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Positive Allosteric Modulators of Alpha4Beta2 Neuronal Nicotinic Receptors: Synthesis and In vitro Studies

Atul Jain
Virginia Commonwealth University

POSITIVE ALLOSTERIC MODULATORS OF ALPHA4BETA2 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS: SYNTHESIS AND IN VITRO STUDIES

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

By

Atul Jain
Master of Science, Bombay College of Pharmacy, Mumbai, India, 2008
Bachelor of Pharmaceutical Sciences, Bombay College of Pharmacy, Mumbai, India, 2005

Director: Dr. Richard A. Glennon, PhD
Professor and Chairman,
Department of Medicinal Chemistry

Virginia Commonwealth University
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List of Abbreviations

%	Percentage
δ	Parts per million
α4β2	Alpha4beta2
μg	Microgram(s)
μL	Microliter(s)
[^{3}H]-nicotine	Tritiated nicotine
°C	Celsius
5-HT_3	Serotonin type 3
9-BBN	Borabicyclo(3.3.1)nonane
Aβ	Beta amyloid
$Aβ_{1-42}$	Beta amyloid with 42 amino acids
A-85380	(*S*-)3-(2-Azetidinylmethoxy)pyridine
A-998679	3-(5-Pyridin-3-yl-1,2,4-oxadiazol-3-yl)benzonitrile
ABT-089	(*S*)-2-Methyl-3-(2-pyrrolidinylmethoxy)pyridine
ABT-107	(*R*)-5-(6-(-1-Azabicyclo[2,2,2]oct-3-yloxy)pyridazin-3-yl)-1*H*-indole
ABT-418	(*R*)-3-Methyl-4-(1-methylpyrrolidin-2-yl)-1,2-oxazole
Acetyl-CoA	Acetyl co-enzyme A
ACh	Acetylcholine
AChE	Acetylcholine esterase
AChRs	Nicotinic acetylcholine receptors
AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
ADME	Adsorption distribution metabolism and elimination
$AlCl_3$	Aluminum chloride
Anal. Calcd	Analysis calculated
Ar	Aromatic
ATP	Adenosine triphosphate
AZD-1446	1-(5-Chloro-2-furoyl)hexahydropyrrolo[3,4-c]pyrrole
BCl_3	Boron trichloride
$BF_3·Et_2O$	Borontrifluoride etharate
$BH_3·S(CH_3)_2$	Borane dimethylsulphide complex
Boc	*tert*-Butylcarbamate
br s	Broad singlet
Br_2	Liquid bromine
Ca^{2+}	Calcium
$CDCl_3$	Deutarated chloroform
CH_2Cl_2	Dichloromethane

CH_3CN	Acetonitrile
CH_3SO_3H	Methanesufonic acid
ClogP	Calculated logP
CNS	Central nervous system
CO_2	Carbon dioxide
CuBr	Copper bromide
CuI	Copper iodide
Cys	Cysteine
d	Doublet
DCC	*N,N'*-Dicyclohexylcarbodiimide
dd	Doublet of doublet
decomp	Decomposition
dFBr	*des*-Formylflustrabromine
DIPEA	Di-*iso*-propylethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	*N,N*-Dimethylformamide
DMF.DMA	*N,N*-Dimethylformamide dimethyl acetal
DMPP	Dimethyl-4-phenylpiperazinium
DMSO	Dimethyl sulfoxide
DMSO-*d6*	Deutarated dimethylslufoxide
DMXB-A/GTS-21	3-(2,4-Dimethoxybenzylidene) anabaseine
EC_{50}	Effective concentration to achieve 50% response
Et_2O	Diethyl ether
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
FDA	Food and Drug administration
Fe	Iron dust
g	Gram
$GABA_A$	γ-Amino butyric acid receptors A
$GABA_C$	γ-Amino butyric acid receptors C
gl. HOAc	Glacial acetic acid
GluR	Glutamate receptor
GlyRs	Glycine receptors
GPCRs	G protein-coupled receptors
h	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
HBr	Hydrogen bromide
HCHO	Formaldehyde
HCl	Hydrochloride
HEPES	4-(2-hydroxyethyl)-1-perazineethanesulfonic acid
HPLC	High performance liquid chromatography
Hz	Hertz

I_2	Molecular iodine
IC_{50}	Inhibition concentration to achieve 50% inhibition
i-PrOH	*iso*-Propanol
IR	Infrared spectroscopy
IUPHAR	International union of pharmacology
J	Coupling constant
K^+	Potassium
KCl	Potassium chloride
K_i	Binding affinity
KI	Potassium iodide
KOH	Potassium hydroxide
K*t*BuO	Potassium *tert*-butoxide
LDE	Di-*iso*-proylethyllithium
L-DOPA	(L)-3,4-Dihydroxyphenylalanine
LGICs	Ligand-gated ion channels
$LiAlH_4$	Lithium aluminum hydride
lit	Literature
LY-2087101	[2-[(4-Fluorophenyl)amino]-4-methyl-5-thiazolyl] thiophen-3-yl-methanone
M	Molar
m	Multiplet
MΩ	Mega ohms
M+1	Molecular base peak
MeOH	Methanol
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
MHz	Mega hertz
min	Minutes
mL	Milliliters
mM/mmol	Millimolar
mol	Molar
mp	Melting point
MS	Mass spectroscopy
MTBE	Methyl *tert*-butyl ether
MW	Microwave
n	Hill co-efficient
N_2	Nitrogen
NA	Nucleus accumbens
Na^+	Sodium
$Na_2S_2O_3$	Sodium thiosulphate
Na_2SO_4	Sodium sulphate
$NaBH_4$	Sodium borohydride
nACh	Neuronal acetylcholine
NaCl	Sodium chloride
NaH	Sodium hydride

$NaHCO_3$	Sodium hydrogen carbonate
NAMs	Negative allosteric modulators
$NaNO_2$	Sodium nitrite
NaOH	Sodium hydroxide
NDP	Nigrostriatal dopaminergic pathway
ng	Nanogram
NH_4Cl	Ammonium chloride
NH_4OH	Ammonium hydroxide
NHRs	Nuclear hormone receptors
nM	Nanomolar
NMR	Nuclear magnetic resonance
NS-1738	*N*-(5-Chloro-2-hydroxyphenyl)-*N*'-[2-chloro-5-trifluoromethylphenyl]urea
P_2O_5	Phosphorous pentoxide
P2X	Purine receptors
PAM	Positive allosteric modulator
PD	Parkinson's disease
$PdCl_2(PPh_3)_2$	*Bis*(triphenylphosphine)palladium(II) dichloride
pM	Pico molar
PNS	Peripheral nervous system
PNU-120596	5-Chloro-2,4-dimethoxyphenyl)-*N*'-(5-methylisoxazol-3-yl) urea
PTMA	Phenyltrimethylammonium
R_f	Retardation factor
s	Singlet
SAR	Structure-activity relationships
SEM	Standard error of mean
sep	Septet
SPR	Structure-property relationship
t	Triplet
TBAB	Tetrabutylammoniumbromide
t-BuONO	*tert*-Butyl nitrite
TC-2429	2-(-3-Pyridinyl)azabicyclo[2,2,2]octane
TC-5619	(*2S, 3S*)-*N*-[2-(Pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]-1-benzofuran-2-carboxamide
TFA	Trifluoro acetic acid
THF	Tetrahydrofuran
TLC	Thin layered chromatography
TMA	Tetramethylammonium
TMS	Tetramethylsilane
TQS	3a,4,5,9b-Tetrahydro-4-(1-naphthalenyl)-3*H*-cyclopentan[*c*]quinoline-8-sulfonamide
Tyr	Tyrosine
v/v	Volume by volume
VGICs	Voltage-gated ion channels
VTA	Ventral tegmental area

W	Watts
Zn	Zinc dust
Zn^{2+}	Zinc
$ZnBr_2$	Zinc bromide
$ZnCl_2$	Zinc chloride

Abstract

POSITIVE ALLOSTERIC MODULATORS OF ALPHA4 BETA2 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS: SYNTHESIS AND IN VITRO STUDIES

By Atul D Jain, Ph.D.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

Virginia Commonwealth University, 2013.

Major Director: Dr. Richard A. Glennon,
Professor and Chairman, Department of Medicinal Chemistry

des-Formylflustrabromine (dFBr), isolated from the marine organism *Flustra foliacea*, is the first selective, positive allosteric modulator (PAM) of α4β2 nicotinic acetylcholine receptors that potentiates the action of the neurotransmitter acetylcholine (ACh). Most agonists for this receptor population are not selective and can activate other nACh receptors. A selective PAM, which activates α4β2 nACh receptors only in the presence of ACh, might find application in the

treatment of of various neurological diseases such as Alzheimer's disease or autism. dFBr was examined and found to produce a biphasic dose-response curve over a wide concentration range (i.e., potentiation at low concentration, but inhibition of the ACh-induced response at high concentrations). Our goal was to examine various structural features of dFBr required for potentiation; a secondary goal was to examine the same for inhibition. To understand the structural requirements of dFBr, a systematic 'deconstruction-reconstruction-elaboration' approach was employed to determine the contribution of various structrual components of dFBr to its activity at α4β2 nACh receptors. Novel compounds were synthesized and characterized. Human α4β2 nACh receptors were expressed in *Xenopus* oocytes and the actions of dFBr and its analogs were measured using a two-electrode voltage clamp technique. Dose-response curves were obtained for the compounds in the absence and presence of 100 μM ACh. Structural features of dFBr optimal and/or required for PAM action at α4β2 nACh receptors were identified. A novel reconstructed analog with all the essential features for PAM action was synthesized and submitted for biological testing. Elaborated analogs of dFBr further helped in identification of various structural features important for PAM action and the inhibition of action of ACh. The 'deconstruction-reconstructio-elaboration' approach identified important structural features of dFBr that modify its actions as a PAM or an antagonist (NAM? or channel blocker?) at α4β2 nACh receptors. This information should be useful for the subsequent design of novel analogs to evaluate their potential for the treatment of neurological disorders associated with ACh.

1. Introduction

Homeostasis is essential to the day-to-day biological functions of all living organisms. For example simple ions (e.g., Na^+, K^+, Ca^{2+}, Cl^-) play a vital role in the regulation of water inside and outside a cell. The phenomenon of transfer of ions across the cell membrane occurs through ion channels. The concept of ion channels evolved because of the discovery of two natural phenomena: the discovery of the *Torpedo* fish and its electrofiring capabilities, and the discovery of α-bungarotoxin, a venom (found in the Taiwanese krait snake).[1] These two spectacular discoveries were the events that attracted the attention of the scientific community to ion channel research. Ion channels are essential for the survival of all organisms including mammals. Malfunction occurs when the normal flow of ions is disrupted which can directly affect the reaction time of the organism to its environment.

Nicotinic acetylcholine receptors are the oldest known ion channels and have been a model for the study of other ligand-gated ion channels.[2] The nicotinic acetylcholine receptors are pentameric in nature with varying subunits (α1-α10, β1-β4, γ, δ and ε) located both in the central nervous system and the peripheral nervous system.[3] The neuronal acetylcholine (nACh) receptors are found to play a modulatory role in the brain where α4β2 and α7 nACh receptors predominate, and have been implicated in various neurological disorders.[4] On binding of the neurotransmitter acetylcholine to the extracellular surface of the ion channel a conformational change in the receptor protein occurs and enables the opening of the channel pore through which

ions pass.[2] The long exposure of the neurotransmitter or agonist causes desensitization of the nACh receptor.[5] The desensitized receptor is the state where the affinity of the neurotransmitter is high but the channels are closed. Problems of desensitization of the receptors and the non-selectivity of various agents across various subtypes of the nACh receptors still pose a considerable challenge to develop agents that can modulate ACh action.[6]

Allosteric agents are compounds that bind to the protein/receptor and stabilize the protein in a given conformation. Monod, Wyman and Changeux proposed the allosteric model in 1965 and the concept has been widely investigated since then.[7] Allosteric molecules can be positive allosteric modulators (PAMs) or negative allosteric modulators (NAMs) depending on whether they activate or inhibit neurotransmitter action.[8]

*des*Formlyflustrabromine (dFBr) was one of 22 alkaloids isolated from the marine bryozoan *Flustra foliacea* and was found to be a selective PAM at the $\alpha4\beta2$ nACh receptors; that is, it was inactive alone, but potentiated the effect of ACh.[9] Thus, dFBr serves as a unique lead for structure-activity relationship studies to understand what structural features are important for its potentiation and/or inhibition of ACh action.

The present investigation is aimed at understanding why dFBr is a PAM of ACh action at $\alpha4\beta2$ nACh receptors.

II. Background

A. RECEPTORS

1. Definition

John Newport Langley, a Cambridge physiologist, was a pioneer in the field of 'receptor research' and was the first to conceptualize the idea of receptors in 1905.[10] He defined the 'receptor' (a receptive substance) as a site where an agonist or antagonist competes with the vehicle for the transmission of a stimulus (transduction) which brings about a physiologic response.[10] Until the 1960s the concept of drug-receptor interactions was a hypothetical scenario. The 1965 development of the receptor-subtype specific β-adrenergic receptor antagonist propranolol established the acceptance of receptors in the pharmacological community.[11]

2. General classification

Now, according to the International Union of Pharmacology (IUPHAR) Committee on Receptor Nomenclature and Drug Classification, a receptor is defined as "a cellular macromolecule, or an assembly of macromolecules, that is concerned directly and specifically in chemical signaling between and within cells".[12] A change in cell function is initiated upon interaction of a hormone, neurotransmitter, drug, or intracellular messenger, with its receptor(s).[12]

The IUPHAR database classifies receptors as:

1. G protein-coupled receptors (GPCRs)
2. Ligand-gated ion channels (LGICs)
3. Voltage-gated ion channels (VGICs)
4. Nuclear hormone receptors (NHRs)
5. Enzymes

3. Classification of ligand-gated ion channels

The concept of ion channels can be dated back to the late 1800s and the early 1900s. Prof. Langley first observed a twitch in the muscle of a dead frog on the application of nicotine and referred to this as fibrillar twitching.[13] Hodgkin and Huxley,[14,15] independently, were the first to study nerve impulses on the neuron of the giant squid (*Loligo*) which is approximately 100 to 1000 times larger than the human neuron in diameter. Later, they also showed that nerve

conductance depends on specific ions outside and inside the nerve cell. They showed that during nerve impulse, or the spike phase, Na^+ ion inflow increases the positive potential in the cell and causes a response while the falling phase involves K^+ ions outflow.[15] LGICs are transmembrane proteins that carry out a physiological response by an ion channel which opens on binding of a ligand.[16] These are termed 'ionotropic' which refers to the ion-channel nature of the receptors. The whole process of channel opening and ion-flow is a display of speed and size. The channel is open for a few milliseconds in which thousands of ions flow through. The channel is a dynamic pore that allows passage of ions. The pore can be a few atoms thick at its narrowest point but still functions as a speedway for ions to go through.[17] LGICs mediate action at the synapse and in a matter of microseconds the neurotransmitter released from the nerve terminal interacts with the target protein, and binds and triggers a physiologic response. LGIC receptors are comprised of the following superfamilies:[17,18]

1. The superfamily that resembles the nicotinic receptors.

2. The ionotropic glutamate receptor superfamily (GluR).

3. Adenosine triphosphate (ATP)-gated purine receptors (P2X).

The classification of LGIC receptor superfamilies is based on molecular architecture, functional behavior, and sequence homology.[19] All of the LGIC receptors share common features, such as their location in the cell membrane, and the three key structural domains that each of them possesses.[19] The superfamily that resembles the nicotinic receptor is distinct because of the presence of a cysteine-loop (Cys-loop) in the extracellular domain of the receptor conserved across all the receptor populations and includes the:

1. Glycine receptors (GlyRs)

2. γ-Amino butyric acid receptors ($GABA_A$ and $GABA_C$)

3. Serotonin type 3 receptors ($5\text{-}HT_3$ Rs)

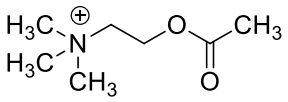
4. Nicotinic acetylcholine receptors (AChRs)

B. ACETYLCHOLINE AND ITS RECEPTORS

1. Acetylcholine

In 1865 Oscar Liebreich (according to Gamgee) isolated and identified a mixture of chemicals from animal brain that was characterized for its elemental content and referred to it as 'protagon'.[20] This finding had a major impact for the next couple of decades and researchers around the world were intrigued and started to study it more closely so as to figure out more about this mystery chemical composite.[20] Later Liebreich[21,22] was able to identify neurin/choline by treating the protagon with a barium hydroxide solution. The finding of Liebreich was consistent with those of Baeyer who had independently synthesized choline.[22] In 1914, Ewins[23,24] synthesized acetylcholine which was also isolated and found to be the major component in the extract of ergot fungus which produced an inhibitory effect on heart muscle and a stimulating effect on intestinal muscle; both responses were abolished by atropine.[24] Henry Dale[25] was responsible for identifying acetylcholine (ACh) in ergot fungus extract. Later, an extensive study was carried out with various ethers and ester of choline and it was found that ACh was the most

active chemical entity that produced the effect.[26] Thus, from this initial literature, it can be seen that ACh was studied and investigated long before it was known to be a neurotransmitter. It was only in the early 1900s that Otto Loewi identified ACh as the first neurotransmitter.[26] Both Henry Dale and Otto Loewi received the Nobel Prize in the field of Physiology or Medicine in 1936.[23,25-27] Acetylcholine is, chemically, 2-acetoxy-*N*,*N*,*N*-trimethylethanamanium (**1**), which is an acetyl derivative of choline.



Acetylcholine (**1**)

The cholinergic system is, phylogenetically, one of the oldest nervous pathways. The neurons that synthesize, store, and release ACh as a neurotransmitter are called cholinergic neurons.[28]

2. Biosynthesis and Metabolism of ACh

In the peripheral nervous system, ACh is involved in smooth and cardiac muscle regulation as well as skeletal muscle movement. In the central nervous system ACh is involved in chemo-electrical transduction in cholinergic synapses. ACh is synthesized (Figure **1**) in the cytosol of nerve terminals and stored in synaptic vesicles prior to exocytotic release.[28] Choline acetyltransferase is an enzyme involved in the synthesis of ACh. Choline is taken up from the synapse by the Na^+-dependent choline transporter.[28] Acetyl-CoA, which is present in the nerve terminal, along with choline, forms ACh in the presence of choline acetyltransferase. CoA is taken up in the mitochondria and is acetylated to acetyl-CoA which is available for ACh synthesis and the cycle continues.[29] The ACh is taken up to the synaptic vesicles by the vesicular

ACh transporter, where it is stored.[29] Upon excitation of the neuron, vesicular fusion occurs and ACh is released into the synapse.[29] The concentration of ACh in the synaptic cleft rises to around 0.1 to 1 mM for less than 1 msec.[28] The released ACh interacts with postsynaptic cholinergic receptors and triggers the relevant physiological response. ACh is metabolized in the synaptic cleft.[29] Acetylcholine esterase (AChE) is the enzyme present in the synapse that metabolizes ACh to choline and acetate.[29] Choline is taken up by the choline transporter present at the presynaptic nerve terminal which is utilized to synthesize ACh.[28] Metabolism, along with reuptake of ACh by diffusion, rapidly allows normal physiological levels of around 10^{-9} M to be attained.[28,29]

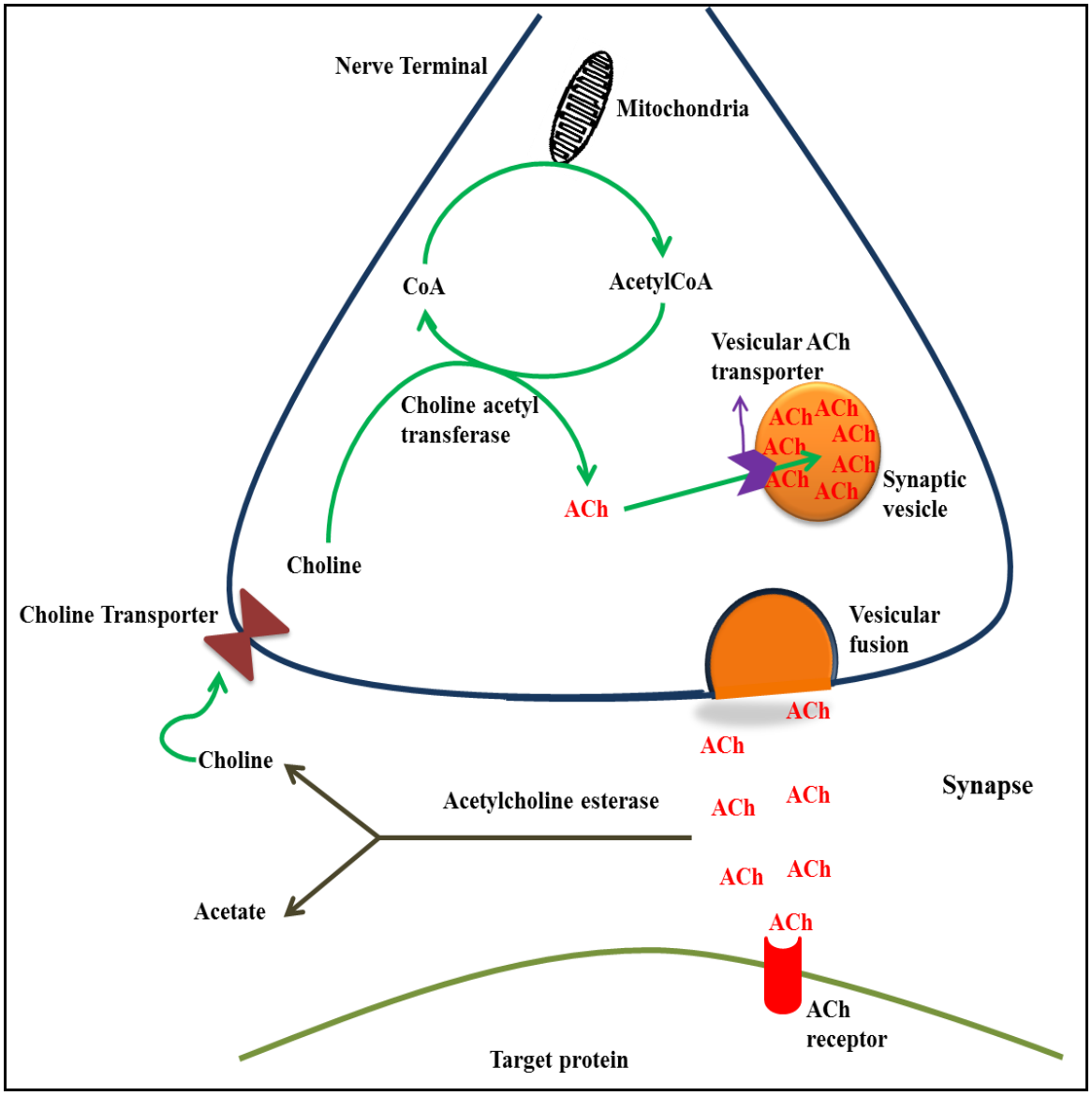


Figure 1. Synthesis and metabolism of ACh adapted from a review by Niewiadomska et al.[30]

3. Classification of ACh receptors

It was in the early 1900s that physiologists were able to identify that not all responses to the ACh stimulus were identical. They then hypothesized that ACh interacts differently with the so-called muscarinic receptors on some receptors and nicotinic receptors on others.[31] These two receptor classes were perceived to have different pharmacological properties along with different interactions with ACh and associated postsynaptic properties.[31] The early classification of ACh receptors involved what ACh imitated, namely, the actions of muscarine and nicotine. This finding was further confirmed with the discovery of selective agents for the two subtypes of ACh receptors. These included nicotinic receptor-selective agents (e.g. tetramethylammonium (TMA), dimethyl-4-phenylpiperazinium (DMPP), phenyltrimethylammonium (PTMA), suxamethonium, and decamethonium) and muscarinic receptor-selective agents (e.g. acetyl-β-methylcholine, oxotremorine, pilocarpine and arecoline).[32] Later, it was found that nicotinic receptors could be classified further as autonomic, which show a preferential affinity towards DMPP, and skeletal muscle, which are more responsive to PTMA. Antagonists for ACh receptors were also investigated to confirm the presence of three different receptor populations.[32] Atropine selectively blocked the effect of muscarinic receptors, whereas hexamethonium and decamethonium differentiated between the autonomic and the skeletal nicotinic receptors.[32] Currently, several receptor subtypes of classical ACh receptor populations have been identified.

The ACh receptors now are classified as shown in Figure **2**.

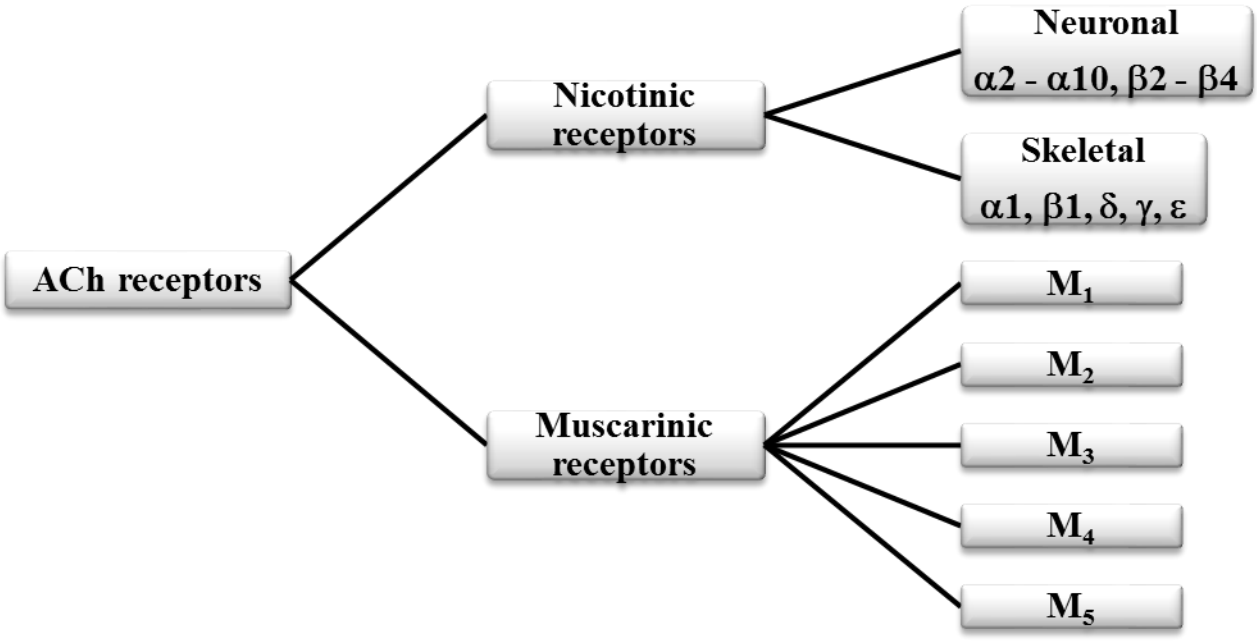
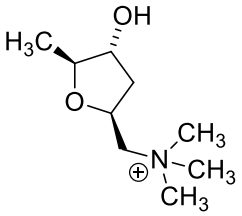


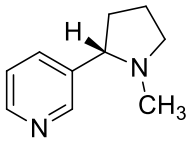
Figure 2. Classification of the ACh receptors.[33,34]

Muscarinic receptors are present in the central nervous system (CNS) and the peripheral nervous system (PNS).[33] The natural ligand that activates these receptor subtypes is muscarine (**2**) which is a plant alkaloid found in various species of mushrooms.[33] The classification of the muscarinic receptors is based on their location and their pharmacological effect.[33] All the subtypes consist of 7 transmembrane-spanning α-helices and are G-protein coupled.[33] The CNS effects associated with muscarinic receptors include motor control, temperature regulation, cardiovascular regulation, and memory.[33] Peripheral effects include smooth muscle contraction, glandular secretion, and modulation of cardiac rate and force.[33] Nicotinic receptors belong to the LGIC receptor superfamily and are ionotropic in nature.[34] The natural ligand that mimics ACh is nicotine (**3**) which is a plant alkaloid found in the tobacco plant,[34] *Nicotiana tabacum*. Nicotinic

receptors are broadly classified on the basis of their location.[34] Nicotinic receptors are present throughout the body and are found, for example, in the PNS, CNS, skeletal muscle, lymphocytes, and fibroblasts.[34] Based on their location, they are broadly classified into neuronal and skeletal subtypes.[34] Nicotinic receptors play a vital role in mediating excitatory neurotransmission at the neuromuscular junction, and modulation of neurotransmitter release.[34]



Muscarine (**2**)



(-)Nicotine (**3**)

In general, nicotinic receptors (Figure **3**) are pentameric in nature consisting of 5 subunits arranged in a bundle that forms a channel in the center.[34] Upon activation by the neurotransmitter ACh, the channel opens to allow ions to flow through. The common feature across all nicotinic receptor subtypes is the extensive N-terminal region of around 200 amino acid residues in the extracellular region.[34] They are all comprised of four transmembrane domains (M1–M4), with the M2 domain lining the ion pore.[34] The M2 domain consists of amino acids lining the pore which is essential for ion transport.[34] A unique intracellular loop between the M3 and M4 subunits consists of 100-200 amino acids, and an extracellular C-terminus.[34] The most prominent feature of all the nicotinic receptors is a loop of 13 amino acids between two cysteine residues forming a disulfide bridge in the N-terminal domain; thus, the name cys-loop LGIC receptors.[34]

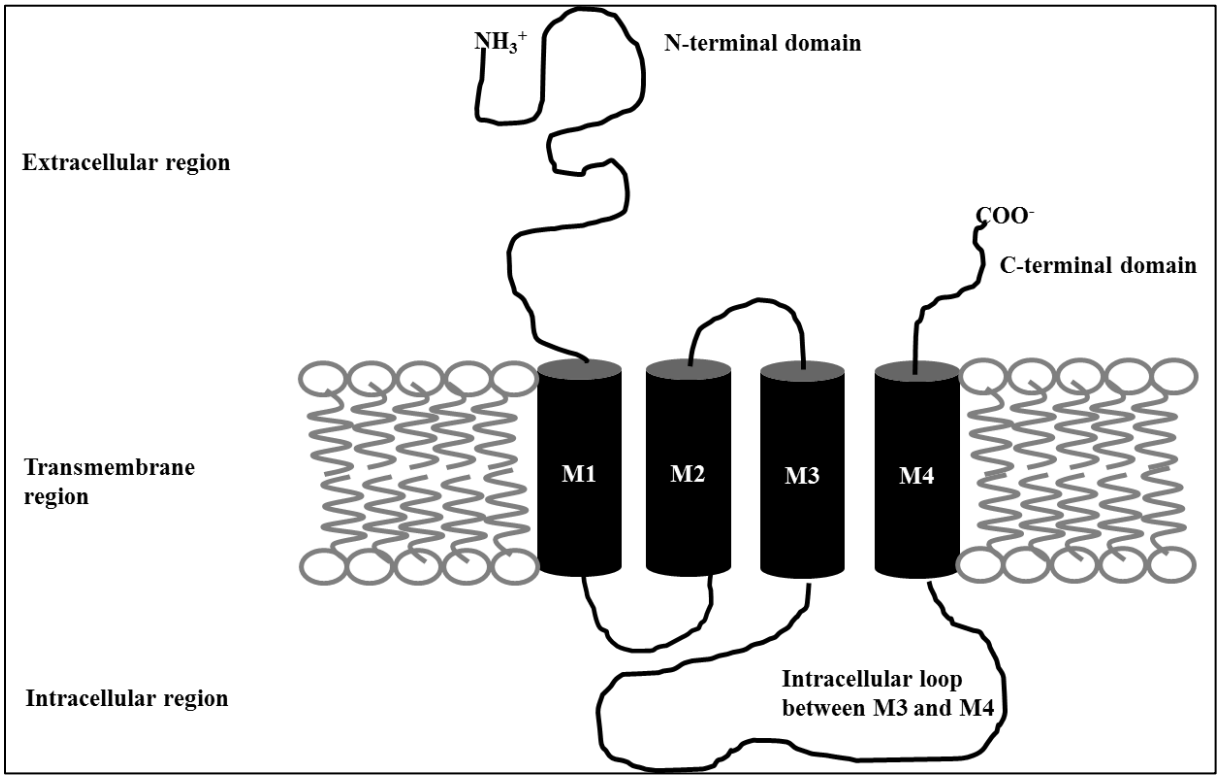


Figure 3. Schematic representation of a subunit of the nicotinic receptor adapted from Lukas et al.[34]

4. Nicotinic ACh receptors

Nicotinic ACh receptors were the first neurotransmitter receptors to be characterized, purified, and cloned.[35] Elliott[36] observed that a hormone present in the motor nerve ending in cross-striated muscle can activate nerve impulses in a manner comparable to adrenaline's action on non-striated muscle. Herring[37] was able to support Elliott's findings in the electric fish, *Torpedo mormorata*, that had a high concentrations of motor nerve endings in their electrical organs that caused an electrical firing caused by ACh due to its depolarizing action at the motor end plates.[38] Also, a very high concentration of AChE was observed at the site of the electrical organ

implicating the presence of ACh.[38] It was found that the fish contain a very high concentration of ACh, around 40-100 μg/g of fresh tissue.[38]

a. Types and sub-types

Unwin reported the first-ever crystal structure of a nicotinic receptor in a closed conformation at 9 Å-resolution using electron microscopy of a tubular crystal from the postsynaptic membrane of *Torpedo mormorata*.[39] Initially, four different skeletal muscle type subunits of nicotinic receptors were identified, namely: α1, β1, γ and δ based on increasing molecular weights of the various subunits.[40] The δ subunit is converted to the ε subunit from the fetal to the adult subtype.[40] Cloning and purification of various nicotinic receptors have revealed many additional subtypes.[40] Neuronal ACh receptors can consist of α2-α10 and β2-β4 subtypes with varying combinations possible between α and β subunits.[40] The α subunit consists of Cys 192 and 193, which is a characteristic and has been shown to be important for ACh binding in all α subunits, a feature not observed in the β subunit.[41] Thus, the β subunit was not able to bind ACh or various agents. To date nine other α subunits have been identified; α2-α10 along with four other β subunits; β2-β4.[41]

C. **Neuronal ACh** (**nACh**) **receptors**

nACh receptors consist of various combinations of α1-α10 and β2-β4 subunits arranged in a pentameric structure (Figure **2**).[42] Functional receptors are a result of the assembly of 5-subunits around a pseudosymmetric axis.[42] Various combinatorial associations of different α and β subunits are possible, and these form functional nACh receptor subtypes of a large variety.[43] It is known that the principal component of the nACh receptor is associated with the α2, α3, α4, α6, α7, α8 or α9 subunits.[42] The **principal component** consists of two adjacent Cys residues, and contributes towards the binding of ligands with three loops from discontinuous sections.[41,44] The **complementary component** of the nACh receptor consists of β2, β4, β7, β8, or α9 subunits. The complementary component provides an additional three discontinuous loops for binding of the ligands.[44] The α10 subunit, although classified as an α-type subunit, can only function as a principal component at the binding site in nACh receptors in conjunction with the α9 subunit.[45] The stoichiometry of $(\alpha x)_2(\beta y)_3$ is, in general, seen with most of the subunits except the α7, α8, α10 subunits that form homopentameric functional receptors when studied by expressing the subunits in *Xenopus laevis* oocytes.[46] The ACh binding site, and the transmembrane (TM) spanning regions, are highly conserved across the various subtypes of nACh receptors, whereas the intra-cellular loop between M3 and M4 (Figure **3**) is conserved across various species.[46] The various subunits of the nACh receptors share a very high homology (91-99%) amongst themselves.[46] Thus, a single amino acid change in the extra-cellular amino terminal (Figure **3**) may affect the property of the nACh receptors.

1. Distribution of nACh receptors

Various techniques, such as in situ hybridization using a subunit mRNA probe, ligand binding studies by means of autoradiography, immunohistochemistry, immunoprecipitation, radioligand binding assays using various ligands such as nicotine, α-bungarotoxin to name a few, and nACh receptor gene knock-out studies, have been performed to map the tissue distribution of nACh receptors.[47]

a. Peripheral Tissues

It is accepted that nACh receptors are located in the ganglionic synapse of the PNS along with broader locations in peripheral tissues.[42] The α3, α5, α7, β2 and β4 subunits are the major subunits identified in peripheral tissue.[48] Chick ganglia and cromaffin cells have α3-, α7-, and β4-containing subtypes of nACh receptors.[48,49] Developing muscles show the presence of the α7-containing subtype.[50] Various subunits, such as α3, α5, α7, β2 and β4, are expressed in keratinocytes,[51] bronchial epithelial cells,[52] and arterial endothelium.[43] Nicotinic receptors have also been found on lymphocytes and polymorphonuclear cells of human blood, and very little is known about their function.[53] Functional nACh receptors containing α3, α5, β2 and β4 subunits are found in endothelial cells lining blood vessels and are quite similar in their channel-open time and, in general, functioning, found in the CNS.[43] α6-Containing nACh receptors are present in the optic pathway which oligomerizes with β4, β2, α3, and β3 subunits to form functional nACh receptors.[54]

b. Central nervous system

The distribution of nACh receptors in primates is less well known when compared to rodents, which have been far more extensively studied to map the distribution of the nACh receptors and the subunits involved.[47] In rodents the most abundant subunits expressed in whole brain are the α4, α7, and β2 subunits (Figure **4**). β2-Containing nACh receptors are highly expressed in the caudate, putamen, and the hippocampal regions of the CNS.[55] The α4 subunit is highly expressed in the cortex, whereas the α7 is expressed in the lateral and medial geniculate nucleus along with the reticular thalamic nucleus.[56] In rodents, as well in humans, α4β2 nACh receptors are the most abundant and widespread in the human brain all along the hippocampus, basal ganglia, and cerebellum.[47] Recent studies have suggested that there is an incorporation of the α5 or α6 subunit in some α4β2 nACh receptors.[47] In rodents, α3β4 nACh receptors are present in the autonomic ganglia, pineal gland, medial habenula, and interpeduncular nucleus,[57] to name a few. α6-Containing nACh receptors are present in the locus coereuleus along with the dopaminergic neurons of the mesolimbic system which control dopamine release.[57] The homopentameric α7 nACh receptors are particularly found in the hippocampus, hypothalamus, cortex, and motor nucleus of the vagus.[47] nACh receptors are anatomically and functionally located in pre- and post-synaptic sites in the CNS where they perform various functions such as, pre-synaptically, regulating the release of various neurotransmitters such as norepinephrine, glutamate and GABA, while post-synaptically, bringing about various voltage-dependent downstream responses.[47,57]

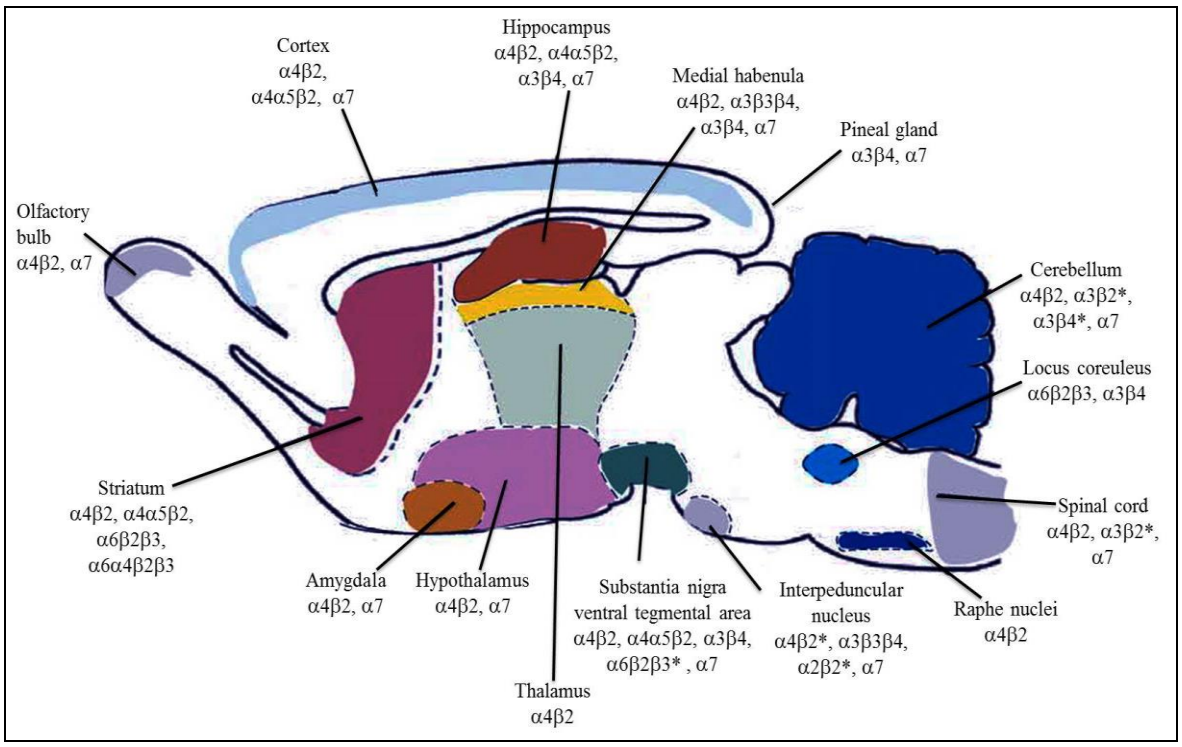


Figure 4. Regional distribution of various receptor subtypes in a rodent CNS adapted from Gotti et al.[57]

2. Importance of α7 and α4β2 nACh receptors in the brain

The two main classes of nACh receptors widely distributed in the brain are the α7 and the α4β2 subtypes.[4] They are thought to be involved in various pathophysiological states such as schizophrenia, Alzheimer's disease (AD), autism, nicotine addiction, attention deficit hyperactivity disorder (ADHD), depression, Parkinson's disease (PD), and pain. Also, non-neuronal nACh receptors present in certain tissues and cells are implicated in various disease states such as lung cell carcinoma, blood pressure, and, inflammatory bowel disease. Due to the vastness of the various disease states, only neuronal disorders are considered here.

a. Schizophrenia

Schizophrenia is a neuropsychiatric disorder associated or characterized by delusions, hallucinations, disorganized speech, and catatonic behavior.[58] A high prevalence of an almost 3-fold increase of smoking in schizophrenic patients led to investigations of the possible involvement of nACh receptors in schizophrenia.[59] Also, the fact that neuroleptic neuronal side effects were fewer among smokers, and a negative correlation between smoking and the symptoms of schizophrenia, established definite involvement of nACh receptors in the pathophysiology of schizophrenia.[60,61] Sensory gating, which is an involuntary process to selectively sense a stimulus, is impaired in schizophrenic patients, was restored to some extent in heavy smokers.[62] It was found that heteromeric nACh receptors are not involved in the pathophysiology of schizophrenia.[63] Experimental studies were unable to show a difference between controls and schizophrenic patients in terms of [^{3}H]-nicotine binding and the levels of $\alpha4$, $\alpha3$, and $\beta2$ subunits in the hippocampus, thalamus, cortex and caudate putamen where most heteromeric nACh receptors are located.[63,64] The homomeric $\alpha7$ nACh receptors, on the other hand, were significantly involved in the pathophysiology of schizophrenia, which was shown by α-bungarotoxin administration to induce sensory gating defects in mice.[65] α-Bungarotoxin was also seen to have reduced binding in temporal lobes along with $\alpha7$ nACh receptor immunoreactivity in the hippocampus,[66,67] reticular nucleus of the thalamus and cingulated frontal cortex of the schizophrenic patients, where $\alpha7$ nACh receptors predominate.[63] Agents approved by the FDA for the treatment of schizophrenia include various atypical antipsychotics such as clozapine, olanzapine and others. Clozapine has been shown to improve gating deficits, and, thus, improve sensory stimulus.[68] The improvement can also be attributed to clozapine-

induced increased levels of ACh in the hippocampus.[68] This observation was experimentally demonstrated when α-bungarotoxin was shown to block clozapine's effects.[69] Clozapine is not a preferred choice for the treatment of schizophrenia due to its severe toxicity profile.[68] Thus, various α7 nACh receptor agonists have been examined as potential therapeutic candidates for the treatment of schizophrenia.[68]

b. Alzheimer's disease (AD)

AD is the most common form of dementia in the elderly population.[70] It is characterized by neuronal cell loss due to the presence of β amyloid ($A\beta_{1-42}$) and progressive accumulation of neurofibrillary tangles caused by aggregates of hyperphosphorylated tau proteins in the neurons and amyloid fibers in senile plaques and in the walls of the blood vessels.[70] To test the neurotoxicity of Aβ, Jay et al.[71] were able to demonstrate that the mortality rates of transgenic animals that overexpressed Aβ were doubled compared to control so as to determine that overexpression of Aβ is sufficient to induce neurotoxicity. This degeneration affects many types of neurons and may account for the numerous neurobiological deficiencies that patients experience with the disease onset. The most notable degeneration occurs in the hippocampus, cerebral cortex, and amygdala.[71] These are the regions that play a major role in memory, cognition and behavior.[71] The vast majority of the people with AD have a late-onset form of the disease in which memory loss occurs at the age of 60 years or more.[70] There are few cases where people are diagnosed with an inherited form of AD at an age as early as 30 or 40 years.[70] In AD patients it has been found that α4 nACh subunits are decreased in the hippocampus and the cortical areas of the brain.[72] Also since the β2 nACh subunit, together with α4, forms the most

abundant heteromeric nACh receptors, a relationship was established between nACh receptors and AD.[73] This relationship was further supported by genetic polymorphism studies of the α4 and β2 subunits in AD patients.[73] The $A\beta_{1-42}$ is seen to inhibit the α4β2 and α7 nACh receptor subtypes acutely at nM and pM concentrations, respectively, which could contribute to the memory and cognitive impairments associated with AD patients.[74] It has been shown that $A\beta_{1-42}$ is a non-competitive antagonist of the α4β2 subtype of nACh receptors. It has been experimentally shown that the β2 subunit isn't affected, suggesting an alternate site of action for the $A\beta_{1-42}$.[75] The $A\beta_{1-42}$ inhibition is not mediated through an open channel block and pretreatment with $A\beta_{1-42}$ is necessary to induce convincing inhibition.[54,75] To combat AD there are two strategies that can help increase cholinergic transmission in the brain: firstly by increasing the amount of ACh at the synapse that could be achieved by blocking acetylcholine esterase (AChE) activity, and secondly by potentiating the action of ACh at nACh receptors. Galantamine, tacrine, memantine, donepezil and rivastigmine are commonly used AChE inhibitors for the treatment of mild to moderate cognitive symptoms of AD of which galantamine, in addition, allosterically potentiates α4β2 and α7 nACh receptors present in the brain.[76] Thus, drugs selectively targeting the nACh receptor subtypes can increase cholinergic tone in the diseased brain and, hence, provide therapeutic potential for the treatment of AD.

