Mild and Convenient Methods to Prepare *N*-Alkyl Tacrines

Jimit Haresh Mehta

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Dr. Paul R. Carlier, Chair
Dr. David G. I. Kingston
Dr. Harry C. Dorn

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ABSTRACT

Alzheimer's Disease (AD) is an irreversible, age-related neurodegenerative disorder which causes cognitive impairment and a wide variety of neuropsychiatric and behavioral disturbances. Acetylcholinesterase inhibitors (AChEI) are the mainstay for the treatment of AD. Acetylcholinesterase (AChE) catalyzes the hydrolysis of acylcholinesters with a relative specificity for acetylcholine (ACh). Observation of a deficiency of cholinergic neurotransmission in AD led to the development of AChEI as the first approved treatment for dementia symptoms. Tacrine (9-amino-1,2,3,4-tetrahydroacridine) is a reversible inhibitor of AChE. It was the first drug approved by the FDA for the treatment of cognitive symptoms of AD.

Tacrine is now rarely prescribed as a drug for the treatment of AD due to its high hepatotoxicity in almost 50% of the patients. However, tacrine derivatives have considerable potential for the palliative treatment of AD. Synthesis of various bivalent tacrines led to the improvement in inhibitory potency and selectivity towards inhibition of AChE. Heptylene-linked bis-tacrine has especially shown immense promise to be an ideal AChEI. Thus dimerization of a lead compound seemed to be an ideal strategy where the compound can bind to both catalytic anionic site (CAS) and peripheral anionic site (PAS) on the AChE enzyme.

However synthesis of *N*-alkyl derivatives of expanded tacrines like 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline by the standard S_NAr methods was unsuccessful and thus alternatives needed to be developed to synthesize *N*-alkylated and bivalent 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline. Upon exploring the alternatives, *N*-arylation by Pd-catalysis seemed to be the most mild and convenient alternative over the standard S_NAr procedures.

Table of Contents

List of Figures

List of Schemes

List of Tables

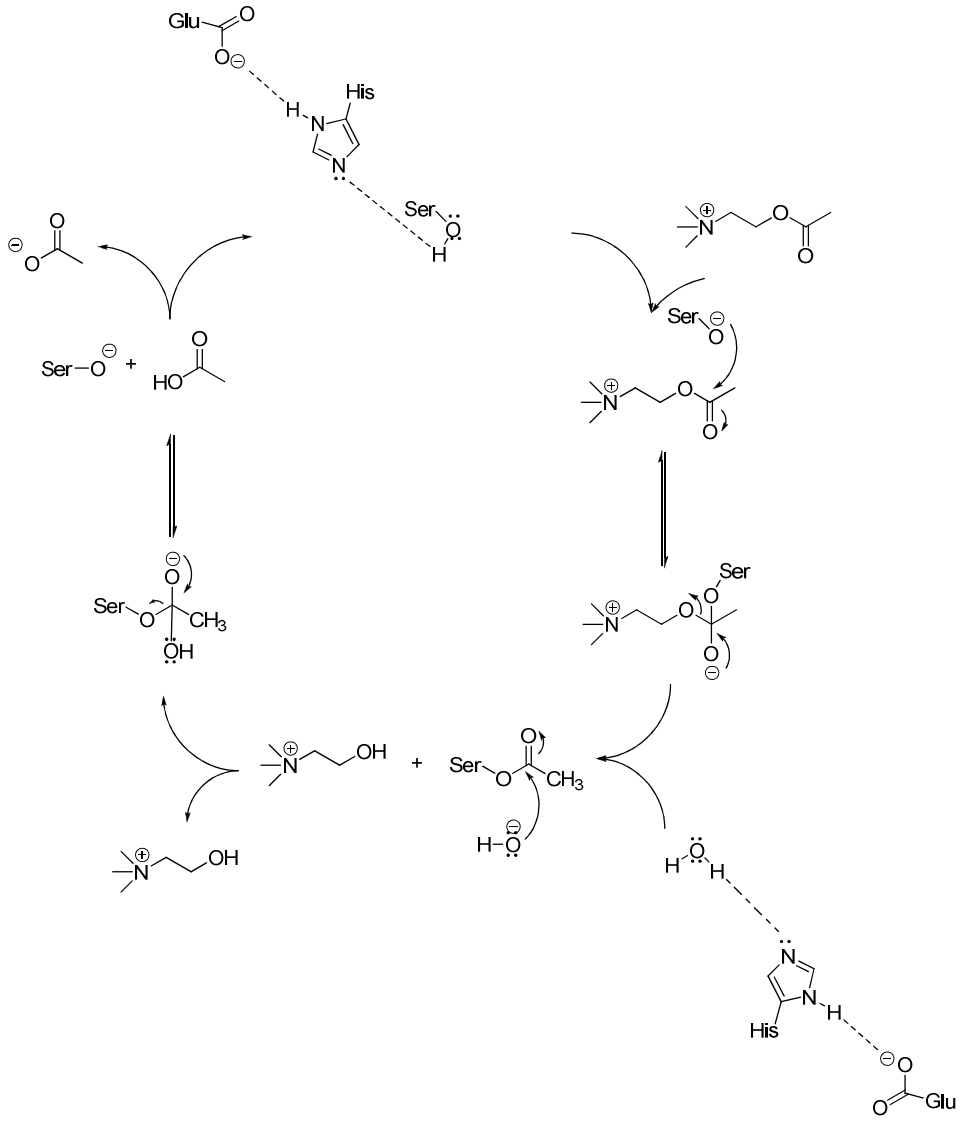
List of Abbreviations

AChE	Acetylcholinesterase
ACh	Acetylcholine
BChE	Butyrylcholinesterase
AD	Alzheimer's disease
CAS	Catalytic anionic site
PAS	Peripheral anionic site
Tc	*Torpedo californica*
ChE	Cholinesterase
AChEI	Acetylcholinesterase inhibitor
NSAIDs	Nonsteroidal anti-inflammatory drugs
CoA	Coenzyme A
OP	Organophosphates
THA	Tacrine

Chapter 1. 9-Amino-1,2,3,4-tetrahydroacridine and its analog use as AChE inhibitors

1.1 Structure and mechanism of catalysis of acetylcholinesterase

The function of acetylcholinesterase (AChE) in the central and peripheral nervous systems, along with the acetylcholine (ACh) receptor, is in the transmission of action potentials across nerve-nerve and neuromuscular synapses. It is an enzyme of the serine hydrolase family, whose physiological task is the hydrolytic degradation of the neurotransmitter acetylcholine (ACh) into choline and an acetate.[1] Acetylcholinesterase has been often characterized as a perfect enzyme because its catalytic properties have been tuned to the highest possible limit.[2] It is one of the most catalytically efficient enzymes in nature,[3] with an exceptionally high turnover number of $>10^4$ s^{-1}.[4] The normal function of AChE is very critical and consists a wide range of biological processes, importantly, critical nervous system, cardiac and respiratory functions. [2]



Scheme 1.1 Mechanism of hydrolysis of acetylcholine by AChE[2]

The mechanism by which AChE hydrolyzes ACh is explained in Scheme 1.1. Initially the serine residue is activated by an activated histidine group. This residue then attacks the carboxyl ester group which leads to an unstable tetrahedral intermediate, which immediately collapses to release choline and give an acyl-enzyme. The acyl-enzyme then undergoes a nucleophilic attack by a water molecule present near the active site, activated again by a histidine group. This leads

again to an unstable tetrahedral intermediate which collapses to give acetic acid, freeing the enzyme.[2]

The first solution of a three dimensional X-ray structure of AChE was derived from the electric fish *Torpedo californica* in 1991 by Sussman J. L. et al.[5] Subsequently other researchers solved the structure of other AChE from insect to mammalian sources,[6] as well as that of butyrylcholinesterase (BChE).[7] These studies enhanced the understanding of the AChE structural elements that provide its underlying specificity and catalytic power. It was observed and reported that surprisingly, for an enzyme which is this rapid and has a high turnover number, the active site is located at a bottom of a very deep narrow gorge. This gorge is labeled as the active site gorge.[8]

The Figure 1.1 is a schematic diagram of AChE. The diagram clearly reveals a deep narrow gorge which leads into the catalytic site. The catalytic site consists of W84 and F330, where as many as 14 conserved aromatic residues are lined up. The rings which make up ~70% of the gorge surface are called the catalytic anionic site (CAS). Figure 1.1 shows the catalytic and the anionic sites in the CAS. The gorge opening consists of Y70, Y121 and W279 among other residues and is called the peripheral anionic site (PAS).[9]

The principal element of the "anionic" site in the CAS was previous assumed to be a cluster of negatively charged residues.[10] But upon its structural elucidation, it was discovered that the indole ring of Trp84 is responsible for it. This Trp84 binds with the quaternary group of the choline moiety of the ACh yielding a cation-π interaction. Similarly, the principle element of the "anionic" site in the PAS is the indole of Trp279. Various bisquaternary and peripheral site ligands interact with this moiety,[8] and will be further described below in Section 1.7

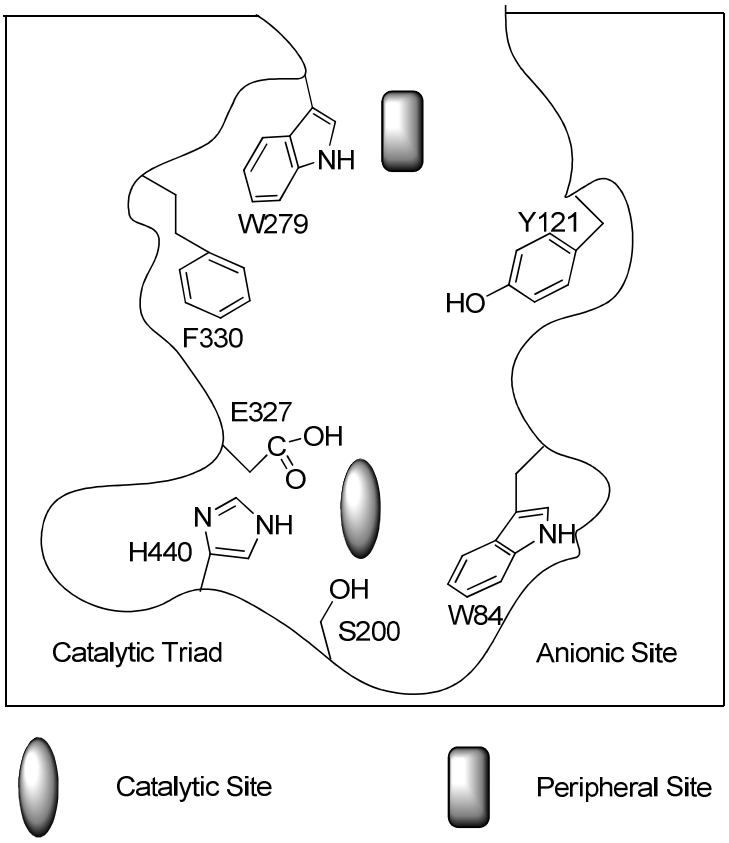


Figure 1.1 Schematic cross-section of the catalytic anionic site and peripheral anionic site present in AChE. Schematic based on graphic in reference [11].

1.2 Role and function of the structure of AChE

Before developing an inhibitor for the enzyme, the role and function of the structure of AChE should be thoroughly studied and understood. So the first question in understanding its role is why is the active site of the enzyme so deeply buried inside? It is very unusual for such an active enzyme to have its active site this deeply buried inside a gorge.[5]

The role of the gorge can be understood a little by two remarkable structural features it possesses. The walls of the gorge are predominantly lined with aromatic rings. Thus the quaternary ammonium ions do not "bind" to the gorge as previously assumed,[10] but they directly

interact with the aromatic side chains of Trp84, Phe330, Trp279.[12] Secondly, the overall distribution of the residual charge created by the aromatic rings precisely lines up with the gorge axis. This arrangement directs the positively charged quaternary ammonium substrates like ACh towards and down the gorge site.[13] Using the knowledge of these two explanations, it is suggested that the gorge basically works as an affinity electrophoresis column, where the dipole provides a motive force for the aromatic groups which act as a series of low affinity binding site for the quaternary ions.[13a, 14] This partly explains the role of the gorge

The role of the deep active site can be found in a study by Silman, Sussman et al[15] who obtained an X-ray of a transition state analog bound to the enzyme. Since the active site is buried, the substrate is surrounded almost entirely by the protein, which yielded multiple substrate-enzyme interactions, which resulted in a low energy transition state. Thus they noted that there is a three pronged interaction in the AChE as opposed to the two-pronged interaction in most serine hydrolases.[9]

1.3 Known AChE inhibitors

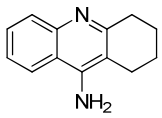
There are two types of cholinesterases, acetylcholinesterase and butyrylcholinesterase. acetylcholinesterase hydrolyzes acetylcholine, thereby terminating the effect of ACh at cholinergic synapses. The function of AChE is relatively well understood, however the physiological function of butyrylcholinesterase (BChE) remains to be established.[16] Cholinesterase (ChE) in the brain is predominantly AChE[17] and is the target of ChE inhibitors used for the cholinergic therapy of Alzheimer's disease.

Memory impairments in patients with Alzheimer's disease (AD) results because of a deficit of cholinergic function in the brain.[18] An important change observed in AD patients as compared to normal humans is the decrease in the levels of neurotransmitter acetylcholine and associated enzymes (choline transferase and acetylcholinesterase) in their brain. An important approach to restoring the balance of acetylcholine to normal levels is inhibiting acetlycholinesterase reversibly. Clinical trials have proven this theory that inhibiting AChE reversibly improves memory in some patients. AD is not the only disease which can be treated by AChE inhibitors (AChEI). AChEI are widely used in treatment of urinary retention, glaucoma, lewy body dementia and myasthenia gravis. We are currently interested in the development of AChEI for the treatment of AD.

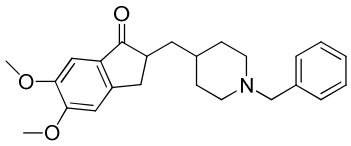
AD is an age-related neurodegenerative disease that affects approximately 4.5 million people in the United States and 25 million across the world.[19] AD is an incurable, degenerative and a terminal disease. It was first described by German psychiatrist and neuropathologist Alois Alzheimer in 1906 and was named after him.[20] There are four major categories of drugs that are used in AD treatment. They are cholinergic treatment, anti-glutaminergic treatment, vitamins and antioxidants and nonsteroidal anti-inflammatory drugs (NSAIDs).[19] Recent advances in the treatment of AD involve use of neuroprotective drugs which are β- and γ- secretase inhibitors,[21] and several of these are under clinical evaluation.

To date, AChEI are the mainstay for the treatment of AD and have become a part of standard care.[22] Compounds which function as reversible competitive or noncompetitive inhibitors of cholinesterase are those most likely to have therapeutic uses. Four AChEI drugs have been approved for the therapeutic treatment of AD by U.S. Food and Drug Administration (FDA). They are tacrine (**1**), donepezil (**2**), rivastigmine (**3**) and galantamine (**4**).[19] Aricept or

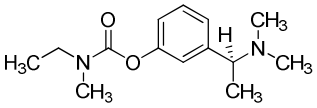
E2020 is the only drug among them used for treatment of AD at all stages – mild, moderate and severe.



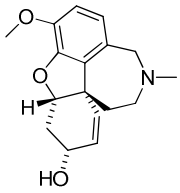
1.1
Tacrine
(THA, Cognex®)



1.2
E2020
(Donepezil, Aricept®)



1.3
Rivastigmine
(Exelon®)



1.4
Galantamine
(Reminyl®)

Figure 1.2. Approved therapeutic AChEI.

1.4 Mechanism of Acetylcholinesterase Inhibitors

The general mechanism of AChEI is to increase the availability of acetylcholine through an inhibition of the catabolic enzyme AChE. Figure 1.3 illustrates how the AChEI works.

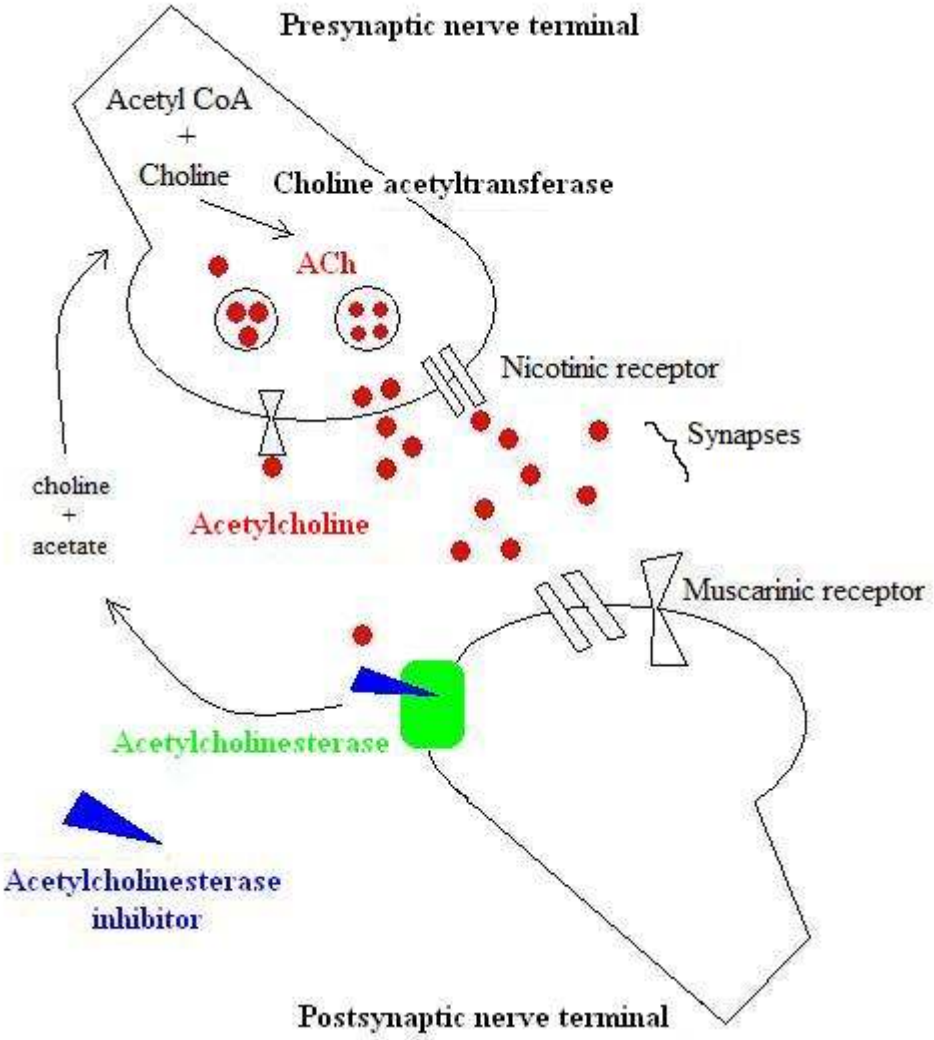


Figure 1.3 Mechanism of inhibition of AChE enzyme. Figure based on graphic in reference[19]

ACh is synthesized from Acetyl coenzyme A (CoA) and choline through the action of the enzyme choline acetyltransferase. ACh is concentrated in vesicles and released into the synaptic cleft where it acts on presynaptic receptors regulating further release of ACh. The postsynaptic receptors regulate the neurotransmission. The AChE then hydrolyzes the ACh back to choline and acetate. The presynaptic nerve terminal then recycles the choline to ACh thus completing the cycle. The AChEI works at the postsynaptic nerve terminal where it limits the degradation of ACh back to choline and acetate by reversibly inhibiting some of the AChE. Thus the synaptic

concentration of ACh increases, increasing the postsynaptic neurotransmission effects. It is important to note that the AChEI does not interfere with the synthesis of ACh.

1.5 Support for the use of AChEI for AD

Tacrine (**1.1**) or 1,2,3,4-tetrahydro-5-aminoacridine is the first centrally acting AChEI approved for the treatment of AD. It was marketed under the brand name Cognex®. The use of tacrine is limited due to poor bioavailability and significant side reactions like diarrhea and severe hepatotoxicity. The other three clinically approved drugs, which are the second generation, are often prescribed as they are much safer and effective. The three recent drugs that were proven safer and more effective for the treatment of AD were donepezil,[23] rivastigmine[24] and galantamine.[25]

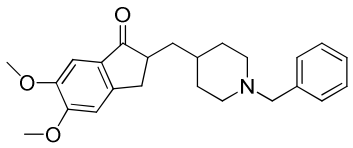
AChEIs appear to have a beneficial impact on the behavioral and neuropsychiatric symptoms.[26] In most of the studies, patients on AChEI's showed small but statistically significant improvements as compared placebo. The use of AChEIs has also been reported to reduce caregiver burden and delay their nursing home placements. Thus AChEIs are the current standard of care for AD.[27]

The approaches approved so far for the treatment of AD are the use of AChEI's or the NMDA antagonist, Memantine. Three types of AChEI have been employed: (1) classical reversible inhibitors, which are generally tertiary amines; (2) irreversible inhibitors, such as organophosphates (OPs), that covalently phosphorylate or phosphonylate the esteratic site of the enzyme; and (3) slow substrates, typified by the carbamates, that also react covalently with the enzyme.[28]

1.6 AChEI's in practice

1.6.1 E2020 (Aricept®) as AChEI

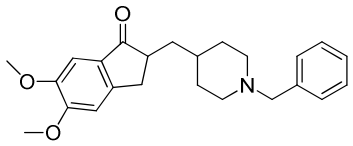
Donepezil hydrochloride(**1.2**) ((±)-2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-indan-1-one) monohydrochloride: E2020: donepezil) is a potent, selective and reversible acetylcholinesterase inhibitor developed for the treatment of Alzheimer's disease.



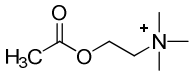
1.2

Figure 1.4. E2020 (Donepezil, Aricept®)

E2020 was a novel piperidine-based AChEI developed in 1993 by Ohnishi *et al.*[29] It was first approved by U.S. FDA in 1996, and is now prescribed worldwide. Donepezil is a reversible inhibitor of AChE, with a high selectivity for AChE over the other major CNS cholinesterase, butrylcholinesterase (BChE). It is 360- to 1200- fold less effective as an inhibitor of BChE.[29b] It has a plasma half-life of 70 hrs. It belongs to a new class of synthetic AChE inhibitors, which contain an *N*-benzylpiperidine and an indanone moiety and is structurally distinct from various AChEI's in use for the treatment of AD. These unique features make it distinctly potent and selective AChEI.[30]



E2020



Acetylcholine

Figure 1.5. Structures of E2020 (**1.2**) and acetylcholine

The similarity between the structures of E2020 and acetylcholine (ACh) (Figure 1.5) is not immediately apparent. However the basic piperidine nitrogen is protonated at physiological pH and thus mimics the charge of the quaternary nitrogen of ACh. On this basis E2020 would be expected to be a competitive inhibitor of AChE.[31] However studies showed that it is a mixed competitive inhibitor of AChE with a K_I value of 4.27 nM.[32] E2020 has an asymmetric center at the 2-position of the indanone ring. Thus there are two possible enantiomers (*R*)-form and (*S*)-form in which the (*R*)-form is six-fold more potent than the (*S*) form.[33] But both the forms have shown similar pharmacokinetics and thus racemic E2020 is developed as a potent therapeutic treatment for palliative treatment of AD. Studies of both enantiomers after docking with the crystal structure of *Torpedo californica* AChE (*Tc*AChE) suggested that both enantiomers of E2020 span the entire AChE gorge with the possibility of multiple sites for each form.

