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ALO RÜÜTEL

Design principles of synthetic molecular receptors for anion-selective electrodes

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Design principles of synthetic molecular receptors for anion-selective electrodes

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Institute of Chemistry, Faculty of Science and Technology, University of Tartu, Estonia.

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TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

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I. Lead author in preparing the manuscript. Carried out part of the synthesis work and structural analysis, performed binding affinity measurements and data treatment.

II. Performed part of the synthesis work and structural analysis. Participated in writing the manuscript.

III. Lead author in preparing the manuscript. Carried out synthesis work and structural analysis, performed binding affinity measurements and data treatment.

ABBREVIATIONS

δ	chemical shift (NMR)
1,2-DCE	1,2-dichloroethane
2-ME-BU	2-methoxyethyl bambus[6]uril
2-Me-THF	2-methyl tetrahydrofuran
Ac_2O	acetic anhydride
AcOH	acetic acid
$_{aq}$	aqueous
Boc	tert-butyloxycarbonyl protecting group
Boc_2O	di-tert-butyl dicarbonate
BzOH	benzyl alcohol
CDI	1,1-carbonyldiimidazole
DCM	dichloromethane
DHI	4,5-dihydroxy-2-imidazolidinone
DMEDA	1,2-dimethylethylenediamine
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
HB	hydrogen bond
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HRMS	high-resolution mass spectroscopy
ISE	ion-selective electrode
K_{ass}	association constant
$\log K_{ass}$	logarithm of association constant
$\log P_{o/w}$	logarithm of octanol-water partition coefficient
NMR	nuclear magnetic resonance
o-NPOE	ortho-nitrophenyl octyl ether
PFA	paraformaldehyde
pK_a	acidity
$pK_a^{o/w}$	biphasic acidity
R_f	retention factor
TBA	tetrabutylammonium
TEA	tetraethylammonium
TEG	triethylene glycol
TFA	trifluoroacetic acid
THF	tetrahydrofuran
UV-Vis	ultraviolet-visible (spectroscopy)

1. INTRODUCTION

The ability of molecules to selectively recognise each other can be considered the fundamental driving force of the universe to form complex matter and, importantly, life. Advances in supramolecular chemistry have marvelled the scientific community, for example, with the development of molecular machines. Despite tremendous research efforts, not many everyday applications stem from this field of chemistry, and the victories of supramolecular chemistry are slow to reach the areas where they are most needed.

One such area is sensor development, which aims to provide selective recognition for medicine, agriculture, water treatment, food industry *etc*. These fields require the accurate detection and quantification of anions. Carboxylates occupy a prominent place among them. Sensing devices for carboxylates are rare outside of laboratory conditions.

In the development of electrochemical sensors, a dead zone exists. Mountains of information are available on different receptor molecules, their synthesis and binding characteristics. However, this research does not usually move on to practical sensor applications or prototype development. At the same time, there is also extensive effort in the research and development of sensor devices. So, the stage from synthesising a receptor molecule to employing it as a real ionophore in a sensor is inhibited. To connect the two research areas, substantial interdisciplinary effort is required.

The aim of this dissertation is to demonstrate the challenges of ionophore and sensor prototype development and contribute to bridging the gap between the two areas of research.

2. LITERATURE OVERVIEW

2.1 Supramolecular chemistry

Supramolecular chemistry studies the discrete non-covalent interactions of molecular systems.[1] Two Nobel prizes in chemistry (1987 and 2016) have been awarded for achievements in supramolecular chemistry.[2,3]

Often deemed host-guest chemistry, supramolecular chemistry explores the non-covalent interactions of two (or more) molecules or ions. Differentiation is possible by the roles in the interaction. The host molecule usually binds the guest inside a binding pocket, forming a supramolecular complex. The host and the guest may have either opposite charges or no charge at all. Three main complexation characteristics are typically studied – affinity of the host towards the guest, selectivity of the host towards a particular guest and the geometry of the complex.[4,5] In this work, the hosts are synthetic anion receptors (or ionophores, if the receptors have been introduced to a sensor membrane) and guest molecules are carboxylate anions.

Affinity describes the strength of binding and is expressed as the association (or also binding) constant K_{ass} (equations 1 and 2). It is often expressed in a logarithmic scale as $\log K_{ass}$.[6]

$$\mathrm{H} + \mathrm{G} \underset{\longleftarrow}{\overset{K_{ass}}{\longrightarrow}} \mathrm{HG} \tag{1}$$

$$K_{ass} = \frac{a_{HG}}{a_H a_G} \tag{2}$$

There is a delicate balance in finding an optimal $\log K_{ass}$ value. If it is too low, the complex that forms may not be observable and such host usually tends to have low selectivity. Too high and dissociation of the formed complex, although perhaps highly selective, will be pushed back to such an extent that it may not be possible to regenerate the host in free form. Such hosts may be applicable for single-use purposes only, *i.e.,* in practical applications, they may not be regenerative.

Binding affinity is directly linked to the Gibbs free energy of binding (equation 3) and is therefore influenced by both enthalpy and entropy contributions (equation 4). Depending on the scenario, either one of them can be the dominant influencer, or they may contribute similarly.

Thermodynamic effects are essential in guiding desolvation, conformational changes and competition with interfering species during binding.

$$\Delta G^{o} = -RT \ln K_{ass} \tag{3}$$

$$\Delta G^{o} = \Delta H^{o} - T\Delta G^{o} \tag{4}$$

Binding processes are competitive since molecular interaction forces are universally compatible with various guests. The most common competitor during binding is the solvent. The first step in binding is the desolvation of the binding pocket and the guest. Therefore, binding effects differ substantially in different environments depending on the solvent.

Due to its molecular properties, water is considered the most challenging environment for binding to be manipulated.[7] Since all solvents contain water to at least some extent (perhaps in trace amounts), hydration is also an essential factor in binding.[8]

Selectivity describes the ability of the host to differentiate a single guest from interferents ($K_{\mathrm{ass}}^{\mathrm{A}} \gg K_{\mathrm{ass}}^{\mathrm{B}}$). Reaching high selectivity, rather than high affinity, is a grand challenge for chemists. At the same time, it is perhaps the single most difficult task in supramolecular chemistry, much more demanding than reaching high affinity. Even nature itself occasionally has difficulties with achieving high selectivity. However, from the perspective of nature, the selectivity issue functions as an essential regulator in living organisms. Insufficient selectivity is exploited in various biological and medical applications, mainly by influencing the inhibition mechanisms of proteins.[9]

Geometry of the host-guest complex reveals information about the "fit" and binding mode (*i.e.,* interaction forces). Visualisation is needed to fully explain both affinity and selectivity of the complex.

Experimental geometry is primarily determined by X-ray crystallography or NMR. The first method requires the possibility of growing a single crystal of the host-guest complex, which is not always possible. However, X-ray crystallography provides the highest accuracy of the obtained structure. NMR methods are more accessible but are not as accurate since the obtained data may not always be conclusive. Geometry can also be estimated using *in silico* calculations. These methods are the most accessible, as no actual compounds are required. Prediction accuracy of the most stable conformers in solution is generally reasonable.[10]

2.2 Anion receptor design

Three types of receptor molecules can be distinguished when considered from the perspective of the analyte – cation, anion and dual binders.[11,12] This dissertation deals exclusively with binding anions, focusing on carboxylates. Throughout this work, all design criteria aim to apply mainly to synthetic anion receptors.

The following design criteria must be addressed:

- interaction forces in the binding pocket,
- geometry of the binding pocket,
- interfering functional groups,
- synthetic accessibility and feasibility,
- lipophilicity.

Interaction forces include hydrogen bonds, Van der Waals forces, ion-ion interactions, π-π interactions between aromatic systems (sometimes called π-stacking) and solvophobic effects.[8,13]

Receptor design has to be based on the specific properties of the target (analyte) anion. All parts of the analyte should be considered. This may mean that different interaction forces must be addressed. HBs are the most common interaction type as they are the second strongest non-covalent interaction force after ion-ion interactions.[13] The introduction of HBD and HBA functional groups is generally more achievable than those necessary for alternative interaction forces (or at least, to a necessary extent). Therefore, HBD groups are the most common interacting groups in carboxylate binding. This is further supported by the property of carboxylates to act as strong HBAs.

An example of a highly selective anion receptor class not relying on HBs in a classical sense is bambusuril. The partially flexible methylene C-H fragments of bambusuril form a surface with a positive partial charge. This, in addition to an enthalpy-driven, highly hydrophobic binding pocket, leads to high affinity and selectivity towards halides.[14,15]

Geometry of the receptor and the binding pocket is the main influencing factor for both affinity and selectivity. For geometry, two types of receptors can be described: flexible and pre-organised. Flexible receptors go through a major conformational change during binding.[12] However, if the structure of the host is not pre-organised, an additional entropic penalty to affinity must be paid for the initial conformation change.[16,17] Too much rigidity of the host is also counter-productive as it may neglect the necessary adaptability for size-matching.[18,19]

In most cases, hosts are much larger in size than guests, especially if the guest is encapsulated during binding (*e.g.,* in the case of macrocycles). With the increasing size of the anion, the surface area of the surrounding binding pocket (if the receptor is planar) increases proportionally to r^2 (where r is the distance from the centre of the anion) and volume (if spatial) increases proportionally to r^3. Since the electrostatic surface of anions extends beyond its physical border, binding pockets need to be large to fully accommodate the anion and not leave it partially bound out of plane with the receptor (publication I). Such partial binding diminishes both affinity and selectivity. When also including the length of HBs, the size requirements for the binding pocket quickly grow.

The binding pocket of the host must contain functionalities that give access to the necessary interaction forces for binding the guest. However, since interaction forces are universal, careful positioning is paramount. In nature, proteins employ filtering of possible interferents by applying selection criteria for access to the binding pocket. One such system is the phosphate binding protein complexed with a fully desolvated HPO_4^{-2} anion.[20] Here, high selectivity is achieved via a single strategically placed carboxylate group in the side chain of Asp56 of the host. This repulses oxoanions that do not have an HBD present, such as SO_4^{2-}, but acts as an HBA for HPO_4^{2-}. However, this introduces a pH requirement for the protein.

In carboxylate binding, the main interacting species is the carboxylate group X-**COO**$^-$, which can form several hydrogen bonds that give good stability to the complex (Figure 1). This is due to a favourable geometry of the carboxylate group in addition to its delocalised negative charge spread across two equivalent oxygen atoms.[21] The residue **X**-COO$^-$ offers possibilities for differentiation by geometry (publications I and III) and size.[22] Common receptor types offering good compatibility with these properties include (thio)ureas, carbazoles, indolocarbazoles and their derivatives. These receptors provide HBDs that favour linear HBs, the strength of which is dependent on the angle of the bond.

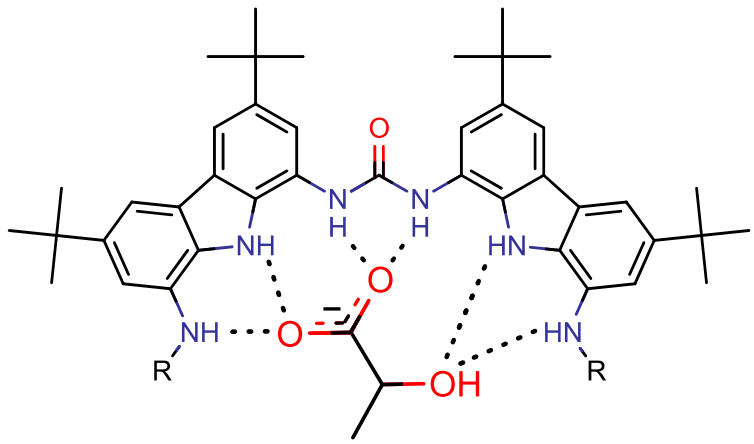


Figure 1. Possible structure of a complex between lactate and a multi-HBD receptor based on a computational prediction presented in publication III.

Achieving the perfect fit for the analyte requires thorough analytical planning. Distance between interaction centres is crucial to prevent intramolecular bonding. Inspiration can again be found in natural systems, *e.g.,* proteins. The vast majority (if not all) of biological systems have binding pockets that approach the target species from all spatial directions. This is not easy to achieve with synthetic receptors, where the binding pockets often have planar structures. An effective strategy is to employ macrocyclic cavitands, such as *e.g.* bambusurils, cyanostars and calixarenes.[14,23,24] Most molecules in these classes are hydrophobic. Although water-soluble receptors are more desired in academic research, hydrophobicity may provide an advantage for sensor building.[7]

Interfering groups of the host during carboxylate binding are proton-donating groups (*e.g.,* R-COOH, R-CO-NH-CO-R) with sufficient acidity to induce proton transfer. These groups may protonate the analyte, thus making it impossible to track the concentration changes of the host and/or guest. However, knowing the concentration of the free guest is a prerequisite in absolute titration methods, and interfering groups may introduce a significant measure-

ment uncertainty source. Interfering groups may also lead to the reduction of both affinity and selectivity.

Synthetic accessibility problems are associated with complicated reaction pathways, where being able to prepare the receptor requires effort and high skill in synthesis. As described, anion receptors are usually large. This often leads to the necessity of multiple reaction steps, and one-pot syntheses are rarely available. Problematic reactions should be addressed before actual synthesis. It is important to know which immobilisation strategy is used for the ionophore in an ISE since the introduction of functional groups necessary for either approach could be accessible in specific synthesis stages. Feasibility issues may not initially prove to inhibit research but may do so in the long run if commercialisation is considered. However, lack of repeatability or low overall yield of the synthesis route is problematic for technology transfer from prototype to commercial output.

Lipophilicity, expressed as $\log P_{o/w}$, is important once the receptor becomes an ionophore. Two main strategies exist for ionophore immobilisation – via a covalent bond onto the carrier surface or by dissolution into the carrier membrane.[25–27] Functional groups necessary for sufficient lipophilicity are often more easily accessible than those required for covalent immobilisation. In this work, the receptors are designed to be used for the dissolution method. Suppose multiple receptor molecules need to be studied during sensor development. In that case, the dissolution method offers an advantage for quickly experimenting with various ionophores as it does not require an additional reaction step for covalent immobilisation. The drawback of this method is the possibility of the ionophore leaching out of the membrane into the measured solution (usually aqueous). This limits the life span of the sensor. High lipophilicity helps counteract this scenario. However, accurately determining high $\log P_{o/w}$ values is challenging (publication II). If receptors are immobilised using covalent bonding, then lipophilicity of the ionophore is not a concern.

2.3 Binding affinity measurements

Two principal measurement strategies exist for determining host-guest binding strength – absolute and relative.[6,28]

In **absolute measurements**, a single guest is measured against a single host. The measurement proceeds essentially according to equation 2, whereby the species' activities (often approximated by concentrations) are measured under equilibrium conditions. As a result, the absolute logK_{ass} value is obtained. Contrary to its name, "absolute" does not describe accuracy or quality. These types of measurements typically have higher metrological uncertainty than relative measurements.[29] However, the vast majority of affinity measurements published in literature are absolute.

In **relative measurements**, equilibrium is achieved between two or more hosts and a single guest, as shown in equation 5. Differences between the logK_{ass} values of two (or more) hosts with the same guest are obtained using equation 6.

$$H_A + H_BG \overset{\Delta K_{ass}}{\rightleftharpoons} H_AG + H_B \tag{5}$$

$$\Delta \log K_{ass} = \log K_{ass}(H_AG) - \log K_{ass}(H_BG) = \log \frac{a_{H_AG} a_{H_B}}{a_{H_BG} a_{H_A}} \tag{6}$$

If the logK_{ass} value for one host is known, logK_{ass} values for other guests can be calculated.

