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<td>Aa</td>
<td>Amino acid</td>
</tr>
<tr>
<td>Ac</td>
<td>Acetyl</td>
</tr>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AChBP</td>
<td>Acetylcholine binding protein</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>BHP</td>
<td>(E)-3-(6-benzylidenecyclohex-1-en-1-yl)pyridine</td>
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<tr>
<td>Boc</td>
<td>t-Butoxycarbonyl</td>
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<tr>
<td>b.p.</td>
<td>Boiling point</td>
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<tr>
<td>Cat.</td>
<td>Catalyst</td>
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<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
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<td>cRNA</td>
<td>Complementary ribonucleic acid</td>
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<td>(CHO)$_n$</td>
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<td>Dichloromethane</td>
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<td>1,2-dimethoxyethane</td>
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<td>Dimethylformamide</td>
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<td>Abbreviation</td>
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<td>Lithium 2,2,6,6-tetramethylpiperidide</td>
</tr>
<tr>
<td>LGIC</td>
<td>Ligand gated ion channel</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>Mol%</td>
<td>Percent molar equivalents</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting point</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>nAChR</td>
<td>Nicotinic acetylcholine receptor</td>
</tr>
<tr>
<td>Et₃N</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser effect</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>o/n</td>
<td>Overnight</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PAB</td>
<td>Pyridinylmethylene anabaseine</td>
</tr>
<tr>
<td>PAM</td>
<td>Positive allosteric modulator</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>PDC</td>
<td>Pyridinium dichlorochromate</td>
</tr>
<tr>
<td>PNU-120596</td>
<td>N-(5-chloro-2,4-dimethylphenyl)-N’-(5-methyl-3-isoxazolyl)-urea</td>
</tr>
<tr>
<td>QN</td>
<td>Quininuclidine</td>
</tr>
<tr>
<td>Red-Al</td>
<td>Sodium bis(2-methoxyethoxy)aluminium hydride</td>
</tr>
<tr>
<td>R&lt;sub&gt;f&lt;/sub&gt;</td>
<td>Retention factor</td>
</tr>
<tr>
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<tr>
<td>S.E.M.</td>
<td>Standard error</td>
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<tr>
<td>TBAF</td>
<td>Tetrabutylammonium fluoride</td>
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<tr>
<td>TBS</td>
<td>Tert-Butyldimethylsilyl</td>
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<td>THF</td>
<td>Tetrahydrofuran</td>
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<td>TLC</td>
<td>Thin layer chromatography</td>
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<td>Tetramethylammonium</td>
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<tr>
<td>TMD</td>
<td>Transmembrane domain</td>
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<tr>
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SYNTHESIS OF NICOTINIC RECEPTOR LIGANDS AND STRIGOLACTONES

By
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December 2013

Chair: Nicole A. Horenstein
Major: Chemistry

The first part of this dissertation is focused on the design and synthesis of molecules that differentially regulate activation and desensitization of the human alpha7 nicotinic acetylcholine receptor (nAChR). Alpha7 nAChR is a pentameric ligand gated ion channel that is currently a drug target for Alzheimer’s disease, schizophrenia and inflammatory disorders. Emerging evidence suggests that alpha7 in non-neuronal cells may inhibit pro-inflammatory cytokine production by a signaling mechanism that does not involve ion channel opening. The term “silent agonists” is introduced to describe receptor ligands that bind to the conventional acetylcholine binding site, do not initiate ion channel activity, and place the receptor in a desensitized state that can be revealed in the presence of a type II positive allosteric modulator. Because these molecules convert the alpha7 nAChR from a resting state selectively into a desensitized state that can be probed by a type II PAM, they are of interest as a tool to study the functions of the alpha7 nAChR that do not involve ion conducting states, and may constitute a new modality for the development of alpha7 nAChR therapeutics.
Three groups of silent agonists are characterized: the first group is represented by newly designed and synthesized KC-1 and KC-5 compounds, the second group features bulky quaternary ammonium compounds of appropriate molecular volume, and the third is exemplified by pyridinylmethylen e anabaseines (PABs).

The second part of this dissertation is focused on the strigolactone synthesis and biosynthesis. Strigolactones are a new class of plant hormones that inhibit shoot lateral branching. Synthesis of the strigolactone ABC-core in one step from linear precursors by an acid-catalyzed double cyclization was proposed and tested. Linear model precursors were prepared and conditions under which these molecules undergo the proposed double cyclization in good yields and with a high degree of stereocontrol were identified. These cyclization results are relevant because they suggest a plausible mechanism along the strigolactone biosynthetic pathway.
This portion of my dissertation is focused on the design, synthesis and characterization of molecules that modulate the function of the human α7 nicotinic acetylcholine receptor (hα7 nAChR), particularly silent agonists. Traditionally, the role of the α7 receptor was linked to its ion channel activity. However, growing evidence supports a metabotropic function independent of ion channel opening that may modulate signal transduction pathways that can regulate inflammatory response and cell death (apoptosis) or survival, especially in non-neuronal cells. Hence, the following section provides background information on ion channels, ligands, and desensitization that might be the active state for signaling beyond ion channel activity.

1.1 Chemical Neurotransmission, Receptors, Ion Channels, Functions of nAChRs

Communication between cells in humans comes from the central nervous system (CNS-brain and spinal column) and the peripheral nervous system (PNS-nerves and ganglia) which connect CNS to the rest of the body. A nerve cell (neuron) possesses a cell body, dendrites and an axon (Figure 1-1 A).¹ Neurons transmit signals by electrical pulses that result from the movement of ions across cell membranes due to different concentrations of ions inside and outside cells established by ion pumps (the concentration of potassium ions inside the cell is larger than the surrounding medium, whereas the concentration of sodium, calcium and chloride ions is smaller).²

Neurons communicate with each other and with other cells through synapses, by passing an electrical signal or a chemical signal.¹³ In the electrical synapses (that are minor in the mammalian nervous system), neurons are extremely close to each other and the signal is passed on directly through tight junctions that involve specialized ion
channels called connexons.\(^4\) In the majority of synapses, the gap (synaptic cleft) between cells is too large for a direct transmission and the communication between cells is mediated by endogenous chemicals called neurotransmitters.\(^1\)

A.

![Diagram A: Structure of a typical nerve cell.](image)

B.

![Diagram B: Scheme of a chemical synapse.](image)

Figure 1-1. Chemical signaling in the nervous system. A) Structure of a typical nerve cell. B) Scheme of a chemical synapse.

Neurotransmitters are stored in vesicles at the axon terminal (Figure 1-1. B). After being released to a synaptic cleft, they diffuse to proteins (called receptors) localized in the membrane of target cells, to which they bind causing a conformational change that leads to a series of secondary effects. For example, a flow of ions across
the cell membrane or the switching on (or off) of enzymes inside the target cells. There are several mechanisms to avoid continuous activation of receptors. The first mechanism is by enzymes in the synaptic cleft that quickly convert neurotransmitters to their inactive metabolites (for example, acetylcholinesterase hydrolyzes acetylcholine to choline). The second mechanism is by reuptake of neurotransmitters by presynaptic cells, the third is by internalization of the receptors, and finally the fourth by desensitization (i.e., induction of a closed state of the receptor that is unresponsive to agonists). The concept of a receptor can be traced to the work of the German physician Paul Ehrlich and especially to the English physiologist John Newport Langley during the period of 1905-1907.

In 1926, drawing from studies of Henry Hallett Dale, Otto Loewi identified acetylcholine (ACh) as the first neurotransmitter by experiments on the heart muscle. Today, there are a large number of neurotransmitters known, for example: biogenic amines (dopamine, norepinephrine, epinephrine, serotonin and histamine), amino acids (glycine, γ-aminobutyric acid-GABA, aspartate and glutamate), purines (ATP, adenosine, and guanosine), and neuropeptides that can be subdivided into opioids (endorphins, enkephalins and dynorphins) and non-opioids (substance P and neuropeptide Y). Although they are not stored in vesicles, and do not bind to the receptors on postsynaptic membranes but rather diffuse right through the postsynaptic membrane, some gaseous molecules (nitric oxide and carbon monoxide), are also classified as neurotransmitters because they are released by neurons and influence the electrochemical state of adjacent cells (Figure 1-2).
A molecule that interacts with receptors is called a ligand. There are two classes of receptors responding to acetylcholine that are named for the exogenous ligands that activate them. The first class, called muscarinic acetylcholine receptors (mAChRs), is activated by a mushroom alkaloid muscarine and inhibited by a plant tropane alkaloid atropine (Figure 1-3). These receptors belong to the superfamily of G-protein coupled receptors (GPCRs), and they mediate the slow metabolic responses to ACh via coupling to second messenger cascades (such as cyclic adenosine monophosphate: c-AMP, diacylglycerol: DAG, and inositol triphosphate: IP$_3$), and are not a subject of this
dissertation. The second class is activated by tobacco alkaloid nicotine (and is thus called nicotinic acetylcholine receptors, nAChRs) and belongs to a superfamily of ligand-gated ion channels (LGIC).²,⁴,¹⁴

![Chemical Structures](image)

Figure 1-3. Structures of acetylcholine, muscarine, atropine, and nicotine.

LGIC are membrane receptors that contain both the binding site for the natural ligand and the ion-conducting pore, which can be opened or closed upon ligand binding, allowing fast ion flux (10⁷ ions per second) across the cell membrane down electrochemical gradients.¹³ There are three subfamilies of LGIC in mammals.⁴,¹⁴ One family is P2X receptors (activated by ATP), another family comprises glutamate receptors (N-methyl-D-aspartate: NMDA, alpha-amino-3-hydroxy-5-methylisoxazole-propionate: AMPA, and kainate receptors), and finally the largest family, called Cys-loop family,¹⁵-¹⁷ includes nicotinic, glycine, serotonin (5HT₃), zinc (ZAC), and γ-aminobutyric acid (GABAₐ, and GABAₐ) receptors.

Nicotinic acetylcholine receptors are pentameric transmembrane glycoproteins that are activated by ACh and nicotinic agonists.¹⁸ They are allosteric, i.e., have multiple conformational states such as open, closed, and desensitized and equilibria between those states are regulated by ligand binding¹⁹ (see 1.6 Allostery and desensitization). Binding of the agonists in the extracellular domain at the interface between two subunits may promote fast opening (within μs) of an ion channel permeable to Na⁺, K⁺ and in some cases Ca²⁺ ions, 50 Å away from the ACh binding
site (Figure 1-4). The open state is intrinsically unstable and on a longer (ms to min) time-scale the receptor is desensitized (i.e., non-conductive and non-activatable by an agonist), in case of heteromeric receptors toward a higher-affinity closed state.20

![Diagram of nAChR](image)

Figure 1-4. Schematic representation of a nicotine acetylcholine receptor (nAChR) from muscle. (Reproduced from Nature Reviews Neuroscience, 3/2, Karlin A., Emerging structure of the nicotinic acetylcholine receptors, 102-114, Copyright (2002), with permission from Macmillan Publishers Ltd).61

The first nAChR characterized in the 1970s, was a degenerate form of a skeletal muscle-type receptor isolated from the electric organ of fish (Torpedo) by Changeux, Kasai and Lee.21,22 Muscle-type nAChR remains the best-characterized LGIC and it serves as a prototype for other ligand-gated ion channels.23 Many other types of nAChRs have been discovered (see 1.2 Subtypes of nAChRs) that are not expressed in muscles, but in neurons and non-neuronal cells, for example in lymphoid tissue (e.g., B- and T-lymphocytes), macrophages (e.g., microglia), skin keratinocytes, lung cells, vascular tissue (e.g., blood vessels), astrocytes (type of glial cells in CNS that provide support and protection for neurons) and certain carcinomas (reviewed in 24-28).

The nAChRs have well established ionotropic function in synaptic transmission of electric signals in the peripheral nervous system.1 However, the function of nAChRs in the brain is more commonly associated with modulatory events than mediation of
synaptic transmission.\textsuperscript{20,29} Since nAChRs can regulate influx of calcium (both directly or indirectly by controlling membrane potential), they may modulate the release of other neurotransmitters (such as dopamine, norepinephrine, serotonin, glutamate, and GABA)\textsuperscript{19,30,31} and also initiate calcium signaling that is involved in many intracellular enzymatic processes linked to cell motility, adhesion, migration, proliferation, differentiation, gene expression and survival.\textsuperscript{29,32} The nAChRs also play a role during development and in neuronal/synaptic plasticity.\textsuperscript{29-30}

Moreover, there is growing appreciation that $\alpha_7$ nAChRs may also have metabotropic function without ion channel opening, and this type of signaling is further described in Section 1.7 (The $\alpha_7$ nAChR and inflammatory response).

1.2 Subtypes of nAChRs

There are 17 different nAChR subunits in vertebrates identified so far ($\alpha_1-\alpha_{10}$, $\beta_1-\beta_{4}$, $\gamma$, $\delta$, and $\epsilon$) that can form many different subtypes of the pentameric nAChR, associated not only with subunit composition, but also subunit stoichiometry and arrangement (reviewed in, \textsuperscript{19,33-38} Figure 1-5). All of these subunits are present in humans except $\alpha_8$ that has only been found in chicken. Even though many potential combinations of nAChRs are possible, a single or a few receptor combinations seem to be preferred.\textsuperscript{19,35} The nAChRs subunits come from a common ancestor and have been highly conserved during evolution (the same subunit has more than 80 % of amino acid identity across vertebrate species\textsuperscript{39} however, it shares less sequence similarity with other subunits). The nAChR subtypes are named according to their known subunit composition (sometimes using an asterisk “*” to indicate possible additional subunits).\textsuperscript{36}
Figure 1-5. Schematic representation of a few nAChR subtypes with acetylcholine binding sites shown.

The nAChR subtypes have similar basic structure but different pharmacological properties (different affinities for ligands, different response to ACh and other ligands, different permeabilities for ions, different kinetics of activation and desensitization and recovery from desensitization). They regulate different physiological processes, and are implicated selectively in some diseases.\textsuperscript{34,40} Therefore, it is possible to some extent to design subtype-selective nAChRs ligands and avoid cardiovascular and gastrointestinal side effects of nicotine and other non-selective nAChRs agonists and potential addiction liability.\textsuperscript{19,41-43}

First four subunits of nAChR from the electric organ of Torpedo were assigned the Greek letters $\alpha$, $\beta$, $\gamma$, $\delta$ on the basis of their increasing apparent molecular weights when resolved on polyacrylamide gels.\textsuperscript{44} Homologous subunits at the neuromuscular junction were renamed later as $\alpha1$, $\beta1$, $\delta$, and $\varepsilon$ (adult form) or $\gamma$ (embryonic form).\textsuperscript{45}
The nAChR subunits are classified as α if they contain vicinal cysteine residues in the C-loop at positions analogous to Cys192 and Cys193 in *Torpedo* α-subunit.\(^{36}\) β-Subunits and other non-alpha subunits lack those vicinal cysteine residues. Based on initial affinity labeling experiments with α-bungarotoxin, it was assumed that α subunits are agonist binding subunits, whereas non-α subunits are structural subunits. Later studies showed that agonists bind at subunit interfaces, both α and some non-α subunits contribute to the agonist binding site, and thus the part of an α subunit that has two vicinal cysteines in the C-loop and forms the main part of the binding site is called primary (or principal, or “+”) face, and the remaining part of the ligand binding domain, formed by either an α or non-α subunit, is called complementary site (“−” face).\(^{37}\) The α5 and α10 subunits appear to not be able to function as primary faces even though they possess vicinal cysteines in the C-loop.\(^{37}\) There are two ligand binding sites in muscular nicotinic receptors, one at the α1-δ interface, and the other at the α1-ɛ (or γ) interface. The β1 subunit is structural (accessory) and is not directly involved in a primary ligand binding.

The nAChRs are traditionally classified as muscle (α1, β1, δ, ε or γ) and neuronal (α2–α10, β2–β4) subunits, however many “neuronal” subunits were also found in muscles and in other non-neuronal tissues.

The nAChR subunits are often classified into those that can form homomeric and those that form heteromeric pentamers. Homomeric receptors (α7, α8, α9) contain five identical subunits and five identical putative binding sites for agonists at the interface between two subunits.\(^{46}\) Even though five binding sites are present, α7 homomeric
receptors can be activated under conditions of submaximal agonist occupancy (one and two ligands present), and higher occupancies of the receptor result in desensitization rather than activation.\textsuperscript{47} Some data suggest that $\alpha7$ subunits can form anomalous heteromeric receptors, (for example, the $\alpha7\beta2$ subtype was reported in basal forebrain in rodents),\textsuperscript{48} but these combinations are rare, and whenever $\alpha7$ is mentioned later on in this dissertation, the $(\alpha7)_5$ subtype is implied.

Most nAChRs are heteromeric and have very diverse combinations. Stoichiometry of many heteromeric receptors is believed to be $(\alpha)_{2} (\beta)_{3}$, arranged clockwise as $\alpha\beta\alpha\beta\beta$ with two binding sites for agonists at the $\alpha-\beta$ interfaces, for example $(\alpha4)_{2}(\beta2)_{3}$ (Figure 1-5). However, receptors with alternative stoichiometries have been also characterized \textit{in vitro} and implicated \textit{in vivo}, such as $(\alpha4)_{3}(\beta2)_{2}$. The $(\alpha4)_{2}(\beta2)_{3}$ receptors have high sensitivity to nicotine and low $\text{Ca}^{2+}$ permeability, whereas $(\alpha4)_{3}(\beta2)_{2}$ has low sensitivity to nicotine and high $\text{Ca}^{2+}$ permeability.\textsuperscript{49,50} There are also nAChR subtypes in which the $\alpha$ and $\beta$ subunits are not identical (for example $(\alpha3)_{2}\beta3(\beta4)_{2}$ and $\alpha3\alpha5\beta2\beta4$).\textsuperscript{37,38}

Early indication that there are significant differences between receptor subtypes was revealed by difference in sensitivity to the snake toxin $\alpha$-bungarotoxin ($\alpha$-Btx).\textsuperscript{18} The $\alpha7-\alpha10$ subunits bind $\alpha$-Btx tightly, while other subunits (except those at the neuromuscular junction) do not.\textsuperscript{38} In 1985, Clarke \textit{et al.} performed an autoradiographic comparison of $[^3\text{H}]$-acetylcholine, $[^3\text{H}]$-nicotine, and $[^{125}\text{I}]$-$\alpha$-bungarotoxin labeled receptors in a rat brain that revealed that nAChRs with different affinities for the studied ligands were located in different parts of the brain.\textsuperscript{51} Much of our current knowledge about nAChR subtypes comes from studies of heterologously expressed receptors,\textsuperscript{37}
knock-out (targeted deletion of specific subunits) and knock-in (mutations in critical receptor domains) mice,\textsuperscript{19,40} studies with subunit-specific antibodies,\textsuperscript{41,25} and subtype-specific ligands, for example $\alpha$-conotoxins.\textsuperscript{52}

The $\alpha7$ subtype was discovered and cloned in 1990.\textsuperscript{53} The $\alpha7$ nAChR is a close existing homolog to an ancestral receptor that was present before the development of nervous system.\textsuperscript{54} Special features of $\alpha7$ receptor include: high permeability to calcium ions (permeability ratio of Ca$^{2+}$ to Na$^+$ is $\sim$10),\textsuperscript{55} low probability of channel opening,\textsuperscript{56} quick and reversible desensitization,\textsuperscript{53} at least two characterized distinct desensitized states: $D_s$ and $D_i$,\textsuperscript{57} activation by choline,\textsuperscript{58,59} and inhibition by $\alpha$-bungarotoxin and methyllycaconitine (MLA). Many of these characteristics will be discussed further.

1.3 Structure of the Receptor

1.3.1 Overview

As mentioned earlier, nAChRs are membrane proteins composed of five pseudo-symmetrically arranged subunits that surround a central ion-conducting pore. Each nAChR subunit has a large N-terminal extracellular domain (ECD, $\sim$200 amino acids), a transmembrane domain (TMD) that is composed of three $\alpha$-helices labeled M1-M3 connected to a fourth $\alpha$-helix (M4) by a large intracellular domain (ICD, $\sim$100-270 amino acids depending on a subunit), and a short extracellular C-terminus (4-30 amino acids) (Figure 1-6). The structure of the nAChRs has been reviewed in.\textsuperscript{19,60-63}
As mentioned before, the nAChRs belong to a Cys-loop family, together with serotonin (5-HT₃), GABAₐ, GABAₐₐ, zinc (ZAC), and glycine receptors. The name of this superfamily comes from a signature sequence of 13 residues flanked by two disulfide-linked cysteines, called Cys loop – a closed loop situated between the extracellular ligand binding domain and the transmembrane domain. 

Although Cys-loop receptors are activated by different ligands and they can be permeable to either cations or anions, they share considerable sequence homology and have similar basic functionality. This similarity of Cys-loop receptors is illustrated by the fact that the ECD and TMD from different Cys loop family receptors can be coupled to form functional receptors, for example, a chimera made of the ECD of the α7 nAChR and the TMD of the 5HT₃ receptor has binding site properties of the α7 and channel domain properties of the 5HT₃.

Our knowledge of the nAChR structure comes from radiolabeling, photolabeling, sequence analysis, site directed mutagenesis, substituted-cysteine accessibility method (SCAM), use of snake venoms, immunological and electrophysiological experiments, computational methods, and three dimensional structures obtained by X-ray or electron
microscopy. The available structures of nAChRs or their homologues are:

1) X-ray structures of molluscan acetylcholine binding proteins (AChBP), which are soluble protein homologues of the extracellular domains of nAChR;

2) the electron microscopy structure of a fish muscle-type nAChR analog at 4 Å resolution;

3) the X-ray structure of a mouse ECD portion of the α1 subunit bound to α-bungarotoxin;

4) the X-ray structures of two prokaryotic LGICs, one from the bacterium *Gleobacter violaceus* (GLIC protein), and the other from the bacterium *Erwinia chrysanthemi* (ELIC protein);

5) the X-ray structure of the ECD hα7 nAChR/AChBP chimera; and

6) the X-ray structure of a pentameric glutamate channel from *C. elegans* – GluCl.

As can be seen from the above list, there is no single high resolution structure of any whole nAChR protein. The available 3D structures do provide valuable information about nAChRs, and have been used to make verifiable experimental hypotheses. However, the use of these structures for design of subtype selective-ligands and elucidation of pharmacological properties for distinct subtypes of the receptor is quite limited and involves a lot of speculation because of the reliance on the homology models (i.e., models created using amino acid sequence of nAChRs and 3D structures of AChBP that has less than 25% sequence identity to nAChRs).

### 1.3.2 Extracellular Domain (Ligand Binding Domain)

The extracellular domain (ECD) of nAChRs contains the ligand binding sites for acetylcholine and is thus also called the ligand binding domain (LBD). Each subunit of the ECD also contains the main immunogenic region (MIR, major target for the nAChR antibodies), at least one glycosylation site, and the Cys loop (Figure 1-6). The
extracellular domain starts at the N-terminus with a three-turn α-helix, followed by a bundle of ten β-stands (β1-10) and several connecting loops. The acetylcholine binding site (also called orthosteric site) is formed by “loops” A, B, and C on the primary face of an α-subunit and “loops” D, E, F on the complementary face (D and E loops are actually parts of β-strands) (Figure 1-7 A). The ligands bind under the C loop (containing two adjacent cysteines residues linked by a disulfide bond), in the cage created by five aromatic residues that interact by cation π-interactions with a positive charge on the nitrogen of the agonist

Figure 1-7. Ligand binding domain. A) Two subunits of the AChBP showing the loops creating the binding site at the interface between the subunits. (Reproduced from Quaterly Reviews of Biophysics, 43/4, AJ Thompson, HA Lester, SCR Lummis, The structural basis of function in Cys-loop receptors, 449-499, Copyright (2010), with permission from Cambridge University Press). B) (S)-Nicotine binding to the AChBP showing the residues participating in the binding of the ligand (Reprinted with permission from Journal of Medicinal Chemistry, 48, JA Jensen, B Frolund, T Liljefors, P. Krogsgaard-Larsen, Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations, 4705-4745, Copyright (2005), American Chemical Society).
It is believed that the binding of an agonist to the nAChR causes the C-loop to close, similar to what is seen in the acetylcholine binding protein (Figure 1-8).

Figure 1-8. Conformational changes in the loop C in AChBPs on ligand-binding. Comparison of the orientation of loop C between two extreme positions, with α-Ctx-ImI (nAChR antagonist) or epibatidine (nAChR agonist). Top view, showing the “open” and “closed” conformations of loop C after binding of α-Ctx-ImI (left, red loop C) or epibatidine (right, blue loop C). (Reprinted from EMBO Journal, 24, Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P, Bourne Y., Structures of aplysia AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations, 3635-3646, Copyright (2005), with permission from Macmillan Publishers Ltd). Note: terms agonist and antagonist have no meaning for the AChBP. Displacement of the C-loop may be a reflection of a larger size of the antagonist.

1.3.3 Transmembrane Domain

The transmembrane domain (TMD) of each nAChR subunit is made of four α-helices (M1-M4). The channel pore is lined with the 40 Å-long M2 segments from each of the five subunits, the M1 and M3 helices form a circle behind them, and the M4s are positioned at the periphery, sequestering the ion channel from the membrane lipids (Figure 1-9). The interface between the TMD helices and the ECD β-sheets is located ~10 Å on the extracellular side of the cell membrane. The M2 helices from all subunits have sets of homologous residues at each level, forming rings of chemically distinct environments facing the lumen of the pore. The narrowest part
of the ion channel is called a gate, which acts as a molecular barrier (both steric and energetic), prohibiting the ions from passing through the channel. The gate is created by interactions between side chains of five leucines ("leucine ring") and another ring of five hydrophobic residues called a "valine/isoleucine ring". A gate can be at different levels in resting and desensitized states.\textsuperscript{19,61}

In the resting state, the channel opening has been estimated to be 6 Å in diameter, thus permeation of hydrated monovalent and divalent cations is impossible (ions cannot readily lose their hydration shells in the absence of polar surfaces that would replace water molecules).\textsuperscript{72} The diameter of an open channel has been estimated to be 9 Å.\textsuperscript{84}

![Figure 1-9](image)

**Figure 1-9.** Cross-section of a nAChR pore in the middle of the transmembrane domain.

The transmembrane domain also contains two negatively charged rings at the ends of the M2 helices (mainly made of glutamate residues), that contribute to ion conductivity through the ion pore by providing an electrostatic potential of opposite sign to that of the permeant ion: one ring on the extracellular domain side, and one ring on the intracellular domain side. The charge selectivity filter is also located in the transmembrane domain.\textsuperscript{19}
1.3.4 Intracellular Domain

The intracellular domain (ICD) of nAChRs consists mainly of a large loop between the M3 and M4 helices that displays high variability in amino acid sequence and length in different subunits (110-270 residues).\textsuperscript{62} It is the least characterized portion of the receptor because there is essentially no structural information about it, except that in case of a Torpedo receptor, part of the intracellular domain forms an $\alpha$-helix, and most of it is intrinsically disordered.\textsuperscript{72,85} However, it is increasingly appreciated that structural disorder is important for the function of some proteins.\textsuperscript{86} The nAChR subunit intracellular domains contain varying numbers of putative phosphorylation sites which have been suggested to play an important role in the nAChRs expression, trafficking, assembly of the subunits, and interactions with the cytoskeleton.\textsuperscript{62,87-89} It also appears that the ICD interacts with many proteins inside the cells,\textsuperscript{90} and it is believed that they play many other important functional roles (such as the regulation of transcription and translation and cellular signal transduction), most of which remain to be clarified.

1.3.5 Three Dimensional Structures of Pentameric LGICs

1.3.5.1 X-ray structures of acetylcholine binding protein (AChBP)

Our knowledge about nAChR structure comes in a large part from X-ray structures of the water soluble pentameric acetylcholine binding protein (AChBP) from snail \textit{aplysia californica} (ac) and \textit{lymnaea stagnalis} (ls).\textsuperscript{91} The AChBP in the mollusks is released by glial cells into a synaptic cleft where it binds acetylcholine to modulate neurotransmission.\textsuperscript{92} The first structure was solved in 2001 by Brejc \textit{et al.} at 2.7 Å with five HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) molecules from the crystallization buffer, 10 Ca$^{2+}$ ions, and 15 water molecules bound (Figure 1-10).\textsuperscript{66} The
AChBP shares 24% sequence identity with the extracellular domain of the h\(\alpha\)7 nAChR, has 80 Å in diameter and a height of 62 Å, contains 210 amino acids in each monomer, and has a glycosylation site at position Asn 66. Each monomer of the protein has a modified immunoglobulin fold, in which two \(\beta\)-sheets are organized in a curled \(\beta\)-sandwich: an inner \(\beta\)-sheet is made of \(\beta1, \beta2, \beta3, \beta5, \beta6, \text{and} \beta8\), and is linked by the Cys-loop disulfide bond to an outer sheet that is formed by \(\beta4, \beta7, \beta9, \text{and} \beta10\). There are also two short 3\(_{10}\) helices (3 amino acids per turn, 2 Å helix translation per residue). Each monomer of the protein contains a pair of adjacent cysteines linked by a disulfide bond in loop C.

![Figure 1-10. The pentameric structure of AChBP. A) top view. B) view perpendicular to the five-fold axis with ligand-binding site shown in ball-and-sticks.](Reprinted from Nature, 411/6835, Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van der Oost J, Smit AB, Sixma TK., Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors, 269-276, Copyright (2001), with permission from Macmillan Publishers Ltd).

There are currently more than fifty structures of AChBP crystallized with a variety of ligands (agonists, antagonists and allosteric modulators), bound at the interface between two monomers, and resolved up to 2.05 Å. The AChBP binds \([^{125}\text{I}]\alpha\)-bungarotoxin and many cholinergic ligands with similar affinities to those of the \(\alpha7\) nAChR and is extensively used as a homology model to predict ligand-receptor interactions. However, the use of the AChBP template is limited because it is not an
ion channel, it lacks an equivalent of the nAChR TMD and ICD, and does not provide information about receptor function.

1.3.5.2 Cryo-electron microscopic structure of the nAChR from the Torpedo electric organ at 4 Å

First images of a muscle-type nAChR in its native lipid surroundings were obtained by Unwin and co-workers by electron microscopy in the late 1980s. Using tubular crystals of postsynaptic membranes from *Torpedo* electric organ, the researchers revealed the structure of the receptor at 18 Å resolution. Since then, Unwin has published several new structures at increasing resolution (up to 4 Å), using the AChBP template for refining of the ECD\textsuperscript{72,94} (Figure 1-11).

A. B. C.

Figure 1-11. Ribbon diagrams of the nAChR from Torpedo electric organ. A) View from the synaptic cleft. B) View parallel with the membrane plane. C) Diagram of a single subunit, view parallel with the membrane plane, the central axis of the pentamer (vertical line) is at the back (the αTrp149 from LBD is shown in gold) (Reproduced from Journal of Molecular Biology, 346/4, Unwin N, Refined structure of the nicotinic acetylcholine receptor at 4 angstrom resolution, 967-989, Copyright (2005), with permission from Elsevier).\textsuperscript{72}
In most of these electron microscopy images, the ion channel is in the closed state, but there are two structures in which the ion channel was freeze-trapped in the open state. The structure is often considered to be the only one of a whole nAChR protein, however a large part of the intracellular domain (M3-M4) is in fact missing. Although the structure is helpful in elucidation of the ion channel functioning, the side chain orientation is quite ambiguous in this low resolution structure.

1.3.5.3 X-ray structure of the ECD of a mouse α1 monomer with α-bungarotoxin bound at 1.94 Å resolution.

In 2007, Dellisanti et al. (Chen group) have published an X-ray structure of the ECD of a mouse α1 monomer with α-bungarotoxin bound at 1.94 Å resolution. The protein contains three mutations that enabled its crystallization. In the structure, there is an oligosaccharide bound from Asn141 to Ser 143 which was suggested to facilitate folding and trafficking of the receptor. The structure is the first atomic-resolution view of a nAChR subunit extracellular domain, but it is not a good template for ligand binding because it is a monomer and its structure is altered by binding bungarotoxin and mutations.

Figure 1-12. Overall structure of the mouse nAChR α1 subunit (cyan) bound to α-Bgtx (orange). The carbohydrate chain is shown as a stick model and colored in magenta. A) Front view between the inner and outer sheets. B) Top view (Reprinted from Nature Neuroscience, 10/8, Dellisanti CD, Yao Y, Stroud JC, Wang Z, Chen L, Crystal structure of the extracellular domain of nAChR alpha 1 bound to alpha-bungarotoxin at 1.94 A resolution, 953-962, Copyright (2007), with permission from Macmillan Publishers Ltd).
X-ray structures of prokaryotic ion channels (GLIC and ELIC)

Analysis of bacterial genome sequences has revealed that 3 % of all bacteria sequenced so far contain a putative pentameric LGIC gene. The available X-ray structures of prokaryotic pentameric ion channels homologous to nAChR are: GLIC (Gloeobacter violaceus) solved at 2.9 Å in an apparently open conformation, and ELIC (Erwinia chrysanthemi) solved at 3.3 Å in a closed conformation. The prokaryotic pentameric LGICs lack the N-terminal α-helix, they contain a sequence homologous to the Cys loop but the disulfide-linked cysteines are actually missing, and the intracellular domain is not present (Figure 1-13).

Figure 1-13. Schematic representation of one monomer of pentameric LGIC in prokaryotes and eukaryotes.

The GLIC protein is permeable to protons and shares 20 % sequence identity with the human α7 nAChR. The ELIC protein is activated by many primary amines such as amino-butanol, cysteamine, putrescine, and by high (mM) concentrations of GABA, and has 16 % amino acid identity with the nAChR α1 subunit. The structures of bacterial proteins have a similar folding pattern to nAChRs (β-sheets in the ECD, and four transmembrane helices) and are used to study the transition from a closed channel to open (Figure 1-14). The structure of a GLIC protein with anaesthetics bound is used to elucidate allosteric potentiation in the transmembrane domain.
Figure 1-14. GLIC and ELIC proteins. A) Ribbon representation of GLIC viewed from the plane of the membrane. B) Top view of GLIC (a) and ELIC (b) M2 helices (Reprinted from Nature, 457/7225, Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux J, Delarue M, Corringer P, X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation, 111-114, Copyright (2009), with permission from Macmillan Publishers Ltd).  

1.3.5.5 X-ray structure of a the ECD of hα7 nAChR and AChBP chimeras

Several X-ray structures of the ECD of hα7 nAChR and AChBP chimeras have been solved in 2011. For example, the Chen lab obtained a structure of a protein that has 64% identity to the human α7 nAChR with and without an agonist at 2.8 and 3.1 Å resolution (Figure 1-15). While the structures have more similarity to the extracellular domain of the α7 nAChR than the AChBP, they still do not contain transmembrane domain and can only be used for predictions of ligand binding.
Figure 1-15. Structures of α7–AChBP chimera. A) Top view of the α7–AChBP chimera pentamer along the five-fold axis of symmetry; each subunit is shown in a different color. B) Structure superposition between the α7–AChBP chimera (blue) and AChBP (orange) pentamers viewed from the side that is normal to the five-fold axis. C) Structure superposition of subunits from the α7–AChBP chimera (blue), α1 extracellular domain (magenta) and AChBP (orange); loops showing substantial differences are labeled (Reprinted from Nat Neurosci, 14/10, Li S, Huang S, Bren N, Noridomi K, Dellisanti CD, Sine SM, Chen L, Ligand-binding domain of an alpha(7)-nicotinic receptor chimera and its complex with agonist, 1253-1260, Copyright (2011), with permission from Macmillan Publishers Ltd).78

1.3.5.6 Structure of a pentameric glutamate LGIC from C. elegans

The last structure that provides some information for studying the nAChR is the eukaryotic pentameric glutamate ligand-gated ion channel from C. elegans (GluCl) in a complex with an antibody at 3.3 Å resolution (human glutamate receptors are tetrameric).80 GluCl is an anionic Cys-loop glutamate receptor. In 2012, Changeux and co-workers started to classify nAChR as pentameric LGIC rather than Cys loop receptors to include the recently discovered ELIC, GLIC, and GluCl into the same family.63
1.4 Studying the nAChR Ionotropic Function

The function of ion channels can be monitored electrophysiologically by measuring ion flow through the cell membrane resulting from activation of the channels. In this research, the synthesized compounds were tested in human nAChRs expressed heterologously in *Xenopus* oocytes, and their function was monitored by two-electrode voltage clamp. All electrophysiological experiments described in this dissertation were performed in Dr. Papke’s Laboratory (University of Florida, Department of Pharmacology and Therapeutics, College of Medicine), using OpusExpress6000A (Molecular Devices, Sunnyvale, CA) – an automated multichannel high throughput system for oocyte recording.\textsuperscript{102,103}

The *Xenopus laevis* (African frog) oocytes (egg cells) are widely used as an expression system for ion channels.\textsuperscript{104} Their large uniform size (approximately 1 mm in diameter), allows direct injection with genetic material (cDNA, cRNA
transcribed *in vitro* from cloned or mutated channel cDNA, or mRNA isolated from tissue of interest). Oocytes express faithfully channel proteins in their cell membrane, and have relatively few endogenous ion channels (expressed at low levels).

Voltage clamping is considered as a gold standard technique for measuring the ion currents across the membrane. There is a direct linear relationship, derived from Ohm’s law (current is the product of the electrical driving force and the conductance), between the response measured and the fraction of the total population of ion channels that opened to create the current measured. In two electrode voltage clamp, glass microelectrodes (current electrode and voltage electrode) and an amplifier are used to inject current in such a way that the cell’s membrane potential is held constant (i.e. is clamped) at a potential set by the operator. Ohm’s law for the ion channel-membrane circuit can be thus written as:

\[ I = (E_m - E_{\text{rev}}) N P_{\text{open}} \gamma, \]

Where \( I \) is the current, \( E_m \) represents the membrane potential (holding potential), \( E_{\text{rev}} \) stands for reversal potential (zero current potential for the ion channel of interest), \( N \) is the total number of channels, \( P_{\text{open}} \) represents probability of a single channel being open, and \( \gamma \) is the conductance of a single open channel (a simplified scheme for two electrodes voltage clamp in *Xenopus* oocytes is shown in Figure 1-17 A).

