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Synthesis towards synthetic adenosine receptor

Rajni Gupta
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ABSTRACT

SYNTHESIS TOWARDS SYNTHETIC ADENOSINE RECEPTOR

by
Rajni Gupta

Adenosine is a biologically important molecule as it acts as local modulator with cytoprotective function in the body. Adenosine accumulates in the extracellular space in response to metabolic stress and cell damage, and elevations in extracellular adenosine level are found in conditions like myocardial ischemia, hypoxia, inflammation and trauma. Its abnormal concentration therefore could be a marker of many diseases. Therefore determining its concentration can help understand the physiological state of the body. However its selective detection by synthetic receptors is not possible till date.

Efforts have been made here to design molecular tweezer for adenosine. Multistep synthesis was done to synthesize two clamps, namely the π-π stacking clamp and the hydrogen bonding clamp for the proposed tweezer. These two clamps in coordination with Zn^{+2} ions intended to form a pre-organized tweezers molecule for more favorable complexation with adenosine.

SYNTHESIS TOWARDS SYNTHETIC ADENOSINE RECEPTOR

by
Rajni Gupta

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APPROVAL PAGE

SYNTHESIS TOWARDS SYNTHETIC ADENOSINE RECEPTOR

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Dedicated to my loving husband

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LIST OF SYMBOLS

DMF	Dimethylformamide
THF	Tetrahydrofuran
TLC	Thin layer Chromatography
NMR	Nuclear Magnetic Resonance
DMSO	Dimethyl sulfoxide
RT	Room Temperature
TBAF	Tetrabutylammonium fluoride
DIPEA	Diisopropylethylamine
TEA	Triethylamine
TMS	Trimethylsilane

CHAPTER 1

INTRODUCTION

1.1 Biological Importance of Adenosine

Adenosine is an endogenous nucleoside and is ubiquitous in mammalian cell types. Adenosine is related both structurally and metabolically to bioactive nucleotides like adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), cyclic adenosine monophosphate (cAMP) and structurally to the coenzymes NAD, FAD and coenzyme A (Figure 1.1). Adenosine and its related compounds are important in the regulation of many aspects of cellular metabolism.[1] Adenosine is produced in many cell types with extracellular concentration in submicromolar range. However, its levels are quite variable depending upon the tissues and degree of stress experienced. There are primarily two pathways for the biosynthesis of adenosine. The first is a cascading hydrolysis pathway, from 5'-ATP to 5'-ADP to 5'-AMP to adenosine both extracellularly and intracellularly. The second is an intracellular enzymatic conversion of S-adenosinesylhomocysteine to adenosine.[2]

Extracellular adenosine is now known to regulate a diverse range of physiological functions, to the extent that almost all mammalian organ systems are affected by adenosine. Adenosine accumulates in the extracellular space in response to metabolic stress, cell damage and elevations are found in conditions like ischaemia hypoxia inflammation and trauma.[3] Adenosine is proposed to function as a paracrine homeostasis modulator. First, extracellular adenosine represents a pre-eminent alarm molecule that report tissue injury to surrounding tissue. Second, extracellular adenosine generates a

range of tissue responses as organ protection thereby mediating homeostasis.[4] The central activities of adenosine are also implicated in the molecular mechanisms underlying sedation, hypnosis, schizophrenia, anxiety, pain, respiration and depression. Adenosine has been proposed to be an endogenous anticonvulsant agent, inhibiting glutamase release from excitory neurons and inhibiting neuronal firing. Also, adenosine is an antiarrythmic agent and is thought to improve the symptoms of Parkinson's disease.

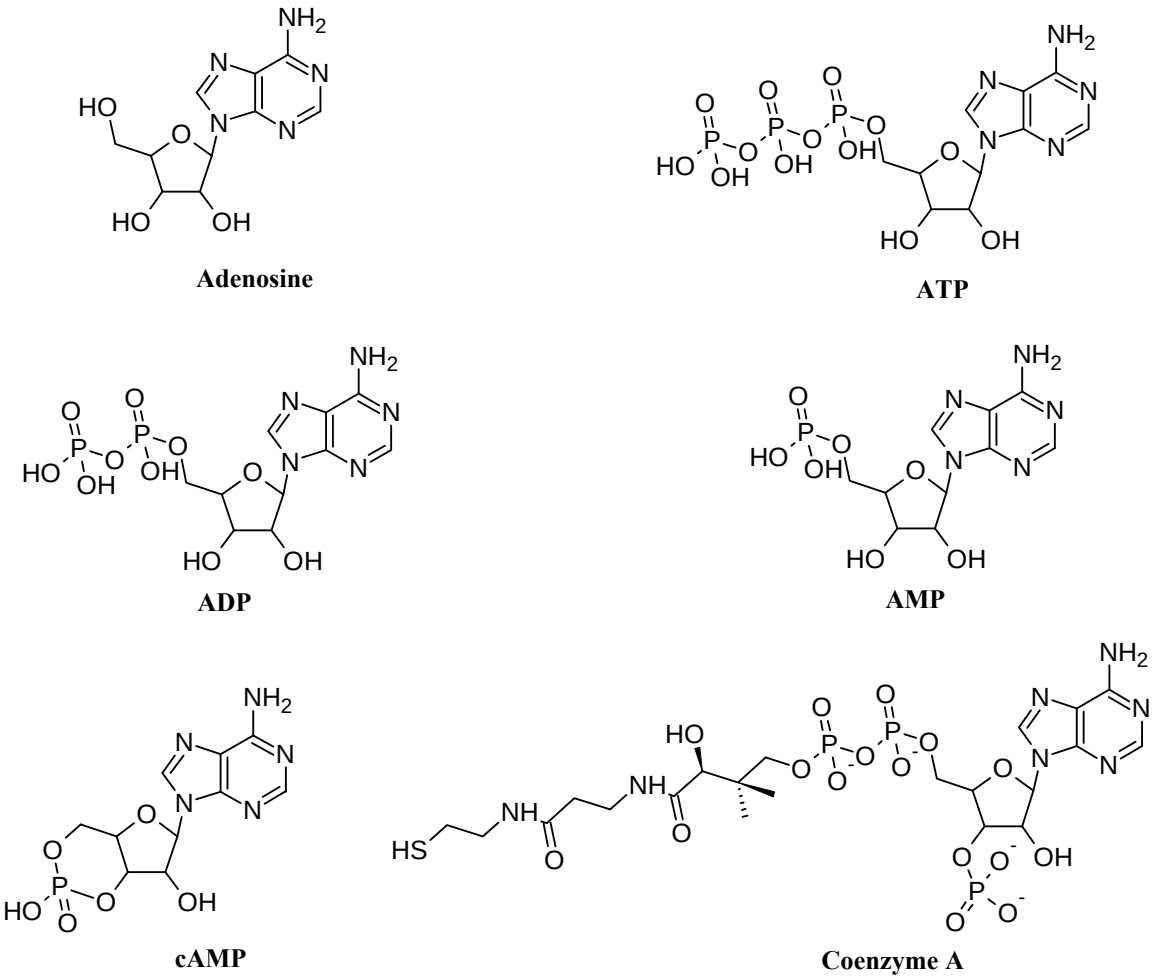


Figure 1.1 Structure of adenosine and related compounds.

The physiological responses to adenosine are complex and exerted through the activation of specific cell surface receptors. Four adenosine receptors A1, A_{2A}, A_{2B} and A_3 are known so far. These are seven transmembrane glycoprotein receptors (Figure 1.2) and belong to the super family of G protein coupled receptor (GPCR).

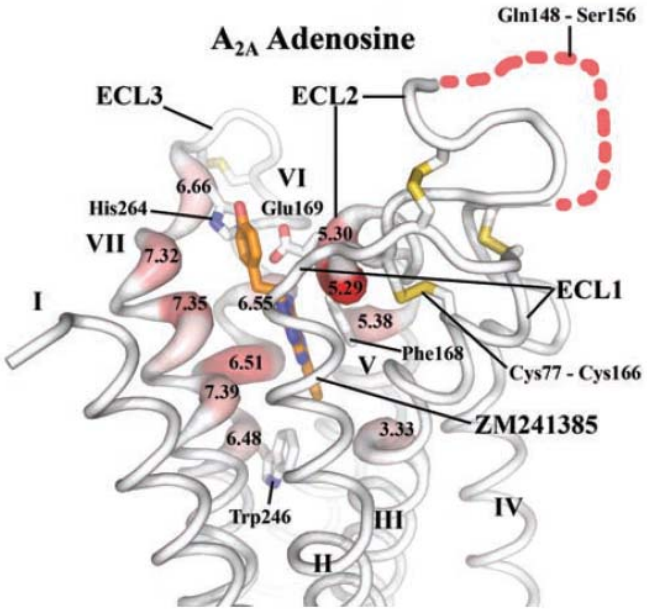
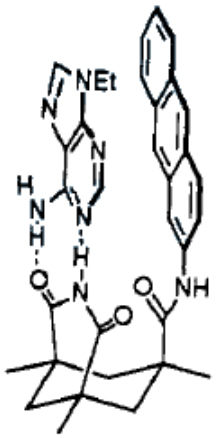


Figure 1.2 Structural motif of A_{2A} adenosine receptor.[5]

Activation of adenosine receptors by adenosine initiates signal transduction mechanisms and various physiological effects are produced depending on the receptor subtype type and the metabolic state of the tissue in our body. It is worth mentioning that these natural receptors can distinguish between adenosine and other similar molecules like adenosine monophosphate (AMP) and adenosine triphosphate (ATP).

If one could determine the concentration of adenosine, underlying cause for some of the diseases can be found out. Therefore it is important to develop receptors that can specifically detect adenosine.

Several attempts were made time to time to develop synthetic receptors for adenine and its derivatives. Rebek et al.[6] had developed a receptor (**1)** for adenine derivatives with an association constant of 11,000 M^{-1} in chloroform. This receptor utilized two hydrogen bonds and a π-stacking interaction to complex 9-ethyladenine. They also described the increased advantage of polarizability of the larger aromatic surface for the interaction of the receptor to the adenine derivatives.



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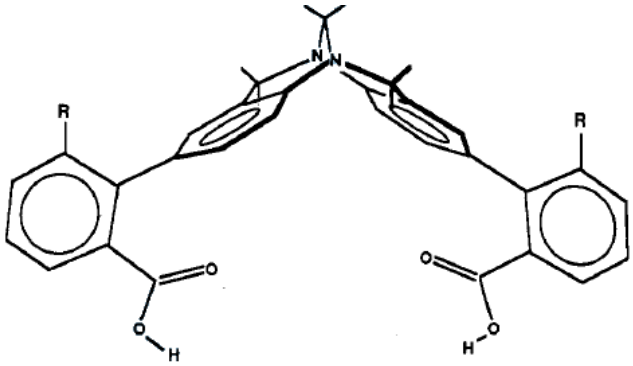

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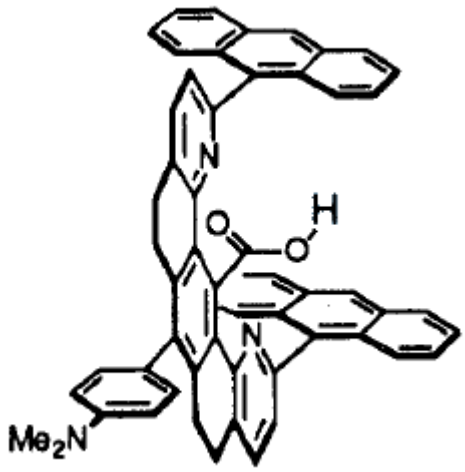
Wilcox et al.[7] reported the importance of orderly interrelationship of functional groups on the receptor (**2)** for binding adenine derivatives and utilized only hydrogen bonding for binding 9-ethyladenine to the receptor with an association constant of 4.5 ± 1.7 x 10^4 M^{-1} in chloroform. Polymer with strong binding interactions to adenine were prepared by copolymerization of cross-linking monomers such as ethylene glycol dimethacrylate and *N,N'*-1,3-phenylenebis (2-methyl-2-propenamide) with methacrylic acid with a comparable association constant.[8] All of the above reported receptors were selective for adenine, had low water solubility and cannot be used for adenosine binding.