In contrast to absolute measurements, relative measurement methods have much higher accuracy since several uncertainty sources decrease or cancel out. Since activity ratios of both hosts in the free and bound state can usually be considered similar, the ratios of activities in equation 6 can be replaced with ratios of equilibrium concentrations. As a result, equation 7 can be derived.[30]

$$\Delta \log K_{ass} = \log K_{ass}(H_AG) - \log K_{ass}(H_BG) = \log \frac{[H_AG][H_B]}{[H_BG][H_A]} \tag{7}$$

Theoretically, there is no limit to how many hosts can be measured simultaneously as long as, for every receptor, the ratio of free and bound receptor can be measured (*e.g.,* in NMR measurements, the signals of different receptors can be differentiated). The output of relative measurements is a binding ladder (Figure 2).

Each measurement in the ladder circle validates all other measurement points. The prerequisite for this is that at least one receptor is shared across different measurements, *i.e.,* different parts of the ladders (from different measurements) must be connected by at least one host. This is usually an anchor molecule (*i.e.*, a reference compound with a known value that is kept constant during the least squares minimisation process), but this is not a strict require-

ment. The results can be mathematically (double) minimised for additional precision to obtain highly accurate logK_{ass} values (publication III).

Binding ladders are excellent for comparing different receptors towards a particular anion. If measurement parameters (temperature, solvent system, binding stoichiometry, *etc*.) are kept constant, then there is also no limit to the number of receptors that can be accommodated in a binding ladder.

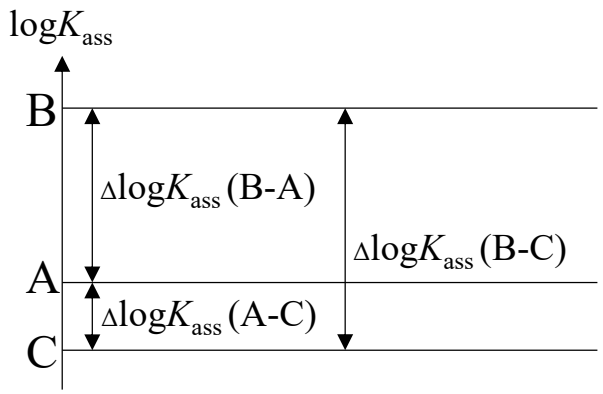


Figure 2. Graphical representation of a binding ladder where, *e.g.,* three different hosts are measured against each other for a single guest.

Absolute binding measurements can be conducted with several different measurement techniques, such as titrations with spectroscopy (mainly UV-Vis and NMR) or isothermal titration calorimetry.[6,31,32] For relative binding measurements, NMR is the preferred method.[30]

2.4 Biphasic logK_{ass}

As described, binding affinity is classically analysed by a titration experiment in a single phase, typically an organic solvent or its mixture with water, as most receptors tend, or are specifically designed, to be insoluble in water. The values from such measurements are used to predict selectivity patterns in practical applications, such as preparing sensors.

As demonstrated in publication I and in literature[26,33], selectivity patterns in real sensors may not match with those predicted from single-phase measurements. This is not surprising, as classical binding affinity titrations fail to consider several factors that influence binding in real-world applications. These can include (but are not limited to):

- significant differences in water content of the analysed sample compared to laboratory titrations[34],
- the lipophilicity of the molecular species involved in complex formation (publication II),
- the physical phase where complex formation occurs, *e.g.,* the semi-liquid polymeric membrane in solid contact ISE-s,
- acidities of the chemical species involved in binding[35,36],
- interfering species present in actual samples,
- influence from the cation.[37]

Some of the shortcomings of classical binding experiments are addressed when employing a biphasic system. To mimic the binding environment of an ISE, it is possible to use a water-immiscible solvent. Although it is not identical to the polymeric sensor membrane, the phase transfer of ions becomes an influencing factor. The analyte ion is present in its "natural" aquatic environment, and the organic phase is saturated with water, further mimicking the conditions present during solid contact ISE measurements.

This dissertation explores the possibility of a new approach (termed biphasic logK_{ass} measurements) for more accurate binding characterisation.

3. EXPERIMENTAL SECTION

3.1 Instruments and equipment

NMR measurements

All structural analysis and logK_{ass} measurements were performed with a Bruker Avance-III 700 MHz spectrometer. The field strength of the superconducting magnet was 16.4 T. Spectra were obtained using TopSpin 3.2 software and calibrated either against the solvent residual signal or tetramethylsilane.

Binding affinity titrations were carried out as described in literature.[30] The solvent used for single-phase logK_{ass} measurements was DMSO-d_6/H_2O (0.5% m/m).

HRMS

Mass spectra were obtained using a hybrid Varian 910-FT-ICR-MS system equipped with an electrospray ion source. Experimental parameters used for HRMS were as described in publication I.

Synthesis

All chemicals used for synthesis were procured from commercial sources with a purity of ≥97% or prepared by the author. Solvents used for synthesis were dried using 3 Å molecular sieves or with a VAC 103991 continuous circulation system. Water was procured from a MilliQ Advantage A10 system. All inert gases had a purity of 5.0.

Flash chromatography was performed using Flash LC Reveleris X2 equipment with normal-phase Reveleris Silica 4g columns.

Detailed synthesis procedures are described in publications I–III and Appendix 1.

3.2 Biphasic measurement method

For the preparation of experimental samples, the receptor molecule is first weighed in a vial and dissolved in a water-immiscible organic solvent previously saturated with water. This solution is then divided into aliquots. For the preparation of the analyte solution, an exact amount of substance is weighed in a vial and dissolved in water. Then, the sample is divided into sub-samples and diluted according to the selected equivalents of the analyte for each titration step. The ion solution's final volume is adjusted so that the aqueous phase is in excess of the organic phase. The organic and aqueous phases for each titration step are added together and mixed thoroughly, *e.g.,* with a Vortex mixer. After separating the phases, the organic phase for each titration step is collected, and the free and bound receptor ratio is measured. For an example titration procedure, please refer to Appendix 2.

NMR was used for analysis. However, biphasic $\log K_{ass}$ measurements could also be done with any other suitable technique. While NMR generally employs deuterated solvents, it is possible to measure biphasic $\log K_{ass}$ values without ^{2}H in either phase if the sample is carefully shimmed and the spectrum internally calibrated. A high resonance frequency of ^{1}H is desirable for acceptable spectrum quality if solubility is a concern.

While not done in this work, there are no apparent reasons why biphasic $\log K_{ass}$ measurements couldn't be carried out as relative measurements.[38]

3.3 Sensor development flowchart

The general workflow of sensor development is outlined in Scheme 1 and is divided into four stages, each subsequently consisting of several steps. Re-visiting a previous (or several) stage may be necessary. The flowchart may be used for a single or several receptor molecules simultaneously.

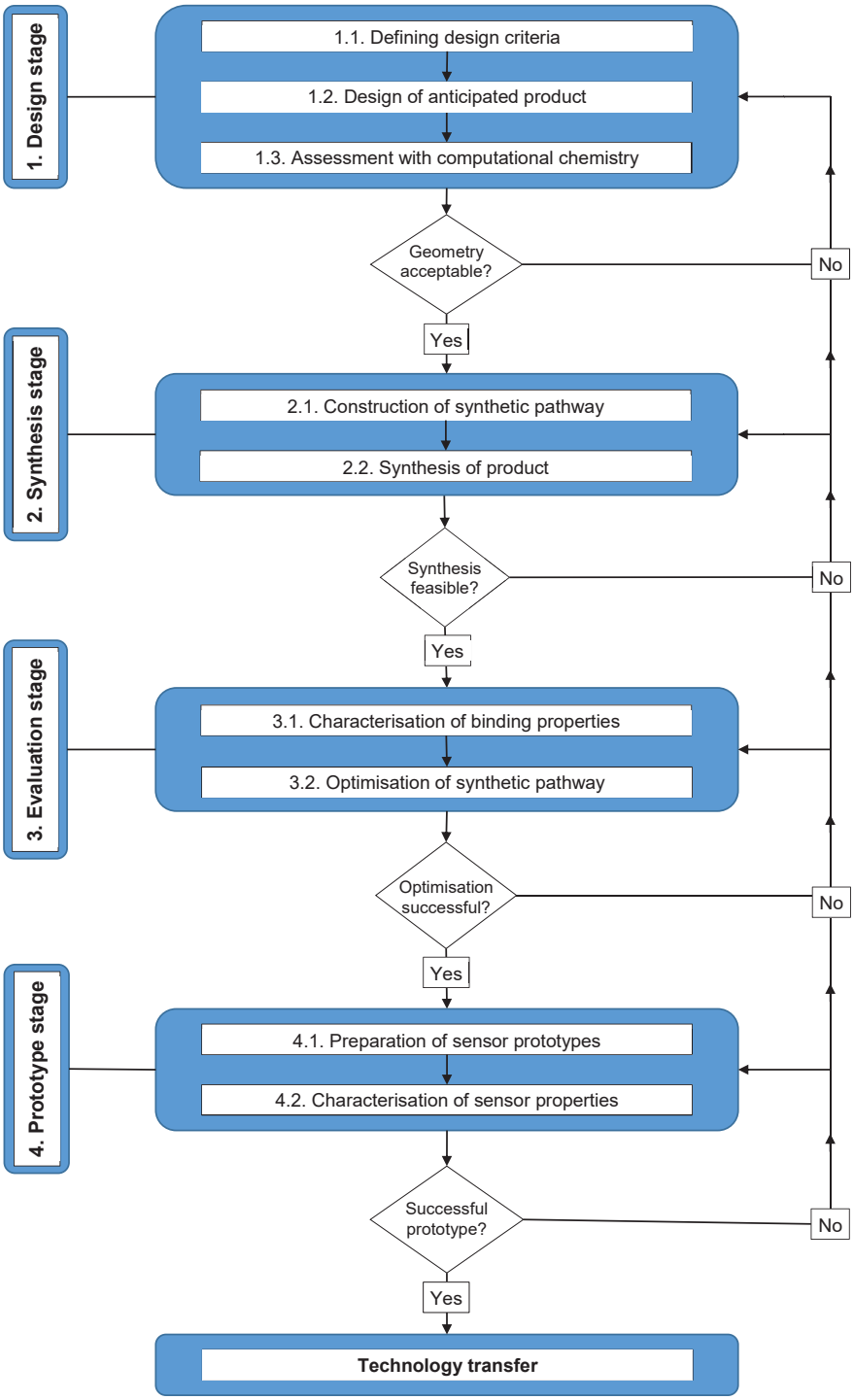
In the **design stage**, the necessary properties for the receptor, highlighted in chapter 2, are addressed. The stage is ideally concluded with *in silico* assessment of the receptor candidate. This decreases the probability of adverse outcomes in the subsequent stages by eliminating host-guest complexes with an unfavourable fit.

The **synthesis stage** requires the construction of an initial synthesis pathway for the receptor candidate. In this stage, reaching a synthesis product with any yield is acceptable as long as the pathway is robust enough to be successfully repeated. Preparative isolation of the purified compound is necessary to proceed to the next stage. Optimising the synthesis pathway is more reasonable after binding parameters have been evaluated as suitable.

During the **evaluation stage**, affinity and selectivity are assessed. Often, thermodynamic binding parameters are determined. Visualisation of the host-guest complex may be carried out by crystallography, NMR or *in silico* methods. This stage concludes with selecting a specific receptor to be used as an ionophore. Optimisation (potentially including scale-up) of the synthetic pathway should be done at this stage, as the commercialisation of a successful prototype requires the ability to mass-produce all parts of the sensor, including the ionophore. Optimisation, however, is not very common, as low target yields are not basis for rejection by academic press.

The space between the 3rd and 4th stages is where the sensor development dead zone is.[39]

In the **prototype stage**, the receptor candidate becomes an ionophore after its introduction into (or onto) the sensor membrane. This stage is mainly hindered by suboptimal results achieved in step 4.2, where sensor properties are characterised. Insufficient selectivity is the single most common issue where a successful prototype cannot be completed.[26,40] However, other factors may potentially also interfere with moving on to technology transfer, such as, *e.g.* inadequate access to a sufficient amount of ionophore or the limited life span of the prototype.



Scheme 1. Flow chart of a sensor development lifecycle.

4. RESULTS AND DISCUSSION

4.1 Investigated anions

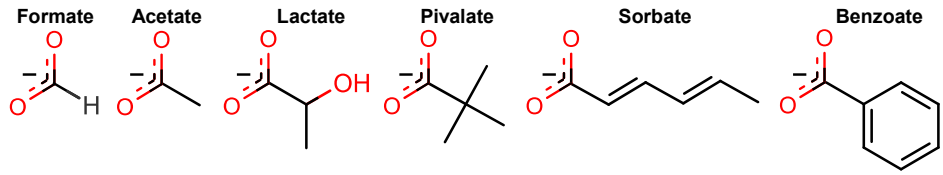
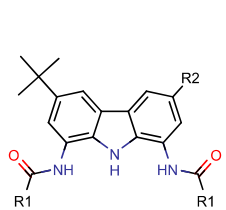
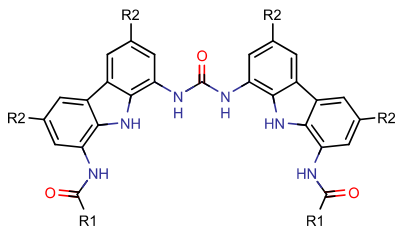


Figure 3. Structures of investigated carboxylates.

The anions investigated in this dissertation (Figure 3) are structurally diverse enough to make deductions for structure-affinity relationships. They range from small (formate) to medium-sized (benzoate, sorbate) anions; from hydrophilic (formate, lactate, acetate) to hydrophobic (sorbate, benzoate) anions; both aliphatic (acetate, pivalate), unsaturated (sorbate) and aromatic (benzoate) anions are represented. They are primarily common carboxylates encountered in research and industrial settings and therefore require accurate sensing methods. Currently, chromatographic methods are favoured for quantifying carboxylates, such as reverse phase liquid chromatography, ion chromatography, hydrophilic interaction chromatography with different detectors and gas chromatography alongside derivatisation techniques.[41–44] These techniques only operate under laboratory conditions and require highly competent personnel and considerable resources to carry out the measurements. Access to portable sensor systems would significantly reduce such requirements and make carboxylate sensing available to a broader population.

The selected anions have also been studied in several references, making them available for the double-minimisation process (publication III).

