POLITECNICO DI MILANO

Ingegneria dei Materiali

Dipartimento di Chimica, Materiali e Ingegneria Chimica

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Design and Synthesis of Halogen Bonding based Receptors for Phosphate Anions

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Astratto:

Il riconoscimento anionico mediante recettori molecolari è uno dei campi più attivi della chimica supramolecolare. In particolare, sali organici dotati di lunghi bracci flessibili hanno mostrato ottime affinità con anioni, caratteristica che li rende particolarmente adatti per applicazioni di sensoristica molecolare. Molti di questi recettori sono costituiti da un anello benzenico sostituito con bracci flessibili opportunamente funzionalizzati per legare specificamente l'anione. Le forze intermolecolari, che guidano la formazione del complesso recettore-anione, possono essere di natura elettrostatica, di tipo legame idrogeno o una combinazione delle due. Gli anioni bersaglio più studiati sono stati ossianioni solfato e fosfato, dato il loro ruolo chiave in molti processi biologici.

In questo lavoro di tesi è stato sintetizzato un recettore per anioni fosfato che sfrutta, per la prima volta, una combinazione di forze intermolecolari di natura elettrostatica e di tipo legame alogeno (XB). Con XB si indicano tutte le interazioni del tipo D···X–Y in cui X è un alogeno elettrofilico (acido di Lewis, donatore XB), D è un donatore di densità elettronica (base di Lewis, accettore XB) ed Y può essere un atomo di carbonio, azoto, ecc. La forza del legame XB risulta particolarmente intensa, se X viene polarizzato da un residuo elettron-attrattore, come l'azoto quaternario del recettore sintetizzato, e D è carico negativamente.

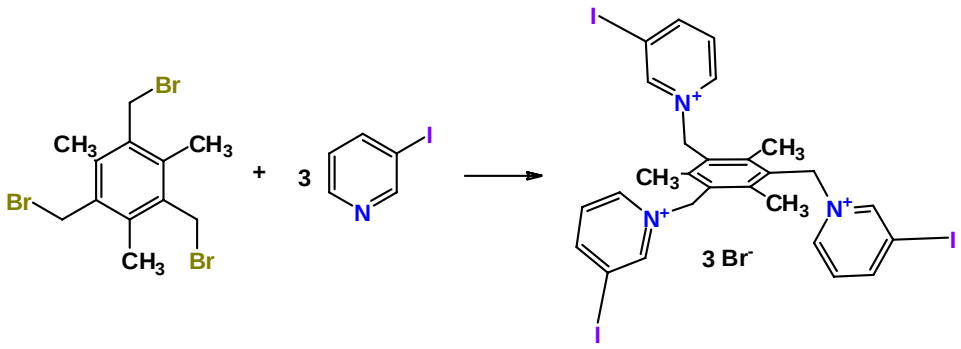


Fig. 1 Schema sintetico del recettore con formula di struttura

Sulla base di queste osservazioni, la realizzazione di un recettore tridentato, in cui l'interazione supramolecolare ($D^{-}\cdots X–Y^{+}$) viene moltiplicata dall'effetto cooperativo di tre bracci, potrebbe risultare in una elevata affinità recettore-anione.

Per valutare la capacità del nuovo recettore di legare anioni fosfato, è stata calcolata la costante di accoppiamento recettore-fosfato in soluzione acquosa mediante misure di fluorescenza. Un anione fosfato legato ad un gruppo cumarinico (*fluoroforo*) è stato utilizzato per generare un segnale di fluorescenza, la cui intensità risulta direttamente proporzionale alla concentrazione della specie in soluzione. La complessazione del fluoroforo "spegne" il segnale di fluorescenza. E', quindi, possibile titolare una soluzione di fluoroforo con aggiunte successive di recettore e calcolare le costanti di accoppiamento *recettore-fluoroforo*.

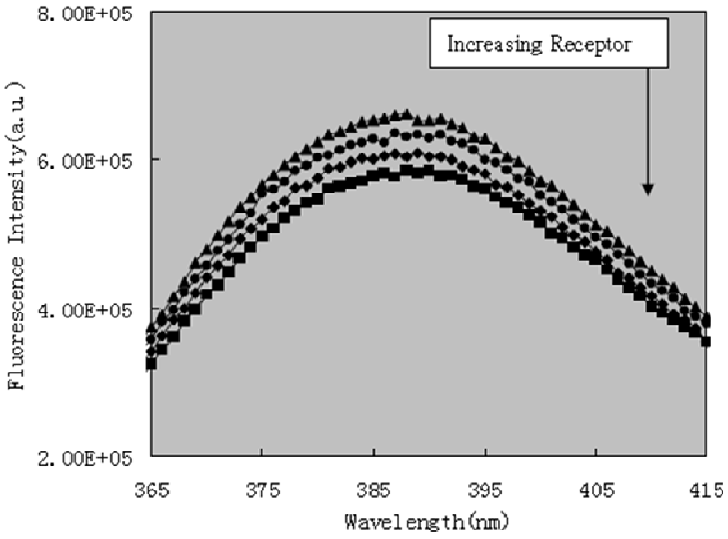
Per misurare la costante di accoppiamento *recettore-fosfato*, si parte da una soluzione 1:1 recettore-fluoroforo, con il segnale di fluorescenza spento dalla complessazione. Aggiungendo quantità crescenti di anione fosfato alla soluzione, si osserva la "riaccensione" del segnale. Si genera in soluzione un equilibrio competitivo tra le due specie supramolecolati recettore-fluoroforo e recettore-fosfato. Il fluoroforo che torna libero in soluzione riaccende il segnale di fluorescenza. Titolando la soluzione con aggiunte successive di fosfato è possibile ricavare la costante di accoppiamento recettore-fosfato.

Reazione supramolecolare	*Costante di accoppiamento*
recettore + $(PO_4)^{3-}$ ↔ recettore-$(PO_4)^{3-}$	$K_p = 0.4\times10^6\ M^{-1}$
recettore + fluoroforo ↔ recettore-fluoroforo	$K_f = 0.2\times10^6\ M^{-1}$
piridinio + fluoroforo ↔ piridinio + fluoroforo	$K_{py} = 0.5\times10^3\ M^{-1}$
recettore-H + fluoroforo ↔ recettore-H-fluoroforo	$K_h = 0.1\times10^5\ M^{-1}$

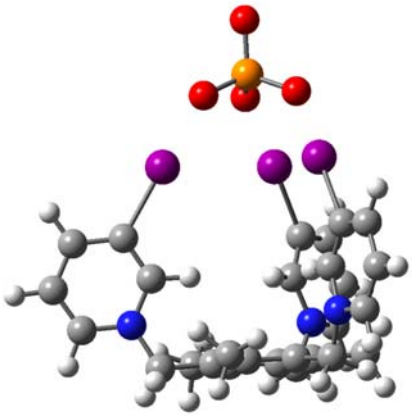
Lo studio è stato approfondito con la sintesi di altri recettori per valutare l'importanza dell'effetto cooperativo dei tre bracci molecolari. Un singolo braccio molecolare, simulato con un piridinio, ha mostrato un'affinità trascurabile per il fosfato.

Il ruolo chiave dell'alogeno nel meccanismo di riconoscimento è stato dimostrato sintetizzando lo stesso recettore privo di iodio. In questo caso l'interazione recettore-fosfato è guidata unicamente da forze di natura elettrostatica. Le costante di accoppiamento è risultata inferiore a quella misurata per il recettore con alogeno.

Sebbene la possibilità di utilizzare XB per il riconoscimento molecolare in fase solida sia stata ampiamente esplorato, non è ancora stata provata la possibilità di sfruttare questa interazione in soluzione. Questo lavoro di tesi dimostra le potenzialità di XB anche in soluzioni altamente competitive, come una soluzione acquosa.



La complessazione del fluoroforo spegne il segnale di fluorescenza



Modellazione molecolare del complesso recettore-fosfato

Parole chiave: Recettore per anione fosfato, Legame ad alogeno, Chimica supramolecolare, Titolazione di Fluorescenza

Abstract:

The anion recognition via molecular receptors is one of the most active areas of supramolecular chemistry. In particular, organic salts with long flexible arms have shown great affinity for anions, making them suitable for application of molecular sensors. Many of these receptors consist of a benzene ring substituted with flexible arms appropriately functionalized to bind specifically to the anion. The intermolecular forces that guide the formation of anion-receptor complex may be electrostatic, hydrogen bond type or a combination of both. The most studied target anions were sulphate and phosphate, given their key role in many biological processes.

This thesis has been synthesized a receptor for phosphate anions that exploits, for the first time, a combination of intermolecular forces of electrostatic interactions and halogen bonding (XB). XB indicates all interactions of D · · · X-Y where X is a halogen electrophilic (Lewis acid, XB donor), D is a donor of electron density (Lewis base, XB acceptor) and Y can be an atom of carbon, nitrogen, etc.. The strength of XB binding is particularly intense, if X is a residue from polarized electron-attractor, such as the quaternary nitrogen synthesized receptor, and D is negatively charged.

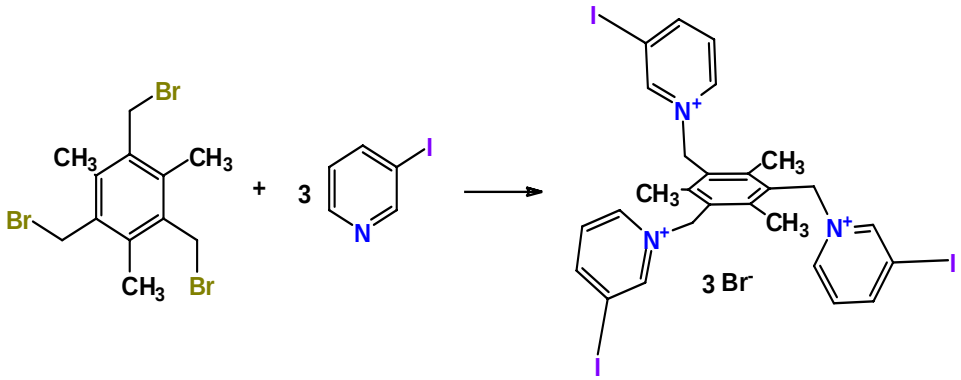


Figure 1 Schematic summary of the receptor with structural formula

Based on these observations, the realization of a receptor tridentate, in which the supramolecular interactions ($D^{-}\cdots X\text{-}Y^{+}$) is multiplied by the effect of cooperative three-arm, could result in a high affinity of receptor-anion.

To evaluate the ability of the new receptor to bind phosphate anions the association constant of receptor-phosphate in aqueous solution was calculated using fluorescence measurements. A phosphate anion bound to a coumarin group (fluorophore) was used to generate a fluorescent signal, whose intensity is directly proportional to the concentration of species in solution. The complexation causes the elimination of the fluorescence signal. According to a fluorophore solution by additions of receptor, we can calculate the association constant of receptor-fluorophore.

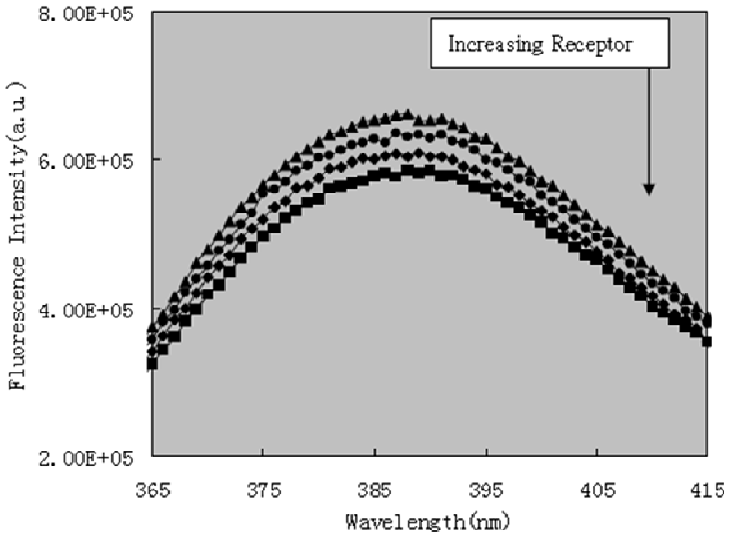
To measure the association constant of receptor-phosphate, we started from a 1:1 receptor-fluorophore solution with the fluorescence signal off .Adding increasing amounts of phosphate anion to the solution, there is the "re" signal. It created a competitive equilibrium in solution between the two species supramolecular fluorophore-receptor and receptor-phosphate. Returning the fluorophore free in solution rekindles the fluorescence signal. Titrating the solution with successive additions of phosphate is possible to get the association constant of receptor-phosphate.

Supramolecular Reaction	*Association Constants*
receptor + $(PO_4)^{3-}$ ↔ receptor-$(PO_4)^{3-}$	$K_p = 0.4\times10^6\ M^{-1}$
receptor + fluorophore ↔ receptor-fluorophore	$K_f = 0.2\times10^6\ M^{-1}$
pyridinium + fluorophore ↔ pyridinium-fluorophore	$K_{py} = 0.5\times10^3\ M^{-1}$
receptor-H + fluorophore ↔ receptor-H-fluorophore	$K_h = 0.1\times10^5\ M^{-1}$

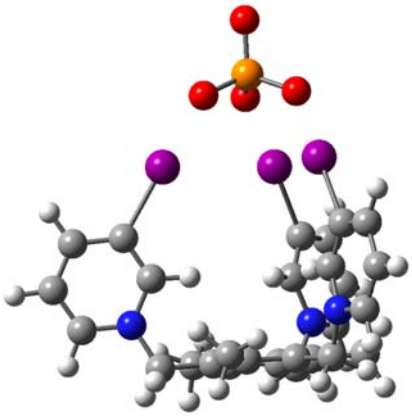
The study was detailed with the synthesis of other receptors to evalue the importance of the effect of the three cooperative molecular arms. A single arm molecular simulated with a pyridinium showed negligible affinity for phosphate.

The key role in halogen recognition mechanism has been demonstrated by synthesizing the same receptor without iodine. In this case, the receptor-phosphate interaction was driven only by electrostatic forces. The coupling constant was less than that measured for the receptor with halogen.

Although the possibility of using XB for molecular recognition in the solid phase has been widely explored, has not proved possible to exploit this interaction in solution. This thesis demonstrates the potential of XB solutions in highly competitive, as an aqueous solution.



The fluorescence signal of the fluorophore ‘off’ complex



Molecular modeling of the complex of receptor with phosphate

Keywords: Phosphate anion receptor, Halogen bonding, Supramolecular chemistry, Fluorescence titration

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1. INTRODUCTION

According to the development of Supramolecular Chemistry, the molecular recognition plays a more important role in the fields of synthetic chemistry, bioscience and so on. The term molecular recognition refers to the specific interaction between two or more molecules through noncovalent bonding such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, p-p interactions, electrostatic and/or electromagnetic[1] effects. The host and guest involved in molecular recognition exhibit molecular complementarity. [2][3]

The ability of XBs to control recognition, self-assembly, and aggregation processes in the solid and gas phases is well documented. While many analytical techniques consistently also prove the existence and the relevance of XBs in solution, supramolecular chemistry based on the formation of XBs in the liquid phase still remains to be fully developed. [4]

Anions as a common ‘guest’ play a fundamental role in a wide range of chemical and biological process. [5] For example, phosphate anions are quite ubiquitous in Nature. Therefore, artificial receptor for selective anion recognition is an area of intensive investigation. Meanwhile receptors based on guest anion induced changes of color or fluorescence seems to be attractive due to their high sensitivity and low detection limit. [6]

1.1 Supramolecular Chemistry

1.1.1 Supramolecular Chemistry

So, what is supramolecular chemistry? The term 'supramolecular' has origins at least to Webster's Dictionary in 1903, but was first applied in the modern sense by Jean-Marie Lehn in 1978 as the '…chemistry of molecular assemblies and of the intermolecular bond'. Lehn shared the 1987 Nobel Prize in Chemistry with Charles Pedersen and Donald Cram for their pioneering work in the field in the late 1960s and subsequent decades.

Supramolecular chemistry has been described as 'chemistry beyond the molecule', whereby a 'supermolecule' is a species that is held together by non-covalent interactions between two or more covalent molecules or ions, such as electrostatic interactions, hydrogen bonding, dispersion interactions and solvophobic effects.

1.1.2 Main Categories of Supramolecular Chemistry

Supramolecular chemistry can be split into two broad categories: **host–guest chemistry** and **self-assembly**. The difference between these two areas is a question of size and shape.

1. Host-guest chemistry:

One definition of hosts and guests was given by Donald Cram, who said 'The host component is defined as an organic molecule or ion whose binding sites converge in the complex…' The guest component is any molecule or ion whose binding sites diverge in the complex. [7]

If one molecule is significantly larger than another and can wrap around it then it is

termed the ‘host’ and the smaller molecule is its ‘guest’, which becomes enveloped by the host (Figure 1.1(a)). A binding site is a region of the host or guest that is of the correct size, geometry and chemical nature to interact with the other species. Commonly the host is larger molecular or aggregate such as an enzyme or synthetic cyclic compound possessing a sizeable, central hole or cavity. The guest may be a monatomic cation, or simple inorganic anion, an ion pair or a more sophisticated molecule such as a hormone, pheromone or neurotransmitter.

On the other hand, the class of solid state inclusion compounds only exhibit host–guest behavior as crystalline solids since the guest is bound within a cavity that is formed as a result of a hole in the packing of the host lattice. Such compounds are generally termed clathrates from the Greek klethra, meaning ‘bars’ (Figure 1.1(b)).

2. Self-assembly

Where there is no significant difference in size and no species is acting as a host for another, the non-covalent joining of two or more species is termed self-assembly. Strictly, self-assembly is an equilibrium between two or more molecular components to produce an aggregate with a structure that is dependent only on the information contained within the chemical building blocks. (Figure 1.1(c)) This process is usually spontaneous but may be influenced by solvation or templation effects or in the case of solids by the nucleation and crystallization processes.

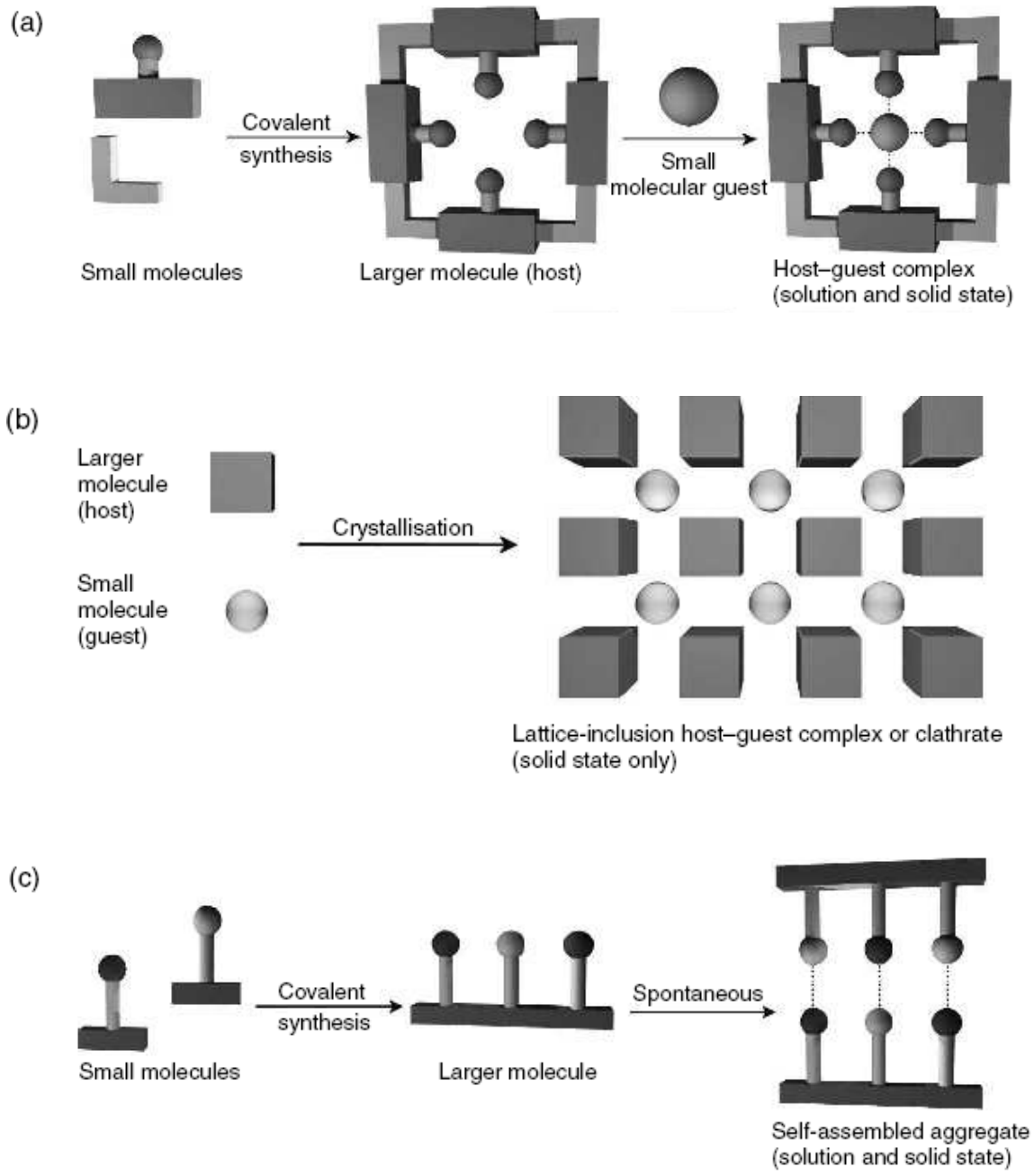


Figure1. 1 The development of a supramolecular system from molecular building blocks (binding sites represented by circles): (a) host–guest complexation; (b) lattice inclusion; (c) self-assembly between complementary molecules. [8]

1.1.3 Nature of Supramolecular Interactions

Non-covalent interactions represent the energies that hold supramolecular species together. Non-covalent interactions are considerably weaker than covalent interactions, which can range between ca. 150 kJ mol^{-1} to 450 kJ mol^{-1} for single bonds. Non-covalent bonds range from 2 kJ mol^{-1} for dispersion interactions to 300 kJ mol^{-1} for 'ion-ion' interactions. However, when these interactions are used in a co-operative manner a stable supramolecular complex can exist. The term 'noncovalent' includes a wide range of attractions and repulsions which are summarized in Table 1.1 and will be described in more detail in the following sub-sections.

