Phosphate complexes of calix[4]pyrroles

Bachelor's thesis

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ABSTRACT

This bachelor’s thesis is about the structure and the properties of different calix[4]pyrroles. Specifically, the binding of calix[4]pyrroles with different phosphate-containing molecules is assessed. These phosphate-binding properties are compared across different substituent-containing calix[4]pyrroles, using for example association constants. Some applications for the use of the complexes are also discussed.

PREFACE

This thesis was written in the spring of 2020 in the department of chemistry of the University of Jyväskylä. The supervisor for this work is Kaisa Helttunen. Literature was found using Google Scholar, Reaxys, SciFinder and JYKDOK. ChemDraw was used to draw molecules.

I would like to thank Kaisa Helttunen for her support and great guidance for this thesis, especially during these unusual times. I would also like to thank my friends for their mental support during this process, and my partner for his help with the maturity essay of this thesis.

CONTENTS

1 INTRODUCTION

Pyrrole is a five membered, planar, and aromatic ring. It is composed of carbon, hydrogen and a single nitrogen.[1] When pyrrole is condensed with acetone in the presence of acid, it forms a macrocycle called calix[4]pyrrole. It is similar to calixarene, and is named in the same fashion.[2]

The individual pyrrole rings are attached to each other by sp^3 hybridized *meso*-carbons. The structure is similar to porphyrin or porphyrinogen. However, calixpyrroles differ from porphyrins as they are not conjugated. They also differ from porphyrinogens in the sense that they are fully *meso*-substituted so they do not undergo oxidation to form the aforementioned porphyrins.[2] *Meso*-octamethylcalix[4]pyrrole, **1** (figure 1), was first synthesized in 1886, but gained attraction around a hundred years later in the 1990s.[3] They bind anions and neutral guest molecules using hydrogen bonding.[2]

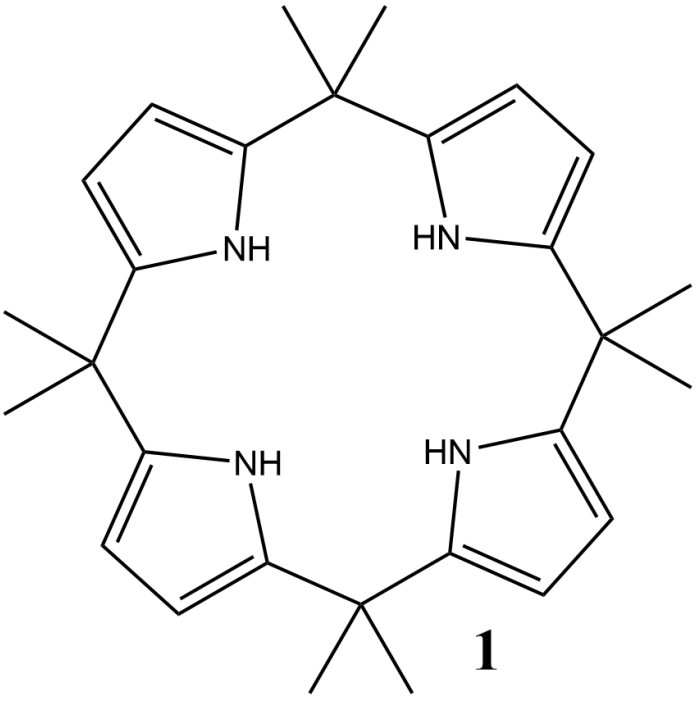

Figure 1. *Meso*-octamethylcalix[4]pyrrole.

2 THE STRUCTURE OF CALIX[4]PYRROLES

2.1 GENERAL STRUCTURE AND OVERVIEW

A calix[4]pyrrole macrocycle is synthesized by a condensation reaction between a pyrrole and a ketone in acidic conditions.[4] This resulting macrocycle can exist in many different conformations: 1,2-alternate, 1,3-alternate, partial cone and cone depending on if the N-H on an individual pyrrole ring is pointing up- or downwards in relation to its adjacent pyrrole rings.[5] In a solid state with no anionic guests, **1** adopts the 1,3-alternate conformation.[2]

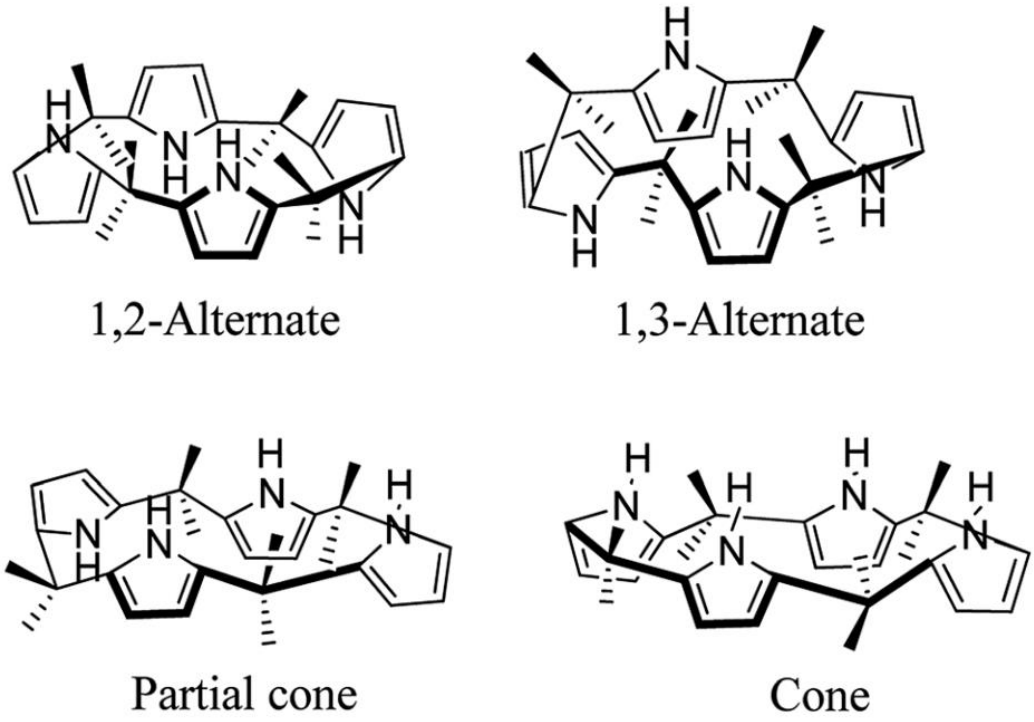

Figure 2. 1,2-alternate, 1,3-alternate, partial cone and cone conformations of **1**. (Published from reference 5 with permission by The Royal Society of Chemistry.)

Sometimes, when calix[4]pyrrole is being synthetized, the synthesis also yields a tiny amount of what is called an *N*-confused calix[4]pyrrole, **2** (figure 3). **2** differs from **1** in that one of the pyrroles is rotated, causing the nitrogen to not point towards the center of the macrocycle. Also, doubly *N*-confused calix[4]pyrroles exist. The ratio of the yields of these different isomers can be modified by changing the acid catalyst and the solvent that is used in the synthesis.[6]

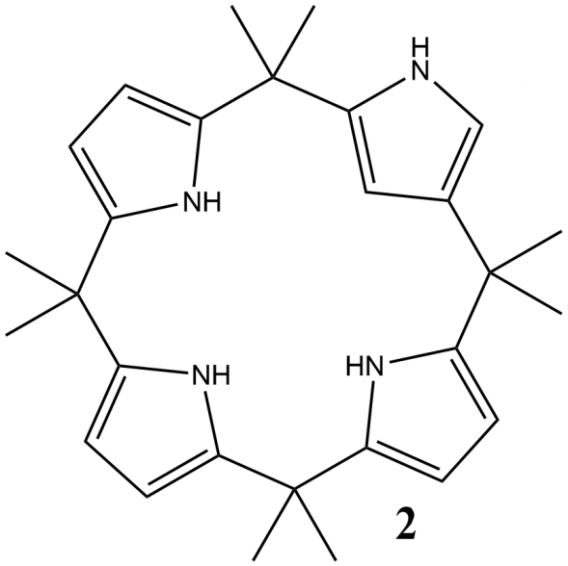

Figure 3. *N*-confused *meso*-octamethylcalix[4]pyrrole, **2**.

A calix[4]pyrrole can also be expanded. The pyrrole units can have a linker between them, other than the alkyl chains. For example, in calix[2]diethynylbenzene[4]pyrrole, **3** (figure 4) the macrocycle has benzene rings in between the pyrrole units. This leads to unique properties and ion selectivity of the host.[7]

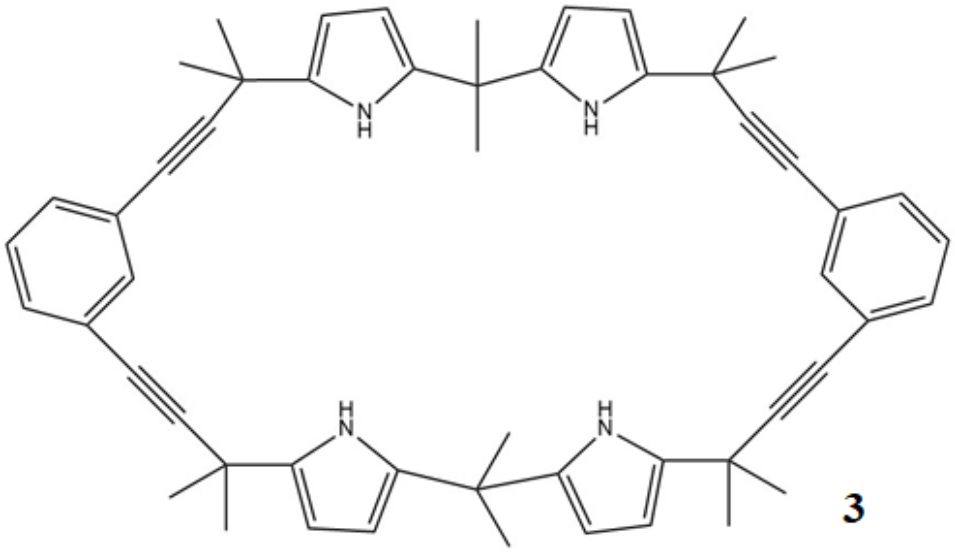

Figure 4. Calix[2]diethynylbenzene[4]pyrrole **3**.