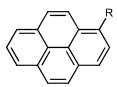




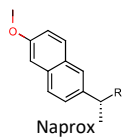
Code	R1	R2
CZ01	-O(t-Bu)	t-Bu-
CZ02	-$(CH_2)_3$-Pyr	t-Bu-
CZ03	-NH-Napht	t-Bu-
CZ04	-NH-Ph	t-Bu-
[CZ05]	-NH-Ph	t-Bu-
CZ06	-NH-(2-NO_2-Ph)	t-Bu-
CZ07	-CH_2-NH-Boc	t-Bu-
CZ17	-NH-Napht	-H

Code	R1	R2
CZ08	-O(t-Bu)	t-Bu-
CZ09	-$(CH_2)_{10}CH_3$	t-Bu-
CZ10	-NH-Napht	t-Bu-
CZ11	-NH-Ph	t-Bu-
CZ13	-O(t-Bu)	-hexyl
CZ18	-$(CH_2)_3$-Pyr	t-Bu-
CZ19	Naprox	t-Bu-
CZ20	-CH_2-(t-Bu)	t-Bu-

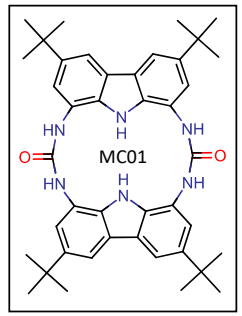
Napht



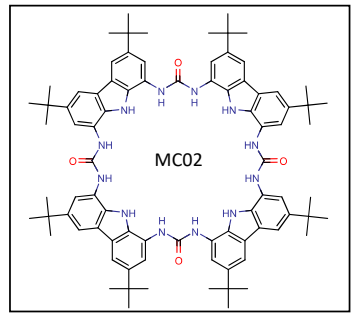
Pyr



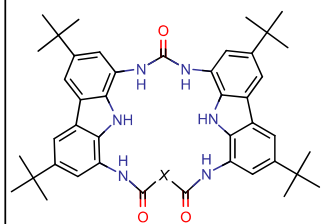
Naprox



MC01

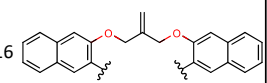
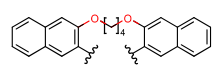


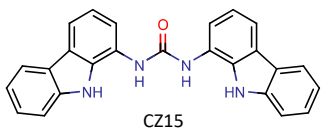
MC02



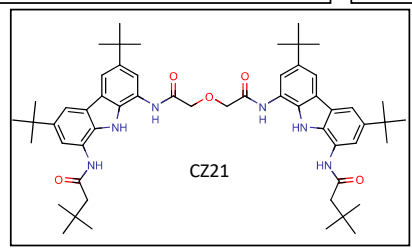
MC03 - MC14

Code	X
MC03	-$(CH_2)_3$-
MC04	-$(CH_2)_4$-
MC05	-$(CH_2)_5$-
MC06	-$(CH_2)_6$-
MC07	-$(CH_2)_7$-
MC08	-$(CH_2)_8$-
MC09	-$(CH_2)_9$-
MC10	-$(CH_2)_{10}$-
MC11	-$(CH_2)_{11}$-
MC12	-$(CH_2)_{12}$-
MC13	-$(CH_2)_{13}$-
MC14	-$(CH_2)_{14}$-

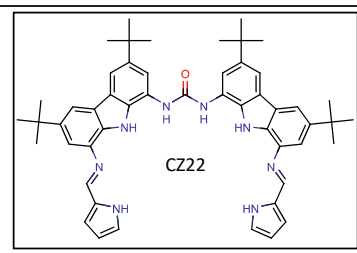
Code	X
MC16	
MC17	



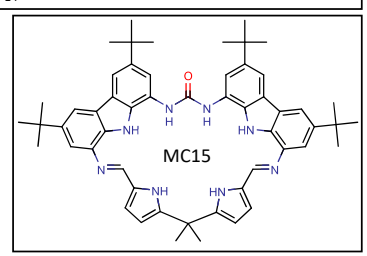
CZ15



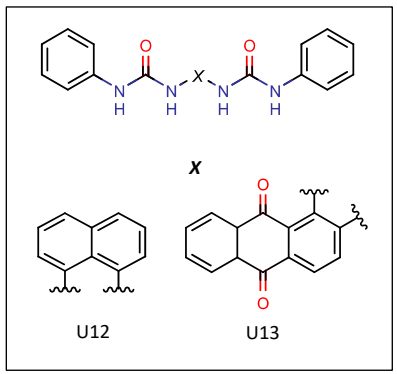
CZ21

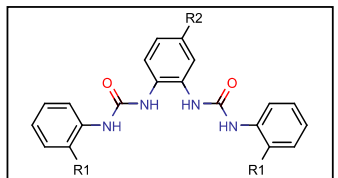


CZ22

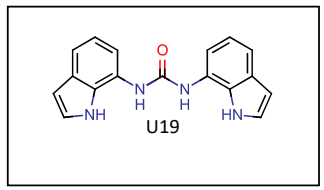


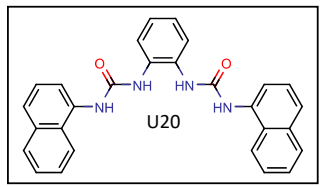
MC15

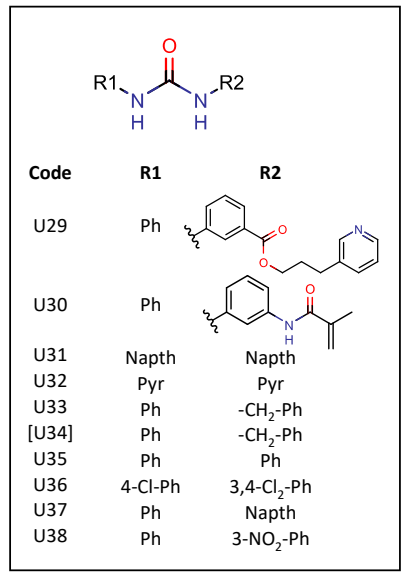




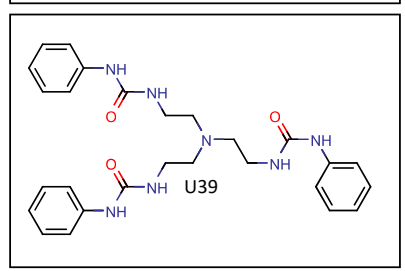
Code	R1	R2
U16	-NH_2	-H
U17	-H	-CH_3
U18	-H	-H
U24	-NH-CO-Ph	-H
U25	-NH-CO-$(CH_2)_2$-Ph	-H
U26	-NH-CO-$(CH_2)_{10}CH_3$	-H

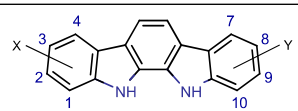






Code	R1	R2
U29	Ph	
U30	Ph	
U31	Napth	Napth
U32	Pyr	Pyr
U33	Ph	-CH_2-Ph
[U34]	Ph	-CH_2-Ph
U35	Ph	Ph
U36	4-Cl-Ph	3,4-Cl_2-Ph
U37	Ph	Napth
U38	Ph	3-NO_2-Ph





Code	X	Y	Code	X	Y	Code	X	Y
IC09	-H	-H	IC20	2-COOBu	9-COOBu	IC31	1-CF_3	-H
IC10	2-COOBu	7-COOBu	IC21	4-COOBu	7-COOBu	IC32	4-COOBu	-H
IC11	2-COO-CH_2-CO-CH_3	-H	IC22	2-Cl	7-Cl	IC33	2-COO-CH_2CH_3	-H
IC12	4-NO_2	-H	IC23	2-OCH_3	9-OCH_3	IC34	2-COO-$CH(CH_3)_2$	-H
IC13	2-OH	-H	IC24	2-CH_3	9-CH_3	IC35	2-NO_2	-H
IC14	2-O-Boc	-H	IC25	4-CH_3	7-CH_3	IC36	4-OCH_3	-H
IC15	2-COO-$(CH_2)_3$-OH	-H	IC26	2-OCH_3	7-OCH_3	IC37	2-OCH_3	-H
IC16	2-COOBu	-H	IC27	1-CF_3	10-CF_3	IC38	2-COO-CH_2-(4-OCH_3-Ph)	-H
IC17	1-Cl	-H	IC28	1-Cl	10-Cl	IC39	2-COO-CH_2-(3-OCH_3-Ph)	-H
IC18	2-COO-CH_2-Ph	-H	IC29	2-Cl	9-Cl	IC40	2-Br	9-Br
IC19	2-COO-CH_2-(4-NO_2-Ph)	-H	IC30	4-Cl	7-Cl	IC41	1-CH_2-OH	10-CH_2-OH

Figure 4. Structures of investigated receptors.

4.2 Design and synthesis

Two families of receptors were synthesised in this dissertation, employing three synthesis routes that were carried out and optimised. Two routes lead to the receptors of the bis-carbazolylurea family, shown in Scheme 2 and Scheme 3. These compounds have previously demonstrated considerable potential as successful anion binders.[5,45–47] The third route investigates the bambusuril moiety, as shown in Scheme 4. The receptors are suitable candidates for binding carboxylates presented in Figure 3.

Bis-carbazolylurea

Most receptors of the CZ and MC families were accessed using the synthetic pathway shown in Scheme 2. [48–50] There are two possible strategies towards the target compound **8**, which acts as a precursor for most high-affinity receptors investigated in this work.

Both pathways start with Friedel-Crafts alkylation of carbazole in nitromethane to yield compound **2**.[48] Reproducing the yield reported in literature is challenging, partially due to a suspension that forms between water, nitromethane and DCM during purification. Alkylation of the 3 and 6 positions ensures that the subsequent substitutions are directed to positions 1 and 8 of carbazole, thereby forming a binding pocket with favourable geometry. Onwards, two possibilities are presented. One method is to nitrate compound **2**. However, the highly acidic environment leads to the loss of one tert-butyl protection group.[50] Subsequent reduction leads to a mixture of diamine **5a** and **5b**, which are difficult to separate due to similar R_f values. A more feasible approach is to brominate compound **2**, which can be aminated to **5a** in higher yield without the need for purification by column chromatography.

Coupling the diamine **5a** to form the bis-carbazolylurea backbone requires first protecting one NH_2 to prevent cyclisation and polymerisation of the reaction mixture. Boc-protecting the diamine leads to an equilibrium reaction where a balance between the unreacted starting material, mono- and di-protected Boc compounds is achieved. This significantly reduces the overall yield of the synthesis pathway and is a limiting step for ionophore production. Coupling and deprotecting the bis-carbazolylurea moiety to achieve target compound **8** is done in good yield and proceeds under easily achievable reaction conditions.

Scheme 3 shows the synthetic pathway to **CZ13**, a highly lipophilic carboxylate host. This molecule was designed to inhibit ionophore leaching from the ISE membrane into solution, thus increasing the prototype's lifespan. To achieve this, positions 3 and 6 (usually substituted by tert-butyl groups) of the carbazole moiety were equipped with hexyl groups to increase the lipophilicity. As shown in Figure 5, this indeed increased the receptor's lipophilicity.

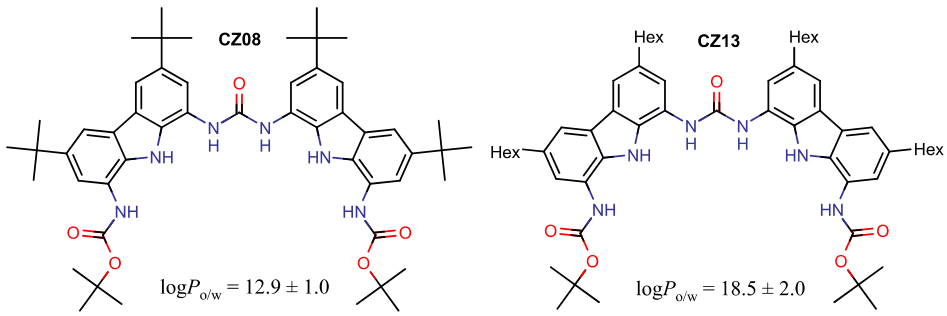
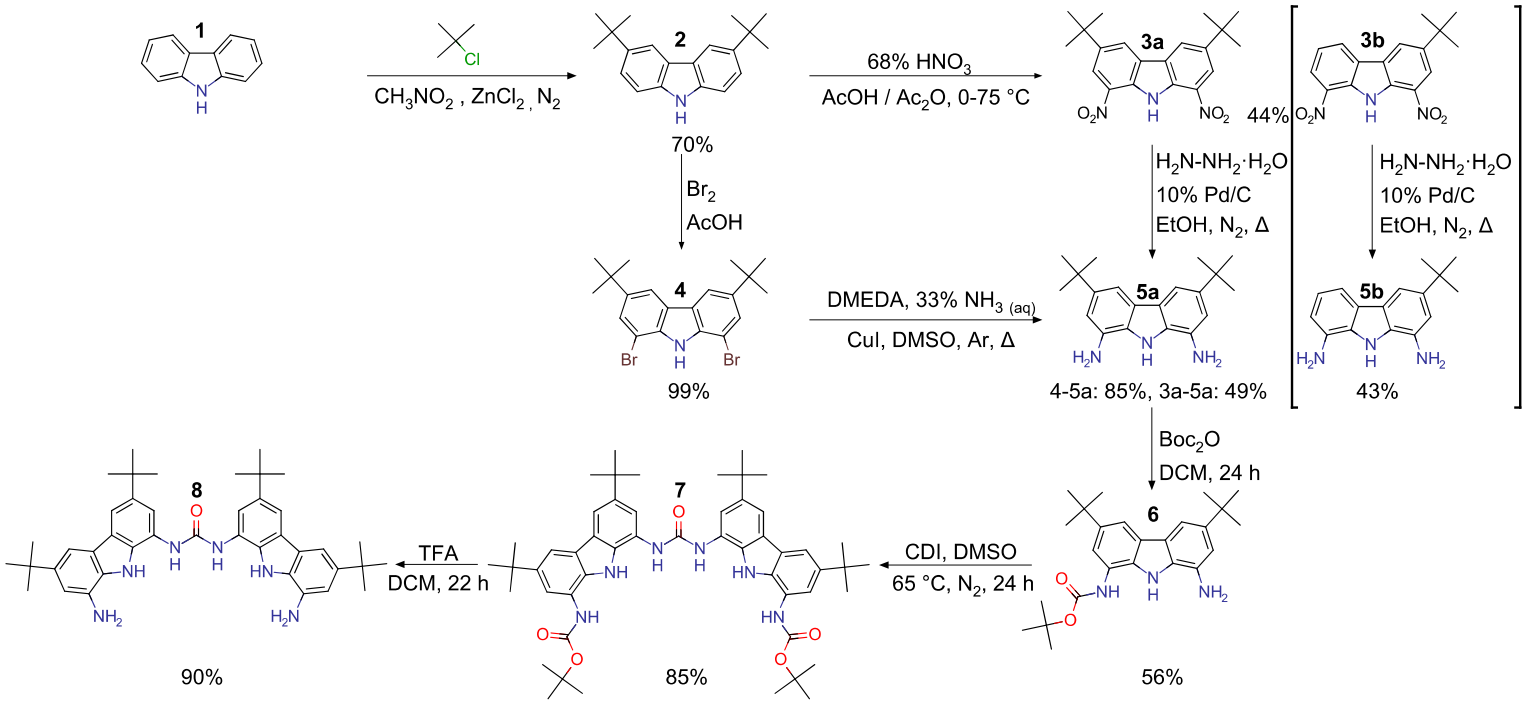


Figure 5. Comparison of log$P_{o/w}$ values of CZ08 and CZ13.

