well as the dispersion forces. These interactions are based on partial charges in molecules (or ions) and polarizability of electron clouds.[13,14] Van der Waals forces can be either attractive or repulsive, depending on the distance between the atoms involved. These interactions are attractive from a distance and become more attractive as the molecules approach each other, but as the molecules become very close together, the interactions become repulsive due to repulsions between the electron clouds of the two molecules.[15] Van der Waals forces are in general weak, but if large parts of molecules are involved – e.g. dispersion forces between large areas of molecules – then they can become quite strong. In the context of binding hosts and guests in supramolecular chemistry Van der Waals interactions usually have a helper role.

2.1.3. Charge-charge interaction

Charge-charge interactions occur between two oppositely charged species.[9] These can generally include ion-ion, ion-dipole interactions. Ion-ion interaction is often the strongest non-covalent interaction with energy around 250 kJ/mol. Ion-ion interaction is independent of direction, while ion-dipole interaction needs particular orientation of the interacting species in order to attain the maximum strength.[9] For example such interaction is observed in complexes between cations and crown ethers[16] and interaction between the cationic guest and electron rich carbonyl-rim of cucurbit[*n*]uril.[17] If ions are involved in supermolecules then charge-charge interactions can be the dominant interactions in keeping them together.

2.1.4. π-π interaction

The term π-π interaction refers to interactions between aromatic rings. The interacting rings can be oriented in different ways (Figure 3). The sandwich interaction is the least favorable because of the electrostatic repulsion of the π-clouds. The perpendicular and parallel-displaced orientations are energetically favorable.[18] In the case of perpendicular geometry the positively polarized CH bonds of one ring are oriented towards the partial negative charge on the π-cloud of the other ring. This stacking geometry is observed in both the solid[19] and liquid[20] benzene.

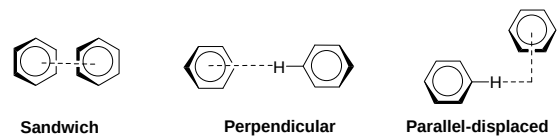


Figure 3. π-π stacking interaction between aromatic rings.

The perpendicular stacking geometry has a calculated stabilization energy of ~3 kcal/mol in the gas phase[21] and 0.5 – 1.5 kcal/mol in the condensed phase.[22] The parallel-displaced stacking geometry has similar interaction energy of ~2.5 kcal/mol in the gas phase, and is mainly attributed to dispersive interactions between the aromatic rings. It is also the preferred type of π-stacking between aromatic rings in water and is encountered in the hydrophobic interaction between π-systems. The π-π interactions are increasingly used as binding interaction in host-guest chemistry and they can be very efficient, especially in polar environments. [23]

2.1.5. The hydrophobic effect

The hydrophobic effect refers to the tendency of nonpolar molecules (or nonpolar parts of molecules) to associate in water. It is not an independent intermolecular interaction but rather an "effect", meaning that different interactions – HB, dispersion forces, and π-π-interaction – are responsible for its occurrence. Energetically, the hydrophobic effect is caused by the large amount of energy required to form a cavity in the aqueous solution. Water molecules interact strongly with other water molecules due to hydrogen bonding. When a hydrophobic solute is dissolved in water then these interaction are interrupted. In addition, a cavity needs to be formed between water molecules in order to accommodate the nonpolar solute. In addition, molecules of water are very small, so that when even a solute molecule of modest size is added to water, a large number of water molecules are affected (Figure 4). Nonpolar molecules especially ones with aromatic rings have remarkable binding affinity with each other in water.[24] The hydrophobic effect is seen as the driving force for a variety of phenomena, such as formation of micelles, protein folding and poor solubility of non-polar solvent in aqueous media.[25,26]

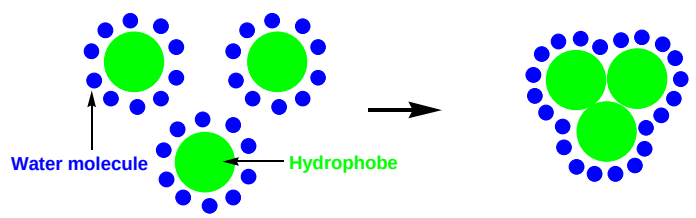


Figure 4. The formation of hydrophobic interaction between three molecules

Hydrophobic effect is highly instrumental in supramolecular chemistry: binding of a nonpolar guest into a hydrophobic receptor site of a host molecule is favorable due to the release of water molecules from the host's receptor pocket, which may result in increase of entropy and/or decrease of enthalpy.[9] The enthalpy change is negative if the water molecules that are released from the

host's binding site can more readily form HBs with other water molecules in bulk water.[9]

2.2. Carboxylate anions

Anions play key roles in many biological, chemical and industrial processes.[27] Carboxylate is a particularly common anionic functional group, both in synthetic and biological molecules.[27] Extremely diverse residues X are found in carboxylates X-COO^-. Carboxylates where X is a short aliphatic chain (often containing substituents) are important metabolites, while carboxylic acids with long aliphatic chains are crucial in the formation of fats. All the amino acids have carboxylate functional groups and are the building blocks of peptides and proteins. Many widely used drugs such as aspirin and ibuprofen are carboxylic acids.[28] There are numerous anions such as succinate, fumarate and malate that take part in citric acid cycle to generate energy.

Under physiological conditions, i.e. pH between 7 and 8, carboxylic acids are predominantly in the anionic form. For this reason the receptors synthesized for them should be capable of binding carboxylates.

While, carboxylate anions can have very different structures and geometries, the geometry of the carboxylate group itself is fairly constant among different anions. The carboxylate group has equal CO bond lengths (1.26 Å in acetate) and the bond angle between the CO bonds close to 120º (120.9° in acetate).[29,30] distance between O atoms is 2.2 Å. The negative charge of carboxylate ions is largely localized on the oxygen atoms making these ions strongly solvated in HBD solvents and especially in water.[32] The importance of carboxylates has triggered the development of a number of different approaches for their recognition. Carboxylic acids are medium strong acids in aqueous media, they deprotonate quite readily and the respective carboxylate anions do not protonate so easily. The geometry of the carboxylate group enables simultaneous formation of two HBs with receptors having suitably positioned HB donor sites, such as urea and indolocarbazole receptors, as shown in Figure 5.

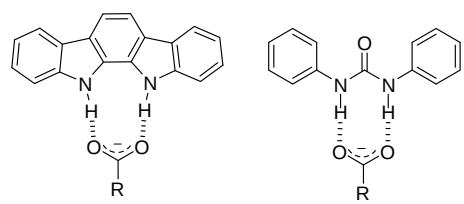


Figure 5. The binding stoichiometry (1:1) of urea and indolocarbazole receptors: carboxylate anions

The design of synthetic receptors for selective and strong binding of carboxylate anions is a challenging task in supramolecular chemistry for two main reasons: (1) the geometry of the carboxylate group is very similar in different carboxylates and (2) in protic solvents, most importantly – water, carboxylates are strongly solvated.

For these reasons simple receptor molecules can be neither sufficiently selective to differentiate between different carboxylates nor sufficiently sensitive to strongly bind carboxylates in protic media. A simple receptor molecule will interact first of all with the carboxylate centre. Although, the binding will be influenced by the rest of the ion (the residue X), such influence and consequently the possibilities of achieving selectivity will be limited by the small size of the receptor. Also, simple receptors, not interacting directly with X cannot achieve very strong binding. It is not difficult to fortify the HB ability of a small receptor by incorporating electron-withdrawing groups, but this will also increase the acidity of the receptor and eventually lead to proton transfer from receptor to carboxylate and thereby loss of selectivity (if these species diffuse apart in the solution).

Such small receptor molecules can, however, be regarded as building blocks of more complex receptors. Understanding the relationship between molecular structures and the binding behavior towards different carboxylate anions is important for designing more complex receptors.

Acetate is one of the simplest and most important carboxylate anions in nature. Acetate together with lactate, benzoate and trimethyl acetate were selected in this work because they have different basicity, hydrophilicity, steric demand and different biological functions and applications.[33]

2.3. Molecular receptors for anion binding

There are many examples in nature of anion binding by proteins with neutral amide functions. The transport of sulphate or phosphate anions through cell membranes is regulated by neutral anion binding proteins. The high specificity is due to a recognition site in which the anion is completely desolvated and bound by binding moieties of suitable properties and orientation. Neutral receptors in both transmembrane and anion sensing such receptor will be required in order to obtain good selectivity. For this reason variety of amide based neutral receptor great interest in anion recognition chemistry.

Synthesis of neutral anion receptors for anion binding is quite a challenging area in supramolecular chemistry. In 1968 the first synthetic anion receptor for inorganic anion (selective binding of Cl^- anion) bind based on macrocyclic diprotonated (1,11-diazabicyclo-[9.9.9]nonacosane) amines was developed by Park and Simmons.[34] The main binding interaction in that receptor was hydrogen bond. HBs are directional, therefore receptor with specific spatial arrangement of hydrogen bond donor fragments can in principle distinguish between anions of different geometry.[35] Several neutral hydrogen bond donor groups/

fragments, such as urea,[36,40,41] thiourea,[37] squaramide,[38] pyrrole,[43] indole,[47] carbazole,[45,46] indolocarbazoles,[39] and others have been used as HB donors for binding different anions.

Hydrogen bond is the most frequently used interaction in anion binding because of its relatively high interaction energy and it can be readily multiplied by incorporating a number of binding centers. The accurate arrangement of hydrogen bonding centres, supported by the rational design, potentially allows the geometry of a binding pocket to be adjusted to the size and topology of a desired anion, leading to its strong and selective binding. Many hydrogen bond donor groups exist, such as -OH, -NH, -CH. However, the -OH groups, especially if attached to CO or SO_2 deprotonate easily. The -CH group are usually not enough polarized to form hydrogen bonds. Thus, although both OH and CH groups are used as HB donor sites, the -NH group is by far the most popular. The NH protons are well polarized and at the same time not strongly acidic. The N-H fragments can be reliably oriented in a suitable way by designing the molecule (as opposed to OH, which can freely rotate) and they are not sterically hindered for anion (as opposed to many CH centers). In order to make the hydrogen of the NH group more polarized there are three possibilities. Firstly, electron withdrawing substituent's ($-NO_2$, -CN, $-CF_3$) increase hydrogen bond donicity. However, with very strong polarization of the NH bond there is a strong risk of deprotonation. Secondly, incorporating N-H atom in to five-member aromatic rings such as pyrrole, indole or carbazole, each of them contains a single NH hydrogen bond donor and the NH protons are strongly polarized. True, this increases also their acidity (pK_a in DMSO: pyrrole 23.0, indole 21.0 and carbazole 19.9) and thus also the danger of deprotonation. Thirdly, as anions are naturally negatively charged an efficient way of achieving anion binding is by the employment of positively charged species. Ammonium and guanidinium groups have been widely used for this purpose.[48]

2.3.1. Amide-based anion receptors

The amide groups contain both hydrogen bond acceptor (carbonyl oxygen) and hydrogen bond donor (NH) sites. A number of types of amide-based receptors have been created and synthesized. The binding centers are based on urea,[36] thiourea,[37] isophthalamide,[48] sulphonamide,[48] squaramide,[38] etc moieties. They have been widely used as hydrogen bond donor sites due to their facile synthesis and easily tunable NH acidity. These amide families have a diverse range of binding geometries and affinities. The urea and thiourea type receptors, as well as squaramide are capable of binding anions in bi-dentate fashion, which by its geometry is very suitable for carboxylate anions. In the isophthalamide structure there are also two NH bonds, but they are spatially much more separated. Sulphonamide NH is more polarized than in the case of carboxylic acid amides, but at the same time also more acidic, leading to the danger of deprotonating the receptor by the anion.

Urea and thiourea are perhaps the most widely used neutral building blocks for easy synthesis of anion receptors.[36,37] Urea and thiourea possess two NH fragments which are able to interact simultaneously with a single anion such as halides, carboxylates, phosphonates, etc. The interaction is especially strong with carboxylates, both because of suitable geometry (leading to formation of an eight-member cyclic complex), as well as the quite high basicity of carboxylate anions. Thiourea is more acidic than urea, meaning that it also has more danger of deprotonation. For achieving high binding affinity introducing strong electron-withdrawing groups is efficient, as this decreases the electron density on nitrogen atom and increases positive polarization of the attached H atom.[36,37,49]

In spite of the high diversity of urea and thiourea as binding fragments, designing suitable receptors is not straight forward. One possible danger is deprotonation of the receptor by the anion and loss of selectivity. This illustrated by receptors **1**–**5**.[36,37] The strong electron-withdrawing groups -NO_2 and -CF_3 lead to high binding affinity but at the same time to deprotonation of some of these molecules (**2**, **3** and **5**) by the acetate anion in the case of polar solvent such as DMSO.[50,51]

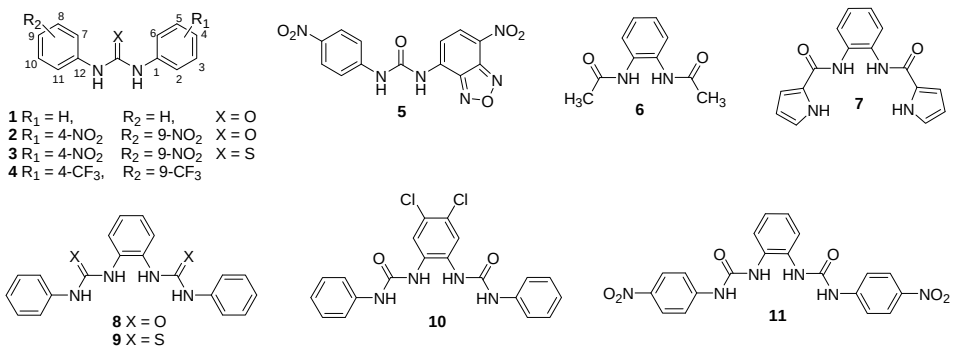


Figure 6. Amide based urea receptors **1**–**11**.

The non-straightforward nature of the binding interactions is illustrated by compounds **8** and **9**. In 0.5% H_2O:DMSO-d_6 compound **8** binds AcO^- and BzO^- with the K_{ass} values 3210 M^{-1} and 1330 M^{-1} respectively.[40-42] the crystal structure of compound **8** with benzoate complex shows that it has the ability to form four complementary HB bind with BzO^- anion. The binding affinity increased by introducing electron withdrawing groups (-Cl, -NO_2), the receptor **10** and **11** the binding affinity towards the AcO^- and BzO^- anions (8079 M^{-1}, 2248 M^{-1}) and (4018 M^{-1}, 1399 M^{-1}) respectively. It shows that compound **10** has higher binding affinity than compound **11**, because the receptor **10** (-Cl) groups on central ring induce formation of intramolecular hydrogen bonds between central ring H atoms and urea carbonyl oxygen atoms. This leads to a

preorganized conformation and decreases electron density on carbonyl groups. In the case of receptor **11** the NO_2 groups introduced to the outer phenyl ring induce similar hydrogen bonds with the outer rings, but their effect remains weaker.[42] Although thioureas are more acidic, the binding is not stronger: in the case of thiourea receptor **9** significantly lower binding affinity towards the carboxylate anions was observed, due to the large sulphur atom altering the shape of the binding site, hindering binding to the outer NH group.

The superiority of substituted ureas against simpler amides as binding moieties can be seen e.g. on the example of compounds **6** and **7**, which have low binding affinity towards BzO^- and AcO^- anions: between 100–200 M^{-1}.[40,42]

2.3.2. Combined anion receptors

A number of receptors containing the pyrrole fragment have been synthesized. Simple pyrrole NH proton does not give strong hydrogen bond but inserting different substituent's or combining pyrrole rings together (e.g. as calix[4]pyrroles[43,44]) leads to significant increase of HB donicity. Recently a number of receptors based on indole and carbazole have been introduced to anion coordination chemistry.[45-47] Carbazole and indole groups are more acidic than the pyrrole group.[49] However, carbazole and indole contain only one HB donor groups. For a more efficient binding multiple HB donor groups would be advantageous (see e.g. Figure 7). In 2005 Beer and coworkers introduced the new family of indolocarbazole type of receptors (Figure 8).[39] In addition to having two HB donor centers indolocarbazole (**20**) has better hydrogen bond donating ability than pyrrole, indole and carbazole also because of the higher acidity of its NH protons.[49] The general way for synthesis of indolocarbazole involves a double Fisher indolization reaction of phenylhydrazine and 1,2-cyclohexanedione refluxed in an acidic medium. Indolocarbazole (**20**) contains a fused ring system with two pyrrole rings that are preorganized very suitably (distance between two H atoms is 2.60 Å) for bidentate binding of anions.

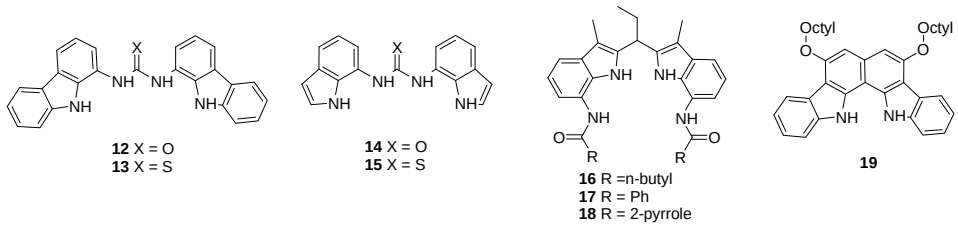


Figure 7. Combined receptors containing indol or carbazole **12–19**.