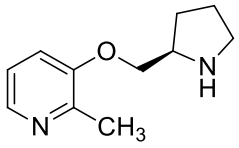
c. Autism

Autism is characterized by impaired social and communications skills along with odd and stereotypical behavior. It is also associated with impaired planning and attention. Autism is also considered as a spectral disorder with severe developmental defects that are apparent beginning

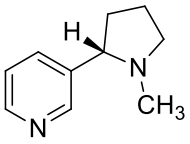
in early childhood.[77] A decreased level of α4 mRNA in the parietal cortex and cerebellum as well as decreased levels of α4β2 nACh receptors with unchanged levels of α7 nACh subtypes can be seen in autistic patients.[78] 'Nicotinic tone' which refers to the overall activation of synapses by nicotine is considered as a factor that could be contributing towards the changes in the etiology of autism.[78] Thus agents that can restore the 'nicotinic tone' and potentiate the α4β2 nACh receptors might play a crucial role in providing therapeutic options for autism. Selective α4β2 nACh receptor potentiators can provide important insight into some of the cellular and molecular mechanisms that can help understand the etiology and pharmacology of autism, which is still unclear.

d. Attention deficit hyperactivity disorder (ADHD)

ADHD is a psychiatric disorder characterized by hyperactivity, inattentiveness and impulsivity symptoms.[79] Factors such as academic, employment and marital difficulties, psychiatric disorders, substance abuse, depression, anxiety and personality disorders can be a result from ADHD in adults.[80] nACh receptors have been implicated and studied for their role in the disease state; and it was found that nicotine and its analogs were efficient in treating ADHD symptoms.[79] α4β2 nACh receptor activation by ABT-089 (**4**), a selective weak agonist, was found to alleviate the symptoms of ADHD in rodents and primates and humans by improving attention, learning and memory.[81] Drugs targeting selectively at the α4β2 nACh receptors subtypes can tackle cognitive disturbances and hence provide therapeutic potential for the treatment of ADHD.



ABT-089 (**4**)



(-)Nicotine (**3**)

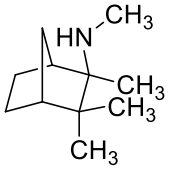
e. Nicotine addiction

Nicotine (**3**), a plant alkaloid, is an active constituent of the leaves of *nicotiana tabacum* that interacts with all the nACh receptor subtypes distributed in the CNS as well as the PNS.[82] Nicotine is the chief component of tobacco, which is widely abused by smoking and chewing of the dried and cured leaf. There is an immediate need in understanding the mechanism of nicotine addiction due to the extreme health risk associated with nicotine abuse.[83] Prolonged exposure to nicotine causes a gradual decrease of the ionic responses called as the high affinity, super-open desensitized state of nACh receptors that is of key importance to understanding nicotine addiction.[84] The brain areas involved in addiction are the dopaminergic neurons in the ventral tegmental area (VTA), the prefrontal cortex, the nucleus accumbens (NA), extended amygdala, hippocampus, and the habenulo-interpeduncular system.[82] Activation followed by desensitization by nicotine causes an alteration in the synaptic response that, in turn, alters synaptic signaling.[82] The dopaminergic system in the VTA and its projections in the NA reinforce the nicotine reward function and nicotine addiction.[85] Presynaptic $\alpha 4\beta 2$ nACh receptors on the dopaminergic neurons cause the release of dopamine which is strongly attributed to the addictive properties on nicotine.[86]

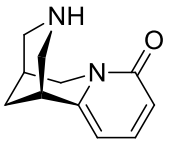
f. Depression

A direct correlation exists between smoking and depression with an almost 3-fold increase in nicotine dependence in depressed patients verses nondepressed.[87] Similar ratios can be found for the population who attempts to quit nicotine consumption, where depressed individuals are 2-3 times less prone to quit versus the nondepressed. This clearly indicates a nicotine component involved in depression and mood.[87,88] The α4β2 nACh receptor subtype has been considered as an important therapeutic target for the potential treatment of depression due to its widespread presence in the brain.[89] α4β2 nACh receptors have been implicated to modulate the levels of dopamine which is an important neurotransmitter involved in reward and reinforcement regulation in the brain through direct actions as well as indirectly through the excitatory glutamatergic and the inhibitory GABAergic neurons.[1,57] The neuronal component of nACh receptors is involved in depression, and not the peripheral nACh receptor subtypes.[90] Smoking has been known to decrease depression through desensitization of nACh receptors; thus, an antagonist at nACh receptors could have antidepressant effects.[90,91] This correlation was observed in studies involving mecamylamine (**5**), a non-selective, non-competitive antagonist at nACh receptors that showed antidepressant-like effects in behavioral tests performed in mice.[92] Cytisine (**6**), a partial agonist at nACh receptors with low efficacy at α4β2 nACh receptors and full agonist action at ganglionic α3β4 ACh receptor and α7 nACh receptors, is used as a smoking cessation agent and was also tested for its antidepressant effect.[93] A partial agonist at the nACh receptor will increase the activity of the nACh receptor but limits the ability of ACh to activate receptors.[94] Hence, if high levels of ACh lead to depression-like effect, a partial agonist

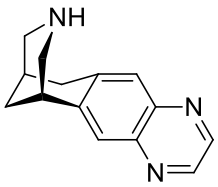
might provide therapeutic benefit towards depression. Studies with cytisine (**6**) in mice have supported this theory.[95]



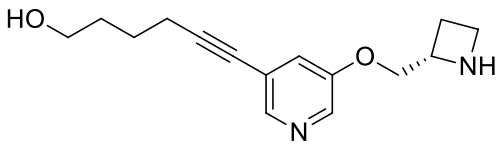
Mecamylamine (**5**)



Cytisine (**6**)



Veranicline (**7**)



Sezetidine-A (**8**)

Similar studies were performed with veranicline (**7**), which is also a partial agonist at nACh receptors with low efficacy at α4β2 nACh receptors, with higher efficacy than cytisine and is a full agonist at ganglionic α3β4 ACh receptors and α7 nACh receptors. Veranicline (**7**) has a similar profile as that of cytisine.[96] The α7 nACh receptor agonist effect in the above drugs could be a possible reason for the antidepressant like effect. This theory was eliminated when sezetidine-A (**8**), a selective α4β2 nACh receptor partial agonist, was able to cause antidepressant-like effect in mice.[97-99] Future directions towards depression and nicotinic receptors as potential targets requires a bidirectional approach where various factors including different receptor subtypes, different brain areas where the ligands might act, and different neurotransmitters that could be influenced by ACh along with different levels of mood, stress and depression levels in patients. Drugs selectively targeting the α4β2 nACh receptor subtype

can potentially target the depressed state of the patient and hence provide therapeutic treatment for depression.

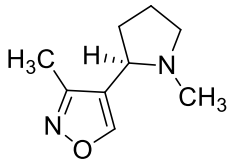
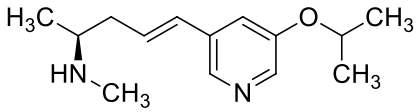
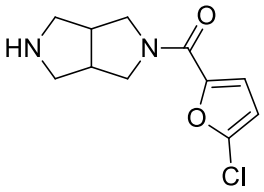
g. Parkinson's disease (PD)

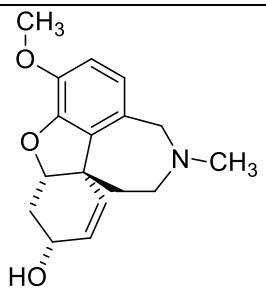
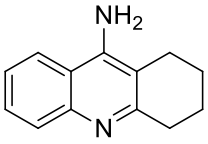
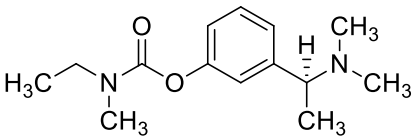
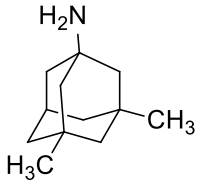
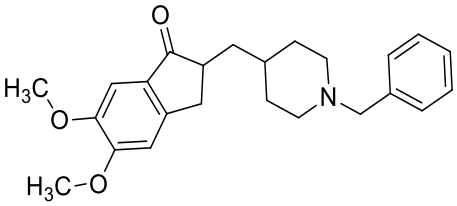
PD is a neurodegenerative disorder marked by tremor, rigidity, postural instability and bradykinesia.[100] The pharmacology involves the degeneration of the nigrostriatal dopaminergic pathway (NDP) which involves neuronal loss in the CNS and PNS. Various neurotransmitters are thought to be affected, but to a lesser extent compared to the NDP. Collectively there is impairment of motor function, cognition/memory and autonomic functions.[100] Currently, dopamine replacement therapy is the preferred treatment for PD with L-DOPA along with other dopamine agonists. These agents are effective for the motor function deficit for the disease state, while ineffective for the nonmotor symptoms in PD.[101] This led to other therapeutic avenues being explored and it was found that nicotine was able to protect the NDP pathway, thus encouraging researchers to explore the cholinergic systems. It has been shown that $\alpha4\alpha6(\beta2)_2\beta3$ and $\alpha4\beta2$ nACh receptors are predominantly present in the corpus striatum with their projections in the substantia nigra region controlling dopaminergic firing.[101] The $\alpha6\beta2$*-containing nACh receptors are almost exclusively expressed on the presynaptic dopaminergic neuronal terminals, whereas $\alpha4\beta2$*-containing nACh receptors are widely distributed on presynaptic dopaminergic and postsynaptic glutamatergic, GABAergic and serotonergic neurons.[44,102,103] Researchers were able to show that the $\alpha4\beta2$* and $\alpha6\beta2$* nACh receptors present on dopaminergic neurons modulate dopamine release and are important for NDP functioning, thus posing as attractive targets for development of selective agents for the treatment of PD.[101]

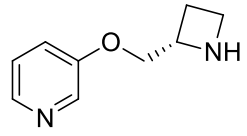
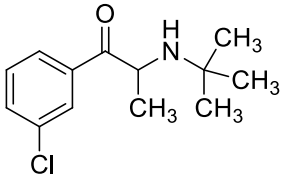
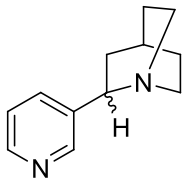
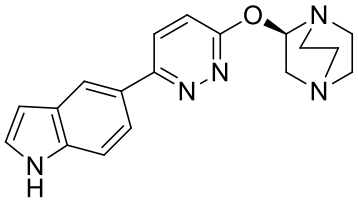
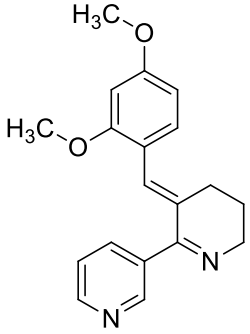
3. Agents of nACh and other receptors in disease state

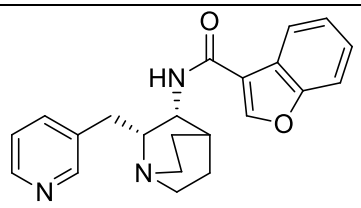
nACh receptor subtypes have been validated as critical drug targets due to their roles in the muscle and ganglionic functions. This has stimulated a wide interest in the research for generation of synthetic analogs for the various nACh receptor subtypes. However there is still a major void in subtype selective agents. Listed below in Table **1** are a few examples of analogs which have been implicated and approved for the use of the various disease states.

Table 1. List of agents implicated in various disease state.

Agents	Targets	Disease state	Additional information
 ABT-418 (**9**)	α4β2, α7 agonist	AD,[104] ADHD[81]	Phase trials; discontinued for AD
 AZD-3480/Ispronicline/ TC-1734 (**10**)	α4β2, α7 partial agonist	AD[105]	Phase IIb; discontinued
		ADHD	Phase II[106]
 AZD-1446 (**11**)	α4β2 agonist	AD	Phase II[107]

 Galantamine (**12**)	AChE inhibitor, α4β2 and α7 agonist	AD[108]	FDA approved
 Tacrine (**13**)	AChE inhibitor	AD[109]	FDA approved
 Rivastigmine (**14**)	AChE inhibitor	AD[110], dementia related to PD[111]	FDA approved
 Memantine (**15**)	NMDA antagonist, α7 non-competitive antagonist	AD[112,113]	FDA approved
 Donepezil (**16**)	AChE inhibitor	AD[114]	FDA approved

 A-85380 (**17**)	α4β2 α7, α1β1δγ agonist	Pharmacological probe[115]	
 Bupropion (**18**)	nicotinic receptor antagonist	Antidepressant,[116] smoking cessation[117]	FDA approved
 TC-2429 (**19**)	α6β2 agonist	PD[118]	Preclinical development
 ABT-107 (**20**)	α7 agonist	AD, schizophrenia[119,120]	Phase trials; discontinued
 DMXB-A/GTS-21 (**21**)	α7 partial agonist	schizophrenia	Phase II[121]

 TC-5619 (**22**)	α7 partial agonist	ADHD, schizophrenia[122]	Phase II;[123] discontinued

4. Desensitization of nACh receptors

Desensitization means decrease of the response in the presence of a stimulus. Katz and Thesleff[5] first noted that continuous application of ACh on frog muscle end-plate resulted in a decreased cell response and almost abolished the response that was quickly recovered upon removal of ACh from the system.[124,125] This phenomenon was thus termed as 'desensitization' and was hypothesized to have resulted from the transition of the receptor into a refractory state.[125] It was also shown that animals exposed to nicotine resulted in an increased density of nACh receptors. This phenomenon allowed scientists to propose a two-state model of nACh receptor desensitization which involved activation and desensitization by nicotine (or any agonist) at the same time.[126] Various factors together provide the net effect of nicotine on nACh receptors such as drug concentration, exposure length, and the state of the receptor.[127] nACh receptors have a complex pharmacology and this can be mostly attributed to the activation and desensitization of the nACh receptors over a short time course of nicotine exposure.[127] The various biological events associated with nACh receptor are initiated by the binding of the ACh, which brings about a conformational change in the receptor shape.[128] As previously discussed the nACh receptors

exist in four states; the closed, the open, the intermediate transition state and the high-affinity channel-inactive desensitized state (Figure **5**).[128,129]

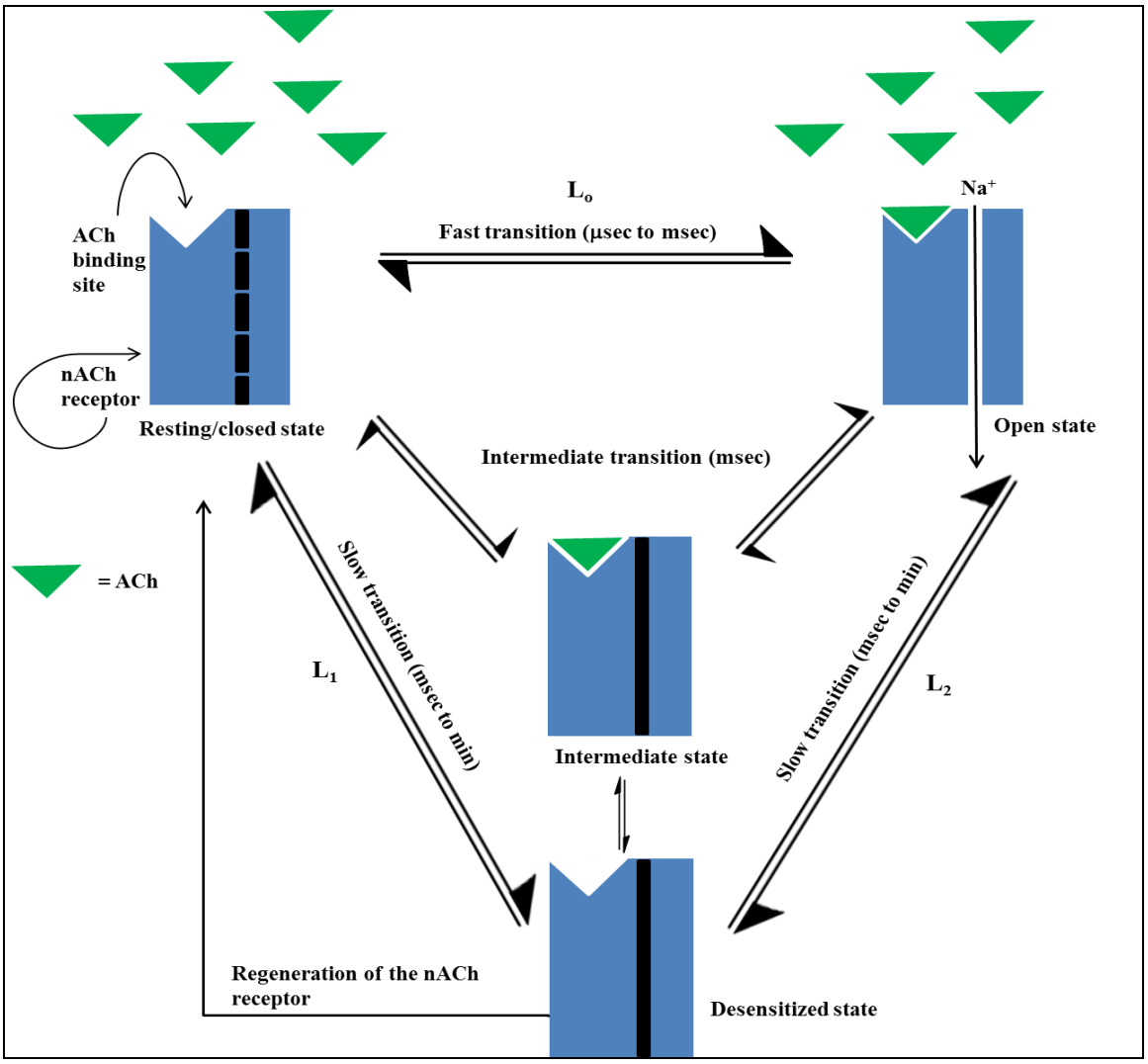


Figure 5. Multiple conformation states of nACh receptors and their transition from one state to the other in the presence of ACh (adapted from Arias[128]).

The closed state is the low-affinity resting state of the receptor and, in the absence of the agonist/ACh, consists of around 80% of the receptor population while the remaining 20% exists in the desensitized state.[130,131] Desensitization of the nACh receptors has been found to be modulated by the activation of protein kinases and receptor phosphorylation.[132] It can be noted that the agonist-induced desensitization of the nACh receptor occurs rapidly. Experiments were performed on the membrane of *Torpedo* species and intrinsic fluorescence measurements showed that the $t_{1/2}$ was 1.9 sec for agonist induced desensitization.[133] The time frame for the activation cycle of the nACh receptor consists of μsec to msec for the fast activation of the receptor from the closed to the open state upon ACh binding. Prolonged presence of agonist/ACh causes the receptor to convert to the intermediate transition state which takes around msec and finally inducing into a high-affinity channel-inactive desensitized state which occurs in a sec to min time frame.[128] Desensitization of the homomeric α7 nACh receptor is associated with a structural change of the binding sites at the orthosteric site and the ion pore.[134,135] Also, mutation of Leu247 in the pore-lining M2 domain of the TM region of the nACh receptor was shown to destabilize the conformation of the desensitized receptor making it conducive to channel opening, thus causing ions to flow through and considerably slow the rate of desensitization of the rapidly fast-desensitizing α7 nACh receptor.[134,136] Desensitization experiments of various heteromeric nACh receptors have shown that the rate of desensitization is faster for the β2 containing than the β4 containing subtypes.[135] The N-terminal region of the β subunit has been shown to confer the different rate of desensitization of the β-containing heteromeric nACh receptors.[134]

D. Allosterism

Receptors are proteins that can adopt multiple tertiary conformations. Receptors can also interact with other cellular proteins that can function as downstream messengers of the signal transduction pathway. The origin of allosterism is in the Greek literature where *allos* means 'other' and *stereos* mean 'solid shapes'. The concept of allosterism was first proposed by Monod and Jacob in 1961[7,137] when it was found that certain bacterial enzymes were feedback inhibited by the end product or regulatory ligand of the biosynthesis pathway, even though they had no structural similarity with the starting material or the substrate.[138] Further experiments were able to demonstrate that the interaction between the substrate and the regulatory ligand were the result of distinct binding sites at which the molecules bind. The binding of the regulatory ligand brings about a change in the conformation of the protein that reversibly modifies the conformation of the active site indirectly.[138] The concept was studied and adopted by Monod, Wyman and Changeux (MWC) in 1965[139] where they showed that allosteric effects do not fit general enzymatic theories, and that a molecular transition, also called the allosteric transition, occurs when an allosteric ligand binds to stabilize the protein.[137] Henceforth, the allosteric model of ligand interaction was also called the MWC model.

1. Theory

In an allosteric modulation various factors play a role such as the dynamic conformational change of the protein in the absence of the ligand, and stabilization of the protein upon ligand binding in a preferred conformation. Thus, in theory, a nACh receptor agonist will preferentially

stabilize the active open state conformation and a nACh receptor competitive antagonist will stabilize the resting or the closed state conformation. As mentioned earlier, different states of the receptor possess different affinity for a ligand (Figure **5**). Transition between the different states can be brought about either by ligand binding or a free energy change between the states (L_0-L_2) (Figure **5**) that involves transition from one state to another. A three-state model for the allosteric properties of the nACh receptor has been proposed that involves the resting state, open state, and the desensitized state of the receptor. Generally the allosteric system involves quaternary protein structures that can cause alteration in the bonding of the subunits of the receptor. Allosteric effects can be broadly classified into two types; homotropic and heterotropic.[137] A homotropic effect occurs when the second molecule of the endogenous ligand (ACh) can bring about an allosteric modulation for ACh binding at the orthosteric site. In the case of the heterotropic effect, the allosteric modulator binds at a site distinct from the orthosteric site and modulates the binding of the orthosteric ligand. Agents that stabilize the open state of the receptor, thus potentiating the effect of the endogenous ligand and further shifting the equilibrium towards the open state, are called as positive allosteric modulators (PAMs) (Figure **6**).[139] Whereas, on the other hand, ligands that bind at an allosteric site and increase the energy barrier along with shifting the equilibrium to the resting state are called negative allosteric modulators (NAMs). PAMs on their own are inactive and don't activate the ion channel. A ligand that binds at an allosteric site and activates the receptor on its own is called an allosteric potentiating ligand or allosteric non-competitive agonist.[140]

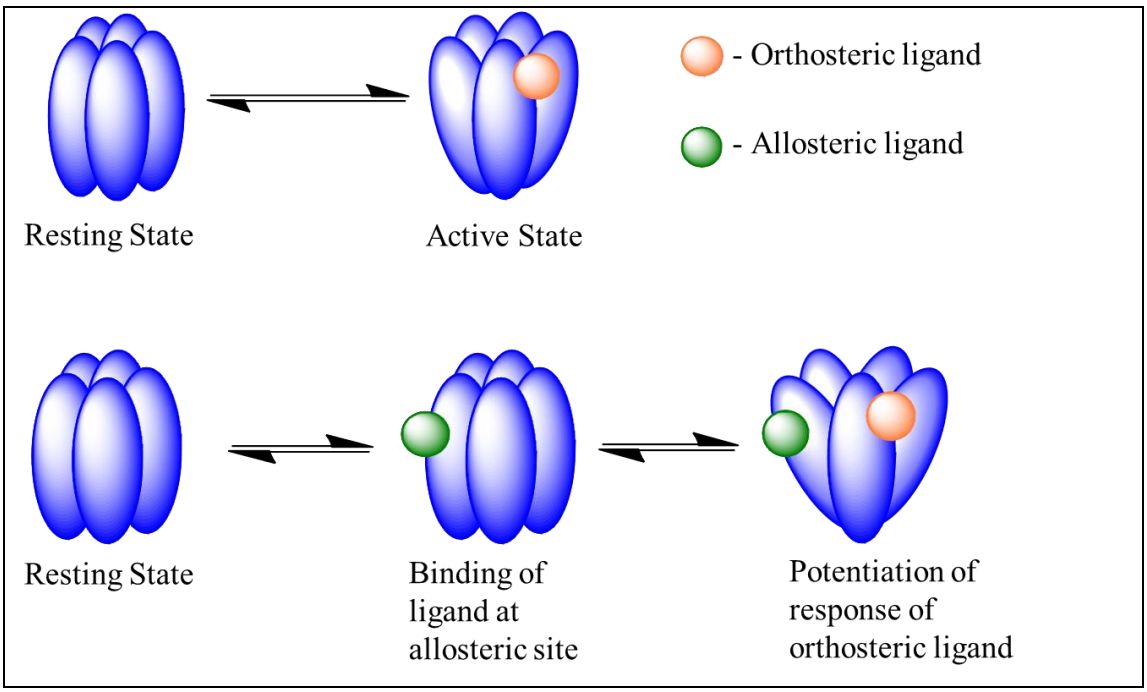


Figure 6. Schematic representation of positive allosteric modulation where the pentameric nACh receptor is depicted in blue (adapted from Bertrand and Gopalakrishnan[139]).

Cooperativity is an important phenomenon where binding of two or more ligands on the same protein at distinct sites generally increases or decreases the rate of binding of the second ligand onto the protein and is termed positive or negative cooperativity, respectively. Cooperativity is measured using a Hill coefficient (*n*) which provides a quantifiable measure of the same. A coefficient of $n > 1$ indicates a positive cooperativity while $n < 1$ indicates negative cooperativity. Kinetic models of the ACh response show that two molecules of ACh are required for the activation of the receptor and that $n = 1.5$ to 2.2, which suggests a homotropic effect.[141] The unbinding of the ACh from the receptor is slow enough to allow the receptor to activate the channel opening more than once, thus suggesting that the channel opens and closes with ACh remaining bound to the receptor.[142] Thus, the allosteric model of ACh binding should include the

unliganded, single liganded and double liganded state of the receptor. The model (Figure **7**) shows the complexity of the transition of the ACh receptor from one state to the other. At resting state, the closed conformation (R) of the receptor is predominant. The closed channel (R) has low affinity for the ligand but possesses a fast rate constant to go to the open state (AR) of the receptor.[143] Once the receptor is bound by 2 molecules of ACh (A_2R), the probability of channel opening is almost a million times more when compared to the closed unliganded state (R) of the receptor. Once in the activated state, the receptor desensitizes (A_2R_D) rapidly; thus, eventually, the burst fades away. Thermodynamically, the desensitized state of the receptor is the high-affinity state with a 100-fold higher affinity for the ACh. With equilibrium favoring the closed state (R) of the receptor, the desensitized receptor (A_2R_D and AR_D) and the receptor reverts to the closed state and the cycle is ready to repeat.[142]

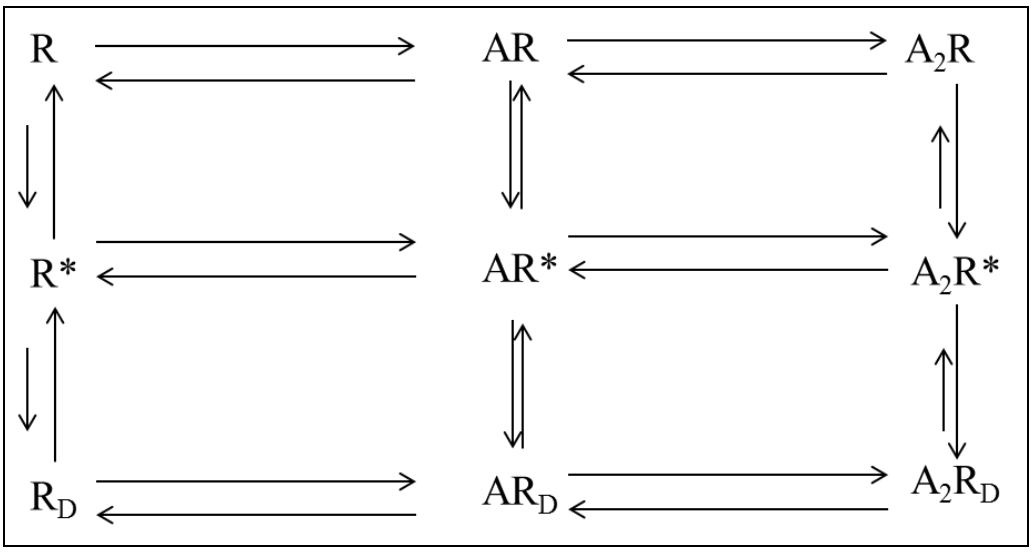


Figure 7. Allosteric model of the various transition states of the nACh receptors. R= Receptor in the open state, AR= 1 molecule of ACh bound to the receptor, A_2R= 2 molecules of ACh bound to the receptor, *= open state of the receptor, $_D$= desensitized state of the receptor.[142]

2. Advantage of allosteric modulators over traditional orthosteric ligands

There are various advantages of allosteric modulators over orthosteric ligands for receptor activation. First, the orthosteric ligand binds to the orthosteric site and activates the receptor; on the other hand, the allosteric modulator doesn't have any intrinsic activation, thus the natural state of the receptor along with the physiological stimulus produced by the endogenous ligand can be maintained. This phenomenon could possibly reduce the likelihood of receptor desensitization even when an allosteric modulator is continuously present, thus providing a tool to escape tolerance.[144] A second major problem associated with the orthosteric ligands is the overdose/toxicity aspect. The allosteric modulators are saturable; thus once all the binding sites for the allosteric modulators are occupied it cannot produce any additional effect and, thus, can be a safer alternative in cases of overdose.[145] The third advantage tries to tackle the most

problematic aspect of drug discovery, i.e., selectivity of the ligands across various receptor subtypes. In the case of nACh receptors the orthosteric site is highly conserved providing very little room for designing selective agents for various nACh receptor subtypes. An allosteric site could be found anywhere on the receptor surface and thus can be specific for a particular receptor subtype population and provide selectivity.[144]

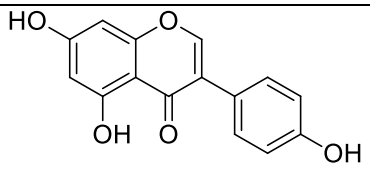
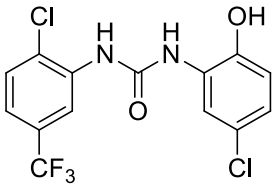
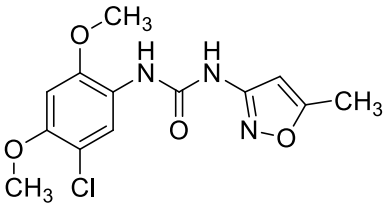
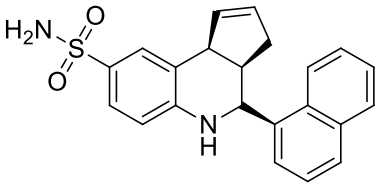
3. Allosteric modulators of nACh receptors

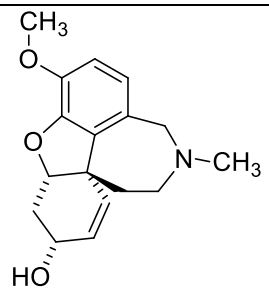
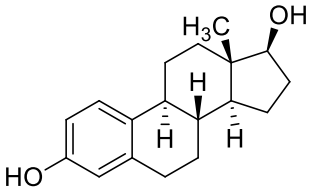
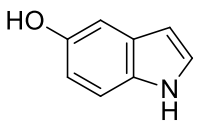
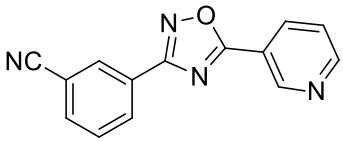
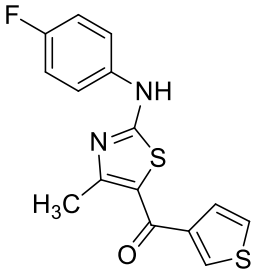
Due to the broad implication of nACh receptors in various neurodegenerative disorders, PAMs can play crucial role in restoration of nicotinic tone.[54] Ivermectin, an anthelminthic agent, was the first agent to be identified as a PAM at α7 nACh receptors. The compound was able to decrease the EC_{50} and increase the slope of the dose response curve, thus potentiating the effect of ACh.[146] Since then, various other agents have come to light as potential PAMs at the α7 nACh receptor subtype.[139] Galantamine, which is an AChE inhibitor, was shown to activate α7 and α4β2 nACh receptors along with other subtype populations, through binding at a site distinct from the orthosteric site for ACh.[147] Thus, co-application of galantamine with ACh in *Xenopus* oocytes expressed with α4β2 nACh receptors was shown to potentiate the effect of ACh.[147] The neuro-steroid 17β-estradiol was shown to potentiate ACh-induced responses by increasing channel opening probability at α4β2 nACh receptors.[148] It was found that the C-terminal region of the α4 subunit was involved in 17β-estradiol binding and that the site was different from other progesterone inhibitions.[148] Various cations, such as zinc (Zn^{2+})[149] and calcium (Ca^{2+}),[150] seem to play a crucial role in the modulation of the various nACh receptor subtype populations in the brain. Zinc co-application (50 μM) with a saturating ACh concentration was shown to potentiate

ACh-evoked currents by 260%. Also, it was observed that zinc potentiates at low concentrations (EC_{50}=16 μM) and inhibits at high concentrations (IC_{50}=440 μM).[151] Through mutagenesis studies it was observed that the Zn^{2+} potentiates low-affinity $(\alpha4)_3(\beta2)_2$ receptors that have a $\alpha4^+/\alpha4^-$ phase while inhibiting both the low- and high-affinity stoichiometry of α4β2 nACh receptor that have a $\beta2^+/\alpha4^-$ phase that is required for Zn^{2+} binding.[149,152]

PAMs have different qualitative effects on the ACh response.[153] They are classified into two types namely, Type I and Type II PAMs. Type I PAMs are agents that increase the receptor sensitivity by increasing the magnitude of the current produced by ACh without affecting the decay rates of the current.[153] Type I PAMs do not affect the onset and the basic decay kinetics of the response. On the other hand Type II PAMs increased the apparent peak current by rapid onset of action and weak current decay kinetics.[154] In the case of α7 nACh receptors Type II PAMs were able to reactivate the desensitized receptors which are considered to be the reason of the prolonged decay rates for the ACh currents.[153] Table **2** summarizes various agents that have been shown to be PAMs at the two major nACh receptor populations in the human brain: the α7 and α4β2 subtypes.

Table 2. A list of agents and their selectivity profile at various nACh receptor subtypes.

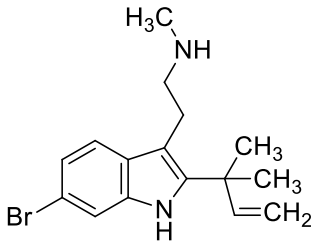
Agent	Target	Type
 Genistein (**23**)	α7 nACh receptor,[153] tyrosine kinase inhibitor	PAM type I
 NS-1738 (**24**)	Non-specific at nACh receptors[155]	PAM type I
 PNU-120596 (**25**)	α7 nACh receptor[156-158]	PAM type II
 TQS (**26**)	α7 nACh receptor[153]	PAM type II

 Galantamine (**27**)	AChE inhibitor,[140] α7 and α4β2 nACh receptor [159]	PAM type II
 17β-Estradiol (**28**)	α4β2 nACh receptor[148] and various nuclear receptors	PAM type II
 5-Hydroxyindole (**29**)	α7 nACh receptor,[160] endogenous ligand	PAM type I
 A-998679 (**30**)	α4β2 nACh receptor[161]	PAM type II
 LY-2087101 (**31**)	α7 and α4β2 nACh receptor[162]	PAM type I

4. *des*-Formylflustrabromine (dFBr)

Marine biology has been for years a great source of novel creatures and a part of the research to identify novel agents for drug discovery. *Flustra foliacea* is a marine bryozoan species found commonly in the North Sea and is now known to biosynthesize a variety of indole-containing natural products as metabolites.[163] To date, about 22 alkaloids and monoterpenes have been isolated from the bryozoan species.[163,164] One of the indolic compounds is dFBr (**32**). Peters et al.[165] performed binding experiments with dFBr (**32**) at α4β2 and α7 nACh receptors and it showed low (K_i = 3,400 nM and >50,000 nM, respectively) affinity compared to (-)nicotine (**3**) (K_i = <1 nM).[165] Some of the metabolites were shown to possess muscle relaxant properties.[166] Several of the *Flustra* metabolites were tested for their biological activity against α3β2, α3β4, α4β2, α4β4 and α7 nACh receptors expressed in oocytes and only dFBr (**32**) showed any significant activity at the α4β2 nACh receptor subtype.[166] dFBr (**32**) was shown to potentiate ACh-induced responses in *Xenopus* oocytes expressed with α4β2 nACh receptors, while a statistically insignificant inhibitory response was measured at the α7 nACh receptor subtype.[166] Various experiments were conducted by Sala et al.[166] and dFBr (**32**) was found to potentiate ACh's effects through a mechanism that does not involve direct activation of α4β2 nicotinic receptors at the orthosteric site. Rather than behaving as an orthosteric agonist, dFBr is a positive allosteric modulator that can potentiate the actions of ACh at α4β2 nACh receptors.[166] It was shown that the action of dFBr (**32**) was not on the pore properties but kept the channel open for a longer time following ACh binding, along with reopening the channel faster, hence increasing the potency and efficacy of the ACh-induced response.[166] The potentiation caused by dFBr on ACh-induced currents was also found to be reversible and concentration-dependent. dFBr was

the first compound shown to possess selectivity for α4β2 receptors as a PAM and could potentially be of great benefit for further understanding the importance of α4β2 nACh receptors in various disease states. Biological testing was performed using the various *Flustra* metabolites as their free bases, which required using DMSO for solubilizing the compounds.[166] Joshi et al.[167] were able to modify the conventional oocyte electrophysiological experiments and incorporated a vertical flow system that was shown to have extended oocyte stability, drug exposure to the complete oocyte surface, and that can be attached to an HPLC autosampler for high throughput style assays. The vertical oocyte chamber was also able to provide a faster exposure thus providing more accurate determination of the results compared to the conventional flow oocyte chamber.[167]



des-Formylflustrabromine (dFBr) (**32**)

III. Specific aims

With the advent of allosteric modulation as a novel route for activation of nACh receptors it is important to identify agents that act selectively so that nACh receptors and their subtypes can be studied in greater detail. The major limiting factor with allosteric modulators is that they are impossible to design *a priori*. Thus, the only way to identify such compounds is through serendipity or through high-throughput screening methodology. Sala et al.[166] were fortunate to find dFBr as a novel agent that was able to potentiate α4β2 nACh receptors allosterically and it, thus, provided a novel lead for further drug development. The previous studies of dFBr (**32**) involved the use of DMSO solutions of the water-insoluble free base for all the biological studies employed. Also, researchers relied on the natural source for obtaining dFBr (**32**) and, hence, its availability was limited. Our laboratory (i.e., Kim et al.)[9] and Lindel et al.[168] independently identified convenient synthetic routes for the synthesis of dFBr (**32**), which was synthesized as a water-soluble HCl salt. The water-soluble HCl salt was tested for its action at the α4β2 and α7 nACh receptor subtypes. It was shown that the potentiating effects of the HCl salt of dFBr was greatly improved over that of its free base.[9] This could be because of better water solubility while performing the biological testing, or due to the non-necessity of using DMSO as solvent. Interestingly, it was observed that contrary to results published by Sala et al.[166] the HCl salt of dFBr (**32**) produced a biphasic response at the α4β2 nACh receptor (Figure **8**) with an EC_{50} of 120 nM for the potentiation component and an IC_{50} of 150,000 nM for the inhibitory component

of the dose-response curve when co-applied with 100 μM ACh.[9] dFBr (**32**) potentiated the maximal ACh response to almost 300%.[9] The inhibition profile at relatively higher concentrations was hypothesized to be due to an open channel blockade mechanism with further studies required to prove it. At α7 nACh receptors, dFBr (**32**) produced only an inhibitory response with an IC_{50} of 44,000 nM (Figure **9**).[9]

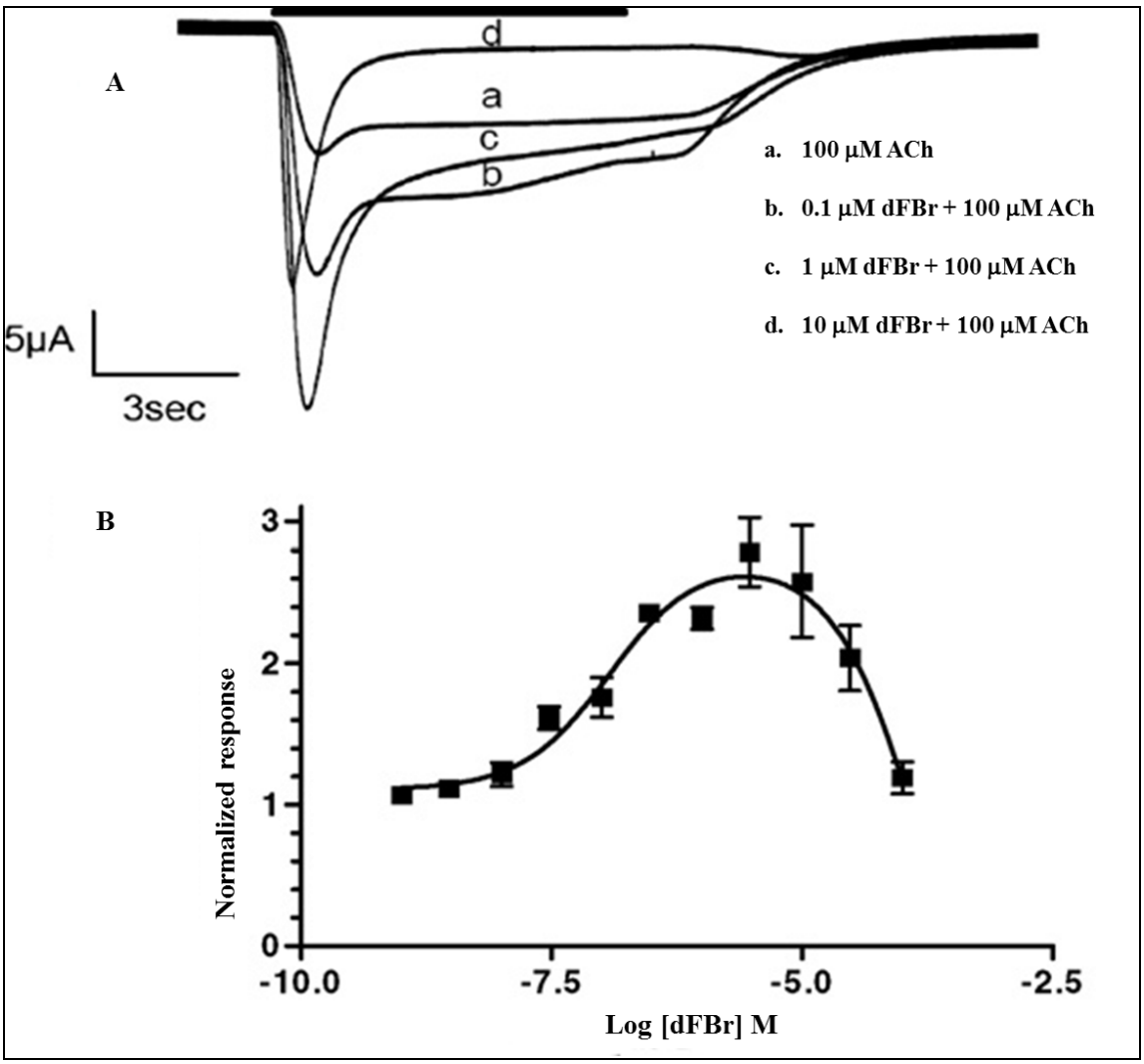


Figure 8. Potentiation and response curve for dFBr at α4β2 nACh receptors. (A) A typical trace of the potentiation of the ACh-evoked response for dFBr (**32**) from a single oocyte expressing human α4β2 nACh receptors. (B) A biphasic dose-response curve for dFBr (**32**) co-applied with 100 μM ACh on human α4β2 nACh receptors.[9]

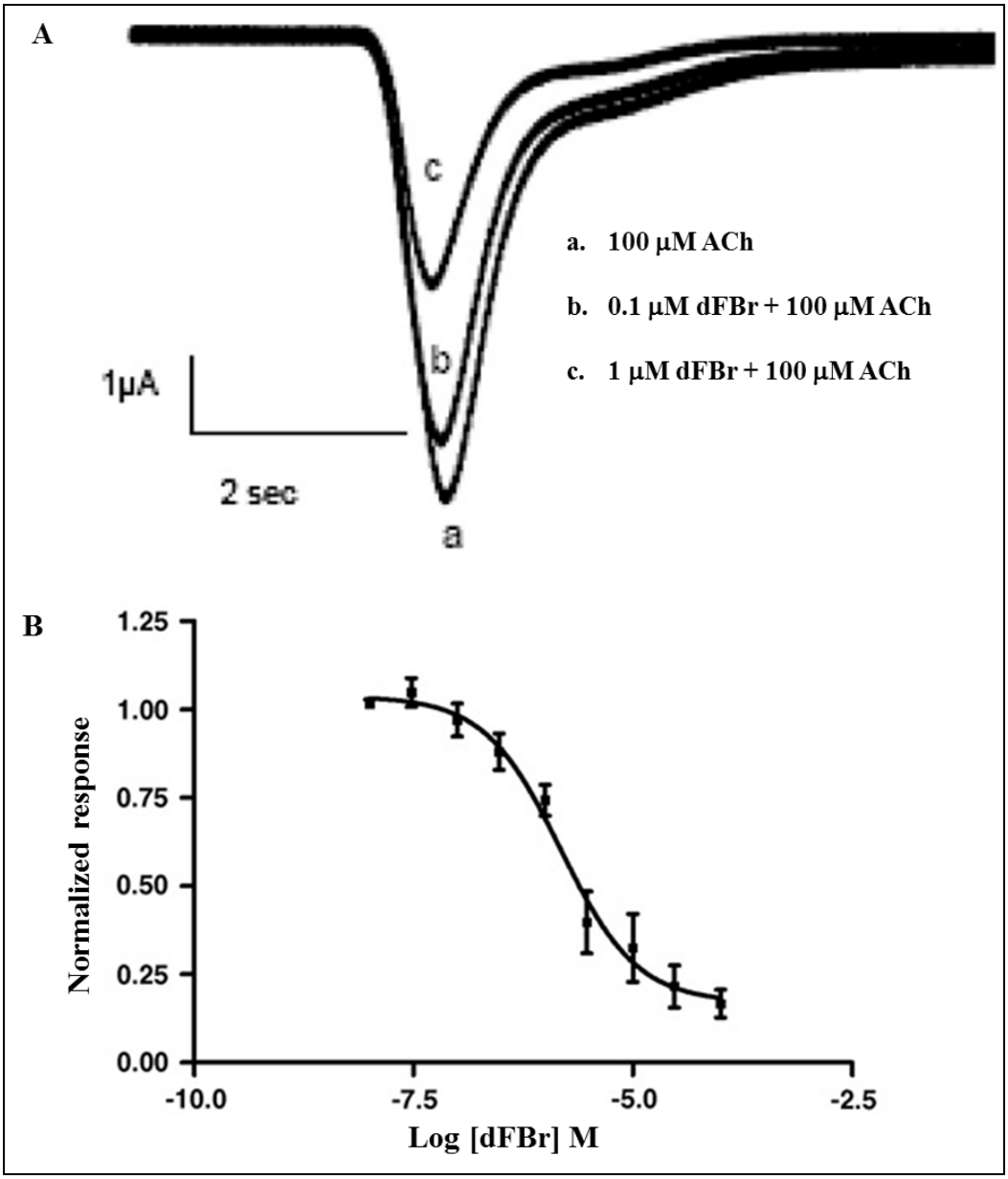


Figure 9. Potentiation and response curve for dFBr at α7 nACh receptors. (A) A typical trace of potentiation of the ACh-evoked response by dFBr (**32**) from a single oocyte expressing human α7 nACh receptors. (B) An inhibitory dose-response curve for dFBr (**32**) co-applied with 100 μM ACh on human α7 nACh receptors.[9]

Few, if any, allosteric ligands show selectivity for α4β2 nACh receptors. dFBr (**32**) is the first selective allosteric modulator that potentiates the actions of ACh at α4β2 nACh receptors. Unfortunately, dFBr (**32**), at high concentrations, will eventually behave (possibly as a channel blocking agent) to reverse its effects on the ACh receptor activation profile at α4β2 nACh receptors.[9] Thus, it would be important to determine what structural requirements are responsible for: a) allosteric activation of α4β2 nACh receptors, and b) antagonism. A study will be undertaken to understand structure-activity relationships (SAR) with dFBr (**32**) as a starting point.