2.2 SUBSTITUENTS IN THE *MESO*-POSITION

The *meso*-position in a calix[4]pyrrole is in the carbon bridge between the pyrrole units. Substituents in the *meso*-position can be added to the molecule during synthesis of the calixpyrrole. For example, in the case of **1**, the ketone used in the synthesis is acetone, which provides the methyl groups in the *meso*-positions. If the ketone is changed to include some functional group (such as cyclohexanone) the functional groups will be present in the molecule. If the ketone is not symmetrical (it has two different substituents, R_1 and R_2) the resulting product has many different configurational isomers (figure 5) based on the stereoisomerism of adjacent *meso*-positions. A pyrrole can also be condensed with two different ketones which results in a mixture of calix[4]pyrroles with different, asymmetrical substituents.[8]

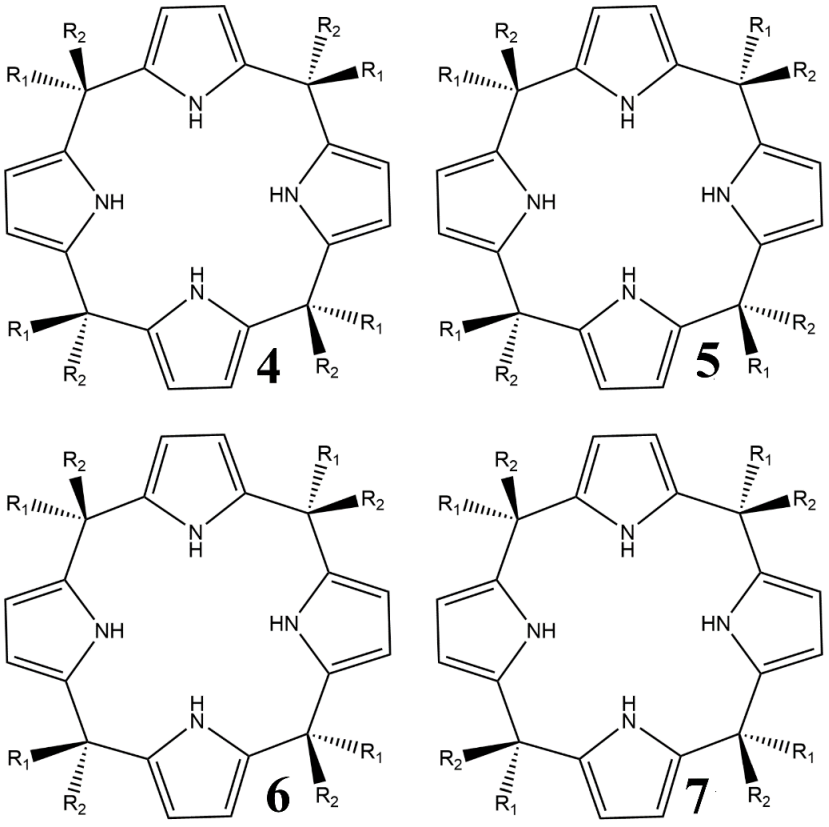

Figure 5. The αβαβ- (**4**), ααββ- (**5**), αααβ- (**6**) and αααα (**7**) isomers of an octasubstituted calix[4]pyrrole with two different substituents.

Substituents can also be added into the *meso*-position using dipyrromethane. The dipyrromethane would contain the desired substituent in its hydrocarbon bridge. The dipyrromethene can then be condensed in acetone resulting in a calix[4]pyrrole with the substituents in the *meso*-position.[3]

Some early work with calix[4]pyrroles included synthesizing the macrocycle to include a strap, producing molecules which look like **3** (figure 6).[9] The strap may include more hydrogen bond donating groups and is added to the macrocycle to improve binding affinity. The synthesis for **9** involves an intramolecular cyclization of a bisdipyrromethane precursor which contains the strap. The length and other features of the strap can be varied as wanted to bind some anions better. For example, a longer strap resulting in a larger binding cavity improved the binding affinity of bromide while chloride preferred a shorter strap. This is due to bromide being a larger ion.[9] This illustrates the effect that cavity size has on the binding of different sized anions. Strap-containing calix[4]pyrroles are also as flexible as other calix[4]pyrrole based molecules, and they can switch between the 1,3-conformation and cone conformation when they bind guests.[8]

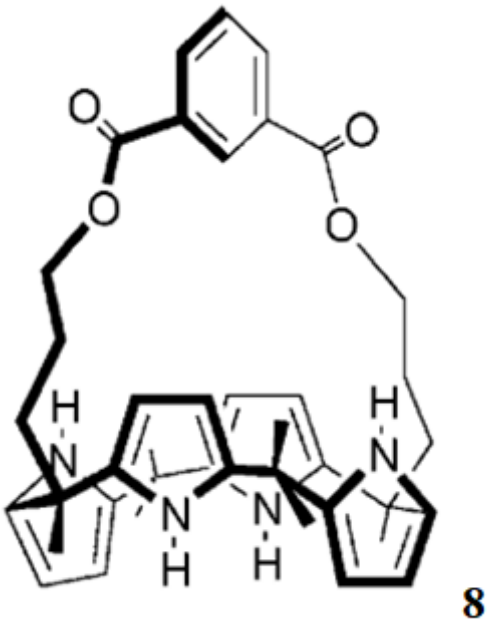

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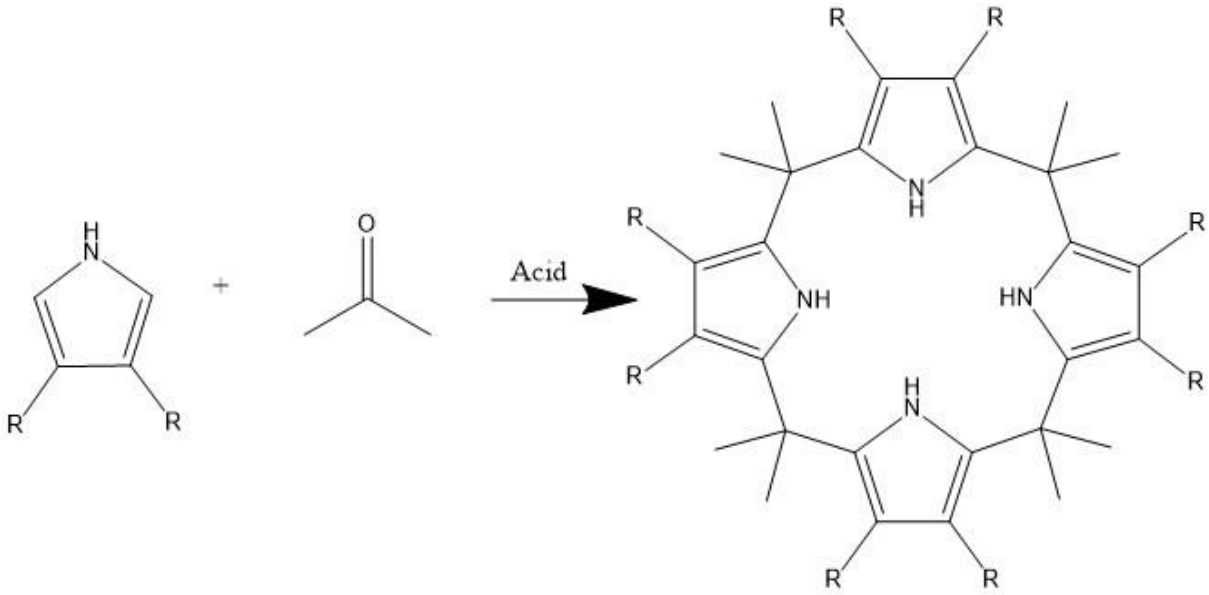
Figure 6. A calix[4]pyrrole which contains a strap, **8**. (Adapted with permission from reference 9. Copyright 2003 American Chemical Society.)

2.3 SUBSTITUENTS IN THE *β*-POSITION

The *β*-position in a calix[4]pyrrole is in the outer edges of the pyrrole units. The substituents in the *β*-position of calix[4]pyrrole are usually added to the pyrrole before the macrocycle is constructed. This is done by having a functional group in the pyrrole and this pyrrole is condensed with a ketone in acidic conditions as usual.[3]



Scheme 1. The reaction between a functional group (R) containing pyrrole and acetone in acidic conditions. This produces a calix[4]pyrrole substituted in all eight *β*-positions.