Some synthetic receptors do show binding with adenosine or its derivatives but the association constants are quite small in comparison for adenine. Zimmerman[9] developed a molecular tweezer (**3)** that is more selective to purines in comparison to pyrimidines. This tweezer forms 1:1 complex **4** with 9-propyl adenine with an association constant of 25,000 M^{-1} in chloroform. It also demonstrated selectivity for nucleoside derivatives, the association constant for adenosine derivative being 15,500 M^{-1} in chloroform.



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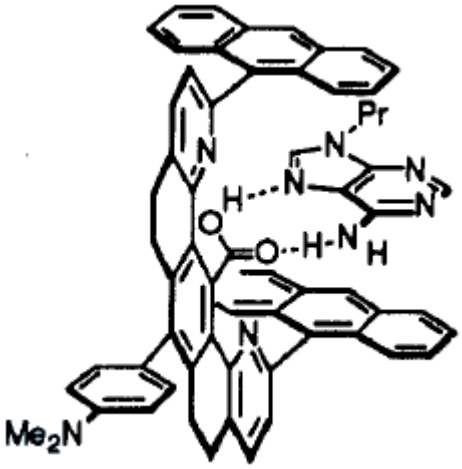

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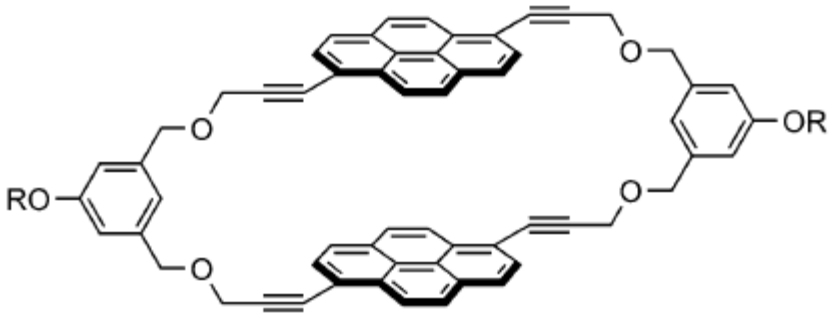
Molecular recognition ability of cyclophanes is also exploited by various groups. Inouye et al.[10] demonstrated how pyreneophanes could be functionalized to alter their selectivities. Amphiphilic poly(oxyethylene)-substituted pyrenophane **(5)** showed good affinity for monopyrenyl guests, regardless of the guest being cationic, anionic or neutral. Polycationic hexaammonium pyrenophane and bis (diazoniacrowm)-substituted showed affinity for anionic guests. The latter one preferred triphosphate nucleotides (1000 times) rather than di- or monophosphate nucleotides or nucleosides.



5

Adenosine aptamers have been developed, although they are not selective for adenosine over ATP. These aptamer sensors have been developed by conjugation of adenosine aptamers with colored nanoparticles, malachite green aptamer, and G-quadruplex DNAzyme.[11-12]

1.2 Molecular Tweezers

Non-covalent interactions are of fundamental importance to all biological processes. This has inspired the study of host-guest chemistry whose goals include the development of artificial receptors and the understanding of complexation phenomena.[13] Host-Guest chemistry involves two or more molecules, a "host" and a "guest", involved in non bonding interactions to form a supramolecular complex. According to Cram hosts and guests as organic molecules or ions whose binding sites converge and diverge, respectively, in forming a complex.[14] This host-guest chemistry encompasses the idea of molecular recognition and thus widely used in the synthesis of receptors/sensors for biological molecules, metal ions and many more. Most common host molecules include calixarenes, crown ethers, cyclodextrin, cryptophanes, cucurbitis and porphyrins. All

these have large rigid cavities in which they encapsulate small molecules. However, these macrocycles are difficult to synthesis and functionalize for selective binding.

Another class of host, called as molecular tweezers, a term coined by Whitlock,[15] was developed and popularized by Steven C. Zimmerman. These are noncyclic macromolecular complexes and contain pre-organized aromatic clefts, which are capable of binding guest's molecules using π-π stacking and hydrophobic interactions. Additional hydrogen bonding and metal co-ordination may be introduced to enhance specific recognition. More sophisticated molecular tweezers carry an additional functional group oriented toward the aromatic cleft so that π-stacking and hydrogen bonding can occur simultaneously. Typically, these hosts contain two aromatic chromophores covalently linked by a single spacer unit. A suitable spacer will allow π-surfaces of the molecular tweezers to converge on the two divergent π-faces of an aromatic guest in a sandwich complex held together by two π-stacking interactions.[16]

In organic solvents, the association of aromatic chromophores is well documented. A favorable interaction generally occurs only when one partner is a π-donor and the other is π-acceptor.[17] The electron donor-acceptor complexes formed between π-donors and π-acceptors are believed to be held together by electrostatic, dispersion and charge transfer forces. These forces are weak with association constant in common organic solvents generally ranging from 0.1 to 1000 M^{-1}, but usually less than 10 M^{-1}. In aqueous environment, enhancement of the association constant occurs by hydrophobic interactions.

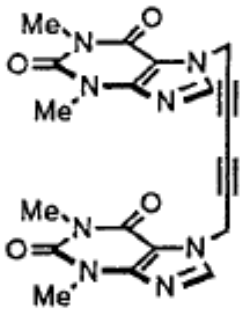
Interest in molecular tweezers relates to their potential for increasing the stability of electron donor acceptor complexes by forming π-sandwiched complex. Whether such a

complex forms and the second chromophore contributes significantly to the stability of the complex depends upon many factors, including the enthalpic strength of the second π-stacking interactions and the enthalpic and entropic loss necessary for adopting the required geometry. During the past 30-35 years, a considerable research effort has been placed toward obtaining molecular tweezer type of receptor molecules specific for an analyte of interest.

1.3 Design of Molecular Tweezers

Supramolecular chemistry has developed the construction of receptors whose shape and structural features allow for selective binding of various guests through non-covalent interactions.[18] Among the rich variety of receptors, molecular tweezers constitute a special recognition platform displaying intercalation of an aromatic substrate inside the cavity of the receptor. In designing molecular tweezers, both complexing chromophores and an appropriate spacer unit must be chosen. The property of the chromophores that is most likely to affect the complexation strength is its π-donor and acceptor strength. The shape and size of the chromophores are also important. However, the critical element of the molecular tweezers is the spacer unit.[16] The optimum spacer acts like a hinge such that the interplanar distance between two aromatic plates (formed by chromophores) forms a pocket suitable for binding planar guest molecules. Also, the spacer must be rigid enough to hold the molecular tweezers in a pre-organized conformation for more favorable complexation. Chen and Whitlock described how increasing rigidity of the spacer in molecular tweezer (**6)** increased the binding power over saturated analogue and thus prevented self association of the caffeine rings.[15] Further, they reported that caffeine-

caffeine distance in syn conformation is about 7 Å, which is perfect for insertion of aromatic system between the rings if the spacer can maintain a syn-orientation. Zimmerman had also described the increase in association constant between the more rigid tweezer **7** and the guest by further increasing the rigidity of the original molecular tweezer.[9]



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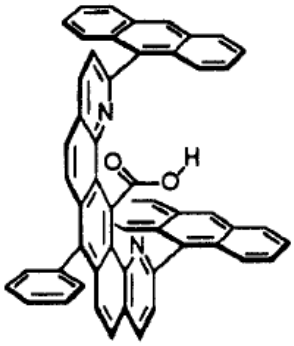

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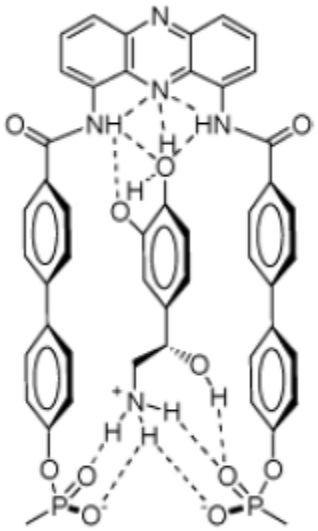
Finally, the molecular tweezers should be easy to synthesize. Ideally, the synthetic route should be sufficiently flexible to allow attachment of different groups for selective binding of guests. Schrader shaped a tweezer **(8)** for noradrenaline[19] which was accessible by a short and convergent synthesis, biphenylic side walls were linked to an aromatic diaminophenazine head group by amide coupling. Intramolecular hydrogen bond between both amide protons and the phenazine ring nitrogen fixed this tweezer at an aromatic distance of 7.0 Å and oriented the amide protons inwards for catechol recognition, phosphonate anions were ideal for recognition of ammonium alcohol moiety.



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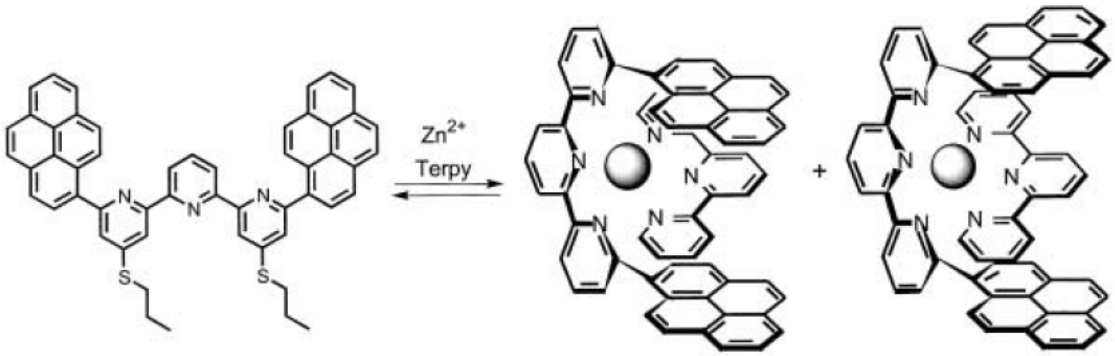
Recently, Lehn[20] et al. reported a terpyridine based molecular tweezer (**9)** which transforms into the desired compact conformation only when zinc is coordinated to the terpyridine moiety. In complex **10**, the two terminal α,α'-linked π-donor pyrene units are situated in a face to face arrangement suitable for the insertion of a flat aromatic π-acceptor between their parallel planes, thus yielding π-π stacking of three overlapping aromatic rings.