To perform the SAR studies that might lead to identification of a possible pharmacophore for PAM action at the α4β2 nACh receptor subtype a 'deconstruction-reconstruction-elaboration' approach will be employed. This approach involves single structural changes being made to a compound and the subsequent evaluation of the new compounds for biological activity.[169] Thus, any change in activity can be associated with a single change in the structure of a molecule, and a rational SAR can be established. The process involves three phases to determine the necessity or contribution to activity of various structural components of the parent molecule. The first phase is the 'deconstruction' phase where the key structural features in the parent molecule are removed one at a time and the new compounds are biologically evaluated to measure the importance of that particular functional group. Also, this might identify the minimum structural features that are required to retain the activity of the parent compound, providing a pharmacophore for designing future analogs. The process of 'deconstruction' is also beneficial in identifying analogs that retain action and simultaneously provide a simpler synthetic procedure

which might ease the scale-up synthesis of a desired final ligand. Once a thorough understanding of the importance of various structural features of the parent molecule is established, the process can move to a 'reconstruction' phase where an analog with all essential structural features is synthesized and biologically evaluated to compare to the parent molecule. The third phase is the 'elaboration' phase where the important structural features are further elaborated to understand their role in producing the action. In this phase, principles of classical medicinal chemistry are applied and an SAR is developed where various substituent parameters, such as electronic, steric, and lipophilic can be studied. A rational approach using the Topliss decision-tree[170] or the Craig Plot[171] can be implemented to minimize the vast number of possible substituents that might be introduced, and the total number of compounds that will be required for synthesis and evaluation. The 'elaboration' phase generally involves lead optimization and allows an understanding of 'why' a particular substituent is important. It also might provide a drug-like molecule with novel structural features.

The specific aims of the current investigation are:

1. Elucidation of critical structural features of dFBr (**32**) required for potentiation of ACh at α4β2 nACh receptors.
2. Determination of what structural features of dFBr (**32**) contribute to the potentiation and inhibition profile of the biphasic dose-response curve of dFBr (**32**) (Figure **8**) which will eventually help better understand the true potentiation potential of dFBr (**32**) analogs at α4β2 nACh receptors.
3. Identification of structurally-simpler PAMs that might result in greater ease of synthesis for scale-up (e.g. for purpose of in vivo studies).
4. Synthesis of a radiolabeled ligand as a selective PAM at α4β2 nACh receptors that might facilitate binding experiments to determine binding profiles along with efficacy studies.

The general goal of this work is to prepare dFBr (**32**) analogs that can help provide an understanding of various structural features and their effects towards α4β2 nACh receptor action. Specifically, the compounds considered for synthesis and evaluation are shown in Figure **10**.

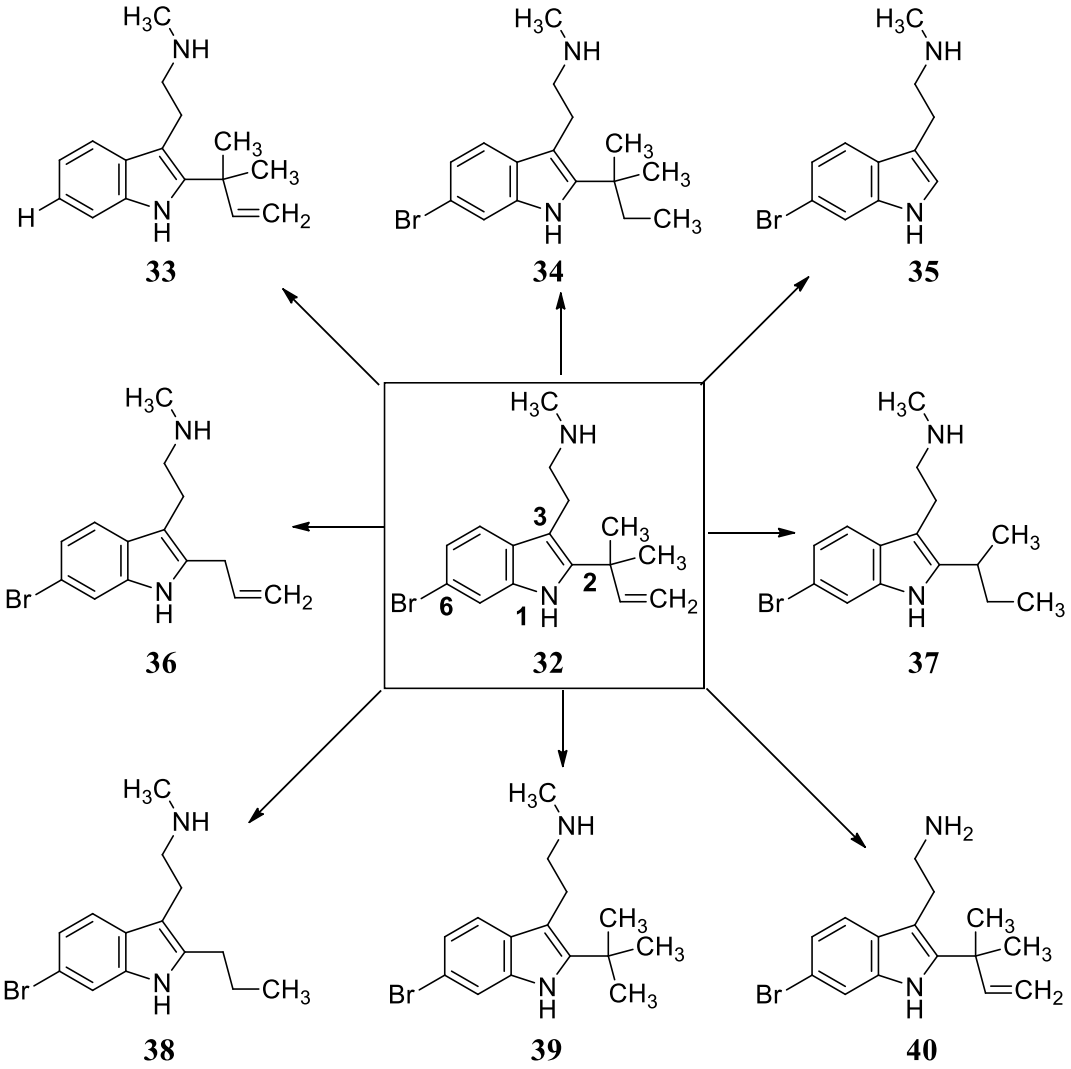


Figure 10. Deconstructed analogs of dFBr (**32**) proposed for synthesis and evaluation. For example, compound **33** can provide information on the necessity of the 6-bromo group of **32**, whereas **34** can provide information on the need for unsaturation. Each structure can provide important information on the contribution of specific substituents.

It might be noted that one of the first studies conducted in our laboratory showed that the 6-bromo substituent of dFBr was an important, but not necessary, contributor to activity (see

discussion to follow). Consequently, compounds **41**-**45** (Figure **11**) will be prepared and evaluated, to determine the contribution of the 2-position chain substituents to α4β2 PAM action. The reason for this is because of the difficulty in preparing 2-alkyl-6-bromotryptamines. The intent is to identify an optimal 2-position substituent, and to then prepare the 6-bromo analog of this compound (i.e., reconstruction).

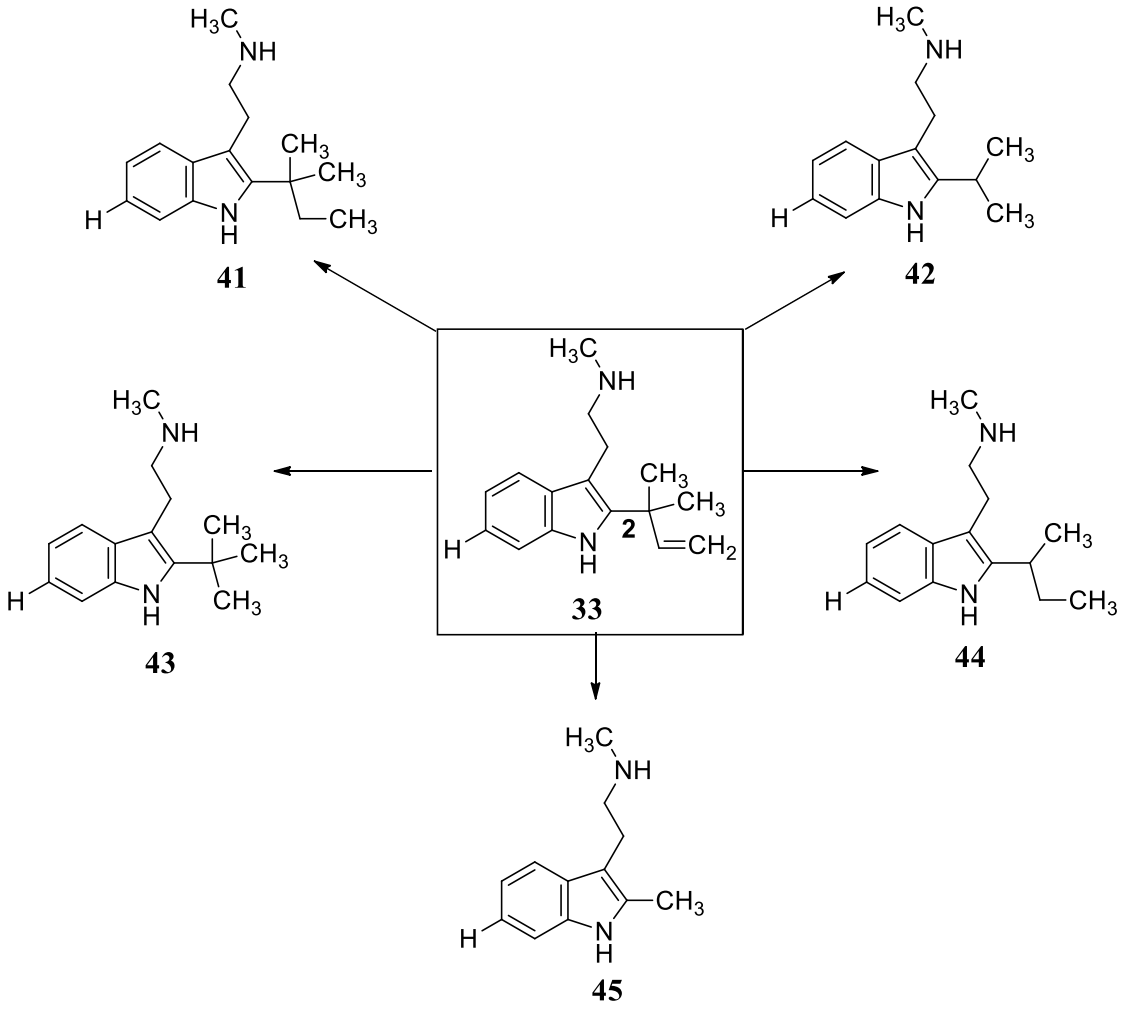


Figure 11. Deconstructed analogs of 6-*des*bromo dFBr (**33**) proposed for synthesis and evaluation.

IV. Results and discussion of deconstruction of dFBr

A. Drug-like properties of dFBr (32)

The drug-like properties of a compound are related to its structure which, in turn, is essential for absorption, distribution, metabolism and elimination (ADME) in humans. The physicochemical properties of a compound, such as its lipophilicity and solubility, might be related to various ADME properties. Medicinal chemists generally engage in the process of structure-activity relationship (SAR) and/or structure-property relationship (SPR) studies to increase the potency and selectivity towards a drug target, to reduce the undesirable effects, and to improve the drug-like properties of a compound. During SAR studies the molecular properties of the compound, such as molecular weight and logP, along with solubility and molecular volume, to name a few, are considered and a multi-dimensional optimization approach is carried out. Lipinski's rule of 5 is a widely accepted guide for understanding the drug's bioavailability, generally for an orally active drug. The rule states that an orally active drug should consist of: 1) no more than 5 hydrogen bond donors; 2) no more than 10 hydrogen bond acceptors; 3) molecular weight under 500 Daltons; and 4) a logP not greater than 5.[172] There are exceptions to these rules and various other parameters such as the polar surface area and number of rotatable bonds in a compound are considered to better predict the oral bioavailability of a drug.[173] Table **3** summarizes the various physical properties of dFBr (**32**) which were calculated using the OSIRIS property explorer (a

web-based molecule editor predicting various drug-relevant properties) and MOLSOFT molecular property calculator.

Table 3. Physicochemical properties of dFBr (**32**) versus ideal values

Physicochemical properties	Predicted values	Ideal values
logP	4.24	< 5
solubility	-4.36	< -4.0
Molecular weight (MW)	320	< 500
Hydrogen bond acceptors	0	< 10
Hydrogen bond donors	3	< 5
Drug likeness	-6.18	High positive value
Drug score	0.26	High positive value

According to the Lipinksi's rule of 5, the molecular weight, logP, hydrogen bond acceptor and hydrogen bond donor are within the requirements, thus dFBr (**32**) can qualify as a potential drug. The drug likeness and the drug score is determined by comparing various marketed drugs.[174] These scores also consider the various fragments in the compound that can be problematic and potentially toxic. A positive value for the drug likeness would mean that the fragments of the compound are what are generally present in commercial drugs. Thus, drug likeness, along with lipophilicity, molecular weight and potential toxicity, can determine the relative drug-like attributes of a compound. From Table **3** it can be observed that the drug likeness and the overall drug score of dFBr (**32**) is poor which suggest that dFBr (**32**) is not an ideal drug candidate. Use

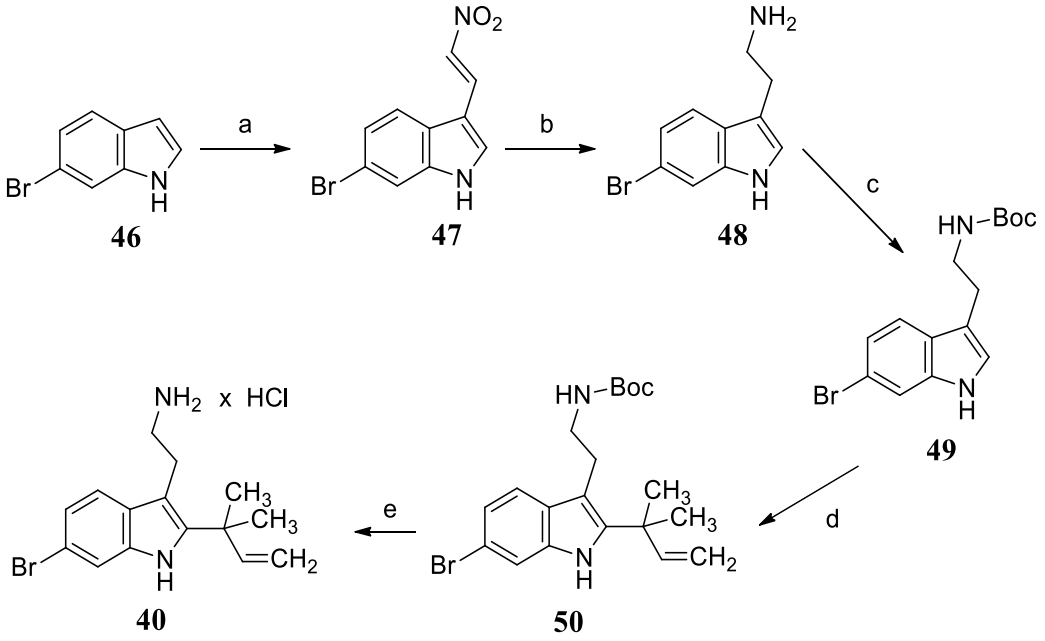
of such tools along with other computational models can help in performing SAR and/or SPR studies on dFBr which can help in determining the structural features which can improve the drug-like property of dFBr (**32**). For example, secondary amines are less lipophilic and more prone to metabolism than, for example, tertiary amine or α-alkyl amines. Hence, such factors should be investigated.

B. Chemistry

Analogs of dFBr (**32**) were synthesized in collaboration with others in our laboratory concurrent with the present investigation. For example the synthesis of compounds **33**-**36**, **38**, **41**, and **42** were synthesized by German et al.[175] and have now been reported. Compound **35** (Figure **10**) was a useful intermediate for analogs where 2-position substitution was introduced as a separate step. Compound **40** (Scheme **1**) was synthesized by performing a modified Henry reaction on an indole nucleus instead of the Speeter-Anthony glyoxylamide synthesis because the reaction and the yield of primary glyoxylamide was poor compared to the secondary glyoxylamide. 6-Bromoindole (**46**) (Scheme **1**) was allowed to react with 1-dimethylamino-2-nitroethylene in the presence of TFA to yield 3-[(*E*)-2-nitroethenyl]-6-bromo-1*H*-indole (**47**), which, upon reduction with $NaBH_4$ in the presence of a Lewis acid, yielded the known 6-bromotryptamine (**48**) in fairly good yield.[176,177] The tryptamine was used for a further reaction where it was protected with di-*tert*-butyl dicarbonate in the presence of Et_3N to yield *tert*-butyl-2-(6-bromo-1*H*-indol-3-yl)ethylcarbamate (**49**). The compound was known as a glassy solid,[178] but a melting point had not been reported. Because of the non-polar nature of the carbamate, it was relatively easy to purify by column chromatography. The Boc-protected tryptamine **49** was subjected to a

prenylation reaction to obtain the 2-substituted indole with freshly prepared prenyl 9-BBN[179] in the presence of *tert*-butyl hypochlorite and Et_3N, which yielded *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethylcarbamate (**50**). This was one of the only reactions that was able to provide the 2-dimethylallyl substitution in the presence of 3- and 6-substitution on the indole nucleus (see below for other routes). Deprotection, along with conversion to an HCl salt, yielded 6-bromo-2-(1,1-dimethylallyl)tryptamine hydrochloride (**40**).

Scheme 1. Synthesis of compound **40**[a]

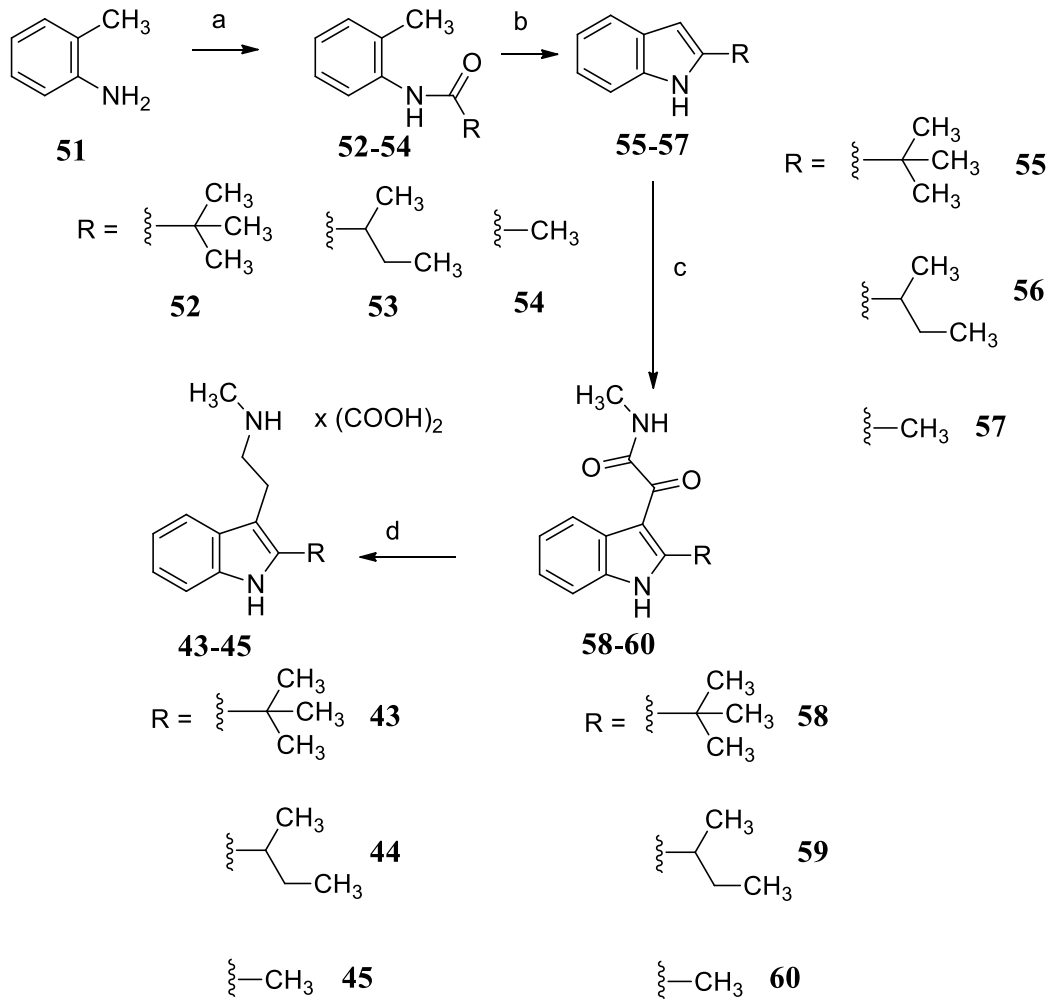


[a]Reagent: (a) 1-dimethylamino-2-nitroethylene, TFA, CH_2Cl_2, room temperature, 1 h; (b) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF, reflux, 2 h; (c) di-*tert*-butyl dicarbonate, Et_3N, DMF, room temperature, 24 h; (d) (1) *tert*-butyl hypochlorite, Et_3N, THF, -78 °C, 45 min; (2) prenyl-9-BBN, THF, room temperature, 2 h; (3) NaOH, H_2O_2, 1 h; (e) HCl, EtOAc, 0 °C, 24 h, recrystallized from MeOH/EtOAc.

Scheme **2** summarizes the various *des*-bromo analogs, **43**-**45**, that were synthesized. *o*-Toludine (**51**) was treated with pivaloyl chloride in the presence of base to yield the corresponding amide

52[180] which underwent cyclization in the presence of *n*-butyllithium to yield the known 2-*tert*-butyl-1*H*-indole (**55**).[181] This is a more convenient synthetic scheme for 2-substituted indoles though the drawback is that halogens are susceptible to lithium exchange so it cannot be used when a 6-bromo substituent is present. The Speeter-Anthony glyoxylamide reaction was performed on 2-*tert*-butyl-1*H*-indole (**55**), followed by $LiAlH_4$ reduction and salt formation, to yield *N*-methyl-2-*tert*-butyltryptamine oxalate (**43**). A similar synthetic route was followed with 2-methylbutanoyl chloride and acetyl chloride to yield **44** and **45**, respectively. The synthesis of compound **44** and **45** was reported by German et al.[175] (a postdoctoral fellow 2007-11 in Dr. Glennon's Lab).

Scheme 2. Synthesis of compounds **43-45**[a]

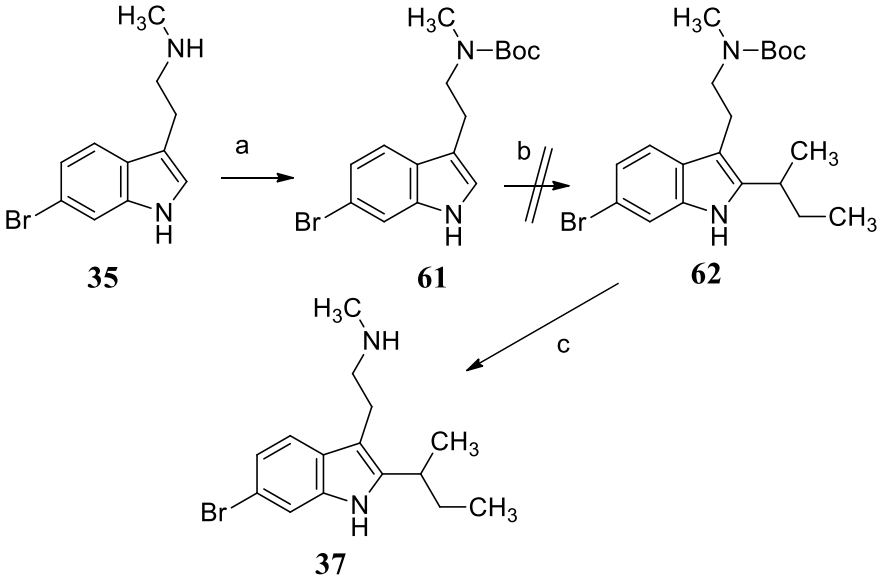


[a]Reagent: (a) trimethylacetyl chloride (for **43**)/2-methylbutanoyl chloride (for **44**)/acetyl chloride (for **45**), Et_3N, CH_2Cl_2; (b) *n*-butyllithium, THF; (c) (1) oxalyl chloride, Et_2O, 0 °C; (2) methylamine, room temperature, overnight; (d) $LiAlH_4$, THF, reflux; (e) oxalic acid, THF, Et_2O, recrystallized from *i*-PrOH.

Various synthetic routes were attempted for the synthesis of compounds **37** and **39**. It was relatively difficult to incorporate the 6-bromo and 2-alkyl substituents while synthesizing the indole scaffold. All trials involved routes that can provide 6-bromo analogs with varying 2-position alkyl substituents. Some of the attempts are shown in the following schemes (Scheme **3**-

7). The route shown in Scheme **3** was attempted based on the synthesis of 3-alkyl indoles.[180] We tried to use a *N*-protected tryptamine, **61**, reacting it with 2-iodobutane in the presence of zinc triflate, which assists in removal of the proton from the indole nucleus. No product (i.e., **62**) was formed suggesting that 2-position alkylation is not possible even with the 3-position being blocked.

Scheme 3. Attempt to achieve synthesis of **37** by alkylation of the *N*-protected tryptamine **61**[a]

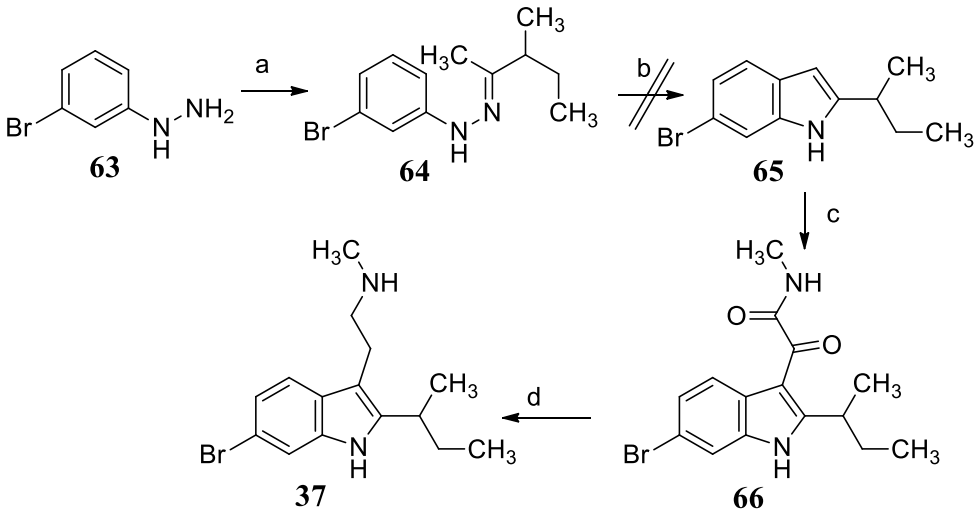


[a]Reagent: (a) di-*tert*-butyl dicarbonate, Et_3N, THF; (b) zinc triflate, TBAB, DIPEA, 2-iodobutane, toluene; (c) (1) oxalyl chloride, Et_2O; (2) methylamine, THF, H_2O; (3) $BH_3 \cdot S(CH_3)_2$, THF; (4) salt formation.

Scheme **4** represents a Fischer indole synthetic route for the synthesis of 2-substituted indoles. It is one of the oldest methods of indole synthesis and involves a rearrangement reaction of a Schiff's base to give 2-substitued indoles. This route, however, is not favorable for obtaining ring-substituted indoles as intra-molecular ring closure can affect the final position of the ring

substituents. Attempts to perform intra-molecular ring closure of intermediate **64** were not successful and only starting material was recovered. We expected some sort of cyclization to occur but, with the recovery of starting material, we are unable to offer any possible explanation.

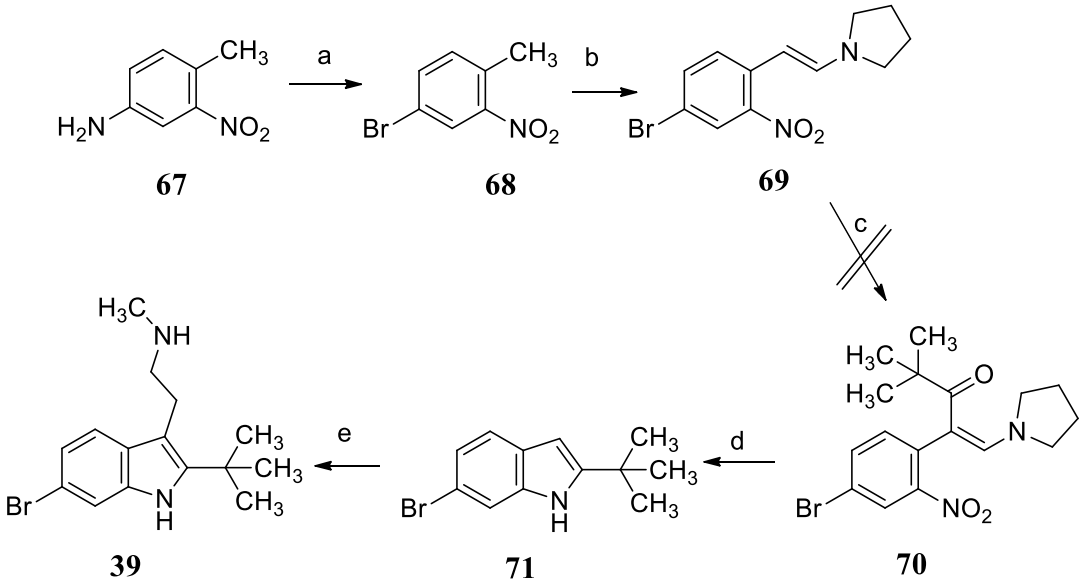
Scheme 4. Fischer indole syntheses for the attempted synthesis of compound **37**[a]



[a]Reagent: (a) 3-methylpentan-2-one, HOAc, MeOH; (b) 10% P_2O_5, CH_3SO_3H; (c) (1) oxalyl chloride, Et_2O; (2) methylamine, THF, H_2O; (d) $BH_3 \cdot S(CH_3)_2$, THF; (4) salt formation.

Another attempt to synthesize **39** was carried out by modifying the Leimgruber-Batcho indole synthesis as shown in Scheme **5**. In this route the 2-nitrostyrene intermediate **70** should undergo hydrolysis to yield the phenyl ketone intermediate which can further undergo cyclization by reduction with Zn/HOAc. To introduce the 2-position alkyl group of **39**, we subjected **69** to alkylation with pivaloyl chloride but the reaction was unsuccessful.[183] A possible reason for the failure to synthesize **70** could be that a bulky substituent cannot be attached to the *β*-styrene portion of **69**.

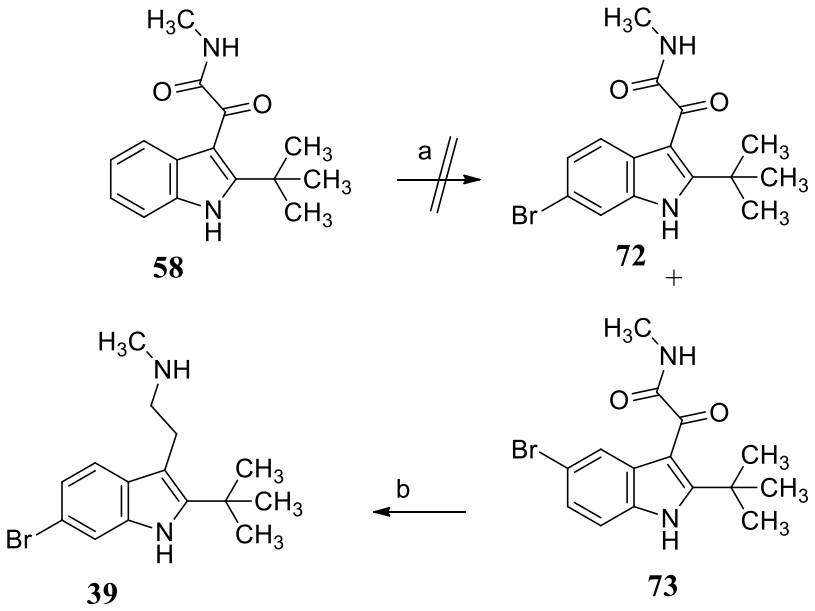
Scheme 5. Modified Leimgruber-Batcho indole syntheses for the attempted preparation of **39**[a]



[a]Reagent: (a) (1) $NaNO_2$, HBr; (2) CuBr, HBr; (b) DMF.DMA, pyrrolidine, DMF; (c) trimethylacetyl chloride, Et_3N; (d) (1) H_2O, reflux; (2) Zn, HOAc; (e) (1) oxalyl chloride, Et_2O; (2) methylamine, THF, H_2O; (3) $BH_3 \cdot S(CH_3)_2$, THF; (4) salt formation.

Direct bromination was attempted to achieve regioselective bromination of the indole ring. Burm et al.[178] reported the total synthesis of (±)-arborescidine, a marine alkaloid in which **61** (see Scheme **4**) was a key intermediate. Intermediate **61** was synthesized by direct bromination of the glyoxylamide, followed by reduction, *N*-protection and chromatographic separation of the 5- and 6-bromo mixture.[178] Scheme **6** summarizes the direct bromination route for the synthesis of **39**. Bromination of **58** resulted in a mixture of 5- and 6-bromo compounds (**73** and **72**, respectively). Separation techniques, including flash column chromatography and preparative TLC, failed to separate the two isomers. A possible explanation could be because the 2-postion alkyl substitution makes the compounds quite lipophilic, resulting in very little difference in polarity of the compounds, making chromatographic separation nearly impossible.

Scheme 6. Direct bromination route for the attempted synthesis of **39**[a]

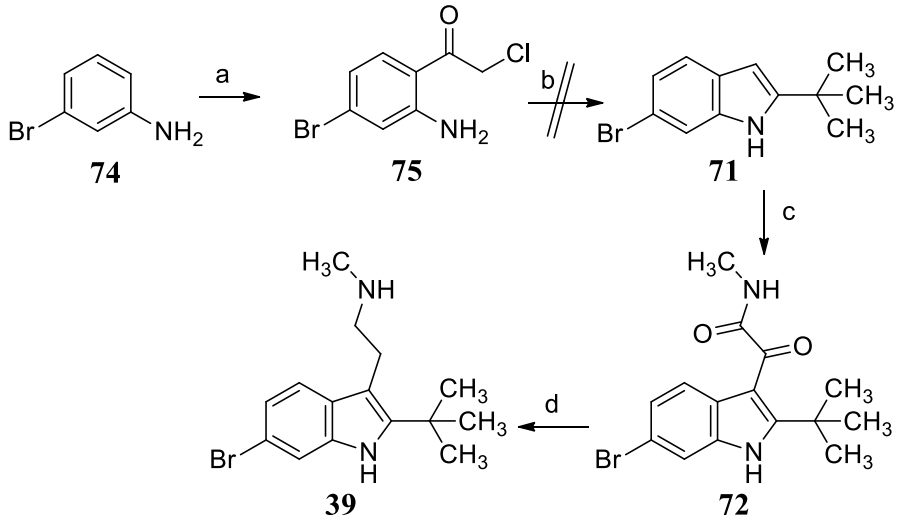


[a]Reagent: (a) Br_2, HOAc; (b) (1) oxalyl chloride, Et_2O; (2) methylamine, THF, H_2O; (3) $BH_3 \cdot S(CH_3)_2$, THF; (4) salt formation.

Scheme **7** shows an attempt to achieve the synthesis of **39** using a Grignard-based reaction for the cyclization. Pei at al.[184] successfully achieved a synthetic route for the synthesis of 2-substituted indoles with ring substitution by a [1,2]-aryl migration shift in α-chloroacetophenones. Intermediate **75** was synthesized using 3-bromoaniline (**74**) by reacting it with chloroacetonitrile in the presence of a Lewis acid, $AlCl_3$ and BCl_3. The substituted α-chloroacetophenone **75** was reacted with *tert*-butyl magnesium bromide to obtain 6-bromo-2-*tert*-butyl-1*H*-indole, but here the reaction yielded total degradation indicated by numerous spots upon the TLC analysis with consumption of the starting material **75**. The Grignard reagent route was successful in the synthesis of the 2-methylpropyl analog (**65**) (see Scheme **8**). This suggests

that the *tert*-butyl Grignard reagent might be unstable in the presence of the aromatic halogens, especially bromine, which is very reactive with the Grignard reagent. There was loss of starting material and no product (i.e., **71**) was formed; hence, finding modified routes for the synthesis of 6-bromo-2-alkyl indoles were explored.

Scheme 7. Grignard reagent route for the attempted synthesis of **39**[a]

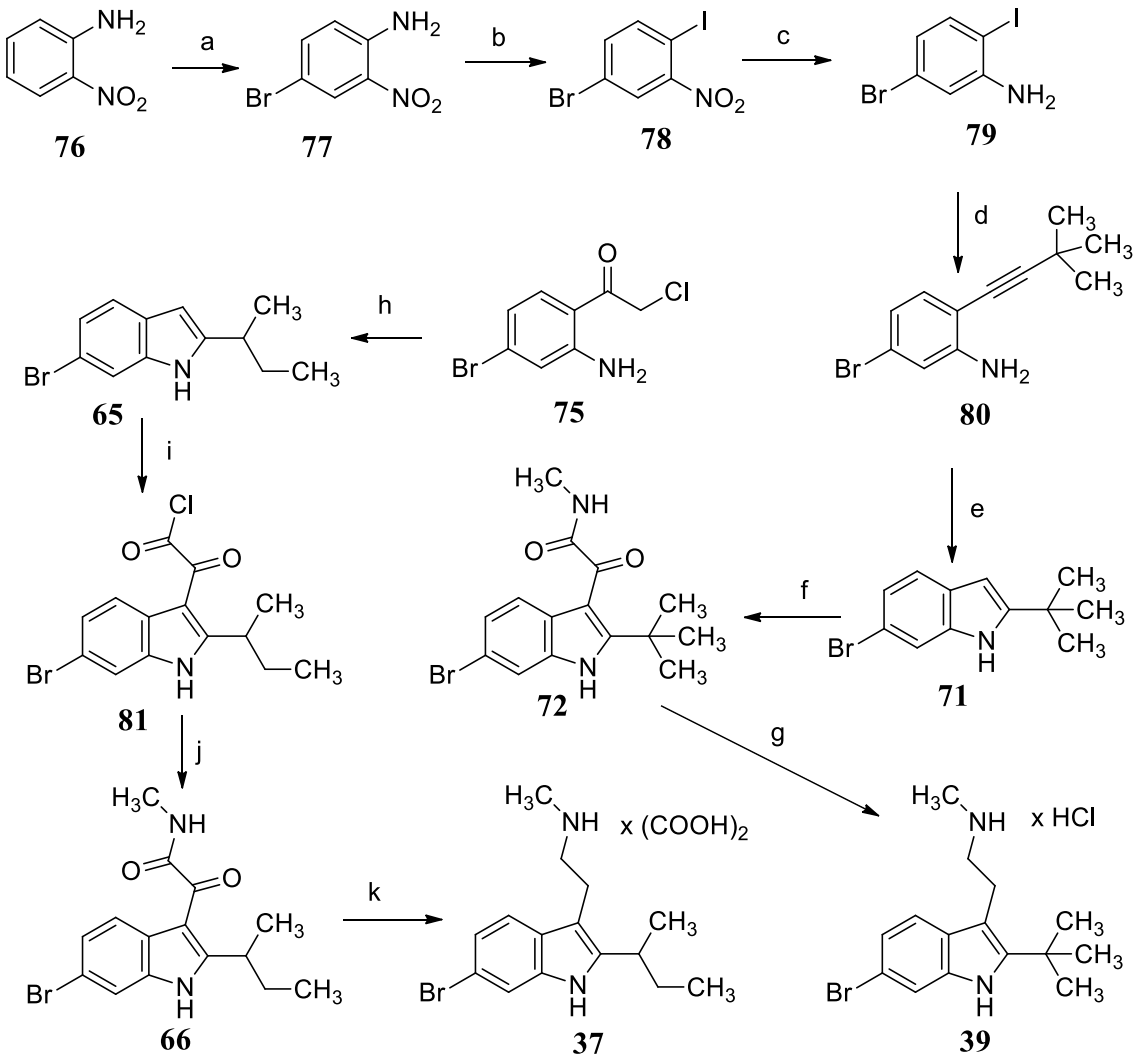


[a]Reagent: (a) chloroacetonitrile, $AlCl_3$, BCl_3, CH_2Cl_2; (b) *tert*-butyl magnesium bromide, Et_2O; (c)) (1) oxalyl chloride, Et_2O; (2) methylamine, THF, H_2O; (d) $BH_3{\cdot}S(CH_3)_2$, THF; (4) salt formation.