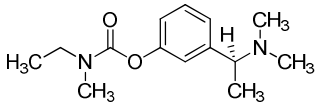
However contradictory data was noted by the Sussman group.[34] They were studying an X-ray structure of a complex of E2020 with *Tc*AChE. They used a racemate in their study and surprisingly found only one conformation of E2020 the (*R*)-form bound to the *Tc*AChE. Even though the (*R*)-form and (*S*)-form have similar binding constants, why would the *Tc*AChE

selectively bind to only the (*R*)-form? It is known that the two form of E2020 interconvert readily in aqueous solutions via the keto-enol tautomerization.[35] Thus it was concluded that the highly chiral environment of not only the active site but of the whole lattice of the crystalline enzyme would cause the epimerization of E2020.

Various binding sites of E2020 are studied by docking with the crystal structure of (*Tc*AChE). The benzyl group on E2020 interacts with the Trp84 at the bottom of the gorge. The piperidine ring interacts with the Tyr70, Asp72, Tyr121 and Tyr334 in the middle of the gorge. The indole ring on E2020 interacts with the Trp279 which is present at the lip of the gorge. It was also noted that the aromatic residue at positions 330 and 279 were responsible for the binding and selectivity of E2020 to AChE.[36] Thus E2020 is a potent and selective inhibitor of AChE having superior inhibition characteristics, has very few side-effects and fast pharmacokinetics. E2020 may prove useful not only for the treatment of AD and other nervous system related dementias, but also for prophylaxis against organophosphate toxicity.[37]

1.6.2 Rivastigmine (Exelon®) as AChEI

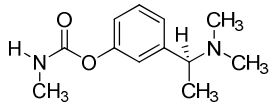
Rivastigmine (**1.3**), an AChEI was approved by the US FDA in 2000 for the treatment of AD under the name of Exelon. Rivastigmine is a relatively newer-generation of inhibitor[38] endowed with a carbamate moiety in its structure. It is a reversible AChE inhibitor with a high brain selectivity.



1.3

Figure 1.6. Rivastigmine (Exelon®)

Rivastigmine belongs to a series of miotine (**1.5**) derivatives. All the miotine derivatives have shown good inhibitory action against AChE. But rivastigmine was the selected candidate for clinical studies because of the 10-fold greater selectivity and good tolerability. Compound **1.3** has a dual inhibitory activity. It inhibits both AChE as well as BChE and has demonstrated broad benefits on the severity of AD.[39] Studies have also shown that **1.3** increases the BChE activity in AD brain and the AChE decreases or remains the same simultaneously. However, its plasmatic half-life is only 2 hrs.



1.5

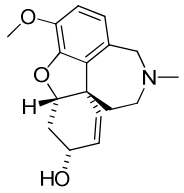
Figure 1.7. Miotine

On observing the crystal structure of rivastigmine/*Tc*AChE complex it was revealed that the carbamyl portion of rivastigmine is positioned to make two H-bonds with the amide nitrogens of A201 and G119 and two non-bonded contacts with F288 and F290 in the acyl pocket. (-)-*S*-3-[1-(dimethylamino)ethyl]phenol (NAP) which is the leaving group of rivastigmine, remains present in the AChE gorge. NAP and three water molecules are within H-bonding distance of each other in the active site. NAP is also within H-bonding distance distance of the amide nitrogen of G118. Rivastigmine also has non-bonding interactions and π-π interactions with W84 and F330.[40]

1.6.3 Galanthamine (Reminyl®) as AChEI

(-)- Galanthamine (**1.4**) is a natural product which belongs to the amaryllidaceae family of alkaloids.[41] It is isolated from the caucasian snow-drop (*Galanthus woronowii*).[42] The

bioactive compound in galanthamine was accidentally discovered in the early 1950s from plant extracts. These plant extracts were then used to treat nerve pain and poliomyelitis. Initially the use of galanthamine was limited to anaesthesia and the treatment of peripheral paralysis syndromes in eastern Europe.[43]



1.4

Figure 1.8. Galanthamine (Reminyl®)

Galanthamine is a highly selective and a reversible competitive inhibitor of AChE. It is 50-fold more selective towards human AChE over BChE. It is long-acting as compared to rivastigmine as its serum half life is 4-6 h. It has been tested in AD patients and found to be readily absorbed. It proved to enhance performance in memory tests in some patients and it was generally well-tolerated.

X-Ray analysis reveals the binding of galanthamine with human AChE. In the x-ray structure, it is observed that the oxygen moiety of the methoxy group in the phenyl ring is in close proximity from Ser200 and His440. Compound **1.4** binds at the base of the active site gorge with the indole ring of Trp84. A hydrogen bonding site is observed between the hydroxyl group of **1.4** and Glu199. The methylene group in the tetrahydroazepine ring makes three different contacts with the indole ring on the Trp84. Also the double bond in the cyclohexene ring of **1.4** stacks against the indole ring of Trp84.[44]

Galanthamine is usually used in conjunction with other AD medicines. Various derivatives of **1.4** have been designed to improve its efficacy. Galanthamine is used for

symptomatic treatment of AD. It has been found that no significant difference is observed in the rate of progression of AD from mild cognitive impairment to severe condition between patients being treated with **1.4** over placebo over a period of two years.[45]

1.6.4 Tacrine (THA, Cognex®) as AChEI

Tacrine (**1.1**) or 1,2,3,4-tetrahydro-9-aminoacridine (THA) was the first AChEI approved by U.S. FDA for the treatment of AD. It was patented by Parke-Davis Pharmaceuticals in 1993. Tacrine is a reversible inhibitor of AChE and ~50% of the patients exposed in clinical trials developed signs of reversible hepatotoxicity which is evidenced by increase in the liver enzyme levels.[46] Thus tacrine is rarely prescribed now. Tacrine is a first generation AChEI which exhibits nonselective inhibition and has significant peripheral toxicity. It also has a short biological half life of 1.6 h to 3 h.

Tacrine was first synthesized in 1945 by Albert and Gledhill.[47] Tacrine was again described in 1953 by Shaw and Bentley.[48] They later on discovered that it inhibits acetylcholinesterase.[49] But in 1974 it was discovered by Maayani et al. that it is an even stronger inhibitor of BChE.[50] More recently it has been found that tacrine possesses a much broader pharmacological profile than just simply being a cholinesterase inhibitor. It is found that tacrine is involved in blockage of potassium channels,[51] inhibition of the neuronal monoamine uptake processes,[52] and inhibition of monoamine oxidase[53]

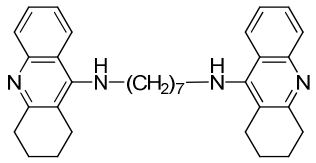
Due to its multiple side-effects, more research needs to be done on improving tacrine by lowering its hepatotoxicity and increasing its efficacy as an AChEI. Various studies have been reported on dual inhibition using tacrine and various other small molecules or tacrine itself as its other partner. These concepts will be discussed further in the chapter.

1.7 Bivalent tacrine inhibitors

As stated before, upon observing the three dimensional structure of AChE, it was revealed that there are two possible target sites on the enzyme. There was a catalytic anionic site (CAS) which could only be accessed through a deep and narrow peripheral anionic site (PAS).[54] The inhibitors of AChE thus act on two target sites on the enzyme, CAS or PAS. The active site on the enzyme contains a catalytic triad (Ser 200, His 440, Glu 327) located at the bottom of a deep narrow gorge which is lined up with aromatic residues and a subsite including Trp 84 located around the bottom of the cavity and Trp 279 located at the opening of the gorge.

Upon discovery of the residues Trp 84 and Trp 279 located at the PAS, new generation of drugs for AD have been developed to take these groups into consideration. Ligands which were able to interact simultaneously with the CAS and PAS of the enzyme would have added advantages over the current generation of inhibitors which bind only to the CAS. These dual site binding inhibitors should greatly improve the inhibitory potency and they would also be involved in the neurotrophic activity.

This idea was first executed and reported by Pang and his co-workers in 1996.[11] They synthesized and reported the first bis-tetrahydroaminoacridine (bis(n)-tacrine) and its derivatives. They devised these molecules using computer modeling. They docked their target molecules on the ligands and developed various derivatives. Their strategy was to connect two tacrine molecues with various tether alkyl chain lengths. Their goal was to obtain the ideal connectivity chain length which would bind at both the catalytic and the peripheral site. Upon experimenting with lengths of 7, 8, 9 and 10, they found that heptylene-linked tacrine (**1.1**) gave them the best results.



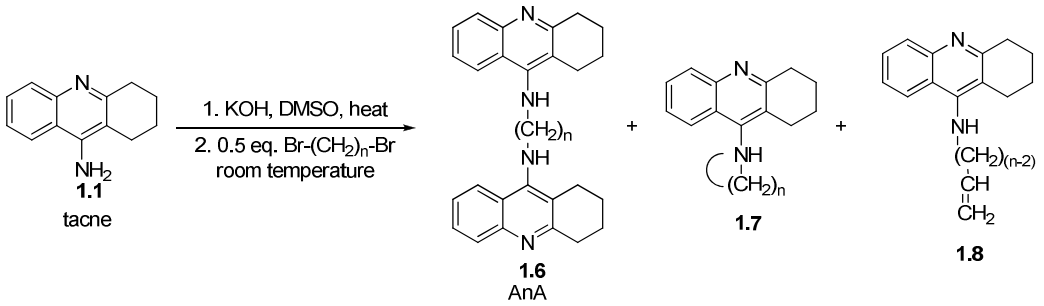
1.6f

Figure 1.9. Heptylene linked tacrine

It was found that **1.6f** is 149-fold more potent and 250-fold more selective for AChE inhibition than tacrine (**1.1**). Thus upon observing these results, it was found that tacrine does have an affinity for the peripheral site of AChE. Observing the success of the bivalent tacrine studies, more studies were needed to carry out which would give the ideal length of tether required as a spacer.

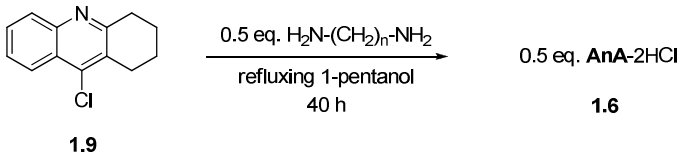
Subsequently Carlier and co-workers[55] in collaboration with Pang, synthesized dimers with tether lengths ranging from 2 to 10 methylene groups. The goal of these studies was two-fold. First, since Pang had shown that the heptamethylene-linked dimer was optimum in the series from 7 to 10, it was possible that a slightly shorter tether might give even better potency and selectivity. Secondly, if the dual-site binding hypothesis is correct, it should be possible to demonstrate that at some point the tether is too short to allow dual-site binding.

It was discovered by Carlier and co-workers that the tacrine alkylation protocol (Figure 1.14) developed by Pang and co-workers gave them low yields and undesired products were formed by intramolecular cyclization (**1.7**) and elimination (**1.8**).



Scheme 1.2. Synthesis of various tacrine dimer having different tether length by alkylation of Tacrine

Thus a new strategy (Scheme 1.3) was developed by Carlier and co-workers to improve yields of bivalent tacrine. They used 9-chloro-1,2,3,4-tetrahydroacridine (**1.9**) with various 1,n-diamine to obtain products. This strategy was successful in obtaining products with tether lengths varying from 2 carbons to 10 carbon atoms.



Scheme 1.3. Synthesis of bivalent tacrine by amination of 9-chloro-1,2,3,4-tetrahydroacridine (**1.9**)

Compd no.	n	Yield (%)
1.6a	2	66
1.6b	3	65
1.6c	4	65
1.6d	5	78
1.6e	6	81
1.6f	7	89
1.6i	10	64

Data taken from reference [55]

Thus upon synthesizing all the tether lengths, all these compounds were tested for its inhibitory potency of AChE. Also the selectivity of the compound for AChE over BChE was necessary to test as tacrine was a greater inhibitor of BChE over AChE.

Table 1.1. AChE and BChE inhibitory potency and selectivity of AnA and tacrine

Compound no.	Drug	AChE IC_{50} (nM)	BChE IC_{50} (nM)	Selectivity for AChE
1.6a	**A2A**·2HCl	711 ± 25	102 ± 4	0.14
1.6b	**A3A**·2HCl	254 ± 55	152 ± 17	0.60
1.6c	**A4A**·2HCl	157 ± 23	252 ± 9	1.60
1.6d	**A5A**·2HCl	28 ± 5	329 ± 21	11.7
1.6e	**A6A**·2HCl	3.8 ± 0.4	119 ± 6	31.6
1.6f	**A7A**·2HCl	1.5 ± 0.3	149 ± 23	99.4
1.6g	**A8A**·2HCl	7.8 ± 0.9	105 ± 13	13.5
1.6h	**A9A**·2HCl	31 ± 3	155 ± 25	4.9
1.6i	**A10A**·2HCl	40 ± 6	167 ± 12	4.2
1.1	**Tacrine**·HCl	223 ± 11	92 ± 2	0.4

Data obtained from reference [55]

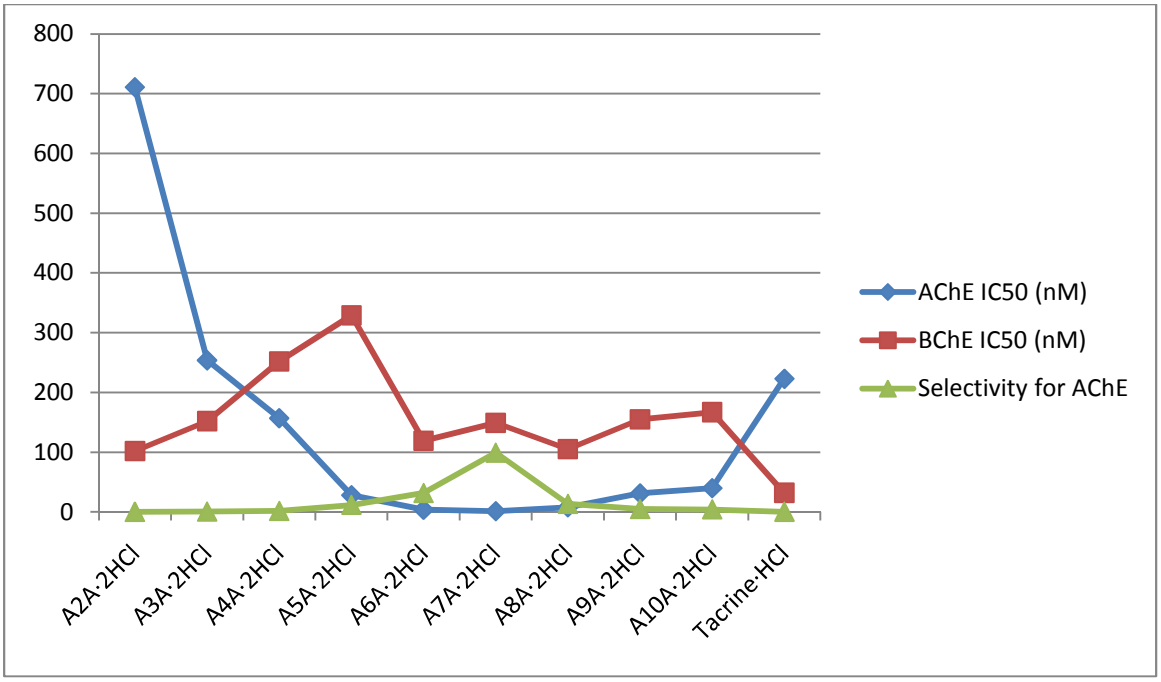


Figure 1.10. Comparison of AChE and BChE potency of tacrine and various bivalent tacrine analogs and selectivity of AChE over BChE

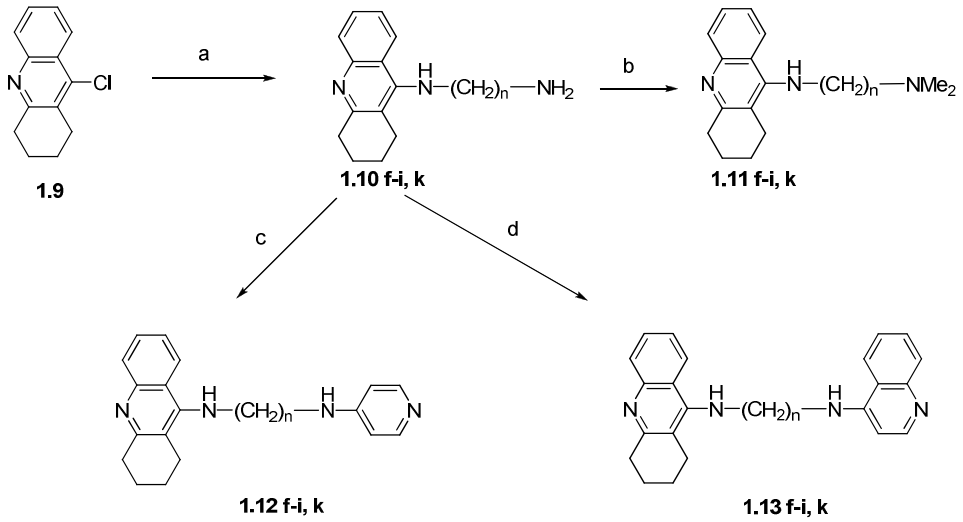
From the above chart (Figure 1.10), it can be seen that the anticipated tether length of 7 methylene groups gives not only the lowest AChE IC_{50} value but also a high BChE IC_{50} value. Thus **A7A**·2HCl offers the best selectivity for AChE and is almost 200 fold more selective over tacrine. Compounds **A5A** and **A6A** showed a significant improvement of AChE inhibitory

potency over tacrine too. But AChE inhibitory potency goes down from **A4A-A2A**. This observation suggests that the improvement in potency of **A5A-A7A** derives from binding to both the CAS and PAS. The tether length is too short from 2-4 methylene groups and thus these inhibitors couldn't bind to both sites simultaneously. Also the inhibitory potency goes down from **A8A-A10A**. This phenomenon likely occurs because the tether length is too long for it to appropriately bind to both CAS and PAS.

Thus it strongly suggests in the above data that tacrine dimers can bind to both CAS and PAS and an appropriate chain length of 7 methylene groups works the best and **A7A** gives the highest inhibitory potency of AChE and the best selectivity for AChE. In 2006, Carlier and co-workers[56] complexed the bis(5)- (**1.6d**) and bis(7)-tacrine (**1.6f**) with *Tc*AChE, thus proving this hypothesis. It was proved that the heptylene-linked spacer in the bis(7)-tacrine (**1.6f**) spans the entire length of the narrow long gorge thus enabling the two tacrine units to bind simultaneously at the CAS and PAS. Also there were no modifications observed in the aromatic lining of the gorge upon binding the AChE with bis(7)-tacrine (**12f**).

1.8 Dual-site binding hypothesis of tacrines with other molecules

As observed from the x-ray crystal structure of AChE, it is clear that the catalytic anionic site and the peripheral anionic site are not identical. Thus it is suprising that bivalent tacrines bind at both the CAS and PAS.[57] Thus to find out if any other substrate would be better suited for binding at the PAS, 4 different molecules were linked with alkyl chain to a tacrine monomer.



f, n=7; **g**, n=8; **h**, n=9; **i**, n=10; e, **k**=12

a. 3.0 equiv. $H_2N(CH_2)_nNH_2$, 1-pentanol, 160 ^oC, 18 h

b. HCOOH, HCHO (Aq), reflux

c. 2.0 equiv. 4-bromopyridine.HCL, 1-pentanol, 160^o C, 40 h

d. 2.0 equiv 4-chloroquinoline, 1-pentanol, 160 ^oC, 40 h

Scheme 1.4. Synthesis of various tacrine heterodimers.[58]

Thus the above 4 tacrine heterodimers were synthesized.[59] The rationale behind the synthesis of these compounds was that they are one step down from tacrine to quinoline to pyridine to simple amino group. Thus maintaining the functionality similar to tacrine but reducing the size would help us better understand what binds better to the PAS. Also various tether lengths varying from 7-10 and 12 methylene groups are prepared to test which is the ideal chain length for which system. The best values of tether chain for a particular dimer are mentioned in Table 1.2

Table 1.2. AChE inhibitory potency of various tacrine-hetero dimer, tacrine dimer and tacrine.[59]

Optimum dimer in series	Tether length (n)	Peripheral site ligand	AChE IC_{50} (nM)	Relative potency
1.10g	8	NH_2	89.4 ± 6.2	2.5
1.11g	8	NMe_2	47.0 ± 1.0	4.7
1.12f	7	4-aminopyridine	12.8 ± 4.0	17
1.13f	7	4-aminoquinoline	10.1 ± 1.4	22
1.6f	7	Tacrine	1.5 ± 0.3	149
Tacrine **1.1**	na	na	223 ± 11	1.0
4-aminoquinoline	na	na	50,700 ± 5,350	0.004
4-aminopyridine	na	na	>500,000	<0.0004

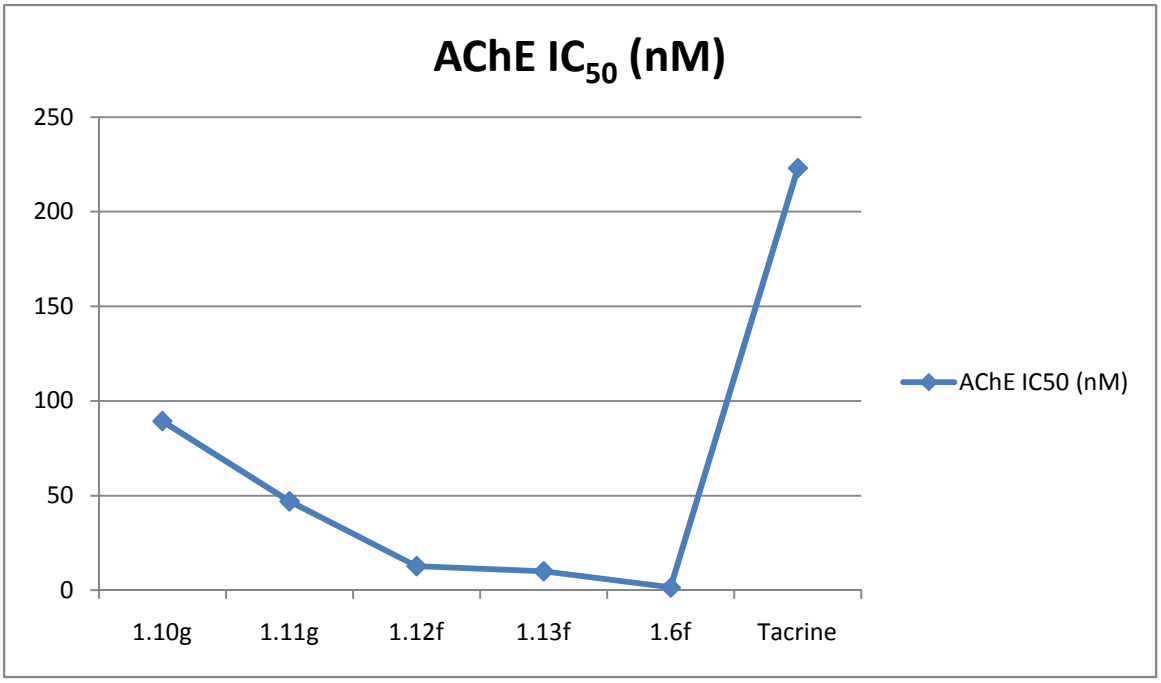


Figure 1.11. AChE inhibitory potency of hetero-tacrine dimer, tacrine dimer and tacrine

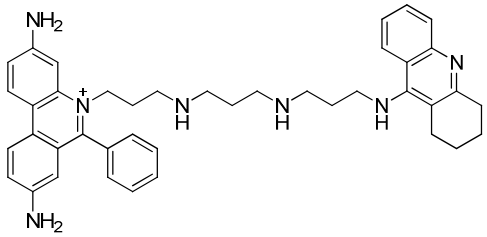
Thus it is observed from the above table (Table 1.2) that tacrine dimer gives the lowest AChE inhibitory potency. Even though all the dimers formed with tacrine have a much lower inhibitory potency as compared to tacrine, none of them matched up to that to tacrine dimer. Also a trend is observed that as the group gets bulkier, the inhibitory potency increases.