9 **10a** **10b**

In the present thesis, efforts have been made to develop a synthetic adenosine receptor. For this purpose, it is proposed to synthesize a molecular tweezer having terpyridine as spacer which is connected to anthracene chromophores via phenyl acetylene linkers. The Lehn tweezer described above formed the framework for the proposed tweezer structure. It will attain a binding conformation when Zn is coordinated in the linker site (Figure 1.3). Another terpyridine unit when sandwiched by the outer clamp can be functionalized desirably. The attachment of specific recognition motif to the tweezed terpyridine will result in another clamp that will be orthogonal to the first one. It was anticipated to adopt a U-shaped conformation, placing the two substituent at 6,6" position of the terpyridine in parallel planes, well adopted for donor/acceptor π-π interactions.

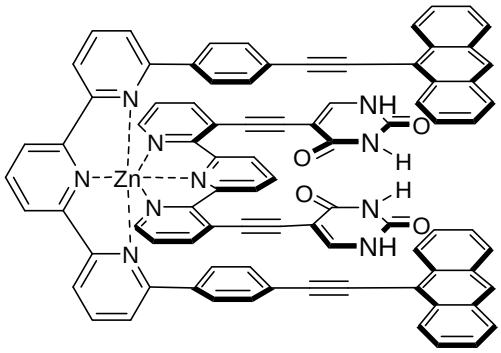
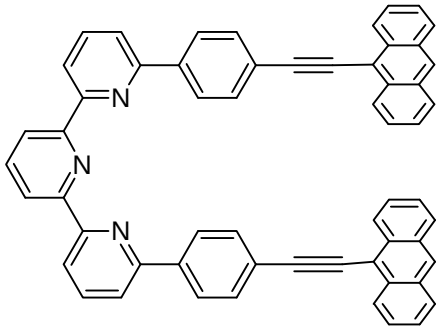


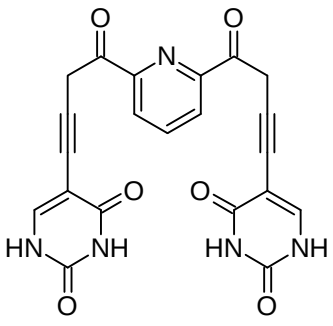
Figure 1.3 Proposed molecular tweezer for adenosine binding.

To make an aromatic cleft for adenosine, two uracil rings are proposed to be attached to a terpyridine via phenyl acetylene linkers. These uracil rings will serve as recognition motif for adenosine as well as will provide extra hydrogen bonding interactions. These extra hydrogen bonding interactions will help stabilizing the U-A-U

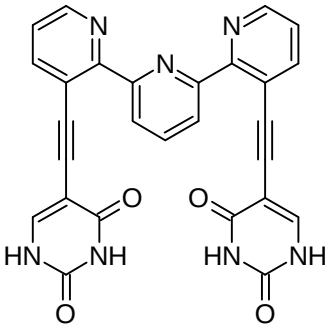
complex formed by the tweezer as they stabilize T-A-T complex in DNA triplex. This uracil-adenosine-uracil complex will have π-π stacking interactions with two anthracene groups. Thus, this molecule tweezer has both, a π-π stacking clamp and a hydrogen bonding clamp. These interactions will greatly stabilize the proposed complex.



Scaffold I



Scaffold IIA



Scaffold IIB

Figure 1.4 Structures of scaffold **I** and **II**.

For the proposed double molecular tweezer, two different molecular clamp synthesis were attempted. One was the π-π stacking clamp (scaffold I) and other being hydrogen bonding clamp (scaffold IIA and IIB) (Figure 1.4). These two clamps were proposed to attain the desired conformation in the presence of zinc ions.

The binding of adenosine to the proposed receptor will be tested by fluorescence experiments. The literature shows that the emission of terpyridine substituted fluorophores is quenched by the addition of metal cations such as zinc. However, adding a second ligand to zinc-terpyridine complex helps to restore the fluorescence emission. It is expected that adding adenosine to the double tweezer will quench the fluorescence again. This pre-organized assembly could therefore serve as adenosine responsive system. Scaffold **IIA** is a simplified structure of scaffold **IIB**. Although this simplification could lead to loss of binding affinity, the synthesis of this tweezer was expected to be much easier than the scaffold **IIB**

CHAPTER 2

SYNTHESIS OF π- π STACKING CLAMP

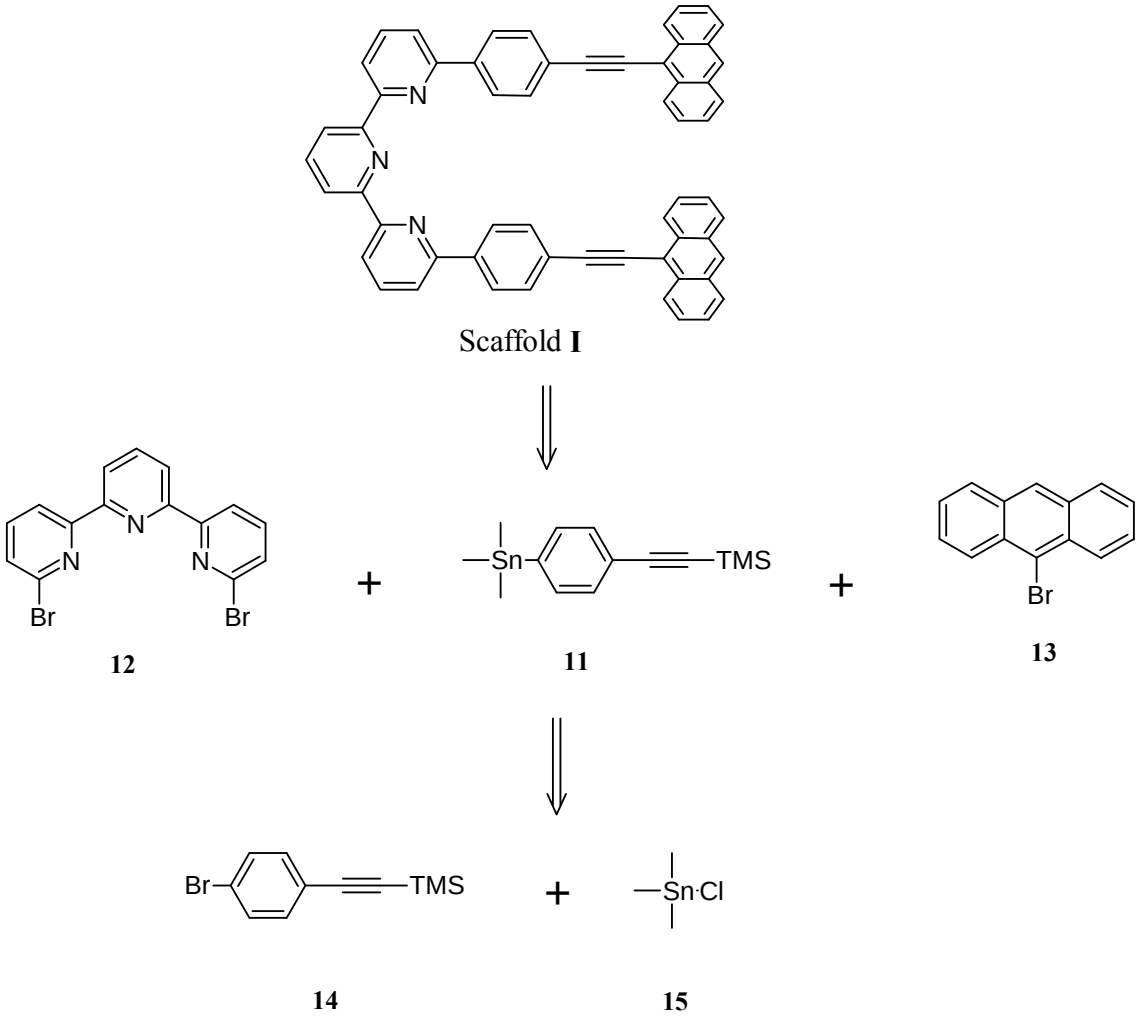
Strong attractive interactions between π-systems have been known for over half a century. They control such diverse phenomena as the vertical base-base interactions which stabilize the double helical structure of DNA, the packing of aromatic molecules in crystal, the tertiary structures of proteins, the conformational preferences and binding properties of polyaromatic macrocycle, complexation in many host-guest systems and porphyrin aggregations.[21] These π-π interactions are controlled by electrostatic as well as other energy contributions. π-π interactions are not due to an attractive electronic interaction between the two π-systems but occur when the attractive interactions between π-electrons and the σ-framework outweigh unfavorable contributions such as π-electron repulsion. Also there are geometric constraints imposed by the structure of the guest as well as the range of relative orientations available to the host and guest is usually limited.

Beside the required electronic complementarity for the binding to occur through stacking interactions, the shape of the tweezer is of paramount importance. As stated previously, the scaffold of a tweezer has to be pre-organized so as to 1) be rigid in order to prevent the tweezer from collapsing, 2) position the two units interacting with the substrate, the "arms", at an optimal distance of 7 Å from each other and 3) orient these arms in a syn conformation.

A scaffold based on 6,6"-difunctionalized 2,2': 6,2"-terpyridine indeed seems to be a potential candidate to fulfill these requirements when complexed to a metal ion. The terpyridine molecule contains three nitrogen atoms and can therefore act as a tridentate