OpusExpress is a high throughput electrophysiology system in which voltage control, data acquisition, fluid delivery, and real-time analysis are all automated (Figure 1-18 B) and it allows for running an experiment on up to 8 oocytes in parallel (Figure 1-18 C).
A response to drug application is illustrated by the current from current electrode that is needed to keep the membrane voltage constant. The height of the peak is called a peak response or peak current, and the area under the peak is called a net charge (Figure 1-18 D). In this research, each oocyte received two pre-controls of acetylcholine, then experimental drug applications (application of an experimental compound to test for agonism, co-application of acetylcholine and experimental compound to test for antagonism, or co-application of PNU-120596 and experimental compound). This was followed by one or two post-controls of acetylcholine (Figure 1-17 E). Responses of the nAChRs were calculated relative to preceding ACh controls to normalize the data (responses to ACh were normalized to 1), compensating for varying levels of receptors’ expression among oocytes.

1.5 Ligands for the nAChRs

Ligands for the nAChRs can be classified as agonists, antagonists, and modulators. Molecules that bind in the same binding site as acetylcholine are often called “orthosteric” ligands and their binding site is an “orthosteric” site, while molecules that bind elsewhere in the protein are classified as “allosteric” ligands and their binding site is an “allosteric” site.

Agonists are molecules, whose binding to the nAChRs activates ion channel opening in a similar manner as acetylcholine and nicotine. Agonists are usually characterized by their efficacy and potency. Efficacy \( (I_{\text{max}}) \) is the maximum response that the ligand can elicit relative to a reference compound, usually acetylcholine in the case of nAChRs.
Figure 1-17. Studying the nAChR ionotropic function. A) Schematic experimental set-up for two electrode voltage clamp in Xenopus oocytes. B) OpusExpress6000A in the Papke Laboratory. C) OpusExpress electrode arrays in baths containing one oocyte each, drug delivery (Reproduced from Methods, 51/1, Papke RL and Stokes C, Working with OpusXpress: Methods for high volume oocyte experiments, 121-133, Copyright (2010), with permission from Elsevier). D) figure representing a response to drug application. E) mock figure illustrating a typical experimental protocol and raw data.
Based on their $I_{\text{max}}$, the agonists can be subdivided into partial ($I_{\text{max}}$ lower than for ACh), full ($I_{\text{max}}$ similar to that of ACh), and super agonists ($I_{\text{max}}$ higher than for ACh).

Potency is often represented by an EC$_{50}$, i.e., a concentration of an agonist that produces a half of a maximum response. The $I_{\text{max}}$ and EC$_{50}$ values can be obtained from a concentration response curve (CRC) that is fitted into a Hill equation:

$$\text{response} = \frac{I_{\text{max}} \times [\text{agonist}]^n}{[\text{agonist}]^n \times (\text{EC}_{50})^n}$$

where $n$ is a Hill coefficient. Hill coefficient might be a measure of cooperativity (for example in case of a muscle nAChR), or have no directly interpretable functional meaning (for example in case of $\alpha_7$).

Antagonists are molecules that inhibit an agonist-evoked activation of the receptor. Antagonists are characterized by an IC$_{50}$, i.e., a concentration of a ligand that reduces the test response by 50%. Antagonists can be divided into competitive and non-competitive. A competitive antagonist binds in the same site as the agonist, thus its action can be overcome by increasing the concentration of the agonist: a competitive antagonist increases the agonist’s EC$_{50}$ but does not lower $I_{\text{max}}$. Binding of a non-competitive antagonist in an allosteric site cannot be overcome by high concentration of an agonist, and both the agonist’s EC$_{50}$ and $I_{\text{max}}$ are affected.

Allosteric modulators can be classified as negative when their binding diminishes a response to agonists (non-competitive antagonists could be called negative allosteric modulators),$^{105}$ and positive allosteric modulators when their binding enhances the response to agonists. Positive allosteric modulators (PAMs) for nAChRs appear promising for development of new nAChR therapeutics.$^{106,107}$
There is a great interest in developing nAChR ligands that are selective for one of the subtypes of the receptor.\textsuperscript{108-111} The selectivity can arise from a big difference in potency for different subtypes, efficacy, or both. There are some nAChR ligands that are considered selective for one subtype of the receptor, though it is important to keep in mind that these ligands are selective only in regards to what they were tested for, and many of the tested compounds were subjected to limited pharmacological characterization (usually nAChR ligands have been tested for α4β2, α3β4, and α7). In addition, a ligand that selectively activates one nAChR subtype may bind to other subtypes and act as an antagonist.\textsuperscript{112-114}

The nAChR ligands have been reviewed in \textsuperscript{19,43,106,115-118}.

1.5.1 Nicotinic Agonists

As mentioned earlier, acetylcholine (ACh) is the endogenous agonist for all nicotinic AChR subtypes. Acetylcholine is susceptible to hydrolysis, especially in biological preparations that contain acetylcholinesterase. Nicotine (Figure 1-18), an alkaloid from tobacco, was used historically to classify the receptors, and distinguishes this subset of AChR from the muscarinic family. Nicotine activates all nAChR subtypes, except α9 and α9α10 for which it acts as an antagonist.\textsuperscript{113,114} \textsuperscript{(-)}-Cytisine\textsuperscript{119} is a toxic alkaloid that occurs naturally in seeds of several plants such as \textit{Laburnum} and \textit{Cytisus}. \textsuperscript{(-)}-Cytisine usually acts as a full agonist for heteromeric receptors containing β4 subunit and a partial agonist for subtypes containing β2.\textsuperscript{120} Anatoxin A is a potent nicotinic agonist first isolated from blue-green algae cyanobacteria, \textit{Anabaena flos aqua}.\textsuperscript{121} Epibatidine was isolated in 1992 from skin of an Ecuadorian frog,\textsuperscript{122} and its both enantiomers are very potent nAChR agonists, especially on heteromeric receptors.
Epibatidine was recognized for analgesic activity.\textsuperscript{123} Anabaseine, isolated from a marine worm\textsuperscript{124} and certain species of ants,\textsuperscript{125} and its reduced analog anabasine found in the tree tobacco (\textit{Nicotiana glauca}) plant, are nicotinic agonists that are more potent on muscle and \(\alpha 7\) nAChR than neuronal heteromeric receptors.\textsuperscript{126} The natural products described above have been extensively used as leads for development of new nAChR ligands.\textsuperscript{19,115-117,127}

![Chemical structures](image)

\textbf{Figure 1-18.} Structures of some non-selective nAChR agonists.

A pharmacophore is defined as a minimal set of structural elements in appropriate arrangement that is required for a molecular recognition of a ligand by the receptor. Based on the known agonists for nAChR (acetylcholine, nicotine, cytisine, anatoxin-A, epibatidine, and anabaseine), it was believed for a long time that a positive charge and an H-bond acceptor were necessary for the molecule to act as an agonist.\textsuperscript{128} However, in the 1990s it was found that tetramethylammonium (TMA) was a full agonist for \(\alpha 7\), \(\alpha 3\beta 4\), and \(\alpha 2\beta 4\),\textsuperscript{58} thus reducing the nAChR agonist pharmacophore.
to a quaternary ammonium ion. Quinuclidine is also a non-selective nAChR agonist that has been found to activate α7 and α3β4 subtypes. The efforts to make ligands selective for a particular subtype of nAChRs have been reviewed in\textsuperscript{108-111}\textsuperscript{117}. Three structural motifs that can be associated with selectivity for α7 nAChR subtype have been defined as the choline, tropane, and benzylidene motifs (Figure 1-19). The choline motif can be defined as an ammonium group separated by two carbons from a hydrogen bond acceptor, such as in choline,\textsuperscript{58} 3-quinuclidinone,\textsuperscript{111} and AR-R17779.\textsuperscript{129} The selectivity of the tropane motif results from small hydrophobic groups affixed on the quaternary nitrogen, such as in tropisetron. Addition of a methyl group to a non-selective agonist quinuclidines, gives α7 selective N-methylquinuclidine.\textsuperscript{111} The benzylidene motif could be described as an extended aromatic ring properly positioned to the ammonium positive charge, such as in 3-[2,4-dimethoxybenzylidene]anabaseine (GTS-21, DMXB),\textsuperscript{130,131} (E)-benzylidene quinuclidine,\textsuperscript{111,132,133} and SSR180711.\textsuperscript{134}

Figure 1-19. Structures of some α7 nAChR selective agonists.
1.5.2 Nicotinic Antagonists

Competitive nAChR antagonists, such as tubocurarine, α-bungarotoxin, α-conotoxins, dihydro-β-erythroidine and methyllycaconitine (MLA) (Figure 1-20) bind at the agonist binding site, stabilizing the receptor in a closed channel conformation and preventing access for agonists. Tubocurarine is the active agent of a South American arrow poison known as curare, it is a non-selective nAChR antagonist that has been used as a skeletal muscle relaxant, and it is believed that it might also have non-competitive interactions with nAChRs.\textsuperscript{135} Alpha-bungarotoxin (α-Bgt), a 57-amino acid peptide isolated from the venom of the snake \textit{Bungarus multicinctus}, is an antagonist that binds selectively to α7, muscle, and \textit{Torpedo} nAChR, it does not appear to bind to heteromeric neuronal receptors with very high affinity. Alpha-bungarotoxin has been used for purification of the \textit{Torpedo} nACh receptor,\textsuperscript{21} and [\textsuperscript{125}I]-α-Bgt can be used for selective labeling of α7 receptors in the brain.\textsuperscript{51} The association and dissociation kinetics of α-bungarotoxin are very slow. Alpha conotoxins, 12-18 amino acid peptides, have been isolated from venoms of various \textit{Conus} snails. They often selectively antagonize one subtype of nAChRs\textsuperscript{136} (for example, α-conotoxin Iml is considered selective for α7 and α9 nAChR).\textsuperscript{137} Dihydro-β-erythroidine (DHβE), an alkaloid from \textit{Erythrina} seeds, is generally non-selective for different nAChR subtypes.\textsuperscript{138} Methyllycaconitine (MLA), an alkaloid produced by \textit{Delphinium} species plants,\textsuperscript{139} is a potent competitive antagonist, selective for α7 nAChR. Inhibition of α7 nAChR by MLA is rapid and reversible.\textsuperscript{140}

Non-competitive antagonists, such as mecamylamine, hexamethonium, TMPH, and tK3BzPB, interact with different sites than the orthosteric ligand binding site, often
the lumen of the nAChR channel. Mecamylamine is a nonselective, non-competitive antagonist, able to cross freely the blood brain barrier. It has been introduced in 1950s as a drug for the treatment of hypertension.\textsuperscript{141} Hexamethonium is also a nonselective, non-competitive antagonist, but unable to cross the blood brain barrier.\textsuperscript{142} 2,2,2,2-Tetramethylpiperidin-4-yl heptanoate (TMPH) is a potent antagonist for heteromeric neuronal nAChRs.\textsuperscript{143} A tetrakis-azaaromatic quaternary ammonium antagonist tkP3BzBP is selective for α7 nAChR.\textsuperscript{144}

Figure 1-20. Structures of some nAChR antagonists.
1.5.3 Positive Allosteric Modulators

A positive allosteric modulator (PAM) binds at the site other than the agonist binding site and enhances the agonist’s channel activation. It typically does not activate the receptor by itself. Positive allosteric modulators of membrane receptors are important tools to control the receptor function, and have been used as therapeutics (eg., valium, a benzodiazepine that acts as a PAM for GABA<sub>A</sub> receptor). Several endogenous PAMs of nAChRs have been identified (such as steroids, calcium and zinc ions, lynx-1<sup>146</sup> (and -2) and SLURP-1 (and -2) proteins). There is great interest in developing synthetic PAMs selective for α<sub>7</sub><sup>106,107</sup> and α<sub>4</sub>β<sub>2</sub>.<sup>147,148</sup>

Grønlien and co-workers have proposed that α<sub>7</sub> PAMs can be divided into two classes: type I and type II (Figure 1-20).<sup>149</sup>

![Structures of some α<sub>7</sub> nACHR PAMs](image)

**Figure 1-20.** Structures of some α<sub>7</sub> nACHR PAMs.

The type I PAMs, such as 5-hydroxyindole<sup>150</sup> act mainly on energy barriers between different conformations of the receptor and predominantly affect the peak current (Figure 1-21 A). Type II PAMs, such as PNU-120596<sup>151</sup> act also on energy levels and largely increase the net charge: they are able to slow down desensitization and even reverse some desensitized states (Figure 1-21 B).<sup>57</sup> PAMs may also act by enhancing binding of agonists and increasing the number of activatable receptors.
Figure 1-21. Representative traces showing the effects of PAMs on ACh-evoked α7 nAChR responses. A) 5-hydroxyindole, type I PAM, was used. B) PNU-120596, type II PAM was used (Reproduced from Molecular Pharmacology, 72, Gronlien JH, Hakerud M, Ween H, Thorin-Hagene K, Briggs CA, Gopalakrishnan M, Malysz J, Distinct profiles of alpha 7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes, 715-724, Copyright (2007), with permission from American Society for Pharmacology and Experimental Therapeutics).149

Closely related structural analogs TQS152 and 4-BP-TQS are also classified as type II PAMs, though 4-BP-TQS is unique because it has also been shown to act as an agonist on its own.153,154 It is believed that the type II PAMs, PNU-120596, TQS and 4-BP-TQS bind in the transmembrane domain.154-156

1.5.4 α7 nAChR Silent Agonists

A new class of α7 nAChR ligands introduced in this dissertation is silent agonists. Silent agonists have little or no ionotrophic activity on their own, appear as competitive antagonists of more efficacious ionotrophic agonists, and cause conformational changes that can be detected with the reference type II PAM PNU-120596.157 In these cases, co-application of the PAM with the silent agonist to the receptor results in a conductive state, thus reporting on what would otherwise be a non-conductive state.
1.5.5 Therapeutic Opportunities of the nAChR Ligands

The nAChRs are implicated in a range of physiological functions related to muscle contraction, cognitive functions, learning and memory, reward, motor control, analgesia, and inflammation and therefore are important for drug research.\(^\text{117,158-160}\) It is believed that nAChR ligands could be used in treatment of neurodegenerative diseases (such as Alzheimer’s disease\(^\text{161}\) and Parkinson’s disease), pain, schizophrenia,\(^\text{162}\) depression, some epilepsies,\(^\text{163}\) attention-deficit hyperactivity disorder (ADHD), Tourette’s syndrome, and inflammatory diseases\(^\text{164}\) (Section 1.7). One important application of nAChR ligands is in nicotine addiction, and the nicotinic drug for smoking cessation on the market is varenicline\(^\text{165}\) (Chantix® US, Champix® EU), a cytisine analog. There are several nAChR ligands that are currently in clinical trials for schizophrenia, Alzheimer’s disease, cognitive disorders, smoking cessation, depression, and pain.\(^\text{158}\) Silent agonists may found use in the treatment of inflammatory disorders (Section 1.7).

1.6 Allosterism and Desensitization

The term “allosteric” was first proposed to account for the observation that bacterial enzymes were inhibited by the end product of the biosynthetic pathways, even though the end product was not structurally similar to the active site substrate.\(^\text{166,167}\) The inhibition was non-competitive with substrate leading to a hypothesis that the non-competitive inhibitor produced conformational alterations in the protein.\(^\text{168}\) The most known model of protein allostery, the Monod, Wyman, Changeux (MWC) model,\(^\text{169,170}\) proposed that proteins are dynamic structures existing in multiple discrete functional states, all of which are accessible to the protein under resting conditions. The binding of
a ligand was predicted to alter the resting equilibrium by reversibly stabilizing the protein in the conformation to which the ligand has greatest affinity.\textsuperscript{171}

Nicotinic acetylcholine receptors are allosteric proteins that can exist in different conformations classified as closed, open, and desensitized.\textsuperscript{172,173} Desensitization can be defined as a decrease or loss of biological response following prolonged or repetitive stimulation.\textsuperscript{174,175,176} Dr. Papke Laboratory has identified that there are at least two different desensitized states of α7 nAChR: Ds that is sensitive to type II positive allosteric modulators (PAM)s such as PNU-120596, and Di that is insensitive to type II PAMs.\textsuperscript{57}

1.7 The α7 nAChR and Inflammatory Response

1.7.1 Cholinergic Anti-inflammatory Pathway

Cholinergic anti-inflammatory pathway is a neural regulatory mechanism comprised of the vagus nerve and the α7 nAChR that attenuates release of pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-1 and 6 (IL-1, IL-6) and high-mobility group box 1 (HMGB1) (Figure 1-22, reviewed in\textsuperscript{177,178}). Hence, the α7 nAChR is a pharmacological target for diseases linked to abnormal inflammatory response, for example: arthritis, asthma, sepsis, atherosclerosis, ulcerative colitis, and psoriasis (reviewed in\textsuperscript{178-181}). The anti-inflammatory effect of α7 ligands may be also extended to treatment of Alzheimer’s disease and type 2 diabetes.\textsuperscript{180,182}
Figure 1-22. Cholinergic anti-inflammatory pathway balances cytokine production. Pathogens, ischemia, and injury activate cytokine production. Efferent signals from the vagus nerve inhibit production of pro-inflammatory cytokines via \( \alpha_7 \) nAChR on macrophages and other cells that are the major source of pro-inflammatory cytokines.

The cholinergic anti-inflammatory pathway was discovered by Kevin J. Tracey and co-workers. In 2000, the group showed that direct stimulation of the vagus nerve during lethal endotoxemia in rats inhibited synthesis of TNF in liver and prevented development of circulatory shock (bodily collapse caused by inadequate oxygen delivery to the cells).\(^{183}\) In 2003, the group further showed that electrical stimulation of the vagus nerve in \( \alpha_7 \) nAChR-deficient mice does not inhibit TNF synthesis, indicating that \( \alpha_7 \) nAChR is essential for inhibiting cytokine synthesis by the cholinergic anti-inflammatory pathway.\(^{184}\)

The exact molecular mechanism by which the \( \alpha_7 \) nAChR inhibits pro-inflammatory cytokine production has not been fully established yet.\(^{177,178,180,185}\) It has
been shown that stimulation of α7 nAChR with agonists acts on the JAK2 (Janus kinase 2)/STAT3 (signal transducer and activator of transcription) pathway\textsuperscript{185-187} and the transcription factor NF-κB (nuclear factor kappa B)\textsuperscript{188,189} that are known regulators of cytokine production.\textsuperscript{190}

1.7.2 Findings Indicating that the α7 nAChR May Have Signaling Function in Inflammation Without Ion Channel Activity

Anti-inflammatory effects of nicotine have been observed in epidemiological studies in tobacco smokers and it is believed that α7 nAChR agonists could be used as anti-inflammatory agents in \textit{vivo}.\textsuperscript{179-181} However, it is known that the net effect of nAChR agonists could depend on many factors, such as drug dose or concentration and the length of the time of exposure, and it has been hypothesized that some effects of nAChR agonists may be due to desensitization of the receptor rather than ion channel activation.\textsuperscript{191} It is thus reasonable to suggest that ligand binding can communicate to the intracellular domain through the non-conductive states, classified as desensitized, or through states that are unique to receptors in non-neuronal cells, and thereby effect metabotropic signaling.

There are several findings indicating that the α7 nAChR may have signaling function in inflammation without ion channel activity. Mainly: 1) The α7 nAChRs are expressed in blood macrophages, microglia, and immune cells\textsuperscript{26,27,184,192,193} and stimulation of these cells with the α7 nAChR agonists does not produce detectable ion currents.\textsuperscript{179,194,195} 2) The α7 nAChR partial agonists (such as GTS-21) with relatively low efficacy have been shown to attenuate cytokine production in human whole blood \textit{ex vivo}, and this effect was more potent than for the full agonist nicotine.\textsuperscript{186,193,196} 3) The partial agonist GTS-21 and NS6740 which has practically no efficacy (classified
in this dissertation as a silent agonist) suppress a pro-inflammatory response by microglia, while more efficacious agonists SSR180711 and A-582941 are ineffective.\textsuperscript{197}

4) Silent agonist ASM-024 (Asmacure), which is currently in clinical trials for asthma, has been shown to attenuate pro-inflammatory cytokine production. 5) Proteomics-based analysis of the α7 nAChR interactome has revealed that the receptor interacts with many intracellular signal transducing proteins (e.g. protein kinase A, protein kinase C, and several phosphatases).\textsuperscript{90} These observations make compounds that can put the receptor selectively into a desensitized state with little or no ion channel activation (silent agonists) of potential interest from both mechanistic and therapeutic perspectives.

1.7.3 Other Possible Signaling Pathways Involving α7 nAChR

It is believed that stimulation of α7 nAChRs in cancer cells with agonists results in acceleration of cancer progression and that it might be possible to design appropriate α7 ligands for anti-cancer therapies.\textsuperscript{28,198,199,200} The exact mechanism by which α7 nAChR mediates survival of cancer cells has not been established, but some evidence indicates that it involves Ras/Raf1/MEK1/ERK (extracellular signal-regulated kinase) and JAK-2/STAT-3 pathways.\textsuperscript{199}
CHAPTER 2
KC COMPOUNDS AS NEW α7 SILENT AGONISTS

2.1 Background

There is growing evidence, especially in non-neuronal cells, that there is α7-mediated signal transduction under conditions when no ion channel activation can be detected (Section 1.7). Two forms of α7 desensitization have been reported. One is sensitive to type II PAMs such as PNU-120596, (termed Ds), and another is induced by strong episodes of activation and high occupancy, and is insensitive to PNU-120596, (termed Di). The pharmacological relevance of desensitized states is likely to extend beyond their lack of ability to conduct an ion-current. These findings make compounds that can put the nAChR in to the Ds state, with little or no apparent agonism, of considerable potential interest from both mechanistic and therapeutic perspectives.

NS6740 is such agent which, although inactive in an α7-sensitive model for cognitive improvement has been shown to be effective at modulating the release of pro-inflammatory cytokines. Several observations lead to a working model for the design of a silent agonist. Compound NS6740 (Figure 2-1) has been characterized by Abbott and Neurosearch as a very weak agonist (<2 % of the response to ACh), whose agonist-like properties were revealed by adding a positive allosteric modulator PNU-120596. We found in our laboratories that 3PAB, also behaves as a silent agonist, leading to the idea that silent agonists may be groupable into structurally distinct families. NS6740 features a basic diaza [3.2.2] bicyclononane group, a central 2,5-disubstituted heterocyclic ring and a phenyl substituent at the 5-position of the central
Further, the compound offers a hydrogen bond acceptor adjacent to the bicyclic ring system (an amide carbonyl). We therefore initiated a synthesis of a molecule whose structural features and biological activity might shed further light on what constitutes a set of features that would confer silent agonism. We considered a minimal pharmacophore to feature a positively charged center, a central ring with hydrogen bonding capability, and a flanking aryl substituent with an angular relationship between these elements as embodied in the molecule KC-1 and the cartoon shown in Figure 2-1. This minimal pharmacophore model does not take into account possible importance of the trifluoromethyl group, amide group and bicyclic ring of NS6740 for conferring silent agonism character. One will note the core anabaseine portion of KC-1 is a non-selective nAChR agonist, and, as will be presented, we show phenyl substitution on the pyridine ring dramatically changes this profile.

![Figure 2-1. Structures of NS6740, KC-1 and one pharmacophore for silent agonists.](image)

### 2.2 Results and Discussion

#### 2.2.1 Synthesis of KC-1

KC-1 (5'-phenylanabaseine) can be considered as an analog of anabaseine, and we decided to make KC-1 by adapting well known anabaseine synthesis protocols. It was first planned to prepare KC-1 from 5'-bromoanabaseine by organometallic coupling (Figure 2-2). That approach would have allowed flexibility in preparation of a series of KC-1 analogs. We envisaged preparing several grams of 5'-bromoanabaseine as its
dihydrochloride salt (1) from bromonicotinic ethyl ester (2), by applying the Zoltewicz anabaseine synthetic protocol\textsuperscript{203} that we use in our lab. That approach is based on a mixed Claisen condensation of nicotinic ester and the amide enolate ion of N-aminomethyl protected 2-piperidone 3, followed by hydrolysis of the resulting sodium salt of the 3-nicotinoyl-2-piperidone intermediate in hot concentrated hydrochloric acid with concomitant decarboxylation and cyclic imine formation. Anabaseine is isolated by crystallization as its stable dihydrochloride salt.

Figure 2-2. KC-1 retrosynthetic analysis

The reaction of 3-bromonicotinic ethyl ester (2) with N-protected 2-piperidone 3\textsuperscript{202,203} and sodium hydride in toluene (Figure 2-3) yielded the sodium salt 4 as a complex mixture of Z- and E- enolates and keto forms as supported by \textsuperscript{1}H-NMR but it could not be isolated from the crude reaction mixture, so the crude product was subjected to the next step, hydrolysis with concentrated hydrochloric acid. The desired 5'-bromoanabaseine dihydrochloride (1) was formed in that reaction as shown by \textsuperscript{1}H-NMR, however extensive degradation was observed, many impurities were present, and the product could not be obtained in a pure form. To circumvent the problems with degradation of brominated pyridine compounds in concentrated HCl, we decided to switch the order of the bond formation and synthesize KC-1 from 5-phenylnicotinic ethyl ester (8), again following the Zoltewicz protocol.
Figure 2-3. Synthesis of 5'-bromoanabaseine 1.

5-Phenylnicotinic ethyl ester (8) is not commercially available and it was prepared from 3-bromonicotinic acid (5) by Suzuki coupling with phenylboronic acid (6), using palladium acetate as catalyst, potassium phosphate as a base, in a 1:1 mixture of water and 2-propanol. The 5-phenylnicotinic acid was then reacted with thionyl chloride, followed by reaction with ethanol to give the product 8 in 63 % yield from 5 (Figure 2-4).

Figure 2-4. Synthesis of 5-phenylnicotinic ethyl ester 8.

The 5-phenylnicotinic ethyl ester (8) was subjected to the mixed Claisen condensation with N-protected 2-piperidone 3, using the same methodology as presented in Figure 2-3. Unfortunately, the intermediate sodium salt did not crystallize from the reaction mixture after removal of excess NaH by filtration, and again the crude reaction mixture was subjected to acidic hydrolysis. After the reaction, 5-phenylcarboxylic acid 7, resulting from hydrolysis of unreacted ethyl ester 8.
crystallized first, then the KC-1 salt, which was impure. The KC-1 salt was thus transformed with 1M sodium hydroxide into its imine free base form, purified by column chromatography, and then converted back into its dihydrochloride salt to yield the pure compound only in 2 % yield over two steps from 5-phenynicotine ethyl ester (8). The results of these two approaches suggested that the synthetic route to anabaseine is not highly tolerant of substitution, though the poor yields might have resulted from the fact that the sodium salts of the Claisen condensation products were not purified. An alternate approach for obtaining synthetically useful yields of KC-1 was needed because the relatively small scale of the reactions and potential instability of the salts would make it difficult for their recrystallization. In addition, the subsequent treatment with concentrated acid would be troublesome to optimize because the reaction is not easily amenable to monitoring by TLC.

The most successful alternate route for synthesis of KC-1 employed addition of 5-phenylpyridinyl lithium generated with nBuLi from the bromide 10 to N-Boc protected 2-piperidone 11 in diethyl ether, followed by deprotection, ring closure, and dehydration (Figure 2-5). Organometallic ring-opening reaction of N-alkoxycarbonyl lactams has been used by Giovanni et al.\textsuperscript{205} to make cyclic imines. 3-Bromo-5-phenylpyridine 10 was prepared from 3,5-dibromopyridine 9 by Suzuki coupling with phenylboronic acid 6, using 2 mol % (triphenylphosphine)palladium tetrakis and potassium carbonate in a mixture of dimethoxyethane and water in 68 % yield following a patent procedure.\textsuperscript{206} The N-Boc 2-piperidone 11 was prepared from 2-piperidone and Boc anhydride following the reported procedure.\textsuperscript{205}
The bromide 10a was added into a solution of nBuLi in diethyl ether at −78 °C, and then the lactam 11 was added to yield the desired N-Boc-ω-aminoketone 12a in 61 % yield. We also generated the anion of 10a using 2 eq. of t-BuLi in diethyl ether, and the desired product 12a was obtained in 31 % yield. When nBuLi was used in diethyl ether at −5 °C no product was obtained, whereas at −40 °C the product was obtained in 12 % yield. When n-BuLi was used in THF instead of diethyl ether at a range of temperatures, no product was formed. The problems with lithium-halogen exchange of bromopyridines in THF have been known in the literature. It has been reported that isopropyl magnesium chloride may be used for metal-halogen exchange instead of alkyl lithiurns to avoid known side reactions of alkyl lithium with pyridines such as deprotonation, addition of nBuLi to pyridine ring, elimination of lithium bromide (to give pyridynes), reaction of 3-lithiopyridine with nBuBr, and even ring opening. However, in our case the use of isopropyl magnesium chloride resulted in a significantly lower yield (4%) of compound 12a.

N-Boc-ω-aminoketone 12a was purified and treated with TFA followed by NaOH to give KC-1 in 80 % yield after silica chromatography. The imine free base was then...
quantitatively transformed into its more stable dihydrochloride salt by rotary evaporation from ethanolic HCl.

### 2.2.2 Activity Profile of KC-1 on the Human α7 Receptor – Silent Agonism

KC-1 was tested on the human α7 nAChR as described in Section 1.4 and Appendix A. The 100 μM and 300 μM applications of KC-1 resulted in no ion channel opening (Figure 2-6 A), showing that KC-1 does not act as a conventional ha7 nAChR ionotropic agonist. When 100 μM KC-1 was co-applied with 60 μM ACh, a diminished response to ACh was observed (Figure 2-6 B) (peak current: 0.17 ± 0.08, net charge 0.29 ± 0.06), suggesting that KC-1 may bind in the same binding site as ACh. Finally, when 100 μM KC-1 was co-applied with 10 μM PNU-120596 a very large response was observed (Figure 2-6 C) (peak current: 4.1 ± 0.7, net charge 18 ± 2), revealing that KC-1 favors the D₃ state without ion channel opening, and thus acts as a silent agonist. Note that PNU-120596 has no detectable activity on its own with the α7 nAChR,¹⁵¹ and that it is just a probe to reveal KC-1 activity. The data indicate that KC-1 binding results in stabilization of an alternate receptor state.

The co-application of 100 μM KC-1 with another type II positive allosteric modulator TQS (30 μM) also resulted in large responses of the α7 nAChR (peak response 2.1 ± 0.2, net charge 4.3 ± 0.3), suggesting that type II PAM in general may be used to detect silent agonism character. The IC₅₀ of KC-1 was determined to be 41 ± 5 μM.
Figure 2-6. Representative traces of the hα7 nAChR response to applications of 100 μM KC-1. Blue bars represent duration of ACh applications, green bars the KC-1 application, and the red bar represents PNU-120596 application. The first two traces represent ACh pre-controls (300 μM ACh in A, 60 μM ACh in B and C).

2.2.3 Synthesis of KC-1 Analogs

After characterizing KC-1 as a new silent agonist, we set out to prepare a series of KC-1 analogs (KC-2 to KC-9, Figure 2-7) to further tease out this compound’s pharmacophore. All the molecules in this series have an aryl group connected to or fused to a pyridine ring, an H-bond acceptor (nitrogen in the pyridine ring) and at physiological pH, a positive charge on the nitrogen in the third ring. KC-2 features a secondary amine that results from the reduction of imine function in KC-1, it thus contains an anabasine core which similarly to anabaseine is an agonist of the nAChR.
KC-3 is a tertiary amine, synthesized by N-methylation of KC-2. KC-4 and KC-7 contain the anabaseine core, similar to KC-1, but the pyridine ring is fused to a benzene ring in different orientations. KC-5 and KC-8 are secondary amines analogous to KC-2. KC-6 and KC-9 are N-methylated amines analogous to KC-3.

![Chemical structures of KC compounds](image)

Figure 2-7. Structures of KC compounds.

We predicted that the compounds would act as α7 nAChR silent agonists, similarly to KC-1 but with differences that would be attributable to their structural variations.

KC-4\textsuperscript{210} and KC-7 were prepared from 4-bromoisoquinoline (10b) and 3-bromoquinoline (10c), respectively, using the same protocol as for the synthesis of KC-1. Therefore, the opening of the lactam 11 by the anions generated from 10b and 10c with nBuLi in diethyl ether, gave N-Boc-ω-aminoketones 12b in 43 % yield and 12c in 42 % yield. The subsequent treatment with trifluoroacetic acid and sodium hydroxide gave KC-4 in 57 % yield and KC-7 in 81 % yield (Figure 2-8).
Figure 2-8. Synthesis of KC-4 and KC-7.

KC-2, KC-5 and KC-8 were prepared from the N-Boc-ω-aminoketones 12a-c by treatment with TFA, NaOH, and sodium borohydride in 60 - 85 % yield. KC-3, KC-6, and KC-9 were prepared by Eschweiler-Clarke methylation\textsuperscript{211,212} of KC-2, KC-5, and KC-8, respectively (Figure 2-9). In this reductive alkylation, the Schiff base formed from an amine and formaldehyde is reduced by a hydride transfer from formic acid.\textsuperscript{213}

Figure 2-9. Synthesis of KC-2, KC-3, KC-5, KC-6, KC-8, KC-9.
2.2.4 Activity Profile of KC-1 Analogs on the Human α7 nAChR

The KC-1 analogs were screened at 100 μM and 300 μM on the human α7 nAChR as described before (Section 1.4. and Appendix A). All KC-1 analogs had no ionotopic activity above the detection limit, as was observed for KC-1, with the exception of KC-7 (Figure 2-10) which was fully characterized and shown to be a weak partial agonist of the α7 nAChR with an $I_{\text{max}} = 0.23 \pm 0.02$ and $EC_{50} = 112 \pm 19$ μM. Therefore, the positioning of the aryl group on the anabaseine core appears to affect the ability of the compound to activate the α7 ion channel as seen in the series of KC-1, KC-4 and KC-7. The relative positioning of the middle ring and positive charge also seem to affect the ability of KC-7 to cause ion channel opening: the positive charge in KC-7 lies practically in the same plane as the quinoline ring, while in KC-8 the conformation in which the positive charge and quinoline ring are in the same plane is disfavored and KC-8 has no ionotropic activity.

![Figure 2-10. Ionotropic agonism alpha 7 KCs series.](image-url)
All KC-1 analogs diminished responses to acetylcholine with the \( IC_{50} \) values in the range from 41 to 117 \( \mu \text{M} \). While in the KC-1 to KC-3 and KC-4 to KC-6 series methylation of the nitrogen resulted in higher \( IC_{50} \) values, the same trend was not seen in the KC-7 to KC-9 series, suggesting that methylation may not be a sole determinant in ability of the compound to inhibit ACh responses

Table 2-1. \( IC_{50} \) values for KC-1 analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>( IC_{50} ) [( \mu \text{M} )]</th>
</tr>
</thead>
<tbody>
<tr>
<td>KC-1</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>KC-2</td>
<td>57 ± 4</td>
</tr>
<tr>
<td>KC-3</td>
<td>77 ± 5</td>
</tr>
<tr>
<td>KC-4</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>KC-5</td>
<td>80 ± 2</td>
</tr>
<tr>
<td>KC-6</td>
<td>117 ± 10</td>
</tr>
<tr>
<td>KC-7</td>
<td>46 ± 9</td>
</tr>
<tr>
<td>KC-8</td>
<td>71 ± 9</td>
</tr>
<tr>
<td>KC-9</td>
<td>60 ± 3</td>
</tr>
</tbody>
</table>

To determine how KC compounds antagonize the \( \alpha 7 \text{nACh} \) receptor, an ACh concentration response curve (CRC) was compared to the curve obtained by co-applying ACh at increasing concentrations with either KC-1 or KC-5 at their \( IC_{50} \) concentrations (40 \( \mu \text{M} \) and 80 \( \mu \text{M} \), respectively). These experiments are consistent with the hypothesis that KC-1 displaces acetylcholine in a competitive way, shifting the ACh \( EC_{50} \) from 30 ± 3 \( \mu \text{M} \) to 165 ± 11 \( \mu \text{M} \), suggesting that KC-1 binds at the same site as acetylcholine (Figure 2-11).
Figure 2-11. A KC-1/ACh competition curve.

The competition curves with KC-5 appear to have a significant competitive component (the ACh EC$_{50}$ increased from 30 ± 3 μM to 74 ± 3 μM) and also a small non-competitive component (I$_{max}$ diminished from 1.32 ± 0.04 to 1.11 ± 0.01) that might be due to a channel block (Figure 2-12).
Finally, the compounds were co-applied at 100 μM with type II positive allosteric modulator 10 μM PNU-120596, revealing that KC-1, KC-5, and KC-7 were putting the α7 nAChR preferentially into a desensitized state that could be made conductive by PNU-120596 (Figure 2-13). KC-4 and KC-8 gave little but significant ionotropic responses when co-applied with PNU-120596. All methylated KC-1 analogs (KC-3, KC-6 and KC-9) that have similar IC₅₀ values to other KC compounds, gave no response at 100 μM when co-applied with type II PAM, indicating that nitrogen methylation in these type of compounds disfavors the receptor from going into a Ds conformation. It is intriguing that reduction of the imine function in the silent agonist KC-1 resulted in a loss of silent agonism activity of KC-2, while reduction of the imine function in a weak silent
agonist KC-4, resulted in an increase in silent agonism of KC-5. It would be interesting to prepare KC-2, KC-5 and KC-8 in their enantiopure forms to check whether there is a difference in activity between the two enantiomers.

Figure 2-13. Desensitized state revealed by co-application with type II PAM.

