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Synthesis and Biological Evaluation of Novel Epibatidine Analogues

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL EPIBATIDINE ANALOGUES

A Thesis

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by

Ying Liu

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Dedicated to:

My husband, Xiao Zhang

My son, Michael Z Zhang

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

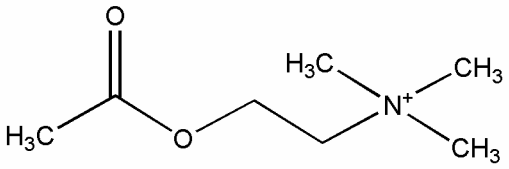
ABSTRACT

In an effect to develop for more selective neuronal nicotinic acetylcholine receptor analgesics that have less toxicity and adverse side effects relative to epibatidine, three new classes of epibatidine analogues were synthesized and evaluated *in vitro* as potential potent selective nAChR ligands. Specifically, three analogues of epibatidine were synthesized to explore the structure-activity relationships of epibatidine relative to neuromuscular blocking activity as well as nAChRs. Both quaternary epibatidine analogues **2** and bis-epibatidine derivative **3** exhibited high binding affinity relative to nicotine. In addition, a new series of 2-(hydroxyalkylpyridyl)-7-azabicyclo[2.2.1]heptane derivatives were synthesized and evaluated as potential ligands for nicotinic acetylcholine receptors. Moreover, two rigid 2-acetoxy-7-azabicyclo[2.2.1]heptane analogues have been prepared to study the binding conformation of acetylcholine at the active sites of the nicotinic acetylcholine receptors.

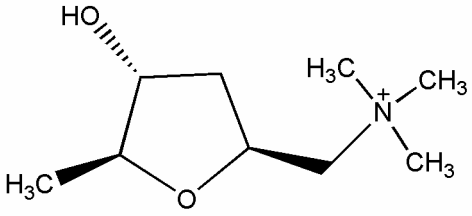
INTRODUCTION

Neuronal Nicotinic Acetylcholine Receptors

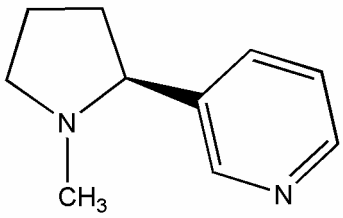
Acetylcholine (**1**) was one of the first neurotransmitters to be discovered. It is produced by the synthetic enzyme choline acetyltransferase that uses acetyl coenzyme A and choline as substrates for the formation of acetylcholine. Acetylcholine receptors in the mammalian central nervous system can be divided into muscarinic **2** (mAChR) and nicotinic **3** (nAChR) subtypes based on the ability of the natural alkaloids, muscarine and nicotine, to mimic the effects of acetylcholine as a neurotransmitter. Neuronal nAChRs are members of the ligand-gated ion channel superfamily that play a key role in the signal transmission between cells at the nerve/muscle synapses.[1] They are distributed both in the peripheral and central nervous systems, and growing evidence from animal and human studies indicated the involvement of nAChRs in high brain functions and in important neurodegenerative pathologies.[2] The nAChRs also are the body's primary targets of nicotine from tobacco products.



Acetylcholine, 1



Muscarine, 2



Nicotine, 3

Over the last decade, neuronal nAChRs have been the target of medicinal chemists. There is evidence that a deficit of nAChRs is implicated in the symptomatology of Alzheimer's disease, and nicotine itself has been shown to be beneficial in the symptomatic treatment of Alzheimer's disease and to exert neuroprotective effects in several *in vivo* models.[4] Preclinical research has demonstrated the therapeutic potential of some nAChR agonists in the treatment of the Parkinson's disease as well.[3] Implications of nAChRs in a number of other relevant physiological and pathological processes, like appetite, schizophrenia, epilepsy, depression and analgesia, have been also suggested.[4, 5]

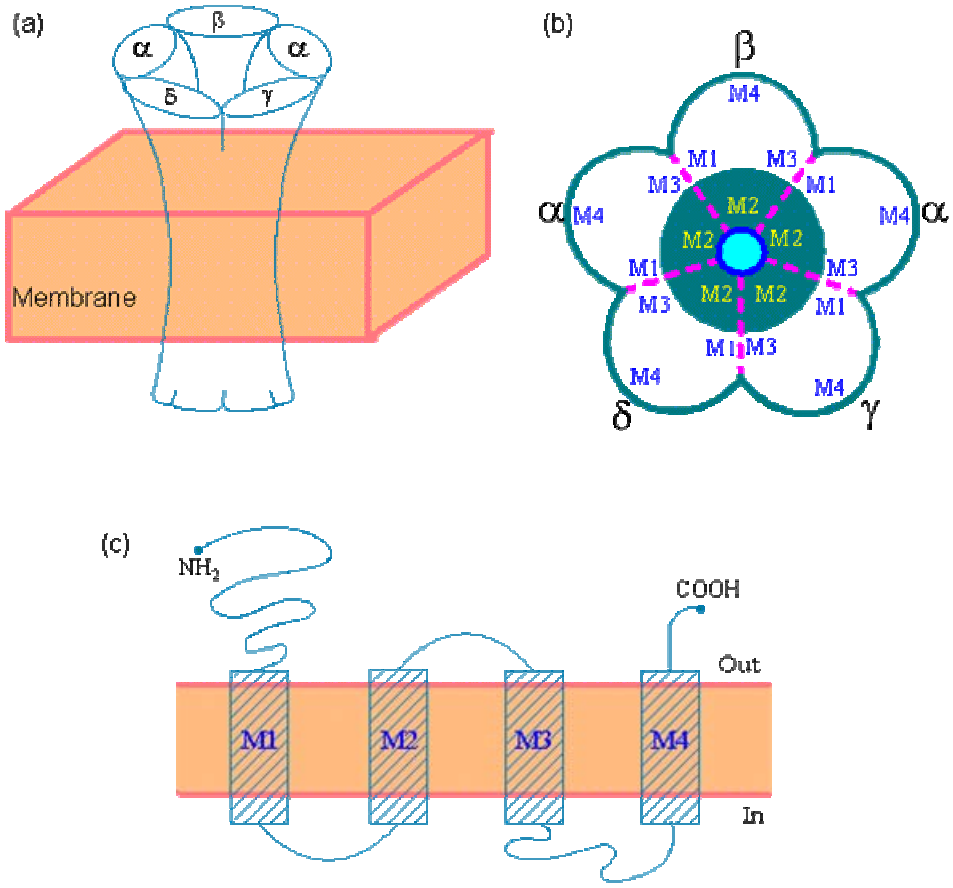
A number of natural products have been disclosed to selectively activate nAChR subtypes, and this has prompted a search for other subtype-selective non-natural nAChR ligands as potential therapeutic agents. The interaction of nicotine with nAChRs is the key step in the process that leads to nicotine addiction. Nicotine (**3**) is an alkaloid contained in the leaves of several species of plants. Defects in or loss of acetylcholine signaling would be expected given the wide variety of important

roles of the receptor in normal brain and body function. The nAChRs also are the body's primary targets of nicotine from tobacco products. It is believed that selective nAChRs agonists and antagonists could prove to be of great therapeutic benefit for the treatment of a variety of disease states and disorders. Unfortunately, side effects such as cardiovascular and gastrointestinal dysfunction, addiction, neuromuscular effects and seizures have limited the use of nAChR agents in drug therapy. Therefore, the search for potent and selective nAChR agents is an extremely important endeavor that will provide pharmacological tools for the study of nAChR function as well as to provide therapeutic agents and medications for the treatment of a variety of neurological disease states.

Nicotinic Acetylcholine Receptor Structure and Subtypes

The structure of peripheral nAChRs is isolated from the electric organ of the Torpedo ray (*Torpedo California*) have has been well characterized. It has been shown to possess five transmembrane protein subunits which assemble to form the ion channel (**Figure 1**).[6] Each subunit consists of four transmembrane α-helical domains (M_1-M_4). The inside wall of the channel is made up by five α-helices coming from the M_2 domains of each five subunits. Various subunits (α, β, γ, δ and ε) have been identified in the nAChRs of the mammalian skeletal muscle that have a pentameric structure constituted by two α-subunits and one each of β, δ, and γ (ε).

Figure 1. Schematic drawing for the nAChR channel which consists of five subunits: α, α, β, γ, and δ. **(a)** Side view. **(b)** Top view. **(c)** The transmembrane topology for each subunit. Five M2 segments (one from each subunit) form the channel pore as shown in (b). Activation of nAChR channel requires binding of two acetylcholine molecules, one on each α subunit.[6]



However, neuronal nAChRs have not been as well characterized, primarily due to the structural diversity of this class of receptors. In general, neuronal nAChRs are also thought to possess a pentameric structure consisting of a combination of nine known α-subunits (α1-α9) with three β-subunits (β2-β4). In addition, functioning homogenous receptors composed of either α7, α8 or α9 subunits have been expressed in oocytes.[7-9] The expressed receptors exhibit a diverse range of pharmacological

activity and function. However, researchers have been not been able to unequivocally correlate the function of expressed receptors with native receptors.

It is believed that the α4β2 subtype (two α4 units and three β2 units) nAChR is the predominant neuronal nicotinic receptor subtype in the mammalian CNS.[10] This subtype binds (-)-nicotine (**3**) with high affinity (K_D = 0.5-5 nM) and is distributed throughout the brain, albeit the distribution of the α4β2 subtype in specific regions of the brain is species dependent. The function of the α4β2 nAChR subtype is not clearly understood at this time, although it is believed to be associated with cognitive function.[11] A unique feature of the α4β2 nAChR subtype is that it is up-regulated by physiologically relevant concentrations of nicotine. This up-regulation was evident in the post-mortem brains of cigarette smokers in which the number of [^{3}H]-(-)-nicotine binding sites were significantly greater than in the brains of non-smokers.[12] Other nAChR subtypes are also up-regulated by nicotine; however, these subtypes require exceedingly large doses of nicotine for up-regulation.

A second nAChR subtype present in the brain has been suggested to be the homogenous α7 nAChRs composed of five α7 units.[13-15] This class of nAChRs has been shown to be distinctly different form the α4β2 subtype in that it binds α-bungarotoxin [α-Bgt is a 75 amino acid protein isolated from snake venom (*Bungarus multicinctus*)] with high affinity (K_D = 0.65-1.7 nM), while nicotine exhibits only micromolar affinity for the α7 nAChR subtype.[13] The concentration of the α7 subtype nAChR in the brain is similar to that of the α4β2 subtype but the distribution is different and species dependent. The function of the α7 is also not

well understood at this time. However, it is thought that this class of receptors may be associated with learning and memory processes as well as neuroprotection.[16-18]

It is important to note, that although a number of compounds have been prepared and characterized as either α4β2 subtype or α7 subtype selective ligands, within a ligand class (e.g. derivatives of nicotine, derivatives of epibatidine) there has been no report of two or more derivatives in which one is α4β2 subtype selective and the other is α7 subtype selective. Rather, the currently available structure-activity relationship data suggests that structural modification of subtype selective ligands results in binding affinity that is affected similarly and proportionally at all nAChR subtypes.

Table 1 summarizes the various nicotinic acetylcholine receptor subtypes discussed, their subunit makeup and location, and perceived primary function. This is only a partial list of the subtypes found in mammals, but describes the subtypes that are most relevant to our research.

Table 1. Nicotinic Acetylcholine Receptor Subtypes

Classification	Subunits	Location	Proposed Function
Neuronal (CNS)	α4β2	CNS	Memory and learning, neurotransmitter release, pain propagation, role in addiction
	α7	CNS	Cognitive functions, neuroprotection
Neuronal (Ganglionic)	α7	PNS	Cellular functions and regulation
Muscular	α1β1δγ	Neuromuscular Junction	Skeletal muscle contractions

Inhibition Constants and Effective Concentrations

Compounds are measured for their binding affinity for the nicotine receptor by determining the concentration at which the compound displaces (or inhibits) 50% of a radiolabeled ligand with known binding affinity. For example, [H^3]nicotine or [^{3}H]cytisine can be used as the standard to measure binding affinity at the nAChR. The concentration of prepared ligand required for the response is reported in molar units as an IC_{50} value-the inhibition concentration. The smaller the value of the IC_{50} is, the stronger the affinity of the displacing drug for the receptor would be. The usefulness of this value is limited due to variances in technique and tissue samples between labs. Cheng and Prusoff derived an equation (**Equation 1**) that incorporates the concentration of the ligand tested [L] and the dissociation constant (K_d) of the standard compound from the receptor.[19] The smaller the value of K_d, the greater the affinity of the ligand for the receptor. This value is also reported in molar units as an inhibition constant (K_i). Inhibition constants are more useful for comparison between labs.

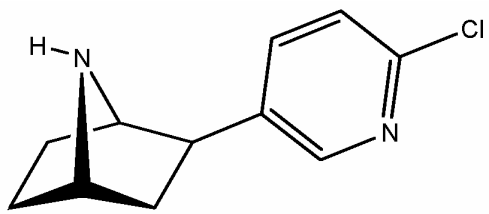
$$K_i = \frac{IC_{50}}{[L]/K_d + 1} \qquad \text{(Eq 1)}$$

Another measurement used to determine the potency of a ligand at a receptor is the effective concentration (EC_{50}). The EC_{50} of a compound is the molar concentration that produces half the maximum biological response observed when acetylcholine, the endogenous neurotransmitter, binds to a receptor. A low EC_{50} value will indicate a potent compound.

Epibatidine

In 1974, John Daly and his coworkers at the National Institute of Health first collected a trace alkaloid from skin extracts of the Ecuadorian poison dart frog (*Epiedobates tricolor*) in the Pacific highlands of Ecuador. However, too little of the compound was isolated to make a structural determination because of lack of the sensitive and sophisticated instruments and methods. The remaining sample was kept in storage for years until 1992, the structure of the novel alkaloid Epibatidine (**4**) [(1*R*, 2*R*, 4*S*) *exo*-2- (2-chloro-5-pyridinyl)-7-azabicyclo[2.2.1]heptane] was finally determined and shown to be a potent analgesic.[19] The structure of epibatidine was found to be a new class of alkaloids possessing a 7-azabicyclo[2.2.1]heptane (7-azanorborane) structure, with a *exo*-oriented 5-(2-chloropyridyl) substituent. The structure of epibatidine (**4**) closely resembles that of nicotine (**3**). Both contain a pyridine ring; both contain a basic nitrogen linked to the pyridine ring by one or two carbons; both basic nitrogen atoms are part of a five-membered ring (in epibatidine, the five membered ring is part of the 7-azabicycloheptane structure). The structural similarity to nicotine suggested that epibatidine would have activity at nicotinic receptors. Indeed, epibatidine along with its singular structure, contributes to its great appeal for the studies by the scientific community.[20] Epibatidine was found to exhibit potent analgesic effects (200 times more potent than morphine) that were not mediated through opioid receptors.[19, 21-22] This is clearly established by the fact that

epibatidine was not blocked by administration of the potent opiate antagonist naloxone. Further studies demonstrated that epibatidine possessed high affinity for neuronal and peripheral nAChRs and was 300 times more potent than (-)-nicotine.[23] Although the analgesic effects of nicotine have been known for more than two decades, the discovery of epibatidine and its exceptionally potent analgesic activity had prompted an intensive study of the pharmacological activity of this unique alkaloid and has renewed the search for non-narcotic nAChR mediated analgesic agents.

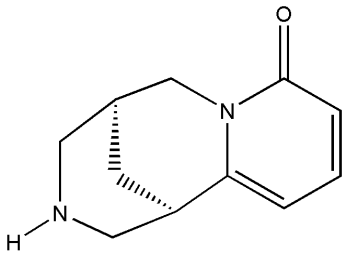


Epibatidine, 4

Epibatidine *In Vitro* Neuronal nAChR Pharmacology

In vitro binding studies, employing radiolabeled competition assays in rat brain, have shown that epibatidine displaced α4β2 subtype selective ligands [^{3}H](-)-nicotine (**3**) and [^{3}H](-)-cytisine (**5**) with K_i's of 55 pM[22] and 43 pM[23], respectively, thus making epibatidine the most potent nAChR ligand known to date. [^{3}H]Cytisine (**5**) binds with high affinity to α4β2 subtype of nAChRs, the major subtype in rodent brain (>90% of (-)-nicotine binding site).[24] However, in binding studies with [^{3}H] α-Bgt, epibatidine was not nearly so potent, having a K_i of 230 nM,

albeit, epibatidine was still 20-fold more potent than (-)-nicotine in this paradigm.[23] This suggests that epibatidine is more selective for the α4β2 nAChR subtype over the α7 nAChR subtype. It has been shown that epibatidine labels more than one binding site in rat brain, human brain and mouse brain.[25] The distribution of [^{3}H]epibatidine sites in rat brain closely matches that of [^{3}H]cytisine, with a few exceptions.[26] One of the sites labeled by epibatidine appears to be cytisine-insensitive, and these sites appear to be located primarily in diencephalic and mesencephalic brain regions.[27] The (+) and (-)-enantiomers of epibatidine displace [^{3}H]nicotine binding with similar affinities and display similar analgesic efficacies.[22]

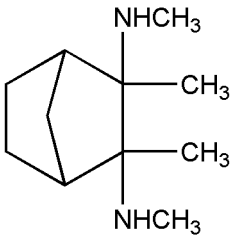


(-)-Cytisine, 5

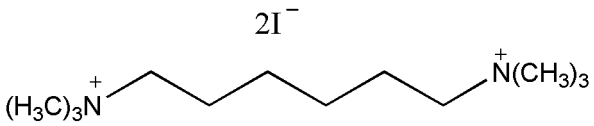
Epibatidine Stimulates Dopamine and Norepinephrine Release

Epibatidine stimulated [^{3}H]dopamine release with an EC_{50} of 0.4 ± 0.1 nM, compared to (-)nicotine, which had an EC_{50} of 60 ± 12 nM.[23] In addition, epibatidine was more efficacious than nicotine at stimulating dopamine release. The stimulated release of dopamine by epibatidine was blocked by both the competitive nAChR antagonist DHβE and the non-competitive nAChR antagonist mecamylamine(**6**).[23] It has been suggested that the subunit involved in dopamine release is the α4β2 subunit,[28] however in mouse striatal synaptosomes the α3 subunit has also been

implicated.[29, 30] In mice lacking the β2 subunit of nAChRs, epibatidine binding was almost completely wiped out, although there was a small amount of residual binding in a few select brain regions including the lateral medial habenula and the dorsal interpeduncular nucleus.[31] Epibatidine stimulated release of norepinephrine in the dentate gyrus region of the hippocampus with an EC_{50} of 19.6 nM (compared to nicotine with an EC_{50} of 34 nM).[32] This value is considerably higher than that seen for dopamine release[22] suggesting the potential involvement of a different receptor subtype. In fact, it is thought that epibatidine stimulated norepinephrine release occurs via the α3β2 subtype of receptor,[32] thus, by measuring the effects of epibatidine analogues on release of both dopamine and norepinephrine, it may be possible to determine differential efficacies and potencies at these two nAChR subtypes.