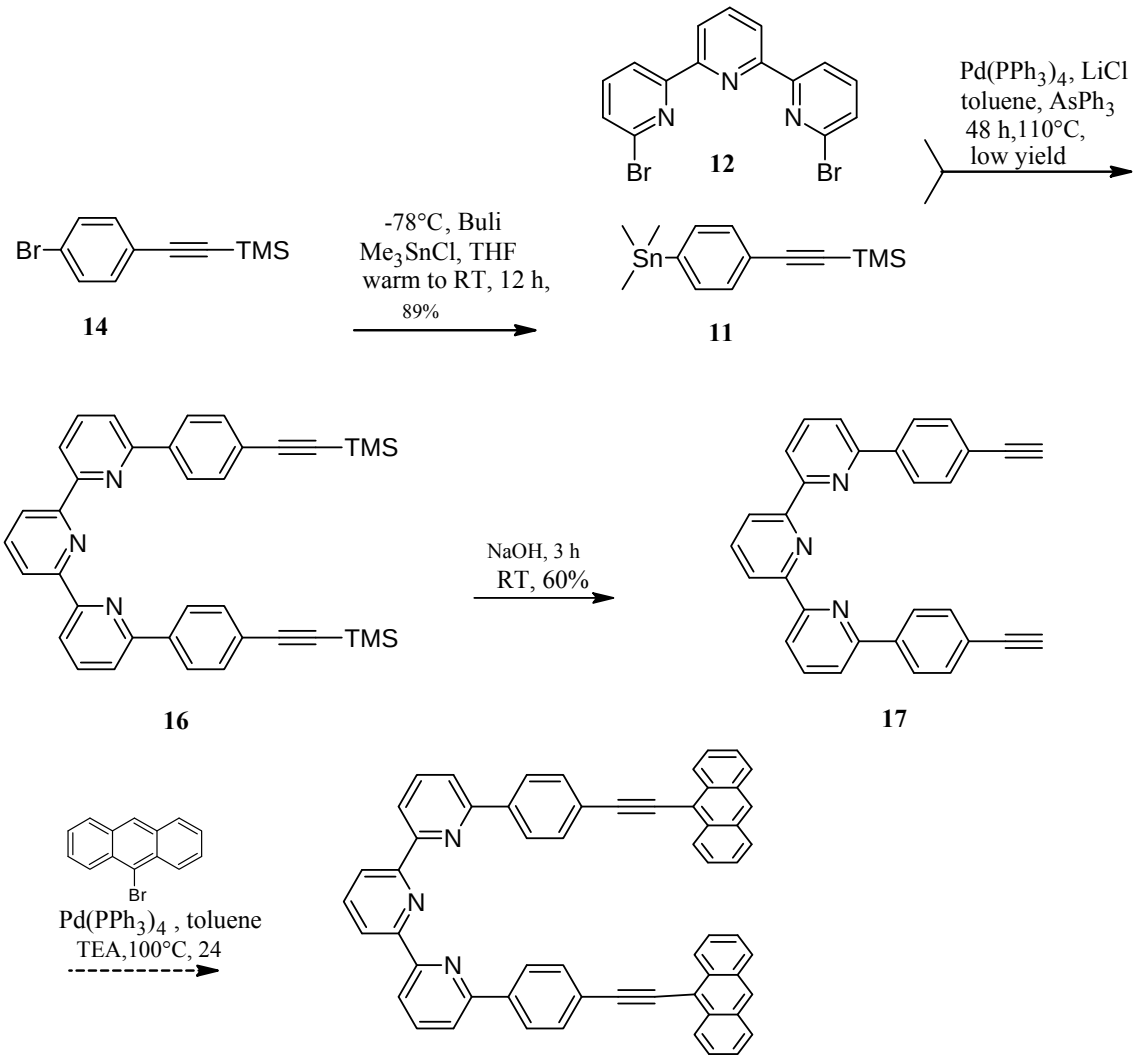
ligand and form stable complexes by chelating a broad variety of transition metal ions. Terpyridine forms distorted octahedral complexes showing different stabilities from kinetically inert (e.g., Ru(II) and Ni(II)) to labile (e.g. Zn(II)).[22] The terpyridine unit could be linked to various π-donor groups to have stacking interactions with the guest molecules. Various linkers can be used; the present study focuses on the use of phenyl acetylene linker so that the cavity formed is big enough to hold the second scaffold. Also, this scaffold has extended conjugated system and therefore is strongly fluorescent.

Based on the structure of scaffold **I**, a retrosynthetic scheme was constructed that led to the recognition of readily available related compounds as starting materials. As shown in Scheme 1, it was envisioned that **11** and **12** would be coupled by Stille coupling which is a versatile palladium catalyzed C-C bond forming reaction between stannanes and organic halides.[23] This coupling is performed under inert atmosphere as oxygen causes the oxidation of palladium catalyst and promotes the homo-coupling of stannyl compounds. The only drawback of the reaction is the toxicity of tin compounds and their low polarity. Stille coupling will be followed by deprotection. The alkyne obtained will be coupled with bromoanthracene (**13)** to provide scaffold **I** by Sonogashira coupling which is also a very known palladium-catalyzed reaction for coupling terminal alkynes with organic halides.[24] The segment **11** could be derived from **14** and **15** by Sonogashira coupling.



Scheme 1: Retrosynthetic analysis of scaffold **I**.

Scheme 2 outlines the attempted synthesis of scaffold **I**. Synthesis of **I** began by stannylation of commercially available (4-bromo-phenylethynyl)-trimethyl-silane (**14**) with trimethyltin chloride (**15**) at the bromide position in presence of n-BuLi to afford **11**. Although inseparable small amounts of byproducts were obtained, it was assumed that these byproducts would not interfere in the following reaction. Stannane **11** was coupled with **12** in presence of lithium chloride by Stille coupling reaction. Initially, ethyl acetate was used for extraction. It was later realized that the product from above reaction did not



Scaffold **I**

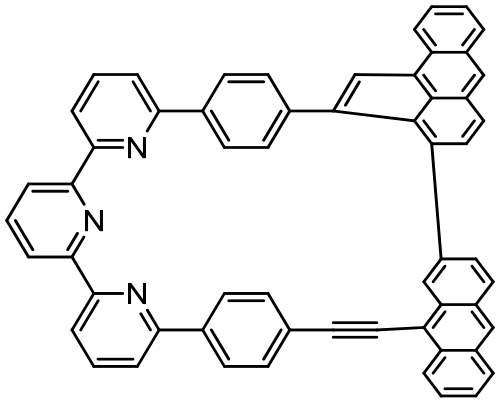
Scheme 2: Attempted synthesis of scaffold **I**.

have good solubility in ethyl acetate. Therefore, in the second attempt, methylene chloride was used instead to obtain crude product. The crude obtained from the above reaction was purified by flash chromatography and analyzed by NMR. However, NMR showed the presence of extra protons in the aromatic region. Probably, coupling did not occur at both the bromide positions of terpyridine. It is possible that hydrogen bromide formed during the reaction interfered with coupling at second bromide position.

Therefore, the product from the above reaction was put back again for the same reaction. Additionally, triphenylarsine was added to activate the palladium catalyst in the zero oxidation state. The reaction was completed in 24 h and again purified by column chromatography and confirmed by NMR analysis. This was then deprotected by using t-butyl ammonium fluoride (TBAF). Unfortunately, NMR spectra showed unexpected peaks in the aliphatic region which indicated the presence of TBAF as impurity. Then, methanolic sodium hydroxide was employed for the deprotection. The product was extracted into the organic the organic layer and was easily separated from sodium hydroxide. This crude product was purified by flash chromatography to give **17** in 60% yield.

Compound **17** reacted with 9-bromoanthracene (**13**) under Sonogashira reaction conditions[25] to yield Scaffold **I**. The reaction was originally carried out in deoxygenated toluene at 100°C employing TEA as a base, later it was also tried in THF at 60°C. Though purification was attempted using flash chromatography, TLC showed mixture of products. Consequently, preparative TLC was done to separate the mixture and two fractions were isolated. Both of them were highly fluorescent as expected. Surprisingly, the ESI Mass spectra of the neither of the two fractions matched the expected molecular ion peak, though one fraction (**I-a**) showed M-2 ion peak (Figure 2.1). Essentially, since Scaffold **I** had only aromatic protons, it was also difficult to identify it with ^{1}H NMR. Fluorometry experiments were done (Figure 2.2b & c) to study the effect of addition of zinc triflate to **I-a** as zinc is known to drive the chelation enhanced quenching of the several fluorescent system.[26] It was observed that addition of zinc triflate at 10^{-9} M conc. of the product quenched the fluorescence but at a higher concentration, results obtained

were different which could be due to self-association of the molecules in solution at higher concentration. However, when fluorometry experiments were performed with **16** (Figure 2.2a), fluorescence emission was increased upon the addition of zinc triflate. It is evident that an unknown reaction happened between **17** and **13**, although the desired product was not finally obtained. The structure shown below could be the expected structure of **I-a**, though it is not characterized yet.



I-a

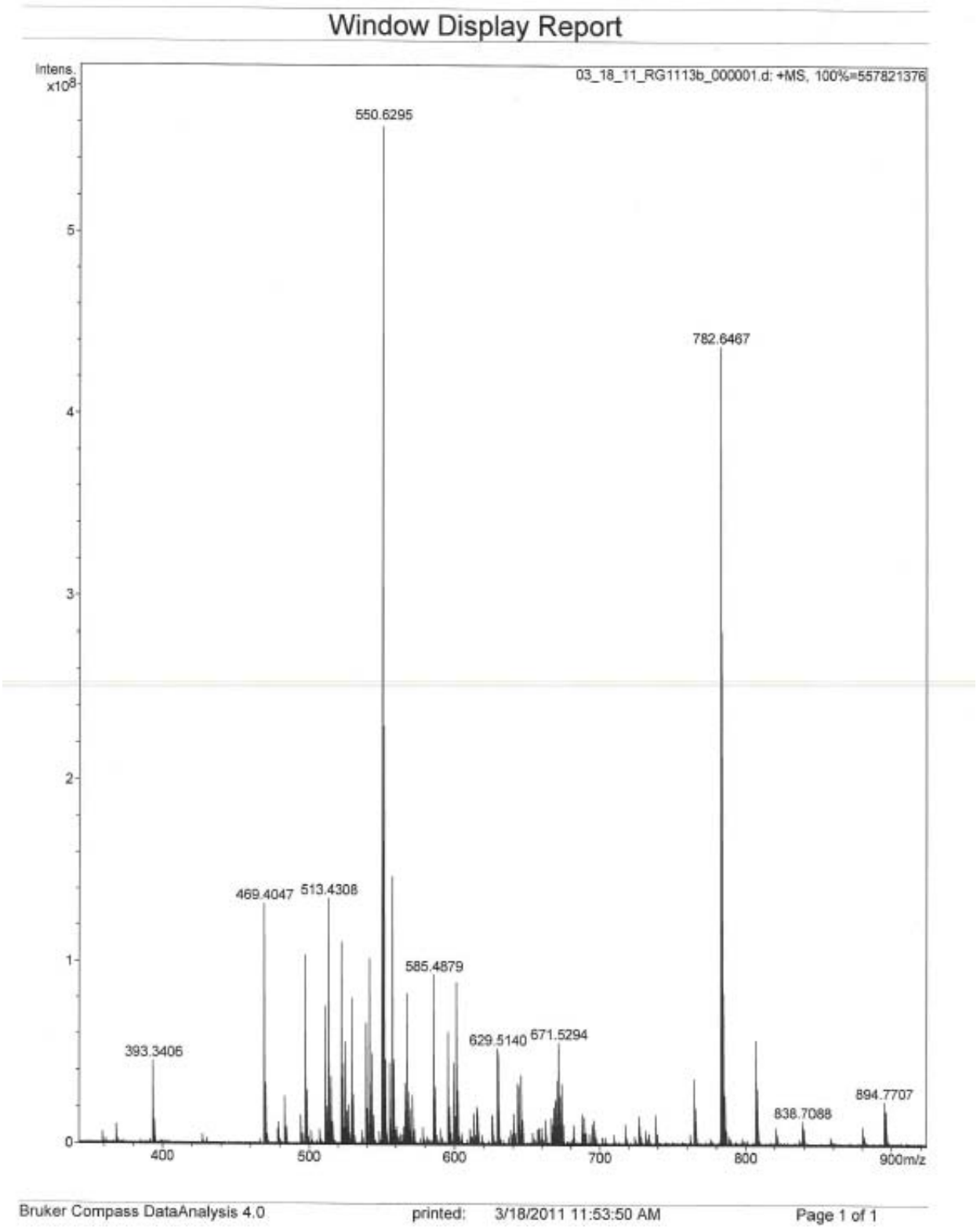


Figure 2.1 Mass spectrum of **I-a**.

