SYNTHESIS AND ANION BINDING AFFINITY OF A TRIPODAL HEXAMIDE LIGAND

DISSERTATION

Submitted in partial fulfilment for the award of the degree of M.Sc. in Inorganic Chemistry

By

Sybil Tina Pereira

Roll No. CH-18-63

To

School of Chemical Sciences

Goa University

April 2020

STATEMENT

I hereby declare that the matter presented in this dissertation entitled **''Synthesis and Anion Binding Affinity of a Tripodal Hexamide Ligand''** is based on the result of investigations carried out by me in the School of Chemical Sciences, Goa University under the supervision of **Dr. Sandeep Kumar Dey** and the same has not been submitted elsewhere for the award of a degree or diploma.

Ms. Sybil Tina Pereira

CH-18-063 (Inorganic Chemistry)

CERTIFICATE

This is to certify that the dissertation entitled **‘‘Synthesis and Anion Binding Affinity of a Tripodal Hexamide Ligand’’** is bonafide work carried out by **Ms. Sybil Tina Pereira** under my supervision in partial fulfillment of the requirements for the award of the degree of Master of Science in Chemistry in the School of Chemical Sciences, Goa University.

Dr. Sandeep Kumar Dey

Project Supervisor

School of Chemical Sciences

CERTIFICATE

This is to certify that the dissertation entitled **‘‘Synthesis and Anion Binding Affinity of a Tripodal Hexamide Ligand’’** is bonafide work carried out by **Ms. Sybil Tina Pereira** under the supervision of **Dr. Sandeep Kumar Dey** in partial fulfillment of the requirements for the award of the degree of Master of Science in Chemistry in the School of Chemical Sciences, Goa University.

Dean,

School of Chemical Sciences

Goa University.

ACKNOWLEDGEMENTS

During the tenure of this project, I have been aided by a number of dedicated, talented people, to whom I wish to express my appreciation for their most valuable help.

Firstly, I would like to express my sincere gratitude to my project guide, Dr. Sandeep Kumar Dey, from whom I have profited by his expertise, good judgment, encouragement and commitment to the project at every step of the way. Dr. Dey's dedication, passionate interest and his impeccable sense of humor shall always be looked up to and remembered.

My sincere thanks, to Dr. V. S. Nadkarni, Dean, School of Chemical Sciences, for providing all necessary facilities during my project work.

I would like to acknowledge the Department of Science and Technology (DST), DST-FIST for X-ray diffractometer and NMR facilities, as well as the Institute of Inorganic and Structural Chemistry, Heinrich-Heine University, Dusseldorf, Germany.

I would also like to extend my sincere thanks to Mr. Sarvesh Harmalkar and Mr. Shashank Mhaldar for their timely help with sample analysis and advice.

I thank lab assistant, Mr. Deepak Chari for readily assisting me during my experimental work.

My indebted gratitude to my parents for their moral support, and encouragement. They have played the biggest role in getting me to where I am today. I would also like to express my thanks to our project group, especially my project partner, Archana, for being a constant source of help, inspiration and being an idol, I will always look up to. In a very special way, I would like to thank Sarvesha for always helping me out in times of difficulty.

In writing a project of this kind, one incurs a mounting indebtedness to several writers on this subject. As I have drawn and assimilated a great amount of material from works of these well-known researchers, I acknowledge with gratitude, my debt to these authors and their publishers whose work I have referred to in the course of my project.

I thank the Almighty for the immense blessings bestowed upon me.

To all these truly outstanding people, and to many others, my deepest gratitude. I truly appreciate your help, guidance and support. Thank you.

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1. Introduction

The study of complexation of anions, from the beginning of the late 1960s, has moved on from being an area solely of academic interest in Supramolecular Chemistry to applications in biochemistry and environmental science in modern era. Over recent years, we have seen the development of a plethora of anion sensors, anion-responsive materials, organocatalytic processes involving anion complexation, as well as development of new systems to selectively bind and extract anions from mixtures.[1] A great deal of attention has recently been focused on the selective recognition of anions by means of synthetic receptors due to their potential applications in many fields, ranging from environmental monitoring, and industrial purposes to clinical diagnostics.[2] Anions like phosphates, halides, nitrates, and carboxylates play important roles in different biological and environmental processes. Environmental and water pollution have raised alarm where different inorganic anions are also responsible. For example, excess use of phosphate containing fertilizers in agriculture is the key reason for eutrophication of water body. Acid rain, which is formed due to NO_2 containing anions like nitrates, causes erosion of our beloved sculptures. On the other hand, higher content of fluoride in drinking water is linked with many health hazards.

1a. Anion Coordination Chemistry

Supramolecular chemistry of anion has grown over the last few decades depending upon receptor dimensionalities, functionalities, platforms, and different non-covalent interactions. The first report on anion recognition with catapinands by Park and Simmons evoked the potentiality of this fields of research.[3]

Unlike transition-metal coordination, the binding of anions with synthetic receptors falls into the realm of "supramolecular chemistry", i.e., interactions between molecular or ionic species in the absence of covalent bond formation. As *Moyer* and *Bonnesen* pointed out, however, it is important to understand the factors influencing anion recognition in the traditional analytical sense, where simple physical properties such as size and hydrophilicity tend to govern selective exchange of one anion over another.[4]

Development and precise arrangement of bonding motifs in the cavity of artificial hosts are of special importance, however an extreme challenge, for efficient anion recognition.[5] The observations in natural systems have inspired researchers to develop numerous

neutral receptors that employ hydrogen bonds offered by specific binding sites from amide, urea/thiourea, pyrrole and indole functionalities for the recognition and binding of anionic guests on suitable frameworks. In contrast, cationic hosts with guanidinium and polyammonium functionalities ensure an adequate electrostatic attraction reinforced by hydrogen bond contacts with the coordinated anions, and the selectivity can be attributed to the charge and basicity factors, rather than true selectivity of the host for anions. Anions generally have very high solvation energies that must be compensated by the host for effective anion recognition. Tripodal scaffolds offer a flexible and structurally preorganized cavity, which has previously been explored in the area of anion coordination chemistry and anion induced formation of capsular assemblies. One of the most fascinating features of molecular capsules is their ability to create a distinct microenvironment that isolates the encapsulated guest from the bulk of the solvent media and, thereby, leads to phenomena such as molecular sorting when formation is possible for different capsules present in the same solution.[6]

The selectivity of a synthetic receptor towards a specific anion is determined by multiple interactions between host and guest in a complementary fashion. The molecular design allows the rational control of binding properties such as complex stability and selectivity. The selectivity of tripodal synthetic receptors relates greatly to the rigidity of its arms and its cavity size. The main aims of designing tripodal anion receptors are: (i) there are a sufficient number of positively charged or neutral electron deficient groups in the ligand to serve as interaction sites, (ii) Receptors with a flexible tripodal structure have a strong affinity for oxyanions, such as phosphate, sulphate and carbonate, because the geometry and orientation of the host molecules favour the formation of stable host guest complexes. The host molecules can be ensured interactions with guest based on non-covalent interactions, which include electrostatic interactions, hydrogen bonding, hydrophobicity, π–πstacking and a combination of these interactions. Theoretical investigation by *Hay et al*. showed that the effect of electron withdrawing substituents on the aryl moiety significantly enhances the stability of anion complexes. Generally, many observable signals such as redox potential changes, NMR and UV-Vis spectral changes, colour changes, and emission fluorescence changes have been used as indication of host-guest interactions. In particular, NMR is the most widely used techniques for measuring interactions between host and guest.[2]

1b. Tripodal amide receptors for anion coordination

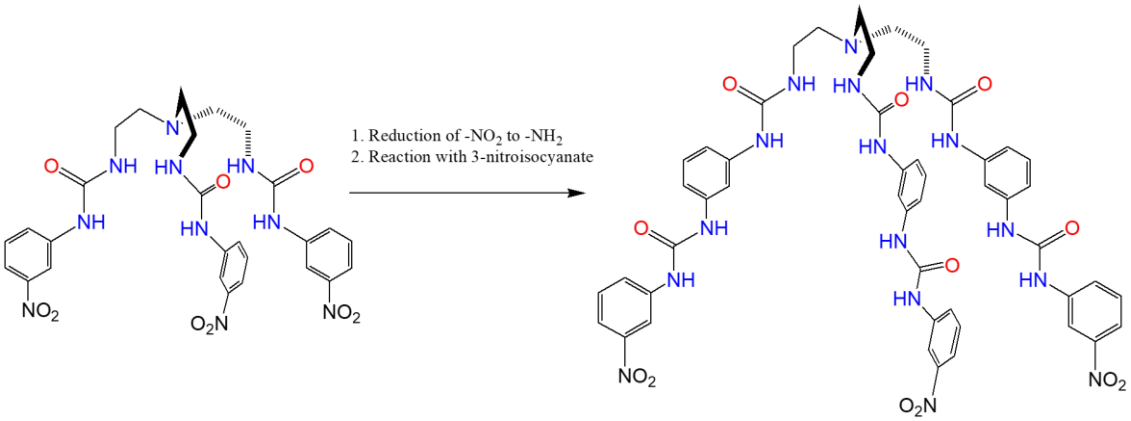
Significant effort has been made to establish the coordination behaviour of anions with synthetic receptor systems due to their critical role in environmental, medicinal and biological issues.[7] Inspired by biological processes of anion recognition and transporting via amide functionality, many artificial receptors incorporating amide functional groups have been reported.[8] Anion binding by tripodal amide receptors started with the pioneering works of *Beer et al*. and *Reinhoudt et al*. in the early 1990s. Such receptors are found to be selective for dihydrogen phosphate. However, in this century, various research groups have shown that tripodal amide receptors can be potential scaffolds for recognition of spherical, planar, as well as hydrated anions. As mentioned earlier, due to the problems caused by phosphates, fluoride, nitrate, etc, designing of receptors for selective recognition of these anions is of prior interest to the researchers around the globe. In this direction, most utilized scaffolds for tripodal amide-based receptors for such anion recognition are Tris(2-aminoethyl) amine and 1,3,5-trialkyl benzene. However, tripodal amides on other platforms have also shown promise for recognition of aforementioned anions.

Tren-based tripodal amide receptors

Tren-based receptors with different binding motifs have been extensively studied from the beginning of anion recognition chemistry. *Beer et al.* have shown halide and ReO_4^- binding by tripodal amide receptors by ^{1}H-NMR titration studies. The work by *Ghosh et al.*and *Das et. al.* dictated that positional isomers play an important role toward guest selectivity. Tren-based amide receptors, due to their small cavity size, mostly recognize a spherical halide F^-/Cl^-within its molecular cavity. If the -NH protons are acidic enough due to the incorporation of a highly electron-withdrawing substituent into the receptor framework; deprotonation may occur in the presence of highly basic anions such as F^- and CH_3COO^-. Notably, a nitro group at the *meta*-position of the peripheral aryl function increases the acidity of the *ortho*-CH protons to participate in anion binding.[3]

Second generation tripodal receptors

In general, second generation tripodal receptors are those that are derived from synthetic modification of first-generation receptors. For example, reaction of tris(2-aminoethylamine) with 3-nitrophenyl isocyante provides a first-generation tris(urea) receptor for anion recognition. Reduction of the nitro groups in the first-generation tris(urea) receptor and subsequent condensation of the amine groups with 3- nitrophenyl isocyanate yields a second generation hexaurea receptor for anion recognition. Both these first and second-generation N-bridged receptors have been studied in detail for anion coordination in the solid and solution state.[9]



First generation receptor Second generation receptor

Figure 1: First generation tripodal receptor and subsequent synthesis of a second generation tripodal receptor.