We were finally successful in achieving the synthesis of **37** and **39** through a relatively long, but simple, route as shown in Scheme **8**. This involved a Sonagashira coupling reaction to synthesize 6-bromo-2-subsituted analogs. Scheme **8** summarizes the synthesis of *N*-methyl-6-bromo-2-*tert*-butyltryptamine hydrochloride (**39**) and *N*-methyl-6-bromo-2-*sec*-butyltryptamine hydrochloride (**37**). Compound **37** was synthesized in collaboration with Dr. German.

2-Nitroaniline (**76**) was brominated with *N*-bromosuccinamide to yield 4-bromo-2-nitroaniline (**77**)[185] which, on diazotization and reaction with KI and I_2, yielded 4-bromo-2-nitroiodobenzene (**78**).[185,186] This was further reduced with Fe and HCl to yield 5-bromo-2-iodoaniline (**79**).[187] Sonagashira coupling in the presence of the corresponding alkyne with ligand-based palladium catalysts was performed on **79** to yield 5-bromo-2-(3,3-dimethylbut-1-yn-1-yl)aniline (**80**). The corresponding alkyne was treated with ZnI_2 to render cyclization to 6-bromo-2-*tert*-butyl-1*H*-indole (**71**). Various Lewis acids can be used to perform the cyclization reaction, such as $ZnBr_2$, $ZnCl_2$, CuI, CuBr and many others.[188] The synthesis of compound **37** was achieved *via* the Grignard reagent route where the corresponding indole was reacted with oxalyl chloride and methylamine via a Speeter-Anthony glyoxylamide reaction to yield *N*-methyl-6-bromo-2-*tert*-butyl-1*H*-indol-3-glyoxylamide (**72**) and *N*-methyl-6-bromo-2-*sec*-butyl-1*H*-indol-3-yl-glyoxylamide (**66**), which followed by reduction and formation of an HCl salt, yielded *N*-methyl-6-bromo-2-*tert*-butyltryptamine hydrochloride (**39**) and *N*-methyl-6-bromo-2-*sec*-butyltryptamine hydrochloride (**37**). The above routes have provided a versatile method for the synthesis of various 2-substituted indoles in the presence of a halogen in the indole nucleus.

Scheme 8. Synthesis of compounds **37** and **39**[a]



[a]Reagents: (a) *N*-bromosuccinamide, EtOH, 40-50 °C, 5 h; (b) (1) $BF_3 \cdot Et_2O$, *t*-BuONO, THF, -30 °C to room temperature, 1 h; (2) KI, I_2, CH_3CN, room temperature, 1 h; (3) $Na_2S_2O_3$; (c) Fe, HOAc, EtOH, reflux, 1.5 h; (d) 3,3-dimethylbut-1-yne, CuI, $PdCl_2(PPh_3)_2$, Et_3N, THF, room temperature, 5 h; (e) ZnI_2, toluene, reflux, 41 h; (f) (1) oxalyl chloride, Et_2O, reflux, overnight; (2) methylamine, THF, H_2O, room temperature, 15 h; (g) (1) $BH_3 \cdot S(CH_3)_2$, THF, reflux, 6 h; (2) HCl, Et_2O, 0 °C, recrystallized from *i*-PrOH; (h) chloroacetonitrile, $AlCl_3$, BCl_3, CH_2Cl_2, 12 h reflux; (i) oxalyl chloride, Et_2O, -5 °C, 30 min; (j) methylamine, THF, H_2O, room temperature, overnight; (k) $BH_3 \cdot S(CH_3)_2$, THF, reflux, overnight; (2) oxalic acid, $CHCl_3$, 0 °C, 1.5 h, recrystallized from *i*-PrOH.

Compound **82**, which is the *des*-bromo analog of **40**, was also synthesized to compare the effect of the 6-bromo substituent with/without the *N*-methyl substituent. Compounds in Figure **12** will provide further evidence towards the importance of the 6-bromo and the *N*-methyl substituent of dFBr (**32**).

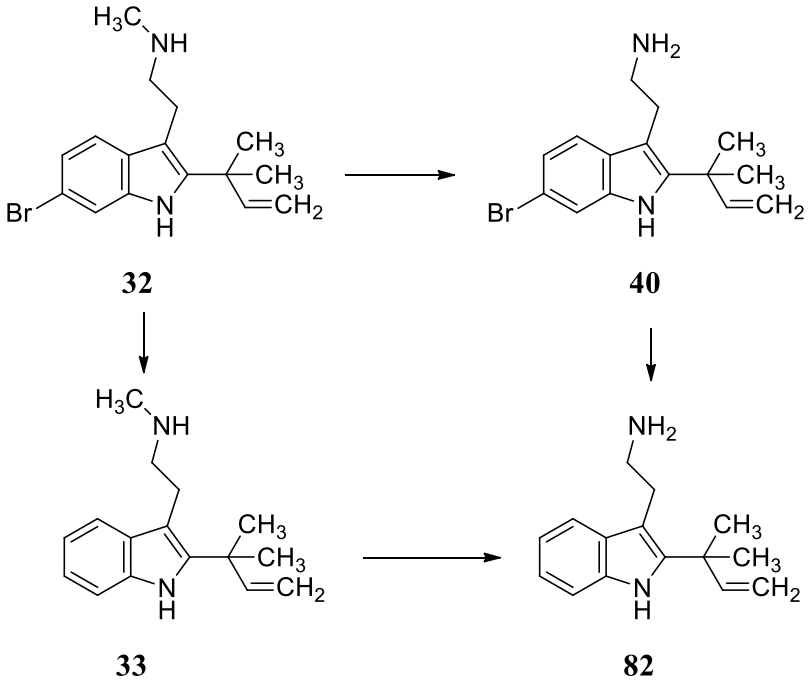
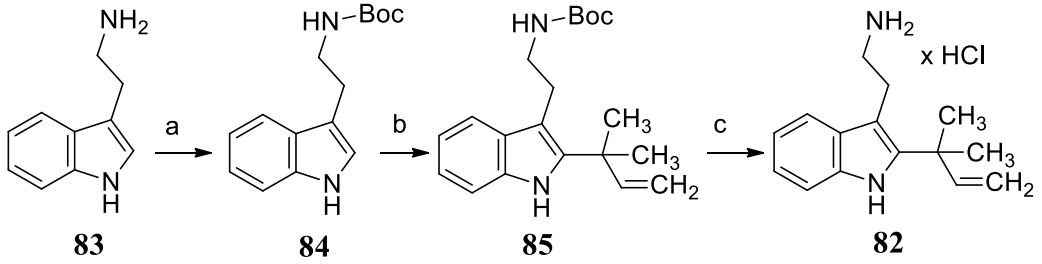


Figure 12. Deconstruction of dFBr (**32**) at the 6-bromo and the *N*-methyl substituents.

Scheme **9** summarizes the synthesis of compound **82**. Compound **82** was synthesized by a relatively simple route from commercially available tryptamine (**83**) which was protected to obtain the Boc-protected tryptamine (**84**).[189] Compound **84** was prenylated to yield *tert*-butyl-2-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethylcarbamate (**85**), that, on deprotection, yielded 2-(1,1-dimethylallyl)tryptamine hydrochloride (**82**) in fairly good yield.

Scheme 9. Synthesis of compound **82**[a]



[a]Reagents: (a) di-*tert*-butyl dicarbonate, Et_3N, DMF, room temperature, 20 h; (b) (1) *tert*-butyl hypochlorite, Et_3N, THF, -78 °C, 45 min; (2) prenyl-9-BBN, THF, room temperature, 3 h; (3) NaOH, H_2O_2, 1 h; (c) HCl, EtOAc, 0 °C, 24 h, recrystallized from *i*-PrOH/Et_2O.

C. Pharmacology

Biological activities of the synthesized compounds were evaluated using two-electrode voltage clamp techniques employing *Xenopus laevis* oocytes with expressed human α4β2 nACh receptors in the absence or presence of 100 μM ACh. A typical dose-response curve for the compounds tested on human α4β2 nACh receptors shows potentiation at lower concentrations and inhibitory activation at higher concentrations, but no effect in the absence of ACh. Table **3** provides the biological results of the potentiation or antagonism (i.e., inhibition) of the action of ACh action at human α4β2 nACh receptors by dFBr (**32**) and its analogs.

Table 4. Effect of dFBr (**32**) and its deconstructed analogs on the action (potentiation or antagonism) of ACh action at the human α4β2 nACh receptors.

Compound	Potentiation	Inhibition	Half maximum potentiation concentration (EC_{50}) (μM) (± SEM)	Half maximum inhibition concentration (IC_{50}) (μM) (± SEM)
32 (dFBr)	+	+	0.33 ± 0.52	150 ± 0.20
33	+	+	7.59 ± 3.50	12.6 ± 3.20
34	+	+	0.14 ± 0.69	NI[a]
35	-	+	NP[b]	39.8 ± 0.87
36	-	+	NP	3.55 ± 0.83
37	+	+	60. 5 (0.6-120)♦	40.7 (0.1-81)♦
38	-	+	NP	72.4 ± 0.79
39			DA[c]	DA
40	+	+	0.51 ± 0.58	NI
41	+	+	3.02 ± 0.09	NI
42	-	-	NP	ND[d]
43	+	+	3.98 ± 0.03	NI
44	-	+	NP	16.6 ± 0.28
45			DA	DA
82			DA	DA

[a]NI = Inhibition potency of the action of ACh could not be reliably determined. [b]NP = No potentiation of the action of ACh. [c]DA = Data unavailable. [d]ND = Not determined. ♦ = 95% confidence limits (CLs). *n* = 4. In some cases, data were provided as ± SEM; in others, data were provided to us with 95% CLs.

D. Discussion

The EC_{50} value obtained for dFBr (**32**) (0.33 μM) (Table **4**) in the current investigation is comparable to a previous report (EC_{50} = 0.12 μM) from our laboratory, and the small difference might be the result of the large number of determinations carried out with **32** and experimental variation. Compounds **33-40** represent the first deconstructed analogs of dFBr (**32**) (Table **4**; Figure **10**). Comparing dFBr (**32**) with its *des*-bromo analog **33** (EC_{50} = 7.59 μM), it is apparent that the 6-bromo substituent makes a substantial contribution to activity. Although removal of the 6-bromo substituent decreased potency by almost 20-fold, compound **33** is still active as a PAM at α4β2 nACh receptors. Thus, the 6-bromo substituent is optimal for potency but is not required for the PAM action of dFBr (**32**). Removal of the 6-bromo substituent improved the inhibitory action (IC_{50} = 12.6 μM). It is notable that the removal of the 6-bromo group decreased the concentration range for the potentiation and the inhibition action at α4β2 nACh receptors (Table **4**, 0.3 to 150 μM for dFBr *vs* 7.6 to 12.6 μM for **33**, respectively).

Next, the importance of the 2-position side chain of dFBr (**32**) was examined. Compounds **34**, **35**, **36**, and **38** were the initial analogs synthesized in our laboratory. Comparing dFBr (**32**) with **34** (EC_{50} = 0.14 μM), it is evident that chain unsaturation is not required for activity. It was found, however, that complete removal of the 2-position chain of dFBr (**32**), affording **35**, resulted in no potentiation of the action of ACh. Compound **35** was, nevertheless, able to produce an inhibitory action at high concentrations (IC_{50} = 39.8 μM). Evidently the 2-position substituent, or a portion thereof, is required for the potentiation of ACh action by dFBr at α4β2 nACh receptors. Because compound **34** was twice as potent as its parent, dFBr (**32**), unsaturation of the side chain is not required for the action of ACh. Compound **36**, retaining the propenyl side

chain was unable to potentiate the action of ACh, but produced relatively potent inhibition (IC_{50} = 3.55 μM). This indicates that at least one of the *gem*-dimethyl groups of dFBr (**32**), and not the unsaturation of the side chain, is important for the potentiating effect of the parent molecule, **32**, as a PAM. Again, supportive of the notion that at least one of the *gem*-dimethyl groups is required for PAM activity, compound **38** lacked this action. Compound **37**, the monomethyl counterpart of **34**, is more than 400-fold less potent than **34** as a PAM. Hence, the *gem*-dimethyl groups seem to play a major role in PAM action.

As in the case of compound **34**, which is twice as potent as dFBr (**32**), compound **38**, although an inhibitor, was almost 24-fold less potent compared to **36** in this regard suggesting a different mode of binding for the potentiation and antagonism at α4β2 nACh receptors.

The *N-des*-methyl analog **40** (EC_{50} = 0.51 μM) was half as potent as dFBr (**32**) (Table **4**) as a PAM showing that the secondary amine of dFBr (**32**) is preferred over the primary amine of **40**. Comparing compounds **32**, **33**, **40** and **82** it can be predicted that compound **82**, which lacks the 6-bromo and the *N*-methyl substituent, will be less potent than **33** and **40** although data have not yet been obtained for compound **82**. It would appear that some steric bulk is tolerated at the terminal amine. Thus, most of the deconstructed analogs of dFBr (**32**) to be examined henceforth will retain the *N*-methyl group as seen in **32** (Figure **13**).

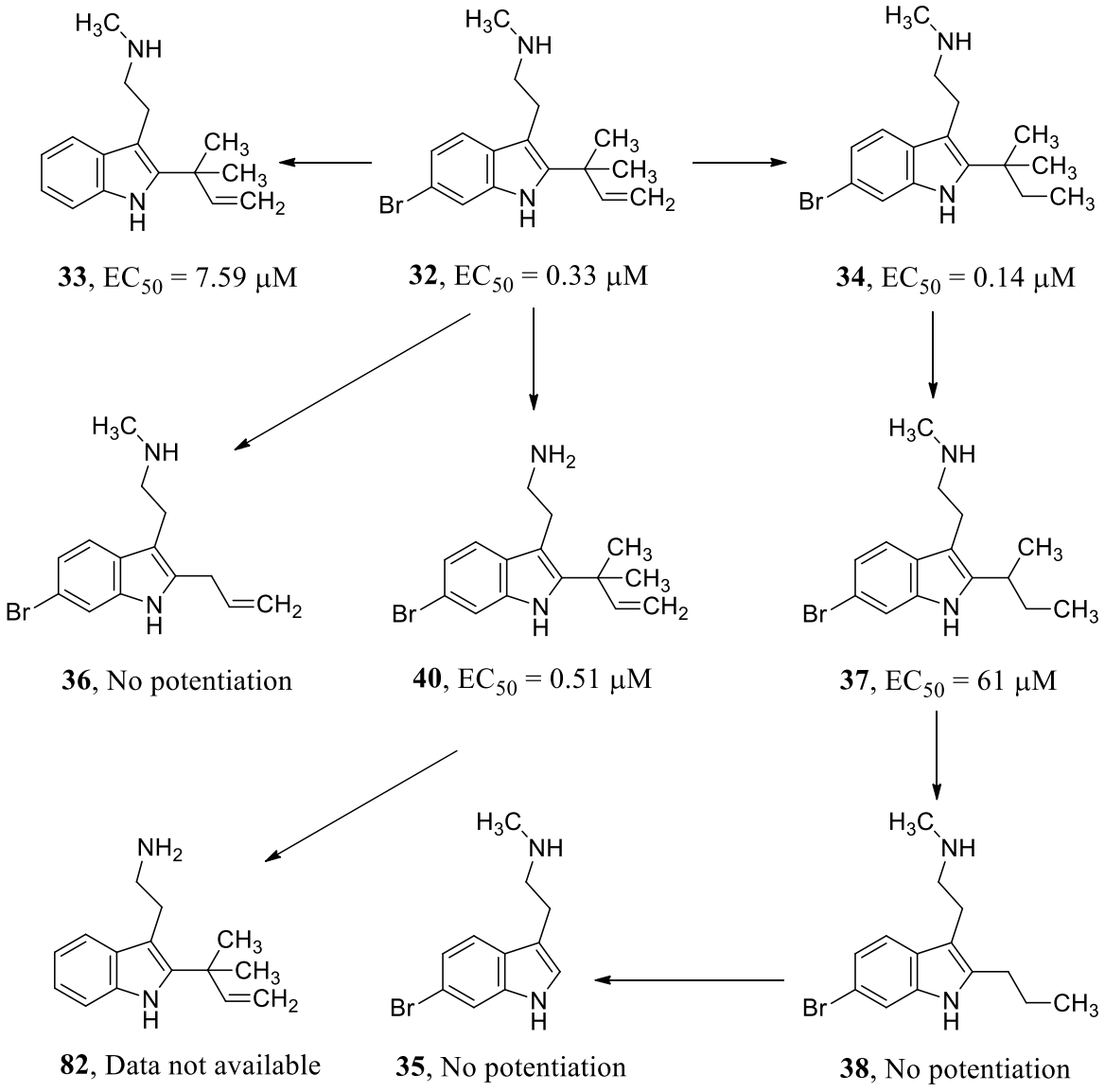
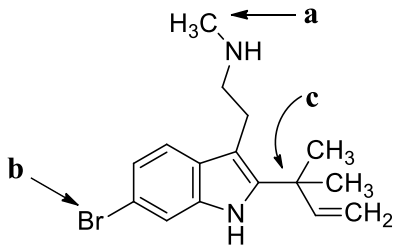


Figure 13. Initial deconstruction of dFBr (**32**). Each arrow symbolizes a single structural change. Data are from Table **4**. Certain analogs did not potentiate the effect of ACh at α4β2 ACh receptors at their highest concentration evaluated (i.e., 100 μM).

Reconstruction. Up to this point, several structural features were found optimal for PAM action: a) an *N*-methyl amine, b) a 6-bromo substituent, and c) a 2-position substituent consisting of a quaternary carbon (Figure**14**).



dFBr (**32**)

Figure 14. Deconstruction of dFBr (**32**) revealing the structural requirement for action. a) A secondary amine is optimal, b), a 6-bromo substituent is required for potency but not action, and c) a 2-position quaternary carbon is required for activity as a PAM at α4β2 nACh receptors.

The next goal was a more detailed examination of 2-position substituents. Synthetically, to incorporate 2-position substituents with a 6-bromo group, is a challenge. Also, the *des*-bromo derivative **33** was active; thus, a relatively simpler route for the synthesis of various analogs of dFBr (**32**) which will help understand what portion of the 2-position substituent is required for the potentiation action of ACh was undertaken. The intent was to optimize the 2-position substituent for a 6-*des*bromo analog, and then to add a bromo group to the optimized compound(s). Compound **33** was further deconstructed to compounds **41**-**45** (Figure **15**; Table **4**) of which only compound **41** and **43** showed potentiation of the activity of ACh (**41**; EC_{50} = 3.02 μM and **43**; EC_{50} = 3.98 μM). Comparing compounds **32** and **33** with **34** and **41**, respectively, it was observed that there is a similar trend where the saturated analogs, **34** and **41**, are twice as potent as the parent molecules, **32** and **33**, respectively. This information allows us to avoid the

unsaturation of dFBr which can prove to be a synthetic advantage for synthesizing simpler analogs. Compounds **42**-**44** (Table **4**) provided information about the *gem*-dimethyl system of the 2-position substituent of dFBr (**32**). It was observed that the *gem*-dimethyl groups are important for the potentiation property of dFBr (**32**). Compound **44**, which has a 2-methylpropyl side chain, was inactive as a PAM, but showed antagonist properties (IC_{50} = 16.6 μM). Comparing compounds **42**, having a 2-*iso*-propyl side chain, and **43** having a 2-*tert*-butyl side chain, it was seen that, although both the analogs possess a *gem*-dimethyl group, the presence of a quaternary carbon at the 2-position in **43** is what provides the potentiation property as a PAM at α4β2 nACh receptors. In simple terms it can be concluded that, for potentiation, the presence of a 2-position quaternary carbon atom is required whereas for the inhibitory/antagonistic effect, a propyl or a mono-substituted propyl substituent is tolerated (Figure **15**).

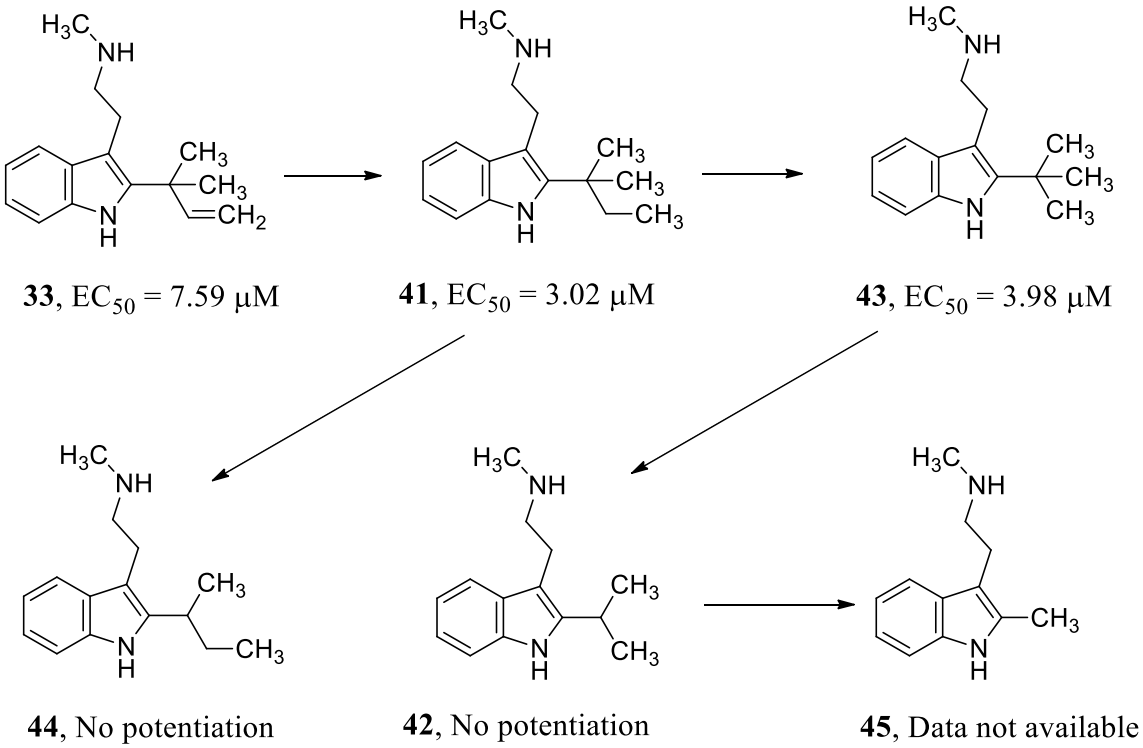
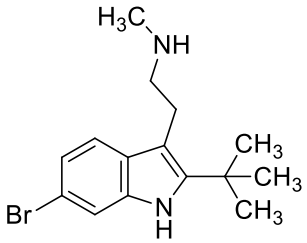


Figure 15. Deconstruction of **33** to determine the role of the 2-position substituent on the PAM action of ACh at nACh receptors. Data are from Table **4**.

In the reconstruction process, we synthesized compound **39** which bears all the regional features shown in Figure **14**. Although it was expected that the 2-*tert*-butyl compound **39** would be more potent than its *des*-bromo parent **43**, data have not yet been obtained for this compound.



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V. Results and discussion of elaboration of dFBr

The elaboration step sought answers to several questions such as, for example: a) is a tertiary amine tolerated, b) is a quaternary amine tolerated (note: ACh is a quaternary amine) (Figure **16**), c) is the indolic N-H a contributor to action (Figure **16**), d) what is the specific role of the 6-bromo group, e) is the position of attachment of the bromo group important for activity (Figure **17**)? That is, the elaboration step introduced substituents not found in dFBr (**32**).

A. Chemistry

Several analogs of dFBr (**32**) were synthesized in collaboration with others in our laboratory concurrent with the present investigation. For example the synthesis of compounds **97**-**100** were synthesized in collaboration with Dr. German (a postdoctoral fellow in Dr. Glennon's lab). Scheme **10** summarizes the synthesis of **86** and **87**. Compound **40** was used as the starting material which was converted to its free base and subjected to Eschweiler-Clarke reaction with formic acid and formaldehyde to yield *N*,*N*-dimethyl-6-bromo-2-(1,1-dimethylallyl)tryptamine (**101**). The latter was converted to the oxalate salt to yield **86**. Compound **101** was further methylated with iodomethane to yield *N*,*N*,*N*-trimethyl-6-bromo-2-(1,1-dimethylallyl)tryptamine methiodide (**87**) (Scheme **10**). Direct quaternization of **40** resulted in the reaction not going to completion and yielded a mixture of mono and di-methylated side products under varying

conditions; from room temperature to reflux and varying equivalence of iodomethane; 3 to 10. Thus, a 2-step approach was adopted for the synthesis of **87**.

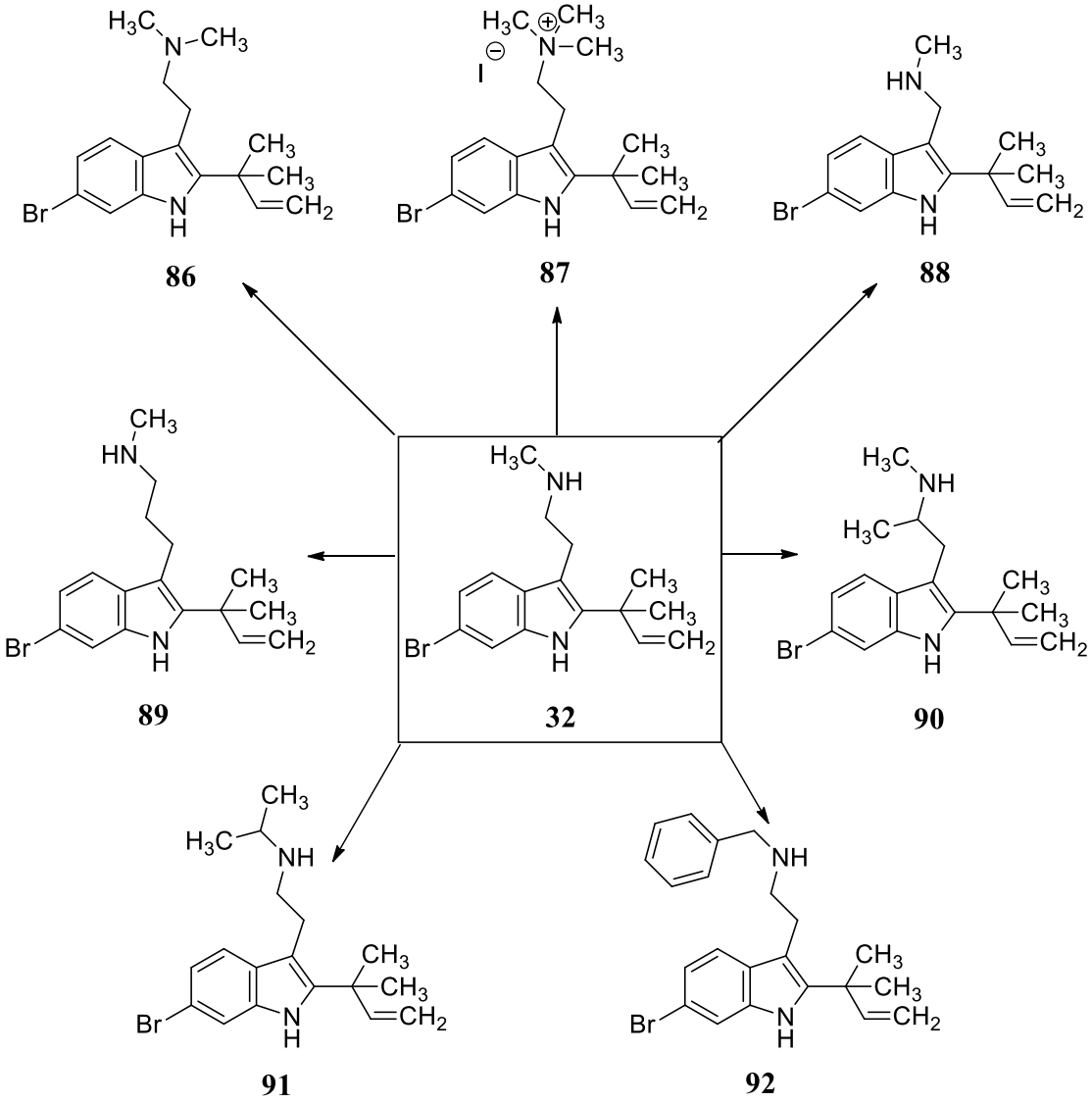


Figure 16. Elaborated analogs of dFBr (**32**) proposed for the synthesis and evaluation. For example compound **86**, **87**, **91** and **92** can provide information on the steric bulk tolerated on the amine, whereas compound **88** and **89** can provide information on the chain length while

compound **90** can provide information about the α-substitution on the tryptamine side chain required for PAM action at the α4β2 nACh receptors.

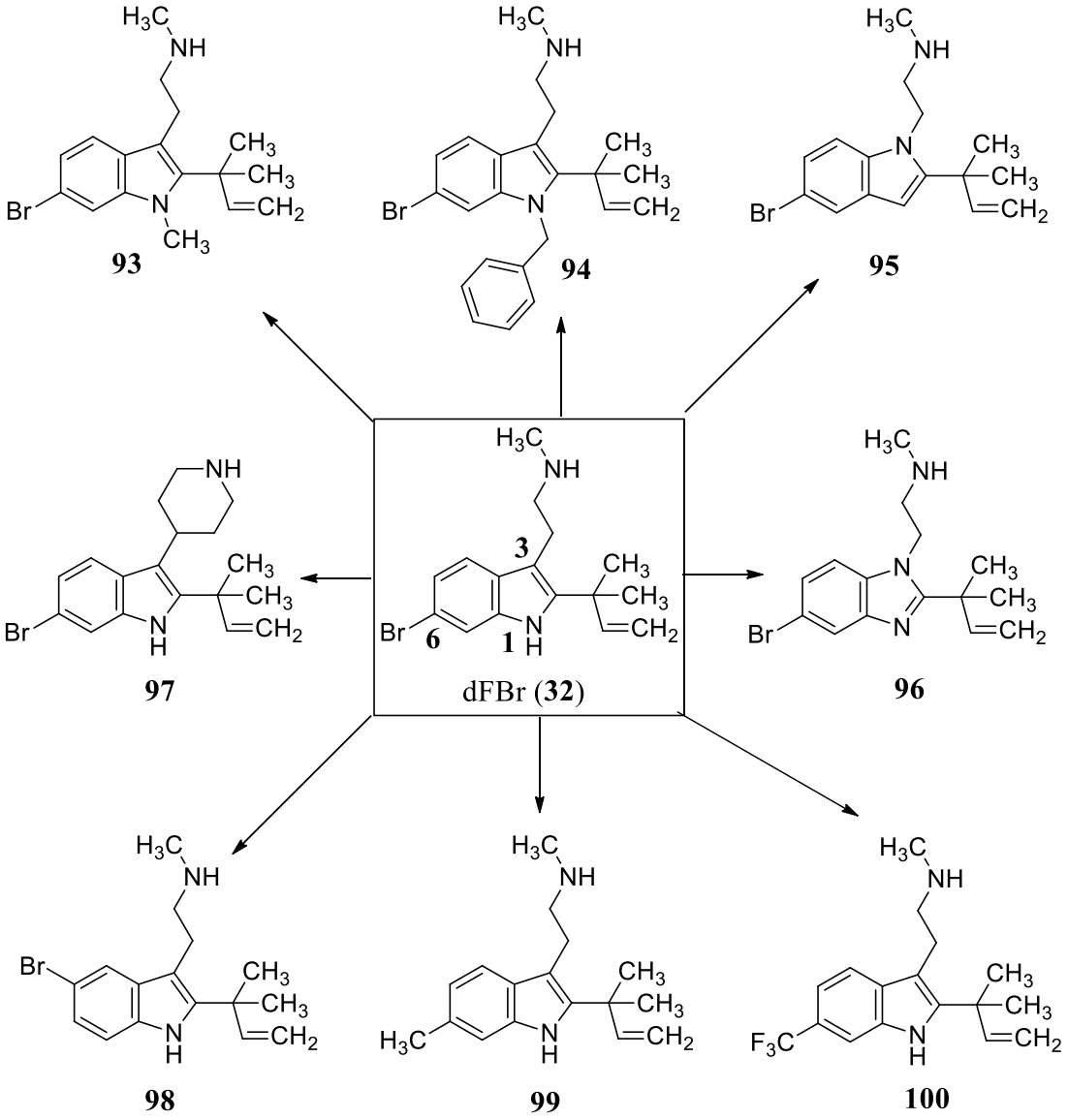
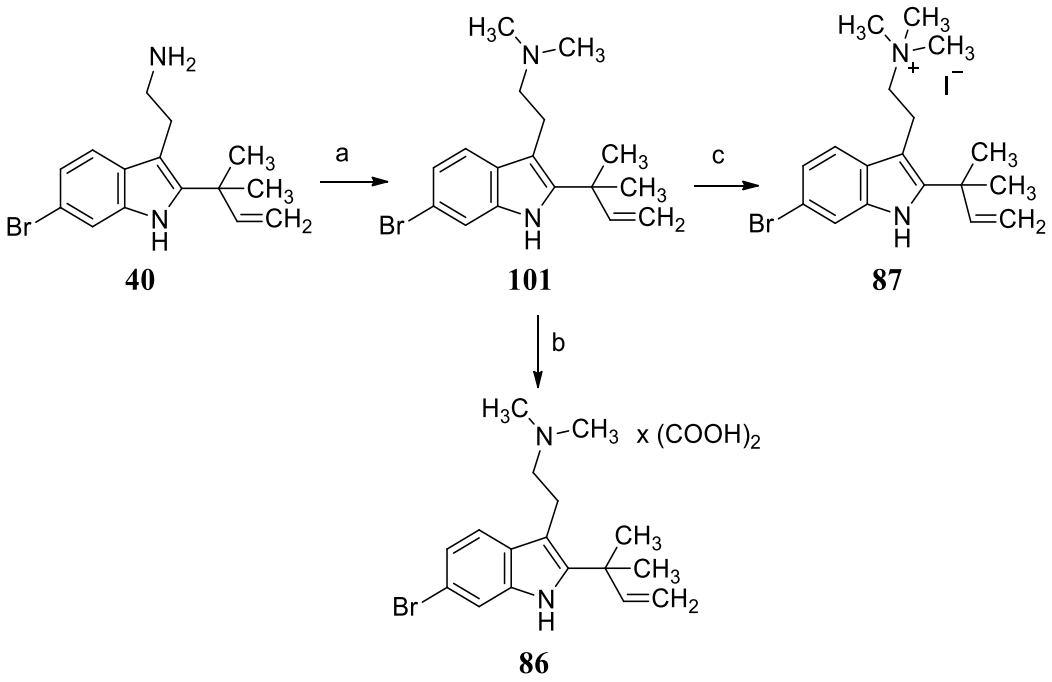


Figure 17. Elaborated analogs of dFBr (**32**) proposed for the synthesis and evaluation. For example, compounds **93-96** can provide information on the importance of indolic N-H, whereas compound **98** can provide information on the position of the bromo substituent and its

importance towards the activity. Compounds **99** and **100** on the other hand can provide information about the specific role of the 6-bromo substituent required for PAM action at the α4β2 nACh receptors.

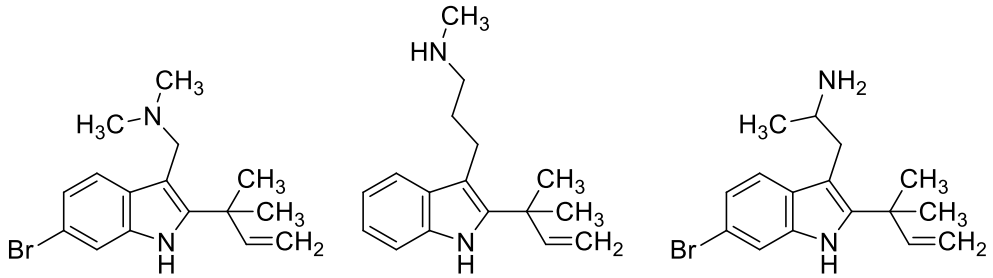
Scheme 10. Synthesis of compounds **86** and **87**[a]



[a]Reagents: (a) Formic acid, formaldehyde (37% v/v), H_2O, reflux, 3 h; (b) Oxalic acid, $CHCl_3$, 0 °C, 1.5 h, recrystallized from *i*-PrOH/Et_2O; (c) iodomethane, *i*-PrOH, 0 °C to room temperature, 22 h, recrystallized from MeOH/*i*-PrOH.

Synthesis of the homotryptamine analog (i.e., **89**) and the 3-position *des*-methyl gramine side chain analog (i.e., **88**) (Figure **16**) was attempted. The instability of the *des*-methyl gramine analog **88** was a major issue, the product could not be isolated and, thus, the compound was not pursued any further. It was found that compound **86** (Scheme **10**) was active (see discussion below) and thus, to see the effect of shortening of the tryptamine chain, compound **102** was

synthesized. Gramines (indole-3-yl-*N*,*N*-dimethylmethylamines) are stable and can be synthesized to obtain substitution on the indole nucleus. Similarly, to understand the role of extension of the tryptamine side chain, compound **103** was considered, which is a *des*-bromo analog of **89**. This particular compound was considered based on the knowledge that the 6-bromo substituent in dFBr (**32**) is required for potency, but not PAM action. Compound **104** was considered instead of **90** (Figure **16**) based on the data that the primary amine (**40**) is only half as potent as dFBr (**32**) (see Table **3**) and also because of the ease of synthesis of **104** compared to **90**.

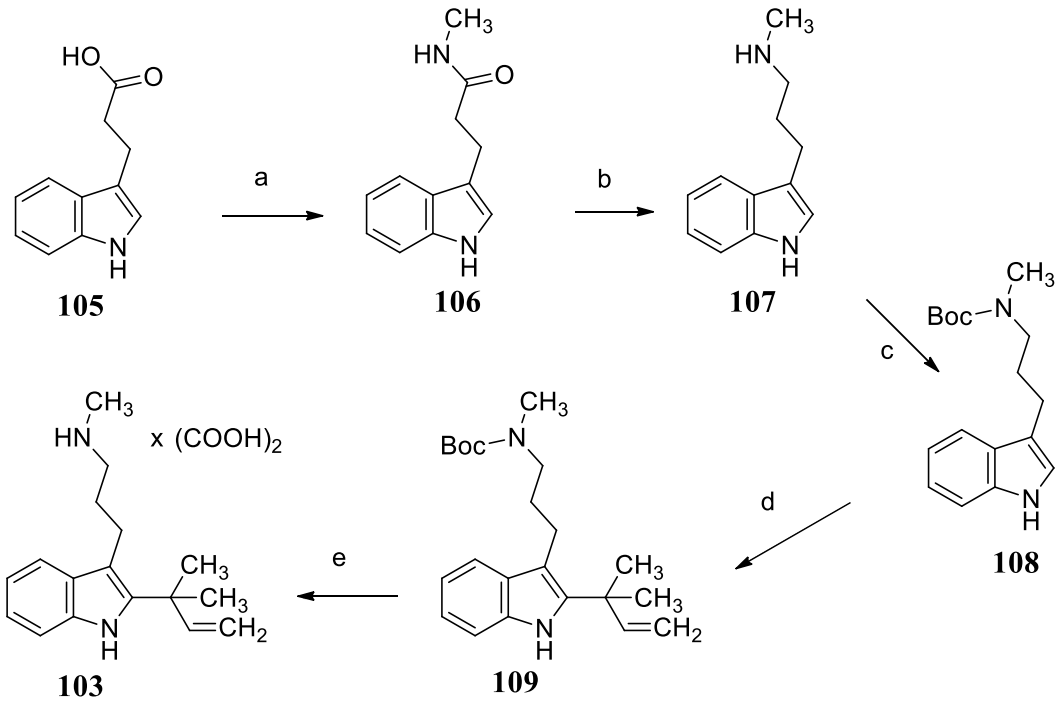


102 **103** **104**

Compound **102** was synthesized by Dr. German. Scheme **11** summarizes the synthesis of compound **103**. Indole-3-propionic acid (**105**) was treated with methyl chloroformate and then with methylamine hydrochloride to yield the known *N*-methyl-3-(1*H*-indol-3-yl)propanamide (**106**)[190] which was further reduced with $LiAlH_4$ to yield *N*-methyl-3-(1*H*-indol-3-yl)propanamine (**107**).[191] The amine was protected by di-*tert*-butyl dicarbonate in the presence of Et_3N to yield *tert*-butyl-3-(indol-3-yl)propyl-*N*-methylcarbamate (**108**). Upon further treatment of **107** with freshly prepared prenyl-9-BBN in the presence of *tert*-butyl hypochlorite and Et_3N, the reaction yielded *tert*-butyl-3-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-*N*-methylcarbamate (**109**), which was deprotected and converted to an oxalate salt to yield *N*-methyl-3-(2-(1,1-

dimethylallyl)-indol-1*H*-yl)propanamine oxalate (**103**). Although homogeneous in thin-layer chromatographic analysis (R_f = 0.4 in CH_2Cl_2/MeOH/NH_3 10:1:01), elemental analysis for **103** was not within the permissible range of 0.4% (Anal. Calcd for ($C_{17}H_{24}N_2 \cdot C_2H_2O_4$) C, 65.87; H, 7.56; N, 8.09. Found: C, 62.95; H, 7.12; N, 7.62). The oxalate salt can exist in different ratios with the free base (e.g., 1 mol of free base and 1 mol of oxalate or 1 mol of free base and 0.5 mol of oxalate). The ^{1}H NMR spectrum didn't indicate the presence of water in the sample. The MS of **103** revealed a molecular base peak of 257.15 (the same as the M+1 of the free base of **103**). The mass peak of the oxalate ion will not be seen in the MS because the salt is ionic and at high temperature will be converted to CO_2. Thus, on the basis of a constant melting point on repeated recrystallization with *i*-PrOH, the molecular base peak in the mass spectrum (all the hydrogen atoms in the molecular structure accounted for in the NMR spectrum) it was concluded that **103** had been obtained.

Scheme 11. Synthesis of compound **103**[a]

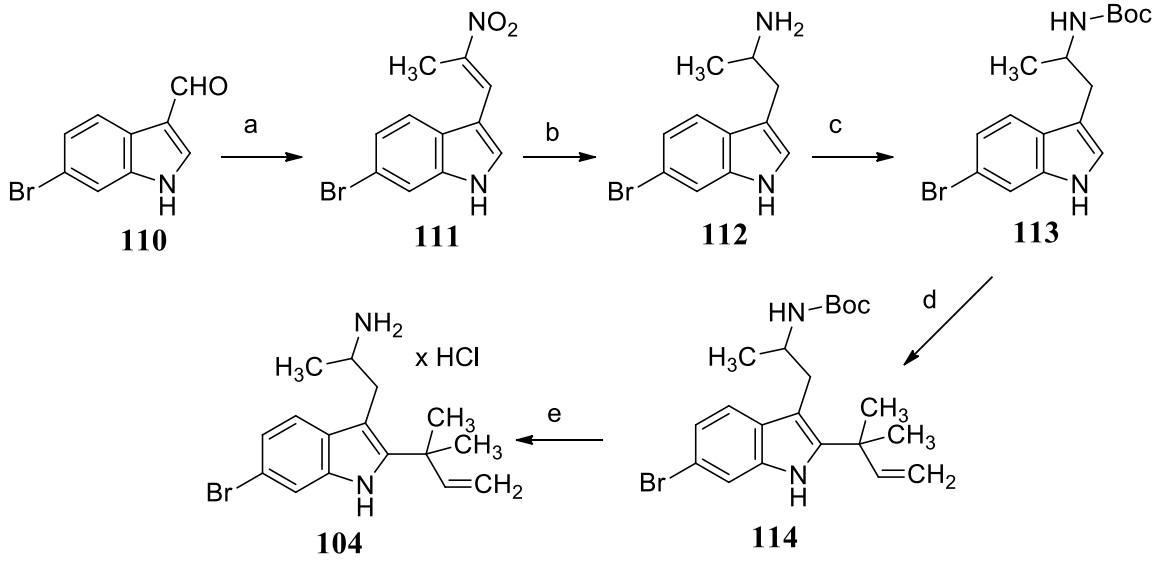


[a]Reagents: (a) (1) methyl chloroformate, Et_3N, THF, 0 °C, 30 min; (2) methylamine HCl, 0 °C, 1.5 h; (b) $LiAlH_4$, dioxane, reflux, overnight; (c) di-*tert*-butyl dicarbonate, Et_3N, CH_2Cl_2, room temperature, 19 h; (d) (1) *tert*-butyl hypochlorite, Et_3N, THF, -78 °C, 45 min; (2) prenyl-9-BBN, THF, room temperature, 2 h; (3) NaOH, H_2O_2, 1 h; (e) oxalic acid, Et_2O, recrystallized from *i*-PrOH.