Mecamylamine, 6



Hexamethonium, 7

Epibatidine *In Vivo* Neuronal Nicotinic Acetylcholine Receptors Pharmacology

In vivo, epibatidine was found to possess potent analgesic activity in rats. From tail-flick and hotplate assays, the analgesic effects of epibatidine were found to be 200-fold more potent than morphine.[19-21] However, the analgesic effects elicited by

epibatidine were not blocked by the opiate antagonist naloxone which suggests a non-opioid mechanism of action. The analgesic effects of epibatidine were blocked by the neuronal nAChR antagonist mecamylamine (**6**).[20] Alternatively, the peripheral nAChR antagonist hexamethonium methiodide (**7**), which is incapable of penetrating the blood-brain-barrier, had no effect on the analgesic effects of epibatidine.[21] This suggests that the analgesia elicited by epibatidine is mediated through occupation of neuronal nAChRs. However, it is uncertain whether the α4β2 nAChR subtype, the α7 nAChR subtype or some other nAChR subtype mediated the analgesic activity.[22,23] In addition, epibatidine was found to lower body temperature in mice and decrease locomotor activity. These effects were also antagonized by mecamylamine (**6**) and not affected by hexamethonium (**7**). Although the analgesic effects of nicotine have been known for more than two decades, the potency of epibatidine is far greater than that observed for any other nAChR ligand. However, it is unlikely that epibatidine itself will ever be developed as an analgesic agent since a therapeutic dosage closely approaches levels which induce severe hypertension, convulsions, respiratory depression and death.[22, 34]

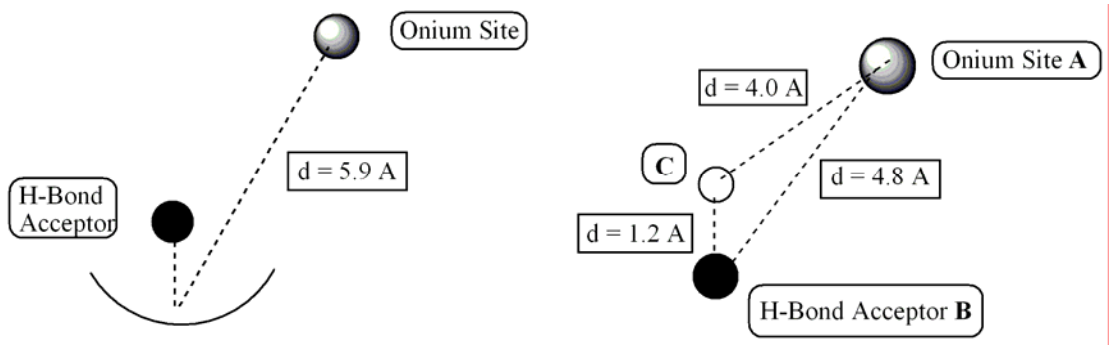
Nicotinic Acetylcholine Receptors Pharmacophore Models

The term pharmacophore was originally described by Paul Ehrilich more than 100 years ago and it is defined as the three-dimensional arrangement of atoms or groups of atoms that are responsible for the biological activity of a drug molecule.

Specifically, a pharmacophore for the nAChR should possess the physiochemical properties necessary to stimulate activity at the receptor. During the last fifty years, several studies have been performed with the aim to define nicotinic cholinergic pharmacophores. Due to the small number of compounds available to investigators and the lack of homogeneity in tissue/receptor preparations used to evaluate activity, pharmacophore models from the early research were of limited significance.

Among the most important studies, around 1970, based on examination of a series of agents using Dreiding and CPK space-filling models, Beers and Reich found nicotinic agents (both agonist and antagonist activity) must be characterized by two common structural features. They are: (1) a couloumbic interaction involving an alkylammonium moiety, and (2) a hydrogen bond that depends upon the presence of an acceptor moiety in the nicotinic agent at a distance of 5.9 Å away from the center of the positive charge (**Figure 2a**). [35] Sixteen years later, Sheridan and co-workers [36] developed a pharmacophore model based on three features; a basic nitrogen atom (corresponding to the pyrrolidine nitrogen in nicotine), a hydrogen bonding acceptor (e.g., the pyridine nitrogen of nicotine or the carbonyl oxygen atom of cytisine), and a third point representing the centroid of the pyridine ring of nicotine or the carbonyl carbon atom of cytisine or other agonists. They also noted a distance of 1.2 Å between the H-bond acceptor and a third atom that resides above the plane of the internitrogen space (**Figure 2b**).

Figure 2. Comparison of the Beers and Reich Pharmacophore with the Sheridan model (a) The Beers and Reich pharmacophore model: Distance d represents the distance between the onium site and the van der Waals surface of a hydrogen bond acceptor atom; optimal d = 5.9 Å. (b) a representation of the pharmacophore produced by Sheridan et al. : Idealized distances are A-B: 4.8 Å, B-C: 1.2 Å, and C-A: 4.0 Å.



(a) Beers and Reich **(b) Sheridan**

Based on X-ray crystallographic data of ligands, Barlow and Johnson (1989)[37] suggested the agonist activity to require a charged nitrogen (i.e., an onium site) and a planar area on the receptor recognizing an aromatic ring or a double bond through hydrophobic or p-p interactions. Glennon *et al.* [38] performed QSAR studies on a series of agents structurally related to nicotine (**3**) and epibatidine (**4**), and found their affinities to parabolically correlate with the respective N-N distances (optimal distance 5.1-5.5 Å). This model was, however, too simplistic to rationalize the agonism of other compounds, such as aminoethoxypyridine derivatives and unsaturated aminoalkylpyridines [39], that displayed an affinity higher than that expected from the calculated internitrogen distances. A relevant contribution has been

given by Livingstone et al. in 1996.[40] Seven semirigid agonist molecules were analyzed through a comparative analysis of the gnomonic projections of surface molecular properties. Three significant features defined the pharmacophore: (i) a cationic head, (ii) a ring centroid of a pyridine ring or the carbon atom of a carbonyl group, and (iii) a dummy atom indicating the location on the receptor of an atom likely making an H-bond with the pyridyl nitrogen or the carbonyl oxygen. A lipophilic region, close to the positions 3' and 4' of the pyrrolidine ring of nicotine, had been considered as an important modulator of the agonistic activity, but it had not been used in the derivation of the pharmacophore model.

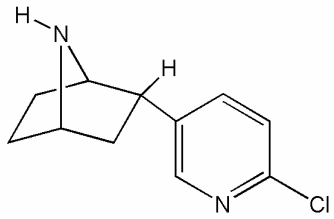
Structure-Activity Relationships at Nicotinic Acetylcholine Receptors

Compounds with similar structures often tend to have similar pharmacological activity. However, they usually exhibit differences in potency and unwanted side effects and in some cases different activities. These structurally related differences are commonly referred to as structure-activity relationships. A study of the structure-activity relationships of a lead compound and its analogues can be used to determine the parts of the structure of the lead compound that are responsible for its biological activity, that is, its pharmacophore and also its unwanted side effects. This information is subsequently used to develop a new drug that has increased activity, and a different activity from an existing drug, fewer unwanted side effects and

improved ease of administration to the patient. The structure-activity relationships of a compound or a class of compounds are usually determined by making minor changes to the structure of the lead compound and assessing the effects of the structural change on biological activity. Traditional structure-activity relationships investigations are carried out by making large numbers of analogues of the lead and testing them for biological activity. Over the years after discovering epibatidine, numerous analogues have been investigated. Such hybrids should take into account the assets of known drugs such as favorable distances between atoms, functional group moieties, and structural similarities resulting in potency with decreased toxicity.

Structure-Activity Relationships of Epibatidine at Nicotinic Acetylcholine Receptors

In light of the high potency and extraordinary analgesic effects of epibatidine, several studies of the structure-activity relationship of this novel alkaloid have been reported. It has been shown that natural (-)-epibatidine and unnatural (+)-epibatidine are equipotent in [^{3}H]nicotine labeled rat brain.[20] However, the C2-epimer **8** exhibited diminished binding affinity.[41] *N*-Methylation of (-)- and (+)-epibatidine (**9**) resulted in slightly reduced binding affinity in rat brain as well as a small enantioselective differential in binding affinity such that the natural analogue was three-fold more potent than the unnatural analogue.[20, 21]



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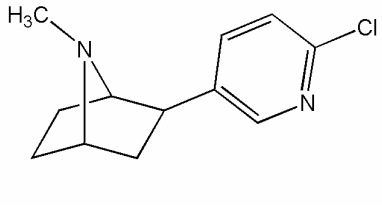
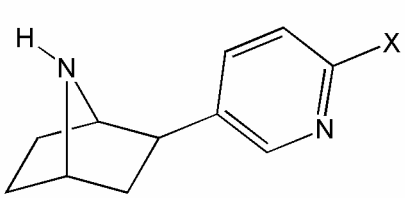


9

Another important structure-activity relationship study for epibatidine was a series of substituent derivatives on the pyridyl ring. It has been found that removal of the chloro substituent (**10a**) did not affect binding potency. Likewise the replacement of the chloro substituent with methyl, fluoro or iodo (**10b-d**) did not affect the binding potency relative to epibatidine.[19-21, 42] The amino moiety **10f** was found to exhibit decreased binding affinity with respect to epibatidine, but maintained potency comparable to nicotine. The 2-position was not tolerant of a hydroxy group **10g**, a dimethyl amino group **10h**, and a triflate **10i** as seen by lower binding affinities for these analogues.[7]



10a, X = H

10b, X = CH_3

10c, X = F

10d, X = I

10e, X = NH_2

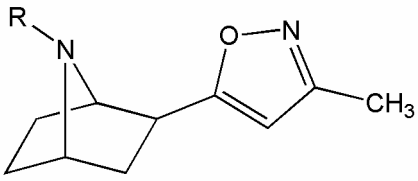
10f, X = OH

10g, X = $N(CH_3)_2$

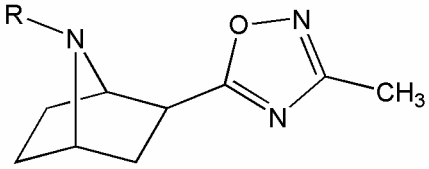
10h, X = CF_3SO_3

There are also a few examples reported in the literature where the pyridine ring of epibatidine has been replaced by an alternative heterocyclic ring. (±)-Epiboxidine (**11**) was reported to exhibit potent binding affinity in [^{3}H]nicotine labeled rat brain but was 10-fold less potent than (-)-epibatidne.[43] The analgesic

activity of **11** was also diminished 10-fold relative to epibatidine in rat hot-plat assays, but **11** was 10-fold less lethal in mice[43]. The *N*-methyl analogue **12** exhibited similar binding affinity and analgesic efficacy to **11**. The oxadiaxolyl derivative **13** was shown to be 30-fold less potent than (±)-epibatidine.[44]

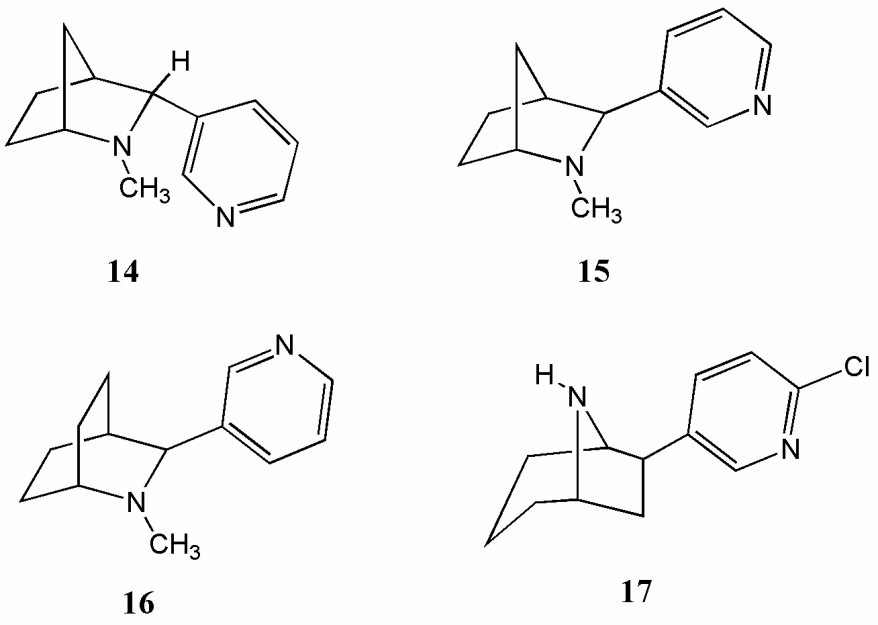


11 R = H
12 R = CH_3



13

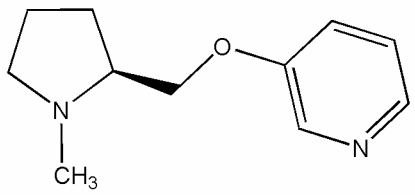
Several bicyclic analogues and homologues of epibatidine have been reported to exhibit binding affinity at nAChRs and elicit analgesic activity. The isomeric 2-azabicyclo[2.2.1]heptane analogues of epibatidine **14** and **15** were found to be similar in potency to nicotine, but were about 50% less efficacious in dopamine release experiments.[45] The 2-azabicyclo[3.2.1]octane homologue **16** was shown to possess binding affinity similar to nicotine and the 8-azabicyclo[3.2.1]octane homologue **17** was reported to exhibit analgesic activity equal to epibatidine at a dose that was only 4-fold greater.[46]



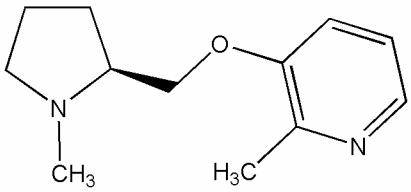
Structure-Activity Relationships of Pyridyl Ethers

Probably the most significant development in the field of nAChR research since the discovery of epibatidine has been the discovery of the 3-pyridyl ethers as a potent nAChR ligand class. In 1996, Holladay and coworkers synthesized a series of methylated and demethylated pyrrolidines and azetidines with 3-pyridyl ether functional groups. The pyrrolidine derivatives A-84543 (**18**) and ABT-098 (**19**) exhibited potent binding affinity at [^{3}H]nicotine labeled binding sites and are selective for α4β2 nAChRs. The azetidine analogue A-85380 (**20**) was reported to exhibit extremely potent binding affinity (K_i = 50 pM), similar to that of epibatidine and also demonstrated selectively for the α4β2 nAChRs.[47] The R-2-chloro analogue ABT-594 (**21**) has a very low affinity for the nicotine receptors in the neuromuscular junction which cause the paralysis effect, but it has a high affinity for the nicotine receptors in the central nervous system which regulate pain perception. Moreover, ABT-594 has

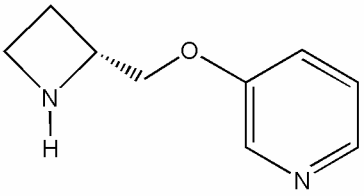
been described as producing orally effective analgesia of similar potency to (±)-epibatidine.[48] The R-isomer **21** was found to be only slightly more potent than its enantiomer. More significantly ABT-594 (**21**) is currently being studied as a potential therapeutic agent for the control of pain.[49, 50]



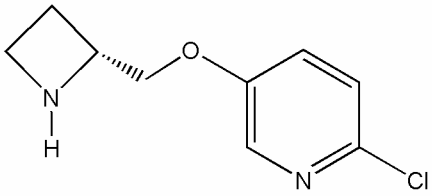
A-84543, **18**



ABT-098, **19**



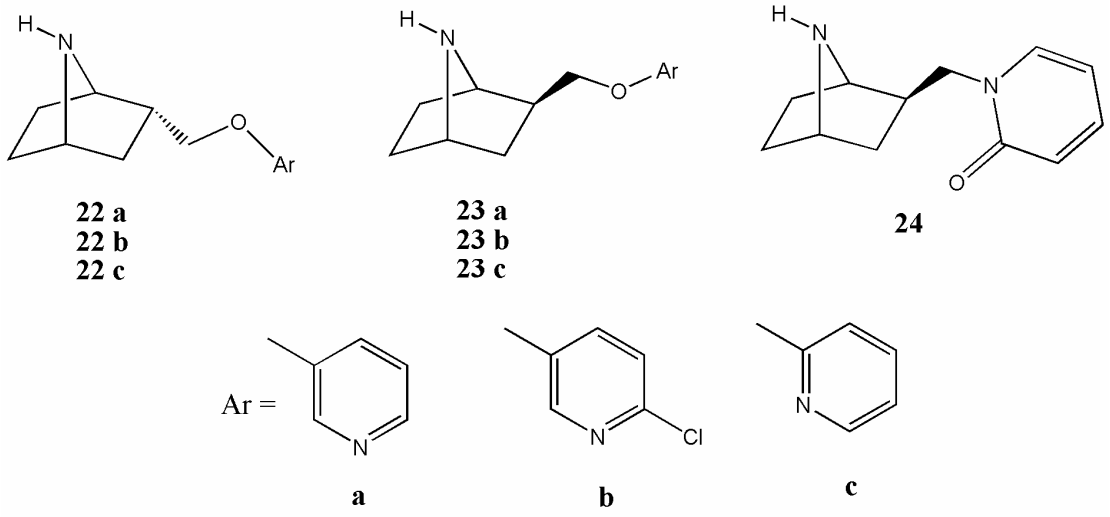
A-85380, **20**



ABT-594, **21**

Recently in our labs, 2-substituted rigid 7-azabicyclo[2.2.1]heptanes **22-24** were synthesized.[51] All compounds showed a lower binding affinity than epibatidine and nicotine, with the *endo* **22a-c** exhibiting low to moderate micromolar concentrations as well as the dechlorinated *exo* compounds **23a, 23c**. The exo 6-chloropyridine **23b** offered the best binding, in the submicromolar range, and though still too low for potent therapeutic use, the structure-activity relationships of the ethers were consistent with those of epibatidine and its analogues. The 2-pyridyl derivatives **22c, 23c** and the amide analog **24** did, however, display comparable potency to the 3-pyridyl homologues, which had not been seen in other series. With the ether linkage in the 2-position of the bicyclic system, the

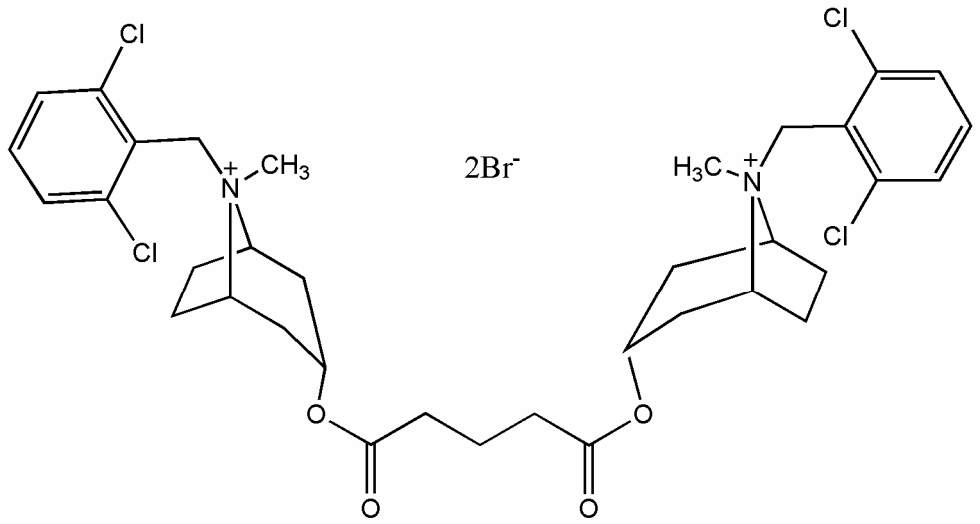
7-azabicyclo[2.2.1]heptane ring does not substitute for the azetidine ring as recognized by the α4β2 receptor.