Most active KC compounds were further characterized in the range of 1 μM to 1 mM to determine their potency when co-applied with PNU-120596. The determination of EC$_{50}$ values for PAM co-application responses appeared infeasible because the data did not fit into the Hill equation. It was observed that a sudden drop in activity for KC-1 occurred between 300 μM and 1000 μM (Figure 2-14), indicating that at higher concentrations, KC-1 was preferentially putting the α7 receptor in a desensitized state that could not be reversed by co-application with PNU-120596. Similar behavior was observed for other KC compounds that had activity when co-applied with PNU-120596 (Table 2-2). KC-2, KC-3, KC-6 and KC-9 were inactive at 100 μM when co-applied with PNU-120596, but had some detectable activity when tested at 300 μM, revealing that they have some weak silent agonism activity or strong induction of D$_i$. 
To help discern functional differences of the KC-1 analogs, the ratio of the PNU-120596 co-application response at 100 μM to the agonism response at 100 μM was calculated for each compound (Figure 2-15). This ratio provides a measure of a compound’s relative preference for stabilizing the Dₘ state over channel opening. The graph shows that silent agonists KC-1, KC-4, KC-5, KC-7, and KC-8 overwhelmingly favor Dₘ desensitization over ion channel activation. KC-1 and KC-5 favor PAM sensitive desensitization over ion channel activation by 1800 and 2300-fold, respectively. KC-7 does so by 200-fold which is due to partial agonism character of this molecule. KC-4 and KC-8 that do not activate opening of the hα7 nAChR ion channel do so by 110 and 70-fold, respectively which results from their small responses when co-applied with PNU-120596.
## Table 2-2. Net charge for KC co-applications with PNU-120596.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug concentration [μM]</th>
<th>Net charge when co-applied with 10 μM PNU-120596</th>
<th>Threshold: Concentration at which 10 % of maximal response was observed [μM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>KC-1</td>
<td>30</td>
<td>0.4 ± 0.2</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>13 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>22 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1.7 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>KC-2</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>KC-3</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.010 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>KC-4</td>
<td>100</td>
<td>1.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>4 ± 2</td>
<td></td>
</tr>
<tr>
<td>KC-5</td>
<td>30</td>
<td>0.6 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12 ± 5</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>12 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>2.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>KC-6</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>KC-7</td>
<td>30</td>
<td>2 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>22 ± 5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>14 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>KC-8</td>
<td>100</td>
<td>0.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>6 ± 3</td>
<td></td>
</tr>
<tr>
<td>KC-9</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.6 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2-15. Ratio of PNU-120596 (10 μM) co-application with KC compounds (100 μM) responses to KC (100 μM) ionotropic agonism responses. To avoid division by 0, when no ionotropic agonism response was observed, the PNU-120596 co-application response was divided by 0.005, which was assumed to be a detection limit for these experiments. The error bars were not calculated because of the inaccuracy resulting from limited detection of small responses.

### 2.2.5 Activity Profile of KC-1 Analogs on the Human α4β2 nAChR

The nine KC compounds were screened at 100 μM on the α4β2 nAChR (Figure 2-16). KC-3, KC-6, KC-9 had no detectable ionotropic activity, KC-1, KC-2, KC-4, KC-5, and KC-8 activated α4β2 nAChR very weakly. KC-7 was the most active compound in the series, similarly to the profile seen on the α7 nAChR, and it was further characterized and determined to be a weak partial agonist for α4β2 nAChR, with an \( I_{\text{max}} = 0.19 \pm 0.02 \) and \( EC_{50} = 51 \pm 3 \) μM for the \((\alpha 4)_2(\beta 2)_3\) receptor and \( I_{\text{max}} = 0.05 \pm 0.01 \) and \( EC_{50} = 65 \pm 5 \) μM for the \((\alpha 4)_3(\beta 2)_2\) receptor.
Figure 2-16. KC-1 to KC-9 agonism on α4β2 nAChR.

When the KC-1 to KC-9 compounds were co-applied with acetylcholine, a diminished response to acetylcholine was observed (Figure 2-17).

Figure 2-17. KC-1 to KC-9 co-application antagonism on α4β2 nAChR.

This antagonism by KC compounds on α4β2 nAChR was even more visible when the receptor was incubated with 100 μM KC compounds for 5 min, and then the response to 100 μM KC co-applied with 30 μM ACh was measured (Figure 2-18).
Taken together, these data suggest that KC compounds are not selective for the α7 nAChR and may also act on α4β2 nAChR.

2.3 Summary

The term silent agonism was introduced in reference to the α7 nAChR subtype to describe compounds that do not activate ion channel opening on their own but favor induction of Dₘ desensitization that might be involved in α7 nAChR non-ionotropic signaling. Silent agonists could potentially be used in the treatment of inflammatory disorders without side effects linked to ion channel activity.

Nine structurally similar compounds, KC-1 to KC-9, were synthesized to test for the importance of hybridization of the positively charged nitrogen, N-methylation, and relative geometry between the aryl group, the hydrogen bond acceptor and the positive charge for pharmacological properties of the ligands.

The molecules were characterized electrophysiologically on the human α7 and α4β2 nAChRs expressed in Xenopus oocytes. KC-1 and KC-5 have fully met the
criteria to be characterized as silent agonists for the α7 nAChR. KC-7 was shown to be a partial agonist for the α7 nAChR, while KC-2, KC-3, KC-4, KC-6, KC-8 and KC-9 appeared to be weak α7 nAChR silent agonists (Figure 2-19).

The systematic structural changes in KC compound series did not result in systematic changes in their activity, suggesting that there are no clear trends between structure and activity in this series. For example, reduction of the imine function in the silent agonist KC-1 gave the weak silent agonist KC-2, while reduction of the imine function in the weak silent agonist KC-4 gave the silent agonist KC-5. It would be interesting to further test the chiral KC compounds in their enantiopure forms to establish whether there is a difference in pharmacological properties between the two antipodes.

![Figure 2-19. KC-1 to KC-9 compounds summary. Red: silent agonists; purple: weak silent agonists; orange: silent agonist with partial ionotropid agonist character.](image)

Although no clear trends were seen between the structure and properties of the KC molecules, and the profile of KC compounds on the hα4β2 nAChR suggested that it
may be challenging to obtain compounds selective for the \( \alpha_7 \) nAChR in this series, new silent agonists with desired pharmacological properties may be found by preparing analogs of these compounds.
3.1 Background

The Papke Laboratory has identified ASM-024 (16) (from Asmacure) (Figure 3-1) as an α7 nAChR silent agonist. That discovery led to a hypothesis that one of the silent agonist pharmacophores may consist of a bulky quaternary ammonium group. Thus, tetraethylammonium (TEA) was tested and it was also determined to act as a silent agonist. Tetramethylammonium (TMA) is a known non-selective agonist of the α7 nAChR, leading to a hypothesis that the size of the quaternary ammonium group may determine whether the molecule acts as an agonist or silent agonist.

Figure 3-1. Structures of ASM-024, TEA and TMA.

We set out to test six series of quaternary ammonium compounds, to determine how their size influences their pharmacological properties (Figure 3-2). To vary the molecular volume of each compound in the series, we replaced N-methyl groups with N-ethyl groups. Following up on earlier published work, the first series features tetramethylammonium, and then each compound has sequential replacement of a methyl with an ethyl group, leading to the tetraethylammonium cation. The second series was based on choline and its analogs in which subsequent methyl groups were
replaced by ethyl groups. Choline is a selective agonist for the α7 nAChR and we were hoping to maintain α7 selectivity in this series. The third series features

![Diagram of quaternary ammonium compounds]

**Figure 3-2.** Structures of bulky quaternary ammonium compounds.
benzylammonium compounds. The next three series (4-6) are represented by pyrroldinium, piperidine, and hexahydroazepinium compounds. In these three series, we varied the ring size to probe for the importance of the 7-membered ring size in ASM-024. While the TMA, pyrroldinium, piperidine and hexahydroazepinium series feature similar structural elements (positively charged nitrogen and alkyl groups), the choline series differs by a hydroxyl group that can act as a hydrogen donor or acceptor, and the benzyl series differs by an aryl group that can be in different positions in relation to the ammonium group.

3.2 Results and Discussion

3.2.1 Synthesis

The compounds were either purchased (TMA, EtMA, diEdiMA, triEA, TEA, choline and BtEA), or prepared by reacting commercially available methyl or ethyl amines with methyl iodide or ethyl iodide in tetrahydrofuran or ethanol and purified by recrystallization. Methylhexahydroazepine (39) was prepared from hexahydroazepine by the Eschweiler-Clarke reaction\textsuperscript{211,212} (formalin and formic acid), and ethylhexahydroazepine (40) was prepared from hexahydroazepine using potassium carbonate and ethyl iodide.

3.2.2 Activity profile of quaternary ammonium compounds on the human α7 nAChR

The quaternary ammonium compounds were screened at 100 μM on the human α7 nAChR, except choline analogs which were tested at 1 mM because of the low potency of choline. The results revealed that small quaternary ammonium compounds act as full agonists of the α7 nAChR, and as their size increases their agonism decreases (Figure 3-3). The only small exception is seen in the choline series where
(2-hydroxyethyl)ethyltrimethyl ammonium (23) is a super agonist and is a stronger agonist than smaller choline (22). The biggest decrease in agonism occurs by changing one methyl to an ethyl in diEdiMA (20), (2HE)-EdiMA (23), EtMePyrr (31), and dMePip (35).

Figure 3-3. Alpha7 agonism bulky ammonium.

The compounds that had an agonist activity in the screening were further tested to determine their \( I_{\text{max}} \) and \( EC_{50} \) (Table 3-1). The results revealed that small quaternary ammonium that acted as full agonists had similar \( EC_{50} \) values (20-50 \( \mu \)M), except...
choline agonists which were much less potent (EC\textsubscript{50} = 272 ± 28 μM for choline (22) and 1500 ± 400 μM for (2HE)-EdiMA (23)). Compounds that had partial agonism character were generally less potent than their full agonist analogs in the series.

Table 3-1. The EC\textsubscript{50} and I\textsubscript{max} for bulky ammonium compounds that have ionotropic agonism activity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC\textsubscript{50} [μM]</th>
<th>I\textsubscript{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMA</td>
<td>30 ± 3</td>
<td>1.07 ± 0.03</td>
</tr>
<tr>
<td>EtMA</td>
<td>26 ± 5</td>
<td>1.20 ± 0.15</td>
</tr>
<tr>
<td>diEdiMA</td>
<td>31 ± 1</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>tEMA</td>
<td>107 ± 43</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>choline</td>
<td>272 ± 28</td>
<td>0.93 ± 0.03</td>
</tr>
<tr>
<td>(2HE)-EdiMA</td>
<td>1500 ± 400</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>BtMA</td>
<td>39 ± 5</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>dMePyrr</td>
<td>18 ± 2</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>EtMePyrr</td>
<td>50.0 ± 0.1</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>dMePip</td>
<td>24 ± 2</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td>diMHHA</td>
<td>183 ± 7</td>
<td>0.29 ± 0.01</td>
</tr>
</tbody>
</table>

The compounds that had little or no ionotropic agonism were further tested to determine their IC\textsubscript{50} values. Benzyl ammonium compounds had IC\textsubscript{50} values in a similar range (26-67 μM), other compounds had generally higher IC\textsubscript{50} values (100 – 400 μM), except diEHHA (38) which had an IC\textsubscript{50} value of 40 ± 8 μM and TEA (80 ± 5 μM) (Table 3-2).
Finally, the quaternary ammonium compounds were co-applied at 100 μM (1 mM for choline analogs series) with type II positive allosteric modulator PNU-120596 (10 μM). Generally, among the compounds tested, those which acted as ionotropic agonists had the largest responses when co-applied with PNU-120596 (because of their ionotropic agonism responses being potentiated by the type II PAM and Ds desensitized state being reversed), though some exceptions are seen in the tetramethylammonium series and pyrroloidinium series (Figure 3-4). Interestingly, TEA (17), 2HE-diEMA (23), and dEtPip (35) that had no ionotropic response on their own, gave big responses when

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ [μM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>tEMA</td>
<td>400 ± 100</td>
</tr>
<tr>
<td>TEA</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>(2HE)-diEMA</td>
<td>14000 ± 5000</td>
</tr>
<tr>
<td>(2HE)-triEA</td>
<td>300 ± 100</td>
</tr>
<tr>
<td>BEdMA</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>BdEMA</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>BtEA</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>dEtPyrr</td>
<td>350 ± 100</td>
</tr>
<tr>
<td>EtMePip</td>
<td>350 ± 100</td>
</tr>
<tr>
<td>dEtPip</td>
<td>200 ± 70</td>
</tr>
<tr>
<td>diMHHA</td>
<td>114 ± 34</td>
</tr>
<tr>
<td>EMHHA</td>
<td>159 ± 36</td>
</tr>
<tr>
<td>diEHHA</td>
<td>40 ± 8</td>
</tr>
</tbody>
</table>
co-applied with PNU-120596 (normalized net charge of 6 ± 2, 24 ± 9, and 11 ± 2, respectively), revealing their silent agonist character. Three other compounds that had no ionotropic agonism when applied alone, BtEA (29), EMHHA (37), and diEHHA (38), gave small but significant responses with PNU-120596 (normalized net charge of 0.18 ± 0.07, 0.14 ± 0.06, and 0.2 ± 0.1, respectively), revealing their weak silent agonism character. BtEA (29), EMHHA (37), and diEHHA (38) are the largest compounds in the quaternary ammonium group, and their properties reveal the limits to the size of quaternary ammonium where a bulky quaternary ammonium silent agonist becomes an antagonist.

The PNU-120596 co-applications with quaternary ammonium compounds were further characterized at a range of concentrations (Table 3-3). Similarly to KC-1 to KC-9 compounds, the determination of EC₅₀ values for PNU-120596 potentiated responses appeared not possible because the values did not fit into the Hill equation. This was due to a big change in the net charge responses at a narrow concentration range, and to a decrease in PNU-potentiated responses at higher concentrations of experimental compounds, possibly caused by preferential induction of a D_s desensitized state.

To illustrate the activity of quaternary ammonium compounds, a ratio of PNU-120596 co-application responses at 100 μM (1mM for choline analogs) to ionotropic agonism responses at 100 μM (1mM for choline analogs) was calculated (Figure 3-5). The graphs show that silent agonists TEA (17), 2HE-diEMA (23), and dEtPip (35) favor induction of D_s desensitization over ion channel activation in the absence of a PAM by 1100, 4800, 1000-fold respectively. The graphs also clearly show that as the size of the quaternary ammonium molecule increases by replacement of methyl by ethyl groups,
their PNU-120596 co-application to agonism ratios increase as well until the molecule becomes too large.

![Graph illustrating desensitized states of quaternary ammonium revealed by co-application with type II PAM.](image)

**Figure 3-4.** Desensitized states of quaternary ammonium revealed by co-application with type II PAM.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug concentration [μM]</th>
<th>Net charge when co-applied with 10 μM PNU-120596</th>
<th>Threshold: Concentration at which 10 % of maximal response was observed [μM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>tEMA</td>
<td>100</td>
<td>68 ± 11</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>78 ± 19</td>
<td></td>
</tr>
<tr>
<td>TEA</td>
<td>100</td>
<td>6 ± 2</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>36 ± 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>37 ± 6</td>
<td></td>
</tr>
<tr>
<td>(2HE)-diEMA</td>
<td>100</td>
<td>3 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>24 ± 9</td>
<td></td>
</tr>
<tr>
<td>(2HE-triEA)</td>
<td>100</td>
<td>0.13 ± 0.06</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>5 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>1.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>BEdMA</td>
<td>10</td>
<td>3 ± 1</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>23 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>103 ± 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>44 ± 16</td>
<td></td>
</tr>
<tr>
<td>BdEMA</td>
<td>10</td>
<td>0.08 ± 0.02</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.9 ± 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>33 ± 14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>34 ± 8</td>
<td></td>
</tr>
<tr>
<td>BtEA</td>
<td>100</td>
<td>0.18 ± 0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.57 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>dMePyrr</td>
<td>10</td>
<td>85 ± 15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99 ± 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>84 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>107 ± 46</td>
<td></td>
</tr>
<tr>
<td>EtMePyrr</td>
<td>10</td>
<td>2.4 ± 0.5</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>25 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>127 ± 30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>137 ± 46</td>
<td></td>
</tr>
</tbody>
</table>
The Connolly solvent excluded volumes for bulky quaternary ammonium compounds were calculated using ChemDraw3D (Figure 3-6). There is a clear correlation between the volume of the quaternary ammonium compounds and their pharmacological properties. The molecules that have a molecular volume of 94-133 Å³ act as agonists, 142-150 Å³ act as silent agonists with partial ionotropic agonism character, 150-163 Å³ are silent agonists, and 167-186 Å³ are weak silent agonists.
Figure 3-5. Ratio of 10 μM PNU-120596 co-application response at 100 μM to an ionotropic response at 100 μM (1 mM for choline analogs). To avoid division by 0, when no ionotropic agonism response was observed, the PNU-120596 co-application response was divided by 0.005 (detection limit). The error bars were not calculated because of the inaccuracy resulting from limited detection of small responses.
Figure 3-6. Bulky quaternary ammonium hα7 nAChR profile summary. The molecular volumes given are Connolly solvent excluded volumes. Green: agonists, orange: silent agonists with partial agonism character, Red: silent agonists, Purple: weak silent agonists.
Benzyl ammonium compounds do not follow the exactly same criteria, most probably because the quaternary ammonium group and the aryl group can be in different orientations to each other, so they do not need to fit into the same space as other quaternary ammonium compounds. However, similarly to other compounds, as their size increases their properties change from ionotropic agonism, through silent agonism to antagonism.

The molecular volume of the α7 silent agonist ASM-024 is 242 Å³ and it might seem that the compound is not following the trends observed in the quaternary ammonium series described in this chapter. Interestingly, the molecular volume of an nAChR agonist dimethylphenylpiperazinium (DMPP) (Figure 3-7) is 207 Å³ and it could be considered as an analog of dimethylpiperidinium (dMePip), characterized here as an agonist. Accordingly to the data presented above, DMPP would be expected to act as an α7 antagonist. Therefore, it appears that the bulky quaternary ammonium constitutes one type of a core pharmacophore for silent agonism, while the addition of an aromatic ring on the side of the molecule opposite to the positively charged nitrogen does not change the character of the molecule (agonist, silent agonist, antagonist) and may allow for optimization of the compound to gain selectivity over other ion channels and for α7 nAChR subtype. For example, TEA is known to block voltage-dependent potassium channels, while ASM-024 is not.

ASM-024 and DMPP possess an additional nitrogen in the ring bearing the quaternary ammonium group and its importance could be investigated by testing diethylphenylpiperazinium (A) and diethylphenylpiperidinium (B). Accordingly to the
quaternary ammonium molecular volume hypothesis and observations made above, both compounds would act as α7 silent agonists.

![Chemical structures](image)

Figure 3-7. Structures of ASM-024, DMPP, diethylphenylpiperazinium and diethylphenylpiperidinium.

### 3.2.3 Activity Profile of Quaternary Ammonium Compounds on the Human α4β2 nAChR

The quaternary ammonium compounds were screened at 100 μM on the hα4β2 nAChR. The compounds had no detectable ionotropic activity, except TMA, EtMA, BtMA, and dMePyrr (small quaternary ammonium with no OH group), that activated the α4β2 receptor only weakly (Figure 3-8).
When the quaternary ammonium compounds were co-applied with ACh, diminished responses to ACh were observed (except for choline) (Figure 3-9), suggesting that quaternary ammonium compounds bind to α4β2 receptors, though many of the compounds appear to do so very weakly.
3.3 Summary

Six series of the quaternary ammonium compounds have been tested on the human α7 nAChR and new α7 silent agonists were identified. A clear correlation between the molecular volume of a quaternary ammonium compound and its α7 pharmacological properties has been found: small quaternary ammoniums act as agonists, and increasing their size results in changing their character from an agonist to silent agonist and finally to an antagonist.
Quaternary ammonium compounds generally do not activate α4β2 receptors, and while they appear to bind to α4β2, there may be a possibility of developing quaternary ammonium compounds selective for α7 nAChR.
CHAPTER 4
SYNTHESIS OF NEW FLUORINATED PYRIDINYL METHYLENE ANABASEINES TO STUDY INTERACTIONS LEADING TO DESENSITIZED STATES OF THE HUMAN \( \alpha_7 \) NICOTINIC ACETYLCHOLINE RECEPTOR

4.1 Background

Dr. Jingyi Wang in Dr. Horenstein’s Laboratory synthesized pyridinylmethylene anabaseines 2-PAB (41), 3-PAB (42), and 4-PAB (43) (Figure 4-1).\(^{202,222}\)

Figure 4-1. Structures of 2-PAB, 3-PAB, and 4-PAB.

The compounds were weak agonists for the \( h_\alpha7 \) nAChR and when they were co-applied with 30 \( \mu \)M PNU-120596, their \( D_s \) stabilizing ability and silent agonism character were revealed (Figure 4-2, Dr. Roger Papke unpublished data).

Figure 4-2. Pharmacological properties of 2-PAB, 3-PAB, and 4-PAB. A. Agonism; B. Co-application with PNU-120596.
Dr. Jingyi Wang also synthesized arylidene anabaseines analogous to PABs (41, 42, 43) but with pyrrole (H-bond donors), furan (H-bond acceptors), and thiophene (non H-bonding, hydrophobic) rings instead of a pyridine (H-bond acceptor) ring, and showed that H-bonding in the α7 nAChR selectivity pocket influences activation and desensitization of the receptor. However, the PABs (41, 42, 43) compounds were generally weaker agonists and much stronger Ds desensitizers than the arylidene anabaseines with pyrrole, furan, and thiophene rings, suggesting that other effects than H-bonding in the α7 nAChR selectivity pocket may also control the state of the receptor. One noticeable difference between pyrrole, furan, thiophene and pyridine is that the first three heterocycles are electron-rich, while pyridine is electron-poor. These observations led to a hypothesis that π-interactions between electron-rich amino acids such as tyrosine or tryptophan in the α7 nAChR benzylidene selectivity pocket and an electron-deficient benzylidene motif may be involved in recognition that promotes the receptor’s Ds desensitized state.

The π-interactions are non-covalent interactions involving π-systems. Dr. Dennis Dougherty and co-workers studied cation-π interactions in the nAChR binding site, and by using fluorinated amino acid mutagenesis, they showed that cation-π interactions between the aromatic amino acids side chains in the nAChR binding site and the positive charge of acetylcholine are very important for acetylcholine binding and activation of the receptor.

Instead of introducing fluorinated amino acids in the binding site of the α7 nAChR, we designed a series of fluorinated 3-PABs and pentafluorinated benzylidene anabaseine [(2-fluoro)-3PAB ((2F)-3PAB, 44), (5-fluoro)-3PAB ((5F)-3PAB, 45), (2,6-
difluoro)-3PAB ((2,6-DF)-3-PAB, 46), (2,4,6-trifluoro)-3PAB ((2,4,6-TF)-3PAB, 47), and (2,3,4,5,6-pentafluoro)-benzylidene anabaseine ((2,3,4,5,6-PF)-AB, 48) (Figure 4-3)], to study whether π-interactions in the α7 nAChR benzylidene selectivity pocket may be responsible for silent agonism character of pyridinylmethylene anabaseines (41, 42, 43).

Figure 4-3. Structures of fluorinated 3-PABs and pentafluorinated benzylidene anabaseine.

Because of the electron-withdrawing effect of fluorine, the fluorinated compounds (44, 45, 46, 47, and 48) are even more electron-poor than the PABs (41, 42, 43). We were expecting that if π-acceptor/donor interactions between the π-system of the ligand and an electron rich donor in the α7 nAChR benzylidene selectivity pocket do control the Ds desensitized state of the receptor, the fluorinated 3-PABs would give higher responses than 3-PAB (42) when co-applied with PNU-120596 because the π-interactions of the ligand with the receptor would be strengthened.

4.2 Results and Discussion

4.2.1 Synthesis of New Fluorinated Arylidene Anabaseines

The general method for the synthesis of arylidene anabaseines consists of a mixed aldol-type condensation between anabaseine dihydrochloride (52) and aryl aldehydes.219,222 Therefore, we decided to make the new fluorinated arylidene
anabaseines 44-48 also by an aldol-type condensation between anabaseine dihydrochloride (52) and an appropriate fluorinated aryl aldehyde.

Anabaseine dihydrochloride (52) was prepared on a four gram scale, following a protocol of Zoltewicz and Cruskie,203 with some modifications of the first step202,222 (Figure 4-4). The NH-function of δ-valerolactam 49 was protected by a base-stable and acid labile aminomethyl group, which was introduced by a Mannich reaction between δ-valerolactam (49), paraformaldehyde and diethylamine in refluxing toluene in a Dean-Stark apparatus to remove water formed. Then, the mixed Claisen condensation of ethyl nicotinate and the 1-(diethylaminomethyl)-2-piperidinone 2 afforded the sodium salt of the 3-nicotinoyl-2-piperidinone 51 in 65 % yield as a mixture of three compounds, most probably the E and Z enolate isomers 51a, 51b and the keto isomer 51c as indicated by $^1$HNMR. The sodium salt 51 was treated with a hot 5:1 mixture of concentrated hydrochloric acid and acetone to yield anabaseine dihydrochloride 52 in 59 % yield by removal of the N-protecting group, hydrolysis of the amide bond, decarboxylation of the β-keto carboxylic acid, and cyclization. Acetone in this reaction facilitates the precipitation of sodium chloride.

Figure 4-4. Synthesis of anabaseine dihydrochloride 52.
The mixed aldol-type condensation between aryl aldehydes and anabaseine dihydrochloride (52) gives arylidene anabaseines most probably via enamine form of anabaseine.\textsuperscript{219,222} Zoltewicz et al. reported that in the case of aryl aldehydes having electron-donating substituents, the acid-catalyzed condensation proceeds well; while in the case of aryl aldehydes having strong electron-withdrawing substituents, a base is needed, for example acetate ion in acetic acid, most probably to facilitate dehydration of an aldol intermediate.\textsuperscript{219} Hence, (2-fluoro)-3PAB (44), (5-fluoro)-3PAB (45), (2,6-difluoro)-3PAB (46), (2,4,6-trifluoro)-3PAB (47), and (2,3,4,5,6-pentafluoro)-benzylidene anabaseine (48) were prepared by reacting suitable fluorinated aryl aldehydes \textsuperscript{53-57} with anabaseine dihydrochloride (52) in methanol in the presence of sodium acetate and acetic acid in a 1:2.5 ratio at room temperature to give the desired products in 80%, 74%, 78%, 70%, and 57 % yield, respectively (Figure 4-5). The reaction between 5-fluoro-3-formylpyridine (54) and anabaseine dihydrochloride (52) required 26 h to go to completion, while more fluorine-substituted aryl aldehydes required longer reaction times (48 h in the case of 2,4,6-trifluoronicotinealdehyde (56) and perfluorobenzaldehyde (57)).
Figure 4-5. Synthesis of new fluorinated arylidene anabaseines.

Only one double bond isomer was formed in the reaction and the aldol intermediate was not detected. According to the molecular models, both E and Z isomer configurations are possible, but the Z isomer of arylidene anabaseine, in which the two aromatic rings are stacked face to face, is higher in energy than the E isomer.\textsuperscript{219,222} All previously reported arylidene anabaseines prepared by aldol-type condensation had an E stereochemistry about the double bond.\textsuperscript{219,222} The NOESY experiments confirmed that the fluorinated arylidene anabaseines retained that previously observed preference for formation of the E-isomer (Figure 4-6, Appendix).
Figure 4-6. 2F-3PAB (44): NOE enhancement used to assign the double bond geometries in fluorinated pyridinylmethylene anabaseines.

The fluorinated aryl aldehydes were purchased from Frontier Scientific, Matrix Scientific, and Sigma-Aldrich except from 2,4,6-trifluoronicotinaldehyde 8 whose synthesis has not been reported in literature. The 2,4,6-nicotinaldehyde 8 was prepared from 2,4,6-trifluoropyridine with n-butyllithium and N-methylformanilide in 58 % yield (Figure 4-7).

Figure 4-7. Synthesis of 2,4,6-trifluoronicotinaldehyde 56.

4.2.2 pKa Values of New Fluorinated Arylidene Anabaseines

The pKₐs of the imine nitrogen determines the concentration of the active protonated forms at physiological pH and thus affects arylidene anabaseines’ potencies. The pKₐs for fluorinated compounds were estimated using the previously reported formula

\[ \text{pK} = (4.29 - \delta H) / 0.055, \]

where \( \delta H \) is the chemical shift of the protons on the carbon adjacent to the imine nitrogen in CDCl₃, with tetramethylsilane as a standard (Table 4-1). The formula to predict the pKa of benzylidene anabaseines from the \(^1\)HNMR was derived based on the
series of seventeen compounds in a study, where the range of chemical shifts for the methylene protons adjacent to the imine group of benzylidene anabaseines was only 0.11 ppm, but a very good linear correlation (r = 0.999) was found using the Hammett substituent constants $\sigma_m$ and $\sigma_p$. The percentages of the iminium cation (% HA) at physiological pH (pH = 7.2) were then calculated via Henderson-Haselbach equation: 

$$\text{pH} = \text{pKa} + \log([A^-]/[HA])$$

Table 4-1. The predicted iminium cation percentages of the fluorinated arylidene anabaseines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>3PAB</th>
<th>(2-F)-3PAB</th>
<th>(5-F)-3PAB</th>
<th>(2,6-DF)-3PAB</th>
<th>(2,4,6-TF)-3PAB</th>
<th>(2,3,4,5,6-PF)-BA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta H [\text{ppm}]$</td>
<td>3.91</td>
<td>3.94</td>
<td>3.93</td>
<td>3.94</td>
<td>3.97</td>
<td>3.98</td>
</tr>
<tr>
<td>pKa</td>
<td>6.91</td>
<td>6.36</td>
<td>6.55</td>
<td>6.36</td>
<td>5.82</td>
<td>5.64</td>
</tr>
<tr>
<td>% HA at pH = 7.2</td>
<td>34 %</td>
<td>13 %</td>
<td>18 %</td>
<td>13 %</td>
<td>4 %</td>
<td>3 %</td>
</tr>
</tbody>
</table>

The 3PAB (42) is predicted to be 34 % protonated at physiological pH, while substitutions with fluorine lower the pK$_a$ of the compounds and the percentage of protonated form (which would be expected based on the structure of these compounds: fluorine stabilizes the positive charge of the iminium ion by electron-withdrawing inductive effect).

4.2.3 Activity Profile of Fluorinated Arylidene Anabaseines on the Human $\alpha_7$ nAChR

The fluorinated arylidene anabaseines (44, 45, 46, 47, and 48) were screened at 30 $\mu$M and 300 $\mu$M on the human $\alpha_7$ nAChR and compared to 3PAB (42). The fluorinated compounds had no ionotropic activity above detection limit (Figure 4-8), indicating that fluorination of 3PAB (42) negatively impacts activation of the receptor.
When co-applied with 60 μM acetylcholine, the fluorinated compounds at 300 μM diminished the response of acetylcholine, suggesting that they bind to the receptor (Figure 4-9). The level of fluorination did not correlate with the ability of the compound to inhibit the ACh response.
Finally, co-application of fluorinated arylidene anabaseines at 300 μM with PNU-120596 at 30 μM, revealed that fluorination of 3PAB (42) also negatively impact $D_s$ desensitization (Figure 4-10), which was contrary to the expected results. Interestingly, there was a big difference in the responses of monofluorinated (2-F)-3PAB (44) and (5-F)-3PAB (45), suggesting that fluorine exerts a local effect with the amino acids in the binding site, possibly such as the interaction described by Parlow at al. where fluorine acted as a hydrogen bond acceptor in a crystal structure of fluorobenzene inhibitor with a tissue factor VIIa.\textsuperscript{221}

![Graph showing normalized net charge](image)

**Figure 4-10.** Desensitized state of fluorinated arylidene anabaseines revealed with PNU-120596.

3-PAB (42) and (2-F)-3PAB (44) were further tested at 30 μM, 100 μM, and 300 μM with PNU-120596 (10 μM) (Figure 4-11). The highest responses were observed at 100 μM for both 3PAB and (2-F)-3PAB (net charge: 72 ± 23 and 9 ± 1 for 3PAB and (2-F)-3PAB, respectively). At 300 μM, the PNU-120596 potentiated responses were lower, suggesting that higher occupancy of the receptor by 3PAB and (2F)-3PAB was favoring
the D₃-desensitized state. This result also indicated that lower activity of (2-F)-3PAB (44) versus 3PAB (42) with PNU-120596 did not result from lower concentration of the fluorinated compound protonated form, otherwise by increasing the concentration of (2F)-3PAB (44), a maximum response similar to that of 3PAB (42) should have been reached.

Figure 4-11. Comparison of PNU-120596 co-application responses for 3PAB and (2-F)-3PAB.

4.3 Summary

Five new fluorinated arylidene anabaseines were synthesized: (2-F)-3PAB (44), (5-F)-3PAB (45), (2,6-DF)-3PAB (46), (2,4,6-TF)-3PAB (47), and (2,3,4,5,6-PF)-BA (48) to test a hypothesis that π-interactions between electron-poor benzylidene motif of the ligand and electron-rich amino acids in the α7 selectivity pocket favor Dₛ desensitization.

It was demonstrated that fluorination of 3PAB (42) negatively impacts activation of the receptor and Dₛ desensitization. Perhaps fluorination enhances entry into Dᵢ state but this remains to be considered after further investigation. The fact that fluorinated 3PABs are less active as α7 activators and Dₛ desensitizers than 3PAB might be due to weaker H-bonding ability of the nitrogen in the fluorinated pyridine ring. However, other
factors also seem to play a role in controlling the state of the receptor, such as local interactions between fluorine atoms on the pyridine ring and amino acids in the binding site. The results do not support the hypothesis stating that \(\pi\)-interactions in the \(\alpha_7\) nAChR benzylidene selectivity pocket influence \(\alpha_7\) nAChR desensitization.

The changes in the pK\(_a\)s of fluorinated compounds did not correlate with their activity, suggesting that lower ionotropic and silent agonist activities of fluorinated 3PABs are not due to lower concentrations of protonated forms of these compounds at physiological pH.

Higher occupancy of the receptor by PAB compounds produces less of the D\(_s\) state and more of the D\(_i\), as observed previously with KC compounds and quaternary ammonium compounds.
CHAPTER 5
SYNTHESIS OF NOVEL ARYLIDENE QUINUCLIDINES TO STUDY THE EFFECT OF H-BONDING IN THE α7 nAChR SELECTIVITY POCKET ON ACTIVATION AND DESENSITIZATION

5.1 Background

As introduced in Chapter 1, anabaseine (52) and quinuclidine (60) are non-selective agonists for nAChRs, and the addition of benzylidene motif, such as in GTS-21 (61) and (E)-benzylidene quinuclidine (62) makes these molecules selective for α7 nAChR (Figure 5-1).¹¹¹

![Structures of non-selective nAChR agonists and hα7 nAChR selective agonists.](image1)

Figure 5-1. Structures of non-selective nAChR agonists (anabaseine, quinuclidine), and hα7 nAChR selective agonists (GTS-21, (E)-benzylidene quinuclidine).

Dr. Jingyi Wang (Horenstein Laboratory) synthesized and characterized aryldene anabaseines 2PyroAB (63) (H-bond donor), 2FAB (64) (H-bond acceptor) and 2 TAB (65) (hydrophobic, non H-bonding) (Figure 5-2) as partial agonists of the hα7 nAChR, and showed that H-bonding interactions in the α7 selectivity pocket could control the state of the receptor (activation, rate of the receptor to enter into different desensitized states, and energy levels of open and desensitized states).²²²,²²³ These results suggested that it may be possible to develop new nAChR agonists that have tailored responses to allosteric modulators.
To further investigate how H-bonding interactions in the α7 selectivity pocket influence the state of the receptor, we designed a series of twelve H-bonding probes: (Z)-2-PyroQN (66a), (E)-2-PyroQN (66b), (Z)-3-PyroQN (67a), (E)-3-PyroQN (67b) (H-bond donors), (Z)-2-FQN (68a), (E)-2-FQN (68b), (Z)-3-FQN (69a), (E)-3-FQN (69b) (H-bond acceptors), and (Z)-2-TQN (70a), (E)-2-TQN (70b), (Z)-3-TQN (71a), (E)-3-TQN (71b) (non H-bonding, hydrophobic) (Figure 5-3).

Figure 5-2. Structures of hα7 nAChR partial agonists 2PyroAB, 2FAB, and 2TAB.

The compounds have the same selectivity motifs as 2-PyroAB, 2-FAB, and 2-TAB, but different pharmacophore cores (quinuclidines vs anabaseines), which would allow us to exploit whether the interactions between the ligand and the receptor in the α7 selectivity pocket could be transferred between different pharmacophore cores. If H-bonding of aryldiene quinuclidines in the α7 selectivity pocket would have same effects on activation and desensitization of the receptor as H-bonding of aryldene anabaseines, it would suggest that their binding modes are the same. If aryldene quinuclidines would show different behavior than their analogous aryldene anabaseines, it would suggest that the molecules have different binding modes. We were also hoping to obtain ligands with better efficacy, potency, and selectivity.
5.2 Results and Discussion

5.2.1 Synthesis of Arylidene Quinuclidines

We decided to synthesize arylidene quinuclidines by Horner-Wadsworth-Emmons olefination\textsuperscript{224,225} using 3-quinuclidinone (72) and appropriate phosphonate esters, similarly to the protocol used for the synthesis of benzyldiene quinuclidines.\textsuperscript{132,133,226}
5.2.1.1 Synthesis of 2-TQN and 2-FQN Using Phosphonate Ethyl Esters

The necessary diethyl(thiophene-2-ylmethyl)phosphonate (77) and diethyl(furan-2-ylmethyl)phosphonate (78) were prepared in 65 % yield by Michaelis-Arbuzov reaction,\textsuperscript{227} using chlorides 75, 76 and triethylphosphite (Figure 5-5). The chlorides were obtained from corresponding alcohols 73, 74 by reaction with thionyl chloride and pyridine in dichloromethane, following published procedures.\textsuperscript{228,229} The phosphonate ethyl esters were treated first with sodium hydride 60 % dispersion in mineral oil to generate the phosphonate anions, and then with 3-quinuclidinone (72) in THF at reflux (no reaction was observed at room temperature). The Horner-Wadsworth-Emmons reactions gave 2-TQN (70ab) and 2-FQN (68ab) in 85 % yield as a mixture of E and Z isomers in 1:1 ratio, that required tedious chromatographic separation on silica gel using hexanes, ethyl acetate and freshly distilled triethylamine (use of DCM, methanol, and triethylamine as an eluent did not afford any separation of isomers).

\[ \text{Horner-Wadsworth-Emmons reactions} \]

Horner-Wadsworth-Emmons reactions normally favor the formation of E isomers,\textsuperscript{225} but since 3-quinuclidinone (72) has similar steric bulk on both sides of the
carbonyl group, no stereoselectivity was seen. The geometry of the double bond was unambiguously established using Nuclear Overhauser Enhancement Spectroscopy (Appendix) and also by analysis of the $^1$HNMR spectra. The most diagnostic NMR feature was the peak for the proton marked by a red arrow in Figure 5-6: it appeared at 2.47 ppm in a Z-isomer and was shifted 0.8 ppm downfield in an E-isomer due to the deshielding from the aryl ring. This shift downfield in the E-isomer was observed for all synthesized arylidene quinuclidines and has been also reported previously for benzylidene quinuclidines.$^{133}$

![Z-isomer and E-isomer](image)

Figure 5-6. Comparison of $^1$HNMR spectra for (Z)-2-TQN (70a) and (E)-2-TQN (70b).
5.2.1.2 Synthesis of 2-TQN and 2-FQN Using Phosphonate Methyl Esters

2-TQN (70ab) and 2-FQN (68ab) were also prepared using phosphonate methyl esters (Figure 5-7). However, the yields were much lower than those obtained using phosphonate ethyl esters and some impurities were difficult to separate, so this approach was not further pursued.