Table1. 1 Summary of Supramolecular Interactions[9]

Interaction	Strength (kJ mol^{-1})	Example
Ion–ion	200–300	Tetrabutylammonium chloride
Ion–dipole	50–200	Sodium [15]crown-5
Dipole–dipole	5–50	Acetone
Hydrogen bonding	4–120	(See Table 1.2)
Cation–π	5–80	K^+ in benzene
π–π	0–50	Benzene and graphite
van der Waals	< 5 kJ mol^{-1} but variable depending on surface area	Argon; packing in molecular crystals
Hydrophobic	Related to solvent–solvent interaction energy	Cyclodextrin inclusion compounds

The hydrogen bond is arguably the most important non-covalent interaction in the design of supramolecular architectures, because of its strength and high degree of directionality. It represents a special kind of dipole–dipole interaction between a proton donor (D) and a proton acceptor (A). There are a number of naturally occurring 'building blocks' that are a rich source of hydrogen bond donors and

acceptors (e.g. amino acids, carbohydrates and nucleobases). Hydrogen bond donors are groups with a hydrogen atom attached to an electronegative atom (such as nitrogen or oxygen), therefore forming a dipole with the hydrogen atom carrying a small positive charge. Hydrogen bond acceptors are dipoles with electron-withdrawing atoms by which the positively charge hydrogen atom can interact, for example, carbonyl moieties (Figure 1.2).

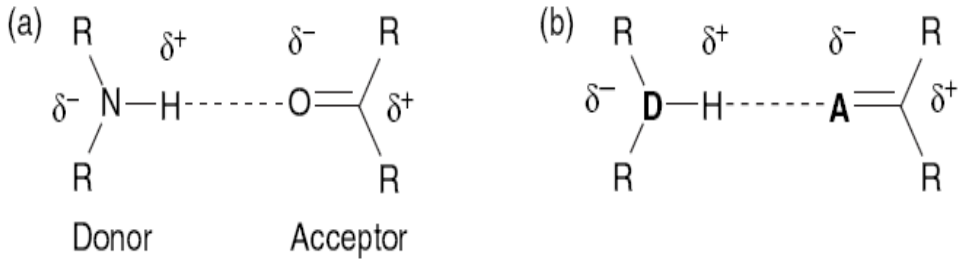


Figure1. 2 A carbonyl accepting a hydrogen bond from a secondary amine donor (a) and (b)the standard way of expressing donor and acceptor atoms (D, donor atom; A, acceptor atom). [10]

But when considering a supramolecuar system, it is vital to consider the interplay of all these interactions and effects relating both to the host and guest as well as their surroundings (e.g. solvation, ion pairing, crystal lattice, gas phase etc.).

1.2 Halogen Bonding

1.2.1 History and Generalities of Halogen Bonding

In these decades, the emergence of new different fields of Chemistry, such as Supramolecular Chemistry, rational drug design and etc, pushes the discovery of new specific intermolecular interactions. As the former paragraph mentioned, the hydrogen bonding is a quite significant non-covalent interaction of molecular

recognition processes not only in the solid, liquid and gas phases, but also in the water.

The second interesting shorter intermolecular contact among non-covalent interactions : the case of Halogen Bonding (XB) which is parallel to hydrogen bonding based on recognition processes. And now it is on the disposal to the chemist as the cement to assemble molecules into supramolecular architectures.

Our group has convincingly demonstrated pointed out that halogen bonding has an impact on all research fields where the control of intermolecular recognition and self-assembly processes. [10]- [11]

Figure1. 3 Comparison between the hydrogen bonding and halogen bonding

The term halogen bonding was first introduced by Dumas et al. [12] for describing the tendency of halogen atoms to interact as Lewis acids with specific Lewis base partners.

In 1896, Remsen and Norris proved the general tendency of amines to form adducts with bromine and chlorine. [13]

Within the framework of Mulliken's theory, [14] the Lewis bases were described as electron donors which possessing one or more lone pairs of electrons (i.e., N, O, S, etc) and the Lewis acids were described as electron acceptors which have the properties of electrophilicity. The mechanism of the halogen bonding was firstly described as electron donor-acceptor complexes.

The first unambiguous report on the ability of halogen atoms to form well-defined adducts with electron donor species was published in 1863 when Guthrie described the formation of the $NH_3 \cdots I_2$ complex. [14]

Sixty years later, thanks to Hassel's splendid work, his crystallographic studies were a

landmark in the understanding of the XB formations and characteristics. In his Nobel lecture, Hassel stressed the similarities between interactions where halogen and hydrogen atoms work as electron acceptors. [14] So the more general definition of halogen bonding was suggested, following closely the analogy to the hydrogen bonding case. Since that moment, a lot of halogen bonded co-crystals have been obtained.

Owing to the hard working of these scientists for years and years, the theory of halogen bonding is becoming more and more perfect constantly. Halogen bonding is the non-covalent interaction between halogen atoms (Lewis acids) and neutral or anionic Lewis bases. XBs can be described in general as D···X-Y, where X is the electrophilic halogen atom (Lewis acid, XB donor), D is a donor of electron density (Lewis base, XB acceptor), and Y is a carbon, nitrogen, or halogen atom (see Figure1.4).

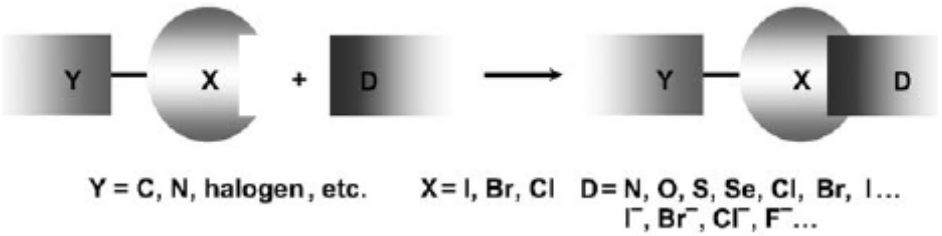


Figure1. 4 General scheme for the formation of halogen bonds [17]

Halogen bonds are strong, directional, and specific interactions that give rise to well-defined structures. The strength of halogen bonds ranges from 5-180 kJ/mol. The shorter distance between the halogen atom and Lewis base, the stronger the XBs interaction. The attractive feature of halogen bonds result in the distance between the donor and acceptor to be shorter than the sum of Van der Waals radii. Meanwhile thanks to its strength, XB can prevail over HB in selecting the modules to be involved in competitive recognition processes. [15] Halogen bonds tend to form at 180° angles, which was shown in Odd Hassel's studies with bromine and 1, 4-dioxane in 1954. [16] In a lot of situations, the angles of halogen bonds tend to be 180°.This could be the

evidence that the halogen bonds are directional interactions. Numerous properties characterizing the halogen bonding run just parallel to those of the hydrogen bonding.[17]

1.2.2 Halogen Bonding in Supramolecular Chemistry

After the seminal papers by Benesi and Hildebrand,[18] and Hassel,[19] halogen bonds entered a dormant period in the1970s and 1980s. This period ended in the 1990s with the gas-phase studies by Legon[20] and solid-phase studies by Metrangolo and Resnati[21]

In the aspect of the crystal engineering, Prof. Resnati's group has demonstrated that the directionality of halogen bonds allows the supramolecular architecture to be anticipated from the structure of the starting molecules.[21]

Halogen bonds also have illustrated the extensive usages in Material Science. The first reported use of halogen bonding in liquid crystal formation was by H. Loc Nguyen.[23] In an effort to form liquid crystals, alkoxystilbazoles and pentafluoroiodobenzene were used. Previous studies by Metrangolo and Resnati demonstrated the utility of pentafluoroiodobenzene for solid-state structures.[24] As we have known, the first example of XBs in conducting molecular materials was reported by Imakubo et al. in 1995—this was the radical cation salt of an iodinesubstituted tetrathiafulvalene (TTF) and $[Ag(CN)_2]^-$ as the halogen bonding acceptor.[25]

Following the fast development of the biological systems, a full understanding of the mechanism that how the halogenated molecules bind to the biological substrates opened a new door to approach the disease recognition and drug design and etc. There are a lot of examples that halogen bonding plays an important role in the biological systems. Thyroid hormones represent a class of naturally iodinated molecules for which halogen bonds appear to play a role in their recognition, as evident by the short I···O contacts between tetraiodothyroxine and its transport protein transthyretin[26].

In 2004, Auffinger et al. reported a comprehensive survey of protein and nucleic acid structures, which revealed that halogen bonds are potentially stabilizing inter- and

intramolecular interactions that can affect ligand binding and molecular folding.[27] One vigorous evidence is that the discovery of the four-stranded DNA Holiday junction in 2003. It revealed that the unusual Br···O contacts has the 12% shorter distance than the Van der Waals radii between Br and O. This survey targeted a data set of protein and nucleic acid structures with short halogen–oxygen distances.

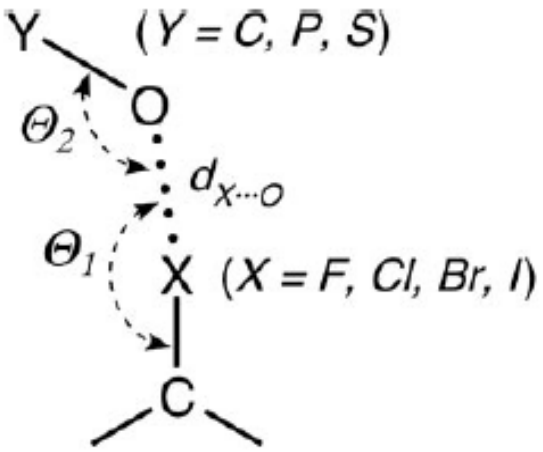


Figure1. 5 Schematic of short halogen (*X*) interactions to various oxygen containing functional groups (where O—*Y* can be a carbonyl, hydroxyl, or carboxylate when *Y* is a carbon; a phosphate when *Y* is a phosphorus; or a sulfate when *Y* is a sulfur). The geometry of the interaction is defined by the normalized *Rx*···o distance [$R_{x}^{\cdots}{}_{o} = d_{X}^{\cdots}{}_{O}/R_{vdw(X...O)}$], the θ_1 angle of the oxygen relative to the C—*X* bond, and the θ_2 angle of the halogen relative to the O—*Y* bond.

More recently, Ho et al. used XBs to manipulate a conformational switch in a four-stranded DNA Holliday junction. [28] A short and directional XB (Br···O-P distance 2.87 Å; C_5-Br···O-P angle 163.28°, Figure1.6) formed between brominated uracil and phosphate oxygen atoms of the DNA backbone competes against the conventional hydrogen bond of cytosine in a similar position.

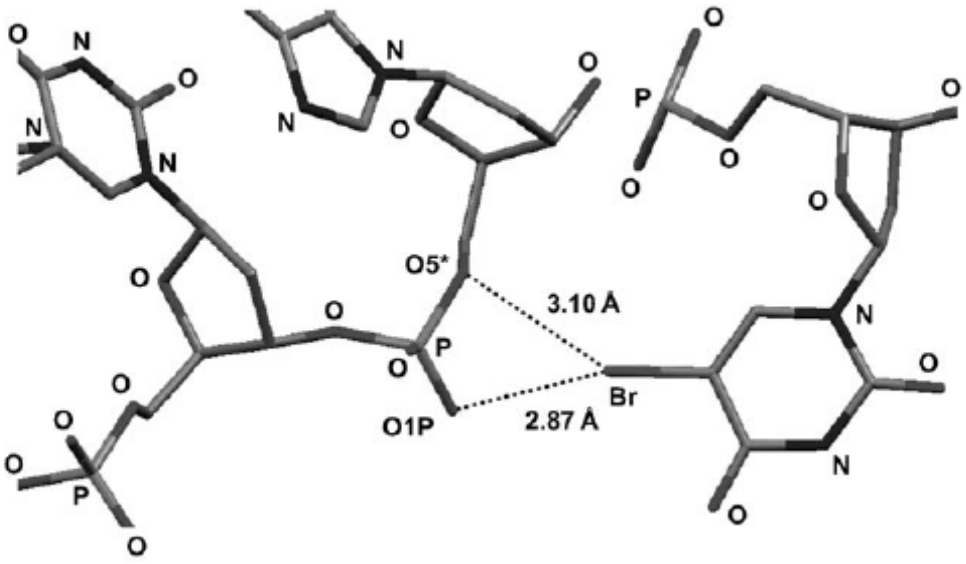


Figure1. 6 The unusually short XB between the bromine atom of a brominated uracil residue and the phosphate oxygen atoms in a four-stranded DNA junction. [28]

The ability of XBs[29] to control recognition, self-assembly, and aggregation processes in the solid and gas phases is well documented, and the interaction of XBs in liquid crystals is also receiving increased attention. According to the utilities of XBs in the biological systems, we realized that the formation of XBs in the liquid phase still remains to be fully developed.

1.3 Anion Receptor

1.3.1 The Importance and Challenge of Anion Recognition

Anions are ubiquitous in the natural world. Chloride anion exists in the ocean, and phosphate and nitrates from agriculture and other human activities, constitute major pollution hazards. It also plays an important role in the biological systems. For example, it is essential in the formation of most enzyme substrates as well as in the interaction between proteins and RNA or DNA. ATP and other high-energy anionic

phosphate derivatives are at the centre of power processes as diverse and important as biosynthesis, molecular transport, and muscle contraction.

The growth of anion recognition is being driven by an increasing appreciation for the importance of non-covalent anion–molecule interactions in biology. Meanwhile anion recognition also has a beneficial role to play in the areas of physiology, medicine, synthetic chemistry, materials development, analyte sensing, and waste remediation.

In contrast to cation binding, however, anion binding was relatively slow to develop. There are some inherent reasons which become the challenge of designing the anion receptors.

1. Anions are relatively larger than the equivalent isoelectronic cation and have lower charge to the radius ratio.
2. Anions have a large range of geometries. Therefore it is harder to design the receptors which satisfy the complementarity of the particular anionic guest than that of the simple cations.
3. Anions are sensitive to pH. Due to this property, the receptors have to operate in a narrow pH window.
4. Anions have higher free energies of salvation compared with those of cations of similar size. A potential anion receptor must, therefore, competes effectively with the solvent environment in which the anion recognition event is to take place.
5. Anions are usually coordinatively saturated and thus they bind only via weak forces such as hydrogen bonding. [30]

1.3.2 Anion Receptor Design

When the researcher understands the nature of the target anion, one starts to design the anion receptor which is complementary to the target anion in terms of its size, shape, chemical properties (acidity, hardness, charge and etc). Other factors also are taken into account in design process, such as the medium in which any competing molecules which must be avoided from binding for the purpose of requiring a more

sensitive anion receptor.

The field of synthetic anion receptor chemistry traces its origins back to the 1968 communication by Simmons and Park from DuPont Central Research in Delawar. [31] In this seminal work, the halide binding properties of several macrobicyclic receptors, consisting of two ammonium bridgehead centers spanned by three alkyl linkers, were reported.(see figure1.7) In general, this work is regarded as the first synthetic anion receptor.

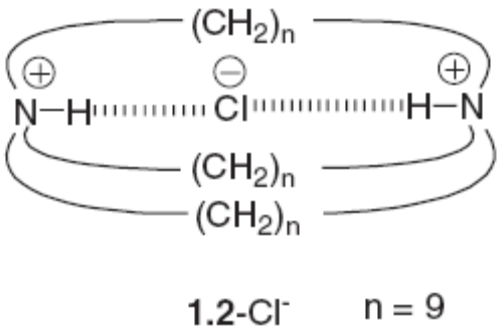


Figure1. 7 The macrobicyclic receptor consisting of two ammonium bridgehead centers spanned by three alkyl likers

Simmons and Park's landmark contribution revealed the development of macrocyclic ammonium-based anion receptors in the following decades. Following Simmons and Park's report, the next step forward came in the mid-1970s, when Lehn and co-workers described the anion-binding properties of a variety of macrobicyclic and macrotricyclic ammonium-based receptors. This research clearly demonstrated how optimizing the fit of an anion for a given charged cavity could lead to a strong binding.

An ellipsoidal hexaprotonated cryptand also synthesized and studied by Lehn and co-workers, was found to be selective for linear anions such as azide N_3^- (added as the sodium salt).(see Figure1.8) This anion was complementary to the shape of the cavity and was found to be bound with high affinity in aqueous media.[32] [33]

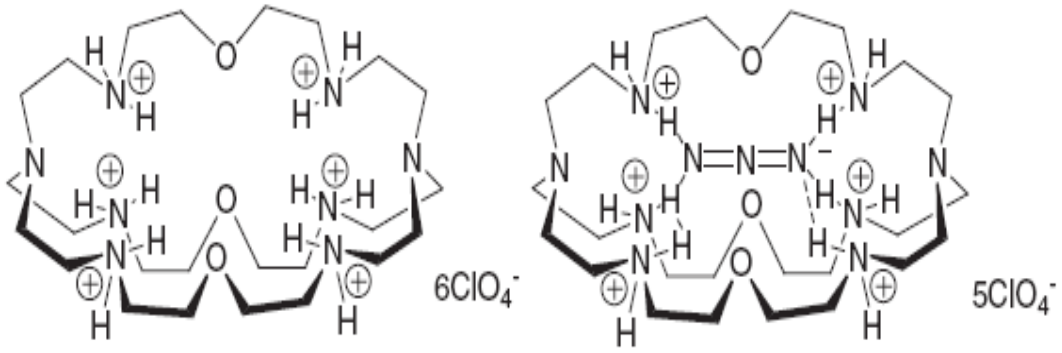
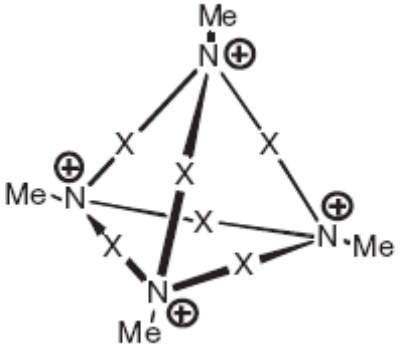


Figure1. 8 An ellipsoidal hexaprotonated receptor complementary to linear anion like azide

Another pioneer Schmidtchen is from the Technische Universität München. He proved that the anion was bound by the cage-like positively charged receptor via electrostatic interactions.(see Figure1.9) According to the different lengths of alkyl chains between ammonium centers, the different anions were complementary to the fit of the receptors. [34]



a) X = —$(CH_2)_6$—
b) X = —$(CH_2)_8$—

Figure1. 9 The cage-like positively charged receptor

Thanks to the contribution of Lehn and co-workers and Schmidtchen, the community understands that anions can be recognized by positively charged concave receptors. Since then, the development of anion coordination chemistry which is similar to the classical coordination chemistry of metals open a new door to reveal that non-covalent interactions play a role between anions and receptors. It means that

anions to be coordinated establish multi-point interactions with the receptors. The classification of anion receptors on the basis of the nature of the interaction is summarized in Figure 1.10.