Scheme **12** summarizes the synthesis of compound **104**. 6-Bromo-1*H*-indole-3-carboxaldehyde (**110**) underwent a Henry reaction in the presence of nitroethane and ammonium acetate as a weak base to yield 3-[(*E*)-2-nitroprop-1-en-1-yl]-6-bromo-1*H*-indole (**111**).[176] Further reduction with $NaBH_4$ in the presence of a Lewis acid yielded the corresponding α-methyltryptamine (**112**), which was Boc-protected to yield *tert*-butyl-1-(6-bromo-1*H*-indol-3-yl)propyl-2-carbamate (**113**). The 2-dimethylallyl substituent was introduced by a prenylation reaction to yield *tert*-butyl-1-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-2-carbamate (**114**)

which, on acidic deprotection, yielded 1-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propan-2-amine hydrochloride (**104**).

Scheme 12. Synthesis of compound **104**[a]

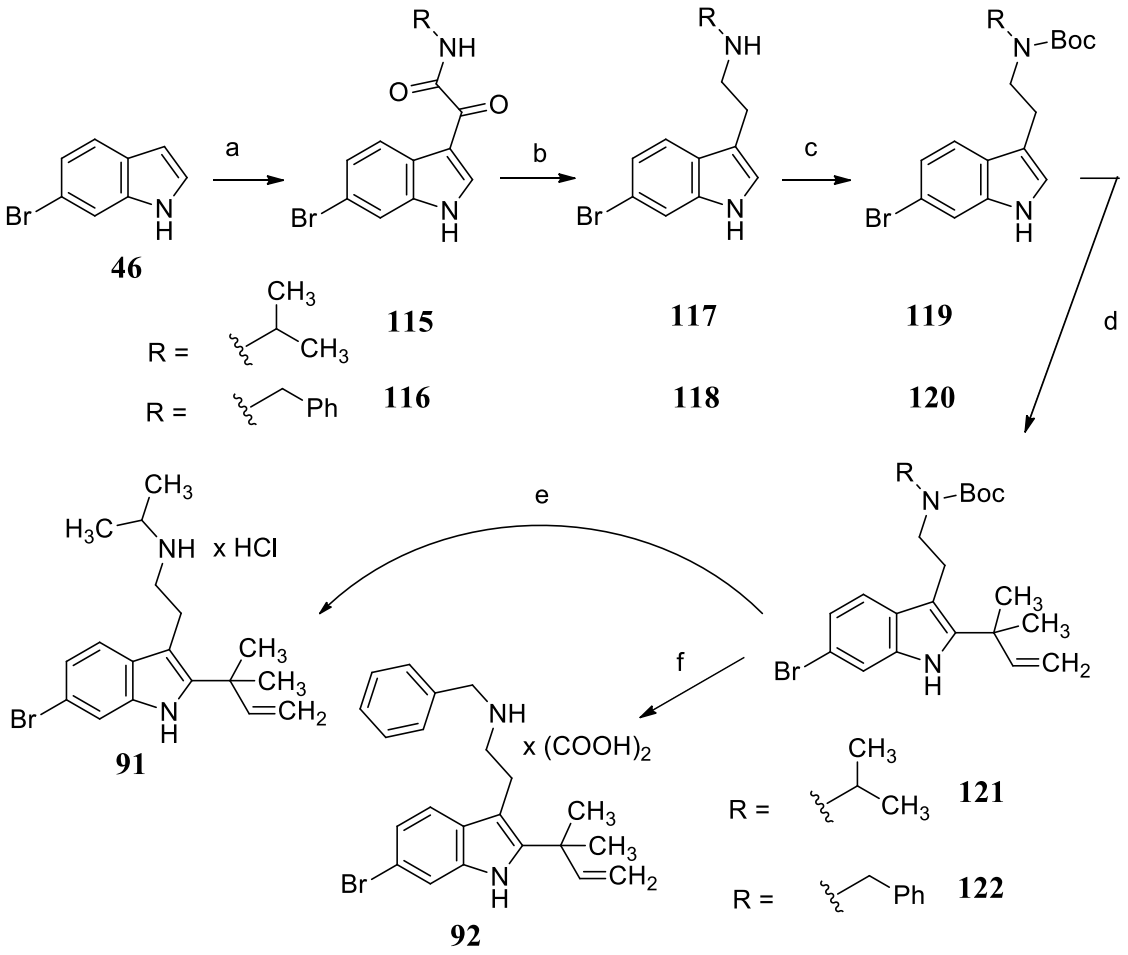


[a]Reagents: (a) Nitroethane, NH_4Ac, reflux, 7 h; (b) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF, reflux, 4 h; (c) di-*tert*-butyl dicarbonate, Et_3N, CH_2Cl_2, room temperature, 24 h; (d) (1) *tert*-butyl hypochlorite, Et_3N, THF, -78 °C, 45 min; (2) prenyl-9-BBN, THF, room temperature, 3 h; (3) NaOH, H_2O_2, 1 h; (e) HCl, Et_2O, 0 °C, 24 h, recrystallized from *i*-PrOH/Et_2O.

Scheme **13** summarizes the synthesis of compounds **91** and **92**. The *N*-alkyl analogs of dFBr (**32**) were synthesized following a similar route where oxalyl chloride was reacted with 6-bromoindole to yield the glyoxylyl chloride, which was further treated with the appropriate amine, *iso*-propylamine and benzylamine, respectively, to yield the corresponding glyoxylamides (**115**, **116**) which, on reduction with borane dimethylsulfide complex, yielded the respective tryptamines (**117**, **118**). Further treatment of **117** and **118** with di-*tert*-butyl dicarbonate in the presence of Et_3N to yielded the Boc-protected tryptamines (**119**, **120**) which were treated with

freshly prepared prenyl-9-BBN in the presence of *tert*-butyl hypochlorite and Et_3N to yield the *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-isopropylcarbamate (**121**) and *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-benzylcarbamate (**122**), respectively. Compound **121** was deprotected and converted to an HCl salt to yield *N*-*iso*-propyl-6-bromo-2-(1,1-dimethylallyl)tryptamine hydrochloride (**91**), while **122** was deprotected by using TFA and converted into a oxalate salt to yield *N*-benzyl-6-bromo-2-(1,1-dimethylallyl)tryptamine hydrogen oxalate (**92**). It was observed that the bulkier *N*-alkyl substituent, benzyl group compared to the isopropyl group, lowered the yield of the various reactions involved in the reaction scheme.

Scheme 13. Synthesis of compounds **91** and **92**[a]

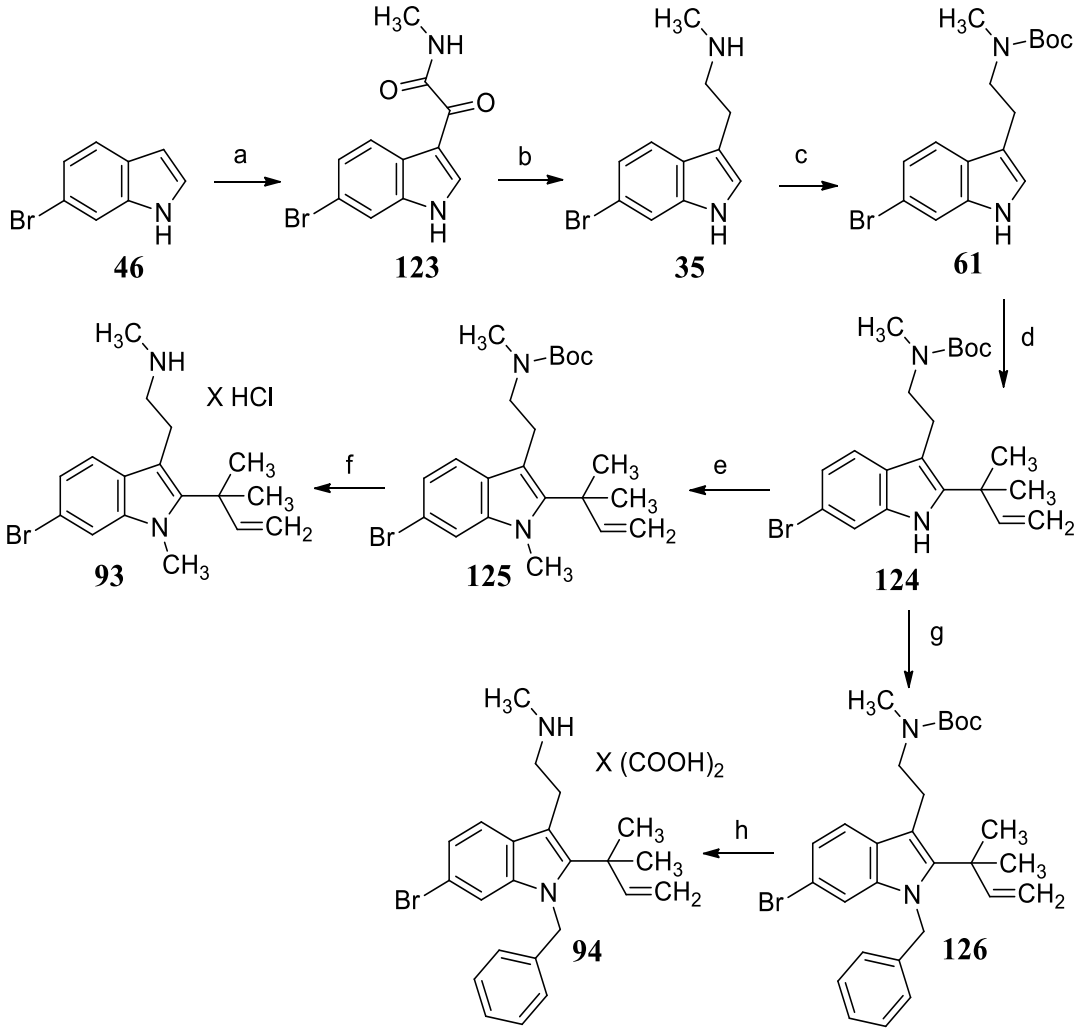


[a]Reagents: Compound **91** (a) (1) oxalyl chloride, Et_2O, -15 °C, 15 min, room temperature, 2 h; (2) *iso*-propylamine, room temperature, overnight; (b) $BH_3 \cdot S(CH_3)_2$, THF, reflux, 5 h; (c) di-*tert*-butyl dicarbonate, Et_3N, DMF, room temperature, overnight; (d) (1) *tert*-butyl hypochlorite, Et_3N, THF, -78 °C, 45 min; (2) prenyl-9-BBN, THF, room temperature, 4 h; (3) NaOH, H_2O_2, 1 h; (e) HCl, EtOAc, 0 °C, 24 h, recrystallized from MeOH.

Compound **92** (a) (1) oxalyl chloride, Et_2O, -10 °C, 5 min, room temperature, 2 h; (2) benzylamine, room temperature, 3 h; (b) $BH_3 \cdot S(CH_3)_2$, THF, reflux, 6 h; (c) di-*tert*-butyl dicarbonate, Et_3N, DMF, room temperature, 24 h; (d) (1) *tert*-butyl hypochlorite, Et_3N, THF, -78 °C, 45 min; (2) prenyl-9-BBN, THF, room temperature, 6 h; (3) NaOH, H_2O_2, 1 h; (f) (1) TFA, anhydrous CH_2Cl_2, room temperature 6 h; (2) oxalic acid, Et_2O, recrystallized from *i*-PrOH.

Scheme **14** summarizes the synthesis of compounds **93** and **94** where oxalyl chloride was reacted with 6-bromoindole (**46**) to yield the glyoxylyl chloride, which was further treated with methylamine to yield *N*-methyl-6-bromo-1*H*-indol-3-glyoxylamide (**123**). This, on reduction with a borane dimethylsulfide complex, yielded *N*-methyl-6-bromotryptamine (**35**). Further treatment with di-*tert*-butyl dicarbonate in the presence of Et_3N to yielded *tert*-butyl-2-(6-bromo-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**61**) which was treated with freshly prepared prenyl 9-BBN[177] in the presence of *tert*-butyl hypochlorite and Et_3N to yield *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**124**). This product was further methylated at the indole 1-position with iodomethane in the presence of NaH to yield *tert*-butyl-2-(6-bromo-1-methyl-2-(1,1-dimethylallyl)-indol-3-yl)ethyl-*N*-methylcarbamate (**125**), which was deprotected and converted to an HCl salt to yield *N*-methyl-6-bromo-1-methyl-2-(1,1-dimethylallyl)tryptamine hydrochloride (**93**). In a similar manner, **124** was alkylated with benzyl bromide in the presence of NaH to yield *tert*-butyl-2-(6-bromo-1-benzyl-2-(1,1-dimethylallyl)-indol-3-yl)ethyl-*N*-methylcarbamate (**126**), which was deprotected by TFA and converted to an oxalate salt to yield *N*-methyl-6-bromo-1-benzyl-2-(1,1-dimethylallyl)tryptamine hydrogen oxalate (**94**).

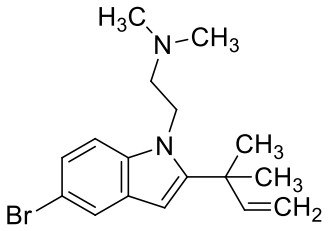
Scheme 14. Synthesis of compound **93** and **94**[a]



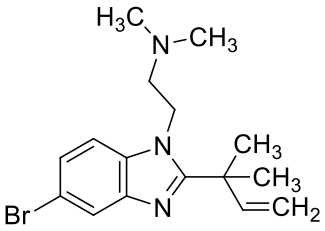
[a]Reagents: (a) (1) oxalyl chloride, Et_2O, 0 °C, 2 h; (2) methylamine, room temperature, 2 h; (b) $BH_3 \cdot S(CH_3)_2$, THF, reflux, 6 h; (c) di-*tert*-butyl dicarbonate, Et_3N, DMF, room temperature, 24 h; (d) (1) *tert*-butyl hypochlorite, Et_3N, THF, -78 °C, 45 min; (2) prenyl-9-BBN, THF, room temperature, 2 h; (3) NaOH, H_2O_2, 1 h; (e) (1) NaH, DMF, 0 °C, 30 min; (2) CH_3I, 1.5 h; (f) HCl, EtOAc, 0 °C, 7 h, recrystallized from MeOH; (g) (1) NaH, DMF, 0 °C, 45 min; (2) benzylbromide, room temperature, 5.5 h; (h) (1) TFA, CH_2Cl_2, 0 °C, 2.5 h; (2) oxalic acid, Et_2O, recrystallized from *i*-PrOH.

Various unsuccessful attempts were made to synthesize compound **95** (Figure **17**). The problem reaction was the attachment of the 2-position dimethylallyl group in the presence of the 1-

position substitution or vice versa. Various conditions were tried but none yielded the *N*-methylethanamine side chain at the 1-position of the indole ring. While attempting the synthesis of **95**, biological results for compound **86** were obtained (Table **4**) which showed that the *N*,*N*-dimethyl analog of **40** was active (see discussion); thus compound **127** was proposed. On similar lines, compound **96** was modified to its *N*,*N*-dimethyl analog, **129**. Scheme **15** summarizes the synthesis of compound **127**.



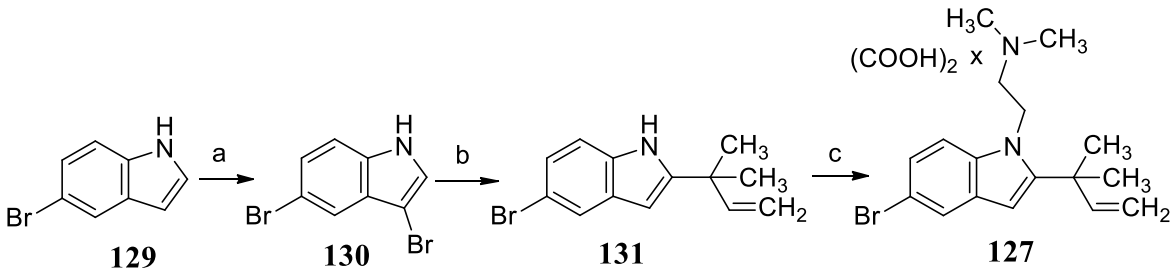
127



128

5-Bromoindole (**129**) was brominated in presence of bromine to yield 3,5-dibromoindole (**130**),[192] which was further treated with freshly prepared prenyl 9-BBN in the presence of Et_3N to yield 5-bromo-2-(1,1-dimethylallyl)-1*H*-indole (**131**)[179,193] Compound **131** was alkylated at the 1-position by *N*,*N*-dimethylaminoethyl chloride hydrochloride in the presence of NaH followed by salt formation to yield *N*,*N*-dimethyl-5-bromo-2-(1,1-dimethylallyl)isotryptamine hydrogen oxalate (**127**).

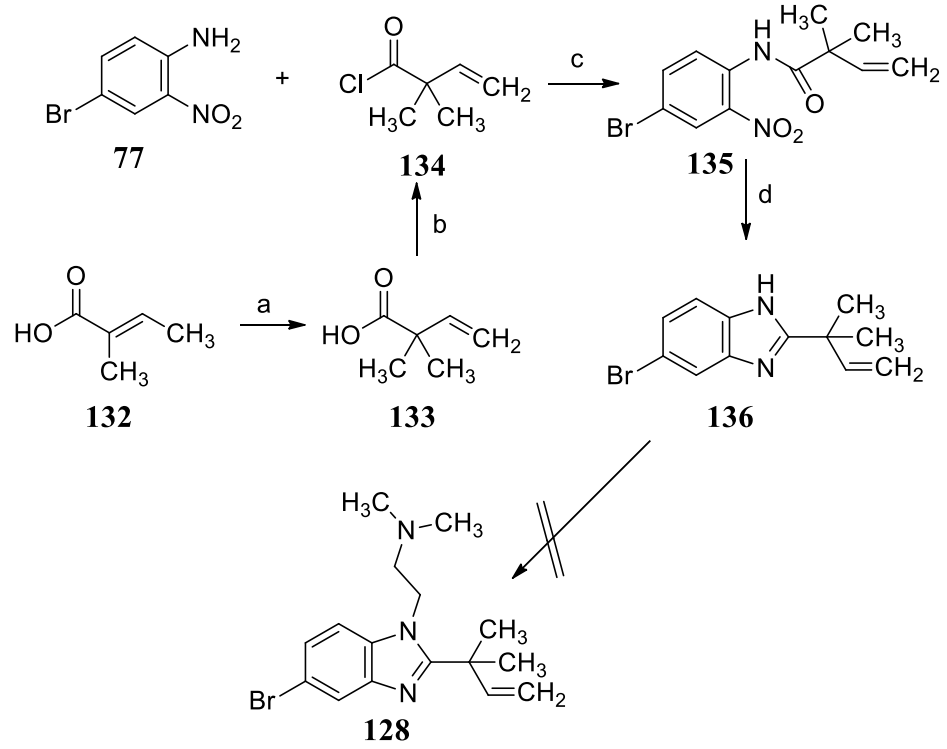
Scheme 15. Synthesis of compound **127**[a]



[a]Reagent: (a) Br_2, DMF, light protected, room temperature, 12 h; (b) prenyl-9-BBN, Et_3N, THF, room temperature, 4 h; (c) (1) NaH, DMF, 0 °C, 30 min; (2) *N*,*N*-dimethylaminoethyl chloride hydrochloride, K*t*BuO, KI, reflux, 17 h; (3) oxalic acid, Et_2O, recrystallized from MeOH/Et_2O.

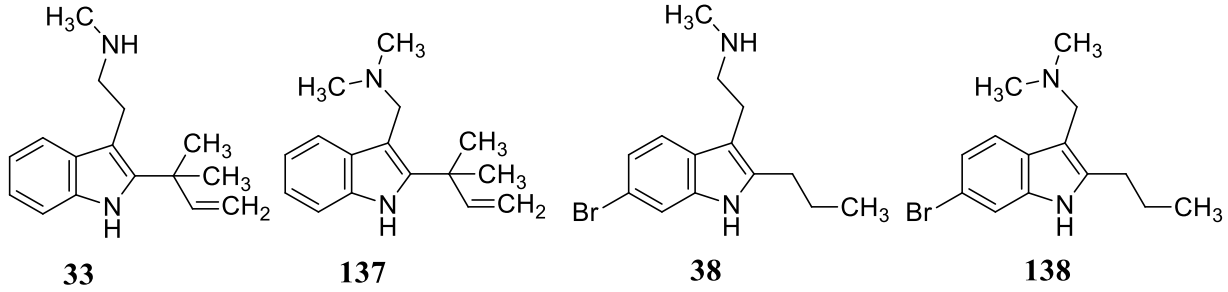
Scheme **16** summarizes the unsuccessful attempt to synthesize the benzimidazole analog **128**. Various unsuccessful attempts were made to attach the *N*,*N*-dimethylethanmine side chain to the 5-bromo-2-(1,1-dimethtylallyl)-benzo[*d*]imidazole moiety. Scheme **16**, however, provided a route for the synthesis of 5-bromo-2-(1,1-dimethtylallyl)-benzo[*d*]imidazole (**136**). The synthesis of the benzimidazole analog (**136**) was discontinued when it was found that the N-H of the indole was important towards the potency at the nACh receptors (see discussion below)

Scheme 16. Attempted synthesis of compound **130**[a]



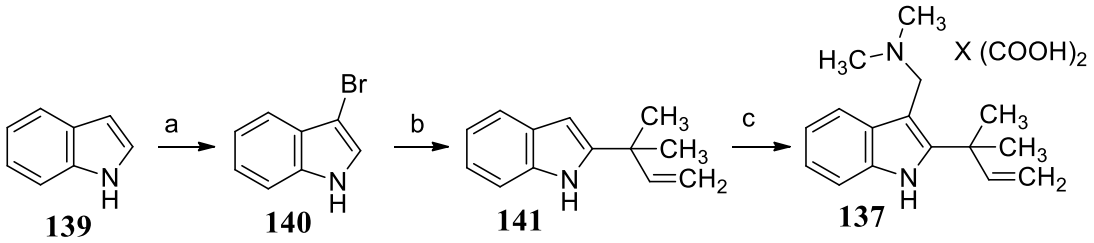
[a]Reagent: (a) LDE, THF, dimethyl sulphate, -78°C to 0°C, 1.5 h; (b) oxalyl chloride, CH_2Cl_2, DMF, room temperature, 16 h; (c) DCC, DMAP, CH_2Cl_2, reflux, 19 h; (d) Fe, HOAc, reflux, 7 h.

Based on the results obtained for compound **102** (see discussion below) two more compounds; **137** and **138**, were synthesized to develop pairs of molecules in different classes of compounds (e.g., **33** and **137**; **38** and **138**).



Scheme **17** summarizes the synthesis of compound **137**. The indole (**139**) was brominated in the presence of bromine to yield 3-bromoindole (**140**) which was further treated with freshly prepared prenyl-9-BBN in the presence of Et_3N which undergoes a nucleophile substitution reaction where the 3-bromo substituent activates the 2-postion of the indole ring to yield 2-(1,1-dimethylallyl)-1*H*-indole (**141**). Compound **141** was further treated with dimethylamine and formaldehyde in a Mannich fashion to yield the corresponding gramine that was converted to the oxalate salt to yield 2-(1,1-dimethylallyl)gramine oxalate (**137**).

Scheme 17. Synthesis of compound **137**[a]

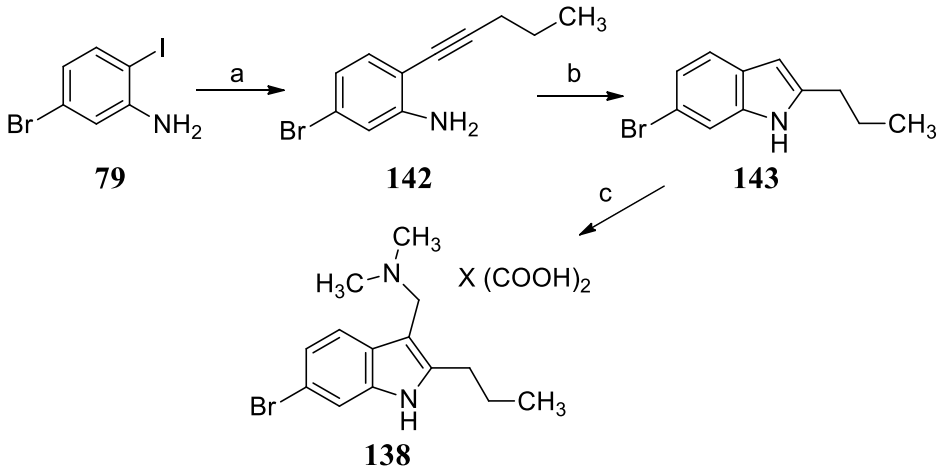


[a]Reagents: (a) Liquid Br_2, DMF, room temperature, 24 h; (b) prenyl-9-BBN, THF, Et_3N room temperature, 5 h; (c) (1) dimethylamine, HCHO, room temperature, overnight; (2) oxalic acid, Et_2O, recrystallized from *i*-PrOH.

Scheme **18** summarizes the synthesis of compound **138**. The synthetic route was similar to Scheme **8** which yielded compounds **37** and **39**. Sonagashira coupling was performed in the presence of the corresponding alkyne and ligand-based palladium catalysts provided 5-bromo-2-(1-pentynyl)aniline (**142**). The corresponding alkyne was treated with ZnI_2 to effect cyclization to yield 6-bromo-2-propyl-1*H*-indole (**143**). Compound **143** was treated with dimethylamine and formaldehyde in the presence of ZnI_2 to undergo a Mannich reaction to yield the corresponding

gramine, which was converted to an oxalate salt to yield 6-bromo-2-propylgramine hydrogen oxalate (**138**).

Scheme 18. Synthesis of compound **138**[a]

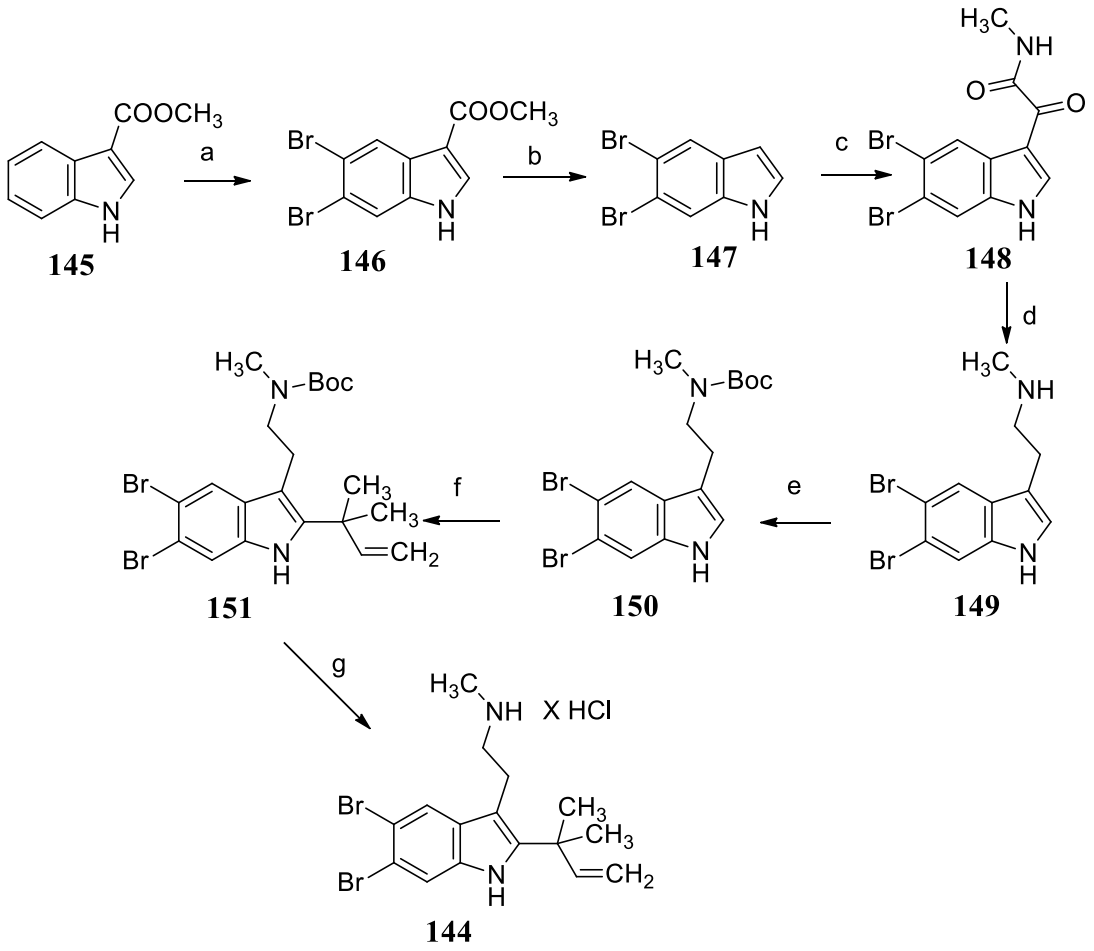


[a]Reagents: (a) 1-pentyne, CuI, $PdCl_2(PPh_3)_2$, Et_3N, THF, room temperature, overnight; (b) ZnI_2, Toluene, reflux, 2.5 h; (c) (1) Formic acid, dimethylamine, ZnI_2, EtOH, room temperature, 24 h; (2) oxalic acid, Et_2O, recrystallized from *i*-PrOH.

Based on the results obtained for compound **98** (see discussion below), compound **144** was prepared. Scheme **19** summarizes the synthesis of compound **144**. Parsons; et al.[194] in 2011, published a method to synthesize 5,6-dibromo indoles as an important building block for the synthesis of various alkaloids. We were able to replicate the two-step reaction where the commercially available methyl-1*H*-indole-3-carboxylate (**145**) was brominated using bromine and HOAc as the solvent to obtain methyl-5,6-dibromo-1*H*-indole-3-carboxylate (**146**) with a melting point matching the literature.[194] This was one of the few reactions ever reported to synthesize a 5,6-dibrominated indoles. Decarboxylation was achieved by performing a

microwave-assisted reaction to obtain 5,6-dibromo-1*H*-indole (**147**).[194] Oxalyl chloride was reacted with 5,6-dibromo-1*H*-indole to yield the glyoxylyl chloride, which was further treated with methylamine to yield *N*-methyl-5,6-bromo-1*H*-indol-3-glyoxylamide (**148**). This compound, upon reduction with a borane dimethylsulfide complex, yielded *N*-methyl-5,6-dibromotryptamine (**149**). Further treatment with di-*tert*-butyl dicarbonate in the presence of Et_3N yielded *tert*-butyl-2-(5,6-dibromo-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**150**), which was treated with freshly prepared prenyl 9-BBN in the presence of *tert*-butyl hypochlorite and Et_3N to yield *tert*-butyl-2-(5,6-dibromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**151**). Compound **151** was deprotected and converted to an HCl salt to yield *N*-methyl-5,6-dibromo-2-(1,1-dimethylallyl)tryptamine hydrochloride (**144**).

Scheme 19. Synthesis of compound **144**[a]



[a]Reagents: (a) Liquid Br_2, HOAc, room temperature, 16 h; (b) KOH, MeOH, H_2O, THF, MW 100 W, 150 °C, 1 h; (c) (1) oxalyl chloride, Et_2O, -5 °C 15 min, reflux 5 h; (2) methylamine, room temperature, overnight; (d) $BH_3{\cdot}S(CH_3)_2$, THF, reflux, 7 h; (e) di-*tert*-butyl dicarbonate, Et_3N, DMF, room temperature, 24 h; (f) (1) *tert*-butyl hypochlorite, Et_3N, THF, -78 °C, 1.5 h; (2) prenyl-9-BBN, THF, room temperature, 6 h; (3) NaOH, H_2O_2, 2 h; (g) HCl, EtOAc, 0 °C, 24 h, recrystallized from *i*-PrOH.

B. Pharmacology

Function data for dFBr (**32**) analogs are provided in Table **5**.

Table 5. Effect of dFBr (**32**) and its elaborated analogs on the action (potentiation or antagonism) of ACh action at the human α4β2 nACh receptors.

Compound	Potentiation	Inhibition	Half maximum potentiation concentration (EC_{50}) (μM) (± SEM)	Half maximum inhibition concentration (IC_{50}) (μM) (± SEM)
dFBr (**32**)	+	+	0.33 ± 0.52	150 ± 0.2
86	+	-	9.7 (2.3-17.1)[b]	146.5 (15.9-280)[b]
87	-	+	-	2.48 (1.6-3.3)[b]
91			DA[a]	DA
92			DA	DA
93	+	+	4.30 ± 1.4	35.0 ± 2.0
94			DA	DA
97	+	+	7.40 ± 0.1	14.0 ± .8
98	+	+	0.90 ± 0.5	32.0 ± 0.6
99	+	-	2.40 ± 0.2	25.0 ± 21.0
100	+	-	0.90 ± 0.1	19.5 ± 0.9
102	-	+	-	1.8 ± 0.7
103			DA	DA
104			DA	DA
127	+	+	11.2 ± 0.5	71.0 ± 1.0
137	-	+	-	17.9 (3.1-32.6)[b]
138	-	+	-	17.8 (3.1-32.6)[b]
144			DA	DA

[a]DA = Data currently unavailable, [b]95% confidence limits, *n* = 4. Note: where both potentiation and inhibition was observed, inhibitory potencies (IC_{50} values) are only estimates.

C. Discussion

Compounds **86**-**100**, **102**-**104**, **127**, **137**, **138** and **144** represent elaborated analogs of dFBr (**32**) (Table **5**; Figure **16** and **17**); that is, they possess substituents uncommon to dFBr (**32**). Comparing dFBr (**32**) with **86** (Figure **18**; EC_{50} = 9.7 μM), it is evident that the *N*,*N*-dimethyl tertiary amine **86** is around 30-fold less potent than the secondary amine of dFBr (**32**). This suggests that either the tertiary amine or steric bulk at the terminal amine may cause the decrease in potency compared to dFBr (**32**). Interestingly with respect to effect, **86** enhanced the action of ACh at α4β2 nACh receptors by 300% (Figure **18**). This is similar to what was found for dFBr (**32**) itself.[9] Hence, N-methylation of dFBr (**32**) decreased its potency, but had no effect on its efficacy.

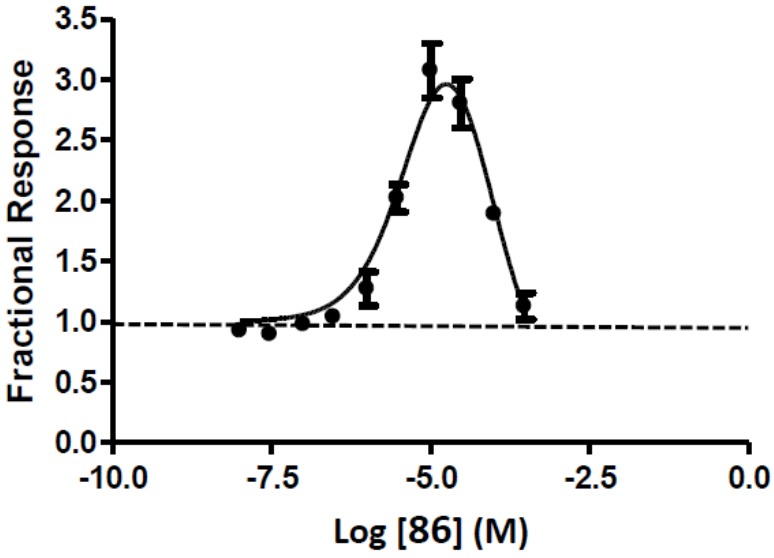


Figure 18. Biphasic dose-response curve for compound **86** co-applied with 100 μM ACh on human α4β2 nACh receptors.

Compounds **91**, which has an *N*-*iso*-propyl substituent, and **92**, which is an *N*-benzyl analog, will help answer questions regarding the tolerance of steric bulk at the amine region of dFBr (**32**). Data have not yet been obtained for compounds **91** and **92**.

The α4β2 nACh receptors are ion channel in nature with ACh being the endogenous ligand. ACh is a quaternary amine; thus, it was worthwhile to check the effect of a quaternary amine analog of dFBr (i.e., **87**) on the activity of ACh at the α4β2 nACh receptors. It was found that compound **87** failed to potentiate the action of ACh, but was a relatively potent antagonist (Table **5**; IC_{50} = 2.5 μM).

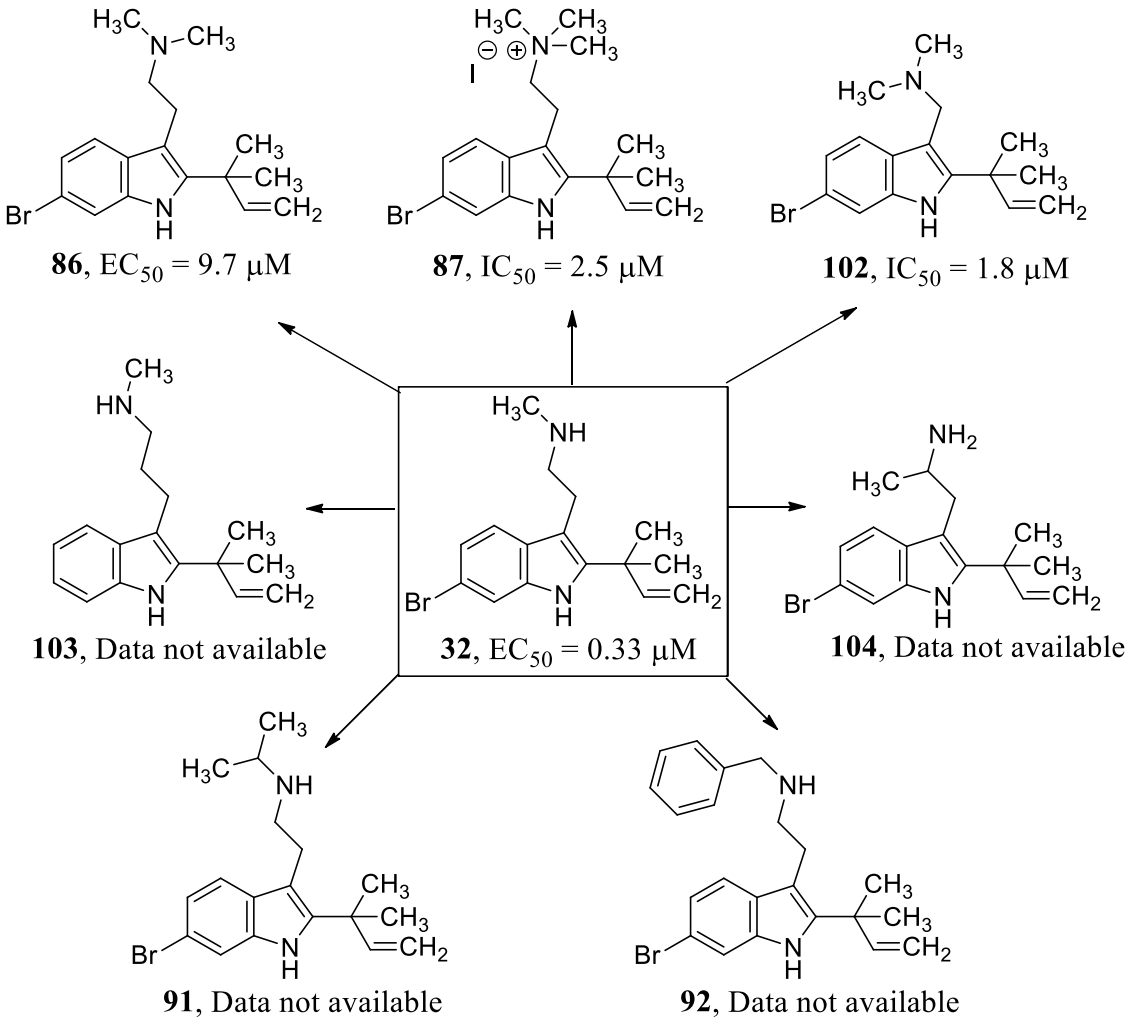


Figure 19. Initial elaboration of dFBr (**32**). Data are from Table **5**. Certain analogs did not potentiate the effect of ACh at nACh receptors, but blocked the effect of ACh.

Given its unusually high potency as an inhibitor, coupled with its quaternary nature, **87** might be a competitive antagonist. Its affinity at α4β2 nACh receptors remains to be measured.

The gramine analog of dFBr (**102**) also showed no potentiation of action, but inhibited ACh action (Table **5**, Figure **19**; IC_{50} = 1.8 μM). It might be proposed that the binding modes of dFBr (**32**) and **102** can be different and that **102** might be a member of an altogether different mechanistic class of compounds. Compound **102** was also further studied to see what substituents can be tolerated and yet retain the inhibition profile of the gramine analog of dFBr (**32**). The two series; **32** and **102** were studied to bring about a parallel structural change. A parallel change in structure should result in a parallel change in potency if the two series are behaving in the same manner. Figure **20** summarizes the compounds that were examined to rationally understand the effect of substituents on the action of ACh. It is rather interesting that gramine **102** is an antagonist that lacks the PAM action of dFBr (**32**). Apparently, the 6-bromo group is neither a requirement for PAM action (comparing **33** with **32**) nor a requirement for blocking action (comparing **137** with **102**) (Figure **20**). But, whereas the *gem*-dimethyl groups appear required for PAM action of **32**, they are not required for the blocking action (i.e., both **38** and **138** behaved only as blockers with no action as PAMs).

It might be concluded that shortening the tryptamine chain of dFBr-like compounds and not the presence of the *N*,*N*-dimethylamine accounts for the elimination of PAM action, because the *N*,*N*-dimethyl counterpart of dFBr (**32**), i.e., **86**, retains such action. Likewise, comparing the *des*-bromo analog **33** with *des*-bromo gramine analog **137**, it is apparent that the 6-bromo substituent is not required for potency as an inhibitor for dFBr-like compounds, whereas the lack of a 6-bromo substituent as in the *des*-bromogramine analog **137** decreases potency as an

inhibitor of ACh action. The tryptamine series and the gramine series appear to act very differently (i.e., some tryptamines lack PAM action, whereas all gramines examined lacked this action) and the SAR for their actions appear independent. Gramine analogs should be further explored to optimize this structurally diverse class of compounds behaving as blockers (potential NAMs?) at the α4β2 nACh receptors.

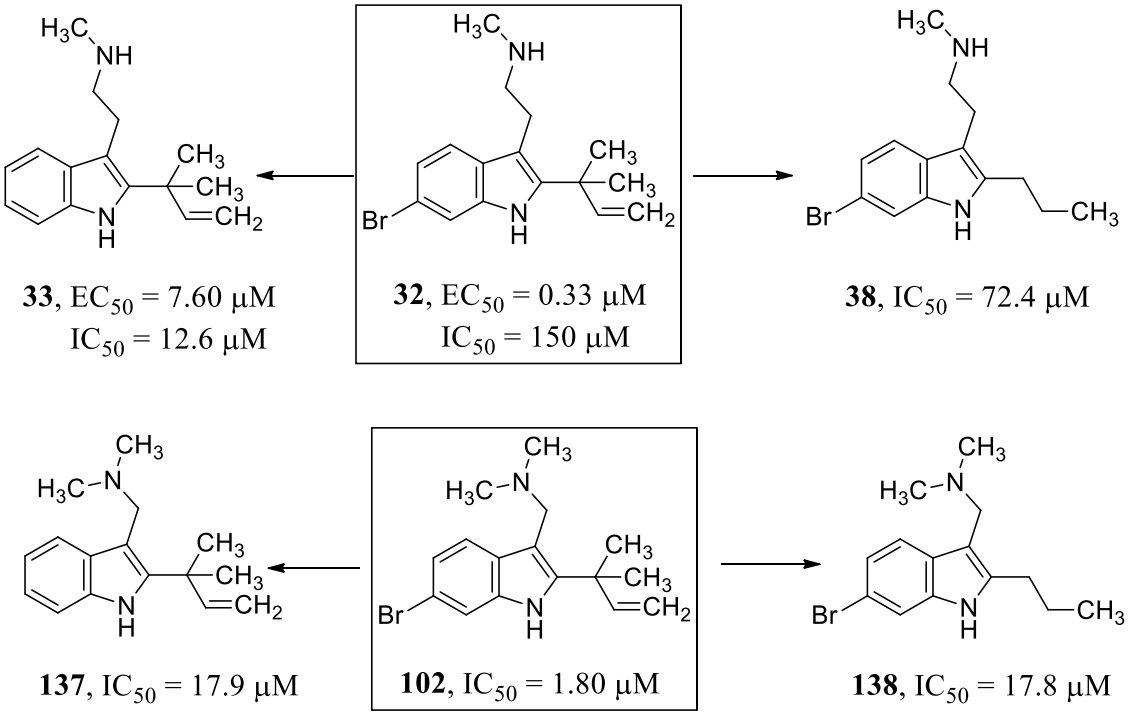


Figure 20. Comparison of the two series of analogs; dFBr (**32**) and **102**. Data for **32**, **33**, and **38** are from Table **4**; data for **102**, **137**, **138** are from Table **5**.