![Synthesis of 2-TQN and 2-FQN](image)

Figure 5-7. Synthesis of 2-TQN (70ab) and 2-FQN (68ab) using phosphonate methyl esters.

5.2.1.3 Synthesis of 3-TQN and 3-FQN

Diethyl(thiophene-3-ylmethyl)phosphonate (85) and diethyl(furan-3-ylmethyl)phosphonate (86) were synthesized by Michaelis-Arbuzov reaction, analogously to the analogues substituted at the position 2 of the heteroaryl ring (Figure 5-8). The Horner-Wadsworth-Emmons reaction of the heteroaryls with phosphonate group at position 3 did not work too well, though. The yields were much lower than those for 2-TQN (70ab) and 2-FQN (68ab), and an excess of phosphonates 85, 86 was necessary to obtain reasonable yields.
Moreover, separation of the Z- and E- isomers of 3-TQN (71ab) and 3-FQN (69ab) was more difficult than for 2-TQN (70ab) and 2-FQN (68ab), with some small impurities remaining after column chromatography, especially in the E-isomers, and the compounds were not satisfactorily pure for biological testing.

5.2.1.4 Attempts to synthesize N-Boc-2-(chloromethyl)pyrrole (90).

The synthesis of pyrrole phosphonates proved to be very problematic, most probably due to instability of the pyrrole ring system in 90 (the chloride can eliminate from the product). The protected 2-hydroxymethylpyrrole 89 was prepared from pyrrole-2-carboxaldehyde (87) following a literature procedure\(^{230}\) (Figure 5-9). Several protocols for transformation of alcohols into chlorides were tried, but all of them met with failure: the substrate was consumed but no product formation was observed.
5.2.1.5 Attempt to synthesize 2-PyroQN (66ab)

To circumvent the instability of the pyrrole chloride, a known 2-(chloromethyl)-1-(methylsulfonyl)pyrrole (93) was prepared following a published procedure with small modifications\textsuperscript{231} (Figure 5-10). The Arbuzov reaction of the chloride 93 with triethyl phosphite gave the phosphonate 94 in a low 12 % yield. Unfortunately, the Horner-Wadsworth-Emmons between the phosphonate 94 and 3-quinuclidinone (72) did not work, probably due to poor nucleophilicity of the anion of 94 (most of the unreacted substrate 94 was found in the crude). Therefore, the preparation of 2-PyroQN (66ab) appeared not possible: the necessary chloride (90) was too unstable, and while the stabilization of the pyrrole chloride by protection with an electron-withdrawing mesyl group enabled the synthesis of a phosphonate 94, the mesyl phosphonate 94 was unreactive in the subsequent Horner-Wadsworth-Emmons reaction.

Figure 5-10. Attempts to synthesize 2-PyroQN.

5.2.2 Activity of QN Compounds

The hydrochloride salts of (Z)-2-TQN (70a), (E)-2-TQN (70b), (Z)-2-FQN (68a), and (Z)-3-TQN (68b) were tested on the \( \alpha_7 \) nAChR in \textit{Xenopus} oocytes as described in Section 1.4 and Appendix A, and appeared to act as partial agonists. (E)-2-TQN (70b) was shown to be a more potent and efficacious agonist than (Z)-2-TQN (70a)
$I_{\text{max}} = 0.71 \pm 0.04$, $EC_{50} = 0.95 \pm 0.24 \mu M$ for (E)-2-TQN; $I_{\text{max}} = 0.20 \pm 0.01$, $EC_{50} = 7 \pm 2 \mu M$ for (Z)-2-TQN. Higher activity of an E isomer has been reported before for benzylidene quinuclidines.\(^{111}\) It was also noticed that (E)-2-TQN (70b) produced large amounts of RID (residual inhibition and desensitization) (10 ± 3 % recovery of the receptor from the desensitized state as indicated by an ACh post-control), while (Z)-2-TQN (70a) did not (86 ± 7 % recovery). It would be interesting to further pursue these observations and study the behavior of (E)-2-TQN (70b) and (Z)-2-TQN (70a) with type II PAMs.

### 5.3 Summary

Eight new aryldiene quinuclidines containing furan and thiophene rings were synthesized: (Z)-2-TQN (70a), (E)-2-TQN (70b), (Z)-2-FQN (68a), (E)-2-FQN (68b), (Z)-3-TQN (71a), (E)-3-TQN (71b), (Z)-3-FQN (69a), (E)-3-FQN (69b), but (E)-3-TQN (71b), (Z)-3-FQN (69a), and (E)-3-FQN (69b) could not be obtained in a pure form.

(Z)-2-TQN (70a), (E)-2-TQN (70b), (Z)-2-FQN (68a), (Z)-3-TQN (71a) were tested on h\(\alpha\)7 nAChR and showed to act as partial agonists.

Quinuclidines containing a pyrrole ring were not synthesized because of the instability of Boc-protected-2-chloromethylpyrrole (90) and lack of reactivity of mesylphosphonate (94) in the Horner-Wadsworth-Emmons reaction.

Due to difficulties with purification of the quinuclidine compounds, problems with synthesis of pyrrole quinuclidines, and higher importance of other projects, this project was abandoned, though some interesting observations about different residual inhibition and desensitization properties of Z- and E-aryldiene quinuclidines were made that may be further investigated in the future.
6.1 Background

6.1.1 Roles of Strigolactones

Strigolactones have long been of interest because they induce germination of seeds of parasitic weeds of the genera *Striga*, *Orobanche* and *Alectra* which cause massive crop losses, especially in the developing world. The seeds of these weeds can remain dormant in the soil for up to 20 years; they do not germinate unless they sense strigolactones secreted by roots of the host plants (Figure 6-1).

Figure 6-1. Strigolactones secreted by host plants induce germination of parasitic weeds (Reproduced from Annual Review of Phytopathology, 48, X. Xie, K. Yoneyama, K. Yoneyama, The strigolactone story, 93-117, Copyright (2010), with permission from Annual Reviews, Inc).

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1 This work was performed in Dr. Nigel G. J. Richards Lab in collaboration with Dr. Aaron Aponick.
Strigol was first isolated in 1966 from cotton root exudates, and later, it gave the name to the strigolactones family (Figure 6-2). Strigolactones are composed of a tricyclic lactone (ABC core) connected via an enol ether linkage to a butyrolactone moiety (D ring). All natural strigolactones contain the same C, D moiety and possess 2′-(R)-stereochemistry except for 2′-epiororobanchol, and same configuration of BC rings, except for fabacyl acetate and solanacol. They display major differences in A and B rings.

Figure 6-2. Structures of natural strigolactones and synthetic analog GR24.

It was not until 40 years after the isolation of the first strigolactone that the true role of these natural products started to emerge. In 2005, it was shown that strigolactones are involved in symbiotic interactions between plant roots and arbuscular mycorrhizal fungi which are important for the growth of 80% of plant species. Finally,
Strigolactones have been identified in 2008 by studies of plant mutants displaying a bushy phenotype as a new class of plant hormones that inhibit shoot branching\(^{235,236}\) (Figure 6-3), which led to renewed interest in strigolactones biosynthesis and their mode of action and resulted in a number of reviews on strigolactones.\(^{237,243}\)

![Figure 6-3. Phenotype of wild type rice plant producing strigolactones (left), and a mutant impaired in strigolactone biosynthesis (right). (Reproduced from Plant Cell, 21, H. Lin, R. Wang, Q. Qian, M. Yan, X. Meng, Z. Fu, C. Yan, B. Jiang, Z. Su, J. Li, Y. Wang, Dwarf27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth, 1512-1525, Copyright (2009), with permission from American Society of Plant Physiologists).](image)

Plant hormones are active at very low concentrations and they can function locally, at or near the site of synthesis, or in distant tissues.\(^{244}\) They regulate plant growth and mediate responses to various stresses. Plant hormones include: auxins, cytokinins, abscisic acid, gibberellins, jasmonates, ethylene, brassinosteroids, and salicylic acid (Figure 6-4).

### 6.1.2 Strigolactone Biosynthetic Pathway

At the time when I started to work on strigolactones, little was known about their biosynthetic pathway. In 2005, Bouwmeester and coworkers have demonstrated that the ABC core of strigolactones is derived from carotenoids by the studies with inhibitors of carotenoid biosynthesis and isoprenoids pathways.\(^{245}\)
The strigolactone biosynthetic pathway has been partially predicted by extensive screening and genetic analysis performed in the last 20 years on various plant mutants (Figure 6-5). Four mutants, max1-max4 (more axillary growth), were described in *Arabidopsis thaliana*. Several mutants have been described in rice (htd and d, high tillering dwarf and dwarf), pea (rms, ramosus) and petunia (dad, decreased apical dominance).
Max3 have been shown to encode a carotenoid cleavage dioxygenase 7 (CCD7) and max4 have been shown to encode a CCD8. It has been reported that in carotenoids accumulating *E. Coli* and *in vitro* assays, CCD7 cleaves β-carotene at the 9',10'-position,\textsuperscript{248,250,251} and the resulting apocarotenoid product, β-apo-10'-carotenal, is further cleaved by the action of CCD8 to produce β-apo-13-carotenone (C18 ketone).\textsuperscript{250,251} However, the natural substrate and enzyme cleavage activities in plants were not reported in the literature at the time when I was actively working on the project.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig6-5.png}
\caption{Partially predicted strigolactones biosynthetic pathway deduced from analysis of plant mutants displaying an increased branching phenotype and studies in carotenoids accumulating *E. Coli* and *in vitro* (the intermediates shown and enzymes activities appeared later to be different).\textsuperscript{252}}
\end{figure}
Research on plant carotenoid dioxygenases started with identification of maize 9-cis-epoxy carotenoid dioxygenase VP14 (viviparous 14), which catalyzes formation of xanthoxin, a precursor of abscisic acid. Carotenoid cleavage dioxygenases are non-heme iron proteins (reviews). These enzymes use dioxygen, but whether one or both oxygen atoms are incorporated into apocarotenoids products and whether the name dioxygenases is correct or not, has not been clarified.

D27 encodes a novel iron-containing protein that is localized in chloroplasts. D27 shares no homology with any functionally identified protein.

Max1 encodes a cytochrome P450 enzyme (CYP711) and it has sequence similarity to Thromboxane A2 synthase, which does not require molecular oxygen or an electron donor for catalysis (it catalyzes an isomerization and fragmentation of prostaglandin H2).

Max2 is not involved in strigolactones biosynthesis and it encodes an F-box protein needed for signal transduction.

The elucidation of strigolactones biosynthetic pathway is not trivial because strigolactones are produced in very low, nano- and picomolar concentrations and they are quite unstable. In addition, the characterization of plastidic CCD7, CCD8 and D27 poses particular problems due to carotenoid hydrophobicity and the difficulty to reproduce the plastid organization outside the plants.

6.1.3 Strigolactone Synthesis

A series of strigolactones have been synthesized because of the need to confirm the proposed structures. For example, total syntheses showed that the initially reported structures for orobanchol, alectrol and solanacol were incorrect. Many synthetic strigolactones analogs have been made in an effort to find an inexpensive compound
for use in agriculture to induce parasites seed germination in the absence of crops.\textsuperscript{267,268}

This goal has not been realized in practice, though some promising results have been obtained in field trials with Nijmegen-1, an analog in which the AB ring system was replaced with phthalimide group.\textsuperscript{238} GR24 (for germination release) (Figure 6-2), is one of the most potent strigolactones analogs and it is now used in bioassays for shoot branching inhibition. Strigolactones synthesis has been reviewed up to 2005 by Humphrey \textit{at al.}\textsuperscript{232}

Strigol was first synthesized in the 1970s by the groups of Charles Sih and Ralph Raphael (Figure 6-6).\textsuperscript{269,270} Both groups made the B and C rings in a stepwise fashion and then they attached the D ring using bromobutenolide. The Sih group used β-cyclocitral as a starting material and the Raphael group started their synthesis from 2,2,2-dimethyl cyclohexanone. Strigol has been synthesized later by modifications of those two routes.\textsuperscript{271-274} 5-Deoxystrigol was first made by Frischmuth \textit{et al.},\textsuperscript{275} and later by Shoji \textit{et al.}\textsuperscript{276} Sorgolactone has been synthesized in the laboratories of Binne Zwanenburg\textsuperscript{277} and Kenji Mori.\textsuperscript{278} Orobranchol synthesis has been published by Matsui \textit{et al.}\textsuperscript{279} (Mori Lab). Most recent synthetic efforts to build the B-ring of strigolactones relied on Diels-Alder, followed by Perkin reaction, alkaline rearrangement and decarboxylation,\textsuperscript{265} reductive carbon-carbon bond formation of an aldehyde in the presence of samarium (II) iodide,\textsuperscript{276} ring-closing metathesis,\textsuperscript{266} and intramolecular Friedel-Crafts.\textsuperscript{280} All the known ABC-core syntheses consist of a stepwise formation of the rings, except samarium iodide-mediated cyclization, in which tricyclic lactone has been obtained as a minor product.\textsuperscript{276} None of the literature strigolactone ABC-ring system syntheses had been biomimetic before this work was published.\textsuperscript{281}
Figure 6-6. First total syntheses of strigol.

Several strigolactones and strigolactones synthetic analogs have been prepared in enantiopure forms.\textsuperscript{232,282,283} (+)-Strigol was first obtained by resolution with a chiral agent.\textsuperscript{284} The first formal asymmetric synthesis has been done by Welzel research group using (S)-malic acid as a starting material (chiral pool approach).\textsuperscript{285}

Strigolactones have also been obtained as pure enantiomers by chromatography on a
chiral column (using cellulose triacetate) (for example\textsuperscript{286,287}), or by kinetic resolution using \textit{Candida Antarctica} lipase B and vinyl acetate or acetic anhydride.\textsuperscript{288,266} Several methodologies have been developed to control the stereochemistry at C-2', such as the use of enantiopure D-ring precursors \textbf{96} and \textbf{97} and liberation of D-ring by retro Diels-Alder reaction\textsuperscript{289,290} (Figure 6-7).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure6-7.png}
\caption{Enantiopure precursors of D-ring.}
\end{figure}

\textbf{6.1.4 Importance of Work on Strigolactones}

Synthetic analogs and biosynthetic inhibitors of enzymes making strigolactones could be used to manage germination of parasitic weeds responsible for massive crop losses in the developing world. They could also be potential tools to control lateral branching and aerial part plant architecture which would be useful in agriculture and food industry (for example by enhancing tillering in rice and thus improving grain yields),\textsuperscript{291} forestry (production of high quality woods with fewer knots), and horticulture (production of ornamental plants).\textsuperscript{262}

\textbf{6.2 Research Design and Specific Aims}

This research has been focused on investigating whether the tricyclic core of strigolactones can be formed in one step by double cyclization of simple linear precursors in model systems (aim 1) and applying that cascade reaction in synthesis of strigolactones (aim 2).
Strigolactones were shown to be derived from carotenoids but only four enzymes involved in strigolactones biosynthesis have been discovered despite extensive studies on plant mutants. In 2005, Bouwmeester and coworkers proposed how strigolactones could be made in plants\textsuperscript{245,292} (Figure 6-8), and their biosynthetic schemes have been repeatedly reproduced in later publications on strigolactones. However, the proposed schemes did not take into account activities of enzymes known to be involved in the biosynthetic pathway and they relied on long sequences of unusual chemical transformations.

![Proposed literature strigolactone biosynthetic pathway](image)

Figure 6-8. Proposed literature strigolactone biosynthetic pathway.\textsuperscript{292}

As an alternative to those chemically complex sets of reactions, we aimed to propose a more rational way in which those natural products could be made in plants and show by chemical synthesis that B and C rings of the ABC ring system might be constructed in one step from a linear precursor type 98 (alcohol oxidation level) by an acid-catalyzed double cyclization. During this research, we also decided to study the feasibility of our proposed cyclization with a linear precursor type 100 (aldehyde...
oxidation level) (Figure 6-9). The demonstration that the ABC ring system of strigolactones can be made in a single step from suitable precursors would provide new insights into strigolactones biosynthesis, obviate the need to involve a large number of unidentified enzymes producing strigolactones, and suggest substrates that could be tested with MAX1.

![Chemical structures](image)

Figure 6-9. Proposed formation of the strigolactone ABC core in one step by an acid-catalyzed double cyclization.

During this research, we also decided to use our cascade cyclization methodology to synthesize natural strigolactones and make solanacol. Solanacol was isolated in 2007 and it is the first natural strigolactone containing an aromatic ring. Solanacol has been initially proposed to have methyl substituents in the para relationship (104) (Figure 6-10), but that structure has been disproved in 2009 by Takikawa *et al.* who suggested that the correct structure of solanacol was 105.
When work on solanacol synthesis was in progress in our lab, Chen et al. demonstrated that 106 is the correct structure for that compound.

6.3 Results and Discussion

6.3.1 First Cyclization Attempts (Aliphatic Substrate)

We set out to study our proposed acid catalyzed double cyclization with a simplified aliphatic-type substrate 107 (Figure 6-11).

We imagined that the necessary substrate 107 could be prepared by a Wittig reaction between the aldehyde 109 and an ylide generated from phosphonium salt 110, followed by deprotection of the TBS group from the Wittig product (Figure 6-12).
It is known that preparation of $\beta,\gamma$-unsaturated acids by this type of Wittig reaction may be problematic, because of possible deprotonation of an $\alpha$-hydrogen from the phosphonium salt, resulting in elimination of triphenylphosphine instead of desired deprotonation of a $\beta$-hydrogen and formation of the ylide. However, Brandsma and co-workers have reported that by using lithium 2,2,6,6-tetramethylpiperidide (LiTMP), they were able to prevent elimination of triphenylphosphine, and they obtained a Wittig product in 70% yield from an enantiomer of phosphonium salt 110 and benzaldehyde reacted in a 1:1 ratio (only E-isomer was formed). It was thus thought that we should be able to make the substrate needed for cyclization studies (107) by our devised route.

Aldehyde 109 was prepared from 3,4,5,6-tetrahydrophthalic anhydride (111) in a low 8% overall yield (Figure 6-13).

![Figure 6-13. Synthesis of the aldehyde 109.](image)

3,4,5,6-tetrahydrophthalic anhydride (111) was reduced with lithium aluminium hydride (LAH) to give the diol 112 in 32% yield after column chromatography. The crude product was quite impure, but based on its mass and the NMR data, the estimated yield would be above 75%. It is thus thought that the product degrades on a silica gel chromatography column. Baldwin et al. reported that the diol 112 can be transformed into an aldehyde 114 under acidic conditions, most probably by a mechanism shown in figure 6-14. It is possible that this reactivity of the diol may be the
cause of its instability on an acidic silica gel column, though no aldehyde 114 was observed.

![Chemical Structure](image)

Figure 6-14. Reported reactivity of the diol 112 under acidic conditions.  

Butina and Sondheimer also reported that the diol 112 was difficult to purify and they obtained it by reduction of dimethyl 1-cyclohexene-1,2-dicarboxylate in 18 % yield after transforming it into a diformate for easier purification and hydrolyzing it back to the diol with methanol and ammonia.

The diol 112 was monoprotected in 32 % yield with one equivalent of tert-butyl dimethylsilyl chloride and 1.5 molar excess of imidazole in dichloromethane. We tried to increase the yield by using a procedure of McDougal et al. for monosilylation of symmetric 1,n-diols, which employs one equivalent of sodium hydride, but the obtained yield was even lower (19 %).

Finally, the alcohol 113 was oxidized to the aldehyde 109 by Swern oxidation and also with activated manganese dioxide. Both oxidation procedures gave the product of satisfactory purity after the work-up (see NMR spectra, appendix D) in 74 % yield. Further purification by chromatography column resulted in a significant yield decrease (to 43 %), and the $^1$HNMR spectrum of the compound indicated that it was not significantly purer than the crude. The aldehyde 109 was rather unstable and gradually decomposed (after 3 weeks of storage under argon in a −20 °C freezer, it turned into a tar, and the $^1$HNMR indicated that it largely degraded). Thus, the aldehyde 109 was prepared fresh, a day before running each Wittig reaction.
Phosphonium salt 110 was prepared following a literature procedure for the synthesis of its enantiomer\(^{297}\) (Figure 6-15).

Figure 6-15. Synthesis of the phosphonium salt 110.

R- (+)-methyl 2-methyl 3-hydroxy-propanoate 115 (the S enantiomer was a bit more expensive than the R enantiomer, and the stereogenic center did not matter to us at that point), was transformed into its tosylate 116 with p-toluenesulfonyl chloride (TsCl) in pyridine in 98 % yield. It was subsequently converted in 86 % yield into the iodide 117, by treatment with lithium iodide in tetrahydrofuran. The cleavage of the methyl group was a bit problematic and the carboxylic acid 118 was obtained in 50 % yield, using chlorotrimethylsilane and sodium iodide in acetonitrile (during this reaction iodonitromethylsilane is generated \textit{in situ}).\(^{301,302}\) Finally, the phosphonium salt 110 was made in 80 % yield, by treating the iodide carboxylic acid 118 with an 8-fold excess of triphenylphosphine in acetonitrile.

The Wittig reaction employing phosphonium salt 110 turned out to be difficult due to preferential triphenylphosphine elimination instead of planned ylide formation, despite the use of lithium tetramethylpiperidide (Figure 6-16).
Figure 6-16. Deprotonation at positions $\beta$ and $\alpha$ of the carboxylate group of 110.

Li-TMP ($pK_a \approx 37$) is the most basic and least nucleophilic of the amide bases. It is reported to be kinetically faster than LDA, allowing some deprotonations that are not possible with LDA.\textsuperscript{303,304,305}

We attempted to repeat Brandsma’s reaction between benzaldehyde and the ylide generated from phosphonium salt 110 with Li-TMP,\textsuperscript{297} but after several tries the alkene product was obtained only in an estimated 30% yield, and it was difficult to purify. To circumvent triphenylphosphine elimination problem, an excess of phosphonium salt can be used (for example 10 fold molar excess).\textsuperscript{295} A test reaction between 1-cyclohexene-1-carbaldehyde and two equivalents of phosphonium salt 110 gave the Wittig product in an estimated 25% yield that was still quite impure after purification by extractions and column chromatography. The reaction between the aldehyde 109 and an ylide made from phosphonium salt 110 (4 equivalents) gave the desired olefin 107 in an estimated 10% yield (the TBS group was removed during the acidic work-up) (Figure 6-17). Again, the product was not obtained pure because of a difficulty to separate the desired carboxylic acid from other multiple impurities (especially methacrylic acid byproduct).
Figure 6-17. Wittig reaction and attempts to cyclize the olefin product 107.

In all the Wittig reactions with phosphonium salt 110 only the E-isomer was formed, the Z isomer has never been detected. It is well known that non-stabilized triphenylphosphine ylides generally react with aldehydes to afford mainly Z alkenes, while stabilized ylides give E-alkenes.\textsuperscript{306} The unusual E-stereoselective behavior of γ-oxido and γ-carboxy ylides have been reported previously.\textsuperscript{307,308,297}

During the Wittig reaction studies, we also tried to use lithium hexamethyldisilazide (Li-HMDS) to generate the ylide, because of its commercial availability as 1M solution in THF, but it resulted in even faster triphenylphosphine elimination, and no Wittig product was ever obtained with that base. We also used methyl ester phosphonium salt instead of carboxylic acid phosphonium salt 110, because it could be directly prepared with triphenylphosphine from methyl ester iodide 117, omitting the troublesome methyl cleavage. However, the methyl ester salt was even more susceptible to triphenylphosphine elimination than the carboxylic acid salt 110.

The cyclization of carboxylic acid alcohol 107 has been attempted using hydrochloric acid, sulfuric acid, PPTS (pyridinium p-toluenesulfonate), and p-toluenesulfonic acid at room temperature but no desired product has been observed and the unreacted substrate was mostly recovered (Figure 6-17).
As the problems with preparing the cyclization precursor 107 piled up (cost of the starting materials, low yields in synthesis of aldehyde 109 and its instability, problems with methyl cleavage from 117, preferential triphenylphosphine elimination instead of formation of an ylide and the need to use the phosphonium salt in excess, low yields in Wittig reaction and difficulty to purify the desired product), a need of a different model substrate to test our proposed acid catalyzed cyclization became evident.

6.3.2 Attempts to Cyclize Aromatic Substrates at the Alcohol Oxidation Level

6.3.2.1 Rationale

To avoid the problems encountered in the preparation of the aliphatic precursor 107 for cyclization studies, a synthesis of an aromatic substrate that could be prepared relatively easily in larger amounts and at reasonable cost was next envisaged. Compound 119 contains all the necessary elements to study the double cyclization without any bulk that would complicate its synthesis or increase the cost of the starting reagents. In addition, the double cyclization of 119 would result in a formation of 120 (Figure 6-18), which is the ABC core of GR24 (Figure 6-2), whose synthesis had been reported in the literature, and thus the cyclization reaction could be monitored using an authentic standard of the product. Since one of the natural strigolactones (solanacol) is aromatic, this reaction could be still considered as valid for biosynthesis of strigolactones.

![Chemical Reaction](image)

Figure 6-18. Aromatic substrate at the alcohol oxidation level - cyclization plan.
The substrate 119 could be prepared by a Wittig reaction between a known aldehyde 121 and a known orthoester phosphonium salt 122 that were used for other reactions in literature, and were stable (Figure 6-19). The orthoester OBO (2,6,7-trioxabicyclo[2.2.2]octane) protecting group was developed by Corey and Raju, it is generally resistant to attack by bases and strong nucleophiles and can be removed by mild acid hydrolysis to give an ester, which can be treated with base to release a carboxylic acid. The protection of the carboxylic acid group as an orthoester would ensure no problems with triphenylphosphine elimination.

![Chemical structure](attachment:image.png)

Figure 6-19. Retrosynthetic analysis of 119.

6.3.2.2 GR24 Synthesis

GR24 (127, 128), a potent strigolactone analog used for bioassays, was made following a Mangnus et al. literature procedure (Figure 6-20) to supply some material for studies in plants (GR24 is expensive, currently priced at €850 for 20 mg), and to prepare lactone 120 needed as a standard for TLC and NMR for cyclization reactions (synthesis of 120 has been published but not its NMR data).
The synthesis of tricyclic lactone 120 started from 1-indanone (123). Ethoxycarbonyl group was introduced with 4-fold excess of diethyl carbonate and sodium hydride (60 % dispersion in mineral oil) to activate the position for alkylation and protect it against dialkylation. The subsequent alkylation with ethyl bromoacetate gave compound 124 in 93 % yield. Acid catalyzed hydrolysis and concomitant decarboxylation yielded the carboxylic acid 125 in 70 % yield. Reduction of the keto acid 125 with sodium borohydride, followed by treatment with catalytic p-toluenesulfonic acid to complete lactonization, afforded the tricyclic lactone in 66 % yield. Formylation was performed with potassium tert-butoxide and ethyl formate. The brominated furanone
was prepared from 3-methyl-2(5H)-furanone (129) with N-bromosuccinimide (NBS), and azobisisobutyronitrile (AIBN) in carbon tetrachloride. Benzoyl peroxide was used in the literature to perform this bromination, but in my experience benzoyl peroxide (75 %, remainder water, Acros Organics, 21178) did not initiate the reaction, and use of AIBN was necessary.

The treatment of potassium enolate 126 with bromofuranone 130 gave GR24 in 75 % yield as a 1:1 mixture of diastereoisomers 127 and 128 that were separated by column chromatography on silica gel. The preference for E-geometry at the enol ether double bond has been reported in the literature (no Z isomer is formed). MacAlpine et al. showed that the resonance for the exocyclic vinyl proton of E-isomer of strigol appears at 7.42 ppm (7.48 ppm for GR24) as a doublet with a coupling constant $^4J = 2.6$ Hz, while the Z-isomer isolated from a mixture obtained by treatment of E-isomer with u.v. light has that proton resonating 0.6 ppm upfield because it is no longer in the deshielding zone of lactone carbonyl group.

6.3.2.3 Synthesis of Aromatic Substrates at Alcohol Oxidation Level and Attempts to Cyclize Them

To make the precursor to test our cyclization, aldehyde 121 and phosphonium salt 122 were needed. The known aromatic aldehyde 26 was synthesized analogously to the aliphatic aldehyde 109 described in the previous section. In contrast to its aliphatic analogs, aromatic intermediates were stable, and all reactions had better yields (Figure 6-21). Phthalic anhydride (131) was reduced with lithium aluminium hydride to give the diol 132. The monoprotection could be achieved using McDougal et al. procedure with sodium hydride and tert-butyldimethylsilyl chloride. The diol and
TBDMSCI were used in a 1:1 ratio to give the alcohol 133 in 90 % yield. Oxidation with activated manganese dioxide afforded the aldehyde 121 in 94 % yield.

Figure 6-21. Synthesis of the aldehyde 121.

The phosphonium salt 122 was prepared from 3-methyl-3-oxetenemethanol (134) and 3-bromopropionyl chloride (135) following a literature procedure (Figure 6-22).

Figure 6-22. Synthesis of the phosphonium salt 122.

The coupling of the aldehyde 121 and an ylide generated from phosphonium salt 122 with Li-HMDS by Wittig reaction gave the olefin product 138 in 93 % yield as mixture of E and Z isomers in a 1:1 ratio (Figure 6-23).
Figure 6-23. Synthesis of linear precursors and attempts to cyclize them.

The alcohol acid 119 could then be produced from 138 by treatment with methanolic sulfuric acid,\textsuperscript{314} followed by aqueous sodium hydroxide to yield the desired product in 82 % yield and a byproduct acid 142 in 12 % yield. The optimum reaction time for the hydrolysis with sodium hydroxide was found to be 20 min at 0 °C, followed by 15 min at room temperature, as at that time most of the substrate was consumed and little byproduct resulting from isomerization to 141 and Michael addition was formed. When the reaction time was increased, mainly the ether carboxylic acid 142 was obtained (Figure 6-24), and when it was run for shorter periods of time, the yield was much lower (below 50 %) and the byproduct 142 was already present.

Figure 6-24. Byproduct 142 formed during prolonged a reaction with sodium hydroxide in water.
The benzylic alcohol 119 was treated with a variety of acids, such as trifluoroacetic acid, polyphosphoric acid, triflic acid, and methanesulfonic acid with phosphorus pentoxide, over a range of temperatures, and also with triphenylphosphine and diisopropyl azodicarboxylate (DIAD) (Mitsunobu conditions), but no cyclization product (120) was ever formed. In these experiments, the starting material was recovered, decomposition was observed, or the ester was formed between the benzylic alcohol and the acid used to induce cyclization (Figure 6-25). Interestingly, the GC-MS-EI analysis of trifluoroacetate ester 143 gave a spectrum that was identical with the spectrum of the standard cyclization product 120, suggesting that the compound can undergo the double cyclization in the GC-MS instrument.

![Figure 6-25. Formation of a cyclic product from the triflic ester 48 in a GC-MS instrument.](image)

Since none of the chemical reactions to cyclize benzylic alcohol carboxylic acid 119 was successful, it was thought that the acidic conditions to form a carbocation were too harsh, or not compatible with other groups in the molecule, and a cyclization of the chloride 140 with silver triflate to induce elimination of chloride was next explored.

Compound 140 was made from 138 by using hydrochloric acid solution in allyl alcohol, followed by conversion of the alcohol function in 139 to a chloride 140 (Figure 6-23). The formation of alcohol 139 needed to be closely monitored because initial attempts to prepare it using H₂SO₄ of HCl in allyl alcohol at reflux, yielded only diallylated compound 144 instead of the desired product. Finally, the alcohol 139 was
prepared in 79 % yield, using 0.25 HCl in allyl alcohol heated at 50 °C for 2.5 h. Chloride 140 and diallylated compound 144 were also formed, but in small amounts and they could be removed by column chromatography (Figure 6-26). Formation of the chloride 140 in this reaction indicates that a benzylic carbocation is formed but the cyclization does not proceed.

Figure 6-26. Compounds formed in the reaction of the orthoester 138 with an HCl solution of allyl alcohol.

Treatment of alcohol 139 with thionyl chloride in pyridine yielded the chloride 140 in 51 % yield. That result could be improved by use of triphenylphosphine and carbon tetrachloride in dichloromethane (68 % yield), and eventually the best result was obtained with methanesulfonyl chloride, triethylamine and lithium chloride in THF (92 % yield) (Figure 6-23).

The chloride 140 was treated with silver triflate and 2,6-lutidine at different temperatures, but formation of the cyclized product was never observed. Since Pd(0) salts are known to ionize allylic carboxylates,\textsuperscript{315,316} it was thought that the use of Pd(0) salt would help to effect the cyclization by facilitating the loss of the allyl group, so the chloride was treated with 5 mol % Pd(PPh\textsubscript{3})\textsubscript{4}, 1.1 equivalents of phthalimide, 2.2 equivalents of Na\textsubscript{2}CO\textsubscript{3}, and 1.05 equivalents of silver triflate. Unfortunately these cyclization attempts also were unsuccessful.
6.3.3 Cyclization of Aromatic Substrates at the Aldehyde Oxidation Level

Since many strigolactones are oxygenated at C4, acid catalyzed cyclization of the aromatic aldehyde 145 was next studied (Figure 6-27). The cyclization of an aldehyde 145 to form the B ring of strigolactones would be a 5-exo-trig cyclization, which is allowed by Baldwin rules, while cyclization of precursors at the alcohol oxidation level was a 5-endo-trig, disfavored by Baldwin rules.\textsuperscript{317,318} Hence, we were hoping that cyclization of the aldehyde precursors would be more feasible under chemical conditions than that of the alcohol 119 or chloride 140.

![Figure 6-27. Cyclization of an aromatic substrate at the aldehyde oxidation level plan.](image)

The cyclization of the first cycle has a precedent in the literature.\textsuperscript{319} Magnus and Mansley studied the cyclization of an aromatic aldehyde 147, using different acids, and were able to obtain cyclized products with SnCl\textsubscript{4}, Sc(OTf)\textsubscript{3}, TfOH and TMSOTf (yields up to 64 \%). They recovered unreacted substrate when Et\textsubscript{2}AlCl, Yb(OTf)\textsubscript{3} or ZnCl\textsubscript{2} were used (Figure 6-28).

![Figure 6-28. Precedent for the first cyclization.](image)

The aldehyde methyl ester cyclization precursors were prepared from **138** by treatment with methanolic sulfuric acid, separation of E and Z isomers by flash column chromatography, and oxidation of **153** and **154** independently with pyridinium chlorochromate to give **153** and **154**, respectively (Figure 6-29).

![Reaction Scheme]

Figure 6-29. Preparation of the linear aldehydes for cyclization.

The double cyclization of **153** and **154** was tested under chemical conditions using both Lewis (trimethylsilyl triflate, TMSOTf) and Brønsted (triflic acid, TfOH) acids in dichloromethane (DCM). The reaction was monitored by TLC and stopped promptly when the substrate was consumed or when TLC analysis indicated no further substrate consumption for prolonged times.

When E-olefin **154** was subjected to catalytic TMSOTf (0.2 equivalent), the cyclized products **155** and **156** were obtained in an 80:20 diastereomeric ratio in 60% combined yield, along with 7% of **157**, resulting from a single cyclization (Table 6-1, entry 3). Interestingly, **155** and **156** were isolated as C4 methyl ethers due to the methyl transfer from methyl ester. The best result in regard to stereo selectivity and isolated yield was obtained with catalytic amounts of a protic acid. Thus, when 0.1 equivalent of triflic acid (TfOH) in dichloromethane was used, **155** and **156** were formed in a 68%
combined yield in a 99:1 diastereomeric ratio along with 2% of 157 (entry 6). When TfOH was used in smaller catalytic amounts (0.05 equivalent) the product 155 and 156 was isolated only in 24 % yield (99:1 diastereomeric ratio), and the unreacted E olefin was recovered in 42 % yield despite prolonged reaction times. When stoichiometric amounts of TMSOTf and TfOH were employed to catalyze the cyclization of 154, the yields were lower (15 %, 33 % depending on temperature, and 12 %), most probably due to decomposition of the substrate and formation of other multiple byproducts using those conditions. Surprisingly, the stereo selectivity was reversed (the mixture of 155:156 in two of those reactions was obtained in a 40:60 ratio).