As said above, for achieving high binding affinity, receptors should have a number of different hydrogen bond donor sites. As a result, almost all high-affinity receptors for anions are designed by combining different binding

moieties in order to support the formation of multiple hydrogen bonds. As an example, 1,3-dicarbazoylurea receptor **12** is designed by combining urea with carbazole. This receptor was titrated with different anions in 0.5% H_2O: DMSO-d_6, followed by ^{1}H NMR. It showed high binding affinity towards the AcO^-, $H_2PO_4^-$ and HSO_4^- with K_{ass} above 10^4.[47] The thiourea-based receptor **13** has somewhat lower binding affinity. The 1,3-diindolylurea and -thiourea type receptors **14** and **15** have high binding affinity towards the AcO^- and $H_2PO_4^-$ anions in 0.5% H_2O: DMSO-d_6 above 10^4 and 10^3, in both cases it is noted that the urea group leads to stronger binding than thiourea. The binding affinity differs by one order of magnitude due to the large S atom altering the shape of the receptor molecule and hindering binding to the outer NH group. It is observed that receptors **14** and **15** have different conformations in solution as confirmed by NOESY experiment. It was observed that the anti-anti conformation is more stable in the free receptor and after formation of complex the syn-syn conformation is more stable.[52]

The receptors **16–18** show high binding affinity towards different oxoanions.[53] In all cases the titration data fit to 1:1 binding stoichiometry in highly polar solvent. The receptor **16** shows remarkably high binding affinity towards the BzO^- and $H_2PO_4^-$ anions. The n-butyl group in the amide substituent turns out to be superior to the other groups used in receptors **17** and **18**.[53] Comparing the binding affinity data for receptors **17** and **18** reveals that introducing additional pyrrole unit does not significantly alter the affinity towards anions. The fused carbazole type receptor **18** to some extent resembles the indolocarbazole (**20**) receptor, but the orientation of the NH groups is different. Receptors **19** and **20** have comparable binding affinities towards carboxylate anions in DMF, for AcO^- and BzO^- anions. Receptor **19** binds AcO^- somewhat stronger than **20**.[54] The main reason is the suitable angle between NH centers of carbazole rings. To somewhat counterbalance this, there is additional interaction between the anions and **19** via the aromatic CH protons in the ortho position relative to the NH groups.

Indolocarbazole-based receptors **20–23** were found promising building blocks in applications for chromogenic and fluorogenic receptor molecules.[55] The binding studied of receptors **20–23** conducted by UV-vis titration, with different solvents ACN and acetone indicated that they bind well with carboxylates as well as halide anions (F^-, Cl^-, Br^-).[39,58] Interestingly, ref 56 reported that some indolocarbazole-based receptor compounds have a higher binding affinity for benzoate anion than for acetate anion, which is unusual. The simple indolocarbazole receptors were studied by fluorescence spectroscopy and significant fluorescence enhancement was observed when simple indolocarbazole receptors bound the F^- or Cl^- anions.[39] The addition of benzoate anion quenched the fluorescence.

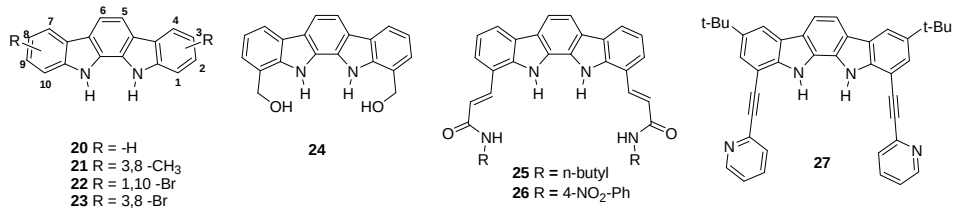


Figure 8. Indolocarbazole type receptors **20–27**.

For improving the binding affinity of receptors indolocarbazole different substituents have been attached to the positions 1 and 10, resulting in receptors **24–27**. The binding affinity of receptors **24–27** in ACN towards different anions has been measured and by logK_{ass} towards BzO^- in ACN these compounds **24**, **25** and **27** increase the unsubstituted indolocarbazole value by 2, 0.7 and 0.8 orders of magnitudes but receptor **26** for AcO^- anion decrease by around one orders of magnitude due to the repulsion of two pyridine rings.[56,57] These data demonstrate that the preorganized indolocarbazole system enables increasing the binding affinity.

Indolocarbazole was chosen as one of the core structures for the receptors in this work, because of the following favorable properties: (1) rigid spatial orientation of the two NH groups, which, in addition, is very suitable for binding carboxylates and leads to good binding affinity towards carboxylates; (2) absence of the possibility of intramolecular hydrogen bond formation (an advantage, compared to e.g. urea-based receptors); (3) good spectral properties: both UV- vis and fluorescence spectroscopy can be used. The disadvantages of indolocarbazole as core structure are (1) limited solubility of many indolocarbazole derivatives in polar solvents (which is a problem for their investigation, but not necessarily for the eventual use); (2) Not all substitution schemes are synthetically easily accessible.

2.3.3. Other anion receptors

As anions are naturally negatively charged, an obvious way of achieving anion binding is by introducing positively charged (cationic) groups (protonated amine, imidazole or guanidinium)[59,60] to receptor molecules. Quaternary ammonium based receptors have important advantages when compared with other cationic receptors. In general, the extent of protonation and thereby also the net charge and overall anion binding ability depend on pH. In contrast, the behavior of receptors based on quaternary ammonium groups is usually pH-independent. On the other hand, quaternary ammonium groups do suffer from the disadvantage that they are unable to interact with the anion via hydrogen bond: they simply lack the NH donor sites.

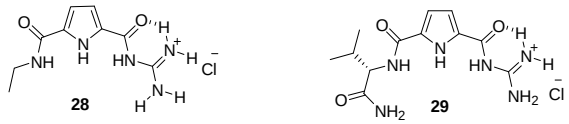


Figure 9. Some examples of charged anion receptors **28** and **29**.

Over the last few years many types of guanidinium-containing receptors have been reported, of which many bind carboxylate anions,[61–63] The unsubstituted or alkyl substituted guanidinium group does not have good affinity towards carboxylate anions in DMSO solvent. The reason is the efficient charge delocalization resulting in low acidity and low HB donicity. However, if the acidity of the guanidinium group is increased by inserting carbonyl, pyrrole, etc groups, then high affinity for carboxylate anions is obtainable, even in highly competitive solvent.[60,64] Receptors **28** and **29** bind carboxylates by ion pairing in combination with multiple hydrogen bonds. The receptor **28** bind N-actylalanyl (amino acid) carboxylate with logK_{ass} value between 2.55 to 3.23 in water: DMSO 40:60. The chiral receptor **29** has an extra amide group which lead to some enantioselectivity.

Besides the tradeoff between the possible deprotonation and HB donating ability of the cationic moiety of the receptor another disadvantage of charged receptors is that they need to be used as salts and the counteranions may potentially interfere with binding the analyte anions.

2.4. Binding affinity and its measurement methods

2.4.1. Definition of binding affinity

Binding constants (association constants) K_{ass} are equilibrium constants that are used as a quantitative measure of the strength of the binding interaction between a particular host and guest (logK_{ass} is often used) in terms of complex formation, under predefined conditions. The more favorable the interaction between a given host and guest is, the higher the binding constant will be. The calculation of the binding constant takes into account the activities of the free guest (that is the unbound guest) and the free host, as well as the activity of the host-guest complex. In the case of 1:1 complex formation the equilibrium is:

$$H + G^- \underset{}{\overset{K_{ass}}{\rightleftharpoons}} HG^- \qquad (1)$$

The binding affinity of a receptor H towards an anion G^- to form a receptor-anion complex HG^- according to 1:1 stoichiometry is quantified by the binding (association) constant K_{ass} according to the following equilibrium:

$$K_{\text{ass}} = \frac{a_{\text{HG}^-}}{a_{\text{H}} a_{\text{G}^-}} \tag{2}$$

where a_{HG^-}, a_{H} and a_{G^-} denote activities of the species in the solution.

Instead of the logK_{ass} value of a given receptor towards a given anion it is possible to measure the $\Delta\log K_{\text{ass}}$ value – relative binding affinity of two receptors towards the same anion. This way of measurement has several advantages, is extensively used in this work and is described in section 4.

2.4.2. Methods for studying receptor-anion interaction

A number of analytical methods have been used for characterization of host-guest interaction in supramolecular chemistry and for the measurement of binding constants, such as NMR spectrometry, UV-visible spectrometry, mass spectrometry, fluorescence, isothermal calorimetric titration etc. The spectroscopic techniques utilize the changes in the spectroscopic features of either the host or the guest, which may be observed upon complexation. Depending on the system studied and the technique used different information can be obtained. Most commonly the stoichiometry of the host-guest system and the binding constant are determined. NMR enables also determining the location of G^- within the host structure. Short descriptions of the two techniques used in this work – NMR and UV-vis spectrometry – are given below, from the point of view of studying anion-receptor interaction.

NMR Spectrometry

NMR spectrometry is widely used in organic chemistry as a multipurpose characterization technique as well as in studies of equilibria and rates of reactions. NMR has been frequently applied to study host-guest interactions in supramolecular chemistry. The host-guest binding is observed by NMR via the changes in chemical shifts (δ values) of the protons directly involved in the interaction. Different protons can be observed in the molecule which gives more information than most techniques about the interaction between the host and guest, e.g. which host protons interact with the guest molecule. The nature of the changes of the δ values of the host (and sometimes also the guest) and intensities of the proton signals can vary depending on how fast the exchange between the host and guest is with respect to the NMR timescale, which is much slower than with most spectroscopic methods.[65] In most cases the proton's exchange rate between two states (i.e. bound and unbound) is faster than the difference in frequencies between the two states and the system called *fast exchange* system in terms of the NMR timescale.[65,66] When the specific proton in a supramolecular system displays fast exchange behaviour, the unbound and bound proton peaks cannot be observed separately and are instead combined

into one single peak. The chemical shift of that peak represents an average of the bound and unbound proton peaks.[65,66] If the host-guest exchange rate is similar to the NMR timescale then the peaks that correspond to the unbound and bound nuclei will become broad, or even disappear. This peak broadening often takes place when the proportion of host and guest is such that there is a mixture of unbound and bound species present. When the species are either predominantly unbound or bound, the peaks are typically sharp.

The main disadvantage of NMR is its low sensitivity compared to the UV-vis or fluorescence. Measuring host-guest binding using the NMR method requires larger quantities of compounds. If absolute $\log K_{ass}$ values are measured then the low sensitivity complicates measuring $\log K_{ass}$ values higher than 4.[67] Use of solutions with high concentrations can also lead to emergence of undesired side processes, especially in solvents of lower polarity, such as ion-ion interaction, homoconjugation etc, which can change the free anion concentration in the solution and make it difficult to calculate the binding constants. The limitation of measuring high $\log K_{ass}$ values can be avoided by measuring the relative binding affinity of two or more receptors in one solution (see section 4). Such relative measurement also eliminates the effect of several side-processes. In addition, it is possible to independently observe the respective receptor proton chemical shift values during titration, which is impossible with, e.g., UV-vis or fluorescence.

UV-Visible spectrometry method

UV-vis spectrometry is broadly used for investigation of different binding affinity measurement in supramolecular chemistry due to the simplicity, robustness and accuracy.[68,69] The sensitivity of UV-visible spectrometry is higher than that of NMR, providing good signal to noise ratio even at low concentrations. In simplified terms, the determination of binding affinity of a receptor towards an anion by UV-vis spectrometry is measured by monitoring the change in absorbance of solution of the receptor in the presence of different concentration of the anion. Receptor concentrations in UV-visible spectrometry are typically on the order of 10^{-4} to 10^{-6} M, depending on the receptor's molar extinction coefficient. UV-visible spectrometry generally does not provide as much information, e.g. about the location of the binding site, as NMR spectrometry and at least one of the species – either the receptor or the anion – needs to absorb radiation in the UV-visible spectral region, i.e. between approximately 200 and 900 nm. In practice this means 250 to 900 nm because at low wavelengths the probability of spectral interferences is high. As a result, compounds with extensive π systems (e.g. aromatic rings) in their structures will more readily be observed by UV-visible spectrometry. Because of the possibility to work with dilute solutions $\log K_{ass}$ values up to 6–7 are measurable by UV-vis spectrometry.

2.5. Solvent influence on Host-Guest complexation equilibria

In the context of anion sensing, the polar solvents generally solvate H and G^- quite strongly, this is especially pronounced in the case of G^- because of its charge. When host-guest complex HG^- is formed in solution a number of solvent molecules previously solvating either H or G^- are freed. Although the formed HG^- is also solvated, the number of solvent molecules is generally smaller and because of the better charge delocalization the interactions are usually weaker. As a result, in broad terms, the higher is the polarity, especially the HB donicity, of the solvent the lower is the binding affinity: DCM > $CHCl_3$ > CH_3CN > DMSO > CH_3OH > H_2O.[70,71]

In order to be able to bind the anion in polar solvent the receptor-anion interaction has to be sufficiently intense to compensate for the loss of solvation. The most common way of achieving this is by formation of multiple hydrogen bonds between H and G^-, but other interactions, such as charge-charge and solvophobic interaction can also be important. In very broad terms, charged receptors tend to have stronger interactions with anions. This advantage is partially offset by the stronger solvation of charged receptors by solvent molecules.

Pure water is the most interesting and at the same time the most challenging medium for anion binding, because it is a highly competing solvent. Careful design of the receptor is needed and their interactions besides HB, such as the hydrophobic effect, are important. In spite of the efforts, synthetic anion receptors that have high affinity in pure water remain relatively underrepresented in the supramolecular literature.

DMSO acts as a both electron-pair donor and hydrogen bond acceptor by virtue of oxygen and sulfur lone pair. DMSO with a small amount of water added is a highly polar solvent, which at the same time dissolves many receptors well and is not too strongly competing. This has been the reason why DMSO, containing 0.5% of water by mass, is used for the majority of the measurements in this work.

3. INSTRUMENTS AND CHEMICALS

Instruments

All the synthesized molecules were characterized on a Bruker Avance II 400 NMR spectrometer or a Bruker Avance II 700 NMR spectrometer. Binding NMR measurements were carried out on the same NMR instruments. Binding UV-vis measurements were carried out on a Thermo Nicolet Evolution 300 double-beam spectrophotometer. Melting points were determined using a BÜCHI B-540 instrument in open capillaries and are uncorrected. The water content of the DMSO and DMSO-d_6 was measured with a Mettler Toledo DL 32 coulometric KF titrator. High resolution mass spectra of all the synthesized molecules were obtained on a Varian 910 FT-ICR mass spectrometer. For ionization ESI or APCI source was used as described in I, II and III (the HRMS measurements were carried out by Tõiv Haljasorg or Charly Mayeux). Purification of the synthesized compounds was carried out by column chromatography on silica gel (pore size 60 Å, 230−400 mesh). Thin layer chromatography (TLC) was conducted on TLC plates (silica gel 60 with fluorescent UV_{254} marker on aluminum sheets).

Solvents and Chemicals

Chemicals used for synthesis were from Sigma-Aldrich, Alfa Aesar or Fluka. The solvents used for synthesis were from Sigma Aldrich, Rathburn, Romil or Lach-Ner. For synthesis dry THF was prepared from the Romil, 99.9% solvent using the VAC (Vacuum Atmospheres Company Inc.) solvent purifier, which utilizes continuous circulation of the solvent through a column filled with alumina and delivers solvent with water content less than 5 ppm according to coulometric Karl-Fisher titration. The solvent is eventually delivered inside a glove-box. DCM, DMF, DMSO, toluene and acetone were dried as described in ref 73. The solvents for binding measurements (DMSO with 0.5% of water) was prepared using anhydrous DMSO 99.9% (for UV-vis measurements) or DMSO-d_6 99.8% (for NMR measurements) and water from MilliQ Advantage A10 system. The original water content in the solvent (as determined by the coulometric KF method) was taken into account when calculating the amount of water to be added to the solvent. The final water content of the solvent varied somewhat between batches (as determined by the coulometric KF titration method) but was always between 0.45% and 0.55%. Titrant solutions were prepared from tetrabutylammonium (TBA) acetate (Sigma-Aldrich), TBA benzoate (Sigma-Aldrich 99%), TBA lactate and TBA trimethylacetate salts were prepared by Kristjan Haav as described in III. The following commercially available receptor molecules were used: 3,4,4′-Cl_3-diphenylurea (Sigma-Aldrich, 99%), 1,3-diphenylurea **(**Sigma-Aldrich, 98 %). Receptors **12** and **14** were obtained from the group of Philip Gale.[72] The synthesis of all the remaining receptors has been described either in papers I, II or III.

4. MEASUREMENT METHODS OF BINDING CONSTANTS

Measurement of relative binding affinity by the NMR method

All the relative measurements were carried on 200 MHz or 700 MHz NMR instruments at 25 °C under fast exchange condition as described in ref II and III. DMSO-d_6 with 0.5% of water was chosen as the solvent for binding affinity measurements. The reasons for this solvent choice were the following: (1) DMSO is a sufficiently polar solvent so that ionic equilibria can be easily investigated; (2) Out of the polar solvents DMSO is solubility-wise one of the best solvents for dissolving the receptors investigated in this work; (3) The 0.5% of water was added for the sake of reproducibility of water content in the solvent (if only trace-level water is present in the solvent then there is a danger for large variability of the results depending on the solvent batch and (4) This solvent composition has been extensively used by other authors.