Comparing compounds **97**, which has a piperidine substituent at the 3-position, **102**, which is a gramine analog, and **103**, which is a homotryptamine analog of dFBr (**32**), will help us understand the effect of changing tryptamine chain length. Comparing dFBr (**32**) with compound

97 (Table **5**; EC_{50} = 7.4 μM), it can be hypothesized that the 20-fold decrease in potency could be attributed to the slight elongation of the distance between the terminal amine and the indole nucleus (2-carbon length for dFBr *vs* the 2.5-carbon length for the piperidine moiety), or the α-substitution on the tryptamine chain. Thus, compound **103**, which is the homotryptamine analog, and **104**, which is a α-methyl substituted analog, respectively, will be able to address each question individually to provide insight into the chain length and α-substitution tolerance on the dFBr (**32**) and potency.

It is known that the 6-bromo substituent of dFBr (**32**) makes a substantial contribution to activity. To further understand whether the effect of the 6-bromo substituent is due to a steric or electronic effect, compounds **99**, which is a 6-methyl analog, and **100**, which is a 6-trifluoromethyl analog, were synthesized. Comparing **32**, **99** and **100** (Figure **21**) it is unclear whether an electronic or steric effect is predominant for activity. Additional compounds will be required to conduct a thorough QSAR study.

Compound **98** in which the position of the bromo substituent was changed, did not substantially affect potency (Figure **21**; Table **5**; EC_{50} = 0.9 μM). This suggests that there could be steric/electronic properties of the 5-position substituent that can be explored. The similarity in potency could be attributed to either steric or electronic properties of the bromo substituent. Also the 5- and 6-bromo compounds can be binding differently to produce their action as a PAM. Data for compound **144** will help answer the question of bulk tolerance at the 5- and 6-position of dFBr (**32**). It will also help understand if the 5- and 6-bromo analogs bind similarly at the receptor site. The 5-bromo analog **98** is an early example of exploration of the positions on the indolic ring that can be tolerated. Also with the 6-bromo and the 5-bromo analog being almost

equipotent (Table **5**; **32**; EC_{50} = 0.33 μM, **98**; EC_{50} = 0.87 μM), by having both substituents in one compound (**146**), an increase in potency might be seen. Data for compound **144** has not yet been obtained.

The importance of the N-H of the indole nucleus was addressed. Compound **93** (Table **5**; EC_{50} = 4.3 μM), N_1-methyl dFBr, was almost 13-fold less potent than dFBr (Table **5**; **32**; EC_{50} = 0.33 μM) suggesting that the N-H of the indole might be involved as a hydrogen-bond donor making an important hydrogen bond interaction with the amino acids in the receptor. This hypothesis was further strengthened when compound **127** (Table **5**; EC_{50} = 11.2 μM) was found to be almost 34 times less potent than dFBr (**32**). However, the lower potency of compound **127** could also be due to the translocation of the indolic nitrogen atom and/or the presence of a substitution on the tertiary amine. However, the potency of **127** was similar to that of N-methyl dFBr (**86**; EC_{50} = 9.7 μM). The comparison of compounds **32**, **93** and **127** helps us understand that a hydrogen bond donor might be important for the potency of dFBr (**32**) (Figure **21**).

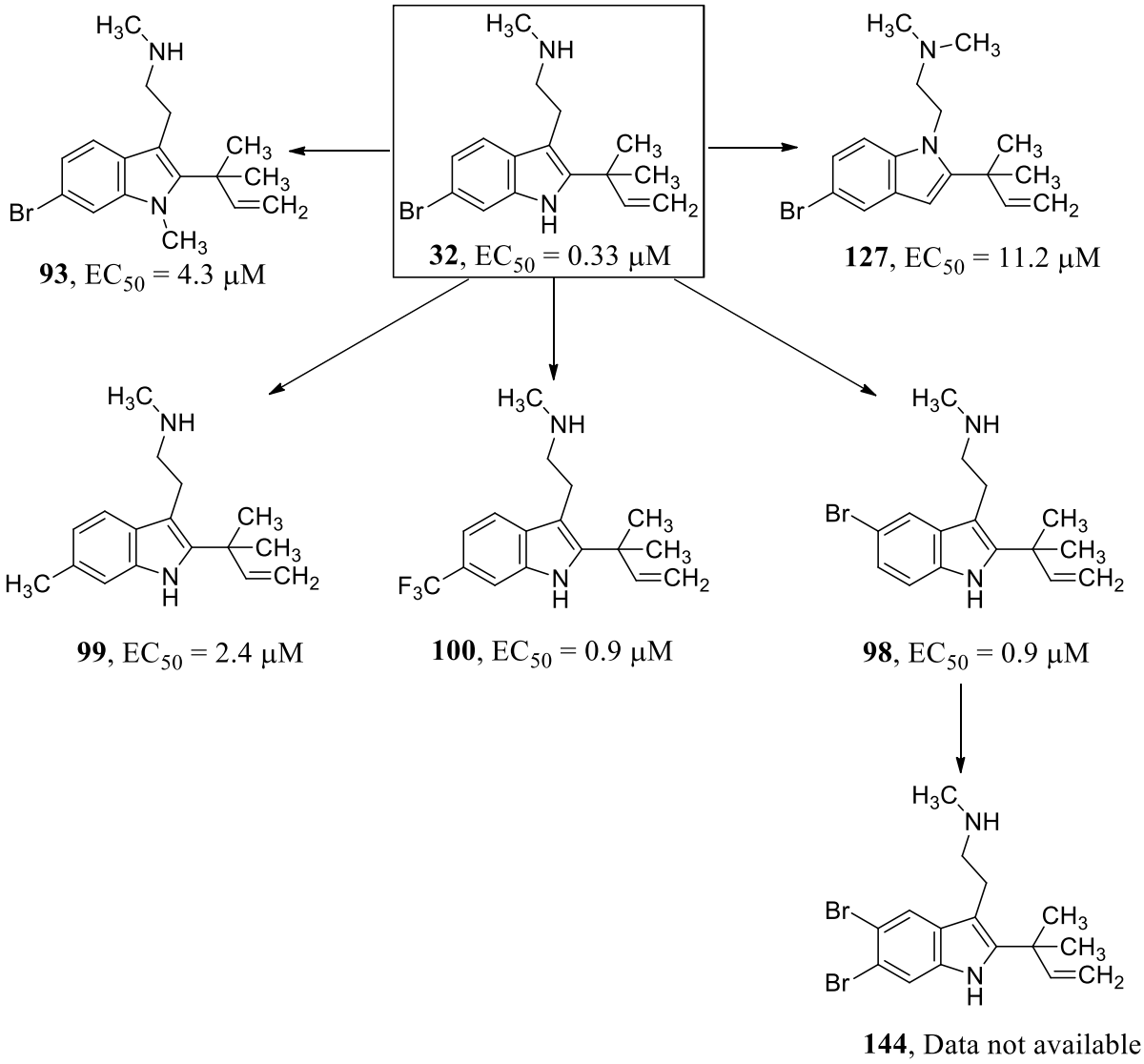
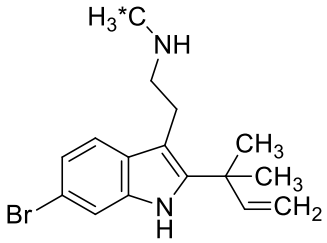


Figure 21. Elaborated analogs of dFBr (**32**). Data are from Table **5**. Certain analogs did not potentiate the effect of ACh at ACh receptors.

Radiolabelled dFBr

Compound **40** was used as an intermediate and submitted for commercial tritiation to obtain [^{3}H]dFBr (in Dr. Jonathan B. Cohen's laboratory at Harvard Medical School).[195] The tritiated dFBr (**152**) is the first radiolabelled positive allosteric modulator at α4β2 nACh receptors. All

the studies performed on dFBr (**32**) involved only the CNS α4β2 nACh receptors, while the peripheral muscle type receptor was not characterized. At the muscle type α4β2γδ ACh receptor, it was found that dFBr (**32**) does not activate nor potentiate the ACh response when expressed in *Xenopus* oocytes. Instead it was found to be a potent inhibitor (IC_{50} = 1 μM). dFBr (**32**) was also found to reversibly inhibit the binding of [^{3}H]phencyclidine (IC_{50} = 4 μM) with higher affinity in the desensitized state than in the resting state ([^{3}H]ACh, IC_{50} = 1 mM).[195] Photolabeling studies performed with [^{3}H]dFBr (**152**) highlight certain amino acids in the ion channel (M2 helix): in the ACh binding orthosteric site on the α-subunit (Tyr93, Tyr190 and Tyr198) as well to a site near the orthosteric ACh binding site on the γ subunit (Tyr105, Tyr111 and Tyr117) in the presence and absence of ACh. This data shows dFBr as a potent inhibitor of muscle-type α4β2γδ ACh receptor that acts as a channel blocker by binding in the channel pore. Studies are underway to identify possible binding site(s) for the PAM at α4β2 nACh receptor.



[^{3}H] dFBr (**152**)

D. Antagonism of the ACh response

In biological studies dFBr (**32**) produces a biphasic dose-response curve (Figure **8**). The biphasic response provides information about the potentiation (EC_{50}) as well as inhibition (IC_{50}) of ACh's effect. The biphasic dose-response curve has an overlay between the potentiation and inhibition profile and sometimes it is relatively difficult to define the two regions. Ideally, the biphasic dose-response curve should have a wide concentration range and a broad plateau to allow a definite demarcation of the potentiation and inhibition profile. Some analogs that were tested did not allow for an accurate estimation of the potentiation and inhibition profile. A more accurate IC_{50} value can be obtained for those compounds that only produced an antagonist effect. For purpose of establishing a preliminary SAR for the antagonistic action of dFBr (**32**), a high standard deviation was accepted and the IC_{50} values were compared. The aim of examining structural analogs of dFBr (**32**) was to determine similarities and differences for agonist *vs* antagonist SAR. Figure **22** summarizes dFBr (**32**) and its analogs. Comparing dFBr (**32**) with **37**, which has a 2-methylpropyl substituent, and **36**, with a 2-propenyl substituent, it is evident that the *gem*-dimethyl groups are not required for the antagonist action at α4β2 nACh receptors. In fact, compound **38** (lacking the methyl group of **37**) was as potent as **37** as an antagonist. Removal of the 6-bromo group of dFBr (i.e., **33**) resulted in almost a 12-fold increase in inhibitory action. The removal of the 6-bromo group of **37** (i.e., **44**) seems to be tolerated and the resulting compound is 3-fold more potent than **37** as an inhibitor. *N*-Methyl dFBr (**86**) showed no change in potency compared to dFBr (**32**) while the gramine analog **102** was almost 80-fold more potent as an inhibitor. Thus, the gramine analog **102** attributes its relative potency as an antagonist to the shortening of the tryptamine side chain rather than to the *N*,*N*-dimethyl amine

found in **86**. In the gramine series (compounds **102**, **137** and **138**), it can be concluded that the removal of the 6-bromo group is tolerated and is not required for action. Similarly the *gem*-dimethyl groups are not essential for potency as an antagonist (comparing **137** and **138**).

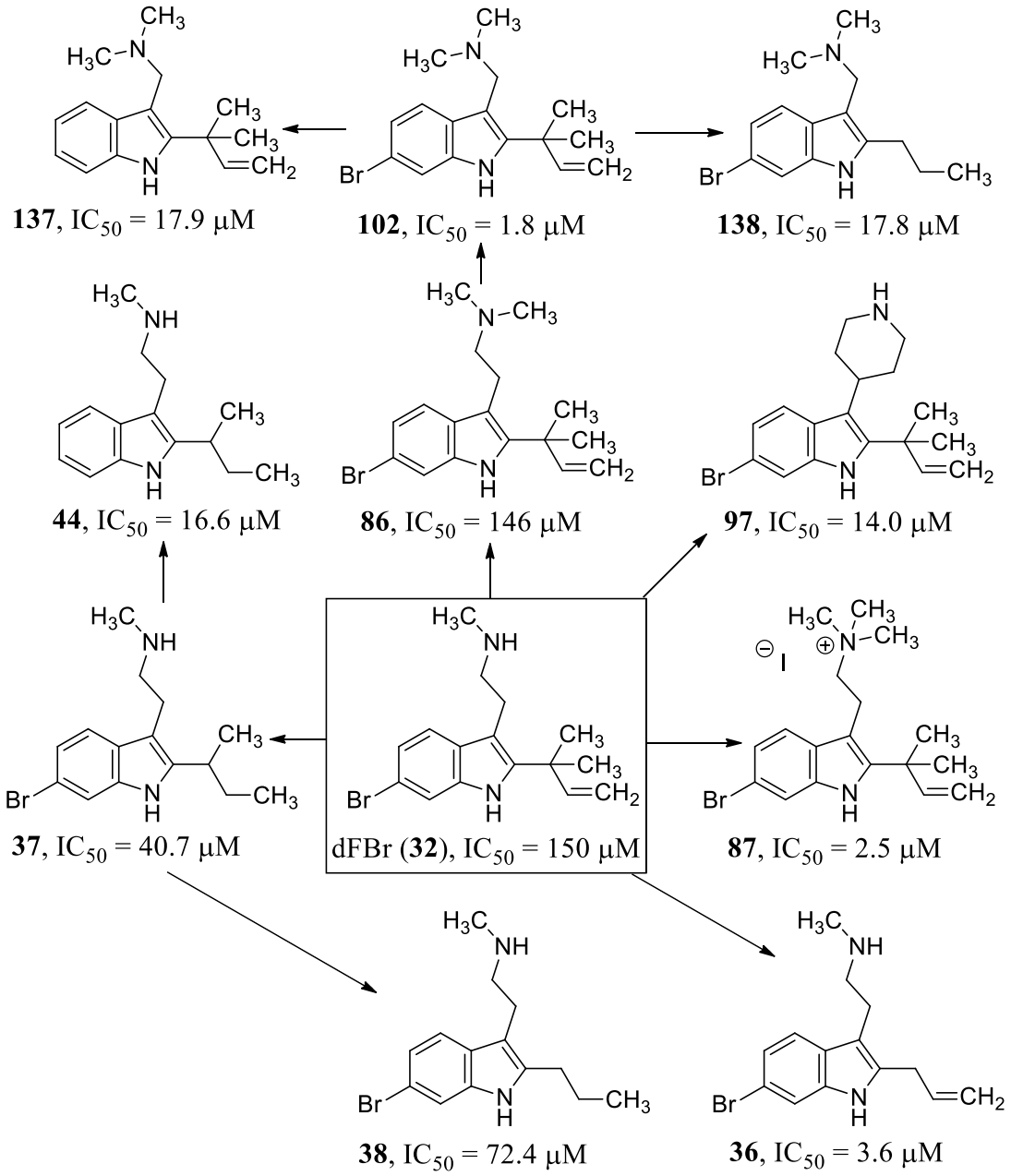


Figure 22. Analogs of dFBr (**32**) comparing the inhibition of the ACh-induced response. Each arrow symbolizes a structural change. Data are from Tables **4** and **5**.

As seen in Figure **22**, comparing dFBr (**32**) and quaternary amine **87**, it is seen that the 60-fold increase in potency of **87** is due to the quaternary amine, suggesting that the electronic nature of the amine is important towards the activity (inhibition), along with greater bulk at the amine region. An observation that needs to be further explored. The 1-methyl analog of dFBr (**93**) was shown to have a 10-fold decrease in PAM potency (Table **6**; EC_{50} = 4.3 μM), but an improved inhibition potency by almost 4-fold (Table **6**; IC_{50} = 35 μM). So it can be predicted that the steric bulk at the N_1-position is not favored for potentiation but it is tolerated for inhibition of ACh action at α4β2 nACh receptors. Comparing dFBr (**32**) and the 1-methyl analog (**93**), it can also be concluded that the hydrogen bond donor property of the indolic N-H might be important for potentiation, while the hydrogen bond acceptor property of the substituted N_1-position of the indole could be favoring the inhibition of the ACh-induced action at the α4β2 nACh receptors.

Table 6. Effect of dFBr (**32**) and its analogs on inhibition of ACh action at the human α4β2 nACh receptors.

Compound	Potentiation	Inhibition	Half maximum potentiation concentration (EC_{50}) (μM)	Half maximum inhibition concentration (IC_{50}) (μM)
dFBr (**32**)	+	+	0.33 ± 0.52*	150 ± 0.2*
86	+	+	9.70 (2.3-17.1)♦	146.5 (15.9-280)♦
87	-	+	NP[a]	2.48 (1.6-3.3)♦
93	+	+	4.30 ± 1.4*	35.0 ± 2.0*
97	+	+	7.40 ± 0.1*	14.0 ± 0.8*
102	-	+	NP	1.8 ± 0.7*
137	-	+	NP	17.9 (3.1-32.6)♦
138	-	+	NP	17.8 (3.1-32.6)♦

[a]NP = No potentiation of the action of ACh; * = SEM; ♦ = 95% confidence limits; *n* = 4.

It can be concluded that the structural features required for potentiation action and inhibition action are not identical, as summarized in Table **7**. Nevertheless, it is realized that the 'inhibitory' effect is in need of further study. That is, the nature of 'inhibition' is unknown at this time. Several dramatic structural changes have been made (e.g., tryptamine *vs* quaternary amine *vs* gramine). Certain compounds might produce their inhibitory effect like dFBr (**32**), presumably by acting as a channel blocker; others might represent NAMs, or perhaps competitive antagonists that bind at the orthosteric site. This needs to be investigated.

Table 7. Summary of structural requirements for potentiation/inhibition of the ACh response at the α4β2 nACh receptors.

Substituent	Potentiators	Inhibitors
6-Bromo	Required for potency but not action	Not required for potency or action
gem-Dimethyl	Required for action	Not required
3-Position carbon chain	2-Carbon (tryptamine)	1-Carbon (gramine)
N-Substituent	Secondary amine optimal	Quaternary amine might be optimal Secondary and tertiary amine are equipotent
N_1-Substitution	Indolic NH required for activity	Bulk tolerated at the indolic NH

The results shown in Table **7** can be a good starting point to develop functionally different series of analogs as potentiators or inhibitors of the ACh response at the α4β2 nACh receptors.

VI. Conclusions

des-Formylflustrabromine (dFBr, **32**) has been identified as a positive allosteric modulator (PAM) at α4β2 nACh receptors that potentiates the actions of the neurotransmitter ACh. It was isolated in small quantities from a marine organism: *Flustra foliacea*. The present investigation was to identify what structural features of dFBr (**32**) are required for the action of dFBr as a PAM. The 'deconstruction-reconstruction-elaboration' approach also identified a possible pharmacophore for a positive allosteric modulator action at α4β2 nACh receptors.

It is worth mentioning that the *in vitro* studies were performed on oocytes. The electrophysiological data obtained from the oocytes studies should be thoroughly examined and various factors such as temperature, experimental conditions, and functioning of the oocytes should be considered. Also the data obtained from electrophysiology should be used as a good starting point for performing *in vivo* studies which has a better correlation with how the drug behaves in humans.

One of the aims of this study was to identify critical structural features of dFBr for potentiation of ACh at α4β2 nACh receptors. Figure **23** summarizes the structural features required for action/potency. The 'deconstructed' analogs of the 2-position showed that double bond saturation (i.e., **34**) is tolerated. This provides a synthetic advantage for future dFBr (**32**) analogs. The *gem*-dimethyl groups are required along with a quaternary carbon at the 2-position (i.e., **39**) for potency. The amine region was investigated and it was shown that the secondary amine of

dFBr (**32**) was optimal. The primary amine counterpart **40** was half as potent as the parent analog. The *N*,*N*-dimethyl tertiary amine analog **86** was almost 30 times less potent than dFBr (**32**). This might be attributed to the basicity of the amine or the steric bulk at the amine region. The quaternary amine **87** was studied to see if the quaternary amine and its cation-π interaction similar to the interaction of ACh would have any effect. It was shown to be an antagonist, and was relatively potent. The antagonistic action of the analog can be because of a different mode of binding. The tryptamine side chain was examined and it was found that shortening of the chain abolishes potentiation of action of ACh, but the compound retains action as an antagonist. A possible explanation could be a different mode of binding. The development of the gramine analogs (i.e., **102**, **137**, and **138**) provides some preliminary information on the structural requirements for their antagonist action (Figure **24**). Additional gramine analogs should be explored for the antagonist action at α4β2 nACh receptors.

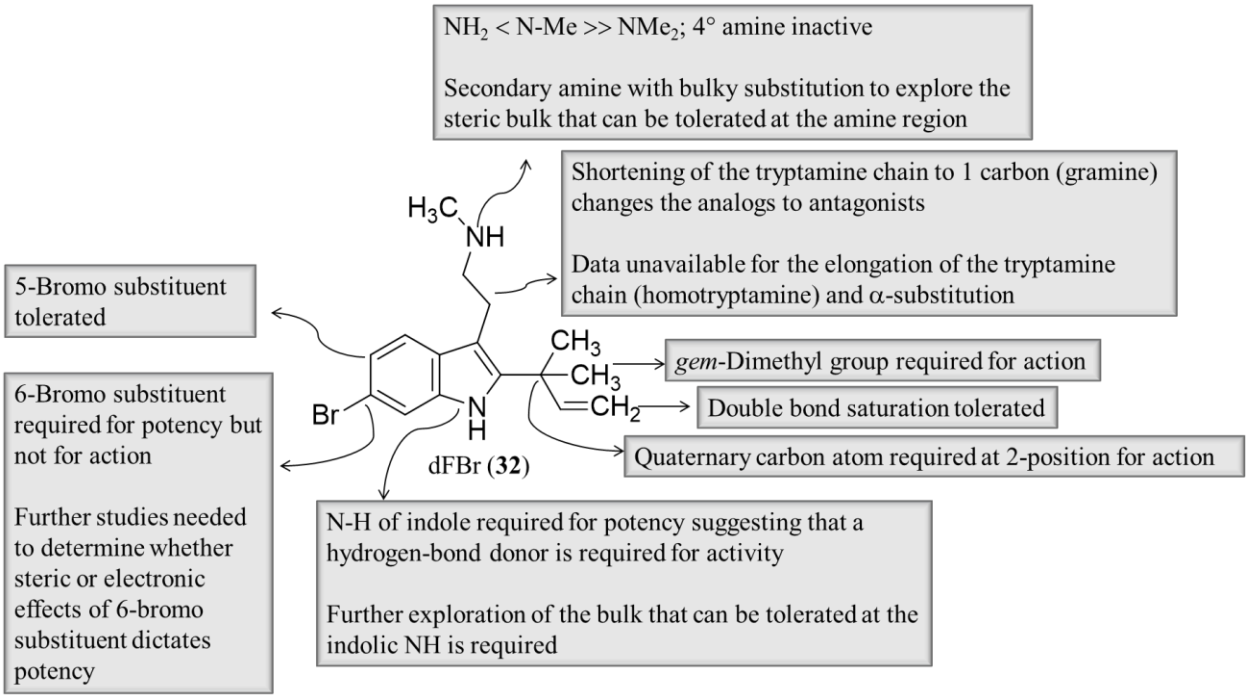


Figure 23. SAR for dFBr (**32**) at α4β2 nACh receptors.

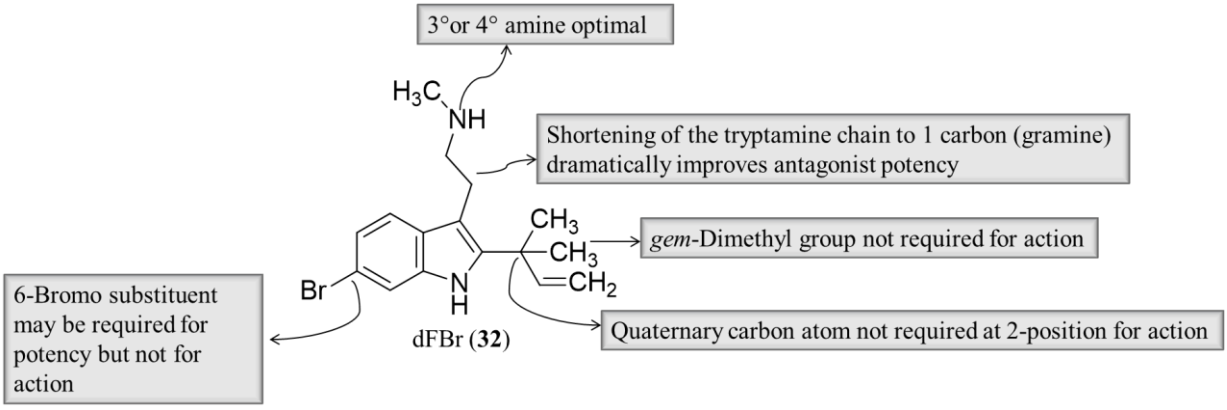


Figure 24. Preliminary SAR for antagonist action of dFBr (**32**) analogs at α4β2 nACh receptors.

The piperidine analog **97** was synthesized to examine a constrained side chain. The piperidine analog **97** possesses two features of interest; the distance of the amine from the indole nucleus, and branching of the 3-position tryptamine side chain. The secondary amines of dFBr (**32**) with *i*-propyl (i.e., **91**) and benzyl (i.e., **92**) substituents were synthesized to explore the region for steric bulk tolerance. The indolic N-H was investigated and it was seen that the hydrogen-bond donor is required at the receptor site, shown by the almost 10-fold decreased potency of **93** compared to **32**. The 1-methyl substitution of **93** provided information about the indolic N-H and steric bulk. Although a methyl substituent is not too bulky in volume, a benzyl substituent (i.e., **94**) was synthesized which might provide information about any region for bulk tolerance at the indolic N-H region. The isotryptamine analog **127** was similar in potency compared to N-methyl dFBr (**86**), which supports data for compound **93** suggesting that the N-H of the indole is optimal for potency. The 6-bromo substituent of dFBr (**32**) was investigated. Removal of the 6-bromo group was a major synthetic advantage for synthesis of 2-position substituted analogs of dFBr (**32**), although removal of the 6-bromo group reduced the activity by 20-fold; thus, the 6-bromo group is required for potency but not for action. The steric and electronic properties of the 6-bromo substituent were further explored. The 6-methyl analog (i.e., **99**) and the 6-trifluoromethyl analog (i.e., **100**) explored the importance of the 6-bromo group, and comparing them, it is still unclear whether electronic or steric properties are important for the potentiation of the ACh-induced response. Additional analogs are required for developing a QSAR for studying the electronic and steric effect for PAM action. The 5-position of dFBr (**32**), which is *para*- to the indolic N-H, was also examined and it was found that a 5-bromo substituent is tolerated; thus, compound **144** can possibly have a synergistic/additive effect towards the potency by having 5,6-

dibromo substituents. Compound **152** will be a useful tool to perform binding experiments to understand the binding sites. In conclusion a pharmacophore of dFBr (**32**) was developed.

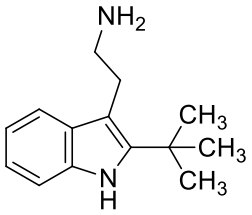
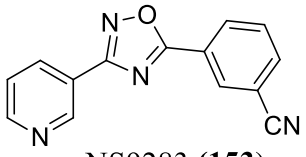


Figure 25. Minimal structural features required for the PAM action of dFBr (**32**). This simplified structure might represent a pharmacophore for such action.

There is growing interest in the discovery of PAMs which are selective for α4-, α2, or β2 containing subunits, and one such compound; NS9283 (**153**) has been found to be selective for the α2- and α4- containing nACh receptors. Compound **153** is a potent PAM at the $(\alpha2)_3(\beta2)_2$, $(\alpha2)_3(\beta4)_2$, and $(\alpha4)_3(\beta2)_2$ nACh receptors (EC_{50} = 1.3 μM, EC_{50} = 1.7 μM, EC_{50} = 3.3 μM respectively).[196] Attaining subunit selectivity and progressing towards subtype selectivity is the need of the hour for understanding the role of various subtypes of the nACh receptors.



NS9283 (**153**)

Binding experiments with tritiated dFBr will further help understand the binding of dFBr (**32**)-like ligands at the α4β2 nACh receptors. Compound **152**, being a radiolabeled ligand, can be used for high-throughput screening to identify other allosteric modulators for the α4β2 nACh receptors thus further helping in identification of possible new leads.

VII. Experimental

A. Synthesis

Melting points (mp) were measured on a Thomas-Hoover melting point apparatus using a glass capillary tube and are uncorrected. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Peak positions are given in parts per million (δ). UHPLC-MS were recorded on a Perkin Elmer Flexar UHLPC with AxION 2 Time of Flight (TOF) Mass Spectrometer and the molecular weight of the compounds was within 0.05% of calculated values. Elemental analysis was performed by Atlantic Microlab Inc. (Norcross, GA) for the elements indicated and the results are within 0.4% of calculated values. Infrared spectra were obtained on a Thermo Nicolet iS10 FT-IR. Flash chromatography was performed on CombiFlash Companion/TS (Telodyne Isco Inc., Lincoln, NE) using packed silica gel (Silica Gel 230-400 mesh) columns (RediSep Rf Normal-phase Silica Flash Column, Teledyne Isco Inc., Lincoln, NE). All reactions were monitored by thin-layer chromatography (TLC) on silica gel GHLF plates (250 μ, 2.5 x 10 cm; Analtech Inc., Newark, DE).

***N*-Methyl-6-bromotryptamine** (**35**). Compound **35** was prepared as described.[9] Borane dimethylsulfide (10.1M in THF, 3.63 mL) was added in a dropwise manner at 60 °C to a stirred solution of *N*-methyl-6-bromo-1*H*-indol-3-gloxylamide (**123**) (3.4 g, 12 mmol) in anhydrous THF (35 mL). The reaction mixture was heated at reflux for 3 h, cooled to room temperature, quenched with HCl (2N, to pH 1), and extracted with EtOAc (2 x 30 mL). The aqueous portion was basified with NaOH (3M, to pH 8) and extracted with CH_2Cl_2 (3 x 40 mL). The combined organic portion was washed with H_2O (2 x 50 mL), brine (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield 1.4 g (49%) of **35** as an off-white colored solid: mp 108-110 °C (lit.[9] mp 112-114 °C); ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 2.85 (m, 4H, CH_2), 6.95-7.41 (m, 4H, Ar), 8.0 (s, 1H, indolic NH).

***N*-Methyl-6-Bromo-2-*sec*-butyltryptamine Hydrogen Oxalate** (**37**). Compound **37** was prepared using a literature procedure for a similar compound.[178] Borane dimethylsulfide (10.1M in THF, 0.4 mL, 4.5 mmol) was added in a dropwise manner at 60 °C to a stirred solution of *N*-methyl-6-bromo-2-*sec*-butyl-1*H*-indol-3-glyoxylamide (**66**) (0.5 g, 1.5 mmol) in anhydrous THF (15 mL). The reaction mixture was allowed to stir at reflux overnight, cooled to 0 °C, quenched with H_2O (4 mL), acidified with HCl (2N, to pH 1), and heated at reflux for 30 min. The THF was evaporated under reduced pressure and the remaining aqueous portion was diluted with H_2O (50 mL) and basified with NaOH (3M, to pH 14) and extracted with CH_2Cl_2 (2 x 20 mL). The combined organic portion was washed with H_2O (2 x 15 mL), dried (Na_2SO_4), and solvent was evaporated under reduced pressure to yield 0.2 g (50%) of the free base of **37** as a yellow oil. Oxalic acid (0.1 g, 0.8 mmol) was added to the solution of free base of **37** in $CHCl_3$ (3 mL) and

the reaction mixture was allowed to stir at room temperature for 30 min. The precipitate was collected by filtration to yield a white-colored solid which upon recrystallization from *i*-PrOH afforded 0.1 g (43%) of **37** as a white solid: mp 126-129 ºC; ^{1}H NMR (DMSO-d_6) δ 0.70 (t, 3H, CH_3), 0.85-0.87 (m, 3H, CH_3), 1.42-1.50 (m, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.76-2.81 (m, 5H, 2 x CH_2, CH), 6.92 (d, 1H, Ar), 7.24-7.28 (m, 2H, Ar), 10.78 (s, 1H, COOH). Anal. Calcd. ($C_{15}H_{21}BrN_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, 50.01; H, 5.92; N, 6.86. Found: C, 49.90; H, 5.73; N, 6.68.

***N*-Methyl-6-Bromo-2-*tert*-butyltryptamine Hydrochloride** (**39**). Compound **39** was prepared using a literature procedure for a similar compound.[178] Borane dimethylsulfide (10.1M in THF, 0.3 mL, 2.5 mmol) was added in a dropwise manner at 60 °C to a stirred solution of *N*-methyl-6-bromo-2-*tert*-butyl-1*H*-indol-3-glyoxylamide (**72**) (0.3 g, 0.8 mmol) in anhydrous THF (15 mL). The reaction mixture was allowed to stir at reflux for 6 h, cooled to 0 °C, quenched with H_2O (4 mL), acidified with HCl (2N, to pH 1), and heated at reflux for 1 h. The THF was evaporated under reduced pressure and the remaining aqueous portion was diluted with H_2O (50 mL) and extracted with Et_2O (2 x 25 mL). The aqueous portion was basified with NaOH (3M, to pH 9) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic portion was washed with H_2O (3 x 70 mL), dried (Na_2SO_4), and solvent was evaporated under reduced pressure to yield 0.2 g (78%) of the free base of **39** as a yellow oil. The oil was dissolved in anhydrous Et_2O (15 mL) and cooled to 0 °C. HCl/Et_2O (10 mL) was added and the reaction mixture was allowed to stir overnight. The solvent was evaporated to yield a white solid which was recrystallized from *i*-PrOH to yield 0.05 g (17%) of **39** as a white solid: mp 246-248 °C; ^{1}H NMR (DMSO-d_6) δ 1.44 (s, 9H, $(CH_3)_3$), 2.61 (s, 3H, CH_3), 2.94-2.98 (m, 2H, CH_2), 3.16-3.19 (m, 2H, CH_2), 7.09-7.12

(dd, *J* = 8.4, 1.7 Hz, 1H, Ar), 7.46 (d, *J* = 1.7 Hz, 1H, Ar), 7.52-7.54 (d, *J* = 8.4 Hz, 1H, Ar), 8.87 (br s, 2H, NH_2^+ aliphatic), 10.76 (s, 1H, indolic NH). Anal. Calcd for ($C_{15}H_{21}BrN_2$·HCl) C, 52.11; H, 6.41; N, 8.10. Found: C, 52.28; H, 6.42; N, 8.01.

6-Bromo-2-(1,1-dimethylallyl)tryptamine Hydrochloride (**40**). Gaseous HCl was bubbled into a solution of *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethylcarbamate (**50**) (90 mg, 0.22 mmol) in anhydrous EtOAc (10 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature for 24 h and the solvent was evaporated to yield a white solid which was recrystallized from EtOAc/MeOH to yield 25 mg (33%) of **40** as white crystals: mp 256-258 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (s, 6H, $(CH_3)_2$), 2.85 (t, 2H, CH_2), 3.05 (t, 2H, CH_2), 5.08 (m, 2H, CH), 6.14 (m, 1H, CH), 7.12 (dd, 1H, Ar), 7.50 (m, 2H, Ar), 7.98 (s, 3H, NH_3^+ aliphatic), 10.76 (s, 1H, indolic NH). Anal. Calcd for ($C_{15}H_{19}BrN_2$·HCl·0.25H_2O) C, 51.74; H, 5.93; N, 8.05. Found: C, 51.90; H, 5.87; N, 8.00.

***N*-Methyl-2-*tert*-butyltryptamine Hydrogen Oxalate** (**43**). Compound **43** was prepared using a literature procedure for a similar compound.[197] Lithium aluminum hydride (0.3 g, 6.5 mmol) was suspended in dioxane (10 mL) and heated at reflux. A solution of *N*-methyl-2-*tert*-butyl-1*H*-indol-3-glyoxylamide (**58**) (0.2 g, 0.7 mmol) was added, and the reaction mixture was allowed to reflux overnight. The reaction mixture was cooled to room temperature, diluted with THF (10 mL) and quenched with MeOH (3 mL) and NaOH (3M, 3 mL). The reaction mixture was filtered and the filtrate was diluted with THF (15 mL) and further heated at reflux for 30 min, the organic solvent was evaporated under reduced pressure to yield a yellow oil. The residue was purified by

column chromatography (silica gel; CH_2Cl_2/MeOH 100:1 to 10:1) to afford 0.05 g (33%) of the free base as a yellow oil. A solution of the free base in anhydrous THF (2 mL) and anhydrous Et_2O (5 mL) was cooled to 0 °C. Saturated oxalic acid solution in anhydrous Et_2O (5 mL) was added and the reaction mixture was allowed to warm to room temperature and stirred overnight. The precipitate was collected by filtration to yield a white-colored solid which upon recrystallization from *i*-PrOH afford 0.03 g (56%) of **43** as white flakes: mp 194-195 °C; ^{1}H NMR (DMSO-d_6) δ 1.43 (s, 9H, $(CH_3)_3$), 2.64 (s, 3H, CH_3), 2.94 (d, 2H, CH_2), 3.15 (d, 2H, CH_2), 6.96-7.51 (m, 4H, Ar), 10.55 (s, 1H, indolic NH). Anal. Calcd for ($C_{18}H_{30}N_2O \cdot C_2H_2O_4 \cdot C_3H_8O$) C, 63.14; H, 8.49; N, 7.19. Found: C, 62.81; H, 8.46; N, 7.18.

3-[(*E*)-2-Nitroethenyl]-6-bromo-1*H*-indole (**47**). Compound **47** was prepared as described.[176] In a 2-neck flask, 1-dimethylamino-2-nitroethylene (1.2 g, 10.2 mmol) was added to a solution of 6-bromoindole (**46**) (2.0 g, 10.2 mmol) in anhydrous CH_2Cl_2 (10 mL) under a N_2 atmosphere. Trifluoroacetic acid (1 mL) was added and the reaction mixture was allowed to stir at room temperature for 1 h, quenched carefully with a saturated aqueous solution of $NaHCO_3$ and the residue was collected by filtration and dried to yield 2.4 g (88%) of **47** as a yellow solid: mp 193-195 °C (decomp) (lit.[176] mp not reported); ^{1}H NMR ($CDCl_3$) δ 7.37 (dd, *J* = 8.64, 1.56 Hz, 1H, Ar), 7.58 (m, 3H, Ar), 7.66 (d, *J* = 13.5 Hz, 1H, CH), 8.17 (d, *J* = 13.5 Hz, 1H, CH), 8.67 (s, 1H, indolic NH).

6-Bromotryptamine (**48**). Compound **48** was prepared as described.[176] To a round bottom flask cooled to 0 °C (ice bath), $NaBH_4$ (0.8 g, 21 mmol) was added followed by addition of anhydrous

THF (18 mL) and $BF_3 \cdot Et_2O$ (5.65 mL, 23 mmol). The ice bath was removed and the contents were allowed to stir at room temperature for 15 min. A solution of 3-[(*E*)-2-nitroethenyl]-6-bromo-1*H*-indole (**47**) (1.0 g, 4 mmol) in anhydrous THF (11 mL) was added in a dropwise manner over 10 min, and the reaction mixture was heated at reflux for 2 h, cooled to room temperature and quenched with careful addition of ice. The mixture was acidified with HCl (2N, to pH 2) and heated at 80-85 °C for 2 h. After cooling, the acidic solution was extracted with Et_2O (2 x 25 mL). The aqueous portion was basified with NaOH (3M, to pH 10) to liberate the amine. NaCl (10 g) was added and the product was extracted with Et_2O (3 x 30 mL). The combined organic portion was washed with H_2O (3 x 50 mL), brine (50 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure to yield 0.33 g (40%) of **48** as a brown oil: ^{1}H NMR ($CDCl_3$) δ 2.81 (t, 2H, CH_2), 2.95 (t, 2H, CH_2), 6.93 (s, 1H, Ar), 7.14 (dd, *J* = 8.4, 1.6 Hz, 1H, Ar), 7.39 (d, *J* = 8.4 Hz, 1H, Ar), 7.43 (s, *J* = 1.5 Hz, 1H, Ar), 8.2 (s, 1H, indolic NH).

***tert*-Butyl-2-(6-bromo-1*H*-indol-3-yl)ethylcarbamate** (**49**). Compound **49** was prepared as described.[9] Di-*tert*-butyl dicarbonate (0.3 g, 1.3 mmol) and Et_3N (0.2 mL, 1.3 mmol) were added to an anhydrous DMF (15 mL) solution of 6-bromotryptamine (**48**) (0.3 g, 1.3 mmol) and the reaction mixture was allowed to stir at room temperature for 24 h. The reaction mixture was quenched with ice-cold H_2O (100 mL) and extracted with EtOAc (3 x 40 mL). The combined organic portion was washed with H_2O (3 x 40 mL), brine (50 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure to afford 0.26 g (60%) of **49** as a white foam which solidified on drying: mp 100-102 °C (lit.[178] mp not reported); ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 9H, $(CH_3)_3$), 2.85 (t, 2H, CH_2), 3.37 (t, 2H, CH_2), 4.51 (s, 1H, NH aliphatic), 6.95 (s, 1H, Ar),

7.15 (dd, *J* = 8.5, 1.6 Hz, 1H, Ar), 7.39 (d, *J* = 8.4 Hz, 1H, Ar), 7.45 (s, *J* = 1.5 Hz, 1H, Ar), 7.94 (s, 1H, indolic NH).

***tert*-Butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethylcarbamate** (**50**). Compound **50** was prepared using a literature procedure for a similar compound.[198] *tert*-Butyl hypochlorite (0.10 g, 0.9 mmol) was added to a solution of *tert*-butyl-2-(6-bromo-1*H*-indol-3-yl)ethylcarbamate (**49**) (0.25 g, 0.7 mmol) and Et_3N (0.90 g, 0.9 mmol) in anhydrous THF (10 mL) at -78 °C and allowed to stir for 45 min. Freshly prepared prenyl 9-BBN[179] (1.5 mmol) was added in a dropwise manner over 20 min while maintaining the temperature below -78 °C. The reaction mixture was allowed to warm to room temperature and stirred for an additional 2 h. NaOH (3M, 3 mL) and H_2O_2 (30% v/v, 3 mL) were added dropwise and stirring was continued for another 1 h at room temperature. The reaction mixture was diluted with Et_2O (100 mL), the organic layer was separated and washed with H_2O (3 x 30 mL), brine (40 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to yield a crude residue which was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 10:1) to afford 0.1 g (33%) of **50** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, $(CH_3)_3$), 1.48 (s, 6H, $(CH_3)_2$), 2.93 (t, 2H, CH_2), 3.29 (t, 2H, CH_2), 5.08 (m, 2H, CH), 6.03 (m, 1H, CH), 7.10 (dd, 1H, Ar), 7.35 (m, 2H, Ar), 7.78 (s, 1H, indolic NH). Compound **50** was used for the preparation of compound **40**.

2,2-Dimethyl-*N*-(*o*-tolyl)propionamide (**52**). Compound **52** was prepared as described.[180] A solution of trimethylacetyl chloride (4.6 mL, 37 mmol) in anhydrous CH_2Cl_2 (10 mL) was added

to a solution of *o*-toludine (**51**) (4.01 mL, 37.3 mol) and Et_3N (5.2 mL, 37.3 mol) in anhydrous CH_2Cl (30 mL) which was previously cooled to 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The reaction was quenched with H_2O (50 mL). The organic layer was washed with H_2O (3 x 50 mL), dried (Na_2SO_4) and evaporated to dryness under reduced pressure to yield a crude white solid which upon recrystallization from CH_2Cl_2/hexanes yielded 5.95 g (83%) of **52** as a white solid: mp 109-111 °C (lit.[180] mp 109-111 °C); ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 9H, $(CH_3)_3$), 2.28 (s, 3H, CH_3), 7.0-7.5 (m, 4H, Ar).

2-*tert*-Butyl-1*H*-indole (**55**). Compound **55** was prepared as described.[181] *n*-Butyllithium (1.6 M in hexane, 19.6 mL, 31 mmol) was added in a dropwise manner at 0 °C to a stirred solution of 2,2-dimethyl-*N*-(*o*-tolyl)propionamide (**52**) (3 g, 16 mmol) in anhydrous THF (20 mL). The reaction mixture was allowed to stir at 0 °C for 1 h, at room temperature for 8 h and heated at reflux for 2 h. The reaction was cooled to room temperature, diluted with EtOAc (35 mL), and quenched with saturated NH_4Cl (20 mL). The organic layer was separated, washed with H_2O (2 x 50 mL), dried (Na_2SO_4) and evaporated to dryness to yield a crude brown oil which on crystallization with MeOH/H_2O yielded 2.0 g (74%) of **55** as a light brown solid: mp 73-75 °C (lit.[181] mp 77 °C); ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, $(CH_3)_3$), 6.32 (s, 1H, CH), 7.15-7.61 (m, 4H, Ar), 7.99 (s, 1H, indolic NH).