Substituents can also be added into **1** after synthesizing it, especially when preparing monosubstituted calixpyrroles.[3] For example, an acyl group can be added to **1** by reacting it with *n*-butyllithium (*n*-BuLi), tetrahydrofuran (THF) and carbon dioxide (CO_2).[10] *n*-BuLi is a strong base which deprotonates the pyrrole, and the CO_2 acts as an electrophile providing the acyl group.[3] Bromide in turn can be added to all eight *β*-positions by reacting **1** with *N*-bromosuccinimide in dry THF.[2]

3 COMPLEX FORMATION OF CALIX[4]PYRROLES

3.1 GENERAL

Calixpyrroles bond to anionic and neutral guests using hydrogen bonds which form between the guest and the pyrrole NH groups. When the macrocycle binds an ion or a molecule, it undergoes a conformation change. The calix[4]pyrrole starts off in a conformation where the adjacent pyrrole rings point “up” or “down”, in what is called a 1,3-alternate conformation.[3] When the guest binds to the host, the molecule adopts a cone-like conformation in which all the pyrrole NH groups are oriented in the same direction toward the guest (figure 7). This causes **1** to establish four individual hydrogen bonds with the guest.[2]

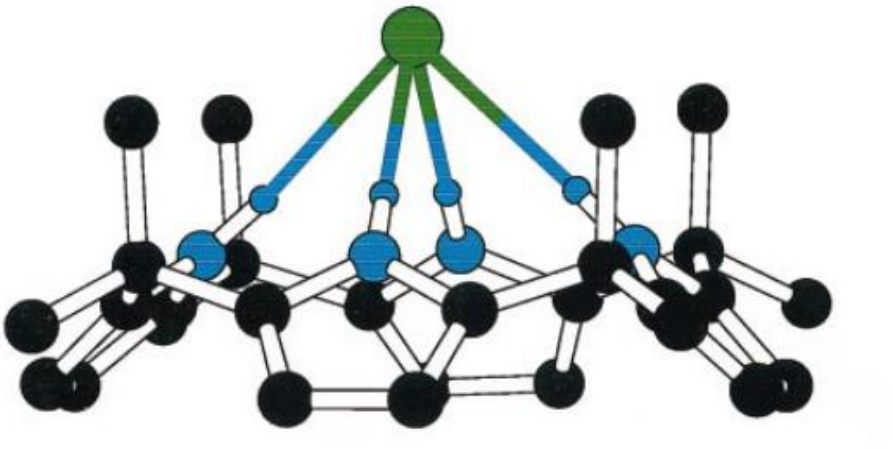


Figure 7. Complex of **1** binding with a chloride ion, which shows the cone-conformation the macrocycle adopts upon binding. (Reproduced from reference 2 with permission from The Royal Society of Chemistry.)

Calix[4]pyrroles also may have a use in cation binding, where they act as ion-pair receptors. For example, a halide upon binding to **1** changes the conformation of the macrocycle to the cone formation as usual. A cavity that forms on the opposite side then binds a cation (figure 8).[8]

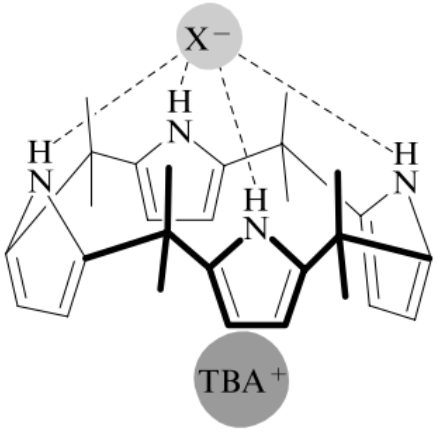


Figure 8. The function of **1** as a ion-pair receptor.[8]

The thermodynamics of the association of a calixpyrrole and a guest can be assessed, and the association constant of a calixpyrrole and its guest can be calculated. They interact according to[12]

$$\mathrm{H} + \mathrm{G}^- \rightleftharpoons \mathrm{HG}^-. \qquad \text{(R1)}$$

If they associate with a 1:1 ratio[11], the association constant K_a is[12]

$$K_a = \frac{[\mathrm{HG}^-]}{[\mathrm{H}][G^-]}, \qquad (1)$$

where [HG^-] is the concentration of the formed host-guest complex, [H] is the concentration of host in the solution and [G^-] is the concentration of the anionic guest. Based on equation 1 and reaction R1, the bigger the association constant, the stronger the association between the host and the guest.

A way to study the association between the ligand and the host is to use nuclear magnetic resonance (NMR). From the ^{1}H NMR data, association constants can be calculated.[13] When a guest is bound to the host, the NH-peaks of the pyrroles shift, for example when $H_2PO_4^-$ or HPO_4^- bind to **1**, the peaks shift to downfield.[11] These shifts in the proton peaks could be used to test qualitatively if the proposed guest is bound to the host.

Solvent plays a significant role in in determining the association constants for different hosts and guests. For example, $H_2PO_4^-$ in dichloromethane as a tetraalkylammonium salt has an association constant of 97 M^{-1} with **1**, while in a water solution of acetonitrile this constant is 1 300 M^{-1}.[14] The effect of the solvent has been studied with **1** complexed with chloride. In the study, it became known that dichloromethane is problematic for anion binding of **1** through ion-pairing effects.[15]

3.2 PHOSPHATE COMPLEXES OF CALIX[4]PYRROLES

3.2.1 ENERGETICS OF THE $H_2PO_4^-$•**1**-COMPLEX

Dihydrogen phosphate ($H_2PO_4^-$) is a very large anion compared to halides, like fluoride.[8] The anion also has many oxygen atoms which each can participate in hydrogen bonding. This gives it an opportunity for two-point interactions.[16] $H_2PO_4^-$ is a very important molecule in the environment and in biology. There is a value in investigating how it binds to different calix[4]pyrrole-based hosts, for example for anion-recognition purposes.

$H_2PO_4^-$ does not bind well to **1** compared to halides. This can be seen in the energetics of the associations. The enthalpy of the reaction between **1** and $H_2PO_4^-$ ($\Delta H^o = -45.77$ kJ mol^{-1}) in dry acetonitrile is much lower than the enthalpy of fluoride reacting with **1** ($\Delta H^o = -34.52$ kcal mol^{-1}) in the same solvent. However, $H_2PO_4^-$ has a much more negative entropy term, which causes a less negative free energy term than fluoride which in turn results in poorer binding between $H_2PO_4^-$ and **1** compared to fluoride.[14]

While that is the case in acetonitrile, in dimethyl formamide (DMF) the entropy term of the binding between **1** and $H_2PO_4^-$ is 29 J K^{-1} mol^{-1}, and the enthalpy term (-18.8 kJ mol^{-1}) is much higher than in acetonitrile. This is due to the $H_2PO_4^-$ being solvated by the DMF. During complex formation, the anion is desolvated, which raises the entropy and lowers the enthalpic stability. F^- still binds to **1** better than $H_2PO_4^-$ in the same solvent based on the association constant.[11]

The association constants between **1** and $H_2PO_4^-$ in literature vary due to the solvent used. In acetonitrile/water mixture the value is 97 M^{-1}.[2] In dry acetonitrile the number rises by many magnitudes to 15100 M^{-1} as a tetrabutylammonium (NBU_4^+) salt and 16800 as a potassium cryptand ({K⊂cryptand}$^+$) salt.[14] This shows that water competes $H_2PO_4^-$ as a guest and binds to **1**, and also that the countercation used can have an influence on determining association constant.

3.2.2 PHOSPHATE COMPLEXES OF *MESO*-SUBSTITUTED CALIX[4]PYRROLES

Substituent groups which contain additional hydrogen bond donor sites allow phosphates to bind better. For example, in macrocycles with a similar structure to **9** (figure 9) bind to phosphates better than to **1** due to the additional hydrogen bonding the NH group can supply to the nucleophilic portions (oxygen atoms) of the anion. The association constants for this type of host can go from 168 300 M^{-1} in acetonitrile up to 682 000 M^{-1} in acetonitrile-water (96:4) based on the R group. This is true for both dihydrogen phosphate and pyrophosphate.[6]

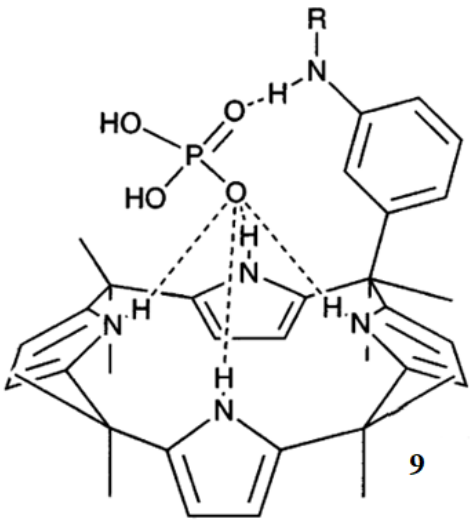


Figure 9. The interaction between $H_2PO_4^-$ and a calix[4]pyrrole containing an NH group, **9**. The two-point interaction of the anion with the receptor can be seen. (Reprinted from reference 6 with permission from Elsevier.)

The configuration of the substituents in the molecule also plays a role in the bonding affinities of ions. In a *meso*-diacylated calix[4]pyrrole it has been found that the *cis*-isomer **10** (where the acyl groups point in the same direction) of the molecule has much greater affinity towards all types of bonding than its *trans*-counterpart **11**. The association constant for **10** with $H_2PO_4^-$ in acetonitrile was 215000 M^{-1}, while for **11** it was 18900 M^{-1}. The difference is significant. In the case of **10**, the enhanced binding is most probably due to the additional hydrogen bonding both of the acyl groups are able to provide to the anion.[17]

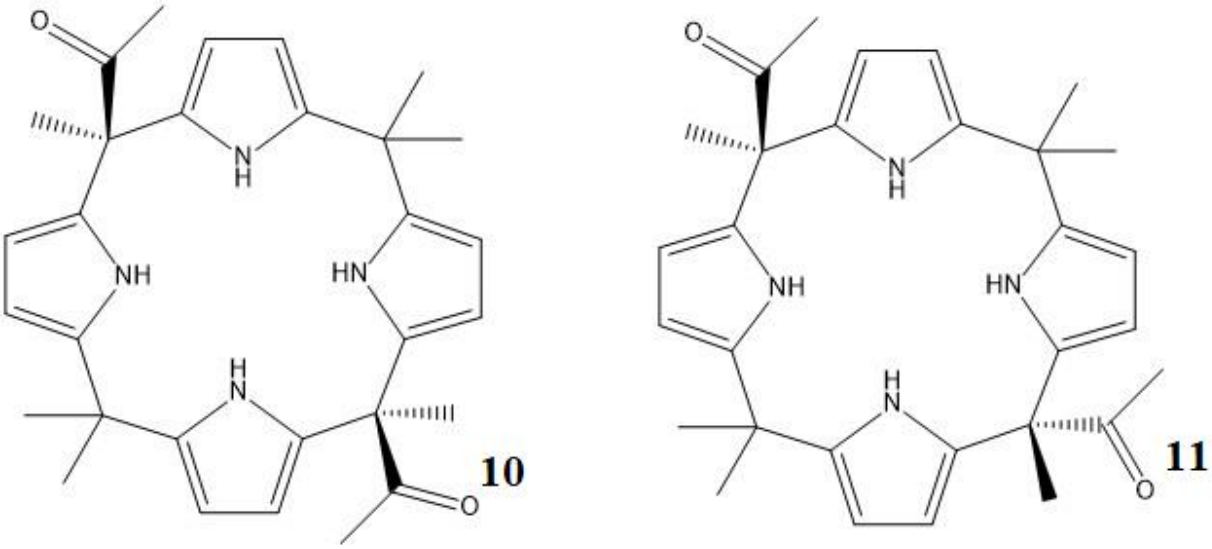


Figure 10. *Cis*- (**10**) and trans- (**11**) diacylated calix[4]pyrroles.