1c. Anion selective tripodal amide ligands

M. Arunachalam and *P. Ghosh* (2009) have reported the synthesis of a bowl-shaped receptor (Figure 2).The single-crystal X-ray crystallographic analysis of four hydrogen bonded anion complexes showed encapsulation of anions or anion-water clusters such as, $(NO_3^-)_2$, $[(AcO)_2(H_2O)_4]^{2-}$, $[F_2(H_2O)_6]^{2-}$, and $[Cl_2(H_2O)_4]^{2-}$ within the staggered dimeric capsular assembly of the receptor **L1**. The amide -NH groups acts as hydrogen bond donor in the capsular assemblies. Upon alteration of $-NO_2$ group substitution from the ortho to the para position in the receptor design, a marked difference in aggregational properties was observed. Though **L1** demonstrates capsule formation upon anion or hydrated anion complexation for all of the anions studied here, its positional isomer with the *o*-nitro-substituted tripodal receptor **L2** selectively formed the dimeric capsular

assembly upon encapsulation of $[F_2(H_2O)_6]^{2-}$ and noncapsular aggregates with other anions.[10]

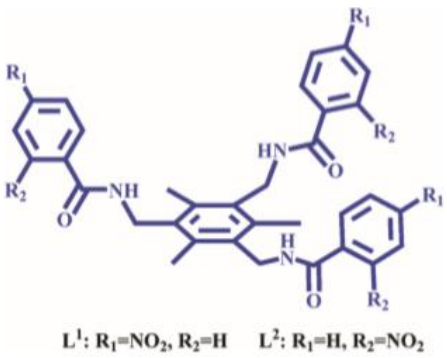


Figure 2: Molecular structure of mesitylene-based tripodal amide receptors.

A. S. Singh and *S. S. Sun* (2012) have reported the following conformationally flexible N-bridged tripodal receptors **1−5** (Figure 3) which forms in situ cone shape conformation upon protonation. The protonation-induced preorganized cavity is capable of selectively recognizing and encapsulating a nitrate anion through amide -NH hydrogen bonds to form discrete nitrate complexes. Receptor **5** represents a unique example of neutral receptor for encapsulation of highly hydrated and weakly coordinated nitrate anion. *Gerber's* group reported a mechanism for nitric oxide in contact with water to form nitric acid. Based on *Gerbers* work, the group envisioned that the high affinity of receptors **1−4** to bind nitrate anion in acidic medium would render the trapping of nitrogen dioxide gas, after water contact by these receptors, highly feasible. Therefore, the fixation of gaseous NO to nitrate was explored with these receptors. Effective trapping of nitrogen oxide upon hydrolysis in aqueous solution by these receptors was seen and thus providing a proof-of-concept method for monitoring the environmental concern of atmosphere smog.[8]

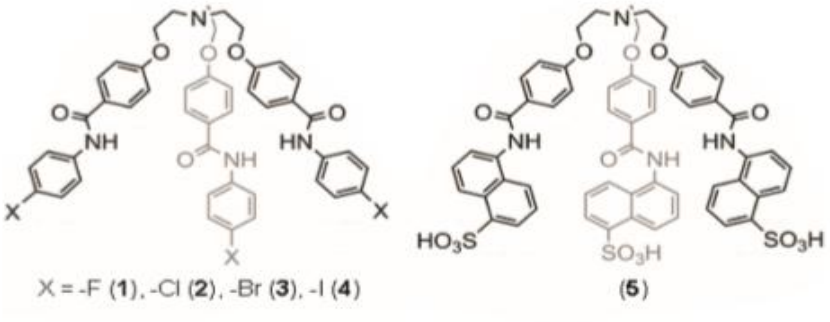


Figure 3: Molecular structures of nitrate selective tripodal amide receptors.

S.K. Dey, *B.K. Datta*, *G. Das* (2012) have reported a fluoride selective dinitrophenyl functionalized tris(amide) receptor **L3** (Figure 4) which showed distinct complexation behaviour towards the F^-anion when tetrabutylammonium fluoride (TBAF) and potassium fluoride (KF) salts were employed for F^- recognition. X-ray crystallography analyses revealed the formation of a F^- encapsulated complex (1:1 host–guest) stabilized by three N–H···F^- and three C–H···F^- hydrogen bonds when TBAF was employed as the F^- source, whereas in the KF complex of **L3** (1:1 host–guest), the receptor is involved in side-cleft binding of a hydrated KF contact ion-pair governed by amide N–H···F^-, aryl C–H···F^- and lp (F^-)···π interactions. In the ^{1}H NMR analyses, a huge downfield shift of the hydrogen bonded –NH and *ortho*–CH protons was observed in the TBAF complex in comparison to the KF complex. Thus, receptor **L3** provides an ideal example of a flexible F^- binding host which adapts its conformation to respond to the demands of the specific counter cation.[11]

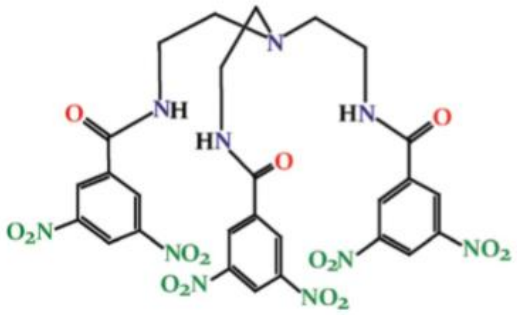


Figure 4: Molecular structure of fluoride selective tripodal amide receptor **L3**.

G. Das and *A. Basu* (2013) have reported the synthesis of a tripodal amide receptor **L4** capable of encapsulating a tetrameric halide water cluster $[X_2(H_2O)_2]^{2-}$ ($X=Cl^-/Br^-$) within its dimeric capsular assembly (Figure 5). X-ray structure determination revealed that a hydrogen bonded rectangular chloride-water cluster $[Cl_2(H_2O)_2]^{2-}$ is encapsulated within the dimeric capsular assembly of the protonated receptor. Interestingly, X-ray crystal structure analysis of the bromide complex established it to be isostructural with the chloride complex**.** Single crystal X-ray structure analysis of the iodide complex revealed a non-capsular 1D polymeric assembly and showed change in conformation of the receptor.[12]

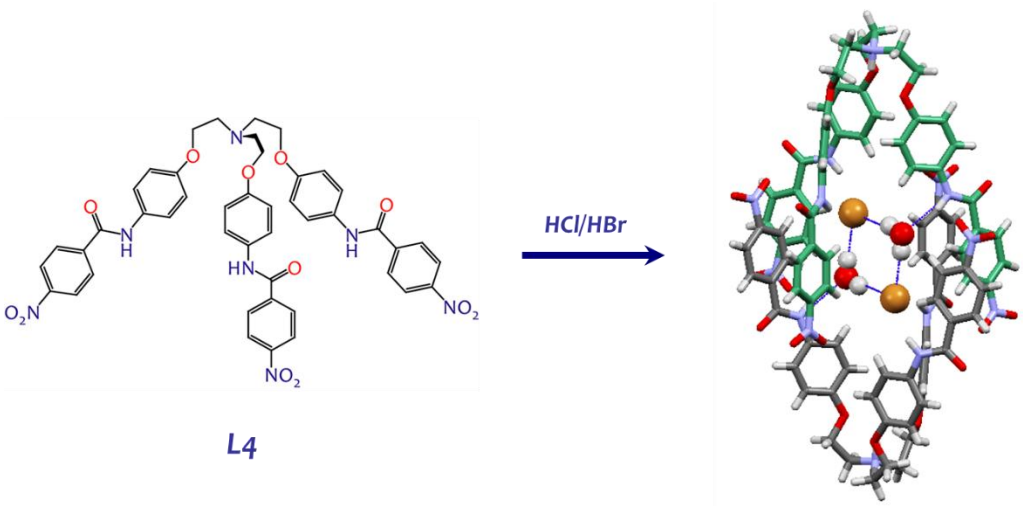


Figure 5: Molecular structure of tripodal amide receptor **L4** and protonation induced capsular assembly formation with halide.

Prohen et. al. investigated the synthesis and characterization of squaramide-based receptors designed for recognition of carboxylates in highly competitive solvents. These receptors were tailored for the binding of certain mono, di and tricarboxylates. Receptor **L5** showed a 1:1 binding ratio for acetate with an 8-10-fold increase in association constant compared with the other identical receptors.

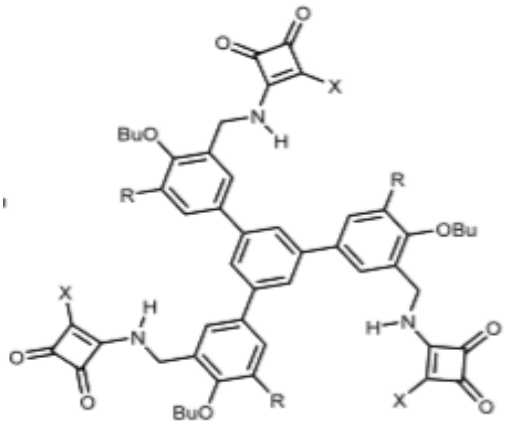


Figure 6: Molecular structure of squaramide-based tripodal amide receptor **L5**.

Zhang et al. prepared a tris-amide receptor functionalized with cyclotriveratrylene, **L6** and immobilized it on gold surface via formation of self-assembled monolayer. Absorption and ^{1}H-NMR titrations indicated that the receptor can selectively bind with acetate anion among the investigated anions (Cl^{-}, Br^{-}, NO_3^{-}, HSO_4^{-}, and $H_2PO_4^{-}$).

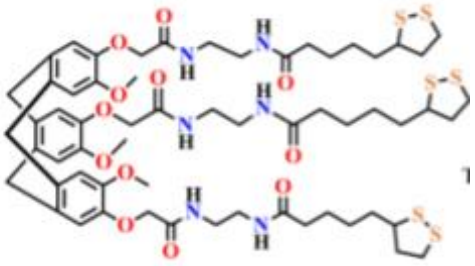


Figure 7: Molecular structure of acetate selective cyclotriveratrylene-based tripodal hexamide receptor **L6**.

Ozturk et al. reported amide-based neutral tripodal anion receptors **T56** and **T57** for recognition of $H_2PO_4^{-}$ and $C_6H_5CO_2^{-}$ anions in presence of other anions such as PF_6^{-}, ClO_4^{-}, HSO_4^{-}, and Br^{-}. Pyridyl appended tripodal receptor **T56** showed higher binding affinity toward anions as compared to its benzene analogue **T57**, however, no selectivity was observed for these receptors.[3]

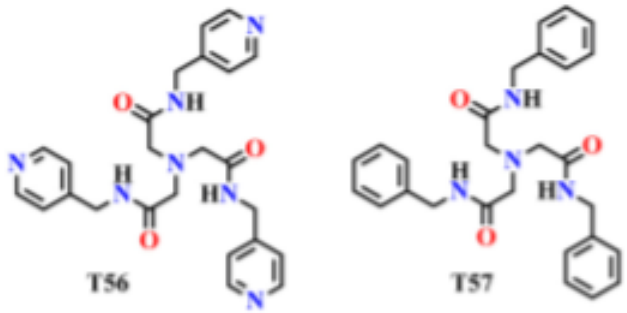


Figure 8: Molecular structures of tripodal amide receptor**s T56 and T57**.

A cationic tripodal receptor, based on amide-pyridinium as recognition sites and nitrobenzene as signalling unit showed high selectivity and strong binding affinity for AcO^{-} over other anions. UV–Vis and ^{1}H NMR experiments indicated that the selectivity can be attributed to synergistic effects arising from hydrogen bonding, electrostatic interactions and conformational change.[13]

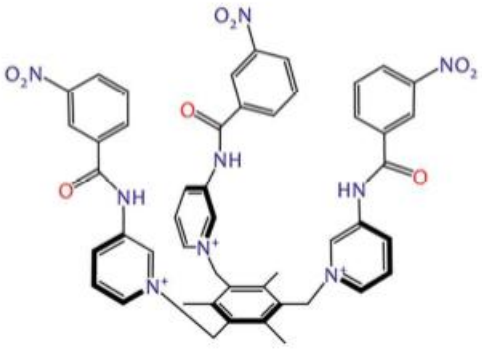


Figure 9: Molecular structure amide-pyridinium-based tripodal receptor.

1d. Applications of tripodal amide receptors

Anion sensing: *Anzenbecher et.al.* utilized phosphate selectivity of the following pyrrole-based amide receptor toward the detection of inorganic phosphates ($H_2PO_4^-$/HPO_4^{2-} present on blood serum) by embedding in hydrophilic polyurethane (biocompatible polymer) matrices. In this direction, they performed component analysis, which revealed that films of these receptors were capable of distinguishing inorganic phosphates from adenosine monophosphate (AMP) and adenosine diphosphate (ADP) in water.[3]

Das et al. utilized **L3** as selective F^-sensor with characteristic solvent dependent absorptions in the optical spectroscopy. Due to addition of F^-, three new peaks were generated in the visible region of the absorption spectrum which showed increase in absorption intensity during gradual addition of F^-. Such deep coloration (colourless to red/blue) attributed to strong anion-π charge-transfer interactions involving F^- and **L3**, similar to the charge transfer interactions in metal complexes.[3,11]

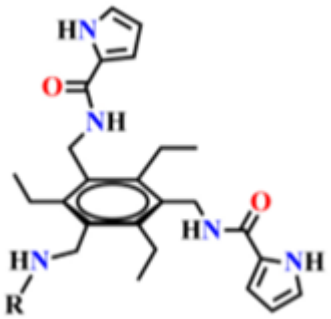


Figure 10: Molecular structure pyrrole-based amide receptor used in phosphate sensing.