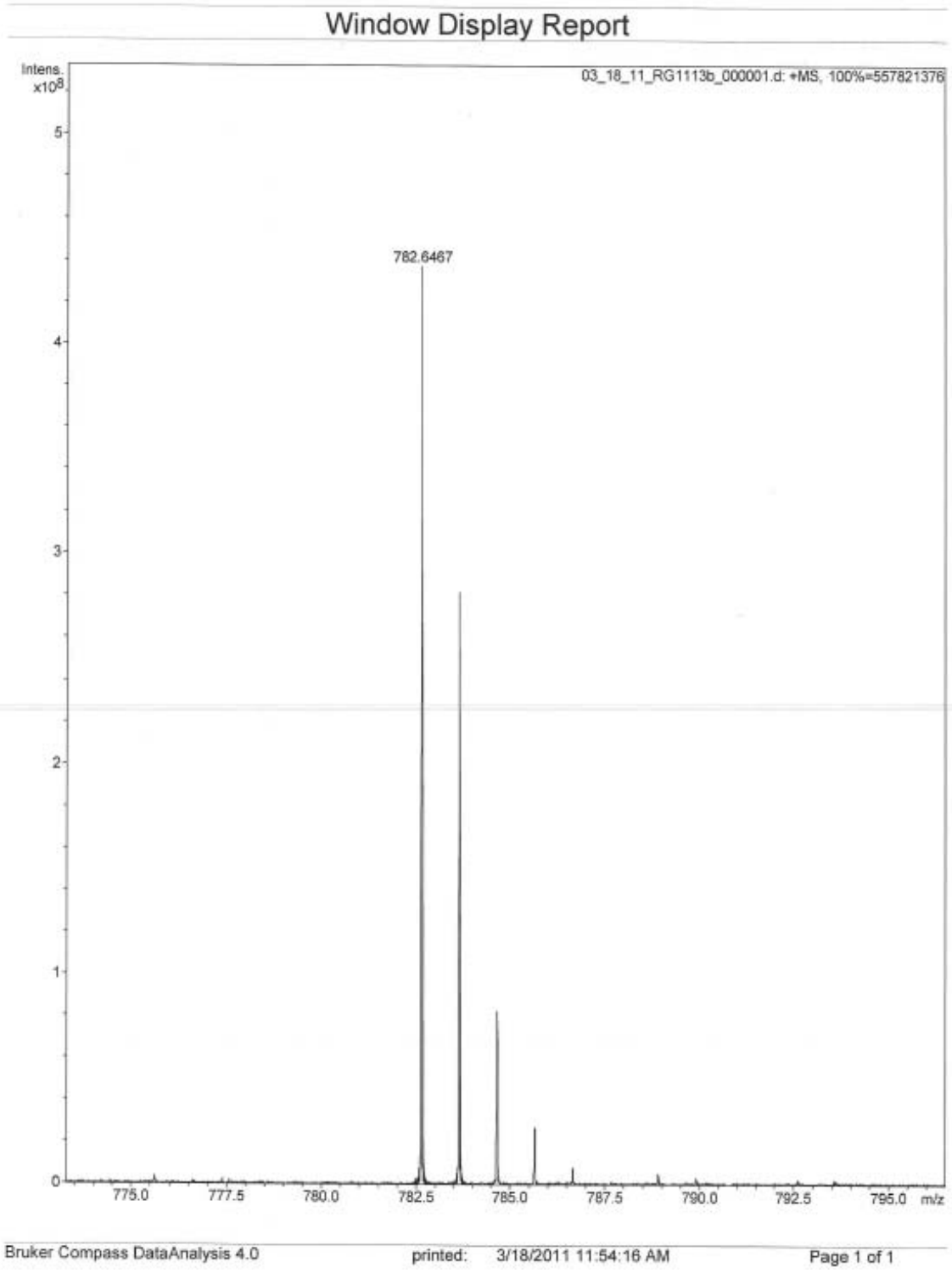


Figure 2.1 Mass spectrum of **I-a** (Continued).

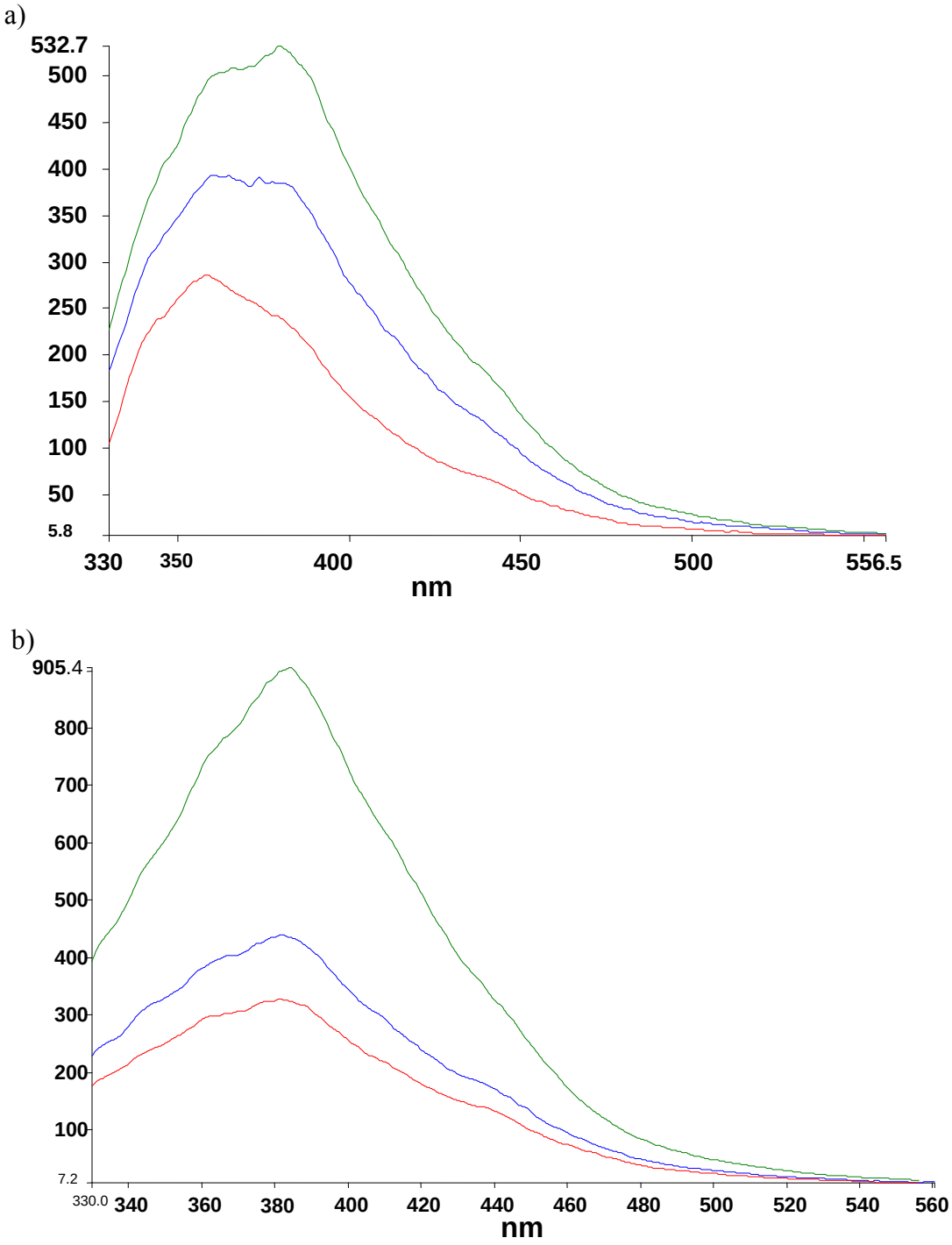


Figure 2.2 (a) Fluorescence spectra of **16** at 10^{-8} M concentration. Fluorophore **16** was excited at 300 nm. Red, **16**. Blue, **16** with 0.5 equivalents of Zn $(OTf)_2$. Green, **16** with 1.0 equivalents of $Zn(OTf)_2$. (b) Fluorescence spectra of **I-a** at 10^{-9} M conc. and 300 nm. Green, **I-a**. Blue, **I-a** with 0.5 equivalents of $Zn(OTf)_2$. Red, **I-a** with 1.0 equivalents of $Zn(OTf)_2$.

c)

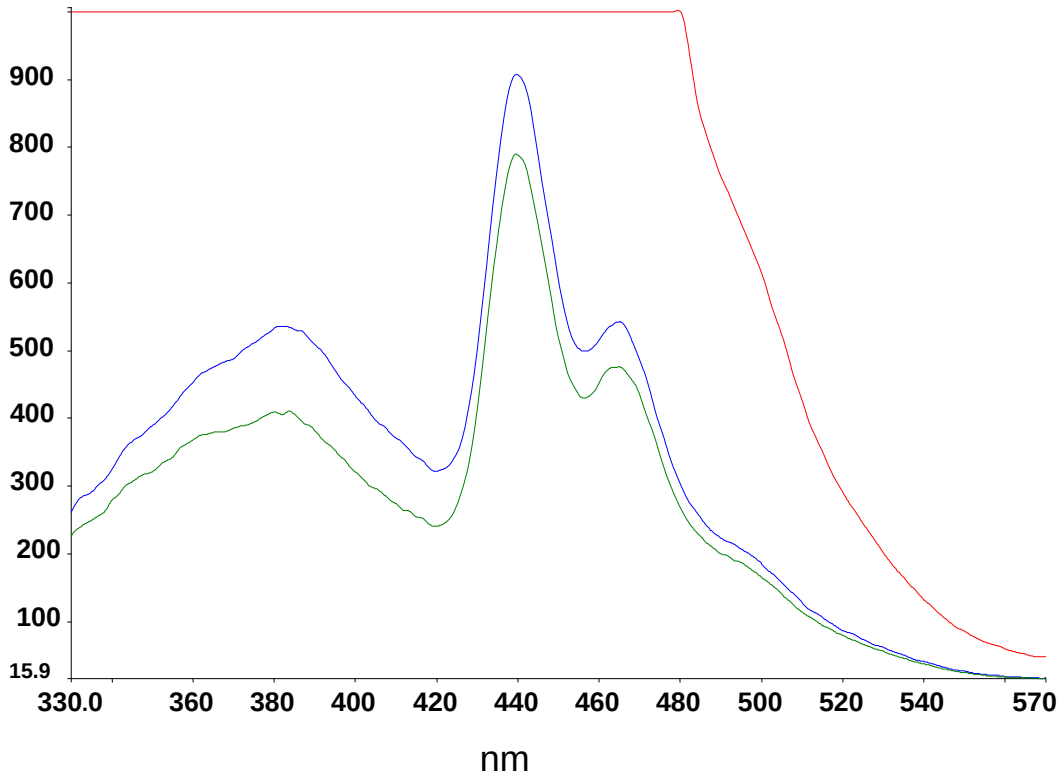


Figure 2.2 (c) Fluorescence spectra of **I-a** at 10^{-7} M conc. and 300 nm. Red, **I-a**. Blue,**I-a** with 0.5 equivalents of $Zn(OTf)_2$. Green, **I-a** with 1.0 equivalents of $Zn(OTf)_2$.