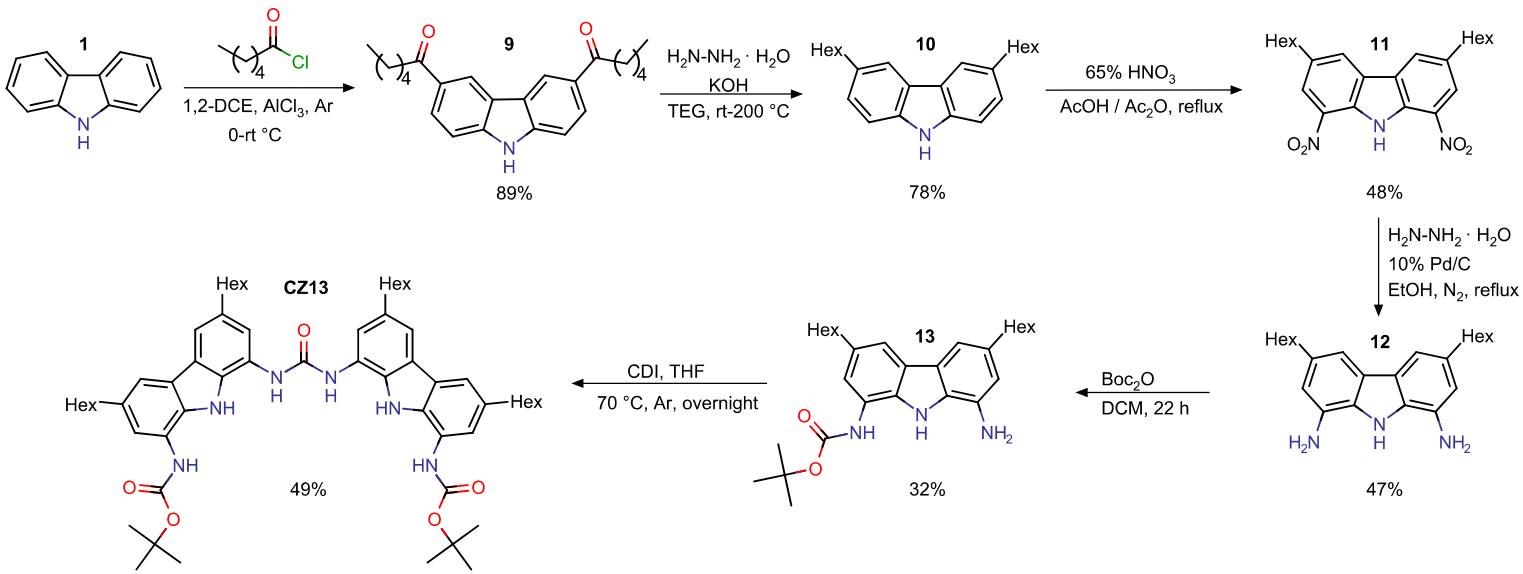
The synthesis pathway starts with Friedel-Crafts acylation of carbazole, which protects positions 3 and 8 of carbazole, similarly to the pathway towards diamine **5a**. Compared to the alkylation process in Scheme 2, acylation towards compound **9** provides better yield and a more straightforward workup procedure, as no suspension forms during extraction. However, it does prompt an additional reaction step for reduction. Compound **10** was obtained using the Huang-Minlon modification of the Wolff-Kishner reaction.[51] Nitrating compound **10** did not remove the protection groups as in Scheme 2 but did not improve yield. Successive reaction steps present similar challenges as in the main pathway (Scheme 2) for the CZ and MC receptors.

Bambus[6]uril

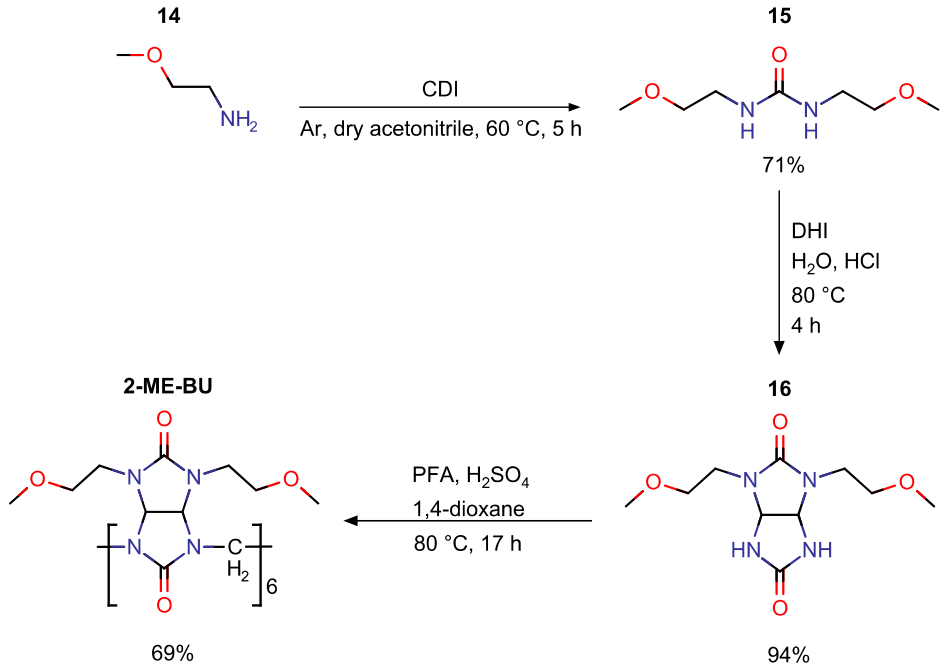
The synthesis of **2-ME-BU** (Scheme 4) was inspired by a similar 6-step reaction pathway published by Fiala *et.al.*[15] The modified route developed in this work stands out as a pathway with generally good yields, few steps and the potential to be advanced into one-pot synthesis. Achieving the desired reaction conditions in all steps are met without difficulties.



Scheme 2. Synthetic pathway to target diamine **8**.



Scheme 3. Synthetic pathway to **CZ13**.



Scheme 4. Synthesis of **2-ME-BU**.

The bambusuril derivative was synthesised employing a 3-step procedure as the starting material was commercially available. First, the bis-substituted urea was prepared from 2-methoxyethylamine using CDI in dry acetonitrile. The obtained 1,3-bis(2-methoxyethyl)urea was reacted with DHI in acidified water without needing an organic co-solvent. This is not common, as most ureas are not readily soluble in water. This reduced the necessary amount of DHI, as the formation of hydantoin, a common side product, was suppressed by the dehydration equilibrium. In the last step, the glycoluril monomer was polycondensated with PFA in 1,4-dioxane. The reaction was catalysed and templated with sulphuric acid. This specific solvent-acid mixture has been shown to guide the reaction towards 6-membered bambusurils effectively.[52]

All reaction products have good solubility in water. Interestingly, although pure 1,3-bis(2-methoxyethyl)urea is solid, the corresponding glycoluril does not seem to solidify even after prolonged periods at -20 °C. Also, the mixture of 1,3-bis(2-methoxyethyl)urea and imidazole (formed as a by-product from CDI) is solid at +4 °C, semi-liquid at room temperature and liquid at approximately 50 °C, well below the melting point of pure imidazole.

Out of the investigated solvents, **2-ME-BU** is soluble in acetonitrile, acetone, ethyl acetate, DMSO, DCM and chloroform. It mostly favours water as the primary solvent since extraction with chloroform or ethyl acetate is ineffective for isolating the compound during reaction workup. This aspect contributes to the challenge of removing the templating anion.

In contrast to a favourable synthesis pathway, one which meets the requirements for ionophore production, the target molecule **2-ME-BU** has only academic potential. It is unlikely to be employed as a champion host due to inaccessibility to the anion-free form.

2-ME-BU serves as an example of an ultra-high affinity host towards SO_4^{2-}. Here, the sulphate anion acts as a reaction template that provides high selectivity of the reaction (in 1,4-dioxane) towards the six-membered homologue. Changing the template results in a significant reduction of reaction selectivity. However, no purification methods were found, which would be able to remove the SO_4^{2-} template, thereby rendering the receptor unusable for any practical applications, including preparing sensors. This illustrates the need for affinity not to be too high, as otherwise, no dissociation of the host-guest complex occurs.

4.3 Selectivity trends

Obtained binding affinity results and their reliability

Table 1 presents $\log K_{ass}$ data for the investigated anions (Figure 3) with 90 receptors (Figure 4) in DMSO-d_6/H_2O (0.5% m/m), which has been double minimised, as explained in publication III. The double minimisation process ensures high reliability of the obtained results by also considering the $\Delta\log K_{ass}$ values between anchor molecules. The high reliability of the measurements is demonstrated by the low consistency standard deviations of the respective binding ladders, ranging from 0.01 (formate) to 0.03 (acetate).

The receptor molecules studied in this work are either fully planar or distorted out-of-plane to some extent (publication I). This introduces the potential for 1:2 host-guest stoichiometry, which would contradict the requirements of the calculation model for $\log K_{ass}$ values. However, no evidence of 1:2 stoichiometry was found during experimental work. 1:1 binding stoichiometry is further supported by considering the following:

- during relative binding experiments with NMR, the obtained titration curves were characteristic of 1:1 binding,
- even if binding of a second anion to the receptor were to occur at a high anion concentration, at that point, it would be much higher than realistically encountered in real-life solutions,
- $\Delta\log K_{ass}$ values are most often calculated from titration points at low analyte concentrations where the binding equilibrium would be shifted towards K_1,
- receptors titrated during the same relative binding experiment generally do not have significant $\log K_{ass}$ differences. Therefore, binding is very competitive, and a single receptor does not have the necessary affinity to bind all of the anion in the solution. Furthermore, such events would be observable in the ^1H spectrum.

Selectivity in DMSO-d_6/H_2O (0.5% m/m)

Table 1. Double minimised logK_{ass} values.

Code	CAS	Formate	Acetate	Lactate	Pivalate	Sorbate	Benzoate
CZ01	1709799-61-0	1.44	2.41	1.70	3.10	2.44	2.33
CZ02	1709799-62-1	2.44	3.39	2.07	3.82	3.48	3.02
CZ03	1709799-63-2	3.65	4.70	3.42	4.95	4.78	3.91
CZ04	2197988-18-2	3.50	4.61	3.63	5.06	4.62	3.97
CZ05	2765272-95-3		3.65	2.51			3.19
CZ06	2765272-96-4		4.45	2.77			4.17
CZ07	2765272-97-5		3.86	2.49			3.31
CZ08	1456532-80-1	3.27	4.97	3.18	5.02	4.43	3.99
CZ09	2484917-56-6	3.80	4.83	3.30	5.22		4.25
CZ10	2098494-22-3	3.64	4.99	3.82	5.39	5.01	4.18
CZ11	2098490-63-0	3.94	4.87	3.76	5.26	4.89	4.29
CZ13	2765272-94-2		4.24				3.79
CZ15	1154733-14-8	3.48	4.58	3.30	5.39	4.62	4.10
CZ17	2839699-26-0		3.95	2.66			3.48
CZ18	2839699-27-1		4.22	3.05			4.09
CZ19	2839699-28-2		4.90	3.48			4.32
CZ20	2763825-59-6	3.77	4.86	3.57		4.87	4.27
CZ21	2763825-60-9	2.82	3.54	2.54		3.67	2.99
CZ22	2763825-61-0	3.70	4.66	3.36		4.50	4.02
MC01	2484917-43-1	2.82	3.29	2.27	3.59		2.89
MC02	2484917-44-2	2.63	3.30	2.68	3.46		2.96
MC03	2484917-45-3	2.68	3.56	2.42	3.75		2.92
MC04	2484917-46-4	3.48	4.48	3.00	4.45		3.71
MC05	2484917-47-5	4.05	5.01	3.37	4.99		4.19
MC06	2484917-48-6	4.43	5.17	3.48	4.97		4.59
MC07	2484917-49-7	4.80	5.70	4.05	5.71		4.90
MC08	2484917-50-0	4.55	5.33	3.62	5.28		4.66
MC09	2484917-51-1	4.51	5.68	4.09	5.88		4.97
MC10	2484917-52-2	4.17	5.36	3.70	5.49		4.61
MC11	2484917-53-3	4.00	5.19	3.77	5.29		4.58
MC12	2484917-54-4	3.86	5.01	3.61	5.24		4.44
MC13	2484917-55-5	3.76	4.98	3.57	5.43		4.35
MC14	2484935-35-3	3.77	4.98	3.54	5.48		4.34
MC15	2763825-62-1	4.28	5.07	3.72		4.94	4.17
MC16	2763825-65-4	3.13	4.12	2.81		4.47	3.92

Code	CAS	Formate	Acetate	Lactate	Pivalate	Sorbate	Benzoate
MC17	2763825-63-2	3.27	4.27	2.73		4.67	3.82
IC09	60511-85-5	2.58	3.27	2.15	3.34	3.20	2.77
IC10	1573116-99-0	3.00	3.76	2.53	3.90	3.74	3.23
IC11	1573117-12-0	2.83	3.58	2.43	3.67	3.51	3.04
IC12	1448617-67-1	3.04	3.87	2.59	3.93	3.77	3.27
IC13	1709799-56-3		3.16	2.01	3.28		2.70
IC14	1709799-57-4		3.36	2.19	3.43		2.82
IC15	1709799-58-5		3.56	2.31	3.61		2.99
IC16	1573117-05-1		3.54	2.42	3.65		3.00
IC17	845620-01-1		2.88	1.88	3.07		2.54
IC18	1573117-08-4		3.55	2.42	3.60		2.99
IC19	1573117-09-5		3.59	2.42	3.65		2.97
IC20	1573116-98-9	3.02	3.76	2.54	3.89		3.22
IC21	1573117-00-6		3.77	2.55	3.88		3.20
IC22	845619-98-9		3.67	2.47	3.78		3.10
IC23	1448617-68-2		3.25	2.18	3.20	3.15	2.69
IC24	1573117-01-7		3.22				
IC25	1573117-02-8		3.28				
IC26	1448617-69-3		3.25				
IC27	1448617-73-9		1.81				
IC28	845619-91-2		2.26				
IC29	845619-99-0		3.51				
IC30	845619-87-6		3.83				
IC31	76255-97-5		2.62				
IC32	1573117-06-2		3.51				
IC33	1573117-13-1		3.57				
IC34	1573117-14-2		3.58				
IC35	1448617-66-0		3.69				
IC36	1448617-72-8		3.26				
IC37	1448617-71-7		3.27				
IC38	1573117-10-8		3.57				
IC39	1573117-11-9		3.55				
IC40	2304964-91-6		3.59	2.52			3.01
IC41	1789717-80-1		3.25	2.03			2.76
U12	614732-77-3	2.91	3.89	2.55	4.47	3.95	3.47
U13	775322-64-0	2.21	3.10	1.92	3.47	3.07	2.55
U16	948047-74-3		3.67	2.39	4.00		3.03
U17	1709799-59-6		3.63	2.46	4.02		3.06

Code	CAS	Formate	Acetate	Lactate	Pivalate	Sorbate	Benzoate
U18	13140-78-8		3.68	2.59	4.09		3.18
U19	1044762-46-0	3.61	4.64	3.30	5.13	4.63	4.04
U20	1709799-60-9	2.67	3.74	2.31	4.28	3.84	3.31
U24	948047-75-4	2.75	3.61	2.45	3.83	3.55	2.94
U25	1709799-52-9	3.07	3.87	2.45	4.06	3.82	3.22
U26	1709799-53-0	3.14	3.88	2.58	4.15	3.98	3.32
U29	1709799-54-1		3.58	2.44	3.72		3.12
U30	1709799-55-2		3.24	2.26	3.30		2.79
U31	607-56-7		2.45	1.63	2.78		2.18
U32	78751-44-7		2.65	1.80			2.42
U33	1467-21-6		2.51	1.63	2.63		2.14
U34	726-25-0		2.80	1.75	2.96		2.37
U35	102-07-8	2.63	3.32	2.31	3.45	3.28	2.88
U36	101-20-2	3.27	4.13	2.90	4.22	4.06	3.58
U37	5031-71-0		2.85	1.90	3.08		2.51
U38	2000-54-6		3.89	2.69	4.04		3.39
U39	171505-24-1	3.13	4.04	2.71	4.28	4.05	3.33

The obtained logK_{ass} values demonstrate the challenge of achieving high selectivity for carboxylate differentiation. None of the 90 presented receptors display high selectivity towards any particular anion.