Anion extraction: *Beer et al*. designed hetero-di-topic 15-crown-5 ether affixed tripodal anion receptor and two controlled receptors having three distinct amide groups as anion binding units. The former showed significant binding with Cl^-, I^-, and ReO_4^-. However, in presence of Na^+ the binding constant values found to increase around 10-folds due to cooperative effect of Na^+ coordination with ethereal unit. Also, efficient extraction and transportation of toxic anions obtained from nuclear waste were carried out using cooperative interaction via sodium ion complexation. Dual host approach had been utilized by *Ghosh et al*. by employing 18-Crown-6 and a pentafluorophenyl-based tripodal amide receptor (Figure 11), for selective removal of KF and KCl via liquid–liquid extraction (chloroform/water) technique. The extracted complexes obtained by the liquid– liquid extraction process was characterized by NMR and X-ray diffraction analysis.

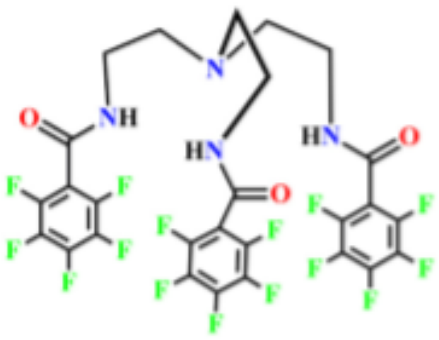


Figure 11: Molecular structure pentafluorophenyl-based tripodal amide receptor used in extraction of fluoride from water.

Anion transportation: Recently, *Gale et al*. Reported Tren-based anion receptors having phenylalanine amino acid residues and amide-urea functionalities **T71–T74** (Figure 12) with potential ability to interact with the anions of biological interest (L-lactate, L-maleate, and L-aspartate, etc.). The ligands were found to bind selectively with Cl^- as compared to other organic anions. The transport of the anions was affected by the nature of the aliphatic central spacer and the side chains. The receptor having shortest aliphatic side chain and longest spacer was found to be the most active receptor.

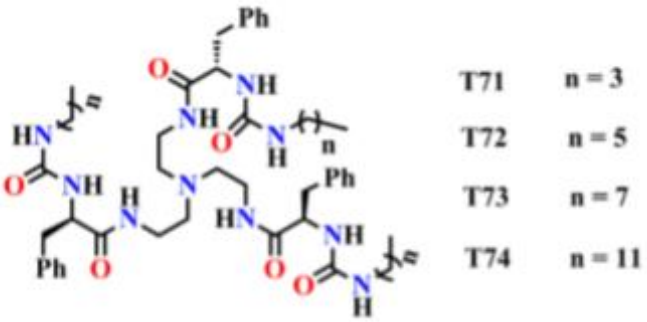


Figure 12: Molecular structure amide and urea functionalized tripodal receptors used in anion transportation studies.

Thus, the amide functional group is easily accessible synthetically and is often used to link structural components. The hydrogen-bonding of a -C(O)NH- group to a substrate anion is easily recognized by significant downfield shifts of the NH proton in ^{1}H-NMR spectroscopy experiments. The degree of hydrogen-bonding and NH shift are both solvent and concentration dependent allowing the measurement of association constants. The amide functional group should continue to be important in anion receptors because of its ease of synthesis and biological precedence.[3]

2. Synthesis and Characterization

2a. Synthesis and characterization of tris(4-amino-N-ethylbenzamide)amine (AL)

Tris(4-amino-N-ethylbenzamide)amine(**AL**) was synthesized by reduction of its nitro analogue (Tris(4-nitro-N-ethylbenzamide)amine**, NL**) which was synthesized by modification of the reported literature procedure (Scheme S1). **NL** was synthesized by the reaction of tris(2-aminoethyl)amine, (Tren) with 4-nitrobenzoyl chloride in 1 : 3.5 molar ratio at room temperature in dry chloroform. In a 100 mL flat bottom flask, 0.73 mL (5 mmol) of tris(2-aminoethyl)amine was dissolved in 25 mL of chloroform and 3.5 g of 4-nitrobenzoyl chloride (17.5 mmol) was added in portions into the above solution with constant stirring at room temperature. The reaction mixture was allowed to stir overnight at room temperature followed by the addition of 3 mL (excess) triethylamine and stirred for another 1 hrs. Reaction of tren with 4-nitrobenzoyl chloride generates HCl in the reaction medium, which eventually protonate the tertiary nitrogen of the formed **NL**.

Triethylamine was added to basify the reaction mixture so that **NL** can be obtained in its neutral form. The precipitate obtained was then filtered, collected in a 250 mL flat bottom flask and washed with 50 mL of methanol in the presence of 1 mL of triethylamine under stirring. The compound was finally filtered again and washed with another 50 ml of methanol over the filter paper to ensure its purity for subsequent reduction reaction.

In a 250 mL flat bottom flask, 1 g of **NL** was dispersed in 100 ml of ethanol and 100 mg of Pd/C and 1 mL of hydrazine hydrate was added into the flask. The reaction mixture was then refluxed overnight at about 80 °C and filtered to remove the heterogeneous Pd/C catalyst. The filtrate was then allowed to evaporate in a beaker at room temperature when colorless crystals of **AL** were obtained in quantitative yield within 2 days. The crystals were collected by decantation/filtration and washed with 10 mL of ethanol to ensure its purity for spectroscopy analysis. The compound was characterized by NMR and FT-IR spectroscopy.

Isolated yield of **AL**: 614 mg (percentage yield 72%). The compound is highly soluble in dimethylformamide, and dimethyl sulfoxide, soluble in methanol/ethanol on heating, and insoluble in tetrahydrofuran, chloroform and acetonitrile.

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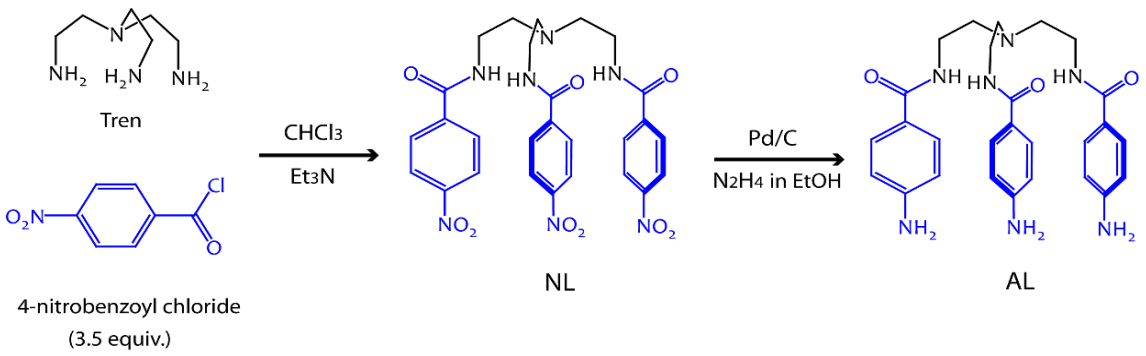


Scheme 1: Synthesis of **AL** from tris(2-aminoethylamine) and 4-nitrobenzoyl chloride.

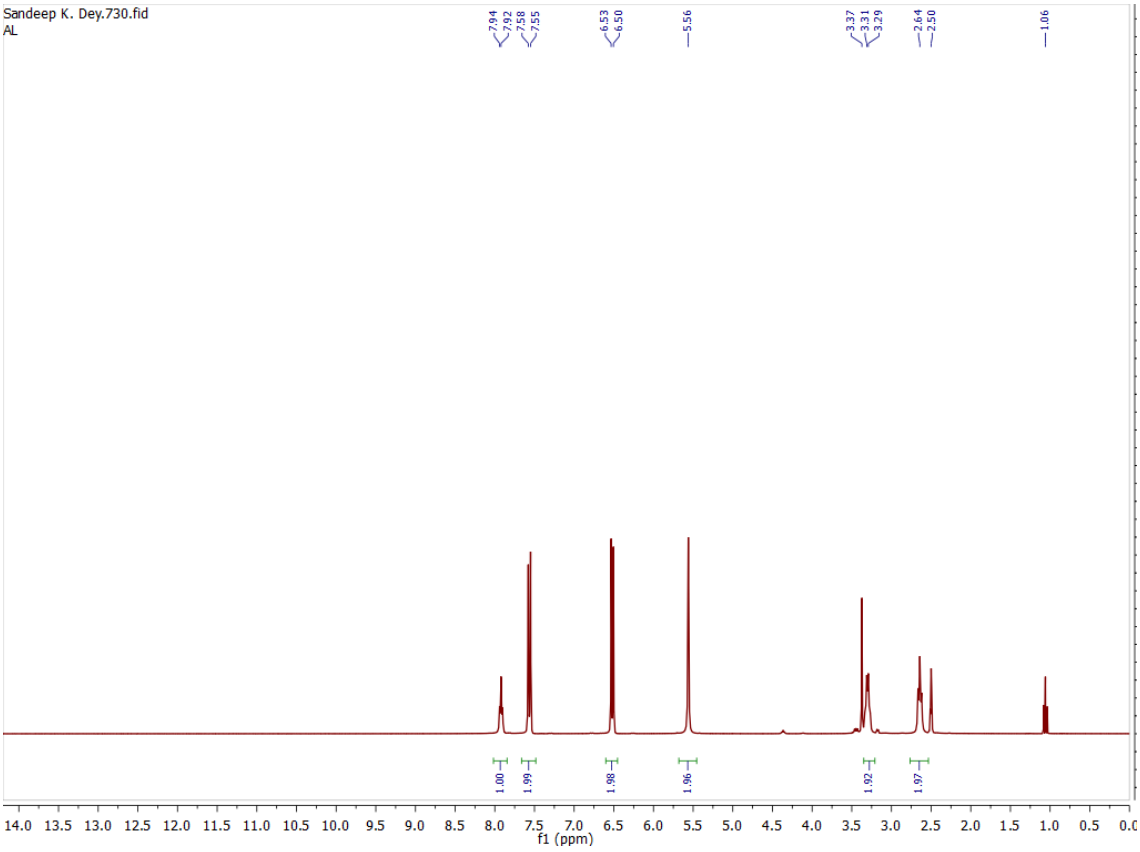


Figure 13: H-NMR spectrum of **AL** in DMSO-d_6.

Characterization of **AL**: ^{1}H-NMR (400 MHz, DMSO-d_6) chemical shift in δ ppm: 2.50 (DMSO-CH_3), 2.64 (t, 6xN**CH_2**), 3.30 (t, 6xNCH_2**CH_2**), 3.37 (HOD), 5.56 (s, 3x**NH_2**), 6.50 (d, 6xCH), 7.55 (d, 6xCH), 7.94 (t, 3x**NH**).

2b. Synthesis and characterization of tripodal hexamide ligand (HAL)

HAL was synthesized by the reaction of **AL** with 4-nitrobenzoyl chloride, in a 1:3.5 molar ratio at room temperature. In a 50 mL flat bottom flask, 0.5 g of **AL** was dissolved in a THF-ethanol binary solvent mixture in 8:2 (v/v) ratio (65 mL THF and 15 mL of ethanol) in the presence of tetrabutylammonium chloride. On complete dissolution, 0.5530 g of 4-nitrobenzoyl chloride was added in small portions to the above solution mixture. The reaction mixture was stirred overnight, followed by the addition of 3 mL (excess) triethylamine and stirred for 1 hour. The precipitate obtained was then filtered, dried and collected in a 250 mL flat bottom flask and washed with 50 mL methanol in the presence of 1 mL triethylamine under stirring. The compound was then filtered again and

washed with 50 mL of methanol over the filter paper to ensure its purity. The compound was characterized by ^{1}H-NMR and FT-IR spectroscopy.