Measuring *relative* binding affinity of two hosts H_1 and H_2 towards the same guest G is described by Equation (3). Importantly, all the species are dissolved in the same solution. The relative binding affinity is expressed by $\Delta\log K_{ass}$ defined in Equation (4).

$$H_1 + H_2G \xrightleftharpoons{\Delta\log K_{ass}} H_1G + H_2 \tag{3}$$

$$\Delta \log K_{ass} = \log K_{ass}(H_1G) - \log K_{ass}(H_2G) = \log \frac{a_{H_1G}\, a_{H_2}}{a_{H_2G}\, a_{H_1}} \tag{4}$$

From equations (3) and (4) it can be seen that the activity of the guest has been eliminated from calculations. This means that the possible side-processes involving the anionic guest, e.g. ion-pairing and homoconjugation, influence binding to both hosts simultaneously, cancel out and thus do not affect the measurement result. The activities of the free and bound hosts enter equation (4) as ratios. Thus, possible effects affecting the hosts also largely cancel and the composition of the solvent is automatically identical for both hosts.

A reasonable assumption to make is that the ratios of activity coefficients of $\gamma(H_x)/\gamma(H_xG)$ are similar for both host molecules.[75,76] As a result the activities in Equation (4) can be replaced with equilibrium concentrations:

$$\Delta \log K_{ass} = \log K_{ass}(H_1G) - \log K_{ass}(H_2G) = \log \frac{[H_1G][H_2]}{[H_2G][H_1]} \tag{5}$$

Because many sources of error cancel with relative binding affinity measurements it is possible to obtain highly accurate results. The proposed method has been extensively used for relative acidity and basicity measurements

(determination of pK_a values) in non-aqueous media,[75,76] and also competition experiments.[77]

The degree of complexation of the anion by the receptor was calculated for every titration point by the following equation.

$$\alpha = \frac{[R_x]}{[R_x]+[R_xA^-]} = \frac{\delta - \delta_{R_x}}{\delta_{R_xA^-} - \delta_{R_x}} \quad (6)$$

Where, δ is the chemical shift of the monitored proton at the respective titration step, δ_{R_x} and $\delta_{R_xA^-}$ are the chemical shifts of the same proton in the free receptor molecule and receptor-anion complex, respectively. By replacing the equilibrium concentrations in equation (5) with the degrees of association H_1 and H_2 (α_1 and α_2) gives the following equation $\Delta\log K_{ass}$

$$\Delta\log K_{ass} = \log\frac{\alpha_1(1-\alpha_2)}{(1-\alpha_1)\alpha_2} \quad (7)$$

The receptors which were measured against each other were chosen according to the chemical shift of the NH protons, avoiding strongly overlapping protons. In some cases the overlap of chemical shifts can be tolerated, if the chemical shifts of the two receptors change in different ways during the titration. The stock solutions of the receptors were prepared in DMSO-d_6 with 0.5 % water (m/m). The concentrations of receptor molecules in the stock solutions were in the range of 0.006–0.009 M. The concentrations of the anionic titrant (TBA lactate, TBA benzoate, TBA acetate, TBA trimethylacetate) solutions were in the range of 0.17 M – 0.44 M and 0.65 M – 1.41 M, for diluted and concentrated titrant solutions, respectively. Initially, dilute titrant solution was used to obtain the association degrees at different levels of anion concentration. Near the titration endpoint concentrated titrant was added, so that eventually virtually all receptor molecules in solution were converted to receptor-anion complexes (9 to 10 equivalents of titrant was eventually added). In each measurement series 12–20 spectra were recorded. The relative binding measurements using NMR were done by the author, except 20-25 relative binding measurements for lactate, benzoate and trimethylacetate, which were done by Kerli Martin.

Measurement of absolute binding affinity by NMR

The solvent and working conditions used for NMR measurements of absolute binding constants were the same as in measurements of relative binding constants. The receptor concentrations were similar to the ones used in measurements of relative binding constants. The concentration of TBA anions in the dilute solution was in the range of 0.15 M – 0.5 M and in concentrated solutions

in the range of 0.6 M – 1.4 M. Over the course of titration 12–17 spectra were recorded. The spectrum of the free receptor was obtained before the first addition of titrant. The spectrum of the receptor-anion complex was obtained at the end of titration by adding a large excess (9–10 equivalents) of titrant. From the weighing data the exact amounts of added titrant were found. The dissociation degree of the complex is expressed through α in equation 6. Three methods were used for calculation of absolute binding affinity. The assigned binding constant values for each run were averaged from the results of the three calculation methods taking into account their internal consistency.

The calculation from every individual titration point. The amount of free anion added and the concentration of the free anion in each titration point were found from titration data. Equation 2 was modified to obtain the equation for finding the logK_{ass} values:

$$\log K_{ass} = \log \frac{(1-\alpha)\cdot \gamma_{RHA^-}}{\alpha \cdot [A^-] \cdot \gamma_{A^-}} \tag{8}$$

γ_{RHA^-} and γ_{A^-} are the activity coefficients of receptor-anion complex and the anion of interest respectively. The activity coefficients were calculated according to the Debye-Hückel equation[82], which for dimethyl sulfoxide reads as follows:

$$\log\gamma = -\frac{1.11 z^2 \sqrt{I}}{1 + 0.43 a \sqrt{I}} \tag{9}$$

Where I is the ionic strength, z is the ion charge and a is the effective radius of the ion. This equation is only a crude approximation under the conditions of our work because the used DMSO contains a considerable amount of water. Also, the ionic species in this work are not really spherical (which is an assumption of the Debye-Hückel theory).

Least squares fitting of the isotherm, without linearization. Based on Equations (2) and (6) it is possible to obtain the following equation of the binding isotherm:

$$\Delta\delta = \Delta\delta_{max} \frac{K_{ass} \cdot \frac{[A^-]\gamma_{A^-}}{\gamma_{RHA^-}}}{1 + K_{ass} \cdot \frac{[A^-]\gamma_{A^-}}{\gamma_{RHA^-}}} \tag{10}$$

K_{ass} was found by fitting the experimental data to this isotherm using the least squares approach and taking $\Delta\delta_{max}$ (equal to $\delta_{RHA^-} - \delta_{RH}$) and K_{ass} as adjustable parameters.

Least squares fitting of the isotherm, with linearization, The Equation 10 was linearized as described by Benesi and Hildebrand [81] to arrive at the following Equation:

$$\frac{1}{\delta^{\lambda}-\delta_{\mathrm{RH}}^{\lambda}}=\frac{\gamma_{\mathrm{RHA^-}}}{K_{ass}(\delta_{\mathrm{RHA^-}}-\delta_{\mathrm{RH}})[\mathrm{A^-}]\gamma_{\mathrm{A^-}}}+\frac{1}{\delta_{\mathrm{RHA^-}}-\delta_{\mathrm{RH}}} \tag{11}$$

By plotting $\frac{1}{\delta^{\lambda}-\delta_{\mathrm{RH}}^{\lambda}}$ vs $\frac{\gamma_{\mathrm{RHA^-}}}{[\mathrm{A^-}]\gamma_{\mathrm{A^-}}}$ K_{ass} can be found from the slope. $\delta_{\mathrm{R_x}}$ is the chemical shift of the non-bound receptor, $\delta_{\mathrm{R_xA^-}}$ is chemical shift of the receptor-anion complex and δ is the absorbance of a solution containing both the free receptor and receptor-anion complex. The absolute binding affinity measurements by NMR were done by the author.

Measurement of absolute binding affinity UV-vis Method

Stock solutions of receptor molecules were prepared by weighing (1–3) mg of the respective compounds and dissolving it in (1–3) ml of 0.5% H_2O:DMSO solvent. Additional dilution was made by transferring (0.1…0.2) g of the initial stock solution to 2 ml vial and diluting it with (1–1.5) ml of 0.5% H_2O:DMSO solvents. The final concentration of the solutions were around (10^{-4}–10^{-5}) M. The concentration of TBA salts in the concentrated solutions was approximately 0.07 M and in diluted solutions approximately (1.5×10^{-3}…2×10^{-3}) M in the case of logK_{ass} value measurement. During titration the spectrophotometric cell was weighed before and after each titrant addition. Over the course of titration around 12–17 spectra were recorded. The spectrum of the free receptor was obtained before the first titrant addition. The spectrum of the receptor-anion complex was obtained as the last stage of titration, after adding a large amount of titrant. From the weighing data the exact amounts of added titrant were found. The dissociation degree of the complex is expressed through α:

$$\alpha=\frac{[\mathrm{RH}]}{[\mathrm{RH}]+[\mathrm{RHA^-}]}=\frac{A^{\lambda}-A_{\mathrm{RHA^-}}^{\lambda}}{A_{\mathrm{RH}}^{\lambda}-A_{\mathrm{RHA^-}}^{\lambda}} \tag{12}$$

Where, A^{λ} is absorbance at the titration step, and $A_{\mathrm{RH}}^{\lambda}$ and $A_{\mathrm{RHA^-}}^{\lambda}$ are the absorbances of free receptor and receptor-anion complex accordingly. The same three methods (above mentioned) were used for calculation of the binding constants. The assigned values for each run were averaged from the results of the three calculation methods taking into account their internal consistency. The absolute binding for UV-vis and fluorescence measurements were done by Kerli Martin.

5. RESULTS

Synthesis of molecular receptors

In very broad terms the two most used (neutral) HB-donating structural fragments for binding anions are amides (especially ureas) and pyrrole derivatives (pyrrole, indole, carbazole). For efficient binding usually multiple HBs are needed and therefore different hybrid molecules, such as indolocarbazole and its derivatives, hybrids of 1,2-phenylene diamines and urea, bis-carbazolyl-urea, etc have been developed.

One of the objectives of this study was to synthesize different families of receptors including indolocarbazole, urea, thiourea, o-phenylenediamine-urea, indole, carbazole as well as other moieties as building blocks. Urea and indolocarbazole were chosen as the main binding sites in this work. Both have two HB donor sites. Urea's are conformationally flexible and inevitably have HB acceptor sites. These two properties together often lead to formation of intramolecular hydrogen bonds and unfavorable orientation of NH groups in the unbound receptors, At the same time urea fragments are easy to synthesize. Indolocarbazole in contrast is rigid and devoid of HB acceptor sites, so that intramolecular HBs cannot form. At the same time the indolocarbazole moiety is more difficult for synthesis.

Urea and thiourea receptors were easily synthesized using different substituted anilines reacting with phenyl isocyanate or phenyl isothiocyanate. Synthesis of unsubstituted indolocarbazole proceeds from phenylhydrazine reacting with 1,2-cyclohexanedione in acidic medium. Substituted indolocarbazoles were prepared in similar way starting from substituted phenylhydrazine. For 2,9-, 2,7- or 4,7- disubstituted indolocarbazoles different 3-substituted phenylhydrazine was used. For preparation of 1,10- or 3,8-disubstituted indolocarbazoles 2- or 4-substituted phenylhydrazines, respectively, were used.

The main purpose for preparation of a large number of different receptor molecules was to apply them for compiling anion binding scales with the relative binding measurement method using UV-vis or NMR technique.

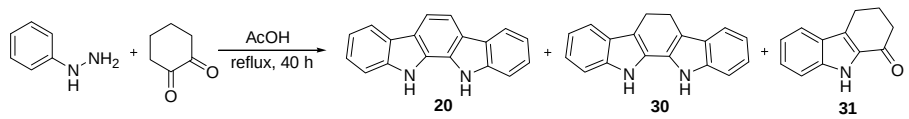
Indolocarbazoles

The preparation of indolocarbazole compounds starts from substituted phenylhydrazine and cyclohexane-1,2-dione,[39] which are refluxed in acidic medium (acetic acid, Conc. HCl, Conc. H_2SO_4 etc) in different protic solvents ethanol, butanol, propanol etc. Scheme 1 demonstrates the synthesis of unsubstituted indolocarbazole **20**, the 5,6-dihydroindolocarbazole (**30**) and the compound **31** (2,3,4,9-tetrahydrocarbazol-1-one). This compound is important for synthesis of unsymmetrical indolocarbazoles and by controlling the reaction conditions either **20** or **31** can be the main product. A similar reaction Scheme 2 is used for synthesis of disubstituted indolocarbazoles. Monosubstituted indolocarbazoles are obtained from **31** and a suitable substituted phenylhydrazine according to Scheme 3.

In literature mostly 2- and 4-substituted phenylhydrazines have been used because single structural isomers are obtained. We were interested in 2- and/or 9-substituted indolocarbazoles and we therefore used mostly 3-substituted phenylhydrazines. As a result, either two (in the case of monosubstituted indolocarbazoles) or three (in the case of disubstituted indolocarbazoles) structural isomers were obtained.

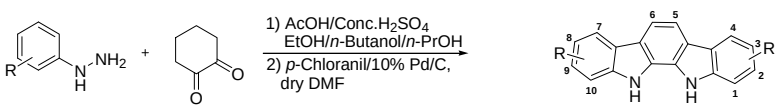
As shown in Scheme 1 the unsubstituted indolocarbazole was prepared from phenylhydrazine and cyclohexane-1,2-dione, dissolved in acetic acid. The mixture was stirred at reflux temperature for 40 h, until the disappearance of the starting materials (monitored by TLC). The reaction mixture was cooled to room temperature, quenched with $NaHCO_3$ solution. The formed precipitate was extracted in ethyl acetate. The organic layer was concentrated under reduced pressure to obtain the crude product as brown solid. The crude product was separated by column chromatography (230–400 mesh silica, eluting with 5–10% ethyl acetate : hexane) into three different compounds **20**, **30** and **31** Scheme 1.

Scheme 1. Synthesis of indolocarbazole.



Slightly modified procedures were used for the synthesis of different mono and di-substituted indolocarbazoles **32–51** (Scheme 2 and Scheme 3). They were prepared from different 2- or 3-substituted (-OCH_3, -CH_3, -COOH, -Cl, -CF_3) phenylhydrazines reacted with 1,2-cyclohexanedione (disubstituted) or compound **31** (monosubstituted) by refluxing in acidic medium (acetic acid or conc. H_2SO_4) in different protic solvents (ethanol, propanol, butanol). In some cases substituted 5,6-dihydroindolocarbazoles were obtained from this step, which were dehydrogenated by 10% Pd/C or *p*-chloranil in dry toluene or DMF (**32–51**).

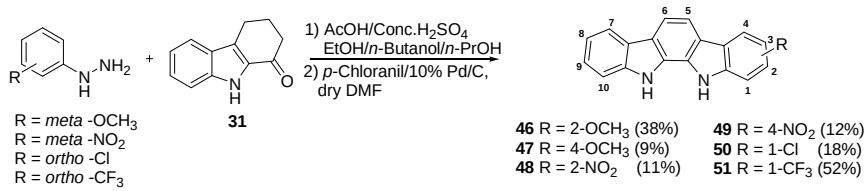
Scheme 2. Synthesis of di-substituted indolocarbazole molecules.



R = *meta* -OCH_3
R = *meta* -CH_3
R = *meta* -COOH
R = *meta* -Cl
R = *ortho* -Cl
R = *ortho* -CF_3

32 R = 2,9-$(OCH_3)_2$ (21%)
33 R = 2,7-$(OCH_3)_2$ (17%)
34 R = 4,7-$(OCH_3)_2$ (3%)
35 R = 2,9-$(CH_3)_2$ (36%)
36 R = 2,7-$(CH_3)_2$ (25%)
37 R = 4,7-$(CH_3)_2$ (7%)
38 R = 2,9-$(COOBu)_2$(18%)
39 R = 2,7-$(COOBu)_2$ (11%)
40 R = 4,7-$(COOBu)_2$ (12%)
41 R = 2,9-$(Cl)_2$ (16%)
42 R = 2,7-$(Cl)_2$ (39%)
43 R = 4,7-$(Cl)_2$ (22%)
44 R = 1,10-$(Cl)_2$ (59%)
45 R = 1,10-$(CF_3)_2$ (28%)

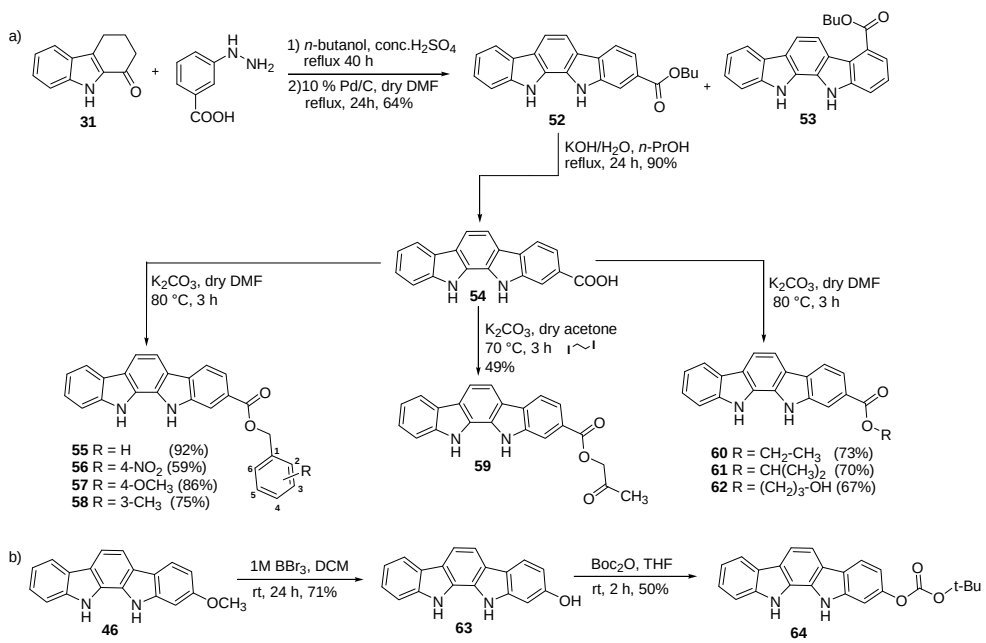
Scheme 3. Synthesis of mono-substituted indolocarbazole molecules.



During the synthesis of -NO_2 or -COOBu substituted indolocarbazole molecules it was observed that electron-withdrawing groups, like -NO_2 and -COOH need high-boiling solvents (i.e. higher temperature).