Table 6-1. Cyclization of the E-aldehyde precursor. The yields given are isolated yields. The ratio of diastereoisomers was determined by $^1$HNMR.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield 155+156 (Ratio 155:156)</th>
<th>Yield 157</th>
<th>Yield unreacted substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TMSOTf (1.0 eq.), 0 °C, 10 min; r.t., 3 h</td>
<td>15 % (40:60)</td>
<td>0 %</td>
<td>0 %</td>
</tr>
<tr>
<td>2</td>
<td>TMSOTf (1.0 eq.), 0 °C, 2 h</td>
<td>33 % (60:40)</td>
<td>5 %</td>
<td>0 %</td>
</tr>
<tr>
<td>3</td>
<td>TMSOTf (0.2 eq.), 0 °C, 2 h</td>
<td>60 % (80:20)</td>
<td>7 %</td>
<td>0 %</td>
</tr>
<tr>
<td>4</td>
<td>TfOH (1.5 eq.), −78 °C, 1h; −78 °C to 0 °C, 1h; 0 °C, 3 h</td>
<td>12 % (40:60)</td>
<td>0 %</td>
<td>0 %</td>
</tr>
<tr>
<td>5</td>
<td>TfOH (0.2 eq.), −78 °C, 1h; −78 °C to 0 °C, 1h; 0 °C, 3 h</td>
<td>60 % 99:1</td>
<td>1 %</td>
<td>1 %</td>
</tr>
<tr>
<td>6</td>
<td>TfOH (0.1 eq.), −78 °C, 1h; −78 °C to 0 °C, 1h; 0 °C, 3 h</td>
<td>68 % (99:1)</td>
<td>2 %</td>
<td>1 %</td>
</tr>
<tr>
<td>7</td>
<td>TfOH (0.05 eq.), −78 °C, 1h; −78 °C to 0 °C, 1h; 0 °C, 6 h; 8 °C, 14 h</td>
<td>24 % (99:1)</td>
<td>0 %</td>
<td>42 %</td>
</tr>
</tbody>
</table>
The relative stereochemistry of the B-ring methoxy group and the C-ring was assigned based on the \(^1\)HNMR data for a signal of the proton on C4 carbon and Karplus relationship, as well as by a comparison to proton assignments in the literature for a similarly oxygenated strigolactone ABC core, whose relative stereochemistry of the B-ring OH and the C-ring was confirmed unambiguously by X-ray.\(^{320}\)

In the 1960s, Karplus deduced that the \(^3\)J coupling constant is largest when the dihedral angle between H-C-C-H is 180° as the orbitals of the two C-H bonds are in the same plane and perfectly parallel. Coupling is nearly as large at 0° when the orbitals are in the same plane but not parallel. The coupling is close to 0 Hz when the dihedral angle is 90° as the orthogonal orbitals do not interact (Figure 6-30).\(^{321}\)

![Figure 6-30. Graph of the Karplus relationship for ethane derivatives: \(^3\)J\(_{HH}\) versus dihedral angle \(\Phi\) (Reproduced from http://www2.chemistry.msu.edu/faculty/reusch/virtxtjml/spectrpy/nmr/nmr2.htm, with permission from Dr. William Reusch, MSU).](http://www2.chemistry.msu.edu/faculty/reusch/virtxtjml/spectrpy/nmr/nmr2.htm)

The structures of lactone methyl ethers 155 and 156 were optimized in Chem 3D Pro 2012 using MM2 (minimize energy, minimum RMS gradient 0.001), and the dihedral angles for the H4 and H3a were measured to be 96.0° for the lactone with trans relationship as shown in 155, and \(-33.7°\) for the lactone with cis relationship as shown
in 156. The signal for H4 proton in 155 was a singlet at 4.72 ppm (no coupling with H3a), while the signal for H4 proton in 156 was a doublet at 4.82 ppm, with $^3J_{H4-H3a} = 6.9$ Hz) (Figure 6-31). This assignment is also in agreement with the $^1$HNMR data for the ABC core of orobanchol (H4 at 4.45 ppm as a singlet for a compound with B-ring OH and C-ring in a trans relationship and H4 at 4.54 ppm as a doublet of doublets and $^3J_{H4-OH} = 7.0$ Hz and $^3J_{H4-H3a} = 6.9$ Hz for a compound with the B-ring OH and the C-ring in a cis relationship). \(^{320}\)

Figure 6-31. $^1$HNMR with proton assignments for 155 (trans) – top spectrum and 156 (cis) – bottom spectrum.
It was thought initially that the high ratio favoring the B-ring methoxy group *trans* to the C-ring might result from equilibration of the product to the most stable diastereomer under reaction conditions. As a control experiment, diastereomers 155 and 156 in 40:60 ratio were treated with either TMSOTf or TfOH to determine if epimerization of the C4 stereocenter was possible. In those reactions, compounds 155 and 156 were recovered in high yields with no detectable interconversion, indicating that there is no equilibration of the product.

The cyclization of the Z-olefin 153 appeared to be much slower than that of the E-olefin 154 requiring 1 equivalent of catalyst to attain a reasonable rate. When 1 equivalent of trimethylsilyl triflate was used, 153 gave exclusively the cis-diastereomer 156 in 46 % yield, along with 2 % of 157 (Table 6-2, entry 1). When catalytic TMSOTf was used, the reaction was slow, even at room temperature, but a mixture of 155 and 156 in a 30:70 ratio was obtained in a higher yield (68 %) (Table 6-2, entry 4). With 1 equivalent of triflic acid, a mixture of 155 and 156 was obtained in a 48 % yield, again favoring 156 in a 13:87 diastereomeric ratio. The use of catalytic triflic acid (0.2 eq.) was not efficient, and it resulted in a further loss of stereoselectivity (43:57), and lower yield of 155 and 156 (20 %) due to incomplete conversion even after several days (24 % of unreacted Z olefin was recovered).
Table 6-2. Cyclization of the Z-aldehyde precursor. The yields given are isolated yields. The ratio of diastereoisomers was determined by $^1$HNMR.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Yield 155+156 (Ratio 155:156)</th>
<th>Yield 157</th>
<th>Yield unreacted substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TMSOTf (1 eq.), 0 °C, 10 min; rt., 6.5 h,</td>
<td>46 % (0:100)</td>
<td>2 %</td>
<td>9 %</td>
</tr>
<tr>
<td>2</td>
<td>TMSOTf (1.1 eq.), 0 °C, 7 h; 8 °C, 15 h</td>
<td>53 % (10:90)</td>
<td>3 %</td>
<td>0 %</td>
</tr>
<tr>
<td>3</td>
<td>TMSOTf (0.5 eq.), 0 °C, 16 h; rt., 3 h</td>
<td>55 % (29:71)</td>
<td>1 %</td>
<td>0 %</td>
</tr>
<tr>
<td>4</td>
<td>TMSOTf (0.2 eq.), 0 °C, 3 h; rt. 22 h</td>
<td>68 % (30:70)</td>
<td>3 %</td>
<td>1 %</td>
</tr>
<tr>
<td>5</td>
<td>TfOH (1.1 eq.), −78 °C, 1h; 0 °C, 2.5 h</td>
<td>48 % (13:87)</td>
<td>0 %</td>
<td>0 %</td>
</tr>
<tr>
<td>6</td>
<td>TfOH (0.2 eq.), −78 °C, 1h; 0 °C, 4 h; 8 °C, 96 h</td>
<td>20 % (43:57)</td>
<td>0 %</td>
<td>24 %</td>
</tr>
</tbody>
</table>

A probable reaction mechanism is shown in Figure 6-32. In this model, oxocarbenium ion 158 is formed by protonation of the aldehyde oxygen. At this stage, the substrate may form both the B- and C-rings in a single concerted step (158→160), but a stepwise mechanism is necessary to explain the formation of 157. Cyclization of the olefin in 154 by addition to the C4 oxocarbenium ion produces the benzylic cation 159 which could further cyclize forming the C-ring (path a) or eliminate to form 161 (path b). Demethylation of 160 may occur by alkylation of a variety of nucleophilic species present in solution such as alcohols 161 or 162. Under these catalytic conditions, the methylated tricyclic strigolactone core 155 is thus obtained and the acid catalyst regenerated.
DFT calculations employing the B3LYP functional\textsuperscript{322,323} (as implemented in the Gaussian03 software package)\textsuperscript{324} were employed to investigate the origin of the stereoselectivity observed in the acid-catalyzed cascade cyclization. These studies assumed that formation of the B and C rings proceeded in a stepwise fashion although we have found that cyclization can be concerted based on the calculated barrier to lactone formation. Standard methods\textsuperscript{325,326} were used to locate transition states (TS) associated with attack of the double bond on the protonated aldehyde moiety, which define the relative stereochemistry of the product if the reaction is under kinetic control. Four transition states were located (Figure 6-31) corresponding to those for cyclization of either the \textit{trans}-olefin 154 or the \textit{cis}-olefin 153 to each of the two possible diastereoisomeric products 155 and 156. The relative energies of these transition states provide a qualitative explanation of the observed cyclization stereoselectivity. Thus, the origin of the reaction selectivity appears to arise from the degree of deviation of the...
protonated aldehyde moiety from co-planarity with the aromatic ring. In the energetically favored transition states (TS$_{\text{trans-olefin to trans-lactone}}$ and TS$_{\text{cis-olefin to cis-lactone}}$) small deviations are observed (~35°). On the other hand, in order to reduce steric hindrance between the protonated aldehyde oxygen and the attacking olefin, the formation of the cis lactone from the trans-olefin and of the trans lactone from the cis-olefin requires a higher deviation from co-planarity in the TS (~80°) with the aldehyde being almost perpendicular to the aromatic ring.

Figure 6-33. Calculated transition states and relative energies for cyclization of the B-ring from trans-olefin 154 and cis-olefin 153 initiated by protonation.

In order to estimate the energetic contribution of this deviation to the increased activation energy barrier, we calculated the rotational barrier of protonated
benzaldehyde using identical methods to those used in locating the cyclization transition states (Figure 6-34). In this case, the barrier to rotation was found to be quite high (24.2 and 20.8 kcal/mol in gas phase and in dichloromethane, respectively), which is consistent with the differences in the energies obtained for the cyclization transition state.

![Calculated rotational barriers for protonated benzaldehyde.](Image)

Figure 6-34. Calculated rotational barriers for protonated benzaldehyde.

The fact that the alcohol 119 and the chloride 140 did not form cyclized products, while aldehydes 153 and 154 easily underwent the double cyclization, might be explained by lack of stabilization of the benzylic carbocation generated from 119 and 140 (the p-orbital system of the aromatic ring and the p-orbital of the carbocation in the transition state are not in the same plane), while the oxygen long electron pair in 158 can stabilize the transition state.

Based on this result, we suggested that a linear aldehyde, such as 98 (Figure 6-35) might be the biosynthetic precursor of the ABC-ring system in strigolactones. The C14 aldehyde 163 could be derived from the C18 ketone, which has previously been proposed as a biosynthetic intermediate based on experimental evidence. After
double cyclization, the product 99 would already contain an oxygenation at C4, and subsequent oxidation of the C6’-methyl group, followed by attachment of D ring, could lead to orobanchol. The cyclized product 99 or orobanchol could thus be precursors for other strigolactones after further biosynthetic transformation.

![Proposed biosynthetic pathway.](image)

Figure 6-35. Proposed biosynthetic pathway.

To synthesize a tricyclic lactone with a free hydroxyl group, we subjected aldehyde methyl esters 153 and 154 to trimethylsilyl iodide, but that resulted in a very complex mixture in which there was no double cyclization product. We also tried to obtain the aldehyde free carboxylic acid 164 by methyl cleavage from 153 and 154, but these attempts were unsuccessful as well. Treatment of methyl ester 153 with sodium hydroxide resulted in aldol-type condensation and aromatization to form a naphthalene derivative, while treatment with TMSCl and NaI in acetonitrile at reflux resulted in a complex mixture in which there was no desired product. Also disappointingly, the synthesis of aldehyde free carboxylic acid by oxidation of alcohol carboxylic acid 119 using PCC yielded the desired product in low yields, partially due to poor solubility of carboxylic acid 119 in many organic solvents and the product could not be isolated in a pure form (Figure 6-36).
Figure 6-36. Synthesis of the aldehyde free carboxylic acid 164.

Hence, we decided to prepare aldehyde benzyl ester 166 and aldehyde allyl ester 170, and cleave the benzyl or allyl group after the double cyclization, as many of removal methods for benzyl and allyl group exist.\(^{327,328}\) The alcohol benzyl ester 165 was obtained by transesterification of the methyl ester 152 in benzyl alcohol in 93 % yield. Oxidation with PCC gave the aldehyde 166 in 87 % yield (Figure 6-37).

Figure 6-37. Synthesis of the aldehyde benzyl ester 166.

The trans olefin aldehyde benzyl ester 166 (containing 4 % of Z isomer impurity), was subjected to the best double cyclization conditions determined for the trans olefin aldehyde methyl ester 153, i.e., 0.1 equivalent of triflic acid. Similarly to the trans olefin aldehyde methyl ester 153, cyclization of the trans olefin aldehyde benzyl ester 166 gave the lactones 167 and 168 in 66 % combined yield, favoring the benzyloxy group and the C-ring trans to each other 95:5. The product resulting from single a cyclization (169) was also isolated in 3 % yield (Figure 6-38).
Figure 6-38. Acid catalyzed double cyclization of the trans olefin benzyl ester aldehyde 166.

The benzyloxy lactone 167 was next treated with hydrogen gas over 10 % palladium on carbon to remove the benzyl group. The $^1$HNMR spectrum of the crude product was very messy and it indicated that there was no unreacted substrate and no desired free alcohol lactone, most probably because there are 3 benzylic positions in the lactone 167 that could be cleaved.

The allyl ester aldehyde 170 was made by oxidation of the alcohol 139 with PCC in 86 % yield. The acid catalyzed double cyclization gave the allyloxy lactone as a mixture of 171 and 172 in various yields and ratios depending on the E:Z ratio of the starting olefin 170 used (Figure 6-39). The stereoselectivity in this reaction seemed to follow the same trend as in cyclization of methyl and benzyl esters, but it was not investigated because the olefin 170 was a mixture of E and Z isomers.

Figure 6-39. Acid catalyzed double cyclization of the allyl ester aldehyde 170.

The cleavage of the allyl group from 171 and 172 was first tried with three equivalents of sodium borohydride, 4 mol% dichloro[propane-1,3-diylbis(diphenyl]
phosphane) nickel(II) ([NiCl₂(dppp)] in THF/EtOH 4:1. The cleaved products 173 and 174 were obtained in an estimated 33% combined yield and they were quite impure. A much better result was obtained by palladium-catalyzed tributyltin hydride reduction \textsuperscript{330,331}: the desired product was obtained in 76% combined yield (Figure 6-40).

![Figure 6-40. Cleavage of the allyl group.](image)

**6.3.4 Synthesis of Proposed Structure for Solanacol (105) Using Acid-catalyzed Double Cyclization as a Key Step to Prepare the Compound’s ABC-Core.**

After demonstrating that the acid-catalyzed double cyclization of the linear aldehyde in a model system proceeds with good yields and stereocontrol, we set out to show that our cyclization can be used as a key step to synthesize natural strigolactones, and prepare a proposed structure for solanacol (105) (Figure 6-41). That proposed structure had the OH B-ring and C-ring trans to each other, as the \textsuperscript{1}HNMR data for natural solanacol indicated, and unknown stereochemistry at C-2’, so four compounds would need to be made to completely prove the structure by comparison to the data for solanacol isolated from plants.\textsuperscript{293} The last steps in the synthesis (formylation and attachment of D-ring) could be done by well preceded methods, analogously to those in the synthesis of GR24 (Figure 6-20). The ABC core of 105 (175) could be prepared by our acid-catalyzed cyclization from an acid aldehyde 176. In case of problems with that cyclization or the preparation of the free carboxylic acid, an allyl ester 177 could be
used, and the allyl group cleaved as shown in our work earlier. The lactone 175 could be separated into its enantiomers by chiral column or kinetic resolution, using one of the literature methods. The aldehyde acid 176 could be made from its corresponding alkyne 178 by hydrosilylation-protodesilylation developed by B. M. Trost and Z. T. Ball. The catalytic hydrosilylation of internal alkynes using a cationic ruthenium complex [Cp*Ru(MeCN)\textsubscript{3}]PF\textsubscript{6} and a silane (for example (EtO)\textsubscript{3}SiH), followed by protodesilylation with catalytic cuprous iodide and TBAF is a protocol for chemoselective reduction of alkynes to (E)-alkenes that is compatible with many sensitive functional groups, for example: ketones, acetals, primary alkyl chlorides, secondary hydroxyl groups that are both benzylic and allylic.

Figure 6-41. Retrosynthesis plan for the synthesis of proposed structure for solanacol (105), using acid-catalyzed double cyclization as a key step.

The alkyne 178 could be prepared by Sonogashira coupling from a triflate 180 and butynoic acid 179, analogously to another literature Sonogashira coupling with butynoic acid, and the triflate 180 could be prepared from readily available and cheap 2,3-dimethylphenol (181) ($26 for 100 g).
Therefore, 2,3-dimethylphenol (181) was transformed into a known aldehyde 182
(Figure 6-42). The published procedure to make 182,\(^{335}\) consists of four steps: conversion of phenol 181 to an allyl ether, Claisen rearrangement, isomerization of the thus formed terminal double bond in aryl allyl phenol, and cleavage with osmium tetroxide and sodium metaperiodate. The authors reported that attempts to prepare the desired aldehyde 182 by Reimer-Tiemann (CHCl\(_3\) and NaOH) and Vilsmeier-Haack (POCl\(_3\), DMF) reactions, as well as formylation with SnCl\(_4\) and paraformaldehyde,\(^{336}\) were not successful. We found that the aldehyde 182 could be easily prepared in one step using the Hofsløkken and Skattebøl monoformylation method.\(^{337}\) That formylation is promoted by electron-donating substituents and proceeds selectively ortho to the hydroxyl group. Thus, 182 was obtained in 74 % yield by refluxing 2,3-dimethylphenol, paraformaldehyde, magnesium chloride, and triethylamine in acetonitrile for 45 min. The aldehyde 182 was converted into a triflate 180 with triflic anhydride and triethylamine.

Figure 6-42. Attempt to synthesize the alkyne aldehyde carboxylic acid 184.

Unfortunately, the coupling between the triflate 180 and 3-butynoic acid (179)\(^{338,339}\) did not work. Gagnon et al. reported that a Sonogashira coupling with 3-butynoic acid (179) proceeded smoothly and in good yield,\(^{334}\) but in our system we did
not observe any coupling product neither under Gagnon coupling conditions (Pd(PPh$_3$)$_4$, CuI, Et$_2$NH in THF) nor with a different catalyst (Pd(PPh)$_3$Cl$_2$) or different base (Et$_3$N), and the starting 3-butynoic acid (179) degraded completely under reaction conditions. The same result was obtained with 3-butynoic acid methyl ester (183). This failure could be explained by the fact that homopropargylic acids and esters rearrange easily to allenes, and can then undergo further undesired reactions or degrade by action of heat.

Since the synthesis of 178 by Sonogashira coupling between the triflate 180 and 3-butynoic acid (179) were unsuccessful, we decided to couple the triflate with 3-butynoic-1-ol (185) to form compound 186 and oxidize the alcohol to the carboxylic acid in the next steps of the synthesis. Thus, alkyne 186 was obtained by Sonogashira reaction in 78 % yield using Pd(PPh$_3$)$_4$ (3 mol %), CuI (7 mol%), and Et$_2$NH (6 eq.) in THF (Figure 6-43). When Pd(PPh)$_3$Cl$_2$, CuI in Et$_3$N were used, the yields for the coupling reaction were much lower (13-31 %). The aldehyde function in 186 was subsequently protected by an acetal group, and the triple bond in 187 was selectively reduced to an E olefin 188 in 95 % yield by use of lithium aluminium hydride (LAH). We also performed that reduction with sodium bis(2-methoxyethoxy)aluminium hydride (Red-Al®) in refluxing tetrahydrofuran, but the yield was lower than that obtained with LAH (65 %). The reduction by Trost-Ball silylation-desilylation did not work; the starting material was recovered.

A direct oxidation of the alcohol 188 to the carboxylic acid 190 with sodium chlorite catalyzed by 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) and bleach resulted in degradation of the substrate and formation of byproducts. Oxidation of
alcohol 188 to aldehyde 189 was troublesome at first: Swern oxidation, pyridinium chlorochromate, pyridinium chlorochromate and ammonium acetate protocol for acid sensitive substrates\textsuperscript{343} all met with failure. Finally, we obtained the desired product 189 in 92 % yield after the work-up, using Dess-Martin periodinane 191 (DMP).\textsuperscript{344} The Dess-Martin oxidation is a reaction of choice for oxidation of primary alcohols to aldehydes and secondary alcohols to ketones, often used in the synthesis of complex molecules because of its high chemoselectivity and tolerance of sensitive functional groups thanks to its mild conditions (room temperature, slightly acidic or neutral pH).\textsuperscript{345,346} In addition, organohypervalent iodine reagents are considered to be environmentally benign.\textsuperscript{346}

Figure 6-43. Synthesis of the linear precursor needed for acid-catalyzed double cyclization to form the ABC core of solanacol.

The Dess-Martin periodinane (1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1\textsubscript{H})-one) (191) is commercially available, but it is rather expensive, and it is known that different batches of DMP show different reactivity due to exposure to moisture.\textsuperscript{347}
Hence, DMP was prepared by conversion of 2-iodobenzoic acid (192) to IBX (193), using 3 equivalents of oxone (2KHSO$_5$-KHSO$_4$-K$_2$SO$_4$)$^{348}$ followed by acylation with acetic anhydride and 0.5 % $p$-toluenesulfonic acid$^{345}$ using literature procedures (Figure 6-44).

![Synthesis of Dess-Martin Periodinane (DMP)](image)

The aldehyde 189 was oxidized to carboxylic acid 190 by Pinnick oxidation,$^{349}$ which is highly suited for substrates with many functional groups (it does not affect any C=C double bonds present, nor alcohols, epoxides, benzyl ethers or halides, and proceeds at constant pH), and is often used in total syntheses of natural products. Therefore, the aldehyde 189 was dissolved in $t$-BuOH with a large excess (40 equivalents) of 2-methyl-2-butene (hypochlorous acid scavenger) and treated with sodium chlorite in potassium dihydrogen phosphate buffer at room temperature giving the acetal carboxylic acid 190 in 70 % yield.

The acetal 190 was treated with TfOH and TMSOTf with intention to form cyclized lactone 175 with the B-ring OH and C-ring trans to each other (Figure 6-45). The $^{1}$HNMR spectra of the crude from each reaction were similar to each other and quite complex (example: Figure 6-46), indicating that at least four cyclization products were formed in different ratios depending on cyclization conditions. In each of those reaction two “trans products” were formed preferentially. Two spots for the products were visible on TLC, and they could be separated on chromatography column, however
even after that purification the $^1$HNMR spectra were often too complex to interpret. One of those spectra that was interpretable indicated that two “trans” products were formed consistent with the structure 194 (Figure 6-47).

![Diagram of chemical reactions](image)

Figure 6-45. Cyclization of the acetal carboxylic acid 190.

The IR spectrum of the mixture of those two “trans” products showed that there was no OH group and HRMS-ESI was also consistent with structure 194 (found 499.2108, calculated for $[C_{29}H_{32}O_6Na]^+$: 499.2093), there was no peak indicating formation of 175. The products degraded during GC-MS analysis.

![HNMR spectrum](image)

Figure 6-46. The $^1$HNMR spectrum of the crude from the reaction of 190 with 0.3 equivalent of TfOH: at least 4 cyclized products are formed.
Figure 6-47. The $^1$HNMR spectrum of two “trans” diastereomers of 194.

Since the attempts to obtain lactone 175 by treatment of acetal 190 with triflic acid and trimethylsilyl triflate met with failure, the acetal was removed with catalytic HCl in acetone and water to afford the aldehyde carboxylic acid 176 in 85% yield (Figure 6-43) (no cyclization was observed under these conditions), and then the aldehyde was subjected to TMSOTf or TfOH with an intent to make 175 (Figure 6-48).

Figure 6-48. Cyclization of the aldehyde carboxylic acid 176.

Again, the crude $^1$HNMR indicated that there were at least 4-6 products formed, separation by column chromatography gave one fraction (A) in which 3 compounds were present (one “trans” lactone and two “cis” lactones, Figure 6-49), and one fraction
(B) whose $^1$HNMR might be the spectrum of 175 (Figure 6-50). However, the IR spectrum of fraction B showed no OH group of alcohol and there was no peak in the HRMS-ESI that would confirm the presence of 175, instead HRMS-ESI indicated that compound 195 was formed (found 441.1691, calculated for [C$_{26}$H$_{26}$O$_5$Na]$^+$: 441.1674).

The products degraded during GC-MS analysis.

Figure 6-49. $^1$HNMR of the fraction A from cyclization of aldehyde carboxylic acid 176 (most probably 3 diasteromers of 195).

Figure 6-50. $^1$HNMR of the fraction B from cyclization of aldehyde carboxylic acid 176 (one “trans” diastereomer of 195).
All reactions to cyclize the acid acetal 190 and acid aldehyde 176 resulted in a formation of complex mixtures of products in 40-70 % yield, depending on exact conditions. The analysis of those products was difficult because they could not be separated and some other small impurities were formed too. All results indicated that the desired free alcohol lactone 175 was not formed and that cyclized products were reacting with the protonated substrate to form “dimers” 194 or 195. Even when dilute concentration of substrates 190 and 176 was used (0.0096 M), only the dimers were detected.

We planned to prepare an allyl ester of 176 but initial tries to make it were not successful. However, an aldehyde methyl ester 197 with E olefin geometry could be easily prepared by oxidation of 189 with pyridinium dichromate (PDC) in the presence of methanol in dimethylformamide in 48 % yield using a Just and O’Connor protocol, followed by removal of acetal protecting group with hydrochloric acid in acetone and water in 75 % yield (Figure 6-51).

![Figure 6-51. Preparation of the E-olefin aldehyde methyl ester 197.](image)

The E-olefin aldehyde methyl ester 197 was then subjected to treatment with triflic acid and trimethylsilyl triflate to form a lactone 198 with the B-ring methoxy group and the C-ring trans to each other (Figure 6-52). The $^1$HNMR spectrum of the isolated product though indicated that two “trans” lactones were formed, and also one “cis”
lactone in smaller quantities (Figure 6-53). This result was very confusing because formation of only two diastereomers was expected: one trans (198) and one cis (199).

Figure 6-52. Cyclization of the E-olefin aldehyde methyl ester 197.

Figure 6-53. ¹HNMR of the products obtained in cyclization of the E aldehyde methyl ester 197.

To tease out that cyclization result, an aldehyde methyl ester 203 with Z olefin geometry was prepared (Figure 6-54). The alkyne 187 was reduced to Z-olefin 200 in 85 % yield with hydrogen gas over Lindlar catalyst in methanol in the presence of quinoline. The Z-olefin methyl ester 203 was then prepared analogously to the E-isomer by Dess-Martin oxidation, treatment with PDC and methanol, and deprotection of acetal.
The Z-olefin aldehyde methyl ester 203 was then treated with 1 equivalent of triflic acid in dichloromethane to form preferentially the cis lactone 199 (Figure 6-55). The crude $^1$HNMR from this reaction was quite complex (Figure 6-56), indicating that four cyclized lactones were formed: 2 cis and 2 trans. Column chromatography allowed separation of one cis lactone ($^1$HNMR Figure 6-57), the other three lactones eluted together. GC-MS (EI) analysis indicated that all four compounds were giving the same molecular ion [M]$^+$=232 and almost identical fragmentation patterns.

Figure 6-55. Cyclization of the Z-olefin aldehyde methyl ester 203.
Figure 6-56. $^1$HNMR of the crude in cyclization of the Z aldehyde methyl ester 203.

Figure 6-57. $^1$HNMR consistent with a structure of 199 formed in cyclization of the Z-aldehyde methyl ester 203.

The nature of the products formed in cyclization of aldehyde methyl ester has not yet been elucidated. It was thought that the lactone in product 199 could reopen and
close after a methyl transfer to form another lactone 204 (Figure 6-58), explaining the formation of two cis lactone methyl ether products, but the same transformation could not occur in the case of the trans lactone 198.

![Figure 6-58. Isomerization of the cis lactone product.](image)

**6.4 Additional Notes**

In 2012, when I was no longer working on the strigolactone project, a paper in Science appeared showing that D27 catalyzes isomerization of all-trans-β-carotene into 9-cis-β-carotene, which is then cleaved with CCD7 to 9-cis-β-apo-10'-carotenal. CCD8 converts 9-cis-β-apo-10'-carotenal into a strigolactone-like compound called carlactone. The authors suggested that carlactone could be oxidized and then converted to 5-deoxystrigol by double cyclization citing our work\textsuperscript{252} (Figure 6-59).

**6.5 Summary**

A new method for constructing the ABC ring system of strigolactones in a single step from simple linear precursors by an acid-catalyzed double cyclization has been reported. The appropriate linear model precursors at alcohol oxidation level and aldehyde oxidation level were prepared and subjected to the proposed reaction. The alcohol and chloride substrates did not undergo the cyclization. However, aldehyde precursors easily underwent the cyclization catalyzed by triflic acid and trimethylsilyl triflate in good yields and with a high degree of stereocontrol. The aldehydic precursors with the E-geometry around the double bond gave tricyclic lactones in which the B-ring
alkoxy group and the C-ring were trans to each other, whereas the precursors with the Z-geometry around the double bond gave tricyclic lactones in which the B-ring alkoxy group and the C-ring were cis to each other. The stereochemical control of the reaction was qualitatively rationalized using DFT calculations. The results suggested a new mechanism that might be operative in the strigolactone biosynthetic pathway.

The cyclization substrate necessary for the synthesis of solanacol was prepared, but its cyclization gave more products than expected. It would be interesting to elucidate the identity of these additional products.

Figure 6-59. Strigolactone biosynthetic pathway proposed in 2012 by Alder et al.252
CHAPTER 7
CONCLUSIONS AND FUTURE WORK

This dissertation has introduced a new class of ligands called “silent agonists”, targeted to the α7 nAChR, which bind in the traditional agonist binding site and induce Ds desensitization without antecedent ion channel opening. The α7 nAChR silent agonists can be used to study the α7 nAChR signaling that does not involve ion channel opening and have been suggested to be implicated in cholinergic anti-inflammatory pathway responsible for inhibition of pro-inflammatory cytokine synthesis. If α7 nAChR silent agonists are established to have anti-inflammatory properties, they would be preferred to regular α7 agonists to treat inflammatory disorders without possible side effects linked to the α7 ionotropic activity (the existing data suggests a hypothesis that the α7 ion channel activity is required for effects on cognition).201

Three different groups of silent agonists were characterized that represented distinct groups of chemical structures.

The first group features compounds KC-1 and KC-5. Closely related analogs of these molecules, KC-2, KC-3, KC-4, KC-6, KC-8, and KC-9 were characterized as weak silent agonists, while KC-7 appeared to have partial agonist character. The putative pharmacophore for this group was described as a positively charged ring, a central ring with hydrogen bonding capability, and a flanking aryl group. However, since small structural changes resulted in significant functional changes that could not be attributed to the structural differences between the molecules, further investigation of what constitutes the silent agonist pharmacophore in this group is needed. It would be interesting to test pure enantiomers of chiral KC compounds to see whether both enantiomers have the same activity, different levels, or activity associated with only one
antipode. This group of silent agonists presents some challenges for rational drug design, because it appears difficult to make predictions on what molecules would act as α7 agonists, silent agonists, and antagonists, as illustrated by properties of KC compounds and closely related NS6740 (silent agonist) versus NS6784 (agonist). Moreover, the KC compounds did not show good selectivity over other nAChR subtypes: they appeared to bind and in some cases weakly activate α4β2 receptors and the weak silent agonist KC-8 acted as an agonist for the α3β4 subtype.

The second group of silent agonists features bulky quaternary ammonium compounds, such as TEA, (2-HE)-diEMA, and dEtPip. A very good correlation between the molecular volume of the quaternary ammonium compounds and their pharmacological activity was found. The molecules that have a molecular volume of 94-133 Å³ act as agonists, 142-150 Å³ act as silent agonists with partial agonism character, 150-163 Å³ are silent agonists, and 167-186 Å³ are weak silent agonists. These results suggest that smaller quaternary ammonium compounds may allow conformational changes, such as full closure of C-loop onto the agonist in the binding site that is then transmitted to the transmembrane domain and causes ion channel opening, while bigger molecules such as silent agonists do not allow for this full movement of the C-loop and instead cause a conformational change, described as Ds desensitized and possibly involving the nearby F-loop, which might be transmitted to the intracellular domain causing metabotropic signaling. Antagonists appear too large to allow the C-loop to close. It would be interesting to further pursue testing of the selectivity of quaternary ammonium compounds for different nAChR subtypes, and design new molecules containing the bulky quaternary ammonium that could be used as
therapeutics. Since in this group a very good correlation between the structure of the molecule and its pharmacological properties was seen, it appears the easiest to optimize bulky ammonium compounds in order to find a therapeutic α7 silent agonist. In addition, the bulky quaternary ammonium compounds presented in this dissertation have a hard positive charge and therefore would be unable to cross the blood-brain barrier, which would be desirable to avoid side effects on the CNS (silent agonists would be expected to act in non-neuronal cells in the PNS, the only exception would be for treatment of Alzheimer’s disease linked to inflammatory abnormalities in the CNS. In that particular case, compounds of appropriate molecular volume with an alkyl group replaced with a hydrogen might be effective).

The third group of silent agonists, drawn from the work of Dr. Jingyi Wang, is exemplified by benzylidene anabaseine type molecules such as 3-pyridinylmethylene anabaseine (3PAB). Fluorination of 3PAB on the pyridine ring that is putatively binding in the α7 nAChR selectivity pocket resulted in decreased activation along with decreased D₅ desensitization of the receptor. This group poses some challenges for rational drug design because it appears difficult to predict the properties of these molecules based on their structure as has been observed with 2PyroAB, 2TAB, 2FAB and 2PAB, though it is not excluded that active α7 silent agonists with good druggable properties are going to be found in this group.

This work could be continued by a thorough analysis of the α7 homology models with silent agonists docked in, obtaining crystals structures of silent agonists bound to the AChBP, and mutations of the amino acids putatively involved in the interactions with the silent agonists or the conformational change of the α7 receptor upon silent agonist
binding. Future studies could also include testing of the silent agonists in biological assays to establish their behavior related to the control of pro-inflammatory cytokine production. Furthermore, it would be interesting to clarify whether Ds, Di, or yet another desensitized state of the α7 nAChR is involved in non-ionotropic signaling.

Additionally, several new arylidene quinuclidines were prepared that acted as partial agonists of the α7 nAChR. The (E)-3-(thiophen-2-ylmethylene)quinuclidine [(E)-2-TQN] produced large amount of residual inhibition and desensitization that could be associated with Di desensitization, while the Z-isomer of 2-TQN did not. The compounds and their analogs should be further tested to establish the interactions on the receptor that lead to this observed difference in behavior for these two geometric isomers.

In the second part of this dissertation, it was presented that the strigolactone ABC-core could be formed in one step from suitable aldehydic precursors by an acid-catalyzed double cyclization. Model linear precursors were prepared and reaction conditions under which the aldehydes undergo the proposed double cyclization in good yields and with a high degree of stereocontrol were successfully identified. The molecules at the alcohol oxidation level did not undergo the cyclization reaction. The synthetic studies on the strigolactone ABC-ring system bear significance for elucidation of the strigolactone biosynthetic pathway. It would be interesting to test the double cyclization precursors and carlactone with a MAX1 enzyme to find the substrates for that protein.
APPENDIX A
EXPERIMENTAL PROCEDURES ELECTROPHYSIOLOGY\textsuperscript{102,103}

All electrophysiology experiments were performed in Dr. Roger Papke Lab (Department of Pharmacology and Therapeutics, College of Medicine, University of Florida) by Clare Stokes, Matthew Kimbrell, Lu Wenchi Corrie, Shehd Abdullah Al Rubaiy, Sara B. Copeland, Matthew D. Isaacson, Thomas F. Pack, Sarah Pinheiro, Akshatha Rao, Khan A. Manther, and Christopher W. Kinter.

\textit{\(\alpha_7\) nAChR clones and site-directed mutants.} The human \(\alpha_7\) clone was obtained from Dr. Jon Lindstrom (University of Pennsylvania, Philadelphia). The human RIC-3 clone, obtained from Dr. Millet Treinin (Hebrew University, Jerusalem, Israel), was co-injected with the \(\alpha_7\) constructs to improve the levels and speed of receptor expression. Amino acids were numbered as for the human \(\alpha_7\) nAChR (vicinal C-loop cysteines at positions 190 and 191). Mutations were introduced using the QuickChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA) following the manufacturer's instructions. All mutations were confirmed with automated fluorescent sequencing at the University of Florida core facility. After linearization and purification of cloned cDNA, cRNA transcripts were prepared in vitro using the appropriate mMessage mMachine kit from Ambion Inc. (Austin, TX).

**Expression in Xenopus laevis oocytes**. Mature (>9 cm) female \textit{X. laevis} African frogs (Nasco, Ft. Atkinson, WI) were used as the source of oocytes. Frogs were maintained in the Animal Care Services facility of University of Florida, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Prior to
surgery, frogs were anaesthetized by placing the animal in a 1.5 g/L solution of MS222 (3-aminobenzoic acid ethyl ester; Sigma, St. Louis, MO) for 30 min. Oocytes were removed from an abdominal incision. In order to remove the follicular cell layer, harvested oocytes were treated with 1.25 mg/mL collagenase (Worthington Biochemical Cooperation, Freehold, NJ) for 2 hours at room temperature in Barth’s solution without calcium (88 mM NaCl, 1 mM KCl, 2.38 mM NaHCO$_3$, 0.82 mM MgSO$_4$, 15 mM HEPES (pH 7.6), 12 mg/L tetracycline). Stage 5 oocytes were isolated and injected with 50 nL (5-20 ng) each of the appropriate cRNAs. Wild type and mutant α7 receptors were routinely co-injected with cDNA for human RIC3, an accessory protein that improves and accelerates α7 expression without affecting the pharmacological properties of the receptor. Recordings were made 1 to 10 days after cRNA injection.