The reason for the choice of binary THF-EtOH solvent mixture for the synthesis of HAL was due to the fact that DMSO and DMF, in which **AL** is readily soluble, instead of acting as a solvent, will react with nitro-benzoyl chloride (an acyl chloride) to give an undesired product (mechanism shown below). Tetrabutylammonium chloride was added to partially solubilize **AL** in THF (otherwise **AL** is not soluble in THF) and addition of ethanol resulted in complete solubilisation of **AL**.

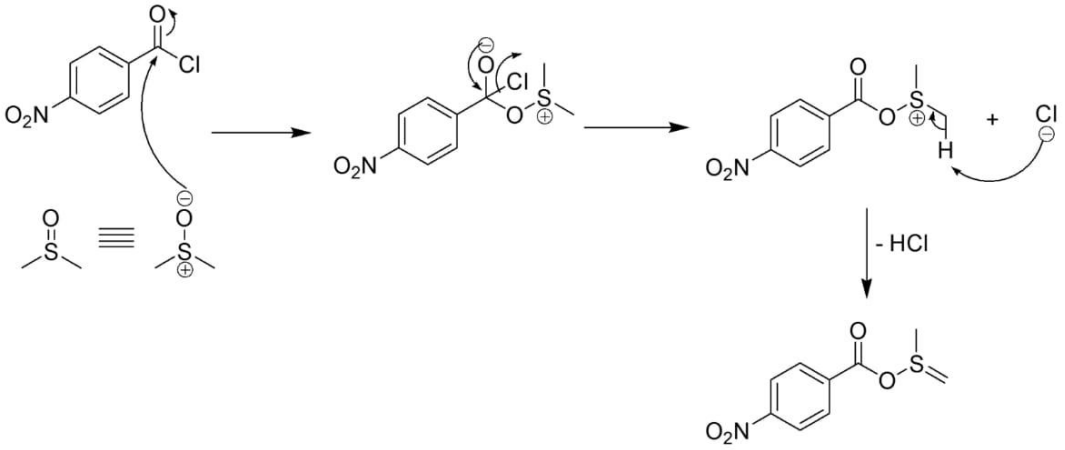
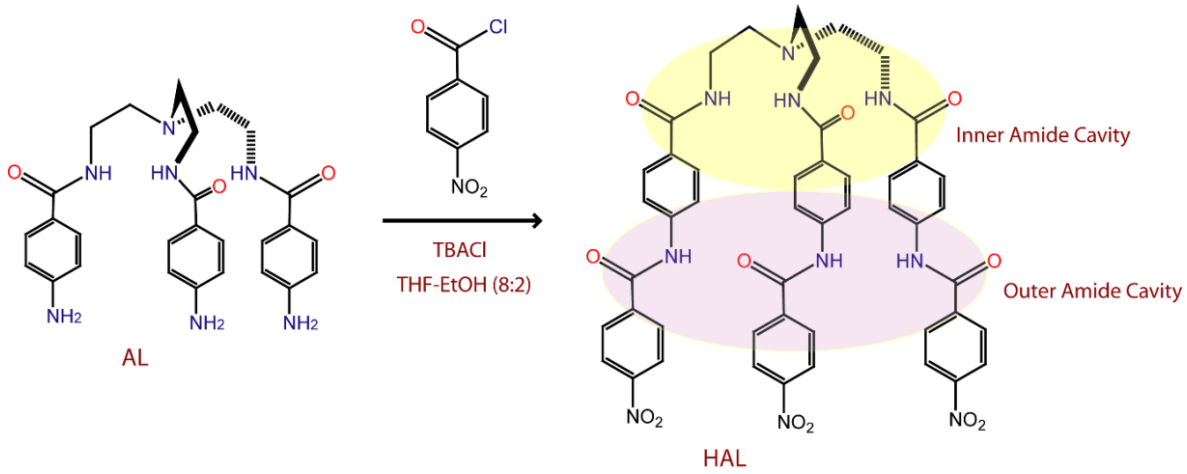


Figure 14: Mechanism of reaction between DMSO and nitro-benzoyl chloride.



Scheme 2: Synthesis of **HAL** from tris(4-amino-N-ethylbenzamide)amine (**AL**) and 4-nitrobenzoyl chloride.

The tripodal hexamide receptor **HAL** was characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR (KBr) and powder X-ray diffraction techniques.

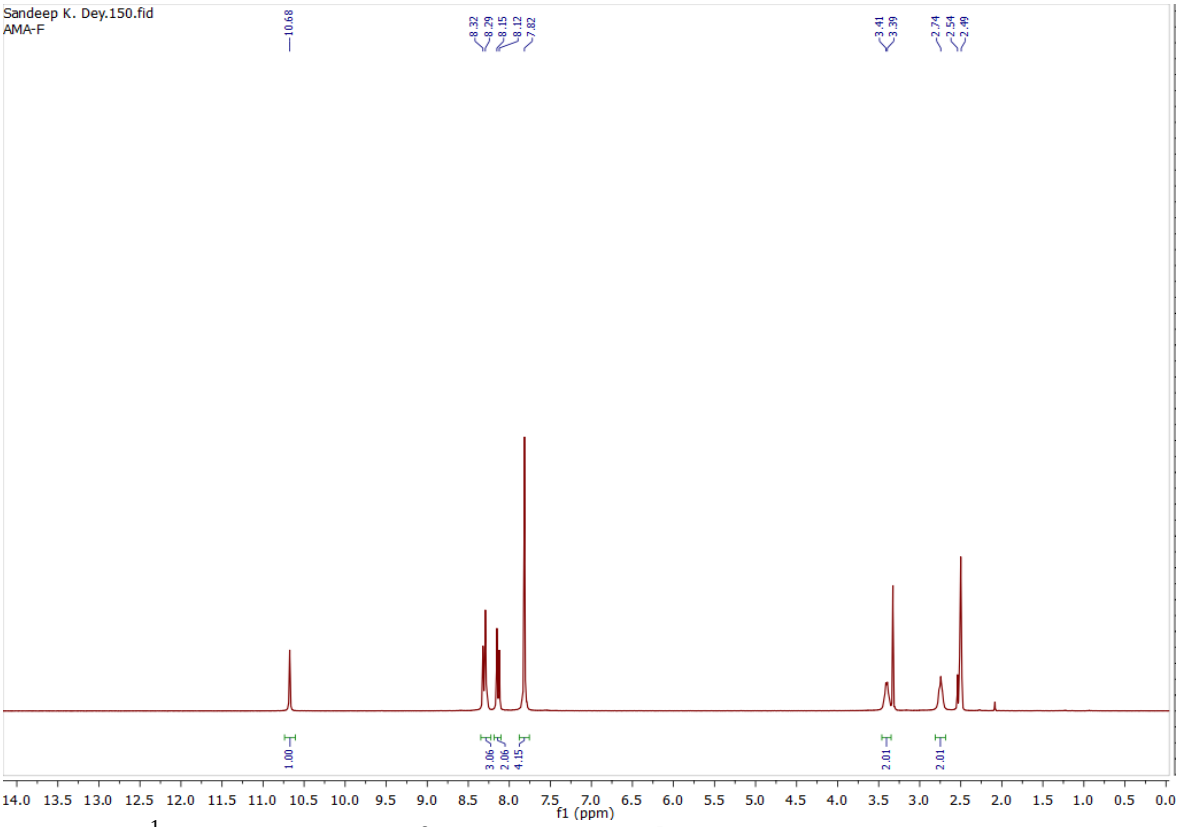


Figure 15: ^{1}H-NMR spectrum of **HAL** in DMSO-d_6.

Characterization of **HAL**: ^{1}H-NMR (400 MHz, DMSO-d_6) chemical shift in δ ppm: 2.50 (DMSO-CH_3), 2.74 (s, 6xN**CH_2**), 3.41 (s, 6xNCH_2**CH_2**), 3.39 (HOD), 7.82 (s, 12x-**CH**), 8.15 (d, 6x-**CH**), 8.32 (d, 6x-**CH** + 3x-**NH**), 10.68 (s, 3x-**NH**).

^{13}C-NMR (100 MHz, DMSO-d_6) chemical shift in δ ppm: 37.56 (3xCH_2), 39.50 (DMSO-CH_3), 53.23 (3xCH_2), 119.60 (6xCH), 123.44 (6xCH), 127.82 (6xCH), 129.24 (6xCH), 129.84 (3xCH), 140.26 (3xCH), 141.15 (3xCH), 149.13 (3xCH), 163.99 (3xC=O), 165.76 (3xC=O).

From the molecular structure of **HAL**, it is evident that the molecule has a C_{3v} symmetry and as such contains two different sets of aliphatic -CH_2 protons, four different sets of aromatic -CH protons and two different types of amide -NH protons. ^{1}H-NMR spectrum of **HAL** in DMSO-d_6 showed the aliphatic -CH_2 peaks at 2.75 and 3.41 ppm whereas, three other peaks in the aliphatic region originated due to the residual solvent molecules. The -CH protons of the inner aromatic ring occurs as a singlet at 7.81 ppm and the -CH

protons of the outer aromatic ring showed two distinct peaks at 8.12 and 8.32 ppm. The inner amide -NH proton is merged with the aromatic -CH signal at 8.32 ppm which is evident from the integral values observed, while the outer amide -NH proton occurs distinctly at 10.68 ppm. The outer -NH proton signal is significantly downfield shifted as compared to the inner amide -NH proton because the outer amide group is bonded to two electron withdrawing aromatic rings. Along the same line, outer aromatic -CH protons are downfield shifted as compared to inner aromatic -CH protons because the outer aromatic ring is bonded to a nitro group and an amide group at the para-positions, while the inner aromatic ring is bonded to two amide groups at the para-positions. Both nitro and amide groups are electron withdrawing functional groups however, nitro is more electron withdrawing than amide group due to the presence of a positive charge on the nitrogen atom.

^{13}C-NMR spectrum of **HAL** showed two peaks for the aliphatic -CH_2 carbons and ten distinct peaks in the aromatic region for the ten different types of carbons present including the two amide carbonyl carbons.

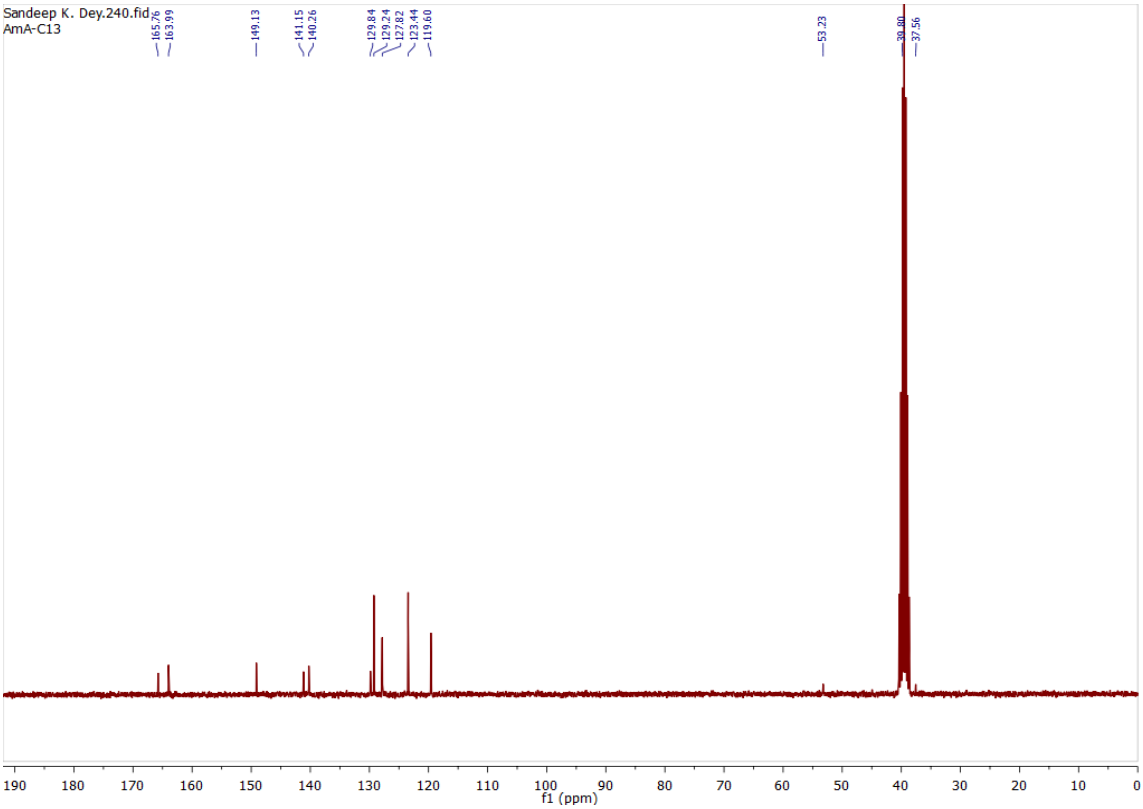


Figure 16: ^{13}C-NMR spectrum of **HAL** in DMSO-d_6.