Scheme 4a presents the synthesis of different ester derivatives of mono-substituted indolocarbazoles (**52–62**). They were prepared from 3-carboxy-phenylhydrazine by reaction with **31** in *n*-butanol to form the butyl ester derivatives of compounds **52** and **53**. The compound **52** was hydrolyzed in KOH to form the acid **54**, which was thereafter coupled with different substituted benzyl halides or alkyl halides (bromomethylbenzene, 1-(bromomethyl)-4-nitrobenzene, 1-(chloromethyl)-4-methoxybenzene, 1-(chloromethyl)-3-methylbenzene, iodoethane, and 2-iodopropane) in K_2CO_3 to form compounds **55–62**.

Scheme 4. Synthesis of ester derivatives of indolocarbazoles.



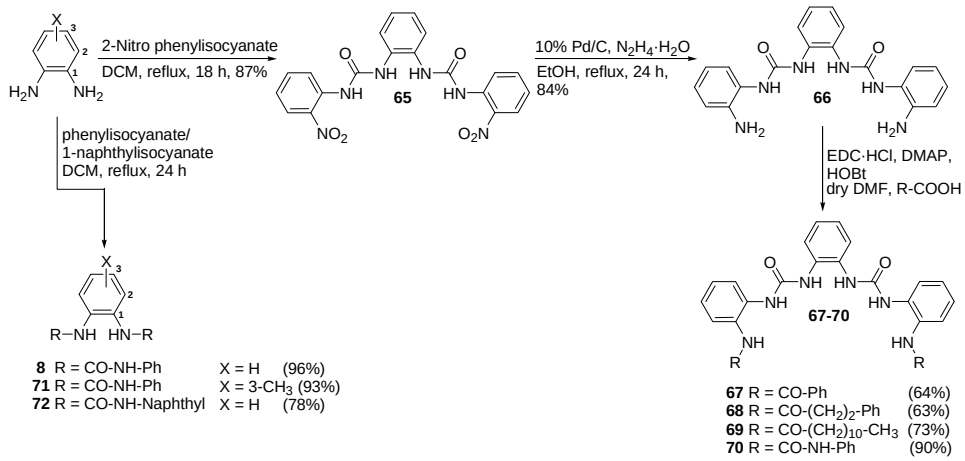
Scheme 4b presents synthesis of compound **64** via demethylating **46** by BBr_3 in dry DCM to form compound **63**, which was coupled with boc-anhydride.

Amide-based receptors

Many procedures have been used for the synthesis of ureas with aryl substituents.[39,58] The most common procedure used for preparation of urea receptors consists in reacting different substituted anilines with phenylisocyanate (which can also be substituted). Depending on the type of aniline different receptor families can be prepared.

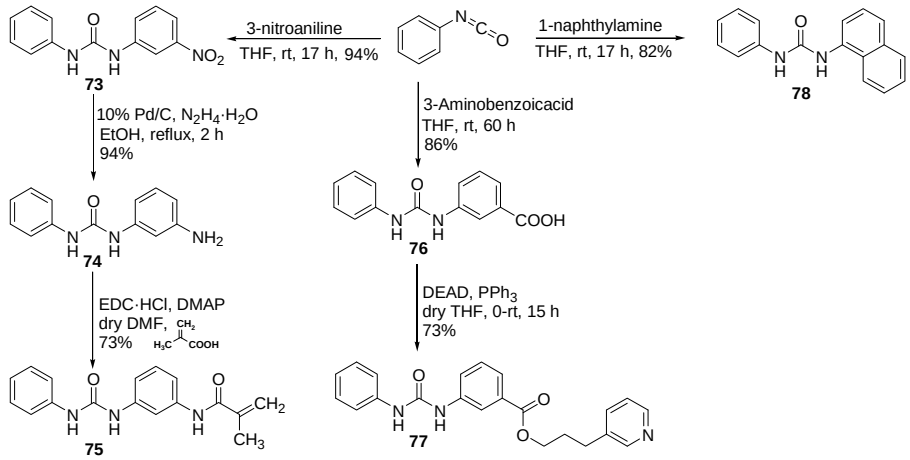
In the case of o-phenylenediamine as aniline, the receptors **67–70** were prepared by coupling with 2-nitro-phenylisocyanate to form compound **65**, which was then reduced with 10% Pd/C and hydrazine hydrate reflux in EtOH to form **66**. After that **66** was coupled with different carboxylic acids (benzoic acid, 3-phenylpropanoic acid, lauric acid) using the coupling reagent EDC·HCl and DMAP to form **67–69**. Compound **66** was also coupled with phenylisocyanate to obtain compound **70**.

Scheme 5. Synthesis of o-phenylenediamine-urea type molecules.



Compounds **8**, **71** and **72** were prepared from substituted o-phenylenediamine coupled with phenylisocyanate and 1-naphthylisocyanate in DCM as shown in Scheme 5.

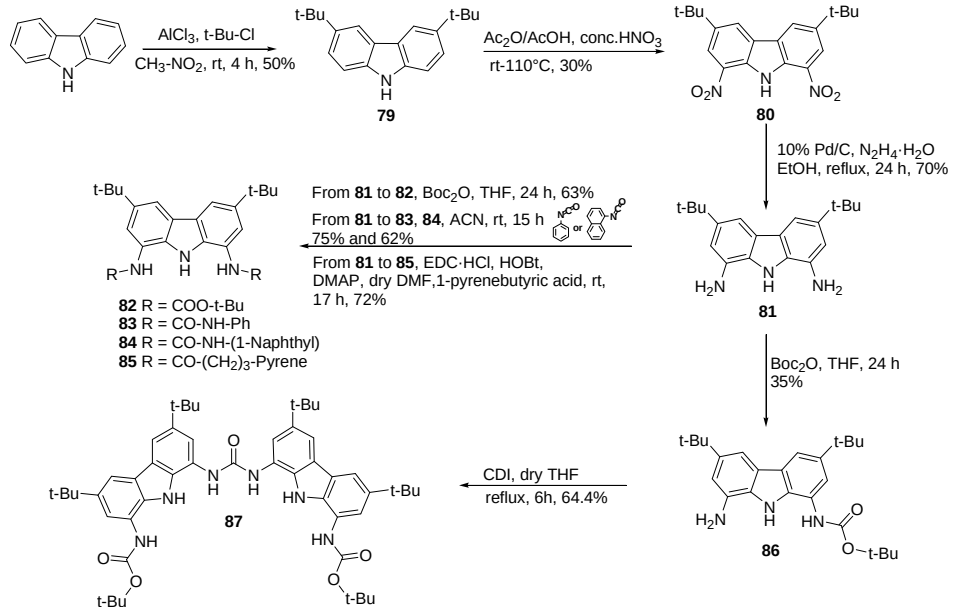
Scheme 6. Synthesis of different substituted urea derivatives.



Different substituted diphenyl urea derivatives **75**, **77** and **78** were prepared. The compound **75** was prepared from 3-nitroaniline reacted with phenylisocyanate to form **73** then reduced in 10% Pd/C and hydrazine hydrate to form **74**, then coupled with methacrylic acid using EDC·HCl and DMAP to form compound **75**. Compound **77** was prepared from 3-aminobenzoic acid reacted with phenylisocyanate in THF to form **76** which is coupled with 3-Pyridin-3-yl-propan-1-ol using DEAD and PPh_3 in dry THF to form compound **77**. Compound **78** was prepared from 1-naphthylamine reacted with phenylisocyanate in THF shown in Scheme 6.

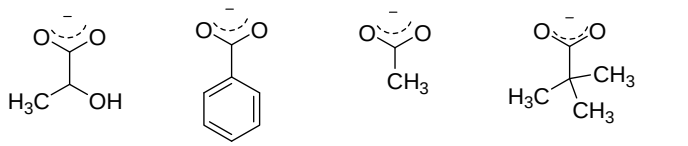
Scheme 7 presents the synthesis of carbazole derivatives **82–85**, **87**. They were prepared from carbazole via several steps. Firstly, carbazole was reacted with *tert*-butylchloride in the presence of $AlCl_3$ in nitromethane to form the compound **79**. This was followed by nitration using conc. HNO_3 to form **80**. Then nitro groups were reduced by 10% Pd/C and hydrazine hydrate reflux in EtOH to form compound **81**.

Scheme 7. Synthesis of different substituted carbazole molecules.

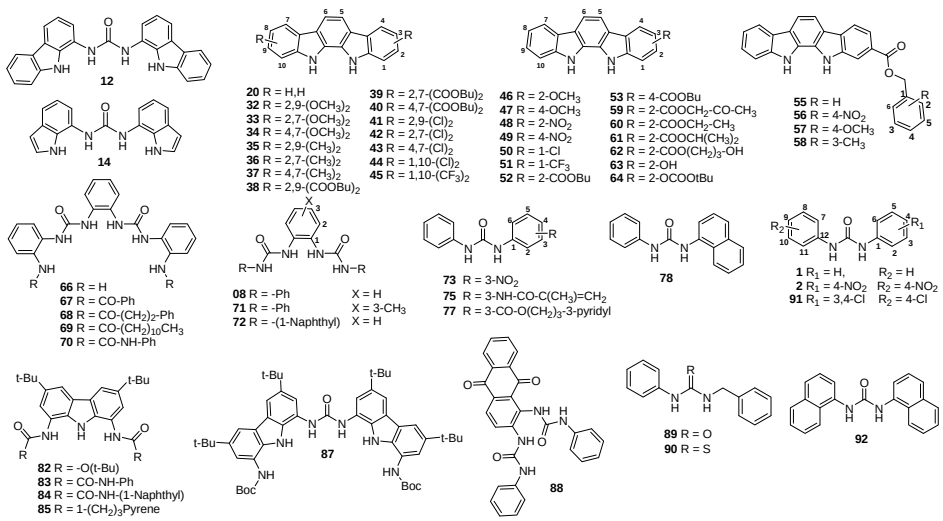


Compounds **82**–**85** were prepared from **81** using different reactants such as Boc_2O, phenylisocyanate, 1-naphthylisocyanate and 1-pyrenebutyric acid as shown in Scheme 7. For preparation of dicarbazolylurea derivatives one amino group in **81** was protected by boc-anhydride to form compounds **82** and **86**. The **86** was coupled with CDI to form the symmetrical boc-protected compound **87**. Compounds **12**,[46] **14**,[47] **88**,[78] **89**,[79] **90**[80], **92**[III] were prepared by literature methods.

Scheme 8. Structures of the investigated anions.



Scheme 9. All the receptors measured towards the lactate, benzoate, acetate and trimethylacetate anions.



Relative binding affinity measurement using NMR method

On the basis of the UV-vis spectrophotometric relative binding measurement method,[I] in this work the NMR-based relative binding affinity measurement method (described in detail in the measurement method section) was developed.[II,III]

It was discovered that while the previously developed UV-vis spectrometric method was able to measure the relative binding affinity between two receptors with consistency parameter 0.04 log units, the NMR method offered significantly better consistency, 0.01 log units, and therefore much better accuracy. Furthermore, it enabled measuring a number of receptors in one solution (still maintaining the excellent consistency). Relative binding ($\Delta\log K_{ass}$ values) between three or four receptors (Figure 10) is routinely measurable, leading to 3 or 6 $\Delta\log K_{ass}$ values, respectively from one measurement series. In one case it was possible to measure the relative binding affinity combinations between six receptors simultaneously, leading to 15 $\Delta\log K_{ass}$ values from a single measure-

ment series! In the NMR method the binding process of each receptor towards the guest can be followed separately from the proton chemical shift of the respective HBD groups, which is not possible by the UV-vis method. As a further advantage of the NMR method, it can give additional information on the investigated process and possible side-processes. It can show whether the anion partially deprotonates the receptor or whether an aromatic CH proton interacts with the anion via a weak hydrogen bond. The NMR technique (although not in the relative measurement mode) can also give information about the orientation of receptor-anion in solution (using the NOESY technique). In addition, differently from the absolute NMR method it is possible to measure very high binding affinities with the relative method.

For the above described reasons it was first of all the NMR method that was used in the subsequent large-scale binding studies with the four carboxylate anions (lactate, benzoate, acetate, trimethylacetate) in DMSO-d_6:H_2O (99.5%:0.5% m/m).

Table 1 presents an example of the so-called binding affinity scale ("ladder") composed of in total 89 relative binding affinity measurements of benzoate anion with 38 receptors. The resulting relative binding affinity scale of benzoate spans for 2 orders of magnitude. Each double-headed arrow in the scale corresponds to a measured difference in absolute binding affinity between two receptor molecules in logarithmic scale expressed as $\Delta\log K_{ass}$ values. Each additional measurement contributes to the circular validation[74] of the whole scale. The absolute binding affinities of the receptors on the scale were obtained by a least squares procedure, by minimizing the sum of the squares of the differences between directly measured $\Delta\log K_{ass}$ values and the differences between the respective assigned $\log K_{ass}$ values, which is denoted as *SS* in the following equation:[76]

$$SS = \sum_{i=1}^{n_m} \left\{ \Delta\log K_{ass}^{i} - \left[\log K_{ass}\left(\mathrm{H_y G}\right) - \log K_{ass}\left(\mathrm{H_x G}\right) \right] \right\}^2 \qquad (13)$$

Each $\Delta\log K_{ass}^{i}$ value corresponds to a directly measured relative binding affinity of the receptors H_y and H_x. The absolute $\log K_{ass}$ values were anchored to the $\log K_{ass}$ values of indolocarbazole (**20**) measured separately using the absolute measurement method (see the Table 3). The internal consistency of the assigned absolute $\log K_{ass}$ values with the measured $\Delta\log K_{ass}$ values was evaluated by the consistency standard deviation (consistency parameter) of the scale *s*,[76] which is found according to the following equation:

$$s = \sqrt{\frac{SS}{n_m - n_c}} \qquad (14)$$

Table 1. Relative binding scale of benzoate in DMSO (d_6):H_2O (99.5%:0.5% m/m)

Receptor number	logK_{ass}	u_c[a]	u_c[b]	ΔlogK_{ass}
1,3-dicarbazolylurea **12**	4.08	0.01	0.04	
1,3-diindolylurea **14**	4.01	0.01	0.04	
Receptor **87**	3.96	0.03	0.05	
Receptor **84**	3.88	0.01	0.04	
Receptor **91**	3.53	0.01	0.04	
1-(3-NO_2-phenyl)-3-phenylurea **73**	3.34	0.01	0.04	
Receptor **69**	3.27	0.01	0.04	
Receptor **72**	3.25	0.01	0.04	
4-NO_2-indolocarbazole **49**	3.22	0.01	0.04	
2,7-(BuOCO)$_2$ indolocarbazole **39**	3.18	0.01	0.04	
Receptor **68**	3.18	0.01	0.04	
2,9-(BuOCO)$_2$-indolocarbazole **38**	3.17	0.01	0.04	
4,7-(BuOCO)$_2$ indolocarbazole **40**	3.16	0.01	0.04	
Receptor **8**	3.11	0.01	0.04	
Receptor **77**	3.08	0.01	0.04	
2,7-Cl_2-indolocarbazole **42**	3.07	0.01	0.04	
Receptor **66**	3.00	0.02	0.04	
Receptor **71**	2.99	0.01	0.04	
Receptor **59**	2.98	0.01	0.04	
Receptor **85**	2.96	0.01	0.04	
Receptor **52**	2.95	0.01	0.04	
Receptor **62**	2.94	0.01	0.04	
Receptor **55**	2.93	0.01	0.04	
Receptor **56**	2.91	0.01	0.04	
Receptor **67**	2.89	0.01	0.04	
1,3-diphenylurea **1**	2.82	0.01	0.04	
Receptor **64**	2.76	0.01	0.04	
Receptor **75**	2.73	0.01	0.04	
Indolocarbazole 20	**2.70**	**0.01**	0.04	
Receptor **63**	2.63	0.01	0.04	
2,9-(MeO)$_2$-indolocarbazole **32**	2.63	0.01	0.04	
Receptor **88**	2.49	0.01	0.04	
1-Cl-indolocarbazole **50**	2.48	0.01	0.04	
1-Naphthalen-1-yl-3-phenyl-urea **78**	2.44	0.01	0.04	
1-Benzyl-3-phenyl-thiourea **90**	2.31	0.01	0.04	
Receptor **82**	2.27	0.01	0.04	
1,3-Di-naphthalen-1-yl-urea **92**	2.11	0.01	0.04	
1-Benzyl-3-phenyl-urea **89**	2.08	0.01	0.04	

[a]Solvent: DMSO-d_6:H_2O (99.5%:0.5% m/m), in all cases 1:1 stoichiometry. [b] Standard uncertainties for comparing logK_{ass} values on the scale. [c] Standard uncertainties for comparing logK_{ass} values with those from other research groups (see II and III for details).

Where n_m = 89 is the number of $\Delta\log K_{ass}$ measurements and n_c = 38 is the number of absolute $\log K_{ass}$ values that were determined. The *s* value of the NMR results was 0.01, which means high consistency of the results and good agreement between obtained data. The high consistency of the results enables differentiating between receptors with binding strength difference less than 0.05 $\log K_{ass}$ unit. This differentiating ability is significantly better than in the case of absolute binding measurement.