Bulky and nonpolar substituents (like an aryl group) in the *meso*-positions of a calix[4]pyrrole result in weak binding of $H_2PO_4^-$ and low stability constants, which are less than 1000 M^{-1} in acetonitrile. These stability constants are very poor compared to F^- complex, for which the stability constants were larger than 10^4 M^{-1}.[10] This is probably due to the steric hindrance the groups in all four *meso*-positions cause on the rather big $H_2PO_4^-$. The aryl group also does not provide any additional stabilizing hydrogen bonding to the $H_2PO_4^-$, which could be a cause for the poor stability constants.

3.2.3 PHOSPHATE COMPLEXES OF *β*-SUBSTITUTED CALIX[4]PYRROLES AND AN EXPANDED CALIXPYRROLE

An amide-linked anthracene substituent has been added to the *β*-position of a calix[4]pyrrole. The resulting stability constants with $H_2PO_4^-$ for these compounds (figure 11) are larger than for **1**. In acetonitrile, for **12** the constant is >10 000 M^{-1}, for **13** it is 3200 M^{-1} and for **14** it is 1200 M^{-1}.[10] **12** is then best at binding $H_2PO_4^-$ out of the three, and **13** and **14** are not very good. The amide group in **12** is at a different distance from the center of the macrocycle than **13** and **14**. That could be a better distance to produce stabilizing hydrogen bonding.

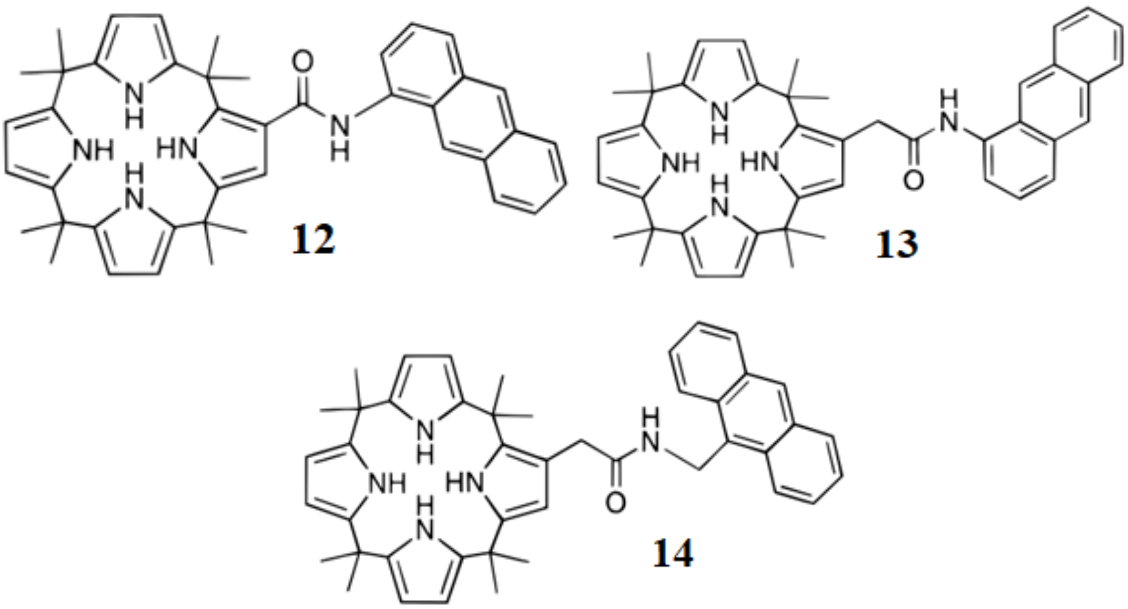


Figure 11. Anthracene-containing calix[4]pyrroles **12**, **13** ja **14**. (Adapted with permission from reference 10. Copyright 2000 American Chemical Society.)

Azo substituents have been added to the *β*-position of a calix[4]pyrrole, producing **15** (figure 12). The substituent makes the pyrrole NH more acidic, which improves the anion binding. The acidity of the NH pyrrole meant that it associates well with more basic guests. $H_2PO_4^-$ is more basic than for example Cl^- so the former binds to an azo substituent containing calix[4]pyrrole better. The association constant for Cl^- binding to a calix[4]pyrrole with an azo substituent in *β*-position is 750 M^{-1}, while for $H_2PO_4^-$ it is 3100 M^{-1} in dimethyl sulfoxide (DMSO). There are more basic anions, like acetate (AcO^-), for which the association constant with the same host is

even better, 8470 M^{-1}. *N*-confusion of the azo substituent containing pyrrole also lowered the association constant between the host and $H_2PO_4^-$ significantly to 295 M^{-1}.[18] In this case *N*-confusion is a significant factor in the binding of $H_2PO_4^-$.

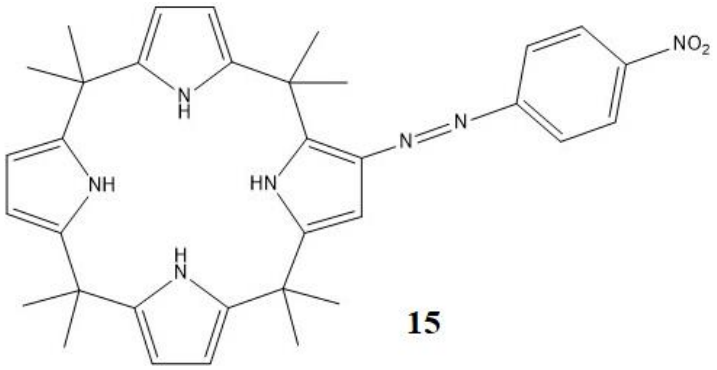


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Figure 12. A monosubstituted calix[4]pyrrole, **15**.

Calix[4]pyrroles were also disubstituted with the same azo-containing group, in the 3,12-positions (**16**, figure 13) and 3,7-positions (**17**, figure 13). The position of the azo substituents has a role in the strength of the association constant between the calixpyrrole and the $H_2PO_4^-$. The association between **16** and $H_2PO_4^-$ (9560 M^{-1}) is slightly larger than in the case of **17** (8497 M^{-1}), but again the acetate anion binds better to the host (~19000 M^{-1}).[18] Therefore these particular hosts are not being very useful for $H_2PO_4^-$-binding in solutions which contain more basic anions.

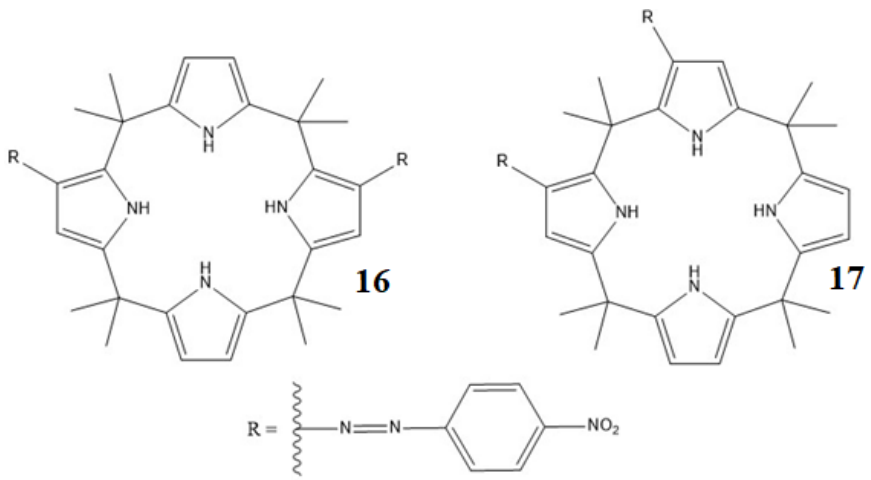


Figure 13. 3,12- (**12**) and 3,7- (**13**) disubstituted calix[4]pyrroles, substituted with the azo-containing group R.

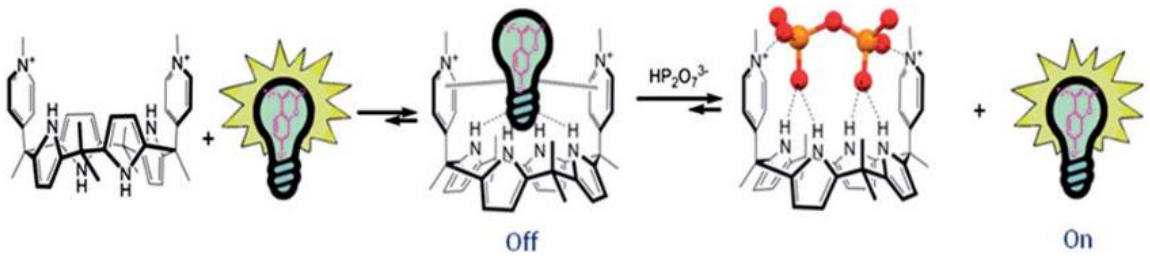
Interestingly, an expanded calix[4]pyrrole, **3** (figure 4) is a calixpyrrole which prefers to bind to dihydrogen phosphate rather than any other tested anion. The association constant between **3** and $H_2PO_4^-$ is 600 M^{-1}, and for F^- it is 450 M^{-1} (both in acetonitrile). This is most probably due to phosphates bigger size.[7] This is very substantial, since most calix[4]pyrrole derivatives seem to have a huge affinity for fluoride. This broadens the horizon for exploring more of the expanded calixpyrroles.