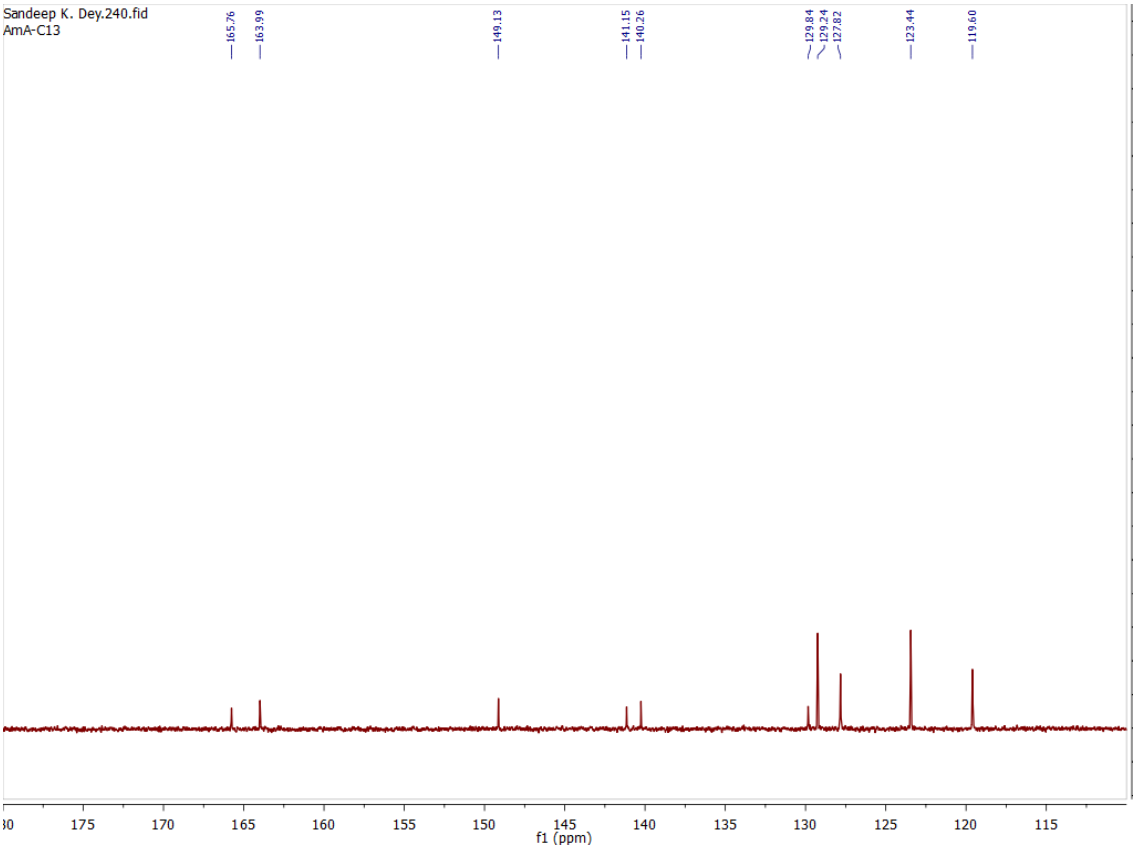


Figure 17: Aromatic region of ^{13}C-NMR spectrum of **HAL** in DMSO-d_6.

3. Results and discussions

3a. Solution state anion binding studies

Solution state anion coordination by hexamide receptor **HAL** was investigated by ^{1}H-NMR spectroscopy. In a typical experiment, 10 mg of HAL was dissolved in 0.5 mL of DMSO-d_6 and 2 equivalents of tetraalkylammonium (Bu_4N^+/Et_4N^+) salts of different anions (halide/oxyanion) was added into the solution. The solution was then sonicated to ensure complete solubility of the receptor and Bu_4N^+/Et_4N^+salts in DMSO-d_6.

Amide -NH protons are known to form hydrogen bonds with electronegative elements such as, F, Cl and O and these hydrogen bonds tend to be much stronger with anions such as F^-, Cl^-, SO_4^{2-} and HPO_4^{2-}. For oxyanions such as SO_4^{2-} and HPO_4^{2-}, O atoms act as hydrogen bond acceptor from the polarized hydrogen bond donor amide -NH. In H-NMR experiments, addition of 2 equivalents of TBAF resulted in disappearance of outer amide -NH signal due to hydrogen bond induced peak broadening, and inner amide -NH proton can now be observed at 8.47 ppm which was merged with an aromatic -CH proton signal

at 8.32ppm ($\Delta\delta$ = 0.15 ppm) in the spectrum of HAL. Observable upfield shift of the aromatic -CH proton signals were observed in the presence of TBAF. In contrast, addition of 2 equivalents of TBACl resulted in slight downfield shift of the outer -NH ($\Delta\delta$ = 0.3 ppm) and inner -NH signals ($\Delta\delta$ = 0.1 ppm), while -CH signals for the outer aromatic ring merged as a singlet at 8.24 ppm and singlet peak observed for the inner aromatic ring protons splits into multiplet at 7.87 ppm in the presence of TBACl. Addition of 2 equivalents of (TBA)H_2PO_4 showed disappearance of outer amide -NH signal due to peak broadening by hydrogen bond formation between -NH and $H_2PO_4^-$ anion. However, the inner amide -NH signal was observed to be upfield shifted by 0.25 ppm and each set of aromatic-CH proton signals can now be distinctly observed in the presence of (TBA)H_2PO_4.

Similar to the NMR spectral changes of HAL in presence of TBAF and (TBA)H_2PO_4, addition of 2 equivalents of TBA(CH_3COO), resulted in the disappearance of the outer amide –NH protons attributed to hydrogen bond induced peak broadening, and slight upfield shift of the aromatic –CH protons was observed. No change in peak position has been observed for the inner -NH proton. Disappearance of the amide -NH signal and changes in peak positions of aromatic -CH protons have also been observed in presence of TEACN. Addition of 2 equivalents of TBABr, $TBABr_3$, TBA(NO_3) and TBA(HSO_4) did not result in any observable changes in the NMR spectrum of HAL, indicating that the hexamide receptor does not interact well with these anions by hydrogen bonds.Thus, from the solution state studies, it has been confirmed that the inner amide cavity is not accessible to anions due to the possible intramolecular -NH···O=C interactions between the amide groups which has been observed in the crystal structure of **AL·H_2O** and thus, no significant shift of the inner amide -NH signals were observed in presence of different anions supplied as tetraalkylammonium salts. Whereas, the outer amide -NH protons can form strong hydrogen bonds with basic anions such as, fluoride, acetate, cyanide and hydrogenphosphate and thus, showed hydrogen bond induced broadening and subsequent disappearance of outer amide -NH signals.

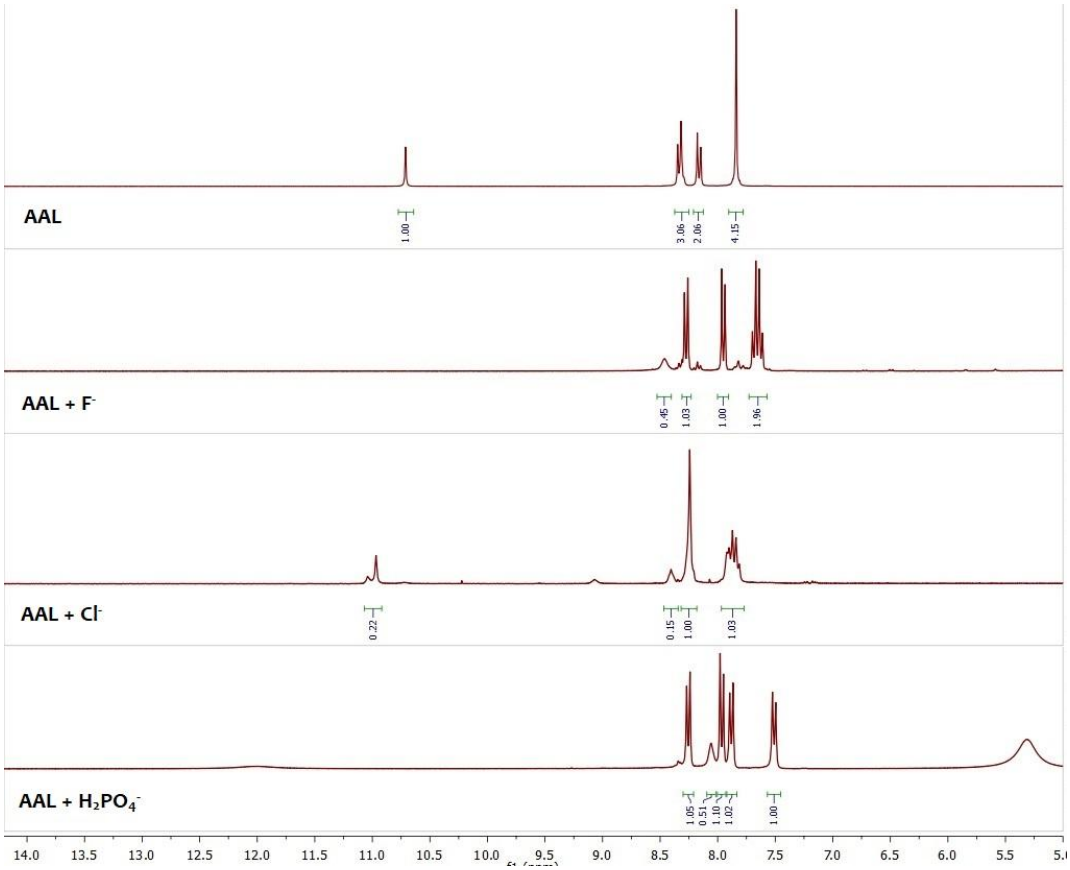


Figure 18: Aromatic region of ^{1}H-NMR spectra of **HAL** in the presence of 2 equivalents of (n-Bu_4N)F, (n-Bu_4N)Cl and (n-Bu_4N)H_2PO_4 in DMSO-d_6.

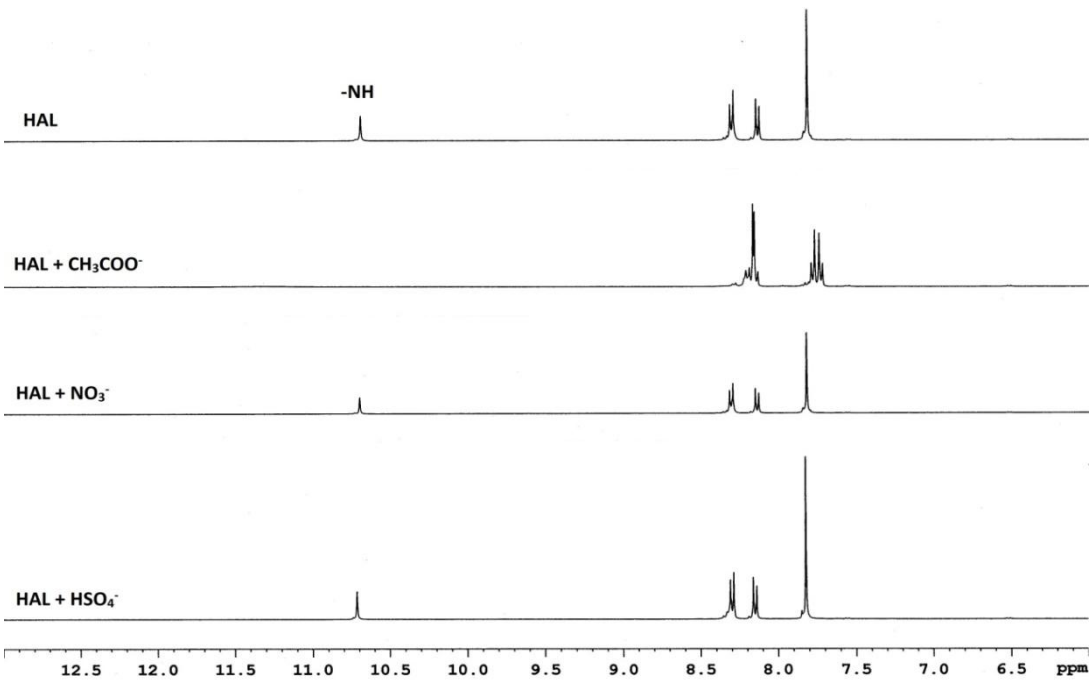


Figure 19: Aromatic region of ^{1}H-NMR spectra of **HAL** in the presence of 2 equivalents of $LiOOCH_3$, (n-Bu_4N)NO_3 and (n-Bu_4N)HSO_4 in DMSO-d_6.