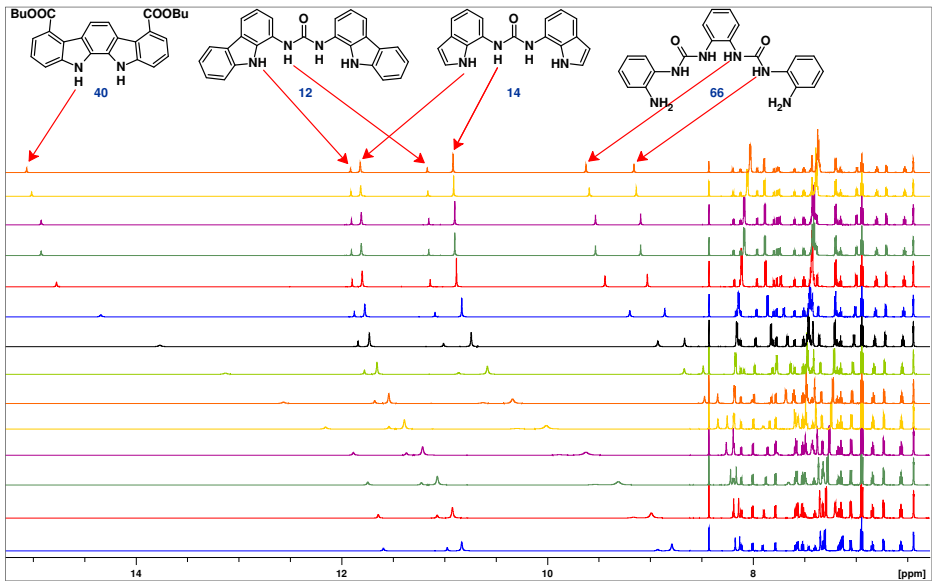


Figure 10. ^{1}H NMR spectra of relative binding affinity measurement between receptors **12**, **14**, **40** and **66** in DMSO-d_6:H_2O (99.5%:0.5% m/m) with benzoate. Titration proceeds from bottom to top. The bottom-most spectrum corresponds to solution without titrant added.

As a demonstration the method's capability of measuring multiple $\Delta\log K_{ass}$ values simultaneously, Figure 10 demonstrates the measurement of relative binding affinity between four different receptors (indolocarbazole, 1,3-dicarbazolylurea, 1,3-diindolylurea, o-phenylene-urea) **12**, **14**, **40** and **66** for benzoate anion in the same solution. In the case of all receptors their NH protons were deshielded upon addition of the benzoate anion. The relative binding affinity difference can be calculated according to equation 7. The ^{1}H NMR spectrum on the bottom of the figure corresponds to the mixture of unbound receptors. Upon the addition of the benzoate anion each receptor's NH protons' chemical shifts increase (i.e. move downfield). Generally, the higher is the binding affinity of a receptor the bigger are the (relative) shifts of the NH protons in the NMR spectrum upon addition of the first portions of the titrant. The Figure 10 demonstrates the receptor **12** has two different NH proton

signals, which exhibit the strongest shifts upon the first additions of benzoate anion. The overall shifts of chemical shift ($\Delta\delta_{max}$) observed for each receptor were the following: **12** ($\Delta\delta$ = 0.95 & 2.25 ppm), **14** ($\Delta\delta$ = 1.49 & 1.06 ppm), **66** ($\Delta\delta$ = 3.5 ppm) and **40** ($\Delta\delta$ = 0.99 & 2.14 ppm). As a result of calculations the binding affinity differences in the receptor pairs **12** and **14**, **12** and **66**, **12** and **40**, **14** and **66**, **14** and **40**, **66** and **40** were found as 0.07, 0.95, 1.06, 0.85, 0.99 and 0.20 respectively. It is observed that receptors **12** and **14** have distinctly higher binding affinity towards the benzoate anion, than **40** and **66**.

Separate ^{1}H NMR measurements with receptors **12** and **14** (in separate solutions) towards the trimethylacetate anion indicated that in receptors **12** and **14** the aromatic CH protons located in position 2 relative to the urea NH group (proton H-3 in Figure 11) are slightly deshielded due to the different structural conformers.[52] The receptor **14** was reported to have three different conformations (syn-syn, anti-syn and anti-anti) with acetate anion in solution.[52] The anti-anti conformation is the most stable one for the free receptor and for the complex the syn-syn conformation is the most stable. The same interaction was observed in the case of receptor **12** with trimethylacetate anion. These observations were confirmed by computations as described in III.

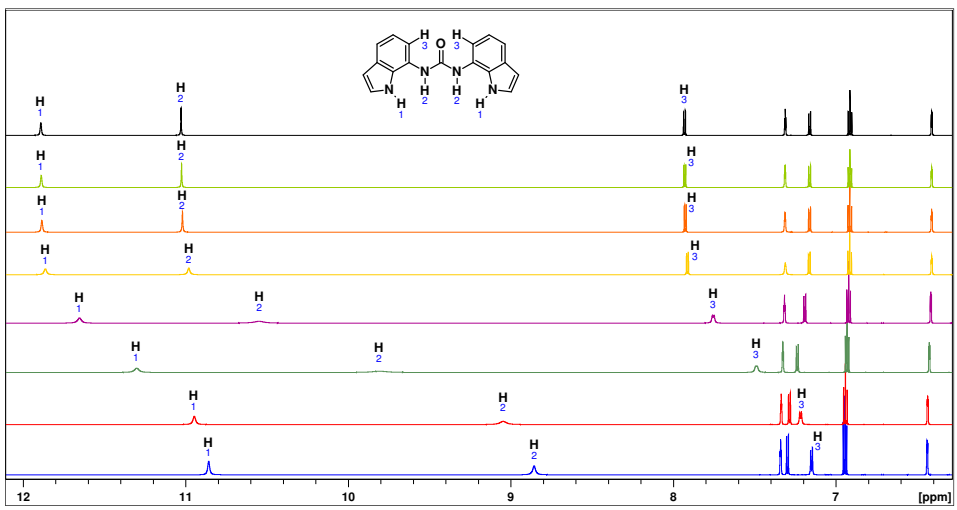


Figure 11. Receptor **14**, ^{1}H NMR spectra towards the trimethylacetate anion in DMSO-d_6:H_2O (99.5%:0.5% m/m), Titration proceeds from bottom to top.

The same relative binding measurement method was applied for lactate, acetate and trimethyl acetate anions.[III] A number of relative binding affinity measurements have been performed: 77, 125 and 86 towards lactate, acetate and trimethyacetate anion, respectively. The binding affinity measurement results are given in table 2. Each scale is anchored to the absolute logK_{ass} value of the respective anion to indolocarbazole (**20**).

Table 2. Binding constants of carboxylate anions in H_2O: DMSO-d_6 (99.5%:0.5% m/m) at 25 °C

Receptor	Lactate anion			Benzoate anion			Acetate anion			Trimethylacetate anion		
	logK_{ass}	u_c^b	u_c^c	logK_{ass}	u_c^b	u_c^c	logK_{ass}	u_c^b	u_c^c	logK_{ass}	u_c^b	u_c^c
Receptor **84**	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 **12**	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 **14**	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 **87**	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 **91**	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 **73**	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 **49**	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 **69**	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 **40**	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 **08**	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 **39**	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 **38**	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 **42**	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 **71**	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 **68**	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 **67**	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 **77**	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 **56**	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 **59**	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 **52**	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 **66**	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 **55**	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 **73**	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 **62**	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 **1**	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 **75**	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 **64**	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 20	**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 **32**	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 **85**	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 **63**	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 **88**	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 **78**	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 **50**	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 **90**	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 **82**	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 **89**	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 **92**	1.62	0.01	0.05	2.11	0.01	0.05	2.45	0.01	0.09	2.72	0.01	0.06

[b] Standard uncertainties for comparing logK_{ass} values within the same scale. [c] Standard uncertainties for comparing logK_{ass} values between different scales or with those from other research groups (see II and III for details).

Absolute binding measurements

All the relative binding measurements ladders were anchored to absolute logK_{ass} values of indolocarbazole (**20**). In order to improve accuracy, for each anion the logK_{ass} value was measured on different days and with most anions different techniques (NMR, UV-vis, and in one case fluorescence) were used. The binding affinity scale of acetate anion was anchored to three different receptors **20**, **40** and **44**. Very good consistency between the absolute and relative measurement results was observed in the case of these receptors. The logK_{ass} value of receptor **20** with lactate was obtained using the UV-vis method and logK_{ass} values of benzoate was obtained using NMR, UV-vis and fluorescence method, for acetate and trimethylacetate the logK_{ass} value were obtained with using both 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 II and III for details). The results of the measurements are presented in Table 3.

Table 3. Results of absolute logK_{ass} value measurements with indolocarbazole (**20**) in DMSO-d_6:H_2O (99.5%:0.5% m/m).

Anion	**Method**	**Abs logK_{ass}**	**s**[a]	**n**[a]	**Assigned logK_{ass}**[b]	**CI (95%)**[b]
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] Standard deviation of values from independent experiments and numbers of independent experiments (on different days). [b] Assigned logK_{ass} values and 95% confidence intervals of the assigned values.

6. DISCUSSION

General

In this work the binding affinity of four carboxylate anions – lactate, benzoate, acetate and trimethylacetate – towards different families of receptors was studied. The observed binding affinity first of all depends on the anion basicity. Table 2 and Figure 12 indicate that in broad terms the binding affinity follows the pK_a values of anions: the higher the pK_a value the stronger the binding affinity. It was observed that the binding affinity decreases in the row trimethylacetate ≥ acetate > benzoate > lactate. In very broad terms the receptors binding strongest to one anion also bind strongly to the three others. However, there are a number of occasions where binding order changes are observed. These small differences can be revealed thanks to the very high accuracy of the NMR-based relative binding measurement method. The most prominent binding affinity order changes are observed in the case of acetate and trimethylacetate anions. It is observed that besides anion basicity the binding affinity also depends on anion's steric demand.

Binding of carboxylates by different receptor families

With most of the indolocarbazole receptors **20**, **32–58** binding affinity order depends on the anion basicity towards the carboxylate anions, but in some cases (e.g. with indolocarbazole receptors **32**, **49**, **55–58**) the binding of acetate is roughly equal towards acetate and trimethylacetate. Figure 12 displays that receptors **32**, **49** and **55** bind acetate even slightly higher than trimethylacetate. However the uncertainties of logK_{ass} values of the anchor compounds are of the same order of magnitude as the differences, thus it is not possible to state this with confidence.

The most prominent binding affinity towards all four anions is displayed by receptors **12**, **14**, **84** and **87**. The high binding affinity is presumably caused by strong hydrogen bonding (facilitated by favourable orientation of the binding NH groups), modulated by solvophobic interaction and suitable binding pocket (cavity) configuration. It can be seen from Figure 12 that these receptors are able to discriminate to some extent between the carboxylate anions. Receptor **12** has the strongest binding affinity towards the benzoate and trimethylacetate anions compared to the rest of the receptors. The binding affinity difference of **12** between acetate and trimethylacetate anion is around 0.70 log units. At the same time in the case of receptor **87** this difference is only around 0.01 log units. Based on the computational geometry analysis the receptor **12** has a suitable cavity for accommodating trimethylacetate anion and partial shielding of its hydrophobic part from the polar solvent without introducing steric stain. Receptor **87**, according to computational geometry analysis it has a cavity, but it is considerably smaller, crowded by the substitutents. As a result, the binding of trimethylacetate anion is sterically hindered, but not the binding of acetate anion, which fits better due to its small size. The receptor **84** is relatively the

strongest lactate binder of all studied receptors. In the case of benzoate and trimethylacetate anions it is surpassed by receptors **12**, **14** and **87**. The computational geometries of the receptors show that the strong binding is caused by numerous suitably located hydrogen bond donor sites leading to tetradentate binding and suitable size of the pocket for fitting the different anions, so that a nearly planar structure with little steric strain is formed.

In the simple phenyl and/or naphthyl substituted urea receptors **1**, **78** and **92** it was found that binding affinity decreases when replacing the phenyl rings by naphthyl rings, although naphthyl rings are more electronegative than phenyl rings. Computational data indicate that this is caused by the steric strain forcing the naphthyl ring out of the plane when binding to the anion. Adding each naphthyl ring to **1** (to get **78** and **92**) decreases the logK_{ass} value by around 0.2–0.5 log units. The reversed situation is observed in the case of the tetradentate system o-phenylenediamine urea type receptors **8** and **72.** Replacing phenyl rings by naphthyl rings leads to binding affinity increase of around 0.2–0.3 log units. Examining the geometries of the receptors and the complexes reveals the reasons: (1) Even the non-complexed receptors are sterically strongly strained and binding the anion does not increase the strain markedly and (2) because of the larger size of the binding pocket the naphthyl rings do not come as close to the anions as in **78** and **92**.

It is interesting to compare the binding affinity differences between acetate and trimethylacetate anions. This difference is 0.06, 0.17 and 0.27 in the case of **1**, **78** and **92**, respectively. Increase of the relative affinity towards the more hydrophobic trimethylacetate anion with increasing the number of naphthyl rings indicates a small share of hydrophobic interaction in binding. Similar situation is observed when comparing binding affinities between acetate and benzoate anions.

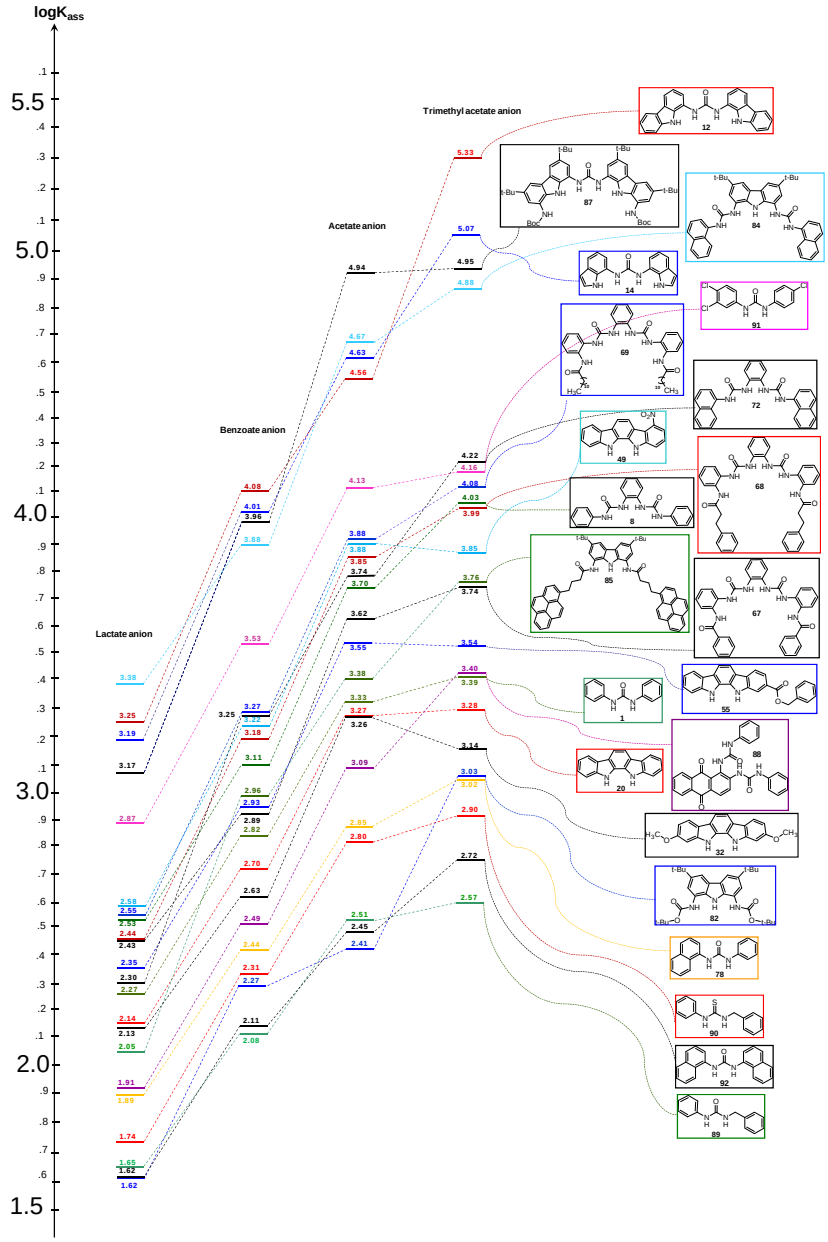


Figure 12. Comparison of the binding affinity of carboxylate anions towards selected receptors.

The binding affinities of the (1,8-disubstituted carbazole) type receptors **82**, **84** and **85** differ widely because of the different substituents. **84** is clearly the strongest binder of the three towards all carboxylate anions by around 2 orders

of magnitude. This is caused by five HB donor sites, which, according to computations all participate in binding. Interestingly, in the case of lactate anion one of the NH groups (near naphthyl ring) gives a hydrogen bond to the lactate OH group (which acts as HB acceptor). The receptors **82** and **85** are tridentate (three NH groups). The data suggest that these receptors have relatively strong binding affinity towards trimethylacetate and benzoate anions and relatively weaker binding affinity towards the acetate and lactate anions. Receptor **82** displays an interesting behaviour – it is the one where the $\log K_{ass}$ difference between acetate and benzoate is only 0.14 log units – the smallest of all receptors – and the difference between trimethylacetate and acetate is 0.62 log units (second highest). Thus, it has disproportionately low binding affinity towards acetate. The origin of this is not fully clear.

The o-phenylenediamine-diphenylurea combined receptors **67–69** have bulky and hydrophobic substituents in the second position of the terminal phenyl rings, linked by NHCO groups. The hydrophobic substituents can be oriented around the large hydrophobic parts of trimethylacetate and benzoate anions, in such way that hydrophobic/solvophobic interactions take place, The binding affinity differences between acetate to trimethylacetate are 0.1–0.2 log units, and between benzoate and acetate around 0.5–0.7 log units. The small difference between acetate and trimethylacetate around 0.1–0.2 log units reveals that either the hydrophobic interaction between trimethylacetate anion and the receptor is weak or the cavity is too small for the trimethylacetate anion, while at the same time being very suitable for the acetate anion.