4 APPLICATIONS OF THE COMPLEXES

4.1 DETECTION AND BINDING OF SPECIFIC ANIONS IN SOLUTION

Calix[4]pyrroles are promising anion sensors in solution. A fluorescent substituent can be added to the macrocycle, which then stops emitting in the presence of a guest anion. For example, the appearance of a cyanide anion turns off the fluorescence of a specific host molecule resulting in a visible change. This indicates the presence of the anion.[5] However, in order for this method to be useful in detecting phosphate from solution the calix[4]pyrrole would need to be very selective for the anion.

A pyrophosphate-specific fluorescence-displacement assay has been proposed. The calix[4]pyrrole in question contains two cationic pyridinium groups, which can interact with Coulombic interactions with the negative oxygens of the pyrophosphate anion. The fluorescent dye (chromenolate anion), in the absence of the pyrophosphate, is bound to the calix[4]pyrrole and does not emit fluorescence. In the presence of the pyrophopsphate anion, the chromenolate is displaced and the solution begins to fluoresce, which acts as the indicator for the pyrophosphate (scheme 2). This calix[4]pyrrole is also very specific for pyrophosphate, which gives promise in the field of specific anion detection.[16]



Scheme 2. The function of the fluorescence-displacement assay. (Reproduced from reference 16 with permission from The Royal Society of Chemistry.)

Another use in phosphate recognition from solution could be that while phosphates are beneficial to plant life in small amounts, in large amounts they lead to the eutrophication of aquatic environments. Eutrophication can in turn lead to hypoxia in water environments, causing the biodiversity to suffer.[19] Solving this problem is very important for aquatic life now and in the future.

The removal of arsenic containing anions from water using **1** has been studied with success. Arsenic, like phosphate, is an environmental pollutant.[20] Arsenate (which shows promise in being extracted from water using a calix[4]pyrrole) is also very similar to phosphate, which means that calix[4]pyrroles could have use in removal of phosphate species from aquatic environments. A problem in this could be that water has potential in disturbing association constant measurements, as discussed previously.

4.2 BIOLOGICAL APPLICATIONS

Calix[4]pyrrole complexes have been studied as anticancer drugs. The phosphates in genetic material, deoxyribonucleic acid (DNA), could theoretically bind to the calix[4]pyrrole. Adding a platinum(II) containing substituent to the calixpyrrole *meso*-position, then could theoretically produce a molecule able to bind to phosphate and deliver the cytotoxic platinum drug to the DNA of a cancer cell. The association between phosphate containing molecule, AMP, was unsuccessful but the platinum-calix[4]pyrrole was cytotoxic on cancer cells it was being tested on. An advantage in using calix[4]pyrrole as a delivery method for a drug is that it has a low cytotoxicity in itself.[21]

Calix[4]pyrroles have a significance in functioning as a transporter, due to their affinity with anions which have a biologically important role. For example, **1** can transport chloride across a lipid bilayer when accompanied by a cesium cation, via a symport mechanism. The macrocycle can also be modified to facilitate its function as a transporter (for example allowing the transport mechanism to switch over to a cation-independent antiport process).[22]

The transport and receptor abilities could also have a use in mononucleotide transport and recognition. The binding of a cytosine-substituted calix[4]pyrrole to phosphate-containing biomolecules has been studied. The cytosine was attached to either the *β*-position or the *meso*-position (**14**, figure 14), which in turn affected the transfer rates of different phosphate-containing molecules, like guanosine-monophosphate (GMP). The transport rate of GMP through the membrane was greater with **14** compared to its *β*-substituted counterpart. This is most probably due to the two-point interaction between the GMP and **14** being formed, while the *β*-substituted calix[4]pyrrole can only bind through one interaction.[23] This shows the specificity that can be achieved purely by modifying the placement of the substituent group.

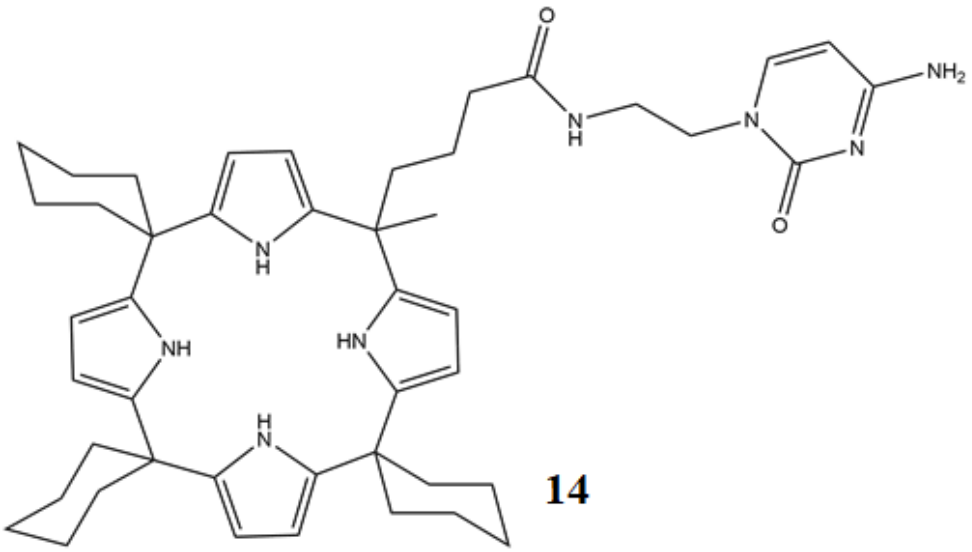


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Figure 14. *meso*-cytosine-substituted calix[4]pyrrole, **14.**

5 CONCLUSIONS

The field of supramolecular chemistry and the study of calix[4]pyrrole-derivatives is rapidly evolving. Calix[4]pyrroles have a role as anion binders, and the addition of different kinds of substituents in the *β*- and *meso*-positions facilitates the anion binding process. Phosphates in turn are important anions in the environment and in biology, so there is a need to investigate the possibilities of calix[4]pyrroles binding them.

The effect of the solvent and countercation used in studying calix[4]pyrrole complexes is an important matter to consider when planning experiments. For example, the use of dichloromethane should probably be avoided. This is due to the ion-pairing effects it causes in the solution, which lowers the reported association constant values. Water in the solution could also form into a problem through being a competitor of the guest.

The low cytotoxicity of calix[4]pyrroles opens the possibility to use them as drug-carriers, especially related to anti-cancer efforts. They could also have a use as an anion-specific detector through a fluorescence-displacement assay. This kind of guest recognition and binding could have a use in solving the problem of water eutrophication.

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Synthesis and a binding study of a four-armed calix[4]pyrrole

Experimental project

University of Jyväskylä

Department of Chemistry

5.11.2020

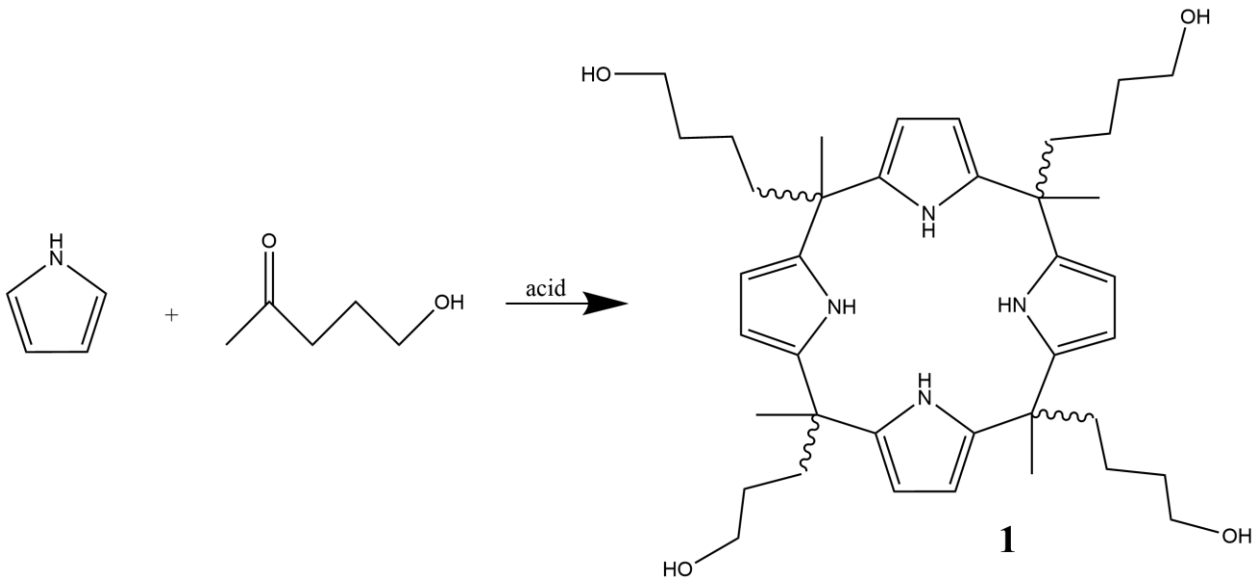
Julia Naulapää

TABLE OF CONTENTS

1 AIM OF WORK

This project was done in the Nanoscience center of University of Jyväskylä during 31.8.-7.9.2020. The work was instructed by Kaisa Helttunen.

The synthesis of a four-armed calix[4]pyrrole **1** was attempted using two different, one-step acid-catalyzed reactions (scheme 1). This molecule has not been synthesized before. The methods used conditions: in method 1 reaction was performed in 50% aqueous ethanol using concentrated HCl[1] and in method 2 the solvent was dry dichloromethane and with 4 M HCl in dioxane[2]. Synthesis was expected to produce multiple different isomers based on the direction of the *meso*-substituents. The synthesis was followed by an anion binding study with tetrabutyl ammonium chloride.