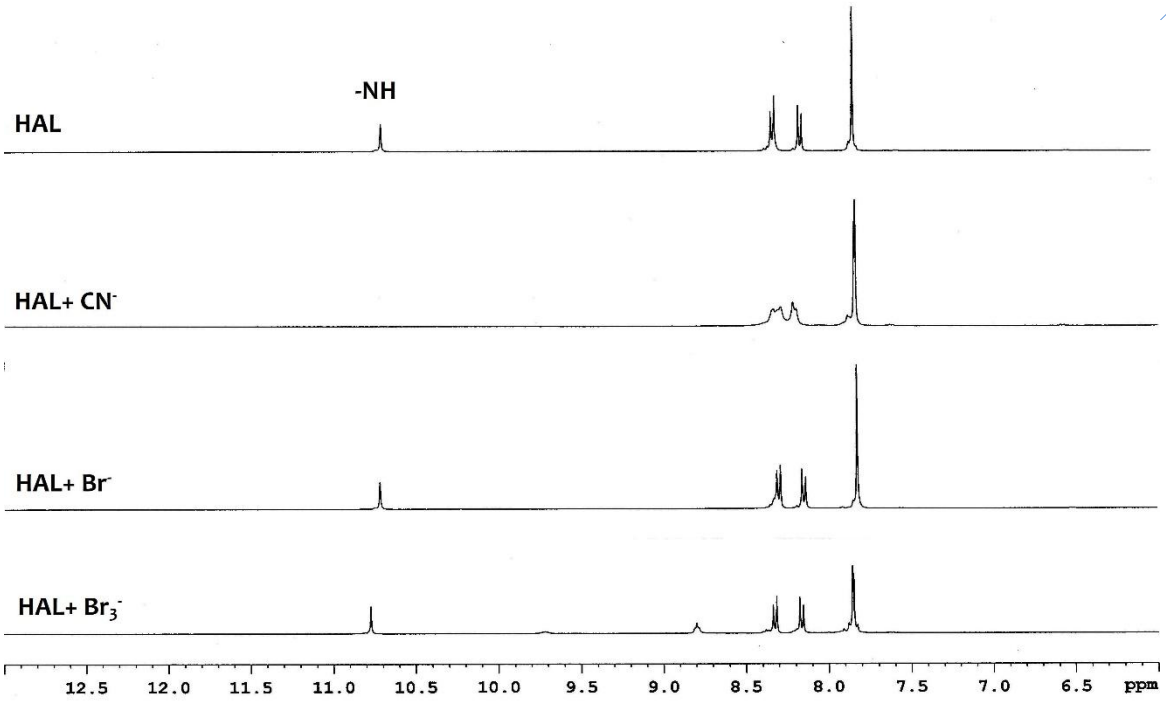


Figure 20: Aromatic region of ^{1}H-NMR spectra of **HAL** in the presence of 2 equivalents of $(Et_4N)CN$, $(n\text{-}Bu_4N)Br$ and $(n\text{-}Bu_4N)Br_3$ in DMSO-d_6.

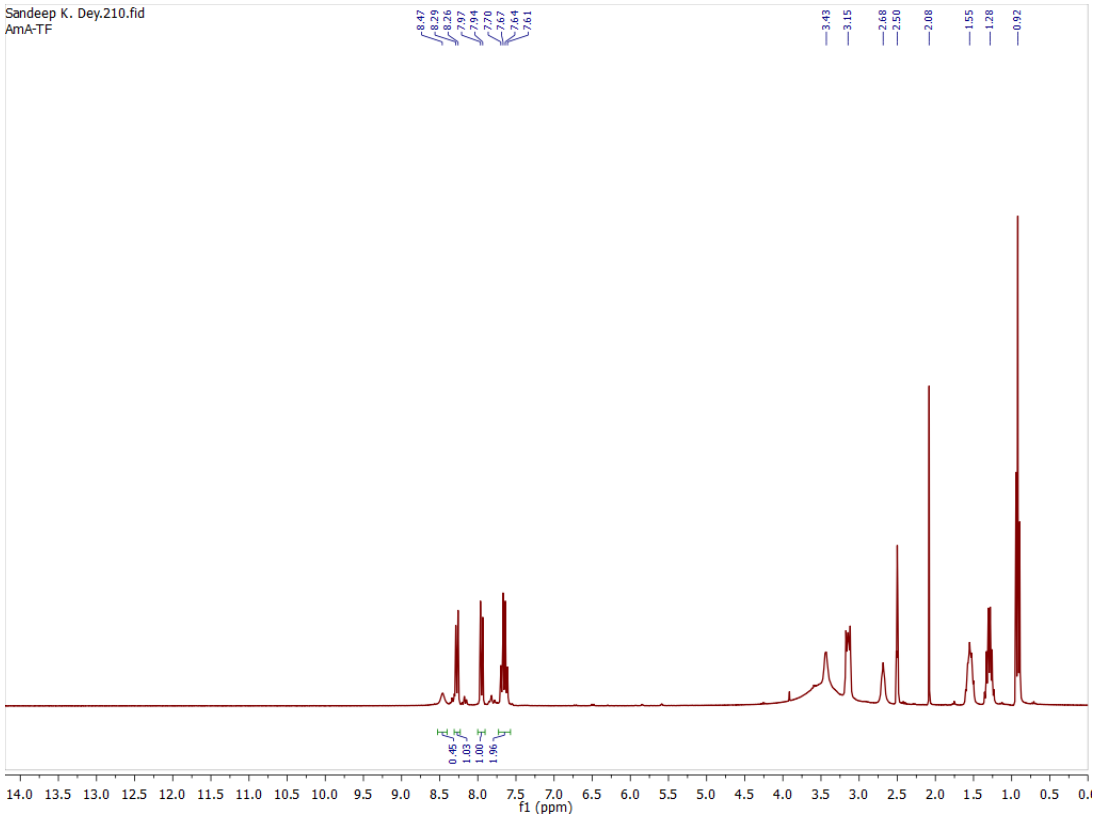


Figure 21: ^{1}H-NMR spectrum of **HAL** in presence of 2 equiv. of $(n\text{-}Bu_4N)F$ in DMSO-d_6.

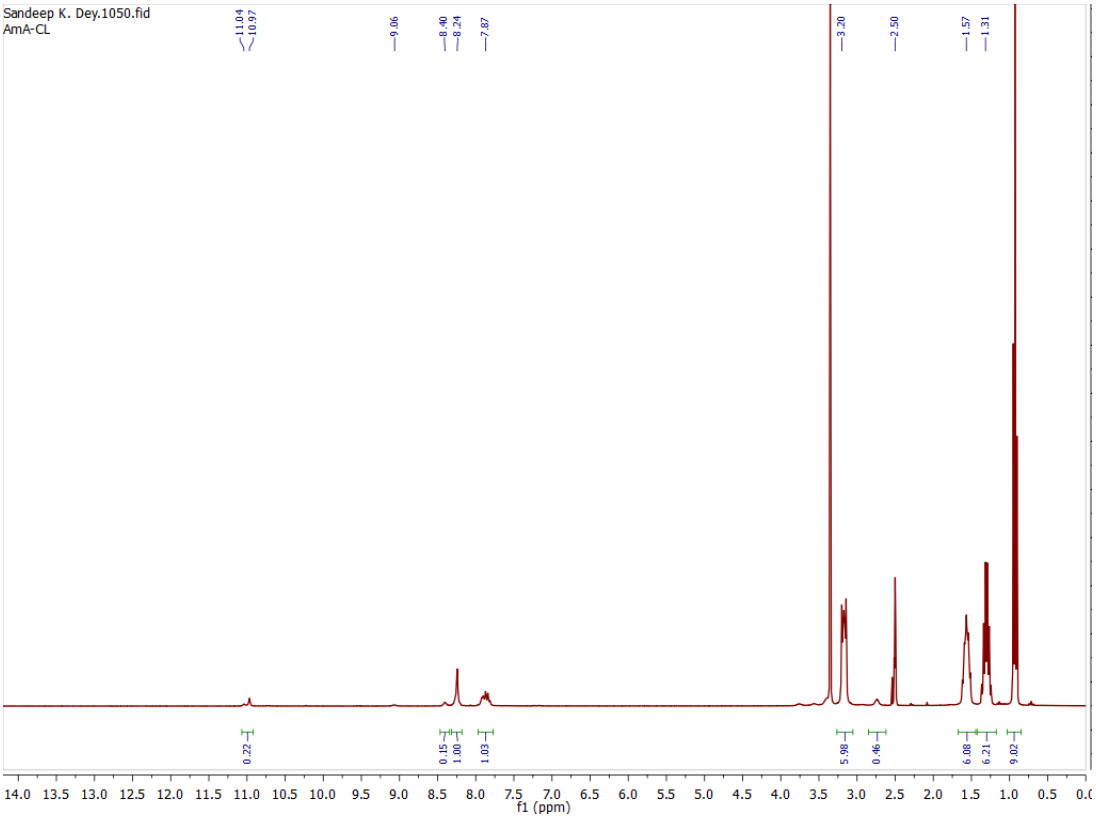


Figure 22: ^{1}H-NMR spectrum of HAL in presence of 2 equiv. of (n-Bu_4N)Cl in DMSO-d_6.

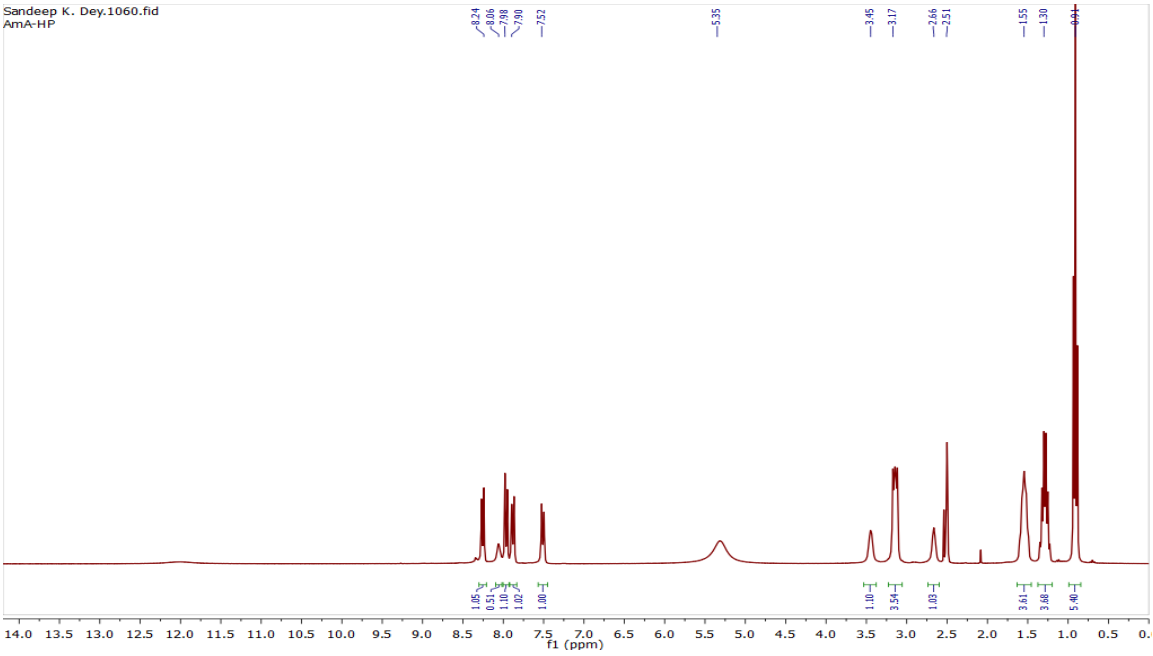


Figure 23: ^{1}H-NMR spectrum of HAL in presence of 2 equiv. of (n-Bu_4N)H_2PO_4 in DMSO-d_6.