CHAPTER 3

SYNTHESIS OF HYDROGEN BONDING CLAMP IIA

Hydrogen bonding plays an important role in determining the three dimensional structure adopted by chemical and biological system as a consequence of their specificity and directionality. The double helical structure of DNA, for example, is due largely to hydrogen bonding between the base pairs which link one complementary strand to the other. The formation of one or multiple hydrogen bonds, especially in combination with other non-covalent forces, such as ionic or hydrophobic interactions, can lead to a dramatic change in the micro- and macroscopic properties of the resulting supramolecular assemblies.[27] In contrast to covalent bonds, which once formed are stable under normal conditions and can only be broken by providing sufficient energy, the formation of hydrogen bonds is reversible and their strength depends on the chemical environment, such as the solvent or temperature. Through a variation of the external parameters, this reversibility allows for the direct control of those physical properties of supramolecular assemblies which are determined by the hydrogen bonds.

Hydrogen bonding forces are cooperative in nature. Based on this principle, chemists have developed wide variety of chemically stable structures. Research groups of Lehn and others have extended this work to synthetic receptors and demonstrated these interactions have an enormous potential for the construction of chemical structures exhibiting a high degree of complexity. However, that can happen only when the synthetic molecules are encoded with the appropriate H-bonding information and

geometry.[28] Most frequently, derivatized heteroaromatic molecules are used as module since their flat shape and rigidity optimally ensures the formation of 2D assemblies.

The proposed scaffold for adenosine binding has two uracil units, the carbonyl oxygen of which can form hydrogen bonds with the guest, adenosine. This hydrogen bonding system resembles a DNA triplex and would make use of Watson-Crick and Hoogsteen base pairing.

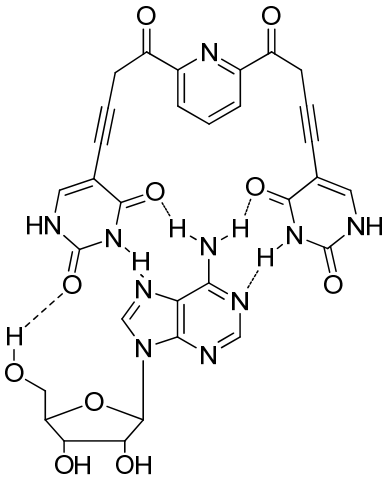
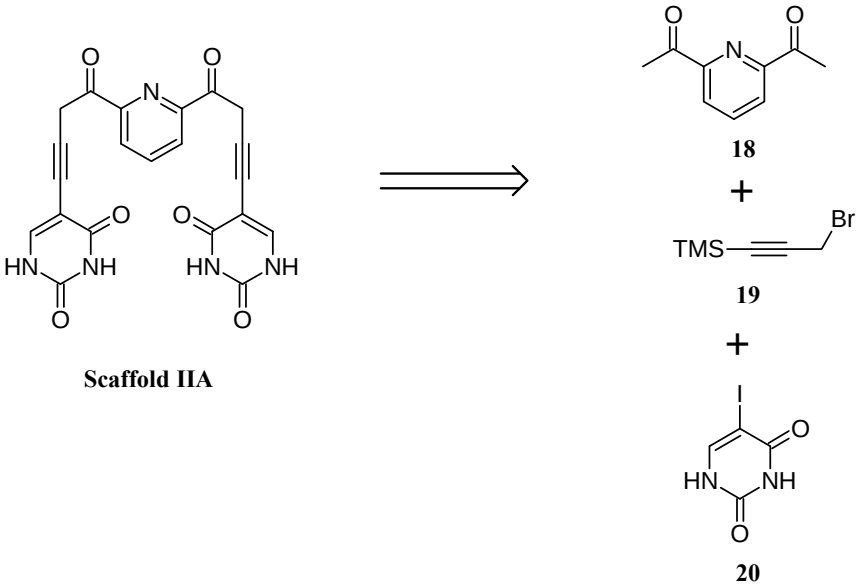
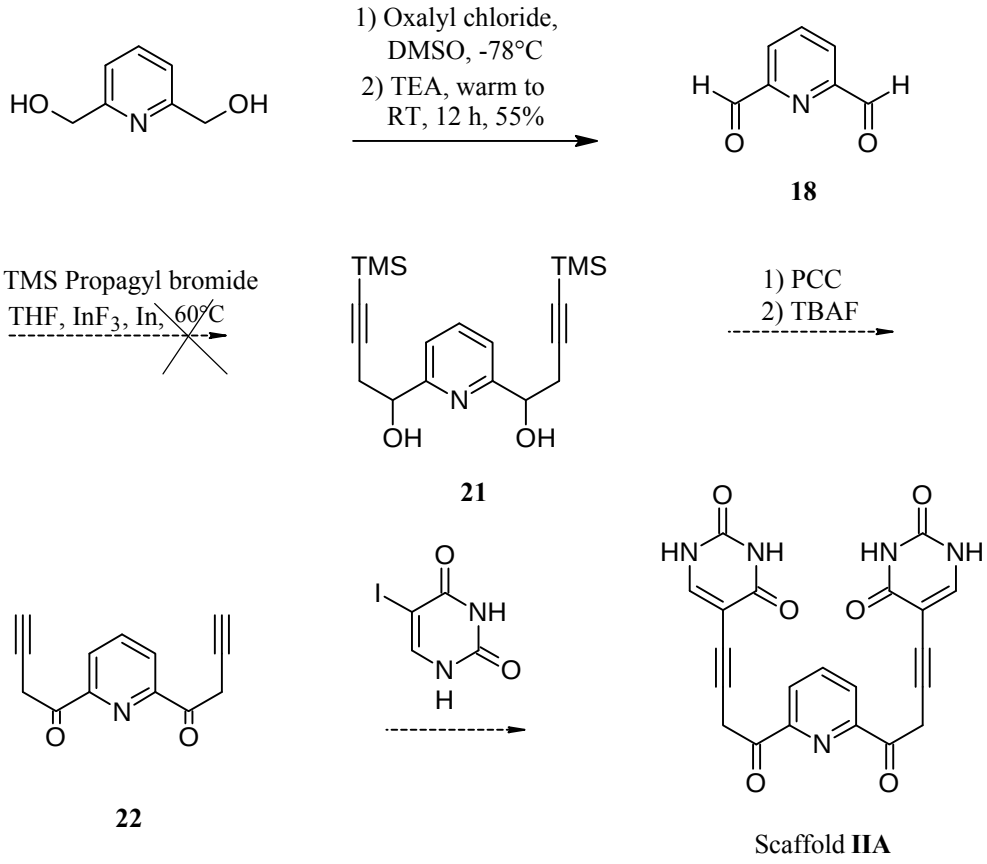


Figure 3.1 Hydrogen bonding interactions between scaffold **IIA** and adenosine.



Scheme 3: Retrosynthetic analysis of scaffold **IIA**.

Based on the retrosynthetic analysis of the scaffold **IIA**, it was envisioned that the scaffold could be made from molecules **18**, **19** and **20**. Initially, the synthesis of pyridinedicarbaldehyde **18** was attempted using dibromopryidine and n-butyllithium (1:2 equivalents, respectively) in DMF. However, NMR did not suggest the expected product. Then **18** was synthesized by Swern oxidation[29] of pyridinedimethanol by oxalyl chloride in DMSO and methylene chloride at -78°C. Later anhydrous triethylamine was added and the reaction flask was warmed to room temperature in 2 h. The reaction yielded the desired product **18** in low yield.



Scheme 4: Attempted synthesis of scaffold **IIA**.

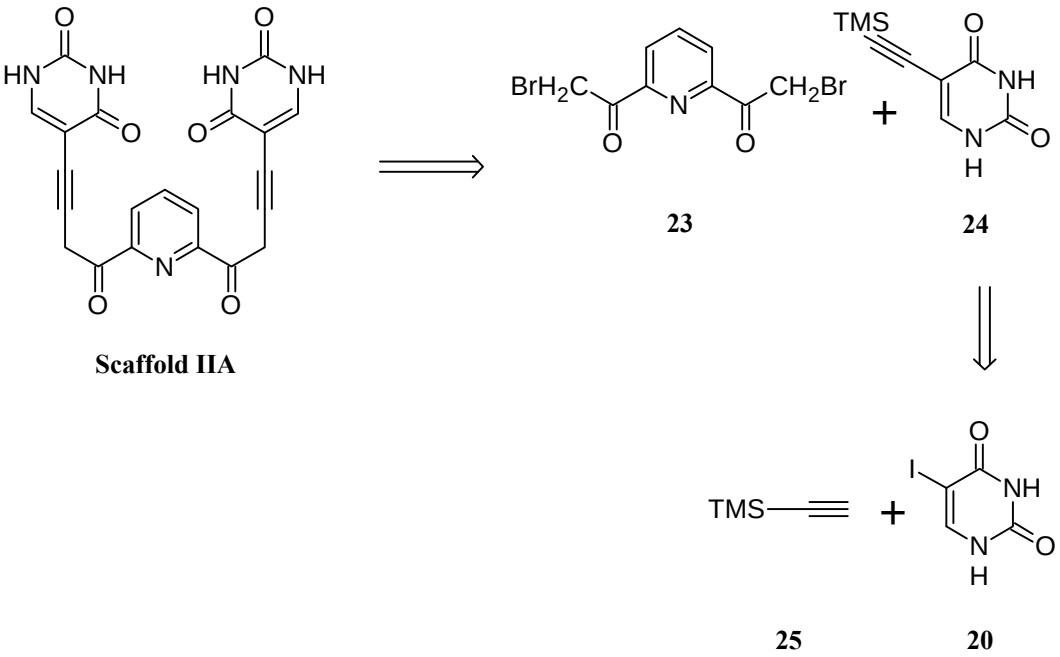
In the second attempt, the reaction flask temperature was slowly raised to room temperature over 12 h. The yield was significantly improved to 55%. Propargylation of

pyridinedicarbaldehyde could be achieved by using TMS propargyl bromide. In this kind of reaction, a mixture of two products, allenic alcohols and homopropargyl alcohols is often obtained. However, regioselectivity of the indium-mediated reaction of TMS propargyl bromide with carbonyl compounds towards the synthesis of either of the alcohols can be controlled by the judicious choice of the reagents and other reaction conditions. It was reported that excellent regioselectivities can be obtained when the reactions were carried out in THF in the presence of catalytic amount of indium trifluoride.[30] Moreover, homopropargylic alcohols were reported at high temperature with excellent selectivities when aldehyde: TMS propargyl bromide: Indium was taken in the ratio of 1:2:2. In contrast, when the reaction was done at room temperature, allenic alcohol was the major product. Efforts have been made to synthesis the homopropargyllic alcohol **21** by the above stated methods. Pyridinedicarbaldehyde, Indium and TMS propargyl bromide were taken in THF in 1:4:4 ratios and 0.2 equivalents of Indium trifluoride were added. The reaction was refluxed overnight under argon, filtered and then quenched with 0.1 M HCl solution. Ammonium acetate buffer was added to avoid the mixture becoming too acidic to protonate pyridine. However, NMR indicated that pyridine structure had changed during the reaction but the desired product was not observed. However, when the reaction was carried at room temperature conditions, allenic alcohol was obtained in 33 % yield.