Scheme 1. The reaction to form a four-armed calix[4]pyrrole **1**.

2 MATERIALS AND METHODS

NMR spectra were measured with Bruker AVIII Spectrospin (300 MHz for ^{1}H) and Bruker Avance III 500 (500 MHz for ^{1}H and 126 MHz for ^{13}C) spectrometers. The measurements were done at 30˚C using DMSO-d6 as the solvent. Flash chromatography was performed using CombiFlash Companion from Teledyne ISCO, and Redisep Gold Silica column. A binding constant for **1**•tetrabutylammonium chloride was calculated using http://app.supramolecular.org/bindfit/. Pyrrole was purified using distillation prior to the syntheses and stored at -20°C.

3 USED REAGENTS

Table 1. Reagents and solvents used in the syntheses

Reagent	Manufacturer	Purity / %
Ethanol	Primalco	Aa
Pyrrole	TCI	> 99
5-hydroxy-2-pentanone	TCI	> 96
Conc. hydrochloric acid	Honeywell	-
Methanol	Honeywell	> 99.8
Dichloromethane	VWR	> 99.5
DMSO-d6	Euriso-top	99.80
Tetrabutylammonium chloride	Aldrich	≥ 98

4 SYNTHESES

METHOD 1

The synthesis was completed under argon atmosphere at room temperature. 50% aqueous ethanol (10 ml) was added to a two-necked flask. With continuous stirring, 5-hydroxy-2-pentanone (1 ml, 9.86 mmol) and pyrrole (0.68 ml, 9.80 mmol) were added. Concentrated hydrochloric acid (0.5 ml) was added over 14 minutes, after which the solution turned quickly from clear to brown. The reaction went on for an hour, during which the solution turned vibrant orange and opaque due to precipitation. The white precipitate was then filtrated and washed with water and a small amount of methanol. The product was dried in vacuum overnight.

FIRST SYNTHESIS

The synthesis followed method 1, producing 364.4 mg (25%) of white solid (figure 1).

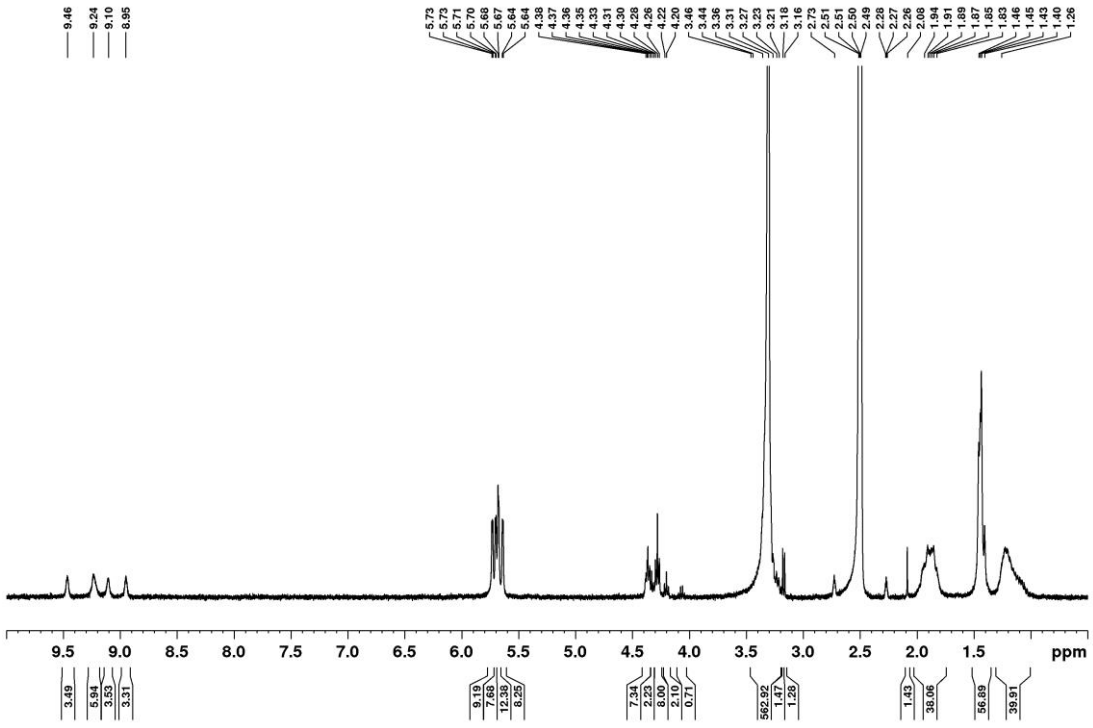


Figure 1. ^{1}H NMR of the white solid in DMSO-d6 at 300 MHz.

The product was recrystallized by dissolving it in methanol, bringing it to boil and then adding dichloromethane. The precipitate was filtered and washed with dichloromethane, giving a white solid. The solid was dried in the vacuum line overnight. The yield was 82.2 mg (6 %).

^{1}H NMR (DMSO-d6, 500 MHz, figure 2)

δ 1.09 (m, 4H), 1.22 (m, 6H), 1.43 (s, 14H), 1.89 (m, 5H), 1.95 (m, 4H), 3.28 (overlapping CH_2 with H_2O), 4.35 (t, 4H), 5.68 (m, 4H), 5.70 (m, 4H), 8.97 (s, 2H), 9.50 (s, 2H)

^{13}C NMR (DMSO-d6, 500MHz), appendix 1.

δ 25.67, 27.84, 36.34, 37.97, 61.40, 102.27, 103.22, 136.89, 138.37

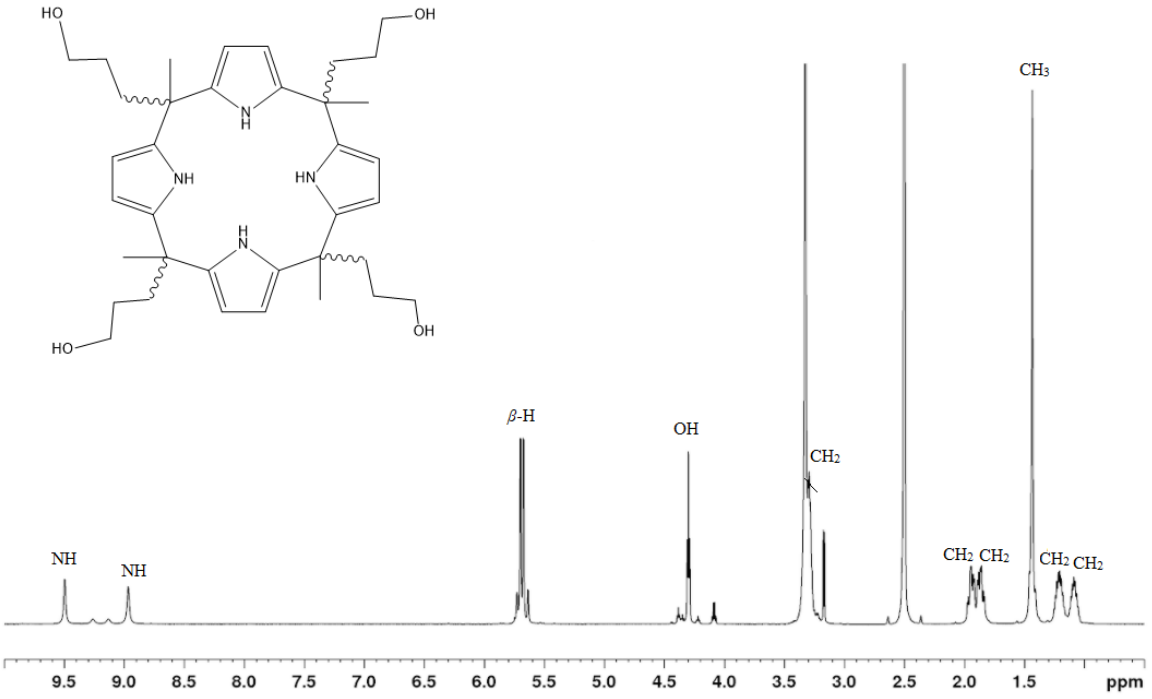


Figure 2. ^{1}H NMR of the recrystallized product in DMSO-d6 at 500 MHz.

SECOND SYNTHESIS

The synthesis followed method 1, producing 687.3 mg (46%) of white solid (figure 3).

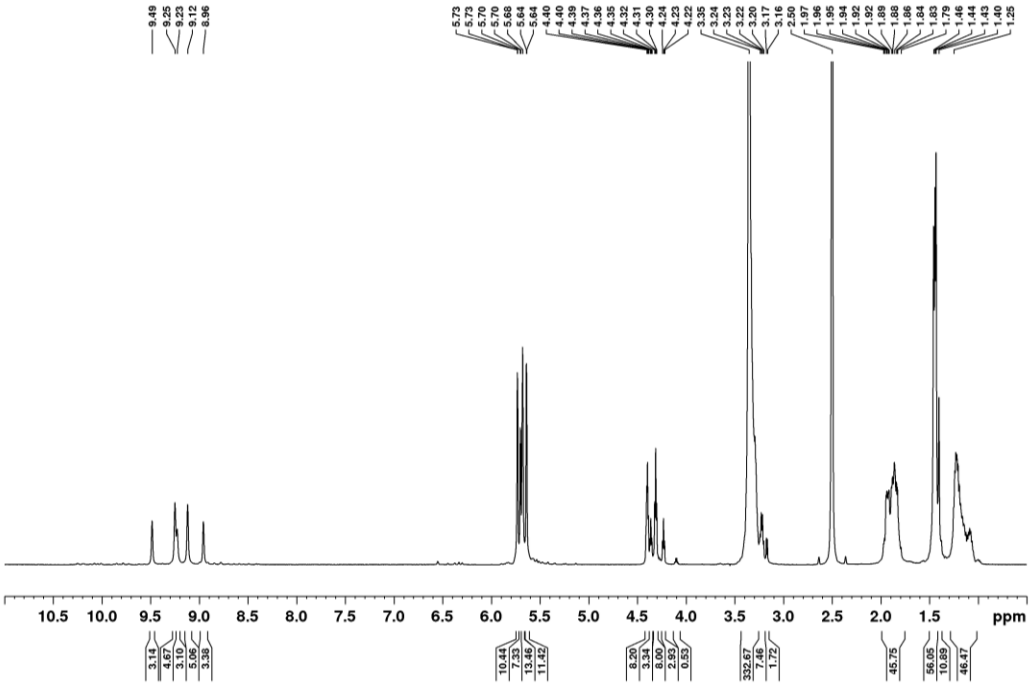


Figure 3. ^{1}H NMR of the white solid in DMSO-d6 at 500 MHz.