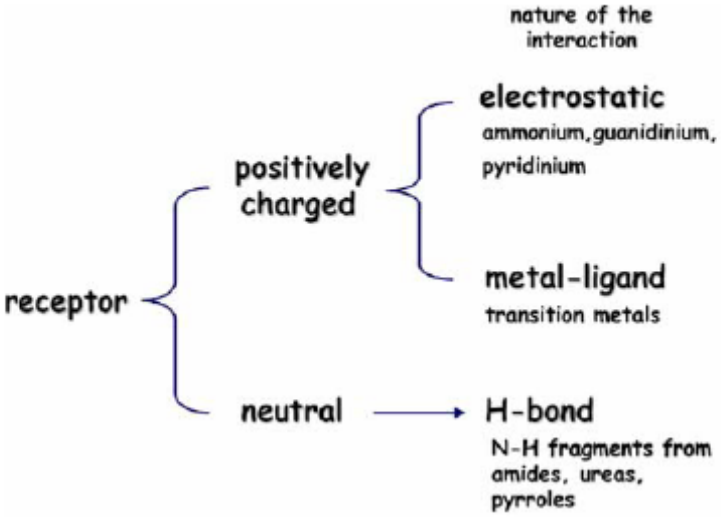
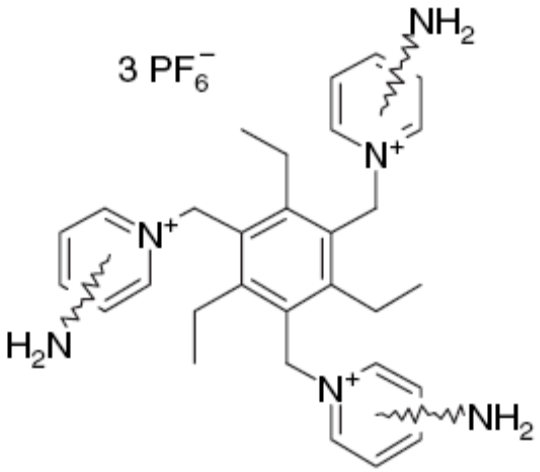


Figure1. 10 Electrical state of anion receptors and nature of the receptor-anion interaction.

It's obvious that if we use a positively charged receptor, all the anions will bind to it to form either a solvent-separated or contact-ion pair due to the non-directional electrostatic interactions. In order to design the ideal receptors for the chosen anions, the attentions of chemists are currently devoted to the neutral receptors via hydrogen bonding. However the limitation of neutral receptors have shown up that the interaction that provided, such as hydrogen bonding , is not strong enough, and it cannot compete with water. To overcome these problems, we get an alternative approach that using more than one non-covalent interaction, in particular, the combination of electrostatic interactions and hydrogen bonding. Many of these receptors consist of a benzene ring substituted with flexible arms appropriately functionalized to bind specifically to the anion. The tripodal species containing protonized nitrogen is a very popular building block in the design of anion receptor. (see Figure1.11)



A= meta

B= para

Figure1. 11 The tripodal Species containing pronated nitrogen

This is a cationic receptor which is based on aminopyridinium moieties distributed around a hexasubstituted triethylbenzene core (receptor A) (see Figure 1.11). The hydrogen bonding sites comprise amine and aryl CH groups, together with the positive charge on the pyridinium moiety. So the anion could be surrounded in a trigonal prismatic array of NH and CH donors. However the analogous para derivative (receptor B) doesn't show a convergent conformation and low anion affinity. [34]

1.3.3 Measurement Methods

In supramolecuar chemistry, it contains not only how to design the receptors but also to quantify the properties of the combination of anions and receptors. So we have to measure the stability constants to evaluate the complex of receptor with anion.

The binding constant is a special case of the equilibrium constant K, such as association constant and dissociation constant. The equilibrium state is the state

between the unbound state, in which the host and the guest are separate from each other, and the bound state, in which there is a structurally defined host-guest complex:

$$H + G \rightleftharpoons HG$$

H= ‘host’, G= ‘guest’ HG= ‘host-guest complex’

So the equations of association and dissociation constants which are associated with the concentrations of each species are:

$$K_a = \frac{[HG]_{eq}}{[H]_{eq}[G]_{eq}} \qquad K_d = \frac{[H]_{eq}[G]_{eq}}{[HG]_{eq}} = \frac{1}{K_a}$$

In the field of anion recognition chemistry, a variety of techniques have been employed to measure the binding constants between the host and guest. The titration may be followed by using one or more of various spectroscopic or calorimetric tools. There are potentiometric titration, nuclear magnetic resonance titration, fluorescence titration, UV/Vis absorption spectroscopy, isothermal titration calorimetry (ITC) and so on. Each of these techniques looks at a different part of the binding process and/or overall equilibrium. But the each technique has different sensitivity range.

In this thesis, we would like to use fluorescence titration due to its high sensitivity. It is based on the proportion of the fluorescence intensity to unbound fluorophore concentration in solution. This is often linked with receptor or anion to be convenient to check the binding result. Fluorescence titration includes quenching titration and enhancing titration. We focus on the fluorescence quenching titration. During analyzing the data, the Stern-Volmer equation and the method of continuous variation (Job Plot) are mainly applied.

Halide collisional quenching, or often referred to as dynamic quenching, requires that a fluorescent dye is sensitive to quenching by a halide ion quencher, which results in a

decrease in fluorescence intensity and lifetime t, which can be described by the Stern-Volmer equation [35], although the quenching of fluorescence was first described by Stokes in 1869, when he observed that the fluorescence of quinine in dilute sulphuric acid was reduced after the addition of hydrochloric acid.

$$\frac{F'}{F} = \frac{\tau'}{\tau} = 1 + k_q \tau' [Q] = 1 + K_{SV}[Q]$$

Here, F', τ' and F, τ are the intensities and lifetimes in the absence and presence of quencher, Q, respectively, K_{SV} is the Stern-Volmer constant, the magnitude of which determines the halide concentration range detectable and kq is the bimolecular quenching constant.

Job's Method, also called the Method of Continuous Variation, is a simple and effective approach to the determination of chemical reaction stoichiometry. We will discuss it in the context of generic reaction (1),

(1): aA + bB <===> dD

which can be rewritten in the form of (2) by dividing all coefficients by "a".

(2): A + kB <===> mD

where k = b/a and m = d/a. Job's method is based on the following fact: if a series of solutions is prepared, each containing the same total number of moles of A and B, but a different ratio, R, of moles B to moles A, the maximum amount of product, D, is obtained in the solution in which R = k (the stoichiometric ratio). To implement Job's Method experimentally, one prepares a series of solutions containing a fixed total number of moles of A and B, but in which the R is systematically varied from large to small, and measures the amount of product obtained in each solution. One then plots amount of product versus R, and obtains a maximum at the initially-unknown value of k. That the maximum amount of product should occur at the stoichiometric ratio can be justified both intuitively and mathematically.

All the other details of preparing the samples and calculating the data will be shown in the follow-up chapters.

2. AIM, RESULT AND DISCUSSION

2.1 Design of Phosphate Anion Receptor Based on Halogen Bonding in Aqueous Phase

2.1.1 Phosphate Anions

Anions are ubiquitous and important in the world. Among the numerous anions, anionic phosphate and its derivatives play the quite important role in fields of biochemistry, physiology, ecology and so on.

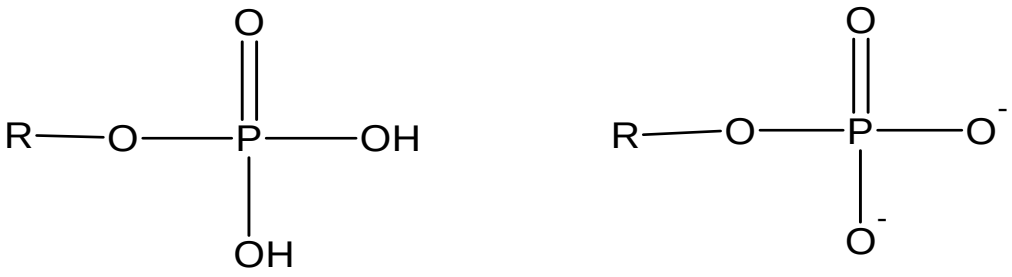
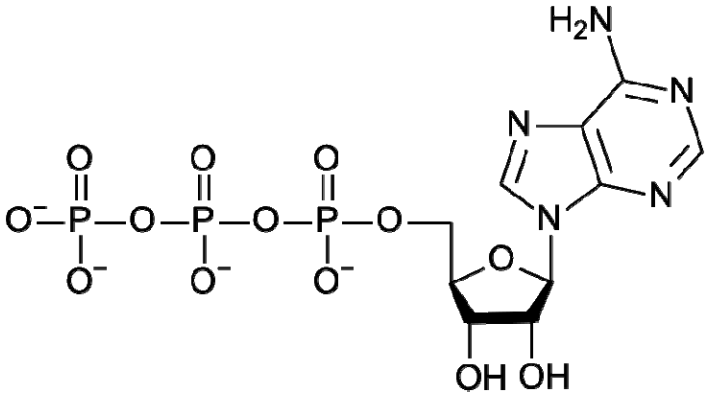


Figure2. 1 The structure formula of anionic phosphate functional group (a) in a acidic aqueous solution and (b) in a basic aqueous solution

Without exaggeration, phosphate groups almost exist everywhere of living organisms, such as bone, teeth, DNA, ATP, protein and so on. The common form of phosphates is adenosine phosphate. For example, ATP stands for Adenosine Triphosphate, and ADP stands for Adenosine Diphosphate. During the process of transduction, ATP which is an unstable molecule in unbuffered water hydrolyses to ADP and phosphate, and accompanies with energy at the same time. Just because of the energy which is created by hydrolysis of ATP, the lives of living organisms could be maintained.



$$ATP + H_2O \rightarrow ADP + P_i + Energy$$

Figure2. 2 The structure formula of ATP anion and the process of transduction from ATP to ADP

Because DNA contains the long-term storage of information of living organisms and virus, its constitution and structure is more eager to be recognized. The backbone of the DNA strand is made from alternating phosphate and sugar residues. Therefore, the negatively charged phosphate site could be recognized via non-covalent interactions. For example, histone octamer, which has numerous positively charged amino acid side chains on the surface of the octamer, displays a good phosphate anion affinity.

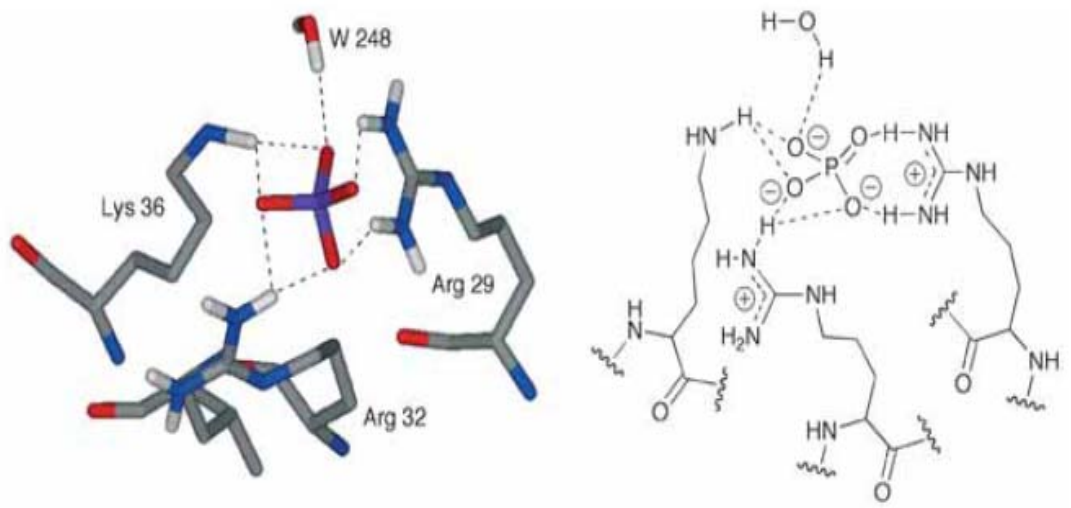
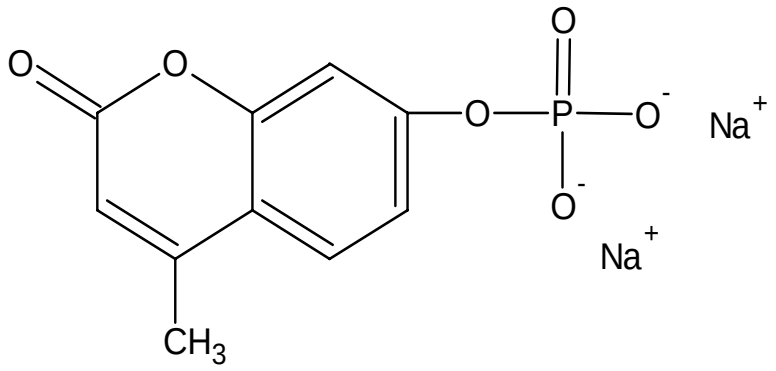


Figure2. 3 The histone octamer—phosphate anion complex. The amino acid residues interact with the phosphate anion via a combination of hydrogen bondings and electrostatic interactions. Only one of five bound phosphates is shown [39]

Due to the important role of phosphate anions in the chemical and biological process, phosphate anions become to be a main target for supramolecular chemists. If binding is to be in a biological situation, it should be established in water, which is highly competitive and attenuates anion binding interactions. Therefore we decided to design and synthesize a phosphate anion receptor which has a good affinity with phosphate anion in aqueous phase.

In order to be convenient to check the process of anion recognition, the phosphate anion should combine with an indicator, such as a fluorophore. It could be tested by the very sensitive fluorescence titration. So we use fluorescent phosphate derivative **1** to assess the affinity of our receptor for phosphate. The structure of **1** is shown as below:



1

Figure2. 4 Fluorescent phosphate derivative **1**

2.1.2 Anion Receptor Based on Halogen Bonding

As the above paragraphs mentioned, halogen bonding is parallel to hydrogen bonding based on recognition processes. We expect that it could work as the cement to assemble molecules into supramolecular architectures just as what hydrogen bonding does. So in this thesis we would like to introduce halogen bonding into the design concept of phosphate anion receptor.

In the last chapter, we saw the tripodal species receptor (see figure1.11) is laden with hydrogen bond-donor moieties which are amine groups. And at the same time the

positive charges on the pyridinium moieties enhance the hydrogen bondings. The tripodal species is a very popular building block in the supramolecular chemistry.

In order to introduce halogen bonding, the way is to use halide elements instead of amine groups of tripodal species. As been well known, Iodine has the largest volume among halide elements and is easy to be polarized. So iodine could be the proper halide element. We build the molecular modeling of the complex of phosphate anion and receptor.

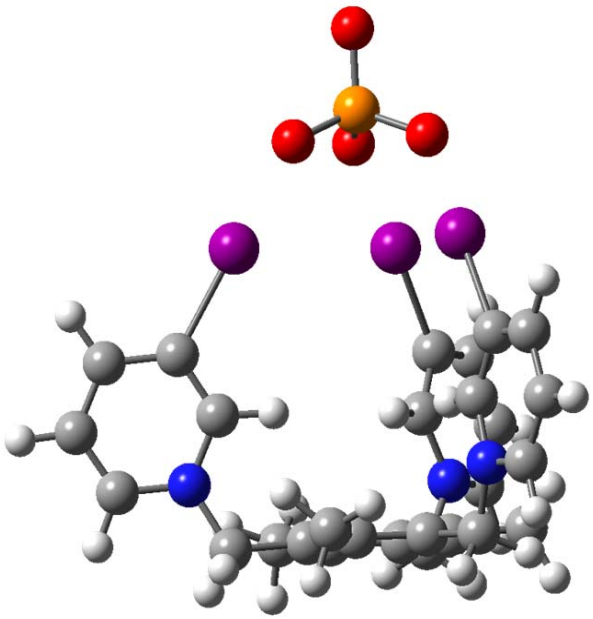


Figure2. 5 Molecular modeling of the complex of receptor with phosphate

The phosphate anion receptor **2** investigated in this thesis is shown as below:

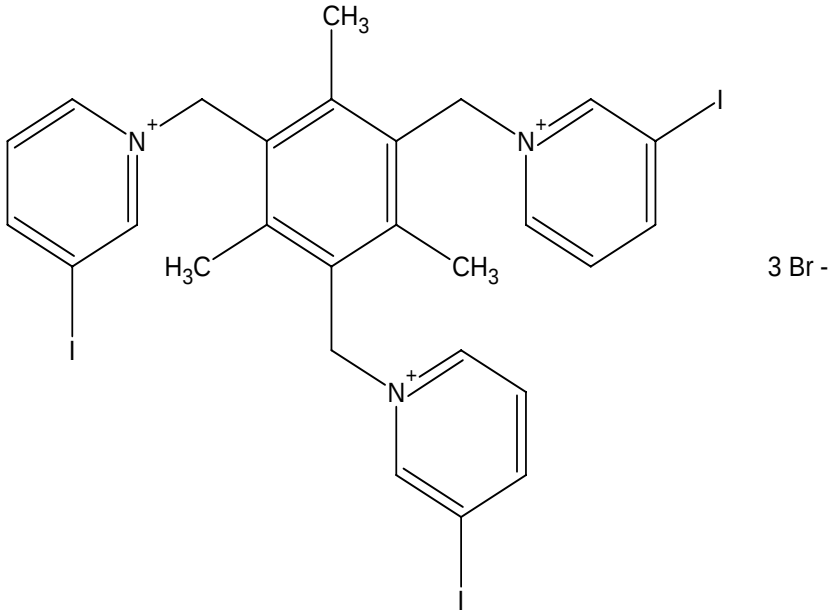


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Figure2. 6 The target structure formula of the phosphate anion receptor **2**

The halogen bonding could occur between the iodine element and the oxygen of phosphate in the process of anion recognition. The association of the phosphate group of **1** and the iodine of receptor **2** triggers a change in the fluorescence spectra. And the change of intensity of the fluorescence spectra correlates with the ratio of the concentrations of **1** and **2**.

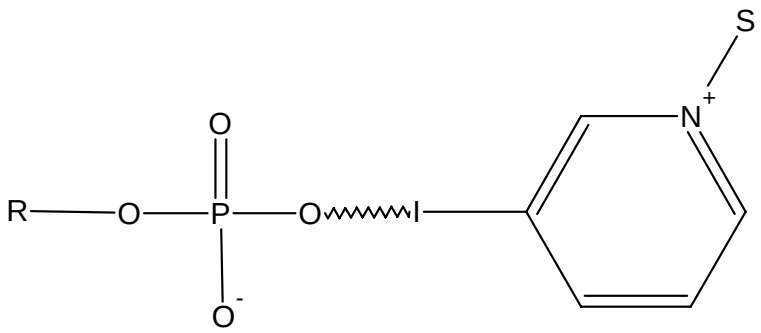
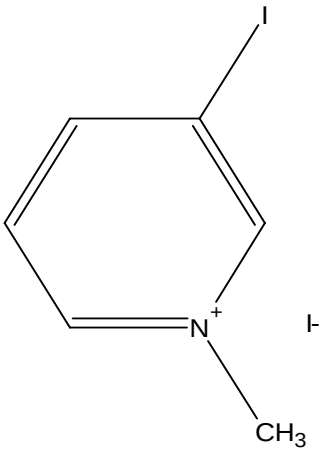


Figure2. 7 The halogen bonding between the iodine element and the oxygen of phosphate

In order to prove that the right halogen bonding occurs at the right position as we expect and to show the advantage of our proposed phosphate anion receptor **2**, we need to synthesize some other receptors to do some contrastive experiments. For example, **3** and **4** only could form only one halogen bond between each receptor and each phosphate. Whereas our target receptor **2** could form multiple halogen bonds between each receptor **2** and phosphate theoretically. Meanwhile compared with **5** and **6** receptors, we can exclude the effect of three bromine anions on the halogen bonding.