**Electrophysiology.** Experiments were conducted by two electrode voltage clamp, using OpusXpress 6000A (Molecular Devices, Sunnyvale, CA). OpusExpress is an integrated system that provides automated impalement and voltage clamp of up to eight oocytes in parallel. Cells were automatically bath-perfused with Ringer’s solution (115 mM NaCl, 10 mM HEPES, 2.5 mM KCl, and 1.8 mM CaCl$_2$, pH 7.2, and 1 μM atropine to block endogenous muscarinic receptors). Both the voltage and current electrodes were filled with 3 M KCl. Cells were voltage-clamped at a holding potential of ~60 mV. Data were collected at 50 Hz and filtered at 20 Hz. Perfusion flow rates were set at 2 mL/min for experiments with α7 receptor and 4 mL/min for other subtypes. Drugs solutions were delivered from a 96-well plate via disposable tips. Drug applications alternated between ACh controls and experimental ligands at varying concentrations.
Unless otherwise indicated, drug applications were 12 s in duration, followed by 181 s washout periods with α7 receptors and 6 s with 241 s washout periods for other subtypes.

**Experimental protocols and data analysis**

Data were analyzed by Clampfit 9.2 (Molecular Devices) and Excel (Microsoft, Redmond, WA), and normalized to the averaged responses of the acetylcholine pre-controls.\(^ {351} \) Data were expressed as means ± SEM from at least four oocytes for each experiment. For the concentration response curves, responses were normalized to the net charge (α7) or peak current (α4β2) of the most adjacent prior control. Data were plotted by Kaleidagraph 3.0.2 (Abelbeck Software, Reading, PA), and curves were generated as the best fit of the average values from the Hill equation.
Strigolactones

These calculations have been done by Dr. Stefano Santoro in Dr. Fahmi Himo Lab (Department of Organic Chemistry, Stockholm University, Stockholm, Sweden).

Geometry optimizations of protonated aldehydes, transition states and cyclized structures employed the 6-31G(d,p) basis set for all atoms. Single-point energy calculations were then performed for each of these optimized structures with the 6-311+G(2d,2p) basis set. Solvation effects were taken into account by performing single-point calculations on the optimized structures using the conductorlike polarizable continuum model (CPCM) method with the UAKS radii. The parameters for CH$_2$Cl$_2$ were used for all solvation calculations. All stationary point structures were confirmed either as minima (no imaginary frequencies) or transition states (only one imaginary frequency) by analytical frequency calculations at the same theory level as the geometry optimizations. All energies reported for the transition states have been corrected for solvation and zero-point vibrational effects.
APPENDIX C
ALTERNATIVE PHARMACOPHORE

For rationale of the project see Jingyi Wang dissertation.\textsuperscript{222}

Structures of 4-OH GTS-21, BHP, and PPP

\textbf{Synthesis of PPP}

Retrosynthetic analysis

\begin{align*}
\text{Suzuki coupling} & \quad \rightarrow \quad \text{bromination} \\
\begin{array}{c}
\text{N}
\
\text{Br}
\
\begin{array}{c}
\text{N}
\
\text{Si}
\
\text{205}
\end{array}
\end{array}
& \quad \rightarrow \quad \begin{array}{c}
\text{Br}
\
\text{N}
\
\text{TIPS}
\end{array}
\end{align*}

Attempts to synthesize PPP 211a

\begin{align*}
\text{Br}
\
\text{N}
\
\text{Si}
\
\text{205}
& +
\begin{array}{c}
\text{N}
\
\text{Br}
\
\text{OH}
\
\text{6: } R = \text{H}
\
\text{206: } R = \text{OMe}
\end{array}
& \xrightleftharpoons[6\text{ mol\%}]{\text{Pd(PPh}_3)_4}\text{Na}_2\text{CO}_3\text{toluene/methanol/H}_2\text{O}
& \begin{array}{c}
\text{N}
\
\text{TIPS}
\
\text{R}
\end{array}
\end{align*}

\begin{align*}
207\text{a: } R = \text{H} & \sim 60 \% \\
207\text{b: } R = \text{OMe} & \sim 30 \%
\end{align*}

\begin{align*}
\text{TBAF} & \quad \rightarrow \quad \begin{array}{c}
\text{N}
\
\text{Br}
\
\text{R}
\end{array}
& \quad \rightarrow \quad \begin{array}{c}
\text{N}
\
\text{Br}
\
\text{R}
\end{array}
\end{align*}

\begin{align*}
208\text{a: } R = \text{H} & \sim 86 \% \\
208\text{b: } R = \text{OMe} & \sim 84 \%
\end{align*}

\begin{align*}
\text{209a: } R = \text{H} \\
\text{209b: } R = \text{OMe}
\end{align*}

\begin{align*}
\text{210} & \quad \rightarrow \quad \\
\text{211a (PPP): } R = \text{H}
\end{align*}

\begin{align*}
\text{211b: } R = \text{OMe}
\end{align*}
PyPyr Synthesis

The free base of PyPyr (217) was synthesized following a literature scheme by Suzuki coupling as a key step. N-(Boc)-pyrrole-2-boronic acid (213) was prepared in 74 % yield using a patent procedure by treating N-(Boc)-pyrrole (212) with LDA, then trimethyl borate, and followed by an acidic work-up. The coupling product 215 was obtained in 66 % yield by heating at reflux a mixture of N-(Boc)-pyrrole-2-boronic acid (213), 3-bromopyridine (214), tetrais-(triphenylphosphine)palladium (4 mol%) in 1,2-dimethoxyethane, with an excess of aqueous sodium carbonate as a base. In that reaction, three other byproducts were observed on a TLC plate, one of them was identified before as a homodimer of 213 resulting from deboronation and self-coupling of N-(Boc)-pyrrole-2-boronic acid (213).

The Boc group was easily removed from 215 by treatment with an excess of sodium methoxide in methanol at room temperature. The free base of PyPyr (216) was then
quantitatively converted into its hydrochloride salt by treatment with hydrochloric acid in ethanol.

<table>
<thead>
<tr>
<th>Test</th>
<th>Receptor</th>
<th>Control (C)</th>
<th>Peak average</th>
<th>Area average</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μM PyPyr</td>
<td>hα7</td>
<td>300 μM ACh</td>
<td>0.0021 ± 0.0005</td>
<td>0.014 ± 0.009</td>
</tr>
<tr>
<td>100 μM PyPyr</td>
<td>hα4β2</td>
<td>30 μM ACh</td>
<td>0.0036 ± 0.0009</td>
<td>0.0010 ± 0.0006</td>
</tr>
<tr>
<td>100 μM PyPyr</td>
<td>hα7W55G</td>
<td>300 μM ACh</td>
<td>0.047 ± 0.007</td>
<td>0.055 ± 0.007</td>
</tr>
<tr>
<td>100 μM PyPyr</td>
<td>hα7Y188F</td>
<td>30 μM 4OH-GTS-21</td>
<td>0.06 ± 0.03</td>
<td>0.05 ± 0.05</td>
</tr>
<tr>
<td>100 μM PyPyr</td>
<td>hα4β2W57Y</td>
<td>30 μM ACh</td>
<td>0.007 ± 0.001</td>
<td>0.0002 ± 0.0006</td>
</tr>
<tr>
<td>100 μM PyPyr</td>
<td>hα4Y109Fβ2</td>
<td>30 μM ACh</td>
<td>0.10 ± 0.01</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>100 μM PyPyr + C</td>
<td>hα7</td>
<td>300 μM ACh</td>
<td>1.00 ± 0.08</td>
<td>0.99 ± 0.02</td>
</tr>
<tr>
<td>100 μM PyPyr + C</td>
<td>hα4β2</td>
<td>30 μM ACh</td>
<td>0.76 ± 0.04</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>100 μM PyPyr + C</td>
<td>hα7W55G</td>
<td>300 μM ACh</td>
<td>1.31 ± 0.09</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>100 μM PyPyr + C</td>
<td>hα7Y188F</td>
<td>30 μM 4OH-GTS-21</td>
<td>0.91 ± 0.08</td>
<td>0.89 ± 0.03</td>
</tr>
<tr>
<td>100 μM PyPyr + C</td>
<td>hα4β2W57Y</td>
<td>30 μM ACh</td>
<td>0.82 ± 0.01</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>100 μM PyPyr + C</td>
<td>hα4Y109Fβ2</td>
<td>30 μM ACh</td>
<td>0.79 ± 0.03</td>
<td>0.89 ± 0.12</td>
</tr>
<tr>
<td>100 μM PyPyr + 10 μM PNU 120596</td>
<td>hα7</td>
<td>60 μM ACh</td>
<td>0.011 ± 0.003</td>
<td>0.011 ± 0.004</td>
</tr>
</tbody>
</table>

**PyPyr activity**
A.1 General:

All reactions requiring anhydrous or oxygen-free conditions were carried out under an atmosphere of argon in oven-dried glassware. Anhydrous solvents were purchased from Sigma-Aldrich in sure-seal bottles and used as received. Thin layer chromatography was performed using 250 µm Silica Gel 60 F\textsubscript{254} pre-coated plates (Whatman) and the plates were visualized with UV or permanganate stain. Flash column chromatography was performed using 230-400 Mesh 60A Silica Gel (Whatman). Proton nuclear magnetic resonance (\textsuperscript{1}H NMR) and carbon-13 nuclear magnetic resonance (\textsuperscript{13}C NMR) spectra were recorded in deuterated chloroform, CDCl\textsubscript{3} at the frequency indicated. Chemical shifts (\textit{\delta}) are reported in parts per million (ppm) relative to tetramethylsilane (TMS, 0.0 ppm) or CDCl\textsubscript{3} (7.27 ppm in \textsuperscript{1}H NMR and 77.0 ppm in \textsuperscript{13}C NMR). Multiplicities are reported using the following abbreviations: s, singlet; d doublet; t, triplet; q, quartet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; br, broad. Infrared spectra were obtained on a Bruker Vector 22 IR spectrometer and are reported in wavenumbers. High resolution mass spectra (HRMS) were obtained by Mass Spectrometry Core Laboratory of University of Florida.

A.2 Silent agonists KC-1 story

\begin{figure}
\centering
\includegraphics[width=0.2\textwidth]{figure.png}
\end{figure}

3-bromo-5-phenylpyridine (10):\textsuperscript{206}

(Triphenylphosphine)palladium tetrakis (125 mg, 0.08 mmol, 2 mol %) was added to a solution of 3,5-dibromopyridine (1.00 g, 4.22 mmol) in dimethoxyethane (13 mL) and
the mixture was stirred for 10 min. A solution of potassium carbonate (1.75 g, 12.7 mmol) in water (6.5 mL) was added, followed by phenyl boronic acid (463 mg, 3.79 mmol), and the mixture was heated at reflux for 4 h. A solution of 1M NaOH (6 mL) was added to the cooled mixture, the mixture was extracted with diethyl ether, the organic extracts were combined, dried over MgSO₄, filtered, and the solvents were evaporated. The crude was purified by column chromatography using dichloromethane as an eluent to give 608 mg of the product (68 % yield) as a white solid. ^1H NMR (CDCl₃, 300 MHz): δ 8.76 (d, 1H, 2.0 Hz); 8.66 (d, 1H, 2.2 Hz); 8.03 (t, 1H, 2.2 Hz, 2.0 Hz); 7.43-7.59 (m, 5H). ^13CNMR (CDCl₃, 75 MHz): δ 149.3, 146.3, 138.2, 136.8, 136.2, 129.1, 128.6, 127.1, 120.8.

**tert-butyl (5-oxo-5-(5-phenylpyridin-3-yl)pentyl)carbamate (12a):**

A solution of 3-bromo-5-phenylpyridine (10) (975 mg, 4.16 mmol) in diethyl ether (10 mL) was added dropwise over 10 min to a solution of nBuLi (1.4 M in hexanes, 2.97 mL, 4.16 mmol) in diethyl ether (15 mL) at −78 ºC and the mixture was stirred at −78 ºC for 20 min. A solution of Boc-protected δ-valerolactam 11) (829 mg, 4.16 mmol) in diethyl ether (8 mL) was added and the mixture was further stirred at −78 ºC for 2 h and at 0 ºC for 5 min. The reaction was quenched with 2M HCl to pH 1-2, and the mixture was extracted with diethyl ether. The combined organic layers were washed twice with 10 % aqueous solution of sodium bicarbonate, dried over Na₂SO₄, filtered, and the solvents were evaporated. The crude was purified by column chromatography, using a gradient of hexanes/ethyl acetate 10:1 to 3:1 as eluent to yield the product as
yellowish solid (900 mg, 61 %). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 9.13 (d, 1H, 2.2 Hz); 9.01 (d, 1H, 2.2 Hz); 8.42 (t, 1H, 2.2 Hz); 7.60-7.64 (m, 2H); 7.42-7.54 (m, 3H); 4.68 (br s, 1H); 3.16-3.22 (m, 2H); 3.09 (t, 2H, 7.0 Hz); 1.78-1.88 (m, 2H); 1.56-1.66 (m, 2H); 1.44 (s, 9H). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 198.6, 155.9, 151.6, 147.8, 136.7, 136.5, 133.4, 131.9, 129.1, 128.5, 127.1, 79.0, 40.1, 38.4, 29.5, 28.3, 20.8. IR (neat): $\nu$ 3369, 1685, 1516, 1248, 1162 cm$^{-1}$. HRMS (ESI): [M+H]$^+$ calculated:355.2016, found: 355.2007; [M+Na]$^+$ calculated: 377.1836, found: 377.1835.

5'phenyl-3,4,5,6-tetrahydro-2,3'-bipyridine (KC-1):

The ketone carbamate (12) (105 mg, 0.30 mmol) was dissolved in dichloromethane (2 mL), trifluoroacetic acid (1 mL) was added at 0 ºC, the mixture was stirred at 0-10 ºC for 2 h, and then quenched with 5 M NaOH (toward the end of addition 1 M NaOH was added to pH 12-13). The mixture was extracted with diethyl ether, dried over Na$_2$SO$_4$, filtered, and the solvents were evaporated. The crude was purified by column chromatography, using a gradient of dichloromethane and methanol 100:1 to 60:1 to give the product as yellowish oil (56 mg, 80 % yield). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.92 (s, 1H); 8.86 (s, 1H); 8.31 (t, 1H, 2.1 Hz); 7.63 (dt, 2H, 7.0 Hz, 1.5 Hz); 7.48 (tt, 2H, 7.0 Hz, 1.5 Hz); 7.41 (tt, 1H, 7.0 Hz, 1.5 Hz); 3.86-3.91 (m, 2H); 2.69 (tt, 2H, 6.5 Hz, 2.2 Hz); 1.85-1.93 (m, 2H), 1.68-1.76 (m, 2H) $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 163.5, 148.8, 146.1, 137.5, 136.1, 135.2, 131.7, 128.9, 128.1, 127.2, 50.1, 27.1, 21.7, 19.5. IR
3-phenyl-5-(piperidin-2-yl)pyridine (KC-2):

The ketone carbamate (12a) (550 mg, 1.55 mmol) was dissolved in dichloromethane (3 mL), trifluoroacetic acid (6 mL) was added at 0 °C, the mixture was stirred at 0-10 °C for 2.5 h, and then quenched with 5 M NaOH (toward the end of addition 1 M NaOH was added to pH 12-13). The mixture was extracted with dichloromethane, dried over Na$_2$SO$_4$, filtered, and the solvents were evaporated. The crude cyclic imine was dissolved in methanol (10 mL) and water (1 mL), sodium borohydride (70 mg, 1.85 mmol) was added at 0°C, and the reaction mixture was stirred at 0-15 °C for 3 h. The reaction was quenched by addition of 1M HCl to pH 1 at 0 °C, the mixture was stirred for 30 min, and then 1M aqueous solution of NaOH was added at 0 °C to pH 13. The mixture was extracted with dichloromethane, dried over Na$_2$SO$_4$, filtered, and the solvents were evaporated. The crude product was purified by column chromatography, using a gradient of dichloromethane and methanol 40:1 to 20:1 to give the product as yellowish oil (221 mg, 60 % yield). $^1$H NMR (CDCl$_3$, 300 MHz): δ 8.74 (dd, 1H, 2.2 Hz, 0.7 Hz); 8.57 (d, 1H, 2.1 Hz); 7.98 (td, 1H, 2.1 Hz, 0.7 Hz); 7.63-7.59 (m, 2H); 7.50-7.44 (m, 2H); 7.43-7.37 (m, 1H); 3.75 (dd, 1H, 10.9 Hz, 2.3 Hz); 3.22 (d, 1H, 11.3 Hz); 2.92 (br s, 1H); 2.83 (td, 1H, 11.5 Hz, 3.0 Hz); 1.95-1.85 (m, 2H); 1.70-1.49 (m, 4H).

$^{13}$CNMR (CDCl$_3$, 75 MHz): δ 147.1, 146.9, 139.6, 137.5, 136.2, 132.5, 128.8, 127.8,
126.9, 59.4, 47.1, 34.2, 25.0, 24.8. IR (neat): ν 3272, 3036, 2930, 2851, 2787, 1439, 1326, 1302, 1107, 1025, 888 cm\(^{-1}\). HRMS (ESI): [M+H]\(^+\) calculated: 239.1543, found: 239.1552; [M+Na]\(^+\) calculated: 261.1362, found: 261.1363.

![Chemical Structure](image)

3-(1-methylpiperidin-2-yl)-5-phenylpyridine (KC-3):

KC-2 (139 mg, 0.58 mmol) in formic acid (0.5 mL) and formalin (0.32 mL) were heated at 90 °C for 3 h. The reaction mixture was cooled to 0 °C and 2M K\(_2\)CO\(_3\) was added to pH 12. The mixture was extracted with dichloromethane and dried over MgSO\(_4\). The crude product was purified by column chromatography using a gradient of dichloromethane and methanol 50:1 to 30:1, to give the product as colorless oil (92 mg, 62 % yield). \(^1\)H NMR (CDCl\(_3\), 300 MHz): δ 8.73 (d, 1H, 2.1 Hz); 8.49 (d, 1H, 1.9 Hz); 7.89 (t, 1H, 2.1 Hz, 1.9 Hz); 7.60 (dt, 2H, 6.9 Hz, 2.1 Hz); 7.45 (t, 2H, 7.1 Hz); 7.40-7.34 (m, 1H); 3.04 (d, 1H, 11.5 Hz); 2.88 (dd, 1H, 11.1 Hz); 2.18-2.09 (m, 1H); 2.03 (s, 3H); 1.84-1.54 (m, 5H); 1.45-1.33 (m, 1H). \(^{13}\)CNMR (CDCl\(_3\), 75 MHz): δ 148.0, 147.0, 140.0, 137.7, 136.5, 133.0, 128.9, 127.9, 127.1, 126.2, 57.3, 44.5, 36.0, 25.9, 24.7. IR (neat): ν 2933, 2853, 2779, 1441, 1272, 1116, 1025, 894 cm\(^{-1}\). HRMS (ESI): [M+H]\(^+\) calculated: 253.1699, found: 253.1706; [M+Na]\(^+\) calculated: 275.1519, found: 275.1519.
**Tert-butyl (5-((isoquinolin-4-yl)-5-oxopentyl)carbamate (12b):**

The compound was prepared from 4-bromoisoquinoline (10b) analogously to (12a).

Yield: 43 %, lightly yellow solid. ¹H NMR (CDCl₃, 300 MHz): δ 9.32 (s, 1H); 8.95 (s, 1H); 8.68 (d, 1H, 8.5 Hz); 7.99 (d, 1H, 8.0 Hz); 7.80 (t, 1H, 8.0 Hz); 7.65 (t, 1H, 8.0 Hz); 4.77 (br s, 1H); 3.18-3.13 (m, 2H); 3.10 (t, 2H, 7.3Hz); 1.87-1.77 (m, 2H); 1.64-1.55 (m, 2H); 1.41 (s, 9H). ¹³CNMR (CDCl₃, 75 MHz): δ 202.3, 156.2, 144.2, 132.7, 132.5, 128.6, 128.3, 128.1, 127.8, 125.0, 79.0, 41.2, 40.1, 29.5, 28.3, 21.4. IR (neat): ν 3383, 1681, 1499, 1248, 1169 cm⁻¹. HRMS (ESI): [M+H]+ calculated: 329.1860, found: 329.1863; [M+Na]+ calculated: 351.1679, found: 351.1684.

**4-(3,4,5,6-tetrahydropyridin-2-yl)isoquinoline (KC-4):**

The compound was prepared from 12b analogously to KC-1. Yield: 57 %, yellow oil.

¹H NMR (CDCl₃, 300 MHz): δ 9.19 (s, 1H); 8.50 (s, 1H); 8.21 (d, 1H, 8.4 Hz); 7.95 (d, 1H, 8.0 Hz); 7.69 (t, 1H, 7.8 Hz, 7.4 Hz); 7.58 (t, 1H, 8.0 Hz, 7.1 Hz); 3.95-3.91 (m, 2H); 2.62-2.58 (m, 2H); 1.94-1.86 (m, 2H); 1.81-1.74 (m, 2H). ¹H NMR was similar to that reported in the literature. ¹³CNMR (CDCl₃, 75 MHz): δ 166.7, 152.8, 141.0, 133.0, 132.8, 130.8, 128.5, 127.8, 127.1, 124.5, 50.0, 31.2, 21.7, 19.7. IR (neat): ν 2931, 2854, 1639, 1499, 1348 cm⁻¹. HRMS (DART): [M+H]+ calculated: 211.1230, found: 211.1222.
4-(piperidin-2-yl)isoquinoline (KC-5):

The compound was prepared from 12b analogously to KC-2. Yield: 85 %, lightly yellow oil. \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 9.15 (s, 1H); 8.69 (s, 1H); 8.24 (d, 1H, 8.6 Hz); 7.97 (d, 1H, 8.2 Hz); 7.75-7.70 (m, 1H); 7.59 (t, 1H, 7.6 Hz); 4.31 (d, 1H, 11.3 Hz); 3.31 (d, 1H, 11.3 Hz); 2.96-2.88 (m, 1H); 2.37 (br s, 1H); 2.00-1.97 (m, 2H); 1.82-1.58 (4H).

\(^1\)\(^3\)CNMR (CDCl\(_3\), 75 MHz): \(\delta\) 151.8, 140.3, 133.3, 129.9, 128.1, 126.4, 122.4, 56.8, 47.8, 33.5, 25.7, 25.3. IR (neat): \(\nu\) 3270, 1105, 895, 857, 848, 786, 745 cm\(^{-1}\). HRMS (ESI): [M+H]\(^+\) calculated: 213.1386, found: 213.1394; [M+Na]\(^+\) calculated: 235.1206, found: 235.1208.

4-(1-methylpiperidin-2-yl)isoquinoline (KC-6):

The compound was prepared from KC-5 analogously to KC-3. Yield: 65 %, colorless oil. \(^1\)H NMR (DMSO-d\(_6\), 500 MHz, 85 °C): \(\delta\) 9.16 (s, 1H); 8.69 (d, 1H, 8.7 Hz); 8.49 (s, 1H); 8.08 (d, 1H, 8.2 Hz); 7.75 (td, 1H, 8.5 Hz, 7.5 Hz, 1.4 Hz); 7.65 (t, 1H, 7.6 Hz); 3.47 (d, 1H, 11.4 Hz); 3.07 (dt, 1H, 12.0 Hz, 3.3 Hz); 2.20-2.14 (m, 1H); 1.95 (s, 3H); 1.88-1.68 (m, 5H); 1.50-1.42 (m, 1H). \(^1\)\(^3\)CNMR (DMSO-d\(_6\), 125 MHz, 85 °C): \(\delta\) 152.1, 142.6, 133.9, 133.4, 130.3, 128.7, 128.6, 127.2, 123.9, 66.9, 57.6, 44.4, 34.6, 26.2, 25.1. IR (neat): \(\nu\) 2934, 2851, 2773, 1620, 1580, 1443, 1371, 1115, 1026, 898, 787 cm\(^{-1}\).

**Tert-butyl (5-oxo-5-(quinolin-3-yl)pentyl)carbamate (12c):**

The compound was prepared from 3-bromoquinoline analogously to (12a). Yield: 42 %, lightly yellow solid. ¹H NMR (CDCl₃, 300 MHz): δ 9.43 (d, 1H, 2.3 Hz); 8.73 (d, 1H, 2.1 Hz); 8.17 (d, 1H, 8.2 Hz); 7.96 (d, 1H, 8.2 Hz); 7.85 (ddd, 1H, 8.6 Hz, 7.8 Hz, 1.3 Hz); 7.64 (ddd, 1H, 8.6 Hz, 7.6 Hz, 1.1 Hz); 4.64 (br s, 1H); 3.24-3.18 (m, 2H); 3.15 (t, 2H, 7.1 Hz); 1.91-1.81 (m, 2H); 1.69-1.59 (m, 2H); 1.45 (s, 9H). ¹³C NMR (CDCl₃, 75 MHz): δ 198.7, 156.0, 149.7, 148.9, 136.9, 131.9, 129.3, 129.0, 127.5, 126.8, 79.1, 40.1, 38.2, 29.5, 28.3, 20.9. IR (neat): ν 3383, 1685, 1525, 1365, 1246, 1164, 1001, 793, 759 cm⁻¹. HRMS (ESI): [M+Na]^+ calculated: 351.1679, found: 351.1669.

**3-(3,4,5,6-tetrahydropyridin-2-yl)quinoline (KC-7):**

The compound was prepared from 12c analogously to KC-1. Yield: 81 %, lightly yellow solid. ¹H NMR (CDCl₃, 300 MHz): δ 9.38 (d, 1H, 2.1 Hz); 8.35 (d, 1H, 2.1 Hz); 8.09 (d, 1H, 8.6 Hz); 7.87-7.84 (m, 1H); 7.69 (ddd, 1H, 8.6 Hz, 6.9 Hz, 1.5 Hz); 7.51 (ddd, 1H, 8.4 Hz, 6.8 Hz, 1.0 Hz); 3.90-3.86 (m, 2H); 2.71-2.65 (m, 2H); 1.90-1.82 (m, 2H); 1.72-1.65 (m, 2H). ¹³C NMR (CDCl₃, 75 MHz): δ 163.5, 148.8, 148.3, 132.8, 132.2, 129.8,
129.1, 128.4, 127.3, 126.7, 50.0, 26.8, 21.7, 19.5. IR (neat): $\nu$ 3052, 2950, 2916, 2848, 1630, 1615, 1570, 1489, 1329, 1122 cm$^{-1}$. HRMS (DART): $[M+H]^+$ calculated: 211.1230, found: 211.1220.

3-(piperidin-2-yl)quinoline (KC-8):

The compound was prepared from 12c analogously to KC-2. Yield: 79 %, lightly yellow solid. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.79 (d, 1H, 1.9 Hz); 8.01-7.99 (m, 2H); 7.67 (d, 1H, 8.0 Hz); 7.56 (t, 1H, 7.4 Hz); 7.41 (t, 1H, 7.4 Hz); 3.66 (d, 1H, 8.4 Hz); 3.13 (d, 1H, 11.5 Hz); 2.76-2.68 (m, 1H); 1.95 (br s, 1H); 1.83-1.75 (m, 2H); 1.60-1.42 (m, 4H). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 150.4, 147.3, 137.8, 132.4, 128.6, 127.8, 127.5, 126.3, 59.5, 47.4, 34.8, 25.4, 25.0. IR (neat): $\nu$ 3303, 2927, 2847, 2789, 1494, 1441, 1317, 1123, 788 cm$^{-1}$. HRMS (ESI): $[M+H]^+$ calculated: 213.1386, found: 213.1395; $[M+Na]^+$ calculated: 235.1206, found: 235.1206.

3-(1-methylpiperidin-2-yl)quinoline (KC-9):

The compound was prepared from KC-8 analogously to KC-3. Yield: 71 %, lightly yellow oil. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.87 (s, 1H); 8.09 (s, 1H); 8.05 (d, 1H, 4.2 Hz); 7.75 (d, 1H, 8.0 Hz); 7.63 (t, 1H, 8.0 Hz, 7.0 Hz); 7.48 (t, 1H, 7.8 Hz, 7.0 Hz); 3.04 (d, 1H, 11.2 Hz); 2.96 (dd, 1H, 11.0 Hz, 2.4 Hz); 2.17-2.08 (m, 1H); 2.00 (s, 3H); 1.83-1.57 (m, 5H); 1.43-1.32 (m, 1H) $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 150.9, 147.6, 137.3, 133.7, 129.0,
128.8, 128.0, 127.5, 126.4, 68.4, 57.3, 44.5, 36.0, 25.9, 24.7. IR (neat): ν 2934, 2852, 2779, 1495, 1321, 1116, 1028, 908, 861, 787 cm⁻¹. HRMS (DART): [M+H]+ calculated: 227.1543, found: 227.1538.

A.3 Silent agonist bulky quaternary ammonium

The quaternary ammonium salts were prepared by reacting commercially available methyl or ethyl amines with methyl iodide or ethyl iodide in tetrahydrofuran or ethanol and purified by recrystallization. Methylhexahydroazepine (39) was made from hexahydroazepine by the Eschweiler-Clarke reaction²¹¹,²¹² (formalin and formic acid), and ethylhexahydroazapine (40) was prepared from hexahydroazepine using potassium carbonate and ethyl iodide.

\[
\text{(2-Hydroxyethyl)-ethylidimethylammonium iodide [(2-HE)-EdiMA] (23):}
\]

From N,N-dimethylethanolamine and ethyl iodide. White hygroscopic solid. \(^1\)H NMR (D₂O, 300 MHz): δ 4.05-4.00 (m, 2H); 3.49-3.42 (m, 4H); 3.10 (s, 6H); 1.35 (tt, 3H, 7.3 Hz, 2.0 Hz). \(^1^3\)CNMR (D₂O, 125 MHz): δ 64.5, 61.1, 55.4, 50.9, 7.8. HRMS (ESI): [M–I]⁻ calculated: 118.1226, found: 118.1232.

\[
\text{(2-Hydroxyethyl)-diethylmethylammonium iodide [(2-HE)-diEMA] (24):}
\]

From N,N-diethylethanolamine and methyl iodide. White solid. \(^1\)H NMR (D₂O, 300 MHz): δ 4.03-3.98 (m, 2H); 3.45-3.37 (m, 6H); 3.02 (s, 3H); 1.31 (tt, 6H, 7.3 Hz, 1.8 Hz).
$^{13}$CNMR (D$_2$O, 75 MHz): $\delta$ 61.6, 57.5, 55.3, 48.1, 7.8. HRMS (ESI): [M−I]$^-$ calculated: 132.1390, found: 132.1390.

(2-Hydroxyethyl)-triethylammonium iodide [(2-HE)-triEA] (25):

From N,N-diethylethanolamine and ethyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 4.00-3.94 (m, 2H); 3.39-3.32 (m, 8H); 1.27 (tt, 9H, 7.2 Hz, 1.5 Hz). $^{13}$CNMR (D$_2$O, 75 MHz): $\delta$ 57.6, 54.9, 53.6, 7.1. HRMS (ESI): [M−I]$^-$ calculated: 146.1539, found: 146.1544.

Benzyltrimethylammonium iodide [BtMA] (26):

From N,N-dimethylbenzylamine and methyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 7.54 (m, 5H); 4.47 (s, 2H); 3.07 (s, 9H). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 135.4, 133.3, 131.7, 129.9, 72.1, 55.0. HRMS (ESI): [M−I]$^-$ calculated: 128.1434, found: 128.1438.

Benzylethyldimethylammonium iodide [BEdMA] (27):

From N,N-dimethylbenzylamine and ethyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 7.52 (m, 5H); 4.44 (s, 2H); 3.36 (q, 2H, 7.2 Hz); 2.96 (s, 6H); 1.40 (t, 3H, 7.2 Hz).
$^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 132.9, 130.7, 129.2, 127.1, 67.3, 60.0, 49.1, 7.8. HRMS (ESI): [M−I]$^-$ calculated: 150.1277, found: 150.1275.

\[
\begin{array}{c}
\text{Benzyldiethylmethylammonium iodide [BdEMA] (28):}
\end{array}
\]

From N-ethyl-N-methylbenzyllamine and ethyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 7.53 (m, 5H); 4.43 (s, 2H); 3.41-3.21 (m, 4H); 2.88 (s, 3H); 1.38 (t, 6H). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 132.9, 130.6, 129.1, 127.1, 64.5, 55.7, 46.4, 7.4. HRMS (ESI): [M−I]$^-$ calculated: 178.1590, found: 178.1591.

\[
\begin{array}{c}
\text{Dimethylpyrrolidinium iodide [dMePyrr] (30):}
\end{array}
\]

From N-methylpyrrolidine and methyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 3.47 (t, 4H, 6.9 Hz); 3.09 (s, 6H); 2.19 (br s, 4H). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 66.0 (t, 2.6 Hz); 51.9 (t, 4.3 Hz); 21.8. HRMS (ESI): [M−I]$^-$ calculated: 100.1121, found: 100.1126.

\[
\begin{array}{c}
\text{Ethylmethylpyrrolidinium iodide [EtMePyrr] (31):}
\end{array}
\]

From N-methylpyrrolidine and ethyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 3.45-3.41 (m, 4H); 3.35 (q, 2H, 7.3 Hz); 2.97 (s, 3H); 2.17-2.14 (m, 4H); 1.32 (tt, 3H, 7.3
Hz, 2.1 Hz). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 66.5, 62.2, 50.3 (t, 3.5 Hz); 24.0, 11.3. HRMS (ESI): [M–I]$^-$ calculated: 114.1277, found: 114.1282.

Diethylpyrrolidinium iodide [dEtPyrr] (32):
From N-ethylpyrrolidine and ethyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 3.43 (t, 4H, 6.8 Hz); 3.28 (q, 4H, 7.3 Hz); 2.13–2.09 (m, 4H); 1.26 (tt, 6H, 7.2 Hz, 1.9 Hz). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 64.4 (t, 3.5 Hz); 56.9, 24.1, 10.7. HRMS (ESI): [M–I]$^-$ calculated: 128.1434, found: 128.1438.

Dimethylpiperidinium iodide [dMePip] (33):
From N-methylpiperidine and methyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 3.29 (t, 4H, 5.9 Hz); 3.05 (s, 6H); 1.83 (br s, 4H); 1.60 (quintet, 2H, 5.9 Hz). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 65.5, 54.3, 23.0, 22.5. HRMS (ESI): [M–I]$^-$ calculated: 114.1277, found: 114.1283.

Ethylmethylpiperidinium iodide [EtMePip] (34):
From N-methylpiperidine and ethyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 3.39 (qd, 2H, 7.2 Hz, 1.0 Hz); 3.29 (t, 4H, 5.4 Hz); 2.98 (s, 3H); 1.86 (br s, 4H); 1.71-
1.57 (m, 2H); 1.31 (td, 3H, 7.2 Hz, 1.8 Hz). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 63.2, 61.5, 49.8, 23.2, 22.2, 9.5. HRMS (ESI): [M−I]$^-$ calculated: 128.1434, found: 128.1437.

Diethylpiperidinium iodide [dEtPip] (35):

From N-ethylpiperidine and ethyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 3.34 (q, 4H, 7.2 Hz); 3.29 (t, 4H, 5.7 Hz); 1.84 (br s, 4H); 1.65 (quintet, 2H, 5.9 Hz); 1.25 (td, 6H, 7.2 Hz, 1.6 Hz). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 58.3 (t, 1.9 Hz); 53.1, 20.8, 19.2, 6.5. HRMS (ESI): [M−I]$^-$ calculated: 142.1590, found: 142.1592.

N-Methylhexahydroazepine (39):

From homopiperidine, formaline, and formic acid. Colorless oil. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 2.50-2.47 (m, 4H); 2.28 (s, 3H); 1.62-1.60 (m, 4H); 1.56-1.54 (m, 4H). $^{13}$CNMR (D$_2$O, 125 MHz): $\delta$ 58.4, 47.2, 27.8, 26.6.

Dimethylhexahydroazepinium [diMHHA] (36):

From N-methylhexahydroazepine (39) and methyl iodide. White solid. $^1$H NMR (D$_2$O, 300 MHz): $\delta$ 3.43-3.41 (m, 4H); 3.07 (s, 6H); 1.86 (m, 4H); 1.69-1.66 (m, 4H). $^{13}$CNMR

Ethylmethylhexahydroazepinium [EMHHA] (37):
From N-methylhexahydroazepine (39) and ethyl iodide. White solid. ¹H NMR (D₂O, 300 MHz): δ 3.44-3.31 (m, 6H); 2.95 (s, 3H); 1.85 (br s, 4H); 1.67-1.65 (m, 4H); 1.32 (tt, 3H, 7.3 Hz, 1.9 Hz). ¹³C NMR (D₂O, 125 MHz): δ 66.4, 62.9, 52.0, 29.8, 23.8, 10.1. HRMS (ESI): [M−I]⁻ calculated: 142.1590, found: 142.1595.

N-Ethylhexahydroazepine (40):
From homopiperidine, ethyl iodide and potassium carbonate. Colorless oil. ¹H NMR (CDCl₃, 300 MHz): δ 2.50 (t, 4H, 4.6 Hz, 5.7 Hz); 2.42 (q, 2H, 7.0 Hz); 1.53-1.50 (m, 8H); 0.94 (t, 3H, 7.0 Hz).