This trend makes one wonder if that if the bulkiness of the tacrine group itself is increased by either substituting its phenyl ring or by increasing the size of the cyclohexyl ring, would the potency of tacrine be increased. These modification were in fact later exploited by Hu and coworkers.[58]

1.9 Multifunctional bivalent inhibitors

Attachment of two moieties known for their inhibitory effects to obtain better pharmacological effects has been known for some time. Thus the class of molecules in vogue, are dual inhibitory drugs which can act on two different targets.

1.7.1 Propidium-tacrine heterodimer



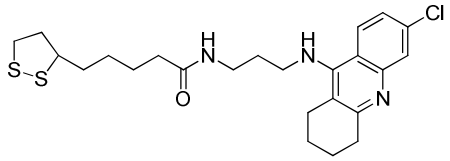
1.14

Figure 1.12. Propidium-tacrine heterodimer

The heterodimer **1.14** is an effective AChE and amyloid-β (Aβ) aggregation inhibitor. Amyloid beta (Aβ or Abeta) is a peptide of 39–43 amino acids that appear to be the main constituent of amyloid plaques in the brains of AD patients. An often recorded neuropathologic hallmark of AD, is a massive deposit of aggregated protein breakdown products, Aβ plaques and neurofibrillary tangles. Aβ aggregates are thought to be mainly responsible for the devastating

clinical effects of the disease. Aβ isolated directly from Alzheimer's disease brains potently and consistently induces several Alzheimer's disease–like phenotypes in normal adult rodents. Thus it is determined that neutralization of these Aβ aggregates can help in treatment of AD.[60] *In vitro* studies revealed that for this heterodimer **1.14** the IC_{50} AChE is 1.55nM while IC_{50} Aβ aggregation is 13.8 μM. Thus compound **1.14** emerged as the most potent heterodimer so far available to inhibit AChE-induced A*β* aggregation. Thus it proves that the dimer strategy works and this compound could be used as a lead compound for developing newer more potent AD drugs.[61]

1.7.2 Lipocrine

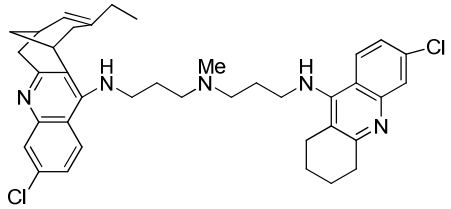


1.15

Figure 1.13 Lipocrine

Lipocrine (**1.15**) is a heterodimer of tacrine and lipoic acid linked by a a tether which contains hetero atoms. This compound is a first of its kind which inhibits AChE, BChE activity and Aβ aggregation and protects neuroblast cells from damage. Lipocrine has been reported to show AChE IC_{50} value of 0.253 nM, BChE IC_{50} value of 10.8 nM and Aβ aggregation IC_{50} value of more than 100 μM. Though lipocrine was three-fold less potent than propidium alone, it shows improvement in biological activity. Further investigation of this compound to develop a lead compound in these lines may bring about a more potent AD drug.[62]

1.7.3 Huprine-tacrine heterodimer



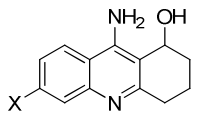
1.16

Figure 1.14. Huperine-tacrine heterodimer

Huprine Y and tacrine are both acetylcholinesterase inhibitors having IC_{50} values of 0.78 nM and 205 nM respectively. But when both of them are linked together to form a heterodimer (**1.16**) with an adequate tether containing hetero atoms, they provide a good inhibitory effect for AChE. The heterodimer **1.16** has shown excellent activity as an AChEI. It has an IC_{50} value of 0.29 nM for human AChE and 31.1 nM for human BChE.[63] Thus tethering two AChEI's together leads to an improvement in the AChE inhibitory potency.

1.10 Modifications to the tacrine nucleus

Developments to improve the tacrine nucleus lead to the discovery of tacrin-1-ol (**1.17x**, velnacrine) which is an active metabolite of tacrine and has been chosen for clinical trials.[64] Similarly 6-fluoro-tacrin-1-ol (**1.17y**) was reported to be slightly more potent than tacrine and also 6-chloro-tacrine-1-ol(**1.17z**) was reported to be atleast 30 fold more potent than tacrine-1-ol.[65] In another studies, it was found that 6-chlorotacrine (**1.18**) is more potent than any other substituted analogs of tacrine and it is shown to improve the binding strength towards AChE.[66]

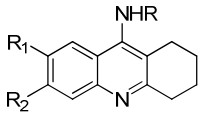


1.17

x, X=H; y, X=F; z, X=Cl

Figure 1.15. Tacrin-1-ol (velnacrine) and analogs

A variety of analogs were prepared by Recanatini and coworkers[66b] to study the structure-activity relationship (SAR). They prepared a wide array of compounds having substitution on the 6 and 7 positions and *N*9 position on tacrine. These substituents were chosen as they consisted of a wide variety of compounds having different uncorrelated properties of hydrophobicity, electronics and sterics. The results of various tacrine analogs and their inhibitory potential of AChE are mentioned in Table 1.3.



1.19-1.40

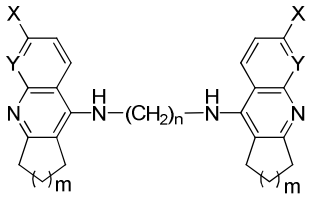
T**able 1.3.** SAR of various 6, 7 and *N*9 substituted analogs of tacrines. Table obtained from reference [66b]

Entry	Compound no.	R (N9 position)	R_1 (7 position)	R_2 (6 position)	AChE IC50 (μM)
1	**1.19**	H	CH_3	H	8.1±0.6
2	**1.20**	H	H	CH_3	0.10±0.001
3	**1.21**	H	Cl	H	0.55±0.02
4	**1.22**	H	H	Cl	0.0099±0.0003
5	**1.23**	H	NO_2	H	3.0±0.2
6	**1.24**	H	H	NO_2	0.028±0.001
7	**1.25**	H	H	OCH_3	0.35±0.01
8	**1.26**	H	NH_2	H	3.8±0.2
9	**1.27**	H	H	F	0.087±0.001
10	**1.28**	H	Cl	Cl	0.47±0.02
11	**1.29**	H	OCH_3	OCH_3	5.2±0.1
12	**1.30**	$CH_2C_6H_5$	CH_3	H	3.7±0.2
13	**1.31**	$CH_2C_6H_5$	H	CH_3	0.75±0.03
14	**1.32**	$CH_2C_6H_5$	H	Cl	0.17±0.01
15	**1.33**	$CH_2C_6H_5$	NO_2	H	1.6±0.1
16	**1.34**	$CH_2C_6H_5$	H	NO_2	4.8±0.4
17	**1.35**	C_7H_{15}	CH_3	H	0.39±0.03
18	**1.36**	C_7H_{15}	H	CH_3	0.13±0.01
19	**1.37**	C_7H_{15}	H	Cl	0.013±0.004
20	**1.38**	C_7H_{15}	H	NO_2	0.29±0.02
21	**1.39**	C_7H_{15}	H	OCH_3	0.46±0.001
22	**1.40**	C_7H_{15}	H	F	0.045±0.002
23	**1.1**	H	H	H	0.25±0.01

Thus from the above table it is evident that both the nature and the position of the substituent would affect its inhibitory potency as compared to the unsubstituted parent compound, tacrine. As stated above, the substitution of chloro group on the 6 position (**1.22**) would increase the potency of tacrine, but it was found that chloro group on the 7 position (**1.21**) would rather lower the potency. The dual substitution, in case of 6,7-dichlorotacrine (**1.28**), seems to have cancelled effects of individual substitution at 6 and 7 positions and the overall

effect is roughly the sum of the two. Usually a trend is observed that substitution at 6 position with electron withdrawing groups have seem to improve the potency of tacrines, while substitution at the 7 position have decreased the potency of tacrines. Thus it seems that the reduced electron density on the tacrine aromatic rings favor the π-interactions with the nearby residue in the active sites of the AChE. This strongly increases the inhibitory potency of tacrines

Since a greatly improved inhibitory potency was observed with the substitution on the 6 position on tacrine, the effect of its dimer should also be greatly enhanced. Also it was observed before, that if the steric bulk of the hetero molecule on the hetero-tacrine dimer was increased, the potency increased. Thus to account for these factors, Hu et al.[58] made a series of homodimeric tacrines which focused on 6-position substitution, changes in the carbocyclic ring size and isosteric modifications on the tacrine nucleus. These were aimed at increasing the inhibitory potency of AChE and also the selectivity of AChE over BChE.



1.41-1.46

Table 1.4. Tacrine dimers and its various analogs

Product	X	Y	m	n	IC50 (nM)		Selectivity for AChE
					AChE	BChE	
1.41e	H	CH	1	6	115±34	273±38	2.4
1.41f	H	CH	1	7	75±11	328±40	4.4
1.41g	H	CH	1	8	22±4	165±21	7.5
1.42e	H	CH	2	6	1.4±0.1	83±19	59
1.42f	H	CH	2	7	0.2±0.1	54±14	221
1.42g	H	CH	2	8	1.6±0.7	53±17	33.1
1.43e	F	CH	2	6	0.9±0.3	45±21	50
1.43f	F	CH	2	7	0.6±0.1	257±35	428
1.43g	F	CH	2	8	0.7±0.2	164±32	234
1.44e	Cl	CH	2	6	0.6±0.2	312±72	520
1.44f	Cl	CH	2	7	0.07±0.01	26±1	371
1.44g	Cl	CH	2	8	0.3±0.2	194±64	647
1.45e	H	N	2	6	4.8±1.3	93±20	19.4
1.45f	H	N	2	7	1.3±0.2	59±14	45.4
1.45g	H	N	2	8	1.9±0.2	23±4	12.1
1.46e	H	CH	3	6	2.5±0.7	3.3±1.4	1.2
1.46f	H	CH	3	7	2.7±0.4	2.6±1.3	1.6
1.46g	H	CH	3	8	1.6±0.4	3.8±0.3	1.5
1.1	H	CH	2	na	333±39	89±7	0.3

All the examples tried above have a minimum of 6 methylene groups and a maximum of 8 as suggested by Carlier *et al*.[55] It is seen from the above table that tacrine dimers having a tether length of 7 have proved the best in all the cases. The presence of a chloro group in the 6 position improved the inhibitory potency as was previously shown. Carbocyclic congeners with contracted rings like products **1.41e**, **1.41f** and **1.41g** showed only a slight increase in potency against AChE inhibition and a slight decrease for BChE inhibition as compared to tacrines. The ring-expanded carbocyclic congeners **1.46e**, **1.46f** and **1.46g** showed a significant improvement in the potency against AChE inhibitions and significant decrease for BChE inhibition as

compared to tacrines. Thus the binding pockets of AChE can accommodate a little more bulkier moiety.[55]

1.11 Conclusion

Even though tacrine is one of the 4 approved therapeutic AChE inhibitors, it is rarely prescribed these days due to high hepatotoxicity. Thus modifications need to be done to improve its AChE inhibitory potency and lower its hepatotoxicity.

Dimerization of tacrine is found to be a key strategy to improve the selectivity towards AChE over BChE. Due to dimerization, we could utilize the dual binding sites present on AChE. The dimer can bind to both the CAS and the PAS on AChE thus improving the selectivity and also inhibitory potency towards AChE.

Extensive studies on SAR have been undertaken to improve the tacrine nucleus. On observing the various SAR, it was found that tacrine containing a ring-expanded carbocyclic congeners show better inhibitory potency towards AChE and decreases the inhibitory potency towards BChE. Thus there is more room in the CAS of AChE for a ring-expanded carbocyclic congener. A substitution at the 6-position improves the inhibitory potency and at the 7-position reduces the inhibitory potency towards AChE.

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Chapter 2. Synthesis and modifications of 9-Chloro-1,2,3,4-tetrahydroacridine for functionalization of S_NAr

2.1 Introduction to S_NAr

Nucleophilic aromatic substitution (S_NAr) proceeds by a mechanism distinct from S_N1 or S_N2 substitution reaction. Neither of those mechanisms is accessible for an aromatic ring system. The back-side attack of an S_N2 reaction is hindered since the back lobe of the σ* orbital is directed towards the center of the aromatic ring (**2.1**). An S_N1 mechanism is very costly in terms of the energy required to form the intermediate.[1] The intermediate in S_N1 reaction is a carbocation. A carbocation on a benzene ring is very high in energy; even a primary carbocation is more stable than a phenyl carbocation (**2.2**). Thus substitution on a benzene ring has to undergo a nucleophilic aromatic substitution.[2]

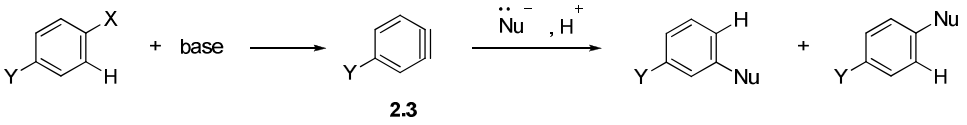
2.1 **2.2**

Figure 2.1. S_N1 and S_N2 are not possible on an aromatic ring

2.2 Mechanism of S_NAr

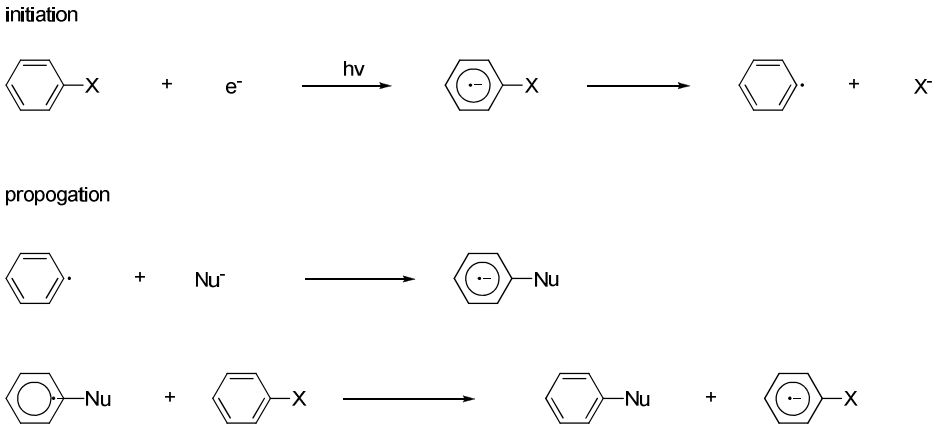
The four principal mechanisms for aromatic nucleophilic substitutions,[3] are 1) addition-elimination, 2) elimination-addition, 3) $S_{RN}1$ which involves a radical intermediate and 4) transition metal-catalyzed processes. The S_NAr mechanism mentioned in the introduction proceeds via addition-elimination and will be described in detail below. The elimination-addition

mechanism (Scheme 2.1) involves formation of a highly unstable dehydrobenzene intermediate, commonly known as benzyne (**2.3**).[4]



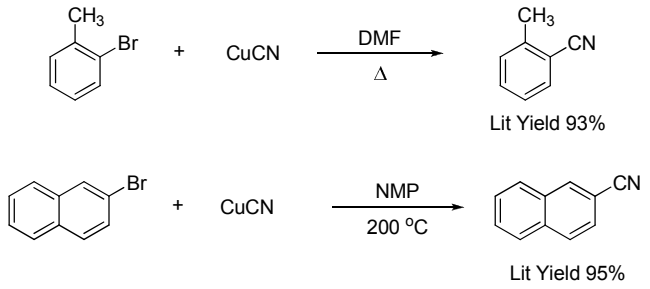
Scheme 2.1. Elimination-addition mechanism showing the formation of benzyne intermediate

Aryl halides can also undergo substitution through a chain mechanism of the $S_{RN}1$ class (Scheme 2.2).[5] Most of these reactions are initiated photochemically, by a strong chemical reductant, or electrochemically.



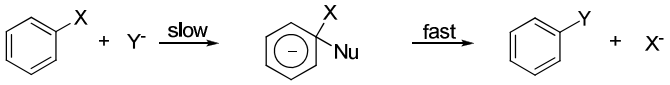
Scheme 2.2. Substitution by $S_{RN}1$ mechanism

Transition metal-catalyzed aromatic substitution reactions can be either copper-catalyzed or palladium-catalyzed reactions. Palladium-catalyzed reactions are discussed in detail in chapter 3. Copper-catalyzed reactions have been known since a long time and can be catalyzed by the presence of copper metal or copper salts.[6] Various aryl nitriles are prepared by this method which involves reaction aryl bromides with Cu(I)CN. These reactions are usually carried out at high temperatures in DMF or similar solvents.



Scheme 2.3. Copper-catalyzed aromatic substitution reactions[7]

Of the first three mechanisms, by far the most important is the addition-elimination mechanism. It is sometimes also called as S_N2Ar or intermediate complex mechanism.[8] It consists of two steps. The first step is the attack of the incoming nucleophile on the ipso carbon (carbon bearing the leaving group) of the aromatic ring. The next step is the elimination of the leaving group and regeneration of the aromatic ring (Scheme 2.1)



Scheme 2.4. Addition-elimination mechanism of nucleophilic aromatic substitution reactions

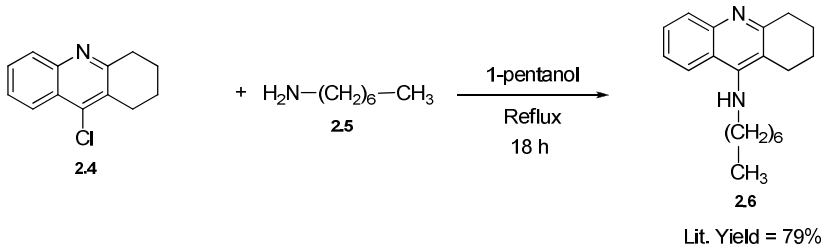
The first step is usually the rate determining step. The IUPAC designation for this mechanism is $A_N + D_N$. But it is commonly referred to as S_NAr mechanism.

The role of the leaving group in S_NAr reaction rate is somewhat different than that in S_N1 or S_N2 reaction rates. In the latter two cases the dominant factor is the strength of the bond of the leaving group with the carbon atom. The weaker the bond, the better is the leaving group ability and the faster would be the rate of the reaction. Thus in both S_N1 and S_N2 reactions, the order of reactivity of the halogens would be I > Br > Cl > F. However the rate order is different for S_NAr reactions. Here the slow step (Scheme 2.4) which is the addition of the nucleophile forming the addition intermediate is the rate determining step. Thus the ease of C—X bond breakage is

irrelevant in the rate of S_NAr reaction. However the carbon of the C—X bond should have a sufficient amount of partial positive charge for the incoming nucleophile to come in quickly, increasing the rate of the first step and thus the reaction. Hence the stronger bond dipoles associated with the more electronegative halogens favor the addition step. Thus the order of reactivity for S_NAr is F > Cl > Br > I.[9]

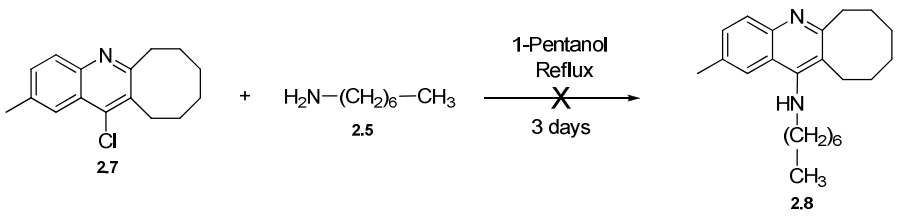
2.3 Functionalization of 9-chloro-1,2,3,4-tetrahydroacridine

Carlier and co workers prepared *n*-heptyl-9-amino-1,2,3,4-tetrahydroacridine using the traditional S_NAr methods by reaction of 9-chloro-1,2,3,4-tetrahydroacridine (**2.4**) and *n*-heptylamine in refluxing 1-pentanol.[10] Thus the reaction of **2.4** is slow and requires high temperatures, providing **2.6** in a moderate 79% yield (Scheme 2.5).[11]



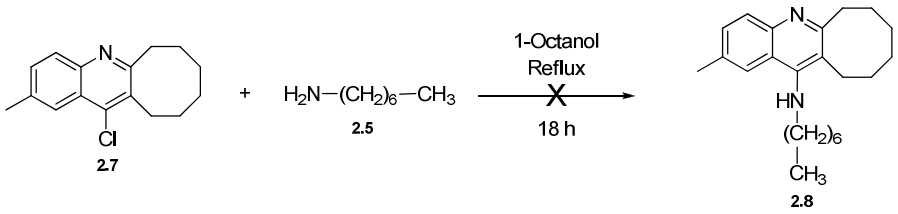
Scheme 2.5. Synthesis of *N*-heptyl-9-amino-1,2,3,4-tetrahydroacridine(**2.3**) by S_NAr method[11a]

But when this procedure was applied in the Carlier group to a slightly more bulky substrate, no reaction was observed. Dr. Ming Ma of the Carlier group treated 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (**2.7**) with *n*-heptyl amine (**2.5**) but it gave no significant change after 3 days (Scheme 2.6).



Scheme 2.6. No reaction was observed when 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (**2.7**) was reacted with n-heptyl amine (**2.5**)

The Carlier group even tried to change the conditions. But when the reaction temperature was increased, the desired product (**2.8**) did not form. The solvent was changed from 1-pentanol to 1-octanol and the reaction mixture was refluxed for 18 h at 195 ^{o}C (Scheme 2.7). Multiple side-products were observed on TLC plate and the desired product (**2.8**) could not be obtained.