Neuromuscular Blocking Agents

The clinical value of an ultra-short-acting nondepolarizing neuromuscular blocking drugs (NDNMBDs) via continuous infusion in the intensive care unit has long been recognized and is gaining in popularity. Savarese and Kitz termed such a compound an "ideal" muscle relaxant.[52] Since then, several new NDNMBDs have been developed; these drugs vary in degree of onset and duration of action, as well as routes of elimination.[53] Examination of the chemical structure of these relaxants reveals that they are either benzylisoquinolinium or aminosteroid compounds. Recently, Gyermek and coworkers have explored the role of ester groups in the neuromuscular blocking property of various quaternary ammonium tropinesters.[53, 54]

Among them, G-1-64 (**25**) exhibited favorable neuromuscular blocking characteristics and modest side effects. Since epibatidine (**4**) exhibits potent analgesic effect and possesses high affinity for neuronal and peripheral nAChRs, structurally similar epibatidine derivatives may have clinical potential.



25

Total Synthesis of Epibatidine

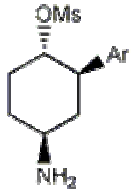
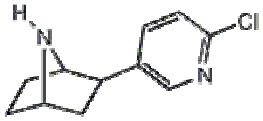
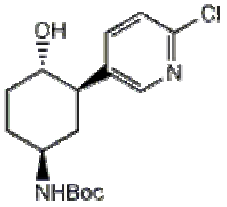
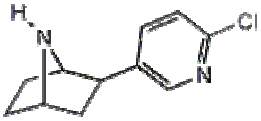
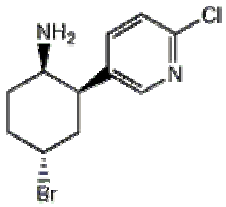
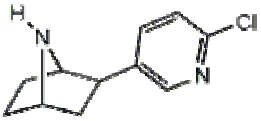
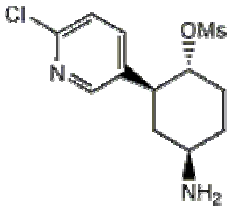
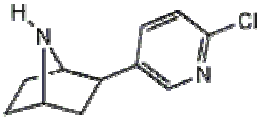
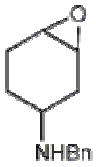
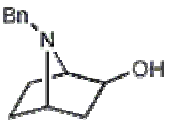
For nearly a decade now, chemical research groups all over the world have been captivated by epibatidine since the elucidation of the structure of epibatidine and its analgesic activity was published in 1992. The azabicycloheptane system of epibatidine is not common among natural products. Synthetic chemists Corey [55], Shen [56], Broka [57], and Clayton and Regan [58] were among the first to report total syntheses

of epibatidine. Many other synthetic routes were later reported (See references 59-61 for reviews). Based on the strategy of forming the azabicyclic ring system, most synthetic approaches can be classified into three categories: (1) intramolecular nucleophilic displacement reaction, (2) Cycloaddition reaction and (3) miscellaneous reactions like ring contraction of the tropinone skeleton via a Favorskii rearrangement and radical cyclization.

1. Intramolecular Nucleophilic Displacement Reaction [62-68]

Intramolecular nucleophilic 1,4-substitution of a *trans*-substituted cyclohexane derivative to construct the 7-azabicyclo[2.2.1]heptane ring system might be the most popular approach in total synthesis of epibatidine and its analogues. Table 2 summarizes the various precursors for epibatidine and its analogues from the recent literature.

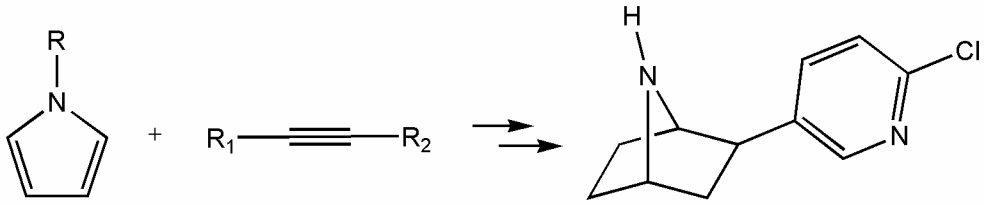
Table 2. Summary of Previous Total Synthesis of Epibatidine

	Precursor	Product	Yield	Ref.
1			78% 84% 90%	62 63 64
2			45%	65
3			97%	66
4			84%	67
5			64%	68

2. Cycloaddition Reaction

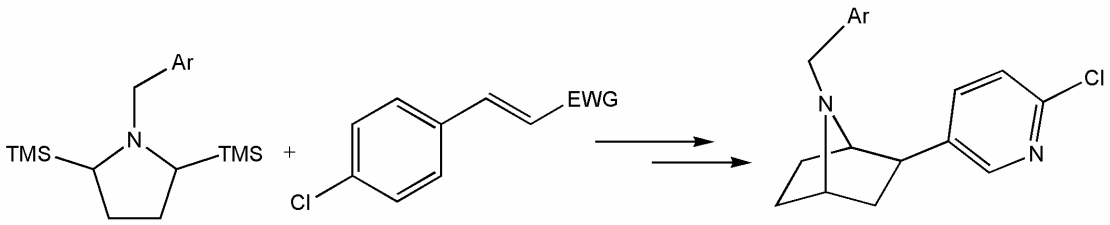
One type of the cycloaddition reaction that has been successful is [4+2] Diels-Alder cycloaddition which employs an N-protected pyrrole as the diene

and a substituted acetylene derivative as the dienophile to prepare the 7-azabicyclo[2.2.1]heptane framework of epibatidine (Scheme 1). Shen and Huang first reported a Diels-Alder based synthesis of epibatidine using the *N*-pyrrole and phenyl sulfonyl 6-chloro-3-pyridyl acetylene.[56] Our group reported an efficient total synthesis of epibatidine by using *N*-Boc pyrrole and methyl 3-bromopropiolate in [4+2] cycloaddition.[69] Our lab also employed allenes in the Diels-Alder reaction to synthesize the important intermediate N-protected 7-azabicyclo[2.2.1] heptan-2-one.[70]



Scheme 1

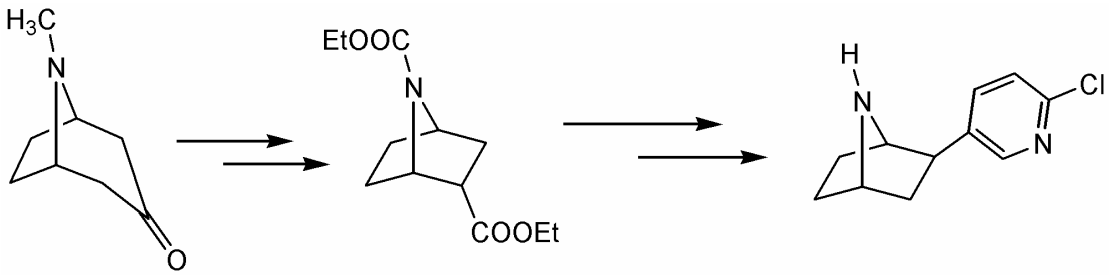
Another strategy to assemble the epibatidine skeleton was [3+2] dipolar Cycloaddition reaction. It was reported by Pandey and coworkers[71] (Scheme 2) that the [3+2] cycloaddition of a nonstabilized azomethine ylide with cis-ethyl-3-(6-chloro-3-pyridyl)-2-propenoate gave the cycloadduct possessing the 6-chloro-3-pyridyl ring with the desired exo-stereochemistry.



Scheme 2

3. Miscellaneous Reactions

One interesting strategy for synthesis of epibatidine is the ring contraction via Favorskii rearrangement (Scheme 3) reported by Bai and coworkers.[48] A N-protected tropane was treated with cupric bromide to give the monobromide, which was subjected to the Favorskii rearrangement with sodium methoxide to afford the contracted bicyclic ester in 56%. The following reductive palladium-catalyzed coupling reaction and radical decarboxylation afforded the N-protected azabicyclohexane, which was subsequent by deprotected reaction furnish epibatidine in 83% yield.



Scheme 3

Specific Aims and Design Strategy

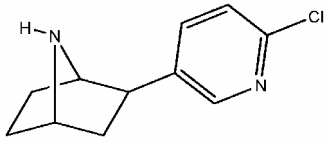
As discussed earlier, the natural product epibatidine (**4**) is one of the most potent nicotinic acetylcholine receptors ligands to date. Its exceptionally potent analgesic activity has prompted an intense study of this unique alkaloid and renewed interest in the search of non-narcotinic nAChR mediated analgesic agents. However, the therapeutic potential of epibatidine is limited due to its acute toxicity at a dose only slightly higher than its effective analgesic dose.

In an effort to develop nicotine acetylcholine receptor selective agonists and antagonists as potential therapeutic agents for nicotine addiction and other central nervous system disorders mediated by the nicotinic acetylcholine receptors, novel analogues of the epibatidine will be synthesized and evaluated in vitro as potential nicotinic acetylcholine receptors ligands

Epibatidine and Quaternary Ammonium Salt Derivatives

Recently, Gyermek and coworkers reported a bisquaternary ammonium tropine ester (G-1-64, **25**) [53] as a new class of neuromuscular blocking (NMB) agents with modest side effects. The exceptionally potent activity of epibatidine has prompted an investigation of the bis-epibatidine ammonium salt derivatives. To this end, the efficient synthesis of (±)-epibatidine (**4**) as well as its quaternary ammonium

salt and bis-epibatidine derivatives (**26-28**) will be investigated to provide useful quantities of these rare compounds for study.



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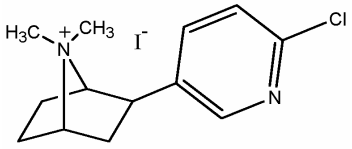

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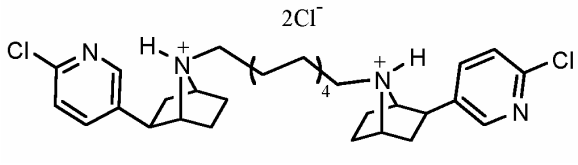

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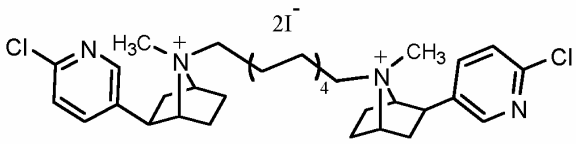
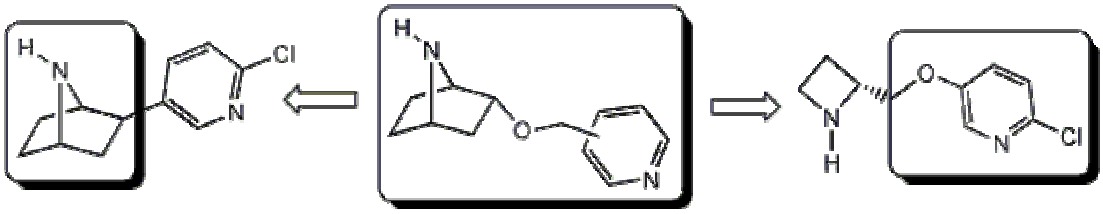


28

Pyridyl ether derivatives

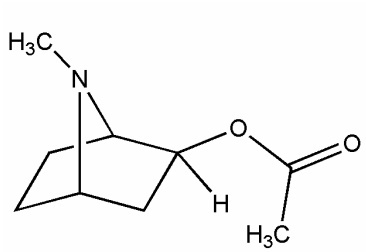
Based on the high potency and extraordinary analgesic effects of epibatidine and ABT-594, it is of interest to study hybrids of these compounds. To this end, a series of pyridyl ether derivatives **29a-c** have been envisaged to explore the structure-activity relationships of epibatidine related compounds. The derivatives were designed to be hybrid structures of epibatidine and ABT-594. In this series, the effect of the position of the nitrogen atom in the pyridyl ring for molecular recognition at the receptor site will also be evaluated.



29a-c

Rigid Acetylcholine Analogues

The nicotinic acetylcholine receptors are responsible for recognition and binding of the neurotransmitter, acetylcholine (**1**). Acetylcholine binds to the receptor activating the ligand gated ion channel and stimulating the central and parasympathetic nervous system. Acetylcholine is flexible molecule with many possible conformations. The topography of the ion-channel of the acetylcholine receptor is not fully known. Likewise, the binding conformation of acetylcholine is unknown. By constructing several rigid conformations of acetylcholine and its derivatives, a better understanding of its spatial geometry within the receptor, and ultimately the three-dimensional receptor structure can be achieved. To this end, a series of rigid 2-acetoxy-7-azabicycloheptane analogues have been prepared. They will be used to study the binding conformation of acetylcholine at the active site of the nAChRs.



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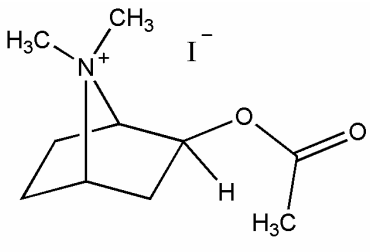


65

RESULTS AND DISCUSSION

Total Synthesis of Epibatidine and Quaternary Ammonium Salt Derivatives

Chemistry

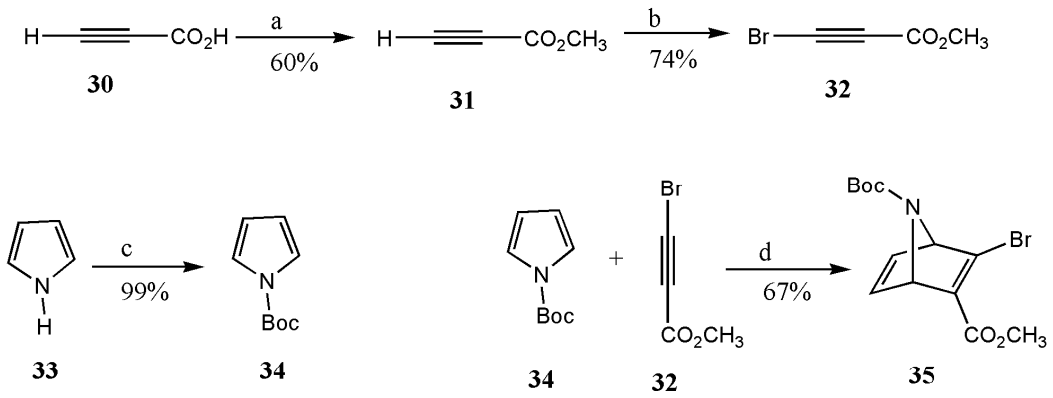
A variety of synthetic approaches to epibatidine have been reported based primarily on three different methods for the construction of the novel azabicyclic system as discussed earily.[55-67] Among those versatile methods, the condensation of *N*-protected azabicyclic ketone with pyridinyl substituent has been shown to be one of the most straightforward methods for the construction of the basic skeleton of epibatidine. The synthetic approach for the total synthesis of epibatidine followed the previous work established in our laboratories.[70] However, the yields of some key steps were significantly improved.

The total synthesis of epibatidine was developed around the [4+2] cycloaddition reaction of *N*-substituted-pyrrole with an appropriate substituted dienophile to provide the 7-azabicyclo[2.2.1]heptane skeleton.[70] As illustrated in **Scheme 4**,[73-75,70] the substituted dienophile was prepared from propiolic acid **30**, which was first esterified (**31**),[73] followed by bromination with *N*-bromosuccinimide (NBS) to furnish **32** in 74% yield.[74] *N*-Pyrrole (**33**) is a poor diene for the [4+2] cycloaddition reaction and usually it can be activated by the addition of alkyloxycarbonyl group.[75] It was found in the literature that the yield of this

protection procedure is 81% after 15 h. However it was found that if the reaction time (**33** to **34**) was increased to 2 days, the yield is almost quantitative (>98%).

With both starting materials in hand, heating **28** with 5 equivalents of **26** at 85-90 °C for 30 h gave the expected cycloaddition adduct **29** in 67% yield. As this is step is very early in the total synthesis of epibatidine, the yield of the cycloaddition reaction is important to the overall yield. The highest previous yield of this step was only 60%. After many attempts to optimize this reaction, it was discovered that temperature was important to this cycloaddition reaction. The reaction temperature should be maintained between 85-90 °C. This improved the yield of this step from 60% to consistent yields approaching 70%.