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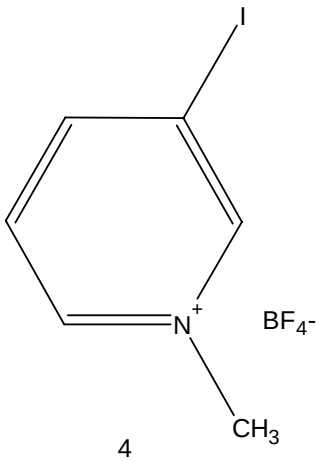

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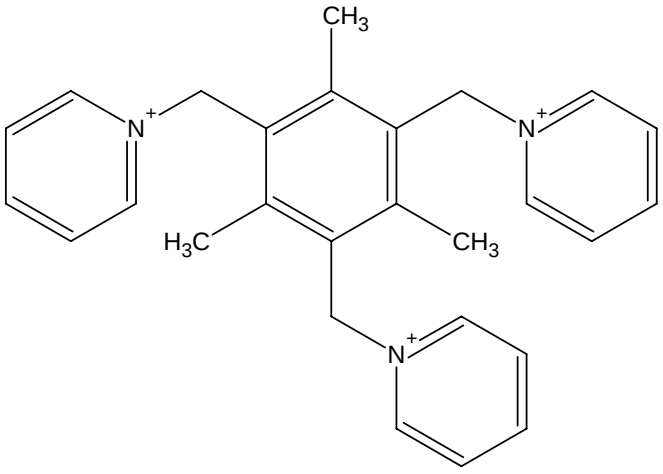

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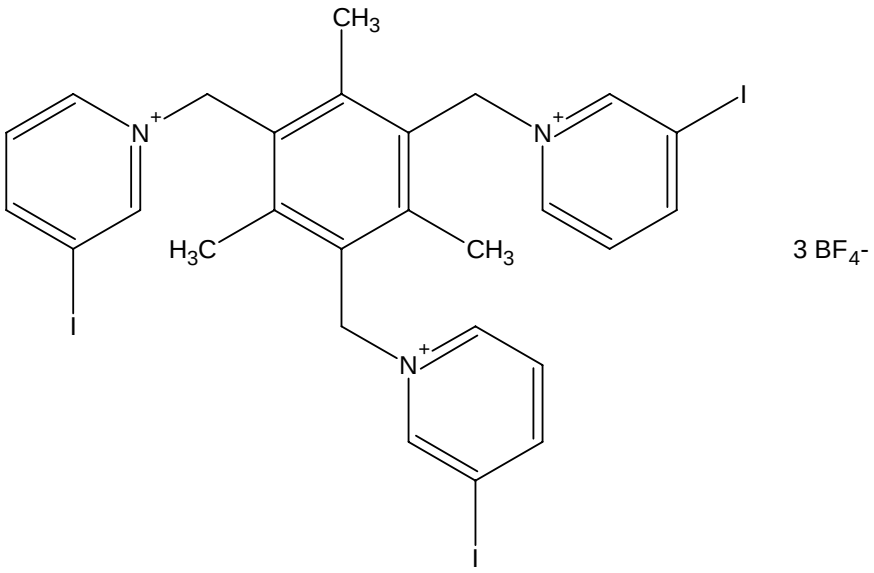

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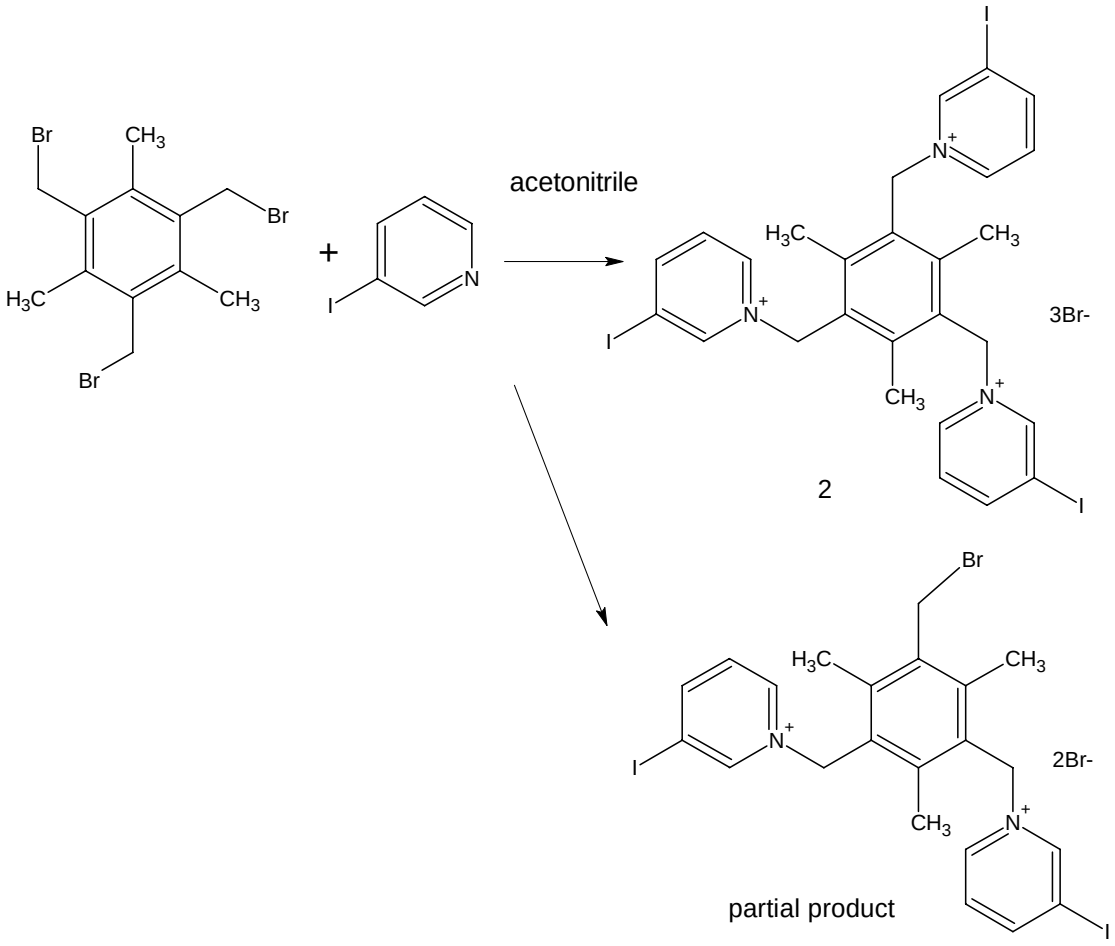
Figure2. 8 The other contrastive anion receptors

2.2 Synthesize the Anion Receptors

2.2.1 Synthesize the Target Halogen Bonding Based Phosphate Anion Receptor **2**

The tripodal species with iodine receptor **2** was synthesized from the mixture of 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene and 3-iodopyridine in acetonitrile. Due to the existence of partial product, **2** was not only purified by dichloromethane and but also recrystalized from methanol/chloroform. Details of the synthetic procedure are described in Experimental Section.

Because the process of synthesis produces some amount of the partial product which is disubstituited, the yield of **2** which is trisubstituited is low. Each **2** carries three cations and could only dissolve in water or methanol. But the solubility of **2** is lower than that of the disubstituited partial product because they carry different amount of cations. So we separated them according to their different solubilities during the recrystallization. The details are shown in Experimental Section.



Scheme2. 1 The synthetic procedure for the target halogen bonding based anion receptor **2**

The final compound was characterized using standard methods, and data were in agreement with the proposed structure. The 3 cations of **2** enhance the power of electron-withdrawing, therefore lead to the significant differences in ^{1}H NMR spectra compared with 3-iodopyridine. In d6-DMSO, the 4 peaks indicated the hydrogen of 3-iodopyridine groups of **2** move to high ppm. For example, the peak at 8.816 which was shown in Figure2.8 moves to 9.431 ppm in Figuire2.9 when the receptor **2** was synthesized.

At the same time, **2** was also characterized by high resolution mass spectrum and DSC. In positive charge mass spectra, we could find the particular peaks which we expected at 257.9, 427.3 and 933.5 m/z, which standed for the receptor **2** with no bromide anion, one bromide anion and two bromide anions. Also in DSC, we could

find the sharp peak which represented the melting point of receptor **2** at 261 ℃ .

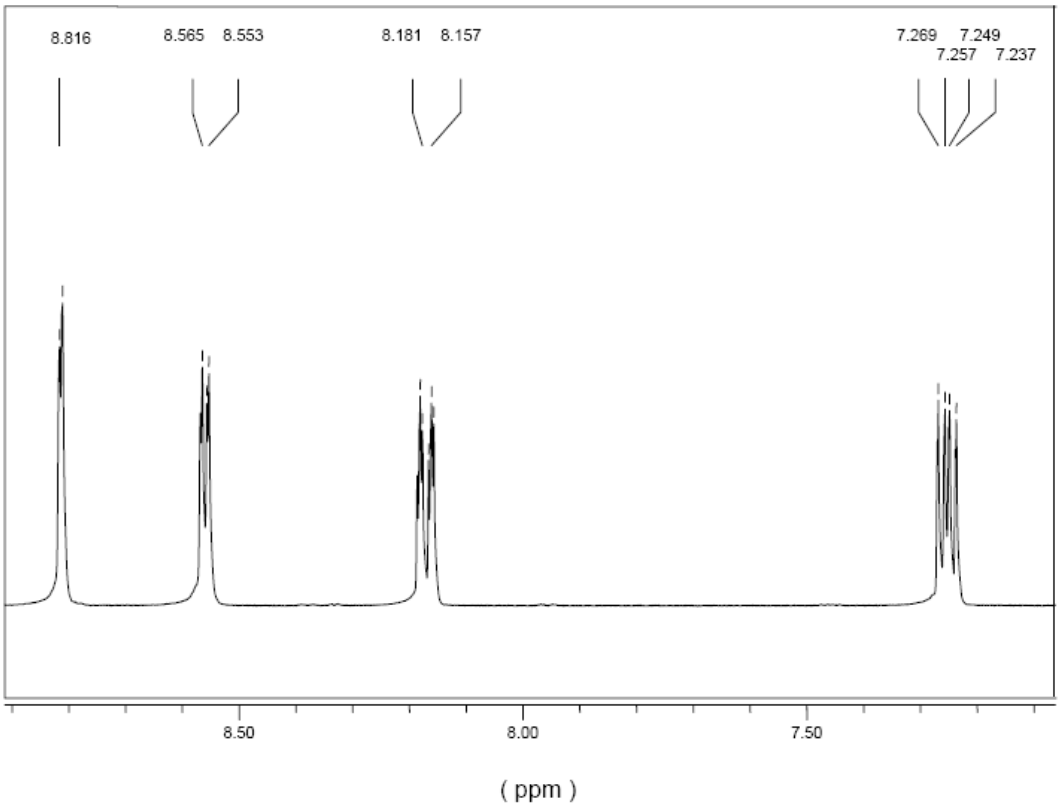


Figure2. 9 NMR spectra of 3-iodopyridine in d_6-DMSO

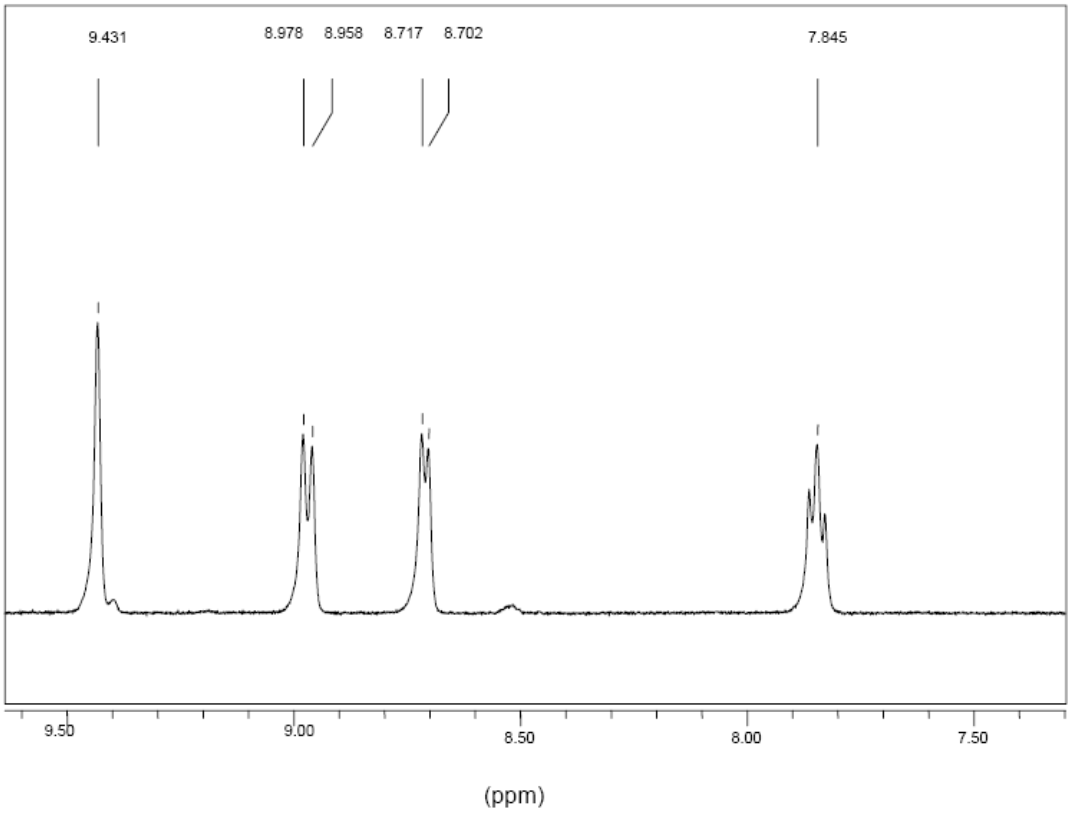


Figure2. 10 NMR spectra receptor **2** in d_6-DMSO

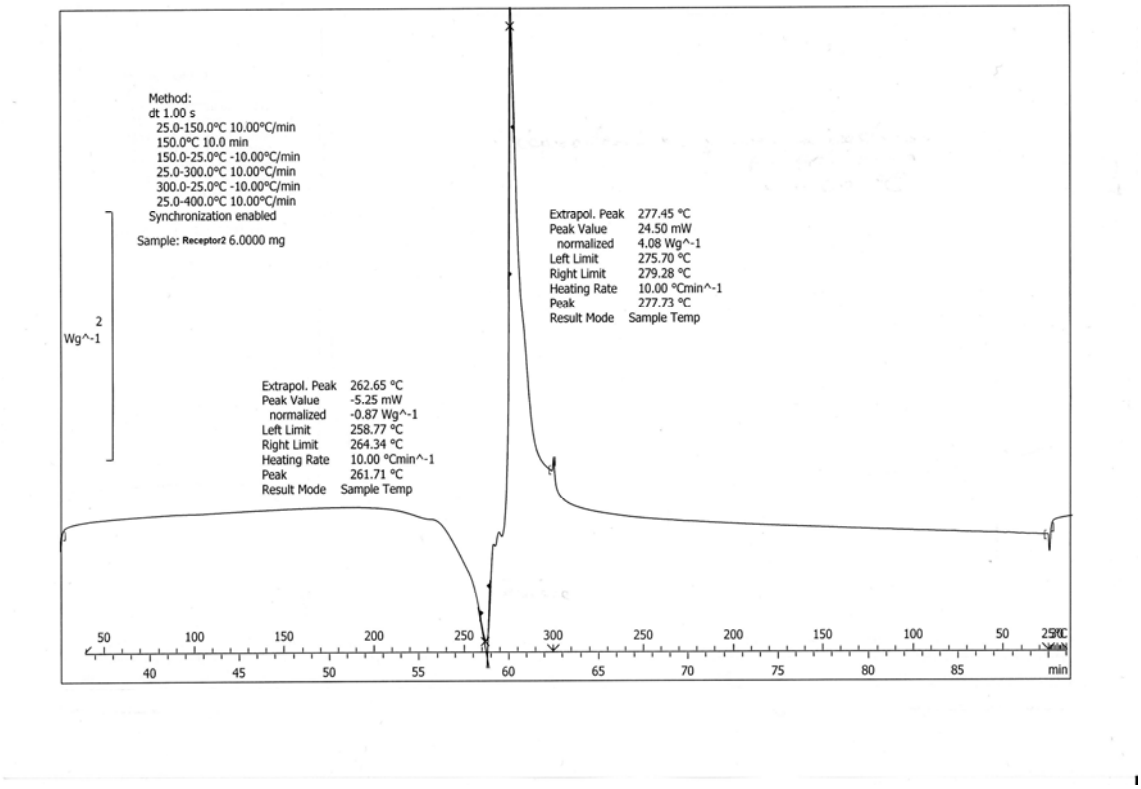


Figure2. 11 DSC spectra of receptor **2**

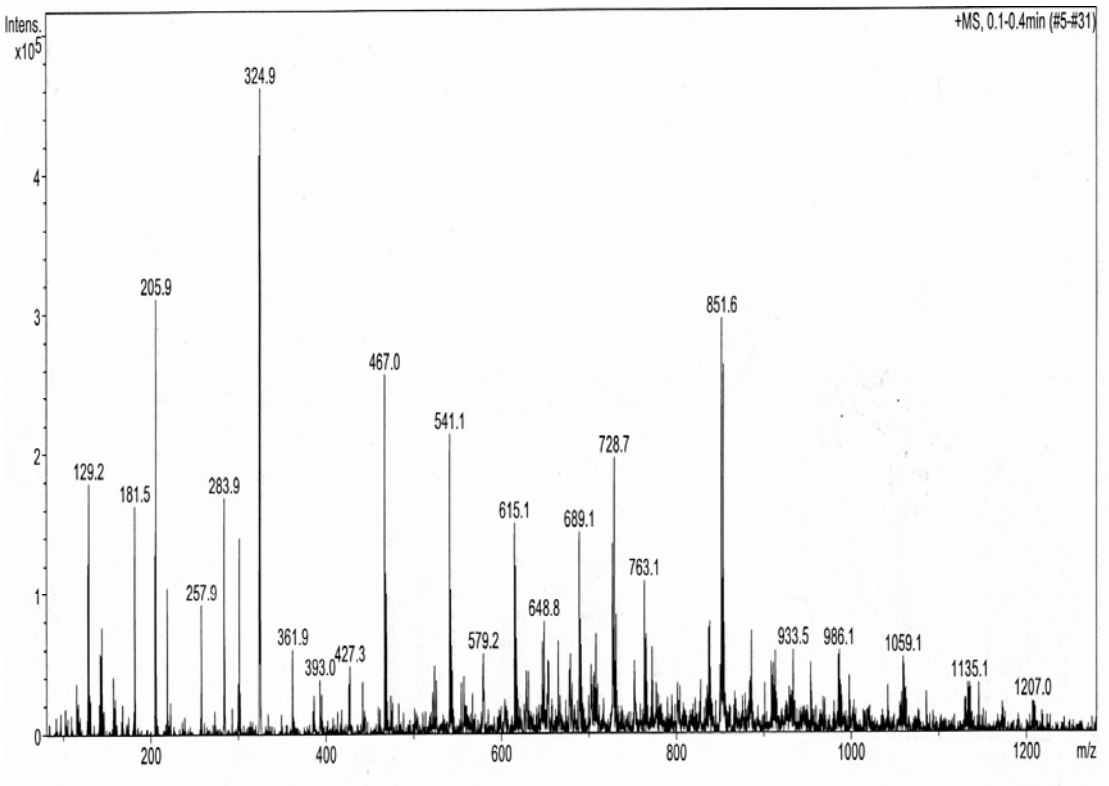
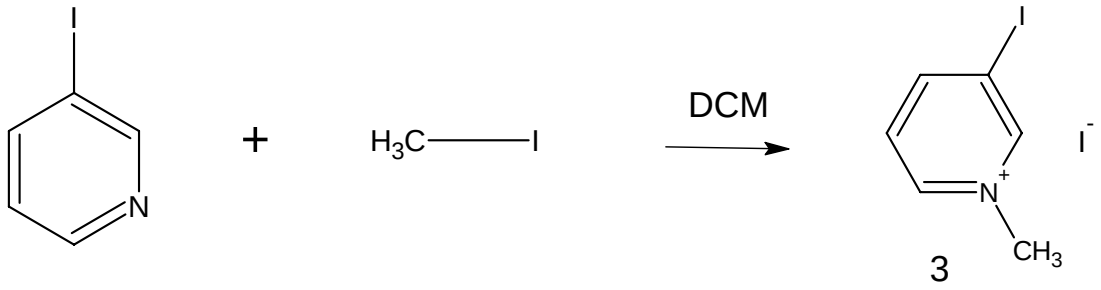


Figure2. 12 Mass Spectra of positive charges of receptor **2** in MeOH

2.2.2 Synthesize the Other Contrastive Phosphate Anion Receptors

The desired 3-iodo-1-methylpyridium iodide **3** was synthesized as shown in Scheme 2.2. Firstly the reagent 3-iodopyridine was dissolved in DCM, and then added iodomethane which is very volatile. After stirring at room temperature, **3** was synthesized. Because **3** was insoluble in DCM, it could be purified by DCM. Then we can use ^{1}H NMR to check. There are 3 sets of typical peaks at 9.394, around 8.9, and around 7.9 ppm.