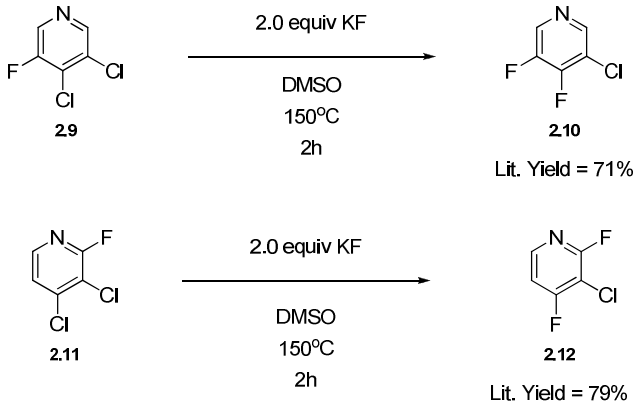


Scheme 2.7. Formation of **2.8** was not observed when solvent was changed to a higher boiling solvent

Thus there was a need to functionalize the starting material so that the reaction would proceed at a faster rate and under more moderate conditions. Since having a fluoride group on the starting material would accelerate the rate of the reaction, our goal was to convert the chloride group to a fluoride group.

2.3.1 Attempts to form 9-fluoro-1,2,3,4-tetrahydroacridine

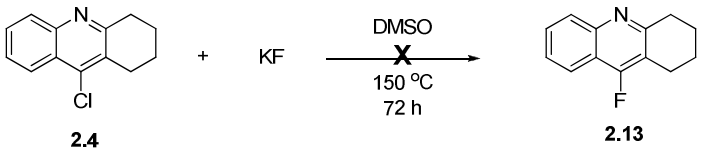
Various literature procedures are available to convert 4-chloropyridine having various electron withdrawing substituents to 4-fluoropyridine. [12] The one that specifically interested us was one by Bobbio et al.[12b] They were able to functionalize various compounds in a regio-selective fashion. They successfully transformed 3,4-dichloro-5-fluoropyridine (**2.9**) to 3-chloro-4,5-difluoropyridine (**2.10**) (Scheme 2.8) and 3,4-dichloro-2-fluoropyridine (**2.11**) to 3-chloro-2,4-difluoropyridine (**2.12**) (Scheme 2.9) in a 71% and 79% yield respectively.



Scheme 2.8. Conversion of a 4-chloro to 4-fluoro analog by KF and DMSO

Even though they had not used a bulky compound as 9-chloro-1,2,3,4-tetrahydroacridine **(2.4)** and they had a lot of electron withdrawing substituents attached to their ring, it was worth applying the same procedure to our compound **2.4**.

A reaction was set up with 9-chloro-1,2,3,4-tetrahydroacridine (**2.4**), KF and DMSO and the reaction was heated to 150 ^{o}C. No starting material was consumed within the first 2 h. So the reaction was stirred at 150 ^{o}C for 72 h. But mostly starting material was observed on the TLC plate. Since the R_f values of the starting material and product could have been similar, the possible product was isolated; however, no peaks were observed in the ^{19}F NMR. (Scheme 2.6).

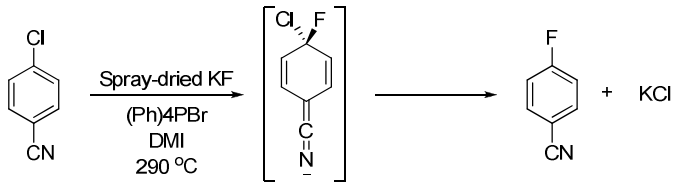


Scheme 2.9. Conversion to 9-fluoro-1,2,3,4-tetrahydroacridine (**2.13**) was unsuccessful by KF/DMSO

The cause of this transformation not working was thought to be bulkiness of **2.4** as compared to **2.9** or **2.11** and the lack to sufficient electron withdrawing groups attached to the substrate.

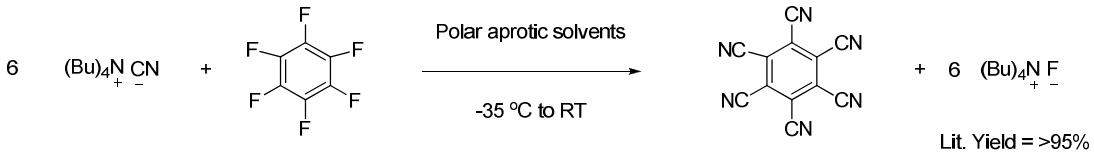
Thus we tried to explore some other procedure to effect the conversion to 9-fluoro-1,2,3,4-tetrahydroacridine (**2.13)**. Upon exploration we found a research paper by Haoran Sun and Stephen G. DiMagno[13] who used anhydrous tetrabutylammonium fluoride ($TBAF_{anh}$) to transform 2-chloropyridine and other such substrates to 2-fluoropyridine and analogs. They developed methods for room-temperature nucleophilic aromatic fluorination.

They noted that fluorination using KF (Scheme 2.10) typically required heating up to 150^{o}C and took up to 10h.[14] Thus they used $TBAF_{anh}$. Their group had previous reported a procedure in which they prepared $TBAF_{anh}$ in situ.[15] They observed that dried TBAF[16] is reported to decompose at room temperature by Hoffman elimination. The salt produced therein is dehydrated and is found to be contaminated with copious amounts of bifluoride ion (HF_2^-) and tributylamine.[17] Thus they discovered that it would be very unlikely to find pure anhydrous tetraalkylammonium salts which can be produced in the case of ammonium ions susceptible to E2 eliminations.[17]



Scheme 2.10. A typical Halex reaction

Thus they used low temperature S_NAr methods to generate highly pure and dry TBAF in-situ prepared in various aprotic solvents, which could be directly used for various transformations.[15] Since the bond C_{sp2}-F in aromatics is exceptionally strong (126 kcal/mol), only charged anionic nucleophiles which are capable of forming strong bonds to carbon would be capable of reacting in S_NAr reactions at low temperatures and polar aprotic solvents. Thus a cyanide ion, which is potent and weakly basic nucleophile, would be able to form strong bonds to sp^2 hybridized carbon in the aromatic system. Thus they selected hexafluorobenzene and tetrabutylammonium cyanide (TBACN) as appropriate candidates for this transformation. These chemicals are also commercially available.



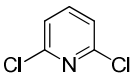
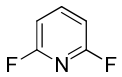
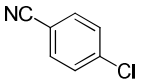
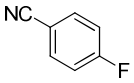
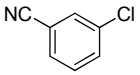
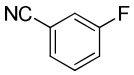
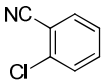
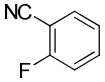
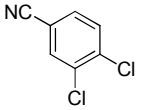
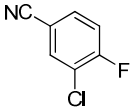
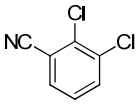
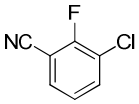
Scheme 2.11. The in-situ generation of pure and anhydrous TBAF in a polar aprotic solvent[15]

Treatment of hexafluorobenzene and TBACN in varying mole ratios from 1:1 to 1:6 and in varying solvents like THF, acetonitrile or DMSO at or below room temperature gave them excellent yields of anhydrous TBAF. ^{19}F NMR spectroscopy proved that they obtained yields of >95% of TBAF.[15]

Once the TBAF anhydrous was produced, they used it in aromatic nucleophilic substitution. They explored the scope of room-temperature reactivity of $TBAF_{anh}$ with chloropyridines and observed that, if the pyridine ring has no electron withdrawing groups, the

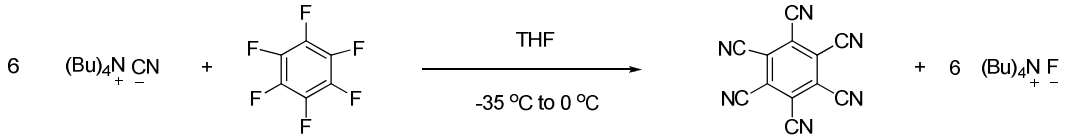
nucleophilic substitution of chloride is sluggish for the ortho position (entry 2) and does not occur at all for the meta position (entry 1). They observed the following results (Table 2.1)

Table 2.1 Reactions of chloro aromatic compounds by $TBAF_{anh}$[13]

Entries	Substrate	Conditions	Product	Yield [%]
1		$TBAF_{anh}$ (4 equiv) 14 days	No reaction	0
2		$TBAF_{anh}$ (4 equiv) 14 days		80
3		$TBAF_{anh}$ (2.5 equiv) 1 h		>95
4		$TBAF_{anh}$ (2.5 equiv) 1.5 h		>95
5		$TBAF_{anh}$ (1.5 equiv) 2 days		80
6		$TBAF_{anh}$ (1.5 equiv) 5 days		2
7		$TBAF_{anh}$ (1.5 equiv) 18 h		>95
8		$TBAF_{anh}$ (2.5 equiv) 20 min		>95
9		$TBAF_{anh}$ (1.4 equiv) 20 min		>85

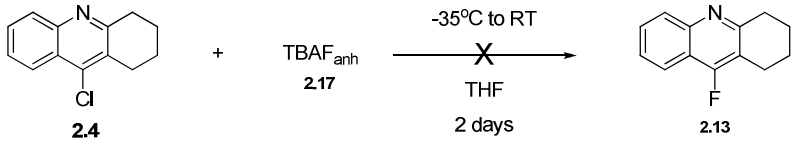
Even though no product was obtained in Table 2.1 entry 1 or product obtained after 14 days in entry 2, what encouraged us was the >95% yield obtained in entry 8 when the chloro group was para to the cyano group. The reaction showed promise and appeared worth an exploration for 9-chloro-1,2,3,4-tetrahydroacridine (**2.4**).

The first reaction was the formation of $TBAF_{anh}$ when hexafluorobenzene and TBACN are mixed together in THF at -35°C (Scheme 2.12). $TBAF_{anh}$ is modestly soluble in THF and thus at low temperatures, a salt of $TBAF_{anh}$ is observed in the solution. The solution also changes its color to a bright yellow solution



Scheme 2.12. Formation of $TBAF_{anh}$ from TBACN and hexafluorobenzene in THF

But when this mixture is added to 9-chloro-1,2,3,4-tetrahydroacridine (**2.4**) and stirred for 2 days, no reaction was observed (Scheme 2.13). Mostly starting material was observed on the TLC plate. Still the possible product was isolated; however no peaks were observed in the ^{19}F NMR.

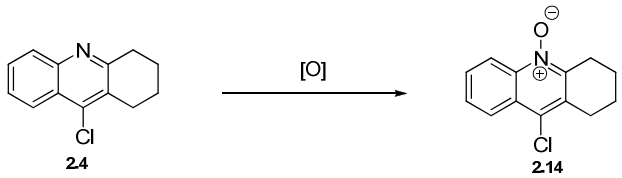


Scheme 2.13. No product **2.13** is observed when the reaction is stirred for 2 days

The reason for the failure is again postulated to be steric bulk of **2.4** and also the lack of sufficient electron withdrawing groups on **2.4**. Since a similar substrate 3-chloropyridine did not react with the $TBAF_{anh}$, probably **2.4** would not have reacted either. It was also noted that Bobbio et al.[12b] had not tried the reaction with 4-chloropyridine which would have been a closer fit to 9-chloro-1,2,3,4-tetrahydroacridine (**2.4**).

2.3.2 Formation of the *N*-oxide

Formation of the *N*-oxide of 9-chloro-1,2,3,4-tetrahydroacridine(**2.4**) would be useful in its functionalization (Scheme 2.14).



Scheme 2.14. Proposed conversion of 9-chloro-1,2,3,4-tetrahydroacridine to 9-chloro-1,2,3,4-tetrahydroacridine 10-oxide

Oxidation of **2.4** would form an *N*-oxide which would form a positive charge on the nitrogen. This would withdraw the electron density on the chlorine bearing carbon and thus enabling it better for S_NAr reaction by enhancing its rate (Figure 2.2).

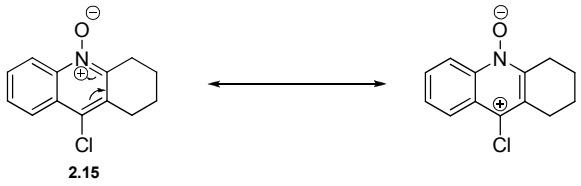
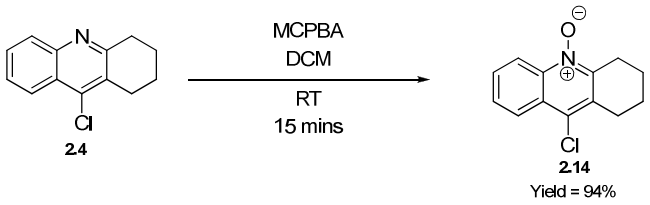


Figure 2.2. Resonance showing the formation of charge on carbon bearing the chlorine

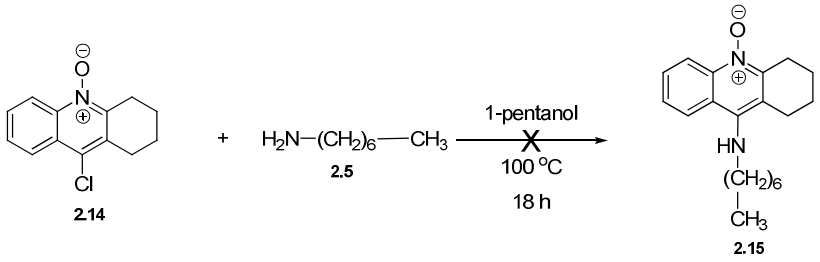
Heterocyclic *N*-oxides can be formed by a variety of ways. They are often obtained by oxidation of heterocycles with acetic acid and hydrogen peroxide ($AcOH/H_2O_2$) [18], m-chloroperbenzoic acid[19] (MCPBA), monoperoxyphthalic acid[20], dioxiranes[21], hydrogen peroxide[22] and Caro's acid (H_2SO_5) [23]. In the recent years, Sharpless and coworkers have reported the synthesis of heterocyclic *N*-oxides using oxides of rhenium ($MeReO_3$)[24].

Pyridine and its derivatives are usually oxidized by peroxyacids. Thus the use of mCPBA was chosen as a simple and effective method for the *N*-oxidation of 9-chloro-1,2,3,4-tetrahydroacridine.[19] Thus we converted **2.4** to 9-chloro-1,2,3,4-tetrahydroacridine 10-oxide (**2.14**) by using mCPBA in DCM at RT and the product **2.14** was formed in 15 min (Scheme 2.15). The product was formed in 94% yield.



Scheme 2.15. *N*-Oxidation of 9-chloro-1,2,3,4-tetrahydroacidine using mCPBA

The 9-chloro-1,2,3,4-tetrahydroacridine 10-oxide was further used for amination with *n*-heptyl amine, in 1-pentanol. Upon heating the reaction mixture to 100°C, multiple spots were observed on a TLC plate (Scheme 2.16). Upon later discovery, it was found that 9-chloro-1,2,3,4-tetrahydroacridine 10-oxide (**2.14**) was reduced even at room temperature back to 9-chloro-1,2,3,4-tetrahydroacridine (**2.4**).



Scheme 2.16. Failure of formation of **2.15** starting from **2.14**

2.4 Conclusion

A variety of methods were tried for the functionalization of 9-chloro-1,2,3,4-tetrahydroacridine. Having fluorine on the 9th position would have definitely given an added advantage over chlorine being there. Lower temperature and faster rates could be expected with amination if fluorine was present on the 9th position. Upon applying this to 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (**2.7)**, it would have enabled the 12-fluoro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline to react with various amines at moderate temperatures. The absence of any electron withdrawing group present near the substituents made the transformation from chloro to fluoro extremely difficult.

The formation of the *N*-oxide of 9-chloro-1,2,3,4-tetrahydroacridine (**2.4**) would have helped too with lowering the temperature of the reaction and increasing its rate. Even though the *N*-oxide of **2.4** was successfully synthesized in high yields, the instability of the *N*-oxide rendered the compound useless for further explorations.

Since functionalization by S_NAr did not successfully work as planned, other means for amination had to be explored for obtaining aminated products at lower temperatures and faster rates of reaction. Many other techniques exist for amination, though the most promising of them all is Buchwald-Hartwig's Pd-catalyzed amination which is discussed in the chapter 3.

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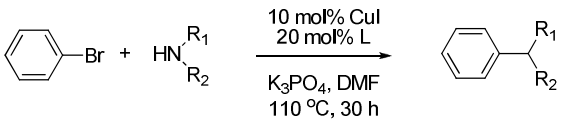
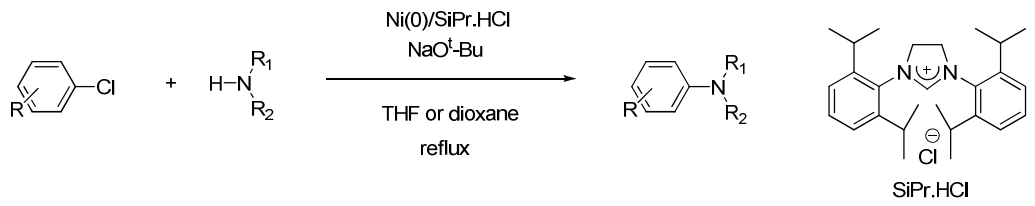
Chapter 3. Mild and convenient methods of *N*-alkylation by organopalladium catalysis

3.1 Introduction to palladium-catalyzed amination

Failure to convert 9-chloro-1,2,3,4-tetrahydroacridine to 9-fluoro-1,2,3,4-tetrahydroacridine by various methods[1], or use the 9-chloro-1,2,3,4-tetrahydroacridine 10-oxide[2] successfully for amination, described in Chapter 2, made us explore into the possibility of using other techniques for the synthesis of *N*-alkyl-1,2,3,4-tetrahydroacridines. Despite the simplicity of the arylamine moieties, synthesis of these materials are often difficult. Procedures which involve nitration, reduction and substitution are incompatible incompatible with many functional groups and require protection and deprotection steps. Goldberg-Ullmann type reactions[3] (Scheme 3.1) have much literature precedence. But it can often be limited by harsh reaction conditions, poor functional group tolerance and undesired side-products. Transition metal arene complexes have shown to accelerate *N*-alkylation of aryl halides,[4] but stoichiometric amounts of these transition metals render these processes less useful (Scheme 3.2). Thus Pd-catalyzed amination was thought as a mild and convenient alternative to *N*-alkylation of 9-chloro-1,2,3,4-tetrahydroacridine.

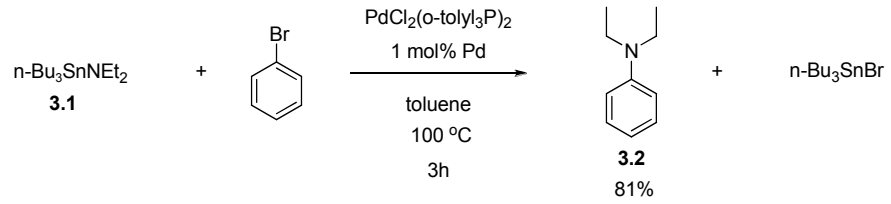


Scheme 3.1. Goldberg-Ullmann type reactions



Scheme 3.2. Transition metal arene complexes

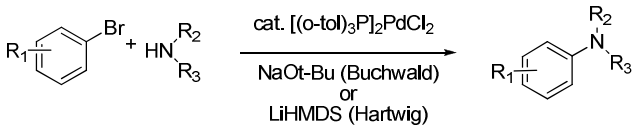
The Pd-catalyzed Buchwald-Hartwig amination is an extremely general and widely used technique for the generation of a C—N bond from an aryl amine. The first paper which showed feasibility for Pd-catalyzed amination was reported by Migita and co-workers in 1983.[5] They demonstrated the amination of aryl bromides with *N*,*N*-diethyl amino-tributyltin catalyzed by $[P(o\text{-}C_6H_4Me)_3]_2PdCl_2$ (Scheme 3.3). But many severe limitations were discovered in this technique. It was not readily extendible to a wide variety of amines as amino stannanes like **3.1** are not typically commercially available and they are thermally as well as moisture sensitive. Also toxic tin alkyl compounds are generated in stoichiometric amounts at the end of the reaction.



Scheme 3.3. *N*-alkylation of bromobenzene as investigated by Migita and co-workers

N-alkylation of aryl bromides without the use of tin was nearly simultaneously discovered by Buchwald and Hartwig groups in 1995.[6,7] Instead of generating the tin amide in

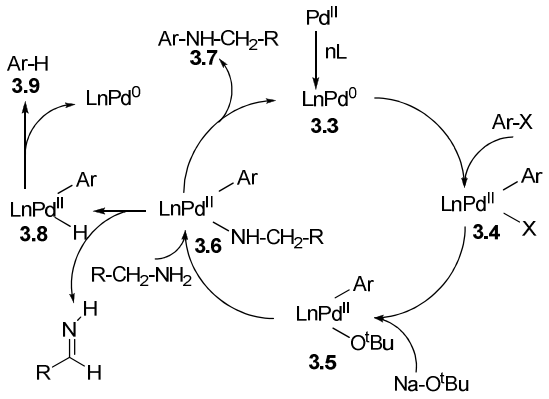
situ, the amination reactions were conducted by reacting an aryl halide with the combination of an amine and either an alkoxide or silylamide base. They used P(o-tolyl)$_3$ as ligand, a Pd-catalyst and a base, commonly NaO*t*-Bu (Scheme 3.4)



Scheme 3.4. *N*-alkylation of aryl bromides as investigated by Buchwald and Hartwig groups

3.2 Mechanism of Pd-catalyzed amination

A reasonable mechanism for Pd-catalyzed amination was suggested by Hartwig (Scheme 3.5).[8] A Pd(0) species (**3.3**) is thought to be the active species involved in the amination process.[9] The first step involved in this process is oxidative addition of the aryl halide to yield **3.4**. A base (Na-O^{t}Bu) is then inserted into the complex **3.4** to yield the intermediate **3.5**. Then the amine is coordinated (**3.6**) and deprotonated. The final step is reductive elimination yielding the aryl amine (**3.7**).[10] Important to note here is, instead of the reductive elimination step to yield *N*-aryl product (**3.7**), the palladium amide species can undergo a β-hydride elimination through the intermediate **3.8** to yield a reduced arene by-product (**3.9**).[11]



Scheme 3.5. Suggested mechanism for Pd-catalyzed amination by Hartwig

This general mechanism for Pd-catalyzed amination mirrors the other Pd-catalyzed cross-couplings mechanisms. The rate of the mechanism depends upon the association of the ligand to the palladium species and the oxidative addition of aryl halide to the palladium species.[12] Various ligands have been developed to improve and generalize this transformation. There have been four generations of ligands developed so far.[13] The first-generation of catalysts involved simple triaryl phosphines like P(o-tolyl)$_3$ (**3.10**). The second-generation of catalysts were DPPF (1,1'-bis(diphenylphosphino)ferrocene) (**3.11**), BINAP (2,2'-bis(diphenylphosphino)-1,1'-binapthyl) (**3.12**), DPEphos (Bis(2-diphenylphosphinophenyl)ether) (**3.13**) and xantphos (4,5-bis(diphenyl-phosphino)-9,9-dimethylxanthene). The third-generation contained hindered alkyl phosphines and trialkyl phosphines like P(*t*-Bu)$_3$ (**3.14**), $Ph_5FcP(t\text{-}Bu)_2$ (**3.15**),[14] *N*-heterocyclic carbenes, (biaryl)PR_2, (heterobiaryl)PR_2 and caged P(NRR´)$_3$. The most recent and fourth-generation of catalyst contains hindered ferrocenyl alkyl biphosphines. The first generation of ligands had β-hydride elimination as one of its major side products. The next three generations has almost eliminated that as one of the major concerns.