Since the propargylation of **18** did not work, efforts were made to directly synthesize **22** by reaction of **18** with substituted thiazolium iodide[31] and TMS-propargyl bromide in the presence of base followed by deprotection. Aldehyde: Thizolium iodide: and NaOH were mixed in 1:2:2 ratios, followed by addition of TMS-propargyl bromide.

The reaction was carried out at room temperature and later purified by flash chromatography. However, only starting material **19** was obtained. It is likely that the intermediate formed between the aldehyde and thiazolium iodide was not reactive to TMS-propargyl bromide.

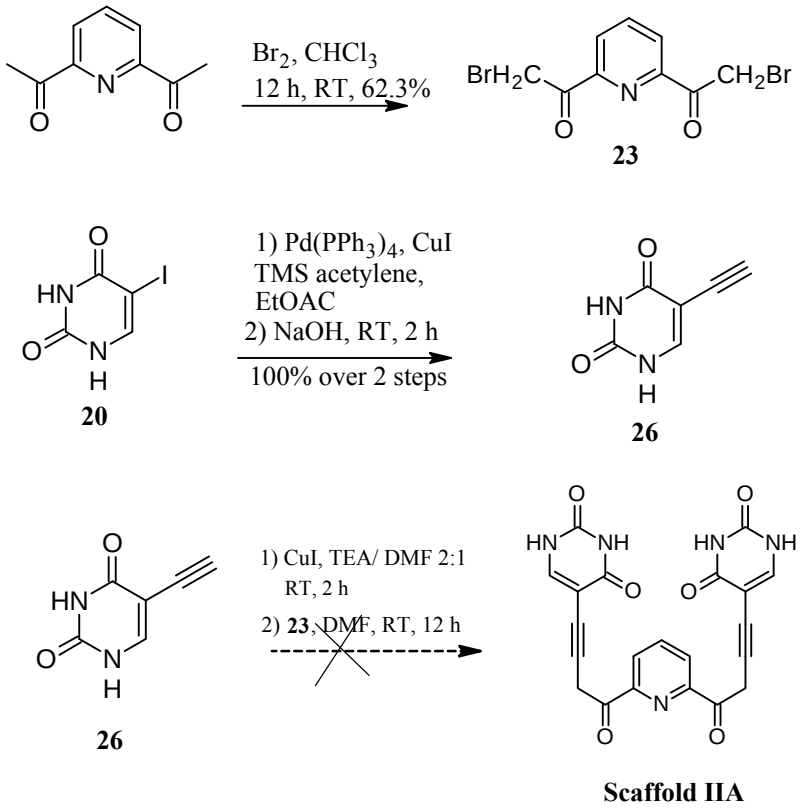
Analyzing scaffold in a different way, it was envisioned that scaffold could also be made from molecules **23** and **24**, **24** in turn can be obtained from **20** and **25**.



Scheme 5: Retrosynthetic Analysis of scaffold **IIA**.

Synthesis of **IIA** was started using commercially available 2,6-diacetylpyridine. Bromination of the above compound was carried out by treating it with two equivalents of bromine in chloroform. The reaction was run for 2 h under room temperature condition and later crystallized in acetone. But the NMR analysis of the product indicated bromination at only one position. In the second attempt, the reaction time was increased

to 48 h but the same monobrominated product was observed. It seems probable that hydrogen bromide generated during the course of the reaction formed an insoluble material with pyridine which stopped further reactions. Thus, the monobrominated compound was again treated with one equivalent of bromine at room temperature. NMR confirmed the formation of the desired bis(bromoacetyl) pyridine **23**.



Scheme 6: Attempted Synthesis of scaffold **IIA**.

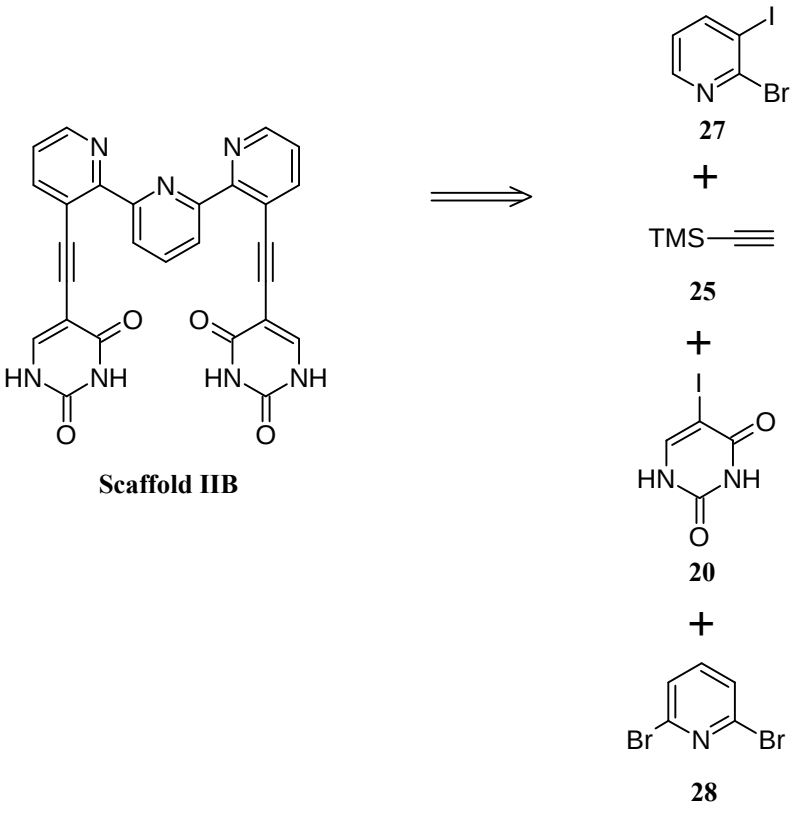
Commercially available 5-iodouracil and acetylene trimethylsilane were coupled together by palladium-catalyzed Sonogashira cross-coupling reaction under inert atmosphere to afford **24.** DMF and triethylamine were used in the ratio of 1:3 as reaction solvent as solubility of uracil compounds is generally a problem. **24** was then deprotected

using methanolic sodium hydroxide to give a terminal alkyne **26**. Since the alkyne proton is weakly acidic, usually strong bases are required for deprotonation. A convenient alternative is to convert them into metal acetylides, the acetylides anions thus obtained are very good nucleophile and thus can react with bromo compounds to give the substitution product. Therefore **26** was treated with cupric iodide to form copper acetylide. Since the acetylide are sensitive to air, they were not isolated and taken as such for further reaction with **23** under argon. Unlikely, the desired scaffold could not be obtained. To avoid the possibility of triethylamine consuming the dibromide starting material by forming quaternary amine and hence 5-iodouracil getting homocoupled, triethylamine was replaced by a bulkier base diisopropylethylamine (DIPEA). However, this change did not give the desired product either. Two distinct fractions were collected from the column. One fraction was quite similar to the ethynyl uracil (**26**) and the other fraction had a lot more aromatic protons than expected and could not be identified. The failure of the reaction could be caused by the poor solubility of the metal acetylide of **26**.

CHAPTER 4

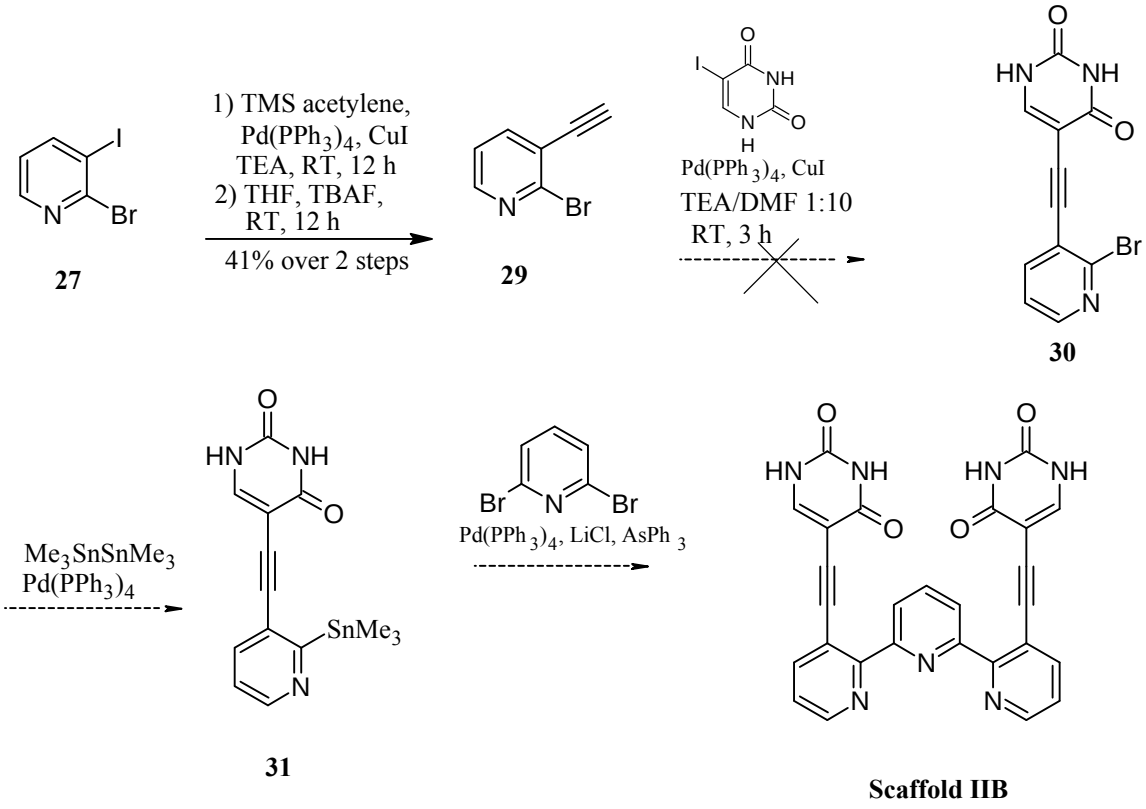
SYNTHESIS OF HYDROGEN BONDING CLAMP IIB

The scaffold described in Chapter 3 could not be made successfully, therefore another hydrogen bonding clamp was proposed for recognition of adenosine. This scaffold involves two uracil units, the carbonyl oxygen atoms of which are expected to form hydrogen bonds with the guest, adenosine. This hydrogen bonding system resembles a DNA triplex and makes use of Watson-Crick and Hoogsteen base pairing. In contrast to scaffold **IIA** which has a pyridine diketone spacer, this scaffold has terpyridine unit as spacer.



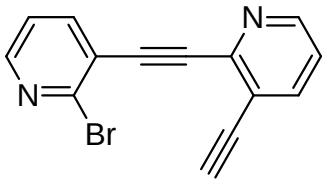
Scheme 7: Retrosynthetic analysis of scaffold **IIB**.