Diethylhexahydroazepinium [diEHHA] (38):
From N-ethylhexahydroazepine (40) and ethyl iodide. White solid. ¹H NMR (D₂O, 300 MHz): δ 3.37-3.27 (m, 8H); 1.86 (m, 4H); 1.65 (m, 4H); 1.27 (t, 6H, 7.1 Hz). ¹³C NMR

A.4 Synthesis of new fluorinated pyridinylmethylene anabaseines to study interactions leading to desensitized stated of the human α7 nicotinic acetylcholine receptor

![Structural formula]

1-(diethylamino)methyl)piperidin-2-one (3):$^{202,203,222}$

Delta-valerolactam (11.223 g, 113 mmol), diethylamine (23.4 mL, 226 mmol), and paraformaldehyde (6.802 g, 226 mmol) in toluene (130 mL) were heated at reflux in a Dean-Stark apparatus for 5 h. The reaction mixture was cooled to room temperature and concentrated on rotary evaporator to a volume of 45 mL. Then, brine was added (45 mL) and the pH of the mixture was adjusted to 11 using 4 M NaOH. The layers were separated and the aqueous layer was extracted twice with ethyl acetate. The combined organic extracts were dried over MgSO$_4$ and concentrated. The crude product was purified by vacuum distillation (b.p. 120-121 °C, 0.4 mmHg) to yield 3 as a colorless oil (18.019 g, 86 % yield). $^1$H NMR (CDCl$_3$, 300 MHz): δ 4.15 (s, 2H); 3.33-3.37 (m, 2H), 2.57 (q, 4H, 7.2 Hz); 2.36-2.41 (m, 2H); 1.78 (m, 4H), 1.01 (t, 6H, 7.2 Hz). $^{13}$CNMR (CDCl$_3$, 75 MHz): δ 170.3, 63.5, 46.0, 45.1, 32.4, 23.1, 21.2, 12.0.
Sodium salt of the aminal of 3-nicotinoyl-2-piperidinone (51):\(^{202,203,222}\)

Sodium hydride 60 % dispersion in mineral oil (4.784 g, 119.6 mmol) was added in 3 portions to a solution of 3 (11.030 g, 59.8 mmol) and ethyl nicotinate 50 (8.20 mL, 60.0 mmol) in toluene (52 mL) at room temperature, and the reaction mixture was heated at reflux. After 4 h, sodium hydride 60 % dispersion in mineral oil (2.35 g, 58.8 mmol) was added and the reaction mixture was stirred at reflux for additional 4 h. Then, the unreacted sodium hydride was removed by filtration of the hot reaction mixture, and washed with hot toluene (NaH was destroyed in ethanol). The filtrate was concentrated to 50 mL and placed in a 8 °C refrigerator overnight to allow precipitation of the product 51. The product was collected and dried to give a slightly yellow solid (12.174 g, 65 % yield).

Anabaseine dihydrochloride (52):\(^{202,203,222}\)

Sodium salt of 51 (9.69 g, 31.1 mmol) was heated at reflux for 12 h in a 5:1 mixture of 12 M hydrochloric acid (100 mL) and acetone (20 mL). The reaction was cooled, concentrated to 70 mL, acetone was added (30 mL), and sodium chloride was removed by filtration. The filtrate was concentrated to 20 mL, a 1:1 mixture of ethanol and 2-propanol (100 mL total) was added, and allowed to stand in a 8 °C refrigerator overnight. The solids were filtered and the product was recrystallized from ethanol: 2-propanol (1:1) to yield the product as a white solid (4.26 g, 59 % yield). \(^1\)H NMR
(DMSO-d$_6$, 500 MHz): δ 9.29 (dd, 1H, 2.1 Hz, 0.6 Hz); 8.97 (dd, 1H, 5.4 Hz, 1.5 Hz); 8.67 (dt, 1H, 8.1 Hz, 1.5 Hz); 7.91 (ddd, 1H, 8.1 Hz, 5.4 Hz, 0.6 Hz); 3.20 (t, 2H, 6.6 Hz); 2.79-2.85 (m, 2H); 1.64-1.71 (m, 4H).

**General procedure for preparing arylidene anabaseines**:\(^{219,222}\)

Anabaseine dihydrochloride (52) (50 mg, 0.21 mmol) was dissolved in a methanolic mixture (2.2 mL) of sodium acetate trihydrate (28 mg, 0.21 mmol) and acetic acid (33 µL, 0.53 mmol). The appropriate aldehyde was added (3 equivalents), and the mixture was stirred at room temperature under argon until the TLC analysis (DCM:methanol 20:1) showed complete consumption of the substrate. Then, water was added (10 mL), the pH was adjusted to 10 with 2 M K$_2$CO$_3$, the product was extracted with ethyl acetate, the organic layers were dried over MgSO$_4$, and the solvents were evaporated. The crude product was purified by a column chromatography (SiO$_2$, gradient of DCM:methanol).

![Chemical Structure](image)

**(E)-3-(2-fluoropyridinylmethylene)-anabaseine [(2-F)-3PAB] (44):**

Reaction time: 30 h. After chromatography (DCM:methanol 100:1 to 50:1 gradient), the product was obtained in 80 % yield. \(^1\)H NMR (CDCl$_3$, 300 MHz): δ 8.76 (d, 1H, 1.5 Hz); 8.65 (dd, 1H, 5.0 Hz, 1.5 Hz); 8.16 (d, 1H, 4.8 Hz); 7.84 (dt, 1H, 7.8 Hz, 1.8 Hz); 7.71-7.78 (m, 1H); 7.35 (ddd, 1H, 7.8 Hz, 5.0 Hz, 0.9 Hz); 7.20-7.25 (m, 1H); 6.61 (s, 1H); 3.94 (t, 2H, 5.7 Hz); 2.69 (td, 2H, 5.7 Hz, 1.2 Hz); 1.85 (qt, 2H, 5.7 Hz). \(^{13}\)CNMR
(CDCl$_3$, 125 MHz): $\delta$ 165.7, 160.5 (d, 240 Hz), 149.8 (d, 31 Hz), 147.0 (d, 15 Hz), 140.4 (d, 4 Hz), 136.2, 135.4 (d, 26 Hz), 126.1, 123.1, 121.1 (d, 4 Hz), 118.5 (d, 29 Hz), 50.5, 25.4, 22.2. HRMS (ESI): [M+H]$^+$ calculated: 268.1244, found: 268.1248.

(E)-3-(5-fluoropyridiniummethylene)-anabaseine [(5-F)-3PAB] (45):

Reaction time: 26 h. After chromatography (DCM:methanol 100:1 to 40:1 gradient), the product was obtained in 74 % yield. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 8.75 (d, 1H, 1.5 Hz); 8.66 (dd, 1H, 4.8 Hz, 1.5 Hz); 8.39 (d, 1H, 3.0 Hz); 8.36 (s, 1H); 7.83 (dt, 1H, 8.0 Hz, 2.0 Hz); 7.35-7.38 (m, 2H); 6.60 (s, 1H); 3.93 (t, 2H, 6.0 Hz); 2.81 (td, 2H, 6.5 Hz, 2.0 Hz); 1.86 (qt, 2H, 6.5 Hz, 6.0 Hz). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 166.0, 159.1 (d, 256 Hz), 149.8 (d, 37 Hz), 146.3 (d, 4 Hz), 137.2, 137.0, 136.2, 135.6, 134.9, 133.1 (d, 4 Hz), 130.0 (d, 2 Hz), 123.2, 122.7 (d, 18 Hz), 50.4, 25.7, 22.2. $^{19}$FNMR (470 MHz, CDCl$_3$ with CFCl$_3$): $\sim$127.1 (d, J= 8.60 Hz). HRMS (ESI): [M+H]$^+$ calculated: 268.1245, found: 268.1247.

(E)-3-(2,6-difluoropyridiniummethylene)-anabaseine [(2,6-DF)-3PAB] (46):

Reaction time: 30 h. After column chromatography (DCM:methanol 100:1 to 60:1 gradient) the product was obtained in 78 % yield. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 8.75
(dd, 1H, 2.0 Hz, 0.5 Hz); 8.65 (dd, 1H, 5.0 Hz, 1.5 Hz); 7.82-7.88 (m, 2H); 7.36 (ddd, 1H, 8.0 Hz, 5.0 Hz, 1.0 Hz); 6.88 (dd, 1H, 8.0 Hz, 2.5 Hz); 6.55 (s, 1H); 3.94 (t, 2H, 5.5 Hz); 2.66 (td, 2H, 6.0 Hz, 2.0 Hz); 1.86 (qt, 2H, 6.0 Hz). ¹³CNMR (CDCl₃, 125 MHz): δ 165.5, 160.7 (dd, 247 Hz, 14 Hz), 158.3 (dd, 248 Hz, 14 Hz), 149.9, 149.6, 144.5 (dd, 7 Hz, 4 Hz), 136.1, 135.4, 135.2, 124.9 (d, 2Hz), 123.1, 115.1 (dd, 27 Hz, 6 Hz), 106.0 (dd, 35 Hz, 6 Hz), 50.5, 25.4, 22.1. HRMS (ESI): [M+H]^+ calculated: 286.1150, found: 286.1156.

(E)-3-(2,4,6-trifluoropyridinylmethylene)-anabaseine [(2,4,6-TF)-3PAB] (47):

Reaction time: 48 h. After column chromatography (DCM: methanol 100:1 to 50:1 gradient), the product was obtained in 70 % yield. ¹H NMR (CDCl₃, 500 MHz): δ 8.77 (s, 1H); 8.65 (dd, 1H, 4.9 Hz, 1.5 Hz); 7.84 (dt, 1H, 8.0 Hz, 1.9 Hz); 7.36 (ddd, 1H, 8.0 Hz, 4.9 Hz, 0.7 Hz); 6.66 (d, 1H, 8.0 Hz); 6.28 (s, 1H), 3.97 (t, 2H, 5.5 Hz); 2.47 (t, 2H, 5.5 Hz); 1.86 (qt, 2H, 5.5 Hz). ¹³CNMR (CDCl₃, 125 MHz): δ 169.7 (ddd, 263 Hz, 12 Hz, 9 Hz); 164.7, 161.4 (dt, 247 Hz, 18 Hz), 158.9 (ddd, 247 Hz, 18 Hz, 12 Hz), 150.0, 149.7, 138.3, 136.2, 135.2, 123.1, 118.8 (d, 2 Hz), 105.0 (ddd, 33 Hz, 19 Hz, 7 Hz), 95.4 (ddd, 39 Hz, 25 Hz, 7 Hz), 50.7, 25.7, 22.1. HRMS (ESI): [M+H]^+ calculated: 304.1056, found: 304.1062.
(E)-3-(2,3,4,5,6-perfluorophenylmethylene)-anabaseine [(2,3,4,5,6-PF)-AB] (48):

Reaction time: 48 h. After chromatography (DCM: methanol 100:1 to 75:1 gradient), the product was obtained in 57 % yield. \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 8.77 (d, 1H, 1.8 Hz); 8.66 (dd, 1H, 5.0 Hz, 1.8 Hz); 7.85 (dt, 1H, 7.5 Hz, 2.1 Hz); 7.35 (ddd, 1H, 7.5 Hz, 5.0 Hz, 0.9 Hz); 6.31 (s, 1H); 3.98 (t, 2H, 6.0 Hz); 2.45-2.50 (m, 2H); 1.86 (qt, 2H, 6.0 Hz)

\(^1^3\)C NMR (CDCl\(_3\), 125 MHz): \(\delta\) 164.5; 150.1; 149.7; 143.7 (dm, 247 Hz); 140.9 (dm, 254 Hz); 138.9; 137.6 (dm, 252 Hz); 136.2; 135.1; 123.1, 118.5-118.8 (m); 110.4 (td, 18 Hz, 4 Hz); 50.8; 25.8; 22.3. HRMS (ESI): [M+H]\(^+\) calculated: 339.0916, found: 339.0925

2,4,6-trifluoronicotinaldehyde (56):

nBuLi (2.5 M in hexanes, 1.40 mL, 3.50 mmol) was added to a solution of 2,4,6-trifluoropyridine (58) (0.33 mL, 3.72 mmol) in THF (18 mL) over 15 min at –78 °C and the mixture was stirred for 30 min. A solution of N-methylformanilide (59) (0.43 mL, 3.48 mmol) in THF (2 mL) was added over 10 min, and the mixture was further stirred at –78 °C for 2 h, and then let warm to room temperature over 2 h. 1M HCl was added, the mixture was extracted with diethyl ether, dried (MgSO\(_4\)) and evaporated. Purification by column chromatography (hexanes: ethyl acetate, 10:1) gave the product as a white solid (325 mg, 58 % yield). \(^1\)H NMR (CDCl\(_3\), 500 MHz): \(\delta\) 10.23 (t, 1H, 1.0 Hz); 6.73 (dd, 1H, 8.5 Hz, 1.8 Hz). \(^1^3\)C NMR (CDCl\(_3\), 125 MHz): \(\delta\) 181.9-181.2 (m), 173.0 (ddd,
278.5 Hz, 13.2 Hz, 6.6 Hz); 164.4 (dt, 253.7 Hz, 19.7 Hz); 162.9 (258.4 Hz, 19.8 Hz, 9.4 Hz); 107.0-106.7 (m), 97.3-96.5 (m). HRMS (GC/Cl): [M+H]⁺ calculated: 162.0167, found: 162.0165.

A.5 Quinuclidines

![Structure of 2-(Chloromethyl)thiophene (75)](image)

2-(Chloromethyl)thiophene (75):²²⁹

A solution of thionyl chloride (5.2 mL, 66 mmol) in dichloromethane (20 mL) was added at 0 ºC to a solution of 2-hydroxymethylthiophene (73, 3.01 g, 26 mmol) and pyridine (3.8 mL, 47 mmol) in dichloromethane (50 mL). The mixture was stirred at room temperature for 2 h, poured into water (60 mL), the layers were separated, the aqueous layer was extracted with dichloromethane, the combined organic layers were washed twice with 10 % aqueous sodium bicarbonate and brine, dried over MgSO₄, and evaporated to give the product as a lightly yellow oil (3.33 g, 95 % yield). ¹H NMR (CDCl₃, 300 MHz): δ 7.31 (dd, 1H, 5.0 Hz, 1.2 Hz); 7.08-7.10 (m, 1H); 6.95 (dd, 1H, 5.0 Hz, 3.5 Hz); 4.82 (s, 2H). The ¹H NMR data matched the reported data.²²⁹ ¹³C NMR (CDCl₃, 75 MHz): δ 140.1, 127.6, 126.9, 126.9, 40.3.

![Structure of 2-(Chloromethyl)furan (76)](image)

2-(Chloromethyl)furan (76):

The compound was prepared from 2-hydroxymethylfuran (74) analogously to 2-(Chloromethyl)thiophene (75) but the reaction was run at −5 ºC. Reaction time: 1 h.
Brown oil. Yield: 64 %. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.42 (dd, 1H, 1.7 Hz, 0.8 Hz); 6.34-6.38 (m, 2H); 4.60 (s, 2H). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 150.0, 143.4, 110.7, 109.7, 37.4. The $^{13}$CNMR for CH$_2$Cl carbon differed from the reported data (37.4 vs 63.4).$^{228}$

![Cl]

3-(Chloromethyl)thiophene (83):

The compound was prepared from 3-hydroxymethylthiophene (81) analogously to 2-(Chloromethyl)thiophene (75). Reaction time: 3 h. Yellow oil. 91 % yield. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.34 (dd, 1H, J= 4.8 Hz, 2.7 Hz); 7.29-7.31 (m, 1H); 7.14 (dd, 1H, J= 4.8 Hz, 1.2 Hz); 4.64 (s, 2H). The $^1$HNMR data matched the literature data.$^{354}$ $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 138.0, 127.5, 126.6, 124.0, 40.6.

![Cl]

3-(Chloromethyl)furan (84):

The compound was prepared from 3-hydroxymethylfuran (82) analogously to 2-(Chloromethyl)thiophene (75). Reaction time: 2 h. Yield: 84 %. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.46-7.47 (m, 1H); 7.41 (t, 1H, 1.5 Hz); 6.45-6.46 (m, 1H); 4.49 (m, 2H). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 143.8, 140.7, 122.4, 110.3, 37.1. The NMR data matched the reported data.$^{355}$

206
Diethyl (thiophene-2-ylmethyl)phosphonate (77):

2-(Chloromethyl)thiophene (75) (1.035 g, 7.80 mmol) and triethylphosphite (1.47 mL, 8.57 mmol) were heated at 140 °C for 4 h. The crude was purified by vacuum distillation to give the product as a colorless oil (1.180 g, 65 % yield, bp. 114-120 °C at 0.5 Torr). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.17-7.26 (m, 1H); 6.94-7.00 (m, 2H); 4.02-4.12 (m, 4H); 3.37 (d, 2H, J= 20.7 Hz); 1.28 (t, 6H, J= 6.9 Hz). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 132.4 (d, J= 10.3 Hz); 127.2 (d, J= 8.3 Hz); 127.0 (d, J= 3.5 Hz); 124.7 (d, J= 3.8 Hz); 62.3 (d, J= 6.5 Hz); 27.9 (d, J= 143.2 Hz); 16.3 (d, J= 6.0 Hz). The NMR data matched the reported data.$^{356}$

Dimethyl (thiophene-2-ylmethyl)phosphonate (79):

2-(Chloromethyl)thiophene (75) (3.35 g, 25.3 mmol) and trimethylphosphite (3.28 mL, 27.8 mmol) were heated at reflux for 3 h. The crude was purified by vacuum distillation to give the pure product as a colorless oil (2.92 g, 56 %, bp. 110-112 °C at 0.5 Torr). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.18-7.21 (m, 1H); 6.94-7.00 (m, 2H); 3.72 (d, 6H, J= 10.8 Hz); 3.38 (dd, 2H, J= 20.7 Hz, 0.7 Hz). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 131.7 (d, J= 10.9 Hz); 127.1 (d, J= 9.1 Hz); 126.9 (d, J= 3.2 Hz); 124.6 (d, J= 3.2 Hz); 52.8 (d, J= 6.9 Hz), 26.7 (d, J= 143.4 Hz). The NMR data matched the literature data.$^{357}$

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Diethyl (furan-2-ylmethyl)phosphonate (78):

The compound was prepared from 2-(chloromethyl)furan (76) analogously to diethyl (thiophene-2-ylmethyl)phosphonate (77). Reflux 1 h. Colorless oil. 65% yield. Bp. 102-110 °C at 0.5 Torr. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.34-7.36 (m, 1H); 6.33-6.34 (m, 1H), 6.23-6.26 (m, 1H); 4.03-4.13 (m, 4H); 3.24 (d, 2H, $J = 20.7$ Hz); 1.29 (t, 6H, $J = 7.2$ Hz). The $^1$HNMR data matched the reported data. $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 145.6 (d, $J = 9.5$ Hz); 141.9 (d, $J = 3.4$ Hz); 110.8 (d, $J = 2.9$ Hz); 108.1 (d, $J = 7.4$ Hz); 62.2 (d, $J = 6.6$ Hz); 26.7 (d, $J = 143.7$ Hz); 16.3 (d, $J = 6.0$ Hz).

Dimethyl (furan-2-ylmethyl)phosphonate (80):

The compound was prepared from 2-(chloromethyl)furan (76) analogously to dimethyl (thiophene-2-ylmethyl)phosphonate (79). Reflux 1 h. Orange oil. 30% yield. Bp. 85-95 °C at 0.4 Torr. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.36-7.37 (m, 1H); 6.33-6.35 (m, 1H); 6.24-6.27 (m, 1H); 3.74 (d, 6H, $J = 10.8$ Hz); 3.27 (d, 2H, $J = 21.0$ Hz). The $^1$HNMR data matched the literature data. $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 144.9 (d, 10.1 Hz); 141.8 (d, 3.6 Hz); 110.6 (d, 3.1 Hz); 108.2 (d, 7.8 Hz); 52.6 (d, 6.9 Hz); 25.4 (d, 143.2 Hz).
Diethyl (thiophene-3-ylmethyl)phosphonate (85):

The compound was prepared from 3-(Chloromethyl)thiophene (83) analogously to diethyl (thiophene-2-ylmethyl)phosphonate (77). Colorless oil. Yield 57 %. Bp. 105-115 °C at 0.5 Torr. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.25 (dd, 1H, J= 5.0 Hz, 3.0 Hz); 7.13 (t, 1H, 3.0 Hz); 7.04 (dt, 1H, 5.0 Hz, 1.4 Hz); 3.95-4.05 (m, 4H); 3.17 (d, 2H, 21.1 Hz); 1.23 (t, 6H, 7.0 Hz). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 130.8 (d, J= 8.8 Hz); 128.9 (d, J= 4.6 Hz); 125.6 (d, J= 1.7 Hz), 123.0 (d, J= 9.7 Hz), 62.0 (d, J= 6.6 Hz); 28.2 (d, J= 140.5 Hz); 16.3 (d, J= 5.7 Hz). The NMR data matched the literature data.$^{359}$

Diethyl (furan-3-ylmethyl)phosphonate (86):

The compound was prepared from 3-(Chloromethyl)furan (84) analogously to diethyl (thiophene-2-ylmethyl)phosphonate (77). Colorless oil. Yield 54 %. Bp. 100-110 °C at 0.5 Torr. $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.37-7.39 (m, 2H); 6.42 (d, 1H, 1.2 Hz); 4.03-4.13 (m, 4H); 2.96 (d, 2H, 20.4 Hz); 1.29 (td, 6H, 6.9 Hz, 0.6 Hz). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 142.8; 140.5 (d, J= 11.1 Hz); 114.6 (d, J= 8.8 Hz); 111.7 (d, 4.9 Hz); 61.9 (d, J= 6.5 Hz); 22.9 (d, J= 143.5 Hz); 16.3 (d, J= 6.0 Hz).
2TQN: (Z)-3-(thiophen-2-ylmethylene)quinuclidine (70a) and (E)-3-(thiophen-2-ylmethylene)quinuclidine (70b):

A solution of diethyl (thiophene-2-ylmethyl)phosphonate (77) (600 mg, 2.56 mmol) in THF (4 mL) was added to a suspension of sodium hydride 60 % dispersion in mineral oil (106 mg, 2.65 mmol) in THF (12 mL) at room temperature and a mixture was stirred at 50 ºC for 1 h. The mixture was cooled to room temperature, a solution of 3-quinuclidinone (72, 247 mg, 1.97 mmol) in THF (4 mL) was added, and the mixture was heated at reflux for 1.5 h. The mixture was cooled to room temperature, water was added, and the mixture was extracted with ethyl acetate, washed with brine, dried (MgSO₄), and evaporated. The crude product was purified by column chromatography on silica gel pretreated with Et₃N in hexanes (gradient of hexanes:ethyl acetate 4:1 + 0.3 % Et₃N to hexanes:ethyl acetate 1:2 + 0.3 Et₃N) to give 170 mg of the Z isomer (42 % yield) and 174 mg of the E isomer (43 % yield) as white solids.

Z isomer: Rᵣ= 0.13 (hexanes: ethyl acetate: Et₃N 1: 1: 0.1). ¹H NMR (CDCl₃, 300 MHz): δ 7.23 (d, 1H, 5.1 Hz); 7.01 (dd, 1H, 3.6 Hz, 5.1 Hz); 6.88 (d, 1H, 3.6 Hz); 6.44 (t, 1H, 2.5 Hz); 3.75 (s, 2H), 2.82-3.01 (m, 4H); 2.47 (quintet, 1H, 3.3 Hz); 1.71-1.84 (m, 4H). ¹³CNMR (CDCl₃, 125 MHz): δ 145.0, 141.1, 127.0, 125.4, 124.4 113.5, 56.0, 47.6, 33.3, 28.1. HRMS (DART): [M+H]⁺ calculated: 206.0998, found: 206.1000.

E isomer: Rᵣ= 0.10 (hexanes: ethyl acetate: Et₃N 1: 1: 0.1). ¹H NMR (CDCl₃, 300 MHz): δ 7.16 (dd, 1H, 5.2 Hz, 0.9 Hz); 6.96 (dd, 1H, 5.2 Hz, 3.6 Hz); 6.85 (d, 1H, 3.6 Hz); 6.31 (t, 1H, 1.8 Hz); 3.55 (s, 2H); 3.30 (quintet, 1H, 3.0 Hz); 2.83-3.00 (m, 4H); 1.69-1.81 (m,
The free base 3-quinuclidinone (72) used in the procedure above was obtained by treating quinuclidine hydrochloride with a 2 M aqueous solution of K$_2$CO$_3$, extraction with diethyl ether, drying (MgSO$_4$), and removal of the solvent on the rotovap.

(Z)-3-(thiophen-2-ylmethylene)quinuclidin-1-ium chloride:

(Z)-3-(thiophen-2-ylmethylene)quinuclidine (70a) was dissolved in ethanol, and a solution of HCl in ethanol was added. The solvents were removed on the rotovap to give the product quantitatively. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 12.98 (s, 1H); 7.38 (d, 1H, 5.2 Hz); 7.08 (dd, 1H, 5.2 Hz, 3.6 Hz); 6.97 (d, 1H, 3.6 Hz); 6.69 (t, 1H, 2.5 Hz); 4.18 (s, 2H); 3.39-3.45 (m, 2H); 3.29-3.35 (m, 2H); 2.84 (quintet, 1H, 3.0 Hz); 2.11-2.15 (m, 4H).

$^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 137.9, 129.1, 127.7, 127.7, 126.7, 118.7, 53.6, 46.7, 31.2, 24.3.

(E)-3-(thiophen-2-ylmethylene)quinuclidin-1-ium chloride:

$^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 12.59 (s, 1H); 7.29 (d, 1H, 5.0 Hz); 7.03 (dd, 1H, 5.0 Hz, 3.5 Hz); 6.98 (d, 1H, 3.5 Hz); 6.52 (s, 1H); 4.03 (s, 2H); 3.63 (t, 1H, 3.0 Hz); 3.42-3.46
(m, 2H); 3.32-3.38 (m, 2H); 2.05-2.17 (m, 4H). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 137.3, 129.9, 128.5, 127.2, 125.7, 118.8, 54.3, 46.5, 25.4, 23.2.

$^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.36 (d, 1H, 1.8 Hz); 6.39 (dd, 1H, 3.6 Hz, 1.8 Hz); 6.08-6.10 (m, 2H); 3.78 (s, 2H); 2.81-3.00 (m, 4H); 2.45 (quintet, 1H, 3.0 Hz); 1.72-1.80 (m, 4H). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 153.2, 145.5, 140.9, 111.2, 109.0, 107.2, 55.9, 47.6, 33.0, 28.0.

2-FQN: (Z)-3-(furan-2-ylmethylene)quinuclidine (68a) and (E)-3-(furan-2-ylmethylene)quinuclidine (68b):

The compounds were prepared from diethyl (furan-2-ylmethyl)phosphonate (78) and 3-quinuclidinone (72) analogously to (Z)-3-(thiophen-2-ylmethylene)quinuclidine (70a) and (E)-3-(thiophen-2-ylmethylene)quinuclidine (70b):

**Z isomer:** white solid, yield: 43 %. $R_f$ = 0.13 (hexanes: ethyl acetate: Et$_3$N 1: 1: 0.1). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.36 (d, 1H, 1.8 Hz); 6.39 (dd, 1H, 3.6 Hz, 1.8 Hz); 6.08-6.10 (m, 2H); 3.78 (s, 2H); 2.81-3.00 (m, 4H); 2.45 (quintet, 1H, 3.0 Hz); 1.72-1.80 (m, 4H). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 153.2, 145.5, 140.9, 111.2, 109.0, 107.2, 55.9, 47.6, 33.0, 28.0.

**E isomer:** brown solid, yield: 42 %. $R_f$ = 0.10 (hexanes: ethyl acetate: Et$_3$N 1: 1: 0.1). $^{1}$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.32 (d, 1H, 1.8 Hz); 6.35 (dd, 1H, 3.3 Hz, 1.8 Hz); 6.12 (d, 1H, 3.3 Hz); 5.96 (t, 1H, 2.1 Hz); 3.54 (s, 2H); 3.44 (quintet, 1H, 3.0 Hz); 2.83-3.00 (m, 4H); 1.69-1.81 (m, 4H). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 152.9, 144.9, 140.7, 110.9, 108.6, 107.3, 56.4, 47.6, 26.7, 26.5.
2-FQN: (Z)-3-(furan-2-ylmethylene)quinuclidin-1-ium chloride:

$^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 12.77 (s, 1H); 7.45 (d, 1H, 1.6 Hz); 6.43 (dd, 1H, 2.8 Hz, 1.6 Hz); 6.28 (d, 2H, 2.8 Hz); 4.35 (s, 2H); 3.39-3.45 (m, 2H); 3.29-3.35 (m, 2H); 2.79 (quintet, 1H, 3.0 Hz); 2.09-2.13 (m, 4H). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 151.0, 143.3, 129.0, 113.4, 111.5, 110.4, 54.1, 46.6, 30.9, 24.3.

3-TQN: (Z)-3-(thiophen-3-ylmethylene)quinuclidine (71a) and (E)-3-(thiophen-3-ylmethylene)quinuclidine (71b):

The compounds were prepared from diethyl (thiophene-3-ylmethyl)phosphonate (85) and 3-quinuclidinone (72) analogously to (Z)-3-(thiophen-2-ylmethylene)quinuclidine (70a) and (E)-3-(thiophen-2-ylmethylene)quinuclidine (70b). The ratio of Z:E isomers based on the crude $^1$H NMR was 3:2. The products were obtained in an estimated 50 % yield (the E isomer was not obtained in a pure form).

**Z-isomer**: white solid. $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 7.29 (dd, 1H, 5.1 Hz, 3.0 Hz); 7.02-7.07 (m, 2H); 6.26 (t, 1H, 2.7 Hz); 3.78 (s, 2H); 2.83-3.01 (m, 4H); 2.46 (quintet, 1H, 3.0 Hz); 1.76-1.81 (m, 4H). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 145.3, 139.0, 128.4, 125.1, 121.4, 114.5, 56.2, 47.7, 33.4, 28.2.

**E-isomer**: $^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 12.82 (s, 1H); 7.36 (dd, 1H, 5.0 Hz, 3.0 Hz); 7.10 (dd, 1H, 3.0 Hz, 1.0 Hz); 7.00 (dd, 1H, 5.0 Hz, 1.0 Hz); 6.51 (t, 1H, 2.5 Hz); 4.18 (s,
2H); 3.29-3.43 (m, 4H); 2.81 (quintet, 1H, 3.0 Hz); 2.10-2.13 (m, 4H). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 136.4, 130.2, 127.8, 126.8, 124.4, 120.3, 54.1, 47.0, 31.5, 24.7.

(Z)-3-(thiophen-3-ylmethylene)quinuclidin-1-iium chloride:
$^1$H NMR (CDCl$_3$, 500 MHz): $\delta$ 12.82 (br s, 1H); 7.36 (dd, 1H, 5.0 Hz, 3.0 Hz); 7.10 (dd, 1H, 3.0 Hz, 1.0 Hz); 7.00 (dd, 1H, 5.0 Hz, 1.0 Hz); 6.51 (t, 1H, 2.5 Hz); 4.18 (s, 2H); 3.29-3.43 (m, 4H); 2.81 (quintet, 1H, 3.0 Hz); 2.10-2.13 (m, 4H). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta$ 136.4, 130.2, 127.8, 126.8, 124.4, 120.3, 54.1, 47.0, 31.5, 24.7.

3-FQN: (Z)-3-(furan-3-ylmethylene)quinuclidine (69a) and (E)-3-(furan-3-ylmethylene)quinuclidine (69b):
The compounds were prepared from diethyl (furan-3-ylmethyl)phosphonate (86) (2.7 equivalent) and 3-quinuclidinone (72) analogously to (Z)-3-(thiophene-2-ylmethylene)quinuclidine (70a) and (E)-3-(thiophen-2-ylmethylene)quinuclidine (70b). The ratio of Z:E isomers based on the crude $^1$H NMR was 3:2. The products were obtained in an estimated 50 % yield (the products were not obtained in a pure form).

Z-isomer: $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.40 (t, 1H, 1.8 Hz); 7.36 (br s, 1H); 6.38 (d, 1H, 1.8 Hz); 6.09 (t, 1H, 2.4 Hz); 3.75 (s, 2H); 3.11-2.92 (m, 4H); 2.55 (quintet, 1H, 3.1 Hz); 1.88-1.82 (m, 4H).
**E-isomer:** $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.39 (t, 1H, 1.7 Hz); 7.36 (br s, 1H); 6.36 (d, 1H, 1.7 Hz); 6.03 (br s, 1H); 3.76 (s, 2H); 3.20-3.06 (m, 5H); 1.95-1.85 (m, 4H).

*Tert-Butyl 2-formyl-1H-pyrrole-1-carboxylate (88).*

4-Dimethylaminopyridine (162 mg, 1.33 mmol), and di-tert-butyldicarbonate (3.557 g, 16.3 mmol) were added to a solution of pyrrole-2-carboxaldehyde (87) (1.50 g, 15.8 mmol) in acetonitrile (25 mL), and stirred at room temperature for 24 h. Solvent was evaporated on the rotovap and the residue was purified by column chromatography using hexanes:ethyl acetate (20:1) as an eluent to give the product (3.05 g, 99 % yield). $R_f=0.37$ (hexanes:ethyl acetate, 10:1). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 10.32 (s, 1H); 7.43 (dd, 1H, 3.1 Hz, 1.8 Hz); 7.19 (dd, 1H, 3.7 Hz, 1.8 Hz); 6.29 (td, 1H, 3.7 Hz, 3.1 Hz, 0.6 Hz); 1.65 (s, 9H).

*Tert-Butyl 2-(hydroxymethyl)-1H-pyrrole-1-carboxylate (89).*

To a solution of aldehyde from above (2.726 g, 14.0 mmol) in methanol (30 mL) at 0 °C was added sodium borohydride (528 mg, 14.0 mg) in portions. The mixture was stirred for 50 min, cold water was added, the mixture was extracted with diethyl ether, and washed with saturated aqueous NaHCO$_3$ solution and brine. The organic layer was dried over MgSO$_4$ and evaporated to give the product as colorless oil which was satisfactory pure by $^1$HNMR (2.47 g, 90 % yield). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.14-
7.16 (m, 1H); 6.15-6.17 (m, 1H); 6.08 (td, 1H, 3.3 Hz, 1.5 Hz); 4.63 (d, 2H, J= 7.2 Hz);
3.61 (t, 1H, J= 7.2 Hz, OH); 1.59 (s, 9H). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 9.67 (t, 1H, 0.85 Hz); 7.58-7.60 (m, 1H); 7.20 (ddd, 1H, 3.7 Hz, 1.8 Hz, 0.6 Hz); 6.40 (ddd, 1H, 3.7 Hz, 3.1 Hz, 0.6 Hz); 3.61 (d, 3H, 0.85 Hz).

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 9.67 (t, 1H, 0.85 Hz); 7.58-7.60 (m, 1H); 7.20 (ddd, 1H, 3.7 Hz, 1.8 Hz, 0.6 Hz); 6.40 (ddd, 1H, 3.7 Hz, 3.1 Hz, 0.6 Hz); 3.61 (d, 3H, 0.85 Hz).

1-(Methylsulfonyl)-1H-pyrrole-2-carbaldehyde (91):$^{231}$

A solution of 2-pyrrolecarboxaldehyde (0.997 g, 10.5 mmol) in THF (10 mL) was added to a stirred suspension of sodium hydride (505 mg, 12.6 mmol of a 60 % dispersion in mineral oil washed twice with hexanes) in THF (30 mL). The resulting mixture was stirred at room temperature for 15 min. A solution of mesyl chloride (1.14 mL, 14.7 mmol) in THF (10 mL) was added dropwise, and the mixture was stirred for 1.5 h at room temperature. Water was added (50 mL), THF was removed on the rotovap, and the resulting mixture was extracted with ethyl acetate, washed with brine, dried over MgSO$_4$, and evaporated. The crude product was purified by column chromatography, using hexanes:ethyl acetate 10:1 as an eluent to give the product as a silver solid (931 mg, 51 %). $R_f$= 0.17 (hexanes:ethyl acetate, 4:1). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 9.67 (t, 1H, 0.85 Hz); 7.58-7.60 (m, 1H); 7.20 (ddd, 1H, 3.7 Hz, 1.8 Hz, 0.6 Hz); 6.40 (ddd, 1H, 3.7 Hz, 3.1 Hz, 0.6 Hz); 3.61 (d, 3H, 0.85 Hz).
[1-(Methylsulfonyl)-1H-pyrrol-2-yl]methanol (92): Sodium borohydride (200 mg, 5.29 mmol) was added at 0 °C to a solution of the aldehyde above (91) (642 mg, 3.71 mmol) in THF (40 mL). After stirring at 0 °C for 1.5 h, water (15 mL) and then acetic acid (7 mL, 10 % solution in water) were carefully added, the mixture was extracted with ethyl acetate, washed with water and brine, dried over MgSO₄, and evaporated. Purification by column chromatography (hexanes:ethyl acetate, 10:1 to 4:1 gradient) afforded the product as an off-white solid (586 mg, 90 %). \( R_f = 0.16 \) (hexanes:ethyl acetate, 2:1). \(^1\)H NMR (CDCl₃, 300 MHz): \( \delta \) 7.16 (dd, 1H, J= 3.3 Hz, J= 1.8 Hz); 6.30 (dd, 1H, J= 3.3 Hz, 1.8 Hz); 6.24 (t, 1H, J= 3.3 Hz); 4.77 (d, 2H, J= 6.0 Hz); 3.30 (s, 3H); 2.41 (t, 1H, 6.0 Hz, OH). \(^13\)CNMR (CDCl₃, 75 MHz): \( \delta \) 133.7, 123.3, 115.4, 111.4, 56.7, 43.0. The NMR data matched the reported data.

2-(Chloromethyl)-1-(methylsulfonyl)-1H-pyrrole (93): Mesyl chloride (0.40 mL, 5.22 mmol) was added to a solution of alcohol (92) (571 mg, 3.26 mmol) and triethylamine (0.74 mL, 5.24 mmol) at 0 °C. The mixture was stirred at 0 °C for 1.5 h, diluted with dichloromethane, and washed with ice-cold water, cold 1 M HCl, saturated NaHCO₃, and brine. The organic phase was dried (MgSO₄) and evaporated to give the product as an orange oil that was satisfactory pure by \(^1\)HNMR and used without further purification (618 mg, 98 %). \(^1\)H NMR (CDCl₃, 300 MHz): \( \delta \) 7.21
(dd, 1H, J= 3.3 Hz, J= 1.8 Hz); 6.42 (dd, 1H, 3.3 Hz, 1.8 Hz); 6.27 (t, 1H, 3.3 Hz); 4.94 (s, 2H); 3.39 (s, 3H). The NMR data matched the literature data.