***N*-Methyl-2-*tert*-Butyl-1*H*-indol-3-glyoxylamide** (**58**). Compound **58** was prepared using a literature procedure for a similar compound.[199] A solution of 2-*tert*-butyl-1*H*-indole (**55**) (0.2 g, 1 mmol) in anhydrous Et_2O (10 mL) in a 2-neck flask was chilled to -5 °C and N_2 was bubbled in

for 5 min. Oxalyl chloride (0.2 mL, 2 mmol) was added in a dropwise manner and the reaction mixture was allowed to stir at 0 °C for 6 h. The organic solvent was evaporated under reduced pressure and to the precipitate was added methylamine (40% in H_2O, 5 mL) and the solution was allowed to stir at room temperature overnight. The precipitate was collected by filtration, washed with H_2O (3 x 50 mL), dried and recrystallized from MeOH to yield 0.2 g (56%) of **58** as a brown solid: mp 190-191 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, $(CH_3)_3$), 3.1 (s, 3H, CH_3), 6.77 (s, 1H, NH aliphatic), 7.07-7.45 (m, 4H, Ar), 7.76 (s, 1H, indolic NH). Compound **58** was employed in synthesis of **43**.

***tert*-Butyl-2-(6-bromo-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate** (**61**). Compound **61** was prepared as described.[9] Di-*tert*-butyl dicarbonate (0.9 g, 4 mmol) and Et_3N (0.5 g, 4 mmol) were added to an anhydrous DMF (15 mL) solution of *N*-methyl-6-bromotryptamine (**35**) (1.0 g, 4 mmol) and allowed to stir at room temperature for 24 h. The reaction mixture was quenched with H_2O (40 mL) and extracted with EtOAc (3 x 40 mL). The combined organic portion was washed with H_2O (3 x 40 mL), brine (50 mL), dried (Na_2SO_4) and evaporated to dryness to afford 1.3 g (94%) of **61** as a white foam: mp 124-126 °C (lit.[9] mp 120-124 °C). The product was used without further purification in the attempted synthesis of **62**.

1-(3-Bromophenyl)-2-(3-methylpentan-2-ylidene)hydrazine (**64**). Compound **64** was prepared according to a literature procedure for a similar compound.[200] To a solution of 3-methylpentan-2-one (0.1 mL, 0.9 mmol) in MeOH (10 mL) was added 3-bromophenylhydrazine (**63**) (0.2 g, 0.9 mmol) along with few drops of gl. HOAc and the reaction mixture was allowed to stir at

room temperature for 2 h. The solvent was removed under reduced pressure and the residue was dried to afford 0.23 g (95%) of **64** as a yellow-colored oil: ^{1}H NMR ($CDCl_3$) δ 0.54 (t, 3H, CH_3), 0.98 (d, 3H, CH_3), 1.45-1.47 (m, 2H, CH_2), 1.56-1.58 (m, 1H, CH), 2.04 (s, 3H, CH_3), 6.69 (d, 1H, Ar), 6.94-6.96 (m, 2H, Ar), 7.30 (m, 1H, Ar). The product was used without further purification in the attempted preparation of **65**.

6-Bromo-2-*sec*-butyl-1*H*-indole (**65**). Compound **64** was prepared according to a literature procedure for similar compound.[184] A solution of *sec*-butyl magnesium chloride in THF (2.0 M, 5 mL) was added in a dropwise manner to a solution of 1-(2-amino-4-bromophenyl)-2-chloroethan-1-one (**75**) (1.0 g, 4.0 mmol) in anhydrous THF (20 mL) at -10 °C and allowed to stir in an ice bath for 15 min. The reaction mixture was allowed to warm to room temperature, and quenched with aqueous saturated NH_4Cl (10 mL), extracted with MTBE (2 x 30 mL) and washed with brine (10 mL). The organic portion was combined, dried (Na_2SO_4) and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography (silica gel) using hexanes/EtOAc (9:1) to afford 0.64 g (38%) of **65** as a yellow solid: mp 37-38 °C; ^{1}H NMR ($CDCl_3$) δ 0.83 (t, 3H, CH_3), 1.25 (d, 3H, CH_3), 1.6-1.65 (m, 2H, CH_2), 2.72-2.75 (m, 1H, CH), 6.14 (s, 1H, ArH), 7.07 (d, J = 8.0 Hz, 1H, ArH), 7.29 (s, 1H, ArH), 7.35 (s, 1H, ArH), 7.78 (br s, 1H, indolic NH).

***N*-Methyl-6-bromo-2-*sec*-butyl-1*H*-indol-3-glyoxylamide** (**66**). Compound **66** was prepared using a literature procedure for a similar compound.[201] A solution of 6-bromo-2-*sec*-butyl-1*H*-indole-3-glyoxylchloride (**81**) (0.6 g, 1.9 mmol) in anhydrous THF (2 mL) was added to

methylamine (40% in H_2O, 25 mL) cooled to °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted with H_2O (100 mL) and the residue was collected by filtration, washed with H_2O (3 x 50 mL) and dried to yield 0.5 g (82%) of **66** as a buff-colored solid: The compound was impure even after repeated attempts at crystallization with various solvents. Compound **66** was used without any further purification in the preparation of compound **37**.

4-Bromo-2-nitrotoluene (**68**). Compound **68** was prepared as described.[202] A solution of 4-methyl-3-nitroaniline (**67**) (10.0 g, 65.7 mmol) in H_2O (25 mL) was added to conc. H_2SO_4 (25 mL) in H_2O (150 mL) in a flask. The mixture was heated until all the starting material was dissolved. On cooling the solid crystals were collected by filtration, washed with cold H_2O (3 x 50 mL) to give the sulfonic acid salt as dark-yellow crystals (A) which was dissolved in H_2O (70 mL) and HBr (62%, 17.5 mL) and the temperature was maintained at -5 °C. In a separate flask, a solution of $NaNO_2$ (8.2 g, 118.3 mmol) in H_2O (26 mL) was added slowly to the above solution while maintaining the temperature below 0 °C (B). In a 3-neck flask, CuBr (9.5 g, 65.7 mmol) was dissolved in HBr (62%, 12.5 mL) and the mixture was heated to reflux. To this heated solution, solution B was added slowly over 15 min and stirring was continued at reflux for 1.5 h. The reaction mixture was allowed to cool to room temperature and extracted with MTBE (3 x 50 mL). The combined organic portion was washed with H_2O (2 x 100 mL), 5% NH_4OH (2 x 150 mL), brine (150 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford 7.1 g (50%) of **68** as yellow, needle-shaped crystals: mp 42-44 °C (lit.[202] mp 43-45 °C), ^1H

NMR ($CDCl_3$) δ 2.55 (s, 3H, CH_3), 7.32 (d, *J* = 8.2 Hz, 1H, Ar), 7.72 (dd, *J* = 8.2, 1.9 Hz, 1H, Ar), 8.22 (d, *J* = 1.9 Hz, 1H, Ar).

(E/Z)-1-(4-Bromo-2-nitrostyryl)pyrrolidine (**69**). Compound **69** was prepared as described.[203] A solution of 4-bromo-2-nitrotoluene (**68**) (1 g, 4.6 mmol), DMF-DMA (0.8 mL, 5.6 mmol) and pyrrolidine (0.5 mL, 5.6 mmol) in DMF (10 mL) was heated to 110 °C until the TLC analysis showed consumption of **68**. The dark red solution was allowed to cool to room temperature, diluted with Et_2O (30 mL) and washed with H_2O (3 x 50 mL). The combined H_2O portion was further extracted with Et_2O (2 x 25 mL) and the combined organic portion was washed with H_2O (3 x 50 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to afford 1 g (70%) of **69** as a red-colored crystals: mp 61-63 °C (lit.[176] mp not reported) ^1H NMR ($CDCl_3$) δ 1.92-1.96 (m, 4H, CH_2), 3.32-3.36 (m, 4H, CH_2), 5.80 (d, *J* = 13.4 Hz, 1H, CH), 7.28 (d, *J* = 13.3 Hz, 1H, CH), 7.34 (d, *J* = 8.3 Hz, 1H, Ar), 7.58 (d, *J* = 1.8 Hz, 1H, Ar), 8.12 (dd, *J* = 8.2, 1.9 Hz, 1H, Ar). Compound **69** was used in the attempted synthesis of **70**.

6-Bromo-2-*tert*-butyl-1*H*-indole (**71**). Compound **71** was prepared using a literature procedure for a similar compound.[188] Zinc iodide (0.2 g, 0.6 mmol) was added in one portion to a solution of 5-bromo-2-(3,3-dimethylbut-1-yn-1-yl)aniline (**80**) (2.9 g, 11.5 mmol) in anhydrous toluene (40 mL) and the reaction mixture was heated at reflux for 41 h. The reaction mixture was allowed to cool to room temperature and washed with H_2O (3 x 50 mL). The aqueous portion was extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic portion was washed with H_2O (3 x 70 mL), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to yield a

crude product which upon recrystallization from hexanes afforded 1.5 g (51%) of **71** as buff-colored flakes: mp 110-111 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H, $(CH_3)_3$), 6.24 (s, 1H, Ar), 7.15-7.18 (dd, *J* = 8.4, 1.2 Hz, 1H, Ar), 7.37-7.40 (d, *J* = 9 Hz, 1H, Ar), 7.46 (d, *J* = 1.2 Hz, 1H, Ar), 7.94 (br s, 1H, indolic NH).

***N*-Methyl-6-bromo-2-*tert*-butyl-1*H*-indol-3-glyoxylamide** (**72**). Compound **72** was prepared using a literature procedure for a similar compound.[201] A solution of 6-bromo-2-*tert*-butyl-1*H*-indole (**71**) (1.3 g, 5 mmol) in anhydrous Et_2O (25 mL) in a 2-neck flask was chilled to -5 °C and N_2 was bubbled in for 5 min. Oxalyl chloride (0.9 mL, 10 mmol) was added in a dropwise manner and the reaction mixture was heated at reflux overnight. The Et_2O was evaporated and the precipitate was dissolved in THF (5 mL) to which was added methylamine (40% in H_2O, 25 mL) and the solution was allowed to stir at room temperature for 15 h. The reaction mixture was diluted with H_2O (100 mL) and the residue was collected by filtration, washed with H_2O (3 x 50 mL), hexanes (3 x 50 mL) and dried to yield 1.4 g (77%) of **72** as a buff-colored solid: mp 190-193 °C (decomp); ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 9H, $(CH_3)_3$), 3.04 (s, 3H, CH_3), 6.85 (br s, 1H, NH), 7.28-7.29 (dd, *J* = 8.4, 1.4 Hz, 1H, Ar), 7.50 (d, *J* = 1.4 Hz, 1H, Ar), 7.54-7.57 (d, *J* = 8.4 Hz, 1H, Ar), 8.66 (br s, 1H, indolic NH). Compound **72** was used in the preparation of compound **39**.

1-(2-Amino-4-bromophenyl)-2-chloroethan-1-one (**75**). Compound **75** was prepared according to a literature procedure for a similar compound.[184] 3-Bromoaniline (**74**) (5.0 g, 29 mmol) and chloroacetonitrile (4.6 mL, 73 mmol) were added sequentially to a mixture of $AlCl_3$ (4.6 g, 35

mmol) and BCl_3 solution (1.0 M in CH_2Cl_2, 35 mL, 35 mmol) in CH_2Cl_2 (30 mL) in an ice bath and the cloudy solution was allowed to stir for 5 h at room temperature and heated at reflux overnight. The reaction mixture was cooled in an ice bath, quenched with 2 N HCl 150 mL, heated at reflux for 20 min, allowed to cool to room temperature, and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic portion was washed with H_2O (3 x 50 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to afford 6.0 g (69%) of **75** as a yellow solid: mp 139-141 °C; ^{1}H NMR ($CDCl_3$) δ 4.54 (s, 2H, CH_2), 6.28 (br s, 2H, NH_2), 6.70 (dd, *J* = 8.68, 1.84 Hz, 1H, Ar), 6.81 (d, *J* = 1.84 Hz ,1H, Ar), 7.40 (d, *J* = 8.68 Hz, 1H, Ar). The product was used without further purification in the attempted synthesis of **71**. It was also used in the preparation of compound **65**.

4-Bromo-2-nitroaniline (**77**). Compound **77** was prepared as described.[185] *N*-Bromosuccinimde (12.9 g, 72 mmol) was added slowly over 10 min to a solution of commercially available 2-nitroaniline (**77**) (10.0 g, 72 mmol) in gl. HOAc (30 mL) and the solution was heated to 55 °C. The reaction mixture was allowed to stir at 40-50 °C for 5 h, upon which it was poured into ice-water (100 mL). The product was collected by filtration, washed with cold H_2O (3 x 50 mL), dried and recrystallized from EtOH to afford 12.4 g (79%) of **77** as orange, needle-shaped crystals: mp 110-111 °C (lit.[185] mp 111-112 °C); ^{1}H NMR ($CDCl_3$) δ 6.01 (br s, 2H, NH_2), 6.65 (d, *J* = 8.9 Hz, 1H, Ar), 7.36 (dd, *J* = 8.8, 2.3 Hz, 1H, Ar), 8.20 (d, *J* = 2.3 Hz, 1H, Ar).

4-Bromo-2-nitroiodobenzene (**78**). Compound **78** prepared as described.[186] A solution of 4-bromo-2-nitroaniline (**77**) (7.3 g, 34 mmol) in anhydrous THF (50 mL) was added in a dropwise

manner to $BF_3 \cdot Et_2O$ (15.7 mL, 125 mmol) at -30 °C. The reaction mixture was allowed to stir for 10 min and then a solution of *t*-BuONO (13.3 mL, 111 mmol) in anhydrous THF (40 mL) was added in a dropwise manner. The reaction mixture was allowed to warm to -10 °C, diluted with Et_2O (100 mL), and stirring was continued at room temperature for 1 h until a pale-yellow solid precipitate was formed. The solid was collected by filtration and washed with Et_2O (50 mL) to afford a pale-yellow solid, which was then slowly added to a mixture of KI (7.1 g, 43 mmol) and I_2 (5.4 g, 21 mmol) in MeCN (100 mL). The reaction mixture was allowed to stir at room temperature for 1 h. A saturated aqueous solution of $Na_2S_2O_3$ (250 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (2 x 90 mL). The combined organic portion was washed with H_2O (2 x 100 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to afford 9.3 g (84%) of **78** as a yellow powder: mp 86-88 °C (lit.[186] mp 87-89 °C); ^1H NMR ($CDCl_3$) δ 7.40 (dd, *J* = 8.4, 2.2 Hz, 1H, Ar), 7.89 (d, *J* = 8.4 Hz, 1H, Ar), 8.0 (d, *J* = 2.2 Hz, 1H, Ar).

5-Bromo-2-iodoaniline (**79**). Compound **79** was prepared as described.[187] A mixture of 4-bromo-2-nitroiodobenzene (**78**) (8.4 g, 26 mmol), Fe powder (6.1 g, 110 mmol) in gl. HOAc (45 mL) and absolute EtOH (40 mL) was heated at reflux for 1.5 h. The reaction mixture was allowed to cool to room temperature and a saturated aqueous solution of $NaHCO_3$ (200 mL) was added. The mixture was extracted with Et_2O (4 x 50 mL) and the combined organic portion was washed with H_2O (3 x 100 mL), brine (100 mL), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to yield a crude residue. The crude product was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 100:5) to afford 4.6 g (60%) of **79** as a

cream-colored solid: mp 54-55 °C (lit.[187] mp 55 °C); ^{1}H NMR ($CDCl_3$) δ 4.14 (br s, 2H, NH_2), 6.60 (dd, *J* = 8.4, 2.2 Hz, 1H, Ar), 6.88 (d, *J* = 2.2 Hz, 1H, Ar), 7.45 (d, *J* = 8.4 Hz, 1H, Ar).

5-Bromo-2-(3,3-dimethylbut-1-yn-1-yl)aniline (**80**). Compound **80** was prepared using a literature procedure for a similar compound.[204] A mixture of 5-bromo-2-iodoaniline (**79**) (4.0 g, 13.4 mmol), CuI (0.1 g, 0.7 mmol) and $PdCl_2(PPh_3)_2$ (0.5 g, 0.7 mmol) was suspended in anhydrous THF (50 mL). Et_3N (5.6 mL, 40.3 mmol) and 3,3-dimethylbut-1-yne (2.2 mL, 17.5 mmol) were added successively and the reaction mixture was allowed to stir at room temperature for 5 h. The reaction mixture was diluted with Et_2O (100 mL), filtered through Celite, and the organic portion was washed sequentially with H_2O (3 x 100 mL), a saturated aqueous solution of NH_4Cl (50 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to yield a crude residue. The crude product was purified by column chromatography (silica gel; hexanes/EtOAc 100:1 to 75:1) to afford 2.9 g (86%) of **80** as a light-yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 9H, $(CH_3)_3$), 4.19 (br s, 2H, NH_2), 6.75-6.79 (dd, *J* = 8.4, 1.9 Hz, 1H, Ar), 6.84 (d, *J* = 1.9 Hz, 1H, Ar), 7.06-7.08 (d, *J* = 8.1 Hz, 1H, Ar). Compound **80** was used in the preparation of compound **71**.

6-Bromo-2-*sec*-butyl-1*H*-indol-3-glyoxylchloride (**81**). Compound **81** was prepared using a literature procedure for a similar compound.[201] A solution of 6-bromo-2-*sec*-butyl-1*H*-indole (**65**) (0.5 g, 1.98 mmol) in anhydrous Et_2O (20 mL) in a 2-neck flask was chilled to -5 °C and N_2 was bubbled in for 5 min. Oxalyl chloride (0.2 mL, 2.40 mmol) was added in a dropwise manner and allowed to stir for 30 min at 0 °C. The Et_2O was evaporated and the residue was washed

with Et_2O (2 x 5 mL) to remove excess oxalyl chloride to afford 0.6 g (94%) of **81** as a yellow colored oil. The product was used without any further purification for the synthesis of compound **66**.

2-(1,1-Dimethylallyl)tryptamine Hydrochloride (**82**). Gaseous HCl was bubbled into a solution of *tert*-butyl-2-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethylcarbamate (**85**) (0.4 g) in anhydrous Et_2O (20 mL) at 0 °C. The reaction mixture was allowed to stir for 24 h and the solvent was evaporated to yield a purple solid which was recrystallized from *i*-PrOH/Et_2O to yield 0.1 g (28%) of **82** as brown crystals: mp 231-232 °C; ^{1}H NMR (DMSO-d_6) δ 1.50 (s, 6H, CH_3), 2.86 (t, 2H, CH_2), 3.07 (t, 2H, CH_2), 5.06-5.11 (m, 2H, CH_2), 6.12-6.18 (m, 1H, CH), 6.95-7.06 (m, 2H, Ar), 7.34 (d, *J* = 7.9 Hz, 1H, Ar), 7.51 (d, *J* = 7.8 Hz, 1H, Ar), 8.05 (br s, 3H, NH_3^+ aliphatic), 10.59 (br s, 1H, indolic NH). Anal. Calcd for ($C_{15}H_{20}N_2$·HCl) C, 68.04; H, 7.99; N, 10.58. Found: C, 67.89; H, 7.99; N, 10.46.

***tert*-Butyl-2-(1*H*-indol-3-yl)ethylcarbamate** (**84**). Compound **84** was prepared as described.[189] Triethylamine (0.9 g, 8.5 mmol) and di-*tert*-butyl dicarbonate (1.9 g, 8.5 mmol) were added to a solution of tryptamine (**83**) (1.4 g, 8.5 mmol) in anhydrous DMF (30 mL) and allowed to stir for 20 h at room temperature. The reaction mixture was quenched with ice and H_2O (80 mL) and extracted with EtOAc (3 x 40 mL). The combined organic portion was washed with H_2O (3 x 40 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a crude oil. The crude product was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 4:1) to afford 2.0 g (91%) of **84** as a white solid: mp 92-94 °C (lit.[189]

mp 96 °C); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, CH_3), 2.96 (t, 2H, CH_2), 3.32 (m, 2H, CH_2), 4.55 (br s, 1H, NH aliphatic), 6.98-7.08 (m, 3H, Ar), 7.21 (d, *J* = 8.0 Hz, 1H, Ar), 7.48 (d, *J* = 7.8 Hz, 1H, Ar), 7.79 (br s, 1H, indolic NH).

***tert*-Butyl-2-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethylcarbamate** (**85**). Compound **85** was prepared using a literature procedure for a similar compound.[198] *tert*-Butyl hypochlorite (0.5 g, 4.6 mmol) was added to a solution of *tert*-butyl-2-(1*H*-indol-3-yl)ethylcarbamate (**84**) (1.0 g, 3.8 mmol) and Et_3N (0.5 g, 4.6 mmol) in anhydrous THF (25 mL) at -78 °C and the solution was allowed to stir for 45 min. Freshly prepared prenyl 9-BBN[179] (7.7 mmol) was added in a dropwise manner over 15 min while maintaining the temperature at -55 °C. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 3 h. Sodium hydroxide (3M, 10 mL) and H_2O_2 (30% v/v, 10 mL) were added in a dropwise manner and the reaction mixture was allowed to stir for 1 h at room temperature. The reaction was diluted with Et_2O (100 mL), the organic layer was separated and washed with H_2O (3 x 60 mL), brine (80 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to yield a crude residue which was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 5:1) to afford 0.5 g (36%) of **85** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, CH_3), 1.47 (s, 6H, CH_3), 2.96 (t, 2H, CH_2), 3.32 (m, 2H, CH_2), 4.53 (br s, 1H, NH aliphatic), 5.06-5.11 (m, 2H, CH_2), 6.01-6.08 (m, 1H, CH), 6.98-7.08 (m, 2H, Ar), 7.21 (d, *J* = 8.0 Hz, 1H, Ar), 7.48 (d, *J* = 7.8 Hz, 1H, Ar), 7.79 (br s, 1H, indolic NH). Compound **85** was used in the preparation of compound **82**.

***N,N*-Dimethyl-6-bromo-2-(1,1-dimethylallyl)tryptamine Hydrogen Oxalate** (**86**). A solution of *N,N*-dimethyl-6-bromo-2-(1,1-dimethylallyl)tryptamine (**101**) (0.05 g) in anhydrous $CHCl_3$ (3 mL) was allowed to stir at 0 °C for 1.5 h. The precipitate was collected by filtration and the white solid obtained was recrystallized from *i*-PrOH/Et_2O to yield 0.02 g (36%) of **86** as a white crystals: mp 172-173 °C; NMR (DMSO-d_6) δ 1.58 (s, 6H, $(CH_3)_2$), 2.73 (s, 6H, $N(CH_3)_2$), 4.39 (m, 4H CH_2), 5.25-5.29 (m, 2H, CH_2), 6.31-6.38 (m, 1H, CH), 7.25 (dd, *J* = 8.4, 1H, Ar), 7.59 (d, *J* = 1.6 Hz, 1H, Ar), 7.70 (d, *J* = 8.4, 1.5 Hz, 1H, Ar), 11.27 (s, 1H, COOH). Anal. Calcd for ($C_{17}H_{23}BrN_2 \cdot C_2H_2O_4$) C, 53.40; H, 6.36; N, 6.55. Found: C, 53.39; H, 6.06; N, 6.50.

***N,N,N*-Trimethyl-6-bromo-2-(1,1-dimethylallyl)tryptamine Methiodide** (**87**). A solution of *N,N*-dimethyl-6-bromo-2-(1,1-dimethylallyl)tryptamine (**101**) (0.05g, 0.2 mmol) in anhydrous *i*-PrOH (4 mL) was cooled to 0 °C (ice-bath). Iodomethane (0.06 g, 0.5 mmol) was added and the reaction mixture was allowed to stir for 22 h at room temperature under a N_2 atmosphere. The precipitate was collected by filtration and washed with anhydrous Et_2O (3 x 15 mL), air dried and crystallized from MeOH/*i*-PrOH to afford 0.02 g (30%) of **87** as yellow crystals: mp 208-210 °C; ^{1}H NMR (CD_3CN) δ 1.54 (s, 6H, $(CH_3)_2$), 3.15 (s, 9H, $N(CH_3)_3$), 3.22-3.38 (m, 4H CH_2), 5.25 (m, 2H, CH_2), 6.22 (m, 1H, CH), 7.22 (dd, *J* = 8.4, 1.7 Hz, 1H, Ar), 7.46 (d, *J* = 8.5 Hz, 1H, Ar), 7.59 (d, *J* = 1.5 Hz, 1H, Ar), 9.35 (br s, 1H, indolic NH). Anal. Calcd for ($C_{18}H_{26}BrIN_2 \cdot C_3H_8O$) C, 46.94; H, 6.38; N, 5.21. Found: C, 46.73; H, 6.29; N, 5.25.

***N*-*iso*-Propyl-6-bromo-2-(1,1-dimethylallyl)tryptamine Hydrochloride** (**91**). HCl gas was bubbled into a 0 °C solution of *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-

N-*iso*-propylcarbamate (**121**) (0.4 g) in dry anhydrous EtOAc (10 mL). The reaction mixture was allowed to stir for 24 h and the solvent was evaporated to yield a white solid which was recrystallized from MeOH to yield 0.1g, (29%) of **91** as white crystals: mp 295-299 °C; ^{1}HNMR (DMSO-d_6) δ 1.25 (d, 6H, CH_3), 1.50 (s, 6H, CH_3), 2.88-2.92 (m, 2H, CH_2), 3.09-3.14 (t, 2H, CH_2), 5.09-5.13 (d, 2H CH_2), 6.12-6.19 (m, 1H, CH), 7.11-7.14 (d, *J* = 8.4, 1.8 Hz, 1H, Ar), 7.49 (d, *J* = 1.7 Hz, 1H, Ar), 7.53-7.55 (d, *J* = 8.4 Hz, 1H, Ar), 8.86 (br s, 2H, NH_2^+ aliphatic), 10.78 (s, IH, indolic NH). Anal. Calcd for ($C_{18}H_{25}BrN_2$·HCl) C, 56.04; H, 6.79; N, 7.26. Found: C, 56.02; H, 6.75; N, 7.32.

***N*-Benzyl-6-bromo-2-(1,1-dimethylallyl)tryptamine Hydrogen Oxalate** (**92**). Trifluoroacetic acid (0.05 mL) was added into a 0 °C solution of *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-benzylcarbamate (**122**) (0.05 g) in anhydrous CH_2Cl_2 (5 mL), and the reaction mixture was allowed to stir for 6 h at room temperature. The reaction was quenched with H_2O (25 mL) and the organic portion was separated. The aqueous portion was further extracted with CH_2Cl_2 (2 x 15 mL) and the combined organic portion was washed with NaOH (3M, 3 x 25 mL), H_2O (3 x 50 mL), dried (Na_2SO_4) and evaporated under reduced pressure to yield a yellow oil. The free base was dissolved in anhydrous Et_2O (15 mL) and converted to the oxalate salt by addition of saturated oxalic acid solution in Et_2O (4 ml). The precipitate was collected by filtration, dried and recrystallized from *i*-PrOH to yield 0.02g (51%) of **92** as a white powder: mp 244-245 °C; ^{1}HNMR (DMSO-d_6) δ 1.47 (s, 6H, $(CH_3)_2$), 2.99 (br s, 2H, CH_2), 3.07 (br s, 2H, CH_2), 4.15 (s, 2H, CH_2), 4.99-5.04 (d, 2H CH_2), 6.03-6.10 (m, 1H, CH), 7.09 (d, *J* = 8.6 Hz, 1H, Ar), 7.38-7.50 (m, 7H, Ar), 10.72 (s, IH, indolic NH). Anal. Calcd

($C_{22}H_{25}BrN_2 \cdot C_2H_2O_4$) C, 59.14; H, 5.58; N, 5.75. Found: C, 56.02; H, 6.75; N, 7.32. UHPLC-MS; [M^+1] calculated for $C_{22}H_{25}BrN_2$ 397.1, found 397.1.

***N*-Methyl-6-bromo-1-methyl-2-(1,1-dimethylallyl)tryptamine Hydrochloride** (**93**). Gaseous HCl was bubbled into a 0 °C solution of *tert*-butyl-2-(6-bromo-1-methyl-2-(1,1-dimethylallyl)-indol-3-yl)ethyl-*N*-methylcarbamate (**125**) (100 mg) in anhydrous EtOAc (10 mL). The reaction mixture was allowed to stir at room temperature for 7 h and the solvent was evaporated under reduced pressure to yield a white solid which was recrystallized from MeOH to yield 35 mg (41%) of **93** as white crystals: mp 251-252 °C; ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 6H, $(CH_3)_2$), 2.53 (s, 3H $NHCH_3$), 2.87 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 3.6 (s, 3H, NCH_3), 4. 88 (d, 1H CH_2), 5.05 (d, 1H, CH_2), 6.15 (m, 1H, CH), 7.12 (d. 1H, Ar), 7.54 (m, 2H, Ar), 8.78 (s, 2H, NH_2^+ aliphatic). Anal. Calcd for ($C_{17}H_{23}BrN_2 \cdot HCl$) C, 54.93; H, 6.51; N, 7.54. Found: C, 55.21; H, 6.70; N, 7.27.

***N*-Methyl-6-bromo-1-benzyl-2-(1,1-dimethylallyl)tryptamine Hydrogen Oxalate** (**94**). Gaseous HCl was bubbled into a 0 °C solution of *tert*-butyl-2-(6-bromo-1-benzyl-2-(1,1-dimethylallyl)-indol-3-yl)ethyl-*N*-methylcarbamate (**126**) (100 mg) in anhydrous EtOAc (10 mL). The reaction mixture was allowed to stir at room temperature for 7 h and the solvent was evaporated under reduced pressure to yield a sticky yellow solid which was converted to an oxalate salt which upon recrystallization from *i*-PrOH yielded 50 mg (60%) of **94** as a yellow solid: mp 184-186 °C; ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 6H, $(CH_3)_2$), 2.53 (s, 3H $NHCH_3$), 2.87 (t, 2H, CH_2), 3.2 (t, 2H, CH_2), 3.6 (s, 3H, NCH_3), 4. 88 (d, 1H CH_2), 5.05 (d, 1H, CH_2), 6.15 (m,

1H, CH), 7.12 (d. 1H, Ar), 7.54 (m, 2H, Ar), 8.78 (s, 2H, NH_2^+ aliphatic). UHPLC-MS; [M^+1] calculated for $C_{23}H_{27}BrN_2$ 410.13, found 410.13.

***N,N*-Dimethyl-6-bromo-2-(1,1-dimethylallyl)tryptamine** (**101**). Compound **101** was prepared using a literature procedure for a similar compound.[205] An aqueous mixture (3N NaOH to pH 10) of 6-bromo-2-(1,1-dimethylallyl)tryptamine hydrochloride (**40**) (0.06 g, 0.2 mmol) was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic portion was washed with H_2O (3 x 15 mL), brine (30 mL), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to yield 6-bromo-2-(1,1-dimethylallyl)tryptamine (**40**, free base). Formic acid (0.04 g, 0.8 mmol), formaldehyde (37%, 0.02 g, 0.5 mmol) and H_2O (3 mL) were added and the reaction mixture was heated at reflux for 3 h. The reaction mixture was allowed to cool to room temperature, H_2O (5 mL) was added, and the reaction mixture was basified with NaOH (3M, to pH 10). The aqueous portion was extracted with EtOAc (3 x 20 mL) and the combined organic portion was washed with H_2O (3 x 40 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield 0.05g (91%) of **101** as a crude product which was used for further reaction without purification in the preparation of compound **86** and **87**.

***N*-Methyl-3-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propanamine Hydrogen Oxalate** (**103**). Gaseous HCl was bubbled into a solution of *tert*-butyl-3-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-*N*-methylcarbamate (**109**) (0.1 g, 0.3 mmol) in anhydrous EtOAc (10 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature overnight. The solvent was evaporated under reduced pressure to yield a brown solid which was converted to the oxalate salt and

recrystallized from *i*-PrOH to yield 0.03 g (28%) of **103** as a yellow solid: mp 135-138 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (s, 6H, $(CH_3)_2$), 1.83 (m, 2H, CH_2), 2.55 (s, 3H, CH_3), 2.76 (t, 2H, CH_2), 2.94 (t, 2H, CH_2), 5.09 (m, 2H, CH_2), 6.16 (m, 1H, CH), 6.95 (t, 1H, Ar), 7.21 (t, 1H, Ar), 7.32 (d, *J* = 8.0 Hz, 1H, Ar), 7.44 (d, *J* = 7.8 Hz, 1H, Ar), 10.47 (s, 1H, COOH). Anal. Calcd for ($C_{17}H_{24}N_2 \cdot C_2H_2O_4$) C, 63.04; H, 7.18; N, 7.50. Found: C, 65.87; H, 7.56; N, 8.09; UHPLC-MS; [M^+1] calculated for $C_{17}H_{24}N_2$ 257.15, found 257.15.

1-(6-Bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propan-2-amine Hydrochloride (**104**). Gaseous HCl was bubbled into a solution of *tert*-butyl-1-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-2-carbamate (**114**) (300 mg, 0.2 mmol) in anhydrous Et_2O (20 mL) at 0 °C. The reaction mixture was allowed to stir at room temperature for 24 h and the solvent was evaporated to yield a white solid which was recrystallized from *i*-PrOH/Et_2O to yield 60 mg (27%) of **104** as white crystals: mp 229-230 °C; ^{1}H NMR (DMSO-d_6) δ 1.09 (d, 3H, CH_3), 1.51 (d, 6H, $(CH_3)_2$), 2.95-3.14 (m, 2H, CH_2), 3.43 (m, 1H, CH), 5.08-5.15 (m, 2H, CH), 6.14-6.21 (m, 1H, CH), 7.14 (dd, 1H, Ar), 7.51 (m, 2H, Ar), 8.07 (br s, 3H, NH_3^+ aliphatic), 10.86 (br s, 1H, indolic NH). Anal. Calcd for ($C_{16}H_{21}BrN_2 \cdot HCl$) C, 53.72; H, 6.20; N, 7.83. Found: C, 53.55; H, 6.25; N, 7.82.

***N*-Methyl-3-(1*H*-indol-3-yl)propanamide** (**106**). Compound **106** was prepared as described.[190] Indole-3-propionic acid (**105**) (1 g, 5.3 mmol) and Et_3N (2.1 g, 21.1 mmol) were dissolved in anhydrous THF (40 mL). The solution was cooled to 0 °C in an ice bath to which was added methyl chloroformate (0.5 mL, 6.3 mmol) in a dropwise manner over 10 min. Stirring was

continued at 0 °C for an additional 30 min. Methylamine hydrochloride (5.5 g, 81.4 mmol) was added and the reaction was allowed to stir further at 0 °C for 90 min upon which the precipitate was removed by filtration and the filtrate was evaporated under reduced pressure to yield a crude yellow oil. The crude product was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 5:1) to afford 0.8 g (76%) of **106** as a cream-colored solid: mp 99-100 °C (lit.[190] mp 99-101 °C); ^{1}H NMR ($CDCl_3$) δ 2.49 (t, 2H, CH_2), 2.66 (d, 3H, CH_3), 3.05 (t, 2H, CH_2), 6.94 (s, 1H, Ar), 7.03-7.06 (dt, 1H, Ar), 7.10-7.14 (dt, 1H, Ar), 7.28 (d, *J* = 8.0 Hz, 1H, Ar), 7.52 (d, *J* = 7.6 Hz, 1H, Ar), 7.95 (br s, 1H, indolic NH).

***N*-Methyl-(1*H*-indol-3-yl)propanamine** (**107**). Compound **107** was prepared as described.[191] Lithium aluminum hydride (1.1 g, 27.7 mmol) was suspended in anhydrous dioxane (12 mL) and a solution of 3-(1*H*-indol-3-yl)-*N*-methylpropanamide (**106**) (0.2 g, 0.65 mmol) was added, and the reaction mixture was heated at reflux overnight. The reaction mixture was allowed to cool to room temperature and diluted with Et_2O (50 mL), H_2O (1 mL), NaOH (15%, 1 mL) and EtOH (2 mL). The reaction mixture was further stirred at room temperature for 2 h and dried (Na_2SO_4). The organic portion was evaporated under reduced pressure to afford 0.5 g (67%) of **107** as a yellow oil which was used without purification for the next step.

***tert*-Butyl-3-(1*H*-indol-3-yl)propyl-*N*-methylcarbamate** (**108**). Compound **108** was prepared using a literature procedure for a similar compound.[9] Triethylamine (0.4 mL, 2.6 mmol) and di-*tert*-butyl dicarbonate (0.6 g, 2.6 mmol) were added to a CH_2Cl_2 (15 mL) solution of indol-3-yl-*N*-methylpropanamine (**107**) (0.5 g, 2.6 mmol) and allowed to stir at room temperature for 19 h.

The reaction mixture was quenched with H_2O (50 mL) and extracted with CH_2Cl_2 (2 x 30 mL). The combined organic portion was washed with H_2O (3 x 50 mL), dried (Na_2SO_4) and evaporated under reduced pressure to obtain a yellow oil. The crude product was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 25:1) to afford 0.4 g (58%) of **108** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, $(CH_3)_3$), 1.83-1.90 (m, 2H, CH_2), 2.68 (t, 2H, CH_2), 2.78 (s, 3H, CH_3), 3.24 (m, 2H, CH_2), 6.93 (s, 1H, Ar), 7.01-7.03 (dt, 1H, Ar), 7.09-7.13 (dt, 1H, Ar), 7.28 (d, *J* = 8.0 Hz, 1H, Ar), 7.52 (d, *J* = 7.8 Hz, 1H, Ar), 7.86 (br s, 1H, indolic NH).

***tert*-Butyl-3-(2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-*N*-methylcarbamate** (**109**). Compound **109** was prepared using a literature procedure for a similar compound.[198] *tert*-Butyl hypochlorite (0.2 g, 1.7 mmol) was added to a solution of *tert*-butyl-3-(indol-3-yl)propyl-*N*-methylcarbamate (**108**) (0.4 g, 1.4 mmol) and Et_3N (0.2 g, 1.7 mmol) in THF (15 mL) at -78 °C and allowed to stir for 45 min. Freshly prepared prenyl-9-BBN[179] (2.8 mmol) was added in a dropwise manner over 10 min while maintaining temperature at -78 °C. The reaction mixture was allowed to warm to room temperature and stirred for additional 2 h. Sodium hydroxide (3M, 5 mL) and H_2O_2 (30% v/v, 5 mL) were added in a dropwise manner and the solution was stirred for 1 h and diluted with Et_2O (100 mL). The organic layer was washed with H_2O (3 x 60 mL), brine (80 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield a yellow oil which was subjected to purification by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 25:1) to afford 0.2 g (40%) of **109** as a glassy solid: mp 116-118 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H, $(CH_3)_3$), 1.45 (m, 8H, CH_2, CH_3), 2.72 (t, 2H, CH_2), 2.81 (s, 3H, CH_3), 3.25 (m,

2H, CH_2), 5.05-5.10 (m, 2H, CH_2), 6.01-6.08 (m, 1H, CH), 6.97-7.06 m, 2H, Ar), 7.20 (d, *J* = 7.8 Hz, 1H, Ar), 7.41 (d, *J* = 7.6 Hz, 1H, Ar), 7.74 (br s, 1H, indolic NH). Compound **109** was used in the preparation of compound **103**.

3-[(E)-2-Nitroprop-1-en-1-yl]-6-bromo-1*H*-indole (**111**). Compound **111** was prepared as described.[176] Ammonium acetate (1.2 g, 15 mmol) was added to a solution of 6-bromo-1*H*-indole-3-carboxaldehyde (**110**) (4.0 g, 17.9 mmol) in nitroethane (25 mL) in a 2-neck flask under an N_2 atmosphere and the solution was then allowed to stir at reflux for 7 h. The reaction mixture was allowed to cool to room temperature and the precipitate was collected by filtration, washed with H_2O (30 mL), nitroethane (2 x 15 mL) and dried to yield 3.7 g (74%) of **111** as a yellow solid: mp 219-220 °C (lit.[176] mp not reported); ^{1}H NMR ($CDCl_3$) δ 2.53 (d, 3H, CH_3), 7.39-7.42 (dd, *J* = 8.48, 1.68 Hz, 1H, Ar), 7.54 (d, *J* = 2.8 Hz, 1H, Ar), 7.63 (d, *J* = 1.4 Hz, 1H, CH), 7.68 (d, *J* = 8.52 Hz, 1H, Ar), 8.43 (s, 1H, Ar), 8.65 (br s, 1H, indolic NH).

1-(6-Bromo-1*H*-indol-3-yl)propan-2-amine (**112**). Compound **112** was prepared using a literature procedure for a similar compound.[176] Sodium borohydride (2.8 g, 73 mmol) was added to a solution of anhydrous THF (40 mL) and $BF_3{\cdot}Et_2O$ (10 mL, 79 mmol) at 0 °C, and the resulting suspension was stirred for 15 min while maintaining the temperature at 0 °C. 3-[(*E*)-2-Nitroprop-1-en-1-yl)-6-bromo-1*H*-indole (**111**) (3.7 g, 13 mmol) was then added, and the reaction mixture was allowed to stir at reflux for 4 h, cooled in an ice bath and quenched by the careful addition of H_2O (2 mL). The mixture was acidified with HCl (2N, to pH 1) and heated at reflux for a further 2 h. After cooling, the acidic solution was extracted with Et_2O (2 x 25 mL).

The aqueous portion was basified with NaOH (3M, to pH 10) and extracted with Et_2O (3 x 50 mL). The combined organic portion was washed with H_2O (3 x 100 mL), brine (50 mL), dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to yield 1.5 g (44%) of **112** as a white foam which was used for next step without purification.

***tert*-Butyl-1-(6-bromo-1*H*-indol-3-yl)propyl-2-carbamate (113)**. Compound **113** was prepared using a literature procedure for a similar compound.[9] Di-*tert*-butyl dicarbonate (1.3 g, 5.8 mmol) and Et_3N (0.6 g, 5.8 mmol) were added to a solution of 1-(6-bromo-1*H*-indol-3-yl)propan-2-amine (**112**) (1.5 g, 1.3 mmol) in anhydrous DMF (20 mL) and the reaction mixture was allowed to stir for 24 h. The reaction mixture was poured into H_2O with ice (100 mL) and extracted with EtOAc (3 x 40 mL). The combined organic portion was washed with H_2O (3 x 40 mL), brine (50 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield the crude product. The crude residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 3:1) to afford 1.5 g (75%) of **113** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.04 (d, 3H, CH_3), 1.36 (s, 9H, $(CH_3)_3$), 2.72-2.89 (m, 2H, CH_2), 3.92 (m, 1H, CH), 4.34 (s, 1H, NH aliphatic), 6.93 (s, *J* = 2.1 Hz, 1H, Ar), 7.14 (dd, *J* = 8.4, 1.6 Hz, 1H, Ar), 7.43 (m, 2H, Ar), 7.95 (s, 1H, indolic NH).

***tert*-Butyl-1-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)propyl-2-carbamate (114)**. Compound **114** was prepared using a literature procedure for a similar compound.[198] *tert*-Butyl hypochlorite (0.4 g, 3.3 mmol) was added to a solution of *tert*-butyl-3-(6-bromo-1*H*-indol-3-yl)propyl-2-carbamate (**113**) (0.9 g, 2.5 mmol) and Et_3N (0.3 g, 3.3 mmol) in anhydrous THF (30 mL) at -78 °C and the solution was allowed to stir for 45 min. Freshly prepared prenyl-9-

BBN[179] (5.1 mmol) was added in a dropwise manner over 20 min while maintaining the temperature at -55 °C. The reaction mixture was allowed to warm to room temperature and was stirred for an additional 3 h. Aqueous NaOH (3M, 7 mL) and H_2O_2 (30% v/v, 7 mL) were added in a dropwise manner and stirring was continued for another 1 h. The reaction mixture was diluted with Et_2O (100 mL), the organic layer was separated and washed with H_2O (3 x 100 mL), brine (80 mL), dried (Na_2SO_4) and evaporated under reduced pressure to yield a crude residue which was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 100:8) to afford 0.5 g (45%) of **114** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.09 (d, 3H, CH_3), 1.36 (s, 9H, $(CH_3)_3$), 1.51 (d, 6H, $(CH_3)_2$), 2.95-3.14 (m, 2H, CH_2), 3.43 (m, 1H, CH), 4.34 (s, 1H, NH aliphatic), 5.08-5.15 (m, 2H, CH), 6.14-6.21 (m, 1H, CH), 7.14 (dd, 1H, Ar), 7.51 (m, 2H, Ar), 7.95 (s, 1H, indolic NH). Compound **114** was employed in the preparation of compound **104**.