Retrosynthetic analysis of scaffold **IIB** revealed that it could be made from **20**, **25**, **27** and **28**. Among several approaches to obtain alkynes, attention has been devoted to palladium-catalyzed cross-coupling reactions, taking into account that the reactivity of halogens towards coupling reactions is known to be I > Br >> Cl and that the positions *ortho* or *para* to the nitrogen of pyridine are usually reactive enough with chlorine to give acceptable yields of the coupled products, whereas meta positions require bromine, iodine or triflates for sufficient reactivity.[32]

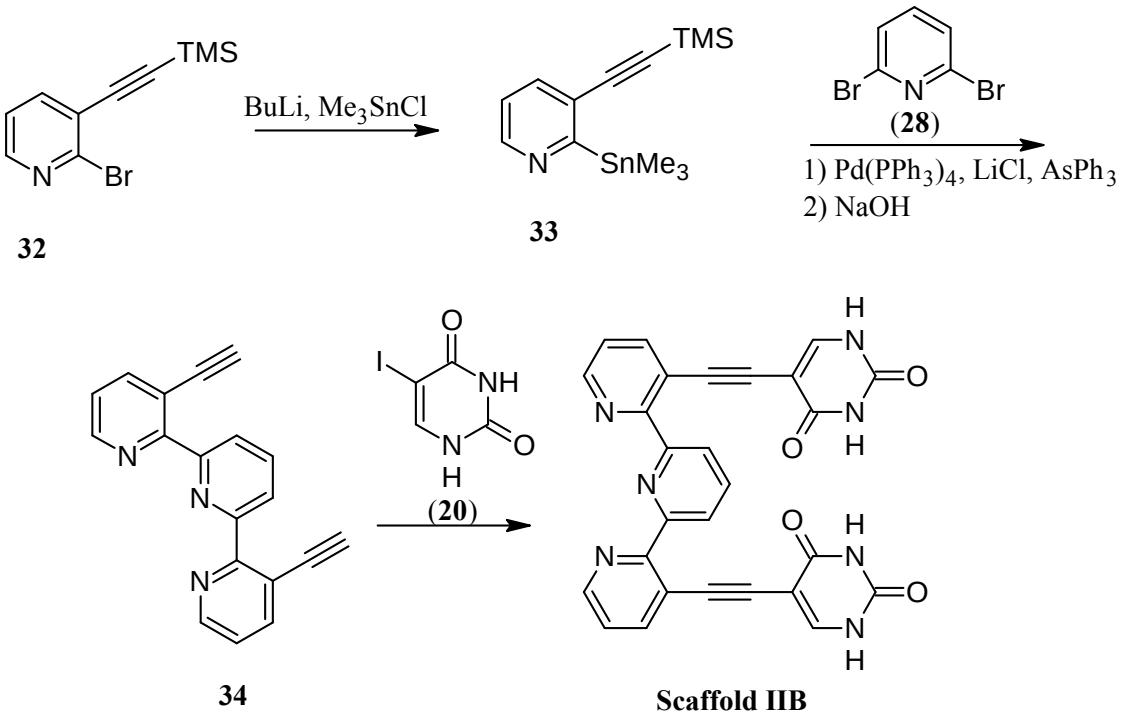


Scheme 8: Attempted Synthesis of scaffold **IIB**.

The Sonogashira reaction of 2-bromo-3-iodopyridine **IIg** with TMS-acetylene **IIf** was examined. The reaction was run overnight under argon atmosphere. Reaction gave the desired product in 50% yield after flash chromatography. TMS group was then removed in an overnight deprotection reaction using tetrabutylammonium fluoride reagent. The product was purified by column chromatography and gave 67% yield. The resulting 2-bromo-3-ethynylpyridine was sought to couple with 5-iodouracil under Sonogashira conditions. DMF was added in addition to the reaction mixture to increase the solubility of 5-iodouracil. The reaction yielded a strongly fluorescent product. However, NMR indicated the absence of uracil moiety in the product. It seemed probable that homo coupling of 2-bromo-3-ethynylpyridine occurred to afford the molecule below and uracil had not participated in the reaction.



Since **23** could not be made because of the presence of bromine, the future plan is to change the sequence of steps. First, **24** will be converted to stannane (**25)**, which will be further coupled with dibromopyridine by Stille coupling followed by deprotection to give **27** which will then be coupled with 5-iodouracil to give the scaffold **IIB**.



Scaffold IIB

Scheme 9: Future synthesis plan for scaffold **IIB**.

CHAPTER 5

CONCLUSION

The synthesis of π-π stacking clamp was completed though the final scaffold **I** structure could not be verified yet. As expected, the synthesized molecule was strongly fluorescent. The addition of zinc triflate to its solution showed quenching in fluorescence. However, the synthesis of hydrogen bonding clamp scaffold **IIA** could not be achieved. Different approaches were tried for its synthesis. The scaffold **IIA** having pyridyl-β-alkynyl-ketone group could be a typical structure that is difficult to synthesize. Synthesis of scaffold **IIB** is currently undergoing.

APPENDIX

EXPERIMENTAL PROCEDURE

The appendix describes the experimental details of the synthesis of the following:

Trimethyl-(4-trimethylstannanyl-phenylethynyl)-silane (11): n-BuLi (2 mL, 5 mmol) was added dropwise at -78°C to a flask containing a solution of (4-bromo-phenylethynyl)-silane (1.01g, 4 mmol) in THF (15mL) under argon. The reaction mixture was stirred for 15 min before adding dropwise trimethyltin chloride (5 mL, 5 mmol). The solution was allowed to warm up at room temperature, stirred overnight and then quenched with methanol. After evaporation of the solvent, the crude material was purified by means of flash chromatography (silica/2% ethyl acetate in hexanes). Purification afforded **11** (1.02g, 89%) as a clear liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.47 (s, 2H), 7.73 (s, 2H), 0.28 (m, 18H).

2,2′:6,6″-(6,6″-diphenylethynyl-trimethyl-silanyl)-terpyridine (16): To a degassed mixture of **11** (390.92 mg, 1.16 mmol), **12** (181.42mg, 0.464 mmol), lithium chloride (491.724mg, 11.6 mmol), tetrakistriphenylphosphine palladium (26.8 mg, 0.0232 mmol) and triphenylarsine (14.21 mg, 0.0464 mmol) was added toluene under argon. The reaction mixture was refluxed for 48 h. The reaction vessel was cooled to room temperature and mixture quenched with water and extracted with methylene chloride. The organic layer was washed with brine, dried over magnesium sulfate and the solvent removed in vacuo. Purification by flash chromatography (silica/hexanes/CH_2Cl_2/ethyl

acetate 80:15:5) afforded the desired product **16** in low yield as cream powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.69-7.61 (m, 17), 0.29 (s, 18H).

2,2′:6,6″-(6,6″-diphenylethynyl)-terpyridine (17): Methanolic NaOH (0.4 g, 10 mmol) was added to a solution of **16** (27 mg, 0.047 mmol) in THF. The reaction mixture was stirred for 3 h and then quenched with water. The product was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over magnesium sulfate and the solvent was removed on rotavap. Purification by flash chromatography (silica/hexanes/CH_2Cl_2 1:2) afforded the desired product **17** in 60 % yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.69-7.6 (m, 17), 3.2 (s, 2H).

2,6 -pyridinedicarbaldehyde (18): A solution of anhydrous DMSO (4.4g, 0.056 mol) in CH_2Cl_2 (6 mL) was added slowly dropwise to a stirred solution of oxalyl chloride (2.62 g, 20.63 mmol) in CH_2Cl_2 at -78°C (the temperature of the reaction was maintained at 78°C until the addition of TEA). After the addition was complete, the solution was stirred for 5 min, and then a solution of 2,6-pyridinedimethanol (1.00 g, 7.2 mmol) in anhydrous DMSO (4 mL) and CH_2Cl_2 (6 mL) was added dropwise over a period of 15 min. The solution was stirred for 20 min, during which time the formation of a white precipitate was noted. Anhydrous TEA (10 mL) was then slowly added dropwise to the reaction mixture. The reaction was let warm to room temperature, stirred overnight, and then poured into 50 mL of water. The layers were separated and the aqueous layer was extracted with 3x 35 mL of CH_2Cl_2. The combined organic extracts were washed with 3x30 mL of brine, dried over magnesium sulfate and filtered. The solvent was then

removed under vacuo, and the tan solid was purified by flash chromatography (silica/ hexanes:ethyl acetate 3:1). Purification afforded 1.07 g of **15** in 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.14 (s, 2H), 8.16 (d, 2H), 8.06 (t, 1H) ppm. The ^{1}H NMR matches the known ^{1}H NMR of 2,6-pyridinedicarbaldehyde.[29]

2,6–bis(bromoacetyl)pyridine (23) : 2,6 –diacetylpyridine (0.163 g, 1 mmol) was dissolved in $CHCl_3$ (10 mL), to it was added bromine (107 µL, 2 mmol) and stirred overnight at room temperature. The reaction mixture was decanted to another flask and the solvent was evaporated on rotavap. Hexanes was added which partially dissolved the crude and then acetone was added at 40°C to make it completely soluble. It was left for crystallization. The crystallized product showed bromination at only one of the two positions. To it was again added bromine (56 µL, 1 mmol) and the same procedure was followed for obtaining crystals of **19** in 62.3 % yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.2(m, 2H), 8.6(m, 1H), 4.8 (m, 4H).

5-ethynyluracil (26): 5-iodouracil (2 g, 8.4 mmol), tetrakistriphenylphosphine palladium (350 mg, 0.303 mmol), copper iodide (130 mg, 0.682 mmol) were degassed and suspended in ethyl acetate (40 mL). To it was added TMS acetylene (2 g, 8.4 mmol) under argon. The reaction mixture was stirred at room temperature for 3.5 h and filtered. The solid was transferred to 50 mL of 1M NaOH and stirred for 2 h and filterd. The filterate was then placed in an ice bath and acetic acid was added until pH reached 4.0. It was filtered and the collected solid was dried in air. The reaction afforded **26** in 100 % yield. ^{1}H NMR (300 MHz, DMSO): δ 11.37 (br., 2H), 7.82 (s, 1H), 4.05 (s, 1H).

2-bromo-3-ethynylpyridine (29): To a degassed suspension of 2-bromo-3-iodopyridine (283 mg, 1.0 mmol), tetrakistriphenylphosphine palladium (35 mg, 0.03 mmol), copper iodide (13 mg, 0.07 mmol) in TEA (5 mL) and ethyl acetate (15 mL), was added TMS acetylene (98.2 mg, 1.0 mmol). The reaction mixture was stirred overnight under argon at room temperature and filtered. The solvent was evaporated on rotavap and the crude was purified by flash chromatography (silica/hexanes: ethyl acetate 9:1) to afford **21**. **21** was then deprotected by dissolving in THF and adding tetrabutylammonium fluoride (315.51 mg, 0.46 mmol) and stirring the reaction mixture overnight. Solvent was evaporated and the crude was purified by flash chromatography (silica/hexanes: ethyl acetate 9:1) to afford **29** in 41.12% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42 (m, IH), 7.86 (m, 1H), 7.34 (m, 1H), 3.6 (s, 1H).

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