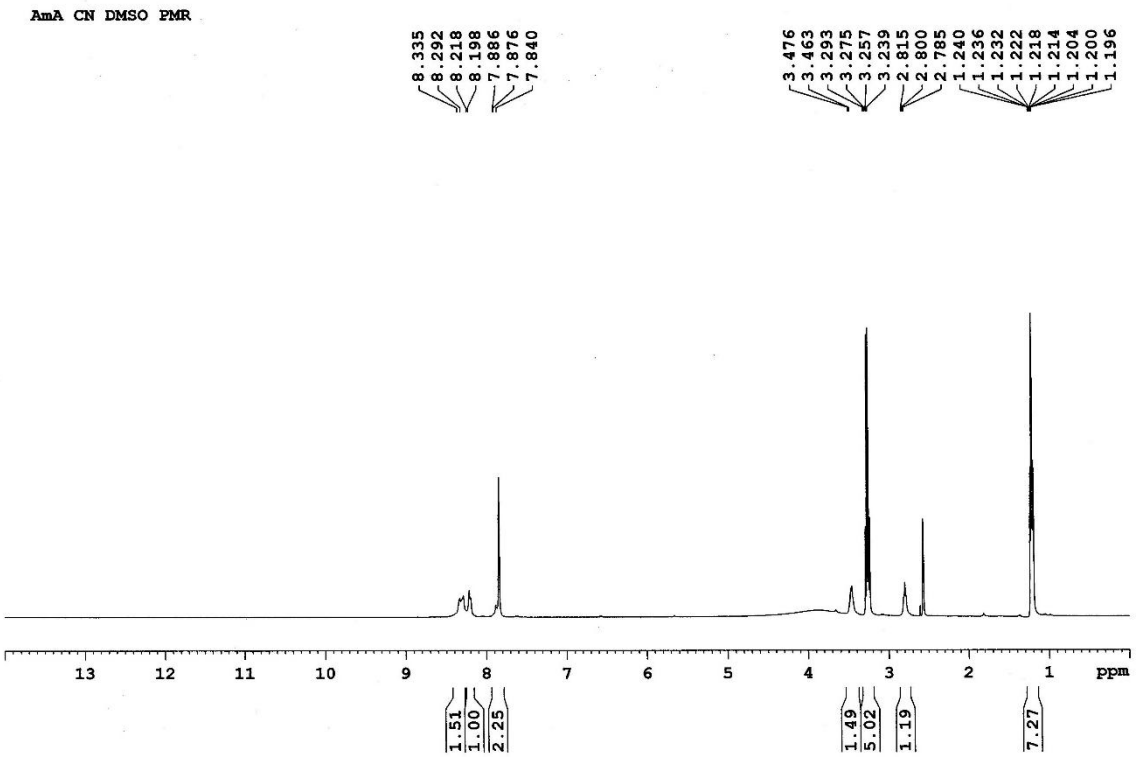


Figure 24: ^{1}H-NMR spectrum of **HAL** in presence of 2 equiv. of $(Et_4N)CN$ in DMSO-d_6.

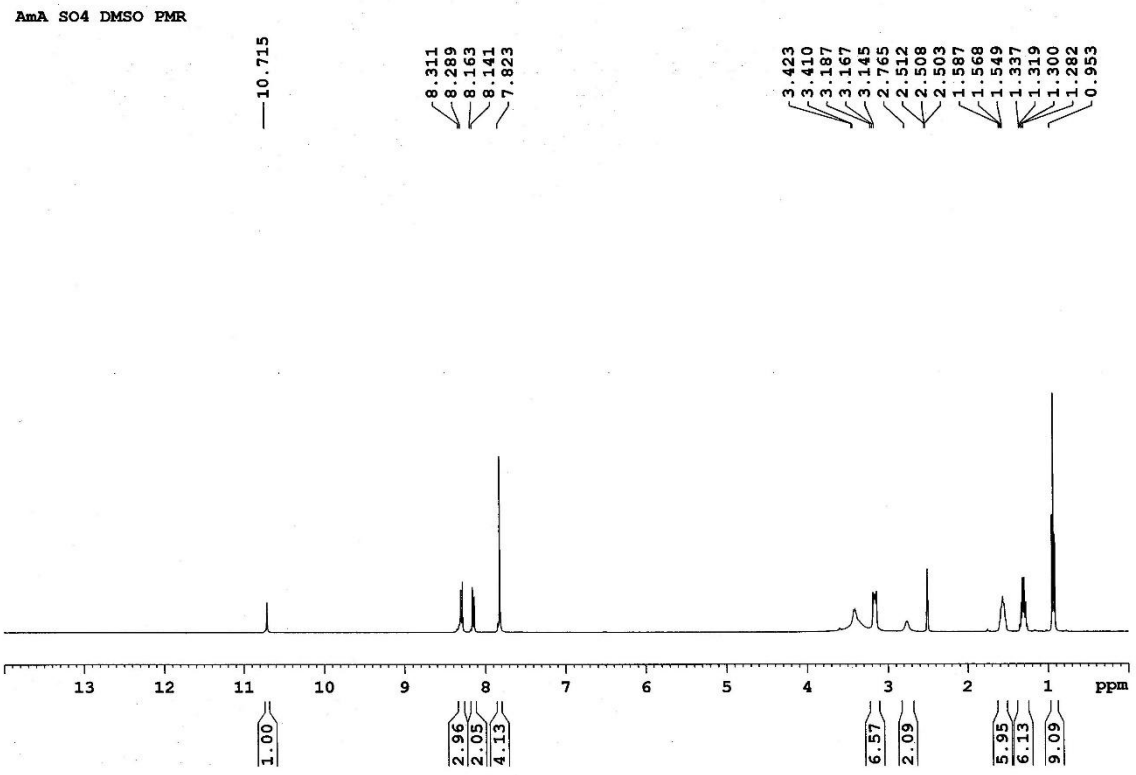


Figure 25: ^{1}H-NMR spectrum of **HAL** in presence of 2 equiv. of $(n\text{-}Bu_4N)HSO_4$ in DMSO-d_6.

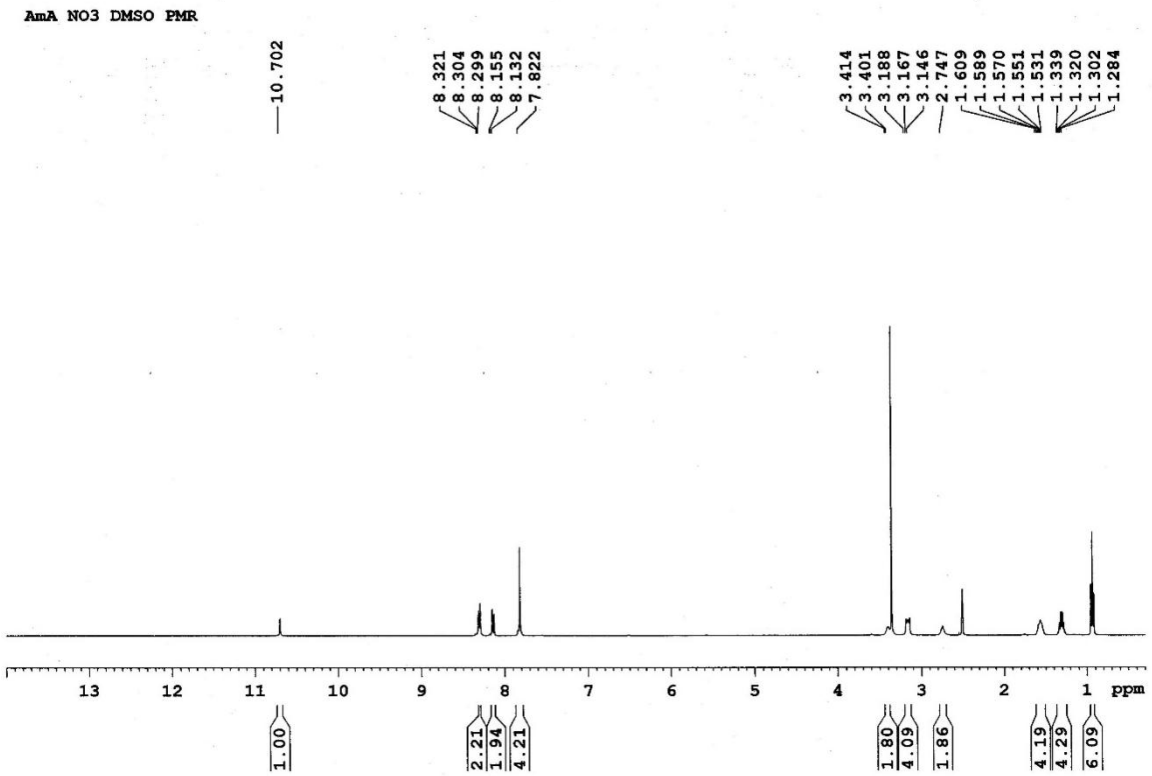


Figure 26: ^{1}H-NMR spectrum of **HAL** in presence of 2 equiv. of (n-Bu_4N)NO_3 in DMSO-d_6.

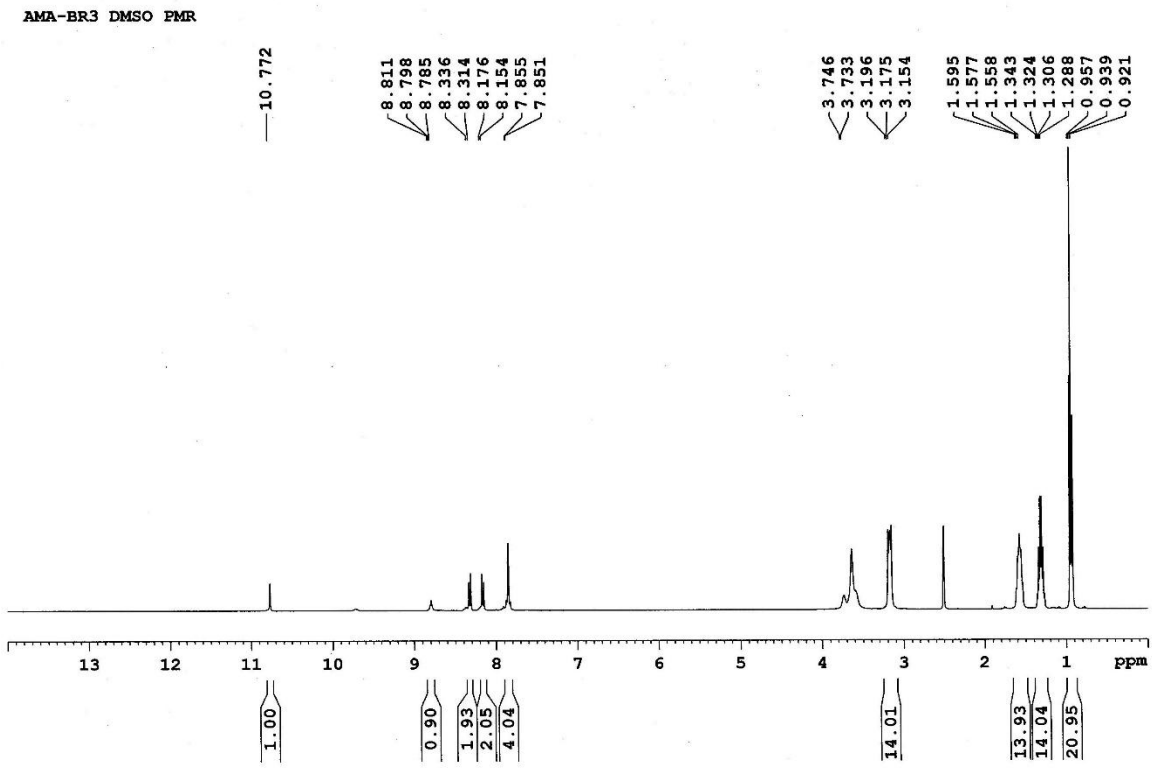


Figure 27: ^{1}H-NMR spectra of **HAL** in presence of 2 equiv. of (n-Bu_4N)Br_3 in DMSO-d_6.