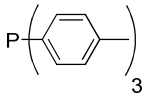
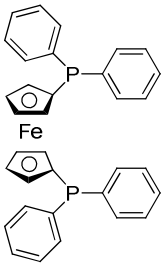
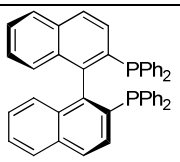
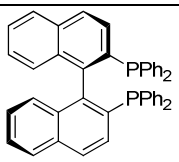
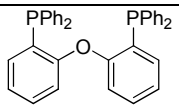
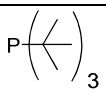
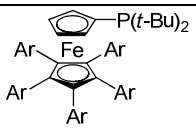
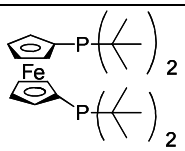
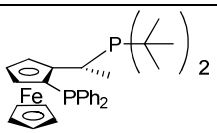
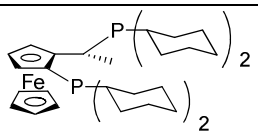
Various Generations of Ligands			
First Generation	 **3.10** p(o-tolyl)$_3$		
Second Generation	 **3.11** DPPF	 **(S)-3.12** (S)-BINAP  **(R)-3.12** (R)-BINAP	 **3.13** DPEphos
Third Generation	 **3.14** P(t-Bu)$_3$	 **3.15** $Ph_5FcP(t\text{-}Bu)_2$	
Fourth Generation (JosiPhos)	 **3.16**	 **3.17**	 **3.18**

Figure 3.1. Various ligands for Pd-catalyzed amination

3.3 Motivation for Pd-catalyzed amination

Additional motivation to explore Pd-catalyzed amination instead of the S_NAr was provided by the work done by Margolis et al. (Scheme 3.7).[15] They were successful in amination

of 4-chloroquinolines and 4-bromoquinolines (**3.19**) with primary amines via the Pd-catalyzed reactions. They observed that it was indeed a mild and convenient alternative to the conventional S_NAr methodology and obtained high yields too.

Their initial results using $Pd(OAc)_2$ as catalyst, DPEphos as ligand and K_3PO_4 as a base gave them encouraging results. Their initial screen of solvents gave them the best results with dioxane. Also, an initial screen of bases gave them good results with K_3PO_4. So they explored the possibility of various ligands. They screened an extensive number of ligands and they obtained the following results.

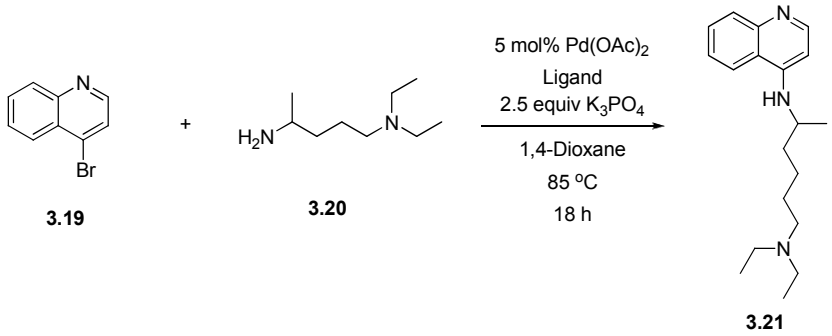


Table 3.1. Pd-catalyzed amination of 4-bromoquinoline with *N*1,*N*1-diethylpentane-1,4-diamine with various ligands[15]

entry[a]	ligand	% yield	entry[a]	ligand	% yield
1	DPEphos	95	10	dtbf	50
2	(R)-BINAP	94	11	PCy_2 biphenyl	50
3	$P(t\text{-}Bu)_3$	92	12	CTC-Q-Phos	49
4	S-Phos	85	13	PPh_2NMe_2 biphenyl	44
5	DPPF	84	14	PAPPSI	40
6	DavePhos	73	15	(IPr)Pd(acac)Cl	37
7	PCy_2Me biphenyl	71	16	X-Phos	18
8	Xantphos	64	17	$P(t\text{-}Bu)_2Me$ biphenyl	11
9	dippf	58	18	PPh_3	5

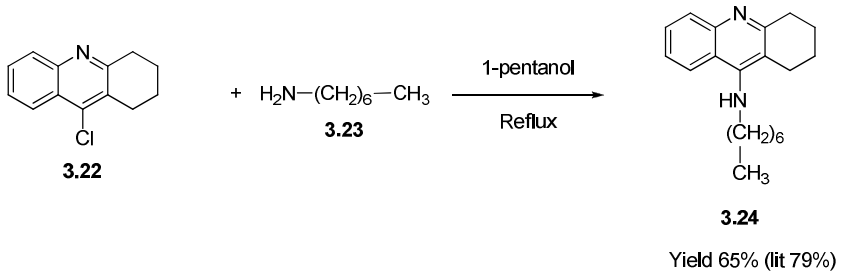
[a] Each reaction ran for 18 h at 85 °C. 20 mol % of ligand was used for monodentate ligands and 10 mol % for bidentate ligands. Yields as detected by quantitative HPLC

Good results obtained by them with these conditions and DPEphos (Table 3.1 entry 1) and (R)-BINAP (Table 3.1 entry 2) as ligands made us explore into the possibility of using these condition for 9-chloro-1,2,3,4-tetrahydroacridine.

3.4 *N*-alkylation by Pd-catalysis

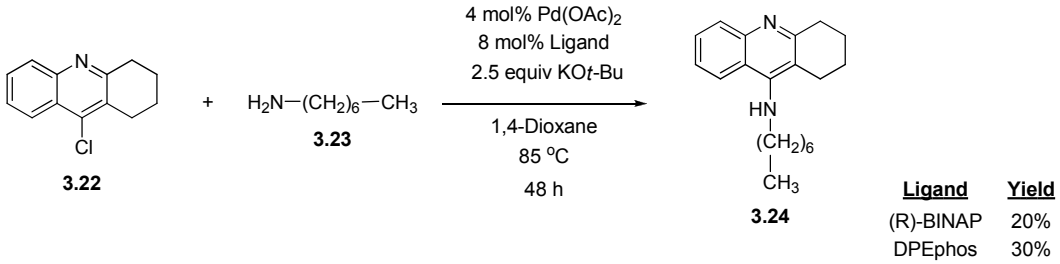
We recognized that there was an exhaustive number of permutations possible involving various solvents, bases and ligands. We therefore took the above mentioned reference as our starting point in determining the suitable combination of catalyst, ligand, solvent and base for our substrates of 9-chloro-1,2,3,4-tetrahydroacridine and *n*-heptyl amine. Initial results obtained with these conditions were encouraging as we did observe some product. Even though low yields were obtained, it gave us some starting point to move ahead from.

Amination of 9-chloro-1,2,3,4-tetrahydroacridine was initially performed by Carlier and co-workers[16] with *n*-heptylamine in refluxing 1-pentanol with a 89% yield. Thus initially a reaction was set up using classical methods of S_NAr using this procedure.[16] 9-chloro-1,2,3,4-tetrahydroacridine was reacted with *n*-heptylamine under refluxing pentanol (Scheme 3.6) and product **3.24** was obtained in a 65% yield.



Scheme 3.6. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *n*-heptylamine by S_NAr methods[16]

Our aim was to improve upon the yield of this reaction and to have much milder conditions too. So using palladium-catalysis using $Pd(OAc)_2$ as catalyst, KO*t*-Bu as base and 1,4-dioxane as solvent, a reaction was set up (Scheme 3.7) and product **3.24** was formed with a yield of 20% with R-BINAP and 30% with DPEphos.



Scheme 3.7. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *n*-heptylamine by Pd-catalysis with R-BINAP and DPEphos as ligands

Since acceptable yields were not obtained with either of the ligands, our next target was to change the base and observe if the change of base has some effect on improvement of the yield of the reaction. So we decided to use the base that gave Margolis et al. a better yield and so we switched to K_3PO_4. Upon examining the reaction with the change of base, and other conditions remaining the same, the reaction still yielded low amounts of product. The yield of **3.24** slightly improved to a 40% but it was still lower than expectation.

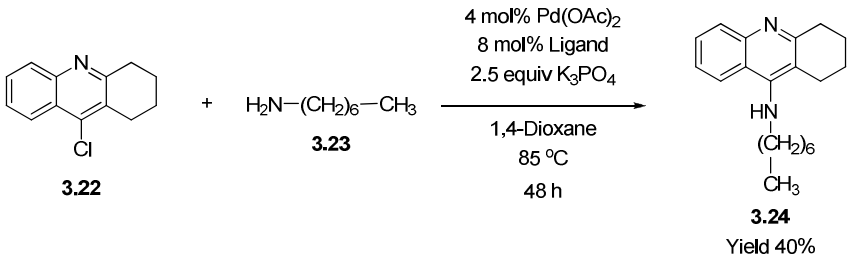
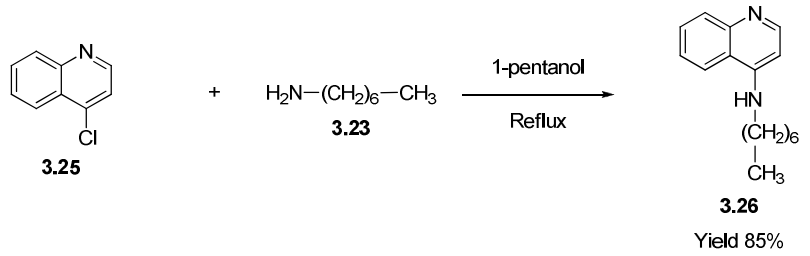


Table 3.2. Reactions of 9-chloro-1,2,3,4-tetrahydroacridine with *n*-heptylamine by S_NAr and Pd-catalysis

Entry	Catalyst	Loading	Ligand	Loading	Base	Solvent	T[°C]	t [h]	yield [%]
1	-	-	-	-	-	1-pentanol	140	18	65
2	$Pd(OAc)_2$	4	R-BINAP	8	K-O^{t}Bu	1,4-dioxane	85	18	20
3	$Pd(OAc)_2$	4	DPEphos	8	K-O^{t}Bu	1,4-dioxane	85	18	30
4	$Pd(OAc)_2$	4	DPEphos	8	K_3PO_4	1,4-dioxane	85	48	40
5	$Pd(OAc)_2$	4	DPEphos	8	K_3PO_4	toluene	85	48	0

Having low success with these results made us wonder why Margolis et al. obtained good yields with the same conditions but gave us lower yields. Our initial assumption was that the 9-chloro-1,2,3,4-tetrahydroacridine was a much more hindered substrate than 4-chloroquinoline. Thus the steric bulk of the 9-chloro-1,2,3,4-tetrahydroacridine was the major difficulty for this reaction. Keeping this in mind, we changed the aryl halide to 4-chloroquinoline to see how well the amine worked with it.

We initially set up the reaction of 4-chloroquinoline (Scheme 3.8) with *n*-heptyamine by the classical technique of S_NAr. Product **3.26** was formed in a better yield of 85% over the 65% obtained with *N*-alkylation of 9-chloro-1,2,3,4-tetrahydroacridine with *n*-heptylamine.



Scheme 3.8. 4-chloroquinoline reacted with *n*-heptylamine by S_NAr methods

Thus we explored this reaction using palladium-catalysis. We obtained just a slight increase in the yield from 40% of **3.24** to 50% of **3.26** with 4-chloroquinoline. This reaction took upto 48 h for the starting material to get completely consumed.

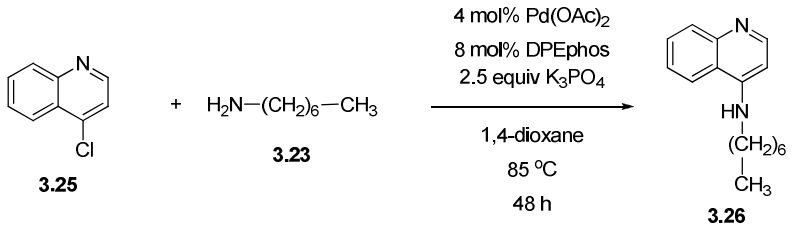
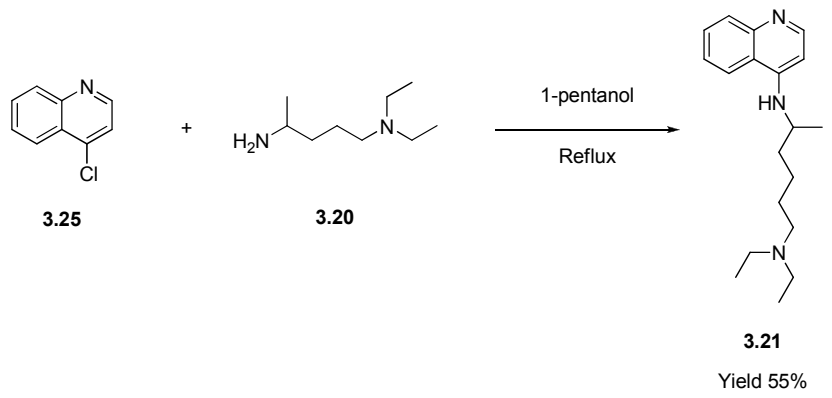


Table 3.3. Reaction of 4-chloroquinoline with *n*-heptylamine

Entry	Catalyst	Loading	Ligand	Loading	Base	Solvent	T [°C]	t [h]	Yield [%]
1	-	-	-	-	-	1-pentanol	140	18	85
2	$Pd(OAc)_2$	4	DPEphos	8	K_3PO_4	1,4-Dioxane	85	18	16
3	$Pd(OAc)_2$	4	DPEphos	8	K_3PO_4	1,4-Dioxane	85	48	50

Since this combination did not give optimum results, we wondered if the structure of the amine was not optimum for these reaction conditions. It is possible that the straight chain primary amine caused diarylation or beta hydride elimination, leading to low yields. Thus we changed the amine to the amine **3.20** mentioned in the paper by Margolis et al. First we tried to synthesize the combination of 4-chloroquinoline with *N*1,*N*1-diethylpentane-1,4-diamine by the classical S_NAr. 55% yield of the desired product **3.21** was obtained, which is somewhat lower that the yield obtained with *n*-heptylamine.



Scheme 3.9. 4-chloroquinoline reacted with *N*1,*N*1-diethylpentane-1,4-diamine by S_NAr methods

We then tried this combination by the Pd-catalyzed process as reported in the work of Margolis *et al.*[15] We were pleased to receive a nearly quantitative yield of the desired product **3.21**, as reported in the original study.[15]

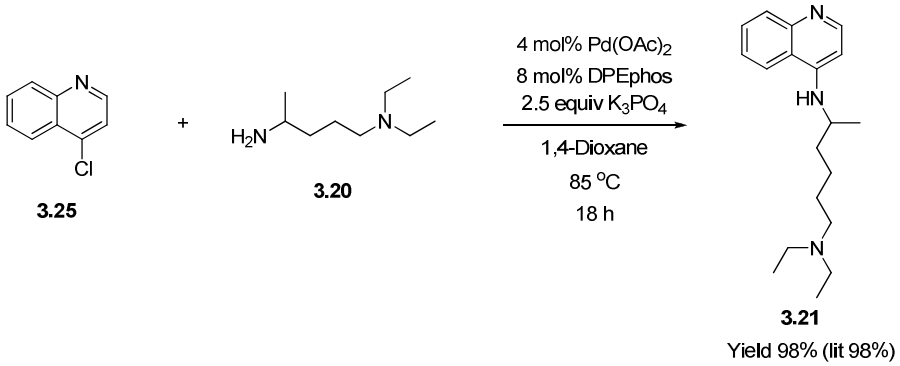
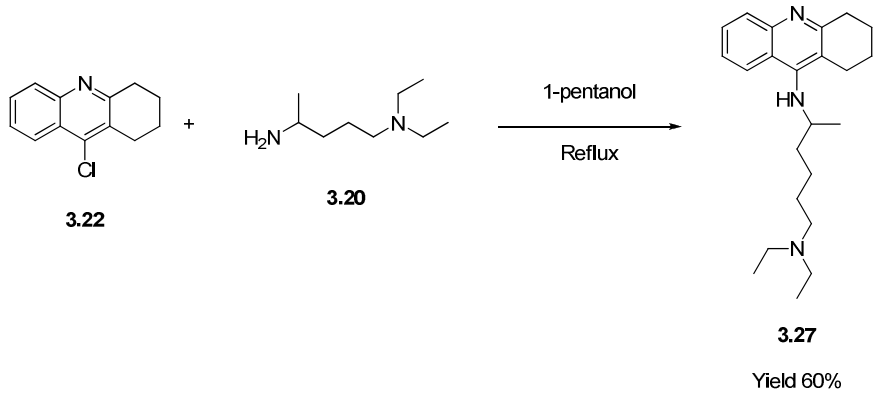


Table 3.4. Reaction of 4-chloroquinoline with *N*1,*N*1-diethylpentane-1,4-diamine

Entry	Catalyst	Loading	Ligand	Loading	Base	Solvent	T [°C]	t [h]	Yield [%]
1	-	-	-	-	-	1-pentanol	140	18	55
2	$Pd(OAc)_2$	4	DPEphos	8	K_3PO_4	1,4-Dioxane	85	48	98

Encouraged by these results, we thought it might be the primary unhindered amine **3.23** that may be causing the lower yields. Thus we changed the amine to *N*1,*N*1-diethylpentane-1,4-diamine for *N*-alkylation of 9-chloro-1,2,3,4-tetrahydroacridine. First we tried amination of

*N*1,*N*1-diethylpentane-1,4-diamine (**3.20**) and 9-chloro-1,2,3,4-tetrahydroacridine by the classical means of S_NAr and obtained a 60% yield of **3.27**.



Scheme 3.10. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *N*1,*N*1-diethylpentane-1,4-diamine by S_NAr method

We then went ahead and tried the new amine with 9-chloro-1,2,3,4-tetrahydroacridine by the Pd-catalyzed method. However the results were not as good as hoped. We obtained the product **3.23** in a low yield of 27%.

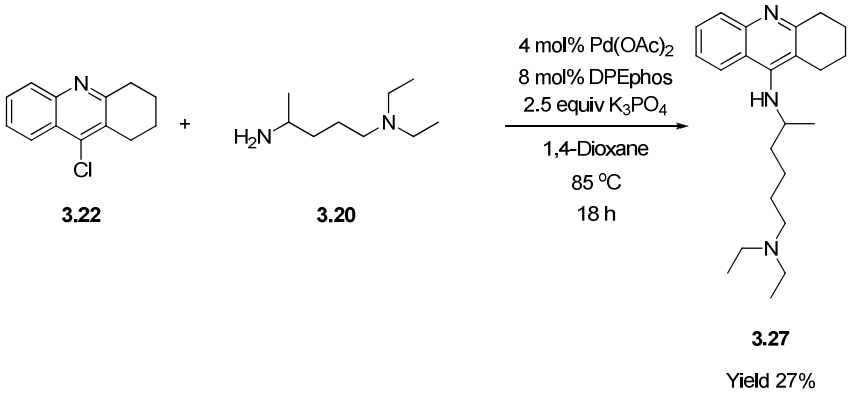
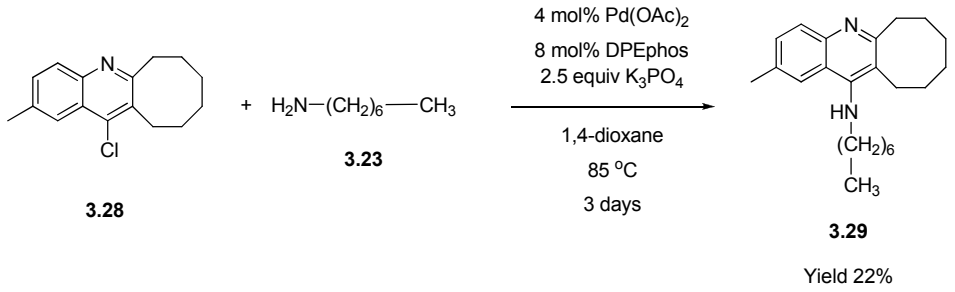


Table 3.5. Reaction of 9-chloro-1,2,3,4-aminoacridine with *N*1,*N*1-diethylpentane-1,4-diamine

Entry	Catalyst	Loading	Ligand	Loading	Base	Solvent	T [°C]	t [h]	Yield [%]
1	-	-	-	-	-	1-pentanol	140	18	60
2	$Pd(OAc)_2$	4	DPEphos	8	K_3PO_4	1,4-dioxane	85	18	27
3	$Pd(OAc)_2$	4	R-BINAP	8	K_3PO_4	1,4-dioxane	85	18	14

Even though bad yields were obtained by this combination of catalyst, ligand, base and solvent, we still wanted to try out how this reaction would work for 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (**3.28**) and *n*-heptylamine (**3.23**). The reaction was run for 3 days and yielded 22% of product **3.29** with a 60% recovery of starting material **3.28** (Scheme 3.11).



Scheme 3.11. 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline reacted with *n*-heptylamine by Pd-catalysis

These results were a bit encouraging because when this reaction was tried out by the classical means of S_NAr, it yielded no product in 1-pentanol reflux after 3 days. To raise the temperature, the solvent was changed to 1-octanol which boils at 195 ^oC. When 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (**3.28**) was reacted with *n*-heptylamine in 1-octanol and refluxed, it gave multiple spots on a TLC plate and we were unable to separate them for analysis. Thus obtaining even a 22% yield of **3.29** with Pd-catalysis meant that there is some scope for this technique.

3.5 Possible reasons for poor results

So after trying every combination of aryl halide and amine and still not obtaining the desired results, we started thinking as to what could be the reasons for not obtaining higher

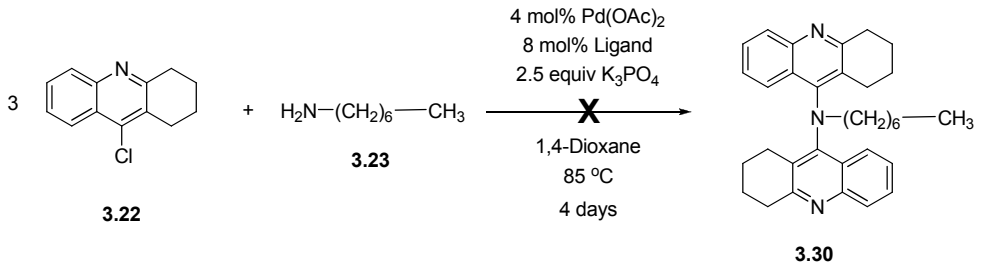
yields. One possibility is that the steric bulk of the 9-chloro-1,2,3,4-tetrahydroacridine was causing yields to be lowered. Di-arylation is also one of the possible outcome of Pd-catalysis which could lower the yield of the desired mono-arylated product. A third reason could be deduced from the mechanism. It could be that β-hydride elimination is taking place and thus yield of the desired product is lowered. The final reason that one could think of was that the choice of ligand was improper and a change of ligand would be needed to obtain higher yields.