Scheme2. 2 The synthesis procedure of the receptor **3**

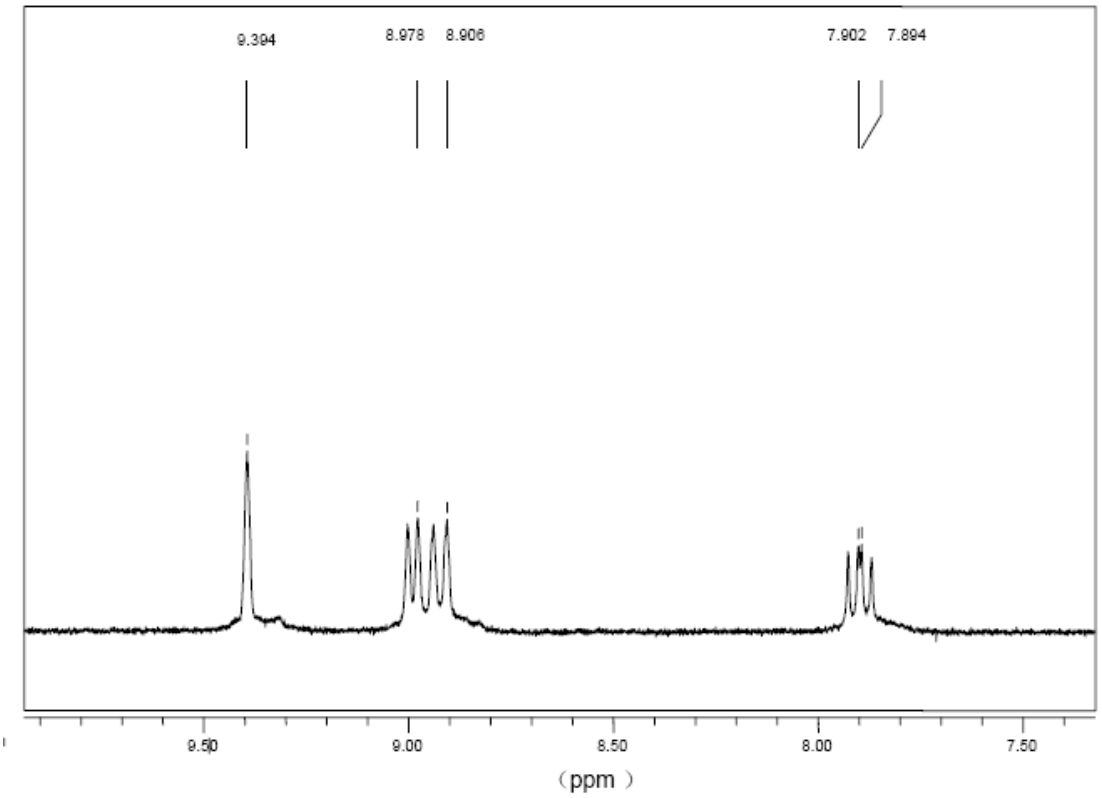
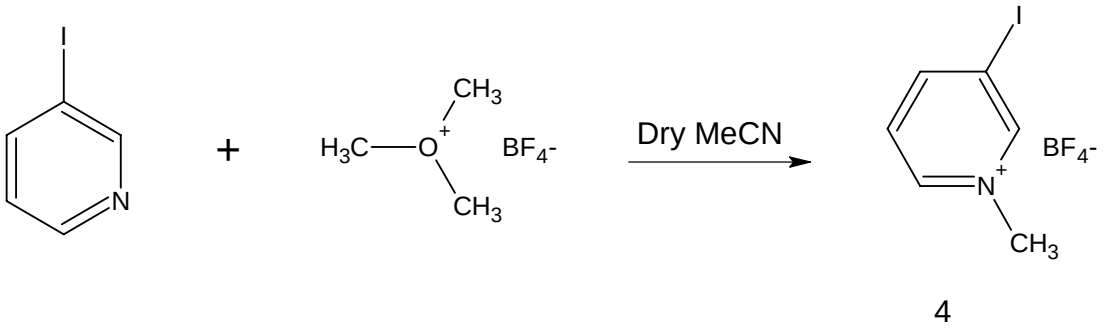


Figure2. 13 NMR spectra receptor **3** in d_6-DMSO

3-iodo-1-methyy-pyridinium tetrafluoroborate **4** was expected to be synthesized according to the Scheme 2.3. The reagents are 3-iodopyridine and trimethyl-oxonium tetrafluoroborate. During the synthesis, all the operations should be in the flux of nitrogen to avoid from forming the by product. And then it was purified by DCM. The ^{1}H NMR spectrum was shown as below.



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Scheme2. 3 The synthesis procedure of the receptor **4**

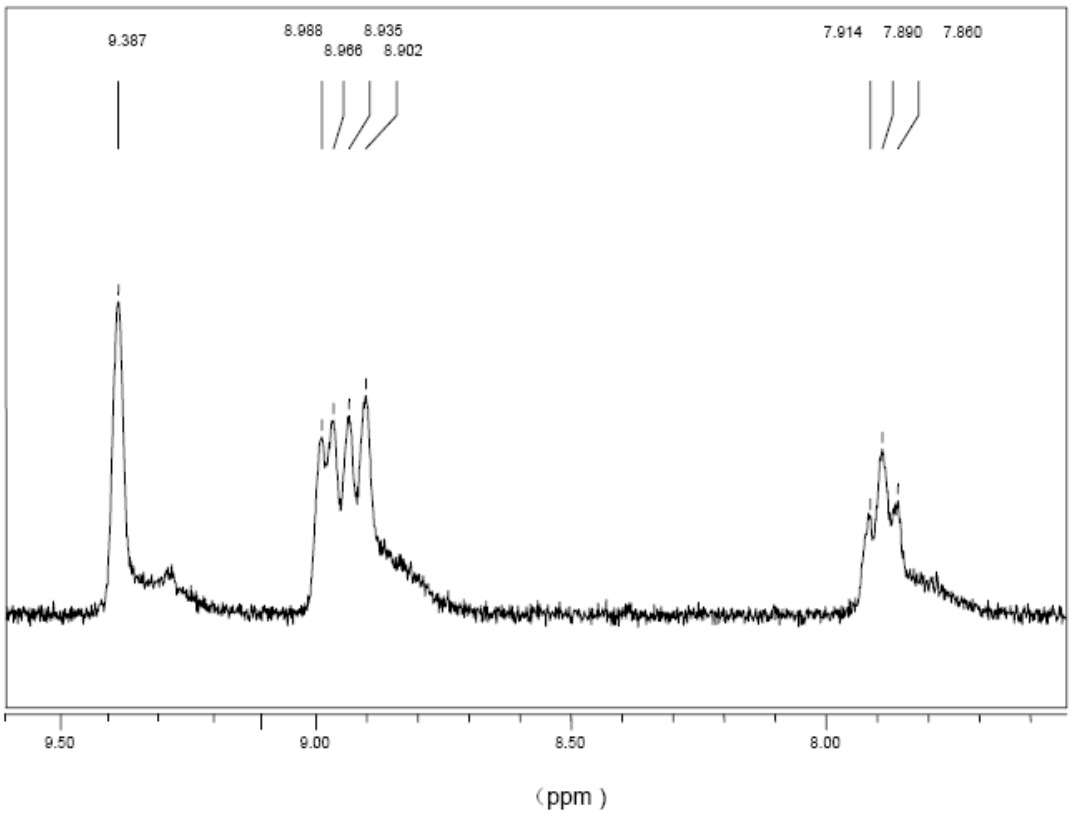
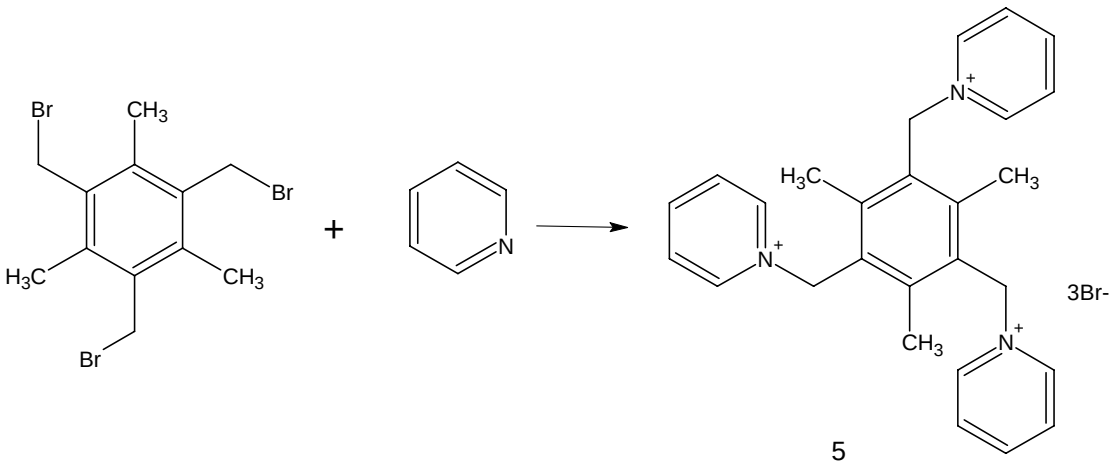


Figure2. 14 NMR spectra receptor **4** in d_6-DMSO

1,3,5-tris(bromomethyl)-2,4,6-trmethylbenzene reacted with a large excess of pyridine according to the procedure of Scheme 2.4. After stirring at the temperature of 90℃, **5** was synthesized. So it that was insoluble in DCM was purified by DCM and tested by ^{1}H NMR.



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Scheme2. 4 The synthesis procedure of the receptor **5**

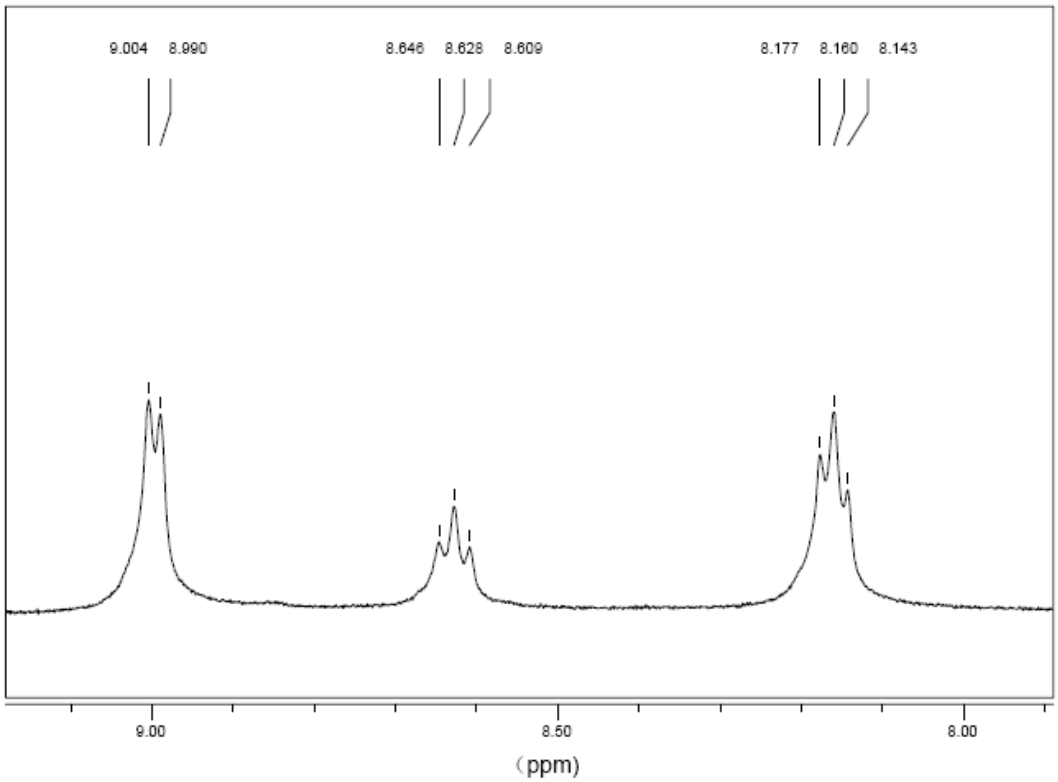
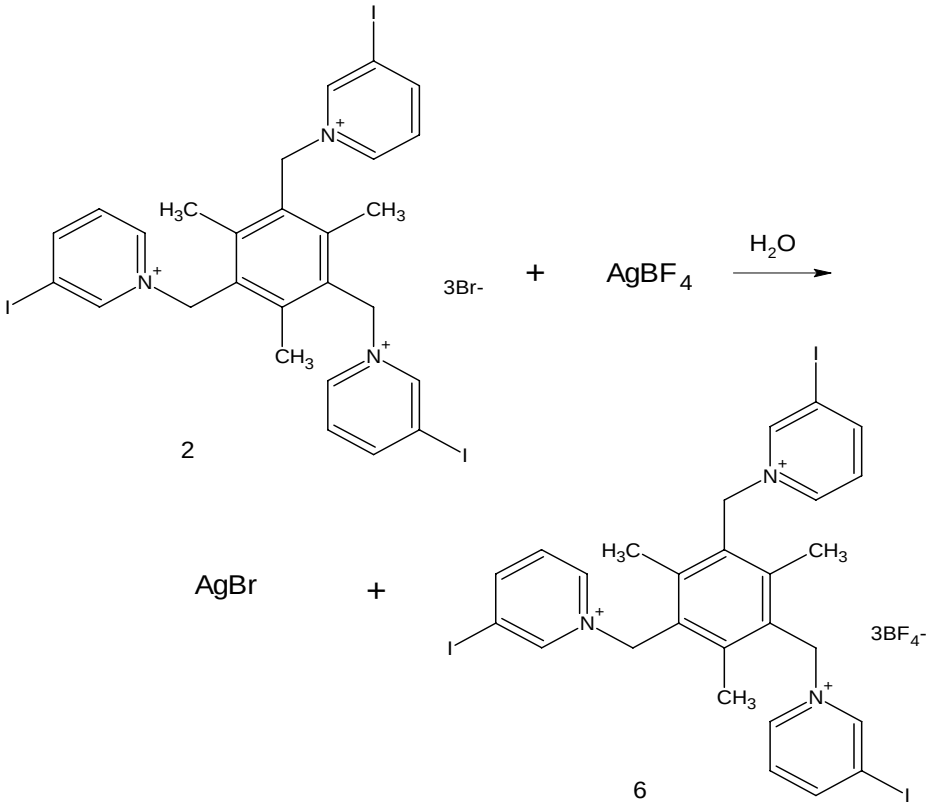


Figure2. 15 NMR spectra receptor **5** in d_6-DMSO

Receptor **6** was synthesized from the reaction of **1** and silver tetrafluoroborate in pure water. Due to the photolysis of silver bromide, there are some silver precipitates which are black. However **6** was soluble in pure water. So we separated the precipitates and solution via centrifugal separation, and then vaporized the solution in vacuum.

Using the same standard methods as above, the configuration and properties of the desired product **6** were characterized. There are ^{1}H NMR spectrum and mass spectrum of **6** which are shown as below.(Figure2.15 2.16) In positive charge mass spectra, we could find the particular peaks at 257.9, 430.0 and 947.8 m/z, which stand for the receptor **2** with no bromide anion, one bromide anion and two bromide anions.There is no difference between receptor **2** and **6** in positive charge mass spectra. So we need to check the negative charge mass spectra in order to exclude the possibility of existing reactant .The peak at 87.3 m/z means the tetrafluoroborate anion. And there is no bromide anion peak. Therefore the pure receptor **6** was gained to do the fluorescence titration on next step.



Scheme2. 5 The synthesis procedure of the receptor **6**

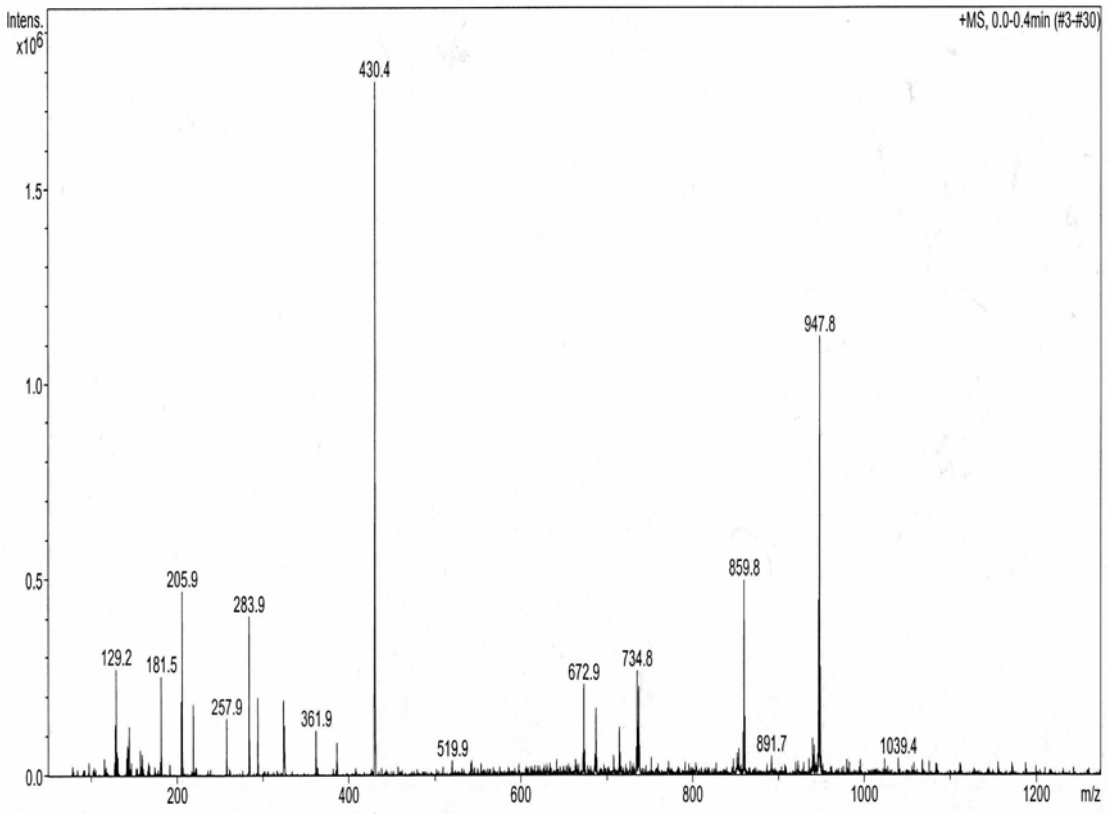


Figure2. 16 Mass spectra of positive charges of receptor **6** in MeOH

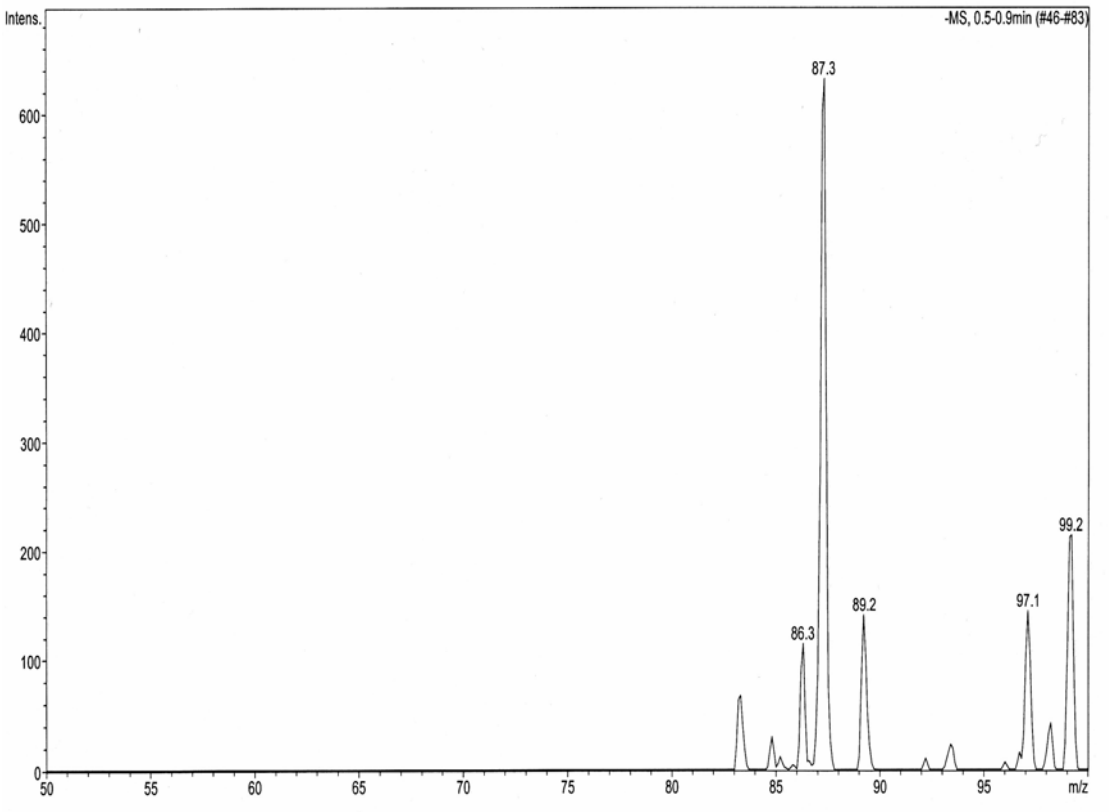


Figure2. 17 Mass spectra of negative charges of receptor **6** in MeOH

2.3 Fluorescence Titration

As above paragraphs mentioned, many anions play important roles in living organisms. Development of anion recognition may lead to the development of identifying the bioorganic molecules containing intramolecularly anionic groups. However, most known anion receptors only were used in organic media so far until now. Because anions are generally larger than cations, anions are more subject to solvation than cations. In organic solvents, it is not so difficult to recognize the anion due to the relatively small solvation energy and electrostatic interaction. In aqueous solvents which are relevant to biological application, it is hard to detect anions because of the strong hydration.