Receptor molecules capable of π-π interactions with benzoate show a slight increase in logK_{ass} values. However, the additional interaction force does not contribute sufficiently to be considered truly beneficial.

The most successful receptors are based on CZ and MC core structures, showing generally high logK_{ass} values. Out of the studied compounds, these receptor types adhere to the design principles highlighted in chapter 2.2 to the furthest extent, *i.e.,* other studied receptor types meet fewer design criteria, especially those concerning geometry. The design of macrocyclic MC-type receptors also puts additional emphasis on considering the structure of the **X-**COO^- residue of carboxylates. These receptors show capping logK_{ass} values roughly near MC09, which seems to be the best fit for the anions studied in this work. However, several open-chain CZ-type receptors compete with the cyclic ones with similar logK_{ass} values while having more conformational flexibility. The need for a delicate balance between structural rigidity and flexibility can be deduced, with no single strategy providing a clear edge.

There is a clear correlation ($R^2 = 0.96$) between the affinity of receptors and the pK_a of the carboxylic acid corresponding to the anion, *i.e.*, the basicity of the anion, as shown in Figure 6.[29,53–56]

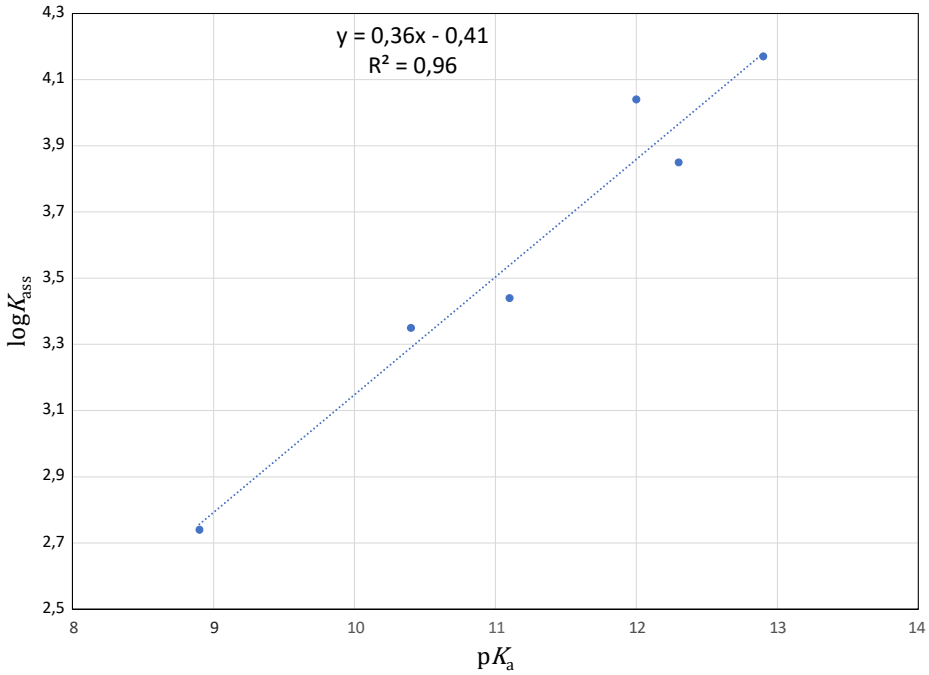


Figure 6. Dependence between logK_{ass} in DMSO-d_6/H_2O (0.5% m/m) and pK_a in pure DMSO.

Selectivity in polymeric ISE membrane at equilibrium with the aqueous phase

MC05, MC09 and MC12 were selected for the ISE prototype development as they showed favourable binding properties. The initial logK_{ass} results from binding experiments in solution suggest the best selectivity towards acetate (except for MC09), although none of the receptors show profound selectivity towards any particulate carboxylate.

The selectivity trend in a lipophilic ISE membrane at equilibrium with an aqueous solution differs from the predictions based on the logK_{ass} measurements. As seen in Figure 7, out of the investigated anions, the selectivity maximum of these receptors shifts towards benzoate instead of pivalate, which in solution was bound by all three receptors with higher logK_{ass} values than benzoate. The stark contrast in selectivity is not surprising since the binding environment is very different from DMSO-d_6/H_2O (0.5% m/m), as described in chapter 2.4.

As shown in publication III, binding affinity does not depend on the lipophilicity of the receptor. However, the distribution and availability of the anion inside the ISE membrane heavily depend on its lipophilicity. This directs the selectivity of the sensor towards more lipophilic anions. As shown in Figure 6, the logK_{ass} values measured in a homogeneous solution are strongly influenced by the acidities of corresponding acids of the carboxylates. Although a rough

approximation of $pK_a^{o/w}$ values being ~3 pK_a units higher than in water is described, specific data on carboxylic acids in a biphasic environment is scarce.[36] Therefore, the selectivity pattern may be distorted due to the differences between $pK_a^{o/w}$ and aqueous pK_a values.

The observed selectivity trend can also, to some extent, be explained by the limitations of the single-phase measurements highlighted in chapter 2.4. The sensor membrane composition also tunes binding characteristics (publication I). However, such mismatches in selectivity patterns are not universal for all sensors and depend on the receptor type, analyte species and ISE membrane composition.[57]

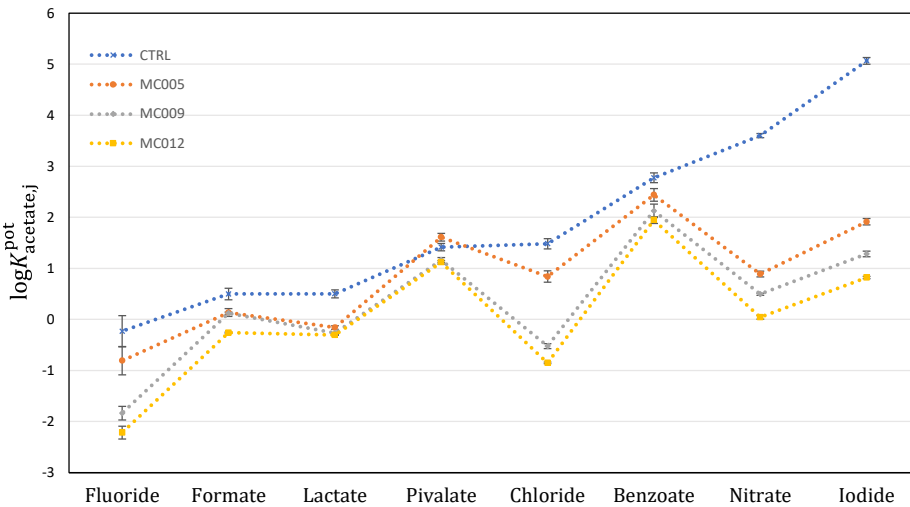


Figure 7. Potentiometric selectivity coefficients displayed by the ISE-s made with the respective receptors. CTRL refers to the ISE membrane without any added ionophore.

4.4 Biphasic logK_{ass}

Binding observations

Successful binding observations in biphasic systems were limited to CZ07+ TBA-acetate in o-NPOE$_{(aq)}$, CZ09+TBA-acetate in BzOH$_{(aq)}$ and U36+TBA-benzoate in 2-Me-THF$_{(aq)}$. No other receptor proved soluble enough in any other investigated biphasic solvent mixture. These included BzOH$_{(aq)}$, octanol$_{(aq)}$, 2-Me-THF$_{(aq)}$ and bis(2-ethylhexyl) sebacate$_{(aq)}$.

The binding of U36 with benzoate was observable in the biphasic system, but the system did not reach a titration plateau, as demonstrated in Figure 8 and Figure 9. The endpoint was not observable even at 20 equivalents of anion (where the molarity of the analyte stock solution exceeds 0.5 M); therefore, the logK_{ass} value was not quantifiable.

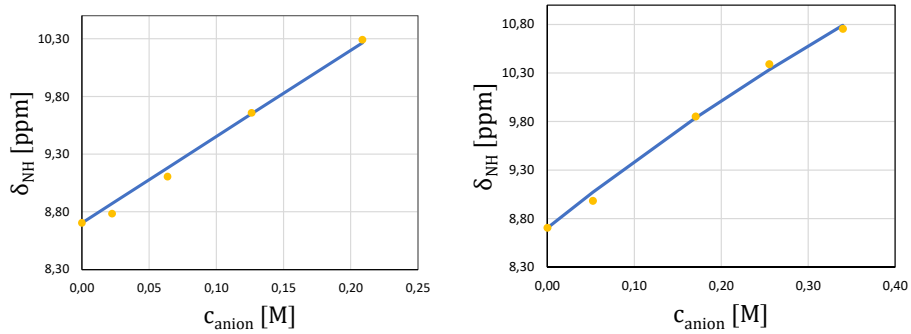


Figure 8. Binding plots of U36 and TBA-benzoate in 2-Me-THF$_{(aq)}$. The left chart shows titration steps of 0, 1, 3, 6, 10 equivalents and the right chart shows titration steps of 0, 3, 15, 20 equivalents.

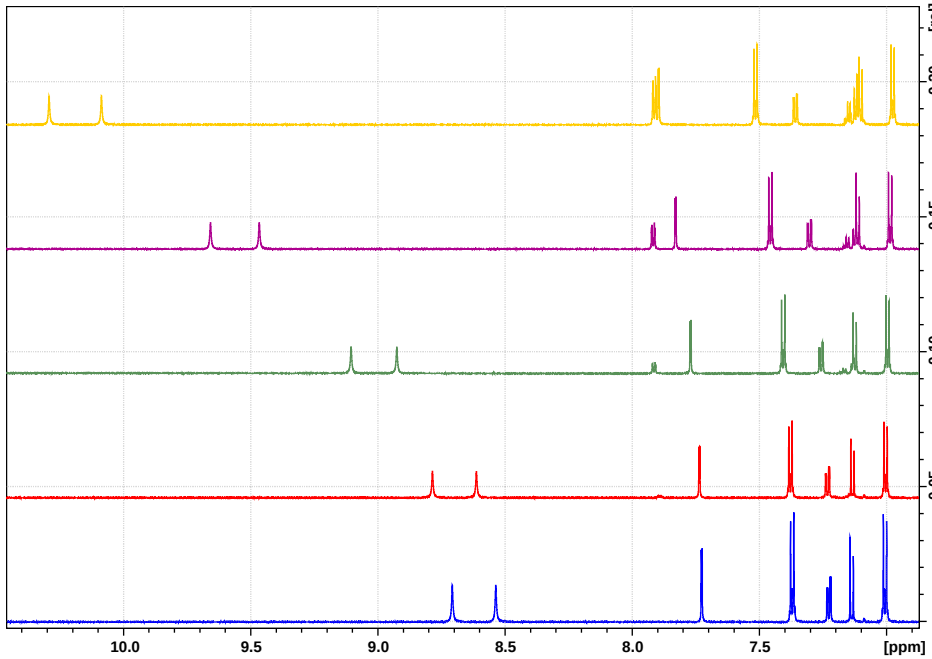


Figure 9. NMR titration spectra of U36 and TBA-benzoate (0, 1, 3, 6 and 10 equivalents) in biphasic 2-Me-THF$_{(aq)}$.

Influence of lipophilicity

In binding experiments done in o-NPOE/water, CZ07 was titrated with TEA-acetate (Figure 10) and TBA-acetate (Figure 11). In the first experiment, the receptor showed no binding with the TEA salt. When titrating with the TBA salt, binding was observable under slow exchange conditions. This is likely due to the difference in cation lipophilicity. Often, in single-phase titration experiments, great effort is undertaken to show that the counter-ion of the analyte does not affect binding affinities. However, this is a considerable shortcoming of single-phase titrations, as it ignores a fundamental influence present in the ISE membranes – lipophilicity of the counter-ion.

Biphasic logK_{ass} measurements have a strong potential to advance the prediction ability of anion-receptor binding in sensor membranes. However, considerable development is needed. Additional soluble receptors in biphasic environments need to be identified. The influence of the cation needs to be better understood, and experimental conditions must be found under which the endpoint of the titration curve can be reached. Working with receptors of limited solubility may require more sensitive detection techniques than NMR.

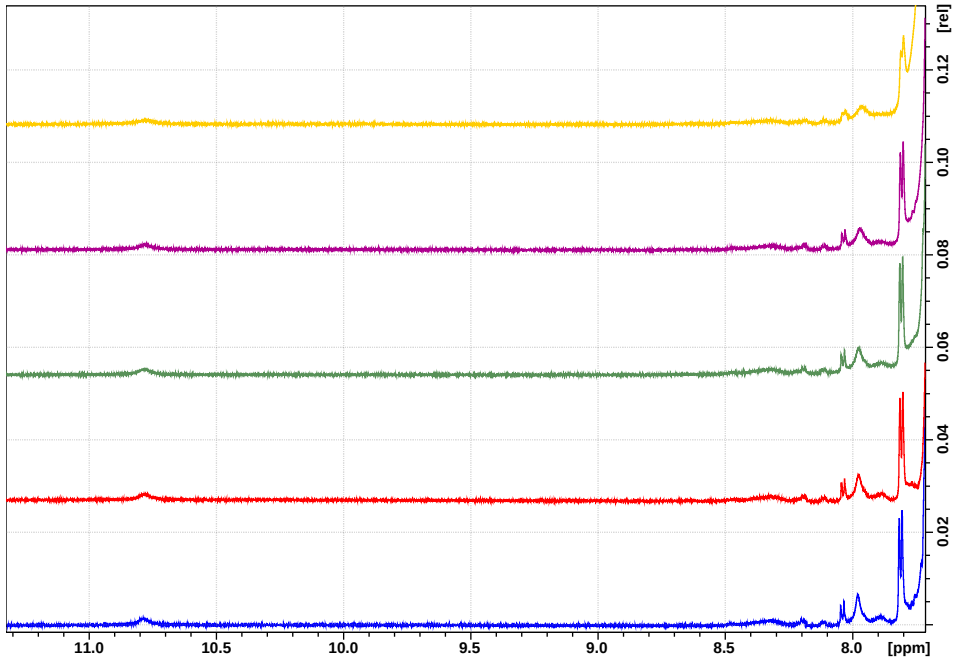


Figure 10. The binding spectrum of TEA-acetate and CZ07 in o-NPOE/water.