The product was dissolved in methanol and brought to a boil. Dichloromethane was added, but the product did not crystallize as expected upon dichloromethane addition. The dichloromethane and methanol containing liquid was put into the fridge overnight and evaporated afterwards using the rotary evaporator. More dichloromethane was added, and the product started to recrystallize. The product was filtered. The filtrate at the recrystallization phase was also collected and concentrated to dryness with a rotary evaporator for NMR analysis. The yield of the solid was 73.7 mg (5%). The yield for the filtrate was 0.7833 g (contains methanol).

^{1}H NMR (DMSO-d6, 500 MHz, figure 4)

δ 1.00-1.30 (m, 10H), 1.41 (t, 14H), 1.94 (m, 9H), 3.29 (overlapping CH_2 with H_2O), 4.27 (t, 4H), 5.68 (m, 4H), 5.70 (m, 4H), 8.95 (s, 2H), 9.47 (s, 2H)

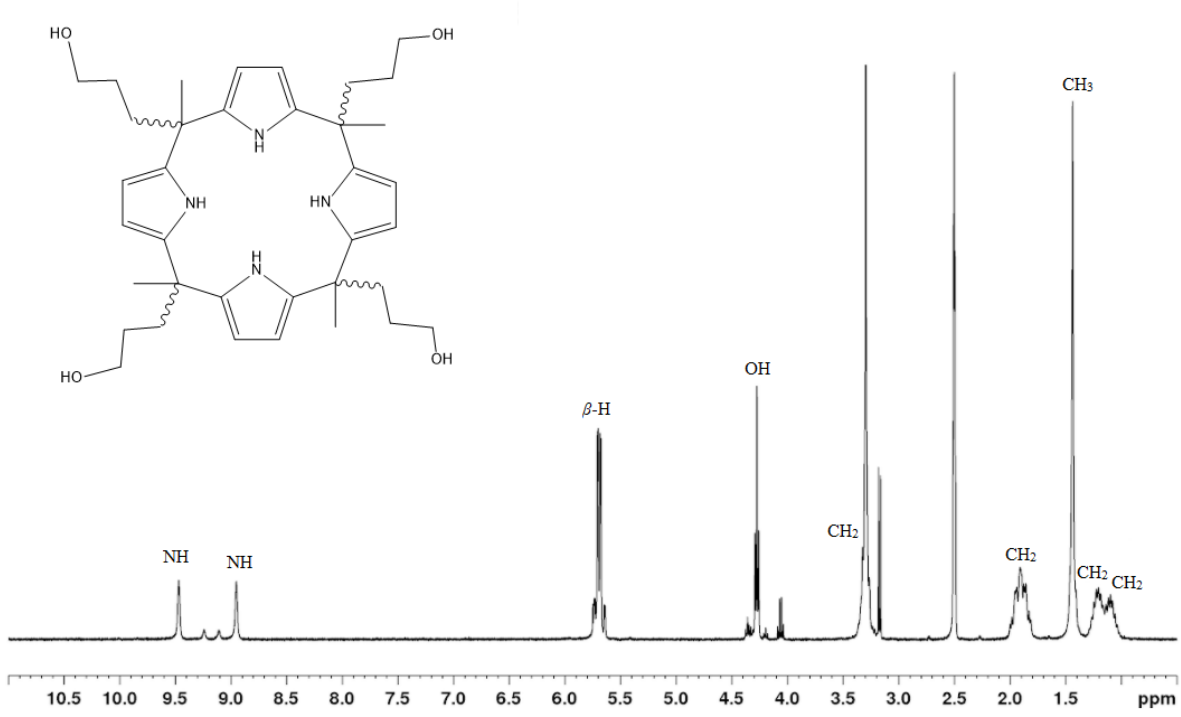


Figure 4. ^{1}H NMR of the recrystallized product in DMSO-d6 at 500 MHz.

METHOD 2

The synthesis was completed in an argon atmosphere at room temperature. To an oven dried two-necked flask, 5-hydroxy-2-pentanone (0.2 ml, 1.97 mmol) and pyrrole (0.15 ml, 2.16 mmol) were diluted in dry CH_2Cl_2 (5 ml). This was covered with foil and the stirring turned on. 4 M HCl (1.5 ml) in dioxane was added in the span of 15 minutes. The mixture was left to react overnight. The contents turned to a sticky, dark brown precipitate, and a pale orange liquid. The precipitate was soluble in ethanol, methanol and dimethyl sulfoxide but did not dissolve in water, dichloromethane, or ethyl acetate.

The liquid was diluted with dichloromethane (50 ml) and washed twice with water (total 30 ml). The organic phase was dried with Na_2SO_4 and filtered and concentrated using rotary evaporator. This left behind a small amount of oily liquid which did not contain the desired product.

The solid was dissolved into methanol and a little dichloromethane was added. Silica gel was added, and the solvent was evaporated in rotary evaporator. The silica-suspended product was purified using flash chromatography. The eluent used was 1:9 mixture of MeOH and dichloromethane. The column used was Redisep Gold. Nine fractions were obtained, of which only the first one contained the product. Solvent was evaporated in the rotary evaporator. The yield was 15.4 mg (5%) of white solid (figure 5).

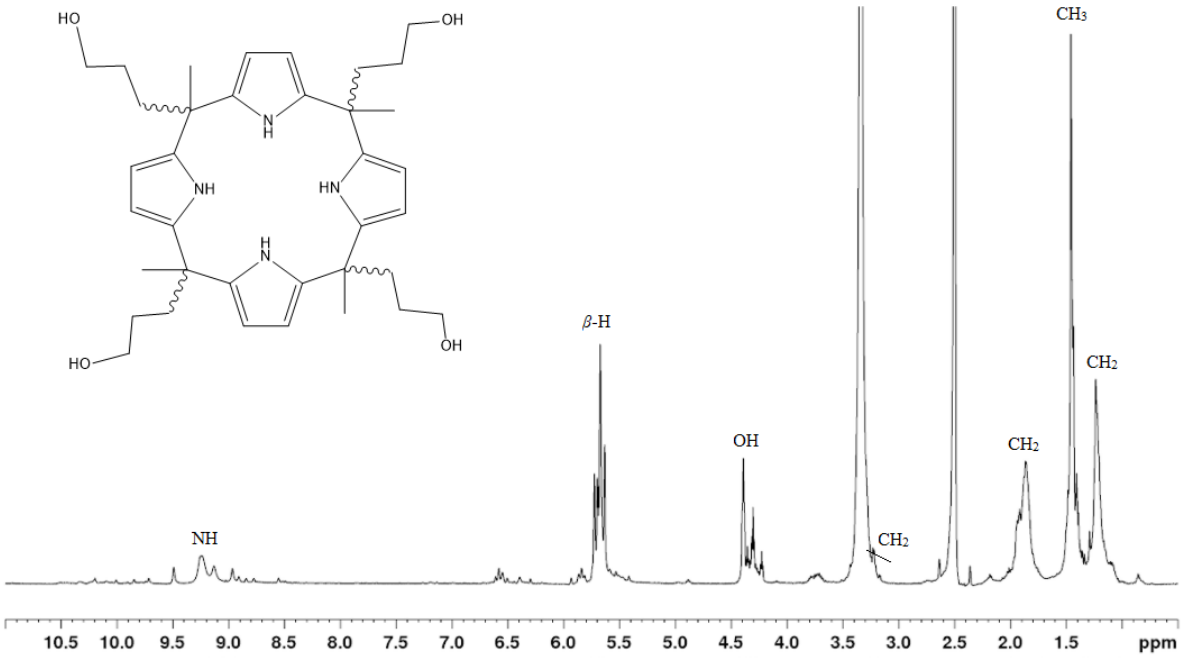


Figure 5. ^{1}H NMR of fraction 1 in DMSO-d6 at 500 MHz.

5 BINDING STUDY

A host solution for the NMR titration was prepared by dissolving **1** (2.9 mg, 4.8 µmol) in DMSO-d6 (2 ml) and transferring 700 µl of it into an NMR tube. The guest was prepared by dissolving 6.3 mg of TBACl (6.3 mg, 22.7 µmol) into 0.5 ml of the host solution. The TBACl-guest solution was added in increments to the host solution of **1** and the ^{1}H NMR spectrum was measured after each addition (volumes and equivalents of additions in table 2).

Table 2. The volumes added in each addition of guest to host, the total volume added and the molar equivalents of the guest to the host.

Addition	Volume added in addition / µl	Molar equivalents of guest to host after addition
1	10	0.25
2	10	0.5
3	20	1
4	40	2
5	120	5
6	200	10

The peaks in the NMR spectrum and their shifts upon guest additions (figure 6) were collected into an Excel sheet, and the association constant between **1** and Cl^- was measured by fitting the titration data to a 1:1 host-guest binding isotherm using http://app.supramolecular.org/bindfit/.

The fit data is shown in figure 7. The association constant was 975 ± 177 M^{-1}. For an octamethyl calix[4]pyrrole the value in DMSO with TBACl is 2200 M^{-1}.[3] This means that **1** has lower affinity for chloride ions than a non-substituted calix[4]pyrrole. There is a potential that **1** could have a higher affinity for some other anion.