So it is expected that there are a good affinity of our receptor for phosphate in aqueous solvent, and this result of binding could transfer to a fluorescence signal to be expressed. In this thesis, to the rapidly access the affinity of our receptors for phosphate in water (pH=12.28, 0.19mM NaOH, at 25℃), we studied the binding of fluorescent phosphate derivative **1**. On the titration of a receptor into a solution of this probe in water (pH=12.28, 0.19mM NaOH, at 25℃), the fluorescence emission was quenched indicating binding.

2.3.1 The Selection of Appropriate pH Aqueous Media for Phosphate Anion

Fluorescence titration is a high sensitive measurement method. It could be used to test the anion with fluorophore at lower concentration. In order to get effective quantitative fluorescence titration, we have to select the appropriate pH environment for phosphate anions. On the one hand, to assure the main form of phosphate anions, the aqueous media should be alkaline solution. Otherwise the other two forms of hydrogen phosphate anion and dihydrogen phosphate anion could be dominant in

neutral or acidic media. On the other hand, the slight difference of pH causes the intense change of fluorescence emission intensity. The tendency is that the fluorescence emission intensity decreases as the pH of aqueous media increases.

Therefore we chose 3 sets of different pH of aqueous media, which are respectively 12.28, 12.38, 12.46 when water was added by different amount of NaOH. The results of intensity are shown below Table2.1

Table2. 1 The changing of average fluorescence emission intensity as the changing of pH

pH	**Average Fluorescence Emission Intensity**
12.28	88
12.38	65
12.46	57

The binding of receptors and phosphate **1** induces the fluorescence quenching. Though all the intensities at different pH environment are stable, at last we chose the pH=12.28 aqueous media which has higher intensity. It's convenient to observe the occurrence of quenching.

All the fluorescence titrations were tested under this situation (pH=12.28, 0.19mM NaOH, at 25℃)

2.3.2 The Fluorescence Titration of Receptor **2**

Firstly we investigated the halogen bonding of our target receptor **2** for **1** to obtain the association constant via fluorescence titration. The intensity of fluorescence emission spectrum from 5μMol*L^{-1} fluorescent phosphate derivative **1** in water (pH=12.28, 0.19mM NaOH, at 25℃) decreased as the concentration of receptor **2** was increased (see Figure2.17), which indicated the association between the receptor **2** and **1** intuitively**.** The Stern-Volmer plot of F_0/F versus the concentration of receptor **2** was

shown in Figure2.18.

When concentration of the receptor **2** is larger than that of the phosphate **1**, the curve tends to be horizontal line and the quench almost stops. In contrast, before that concentration, the curve is nearly linear and useful for binding determination in Figure2.19. The linear part of Stern-Volmer plot further confirms the formation of one type complex between receptor **2** and phosphate **1**. According to the calculation of Stern-Volmer equation, the association constant (Ka) of our target receptor **2** for phosphate is around 0.2×10^{6} M^{-1}. The stoichiometry between receptor **2** and phosphate **1** was determined by fluorescence Job Plot, which showed evident 1:1 stoichiometry.

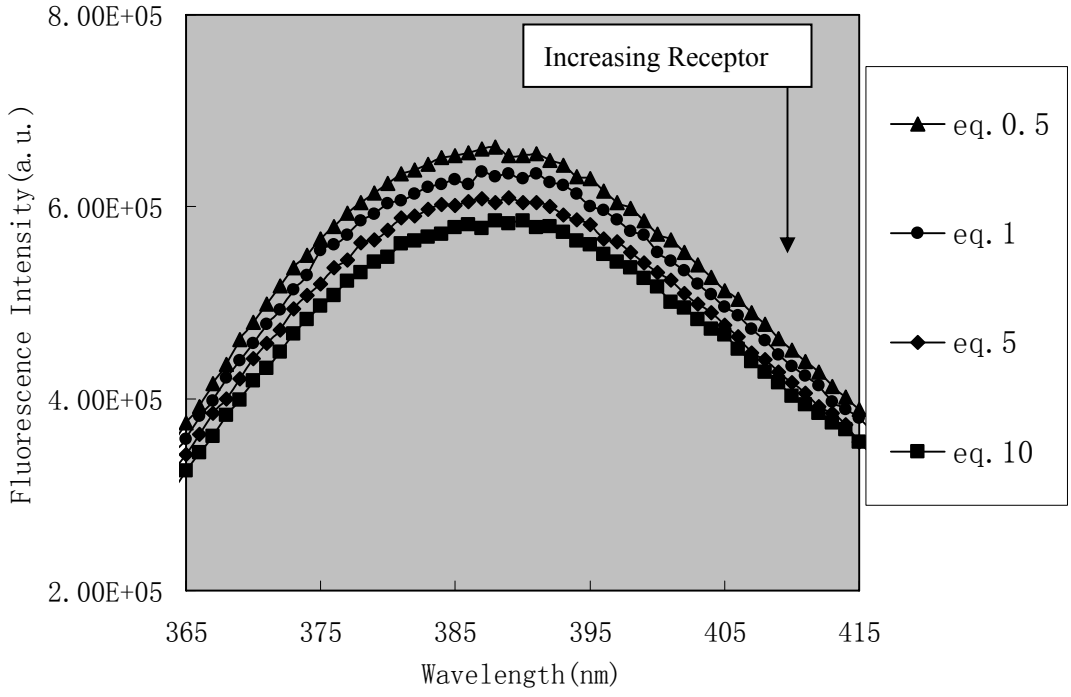


Figure2. 18 The change of fluorescence spectra in the fluorescent phosphate derivative **1** when receptor **2** was added.

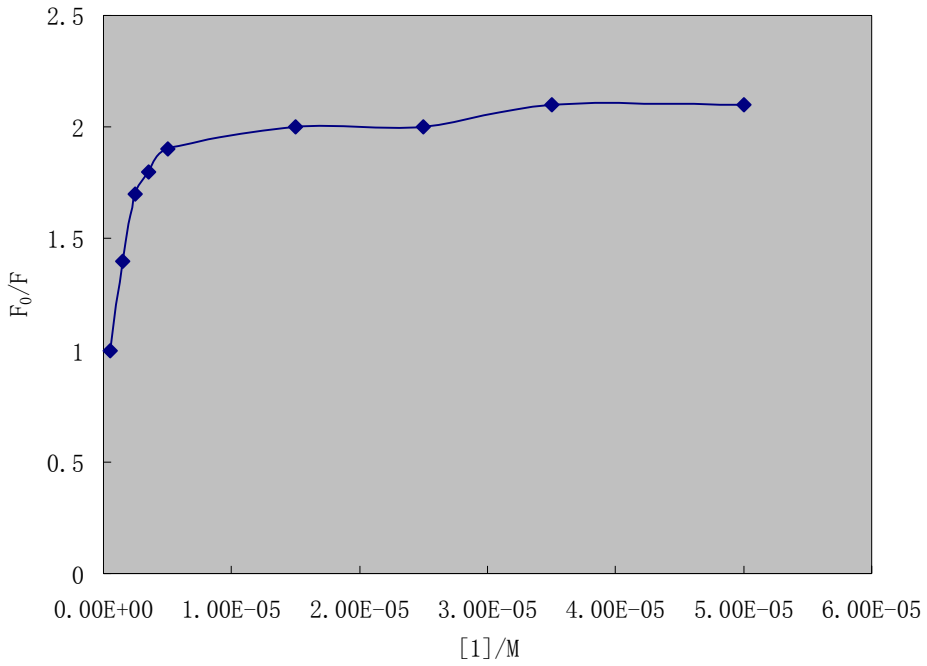


Figure2. 19 The Stern-Volmer plot for phosphate **1** quenched by receptor **2**.The concentration of receptor **2** changes from equivalent 0.1 to equivalent 10 to that of phosphate **1**

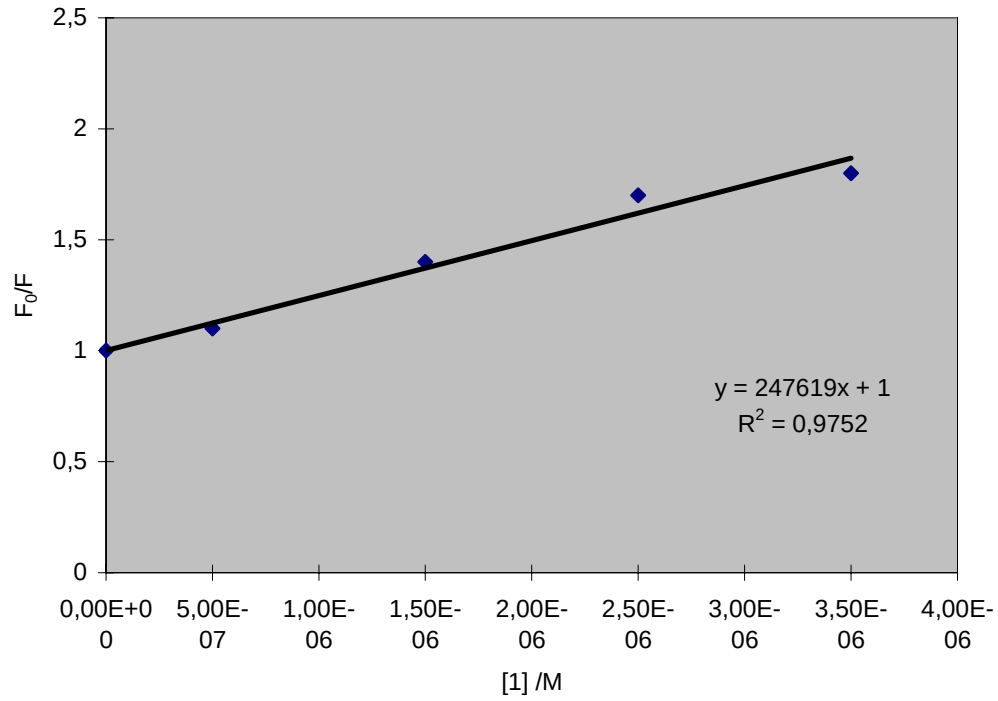


Figure2. 20 The Stern-Volmer plot for the association of receptor **2** and phosphate

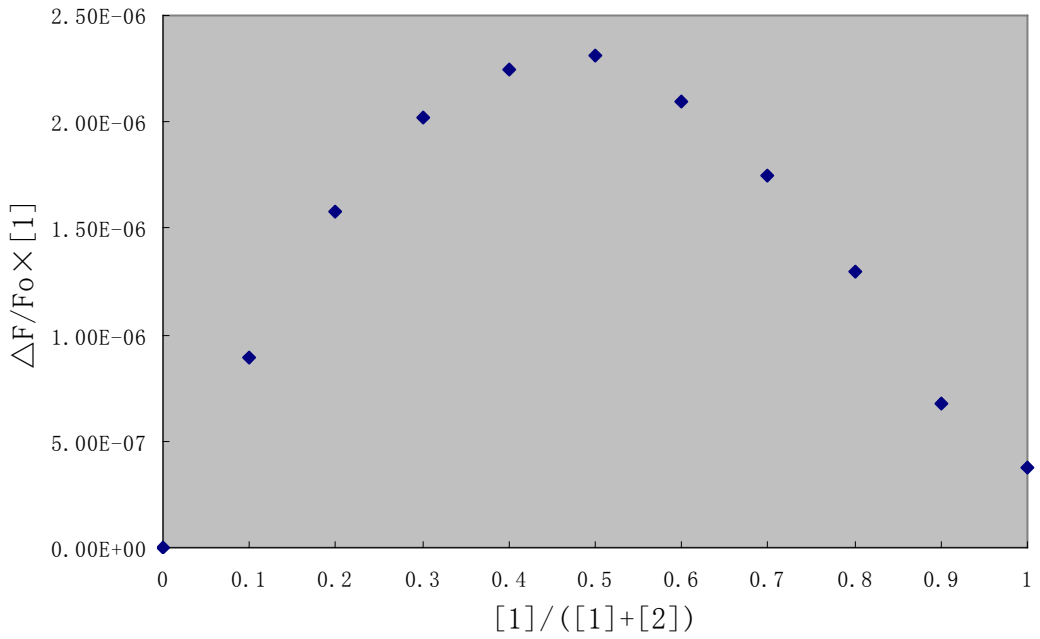


Figure2. 21 Job plot between receptor **2** and phosphate 1

2.3.3 The Fluorescence Titration of Other Contrastive Receptors

To identify the binding site and possible anion binding mode, we also investigated the binding of other receptors with phosphate **1** by fluorescence titration.

The receptor **3** and receptor **4** have only one iodine group and different anion moieties respectively. It was found that along with the addition of receptor **3** and receptor **4,** there is no significant quenching of fluorescence emission and association constants is quite low, around 0.5×10^{3} M^{-1}. This indicates that the receptor **3** and **4**, which have only one iodine group arm, are difficult to form good binding affinity to phosphate **1.** On the other hand, no matter what anion moieties of receptors are, the results didn't show any different quenching effect.

To further identify the binding site and to check the quenching effect of bromide anion moiety of **2,** we introduce receptor **5** which also contains bromide anion moiety but without iodine group to do the contrastive fluorescence quenching test. The result is shown in Figure2.21.

It indicates that the fluorescence change by various receptors which contain iodine group or not. Due to without iodine group, the receptor-phosphate interaction is driven only by electrostatic forces. The coupling constant was less than that measured for the receptor with halogen. In this case, the slope of the curve of receptor **5** in Stern-Volmer plot tends to be much less minor, giving the association constant of $0.1\times10^{5}\ M^{-1}$ in order of magnitude. In contrast, the association constant of receptor **2** with iodine group is $0.2\times10^{6}\ M^{-1}$ in order of magnitude as we calculated before. It is obvious that the main binding site is iodine group and the bromide moiety doesn't affect the quenching a lot.

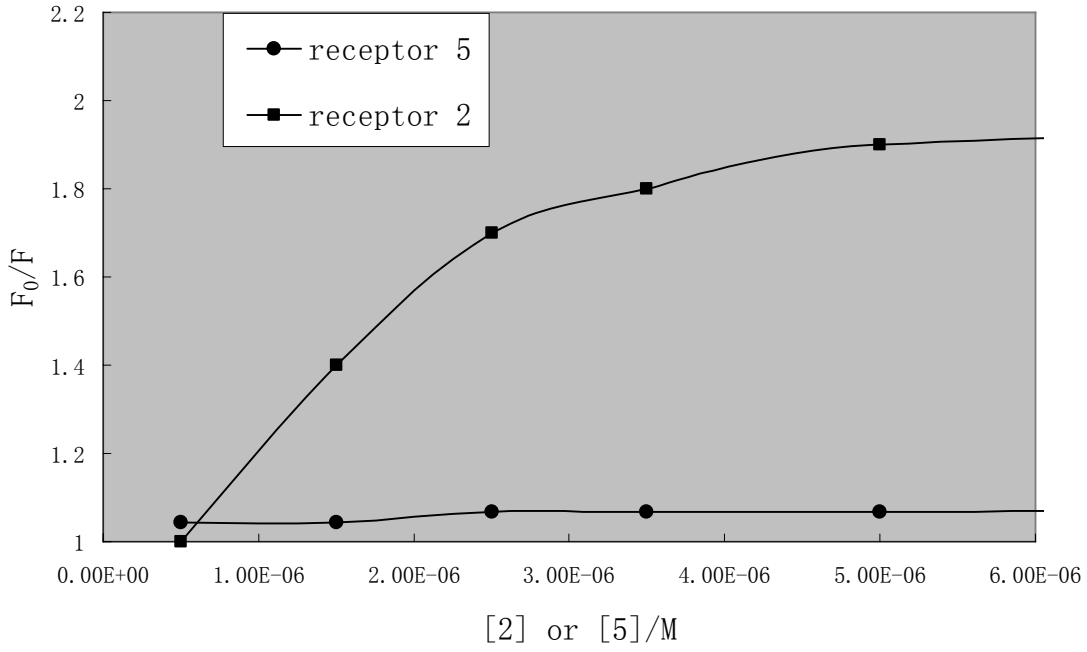


Figure2. 22 The Stern-Volmer plots for phosphate **1** quenched by receptor **2** and receptor **5**

At the same time the receptor **6** was synthesized, in which the bromide anion moiety was replaced by tetrafluoroborate anion moiety. The quenching effect of bromide moiety could be more definite if we use receptor **6** to do the double check via fluorescence titration. This test we will do it on next step.

All the association constants for the receptors **2-6** are reported in the Table 2.2.

Table 2.2. Association constants for receptors used in this thesis

Receptors	Counterions	Association Constant
2	Br^-	$0.2\times10^6\ M^{-1}$
3	I^-	$0.5\times10^3\ M^{-1}$
4	BF_4^-	$0.5\times10^3\ M^{-1}$
5	Br^-	$0.1\times10^5\ M^{-1}$
6	BF_4^-	Work in progress

2.3.4 The Fluorescence Displacement Assay for Phosphate anions

The fluorescence displacement assay is an increasingly popular strategy for anion recognition. So now our task is to evaluate our receptor for phosphate in this way again. As the above paragraph mentioned, the target anion receptor **2** for phosphate anions has a good affinity, and the iodine group of receptor **2** plays an important role in forming the halogen bonding with phosphate oxyaanion. According to the Job Plot method, the 1:1 binding model is proved.

Therefore we prepared a 1:1 receptor **2**–fluorescent phosphate derivative **1** complex in the same aqueous solution (pH=12.28, at 25℃，0.19mM NaOH).The phosphate **1** contains a coumarin moiety as an indicator. Then aliquots of the sodium phosphate were titrated into this solution. As Figure 2.22 was shown, the sodium phosphate was able to displace the indicator from the receptor **2**–fluorescent phosphate derivative **1** complex. Fluorescence emission intensities were then analyzed by the standard method for competition assays to determine the association constants.[35] Firstly we need to know the dependence of indicator concentration on the fluorescence signal and in this way we can calculate the association constant for receptor **2**-sodium phosphate (inorganic salt). (Figure2.23) At eq.1, there is no displacement which occurred. At equivalent 10, the association constant of receptor **2**-sodium phosphate (inorganic salt) complex in this competition assay is $0.4\times10^6 M^{-1}$. If we increase the

amount of sodium phosphate until equivalent 100, no more special increase occurred.

In this series of fluorescent displacement assay, we obtained the important information that our target receptor **2** could be used in combination with different phosphate types, inorganic too.

The comparison of the association constants for two phosphates, the organic and the inorganic one, shows that the Ka for the PO_4^{-3} is twice higher than the one for the binding of receptor organic phosphate. This suggest a slightly higher affinity of the receptor **2** for the inorganic phosphate and this can be due to the co-operative effect of the three arms, halogen bonding donors, towards the three halogen bonding acceptors site, namely the three oxygen anions, which provide better coordination in solution.