Scheme 4

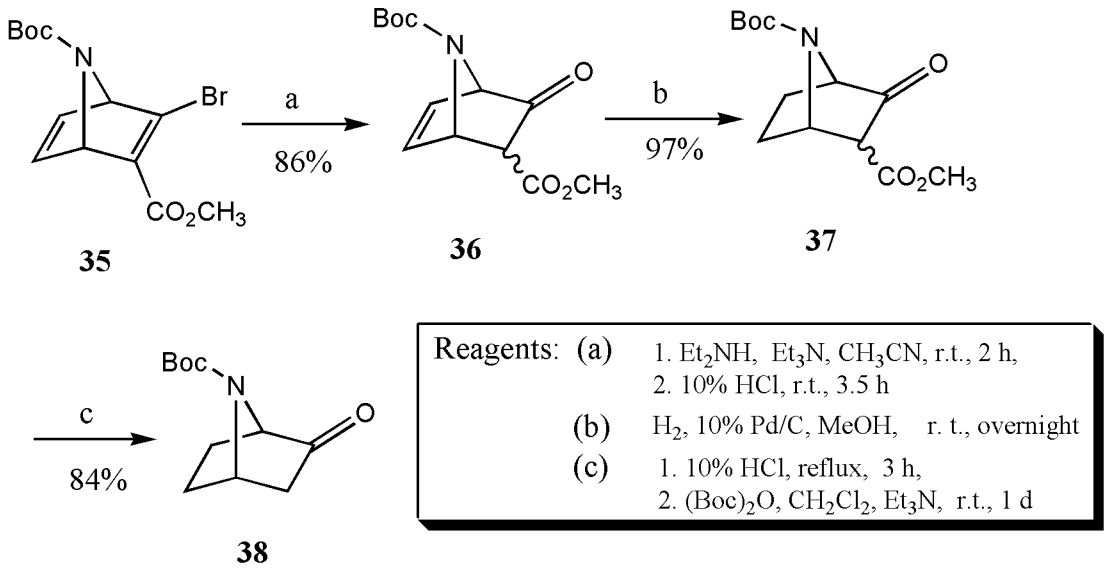


Reagents: (a) MeOH, $BF_3{\cdot}Et_2O$, reflux 1.5 h r. t,. 4 h (b) NBS/Me_2CO, $AgNO_3$(cat.) r.t., 1 h
(c) $(Boc)_2O$, DMAP, CH_3CN, r.t., 2 d (d) 85-90°C, 30 h

As illustrated in **Scheme 5**, treatment of **35** with diethylamine, followed by hydrolysis with 10% HCl, afforded the desired *β*-keto ester **36** in 86% yield as a mixture of isomers (*endo/exo*, 7:1). Initially, yields for this reaction were lower than

yields reported in the literature. It was found that 10% HCl should be added slowly but not dropwise. In addition, the reaction time was decreased to 3.5 h compared to 4 h previous reported in the literature. This gave consistent yields of almost 90%. Hydrogenation of the carbon-carbon double bond of **36** with 10% Pd/C (H_2, 1 atm) furnished **37** as a mixture of isomers (*endo/exo*, 3:2) in quantitative yield. The *β*-keto ester **37** was then decarboxylated followed by re-introduction of the Boc protecting group to provide the desired ketone **38** in 84% yield (**Scheme 5**).[70] The total yield of both the decarboxylation and Boc-protection reactions was improved from 77% to 84% by complete removal of residual water via azeotropic distillation with ethanol and then dried thoroughly under vacuum.

Scheme 5

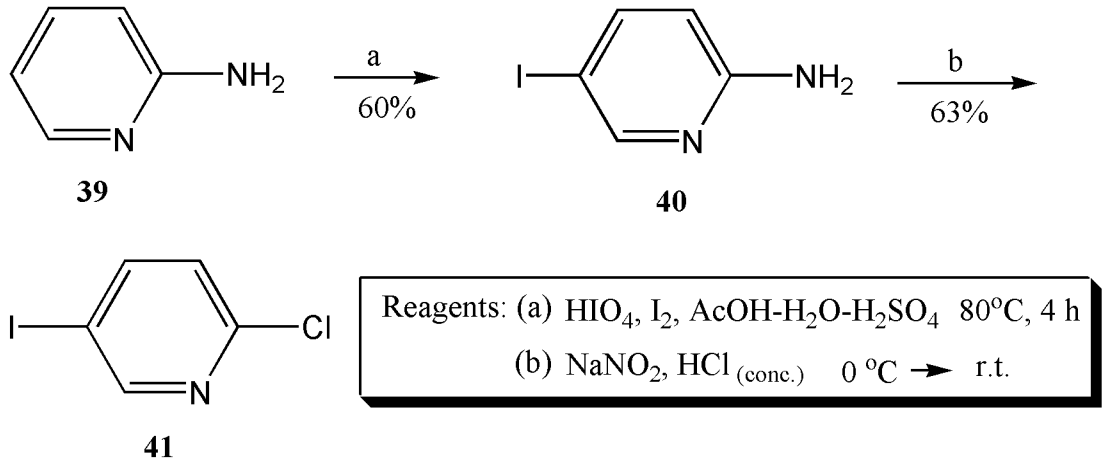


It is noteworthy that the 7-azabicyclo[2.2.1]heptan-2-one **38** is of potential value for the synthesis of epibatidine analogues as it contains the basic epibatidine skeleton. The ketone **38** could be obtained in 7 steps from commercially available

starting materials in 21% overall yield. This was a significant improvement from previous reports as well as providing multigram quantities for further transformations.

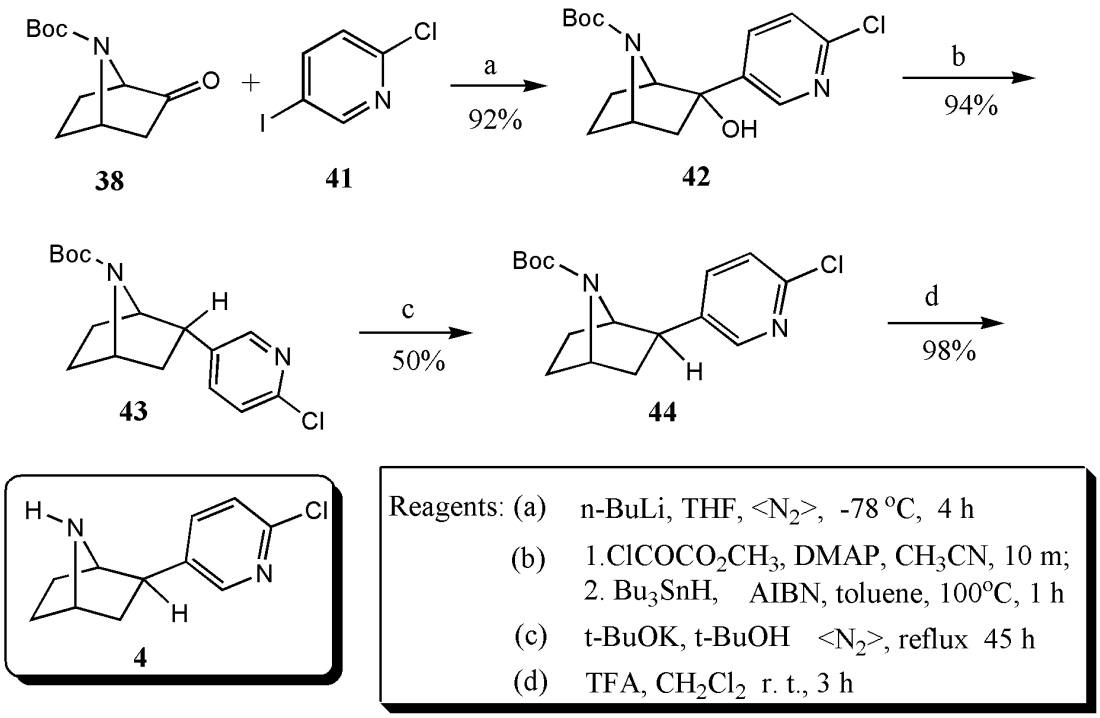
The preparation of the pyridine substituents has long been known but was prepared by an improved procedure from the recent literature[76] in two steps from 2-aminopyridine **33** (**Scheme 6**). Direct iodination of **39** in the presence of periodic acid gave 2-amino-5-iodopyridine **40** in 60% yield.[76] Diazonium salt formation in concentrated hydrochloric acid then afforded 2-chloro-5-iodopyridine **41** in 63% yield.

Scheme 6



Treatment of ketone **38** with 5-lithio-2-chloropyridine, which was generated by lithiation of 2-chloro-5-iodopyridine **41** with *n*-butyllithium at –78 °C under nitrogen, afforded the tertiary alcohol **42** in 92% yield as a single diastereoismer (**Scheme 7**).

Scheme 7



This step was improved from 88% to 92%. Due to the sensitivity of *n*-BuLi to water and air, this reaction required several considerations. In this reaction, even a trace of water can influence the yield significantly. First, all glassware was flame dried. Secondly, the amount of *n*-BuLi was increased slightly from 1.05 equivalent to 1.10 equivalent. Thirdly, the time of the exchange reaction (*n*-BuLi reacting with 2-chloro-5-iodopyridine to make 2-chloro-5-lithiumpyridine) was increased from 30 min to 45 min to ensure that the exchange reaction was complete.

Treatment of alcohol **42** with methyl oxalyl chloride afforded the corresponding methyl oxalyl ester which, without purification, was subjected to radical deoxygenation with tributyltinhydride (Bu_3SnH) in the presence of 2,2'-azobisisobutyronitrile (AIBN). This afforded the deoxygenated product

stereoselectively as the *endo*-isomer **43** in 94% yield (**Scheme 7**). Again, the yield of this step was improved from 88% to 94%. This improvement was due to freshly recrystallizing the initiator AIBN just before use. Epimerization of **43** using potassium *tert*-butoxide in refluxing *tert*-butyl alcohol afforded the desired *exo*-isomer **44** in 50% yield (**Scheme 7**). The yields of this step were substantially lower if the reagents (even *t*-BuOH) were not dry. Deprotection of the *N*-Boc-protected *exo*-isomer **44** using trifluoroacetic acid in CH_2Cl_2 at room temperature furnished (±)-epibatidine (**4**) as a white solid in almost quantitative yield (98%) (**Scheme 7**).[70]

As shown in Table 3, the yields of some key steps for the total synthesis of epibatidine have been significantly improved. The overall yield resulting from the improved steps was increased to 48%.

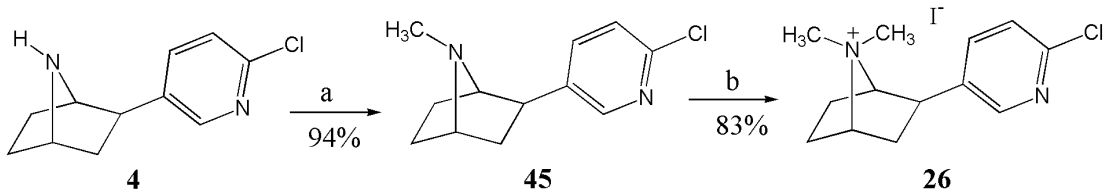
Table 3. Summary of the Yields of the Improved Steps

Reaction Scheme	Scheme 4 **34**	Scheme 4 **35**	Scheme 5 **38**	Scheme 7 **42**	Scheme 7 **43**	**Overall**
Literature Yields	81%	60%	77%	88%	88%	**29%**
Improved Yields	**99%**	**67%**	**84%**	**92%**	**94%**	**48%**

Once epibatidine (**4**) had been generated successfully, the syntheses of quaternary ammonium salts were carried out. For the *N*,*N*'-dimethyl epibatidinium iodide (**26**), several methods were attempted. Initial attempts to provide **26** in one step failed due to difficulty in isolation and purification of the quaternary salt. The studies were then focused on the preparation of **26** in two steps. The first step was to make the *N*-methyl tertiary amine. Initial attempts to reduce the *N*-Boc precursor **44** to

N-methyl tertiary amine also failed due to decomposition of the epibatidine ring system. Alternatively, the direct methylation from epibatidine worked well. After trying numerous conditions for the reductive *N*-methylation, as illustrated in **Scheme 8**, epibatidine (**4**) was methylated using 37% aqueous formaldehyde to provide *N*-methyl epibatidine **45** in 94% yield.[77] Subsequently, treatment of **45** with 25 equivalents of iodomethane for 3 h afforded the final salt **36** in 83% yield.

Scheme 8



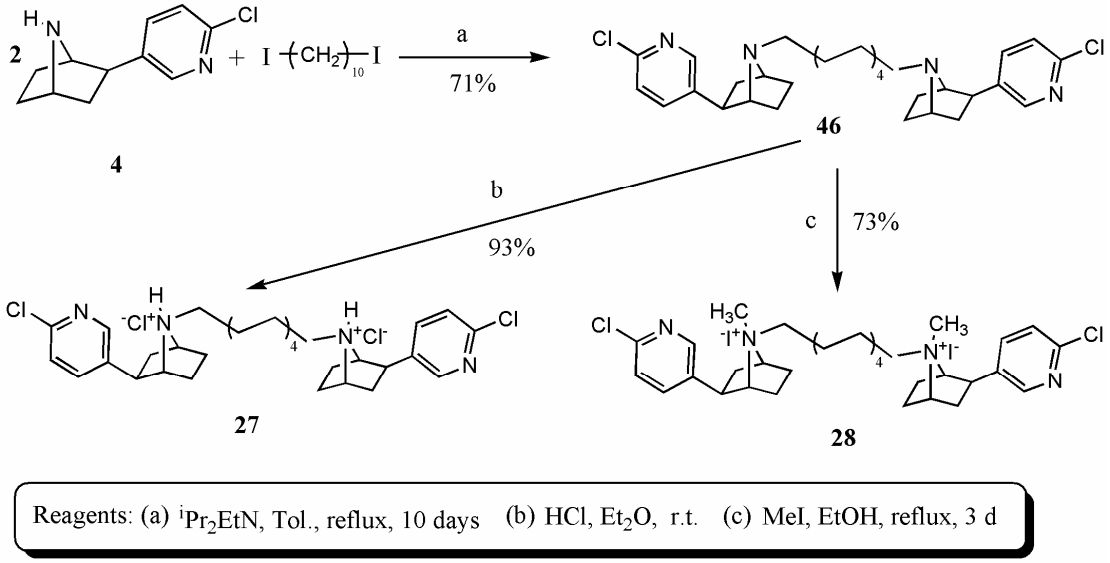
4 **45** **26**

Reagents: (a) 37% HCHO, $NaBH_3CN$, CH_3CN, r.t., 1 h (b) CH_3I, reflux, 3 h

The bis-ammonium salts were prepared in two steps. Epibatidine was treated with 1, 10-diiododecane for 10 d to provide bis-tertiary eqibatidine **46** in 71% yield. Treatment of **46** with HCl gave the final expected salt *N,N'*-decamethylene bisepibatidinium dihydrochloride **27** in 93% yield (**Scheme 7**). It was hoped that treatment of **46** with methyliodide in absolute ethanol would provide *N,N'*-decamethylene bisepibatidinium dimethyliodide **28**. Since compound **28** has conformation isomers due to the ten-carbon long chain, a very complex ^1H NMR was obtained. After purification of methyliodide by passing it through a pipet of anhydrous potassium carbonate. The compound 28 was obtained in 73% yield as a

brown solid.

Scheme 9



Overall, the novel NMB agents with epibatidinum substituents have been successfully synthesized. The biological activity of desired pure compounds **26-27** have been evaluated at nAChRs.

Biological Testing

The *in vitro* binding affinities (*K*i) of the 7-azabicyclo[2.2.1]heptane quaternary derivatives **26-27** summarized in Table 6, were measured by inhibition of [^{3}H]cytisine binding in homogenates of rat brain tissue. There are a variety of nAChRs subtypes that exist in the central nervous system; however, the α4β2 subtype is the predominant nAChR in rat striatum tissue. Therefore, the binding affinities (Ki)

reported in Table 6 correspond to the α4β2-subtype affinity of epibatidine and related compounds.

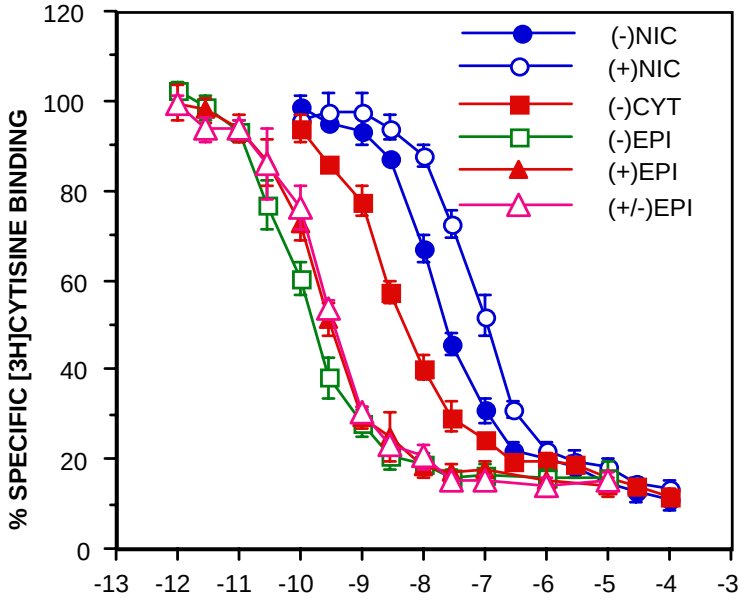
As shown in Table 4, both epibatidium quaternary methylioidide salt **26** and bis-epibatidine hydrochloride salt **27** exhibited equipotent binding affinity with (-)-cytisine and about twenty-fold more potent than unnatural (+)-nicotine. Though they exhibited lower binding affinity when compared to (±)-epibatidine. This result is still very exciting since it gives a new class of novel quaternary compounds that exhibit high affinity binding nAChRs. Between these two compounds, the long chain epibatidium hydrogen chloride **27** was found to possess similar potency with the quaternary epibatidium methiodide **26**.

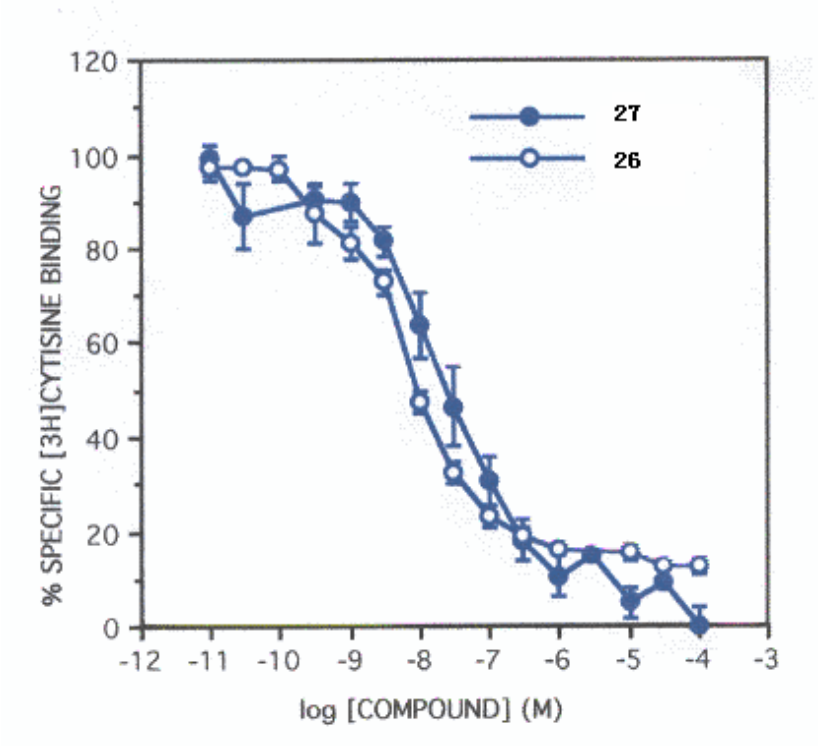
Table 4. Inhibition of [^{3}H]Cytisine Binding at α4β2 Subtype nAChRs.