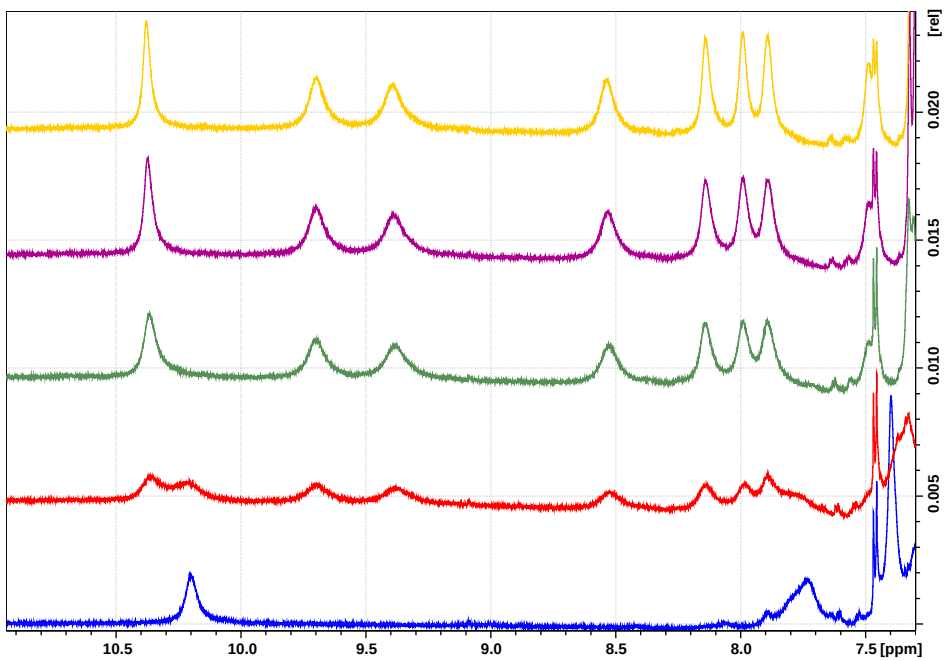


Figure 11. The binding spectrum of TBA-acetate and CZ07 in o-NPOE/water.

SUMMARY

The supramolecular community, especially its branch involved in designing synthetic receptors for anions, has been criticised for the lack of true sensors (ISE-s). At the same time, a vast selection of diverse families of receptors is available that display good anion-binding properties in solution and thus could work as potential ionophore candidates.

While all design stages of ISE development have choke points, one main fundamental challenge is most difficult to overcome – the selectivity issue. The selectivity issue is a grand challenge of supramolecular chemistry and receives considerable attention from the scientific community.

Characterising receptor properties in solution can be of limited use when the receptor is used as an ionophore inside a sensor membrane. Binding measurements in solution can be highly accurate. However, their results are not transferable to the biphasic environment composed of a polymeric membrane and the measured solution. Fundamental differences compared to the homogeneous binding environment in solution may lead to inconsistencies with observations in lipophilic sensor membranes. Compared to the selectivity issue, problems with prediction models of binding and selectivity have not been demonstrated to a similar extent. They, therefore, receive less attention from the scientific community. This dissertation highlighted these issues and explored a possible solution with the concept of biphasic $\log K_{ass}$ measurements.

The main aim of this dissertation was to demonstrate and explain these shortcomings by addressing all design stages of ISE development. Following strict design criteria, anion-selective receptor molecules were chosen as potential ionophore candidates. Investigation of their binding properties was conducted with high accuracy. The developed receptors were employed as ionophores in anion-selective ISE sensor prototypes. Doing so bridged the gap between receptor and sensor development. However, the obtained sensor prototypes showed modest selectivity towards specific carboxylates. Further development of synthetic anion receptors with more sophisticated structures is necessary to achieve a truly selective carboxylate binding.

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SUMMARY IN ESTONIAN

Sünteetiliste molekulaarretseptorite disainiprintsiibid anioon-selektiivsetele elektroodidele

Supramolekulaarkeemia valdkonda, eriti sünteetiliste aniooniretseptorite disainimisega tegelevat haru, on kritiseeritud vähese edu eest tõeliste sensorsüsteemide loomisel. Seejuures on publitseeritud märkimisväärses koguses mitmekesiste struktuuridega retseptormolekule, mis võiksid olla sobilikud ionofoorikandidaadid anioonide sidumiseks.

Kuigi ioonselektiivsete elektroodide arendamisel on mitmeid kitsaskohti, tõuseb esile üks fundamentaalteaduslik väljakutse, mis on ülejäänutest oluliselt keerulisem – raskused piisava selektiivsuse saavutamisel. Selektiivsuse probleemi lahendamine on supramolekulaarkeemia üks olulisemaid väljakutseid, mistõttu leiab see laialdast teaduslikku tähelepanu.

Lahuses uuritud retseptormolekulide omadused ei kajasta tingimata ionofooride omadusi sensormembraanis. Olemasolevad meetodid selektiivsuse hindamiseks jätavad arvestamata mitmeid füüsikalisi ja keemilisi omadusi, olgugi, et metroloogilisest vaatepunktist on sellised mõõtmised kõrge usaldusväärsusega. Peamiseks erinevuseks lahuses ja sensor-membraanis teostatud mõõtmiste vahel on märkimisväärselt muutunud seondumise keskkond, eriti aniooni seisukohast. Ennustuslike seondumis-mudelite puudujääkide üle ei ole arutletud samaväärse tähelepanuga nagu selektiivsuse probleemi puhul. Seetõttu on supramolekulaarkeemias pühendatud ka vähem tähelepanu nende meetodite arendamisele. Käesolev dissertatsioon tõi esile selliseid kitsaskohti ja uuris kontseptuaalsel tasemel ühe võimaliku lahendusena kahefaasiliste afiinsuste mõõtemeetodit.

Käesoleva dissertatsiooni peamiseks eesmärgiks oli demonstreerida sensoriarenduse väljakutseid kõikides selle etappides. Rangeid disainiprintsiipe järgides valiti erinevaid retseptormolekule võimalikeks ionofoorideks. Seondumisomadused lahuses mõõdeti kõrge täpsusega. Uuritud retseptormolekule kasutati ionofooridena anioonselektiivsete sensorite prototüüpide loomisel. Uurimustöö tulemusena suudeti ühendada sünteetiliste retseptormolekulide disainimise ja sensorarenduse valdkonnad. Loodud sensorite prototüübid näitasid siiski vaid keskpärast edukust konkreetsete karboksülaatide eristamisel üksteisest. Karboksülaatide eristamises tõeliselt eduka sensori loomine nõuab veel täiendavaid pingutusi.

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I thank student corporation Vironia for the financial aid during my studies and for helping me keep my motivation for achieving my PhD degree.

APPENDICES

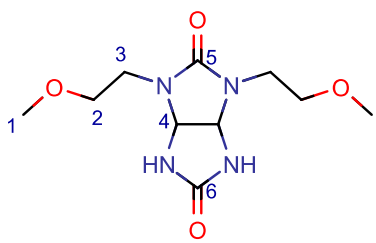
Appendix 1 – Synthesis of 2-ME-BU

1,3-bis(2-methoxyethyl)urea

Before starting the reaction, all glassware was heated at 130 °C for 18 h. The acetonitrile was dried with molecular sieves before use.

CDI (1.5 g) was dissolved in 50 ml dry acetonitrile under an argon atmosphere. 1.96 ml of 2-methoxyethylamine was added. The apparatus was equipped with a condenser, sealed, and the mixture was stirred at 60 °C for 5 h. Then, the solution was concentrated under rotary evaporator. The resulting liquid was dissolved in 5% methanol/DCM and purified with flash chromatography using gradient elution with 3-8% methanol/DCM as the mobile phase. 1.41 g of product was collected as a white solid (yield: 71%). Structural analysis conformed to data published in literature.[58]

2,4-di(2-methoxyethyl)glycoluril



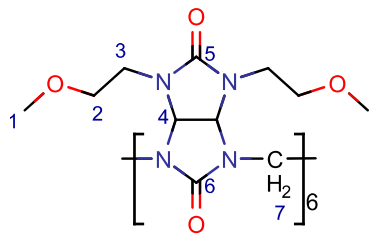
300 mg of 1,3-bis(2-methoxyethyl)urea was weighed in a round bottom flask alongside 400 mg (2 eq) of DHI. The solids were dissolved in 10 ml MilliQ grade water, and the solution was acidified with 163 µl 35% HCl. The mixture was heated to 80 °C and stirred for 4 h. Then, the mixture was allowed to cool to room temperature. 5 ml of brine was added, and the solution was extracted 5 times with 20 ml chloroform. The obtained liquid was concentrated and dried under vacuum. 414 mg of transparent gel was collected (yield 94%).

^{1}H NMR (700.1 MHz, $CDCl_3$, +25 °C) δ: 5.80 (s, NH, 2H), 5.24 (s, 4-CH, 2H), 3.76 (ddd, J = 15.2, 5.1, 1.9 Hz, 3-CH, 2H), 3.55 (ddd, J=10.3, 9.2, 1.9 Hz, 2-CH, 2H), 3.49 (ddd, J=10.1, 4.9, 2.3 Hz, 2-CH, 1H) 3.2 (ddd, J=15.1, 8.7, 2.3 Hz, 3-CH, 1H) ppm.

^{13}C NMR (176.0 MHz, $CDCl_3$, +25 °C) δ: 160.8 (6-CO), 158.1 (5-CO), 72.4 (2-CH_2), 68.2 (4-CH), 58.9 (1-CH_3), 43.3 (3-CH_2) ppm.

ESI-FT-ICR(+): solvent acetonitrile, *m/z* of [M+Na] calculated for $C_{10}H_{18}N_4O_4Na$ 281.12203 found 281.12193.

2-ME-BU



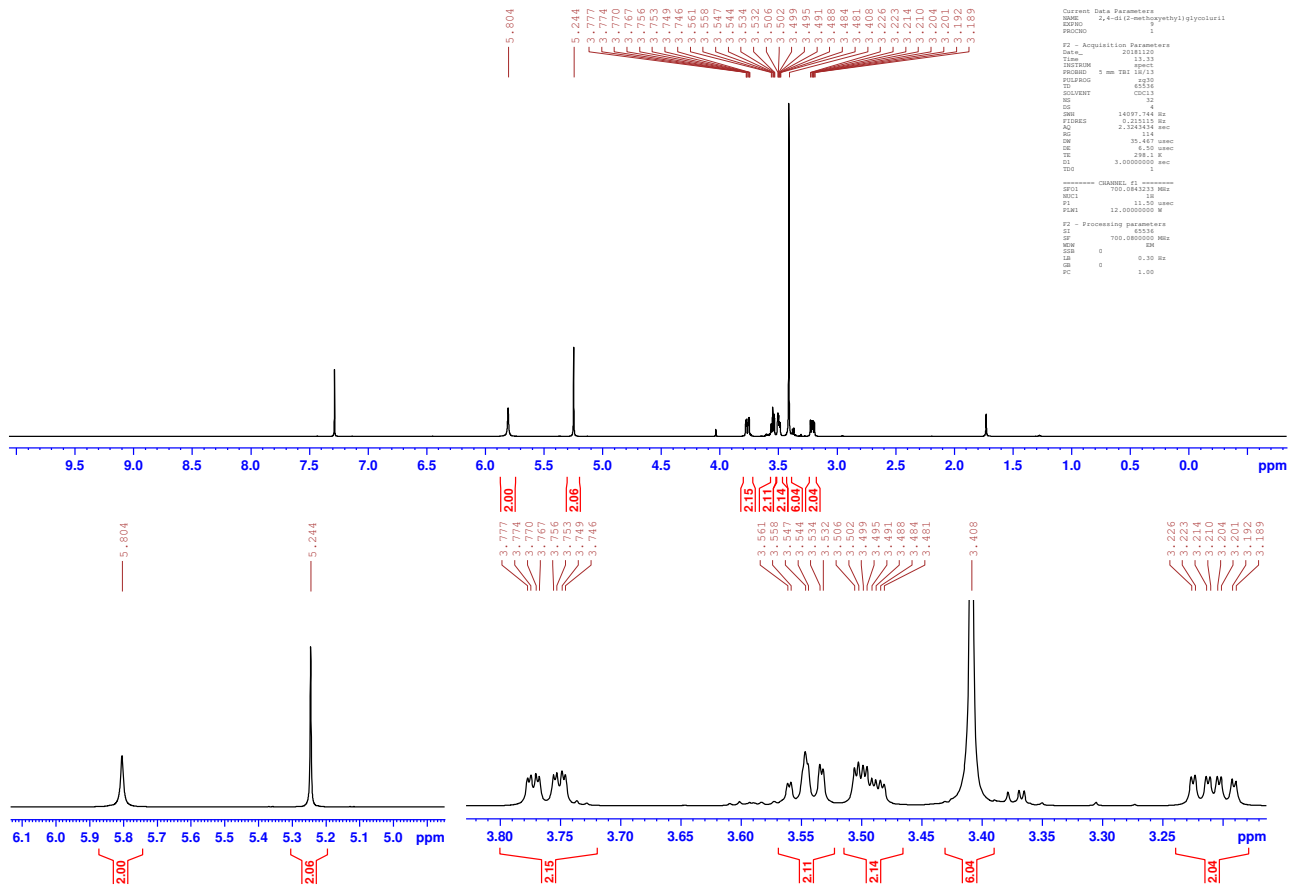
285 mg of 2,4-di(2-methoxyethyl)glycoluril was dissolved in 7.8 ml 1,4-dioxane alongside 40.8 mg of PFA. The mixture was heated to 60 °C and acidified with 93.2 µl H_2SO_4. The temperature was adjusted to 80 °C and the solution was stirred for 17h. The mixture was cooled to room temperature, and 2 eq of NaOH (dissolved in 1 ml water) was added. The mixture was filtered on a glass funnel and washed with 10 ml ether. The obtained white solid was vacuum dried, yielding 223 mg of product as its Na_2SO_4 complex (yield 69%).

^{1}H NMR (700.1 MHz, DMSO-*d*$_6$, +25 °C) δ: 5.46 (s, 4-C<u>H</u>, 12H), 4.93 (s, 7-C<u>H</u>$_2$, 12H), 3.83 (quint, J=6.7 Hz, 3-C<u>H</u>, 12H), 3.54 (m, 2-C<u>H</u>, 12H), 3.49 (m, 2-C<u>H</u>, 12H) 3.41 (m, 3-C<u>H</u>, 12H), 3.20 (s, 1-C<u>H</u>$_3$, 36H) ppm.