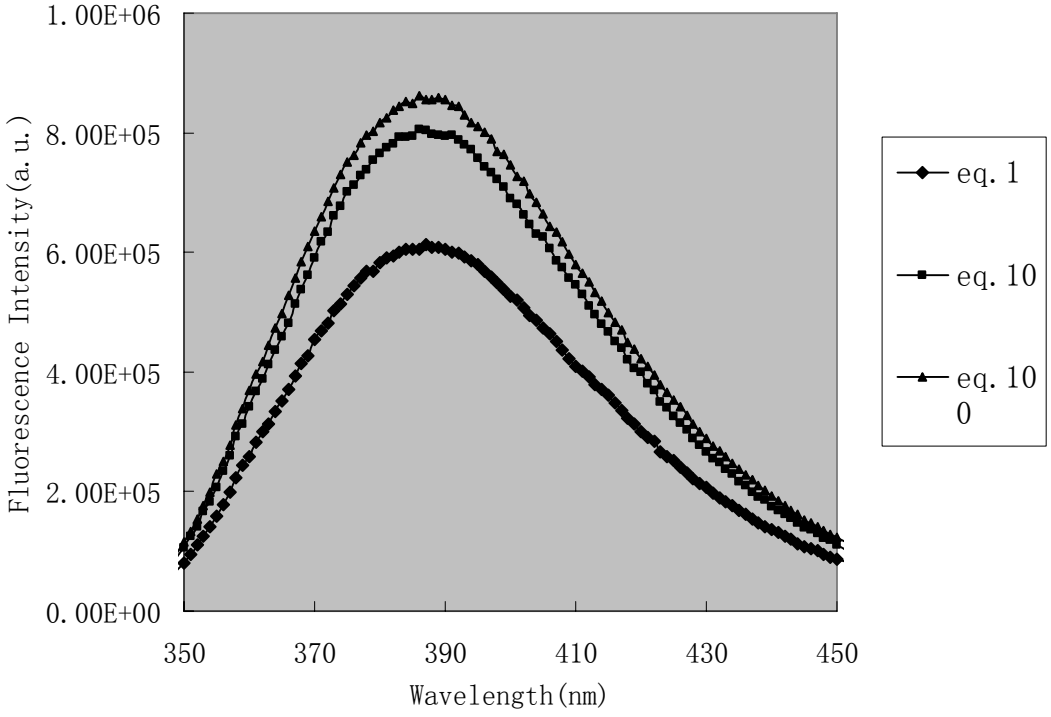


Figure2. 23 The fluorescence emission spectra of fluorescent phosphate derivative **1** after addition of increasing amounts of sodium phosphate to a 1:1 mixture of **1** and **2**

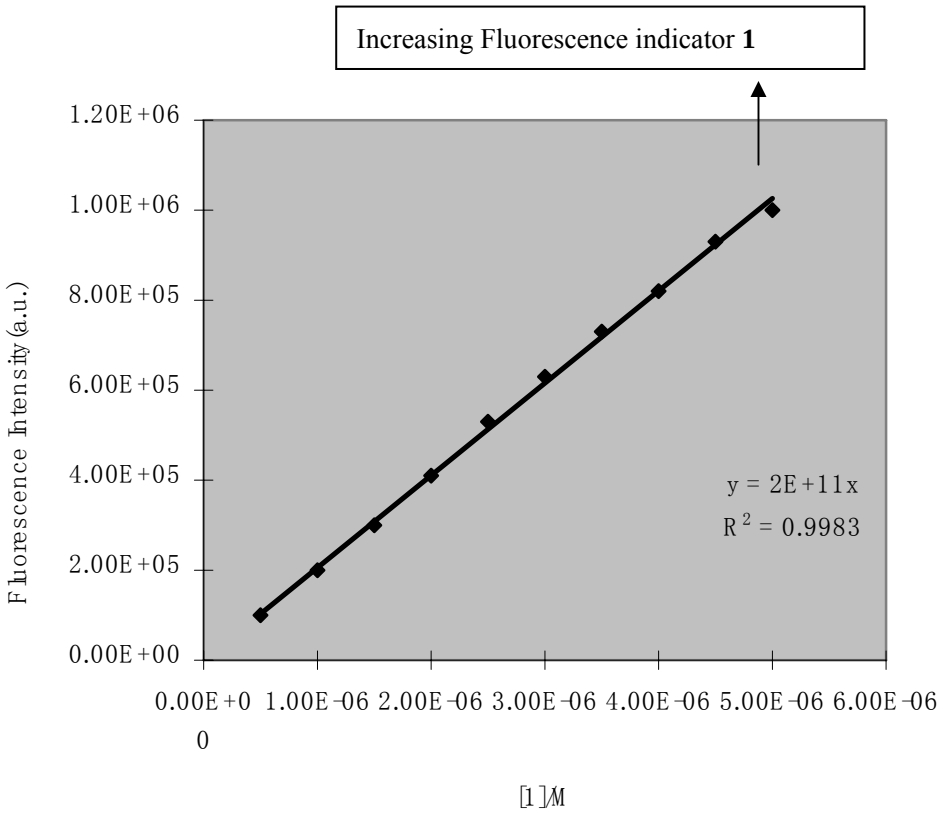


Figure2. 24 Calibration chart for fluorescence indicator **1**

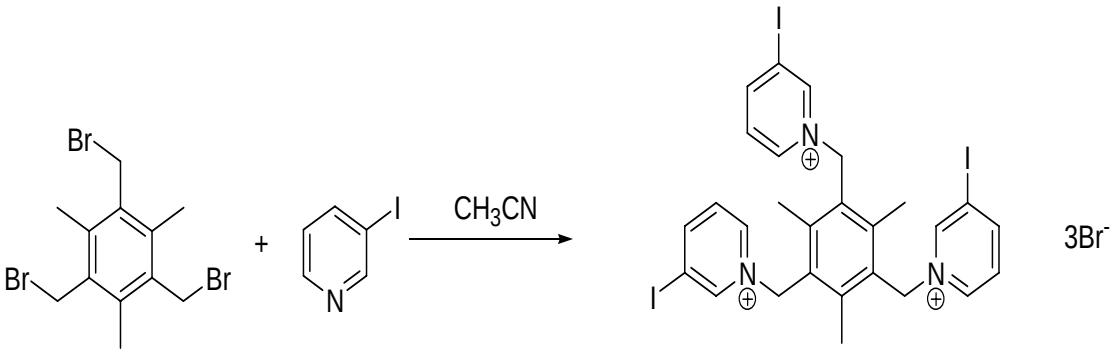
3. EXPERIMENTAL SECTION

3.1 Materials and Methods:

Commercial HPLC-grade solvents were used without further purification. Starting materials (4-methylumbelliferyl phosphate disodium salt, 1,3,5-tris (bromomethyl) -2,4,6-trimethylbenzene, 3-iodopyridine, iodomethane, trimethyl-oxonium tetrafluoro borate, silver tetrafluoroborate) were purchased from Sigma–Aldrich, Acros Organics, and Apollo Scientific. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded at ambient temperature with a Bruker 250 MHz, 400 MHz and 500 MHz spectrometer. Unless otherwise stated, DMSO-d_6 was used as both solvent and internal standard in ^{1}H NMR spectra. All chemical shift values are given in ppm. The mass spectra were recorded on a GC-MS AGILENT GC-MSD5975. Differential Scanning Calorimetry (DSC) analysis was performed on a Mettler Toledo DSC823^{e} instrument, aluminium light 20 µl sample pans and the Mettler: STARe software for calculation. Melting points were also determined Reichert instrument by observing the melting and crystallizing process through an optical microscope. Emission spectra were recorded using a Jobin-Yvon Fluorolog-3 spectrometer equipped with double monochromators and Hamamatsu-928 photomultiplier tube (PMT) as the detector. All complexes were excited at 320 nm.

3.2 Synthesis of Receptors

Receptor **2**:

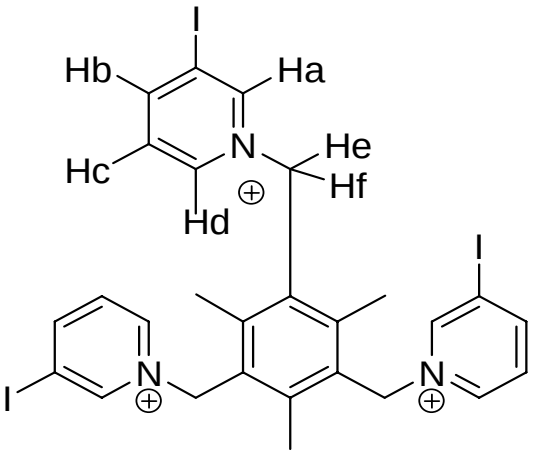


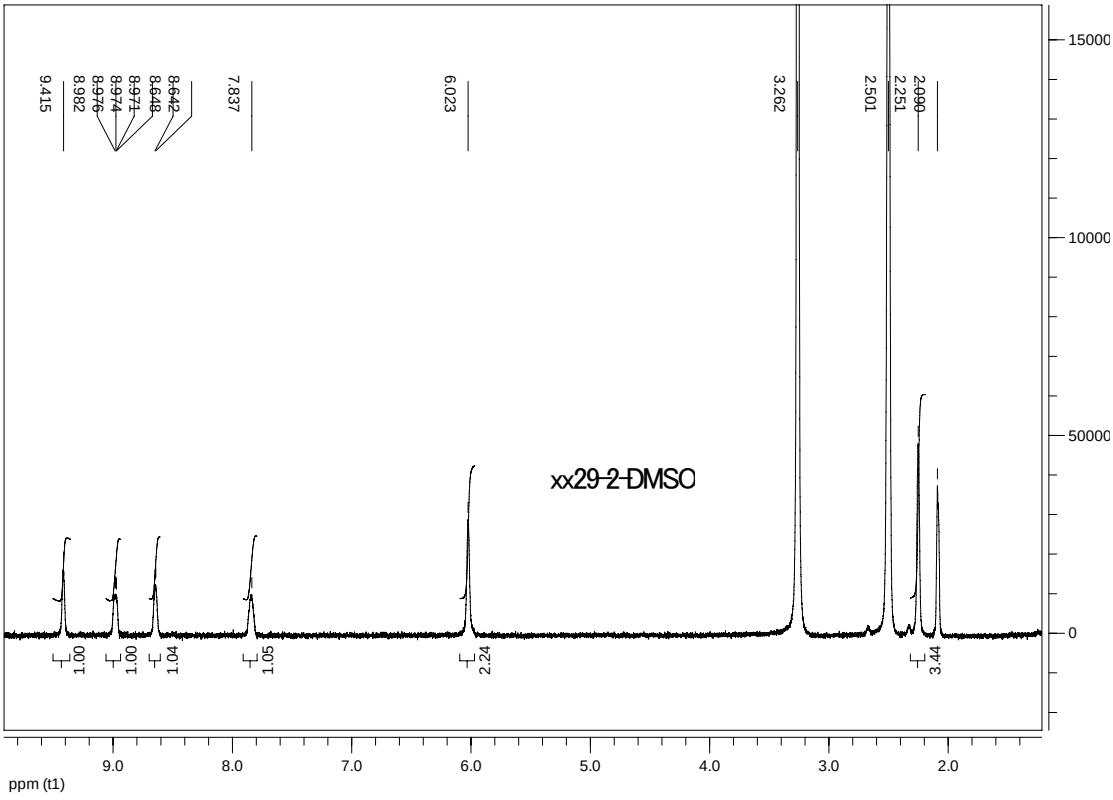
2

The receptor **2** was synthesis from 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene and 3-iodopyridine. In order to improve the yield and avoid from the by-product, at the beginning more than 3.5 times molar amount of 3-iodopyridine was dissolved in acetonitrile in the 25ml flask. And then we added 0.7mMol 1,3,5 -tris(bromomethyl)-2,4,6- trimethylbenzene into the solution. stirring the mixed solution for 72 hours at 70℃.

The product was washed by DCM 4 times. During the slow evaporation of the residual solvents, white color precipitations was gained. Due to hard to exclude the partial product, we used the method of recrystalization to get the pure receptor **2**. The procedure is that white color precipitations were dissolved into MeOH with heating, Afterwards the solution was added some amount $CHCl_3$ slowly and along the wall of flask, and was putted into the fridge for 24 hours. After the slow evaporation we can get the pure receptor **2** with yield 50%**.**

NMR proton label:



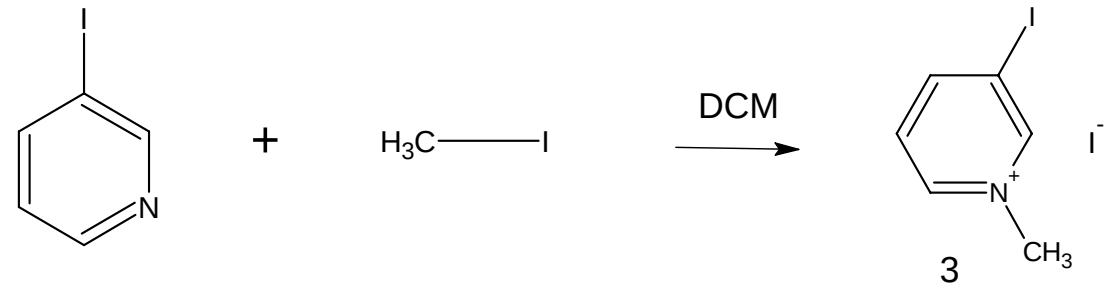


^{1}H NMR (DMSO-d_6, 400 MHz) : 9.41 (3H, s, Ha), 8.98-8.97 (3H, m, Hd), 8.70-8.64 (3H, m, Hb), 7.84 (3H, t, J = 7.0 Hz, Hc), 6.02 (6H, s, He and Hf), 2.25 (9H, s, CH_3)

m.p.: 261.7 °C

m.s: positive: 933.5 $[M-2Br]^+$, 467.0 $[M-Br]^{2+}$, 257.9 $[M]^{3+}$

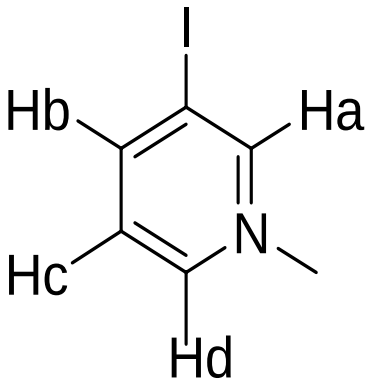
Receptor **3**:

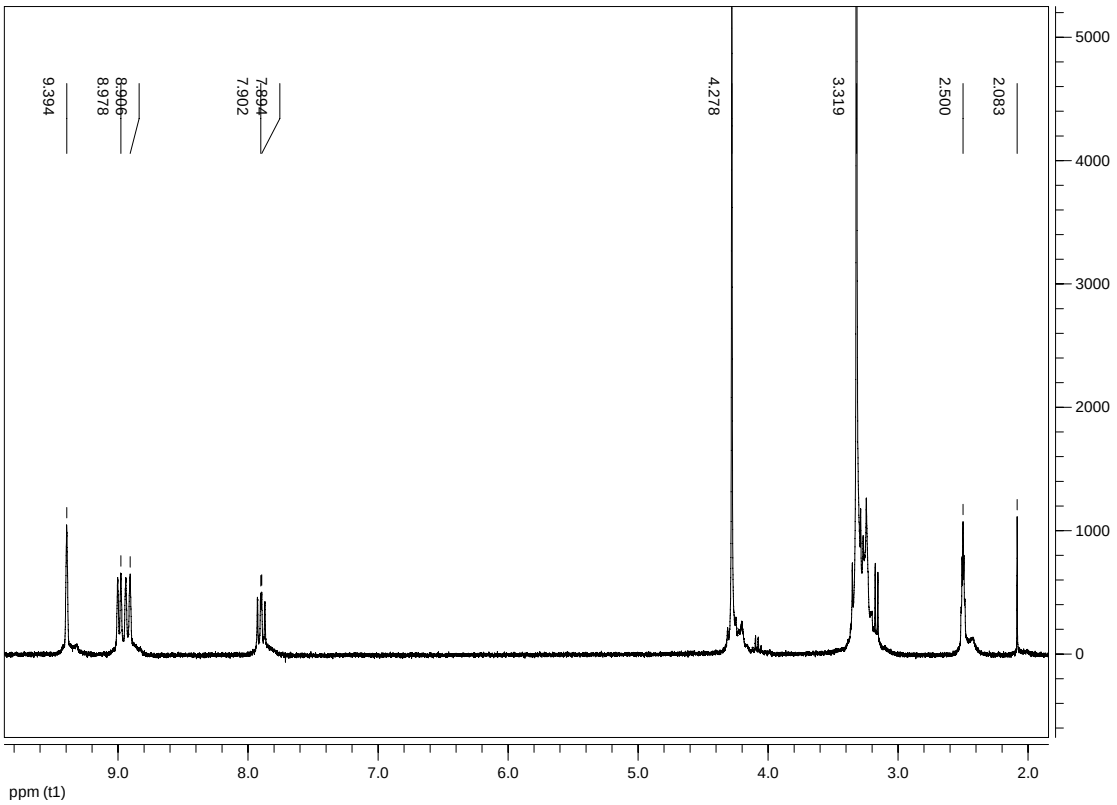


The receptor **3** was synthesis from 3-iodopyridine and iodomethane. Firstly preparing the solution of 1.46mMol 3-iodopyridine in DCM and injecting more than 1.5 equivalent molar amount of iodomethane in the reaction flask because iodomethane is liquid and very volatile. Strirring the solution for 24 hours at room temperature.

Take care to completely isolate the reaction flask for avoiding the iodomethane volatile. Due to product in the form solid and insoluble in DCM, the precipitants was washed by DCM 4 times to eliminate the excess of reactants. After the slow evaporation, we can get the pure receptor **3** with quantitative yield.

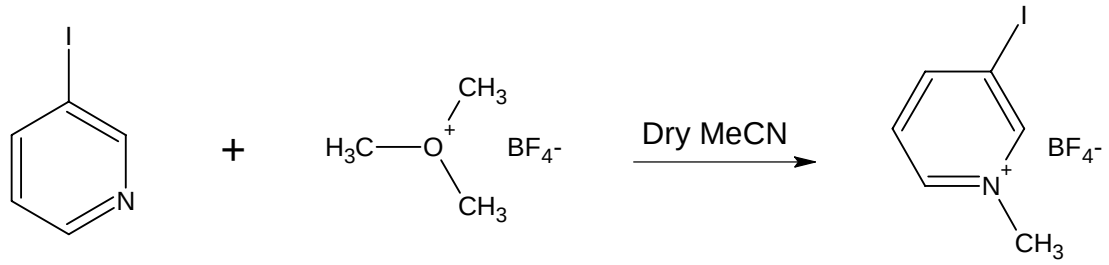
NMR proton label:





^{1}H NMR (DMSO-d_6, 400 MHz) : 9.4 (1H, s, Ha), 8.98 (1H, d, J = 7.0 Hz, Hd), 8.90 (1H, d, J = 7.0 Hz, Hb), 7.90 (1H, t, J = 7.0 Hz, Hc), 4.28 (3H, s, CH_3)

Receptor **4**:

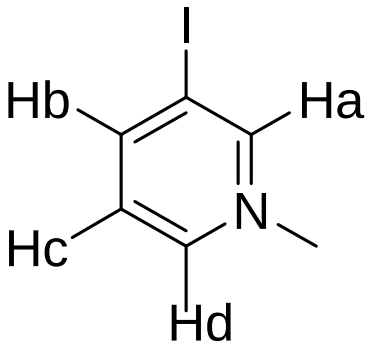


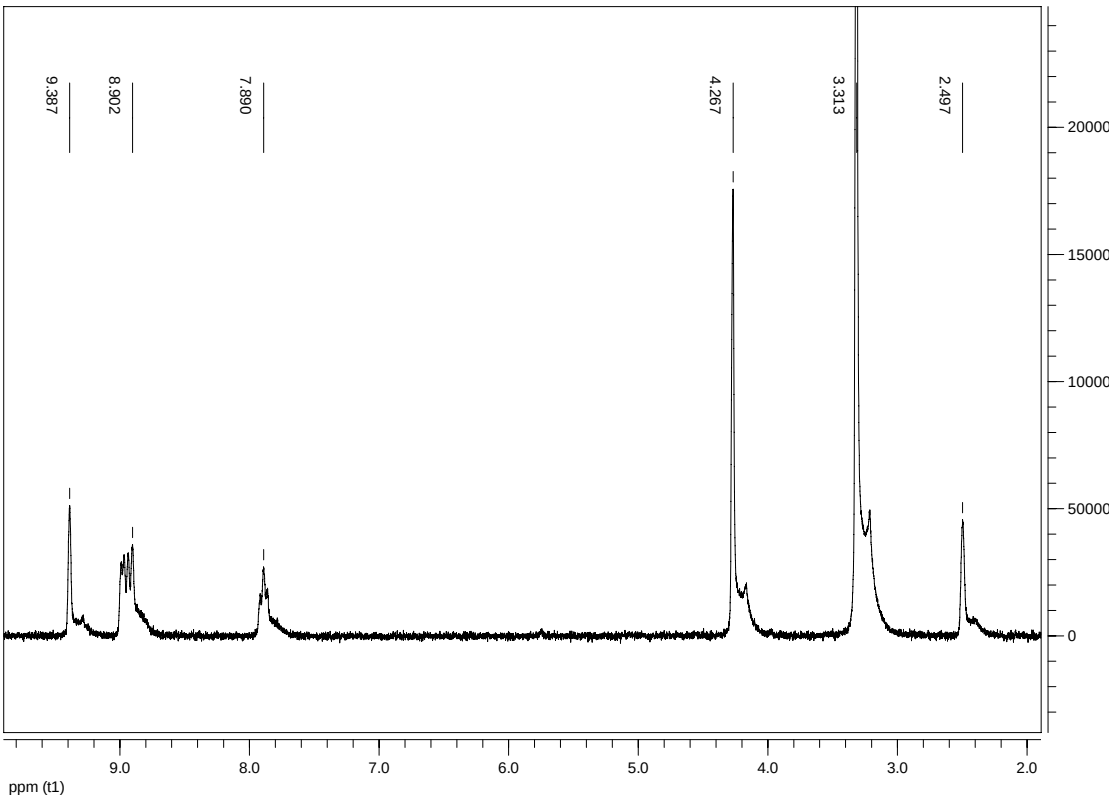
4

The receptor **4** was synthesis from 3-iodopyridine and trimethyl-oxonium tetrafluoro borate. Firstly trimethyl-oxonium tetrafluoro borate was dissolved in dry acetonitrile under nitrogen flow, because trimethyl-oxonium tetrafluoroborate is quite easy to react with water. And then preparing the solution of 1.46mMol 3-iodopyridine in the solution. Keep stirring for 12 hours at room temperature.