Compound	K_i (nM)
(-)-epibatidine	0.079±0. 016
(+)-epibatidine	0.14±0. 02
(±)-epibatidine	0.16±0.03
(-)-cytisine	4.2±0.54
(-)-nicotine	8.0±4.5
(+)-nicotine	87±42
26	4.1±0. 6
27	6.6±1.3

All values are the mean ±SEM of data from three experiments performed in triplicate

Figure 3. Inhibition of [^{3}H]Cytisine Binding at α4β2 Subtypes nAChRs

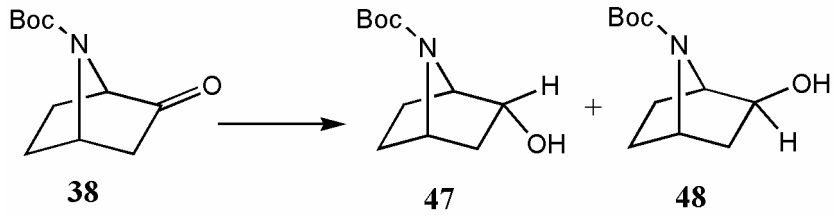




Synthesis of *endo*-2-(Hydroxymethylpyridyl)-7-azabicyclo[2.2.1]heptane Derivatives

Based on the high binding affinity and potent activity observed for the 3-pyridyl azetidine ester ABT-594 (**13**), the hybrid 7-azabicyclo[2.2.1]heptane analogues were envisaged as potential nicotinic acetylcholine receptors ligands. The synthesis of the hybrids **29a-c** was envisaged to proceed via the important intermediate developed from the total synthesis of epibatidine. The preparation of *endo* alcohol **47** was achieved by the reduction of the corresponding ketone **38** (**Scheme 10**). The 7-azabicyclo[2.2.1]heptane-2-one **38** was synthesized as in the previously described. Initially, reduction of the ketone **38** with DIBAl-H was attempted. Though the yield of this reaction is 81%, a mixture of *endo* **47** and *exo* **48** isomers was obtained in a ratio of **47:48** / 7:3. Alternatively, when the bulkier reducing reagent, lithium (*tri*-*tert*-butoxyallumino)hydride was applied, favorable stereoselective results had been obtained. The first attempted reduction was performed in diglyme only gave 39% yield. However, when the solvent was changed to THF, the pure *endo* alcohol **47** was obtained stereoselectively (>99%) in 78% yield.

Scheme 10



38 **47** **48**

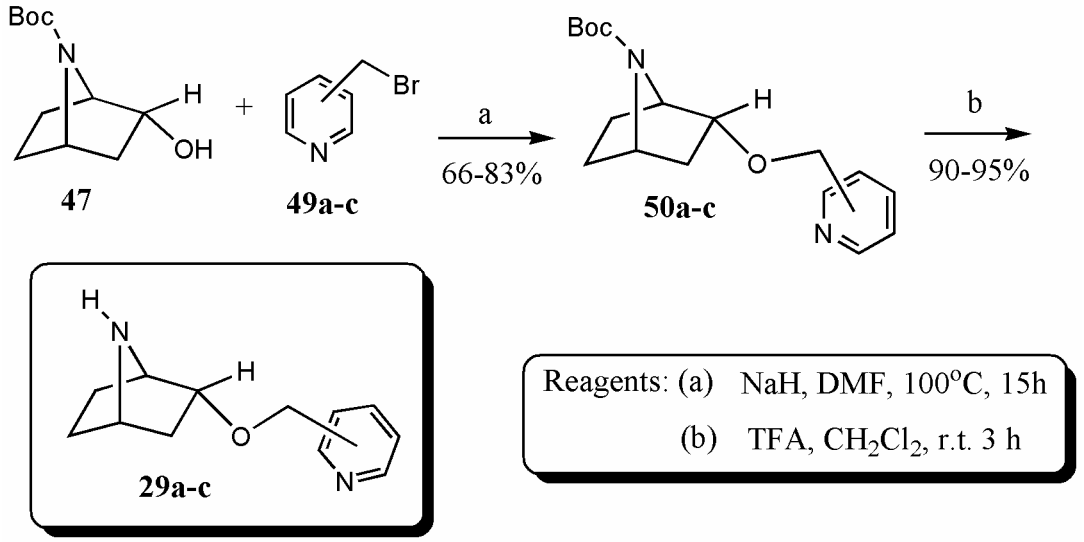
Reagents	**47**	**48**	Yield
DIBAl-H, THF, -78^{o}C, r. t., 10 h	70%	30%	81%
LiAlH(^{t}BuO)$_3$ (0.5M in diglyme), 0^{o}C, 2 h, r. t., 2 h	99%	trace	39%
LiAlH(^{t}BuO)$_3$ (1M in THF), 0^{o}C, 2 h, r. t., 2 h	99%	trace	78%

With pure *endo* alcohol (**47**) in hand, synthesis of the epibatidine pyridyl ether series **29a-c** proceeded. In a process as shown in **Scheme 11**, the *endo* alcohol **47** can be treated with the corresponding bromoalkylpyridine under Williamson ether synthesis conditions. It seemed that this particular reaction was very sensitive to the reaction conditions (temperature and time). After optimizing the reaction temperature and time, it was found that **47** when treated with the bromomethylprydine **49a-c** at 100^{o}C for 15 h furnished the corresponding ester **50a-c** in 66-83% yield. Table 5 summarized the optimized reaction conditions for synthesis of compound **49b**. It was found that when the reaction temperature is higher than 120^{o}C, most of the azabicyclic agent would be decomposed. The desired 2(-hydroxyalkylpyridyl)-7-azabicyclo[2.2.1]heptane derivatives **29a-c** were finally obtained by removal of the Boc protectiving group with TFA (**Scheme 11**).

Table 5. Optimized Williamson Ether Synthesis Conditions

	1	2	3	4	5
Temperature	80^{o}C	100^{o}C	100^{o}C	120^{o}C	130^{o}C
Time	4 h	4 h	15h	15 h	15 h
Yield	------	36%	77%	50%	45%

Scheme 11

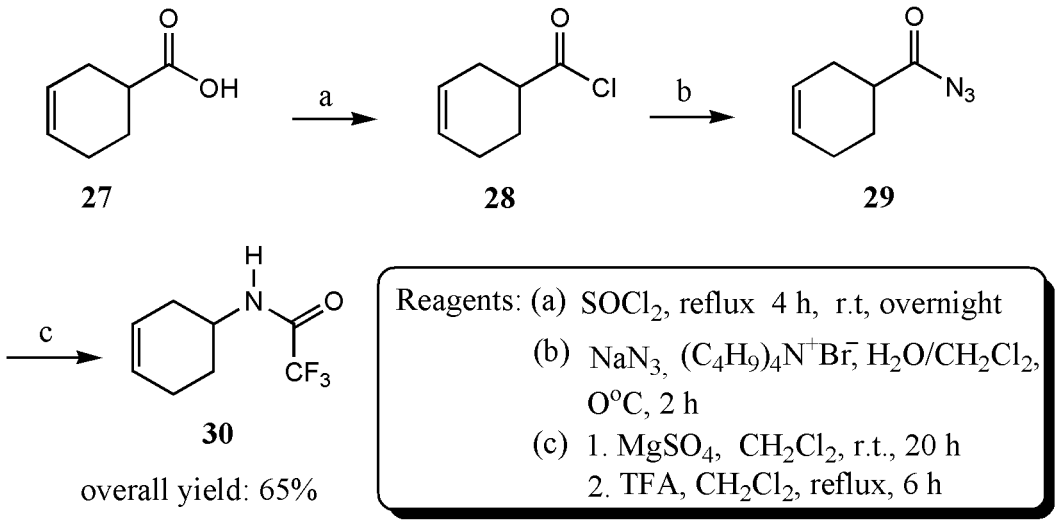


Overall, a series of epibatidine pyridyl ethers have been successfully synthesized. The binding affinity of these compound at nAChRs is currently under investigation and will be reported elsewhere.

Synthesis of *exo*-2-(Hydroxymethylpyridyl)-7-azabicyclo[2.2.1]heptane Derivatives Efforts

A synthetic route to prepare exo-*N*-methyl-1-azabicylco-[2.2.1]heptane **51a-c** was also investigated. As illustrated in **Scheme 12**, the acid chloride **53** was prepared from the acid **52** using thionyl chloride in high yield. The reaction of acid chloride **53** with sodium azide under phase transfer conditions afforded the acyl azide **54**. The rearrangement of acyl azide **54** was done by letting it stand overnight in dry dichloromethane over a drying agent. A slight excess of trifluoroacetic acid was then added. This resulted the amide **55** in 65% overall yield.[78]

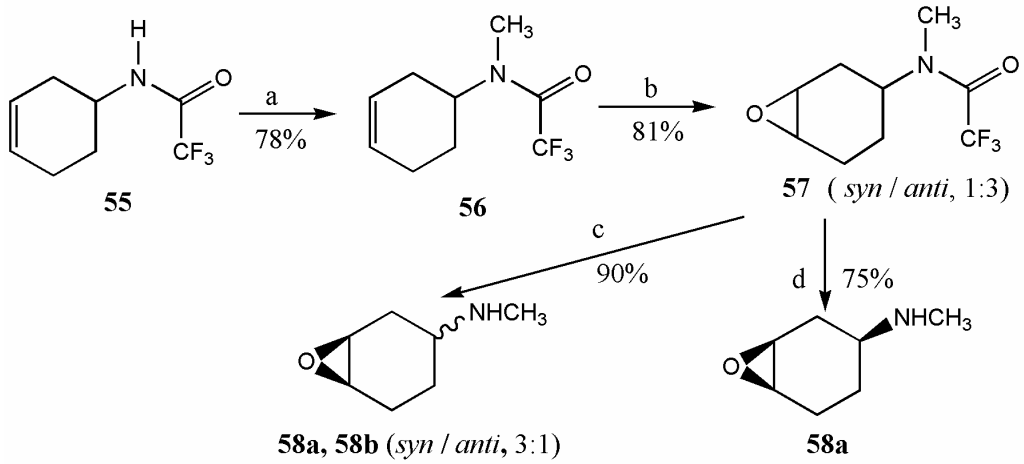
Scheme 12



Alkylation of **55** with methyl iodide and sodium hydride gave the *N*-methyl derivative **56** in 78% yield, which was then transformed into the epoxides **57** (*syn*/*anti* ratio ~ 1:3) with *m*-chloroperoxybenzoic acid in 81% yield. The mixture of epoxides was then treated with potassium carbonate to remove the trifluoroacetyl protecting

group in 75% yield to afford the *syn*-*N*-methylamino epoxide **58a** as the sole product (**Scheme 13**).[79, 80] The yield of this step was improved from 65% to 75% by a rigorous work-up of this reaction. Both dichloromethane and ether were used to extract the product as it was found that if just ether was used as described in the literature, product was still found in the water layer as seen by T.L.C. It is noteworthy that a single diastereoisomer **58a** was obtained from the hydrolysis reaction of **57**. Alternatively, if the mixture of epoxides **57** was treated with methanolic potassium hydroxide, a mixture of *syn* and *anti* epoxides **58a : 58b** (3:1) was obtained.[38] In a similar manner as described above, dichloromethane and ether were both used for extraction to improve the yield from 70% to 91%.

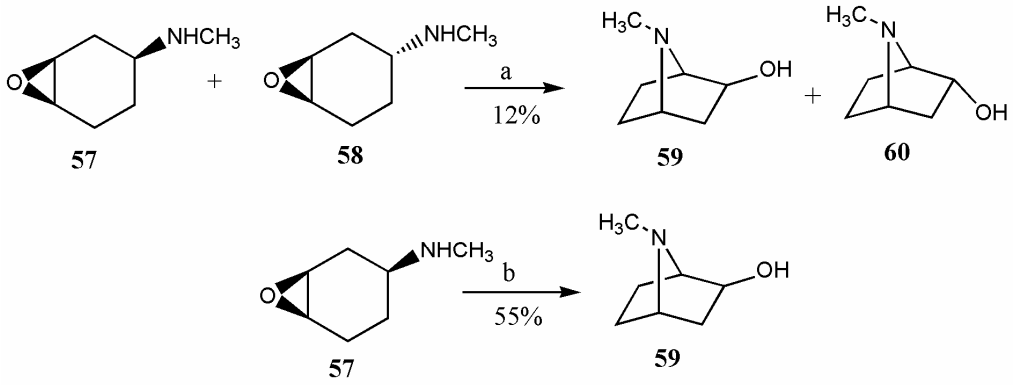
Scheme 13



The mixture of epoxides **58a** and **58b** was heated at 160°C in *N*-methylpyrrolidone (NMP)/potassium carbonate for 72 h and then allowed to cool to room temperature over 12 h. After work-up, a mixture was expected to be obtained

corresponding to the ring closure of *exo*-isomer **59** and *endo*-isomer **60** (**Scheme 14**).[80]

Scheme 14



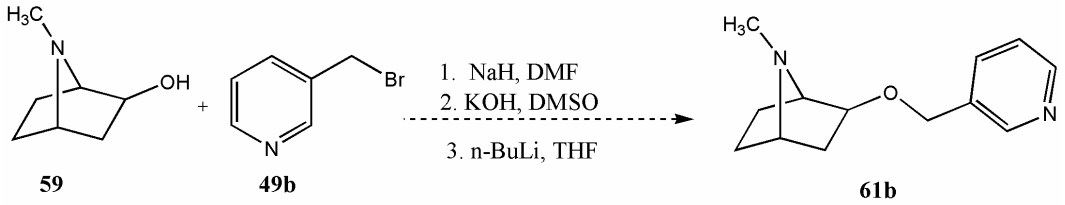
Reagents: (a) NMP, K_2CO_3, 160°C, 3 d (b) NMP, K_2CO_3, 150°C, 3 d

The best yield of this step was only 12%. However, it was determined that the *exo*-isomer **59** was formed by intramolecular cyclization reaction if the pure epoxide **57** was cyclized, the yield was increased to 55% this way.

Some difficulties were encountered with the attempted Williamson ether synthesis of the *exo*-*N*-methyl alcohol **59** and the corresponding bromalkylpyridine. The first attempted ether synthesis of *exo*-*N*-methyl alcohol **59** was performed with the same ether synthesis condition of *endo*-*N*-Boc alcohol **47** using NaH in DMF. A very complex mixture resulted as determined by TLC. Alternatively, a weaker base KOH was attempted exploiting the formation of the ether in DMSO. Unfortunately, none of the desired ethers were obtained. Competing *N*-alkylation gave a complex mixture of intractable materials. The third attempt of a Williamson ether synthesis was performed with stronger base *n*-BuLi in THF at room temperature only gave starting material. However, the decomposition of azabicyclic ring was observed when the

temperature was increased to 60°C. The lack of reactivity of *exo*-*N*-methyl alcohol was surprising. Though the *N*-methyl group has been identified as a good protecting group for amines, there is a fine line between the stability and the decomposition of the azabicyclic ring in basic environment.

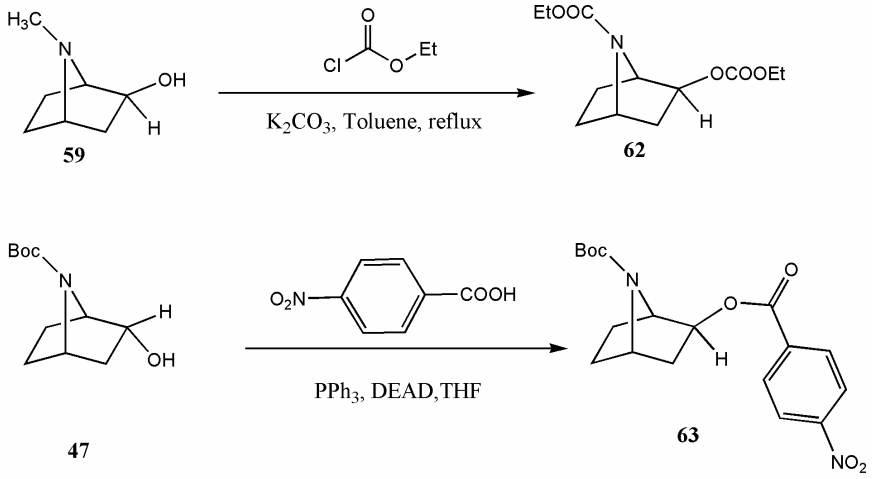
Scheme 15



59 **49b** **61b**

Two alternative routes can be attempted. The first is the conversion of the *N*-methyl group to a *N*-carbonyl group. This will reduce the nucleophilicity of the nitrogen atom and prevent quaternization (**Scheme 16**). The second route started from *endo*-*N*-Boc alcohol **47**. Treatment of **47** under Mitsunobu reaction condition should then afford the compound **63** (**Scheme 16**). Although these two methods were exploited, the yields were relatively low with the tiny scale after separation and purification. Further attempts will be reinvestigated at a later date.

Scheme 16



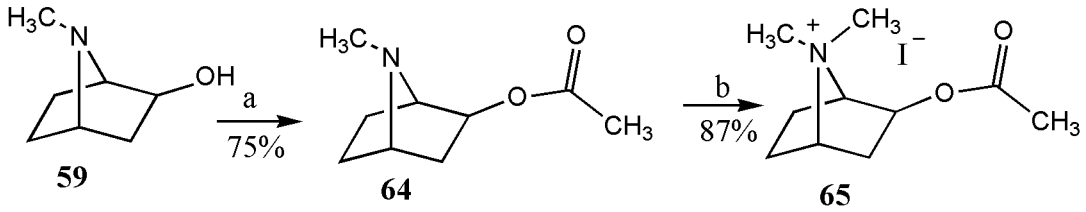
59 **62** **47** **63**

Synthesis Acetylcholine Analogues

Acetylcholine is a flexible molecule with many possible conformations. The topography of the ion-channel of the acetylcholine receptor is not fully known. Likewise, the binding conformation of acetylcholine is unknown. By constructing several rigid conformations of acetylcholine and its derivatives, a better understanding of its spatial geometry within the receptor, and ultimately the three-dimensional receptor structure can be achieved.

With the important intermediate azabicyclic alcohol **59** in hand, the syntheses of the rigid acetylcholine analogues were also carried out (**Scheme 17**). The *exo* alcohol **59** was treated with freshly distilled acetic anhydride (Ac_2O) in the presence of dry pyridine at room temperature. High yield was achieved if the chromatography solvent system contained NH_4OH in order to neutralize the acidic sites of the silica gel. The *exo*-2-acetoxy-7-methyl-7-azabicyclo[2.2.1]heptane **64** was converted into the oxalic acid salts as white solid for biological testing. The quaternary *exo*-2-acetoxy-7-methyl-7-azabicyclo[2.2.1]heptane methiodide **65** was synthesized by treating the starting material **64** with methyl iodide (CH_3I) in refluxing THF. Upon completion of the methylation, the solvent was removed and the resultant yellow solid was washed with ether to afford the quaternary salt **65** in high yield. The purity of the resulting salt **65** was suitable for biological testing. The biological activity of **64-65** is currently under investigation and will be reported elsewhere in due course.