***N-iso*-Propyl-6-bromo-1*H*-indol-3-glyoxylamide** (**115**). Compound **115** was prepared using a literature procedure for a similar compound.[201,206] In a 2-neck flask a solution of 6-bromoindole (**46**) (4.0 g, 20.4 mmol) in anhydrous Et_2O (25 mL) was chilled to -15 ^{o}C and N_2 was bubbled for 5 min. Oxalyl chloride (3.6 mL, 40.8 mol) was added in a dropwise manner with continued stirring for 2 h, after which the precipitate was collected by filtration, washed with Et_2O (2 x 25 mL) and dried over vacuum. The solid was dissolved in THF (10 mL) and cooled to 0 °C. *iso*-Propylamine (3 mL) was added in a dropwise manner and the reaction mixture was allowed to stir at room temperature overnight. The organic portion was evaporated and the residue was washed with H_2O (100 mL), Et_2O (3 x 35 mL) to afford 2.7 g (69%) of **115** as a white solid: mp 268-269 °C; ^{1}H NMR (DMSO-d_6) δ 1.16-1.18 (d, 6H, CH_3), 4.00-4.06 (sep, 1H, CH), 7.40-7.42

(dd, *J* = 8.4, 1.8 Hz, 1H, Ar), 7.44 (d, *J* = 1.8 Hz, 1H, Ar), 8.14-8.16 (d, *J* = 8.5 Hz, 1H, Ar), 8.53-8.55 (d, *J* = 8.2 Hz, 1H, Ar), 8.74 (s, 1H, NH aliphatic), 12.27 (br s, 1H, indolic NH).

***N*-Benzyl-6-bromo-1*H*-indol-3-glyoxylamide** (**116**). Compound **116** was prepared using a literature procedure for a similar compound.[201,206] In a 2-neck flask a solution of 6-bromoindole (**46**) (1.7 g, 8.7 mmol) in anhydrous Et_2O (25 mL) was chilled to -10 °C and N_2 was bubbled for 5 min. Oxalyl chloride (1.5 mL, 17.4 mmol) was added in a dropwise manner and the stirring was continued for 2 h. The glyoxyl chloride formed was collected by filtration, washed with Et_2O (2 x 25 mL), dried under reduced pressure, dissolved in anhydrous THF (18 mL), and the solution cooled to 0 °C. Benzylamine (1.4 mL, 13.1 mmol) was added in a dropwise manner and the reaction was allowed to stir at room temperature for 3 h. The precipitate was collected by filtration and washed with H_2O (100 mL). The crude product was recrystallized from MeOH to afford 1.6 g (51%) of **116** as buff-colored, needle-shaped crystals: mp 232-233 °C; ^{1}H NMR (DMSO-d_6) δ 4.42-4.44 (d, 2H, CH_2), 7.26-7.28 (m, 1H, Ar), 7.33-7.35 (m, 4H, Ar), 7.40-7.43 (dd, *J* = 8.5, 1.8 Hz, 1H, Ar), 8.16-8.18 (d, *J* = 8.4 Hz, 1H, Ar), 8.78 (s, 1H, Ar), 9.30-9.33 (t, 1H, NH aliphatic), 12.30 (br s, 1H, indolic NH).

***N-iso*-Propyl-6-bromotryptamine** (**117**). Compound **117** was prepared using a literature procedure for a similar compound.[178] Borane dimethylsulfide (10.1M in THF, 1.4 mL) was added in a dropwise manner at 60 °C to a stirred solution of *N-iso*-propyl-6-bromo-1*H*-indol-3-glyoxylamide (**115**) (1.5 g, 4.9 mol) in dry THF (35 mL). The reaction mixture was heated at reflux for 5 h, cooled to room temperature and quenched with HCl (2N, to pH 1). The THF was

evaporated under reduced pressure and the remaining aqueous portion was diluted with H_2O (100 mL), basified with NaOH (3M, to pH 9) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic portions were washed with H_2O (2 x 50 mL), dried (Na_2SO_4), and solvent was evaporated under reduced pressure to yield 1.2 g (89%) of **117** as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 1.07-1.09 (d, 6H, CH_3), 2.86-2.90 (m, 2H, CH_2), 3.21-3.23 (t, 2H, CH_2), 6.91 (s, 1H, Ar), 7.13-7.15 (d, 1H, Ar), 7.43 (s, 2H, Ar), 8.01 (s, IH, indolic NH). Compound **117** was used without further purification in the synthesis of compound **119**.

***N*-Benzyl-6-bromotryptamine** (**118**). Compound **118** was prepared using a literature procedure for a similar compound.[178] Borane dimethylsulfide (10.1M in THF, 1.3 mL) was added in a dropwise manner at 60 °C to a stirred solution of *N*-benzyl-6-bromo-1*H*-indol-3-glyoxylamide (**116**) (1.5 g, 4.2 mmol) in anhydrous THF (35 mL). The reaction mixture was heated at reflux for 5.5 h, cooled to room temperature and quenched with HCl (2N, to pH 1). The organic solvent was evaporated under reduced pressure and the remaining aqueous mixture was diluted with H_2O (100 mL), basified with NaOH (3M, to pH 9) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic portion was washed with H_2O (2 x 50 mL), dried (Na_2SO_4), and solvent was evaporated under reduced pressure to yield 1.4 g (61%) of **118** as a yellow foam. Compound **118** was used without further purification for synthesis of compound **120**.

***tert*-Butyl-2-(6-bromo-1*H*-indol-3-yl)ethyl-*N*-*iso*-propylcarbamate** (**119**). Compound **119** was prepared using a literature procedure for a similar compound.[9] Triethylamine (0.60 mL, 4.3 mmol) and di-*tert*-butyl dicarbonate (0.93 g, 4.3 mmol) were added to a CH_2Cl_2 (20 mL) solution

of *N-iso*-propyl-6-bromotryptamine (**117**) (1.20 g, 4.3 mmol) and allowed to stir overnight. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with H_2O (3 x 40 mL), dried (Na_2SO_4), and evaporated to yield a crude residue. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 100:24) to afford 1.04 g (64%) of **119** as white foam: ^{1}H NMR ($CDCl_3$) δ 1.07-1.09 (d, 6H, CH_3), 1.43 (s, 9H, CH_3), 2.86-2.90 (m, 2H, CH_2), 3.21-3.23 (t, 2H, CH_2), 4.04-4.06 (sep, 1H, CH), 6.91 (s, 1H, Ar), 7.13-7.15 (d, 1H, Ar), 7.43 (s, 2H, Ar), 8.01 (s, IH, indolic NH).

***tert*-Butyl-2-(6-bromo-1*H*-indol-3-yl)ethyl-*N*-benzylcarbamate** (**120**). Compound **120** was prepared using a literature procedure for a similar compound.[9] Triethylamine (0.3 g, 2.6 mmol) and di-*tert*-butyl dicarbonate (0.6 g, 2.6 mmol) were added to an anhydrous CH_2Cl_2 (15 mL) solution of *N*-benzyl-6-bromotryptamine (**118**) (0.8 g, 2.6 mmol) and allowed to stir at room temperature for 24 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with H_2O (3 x 40 mL), dried (Na_2SO_4), and evaporated to yield a crude residue. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 100:24) to afford 0.6 g (50%) of **120** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H, $(CH_3)_3$), 2.83 (br s, 2H, CH_2), 3.36 (br s, 2H, CH_2), 4.31 (s, 2H, CH_2), 6.84 (br s, 1H, Ar), 7.10-7.30 (m, 8H, Ar), 7.91 (br s, IH, indolic NH).

***tert*-Butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-*iso*-propyl carbamate** (**121**). Compound **121** was prepared using a literature procedure for a similar compound.[198] *tert*-Butyl hypochlorite (0.24 g, 2.2 mmol) was added to a solution of *tert*-butyl-2-(6-bromo-1*H*-

indol-3-yl)ethyl-*N*-*iso*-propylcarbamate (**119**) (0.70 g, 1.8 mmol) and Et_3N (0.22 g, 2.2 mmol) in THF (15 mL) at -65 ^oC and allowed to stir for 45 min. Freshly prepared prenyl 9-BBN[179] (3.7 mmol) was added in a dropwise manner over 30 min while maintaining the temperature at -50 ^oC. The reaction mixture was allowed to warm to room temperature and stirred for an additional 4 h. Sodium hydroxide (3M, 7 mL) and H_2O_2 (30%, 7 mL) were added in a dropwise manner and stirring was continued for 1 h. The reaction mixture was diluted with Et_2O (50 mL), and the organic layer was washed with H_2O (3 x 60 mL), brine (80 mL), dried (Na_2SO_4), and evaporated to yield a crude residue. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 10:1) to afford 0.5 g (62%) of **121** as a white foam: ^{1}HNMR ($CDCl_3$) δ 1.25 (d, 6H, CH_3), 1.43 (s, 9H, CH_3), 1.50 (s, 6H, CH_3), 2.86-2.90 (m, 2H, CH_2), 3.21-3.23 (t, 2H, CH_2), 5.09-5.13 (d, 2H CH_2), 6.12-6.19 (m, 1H, CH), 7.13-7.15 (d, J = 8.4, 1.8 Hz, 1H, Ar), 7.43 (s, 2H, Ar), 8.01 (s, IH, indolic NH). Compound **121** was used in preparation of **91**.

***tert*-Butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-benzylcarbamate** (**122**). Compound **122** was prepared using a literature procedure for a similar compound.[198] *tert*-Butyl hypochlorite (0.09 g, 0.8 mmol) was added to a solution of *tert*-butyl-2-(6-bromo-1*H*-indol-3-yl)ethyl-*N*-benzylcarbamate (**120**) (0.30 g, 0.7 mmol) and Et_3N (0.08 g, 0.8 mmol) in anhydrous THF (10 mL) at -65 °C and allowed to stir for 45 min. Freshly prepared prenyl 9-BBN[179] (1.4 mmol) was added in a dropwise manner over 10 min while maintaining the temperature at -50 °C. The reaction mixture was allowed to warm to room temperature and stirred for an additional 5.5 h. Sodium hydroxide (3M, 3 mL) and H_2O_2 (30%, 3 mL) were added in a dropwise manner and stirring was continued for 1 h. The reaction mixture was diluted with Et_2O (50 mL), and the

organic layer was washed with H_2O (3 x 30 mL), brine (50 mL), dried (Na_2SO_4), and evaporated to yield a crude residue. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 10:1) to afford 0.06 g (17%) of **122** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H, $(CH_3)_3$), 1.43 (s, 6H, $(CH_3)_2$), 2.84 (br s, 2H, CH_2), 3.36 (br s, 2H, CH_2), 4.31 (s, 2H, CH_2), 4.99-5.03 (d, 2H CH_2), 6.02-6.08 (m, 1H, CH), 6.84 (d, *J* = 8.4 Hz, 1H, Ar), 7.10-7.30 (m, 7H, Ar), 7.43 (s, 2H, Ar), 8.01 (s, IH, indolic NH). This compound was employed in the preparation of compound **92**.

***N*-Methyl-6-bromo-1*H*-indol-3-glyoxylamide** (**123**). Compound **123** was prepared as described.[201] In a 2-neck flask a solution of 6-bromoindole (**46**) (4.0 g, 20 mmol) in anhydrous Et_2O (20 mL) was chilled to -5 °C and N_2 was bubbled for 5 min. Oxalyl chloride (3.6 mL, 41 mmol) was added in a dropwise manner with continued stirring and the reaction was stirred at 0 °C for 2 h, after which the solvent was evaporated, the residue was washed with cold Et_2O (25 mL) and dried under vacuum. The solid was added to cold aqueous methylamine (40%, 35 mL) and was allowed to stir at room temperature for 2 h after which the precipitate was collected by filtration to yield a brown solid which, upon recrystallization from MeOH, yielded 4.3 g (73%) of **123** as a white solid: mp 252-253 °C (lit.[9] mp 249-252 °C); ^{1}H NMR (DMSO-d_6) δ 2.75 (s, 3H, CH_3), 7.4 (d, *J* = 8.48 Hz, 1H, Ar), 7.73 (s, 1H, Ar), 8.15 (d, *J* = 8.48 Hz, 1H, Ar), 8.68 (s, 1H, NH aliphatic), 8.79 (s, 1H, Ar), 12.26 (s, 1H, indolic NH). Compound **123** was used in the preparation of compound **35**.

***tert*-Butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (124)**. Compound **124** was prepared as described.[198] *tert*-Butyl hypochlorite (0.5 g, 4.1 mmol) was added to a solution of *tert*-butyl-2-(6-bromo-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**61**) (1.2 g, 3.4 mmol) and Et_3N (0.4 g, 4.1 mmol) in anhydrous THF (15 mL) at -78 °C and allowed to stir for 45 min. Freshly prepared prenyl 9-BBN[179] (6.8 mmol) was added in a dropwise manner over 30 min while maintaining the temperature at -78 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. Sodium hydroxide (3M, 5 mL) and H_2O_2 (30% v/v, 5 mL) were added in a dropwise manner and stirring was continued for 1 h. The reaction mixture was diluted with Et_2O (100 mL), and the organic layer was washed with H_2O (3 x 60 mL), brine (80 mL), dried (Na_2SO_4), and evaporated to yield a crude residue which was purified by column chromatography (silica gel; hexanes/EtOAc 100:1 to 10:1) to afford 0.4 g (27%) of **124** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H, $(CH_3)_3$), 1.54 (s, 6H, $(CH_3)_2$), 2.78 (t, 3H, CH_3), 2.92 (t, 2H, CH_2), 3.32 (t, 2H, CH_2), 5.09 (m, 2H, CH_2), 6.03 (m, 1H, CH), 7.10-7.35 (m, 3H, Ar), 7.77 (s, 1H, indolic NH).

***tert*-Butyl-2-(6-bromo-1-methyl-2-(1,1-dimethylallyl)-indol-3-yl)ethyl-*N*-methyl carbamate (125)**. Compound **125** was prepared according to a literature procedure for a similar compound.[207,208] In a dry, round-bottom flask NaH (60% oil dispersion) (0.04 g, 0.9 mmol) was allowed to stir with anhydrous toluene at 0 °C (ice bath) for 10 min. After removal of the toluene, a solution of *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**124**) (0.3 g, 0.7 mmol) in anhydrous DMF (5 mL) was added and stirring continued at 0 °C (ice bath) for 30 min. Iodomethane (0.15 g, 1.1 mmol) was added to the cold

solution and the reaction mixture was allowed to stir at room temperature for 1.5 h. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic portions were washed with H_2O (3 x 25 mL) and brine (40 mL), dried (Na_2SO_4) and evaporated to dryness under reduced pressure to yield a crude, yellow-colored oil which was purified by column chromatography (silica gel; hexanes/EtOAc 100:1 to 10:1) to afford 0.2 g (79%) of **125** as a light-yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, $(CH_3)_3$), 1.56 (s, 6H, $(CH_3)_2$), 1.97-2.11 (m, 2H, CH_2), 2.51 (br s, 5H, NCH_3, CH_2), 5.18-5.26 (m, 2H, CH_2), 6.28-6.35 (m, 1H, CH), 6.77 (d, 2H, $ArCH_2$), 7.13-7.18 (m. 7H, Ar), 7.72 (s, 1H, Ar). The product was used in the preparation of compound **93**.

***tert*-Butyl-2-(6-bromo-1-benzyl-2-(1,1-dimethylallyl)-indol-3-yl)ethyl-*N*-methyl carbamate (126)**. Compound **126** was prepared according to a literature procedure for a similar compound.[207,208] In a dry, round-bottom flask NaH (60% oil dispersion) (0.02 g, 0.6 mmol) was allowed to stir with anhydrous toluene at 0 °C (ice bath) for 10 min. After removal of the toluene, a solution of *tert*-butyl-2-(6-bromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**124**) (0.2 g, 0.5 mmol) in anhydrous DMF (2 mL) was added and stirring continued at 0 °C (ice bath) for 45 min. Benzyl bromide (0.09 g, 0.6 mmol) was added to the cold solution and the reaction mixture was allowed to stir at room temperature for 5.5 h. The reaction was quenched with H_2O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic portions were washed with H_2O (3 x 25 mL) and brine (40 mL), dried (Na_2SO_4) and evaporated to dryness under reduced pressure to yield a crude, yellow-colored oil which was purified by column chromatography (silica gel; hexanes/EtOAc 100:1 to 100:5) to afford 0.13 g

(55%) of **126** as a white foam: ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 9H, $(CH_3)_3$), 1.47 (s, 6H, $(CH_3)_2$), 1.97-2.11 (m, 2H, CH_2), 2.51 (br s, 5H, NCH_3, CH_2), 5.18-5.26 (m, 2H, CH_2), 6.28-6.35 (m, 1H, CH), 6.7 (s, 2H, CH_2), 7.1-7.2 (m, 7H, Ar), 7.37 (s, 1H, Ar). Compound **126** was used in the preparation of compound **94**.

***N,N*-Dimethyl-5-bromo-2-(1,1-dimethylallyl)isotryptamine Hydrogen Oxalate** (**127**). Compound **127** was prepared according to a literature procedure for a similar compound.[207,208] In a dry, round-bottom flask NaH (60% oil dispersion) (0.06 g, 1.4 mmol) was allowed to stir with anhydrous toluene in an ice bath for 10 min. After removal of the toluene, a solution of 5-bromo-2-(1,1-dimethylallyl)-1*H*-indole (**131**) (0.15 g, 0.6 mmol) in anhydrous DMF (5 mL) was added and stirring continued in an ice bath (0 °C) for another 20 min. In a separate beaker a solution of *N*,*N*-dimethylaminoethyl chloride hydrochloride (0.2 g, 1.1 mmol), K*t*BuO (0.2 g, 1.4 mmol) and KI (0.08 g, 0.6 mmol) in cold anhydrous DMF (3 mL) was then added to the round-bottom flask and the reaction mixture was heated at reflux for 17 h. The reaction mixture was quenched with H_2O (20 mL) and extracted with EtOAc (2 x 25 mL) and CH_2Cl_2 (2 x 25 mL). The combined organic portions were washed with H_2O (2 x 50 mL), brine (50 mL), dried (Na_2SO_4) and evaporated to dryness to yield 0.1 g (63%) of free base as a brown-colored oil. Saturated oxalic acid solution in anhydrous Et_2O (5 mL) was added to a solution of the free base (0.1 g) in anhydrous Et_2O (5 mL) at 0 °C and allowed to stir overnight. The precipitate formed was collected by filtration and recrystallized from MeOH/Et_2O to afford 0.04 g (32%) of **127** as white flakes: mp 185-188 °C [The compound was prepared as previously described by D. Kim (postdoctoral fellow in Dr. Glennons lab) as **JSK-087** (mp 191-194 °C)]; ^{1}H NMR (DMSO-d_6) δ

1.49 (s, 6H, $(CH_3)_2$), 2.69 (s, 6H $N(CH_3)_2$), 3.03 (t, 2H, CH_2), 4.42 (t, 2H, CH_2), 5.17 (m, 2H, CH_2), 6.09 (m, 1H, CH), 6.37 (s,1H, CH), 7.25 (d, 1H, *J* = 8.7 Hz, Ar), 7.43 (s, 1H, *J* = 8.7 Hz, Ar), 7.70 (d, 1H, Ar).

3,5-Dibromo-1*H*-indole (**130**). Compound **130** prepared as described.[192] A solution of liquid Br_2 (0.2 mL, 4 mmol) in anhydrous DMF (5 mL) was added in a dropwise manner to a solution of 5-bromoindole (**129**) (1.0 g, 4 mmol) in anhydrous DMF (5 mL). The light-protected mixture was allowed to stir at room temperature for 12 h and then poured into water and ice (100 mL) containing NH_4OH (2 mL) and saturated aqueous NaS_2O_3 (0.3 mL). The product was collected by filtration and was washed with water, dried under reduced pressure to yield 0.6 g (41%) of **130** as a brown solid: mp 90-92 °C (decomp) (lit.[192] mp 94 °C); ^{1}H NMR (DMSOd_6) δ 7.41 (dd, 1H, Ar), 7.29 (dd, 1H, Ar), 7.62 (s, 1H, Ar), 7.53 (s, 1H, Ar), 11.69 (br s, 1H, indolic NH).

5-Bromo-2-(1,1-dimethylallyl)-1*H*-indole (**131**). Compound **131** was prepared as described.[193] 3,5-Dibromo-1*H*-indole (**130**) (0.5 g, 1.8 mmol) was added in one portion to a freshly prepared prenyl 9-BBN[177] (5.4 mmol) and Et_3N (0.9 mL, 6.3 mmol) in anhydrous THF (10 mL) at room temperature. The reaction mixture was allowed to stir at room temperature for 4 h and then quenched with a saturated solution of $NaHCO_3$ (20 mL). The organic layer was separated and the aqueous portion was extracted with Et_2O (2 x 20 mL). The combined organic portion was washed with H_2O (2 x 30 mL), brine (30 mL), dried (Na_2SO_4) evaporated to dryness under reduced pressure to yield a crude, dark-yellow oil. The residue was purified by column chromatography (silica gel; hexanes/EtOAc 100:1 to 30:1) to afford 0.3 g (78%) of **131** as a

yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 6H, CH_3), 5.10 (m, 2H, CH_2), 6.01 (m, 1H, CH), 6.24 (s, 1H, Ar), 7.20 (dd, 2H, Ar), 7.89 (br s, 1H, indolic NH). Compound **131** was used in the preparation of **127**.

2,2-Dimethylbut-3-enoic acid (**133**). Compound **133** was prepared as described.[209] n-Butyllithium (2.5 M in hexane, 4.6 mL, 11.5 mmol) was added to a 3-neck flask and the solvent was evaporated to dryness under N_2. Anhydrous THF (5 mL) was added and the resulting solution was cooled to -78 °C. Diethylamine (1.1 mL, 10.9 mmol) was added and the solution was stirred for 15 min at 0 °C and cooled to -78 °C after which a solution of 2-methylbut-2-enoic acid (**132**) (0.5 g, 4.9 mmol) in THF (3 mL) was added over 5 min with continuous stirring and stirred additionally for 0.5 h at 0 °C. The solution was cooled again to -78 °C and dimethyl sulfate (0.5 mL, 4.9 mmol) in THF (5 mL) was added over 5 min and stirring was continued for 0.5 h at the temperature, and then for additional 1.5 h at room temperature. The reaction mixture was quenched with H_2O (10 mL) and the mixture was washed with Et_2O (3 x 15 mL). The aqueous portion was acidified with conc. HCl (to pH 1) and extracted with EtOAc (3 x 10 mL). The combined organic portion was washed with H_2O (3 x 25 mL), brine (30 mL), dried (Na_2SO_4) and evaporated to dryness to afford 0.35 g (61%) of **133** as a colorless oil: bp 184-187 °C (lit.[209] bp not reported); ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 6H, $(CH_3)_2$), 5.03-5.11 (m, 2H, CH_2), 5.95-6.02 (m, 1H, CH), 6.93 (s, 1H, COOH).

2,2-Dimethylbut-3-enoyl chloride (**134**). Compound **134** was prepared as described.[210] Oxalyl chloride (0.3 mL, 2.9 mmol) was added dropwise to a solution of 2,2-dimethylbut-3-enoic acid

(**133**) (0.3 g, 2.6 mmol) in anhydrous CH_2Cl_2 (5 mL) and few drops of DMF at 0 °C and stirred for 16 h at room temperature. On completion the reaction mixture was evaporated to dryness under reduced pressure to afford 0.3 g (90%) of **134** as a colorless oil. The material was used without further purification for synthesis of compound **135**.

***N*-(4-Bromo-2-nitrophenyl)-2,2-dimethylbut-3-enamide** (**135**). Compound **135** was prepared according to a literature procedure for a similar compound.[211] A solution of 2-butyl-2-methylbut-3-enoyl chloride (**134**) (0.3 g, 2.4 mmol) in CH_2Cl_2 (30 mL) was cooled to 0 °C to which DMAP (0.1 g, 0.8 mmol), 4-bromo-2-nitroaniline (**77**) (0.3 g, 1.5 mmol) and DCC (0.3 g, 1.5 mmol) was added sequentially, stirred for 10 min at 0 °C and heated at reflux for 19 h. The reaction mixture was allowed to cool to room temperature and washed with HCl (2M, 3 x 30 mL), saturated $NaHCO_3$ (40 mL), brine (40 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a crude, yellow oil. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 100:15) to afford 0.2 g (52%) of **135** as a yellow solid: mp 98-99 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 6H, $(CH_3)_2$), 5.33-5.37 (m, 2H, CH_2), 5.98-6.06 (m, 1H, CH), 7.65 (dd, *J* = 9.1, 2.4 Hz, 1H, Ar), 8.28 (d, *J* = 2.4 Hz, 1H, Ar), 8.68 (d, *J* = 9.1 Hz, 1H, Ar), 10.51 (br s, 1H, NH).); IR (Diamond): 1698 cm^{-1} (C=O).

5-Bromo-2-(1,1-dimethtylallyl)-benzo[*d*]imidazole (**136**). Compound **136** was prepared according to a literature procedure for a similar compound.[212] Iron (0.07 g, 1.3 mmol) was added to a solution of *N*-(4-bromo-2-nitrophenyl)-2,2-dimethylbut-3-enamide (**135**) (0.2 g, 0.6 mmol) in gl. HOAc (4 mL) and the reaction mixture was heated at reflux for 5 h. The reaction mixture

was allowed to cool to room temperature and diluted with H_2O (25 mL). The aqueous mixture was washed with EtOAc (2 x 25 mL) and basified with NaOH (3M, to pH 8). The basified aqueous portion was extracted with EtOAc (3 x 30 mL), and the combined organic portions were washed with H_2O (2 x 30 mL), brine (30 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield 0.15 g (86%) of **136** as a cream-colored solid: mp 231-233 °C; ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 6H, $(CH_3)_2$), 5.15-5.20 (m, 2H, CH_2), 6.04-6.11 (m, 1H, CH), 7.44 (d, *J* = 1.8 Hz, 1H, Ar), 7.55 (dd, *J* = 8.5, 1.8 Hz, 1H, Ar), 7.82 (d, *J* = 8.6 Hz, 1H, Ar), 8.73 (br s, 1H, NH).

2-(1,1-Dimethylallyl)gramine Hydrogen Oxalate (**137**). Compound **137** was prepared following a literature procedure for a similar compound.[213] Dimethylamine (40%, 0.1 mL, 0.8 mmol) and HCHO (37%, 0.7 mL, 0.8 mmol) were added to a solution of 2-(1,1-dimethylallyl)-1*H*-indole (**141**) (0.10 g, 0.5 mmol) in gl. HOAc (3 mL) maintained at 5 °C. When the vapors ceased, MeOH (4 mL) was added to make a clear solution and the reaction mixture was allowed to stir overnight at room temperature. The organic solvent was removed by evaporation under reduced pressure and the solution was basified with NaOH (3M, to pH 10). The aqueous portion was extracted with Et_2O (3 x 30 mL) and the combined organic portion was washed with H_2O (3 x 50 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield the gramine as a free base which was converted to an oxalate salt by addition of a saturated oxalic acid solution in anhydrous Et_2O (5 mL) to a solution of crude 2-(1,1-dimethylallyl)gramine in anhydrous Et_2O (5 mL) at 0 °C. The precipitate was collected by filtration, dried and recrystallized from *i*-PrOH to afford 0.04 g (22%) of **137** as white crystals:

mp 155-158 °C; ^{1}H NMR (DMSO-d_6) δ 1.54 (s, 6H, $(CH_3)_2$), 2.72 (s, 6H, $N(CH_3)_2$), 4.39 (s, 2H, CH_2), 5.22 (m, 2H, CH_2), 6.33 (m, 1H, CH), 7.04-7.68 (m, 4H, Ar), 11.13 (br s, 1H, COOH). Anal. Calcd for ($C_{16}H_{22}N_2 \cdot C_2H_2O_4 \cdot 0.5\ H_2O$) C, 63.33; H, 7.38; N, 8.21. Found: C, 63.09; H, 7.40; N, 8.42.

6-Bromo-2-propylgramine Hydrogen Oxalate (**138**). Compound **138** was prepared following a literature procedure for a similar compound.[213] Dimethylamine (40% in H_2O, 0.12 mL, 1.0 mmol), formaldehyde (37%, 0.07 mL, 0.9 mmol) and zinc iodide (0.30 g, 1.0 mmol) were added to a solution of 6-bromo-2-propyl-1*H*-indole (**143**) (0.15 g, 0.6 mmol) in absolute EtOH (20 mL) and allowed to stir at room temperature for 24 h. The organic solvent was evaporated under reduced pressure, and the aqueous portion was extracted with CH_2Cl_2 (2 x 40 mL). The combined organic portions were washed with H_2O (3 x 50 mL), dried (Na_2SO_4) and evaporated under reduced pressure to yield the free base as a yellow oil. Saturated oxalic acid solution in anhydrous Et_2O (5 mL) was added to a solution of crude 6-bromo-2-propylgramine (0.1 g) in anhydrous Et_2O (5 mL) at 0 °C and allowed to stir overnight. The precipitate formed was collected by filtration and recrystallized from *i*-PrOH to afford 0.05 g (21%) of **138** as white crystals: mp 181-183 °C; ^{1}H NMR (DMSO-d_6) δ 0.93 (s, 3H, CH_3), 1.69 (m, 2H, CH_2), 2.74 (s, 6H, CH_3), 2.79 (t, 2H, CH_2), 4.39 (s, 2H, CH_2), 7.20 (dd, J = 8.4, 1.8 Hz, 1H, Ar), 7.50 (d, J = 1.6 Hz, 1H, Ar), 7.65 (d, J = 8.4 Hz, 1H, Ar), 9.17 (br s, 1H, indolic NH), 11.47 (s, 1H, COOH). Anal. Calcd for ($C_{14}H_{19}BrN_2 \cdot C_2H_2O_4$) C, 49.88; H, 5.49; N, 7.27. Found: C, 49.85; H, 5.44; N, 7.26.

3-Bromo-1*H*-indole (**140**). Compound **140** was prepared as described.[192,214] A solution of liquid Br_2 (0.7 mL, 14 mmol) in anhydrous DMF (20 mL) was added in a dropwise manner to a solution of indole (**139**) (2.0 g, 17 mmol) in anhydrous DMF (20 mL). The light-protected mixture was allowed to stir at room temperature for 24 h and then poured into water and ice (100 mL) containing NH_4OH (2 mL) and a saturated aqueous solution of $Na_2S_2O_3$ (0.5 mL). The aqueous portion was extracted with EtOAc (3 x 40 mL). The combined organic portion was washed with 5% aqueous LiCl (3 x 40 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a crude product which upon recrystallization from petroleum ether/Et_2O yield 1.6 g (48%) of **140** as an off-white solid: mp 66-67 °C (decomp) (lit.[214] mp 65 °C).

2-(1,1-Dimethylallyl)-1*H*-indole (**141**). Compound **141** was prepared as described.[215] 3-Bromo-1*H*-indole (**140**) (0.3 g, 1.3 mmol) was added in one portion to freshly prepared prenyl 9-BBN[177] (3.8 mmol) and Et_3N (0.6 mL, 4.5 mmol) in anhydrous THF (10 mL) at room temperature. The reaction mixture was allowed to stir at room temperature for 5 h and then quenched with saturated $NaHCO_3$ (20 mL). The organic layer was separated and the aqueous portion was extracted with Et_2O (3 x 40 mL). The combined organic portion was washed with H_2O (2 x 30 mL), brine (30 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a crude, yellow oil. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 50:1 to 10:1) to afford 0.2 g (85%) of **141** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 6H, $(CH_3)_2$), 5.05 (m, 2H, CH_2), 5.97 (m, 1H, CH), 6.24 (s, 1H, Ar), 6.97-7.48 (m, 4H, Ar), 7.8 (br s, 1H, indolic NH). Compound **141** was used in the preparation of compound **137**.

5-Bromo-2-(1-pentynyl)aniline (**142**). Compound **142** was prepared following a literature procedure for a similar compound.[204] A mixture of 5-bromo-2-iodoaniline (**79**) (1.0 g, 3.4 mmol), CuI (0.03 g, 0.2 mmol) and $PdCl_2(PPh_3)_2$ (0.12 g, 0.2 mmol) were suspended in anhydrous THF (30 mL). Et_3N (1.4 mL, 10.1 mmol) and 1-pentyne (0.4 mL, 4.4 mmol) were added successively and the reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was diluted with Et_2O (100 mL), filtered through Celite, and the organic portion was washed sequentially with H_2O (3 x 100 mL), a saturated aqueous solution of NH_4Cl (50 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to yield a crude residue. The crude product was purified by column chromatography (silica gel; hexanes/EtOAc 100:1 to 75:1) to afford 1.4 g (88%) of **142** as a light-yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H, CH_3), 1.57 (m, 2H, CH_2), 2.36 (t, 2H, CH_2), 4.14 (br s, 2H, NH_2), 6.70 (dd, *J* = 8.2, 1.9 Hz, 1H, Ar), 6.76 (d, *J* = 1.9 Hz, 1H, Ar), 7.01 (d, *J* = 8.2 Hz, 1H, Ar).

6-Bromo-2-propyl-1*H*-indole (**143**). Compound **143** was prepared following a literature procedure for a similar compound.[188] Zinc iodide (0.9 g, 0.3 mmol) was added in one portion to a solution of 5-bromo-2-(1-pentynyl)aniline (**142**) (1.4 g, 5.9 mmol) in anhydrous toluene (20 mL) and the reaction mixture was heated at reflux for 3 h. The reaction mixture was allowed to cool to room temperature, quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 X 50 mL). The combined organic portion was washed with H_2O (3 x 70 mL), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to afford a crude product which after recrystallization from hexanes yielded 1.2 g (87%) of **143** as yellow crystals: mp 85-86 °C; ^{1}H NMR ($CDCl_3$) δ

0.93 (t, 3H, CH_3), 1.67 (m, 2H, CH_2), 2.64 (t, 2H, CH_2), 6.14 (s, 1H, Ar), 7.09 (dd, *J* = 8.4, 1.7 Hz, 1H, Ar), 7.29 (d, *J* = 8.4 Hz, 1H, Ar), 7.36 (s, 1H, Ar), 7.77 (br s, 1H, indolic NH). Compound **143** was employed in preparation of compound **138**.

***N*-Methyl-5,6-Dibromo-2-(1,1-dimethylallyl)tryptamine Hydrochloride** (**144**). Gaseous HCl was bubbled through a solution of *tert*-butyl-2-(5,6-dibromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**151**) (280 mg) in dry anhydrous EtOAc (10 mL) at 0 °C. The reaction mixture was allowed to stir for 24 h and the solvent was evaporated to yield a white solid which upon recrystallization from *i*-PrOH, yielded 60 mg (25%) of **144** as white crystals: mp 238-239 °C; ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 6H, CH_3), 2.62 (s, 3H NH(CH3), 2.95 (t, 2H, CH_2), 3.09 (t, 2H, CH_2), 5.10-5.16 (m, 2H, CH_2), 6.12-6.19 (m, 1H, CH), 7.71 (s, 1H, Ar), 8.03 (s, 1H, Ar), 8.81 (br s, 1H, NH^+ aliphatic), 10.98 (s, 1H, indolic NH). Anal. Calcd for ($C_{16}H_{20}Br_2N_2$·HCl) C, 44.01; H, 4.85; N, 6.35. Found: C, 43.99; H, 4.74; N, 6.35.

Methyl-5,6-dibromo-1*H*-indolyl-3-carboxylate (**146**). Compound **146** was prepared as described.[194] Liquid Br_2 (3.9 mL, 75 mmol) was added to a suspension of commercially available methyl-1*H*-indolyl-3-carboxylate (**145**) (6 g, 34 mmol) in HOAc (45 mL) and the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was filtered and the precipitate was collected and washed with EtOH (2 x 50 mL) and petroleum ether (50 mL), and dried in air to yield 7.7 g (68%) of **146** as a grey powder: mp 246-252 °C (lit.[194] mp 237-242 °C); ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H, CH_3), 7.65 (s, 1H, Ar), 7.83 (s, 1H, Ar), 8.39 (s, 1H, Ar), 8.45 (br s, 1H, indolic NH).

5,6-Dibromo-1*H*-indole (**147**). Compound **146** was prepared as described.[194] Methyl-5,6-dibromo-1*H*-indolyl-3-carboxylate (**146**) (5 g, 15 mmol), KOH (3 g, 54 mmol), MeOH (9 mL), THF (9 mL) and H_2O (4 mL) was added to a 50 mL glass tube. The solution was microwave (100 W, 150 °C, 1 h) irradiated. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and the organic phase was separated and washed with H_2O (2 x 50 mL), and dried (Na_2SO_4) to obtain a crude brown solid which, on recrystallization with toluene/hexanes, yielded 3.2 g (68%) of **147** as buff-colored flakes: mp 150-152 °C (lit.[192] mp 150-151°C); ^{1}H NMR ($CDCl_3$) δ 6.41 (m, 1H, Ar), 7.14 (t, 1H, Ar), 7.63 (s, 1H, Ar), 7.84 (s, 1H, Ar), 8.09 (br s, 1H, indolic NH).

***N*-Methyl-5,6-dibromo-1*H*-indol-3-glyoxylamide** (**148**). Compound **148** was prepared according to a literature procedure for a similar compound.[201] A solution of 5,6-dibromo-1*H*-indole (**147**) (1.8 g, 6.6 mmol) in anhydrous Et_2O (20 mL) in a 2-neck flask was chilled to -5 °C and N_2 was bubbled in for 5 min. Oxalyl chloride (1.2 mL, 13.1 mmol) was added in a dropwise manner and the reaction mixture was heated at reflux for 5 h. The reaction mixture was filtered and the precipitate was washed with cold Et_2O (2 x 10 mL) and air dried. The solid was added to cold methylamine (40% in H_2O, 25 mL) and the solution was allowed to stir at room temperature overnight. The reaction mixture was diluted with H_2O (100 mL) and the residue was collected by filtration and air dried to yield 1.8 g (76%) of **148** as a buff-colored solid: mp 263-264 °C (decomp); ^{1}H NMR (DMSOd_6) δ 2.75 (s, 3H, CH_3), 7.14 (s, 1H, Ar), 7.63 (s, 1H, Ar), 7.84 (s, 1H, Ar), 8.09 (br s, 1H, indolic NH).

***N*-Methyl-5,6-Dibromotryptamine** (**149**). Borane dimethylsulfide (10.1M in THF, 1.4 mL, 14 mmol) was added in a dropwise manner at 60 °C to a stirred solution of *N*-methyl-5,6-dibromo-1*H*-indol-3-glyoxylamide (**148**) (1.7 g, 5 mmol) in dry THF (35 mL). The reaction mixture was allowed to stir at reflux for 7 h, cooled to 0 °C, quenched with H_2O (4 mL), acidified with HCl (2N, to pH 1), and heated at reflux for 1 h. The THF was evaporated under reduced pressure and the remaining aqueous portion was diluted with H_2O (100 mL), basified with NaOH (3M, to pH 9) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic portions were washed with H_2O (2 x 50 mL), dried (Na_2SO_4), and solvent was evaporated under reduced pressure to yield 1.5 g (96%) of **149** as a white solid: mp 107-111 °C; ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, $NHCH_3$), 2.71 (t, 2H, CH_2), 2.76 (t, 2H, CH_2), 7.25 (s, 1H, Ar), 7.73 (s, 1H, Ar), 7.91 (s, 1H, Ar), 11.09 (br s, 1H, indolic NH). The material was used without further purification for synthesis of compound **150**.

***tert*-Butyl-2-(5,6-dibromo-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate** (**150**). Compound **150** was prepared based on a literature procedure for similar compound.[9] Triethylamine (0.5 g, 4.5 mmol) and di-*tert*-butyl dicarbonate (1.0 g, 4.5 mmol) were added to a solution of 5,6-dibromo-1*H*-indol-3-yl-*N*-methylethanamine (**149**) (1.5 g, 4.5 mmol) in DMF (20 mL) and the solution was allowed to stir at room temperature for 24 h. The reaction mixture was quenched with H_2O (40 mL) and extracted with EtOAc (3 x 40 mL). The combined organic portions were washed with H_2O (3 x 40 mL), brine (50 mL), dried (Na_2SO_4) and the solvent was evaporated under reduced pressure to yield a crude residue. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 3:1) to afford 1.2 g (60%) of **150** as a white foam: ^{1}H NMR

($CDCl_3$) δ 1.40 (s, 9H, CH_3), 2.84 (s, 3H, $NHCH_3$), 2.91 (t, 2H, CH_2), 3.48 (t, 2H, CH_2), 7.01 (s, 1H, Ar), 7.66 (s, 1H, Ar), 7.87 (s, 1H, Ar), 8.07 (br s, 1H, indolic NH).

***tert*-Butyl-2-(5,6-dibromo-2-(1,1-dimethylallyl)-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate** (**151**). Compound **151** was prepared based on a literature procedure of a similar compound.[198] *tert*-Butyl hypochlorite (0.3 g, 3.0 mmol) was added to a solution of *tert*-butyl-2-(5,6-dibromo-1*H*-indol-3-yl)ethyl-*N*-methylcarbamate (**150**) (1.0 g, 2.3 mmol) and Et_3N (0.3 g, 3 mmol) in THF (25 mL) at -78 ^oC and the solution was allowed to stir for 90 min. Freshly prepared prenyl 9-BBN[179] (4.6 mmol) was added in a dropwise manner over 20 min while maintaining the temperature at -55 ^oC. The reaction mixture was allowed to warm to room temperature and stirred for an additional 6 h. Sodium hydroxide (3M, 10 mL) and H_2O_2 (30%, 10 mL) were added in a dropwise manner, and reaction mixture was stirred for an additional 2 h, and then diluted with Et_2O (100 mL). The organic layer was washed with H_2O (3 x 60 mL), brine (80 mL), dried (Na_2SO_4), and evaporated under reduced pressure to yield a crude residue. The residue was purified by column chromatography (silica gel; hexanes/EtOAc; 100:1 to 10:1) to afford 0.4 g (33%) of **151** as a white foam: mp 55-57 °C; ^1H NMR ($CDCl_3$) δ 1.44 (s, 9H, CH_3), 2.71-2.90 (m, 5H, CH_2, CH_3), 3.29 (m, 2H, CH_2), 5.07-5.12 (m, 2H, CH_2), 5.99-6.06 (m, 1H, CH), 7.49 (s, 1H, Ar), 7.72 (s, 1H, Ar), 7.82 (br s, 1H, indolic NH). Compound **151** was employed in the preparation of compound **144**.

B. Electrophysiology

The electrophysiological studies were performed in Dr. Shulte's lab at the University of Alaska (Fairbanks). The cDNA sequences for the human α4 (NCBI Reference Sequence: NM_000744.5) and β2 (NCBI Reference Sequence: NM_000748.2) nACh receptors were used for the synthesis of the cDNA for for each of the subunits (performed in Burlingame, CA). The cDNA for the β2 subunit was inserted in the pcDNA3.1/Zero (+) expression vector and the α4 subunit was inserted in the pcDNA3.1/hygromyocin expression vector (both the vectors obtained from Invitrogen, Carlsbad, CA). Ovarian lobes from the *Xenopus laevis* frogs were obtained by surgically removing and washing twice with Barth's buffer (Ca^{2+} free) (82.5 mM NaCl/2.5 mM KCl/1 mM $MgCl_2$/5 mM HEPES, pH 7.4). The oocytes were then shaken with collagenase (1.5 mg/mL, Sigma Type II obtained from Sigma-Aldrich) for 1 h at 20-25 °C. Stage IV oocytes were selected for the microinjection and injected with 50 nL of the appropriate ratios of the synthetic cRNA transcripts of α4 and β2 subunits (prepared using mMESSAGE mMACHINE High Yield Capped RNA Transcription Kit obtained from Ambion, TX) at a concentration of 0.2 ng/mL. Later the injected oocytes were incubated at 19 °C for 24-72 h before being used for the voltage clamp experiments. The incubated oocytes were used for recordings were obtained using an automated two-electrode voltage-clamp system incorporating an OC-725C oocyte amplifier (obtained from Warner Instruments, CT) which was coupled to a computerized data acquisition system; Datapac 2000 (obtained from RUN technologies) and an autoinjection system (Gilson) for the injection of solution of test compounds. The electrodes used for recording and the one providing the current (with a resistance of 1-4 MΩ) were filled with 3 M KCl solution. The oocytes were held in a vertical flow chamber of 200 μL volume and perfused with ND-96 buffer

(96 mM NaCl/ 2 mM KCl/ 1.8 mM $CaCl_2$/ 1 mM $MgCl_2$/5 mM HEPES; pH 7.4) at a rate of 20 mL/min. The test compound solution (using ND-96 buffer) were injected into the chamber (20 mL/min) using the autoinjection system. The test compounds were applied alone or co-applied with 100 μM (EC_{75}) of ACh.[9] All assays were performed at least in triplicate. The EC_{50} values were calculated by fitting the dose-response curve by non-linear cruve fitting. Built-in algorithms of GraphPad prism (San Diego, CA) was used for the calculation. For IC_{50} determination dose-response curve data were fit to a single-site competition model. For compounds with the biphasic dose-response curve the data were fit to a hormetic model which generates the EC_{50} and IC_{50} simultaneously. Data was expressed in ± SEM or 95% confidence limits. All statistical differences were tested using student's *t*-test with the P values ($P < 0.05$ representing significant statistical difference).[9,167,216]

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Vita

Atul Dilip Jain was born on June 28, 1983 in Mumbai, India to the parents Dilip and Manjula Jain. He received a Bachelor of Pharmaceutical Science degree from Bombay College of Pharmacy, Mumbai University, India in August 2005 and a Master of Science with major in Pharmaceutical Chemistry from Bombay College of Pharmacy, Mumbai University, India in May 2008. Subsequently he was enrolled in the Virginia Commonwealth University's School of Pharmacy, Pharmaceutical Sciences graduate program with a concentration in Medicinal Chemistry in August 2008.