In the context of the receptors and anions studied in this work the most important structural aspects for high binding affinity for carboxylate ions are the following:

- The negative charge in carboxylate anions is quite strongly localized on the carboxylate group and the HB interaction with it is the main binding force. It is therefore important that the HB donating groups of the receptor form strong HBs with the carboxylate group. The urea derivatives seem to have a slight advantage over indolocarbazole – diphenyl urea (**1**) binds all anions slightly stronger than indolocarbazole (**20**). However, this binding strength can be strongly modified with substituents. Inserting just one -NO_2 group in indolocarbazole (receptor **49**) increases its binding affinity to all anions by almost an order of magnitude. Similar effect is achieved on adding three -Cl groups to diphenyl urea (receptor **91**).
- It is critical that the HB donating NH bonds are oriented in a suitable way so that multiple HBs with the anion can indeed form. Both urea and indolocarbazole building blocks are very suitable in this respect. Out of the more complex receptors 1,3-diindolylurea (**14**) and 1,3-dicarbazolylurea (**12**) feature (in the receptor-anion complex) four suitably positioned NH bonds and four HBs are formed, leading to high binding affinity. At the same time in receptors based on 1,2-phenylenediamine (e.g. **72** and **69**) the two NH fragments attached to the phenyl ring suffer from steric repulsion and the

placement of the NH bonds in the complex is not optimal (they are twisted with respect to each other).

Substituent effects on binding affinity of indolocarbazole towards carboxylate anions

Binding affinity depends on the hydrogen bond donicity of the receptor. Introducing electron withdrawing groups into the receptor increases the positive partial charge on the NH proton and thereby also the binding affinity. The opposite holds for the electron donating groups. Table 2 enables comparing indolocarbazole (**20**) with substituted indolocarbazoles, both with electron-donating and electron-withdrawing groups. The receptors with electron-donating groups, such as -CH_3 and -OCH_3 (receptors **32–37**, **46–47),** display binding affinity almost equal to indolocarbazole (**20**). The receptors with electron withdrawing groups such as -Cl, -CF_3, -NO_2 and -COOBu (receptors **38–45**, **48–62**) including mono or disubstituted indolocarbazole receptors have binding affinities higher than **20** by around 0.3–0.8 log units. The binding affinity of indolocarbazoles disubstituted with -COOBu or -Cl groups (**38–43**) is almost equal to mono-NO_2-substituted indolocarbazole receptors **48** and **49**. In broad terms one -NO_2 group is as powerful as two -Cl and -COOBu groups because of the very strong electron-withdrawing ability of the NO_2 group. In the case of mono substitution by a -COO-spacer (receptors **52–58**) the binding affinity does not differ from that of -COOBu.

The position of the substituent in the indolocarbazole moiety is also important. In our case if the EWGs are in 1 and 10 positions of indolocarbazole (receptors **44** and **45**, -Cl and -CF_3 groups, respectively) then the binding with 2 NH groups is crowded, which hinders anion binding, so that instead of increasing the binding affinity decreases by around 1–1.5 log units compared to indolocarbazole (**20**). When replacing one -Cl in position 1 by -CF_3 then logK_{ass} with acetate decreases by 0.27 log units in DMSO-d_6:H_2O (99.5%:0.5% m/m). When replacing two -Cl substituents in 1 and 10 positions by -CF_3 groups then the binding affinity decreases by 0.46 log units.

Differences between logK_{ass} in 0.5% H_2O:ACN and 0.5% H_2O:DMSO

The binding affinity differences of simple receptors in 0.5%H_2O:ACN and 0.5% H_2O:DMSO were investigated on the example of substituted indolocarbazoles and two urea-based receptors Table 4. Generally the anion-binding ability of receptors becomes weaker upon increasing solvent polarity. The same is observed here – the logK_{ass} values in the more polar DMSO:H_2O mixture are lower by 1–1.5 orders of magnitude. The correlation between the logK_{ass} values in these two media (logK_{ass} in ACN against logK_{ass} in DMSO) is good, with R^2 = 0.96 and slope = 0.9. The slope value means that the DMSO:H_2O mixture has ca 10% better ability to differentiate between the binding affinities. Table 4 demonstrates that the difference of binding affinities between substituted indolocarbazoles in ACN:H_2O and DMSO:H_2O increases with increasing number of electron-withdrawing groups (EWGs) and thus also with increasing

positive partial charge on the NH groups. This can be interpreted as follows. As the number of EWGs increases, the HB donicity of the NH protons also increases, leading to stronger binding of anions. On the other hand, this also increases the binding efficiency of solvent molecules competing with anions. This effect is more pronounced with DMSO as DMSO is a stronger HB acceptor than ACN. Thus, the stronger is the receptor, the more of its binding strength is "taken away" by DMSO and the difference between the logK_{ass} values in ACN and DMSO increases.

Table 4. Binding affinity difference towards the acetate anion in ACN and DMSO.

Receptor	$logK_{ass}$		
	0.5 H_2O:DMSO	**0.5 H_2O:ACN**	**Difference**
1,3-diphenylurea **1**	3.33	4.28	0.95
Receptor **91**	4.13	5.20	1.07
Indolocarbazole 20	3.27	4.46	1.19
2,7-$(MeO)_2$-indolocarbazole **33**	3.26	4.46	1.20
2-MeO-indolocarbazole **46**	3.27	4.50	1.23
1-Cl-indolocarbazole **50**	2.89	4.24	1.35
4-NO_2-indolocarbazole **49**	3.88	5.24	1.36
2,7-Cl_2-indolocarbazole **42**	3.67	5.05	1.38
2-NO_2-indolocarbazole **48**	3.69	5.09	1.40
2,9-$(Cl)_2$-indolocarbazole **41**	3.52	4.95	1.43
4,7-$(Cl)_2$-indolocarbazole **43**	3.74	5.20	1.46
1,10-$(CF_3)_2$-indolocarbazole **45**	1.81	3.36	1.55
1,10-$(Cl)_2$-indolocarbazole **44**	2.27	3.84	1.57

SUMMARY

This thesis focuses on synthesis and accurate binding affinity measurement of molecular receptors towards carboxylate anions. The work is divided in two major sections: in the first section synthesis different receptor families (indolocarbazoles, urea, thiourea, carbazoles, amide based receptors and their combinations) is described. In the second section the measurements of the binding affinities of all the receptors towards carboxylate anions using the NMR based relative binding affinity measurement method are presented.

In the first section the synthesis of different substituted indolocarbazole receptors as well as receptors based on other binding moieties (urea's, thiourea, o-phenylene bis-urea, carbazole, indole) and/or their combinations is presented.

In the second section the relative binding affinity measurements method by NMR developed in this work is presented. By measuring the binding affinity differences instead of absolute binding affinities it is possible to obtain highly accurate results, because many sources of uncertainties (ion pairing, homoconjugation, deviation of the actual water content in solution from the nominal one, etc) cancel out (either fully or partially). The method was applied to the measurement of 38 synthetic receptors towards the acetate, benzoate, lactate and trimethylacetate anions in 0.5% H_2O:DMSO-d_6 with excellent consistency parameters of the order of 0.01. The obtained scales were anchored to directly measured the logK_{ass} values of indolocarbazole. As a result such binding scales for different anions can be constructed under the same experimental conditions which allow direct comparison of their binding affinity. The scales serve also as tools for determination of binding constants for new receptors. The results reveal that binding affinity depends on the anion basicity, anion size and properties of the receptor (number and donicity of the HB donor centres and their spatial positions in the receptor molecule).

The applicability of NMR method for measuring the relative binding measurement has been demonstrated and its advantages have been outlined: No need to measure the anion and receptor concentration, high accuracy (many error sources cancel), robustness, possibility to detect deprotonation and the possibility to measure up to six receptors in one solution with high consistency. The scales created in this work can be helpful as anchoring systems for future measurements of accurate binding affinities of newly developed receptors.

SUMMARY IN ESTONIAN

Anioonide retseptorid: Süntees ja seondumise täppismõõtmised

Käesolev töö keskendub karboksülaatanioonide molekulaarretseptorite sünteesile ja seondumise mõõtmistele. Töö on jagatud kahte ossa. Esimene osa kirjeldab erinevate retseptoriperekondade (indolokarbasoolid, uuread tiouuread, karbasoolid, amiidid ja nende erinevad kombinatsioonid) sünteesi. Teine osa kirjeldab NMR-põhist seondumiskonstantide täpsete väärtuste mõõtemeetodit ning selle abil loodud retseptorite ja nelja karboksülaatanioon vahelise seondumise skaalasid.

Mõõtes absoluutsete seondumiskonstantide asemel nende erinevusi on võimalik saada väga kõrge täpsusega andmeid, sest paljud mõõtemääramatuse allikad (ioonpaardumine, homokonjugatsioon, veesisalduse mõningane erinevus etteantust, …) taanduvad täielikult või osaliselt välja. Välja töötatud mõõtemeetodit rakendati 38 sünteetilise retseptori ja nelja karboksülaatanioon vaheliste seondumiskonstantide määramiseks 0.5% H_2O:DMSO-d_6 lahuses. Kõigi nelja skaala kooskõlalisuse standardhälve on väga hea, suurusjärgus 0.01 log ühikut. Skaalad on ankurdatud absoluutsel meetodil mõõdetud indolokarbasooli logK_{ass} väärtuste külge. Sellised skaalad, mis on koostatud eri anioonide jaoks samadel tingimustel ning mille sisemised kooskõlalisused on väga kõrged, on hästi kasutatavad retseptorite võrdlemiseks sama aniooni seondumisel ning ka erinevate anioonide seondumiste võrdlemiseks erinevate retseptoritega. Sellised skaalad on väga kasulikuks tööriistaks, et mõõta uute retseptorite logK_{ass} väärtusi. Tulemused näitavad, et logK_{ass} väärtused sõltuvad aniooni aluselisusest ja suurusest ning retseptori HB tsentrite donoorsusest, arvust ja paigutusest.

Loodud NMR meetodi rakendatavus suhteliste seondumisafiinsuste mõõtmisel on saanud kinnituse ja selle eelised on välja toodud: ei ole vaja teada anioonide ega retseptorite täpseid kontsentratsioone lahuses, kõrge täpsus (mitmed määramatuse allikad taanduvad välja), lihtsus kasutuses, võimalus detekteerida retseptorite deprotoneerumist ja võimalus mõõta mitme (kuni kuue) retseptori suhtelist seondumist samas lahuses. Loodud skaalad on kasulikud kui ankur-süsteemid tulevasteks seondumise mõõtmisteks uute retseptoritega.

REFERENCES

1. Lehn, J.-M., *Pure Appl. Chem.* **1978**, *50*, 871–892.
2. Steed, J. W.; Atwood, J. L. *Supramolecular Chemistry*, 2nd ed; John Wiley and Sons, Ltd: Chichester, **2009**.
3. Pedersen, C. J., Cyclic polyethers and their complexes with metal salts. *J. Am. Chem. Soc.* **1967**, *89*, 2495–2496.
4. Lehn, J.-M. Toward Self-Organization and Complex Matter. *Science* **2002**, *295*, 2400–2403.
5. Lehn, J.-M. Supramolecular Chemistry–Scope and Perspectives Molecules, Supermolecules, and Molecular Devices. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89–112.
6. Cram, D.J., *Science*, **1983**, *219*, 1177.
7. Cram, D.J.; Karbach, S.; Kim, Y.H.; Baczynskyj, L.; and Kalleymeyn, G. *J. Am. Chem. Soc.*, **1985**, *107*, 2575.
8. Cram, D.J.; Karbach, S.; Kim, Y.H.; Baczynskyj, L.; Marti, K.; Sampson, R.M.; and Kalleymeyn, G.M. *J. Am. Chem. Soc.*, **1988**, *110*, 2554.
9. Beer, P.D.; Gale, P.A.; Smith, D. K. *Supramolecular Chemistry*, Oxford Chemistry, Primers, 74, Oxford University Press: Oxford, **2003**, 1–14.
10. a) Frenking, G.; Shaik, S. (ed), *The chemical bond*, Wiley-VCH, **2014**. b) Parthasarathi, R.; Subramanian, V. Hydrogen Bonding — New Insights (*Ed.*: *S. J. Grabowski*), *Springer*, *Dordrecht*, **2006**, 1–50.
11. Jeffrey, G.A., *An Introduction to Hydrogen Bonding*, Oxford University Press: New York, **1997**, 11–32.
12. Desiraju, G.R. *Hydrogen Bonding*, In *Encyclopedia of Supramolecular Chemistry*, Marcel Dekker: New York, **2004**, 658–665.
13. Winterton, R.H.S, *Contemp. Phys.*, **1970**, *11*, 559.
14. Schneider, H.-J. *van der Waals Forces*, In *Encyclopedia of Supramolecular Chemistry*, Marcel Dekker, Inc.: New York, **2004**, 1550–1556.
15. Kim, D. H.; Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D.; Vogtle F. New York, **1996**, 503–526.
16. (a) Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J. *Chem. Rev.* **1985**, *85*, 271–339. (b) Gale, P. A.; Sessler, J. L.; Kral, V. *Chem. Commun.* **1998**, 1–8.
17. a) Day, A. I.; Blanch, R. J.; Arnold, A. P.; Lorenzo, S.; Lewis, G. R.; Dance, I. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 275−277. b) Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 4844−4870.
18. Janiak, C. J. Chem. Soc., *Dalton* Trans. **2000**, 3885–3896.
19. Cox, E. G.; Cruickshank, D. W. J.; Smith, J. A. S. *Proc. R. Soc. London*, *A* **1958**, *247*, 1–21.
20. Laatikainen, R.; Ratilainen, J.; Sebastian, R.; Santa, H. *J. Am. Chem. Soc.* **1995**, *117*, 11006–11010.
21. Neusser, H. J.; Krause, H. *Chem. Rev.* **1994**, *94*, 1829–1843.
22. Jorgensen, W. L.; Severance, D. L. *J. Am. Chem. Soc.* **1990**, *112*, 4768–4774.

23. Kearney, P. C.; Mizoue, L. S.; Kumpf, R. A.; Forman, J. E.; McCurdy, A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1993**, **115**, 9907–9919.
24. Saenger, W. Cyclodextrin Inclusion Compounds in Research and Industry. *Angew. Chem., Int. Ed. Eng.* **1980**, *19*, 344–362.
25. Thangavel, A.; Rawashdeh, A. M. M.; Sotirou-Leventis, C.; Leventis, N. *Org. Lett.*, **2009**, *11*, 1595.
26. Anslyn, E. V.; Dougherty, D. A. *Modern Physical Organic Chemistry*; University Science Books: Sausalito, **2006**, pp 189–194, 207–241.
27. (a) Kroschwitz, J. I.; Seidel, A. Kirk-Othmer Encyclopedia of Chemical Technology, 5th ed.; Wiley-Interscience: Hoboken, N. J.; New York, **2007**; Vol. 5. (b) Ullmann, F.; Gerhartz, W.; Yamamoto, Y. S.; Campbell, T.; Pfefferkorn, R.; Rounsaville, J. F. Ullmann's Encyclopedia Industrial Chemistry, 7th ed.; *Wiley-VCH*: Weinheim, **2011**.
28. (a) Nonsteroidal Anti-Inflammatory Drugs: Mechanisms and Clinical Uses, 2nd ed,; Lewis, A. J.; Furst, D. E., Eds. Marcel Dekker: New York, **1994**; (b) Lemke, T. L.; Williams, D. A. Nonsteroidal Anti-Inflammantory Drugs Foye's Principles of Medicinal Chemistry, 6th ed.; Lippincott, Williams, & Wilkins: Philadelphia, **2008**. (c) Pharmacotherapy Handbook, 8th ed.; Wells, B. G., DiPiro, J. T., Schwinghammer, T. L., DiPiro, C., Eds.; McGraw Hill: New York, **2012**.
29. Vollhardt, P.; Schore, N. Carboxylic acids. In *Organic Chemistry: Structure and Function*, 6th ed.; W. H. Freeman: New York, **2011**; pp 879–881.
30. Wang, L.Y.; Zhang, Y.H.; Zhao, L. J. *J. Phys. Chem. A* **2005**, *109*, 609–614.
31. (a) Potter, B. V. L.; Lampe, D. *Angew. Chem., Int. Ed.* **1995**, *34*, 1933. (b) Saenger, W. Principles of Nucleic Acid Structure; Springer-Verlag: New York, **1984**; pp 343–346.
32. Kelly, C. P.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B,* **2006**, *110*, 16066–16081.
33. Teymourian, H.; Salimi A.; Hallaj, R. *Biosens. Bioelectron.*, **2012**, *33*, 60–68.
34. Park, C. H.; Simmons, H. E. *J. Am. Chem. Soc.*, **1968**, *90*, 2429–2431
35. Beer, P. D.; Gale, P. A. *Angewandte Chemie-International Edition* **2001**, *40*, 486–516
36. Amendola, V.; Fabbrizzi, L.; Mosca, L. Anion Recognition by Hydrogen Bonding: Urea-based Receptors. *Chem. Soc. Rev.* **2010**, *39*, 3889–3915.
37. Li, A.-F.; Wang, J.-H.; Wang, F.; Jiang, Y.-B. Anion Complexation and Sensing Using Modified Urea and Thiourea-based Receptors. *Chem. Soc. Rev.* **2010**, *39*, 3729–3745.
38. Ramalingam, V.; Domaradzki, M. E.; Jang, S.; Muthyala, R. S. *Org. Lett.*, **2008**, *10*, 3315–3318.
39. Curiel, D.; Cowley, A.; Beer, P. D. *Chem. Commun*, **2005**, *2*, 236–238.
40. Brooks, S. J.; Gale, P. A.; Light, M. E. *Chem. Commun*, **2005**, 4696–4698.
41. Brooks, S. J.; Gale, P. A.; Light, M. E. *Superamolecular Chemistry,* **2007**, *19*, 9–15.
42. Brooks, S. J.; Edwards, P. R.; Gale, P. A.; Light, M. E. *New J. Chem.* **2006**, *30*, 65–70.