3.5.1 Steric bulk

So we set out to explore which problem was lowering our yields. Nothing could be done about the steric bulk as 9-chloro-1,2,3,4-tetrahydroacridine is our aryl halide and the final goal is the *N*-alkylation of 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (**3.28**) which is even more sterically demanding. Thus we moved on to exploring our next concern.

3.5.2 Di-arylation

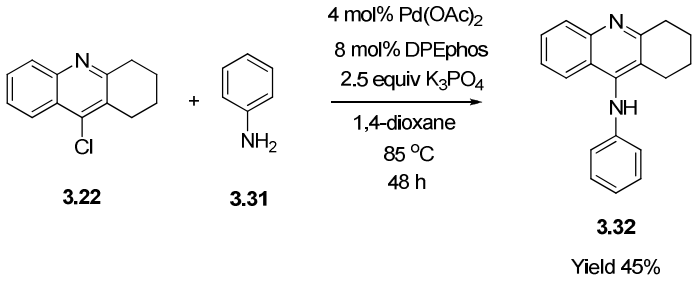
Diarylation was one of the major concerns when Pd-catalyzed amination was first discovered and tried out with primary amine. This problem arises as the mono-arylated species is more reactive than the starting material. Thus the reaction would not stop at the mono-arylated stage but further go on to for the di-arylated product. Although this seems highly unlikely to happen in our case as our starting material, 9-chloro-1,2,3,4-tetrahydroacridine is bulky to undergo diarylation. But still we explored the possibility (Scheme 3.12) and confirmed that indeed diarylated product **3.30** is definitely not forming with 9-chloro-1,2,3,4-tetrahydroacridine.



Scheme 3.12. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *n*-heptylamine when trying to diarylate

3.53 β-hydride Elimination

So our next problem was β-hydride elimination. As previously noted, since the development of the second generation of catalysts, the problem of β-hydride elimination was almost completely solved.[17] But in cases of some amine and aryl chloride combinations β-hydride elimination still remains a concern. To eliminate any possibility of this in our reaction, we used an amine without any α-proton and saw the effects on the yield of the reaction. If this reaction gave higher yields, it would mean that the other reactions suffered through this problem. But it was not the case. Reaction of 9-chloro-1,2,3,4-tetrahydroacridine with aniline (Scheme 3.13) yielded a 45% yield of **3.32** which is not a big improvement over the reaction with *n*-heptylamine.

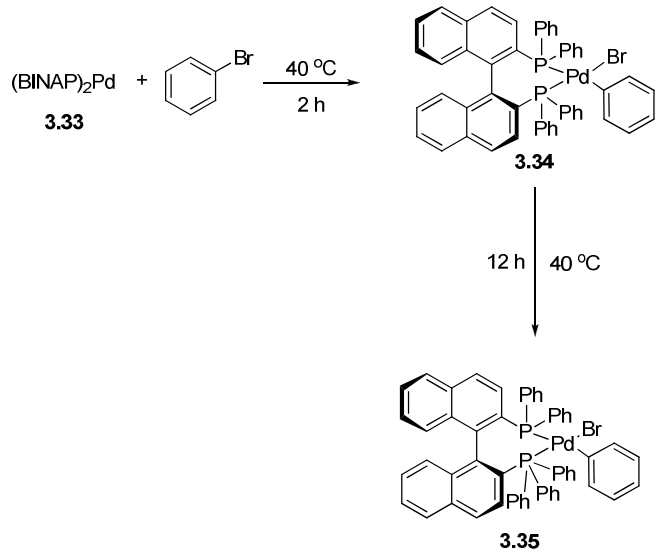


Scheme 3.13. 9-chloro-1,2,3,4-tetrahydroacridine reacted with aniline by Pd-catalysis

We ruled out di-arylation as no di-arylated product (**3.30**) (Scheme 3.13) was obtained after 3 days. Similarly a 45% yield of **3.32** with aniline (Scheme 3.14) showed that β-hydride elimination was not the actual problem. The only one remaining was that the ligand used was not proper. A wide variety of ligands have been described in the literature. To narrow it down to the exact ligand is almost an impossible task. But we figured that since the second generation of ligands did not work for the *N*-alkylation, why not try the latter generations. As described earlier, the fourth generation of ligands contain hindered ferrocenyl alkyl bisphosphines.[13]

3.6 Change in strategy

The ferrocenyl ligands (**3.16-3.18**) were known since 1983,[18] and are readily prepared by dilithiation of ferrocene, followed by quenching with $ClP(t\text{-}Bu)_2$. But they were rarely studied, prior to 1998, for the Pd-catalyzed amination processes. Aryl halide aminations were some of the first coupling reactions of aryl chlorides at low temperatures.[19] Very few mechanistic studies were conducted prior to 2001 on Pd-catalyzed reactions. The only studies available were on oxidative addition of aryl chlorides to a modestly active Pd(0) species.[20] Oxidative addition is usually the turnover limiting step for the Pd-catalysis and hence the rate determining step.[21] Studies by the Hartwig group have shown that oxidative addition involves a complete dissociation of the chelating phosphine ligands to produce a bent Pd(0)L (**3.35**) chelate complex.[22] Thus, we do not need a tight binding of alkyl phosphines to the palladium species as it will result in deceleration of the oxidative addition step since it would disfavor generation of active Pd(0)L. Sterically hindered alkylphosphine ligands will provide the requisite electron rich metal center and thus favouring the generation of the active Pd(0)L chelate complex.[19a]



Scheme 3.14. Oxidative addition step of Pd-catalysis

Hamann and Hartwig were among the first ones to study the use of ferrocene ligands for Pd-catalyzed amination.[19a] They observed the following results (Table 3.6).

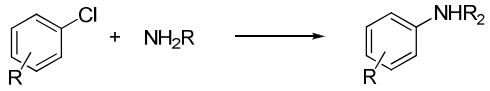
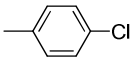
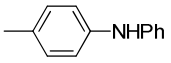
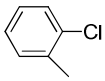
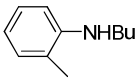
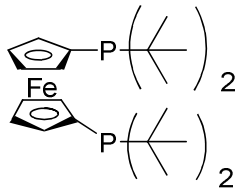


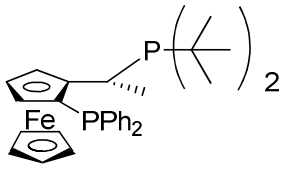
Table 3.6. Hindered chelating alkylphosphines in the Pd-catalyzed amination of aryl chlorides

Entry	Amine	ArX	Product	Catalyst	Conditions	Yield
1	NH_2Ph			3 mol% Pd(dba)/**3.16**	110 °C, 24 h	93%
2	"			3 mol% Pd(dba)/**3.16**	110 °C, 4 h	93%
3	"			2 mol% Pd(dba)/**3.17**	110 °C, 16 h	99%
4	"			1 mol% Pd(OAc)/**3.17**	85 °C, 12 h	92%
5	"			2 mol% Pd(dba)/**3.18**	110 °C, 16 h	96%
6	NH_2Bu			1 mol% Pd(dba)/**3.16**	110 °C, 24 h	57%
7	"			1 mol% Pd(OAc)/**3.17**	85 °C, 2 h	89%
8	"			1 mol% Pd(dba)/**3.18**	85 °C, 2 h	94%

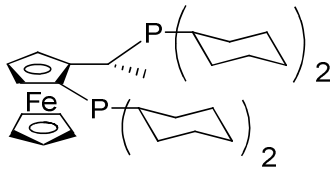
1.0 equiv of aryl halide, 1.2 equiv of amine, and 1.2 equiv of NaO*t*-Bu were used alongwith above specified catalysts in toluene solvent.



3.16



3.17



3.18

Using the above mentioned catalysts, they tested for selectivity of monoarylated product vs the diarylated product. They observed that catalyst 2 (**3.17**) was the best for selectivity of monoarylated product (Table 3.7)

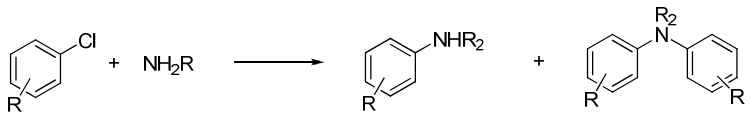


Table 3.7. Effect of ligand on monoarylation vs diarylation

Ligand	Amine (RNH_2)	HNArR : NAr_2R
3.16	R=Bu	3.3:1
3.17	R=Bu	130:1
3.18	R=Bu	30:1
3.17	R=Ph	Diarylation product not detected

Thus we were encouraged by these results and thought a hindered chelating alkylphosphine (JosiPhos) ligand would still be suited for our starting material. But we were looking for a JosiPhos catalyst that would work for a pyridinyl or quinolinyl starting material.

An exceptionally hindered and an electron donating version of a JosiPhos ligand was considered as an ideal ligand for our substrate. Ferrocenyl-1-ethyl backbone (CyPF*t*Bu) (**3.17**) is such a ligand which is air stable in solution and also as a solid. The steric property of this ligand, as considered by Hartwig, would improve the selectivity for monoarylation of primary amines.[23] Its electron donating capacity would improve chelation which could create a catalyst that would be more stable to displacement of the ancillary ligand by primary amines and improve their overall turnover number. Also this chelation would prevent the displacement of the phosphine by

basic heterocycles such as pyridines, quinolines and tacrine in our case. Thus if a ligand was bidentate and one which possessed a backbone that preorganized the two phosphines towards metal binding, then it would stay bound to the palladium species despite the steric demands of the substrate, which is also important in our case as 9-chloro-1,2,3,4-tetrahydroacridine is very sterically demanding. This preorganization was provided by CyPF*t*Bu (**3.17**).

Thus CyPF*t*Bu (**3.17**) was chosen for three basic advantages it provided over several other ligands. First, it would provide a high turnover number, which would result in lowering the cost of the process. Second, since primary amines are used in our research, we wanted a ligand that would have a high selectivity for monoarylation over diarylation. The bulky CyPF*t*Bu was perfect for this job. Third, it was sought that the ligand species should work well with pyridinyl or quinolinyl type of system as tacrine had a similar framework. This ligand was suitable for such basic heteroaryl halides.

A study by Hartwig and co-workers determined what catalyst, ligand, base and solvent combination would be ideal for a basic heteroaryl halide as in pyridines and quinolines (Scheme 3.23).[24] They noted that the reactions catalyzed by complex generated from $Pd(OAc)_2$ were much faster than those generated from $Pd(dba)_2$ or $PdCl_2(PhCN)_2$. A reaction which was catalyzed by $Pd(OAc)_2$ and CYPF*t*Bu finished within an hour and was needed a low catalyst loading of only 0.05%. For the base, they suggested using NaO*t*-Bu. Reaction which used NaO*t*-Bu as base occurred in high yield, while the reactions of the heteroaryl chloride with weaker bases as Cs_2CO_3, K_2CO_3, K_3PO_4 led to low or no product conversions. This result gave us a confirmation why our reaction was not yielding higher conversions with K_3PO_4. Also, reactions which used NaO*t*-Bu as base, were faster in DME (dimethoxyethane) as compared to those run

in THF or 1,4-dioxane. Again, these results were not encouraging as we used 1,4-Dioxane as a solvent. Results obtained by them were as follows (Table 3.8).

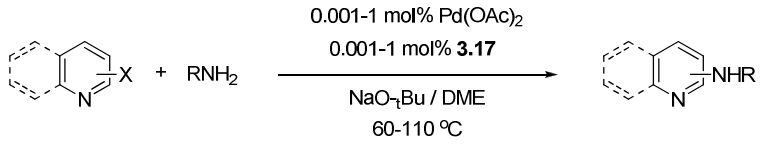


Table 3.8. Coupling of heteroaryl halides with primary amines catalyzed by $Pd(OAc)_2$ and CyPF*t*Bu[24b]

Entry	Ar	X	R	Cat. (%)	Conditions	Yield (%)
1	2-Py	Cl	Octyl	0.001	110 °C, 48 h	86
2	3-Me-2-Py	Cl	Octyl	0.005	90 °C, 24 h	96
3	3-Py	Cl	Octyl	0.005	90 °C, 24 h	93
4	4-Py	Cl	Octyl	0.01	90 °C, 24 h	83
5	3-quinolinyl	Cl	Cyclohexyl	1.0	100 °C, 48 h	60
6	1-iso-quinolinyl	Cl	Octyl	0.005	90 °C, 15 h	91

Reactions were conducted with 1:1 ratio of metal to ligand. 1mmol of aryl halide, 1.2 equiv amine, and 1.4 equiv NaO*t*-Bu in 1 mL DME.

Also a study of various Josiphos ligands yielded that CyPF*t*Bu is the best JosiPhos ligand among the others (Table 3.9)

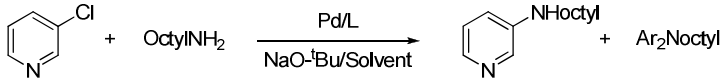
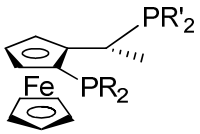


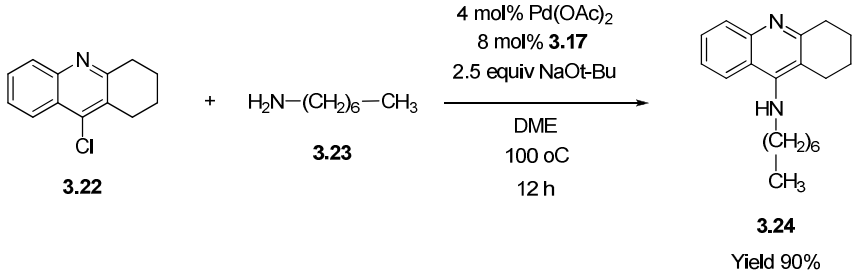
Table 3.9. Comparison of activity of Josiphos analogs for coupling of heteroaryl chloride with primary alkylamine[24b]



Compound no.	R	R'	Common name
3.17	Cy	^{t}Bu	CyPF-^{t}Bu
3.36	Ph	^{t}Bu	PPF-^{t}Bu
3.37	Me	^{t}Bu	MePF-^{t}Bu
3.38	Et	^{t}Bu	EtPF-^{t}Bu
3.39	Cy	Cy	CyPF-Cy
3.40	Cy	Ph	CyPF-Ph
3.41	^{t}Bu	Cy	^{t}BuPF-Cy

Entry	Ligand	Loading	Temp. (^{o}C)	Time (h)	Yield (%)
1	**3.17**	0.005	90	24	93
2	**3.36**	1.0	90	24	67
3	**3.37**	0.005	90	24	<5
4	**3.38**	0.005	90	24	<5
5	**3.39**	1.0	90	24	46
6	**3.40**	1.0	90	24	48
7	**3.41**	0.005	90	24	62

So we discovered that there is a great scope in CyPF*t*Bu (**3.17**) as a catalyst, NaO*t*-Bu as base and DME as solvent. These were the ideal conditions to use for a basic heteroaryl halide, specifically chloride, which we have in our starting material. So having discovered this, we tried out this new combination. The reaction yielded a satisfyingly high 90% yield of **3.19** with these new reagents.

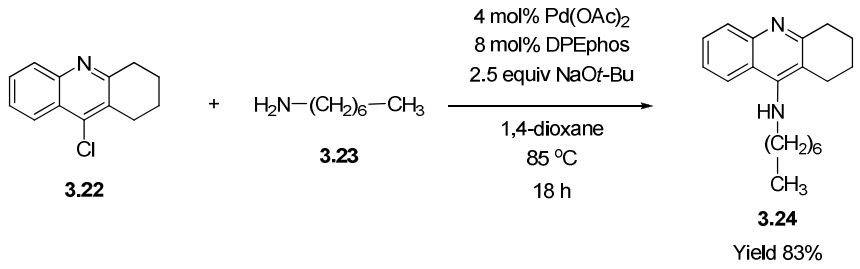


Scheme 3.15. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *n*-heptylamine by Pd-catalysis with CyPF*t*Bu as ligand, DME as solvent and NaO*t*-Bu as base

Thus being encouraged by these results, we knew this combination worked the best for our starting material. However the change in ligand, base or the solvent could have resulted in better yields. To figure out which of these three conditions resulted in the high yields, one of them is changed at a time.

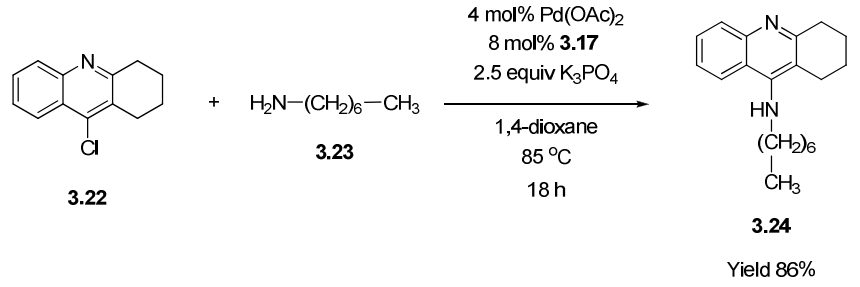
Initially the base was changed from K_3PO_4 to NaO*t*-Bu. As suggested by Hartwig,[17] K_3PO_4 was a much weaker base to be useful for palladium catalysis and thus a stronger base

such as NaO*t*-Bu was needed for this reaction. Keeping the other conditions the same as before and just changing the base to NaO*t*-Bu resulted in a yield of 83% of **3.24**.



Scheme 3.16. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *n*-heptylamine by Pd-catalysis with NaO*t*-Bu as base

Next the ligand was changed to CyPF*t*Bu (**3.17**) and all other conditions were kept exactly the same to note the effects of change in ligand on the reaction. And the reaction did yield a high 86% yield (Scheme 3.17) of product **3.24**.

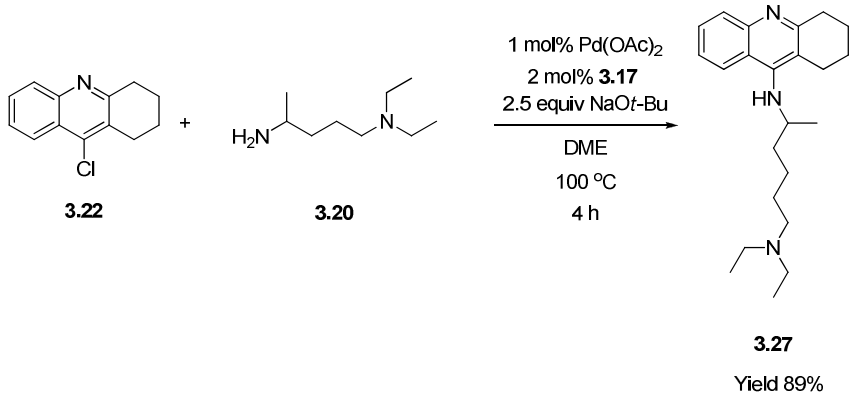


Scheme 3.17. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *n*-heptylamine by Pd-catalysis with CYPF-^{t}Bu as ligand

Table 3.10. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *n*-heptylamine by Pd-catalysis

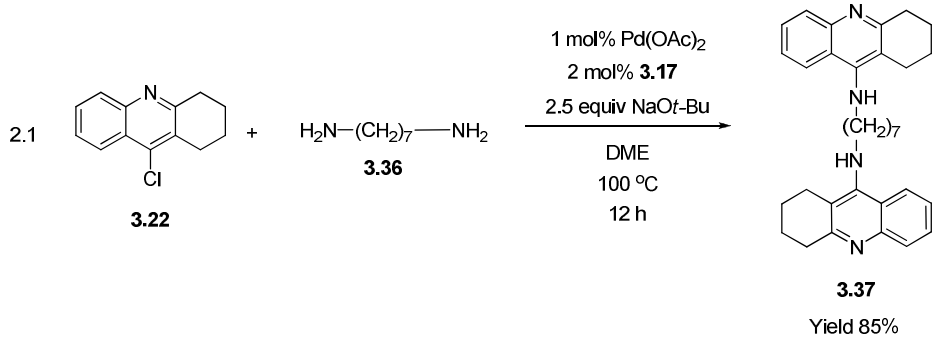
Entry	Catalyst	Loading	Ligand	Loading	Base	Solvent	T[^{o}C]	t [h]	yield [%]
1	$Pd(OAc)_2$	4	**3.13**	8	Na-O^{t}Bu	1,4-Dioxane	85	48	83
2	$Pd(OAc)_2$	4	**3.17**	8	K_3PO_4	1,4-Dioxane	85	48	86
3	$Pd(OAc)_2$	1	**3.17**	2	Na-O^{t}Bu	DME	100	12	90

Thus even though just a change in base (Scheme 3.16) or ligand (Scheme 3.17) affected the yield a lot, the best combination was where the ligand, base and solvent were changed simultaneously (Scheme 3.15). Now this new combination was tried with *N*1,*N*1-diethylpentane-1,4-diamine (**3.20**). The reaction which yielded only a 27% yield of **3.27** in 18 h with the previous combination (Scheme 3.18) yielded a 89 % of the product **3.27** in only 4 h. Thus this combination of ligand, base and solvent is best suited for tacrines.



Scheme 3.18. 9-chloro-1,2,3,4-tetrahydroacridine reacted with *N*1,*N*1-diethylpentane-1,4-diamine by Pd-catalysis with CyPF*t*Bu as ligand, DME as solvent and NaO*t*-Bu as base

After finding an ideal combination for Pd-catalysis, the next task was to dimerize and see how two tacrine units would attach to a diamine. 2.1 equiv of tacrine were reacted with 1 equiv of the 1,7-diaminoheptane (**3.36**) to see if it dimerizes. The reaction was done in 12 h and yielded an excellent 85 % yield of product **3.37**.



Scheme 3.19. 9-chloro-1,2,3,4-tetrahydroacridine reacted with 1,7-diaminoheptane by Pd-catalysis with CyPF*t*Bu as ligand, DME as solvent and NaO*t*-Bu as base

3.7 Conclusion

Even though Pd-catalyzed amination results did not turn out as well as expected initially, a change in ligand, base and solvent did eventually yield good results. Proper choice of ligand is the key to successful Buchwald-Hartwig amination. Hindered chelating ferrocenyl ligands would be a proper choice for *N*-arylation of primary amines. Even though ligand plays a significant role in increasing the yields of the product, it is not the only factor that needs to be correct. The ideal combination of catalyst, ligand, base and solvent should be worked out for your own respective starting material.

Thus at the end, we were successful in obtaining the desired product in very good yields and a reasonable time. Amination of chloro compounds using S_NAr techniques requires high temperature and longer reaction times. As described in chapter 2, conversion of chloro analogs into fluoro analogs is not suitable for all starting material and requires an additional step which can lower the overall yield of the reaction. Formation of 9-chloro-1,2,3,4-tetrahydroacridine-*N*-oxide appeared to be a suitable technique to make the starting material more reactive for amination. However, as described in chapter 2, the *N*-oxide was not chemically stable and did not provide superior yields in S_NAr reactions with amine nucleophiles.

Thus our results confirm that Pd-catalyzed amination is a mild and convenient alternative to S_NAr methodology.