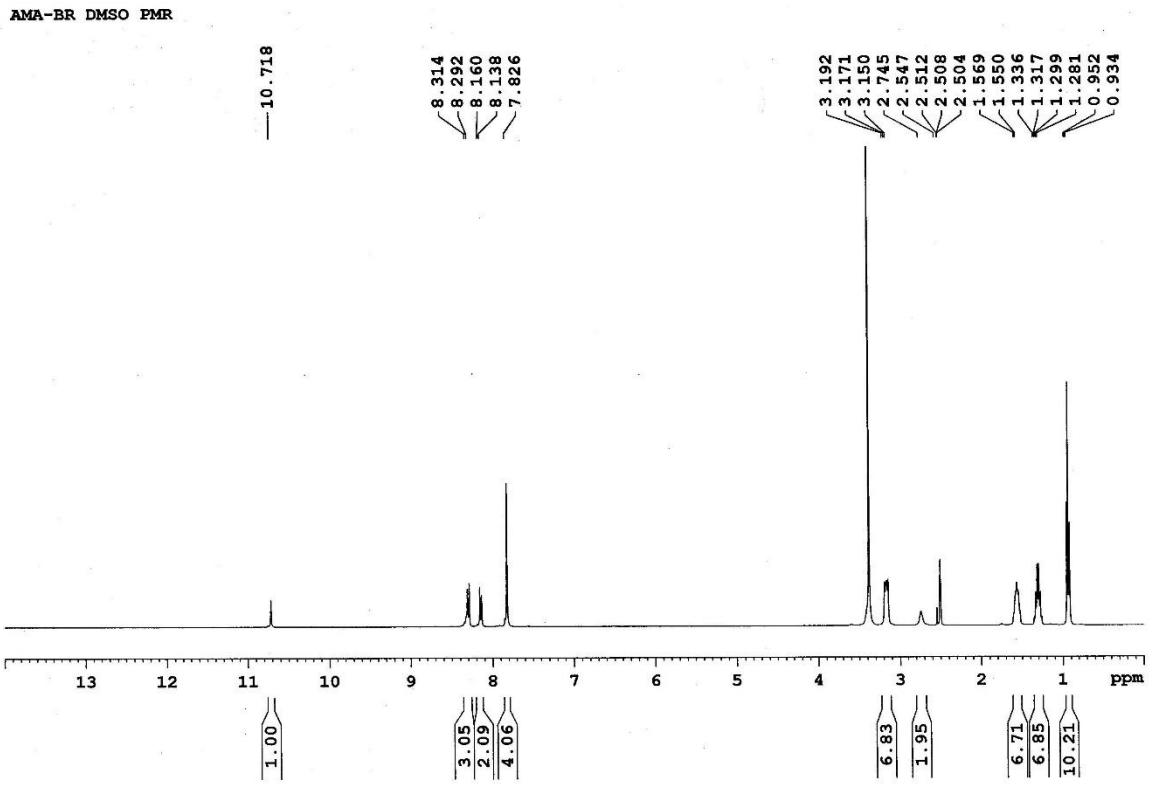


Figure 28: ^{1}H-NMR spectra of **HAL** in presence of 2 equiv. of (n-Bu_4N)Br in DMSO-d_6.

3b. Crystallization of hexamide ligand in presence of tetraalkylammonium salts

Solution state anion binding studies showed strong hydrogen bonding interactions of outer amide -NH protons with fluoride, acetate, cyanide and dihydrogenphosphate and weak interactions with chloride. Thus, in order to obtain hydrogen bonded complexes in the solid state, we have crystallized **HAL** in the presence of different tetraalkylammonium salts (fluoride, chloride, acetate, cyanide and hydrogenphosphate) in DMSO. In a typical crystallization experiment, 100 mg of **HAL** was dissolved in 5 mL of DMSO and an excess of tetraalkylammonium salt (5 equivalents) was added into it followed by stirring at room temperature for about an hour. The solution was then kept undisturbed at room temperature in a 10 mL beaker for crystallization. It is important to mention that, tetrabutylammonium salts of fluoride, chloride, acetate, cyanide and hydrogenphosphate are highly hygroscopic in nature and thus, the chances of them being crystallized from solution do not arise. That is the reason we have employed an excess of these salts in the crystallization experiments to increase the possibility of getting hydrogen bonded receptor-anion complexes.

However, no crystals were formed in any of the above solution mixtures containing **HAL** and a tetraalkylammonium anion (fluoride, chloride, acetate, cyanide and hydrogenphosphate). Instead, yellow crystalline powders were observed to be precipitated from each DMSO solution mixtures. The precipitates were then collected by filtration in each case and washed repeatedly with methanol before air drying at room temperature. The samples thus collected were analyzed by H-NMR spectroscopy, FT-IR spectroscopy and powder X-ray diffraction. H-NMR analysis of these precipitated compounds in DMSO-d_6 revealed the absence of tetraalkylammonium (butyl or ethyl peaks) signals in the aliphatic region and the H-NMR spectrum in each case matches perfectly with the spectrum of **HAL** recorded in DMSO-d_6. Therefore, it is confirmed that the hexamide receptor has precipitated in all cases and no hydrogen bond receptor-anion complex was formed from the crystallization experiments. The powder X-ray diffraction patterns of all samples were observed to be identical to the PXRD pattern of **HAL** crystallized from DMSO in the absence of tetraalkylammonium salt. The FT-IR spectra of all samples were also observed to be identical to the FT-IR spectrum of HAL crystallized from DMSO in the absence of tetraalkylammonium salt.

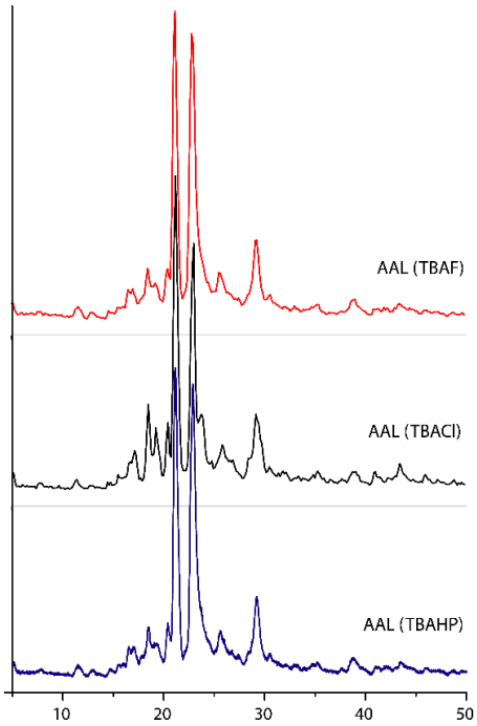


Figure 29: Powder XRD patterns of precipitated compounds (**HAL**) obtained from the crystallization experiments of **HAL** with tetraalkylammonium salts of F^-, Cl^- and $H_2PO_4^-$.

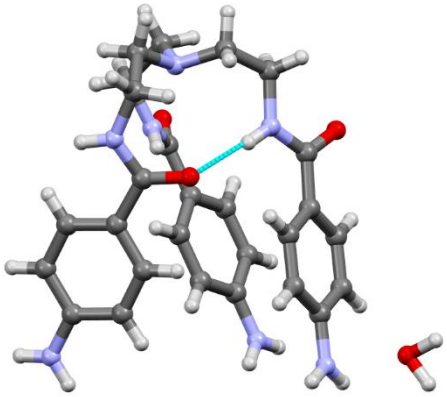
Thus, from the crystallization experiments it is evident that the hexamide ligand is unable to form a stable hydrogen bonded complex with anion in the solid state. The results are very surprising given the fact that we have attempted crystallization of **HAL** with anions of different sizes and shapes such as, spherical fluoride, linear cyanide, planar acetate and tetrahedral hydrogenphosphate but receptor-anion complementarity could not be achieved with any of these tested anions. Receptor-anion complementarity is fundamental in obtaining a stable hydrogen bonded anion complex in the solid state, where the total number of hydrogen bond donors and cavity size of the receptor play an important role in stabilizing the complex.

The inability of **HAL** to encapsulate anion by hydrogen bond formation can possibly be explained by considering the tripodal cavity size and number of hydrogen bond donors. It is assumed that the outer amide cavity is too large to encapsulate smaller anions (such as fluoride, chloride and cyanide) to form stable hydrogen bonded complex in the solid state. On the other hand, due to the insufficient number of hydrogen bond donors in the outer amide cavity larger tetrahedral anions are not stabilized well by the ligand in the solid state.

3c. Single crystal X-ray structure of AL

Single crystals of AL suitable for X-ray analysis were obtained from ethanol solution within a day. Structural elucidation revealed that AL crystallized in orthorhombic $P2_12_12_1$ space group with a water molecule in the crystal lattice AL·H_2O. Two amide groups of AL are involved in strong intramolecular N-H···O=C hydrogen bonding (C=O···NH = 3.015 Å) and the lattice water molecule is hydrogen bonded to an amine -NH_2 proton (H_2O···NH_2 = 3.326 Å). Amine -NH_2 groups are also involved in N-H···O=C hydrogen bonding with carbonyl oxygen of adjacent molecules generating a hydrogen bonded network.

Crystal data: a=10.3677(3), b=11.6016(3), c=23.4291(6), α = β = γ =90, Volume 2818.10(13), Space group = $P2_12_12_1$, Sum formula = $C_{27}H_{35}N_7O_4$, Mr = 521.62, Dx = 1.230g cm^{-3}, Mu (mm^{-1}) = 0.085, T_{min} & T_{max} = 0.968 & 0.982, S = 1.029, N_{par} = 352, Theta (max)= 28.285.



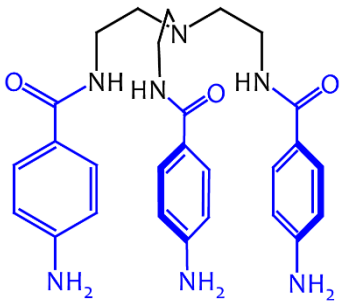


Figure 30: Single crystal X-ray structure of **AL-H_2O** and molecular structure of **AL**.

4. Conclusion

A hydrogen bond donor tripodal hexamide based ligand (**HAL**) has been synthesized for selective recognition of anions. **HAL** consists of a smaller inner cavity and a larger outer cavity both functionalized with amide –NH hydrogen bond donor. On ^{1}H-NMR investigation of solution state anion binding by **HAL**, it has been observed that no significant shift of the inner –NH signals occur thus confirming that the inner amide cavity is not accessible to anions due to the possible –NH···O=C interactions, between amide groups. The outer amide –NH signal, in the presence of TBA salts of F^-, CN^-, $H_2PO_4^-$, and CH_3COO^-disappeared due to significant broadening. This indicated strong hydrogen bond formation between outer –NH protons and the above anions, which led peak broadening and subsequent disappearance of the outer –NH signal. However, in the case of TBACl, the outer –NH proton showed a slight downfield shift whereas in the cases of TBABr, $TBABr_3$, $TBA(NO_3)$ and $TBA(HSO_4)$ no shift of the outer –NH proton was observed.

Crystallisation in the presence of tetraalkylammonium salts led to the formation yellow crystalline powder in each case. ^{1}H-NMR studies and PXRD analysis of the powder revealed that the spectrum and pattern in each case matches with that of **HAL** recorded in the absence of an anion. This confirmed that no hydrogen bond anion complex formed in the crystallization experiments, and the hexamide ligand has precipitated in all cases. FT-IR also showed similar results.

Thus, it has been seen that in the solid state, the hexamide ligand is unable to form a stable hydrogen bonded complex with anions of different sizes and shapes, as the acidity of the -NH protons of ligand was unable to complement the basicity of the studied anions i.e., receptor-anion complementarity was unable to be achieved, although receptor-anion interaction was observed in NMR studies. The inability to encapsulate smaller and larger size anions could also be explained due to the large size of the outer cavity and insufficient number of hydrogen bond donors present in the outer cavity respectively.

The present concept can be explored in future by substituting the inner amide cavity with urea groups (a greater number of H-bond donors), or replacing 4-nitrobenzoyl chloride with its positional meta isomer .Once selective encapsulation is achieved, future advances should focus on binding in solvents, such as water.

5. References

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