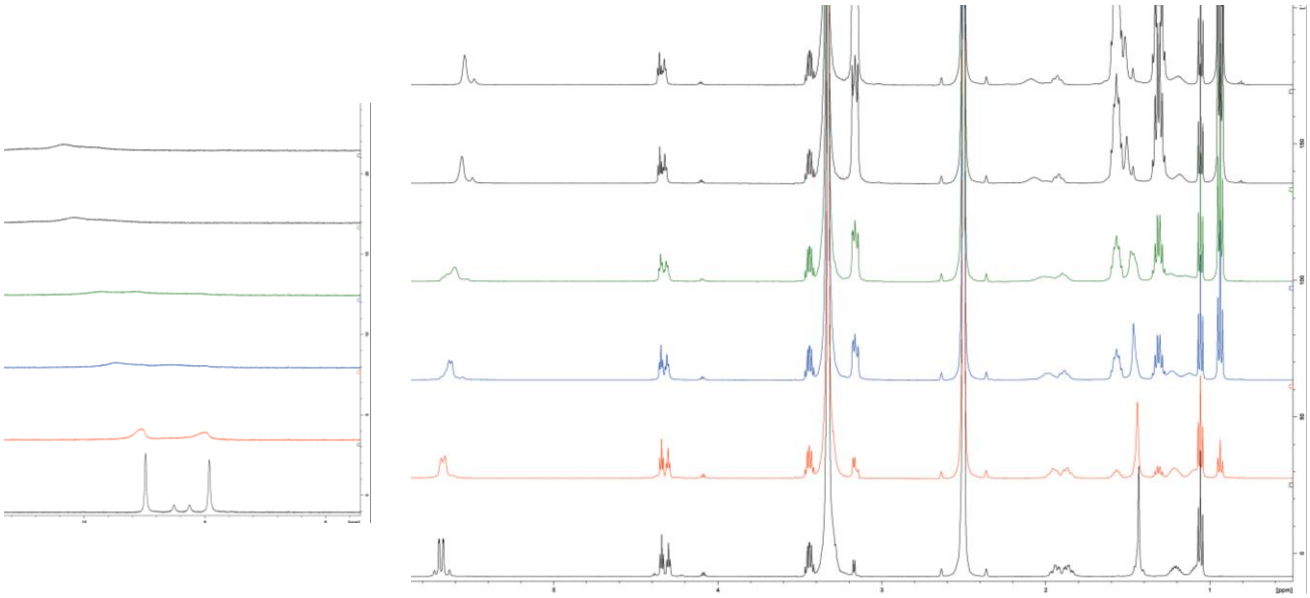


Figure 6. The spectra of the NMR titration. In the bottom the spectrum of the free host, and the additions (1-6) are stacked on top in order. On the left are the NH-peaks, and on the right is the upfield region. The movement of the peaks can be seen clearly, especially with the NH peaks.

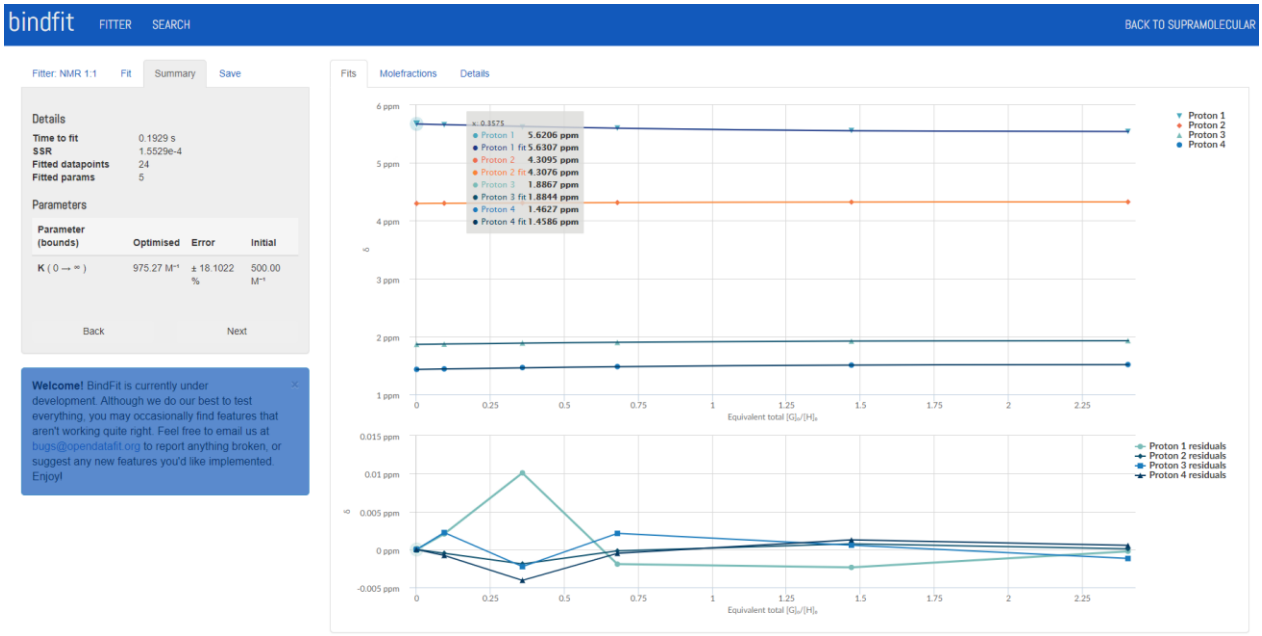


Figure 7. The bindfit-data for the determination of binding constant between **1** and Cl^- (1:1 stoichiometry).

6 CONCLUSIONS

A four-armed calix[4]pyrrole containing *meso*-hydroxypropyl substituents was synthetized. All the attempted syntheses resulted in product formation, but the yields of the purified solid product were low. The two methods did have a significant difference in the yield if the crude products are compared containing a mixture of the αααα, ααββ, αβαβ and αααβ isomers. The first method gave hundreds of milligrams of relatively pure product without recrystallization or any other purification (as is seen in the NMR).

The recrystallization process caused some of the isomers of the calix[4]pyrrole to be eliminated from the solid product. When the filtrate from second synthesis in method 1 was analyzed with NMR, it became evident that most of the isomers of the product did not recrystallize back into a solid but remained in the filtrate. The NMR spectrum for the filtrate has some NH-peaks more intense than the solid and vice versa (appendix 2). This means that recrystallization may have a role in separating isomers of *meso*-substituted calix[4]pyrroles.

The first method, based on the N-H peaks on the ^{1}H NMR spectra, produced a purer product. It is likely also due to the difference in the purification method. The first method is also less laborious so it could be favored over the second one. It is not sure why the second synthesis of the first method did not crystallize as expected.

The association constant of **1** and Cl^- was determined using NMR titration to be 975 ± 177 M^{-1} which is lower than the value for a non-substituted calix[4]pyrrole. The error is quite large due to the broadening of the NMR signals upon binding. In addition, ethanol was discovered from the NMR spectra due to it being used to clean the used glassware. The NMR titration should be measured again with properly cleaned and dried glassware to get a more accurate result. More association constants for **1** could also be determined, in order to see if the molecule could have a use in for example selective ion recognition and to compare its ion binding abilities to other calix[4]pyrroles.

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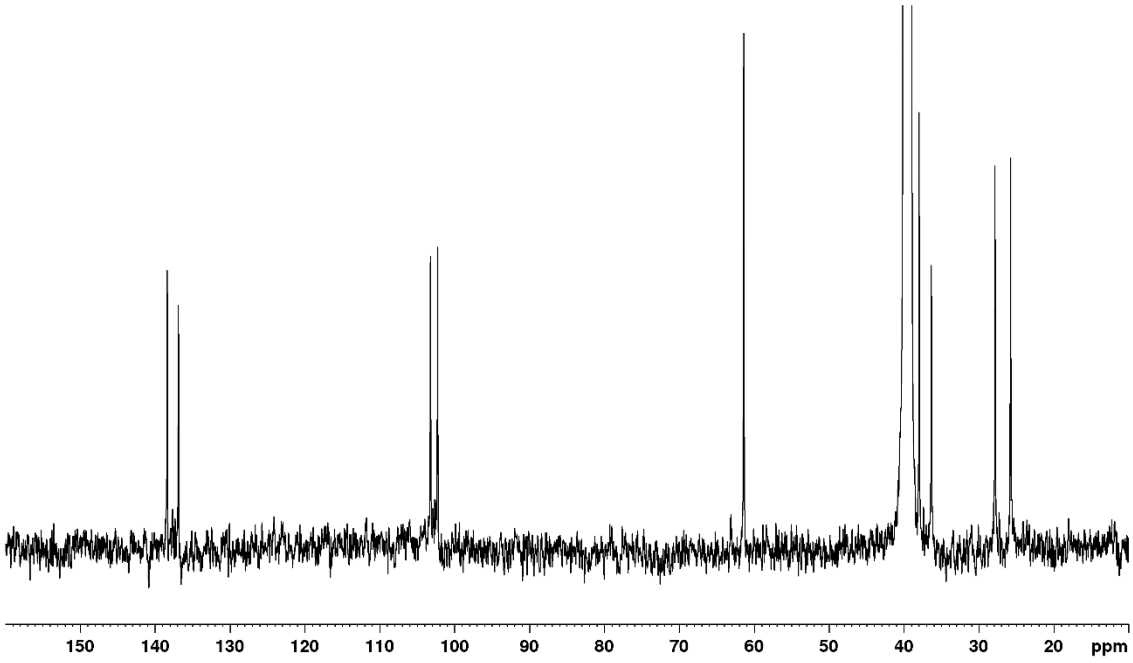
APPENDICES

APPENDIX 1.

^{13}C NMR (DMSO-d6, 500 MHz), method 1, synthesis 1.



APPENDIX 2

Comparison between the filtrate and the solid synthesis 2 of method 1. In the bottom the spectrum of the product pre-crystallization, in the middle the recrystallized solid product and at the top the filtrate.