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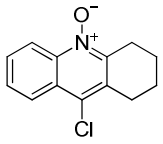
Chapter 4. Experimental Procedures

4.1 General information

9-chloro-1,2,3,4-tetrahydroacridine was synthesized in the Carlier group by Dr. Larry Williams. All other reagents were received from commercial sources and used directly. All the known compounds were compared with the literature data. NMR analysis was performed at 500MHz for ^{1}H NMR and at 125MHz for ^{13}C NMR. The mass analysis was done using Agilent LC-ESI-TOF with accurate mass capability.

4.2 Synthesis of functionalized 9-chloro-1,2,3,4-tetrahydroacridine



2.14

9-chloro-1,2,3,4-tetrahydroacridine-10-oxide (**2.14**): To a 50 mL round bottom flash equipped with a magnetic stirrer was added 9-chloro-1,2,3,4-tetrahydroacridine (0.5 g, 2.3 mmol) and m-chloroperoxybenzoic acid (mCPBA) (1.6 g, 4.6 mmol) and stirred in dichloro methane (DCM) for 15 mins. The reaction mixture was then added to a 0.5 M sodium hydroxide solution and extracted with ethyl acetate. Two brine washes are given to the extracted mixture and the solvent

is evaporated using rotary evaporator. The crude reaction mixture was re-crystallized in hexanes to yield 0.503 g (94%) of a bright crystalline solid.

^{1}H NMR (500 MHz, CD_3OD) δ 8.75 (d, *J* = 8.3 Hz, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 7.73 (t, *J* = 7.8 Hz, 1H), 7.65 (t, *J* = 7.6 Hz, 1H), 3.18 (t, *J* = 6.5 Hz, 2H), 2.99 (t, *J* = 6.3 Hz, 2H), 1.98 – 1.91 (m, 2H), 1.90 – 1.83 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 147.63, 140.49, 130.23, 130.14, 129.11, 128.59, 126.29, 124.58, 119.88, 27.75, 26.59, 21.74, 21.53; MS (FAB+): Calc for $C_{13}H_{12}ClNO^{+}$: 233.06074. Found: 233.0593 (-6.36 ppm)

4.3 Synthesis of *N*-alkyltacrines and *N*-alkylquinolines

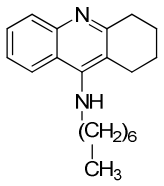
General procedures for the synthesis of N-alkyltacrines and N-alkylquinolines by S_NAr methods.[1]

To a 25 mL round bottom flask equipped with a magnetic stirrer, condenser and N_2 inlet, were added an appropriate amount of 9-chloro-1,2,3,4-tetrahydroacridine or 4-chloro-quinoline (1 equiv), below mentioned amine (1.5 equiv) and 1-pentanol. The reaction is stirred at reflux in an oil bath kept at 140 ^{o}C for 18 h. After the reaction is complete, the mixture is cooled down to room temperature. The reaction mixture is added to 0.5 M sodium hydroxide to neutralize the acids. Then the reaction mixture is extracted using ethyl acetate. This extract is washed twice with brine solutions. The solvent is then separated and evaporated to obtain the crude product. This crude product is chromatographed with 5% of methanol in dichloromethane and 7mL of ammonium hydroxide per liter of solvent to yield pure product.

General procedures for the synthesis of N-alkyltacrines and N-alkylquinolines by Pd-catalysis[2]

To a 10 mL round bottom flask equipped with a magnetic stirrer, condenser and N_2 inlet, were added an appropriate amount of 9-chloro-1,2,3,4-tetrahydroacridine (1 equiv), below mentioned

amine (1.5 equiv), palladium acetate (1 mole%), ligand (2 mole%), a base (2.5 equiv) and a solvent (4mL/mmol). This mixture is heated to reflux for the required time. Once the reaction is complete, the mixture is loaded directly on the chromatography column with 5% of methanol in dichloromethane and 7 mL of ammonium hydroxide per liter of solvent to yield pure product.



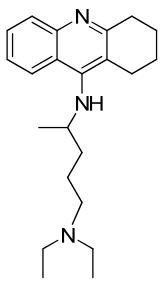
3.24

N-heptyl-1,2,3,4-tetrahydroacridin-9-amine (**3.24**):

By S_NAr methods: 9-chloro-1,2,3,4-tetrahydroacridine (0.1 g, 0.46 mmol) and n-heptylamine (0.08 g, 0.69 mmol) was combined in refluxing 1-pentanol. Work up and column chromatography yielded 0.1 g (65 %) of **3.24**.

By Pd-catalysis: 9-chloro-1,2,3,4-tetrahydroacridine (0.218 g, 1mmol), n-heptylamine (0.175 g, 1.5mmol), palladium acetate (0.002 g, 1 mol%), CyPF*t*Bu (0.01 g, 2 mol%), Na-O*t*Bu (0.24 g, 2.5 mmol) was combined in refluxing DME (4mL). Column chromatography yielded 0.264 g (90 %) of **3.24**

^{1}H and ^{13}C NMR data matched the literature values.[1]



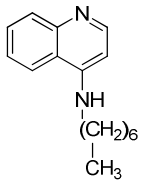
3.27

N1,N1-diethyl-N4-(1,2,3,4-tetrahydroacridin-9-yl)pentane-1,4-diamine (**3.27**):

By S_NAr methods: 9-chloro-1,2,3,4-tetrahydroacridine (0.218 g, 1 mmol) and *N*1,*N*1-diethylpentane-1,4-diamine (0.237 g, 1.5 mmol) was combined in refluxing 1-pentanol. Work up and column chromatography yielded 0.198 g (60 %) of **3.27**.

By Pd-catalysis: 9-chloro-1,2,3,4-tetrahydroacridine (0.218 g, 1mmol), *N*1,*N*1-diethylpentane-1,4-diamine (0.237 g, 1.5 mmol), palladium acetate (0.002 g, 1 mol%), CyPFtBu (0.01 g, 2 mol%), Na-O*t*Bu (0.24 g, 2.5 mmol) was combined in refluxing DME (4mL). Column chromatography yielded 0.288 g (85 %) of **3.27**.

^{1}H NMR (500 MHz, CD_3OD) δ 8.06 (d, *J* = 8.5 Hz, 1H), 7.77 (d, *J* = 9.0 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 8.1 Hz, 1H), 3.96 – 3.86 (m, 1H), 2.99 (t, *J* = 6.2 Hz, 2H), 2.78 (t, *J* = 6.0 Hz, 2H), 2.49 – 2.30 (m, 6H), 1.91 (tt, *J* = 13.3, 6.6 Hz, 4H), 1.72 – 1.38 (m, 4H), 1.28 (d, *J* = 6.4 Hz, 3H), 0.94 (t, *J* = 7.2 Hz, 5H). ^{13}C NMR (125 MHz, CD_3OD) δ 187.82, 157.64, 151.93, 146.04, 128.66, 126.29, 123.00, 122.94, 120.39, 116.57, 53.52, 52.07, 36.18, 32.56, 25.06, 22.65, 22.42, 20.96, 20.91, 9.54, 9.51; MS (FAB+): Calc for $C_{22}H_{33}N_3^+$: 339.27. Found: 339.26745 (+5.2 ppm)



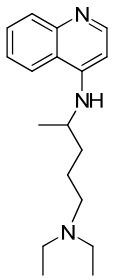
3.26

N-heptylquinolin-4-amine (**3.26**):

By S_NAr methods: 4-chloro-quinoline (0.164 g, 1 mmol) and n-heptylamine (0.173 g, 1.5 mmol) was combined in refluxing 1-pentanol. Work up and column chromatography yielded 0.202 g (85 %) of **3.26**.

By Pd-catalysis: 4-chloro-quinoline (0.164 g, 1mmol), n-heptylamine (0.173 g, 1.5 mmol), palladium acetate (0.009 g, 4 mol%), DPEphos (0.04 g, 8 mol%), K_3PO_4 (0.53 g, 2.5 mmol) was combined in refluxing 1,4-dioxane (4 mL). Colum chromatography yielded 0.12 g (50 %) of **3.26**.

^1H and ^{13}C NMR data matched the literature values.[1]



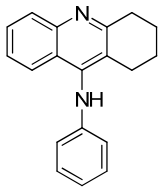
3.21

N1,N1-diethyl-N4-(quinolin-4-yl)pentane-1,4-diamine (**3.21**):

By S_NAr methods: 4-chloro-quinoline (0.164 g, 1 mmol) and *N*1,*N*1-diethylpentane-1,4-diamine (0.237 g, 1.5 mmol) was combined in refluxing 1-pentanol. Work up and column chromatography yielded 0.18 g (55 %) of **3.21**.

By Pd-catalysis: 4-chloro-quinoline (0.164 g, 1 mmol), *N*1,*N*1-diethylpentane-1,4-diamine (0.237 g, 1.5 mmol), palladium acetate (0.009 g, 4 mol%), DPEphos (0.04 g, 8 mol%), K_3PO_4 (0.53 g, 2.5 mmol) was combined in refluxing 1,4-dioxane (4mL). Column chromatography yielded 0.235 g (98 %) of **3.21**.

^1H and ^{13}C NMR data matched the literature values.[2a]

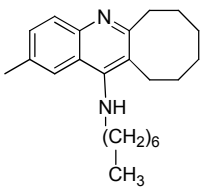


3.32

N-phenyl-1,2,3,4-tetrahydroacridin-9-amine (**3.32**):

By Pd-catalysis: 9-chloro-1,2,3,4-tetrahydroacridine (0.218 g, 1 mmol), aniline (0.14 g, 1.5 mmol), palladium acetate (0.009 g, 4 mol%), DPEphos (0.09 g, 8 mol%), K_3PO_4 (0.53 g, 2.5 mmol) was combined in refluxing 1,4-dioxane (4 mL). Column chromatography yielded 0.12 g (45 %) of **3.32**.

^1H and ^{13}C NMR data matched the literature values.[3]



3.29

N-heptyl-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinolin-12-amine (**3.29**)

By Pd-catalysis: 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (0.261 g, 1mmol), n-heptylamine (0.175 g, 1.5mmol), palladium acetate (0.009 g, 4 mol%), ligand (8 mol%), base (2.5 mmol) was combined in appropriate refluxing solvent (4 mL/mmol).

^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (s, 1H), 7.71 (d, *J* = 8.5 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 3.43 – 3.29 (m, 2H), 3.06 – 2.93 (m, 2H), 2.93 – 2.78 (m, 2H), 1.76 (s, 2H), 1.64 (d, *J* = 14.4 Hz, 2H), 1.59 (dd, *J* = 14.4, 7.5 Hz, 2H), 1.39 (s, 2H), 1.22 (dd, *J* = 17.4, 10.2 Hz, 11H), 0.82 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 161.30, 151.21, 145.29, 133.54, 130.51, 126.62, 122.64, 120.88, 119.71, 49.71, 34.96, 31.60, 30.97, 30.90, 29.51, 28.83, 26.72, 26.10, 25.85, 24.54, 22.34, 20.60, 13.19; MS (FAB+): Calc for $C_{22}H_{32}N_2^+$: 338.2722. Found: 338.27.15 (-1.95 ppm).

4.4 Synthesis of bivalent AChEI

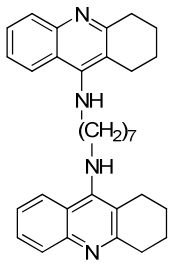
General procedures for the synthesis of bivalent AChEI by S_NAr methods.[4]

To a 25 mL round bottom flask equipped with a magnetic stirrer, condenser and N_2 inlet, were added an appropriate amount of 9-chloro-1,2,3,4-tetrahydroacridine or 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (2.5 equiv), 1,7-diaminoheptane (1 equiv) and 1-pentanol. The reaction is stirred at reflux in an oil bath kept at 140 ^{o}C for 18 h. After the reaction

is complete, the mixture is cooled down to room temperature. The reaction mixture is added to 0.5 M sodium hydroxide to neutralize the acids. Then the reaction mixture is extracted using ethyl acetate. This extract is washed twice with brine solutions. The solvent is then separated and evaporated to obtain the crude product. This crude product is chromatographed with 5% of methanol in dichloromethane and 7 mL of ammonium hydroxide per liter of solvent to yield pure product.

General procedures for the synthesis of bivalent AChEI by Pd-catalysis[2]

To a 10 mL round bottom flask equipped with a magnetic stirrer, condenser and N_2 inlet, were added an appropriate amount of 9-chloro-1,2,3,4-tetrahydroacridine or 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (2.5 equiv), 1,7-diaminoheptane (1 equiv), palladium acetate (2 mole%), ligand (4 mole%), a base (2.5 equiv) and a solvent (4mL/mmol). This mixture is heated to reflux for the required time. Once the reaction is complete, the mixture is loaded directly on the chromatography column with 5% of methanol in dichloromethane and 7mL of ammonium hydroxide per liter of solvent to yield pure product.



3.37

N1,N7-bis(1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (**3.37**)

By S_NAr methods: 9-chloro-1,2,3,4-tetrahydroacridine (0.3 g, 1.38 mmol) and 1,7-diaminoheptane (0.06 g, 0.46 mmol) was combined in refluxing 1-pentanol. Work up and column chromatography yielded 0.22 g (85 %) yield of **3.37**.

By Pd-catalysis: 9-chloro-1,2,3,4-tetrahydroacridine (0.218 g, 1mmol), 1,7-diaminoheptane (0.06 g, 0.46 mmol), palladium acetate (0.002 g, 2 mol%), CyPF*t*Bu (0.01 g, 4 mol%), Na-O*t*Bu (0.24 g, 2.5 mmol) was combined in refluxing DME (4mL). Column chromatography yielded 0.48 g (85 %) of **3.37**.

^{1}H and ^{13}C NMR data matched the literature value.[5]

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2. (a) Margolis, B. J.; Long, K. A.; Laird, D. L. T.; Ruble, J. C.; Pulley, S. R., Assembly of 4-aminoquinolines via palladium catalysis: A mild and convenient alternative to S_NAr methodology. *The Journal of Organic Chemistry* **2007,** *72*, 2232-2235; (b) Hamann, B. C.; Hartwig, J. F., Sterically hindered chelating alkyl phosphines provide large rate accelerations in palladium-catalyzed amination of aryl iodides, bromides, and chlorides, and the first amination of aryl tosylates. *Journal of the American Chemical Society* **1998,** *120*, 7369-7370.

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Chapter 5. Summary of thesis and future directions

5.1 Summary of thesis

A variety of methods were tried for the functionalization of 9-chloro-1,2,3,4-tetrahydroacridine. Having fluorine on the 9th position, in place of chlorine, would have expected to allow S_NAr at lower temperature and with faster rates. Upon applying this substitution to 12-chloro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline (**2.7)**, it would have enabled the 12-fluoro-2-methyl-6,7,8,9,10,11-hexahydrocycloocta[b]quinoline to react with various amines at moderate temperatures. However the absence of any electron withdrawing group present near the substituents made the transformation from chloro to fluoro extremely difficult and ultimately I did not achieve this goal.

The formation of the *N*-oxide of 9-chloro-1,2,3,4-tetrahydroacridine (**2.4**) would also have helped to accelerate S_NAr reaction. Even though the *N*-oxide of **2.4** was successfully synthesized in high yields, the instability of the *N*-oxide rendered the compound useless for further explorations. We thus abandoned this approach.

Since functionalization by S_NAr did not successfully work as planned, other means for amination had to be explored for obtaining aminated products at lower temperatures and faster rates of reaction. Many other techniques exist for amination, though the most promising of them all is Buchwald-Hartwig's Pd-catalyzed amination which is discussed in the chapter 3. Buchwald-Hartwig amination did prove out to be a successful technique for the amination of 9-chloro-1,2,3,4-tetrahydroacridine. Even though the results did not turn out as well as they were

expected initially, a change in ligand, base and solvent did yield good results. Proper choice of ligand is the key to successful Buchwald-Hartwig amination. Hindered chelating ferrocenyl ligands would be a proper choice for *N*-arylation of primary amine. Even though ligand plays a significant role in increasing the yields of the product, it is not the only factor that needs to be correct. The ideal combination of catalyst, ligand, base and solvent should be worked out for your own respective starting material. Thus at the end, we were successful in obtaining the desired product in very good yields and a reasonable time. These results confirm that Pd-catalyzed amination is a mild and convenient alternative to S_NAr methodology.

5.2 Future direction

It was established earlier that substitution at 6 position on the tacrine nucleus would increase the potency again human AChE inhibition and substitution at 7 position would decrease the inhibitory potency. [1]

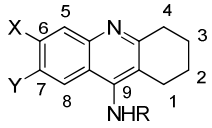
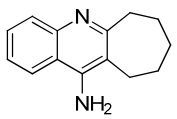


Figure 5.1. SAR of tacrine nucleus

Substitution at both the positions simultaneously would cancel out each other effect and the inhibitory potency would roughly be the same. Thus it is important to have substitution at the 6 position for increase in inhibitory potency of human AChE. Also it was established that the AChE can accommodate a more bulkier moiety.[1] Thus expanded carbocyclic congener like **5.1** would significantly increase the inhibitory potency again human AChE.

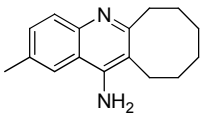


5.1

Figure 5.2. Expanded carbocyclic congener

Thus having both a substitution at 6 position and an expanded carbocyclic congener should significantly increase the inhibitory potency against human AChE. Also since dimerization with heptylene-linked alkyl tether drastically increases the inhibitory potency, I would dimerize these lead compounds using Pd-catalyzed amination. I would vary the various groups at 6 position ranging from a strong electron withdrawing group like $–NO_2$ to a strong electron releasing group like $–OCH_3$. I would also like to have different group of varying bulkiness at the 6-position, thus reflecting how much binding space is further available at the active site.

An interesting compound **5.2** was synthesized by Dr. Larry Williams in the Carlier group[2] which had a very low inhibitory potency against human AChE but a much greater inhibitory potency against *Anopheles gambiae* AChE (*Ag*AChE).



5.2

Figure 5.3. Interesting compound showing a high inhibitory potency against *Ag*AChE

Substitution at 7 position on the ring and having an eight membered expanded carbocyclic congener decreases the inhibitory potency against human AChE but it significantly increases the inhibitory potency against *Ag*AChE. This strategy can used to used synthesize a series selective AChEI. Having varying substitutions at 7 position ranging from electron withdrawing group like $–NO_2$ to a strong electron donating group $–OCH_3$ would give a wide

variety of compounds to try out. Also groups having different bulkiness would be tried out to find out how much extra space is available in the binding pockets of *Ag*AChE.

Thus I would be interested in developing a whole series of species selective AChEI which would be synthesized using the basic understanding of the different AChE in various species. Inhibition of human AChE would lead to the development of a palliative treatment for patients with AD. Inhibition of *Ag*AChE would help synthesizing a new form of insecticide which would be highly selective for the *Ag*AChE over human AChE, thus making it safe for human to use. This should decrease the rapid spread of malaria through the mosquito Anopheles gambiae.

1. Recanatini, M.; Cavalli, A.; Belluti, F.; Piazzi, L.; Rampa, A.; Bisi, A.; Gobbi, S.; Valenti, P.; Andrisano, V.; Bartolini, M.; Cavrini, V., SAR of 9-amino-1,2,3,4-tetrahydroacridine-based acetylcholinesterase inhibitors: Synthesis, enzyme inhibitory activity, QSAR, and structure-based CoMFA of tacrine analogues. *Journal of Medicinal Chemistry* **2000,** *43*, 2007-2018.

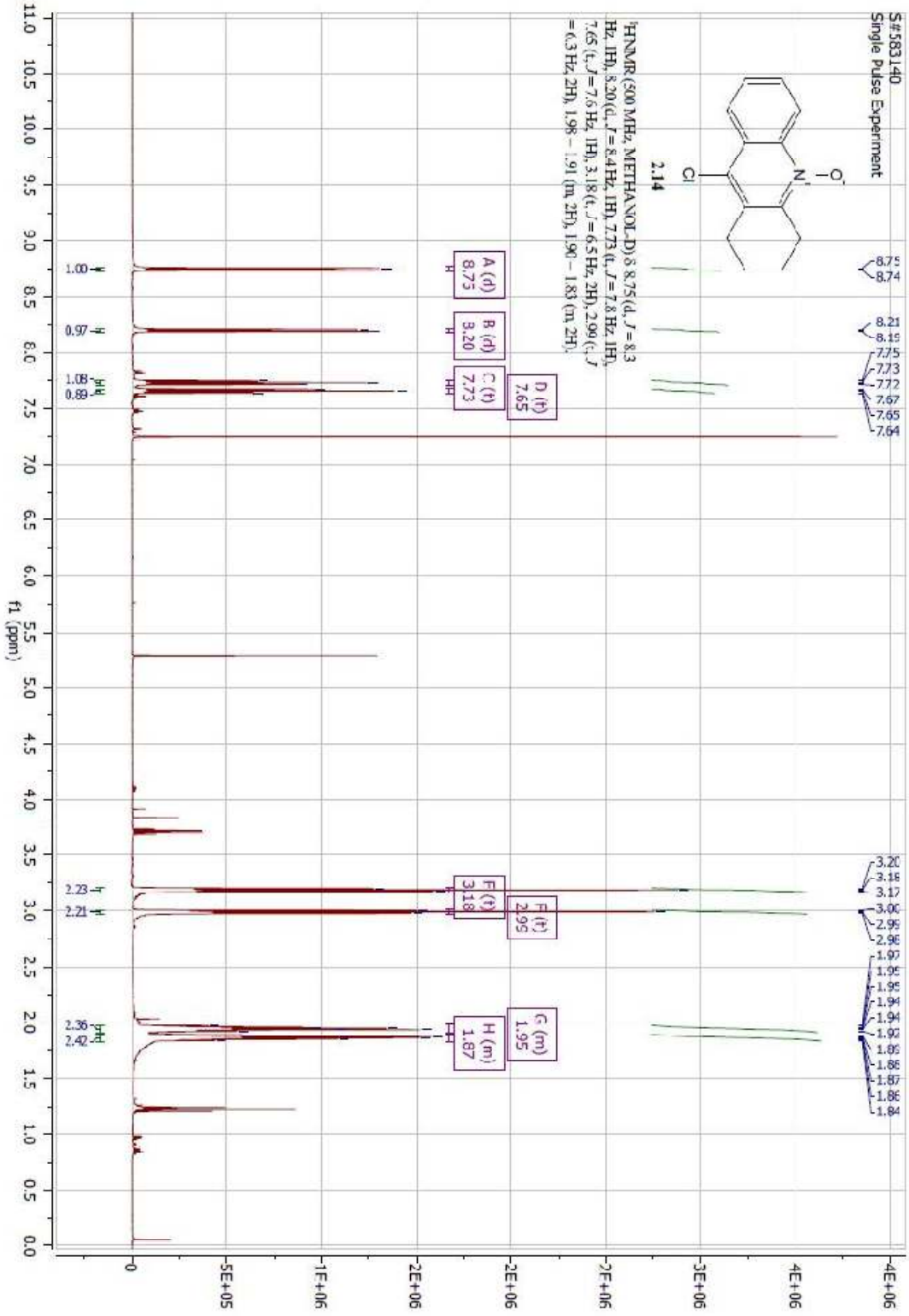
2. Williams, L. D. Experimental and computational investigation of tacrine-based inhibitor of acetylcholinesterase. Virginia Polytechnic Institute and State University, Blacksburg, 2008.