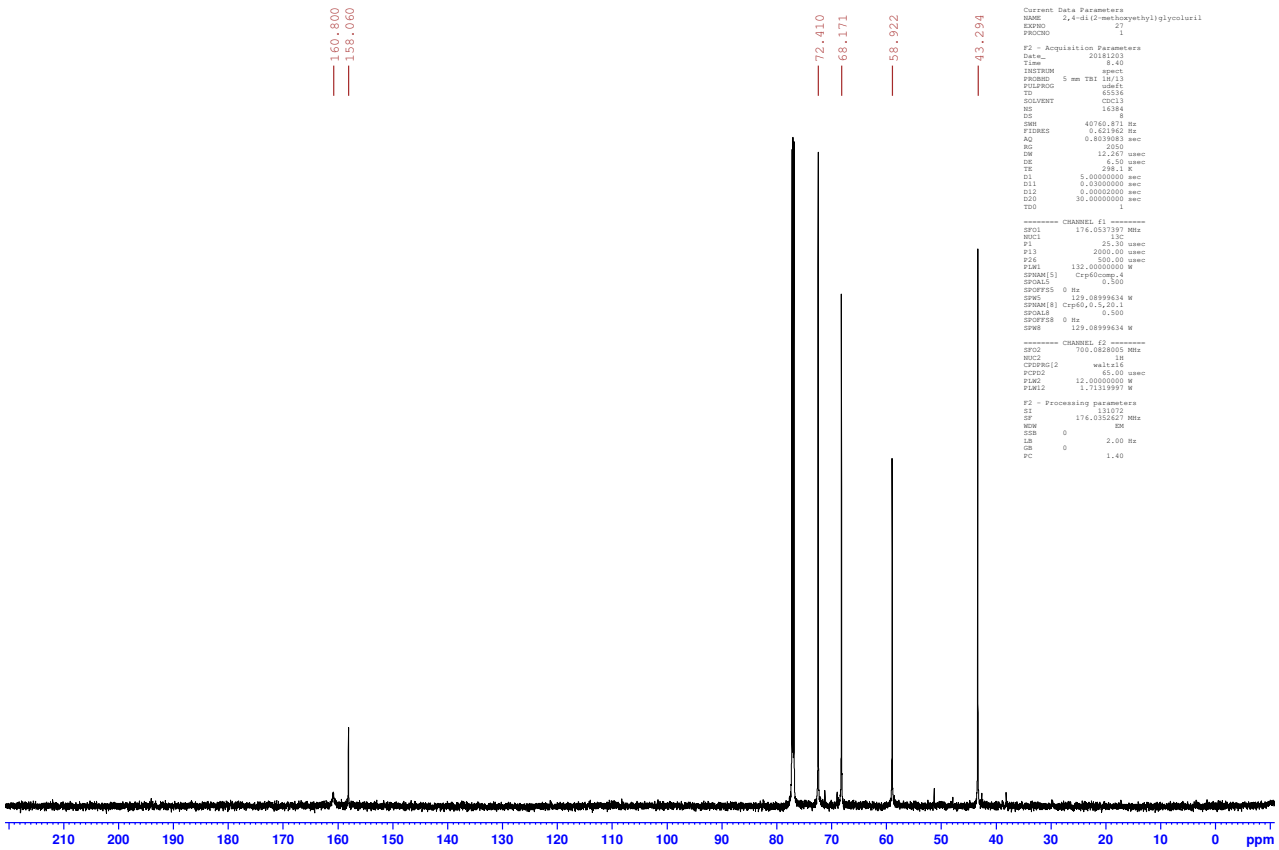
^{13}C NMR (176.0 MHz, DMSO-*d*$_6$, +25 °C) δ: 159.6 (5-CO), 158.8 (6-CO), 69.7 (2-CH_2), 68.0 (4-CH), 57.9 (1-CH_3), 46.4 (7-CH_2), 43.0 (3-CH_2) ppm.

ESI-FT-ICR(-): solvent MilliQ, *m/z* of [M+HSO_4^-] calculated for $C_{66}H_{109}N_{24}O_{28}S$ 1717.75693 found 1717.75693.

^{1}H spectrum of 2,4-di(2-methoxyethyl)glycoluril



^{13}C spectrum of 2,4-di(2-methoxyethyl)glycoluril



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PROCNO 1

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PROBHD 5 mm TBI 1H/13
PULPROG udeft
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SOLVENT CDCl3
NS 16384
DS 8
SWH 40760.871 Hz
FIDRES 0.621962 Hz
AQ 0.8039083 sec
RG 2050
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D12 0.00002000 sec
D20 30.00000000 sec
TD0 1

======== CHANNEL f1 ========
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NUC1 13C
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P13 2000.00 usec
P26 500.00 usec
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SPOAL5 0.500
SPOFFS5 0 Hz
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SPNAM[8] Crp60,0.5,20.1
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SPW8 129.08999634 W

======== CHANNEL f2 ========
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NUC2 1H
CPDPRG[2 waltz16
PCPD2 65.00 usec
PLW2 12.00000000 W
PLW12 1.71319997 W

F2 - Processing parameters
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WDW EM
SSB 0
LB 2.00 Hz
GB 0
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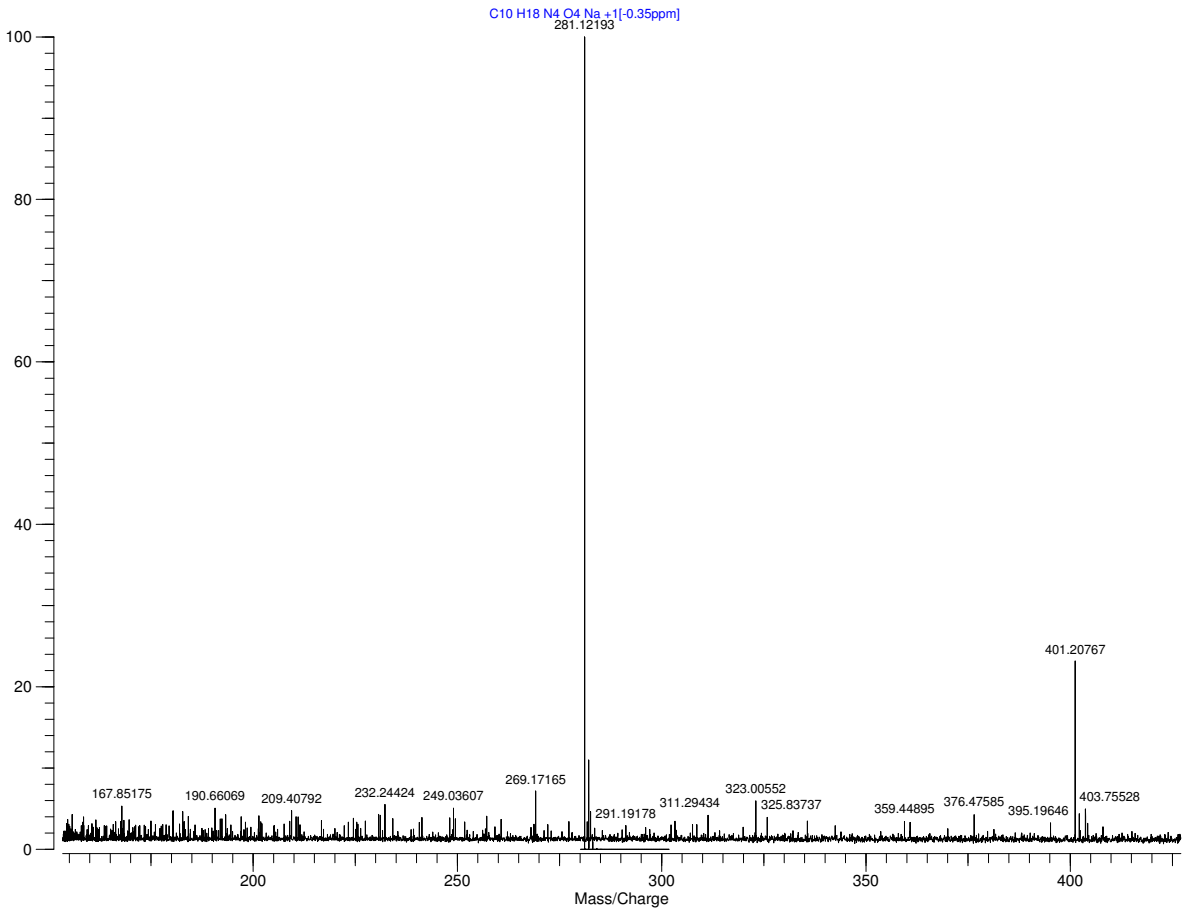
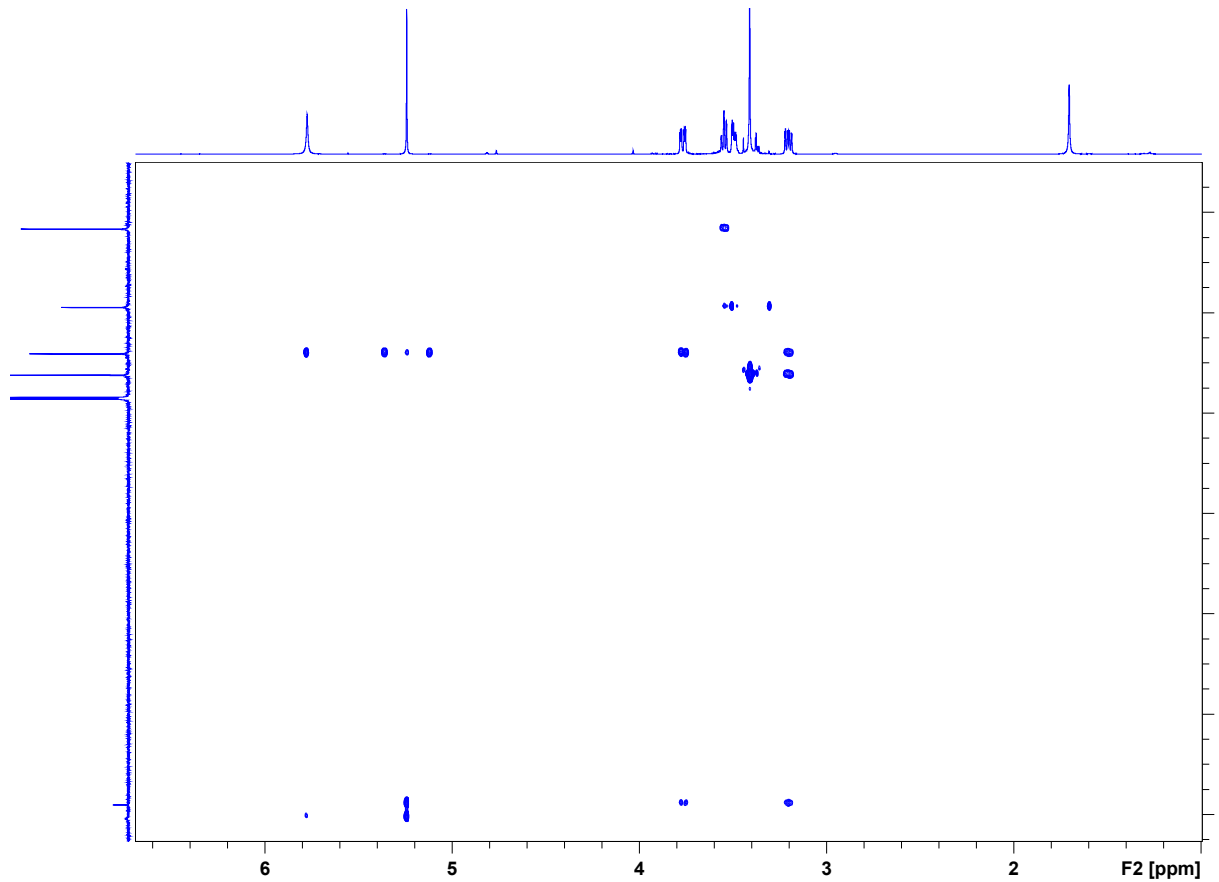
HRMS spectrum of 2,4-di(2-methoxyethyl)glycoluril



HMBC spectrum of 2,4-di(2-methoxyethyl)glycoluril

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D6             0.05000000 sec
D16            0.00020000 sec
IN0            0.00002030 sec

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NUC1                  1H
P1                 11.50 usec
P2                 23.00 usec
PLW1          12.00000000 W

======== CHANNEL f2 ========
SFO2          176.0528595 MHz
NUC2                  13C
P3                 25.30 usec
PLW2         132.00000000 W

====== GRADIENT CHANNEL =====
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GPNAM[2]      SMSQ10.100
GPNAM[3]      SMSQ10.100
GPZ1               50.00 %
GPZ2               30.00 %
GPZ3               40.10 %
P16              1000.00 usec

F1 - Acquisition parameters
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FIDRES        246.305420 Hz
SW            139.904 ppm
FnMODE                QF