Scheme 17



Reagent: (a) Ac_2O, Pyridine, $CHCl_3$, r.t. (b) CH_3I, THF, reflux, overnight

CONCLUSION

Three novel series of epibatidine analogues have been prepared and evaluated as potential nAChR ligands. Among them, the novel quaternary epibatidinium salt **26** and bis-epibatidine derivative **27** exhibited significantly high binding affinity at nAChRs relative to nicotine and cytisine. It is apparent from this study that a new class of bis-epibatidine derivatives would act as a potential potent nAChR ligand. In addition, a series of pyridyl ether epibatidine analogues have been identified and three endo-2-(hydroxymethylpyridyl)-7-azabicyclo[2.2.1]heptane compounds have been successfully synthesized. The structure-activity relationship studies of these analogues should offer some interesting insights into the elucidation of the neuronal nAChR pharmacophore and will be useful in the further studies aimed a development of selective nAChR therapeutic agent. Moreover, two more new rigid 2-acetoxy-7-azabicyclo[2.21]heptane derivatives have been prepared. These studies will certainly provide additional information with regard to the structure-activity relationships and pharmacology of the nAChR ligand epibatidine. Overall, these studies may lead to the development of new pharmacological strategies and therapeutic agents for nAChR research.

EXPERIMENTALS

General Information

- All chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, unless otherwise noted. Ethyl alcohol, absolute-200 proof, was purchased from AAPER Alcohol and Chemical Co., Shelbyville, KY. Bakerdry® THF, Bakerdry® dichloromethane, Bakerdry® acetonitrile and Bakerdry® mechanol purchased from Mallinkrodt Baker Inc., Philisburg, NJ, and stored under argon. Benzene, hexane and toluene were dried by distillation over sodium pieces using benzophenone as the indicator and stored under nitrogen. Anhydrous DMF was purchased in a sure-seal bottle from Aldrich Chemical Co. Acetic acid (100 mL) was dried by simple distillation from acetic anhydride (1 mL).

- Chromatography was accomplished on silica gel (Silica Gel 60, 200-400 mesh, Natland International Corp, Morrisvile, NC). Petroleum ether refers to pentanes with a boiling point range of 30-60 °C.

- NMR spectra were recorded on Varian-Gemini 400MHz and Varian Gemini 300 MHz multiprobe spectrometers as indicated. Both ^{1}H NMR spectra and ^{13}C NMR spectra were recorder for the freebase unless otherwise indicated. Chemical shifts

are reported as δ values from Deuterated chloroform ($CDCl_3$) or as noted, tetramethylsilane (TMS) (Cambridge isotope Laboratories) were employed as the internal standards.

- Elemental analyses were determined by Atlantic Microlabs Inc., Norcross, GA for the corresponding oxalate salts and hydrochloride salts.

- Reported melting points were recorded on a Hoover Mel-Temp apparatus and are uncorrected.

General Procedure for the Preparation of Hydrochloride Salts. The freebase compound (50 mg) was dissolved in THF (1-2 mL). This solution was added to a cooled solution of saturated hydrogen chloride in ether. The hydrogen chloride salt precipitated instantaneously with the mixing of the two solutions. The precipitate was immediately collected by vacuum filtration to prevent decomposition of acid sensitive compounds. The salt was washed with anhydrous ether three times. Some analytical samples contained minute amounts of water despite drying (48 h under vacuum).

Methyl Propiolate (31): To a solution of propiolic acid (25 g, 0.36 mol) in dry methanol (200 mL) was added via syringe freshly distilled boron trifluoride etherate (93 mL, 0.76 mol). The solution was refluxed for 1.5 h and further stirred at room temperature for 4 h. Water (200 mL) was added, the mixture was extracted with

dichloromethane (100 mL). The organic layer was removed, and the aqueous portion was extracted with 3 × 100 mL of dichloromethane. The combined organic layers were washed sequentially with 200 mL of water and 100 mL of brine. The organic solution was dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was distilled in vacuo to give pure **31** (19 g, 60%). bp 42-44°C/ 30 mmHg; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (3H, s), 2.90 (1 H, s).

Methyl 3-bromopropiolate (32): To a magnetically stirred solution of compound **31** (10 g, 0.12 mol) in freshly distilled acetone (350 mL) at room temperature, silver nitrate (2 g, 12 mol) was added, followed by *N*-bromosuccinimide (24 g, 0.14 mol) all at once. Stirring was continued for 3 h while the homogeneous solution turned cloudy and then a grayish precipitate developed. Careful removal of acetone by rotary evaporation under water pump pressure at room temperature gave a yellow oil. The residue distilled by vacuum bulb-to-bulb distillation to afford a yellow solid (14 g, 74%) ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 3.73 (3 H, s).

***N*- (*tert*-Butoxycarbonyl)-pyrrole (34):** To a stirred solution of pyrrole (7 mL, 0.1 mol) in dry CH_3CN (100 mL) was added DMAP (1.2 g , 0.01 mol) and Boc_2O (26 g, 0.12 mol) at room temperature. Evolution of gas commenced, and after 0.5 h a clear solution was obtained. The whole reaction mixture was stirred at room temperature for 48 h to ensure complete reaction. The solvent was carefully removed under reduced pressure to give the product **34** as yellow oil (16 g, 99%) ^{1}H NMR (400

MHz, $CDCl_3$/TMS) δ 7.24 (2 H, d, J = 2.1Hz), 6.21 (2 H, d, J = 2.1Hz), 1.59 (9 H, s).

Methyl 2-bromo-7-(*tert*-butoxycarbonyl)-7-azabicyclo[2.2.1]hepta-2,5-diene-2-carboxylate (35): A mixture of methyl 3-bromopropiolate (**32**) (8.2 g, 0.05 mol) and *N*-Boc-pyrrole (42 g, 0.25 mol) was stirred at 85-90^{o}C under nitrogen for 30 h. The resulting mixture was cooled to room temperature and subjected to column chromatography (EtOAc : Hexane, 1 : 15) to give the desired product **35** as a slightly yellow oil (22 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 7.12 (2 H, br s), 5.47 (1 H, s), 5.12 (1 H, s), 3.79 (3 H, s), 1.41 (9 H, s).

7-(*tert*-Butoxycarbonyl)-3-(methoxycarbonyl)-7-azabicyclo[2.2.1]hept-5-en-2-one (36): To a solution of **35** (10 g, 30 mmol) and triethylamine (20 mL, 150 mmol) in dry acetonitrile (60 mL) was added dropwise a solution of diethylamine (3.5 mL, 33 mmol) in dry acetonitrile (40 mL) under a nitrogen atmosphere. The mixture was stirred at room temperature for 2 h. A 10% HCl (100 mL) solution was then added slowly. The reaction mixture was stirred for another 3.5 h. Water (100 mL) was added, and the mixture was extracted with dichloromethane (3 × 60 mL). The dichloromethane was dried with sodium sulfate and concentrated under reduced pressure. The residue was chromatographed (silica gel, EtOAc : Hexane, 1: 6) to afford the β-keto ester **36** (6.9 g, 86%) as an yellow oil (*endo* : *exo*, 7 : 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (0.88 H, dd, J = 3.6 Hz), 6.77 (0.12 H, s), 6.36 (0.12H,

s), 6.35 (0.88H, d, J = 2.8 Hz), 5.42 (0.12 H, s), 5.09 (0.88 H, s), 4.69 (1 H, s), 3.75 (2.64 H, s), 3.73 (0.36 H, s), 3.40 (0.88 H, d, J = 3.2 Hz), 2.93 (0.12 H, s), 1.44 (9 H, s).

7-(*tert*-Butoxycarbonyl)-3-(methoxycarbonyl)-7-azabicyclo[2.2.1]heptan-2-one (37): A suspension of **36** (4.0 g, 15 mmol) and 10% Pd / C (500 mg) in dry methanol (45 mL) was vigorously stirred under a hydrogen atmosphere (1 atm) at room temperature overnight. The catalyst was removed by filtration through a pad of celite and the filtrate was concentrated under reduced pressure. Flash chromatograph on silica gel (EtOAc : Hexane, 1: 6) afforded **37** (3.9 g, 97 %) as colorless oil (*endo* : *exo*, 3 : 2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.84 (0.6 H, d, J = 3.6 Hz), 4.73 (0.4 H, t, J = 4.4 Hz), 4.36 (0.6 H, d, J = 4.5 Hz), 4.32 (0.4 H, d, J = 6.0 Hz), 3.76(1.2 H, s), 3.73 (1.8 H, s), 3.45(0.4 H, d, J = 4.2 Hz), 2.99 (0.6 H, s), 2.00-2.07 (2 H, m), 1.60-1.73 (2 H, m), 1.45(9 H, s).

7-(*tert*-Butoxycarbonyl)-7-azabicyclo[2.2.1]heptan-2-one (38): A solution of the β-keto ester **37** (1.4 g, 5 mmol) in 10% HCl (110 mL) was heated at 100-110 ^{o}C for 3 h under nitrogen atmosphere. The solution was allowed to cool to room temperature and evaporated under reduced pressure. The trace of remaining water was removed by azeotropic distillation with EtOH and then dried thoroughly under vacuum. The gray residue was dissolved in dichloromethane (60 mL) and Et_3N (2.8 mL, 20 mmol) and Boc_2O (2.2 g, 10 mmol) were added. The solution was stirred for 24 h at room

temperature and then washed with saturated Na_2CO_3 solution. The organic layer was dried (Na_2SO_4), filtered, and concentrated to provided a yellow oily residue which was chromatographed (SiO_2, EtOAc : Hexane, 1 : 5) to afford the ketone **38** (0.88g, 84%) as a white solid. mp 60-62^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 4.56 (1 H, t, J = 4.4 Hz), 4.25 (1 H, d, J = 5.2 Hz), 2.46 (1 H, dd, J = 5.2 Hz), 1.99-2.04 (3 H, m), 1.59-1.68 (2 H, m), 1.46 (9 H, s).

2-Amino-5-iodopyridine (40): A mixture of 2-aminopyridine **39** (9.4 g, 0.1 mol), periodic acid dehydrate (4.5 g, 0.02 mol) and iodine (10 g, 0.04 mol) was heated in a mixed solution of acetic acid (60 mL), water (12 mL) and H_2SO_4 (1.8 mL) at 80^oC for 4 h. The mixture was then poured into aqueous $Na_2S_2O_3$ to remove unreacted iodine and extracted with ether. The extract was washed with aqueous diluted NaOH, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography on silica gel with ethyl acetate as eluent and then recrystallized from ethanol to afford the colorless prisms of **40** (13 g, 60%): ^1H NMR (400 MHz, $CDCl_3$) δ 8.21 (1 H, d, J = 1.5 Hz), 7.64 (1 H, dd, J = 2.1, 1.8 Hz), 6.38 (1 H, d, J = 3.6 Hz), 4.24 (2 H, br s).

2-Chlolo-5-iodopyridine (41): To a stirred solution of 2-amino-5-iodopyridine **40** (11 g, 0.05 mol) in 37% concentrated HCl (100 mL) were added sodium nitrite (17 g, 0.25 mol) at ice bath, the temperature being kept around 0^oC during the addition. The reaction was then continued for 1 h at room temperature and then quenched by

addition of aqueous solution of NaOH (20 mL). The mixture was extracted with ether and the extract was washed with water, dried and concentrated. The residue was purified by column chromatography on silica gel and then recrystallized from ethanol to give pale yellow plates of **41** (7.5 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ8.60 (1 H, d, J = 2.1 Hz), 7.94 (1 H, d, J = 11.1 Hz), 7.14 (1H, d, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$) δ151.1, 146.4, 142.2, 121.6, 86.2.

***exo*-2-(2-Chloro-5-pyridinyl)-7-(*tert*-butoxycarbonyl)-7-azabicyclo[2.2.1]heptan-2-ol (42):** A solution of 2-chloro-5-iodopyridine **41** (260 mg, 1.1 mmol) in dry ether (5 mL) and dry THF (2.5 mL) at -78 ^{o}C was added n-BuLi (0.80 mL, 1.4 M solution in hextane, 1.2 mmol) dropwise. The mixture was stirred at -78^{o}C for 45 min before a solution of ketone **38** (210 mg, 1.0 mmol) in ether (3 mL) was added dropwise. The mixture was stirred at -78^{o}C for 3 h and then warmed to -50^{o}C and stirred for another 30 min. Saturated aqueous NH_4Cl (2 mL) was added, and the mixture was allowed to warm to room temperature. Water (5 mL) was added, and the organic layer was separated. The aqueous phase was extracted with EtOAc (10 mL), and the combined organic layers were dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel (EtOAc : Hexane, 1 : 3) to give the tertiary alcohol **42** (0.3g, 92%) as a white solid. mp 147-149^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ8.59 (1 H, d, J = 2.4 Hz), 7.85 (1 H, dd, J = 2.4, 6.0 Hz), 7.26 (1 H, d, J = 6.0 Hz), 4.28 (1 H, s), 4.20 (1 H, s), 2.71 (1 H, br s), 2.36 (2 H, m), 1.64-1.9 (4 H, m), 1.44 (9 H, s).

***endo*-(2-Chloro-5-pyridinyl)-7-(*tert*-butoxycarbonyl)-7-azabicyclo[2.2.1]heptane (43):** To a solution of the tertiary alcohol **42** (130 mg, 0.4 mmol) and 4-(dimethylamino)pyridine (DMAP, 73 mg, 0.6 mmol) in dry CH_3CN (5 mL) was added methyl oxalyl chloride (0.055 mL, 0.6 mL). The mixture was stirred for 10 min at room temperature under nitrogen and then diluted with EtOAc (20 mL). The mixture was then washed successively with saturated aqueous $NaHCO_3$ (10 mL) and H_2O (10 mL). The organic portion was dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was evaporated twice with toluene to afford the methyl oxalyl ester. Without further purification, the crude ester was added to a mixture of Bu_3SnH (0.18 mL, 0.63 mmol) and freshly recrystalized 2, 2'-azobis(isobutyronitrile) (AIBN, 10 mg) in dry toluene (5 mL) under nitrogen. The mixture was heated at 100^oC for 1 h. The solvent was removed under reduced pressure and the residue was purified by chromatography (EtOAc : Hexane, 1 : 9) to afford the endo-isomer (116 mg, 94%) as a white solid. mp 80-82^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (1 H, d, J = 2.4 Hz), 7.46 (1 H, dd, J = 3.2, 8.4 Hz), 7.27 (1 H, d, J = 2.4 Hz), 4.3 (2 H, m), 3.44 (1H, m), 2.28 (1 H, m), 1.83 (1 H, m), 1.49-1.85 (2 H, m), 1.46 (9 H, s), 0.94-1.43 (2 H, m).

***exo*-2-(2-Chloro-5-pyridinyl)-7-(*tert*-butoxycarbonyl)-7-azabicyclo[2.2.1]heptane (44): Epimerization of 43.** A mixture of **37** (100 mg, 0.33 mmol) and *t*-BuOK (190 mg, 1.7 mmol) in *t*-butyl alcohol (5 mL) was heated to reflux for 45 h under nitrogen. The solvent was evaporated, and the residue was chromatographed (EtOAc : Hexane,

1 : 9) to afford the *endo*-isomer **43** and the *exo*-isomer **44** (33 mg, 50% based on recovered **43**) as a white solid. mp 67-69°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (1 H, d, J = 1.2 Hz), 7.62 (1 H, d, J = 7.6 Hz), 7.22 (1 H, d, J = 1.2 Hz), 4.35 (1 H, br s), 4.13 (1 H, br s), 2.84 (1 H, dd, J = 4.8, 4.0 Hz), 1.97 (1 H, d, J = 8.8 Hz), 1.80 (3 H, m), 1.55 (2 H, m), 1.45(9 H, s).

***exo*-2-(2-Chloro-5-pyridinyl)-7-azabicyclo[2.2.1]heptane (4):** To a solution of **44** (156 mg, 0.5 mmol) in dichloromethane (5.0 mL), trifluoroacetic acid (0.5 mL, 6.5 mmol) was added dropwise with stirring under nitrogen. The mixture was stirred for 3 h at room temperature and rendered basic with saturated Na_2CO_3 (10 mL). The organic layer was separated, and the water phase was extracted with dichloromethane. The organic layers were combined, dried and concentrated under reduced pressure. The residue was purified by chromatography (SiO_2, CH_2Cl_2 : MeOH : Et_3N, 90 : 10 : 1) to give **2** (133 mg, 98%) as a white solid. mp 50-51°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (1 H, d, J = 3.6 Hz), 7.76 (1 H, dd, J = 3.6, 8.0 Hz), 7.22 (1 H, d, J = 3.6 Hz), 3.79 (1 H, br s), 3.56 (1 H, br s), 2.77 (1 H, dd, J = 6.4 Hz), 1.91 (1 H, m), 1.50-1.63 (5 H, m)

***exo*-2-(2-Chloro-5-pyridinyl)-7-methyl-7-azabicyclo[2.2.1]heptane (45):** To a stirred solution of **4** (42 mg, 0.2 mmol) and 37% aqueous formaldehyde (0.16 mL, 2 mmol) in 1.5 mL of acetonitrile under an atmosphere of nitrogen was added sodium cyanoborohydride (38 mg, 0.6 mmol). An exothermic reaction ensued and the white

gray solid precipitated. The reaction mixture was stirred for 10 minutes, and then glacial acetic acid was added dropwise until the solution tested neutral on pH paper. Stirring was then continued for an additional 30 minutes. The whole solution was evaporated under reduced pressure, and 1 mL of 10% NaOH solution was added to the residue. The resulting mixture was extracted with ether (3 × 2 mL). The combined ether extracts were washed with 5% NaOH solution (2 mL), and then with 10% HCl (3 × 1 mL). The acid extracts were combined and neutralized with solid NaOH and then extracted with ether (3 × 2 mL). The combined ether extracts were dried with sodium sulfate. After filtering, the solvent was removed under reduced pressure. Purification of the residue was achieved by flash chromatography (SiO_2, CH_2Cl_2: MeOH: Et_3N, 180:20:1) to yield **45** as white solid (40 mg, 94% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 8.28 (1 H, d, J = 2.7 Hz), 7.88 (1 H, dd, J = 2.7 Hz), 7.21 (1 H, d, J = 6.4 Hz), 3.33 (1 H, t, J = 4.5 Hz), 3.13 (1 H, d, J = 3.9 Hz), 2.65 (1 H, dd, J = 4.5 Hz), 2.25 (3 H, s), 1.82-1.95 (3 H, m), 1.68 (1 H, m), 1.42 (2 H, m). ^{13}C NMR ($CDCl_3$) δ 148.9, 148.8, 141.7, 138.1, 123.8, 67.6, 61.3, 45.4, 41.5, 34.7, 26.5, 25.5.