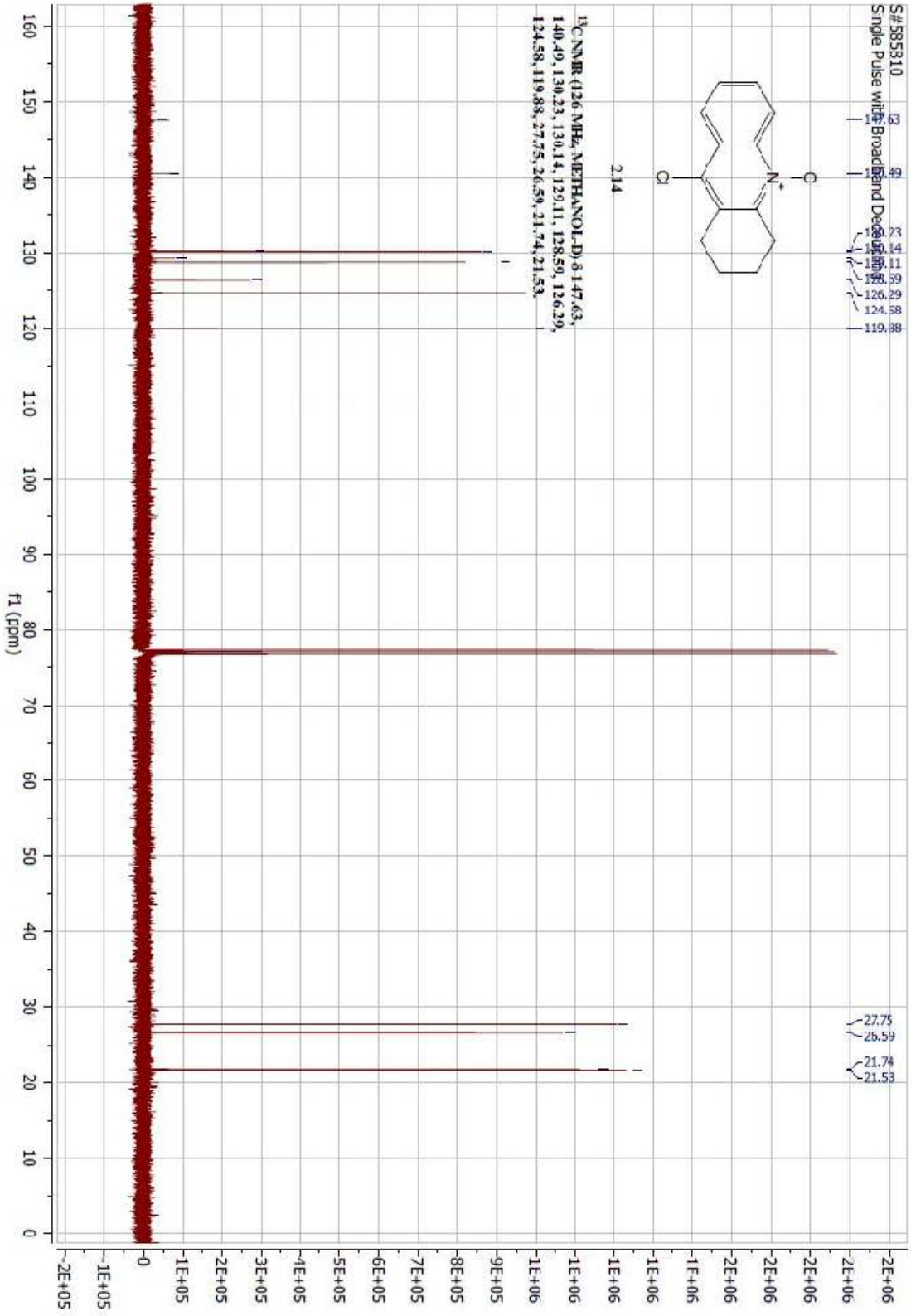
Chapter 6. Appendix

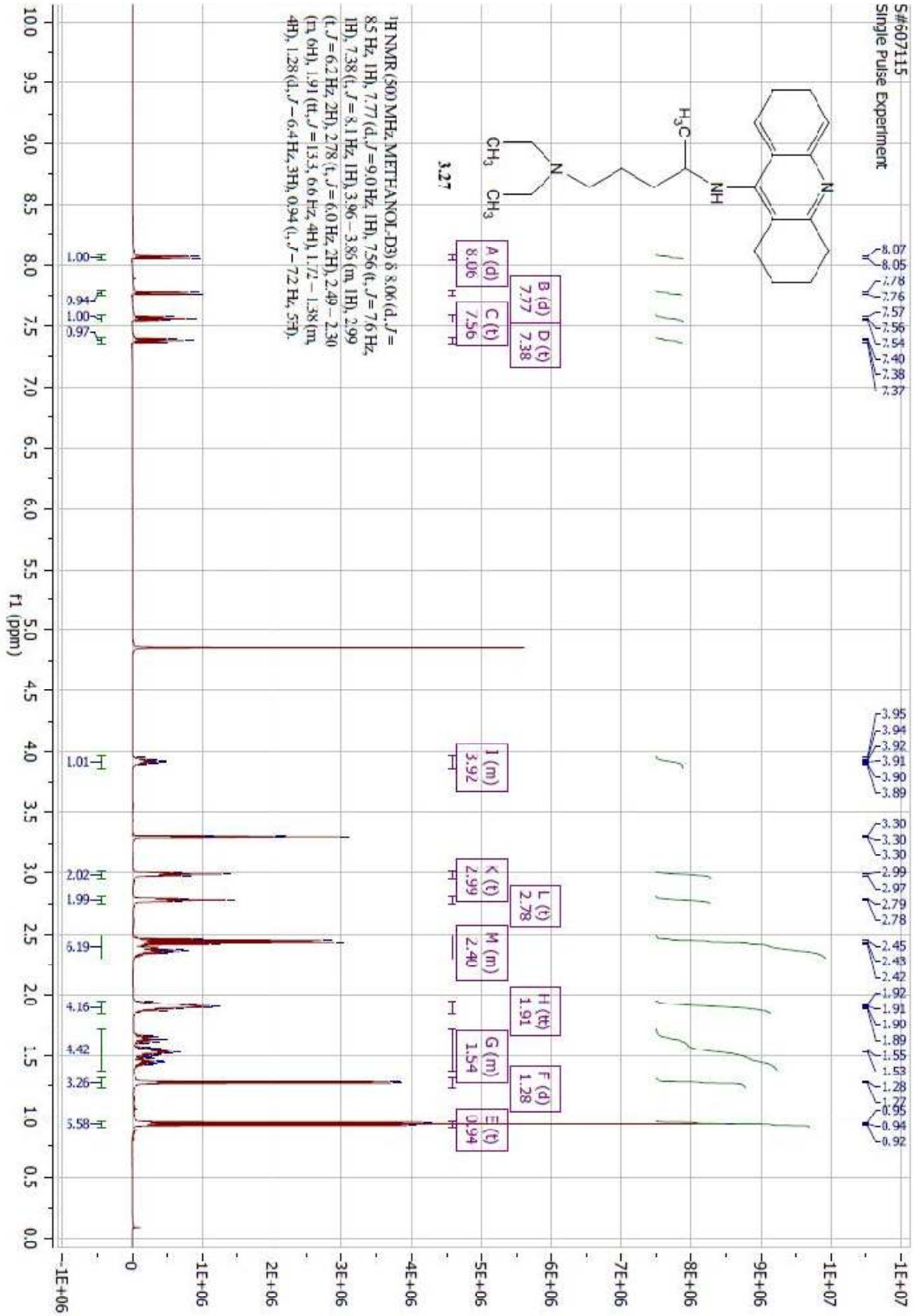
A) ^{1}H and ^{13}C NMR spectra of compound **2.14**

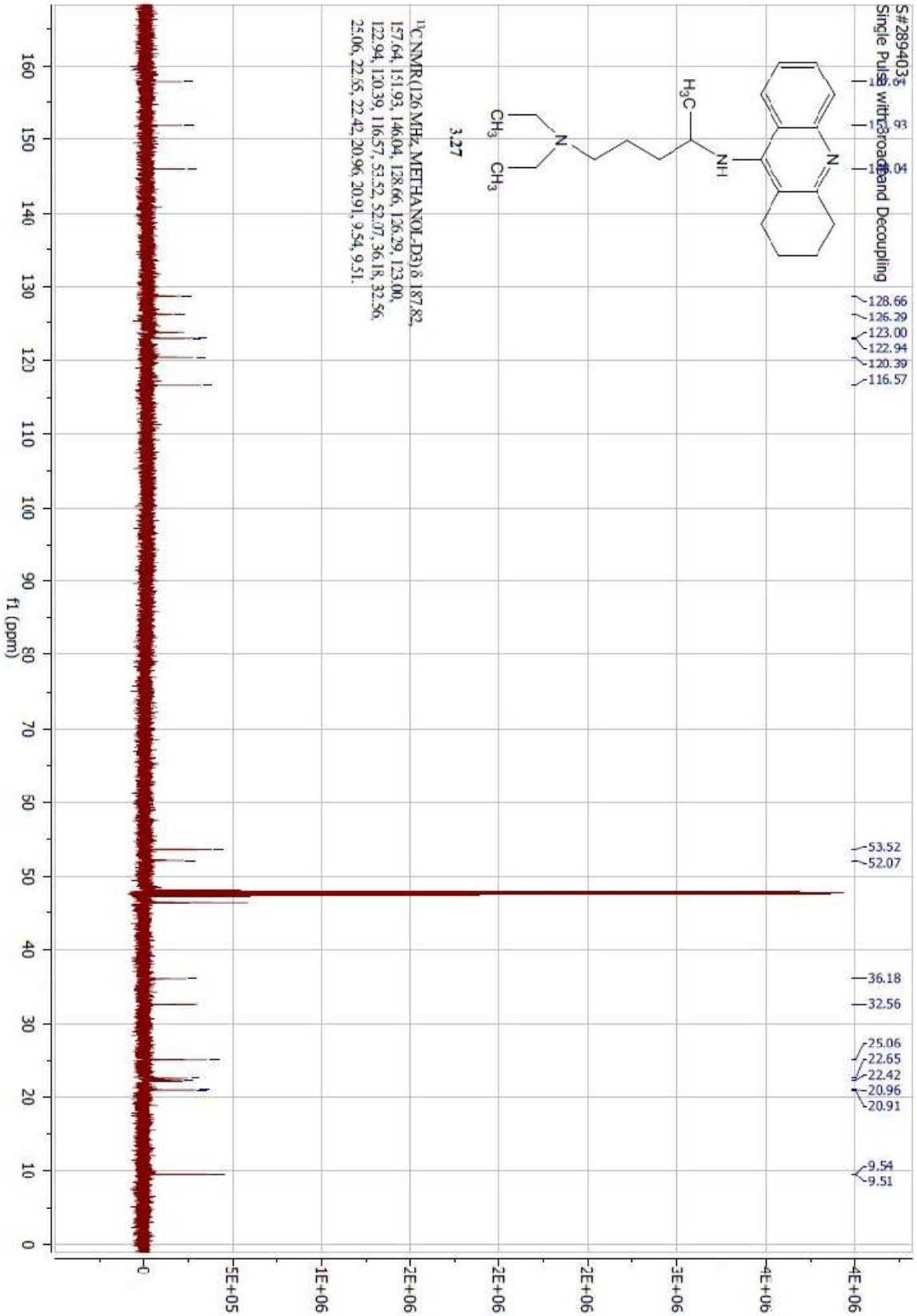
B) ^{1}H and ^{13}C NMR spectra of compound **3.27**

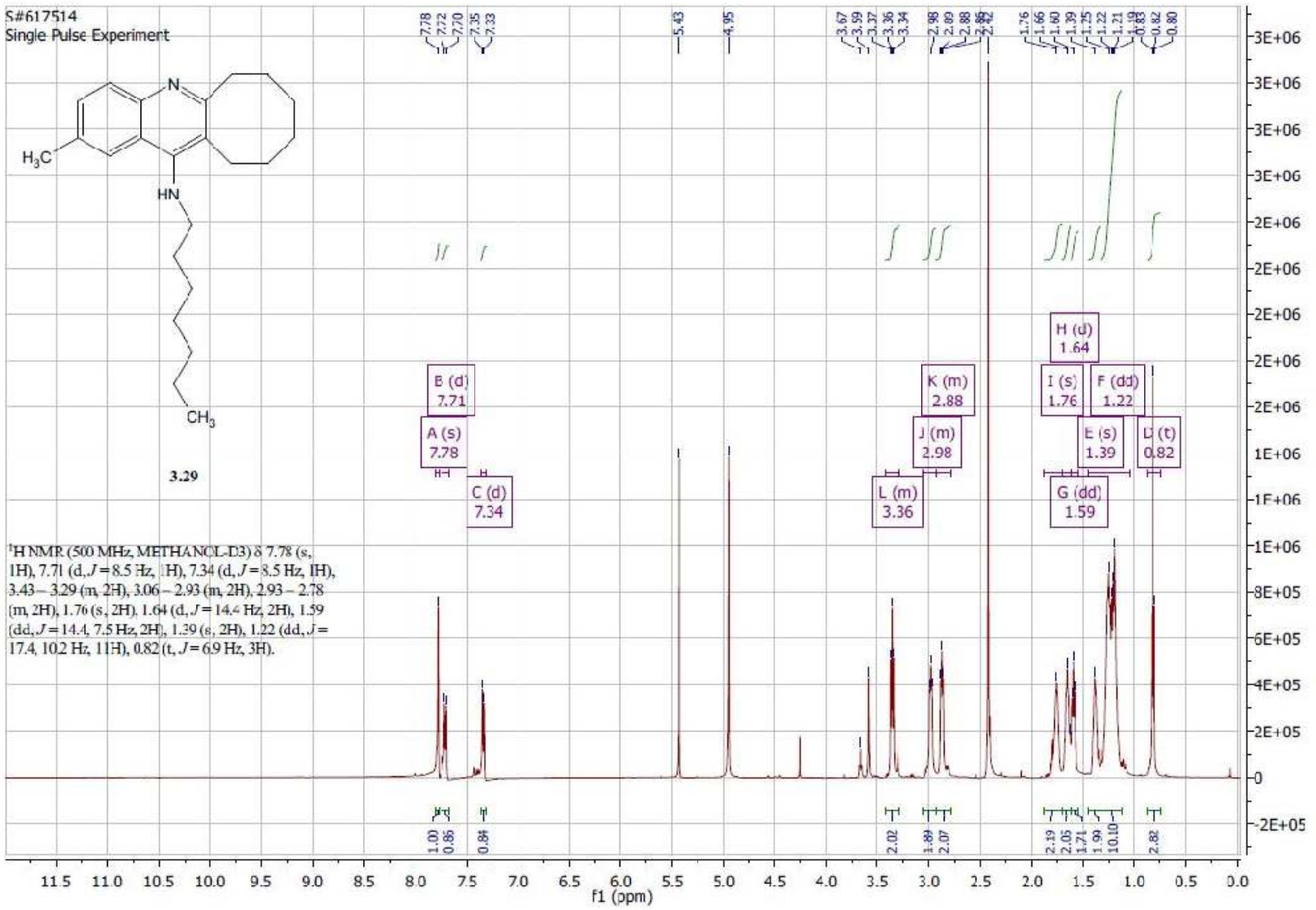
C) ^{1}H and ^{13}C NMR spectra of compound **3.29**

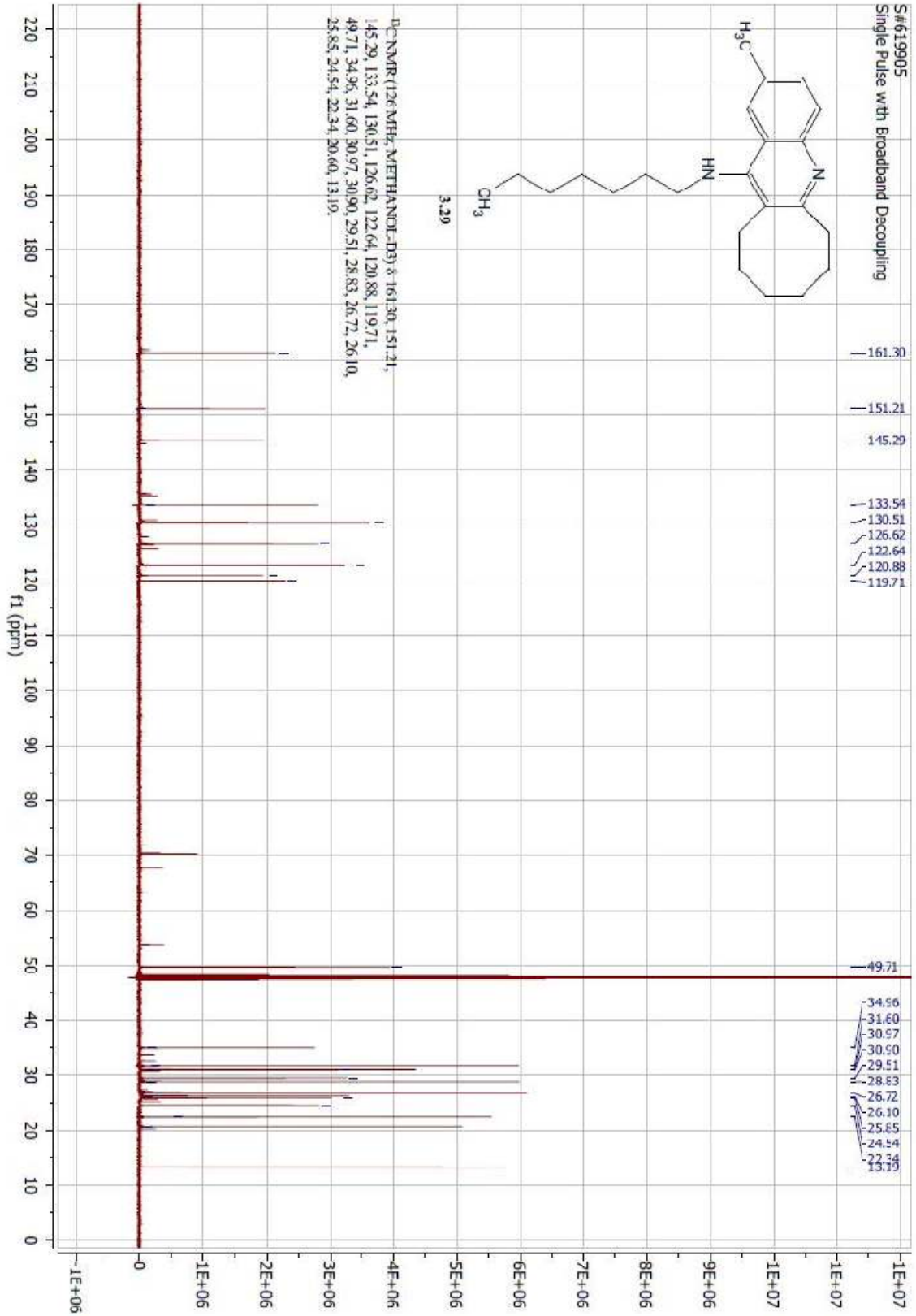

S#583140
Single Pulse Experiment
Cl
2.14
¹HNMR (500 MHz, METHANOL-D) δ 8.75(d, J = 8.3 Hz, 1H), 8.20(d, J = 8.4 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 3.18(t, J = 6.5 Hz, 2H), 2.99 (t, J = 6.3 Hz, 2H), 1.98 – 1.91 (m, 2H), 1.90 – 1.83 (m, 2H).
A (d) 8.75
B (d) 3.20
C (t) 7.73
D (t) 7.65
F (t) 3.18
E (t) 2.99
G (m) 1.95
H (m) 1.87
f1 (ppm)


S#585310
Single Pulse with Broadband Decoupling
147.63
140.49
130.23
130.14
129.11
128.59
126.29
124.58
119.88
27.75
26.59
21.74
21.53
2.14
13C NMR (126 MHz, METHANOL-D) δ 147.63, 140.49, 130.23, 130.14, 129.11, 128.59, 126.29, 124.58, 119.88, 27.75, 26.59, 21.74, 21.53.
f1 (ppm)


S#607115
Single Pulse Experiment
3.27
¹H NMR (500 MHz, METHANOL-D3) δ 8.06 (d, J = 8.5 Hz, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.56 (t, J = 7.6 Hz, 1H), 7.38 (t, J = 8.1 Hz, 1H), 3.96 – 3.85 (m, 1H), 2.99 (t, J = 6.2 Hz, 2H), 2.78 (t, J = 6.0 Hz, 2H), 2.49 – 2.30 (m, 6H), 1.91 (tt, J = 13.5, 6.6 Hz, 4H), 1.72 – 1.58 (m, 4H), 1.28 (d, J = 6.4 Hz, 3H), 0.94 (t, J = 7.2 Hz, 5H).
f1 (ppm)


S#289403
Single Pulse with Broadband Decoupling
128.66
126.29
123.00
122.94
120.39
116.57
53.52
52.07
36.18
32.56
25.06
22.65
22.42
20.96
20.91
9.54
9.51
3.27
13CNMR(126.MHz,METHANOL-D3)δ 187.82,
157.64, 151.93, 146.04, 128.66, 126.29, 123.00,
122.94, 120.39, 116.57, 53.52, 52.07, 36.18, 32.56,
25.06, 22.65, 22.42, 20.96, 20.91, 9.54, 9.51.
f1 (ppm)


S#617514
Single Pulse Experiment
N
H3C
HN
CH3
3.29
A (s) 7.78
B (d) 7.71
C (d) 7.34
H (d) 1.64
I (s) 1.76
F (dd) 1.22
E (s) 1.39
D (t) 0.82
K (m) 2.88
J (m) 2.98
L (m) 3.36
G (dd) 1.59
1H NMR (500 MHz, METHANOL-D3) δ 7.78 (s, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 3.43 – 3.29 (m, 2H), 3.06 – 2.93 (m, 2H), 2.93 – 2.78 (m, 2H), 1.76 (s, 2H), 1.64 (d, J = 14.4 Hz, 2H), 1.59 (dd, J = 14.4, 7.5 Hz, 2H), 1.39 (s, 2H), 1.22 (dd, J = 17.4, 10.2 Hz, 11H), 0.82 (t, J = 6.9 Hz, 3H).
f1 (ppm)


S#619905
Single Pulse w/th Broadband Decoupling
^{13}C NMR (126 MHz, METHANOL-D3) δ 161.30, 151.21, 145.29, 133.54, 130.51, 126.62, 122.64, 120.88, 119.71, 49.71, 34.96, 31.60, 30.97, 30.90, 29.51, 28.83, 26.72, 26.10, 25.85, 24.54, 22.34, 20.60, 13.19.
3.29
161.30
151.21
145.29
133.54
130.51
126.62
122.64
120.88
119.71
49.71
34.96
31.60
30.97
30.90
29.51
28.83
26.72
26.10
25.85
24.54
22.34
13.19
f1 (ppm)