43. (a) Baeyer, A. *Ber*. *Dtsch*. *Chem*. *Ges*. **1886**, *19*, 2184. (b) Gale, P. A.; Sessler, J. L; Kral, V.; Lynch, V. *J*. *Am*. *Chem*. *Soc*. **1996**, *118*, 5140.
44. Piaütek, P.; Lynch, V. M.; Sessler, J. L. *J*. *Am*. *Chem*. *Soc.* **2004**, *126*, 16073–16076.
45. Chmielewski, M. J.; Charon, M.; Jurczak, J. *Org*. *Lett*., **2004**, *6*, 20, 3501–3504.
46. Hiscock, J. R.; Caltagirone, C.; Light, M. E.; Hursthouse, M. B.; Gale, P. A. *Org*. *Biomol*. *Chem*., **2009**, *7*, 1781–1783.
47. Caltagirone C.; Gale P. A.; Hiscock J. R.; Brooks S. J.; Hursthouse M. B.; Light M. E. *Chem*. *Commun*. **2008**, 3007–3009.
48. Gale, P. A.; Busschaert, N.; Haynes, C. J. E.; Karagiannidis, L. E.; Kirby, I. L. *Chem. Soc. Rev.,* **2014**, *43*, 205.
49. Bordwell, F. G. Equilibrium Acidities in Dimethyl Sulfoxide Solution. *Acc. Chem. Res.* **1988**, *21*, 456–463.
50. Boicchi, M.; Del Boca, L.; Gomez, D. E.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *J. Am. Chem. Soc,* **2004***, 126,*16507–16514.
51. Boiocchi, M.; Del Bocca, L.; Esteban-Gomez, D.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *Chem*.,*Eur*. *J*. **2005**, *11*, 3097–3104.
52. Makuc, D.; Hiscock, J. R.; Light, M. E.; Gale, P. A. J. Plavec, *Beilstein J. Org*. *Chem*. **2011**, *7*, 1205–1214.
53. Dydio,P.; Lichosyt, D.; Zielinski, T.; Jurczak, J. *Chem*. *Eur*. *J*. **2012**, *18*, 13686–13701.
54. Curiel, D.; Mas-Montoya, M.; Sanchez, G.; Orenes, R. A.; Molina, P.; Tarraga, A. *Org. Biomol. Chem.*, **2010**, *8*, 4811–4814.
55. Gale, P. A. *Chem. Commun.* **2008**, 4525–4540.
56. Sánchez, G.; Curiel, D.; Tárraga, A.; Molina, P. *Sensors* 2014, *14*, 14038–14049.
57. Kwon, T. H.; Jeong K.-S. *Tetrahedron Letters,* **2006**, *47,* 8539–8541.
58. Wang, T.; Bai, Y.; Ma, L.; Yan, X. *Org. Biomol. Chem*., **2008**, *6*, 1751–1755.
59. Fitzmaurice, R. J.; Kyne, G. M.; Douheret D.; Kilburn, J. D. *J. Chem*. *Soc*., *Perkin Trans*. **2002**, *1*, 841–864.
60. Hargrove, A. E.; Nieto, S. Zhang, T. Sessler, J. L. Anslyn, E. V. *Chem*. *Rev*. **2011**, *111*, 6603–6782
61. Schmuck, C.; Machon, U. *Chem*. *Eur*. *J*., **2005**, *11*, 1109.
62. Schmuck, C.; Machon, U. *Eur*. *J*. *Org*. *Chem*., **2006**, 4385.
63. Schmuck, C. *Coord*. *Chem*. *Rev*., **2006**, *250*, 3053.
64. Blondeau, P.; Segura, M.; Pe´rez-Ferna´ndez, R.; Mendoza, J. d. *Chem*. *Soc*. *Rev*., **2007**, *36*, 198–210.
65. Sanders, J. K. M.; Hunter, B.K. *Modern NMR Spectroscopy. A Guide for Chemists*; Oxford University Press: Oxford, **1988**, 208–211.
66. Connors, K. A. *Binding Constants. The Measurement of Complex Stability*, John Wiley & Sons: *New York*, **1987**, 189–193.
67. Sessler, J. L.; Gross, D. E.; Cho, W.-S.; Lynch, V. M.; Schmidtchen, F. P.; Bates, G. W.; Light, M. E.; Gale, P. A. *J. Am. Chem. Soc.* **2006**, *128*, 12281–12288.
68. Gunnlaugsson, T.; Glynn, M.; Tocci, G.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006,** *250*, 3094–3117.

69. Hirose, K. Quantitative Analysis of Binding Properties. In *Analytical Methods in Supramolecular Chemistry*; Schalley, C. A., Ed.; *Wiley-VCH*: *Weinheim*, **2012**; pp 27–66.
70. Kubik, S.; Reyheller, C.; Stu¨we, S. *J. Inclusion Phenom. Macrocyclic chem.* ***2005****, 52,* 137–187.
71. Jorgensen, W. L. *J. Am. Chem. Soc.* **1989**, *111*, 3770–3771.
72. Hiscock, J. R.; Gale, P. A.; Lalaoui, N.; Light, M. E.; Wells, N. *J. Org. Biomol. Chem*., **2012**, *10*, 7780.
73. Armarego, W. L. F.; Chai, C. L. L. Purification of laboratory chemical, 6th Ed. **2009**.
74. Leito, I.; Herodes, K.; Huopolainen, M.; Virro, K.; Künnapas, A.; Kruve, A.; Tanner, R. *Rapid Commun. Mass Spectrom*. **2008**, *22*, 379–384.
75. Sooväli, L.; Kaljurand, I.; Kütt, A.; Leito, I. *Anal. Chim. Acta* **2006**, *566*, 290–303.
76. Kütt, A.; Leito, I.; Kaljurand, I.; Sooväli, L.; Vlasov, V. M.; Yagupolskii, L. M.; Koppel, I. A. *J. Org. Chem.* **2006**, *71*, 2829–2838.
77. You, L.; Anslyn, E. V. In *Supramolecular Chemistry: From Molecules to Nanomaterials*; Gale, P. A., Steed J. W., Eds.; Wiley, Chichester, **2012**, *1*, 135–160.
78. Jose, D. A.; Kumar, D. K.; Ganguly, B.; Das, A. *Org. Lett*. **2004**, *6*, 20, 3445–3448.
79. Figlus, M.; Tarruella, A. C.; Messer, A.; Sollis, S. L.; Hartley, R. C. *Chem. Commun*. **2010**, *46*, 4405–4407.
80. Štrukil, V.; Igrc, M. D.; Fábián, L.; Eckert-Maksić, M.; Childs, S. L.; Reid, D. G.; Duer, M. J.; Halasz, I.; Mottillo, C.; Friščić, T. *Green Chem*. **2012**, *14*, 2462–2473.
81. Benesi, H.; Hildebrand, J. *J. Am. Chem. Soc*. **1949**, *71*, 2703–2707.
82. Coetzee, J. F. *Prog. Phys. Org. Chem.* **1967**, *4*, 45–92.
83. Fielding, L. *Progress in Nuclear Magnetic Resonance Spectroscopy*. **2007**, *51*, 219–242.
84. Sanchez, G.; Espinosa, A; Curiel, D.; Tarraga, A.; Molina, P. *J. Org. Chem.* **2013**, *78*, 9725–9737.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor Prof. Ivo leito for the kind support of my Ph.D. study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of the thesis. I could not have imagined having a better advisor and mentor for my Ph.D. study.

I am thankful to Tõiv Haljasorg and Charly Mayeux for HRMS spectra of molecules. I am thankful to Astrid Pung for the computational data. I am thankful to my labmates who were always willing to help: Kristjan Haav, Lauri Toom, Agnes Kütt and Kerli Martin. I am also thankful to my collaborators and co-authors for their help, suggestions and scholarly inputs during manuscript preparation.

Thanks for supported by European Social Fund's Doctoral Studies and Internationalisation programme DoRa, which is carried out by Foundation Archimedes. Also thanks to all my B.Sc. and M.Sc. friends for support.

Last but not the least, I would like to thank my family members, my parents (Pushpa and Ashokrao) unconditional love and support during my PhD research and for allowing enhancing my carrier and my brother and sister Pradip and Sonali always cheering me all the time. Finally, I would like to thank my wife Kartiki, she was always there cheering me up and standing by me through the good times and bad.

PUBLICATIONS

CURRICULUM VITAE

Name: Sandip Ashokrao Kadam
Born: December 25th 1984, Lonand, Maharashtra, India
E-mail: Sandip.Kadam@ut.ee
Citizenship: Indian

Education:

2011–present	University of Tartu, *Ph.D* student in Chemistry
2005–2007	University of Pune, *M.Sc* in Organic Chemistry
2002–2005	University of Kolhapur, *B.Sc* in Chemistry

Professional Employment:

2013–present	University of Tartu, 0.5 Chemist
2008–2011	Research Chemist in Chembiotek (TCG Lifesciences), Pune, India
2007–2008	Research Associate in Calyx Chemical and Pharmacuitical Ltd, Mumbai, India

Scientific publications:

1. Upadhayaya, R. S.; Shinde, P. D.; Sayyad, A. Y.; Kadam, S. A.; Bawane, A. N.; Poddar, A.; Plashkevych, O.; Foldesi, A.; Chattopadhyaya. J. Synthesis and structure of azole-fused indeno[2,1-c]quinolines and their antimicobacterial properties. *Org. Biomol. Chem.*, **2010**, *8*, 5661−5673.
2. Upadhayaya, R. S.; Shinde, P. D.; Kadam, S. A.; Bawane, A. N.; Sayyad, A. Y.; Kardile, R. A.; Gitay, P. N.; Lahore, S. V.; Dixit, S. S.; Foldesi, A.; Chattopadhyaya, J. Synthesis and antimycobacterial activity of prodrugs of indeno[2,1-c]quinolines derivatives. *European Journal of Medicinal Chemistry.* **2011**, *46*, 1306−1324.
3. Haav, K.; [#] Kadam, S. A.;[#] Toom, L.; Gale, P. A.; Busschaert, N.; Wenzel, M. Hiscock, J. R.; Kirby, I. L.; Haljasorg, T.; Lokov, M.; Leito, I. Accurate method to quantify binding in supramolecular chemistry. *J. Org. Chem.* **2013**, *78*, 7796−7808. [#] Equal contribution
4. Kadam, S. A.; Haav, K.; Toom, L.; Haljasorg, T.; Leito, I. NMR method for simultaneous host-guest binding constant measurement. *J. Org. Chem.,* **2014**, *79*, 2501−2523.
5. Kadam, S. A.; Martin, K.; Haav, K.; Toom, L.; Mayeux, C.; Pung, A.; Gale, P. A.; Hiscock, J. R.; Brooks, S. J.; Kirby, I. L.; Busschaert, N.; Leito, I. Towards discrimination of carboxylate by hydrogen-bond donor anion receptors. *Chemistry A European Journal,* **2015**, *21*, 5145−5160.
6. Kadam, S. A.; Haav, K.; Toom, L.; Pung, A.; Mayeux, C.; Leito, I. Synthesis and binding affinity towards the glyphosate anion in aqueous media. (**2015**, Manuscript under preparation)
7. Kadam, S. A.; Pung, A.; Leito, I. Novel synthesis of macrocyclic carbazole receptor for different anions. (**2015**, Manuscript under preparation)

ELULOOKIRJELDUS

Nimi: Sandip Ashokrao Kadam
Sünniaeg: 25. detsember, 1984, Lonand, Maharashtra, India
E-mail: Sandip.Kadam@ut.ee
Kodakondsus: India

Haridus:

2011–tänaseni	Tartu Ülikool, keemia eriala doktorant
2005–2007	Pune Ülikool, *M.Sc* (orgaaniline keemia)
2002–2005	Kolhapuri Ülikool, *B.Sc* (keemia)

Teenistuskäik:

2013–tänaseni	Tartu Ülikool, keemik (0.5)
2008–2011	Chembiotek (TCG Lifesciences), keemik
2007–2008	Calyx Chemical and Pharmacuitical Ltd, assistent

Teaduspublikatsioonid:

1. Upadhayaya, R. S.; Shinde, P. D.; Sayyad, A. Y.; Kadam, S. A.; Bawane, A. N.; Poddar, A.; Plashkevych, O.; Foldesi, A.; Chattopadhyaya. J. Synthesis and structure of azole-fused indeno[2,1-c]quinolines and their antimicobacterial properties. *Org. Biomol. Chem.*, **2010**, *8*, 5661–5673.
2. Upadhayaya, R. S.; Shinde, P. D.; Kadam, S. A.; Bawane, A. N.; Sayyad, A. Y.; Kardile, R. A.; Gitay, P. N.; Lahore, S. V.; Dixit, S. S.; Foldesi, A.; Chattopadhyaya, J. Synthesis and antimycobacterial activity of prodrugs of indeno[2,1-c]quinolines derivatives. *European Journal of Medicinal Chemistry.* **2011**, *46*, 1306–1324.
3. Haav, K.; [#] Kadam, S. A.;[#] Toom, L.; Gale, P. A.; Busschaert, N.; Wenzel, M. Hiscock, J. R.; Kirby, I. L.; Haljasorg, T.; Lokov, M.; Leito, I. Accurate method to quantify binding in supramolecular chemistry. *J. Org. Chem.* **2013**, *78*, 7796–7808. [#] Equal contribution
4. Kadam, S. A.; Haav, K.; Toom, L.; Haljasorg, T.; Leito, I. NMR method for simultaneous host-guest binding constant measurement. *J. Org. Chem.,* **2014**, *79*, 2501–2523.
5. Kadam, S. A.; Martin, K.; Haav, K.; Toom, L.; Mayeux, C.; Pung, A.; Gale, P. A.; Hiscock, J. R.; Brooks, S. J.; Kirby, I. L.; Busschaert, N.; Leito, I. Towards discrimination of carboxylate by hydrogen-bond donor anion receptors. *Chemistry A European Journal,* **2015**, *21*, 5145–5160.
6. Kadam, S. A.; Haav, K.; Toom, L.; Pung, A.; Mayeux, C.; Leito, I. Synthesis and binding affinity towards the glyphosate anion in aqueous media. (**2015**, Manuscript under preparation)
7. Kadam, S. A.; Pung, A.; Leito, I. Novel synthesis of macrocyclic carbazole receptor for different anions. (**2015**, Manuscript under preparation)

DISSERTATIONES CHIMICAE UNIVERSITATIS TARTUENSIS

1. **Toomas Tamm.** Quantum-chemical simulation of solvent effects. Tartu, 1993, 110 p.
2. **Peeter Burk.** Theoretical study of gas-phase acid-base equilibria. Tartu, 1994, 96 p.
3. **Victor Lobanov.** Quantitative structure-property relationships in large descriptor spaces. Tartu, 1995, 135 p.
4. **Vahur Mäemets.** The ^{17}O and ^{1}H nuclear magnetic resonance study of H_2O in individual solvents and its charged clusters in aqueous solutions of electrolytes. Tartu, 1997, 140 p.
5. **Andrus Metsala.** Microcanonical rate constant in nonequilibrium distribution of vibrational energy and in restricted intramolecular vibrational energy redistribution on the basis of slater's theory of unimolecular reactions. Tartu, 1997, 150 p.
6. **Uko Maran.** Quantum-mechanical study of potential energy surfaces in different environments. Tartu, 1997, 137 p.
7. **Alar Jänes.** Adsorption of organic compounds on antimony, bismuth and cadmium electrodes. Tartu, 1998, 219 p.
8. **Kaido Tammeveski.** Oxygen electroreduction on thin platinum films and the electrochemical detection of superoxide anion. Tartu, 1998, 139 p.
9. **Ivo Leito.** Studies of Brønsted acid-base equilibria in water and non-aqueous media. Tartu, 1998, 101 p.
10. **Jaan Leis.** Conformational dynamics and equilibria in amides. Tartu, 1998, 131 p.
11. **Toonika Rinken.** The modelling of amperometric biosensors based on oxidoreductases. Tartu, 2000, 108 p.
12. **Dmitri Panov.** Partially solvated Grignard reagents. Tartu, 2000, 64 p.
13. **Kaja Orupõld.** Treatment and analysis of phenolic wastewater with microorganisms. Tartu, 2000, 123 p.
14. **Jüri Ivask.** Ion Chromatographic determination of major anions and cations in polar ice core. Tartu, 2000, 85 p.
15. **Lauri Vares.** Stereoselective Synthesis of Tetrahydrofuran and Tetrahydropyran Derivatives by Use of Asymmetric Horner-Wadsworth-Emmons and Ring Closure Reactions. Tartu, 2000, 184 p.
16. **Martin Lepiku.** Kinetic aspects of dopamine D_2 receptor interactions with specific ligands. Tartu, 2000, 81 p.
17. **Katrin Sak.** Some aspects of ligand specificity of P2Y receptors. Tartu, 2000, 106 p.
18. **Vello Pällin.** The role of solvation in the formation of iotsitch complexes. Tartu, 2001, 95 p.