***exo*-2-(2-Chloro-5-pyridinyl)-7-methyl-7-azabicyclo[2.2.1]heptane methiodide (26):** To a stirred solution of **45** (34 mg, 0.15 mmol) in freshly distilled THF (3 mL) under an atmosphere of nitrogen was added CH_3I (0.3 mL, 4.8 mmol) dropwise. The solution was heated to reflux for 3 h. The solution was removed under reduced pressure and the resulting fine powder was triturated with warm ether to yield **39** as a

yellow powder (42 mg, 83% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.45 (1 H, d), 7.88 (1 H, dd), 7.51 (1 H, d), 4.83 (1 H, d), 4.22 (1 H, t), 3.80 (1 H, t), 3.21 (3 H, s), 2.87 (3H, s), 2.70 (2 H, m), 2.548(2 H, m), 2.14 (2 H. m). ^{13}C NMR (CD_3OD) δ 149.5, 147.2, 137.4, 136.3, 124.3, 73.7, 72.5, 45.6, 44.9, 42.9, 34.7, 27.9, 26.1 . Anal. Calc. for $C_{13}H_{18}N_2ClI$•0.5 H_2O: C, 41.79%; H, 5.12%; N, 7.50%. Found: C 42.05%, H, 4.95%, N, 7.36%

***N, N'*-Decamethylene bis-*exo*-2-(2-chloro-5-pyridinyl)-7azabicyclo[2.2.1]heptane (46):** A mixture of epibatidine **4** (52 mg, 0.25 mmol) and 1, 10-diiododecane (50 mg, 0.125 mmol) in 5 mL of freshly distilled toluene in the presence of *N, N'*-diisopropylethylamine (0.11 mL, 0.75 mmol) was heated with stirring at 110^{o}C under nitrogen for 10 days. The solvent was removed under reduced pressure and water (5 mL) was added to the residue. The aqueous layer was then extracted with dichloromethane. The extract was dried with sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2 : MeOH, 30:1) to yield **46** as thick, brown oil (49 mg, 71% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (2 H, d, J = 2.4 Hz), 7.90(2 H, dd, J = 2.4, 6.4 Hz), 7.19 (2 H, d, J = 8.0 Hz), 3.45 (2 H, s), 3.20 (2 H, s), 2,63 (2 H, t, J = 4.4 Hz), 2.41 (2 H, m), 2.32 (2 H, m), 1.82-1.89 (6 H, m), 1.70 (2 H, m), 1.40-1.48 (8 H, m), 1.27 (12 H, m). ^{13}C NMR ($CDCl_3$) δ 149.0 (2), 148.8 (2), 142.1 (2), 138.3 (2), 123.7 (2), 65.5 (2), 59.4 (2), 47.3 (2), 45.2 (2), 41.5 (2), 29.6 (6), 27.7 (2), 26.8 (2), 26.1 (2) .

The free base (**46**) was converted into the HCl salt.

***N, N'*-Decamethylene bis-*exo*-2-(2-chloro-5-pyridinyl)-7-azabicyclo[2.2.1]heptane hydrochloride (27):** Anal. Calc. for $C_{32}H_{44}N_4Cl_2$•2HCl•3H_2O: C, 56.31%; H, 7.68%; N, 8.21%. Found: C, 56.54%, H, 7.47%, N, 7.89%.

***N, N'*-Decamethylene bis-*exo*-2-(2-chloro-5-pyridinyl)-7-azabicyclo[2.2.1]heptane methiodide (28):** Compound **46** (30 mg, 0.054 mmol) was dissolved in 3.0 mL of absolute ethanol. Under an atmosphere of nitrogen, iodomethane (3.0 mL, 48 mmol, passed through a small column of anhydrous potassium carbonate) was added, and the reaction mixture was allowed to stir and reflux for 3 days. The solvent was removed under reduced pressure and the residue was washed with ethyl ether to give **28** as crude brown solid (45 mg, 73% yield).

***endo*-7-[(1,1-Dimethylethoxy)carbonyl]-7-azabicyclo[2.2.1]heptan-2-ol (47):** To a stirred solution of ketone **38** (211 mg, 1 mmol) in freshly stilled THF (2 mL) was added dropwise lithium(*tri-tert*-butoxyalumino)hydride (1.0 M in THF) (3 mL, 3 mmol). The solution was stirred for 2 h at 0^oC and then for another 2 h at room temperature. The reaction was quenched by addition of water (5 mL). The mixture was filtered through a pad of silica gel (3 cm), which was then washed with ethyl ether. After evaporation of the solvent from the filtrate, the residue was subsequently chromatographed over silica gel (ethyl acetate : hexane = 1 : 5) to furnish **47** as a white solid (166 mg, 78% yield). mp 44-45.5^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 4.35

(1 H, t, J = 4.8 Hz), 4.13 (2 H, dd, J = 7.2 Hz), 2.15-2.22 (2 H, m), 1.75-1.80 (1 H, m), 1.60-1.66 (2 H, m), 1,45 (9 H, s)，1.27 (1 H, d, J = 2.8 Hz). ^{13}C NMR ($CDCl_3$) δ 155.7, 79.8, 70.5, 59.9, 57.3, 38.9, 29.8, 28.2 (3), 20.7.

***endo*-2-[Hydroxymethyl-(2-pyridinyl)]-7-[(1,1-dimethylethoxy)carbonyl]-7-azabi cyclo[2.2.1]heptane (50a):** To a stirred solution of *endo*-alcohol **47** (85 mg, 0.4 mmol) in anhydrous DMF (1 mL) at ice bath was added NaH (60% in oil, 40 mg, 1 mmol) slowly. The whole mixture was stirred at room temperature for 1 h. Subsequently, a solution of 2-(bromomethyl)pyridine hydrobromide (202 mg, 0.8 mmol) in anhydrous DMF (3 mL) was added slowly. The reaction mixture was then stirred at 90^{o}C-100^{o}C for 15 h. After the mixture was cooled, the solvent was removed under reduced pressure. The residue was partitioned between ether and water. The organic layer was then washed with saturated Na_2CO_3 solution and water, dried (Na_2SO_4) and evaporated. The crude product was purified by silica gel column chromatography (ethyl acetate : hexane = 1 : 1) to give the desired product **50a** (94 mg, 83% yield) as white solid. mp. 65-68^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (1 H, d, J = 0.8 Hz), 7.68 (1 H, t, J = 2.0 Hz), 7.42 (1 H, d, J = 8.0 Hz), 7.18 (1 H, t, J = 4.8, 2.0 Hz), 4.56 (2 H, s), 4.32 (1 H, s), 4.16 (1 H, s), 4.09 (1 H, t, J = 4.8 Hz), 2.15 (2 H, m), 1.78 (1 H, m), 1.64 (1 H, m), 1.45 (9 H, s), 1.26 (2 H, m). ^{13}C NMR ($CDCl_3$) δ 158.2, 155.6, 149.1, 136.6, 122.4, 121.4, 79.7, 78.4, 72.6, 58.2, 56.8, 37.0. 29.7, 28.2 (3), 21. 2.

***endo*-2-[Hydroxymethyl-(3-pyridinyl)]-7-[(1,1-dimethylethoxy)carbonyl]-7-azabi cyclo[2.2.1]heptane (50b):** To a stirred solution of endo-alcohol **47** (85 mg, 0.4 mmol) in anhydrous DMF (1 mL) at ice bath was slowly added NaH (60% in oil, 40 mg, 1 mmol). The mixture was stirred at room temperature for 1 h. Subsequently, a solution of 3-(bromomethyl)pyridine hydrobromide (200 mg, 0.8 mmol) in anhydrous DMF (3 mL) was added slowly. The reaction mixture was then stirred at 90^{o}C-100^{o}C for 15 h. After the mixture was cooled, the solvent was removed under reduced pressure. The residue was partitioned between ether and water. The organic layer was washed with saturated Na_2CO_3 solution and water, dried (Na_2SO_4) and evaporated. The crude product was purified by silica gel column chromatography (ethyl acetate : hexane = 1 : 1) to give the desired product **50b** (83 mg, 73% yield) as white solid. mp 73-74^{o}C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (1 H, s), 8.55 (1 H, d, J = 3.6 Hz), 7.65 (1 H, d, J = 2.0 Hz), 7.27 (1 H, dd, J = 4.8, 2.4 Hz), 4.45 (2 H, s), 4.29 (1 H, s) , 4.13 (1 H, s), 4.03 (1 H, t), 2.16 (1 H, m), 2.06 (1 H, m), 1.76 (1 H, m), 1.61 (1 H, m), 1.43 (9 H, s), 1.24 (1 H, m). ^{13}C NMR ($CDCl_3$) δ 155.6, 149.2, 149.1, 135.3, 133.5, 123.4, 79.8, 78.3, 69.3, 58.2, 56.9, 37.1, 29.7, 28.3 (3), 21.2.

***endo*-2-[Hydroxymethyl-(4-pyridinyl)]-7-[(1,1-dimethylethoxy)carbonyl]-7-azabi cyclo[2.2.1]heptane (50c):** To a stirred solution of endo-alcohol **47** (85 mg, 0.4 mmol) in anhydrous DMF (1 mL) at ice bath was added NaH (60% in oil, 40 mg, 1 mmol) slowly. The whole mixture was stirred at room temperature for 1 h.

Subsequently, a solution of 4-(bromomethyl)pyridine hydrobromide (202 mg, 0.8 mmol) in anhydrous DMF (3 mL) was added slowly. The reaction mixture was then stirred at 90°C-100°C for 15 h. After the mixture was cooled, the solvent was removed under reduced pressure. The residue was partitioned between ether and water. The organic layer was washed with saturated Na_2CO_3 solution and water, dried (Na_2SO_4) and evaporated. The crude product was purified by silica gel column chromatography (ethyl acetate : hexane = 1 : 1) to give the desired product **50c** (75 mg, 66% yield) as white solid. mp. 78-80°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (2 H, d, J = 4.4 Hz), 7.17 (2 H, d, J = 4.8 Hz), 4.41 (2 H, s), 4.26 (1 H, s), 4.11 (1 H, s), 4.00 (1 H, t, J = 4.8 Hz), 2.14 (1 H, t, J = 2.8 Hz), 2.02 (1 H, t, J = 4.4 Hz), 1.75 (1 H, s), 1.58 (1 H, m), 1.50 (1 H, m), 1.42 (9 H, s), 1.18 (1 H, m).

***endo*-2-[Hydroxymethyl-(2-pyridinyl)]-7-azabicyclo[2.2.1]heptane (29a):** To a solution of ester **50a** (60 mg, 0.2 mmol) in dry CH_2Cl_2 (2 mL) was added trifluoroacetic acid (0.2 mL, 2.6 mmol) dropwise with stirring under nitrogen. The mixture was stirred for 3 h at room temperature and rendered basic with saturated Na_2CO_3 (3 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by chromatography (CH_2Cl_2 : MeOH : NH_4OH, 100 : 10 : 1) to give **29a** as colorless solid (37 mg, 93% yield). mp 132-134°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (1 H, d, J = 4.4 Hz), 7.70 (1 H, t, J = 7.6 Hz), 7.45 (1 H, d, J = 7.6 Hz), 7.18 (1 H, t, J = 6.0 Hz), 4.58 (2 H, s), 4.03 (1 H, t , J = 5.2 Hz), 3. 74 (1 H, t, J = 4.4 Hz), 3.60 (1 H, t, J = 4.4 Hz), 2.12 (1 H,

m), 2.01 (1 H, m), 1.78 (1 H, s), 1.58 (1 H, m), 1.44 (1 H, m), 1.25 (1 H, s). ^{13}C NMR ($CDCl_3$) δ 158.8, 149.1, 136.6, 122.3, 121.4, 80.1, 72.7, 58.5, 57.1, 37.1, 30.9, 22.3.

***endo*-2-[Hydroxymethyl-(3-pyridinyl)]-7-azabicyclo[2.2.1]heptane (29b):** To a solution of ester **50b** (72 mg, 0.24 mmol) in dry CH_2Cl_2 (2 mL) was added trifluoroacetic acid (0.2 mL, 2.6 mmol) dropwise with stirring under nitrogen. The mixture was stirred for 3 h at room temperature and rendered basic with saturated Na_2CO_3 (3 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by chromatography (CH_2Cl_2 : MeOH : NH_4OH, 100 : 10 : 1) to give **29b** as yellow solid (43 mg, 90% yield). mp. 136-138^oC ^1H NMR (400 MHz, $CDCl_3$) δ 8.57 (1 H, s), 8.53 (1 H, d, J = 4.4 Hz), 7.69 (1 H, d, J = 7.6 Hz), 7.30 (1 H, dd, J = 4.4, 2.4 Hz), 4.47 (2 H, s), 4.03 (1 H, m,), 3.77 (1 H, t, J = 4.8 Hz), 3.65 (1 H, t, J = 4.8 Hz), 3.02 (N-H, s), 2.11 (1 H, m), 2.05 (1 H, m), 1.65 (1 H, m), 1.57 (1 H, m), 1. 51 (1 H, m), 1.16 (1 H, dd, J = 3.6 Hz). ^{13}C NMR ($CDCl_3$) 149.03, 149.00, 135.4, 133.7, 123.4, 79.4, 69.3, 58.5, 57.2, 36.9, 30.6, 21.9.

***endo*-2-[Hydroxymethyl-(4-pyridinyl)]-7-azabicyclo[2.2.1]heptane (29c):** To a solution of ester **50c** (75 mg, 0.25 mmol) in dry CH_2Cl_2 (2 mL) was added trifluoroacetic acid (0.2 mL, 2.6 mmol) dropwise with stirring under nitrogen. The mixture was stirred for 3 h at room temperature and rendered basic with saturated

Na_2CO_3 (3 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by chromatography (CH_2Cl_2 : MeOH : NH_4OH, 100 : 10 : 1) to give **29c** as white solid (48 mg, 95% yield). mp. ^1H NMR (400 MHz, $CDCl_3$) δ 8.55 (2 H, d, J = 8.0 Hz), 7.27 (2 H, d, J = 7.2 Hz), 4.47 (2 H, s), 4.00 (1 H, m), 3.73 (1 H, t, J = 6.0 Hz), 3.62 (1 H, t, J = 6.0 Hz), 2.06 (2 H, m), 1.60 (2 H, m), 1.46 (1 H, m), 1.16 (1 H, dd, J = 4.4 Hz).

General Procedure for the Preparation of Oxalate Salts. The free base compound (50 mg) was dissolved in ether or THF (1-2 mL). This solution was added dropwise to a solution of the oxalic acid (2.4 eq.) in ether (1-2 mL). The oxalate salts precipitated instantaneously with the mixing of the two solutions. The salt was rinsed with cold anhydrous ether three times to afford the salt as an analytically pure crystalline solid. Some analytical samples contained minute amounts of water despite drying (48 h under vacuum).

***endo*-2-[Hydroxymethyl-(2-pyridinyl)]-7-azabicyclo[2.2.1]heptane oxalate (51a):** White solid, mp 96-98°C. Anal. Calc. for $C_{12}H_{16}N_2O \cdot 2(COOH)_2$: C, 50.00%; H, 5.24%; N, 7.29%. Found: C, 49.53%, H, 5.52%, N, 7.48%

***endo*-2-[Hydroxymethyl-(3-pyridinyl)]-7-azabicyclo[2.2.1]heptane oxalate (51b):** Light yellow solid, mp 130-132°C. Anal. Calc. for $C_{12}H_{16}N_2O \bullet (COOH)_2 \bullet H_2O$: C, 53.78%; H, 6.45%; N, 8.97%. Found: C, 53.19, H, 6.20%, N, 8.81%.

***endo*-2-[Hydroxymethyl-(4-pyridinyl)]-7-azabicyclo[2.2.1]heptane oxalate (51c):**

Brown solid**.** Anal. Calc. for $C_{12}H_{16}N_2O \cdot 2(COOH)_2$: C, 50.00%; H, 5.24%; N, 7.29%. Found: C, 50.47%, H, 5.65%, C, 7.79%.

Cyclohexene-4-carbonyl chloride (53): To a stirred solution of 3-cyclohexene-1-carboxylic acid (98%, 5 g, 0.04 mol) was added a solution of thionyl chloride (9 mL, 0.12 mol) dropwise in all-glass apparatus. The mixture was refluxed for 3 h until no further bubbles were observed. The whole reaction mixture was then stirred overnight at room temperature. The excess $SOCl_2$ was carefully removed under reduced pressure to provide the crude product **53**, which could be further reacted without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69(2 H, m), 3.01 (1 H, m), 2.41 (2 H, m), 2.16 (3 H, m), 1.83 (1 H, m).

4-(*N*-Trifluoroacetylamino)cyclohexene (55): A solution of crude **53** (5.7 g, 0.04 mol) in CH_2Cl_2 (60 mL) containing tetrabutylammonium bromide (40 mg, 0.124 mmol) was cooled in an ice bath. A solution of sodium azide (3.1 g, 0.048 mol), in water (10 mL) was added and the reaction mixture was stirred vigorously at 0^{o}C for 2 h. The organic phase was separated, washed with water and dried with Na_2SO_4 for 20 h. Continued evolution of nitrogen as very small bubbles was observed during this period. Trifluoroacetic acid (4.1 mL, 0.054 mol) was added dropwise to the filtered solution which was thereafter refluxed for 6 h. The cooled reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution, dried, and concentrated. The crystalline residue is distilled in a Kugelrohr apparatus

(bulb-to-bulb) at 90-100^{o}C / 1 torr to give **55** (5 g, 65% from the starting reagent **52**) as a white crystalline solid. mp 60-63^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.24 (1 H, br s), 5.73 (1 H, m), 2.63 (1 H, m), 4.18 (1 H, m), 2.44 (1 H, d, J = 17.2 Hz), 2.16 (2 H, m), 1.93 (2 H, m), 1.72 (1 H, m).

4-(*N*-Methyl-*N*-trifluoracetylamino)cyclohexene (56): A stirred solution of **55** (9.7 g, 0.05 mol) and methyl iodide (10 mL, 0.16 mol) in dry DMF (60 mL) was cooled in an ice bath. Sodium hydride (60% oil dispersion in mineral oil, 2.5 g, 0.06 mol) was added in portions. The ice bath was removed, and stirring was continued at room temperature for 1 h. The mixture was poured into water (400 mL) containing acetic acid (5 mL) and extracted with ether. The organic phase was washed four times with water, dried over sodium sulfate, filtered, and concentrated by distillation at normal pressure on a steam bath. The residue was distilled at the aspirator through a short Vigreux column to afford **56** (8.1 g, 78%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.67 (2 H, m), 4.26 (1 H, d), 4.62 (0.55 H, m), 4.05 (0.45 H, m), 3.00 (1.65 H, s), 2.93 (1.35 H, s), 2.15-2.25 (4 H, m), 1.75-1.82 (2 H, m).