The product which is white powder was washed with DCM to eliminate the reactants. After the slow evaporation, we can get the pure receptor **4** with quantitative yield.

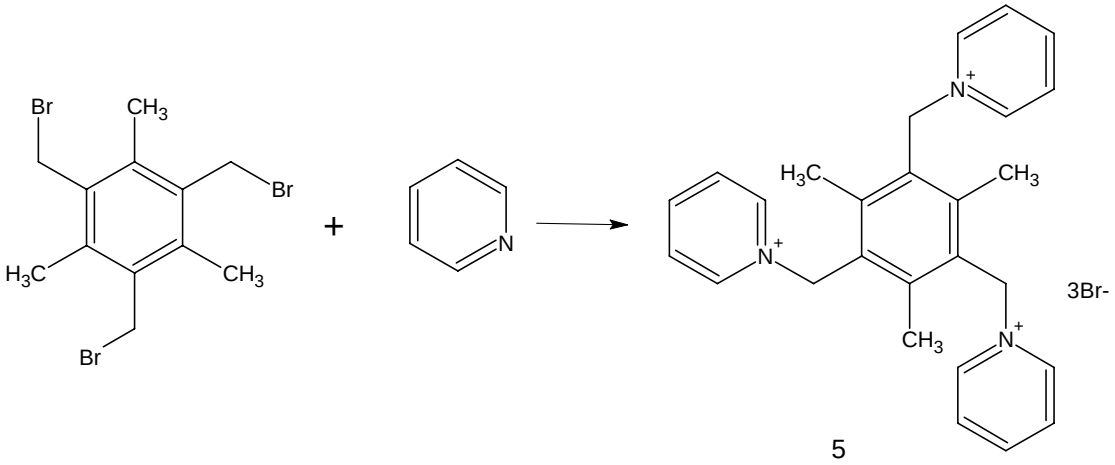
NMR proton label:





^{1}H NMR (DMSO-d_6, 400 MHz) : 9.4 (1H, s, Ha), 8.98 (1H, d, J = 7.0 Hz, Hd), 8.90 (1H, d, J = 7.0 Hz, Hb), 7.90 (1H, t, J = 7.0 Hz, Hc), 4.27 (3H, s, CH_3)

Receptor **5**:

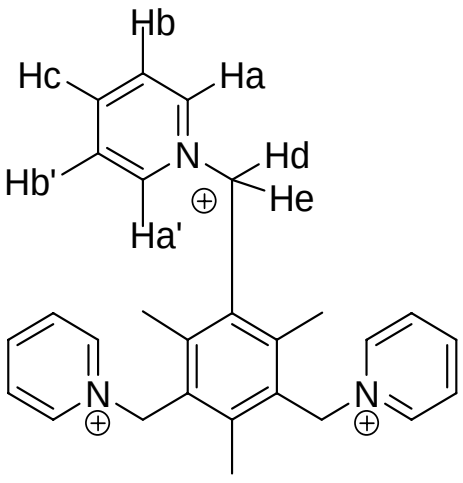


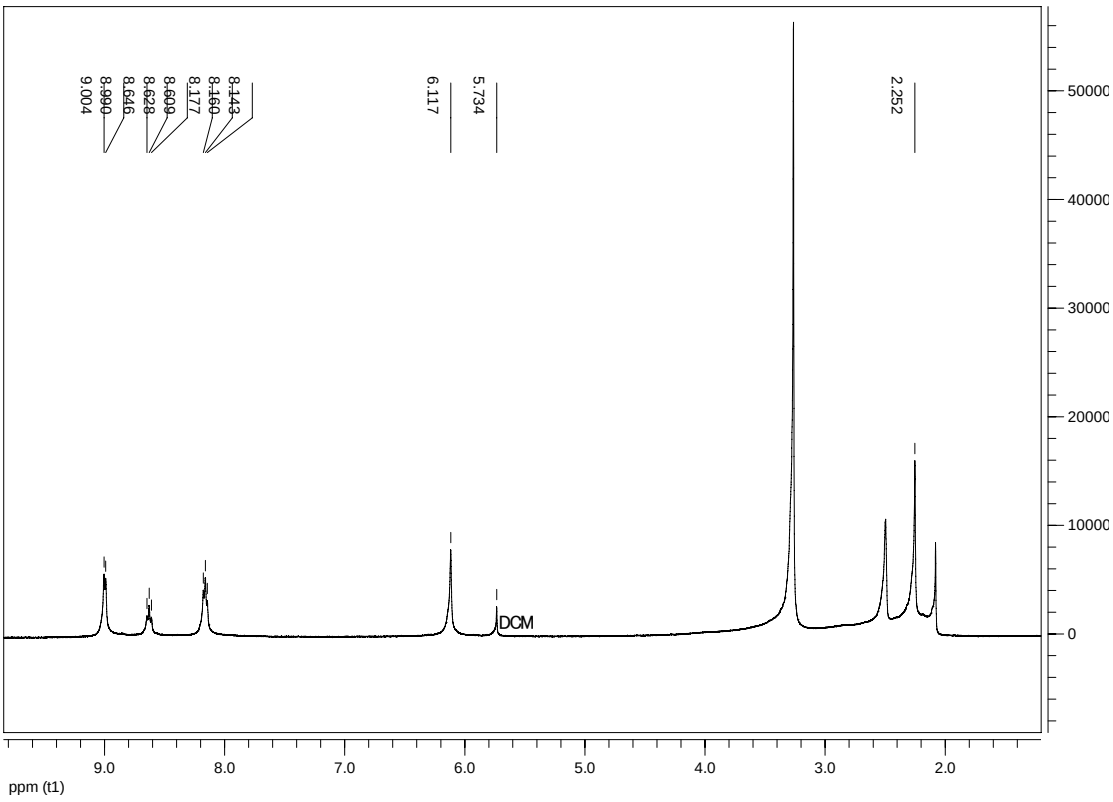
5

The receptor **5** was synthesis from 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene and pyridine 1mMol 1,3,5-tris(bromomethyl)-2,4,6-trimethylbenzene began to react in a large excess of pyridine. Let the reaction keep vigorous stirring for 12 hours at 70℃.

As usual in the purification procedure, DCM was used for washing the reaction product to eliminate the reactants. Finally the white pure receptor **5** will be obtain by the slow evaporation with 50% yield.

NMR proton label:

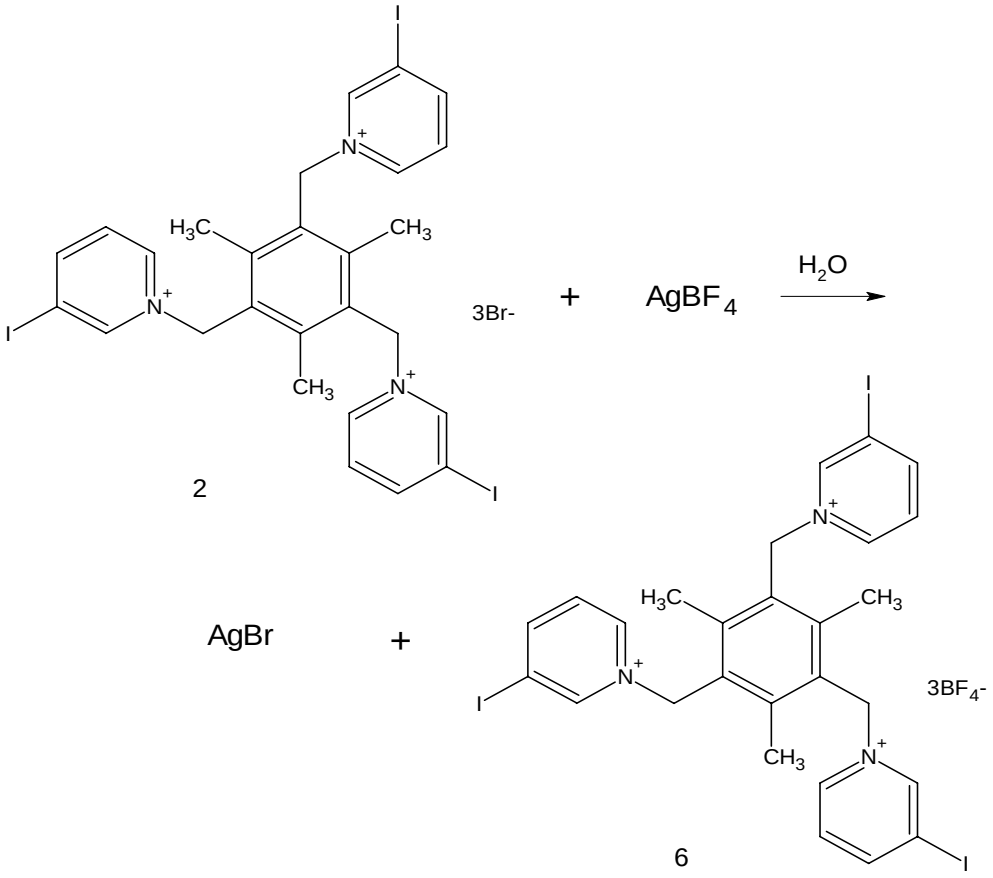




^{1}H NMR (DMSO-d_6, 400 MHz) : 9.01 (6H, d, J = 6.5 Hz, Ha and Ha'), 8.63 (6H, t, J = 7.2 Hz, Hb and Hb'), 8.16 (6H, t, J = 7.5 Hz, Hc), 6.02 (6H, s, He and Hf), 2.25 (9H, s, CH_3)

m.p.: 290.3 °C

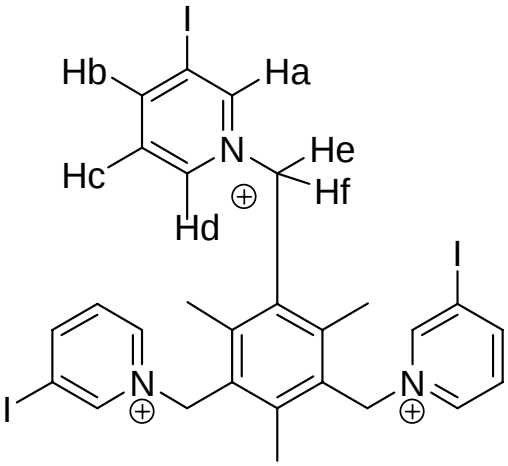
Receptor **6**:

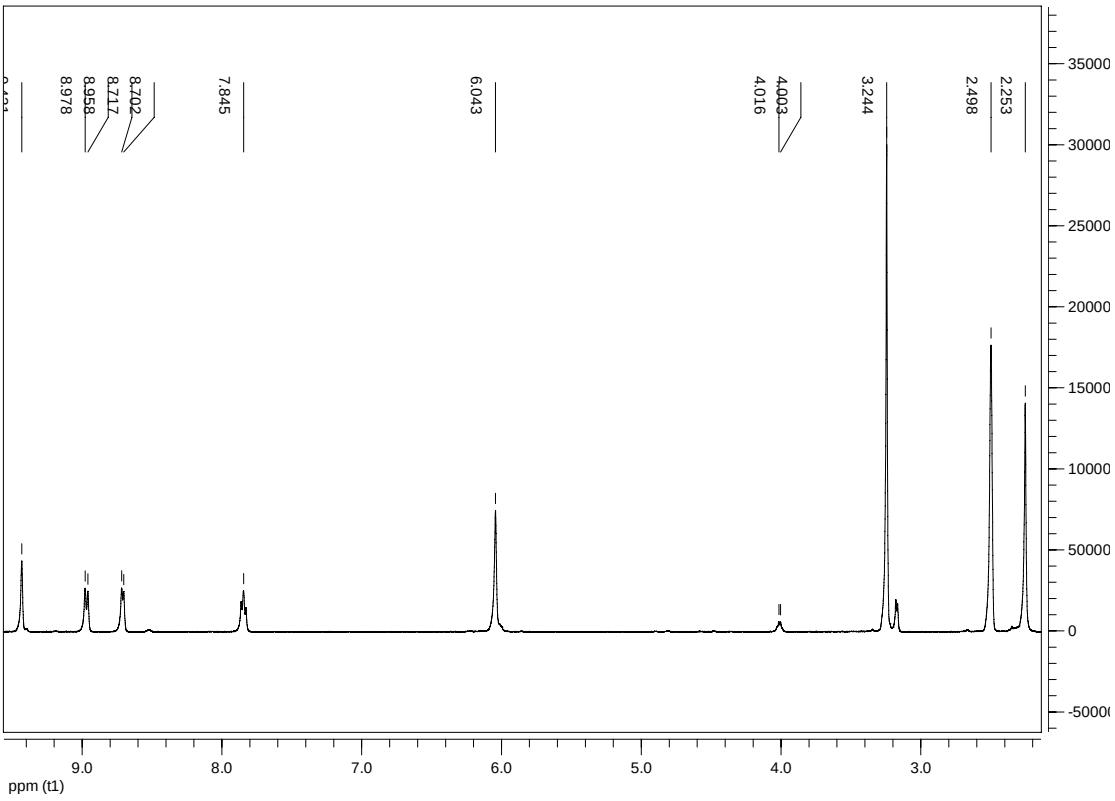


In order to synthesize receptor **6**, we use 0.148mMol receptor 2 as the reactant which reacted with 3 times molar amounts of silver tetrafluoroborate in pure water. Keep the reaction stirring for 48 hours at 70℃.

Our product was in the solvent, so we separate the precipitants and solvent by the centrifugal separator. Then the solvent was collected and evaporated at high temperature. Finally the white pure receptor **6** was obtained with 40% yield.

NMR proton label:





^{1}H NMR (DMSO-d_6, 400 MHz) : 9.41 (3H, s, Ha), 8.98-8.97 (3H, m, Hd), 8.70-8.64 (3H, m, Hb), 7.84 (3H, t, J = 7.0 Hz, Hc), 6.02 (6H, s, He and Hf), 2.25 (9H, s, CH_3)

m.s: positive 947.8.5 $[M\text{-}2BF_4]^+$, 430.4 $[M\text{-}BF_4]^{2+}$, 257.9 $[M]^{3+}$; negative 87.3$[BF_4]^-$

3.3 The Preparation of Fluorescence Titration

The general step of preparation of Fluorescence Titration of receptors for phosphate **1** is as shown below:

1. Add 20g NaOH into a tank with 26L water in order to obtain the aqueous media at pH=12.28.
2. Use pH=12.28 aqueous solvent to clean all flasks
3. Add some amount of the receptor into 500ml flask. And fill the pH=12.28 aqueous solvent to the flask until the scale mark and dissolve all receptors.
4. Add some amount of phosphate **1** into 100ml flask. And fill the pH=12.28 aqueous solvent to the flask until the scale mark and dissolve all phosphate **1**.
5. According to the various equivalents between receptors and phosphate **1**, we took and mixed them in 1L flask. Then we filled the mixed solution into 2ml bottle as sample.

3.4 The Preparation of Fluorescent Displacement Assay

Following the step from 1 to 4，add some amount of sodium phosphate into 100 flask. And fill the pH=12.28 aqueous solvent to the flask until the scale mark. Sodium phosphate was dissolved. Afterwards according to 1:1 stoichiometry of receptor and phosphate **1**, we added the various equivalent amount of sodium phosphate to check the displacement assay.

4. CONCLUSION

Phosphate anions are ubiquitous in the world, especially in the field of biochemistry and physiology. The selective recognition of phosphate anions in water plays a more and more role and is a topic of current interest to both chemists and biologists. In this thesis, we would like to design and synthesize receptors which have good affinities with phosphate anions. As we known, halogen bonding is regarded as a world parallel to hydrogen bonding on recognition process. The ability of XBs to control recognition, self-assembly, and aggregation processes in the solid and gas phases is well documented, and the interaction of XBs in liquid crystals is also receiving increased attention. According to the utilities of XBs in the biological systems, we realized that the formation of XBs in the liquid phase still remains to be fully developed.[33] Therefore our aim is to design and synthesize halogen bonding based receptors for phosphate anions, which work in high competitive environment such as water.

We have developed and fully characterized a new receptor **2** based on halogen bonding for phosphate anions with relatively simple synthetic procedures. We have also set up a robust methodology to detect its affinity with phosphate anion in solution using fluorescence titration. Its association constant for phosphate anion in water (pH=12.28, 0.19mM NaOH, at 25°C) is around 0.2×10^{6} M^{-1} via fluorescence titration. In addition, using Job Plot fluorescence experiments, we have addressed the stoichiometry of the supramolecular assembling (receptor **2** and phosphate **1**) in solution which showed evident 1:1 stoichiometry.

A systematic studies, in order to prove the fundamental importance of the co-operative role of the three arms of the receptor **2** and that the binding driving force was the halogen bonding, have been carried out synthesizing several other receptors with different functionalities.

Receptor **3** confirms the key role of the co-operative effect, the association constant for the system was several order of magnitude below **2**, 0.5×10^{3} M^{-1}.

The formation of receptor **5**, system without iodine atoms on the pyridinium moiety, highlights that the presence of halogen bonding donors are essential for the recognition and binding process, in fact the association constant for this receptor is still lower that **2**, 0.1×10^5 M^{-1}.

These results strongly suggest that the synthesized receptor **2** has very good affinity with phosphate anions in solution compared to the others reported in literature and, more important, these studies open a new concept in development anion receptors.

If fact, until now the main anion receptors were based on cation or hydrogen bonding interactions, while in this thesis we have shown how halogen bonding can be used as new interaction for binding an important anion moiety.

This statement is further verified by the displacement experiment between organic and inorganic phosphates with the receptor **2**. The association constant for the inorganic phosphate, PO_4^{3-}, is twice higher than the organic phosphate **1,** 0.4×10^6 M^{-1} and this opens the possibility to apply this receptor in a biological assay.

In conclusion, we synthesized the receptor for phosphate anion with a good affinity. It also demonstrated the potential of XB solutions in highly competitive, as an aqueous solution. In the future, we would like to know how the configuration of complex of receptor-phosphate anion via halogen bonding.

5. ACKNOWLEDGEMENT

Here I avail myself of this opportunity to express my heartfelt gratitude to all those who have helped me in the course of writing this thesis.

First and foremost, I would like to thank my dear supervisor, Prof. Giuseppe Resnati and co-supervisor, Prof. Pierangelo Metrangolo, without their patient and precious supervision, this thesis would never come to its present form.

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7. FIGURE INDEX