19. **Katrin Kollist.** Interactions between polycyclic aromatic compounds and humic substances. Tartu, 2001, 93 p.
20. **Ivar Koppel.** Quantum chemical study of acidity of strong and superstrong Brønsted acids. Tartu, 2001, 104 p.
21. **Viljar Pihl.** The study of the substituent and solvent effects on the acidity of OH and CH acids. Tartu, 2001, 132 p.
22. **Natalia Palm.** Specification of the minimum, sufficient and significant set of descriptors for general description of solvent effects. Tartu, 2001, 134 p.
23. **Sulev Sild.** QSPR/QSAR approaches for complex molecular systems. Tartu, 2001, 134 p.
24. **Ruslan Petrukhin.** Industrial applications of the quantitative structure-property relationships. Tartu, 2001, 162 p.
25. **Boris V. Rogovoy.** Synthesis of (benzotriazolyl)carboximidamides and their application in relations with *N*- and *S*-nucleophyles. Tartu, 2002, 84 p.
26. **Koit Herodes.** Solvent effects on UV-vis absorption spectra of some solvatochromic substances in binary solvent mixtures: the preferential solvation model. Tartu, 2002, 102 p.
27. **Anti Perkson.** Synthesis and characterisation of nanostructured carbon. Tartu, 2002, 152 p.
28. **Ivari Kaljurand.** Self-consistent acidity scales of neutral and cationic Brønsted acids in acetonitrile and tetrahydrofuran. Tartu, 2003, 108 p.
29. **Karmen Lust.** Adsorption of anions on bismuth single crystal electrodes. Tartu, 2003, 128 p.
30. **Mare Piirsalu.** Substituent, temperature and solvent effects on the alkaline hydrolysis of substituted phenyl and alkyl esters of benzoic acid. Tartu, 2003, 156 p.
31. **Meeri Sassian.** Reactions of partially solvated Grignard reagents. Tartu, 2003, 78 p.
32. **Tarmo Tamm.** Quantum chemical modelling of polypyrrole. Tartu, 2003. 100 p.
33. **Erik Teinemaa.** The environmental fate of the particulate matter and organic pollutants from an oil shale power plant. Tartu, 2003. 102 p.
34. **Jaana Tammiku-Taul.** Quantum chemical study of the properties of Grignard reagents. Tartu, 2003. 120 p.
35. **Andre Lomaka.** Biomedical applications of predictive computational chemistry. Tartu, 2003. 132 p.
36. **Kostyantyn Kirichenko.** Benzotriazole – Mediated Carbon–Carbon Bond Formation. Tartu, 2003. 132 p.
37. **Gunnar Nurk.** Adsorption kinetics of some organic compounds on bismuth single crystal electrodes. Tartu, 2003, 170 p.
38. **Mati Arulepp.** Electrochemical characteristics of porous carbon materials and electrical double layer capacitors. Tartu, 2003, 196 p.

39. **Dan Cornel Fara.** QSPR modeling of complexation and distribution of organic compounds. Tartu, 2004, 126 p.
40. **Riina Mahlapuu.** Signalling of galanin and amyloid precursor protein through adenylate cyclase. Tartu, 2004, 124 p.
41. **Mihkel Kerikmäe.** Some luminescent materials for dosimetric applications and physical research. Tartu, 2004, 143 p.
42. **Jaanus Kruusma.** Determination of some important trace metal ions in human blood. Tartu, 2004, 115 p.
43. **Urmas Johanson.** Investigations of the electrochemical properties of polypyrrole modified electrodes. Tartu, 2004, 91 p.
44. **Kaido Sillar.** Computational study of the acid sites in zeolite ZSM-5. Tartu, 2004, 80 p.
45. **Aldo Oras.** Kinetic aspects of dATPαS interaction with $P2Y_1$ receptor. Tartu, 2004, 75 p.
46. **Erik Mölder.** Measurement of the oxygen mass transfer through the air-water interface. Tartu, 2005, 73 p.
47. **Thomas Thomberg.** The kinetics of electroreduction of peroxodisulfate anion on cadmium (0001) single crystal electrode. Tartu, 2005, 95 p.
48. **Olavi Loog.** Aspects of condensations of carbonyl compounds and their imine analogues. Tartu, 2005, 83 p.
49. **Siim Salmar.** Effect of ultrasound on ester hydrolysis in aqueous ethanol. Tartu, 2006, 73 p.
50. **Ain Uustare.** Modulation of signal transduction of heptahelical receptors by other receptors and G proteins. Tartu, 2006, 121 p.
51. **Sergei Yurchenko.** Determination of some carcinogenic contaminants in food. Tartu, 2006, 143 p.
52. **Kaido Tämm.** QSPR modeling of some properties of organic compounds. Tartu, 2006, 67 p.
53. **Olga Tšubrik.** New methods in the synthesis of multisubstituted hydrazines. Tartu. 2006, 183 p.
54. **Lilli Sooväli.** Spectrophotometric measurements and their uncertainty in chemical analysis and dissociation constant measurements. Tartu, 2006, 125 p.
55. **Eve Koort.** Uncertainty estimation of potentiometrically measured ph and pK_a values. Tartu, 2006, 139 p.
56. **Sergei Kopanchuk.** Regulation of ligand binding to melanocortin receptor subtypes. Tartu, 2006, 119 p.
57. **Silvar Kallip.** Surface structure of some bismuth and antimony single crystal electrodes. Tartu, 2006, 107 p.
58. **Kristjan Saal.** Surface silanization and its application in biomolecule coupling. Tartu, 2006, 77 p.
59. **Tanel Tätte.** High viscosity $Sn(OBu)_4$ oligomeric concentrates and their applications in technology. Tartu, 2006, 91 p.

60. **Dimitar Atanasov Dobchev**. Robust QSAR methods for the prediction of properties from molecular structure. Tartu, 2006, 118 p.
61. **Hannes Hagu**. Impact of ultrasound on hydrophobic interactions in solutions. Tartu, 2007, 81 p.
62. **Rutha Jäger.** Electroreduction of peroxodisulfate anion on bismuth electrodes. Tartu, 2007, 142 p.
63. **Kaido Viht.** Immobilizable bisubstrate-analogue inhibitors of basophilic protein kinases: development and application in biosensors. Tartu, 2007, 88 p.
64. **Eva-Ingrid Rõõm.** Acid-base equilibria in nonpolar media. Tartu, 2007, 156 p.
65. **Sven Tamp.** DFT study of the cesium cation containing complexes relevant to the cesium cation binding by the humic acids. Tartu, 2007, 102 p.
66. **Jaak Nerut.** Electroreduction of hexacyanoferrate(III) anion on Cadmium (0001) single crystal electrode. Tartu, 2007, 180 p.
67. **Lauri Jalukse.** Measurement uncertainty estimation in amperometric dissolved oxygen concentration measurement. Tartu, 2007, 112 p.
68. **Aime Lust.** Charge state of dopants and ordered clusters formation in CaF_2:Mn and CaF_2:Eu luminophors. Tartu, 2007, 100 p.
69. **Iiris Kahn**. Quantitative Structure-Activity Relationships of environmentally relevant properties. Tartu, 2007, 98 p.
70. **Mari Reinik.** Nitrates, nitrites, N-nitrosamines and polycyclic aromatic hydrocarbons in food: analytical methods, occurrence and dietary intake. Tartu, 2007, 172 p.
71. **Heili Kasuk.** Thermodynamic parameters and adsorption kinetics of organic compounds forming the compact adsorption layer at Bi single crystal electrodes. Tartu, 2007, 212 p.
72. **Erki Enkvist.** Synthesis of adenosine-peptide conjugates for biological applications. Tartu, 2007, 114 p.
73. **Svetoslav Hristov Slavov**. Biomedical applications of the QSAR approach. Tartu, 2007, 146 p.
74. **Eneli Härk.** Electroreduction of complex cations on electrochemically polished Bi(*hkl*) single crystal electrodes. Tartu, 2008, 158 p.
75. **Priit Möller.** Electrochemical characteristics of some cathodes for medium temperature solid oxide fuel cells, synthesized by solid state reaction technique. Tartu, 2008, 90 p.
76. **Signe Viggor.** Impact of biochemical parameters of genetically different pseudomonads at the degradation of phenolic compounds. Tartu, 2008, 122 p.
77. **Ave Sarapuu.** Electrochemical reduction of oxygen on quinone-modified carbon electrodes and on thin films of platinum and gold. Tartu, 2008, 134 p.
78. **Agnes Kütt.** Studies of acid-base equilibria in non-aqueous media. Tartu, 2008, 198 p.

79. **Rouvim Kadis.** Evaluation of measurement uncertainty in analytical chemistry: related concepts and some points of misinterpretation. Tartu, 2008, 118 p.
80. **Valter Reedo.** Elaboration of IVB group metal oxide structures and their possible applications. Tartu, 2008, 98 p.
81. **Aleksei Kuznetsov.** Allosteric effects in reactions catalyzed by the cAMP-dependent protein kinase catalytic subunit. Tartu, 2009, 133 p.
82. **Aleksei Bredihhin.** Use of mono- and polyanions in the synthesis of multisubstituted hydrazine derivatives. Tartu, 2009, 105 p.
83. **Anu Ploom.** Quantitative structure-reactivity analysis in organosilicon chemistry. Tartu, 2009, 99 p.
84. **Argo Vonk.** Determination of adenosine A_{2A}- and dopamine D_1 receptor-specific modulation of adenylate cyclase activity in rat striatum. Tartu, 2009, 129 p.
85. **Indrek Kivi.** Synthesis and electrochemical characterization of porous cathode materials for intermediate temperature solid oxide fuel cells. Tartu, 2009, 177 p.
86. **Jaanus Eskusson.** Synthesis and characterisation of diamond-like carbon thin films prepared by pulsed laser deposition method. Tartu, 2009, 117 p.
87. **Marko Lätt.** Carbide derived microporous carbon and electrical double layer capacitors. Tartu, 2009, 107 p.
88. **Vladimir Stepanov.** Slow conformational changes in dopamine transporter interaction with its ligands. Tartu, 2009, 103 p.
89. **Aleksander Trummal.** Computational Study of Structural and Solvent Effects on Acidities of Some Brønsted Acids. Tartu, 2009, 103 p.
90. **Eerold Vellemäe.** Applications of mischmetal in organic synthesis. Tartu, 2009, 93 p.
91. **Sven Parkel.** Ligand binding to 5-HT_{1A} receptors and its regulation by Mg^{2+} and Mn^{2+}. Tartu, 2010, 99 p.
92. **Signe Vahur.** Expanding the possibilities of ATR-FT-IR spectroscopy in determination of inorganic pigments. Tartu, 2010, 184 p.
93. **Tavo Romann**. Preparation and surface modification of bismuth thin film, porous, and microelectrodes. Tartu, 2010, 155 p.
94. **Nadežda Aleksejeva.** Electrocatalytic reduction of oxygen on carbon nanotube-based nanocomposite materials. Tartu, 2010, 147 p.
95. **Marko Kullapere.** Electrochemical properties of glassy carbon, nickel and gold electrodes modified with aryl groups. Tartu, 2010, 233 p.
96. **Liis Siinor.** Adsorption kinetics of ions at Bi single crystal planes from aqueous electrolyte solutions and room-temperature ionic liquids. Tartu, 2010, 101 p.
97. **Angela Vaasa.** Development of fluorescence-based kinetic and binding assays for characterization of protein kinases and their inhibitors. Tartu 2010, 101 p.

98. **Indrek Tulp.** Multivariate analysis of chemical and biological properties. Tartu 2010, 105 p.
99. **Aare Selberg.** Evaluation of environmental quality in Northern Estonia by the analysis of leachate. Tartu 2010, 117 p.
100. **Darja Lavõgina.** Development of protein kinase inhibitors based on adenosine analogue-oligoarginine conjugates. Tartu 2010, 248 p.
101. **Laura Herm.** Biochemistry of dopamine D_2 receptors and its association with motivated behaviour. Tartu 2010, 156 p.
102. **Terje Raudsepp.** Influence of dopant anions on the electrochemical properties of polypyrrole films. Tartu 2010, 112 p.
103. **Margus Marandi.** Electroformation of Polypyrrole Films: *In-situ* AFM and STM Study. Tartu 2011, 116 p.
104. **Kairi Kivirand.** Diamine oxidase-based biosensors: construction and working principles. Tartu, 2011, 140 p.
105. **Anneli Kruve.** Matrix effects in liquid-chromatography electrospray mass-spectrometry. Tartu, 2011, 156 p.
106. **Gary Urb.** Assessment of environmental impact of oil shale fly ash from PF and CFB combustion. Tartu, 2011, 108 p.
107. **Nikita Oskolkov.** A novel strategy for peptide-mediated cellular delivery and induction of endosomal escape. Tartu, 2011, 106 p.
108. **Dana Martin.** The QSPR/QSAR approach for the prediction of properties of fullerene derivatives. Tartu, 2011, 98 p.
109. **Säde Viirlaid.** Novel glutathione analogues and their antioxidant activity. Tartu, 2011, 106 p.
110. **Ülis Sõukand.** Simultaneous adsorption of Cd^{2+}, Ni^{2+}, and Pb^{2+} on peat. Tartu, 2011, 124 p.
111. **Lauri Lipping.** The acidity of strong and superstrong Brønsted acids, an outreach for the "limits of growth": a quantum chemical study. Tartu, 2011, 124 p.
112. **Heisi Kurig.** Electrical double-layer capacitors based on ionic liquids as electrolytes. Tartu, 2011, 146 p.
113. **Marje Kasari.** Bisubstrate luminescent probes, optical sensors and affinity adsorbents for measurement of active protein kinases in biological samples. Tartu, 2012, 126 p.
114. **Kalev Takkis.** Virtual screening of chemical databases for bioactive molecules. Tartu, 2012, 122 p.
115. **Ksenija Kisseljova**. Synthesis of aza-β^3-amino acid containing peptides and kinetic study of their phosphorylation by protein kinase A. Tartu, 2012, 104 p.
116. **Riin Rebane.** Advanced method development strategy for derivatization LC/ESI/MS. Tartu, 2012, 184 p.

117. **Vladislav Ivaništšev.** Double layer structure and adsorption kinetics of ions at metal electrodes in room temperature ionic liquids. Tartu, 2012, 128 p.
118. **Irja Helm.** High accuracy gravimetric Winkler method for determination of dissolved oxygen. Tartu, 2012, 139 p.
119. **Karin Kipper.** Fluoroalcohols as Components of LC-ESI-MS Eluents: Usage and Applications. Tartu, 2012, 164 p.
120. **Arno Ratas.** Energy storage and transfer in dosimetric luminescent materials. Tartu, 2012, 163 p.
121. **Reet Reinart-Okugbeni**. Assay systems for characterisation of subtype-selective binding and functional activity of ligands on dopamine receptors. Tartu, 2012, 159 p.
122. **Lauri Sikk.** Computational study of the Sonogashira cross-coupling reaction. Tartu, 2012, 81 p.
123. **Karita Raudkivi.** Neurochemical studies on inter-individual differences in affect-related behaviour of the laboratory rat. Tartu, 2012, 161 p.
124. **Indrek Saar.** Design of GalR2 subtype specific ligands: their role in depression-like behavior and feeding regulation. Tartu, 2013, 126 p.
125. **Ann Laheäär.** Electrochemical characterization of alkali metal salt based non-aqueous electrolytes for supercapacitors. Tartu, 2013, 127 p.
126. **Kerli Tõnurist.** Influence of electrospun separator materials properties on electrochemical performance of electrical double-layer capacitors. Tartu, 2013, 147 p.
127. **Kaija Põhako-Esko.** Novel organic and inorganic ionogels: preparation and characterization. Tartu, 2013, 124 p.
128. **Ivar Kruusenberg.** Electroreduction of oxygen on carbon nanomaterial-based catalysts. Tartu, 2013, 191 p.
129. **Sander Piiskop.** Kinetic effects of ultrasound in aqueous acetonitrile solutions. Tartu, 2013, 95 p.
130. **Ilona Faustova**. Regulatory role of L-type pyruvate kinase N-terminal domain. Tartu, 2013, 109 p.
131. **Kadi Tamm.** Synthesis and characterization of the micro-mesoporous anode materials and testing of the medium temperature solid oxide fuel cell single cells. Tartu, 2013, 138 p.
132. **Iva Bozhidarova Stoyanova-Slavova.** Validation of QSAR/QSPR for regulatory purposes. Tartu, 2013, 109 p.
133. **Vitali Grozovski**. Adsorption of organic molecules at single crystal electrodes studied by *in situ* STM method. Tartu, 2014, 146 p.
134. **Santa Veikšina**. Development of assay systems for characterisation of ligand binding properties to melanocortin 4 receptors. Tartu, 2014, 151 p.
135. **Jüri Liiv.** PVDF (polyvinylidene difluoride) as material for active element of twisting-ball displays. Tartu, 2014, 111 p.

136. **Kersti Vaarmets.** Electrochemical and physical characterization of pristine and activated molybdenum carbide-derived carbon electrodes for the oxygen electroreduction reaction. Tartu, 2014, 131 p.
137. **Lauri Tõntson**. Regulation of G-protein subtypes by receptors, guanine nucleotides and Mn^{2+}. Tartu, 2014, 105 p.
138. **Aiko Adamson.** Properties of amine-boranes and phosphorus analogues in the gas phase. Tartu, 2014, 78 p.
139. **Elo Kibena**. Electrochemical grafting of glassy carbon, gold, highly oriented pyrolytic graphite and chemical vapour deposition-grown graphene electrodes by diazonium reduction method. Tartu, 2014, 184 p.
140. **Teemu Näykki.** Novel Tools for Water Quality Monitoring – From Field to Laboratory. Tartu, 2014, 202 p.
141. **Karl Kaupmees.** Acidity and basicity in non-aqueous media: importance of solvent properties and purity. Tartu, 2014, 128 p.
142. **Oleg Lebedev**. Hydrazine polyanions: different strategies in the synthesis of heterocycles. Tartu, 2015, 118 p.
143. **Geven Piir.** Environmental risk assessment of chemicals using QSAR methods. Tartu, 2015, 123 p.
144. **Olga Mazina.** Development and application of the biosensor assay for measurements of cyclic adenosine monophosphate in studies of G protein-coupled receptor signalinga. Tartu, 2015, 116 p.