4-(*N*-Methyl-*N*-trifluoracetylamino)-1,2-epoxy-cyclohexane (57): To a solution of **56** (10 g, 0.05 mol) in CH_2Cl_2 (200 mL), stirred in an ice bath, was added *m*-chloroperoxybenzoic acid (70-75%, 12 g, 0.05 mol) in portions. After stirring for 4 h at room temperature, the excess peracid was destroyed by addition of aqueous potassium iodide, followed by sodium sulfite. The organic phase was separated,

washed three times with $NaHCO_3$, and dried over Na_2SO_4. Filtration and evaporation left crude oil **57** (10.6 g, 95%), which was further purified by kugelrohr distillation at 75-90^oC (0.3 mm) to provide **57** (9.0 g, 81%) as a brown oil consisting of a mixture of (66.7%) *anti*- and (33.3%) *syn*-isomers. ^1H NMR (400 MHz, $CDCl_3$) δ 4.40 (0.75 H, m), 3.79 (0.25 H, d, J = 7.2 Hz), 3.20 (2 H, m), 2.93 (1.25 H, s), 2.86 (0.75 H, s), 2.28 (1 H, m), 2.11 (1 H, m), 1.92 (2 H, m), 1.71 (1 H, m), 1.36 (1 H, m).

***Cis*-4-(*N*-Methylamino)-1,2-epoxycyclohexane (58a):** To a stirred solution of **57** (6.4 g, 29 mmol) in methanol (18 mL) was added dropwise a solution of potassium hydroxide (4.7 g, 34 mmol) in water (15 mL). After stirring at room temperature for 5 h, most of the methanol was removed under reduced pressure. The residue was extracted with ether (3 × 30 mL) and dichloromethane (3 × 30 mL). The extract was dried and concentrated to provide a single product **58a** (2.66g, 75%) as an oil. ^1H NMR (400 MHz, $CDCl_3$) δ 3.12 (1 H, d, J = 2.0 Hz), 2.38 (3 H, s), 2.17 (2 H, m), 1.79 (1 H, m), 1.64 (1 H, dd), 1.51 (2 H, m), 1.29 (1 H, m).

***exo*-7-Methyl-7-azabicyclo[2.2.1]heptan-2-ol (59):** A solution of **58a** (2.2g, 17.3 mmol) and K_2CO_3 (69 mg, 0.5 mmol) in dry *N*-methylpyrrolidone (30 mL), was heated under N_2 atmosphere at 160^oC in oil bath for 72 h. The dark mixture was subjected to distillation under reduced pressure and then distillate was made slightly acidic with HCl (conc.). The solvent was distilled and the residue was washed with

hot ether to furnish **58a** hydrochloride salt. The salt was dissolved in aqueous sodium carbonate followed by continuous extraction with dichloromethane (24 h). the solvent was then carefully removed under reduced pressure and the residue was purified by column chromatography (SiO_2, CH_2Cl_2 : CH_3OH : NH_4OH, 100 : 10 :1) to afford **55** as a white solid (1.17 g, 55%). mp 46-47^oC. ^1H NMR (400 MHz, $CDCl_3$) δ 3.63 (1 H, d, J = 7.2 Hz), 3.20 (1 H, t, J = 3.6 Hz), 3.11 (1 H, d, J = 4.0 Hz), 2.78 (1 H, br s), 2.25 (3 H, s), 1.68-1.77 (3 H, m), 1.52 (1 H, m), 1.12-1.20 (2 H, m).

2-Acetoxy-7-methyl-exo-7-azabicyclo[2.2.1]heptane (64) : To a stirred solution of alcohol **59** (50 mg, 0.4 mmol) in freshly distilled $CHCl_3$ (3 mL) under an atmosphere of nitrogen was added dry pyridine (100 μL, 1.2 mmol) and dry acetic anhydride (110 μL, 1.2 mmol). The solution was stirred at room temperature overnight. The reaction was quenched with a saturated solution of $NaHCO_3$ (5 mL) and the organic layer was removed. The aqueous layer was washed with $CHCl_3$ (3 × 5 mL) and the organic fractions were combined and concentrated. Purification was achieved by flash chromatography (SiO_2, CH_2Cl_2 : MeOH : NH_4OH, 90 : 10 : 1) to yield **64** as a colorless oil (51 mg, 75% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 4.59 (1 H, dd, J = 2.8, 4.4 Hz), 2.28 (2 H, t, J = 4.8 Hz), 2.28 (3 H, s), 2.04 (3 H, s), 1.70-1.83 (4 H, m), 1.19-1.28 (2 H, m). ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.2, 65.6, 60.3, 40.0, 34.6, 29,7, 24.8, 21.9, 21.4. Anal. Calcd for $C_{11}H_{17}NO_2 \bullet C_2H_2O_4 \bullet H_2O$: C, 47.60; H, 6.85; N, 5.04. Found: C, 47.39, H, 6.13; N, 4.57.

2-Acetoxy-7-methyl-exo-7-azabicyclo[2.2.1]heptane methyliodide (65): To a stirred solution of the ester **64** (34 mg, 0.2 mmol) in dry THF (3 mL) under an atmosphere of nitrogen was added CH_3I (1 mL). The reaction was heated to reflux overnight to give **65** as a light yellow solid (53 mg, 87% yield). Anal. Calcd. For $C_{10}H_{18}NO_2I \cdot H_2O$: C, 36.45; H, 6.07; N, 4.25. Found: C, 36.60; H, 5.43; N, 3.98.

REFERENCES

1 McDonald, I A.; Cosford, N.; Vernier, J. M. *Ann. Rep. in Med. Chem.*, **1995**, *5*, 41.

2 Holladay, M.W.; Dart, M. J.; Lynch., J. K. *J. Med. Chem.*, **1997**, *40*, 4169.

3 Levin, E. D. *Drug Dev. Res.*, **1996**, *38*, 188.

4 Gualtieri, F. *Pharm. Acta Helvet.*, **2000**, *74*, 85.

5 Newhouse, P. A.; Kelton, M. *Pharm. Acta Helvet.*, **2000**, *74*, 91.

6 Shacka, J.J.; Robinson, S.E. *Med. Chem. Res.*, **1996**, *6*, 444.

http://www.web-books.com/Mobio/Free/Ch2F4.htm

7 Patrick, J. Sequela, P.; Verino, S.; Amador, N.; Luetje, C; Dani, J. A. *Prog. Brain Res*. **1993**, *98*, 113-120.

8 McGehee, D. S.; Role, L. W. *Ann. Rev. Physiol* **1995**, *57*, 521-546.

9 Puchacz, E.; Buisson, B.; Bertrand, D.; Lukas, *R. FEBS Lett.* **1994**, *354*, 155-159.

10 Ordberg, A. *Med. Chem. Res.* **1993**, *2*, 522-529.

11 Picciotoo, M. R.; Zoli, M.; Lena, C.; Bessis, A.; Lallemand, Y.; LeNovere, N.; Vincent, P.; Pich, E. M.; Brulet, P.; changeaux, J.-P *Nature* **1995**, *374*,65-67.

12 Benwell, M. E.; Balfour, D. J. K.; Anderson, J. M. J. *Neurochem*. **1988**, *50*, 1243-1247.

13 Marks, M. J.; Stitizel, J. A.; Romm, E.; Wehner, J. M.; Collins, A. C. *Mol. Pharmacol.* **1986**, *30*, 427-436.

14 Clark, P. B. S.; Schwartz, R. D.; Paul, S. M.; Pert, C. B.; Pert, A. *J. Neurosci*. **1985**, *5*, 1307-1315.

15 Seguela, P.; Wadiche, J.; dineley-Miller, K.; Dani, J. A.; Patrick, J. W. *J. Neurosci.* **1993**, *13*, 596-604.

16 Stevens, C. F. *Cell*, **1996**, *87*, 1147-1148.

17 Tsien, J. Z.; Huerta, P. T.; Tonegawa, S *Cell*, **1996**, *87*, 1327-1338.

18 McHugh, T. J.; Blum, K. I.; Tsien, J. Z.; Tonegawa, S.; Wilson, M. A. *Cell*, **1996**, *87,* 1339-1349.

19 Badio, B. Garraffo, H.; Spande, T. F; Daly, J. W.. *Med. Chem. Res.* **1994**, *4,* 440-448.

20 Srauss: Evelyn *Science* **1998**, *279*, 32.

21 Badion, B.; Daly, J. W. *Mol. Pharmacol*. **1994**, *45*, 563-569.

22 Qian, C.; Li, T.; Shen, T. Y.; Libertine-Garahan, L.; Eckman, J.; Biftu, T.; Ip, S. *Eur. J. Pharmacol*. **1993**, *250*, R13-R14.

23 Sullivan, J. P.; Decker, M. W.; Brioni, J. D.; Donnelly-Roberts, D.; Anderson, D. J.; Bannon, A. W.; Kang, C.-H,; Adams, P.; Piattoni-Kaplan, M.; Buckley, J. J.; Gopalakrishnan, M.; Williams, M.; Arneric, S. P. *J. Pharmacol Exp. Ther.* **1994**, *271*, 624-631.

24 Whiting, P. J.; esch, F.; Shimassaski, S.; Lindstrom, J. M. *FEBS Lett.* **1987**, *219*, 459-463.

25 Houghtling, R. A.; Davila-Garcia, M. I.; Keller, K. *J. Mol. Pharmacol,* **1995**, *48*, 280-287.

26 Marks, M. J.; Smith, K. W.; Collins, A. C. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 377-386.

27 Perry, D. C.; Kellar, K. *J. Pharmacol. Exp. Ther,* **1995**, *275,* 1030-1034.

28 Wonnacott, S.; Drasdo, A.; Sanderson, E.; Rowell, P. CIBA Foundation Symposium 152, John Wiley and Sons: New York **1990**, pp87-101.

29 Grady, S.; Marks, Wonnacott, S.; Collins, A. C. *Neurochem*. **1992**, *59*, 848-856.

30 Keiser, S. A.; Soliakov, L.; Harvery, S. C.; Luetje, C. W.; Wonnacot, S. J. *Neurochem*. **1998**, *70*, 1069-1076.

31 Zoli, M.; Lena, c.; Picciotto, M. R.; Changeux, J.-P. *J. Neurosc*i, **1998**, *18*, 4461-4472.

32 Serschen, H.; Balla, A.; Lajtha, A.; vizi, E. *Neuroscience* **1997**, *77*, 121-130.

33 Damaj, M. I.; Creasy, K.; Welch, S. P.; rosecrans, J.; Aceto, M.; Martin,B. R *Brain Res.* **1994**, *64*, 34-40.

34 Bonhaus, D. W.; Bley, K. R. Broka, C. A.; Fontana, D. J.; Leung, E.; Lewis, R.; Shieh, A.; Wong, E. H. f. *J. Pharmacol. Exp. Ther.* **1995,** *272*, 1199-1203.

35 Beers, W.H.; Reich, E. *Nature*, **1970**, *225*, 917.

36 Sheridan, R.P.; Nilakantan, R.; Dixon, J.S.; Venkataraghavan, R. J. *J. Med. Chem*, **1986**, *29*, 899.

37 Barlow, R.B. Johnson, O. *Br. J. Pharmacol*., **1989**, *98*, 799.

38 Glennon, R.A.; Herndon, J.L; Dukat, M. *Med. Chem. Res*., **1994**, *4*, 461.

39 Glennon, R.A.; Dukat, M. in ref. 15, pp 271-284.

40 Manallack, D.T.; Gallagher, T.; Livingstone D.J. in *Neural networks in QSAR and Drug Design*, James Devillers, Ed.: Rillieux la Pape, France, **1995.**

41 Li, T.; Qian, C.; Eckman, J.; Huang, D. F.; shen, T. Y. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2759-2762.

42 Badio, B.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. *Med. Chem. Res*. **1994**, *4*, 440-448.

43 Carroll, F.; Lee, J.; Hernan, A. Navarro, H.; Wei, M.; Brieaddy, L.; Abraham, P.; Damaj, M; Martin, B.;. *J. Med. Chem.* **2002**, *44*, 4755-4761.

44 Bannon, A. W.; Gunther, K. L.; Decker. M. W. *Pharmacol Biochem. Behav.* **1995**, *51*, 693-698.

45 Decker, M. W.; Buckley, M. J.; Brioni, J. D. *Drug Dev. Res*. **1994,** *31*, 52-58.

46 Li, T.; Qian, C.; Eckman, J.; Huang, D. F.; shen, T. Y. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2759-2762.

47 Badio, B.; Garraffo, H. M.; Spande, T. F.; Daly, J. W. *Med. Chem. Res*. **1994**, *4*, 440-448.

48 Badio, B.; Garraffo, H. M.; Plummer, C. V.; Padgett, W. L.; Daly, J. W. *Eur. J. Pharmacol*. **1997**, *321,* 1890194.

49 Shen, T. Y.; Huang, D. F.; WO96/06093 (**1996**).

50 Bencherif, M.; Caldwell, W. S.; dull, G. M.; Lippiello, P. M. US Patent 5, 583, 140 (**1996**).

51 Xu, R.; Bai, D.; Chu, G.; Tao, J.; Zhu,X. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 279-282.

52 Dukat, M.; Fielder, W.; Dumas, D.; Damaj, I.; Martin, B. R.; Rosencrans, J. A.; James, J. R.; Glennon, R. A. Eur. *J. Med. Chem*. **1996**, *31*, 875-888.

53 Gyermek, L.; Lee, C.; Nguyen, N.; Cho, Y. M. *Life Science* **1998,** *63*, 303-309.

54 Gyermek, L.; Lee, C.; Nguyen, N. *Acta Anaethesiol Scand*, **1999**, *43*, 651-657.

55 Corey, E. J.; Loh, T. E.; Achyutha-Rao, S., Daley, D. C.; Sarshar, S. J. Org. Chem. 1993, 58, 5600-5603.

56 Huang, D. F.; Shen, T. Y. *Tetrahedron Lett.* **1993**, *34*, 4477-4480.

57 Broka, C. A. *Tetrahedron Lett*. **1993,** *34*, 3251-3254.

58 Clayton, S. C.; Regan, A. C. *Tetrahedron Lett.* **1993**, *34*, 7493-7496.

59 Broka, C. A. *Med. Chem. Res*. **1994**, *4,* 449-460.

60 Chen, Z.; Trudell, M. L. *Chem. Rev.* **1996**, *96,* 1179-1193.

61 Szantay, C.; Kardos-Balogh, Z.; Szantay, C. Jr. Epibatidine. In: The Alkaloids, Vol. *46*. G. A. Cordell (Ed.), Academic Press, San Diego, **1995**, 95-125.

62 Ko, S. Y.; Lerpiniere, J; Linney, I Detal *J. Chem. Soc; Chem.* **1994**, 1775.

63 Cosford, N. D. P.; Bleicher, L.; Herbaut, A.; McCallum, J. S.; Vernier, J.-M; Dawson, H.; Whitten, J. P.; Adams, P.; Chavez-Noriega, L.; Correa, L. D.; Crona, J. H.; Mahaffy, L. S.; Menzaghi, F. M.; Rao, T. S.; Reid, R.; Sacaan, A. I.; Santori, E.; Stauderman, K.; Whelan, K.; Lloyd, G. K.; McDonald, I. A. *J. Med. Chem.* **1996**, *39*, 3235-3237.

64 Kim, K. D.; Lerner-Marmarosh, N.; Saraswatti, M.; Kende, A.; Abood, L. G. *Biochem Pharm*. **1994,** *47*, 1965-1967.

65 McDonald, I. A.; Vernier, J.-M.; Cosford, N.; Corey-Naeve, J. *Curr. Pharm. Des.* **1996**, *2*, 357-366.

66 Whitten, J. P.; McDonald, I. A.; Vernier, J.-M. WO 96/11931 (**1996**).

67 Devlin, J. P.; Edwards, O. E.; Gorham, P. R.; Hunter, N. R.; Pike, R. K.; Stavric, B. *Can. J. Chem*. **1977**, *55*, 1367-1371.

68 Wright, E.; Gallagher, Sharples, C. G. V.; Wonnacott, S. *Bioor. Med. Chem. Lett*. **1998**, *7*, 2867-2870.

69 De Fiebre, C. M.; Meyer, E. M.; Henry, J. C.; Muraskin, S. I.; Kem, W. R.; Papke, R. L. *Mol. Pharmacol*. **1995**, *47*, 164-171.

70 Zhang, C.; Trudell, M. L. *J. Org. Chem.* **1996**, *61*, 7989-7191.

71 Pandey, G..; Bagul, T. D.; Lakshmaiah, G. *Tetra. Lett.* **1994**, *35*, 7339.

72 Schneider, F. H. Olsson, T. *Med. Chem. Res*. **1996**, 562-570.

73 Jung, M. E.; Buszek, K. R. *J. Am. Chem. Soc.*, **1988**, *110*, 3969.

74 Leroy, J. A *Synthetic Communications*, **1992**, *22* (4), 567-572.

75 Grehn L.; Ragnarsson, U. Angew. *Chem. Int. Ed. Engl.* **1984**, *23,* 296-301.

76 Hama, Y.; Nobuhara, Y.; Aso, Y.; Oetsubo, T.; Ogura, F. Bull. *Chem. Soc. Jpn.* **1988***, 61,* 1683-1686.

77 Richard F. B.; Aviv, I. H. *J. Org. Chem.* **1972,** *37*, 1673-1674.

78 Pfister, J. R.; Wymann, W. E. *Synthesis*, **1983,** 38-40.

79 Pfister, J. R.; Wymann, W. E.; Weissberg, R. M.; Strosberg, A. M. S *J. Pharm. Sci*. **1985**, *74*, 208-210.

80 Ramanaiah, K. C. V.; Zhu N.; Klein-Stevens, C.; Trudell M. L.; Syntheis and Stereochemical Assignment of *exo*- and *endo*-7-Methyl-7-azabicyclo [2.2.1] heptan-2-ol, *Org. Lett*. **1999**, *1*, 1439-1441.

APPENDIX

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Ying Liu

VITA

The author was born in Nanchang, Jiangxi Province, China. She began her undergraduate study at University of Science and Technology of China (USTC) in 1991. After 5 years studying at USTC, she earned the B.S. degree in Polymer Science in July of 1996. She continued for her graduate study in Chemistry and Physics Department at USTC before she came to University of New Orleans, USA in the Spring of 2000 and pursued her M.S. degree in organic chemistry, under the supervision of Prof. Mark. L. Trudell.

UNIVERSITY OF NEW ORLEANS

MASTER'S EXAMINATION REPORT
Thesis

CANDIDATE: Ying Liu

MAJOR PROGRAM: Chemistry

TITLE OF THESIS: Synthesis and Biological Evaluation of Novel Epibatidine Analogues

APPROVED

Mark L. Trudell — Signature
Major Professor (typed)

Guijun Wang — Signature
Committee Member (typed)

Jiye Fang — Signature
Committee Member (typed)

Committee Member (typed) — Signature

Robert C. Cashner — Signature
Dean of the Graduate School

DATE OF EXAMINATION:

December 11, 2003