F2 - Processing parameters
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GB        0
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F1 - Processing parameters
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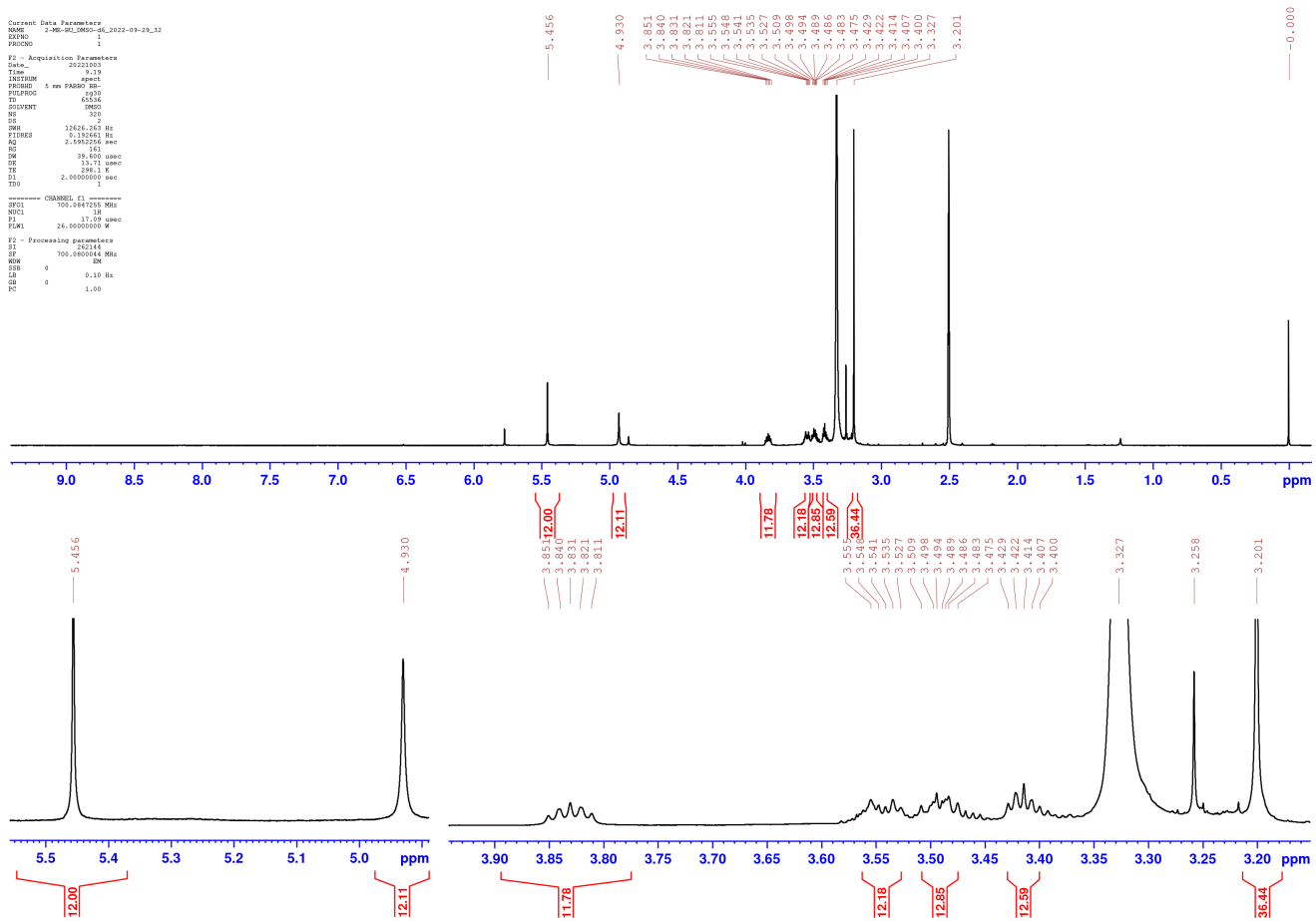
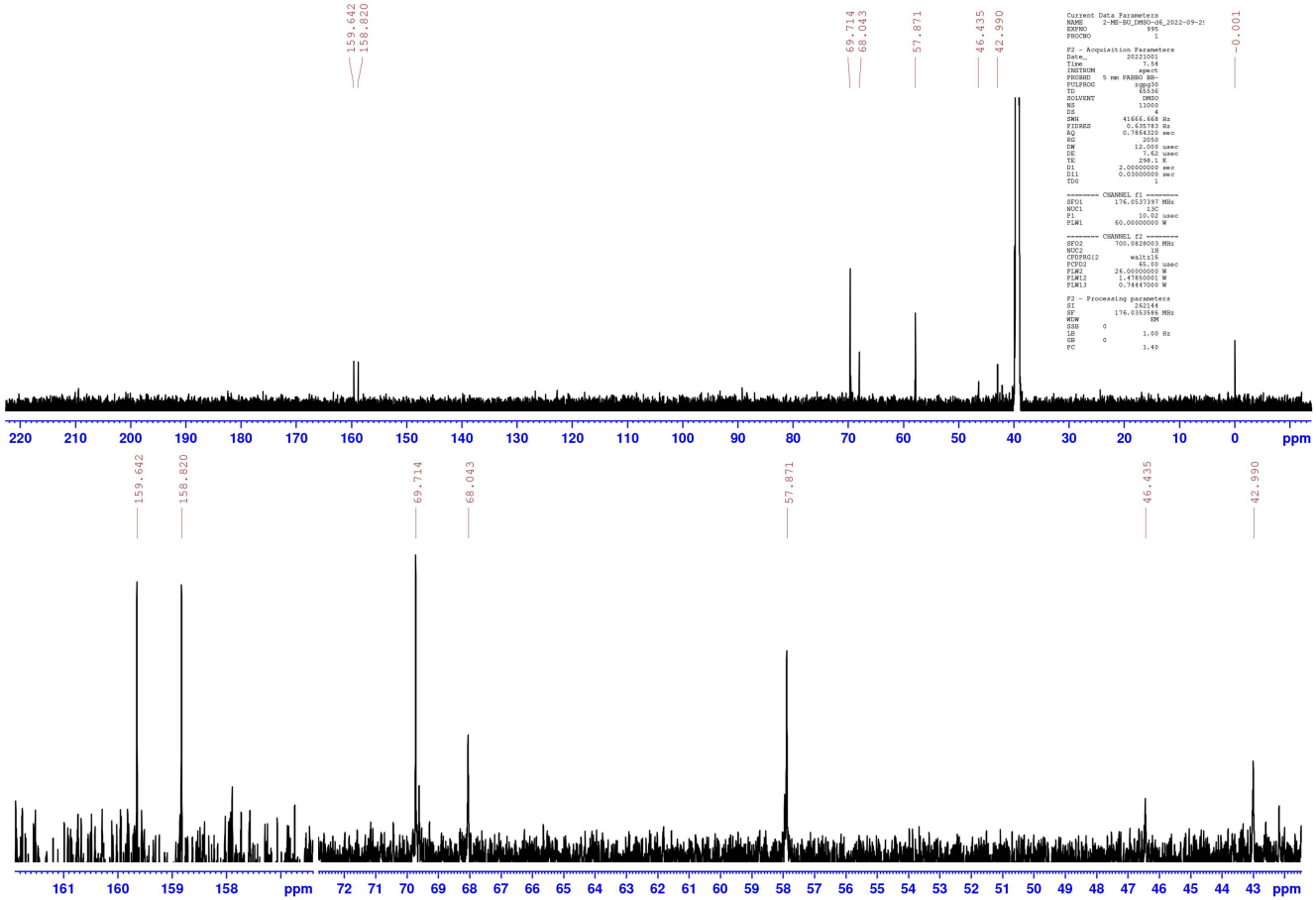
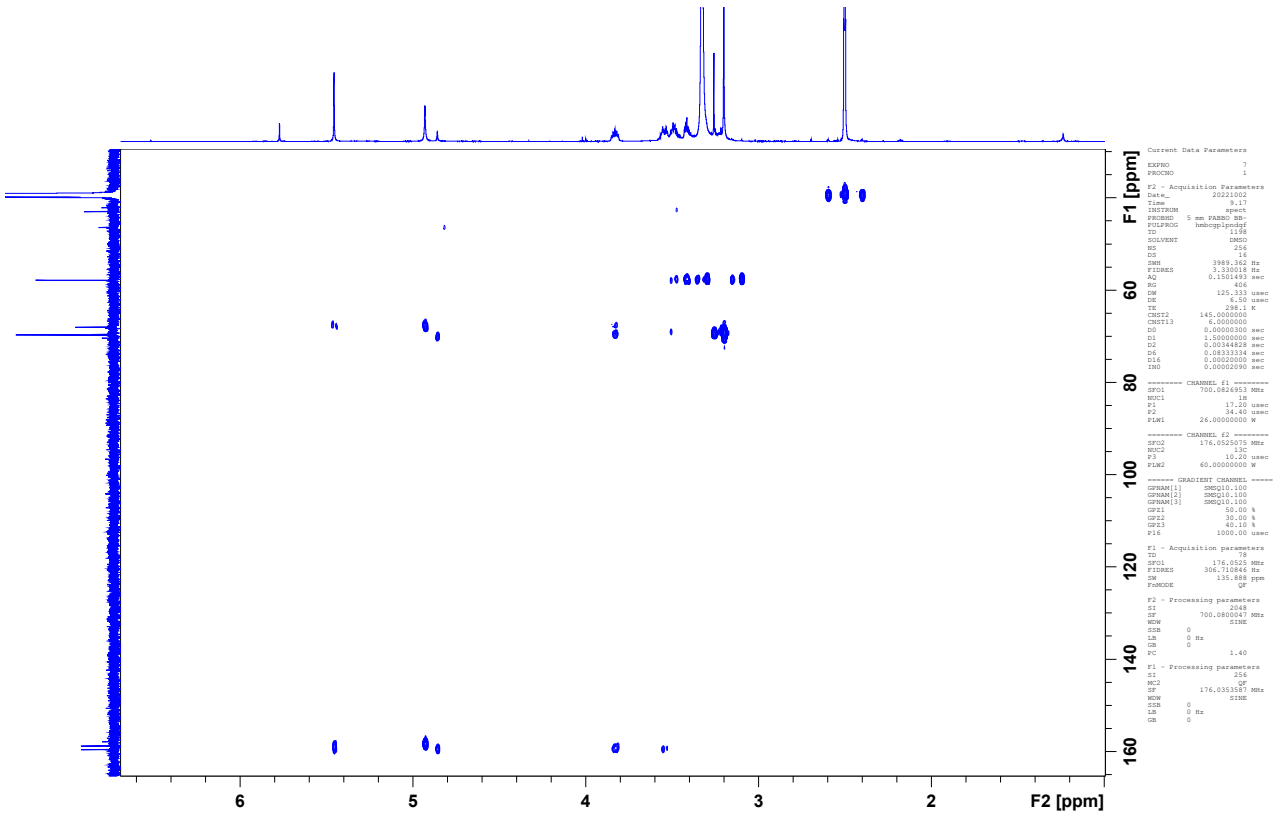
^{1}H spectrum of 2-ME-BU



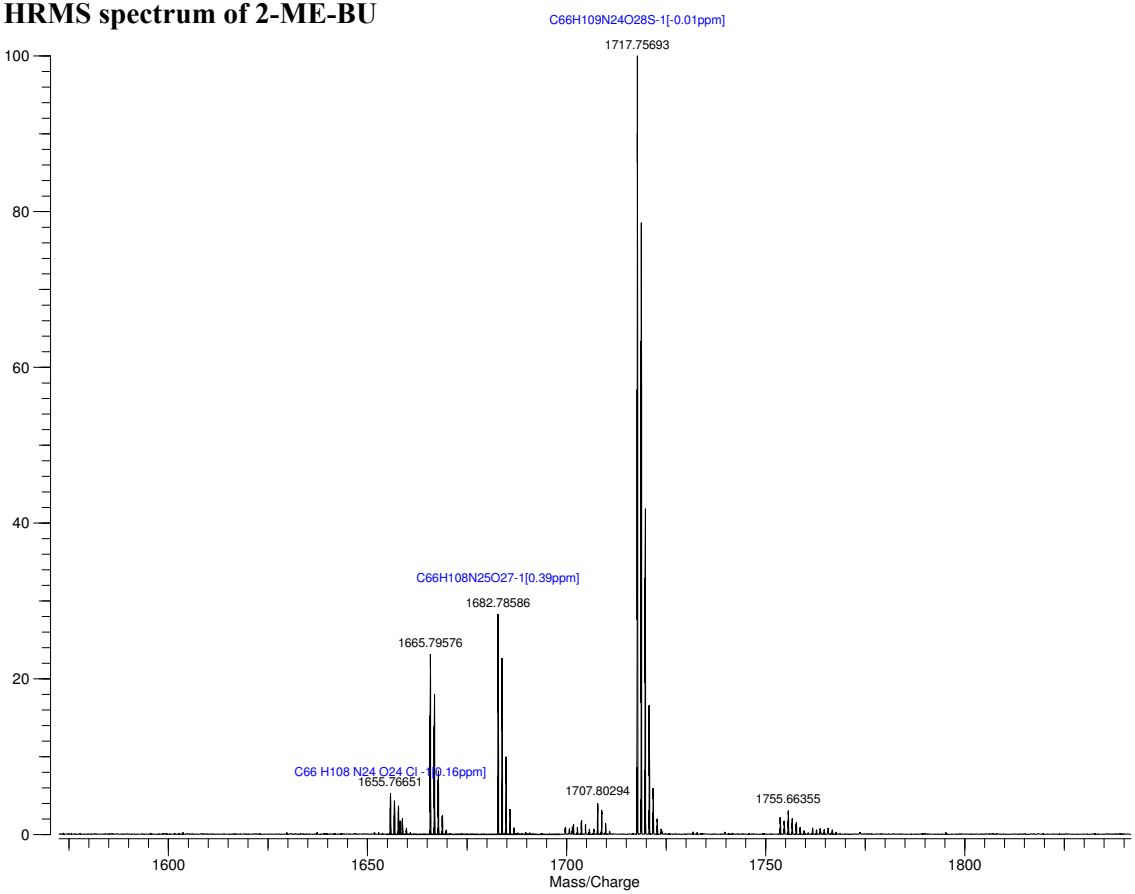
^{13}C spectrum of 2-ME-BU



HMBC spectrum of 2-ME-BU



HRMS spectrum of 2-ME-BU



Appendix 2 – Biphasic titration protocol example

1. Using a separatory funnel, saturate the organic solvent with MilliQ-grade water and collect both saturated phases.
2. Weigh 20-40 mg[a] of receptor into a vial. It is important to record the exact mass accurately.
3. Dissolve the receptor in 4 ml[b] $organic_{aq}$ solvent.
4. Separate the receptor solution into 5 aliquots (0.8 ml each): 1 blank + four measurement points.
5. Weigh the sum total amount of equivalents (*e.g.* 1, 3, 6 and 10 eq) of analyte (corresponding to the receptor mass) into a vial. It is important to record the exact mass accurately.
6. Dissolve the analyte in 1 ml MilliQ grade water.
7. Dilute the analyte solutions to match the equivalents of each titration point. It is allowed to add the necessary amount of analyte solution straight to the receptor aliquots and dilute them in the same vial. Add MilliQ grade water as follows:
   a. 2 ml MilliQ for the blank solution (for finding the δ of the fully dissociated receptor)
   b. match the total volume of the aqueous analyte solution to 2 ml.
8. Vigorously shake all solutions using a Vortex mixer for 60 s and let the phases set for ~10 min.
9. *Optional: transfer the organic phase to an Eppendorf vial and centrifuge for ~60 s.*
10. Transfer the organic phases into NMR tubes.
11. Measure ^1H spectra of the organic phases in the NMR tubes.

[a] – the necessary mass depends on the molecular mass and purity of the receptor as well as the detection limit of NMR. The final concentration of receptor stock solution should be ~0.5 mM.
[b] – it may be necessary to use different amounts of solvents as contraction can be encountered with some biphasic mixtures, *e.g.* o-NPOE/H_2O. Optional step 9 may help recover additional solvent volume to meet NMR requirements specific to the spectrometer.

PUBLICATIONS

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SCIENTIFIC PUBLICATIONS

1. A. Rüütel, V. Yrjänä, S. A. Kadam, I. Saar, M. Ilisson, A. Darnell, K. Haav, T. Haljasorg, L. Toom, J. Bobacka, I. Leito, "Design, synthesis and application of carbazole macrocycles in anion sensors". *Beilstein J. Org. Chem.* **2020**, *16*, 1901–1914.
2. S. Tshepelevitsh, S. A. Kadam, A. Darnell, J. Bobacka, A. Rüütel, T. Haljasorg, I. Leito, "LogP Determination for Highly Lipophilic Hydrogen Bonding Anion Receptor Molecules". *Anal. Chim. Acta.* **2020**, *1132*, 123–133.
3. S. Kheirjou, A. Rüütel, A. Darnell, T. Haljasorg, I. Leito. "Macrocyclic versus open-chain carbazole receptors for carboxylate binding." *Org. Biomol. Chem.* **2022**, *20*, 2121–2130.
4. A. Rüütel, S. Tshepelevitsh, I. Leito, "One Hundred Carboxylate Receptors". *J. Org. Chem.* **2022**, ahead of print.

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1. A. Rüütel, M. Ilisson, "Optimisation of the reaction pathway for the synthesis of substituted 1,3-bis(carbazolyl)urea anion receptors". Suprachem 2019, Germany, Würzburg (24–26.02.2019). Poster presentation.
2. A. Rüütel, I. Leito, "Towards the Synthesis and Isolation of Homologues of Phenyl-Substituted Bambusuril". The 5th International Conference on Cucurbiturils (ICCB 2017), Czech Republic, Brno (27–30.06.2017). Poster presentation.

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2013	Tervise Arengu Instituut, keemik-laborant

ERIALANE ENESETÄIENDUS

2021	Juhtimissüsteemide siseauditite läbiviimine

TEADUSPUBLIKATSIOONID

1. A. Rüütel, V. Yrjänä, S. A. Kadam, I. Saar, M. Ilisson, A. Darnell, K. Haav, T. Haljasorg, L. Toom, J. Bobacka, I. Leito, "Design, synthesis and application of carbazole macrocycles in anion sensors". *Beilstein J. Org. Chem.* **2020**, *16*, 1901–1914.
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3. S. Kheirjou, A. Rüütel, A. Darnell, T. Haljasorg, I. Leito. "Macrocyclic versus open-chain carbazole receptors for carboxylate binding." *Org. Biomol. Chem.* **2022**, *20*, 2121–2130.
4. A. Rüütel, S. Tshepelevitsh, I. Leito, "One Hundred Carboxylate Receptors". *J. Org. Chem.* **2022**, avaldatud veebis 10.10.2022.

KONVERENTSID

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2. A. Rüütel, I. Leito, "Towards the Synthesis and Isolation of Homologues of Phenyl-Substituted Bambusuril". The 5th International Conference on Cucurbiturils (ICCB 2017), Tšehhi Vabariik, Brno (27–30.06.2017). Stendiettekanne.

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