Diethyl {[1-(methylsulfonyl)-1H-pyrrol-2-yl]methyl}phosphonate (94):

2-(Chloromethyl)-1-(methylsulfonyl)-1H-pyrrole (93) (4.140 g, 21.4 mmol) and triethyl phosphite (3.68 mL, 21.4 mmol) were stirred at 130 ºC for 50 min. The mixture was cooled and purified by column chromatography (hexanes:ethyl acetate, 10:1 to 3:1 gradient) to give the product as a lightly yellow solid of acceptable purity (see NMR, estimated 12 % yield). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.13-7.15 (m, 1H); 6.25-6.30 (m, 2H); 4.06-4.15 (m, 4H); 3.58 (d, 2H, J$_{P-CH_2}$= 21.6 Hz); 3.46 (s, 3H); 1.32 (t, 6H, J= 7.1 Hz). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 124.0 (d, J= 8.8 Hz); 122.2 (d, J= 3.7 Hz); 114.8 (d, J= 7.4 Hz); 111.3 (d, J= 3.7 Hz); 62.1 (d, J= 6.8 Hz); 42.9; 24.2 (d, J= 143.6 Hz); 16.1 (d, J= 8.0 Hz).

A.6 Strigolactones

Cyclohex-1-ene-1,2-diyldimethanol (112):

Solution of 3,4,5,6-tetrahydrophthalic anhydride (985 mg, 6.5 mmol) in THF (15 mL) was added slowly to a suspension of lithium aluminium hydride (401 mg, 10.6 mmol) in THF (35 mL) at 0 ºC and then stirred for 30 min at room temperature and for 2 h at reflux. The reaction mixture was cooled down to 0 ºC and water (0.4 mL), 15 % sodium
hydroxide (0.4 mL), and water (1.2 mL) were carefully added. The resulting mixture was
diluted with ethyl acetate (50 mL), filtered through celite, eluted with ethyl acetate (100
mL), dried (MgSO₄), and evaporated under reduced pressure. The crude product was
purified by column chromatography (hexane-ethyl acetate 5:1 to 1:2 gradient) to yield
289 mg (31 % yield) of the pure product. Rᵣ = 0.08 (petroleum ether-ethyl acetate 1:1,
KMnO₄). ¹H NMR (CDCl₃, 300 MHz): δ = 4.05 (4H, s); 3.75 (2H, br s); 2.11 (4H, s); 1.59
(4H, s). ¹³C NMR (CDCl₃, 75 MHz): δ = 135.0, 63.0, 28.6, 22.7.

(2-(((tert-butyldimethylsilyl)oxy)methyl)cyclohex-1-en-1-yl)methanol (113):
Solution of tert-butyldimethyl silyl chloride (687 mg, 4.6 mmol) in dichloromethane
(3 mL) was added at 0 °C to the solution of diol (650 mg, 4.6 mmol) and imidazole (454
mg, 6.7 mmol) in dichloromethane (23 mL) and stirred at room temperature for 3 h. 1M
hydrochloric acid was added and the mixture was extracted with dichloromethane,
washed with brine, dried (MgSO₄), and evaporated. The crude was purified by column
chromatography (hexane-ethyl acetate 50:1 to 10:1 gradient) to yield 373 mg (32 %
yield) of the pure product. Rᵣ = 0.20 (hexanes-ethyl acetate 10:1, KMnO₄). ¹H NMR
(CDCl₃, 300 MHz): δ = 4.15 (2H, s); 4.06 (2H, s); 2.13 (2H, s); 2.05 (2H, s); 1.60 (4H, m);
0.90 (9H, s); 0.09 (s, 6H). ¹³C NMR (CDCl₃, 75 MHz): δ = 134.4, 134.0, 63.4, 63.0, 28.5,
28.1, 22.7, 22.6, 25.9, −5.3.
2-((tert-butyldimethylsilyl)oxy)methyl)cyclohex-1-ene-1-carbaldehyde (109):
DMSO (51 mg, 0.65 mmol) in dichloromethane (2 mL) was added over 15 min to a
solution of oxalyl chloride (113 mg, 0.89 mmol) in dichloromethane (1 mL) at -78 °C and
the reaction mixture was stirred for 20 min (making sure that the temperature of the
reaction mixture did not exceed ~60 °C as the chlorosulfonium intermediate
decomposes above that temperature). The monoprotected diol (155 mg, 0.60 mmol) in
dichloromethane (1 mL) was added at -78 °C and the reaction mixture was stirred for 40
min. Triethylamine (220 µL, 1.6 mmol) was added in one portion and the reaction
mixture was stirred at -78 °C. After 15 min the mixture was allowed to warm to room
temperature and diluted with dichloromethane. The organic layer was washed with
saturated ammonium chloride solution and brine, dried (MgSO₄), and evaporated to
give 115 mg (74 % yield) of almost pure product as colorless oil. Rᵢ= 0.61
(hexanes:ethyl acetate 10:1). ¹HNMR (CDCl₃, 300 MHz): δ= 10.19 (1H, s); 4.57 (2H, s);
2.32 (2H, m); 2.21 (2H, m); 1.61 (4H, m); 0.90 (9H, s); 0.09 (6H, s). ¹³CNMR (CDCl₃, 75
MHz): δ= 190.6, 156.9, 134.5, 61.2, 28.8, 25.8, 22.3, 21.8, 21.4, -5.4.

Methyl (R)-2-methyl-3-(tosyloxy)propanoate (116):
To the solution of methyl-(R)-3-hydroxy2-methylpropionate (10.1 g, 85.5 mmol) in
pyridine (110 mL) at -5 °C tosyl chloride (17.3 g, 90.6 mmol) was added and the
reaction mixture was stirred at 0 °C for 4 h and then kept in the refrigerator (8 °C) for 2
days. Ice and water were added and the resulting milky suspension was extracted with ether. The combined organic layers were washed with 1 M HCl, water, and brine, and dried over MgSO₄. The solvents were removed under reduced pressure to give the tosylate in 98 % yield (22.8 g) as colorless oil. Rᵣₐ₇ = 0.54 (petroleum ether-ethyl acetate 2:1). ¹HNMR (300 MHz, CDCl₃): δ = 7.78 (2H, d, 7.8 Hz); 7.36 (2H, d, 7.8 Hz); 4.19 (1H, dd, 9.8 Hz, 6.7 Hz); 4.06 (1H, dd, 9.8 Hz, 6.0 Hz); 3.64 (3H, s); 2.83 (1H, quintet, 7.2 Hz, 6.3 Hz); 2.46 (3H, s); 1.17 (3H, d, 7.2 Hz).

![Methyl (S)-3-iodo-2-methylpropanoate](image)

Methyl (S)-3-iodo-2-methylpropanoate (117):²⁹⁷

A solution of tosylate 116 (23.3 g, 85.5 mmol) in THF (50 mL) was added to a solution of lithium iodide (13.5 g, 101 mmol) in THF (110 mL) and the reaction mixture was heated at reflux for 20 min. A yellow suspension formed. Ether was added to a cooled mixture to precipitate more solids and the solids were filtered off and the residue was washed with ether. The organic solution was washed with saturated ammonium chloride solution, 5 % sodium thiosulfate solution, brine and dried (MgSO₄). The solvents were removed under reduced pressure to yield iodide ester 117 in 86 % yield (16.7 g).

¹HNMR (300 MHz, CDCl₃): δ = 3.71 (3H, s); 3.36 (1H, dd, 9.9 Hz, 6.6 Hz); 3.24 (1H, dd, 9.9 Hz, 6.2 Hz); 2.79 (1H, sextet, 6.7 Hz); 1.26 (3H, d, 7.0 Hz).
(S)-3-iodo-2-methylpropanoic acid (118):\textsuperscript{297}

Iodide ester 117 (5.97 g, 15.7 mmol) was added to a solution of sodium iodide (15.6 g, 104 mmol) in acetonitrile (150 mL), after which trimethylsilyl chloride (13.2 mL, 104 mmol, freshly distilled from CaH\textsubscript{2}) was added. The mixture became very cloudy immediately due to precipitation of sodium chloride. The reaction mixture was heated under reflux for 86 h. Water (40 mL) was added at room temperature and the resulting clear solution was stirred for 2 h. The mixture was extracted with ether and washed with 15 \% sodium thiosulfate. The acid iodide was extracted with 10 \% sodium carbonate solution, acidified to pH 2 with 2 M HCl, extracted with ether, washed with brine, dried (MgSO\textsubscript{4}) and evaporated to yield 2.80 g (50 \% yield) of the product. \textsuperscript{1}HNMR (300 MHz, CDCl\textsubscript{3}): $\delta$ = 7.34 (s, broad, 1H); 3.39 (1H, dd, 10.0 Hz, 6.2 Hz); 3.27 (1H, dd, 10.0 Hz, 6.2 Hz); 2.82 (1H, sextet, 7.0 Hz, 6.8 Hz, 6.2 Hz); 1.31 (3H, d, 7.1 Hz).

(S)-(2-carboxypropyl)triphenylphosphonium iodide (110):\textsuperscript{297}

A solution of acid iodide (118, 1.42 g, 6.63 mmol) in acetonitrile (25 mL) was added to a suspension of triphenylphosphine (13.76 g, 52.4 mmol) in acetonitrile (20 mL) and the reaction mixture was heated under reflux for 40 h. The solvent was distilled off under reduced pressure, 20 mL of ether was added and it was stirred for 2 h. The solvent was syringed out to give a gummy solid which was again stirred with ether (20 mL) for 2 h. This procedure was repeated until fine white powder remained (10 times) to yield 2.60 g
(S)-(3-methoxy-2-methyl-3-oxopropyl)triphenylphosphonium iodide:

Iodide ester 117 (1.374 g, 6.02 mmol) in acetonitrile (15 mL) was added to a suspension of triphenylphosphine (12.334 g, 47.0 mmol) in acetonitrile (45 mL) and the reaction mixture was heated at reflux for 40 h. The solvent was removed under reduced pressure, ether (12 mL) was added and it was stirred for 2 h. Ether was syringed out to give a sticky solid. Ether was added (12 mL) and this procedure was repeated until fine white powder remained. Yield: 1.394 g, 47 %. $^1$HNMR (300 MHz, CDCl$_3$): $\delta$ = 7.66-7.88 (15 H, m); 4.50 (1H, m); 3.56 (1H, m); 3.21 (3H, s); 2.96 (1H, m); 1.60 (3H, dd, 7.3 Hz, 1.9 Hz).

(R,E)-4-(cyclohex-1-en-1-yl)-2-methylbut-3-enoic acid:

Li-TMP was prepared by adding nBuLi (1.6 M, 8.41 mL, 13.46 mmol) to 2,2,6,6,-tetramethylpiperidine (2.40 mL, 14.14 mmol, freshly distilled from CaH$_2$ by vacuum distillation) in THF (9.00 mL) at −78 °C and stirring at −78 °C for 15 min and at −5 to 0 °C for 35 min. The ylide was prepared by adding Li-TMP (5.36 mL, 3.64mmol) over 1 h to a suspension of phosphonium salt 110 (880 mg, 1.85 mmol) in THF (10 mL) at −30 to −20 °C and stirring at −10 to 0 °C for 1 h and at room temperature for 2 h. The
aldehyde (97 mg, 0.88 mmol) in THF (2 mL) was added to the ylide at −30 °C and the reaction mixture was stirred at −30 °C for 1 h and at room temperature overnight. Water was added (5 mL) and the mixture was acidified to pH 3 with 1M HCl, and extracted with ether. The ether layer was then extracted with 10 % sodium carbonate solution. The aqueous layer was acidified to pH 2 with 1M HCl, extracted with ether, dried (MgSO₄) and evaporated to give orange oil (78 mg) that was further purified by column chromatography (hexane-ethyl acetate 10:1 to 3:1 gradient) to give the product (47 mg, 25 % yield) containing small impurities (2-methylacrylic acid).

1H NMR (300 MHz, CDCl₃): δ= 11.30 (br s, 1H); 6.15 (d, 1H, 15.6 Hz); 5.58 (dd, 1H, 15.6 Hz, 8.0 Hz); 3.20 (quintet, 1H, 7.1 Hz); 2.15-2.12 (m, 4H); 1.71-1.56 (m, 4H); 1.31 (d, 3H, 7.1 Hz).

2-methylacrylic acid: 1H NMR (300 MHz, CDCl₃): δ= 6.24-6.23 (m, 1H); 5.68-5.66 (m, 1H); 1.95 (dd, 3H, 1.6 Hz, 1.1 Hz).

(R,E)-4-(2-(hydroxymethyl)cyclohex-1-en-1-yl)-2-methylbut-3-enoic acid (107):

Compound was prepared from 2-((tert-butylidimethylsilyl)oxy)methyl)cyclohex-1-ene-1-carbaldehyde (109) analogously to (R,E)-4-(cyclohex-1-en-1-yl)-2-methylbut-3-enoic acid above. Four equivalents of phosphonium salt 110 were used. Estimated yield: 10 % (2-methylacrylic acid, and other small impurities still present after purification).

1H NMR (300 MHz, CDCl₃): δ= 6.67 (d, 1H, 15.6 Hz); 5.73 (dd, 1H, 15.6 Hz, 8.2 Hz); 4.24 (s, 2H); 3.24 (quintet, 1H, 7.1 Hz); 2.24 (m, 2H); 2.19 (m, 2H); 1.65-1.61 (m, 4H); 1.32 (d, 3H, 7.1 Hz).
**GR24 Synthesis (127, 128):** The compound was prepared following a literature procedure, with modifications described below.

**Ethyl 2-(2-ethoxy-2-oxoethyl)-1-oxo-2,3-dihydro-1H-indene-2-carboxylate (124):**

Addition of indanone (8.7 g) to a solution of NaH 60 % dispersion and diethyl carbonate in DMF, and the subsequent addition of ethyl bromoacetate are very exothermic, especially the first addition. The reaction mixture can heat up to 65 °C spontaneously, without external heating. Vacuum distillation of the crude resulted in a product that was much less pure than the crude. The product was obtained pure by column chromatography instead (hexanes:ethyl acetate 8:1).  $R_f = 0.55$ (hexanes:ethyl acetate, 2:1).  $^1$HNMR (300 MHz, CDCl$_3$): $\delta = 7.77$ (d, 1H, 7.6 Hz); 7.63 (td, 1H, 7.5 Hz, 1.1 Hz); 7.50 (d, 1H, 7.9 Hz); 7.39 (t, 1H, 7.6 Hz); 4.17-4.06 (m, 4H); 3.89 (d, 1H, 17.6 Hz); 3.33 (d, 1H, 17.3 Hz); 3.20 (d, 1H, 17.6 Hz); 2.79 (d, 1H, 17.3 Hz); 1.17 (td, 6H, 7.1 Hz, 0.7 Hz).  $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta = 200.9$, 170.8, 169.7, 153.4, 135.4, 134.7, 127.7, 126.3, 124.8, 61.9, 60.8, 58.0, 38.8, 37.8, 14.0, 13.9.

**2-(1-oxo-2,3-dihydro-1H-inden-2-yl)acetic acid (125):**

The reaction was run for 10 h, because after 3 h there was still a lot of unreacted substrate.  $^1$HNMR (300 MHz, acetone-$d_6$): $\delta = 10.77$ (br s, 1H); 7.69-7.64 (m, 2H); 7.56 (d, 1H, 7.6 Hz); 7.43 (t, 1H, 7.3 Hz); 3.46 (dd, 1H, 18.4 Hz, 8.8 Hz); 3.01-2.88 (m, 3H); 2.69 (dd, 1H, 17.3 Hz, 8.0 Hz).  $^{13}$C NMR (methanol-$d_4$, 75 MHz): $\delta = 209.7$, 175.5, 155.5, 137.7, 136.3, 128.6, 127.9, 124.7, 45.1, 35.6, 34.0.
Racemic \((3aR,8bS)-3,3a,4,8b\)-tetrahydro-2H-indeno[1,2-b]furan-2-one (120):

To a solution of sodium borohydride (214 mg, 5.64 mmol) in 0.2 M NaOH (3 mL) was added dropwise a solution of 2-(1-oxo-2,3-dihydro-1H-inden-2-yl)acetic acid (125) (507 mg, 2.66 mmol) in 1M NaOH (5 mL), and the mixture was stirred at room temperature for 20 h. The mixture was cooled to 0 °C, acidified to pH 1 with 6M HCl, stirred for 30 min, extracted with diethyl ether, washed with brine, and dried over MgSO₄. The \(^1\)HNMR analysis of the crude has indicated that there was substantial amount of hydroxy acid, and the crude product was heated at reflux with a crystal of p-TsOH in tetrahydrofuran (25 mL) for 20 h to complete lactonization. The solvent was removed on the rotovap, and the crude was purified by column chromatography (hexanes: ethyl acetate 10:1 to 4:1) to yield the product as an oil that turned into white plates on standing (308 mg, 66 % yield). \(R_f = 0.33\) (hexanes: ethyl acetate, 2:1). \(^1\)HNMR (300 MHz, CDCl₃): \(\delta = 7.47\) (d, 1H, 7.4 Hz); 7.38-7.32 (m, 1H); 7.28 (t, 2H, 7.4 Hz); 5.88 (d, 1H, 7.4 Hz); 3.40-3.27 (m, 2H); 2.94-2.85 (m, 2H); 2.38 (dd, 1H, 18.0 Hz, 5.4 Hz). \(^13\)C NMR (CDCl₃, 75 MHz): \(\delta = 176.8, 142.5, 138.7, 129.9, 127.5, 126.3, 125.3, 87.6, 37.8, 37.3, 35.6\). IR (neat): 1771 cm\(^{-1}\), 1167 cm\(^{-1}\).

Potassium salt of racemic (E)-\(((3aR,8bS)-2-oxo-4,8b\)-dihydro-2H-indeno[1,2-b]furan-3(3aH)-ylidene)methanolate (126):

Ethyl formate was used instead of methyl formate (Either can be used).
5-bromo-3-methylfuran-2(5H)-one (130):

3-methyl-2(5H)-furanone (129, 225 μL, 2.6 mmol), N-bromosuccinimide (520 mg, 2.9 mmol), and azobisisobutyronitrile (15 mg, 0.09 mmol) in carbon tetrachloride (5 mL) were heated at reflux for 4 h. The mixture was cooled to room temperature and then kept in the −20 °C freezer for 2 h. The solids were filtered off and the crude was purified by Kugelrohr distillation (105 °C, 2 mmHg) to give the product as lightly yellow oil (371 mg, 82 % yield). $^1$H NMR (CDCl$_3$, 300 MHz): δ 7.21 (quintet, 1H, 1.6 Hz); 6.84 (quintet, 1H, 1.6 Hz), 2.03 (t, 3H, 1.6 Hz).

GR24 (127, 128).

Hexanes:ethyl acetate:diethyl ether in a 10:1:1 ratio was used as an eluent instead of diisopropyl ether/ethyl acetate.

First diastereomer: white solid, 89 mg, 37 % yield. R$_f$ = 0.20 (hexanes:ethyl acetate 2:1). $^1$H NMR (CDCl$_3$, 300 MHz): δ = 7.50 (d, 1H, 7.2 Hz); 7.48 (d, 1H, 2.6 Hz); 7.37-7.22 (m, 3H); 6.97 (quintet, 1H, 1.5 Hz); 6.18 (quintet, 1H, 1.5 Hz); 5.95 (d, 1H, 7.9 Hz); 3.99-3.91 (m, 1H); 3.44 (dd, 1H, 16.7 Hz, 9.4 Hz); 3.11 (dd, 1H, 16.9 Hz, 3.4 Hz); 2.04 (t, 3H, 1.5 Hz). $^{13}$C NMR (CDCl$_3$, 75 MHz): δ 171.2, 170.1, 150.9, 142.5, 140.9, 138.8, 136.0, 130.0, 127.5, 126.4, 125.1, 113.2, 100.5, 85.9, 38.8, 37.3, 10.7. IR (neat): 1789, 1748, 1684 cm$^{-1}$. HRMS (DART): calculated for [M+H]$^+$: 299.0912, found: 299.0919.

Second diastereomer: white solid, 91 mg, 38 % yield. R$_f$ = 0.18 (hexanes:ethyl acetate 2:1). $^1$H NMR and $^{13}$C NMR were the same as for the first diastereomer.
1,2-phenylenedimethanol (132):
A solution of phthalic anhydride (131) (5.04 g, 34.0 mmol) in tetrahydrofuran (50 mL) was added at 0 °C to the suspension of lithium aluminium hydride (2.37 g, 62.4 mmol) in tetrahydrofuran (80 mL), and the mixture was stirred at room temperature for 30 min and then at reflux for 3 h. The mixture was cooled to 0 °C and water (2.4 mL) was added in a dropwise fashion at 0 °C, followed by 15 % aqueous sodium hydroxide solution (2.4 mL) and water (6 mL). The mixture was diluted with ethyl acetate (100 mL), filtered through a plug of celite, and eluted with ethyl acetate. The solvents were evaporated and the crude was purified by column chromatography with hexanes-ethyl acetate (5:1 to 1:1 gradient) to yield 3.36 g (71 %) of the product as a white solid. R_f = 0.20 (hexanes: ethyl acetate 1:1, KMnO_4). ^1H NMR (CDCl_3, 300 MHz): δ 7.33 (m, 4H); 4.68 (s, 4H), 3.43 (s, 2H); ^13C NMR (CDCl_3, 75 MHz): δ 139.3, 129.7, 128.5, 64.1.

(2-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)methanol (133):
Using a literature procedure, a solution of 1,2-phenylenedimethanol (132) (539 mg, 3.90 mmol) in tetrahydrofuran (10 mL) was added to a suspension of sodium hydride (154 mg of a 60 % dispersion in mineral oil, 3.85 mmol) in tetrahydrofuran (25 mL) at room temperature and the mixture was stirred overnight. The resulting solution was then cooled to 0 °C and tert-butyldimethylsilyl chloride (580 mg, 3.85 mmol) was added in one portion. After 10 min at 0 °C, the reaction mixture was stirred at room temperature for 1.5 h. Saturated aqueous ammonium chloride (40 mL) was added and the mixture was
extracted with ether. The combined extracts were washed with brine, dried (MgSO₄) and evaporated. The crude product was purified by column chromatography with hexanes:ethyl-acetate 10:1 as eluent to yield 877 mg (90 %) of the title compound. Rᵢ= 0.38 (hexanes: ethyl acetate 10:1, KMnO₄). ¹H NMR (CDCl₃, 300 MHz): δ 7.25-7.37 (m, 4H); 4.80 (s, 2H); 4.67 (d, 2H); 3.25 (t, 1H); 0.92 (s, 9H); 0.12 (s, 6H); ¹³CNMR (CDCl₃, 75 MHz): δ 139.8, 138.6, 129.4, 128.6, 127.9, 64.7, 63.8, 25.8, 18.2, -5.3.

2-(((tert-butyldimethylsilyl)oxy)methyl)benzaldehyde (121): ³¹¹
Activated manganese dioxide (2.0 g, 90 % technical oxidation grade, 63548 Fluka) was added to the solution of the alcohol (133) obtained above (237 mg, 0.94 mmol) in petroleum ether (10 mL) at 0 °C, and the mixture was stirred at 0-10 °C for 3 h. The solids were then removed by filtration through a celite plug and the solvent evaporated to yield 220 mg (94 %) of the title compound as colorless oil. Rᵢ= 0.54 (hexanes: ethyl acetate 10:1). ¹H NMR (CDCl₃, 300 MHz): δ 10.18 (s, 1H); 7.82 (m, 2H); 7.63 (m, 1H); 7.45 (m, 1H); 5.16 (s, 2H); 0.97 (s, 9H); 0.14 (s, 6H); ¹³CNMR (CDCl₃, 75 MHz): δ 193.3, 144.3, 133.9, 133.4, 132.6, 127.0, 126.6, 62.9, 25.9, 18.4, -5.3.

(2-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)ethyl)triphenylphosphonium bromide (122):
The compound was prepared following a literature procedure.³¹³
Tert-butyl(dimethyl)((2-(3-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)prop-1-en-1-yl)benzyl)oxy)silane (138). Lithium hexamethyldisilazide 1 M in THF (53.0 mL, 53.0 mmol) was added at room temperature to a suspension of phosphonium bromide salt 122 (27.35 g, 54.8 mmol) in tetrahydrofuran (200 mL) and the mixture was heated to reflux for 1 h. The mixture was then cooled down to −78 °C and a solution of aldehyde 121 (9.14 g, 36.5 mmol) in tetrahydrofuran (100 mL) was added. The reaction mixture was then stirred at −78 °C for 1 h, and room temperature for 2 h. Water (70 mL) was added and the mixture was extracted with diethyl ether. The combined organic layers were washed with brine, dried (MgSO₄) and evaporated. The crude product was purified by column chromatography pretreated with 3 % triethylamine in hexane and eluted with 0.5 % triethylamine in hexane to yield 13.83 g of the product as 1:1 mixture (97 % yield) of E/Z isomers. Z isomer: Rᵣ = 0.26 (hexanes: ethyl acetate 10:1); E isomer: Rᵣ = 0.22 (hexanes: ethyl acetate 10:1). ¹H NMR (CDCl₃, 300 MHz): δ 7.22-7.55 (m, 4H); 6.60 (d, J = 11.3 Hz) and 6.65 (d, 1H, J = 15.9 Hz); 5.89 (dt, J = 7.1, 11.3 Hz) and 6.17 (dt, 1H, J = 7.1, 15.9 Hz); 4.72 and 4.82 (s, 2H); 3.93 and 3.94 (s, 6H); 2.58 and 2.68 (dd, 2H, J = 7.1, 1.7 Hz); 0.98 (s, 9H); 0.81 and 0.82 (s, 3 H); 0.13 (s, 6H). ¹³C NMR (CDCl₃, 75 MHz): δ 139.1, 137.7, 135.2, 134.2, 133.8, 133.5, 129.4, 129.0, 128.8, 128.6, 128.5, 128.4, 126.9, 126.6, 126.3, 125.8, 125.7, 125.6, 125.3, 108.6, 108.4, 108.2, 72.7, 63.0, 62.8, 40.7, 35.9, 30.4, 30.3, 25.9, 18.3, 14.5, −5.3, −5.4. IR (neat): 3018 cm⁻¹, 2931 cm⁻¹, 2881 cm⁻¹, 1472 cm⁻¹, 1397 cm⁻¹, 1356 cm⁻¹, 1259 cm⁻¹, 1119 cm⁻¹, 994 cm⁻¹, 839 cm⁻¹; HRMS (DART): calculated for C₂₂H₃₅O₄Si [M+H]⁺: 391.2299, found: 391.2300.
4-(2-(hydroxymethyl)phenyl)but-3-enoic acid (119):

*Tert*-butyldimethyl((2-(3-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)prop-1-en-1-yl)benzyl)oxy)silane (138) (500 mg, 1.28 mmol) was dissolved in methanol (20 mL), concentrated H$_2$SO$_4$ was added (0.25 mL), and the mixture was stirred at room temperature for 1h. The reaction mixture was cooled to 0 °C, 1 M NaOH (15 mL) was added, the mixture was stirred at 0 °C for 20 min, and at room temperature for 15 min. Water was added (40 mL), the aqueous layer was washed 3 times with diethyl ether, then acidified to pH 1, extracted with DCM, dried (MgSO$_4$), and evaporated to yield the product as a white solid (231 mg, 94 % yield). Based on $^1$HNMR the desired product was obtained in 82 % yield, and the 2-(isochroman-3-yl)acetic acid (142) impurity in 12 % yield. $^1$H NMR (acetone-$d_6$, 300 MHz): $\delta$ = 7.53-7.49 (m, 2H); 7.43-7.40 (m, 1H); 7.32-7.19 (m, 5H); 6.86 (dt, 1H, 15.7 Hz, 1.4 Hz); 6.74 (dt, 1H, 11.4 Hz, 1.4 Hz); 6.26 (dt, 15.7 Hz, 7.2 Hz); 5.95 (dt, 11.4 Hz, 7.4 Hz); 4.69 (s, 2H); 4.60 (s, 2H); 3.27 (dd, 6.7 Hz, 1.7 Hz). $^{13}$CNMR (acetone-$d_6$, 75 MHz): $\delta$ = 173.0, 172.9, 141.0, 139.7, 136.5, 135.5, 130.7, 130.4, 129.6, 128.6, 128.2, 128.2, 128.1, 128.0, 127.5, 126.4, 125.9, 125.4, 62.7, 62.5, 38.7, 34.3. IR (neat): 3300-2750 cm$^{-1}$, 1716 cm$^{-1}$.

2-(isochroman-3-yl)acetic acid (142):

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 7.82 (br s, 1H); 7.17-7.13 (m, 2H); 7.08 (dd, 1H, 5.1 Hz, 3.8 Hz); 6.98 (dd, 1H, 5.1 Hz, 3.8 Hz), 4.83 (s, 2H); 4.19-4.10 (m, 1H); 2.79 (d, 2H, 6.7
Hz); 2.74-2.56 (m, 2H). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ = 176.0, 134.0, 132.3, 128.7, 126.5, 126.1, 124.1, 71.2, 68.1, 40.6, 33.4.

4-(2-((2,2,2-trifluoroacetoxy)methyl)phenyl)but-3-enoic acid (143):
$^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 10.58 (br s, 1H); 7.53-7.23 (m, 4H); 6.76 (d, 0.6 H, 11.1 Hz); 6.73 (d, 0.4 H, 15.8 Hz); 6.22 (dt, 0.4 H, 15.8 Hz, 7.3 Hz); 6.02 (dt, 0.6H, 11.1 Hz, 7.4 Hz); 5.42 (s, 0.8H); 5.33 (s, 1.2H); 3.34 (dd, 0.8H, 7.3 Hz, 1.5 Hz); 3.17 (dd, 1.2H, 7.4 Hz, 1.5 Hz). IR (neat): 3500-2500 cm$^{-1}$, 1784 cm$^{-1}$, 1713 cm$^{-1}$, 1173 cm$^{-1}$, 1145 cm$^{-1}$.

Allyl 4-(2-(hydroxymethyl)phenyl)but-3-enoate (139):
*Tert*-butyldimethyl((2-(3-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)prop-1-en-1-yl)benzyl)oxy)silane (138) (1.435 g, 3.67 mmol) was dissolved in acidic allyl alcohol (20 mL + 0.45 mL concentrated HCl) and stirred at 50 °C for 2.5 h. The mixture was cooled to room temperature, DCM (80 mL) was added, the organic layer was washed with water and brine, dried (MgSO$_4$), and evaporated. The crude was purified by column chromatography (hexanes: ethyl acetate 20:1 to 5:1) to give 677 g of the product (79 % yield). $R_f$ = 0.17 (hexanes: ethyl acetate 4:1). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 7.51-7.48 (m, 0.4H); 7.45-7.42 (m, 1H); 7.36-7.23 (m, 3.2 H); 7.19-7.15 (m, 1H); 6.85 (d, 0.4H,
Allyl 4-(2-chloromethyl)phenyl)but-3-enoate (140):

Allyl 4-(2-(hydroxymethyl)phenyl)but-3-enoate (139) (476 mg, 2.05 mmol) was dissolved in THF (25 mL). At 0 °C triethylamine (0.46 mL, 3.30 mmol) was added, followed by methanesulfonate chloride (0.25 mL, 3.23 mmol), and the mixture was stirred at 0 °C for 15 min. Lithium chloride (287 mg, 6.76 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 6 h, washed with water, dried (MgSO₄), and evaporated. Purification by column chromatography (hexanes: ethyl acetate, 20:1) yielded the pure product (473 mg, 92 % yield). Rf = 0.55 (hexanes: ethyl acetate 4:1). $^1$H NMR (CDCl₃, 300 MHz): δ = 7.53-7.50 (m, 0.6H); 7.43-7.40 (m, 1H); 7.36-7.28 (m, 3.6H); 7.26-7.21 (m, 1.2H); 6.90-6.83 (m, 1.6H); 6.30 (dt, 0.6 H, 15.5 Hz, 7.3 Hz); 6.06 (dt, 1H, 11.4 Hz, 7.3 Hz); 6.00-5.85 (m, 1H); 5.39-5.23 (m, 2.8H); 4.66-4.60 (m, 4.2H); 4.65 (s, 1.2H); 4.57 (s, 2H); 3.36 (dd,1.2H, 7.0 Hz); 3.19 (dd, 2H, 7.3 Hz,1.8 Hz). $^{13}$CNMR (CDCl₃, 75 MHz): δ = 171.4, 171.2, 138.8, 137.4, 135.5, 134.6, 131.8, 131.7, 130.5, 130.4, 128.8, 128.1, 127.8, 127.7, 127.5, 127.5, 127.2, 126.0, 124.3, 123.8, 118.3, 65.3, 62.9, 62.7, 38.3, 33.7. IR (neat): 3448 cm⁻¹, 1733 cm⁻¹, 1165 cm⁻¹. HRMS (ESI): calculated for [M+Na]^+: 255.0992, found: 255.0988.
MHz): δ = 171.1, 171.0, 136.5, 136.0, 135.4, 134.2, 131.9, 130.1, 129.9, 129.9, 129.8, 129.5, 129.1, 128.6, 127.9, 127.9, 126.7, 125.4, 125.1, 118.5, 118.4, 65.5, 65.4, 44.4, 38.5, 34.0. IR (neat): 1734 cm⁻¹, 1160 cm⁻¹. HRMS (ESI): calculated for [M+H]+ = 251.0833 found 251.0837.

(E)-methyl 4-(2-hydroxymethyl)phenyl)but-3-enoate (152) and (Z)-methyl 4-(2-hydroxymethyl)phenyl)but-3-enoate (151):

The orthoester 138 (9.00 g, 23.0 mmol) was stirred in 0.2 M sulfuric acid methanolic solution (200 mL) at room temperature for 1 h. Water was then added and the resulting mixture was extracted with methylene chloride. The combined organic layers were washed with brine, dried (MgSO₄), and evaporated. The crude product was purified by column chromatography (hexane/ethyl acetate 4:1) to give 1.646 g of 152 and 1.474 g of 151 (66 %). Data for 152: Rf = 0.21 (hexane:ethyl acetate :4:1); ¹H NMR (CDCl₃, 300 MHz): δ 7.47 (m, 1H); 7.19-7.32 (m, 3H); 6.78 (d, 1H, J= 15.9 Hz); 6.19 (m, 1H); 4.66 (s, 2H); 3.68 (s, 3H); 3.25 (dd, 2H, J= 7.2, 1.5 Hz); 2.01 (br s, 1H); ¹³C NMR (CDCl₃, 75 MHz): δ 172.0, 137.4, 135.6, 130.3, 128.2, 127.9, 127.5, 126.1, 123.9, 63.0, 51.8, 38.2; IR (neat): 3461 cm⁻¹, 1733 cm⁻¹; HRMS (DART): calculated for C₁₂H₁₅O₃ [M+H]⁺: 207.1016, found: 207.1018.

Data for 151: Rf = 0.27 (hexane:ethyl acetate :4:1); ¹H NMR (CDCl₃, 300 MHz): δ 7.39-7.42 (m, 1H); 7.23-7.31 (m, 2H); 7.12-7.15 (m, 1H); 6.76 (d, 1H, J= 11.1 Hz); 5.92 (dt, 1H, J= 7.3, 11.1 Hz); 4.59 (d, 2H, J= 5.4 Hz); 3.64 (s, 3H); 3.14 (dd, 2H, J= 7.3, 1.5 Hz); 2.11 (br s, 1H); ¹³C NMR (CDCl₃, 75 MHz): δ 172.2, 138.9, 134.7, 130.6, 128.9, 127.9,
127.6, 127.4, 124.5, 62.9, 51.9, 33.6; IR (neat): 3466 cm\(^{-1}\), 1726 cm\(^{-1}\); HRMS (DART): calculated for [M+H]\(^+\): 207.1016, found: 207.1010.

(E)-methyl 4-(2-formylphenyl)but-3-enoate (154):

A solution of alcohol 152 (470 mg, 2.28 mmol) in DCM (4 mL) was added to a mixture of PCC (688 mg, 3.19 mmol) and celite (688 mg) in DCM (13 mL) at room temperature. After 1 h, the mixture was diluted with diethyl ether, filtered through celite, and the solvents were evaporated. Flash column chromatography (hexane-ethyl acetate 10:1) provided 432 mg of 154 as a colorless oil (93 %); \(^1\)H NMR (CDCl\(_3\), 300 MHz): \(\delta\) 10.26 (s, 1H); 7.81 (m, 1H); 7.34-7.59 (m, 4H); 6.28 (dt, 1H, J= 7.2, 15.9 Hz); 3.74 (s, 3H); 3.35 (dd, 2H, J= 7.2, 1.6 Hz); \(^{13}\)C NMR (CDCl\(_3\), 75 MHz): \(\delta\) 192.4, 171.6, 139.3, 133.7, 132.6, 131.7, 129.6, 127.7, 127.6, 127.2, 51.9, 38.2; IR (neat): 2849 cm\(^{-1}\), 2745 cm\(^{-1}\), 1735 cm\(^{-1}\), 1698 cm\(^{-1}\); HRMS (DART): calculated for C\(_{12}\)H\(_{13}\)O\(_3\) [M+H]\(^+\): 205.0859, found: 205.0868.

(Z)-methyl 4-(2-formylphenyl)but-3-enoate (153):

A solution of alcohol 151 (1.074 g, 5.21 mmol) in DCM (15 mL) was added to a mixture of PCC (1.590 g, 7.38 mmol) and celite (1.590 mg) in DCM (35 mL) at room temperature. After 1 h, the mixture was diluted with diethyl ether, filtered through celite, and the solvents evaporated. Flash column chromatography (hexane-ethyl acetate
10:1) yielded 994 mg of 153 as a colorless oil (93%); $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 10.21 (s, 1H); 7.90 (d, 1H, $J$ = 7.8 Hz); 7.58 (m, 1H); 7.44 (m, 1H); 7.29 (d, 1H, $J$ = 7.5 Hz); 7.06 (d, 1H, $J$ = 11.7 Hz); 6.07-6.16 (m, 1H); 3.68 (s, 3H); 3.11 (dd, 2H, $J$ = 7.6, 1.1 Hz); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 191.5, 171.1, 138.8, 133.4, 133.4, 129.9, 129.2, 128.9, 127.6, 126.1, 51.6, 33.5; IR (neat): 2854 cm$^{-1}$, 2740 cm$^{-1}$, 1731 cm$^{-1}$, 1695 cm$^{-1}$; HRMS (DART): calculated for C$_{12}$H$_{13}$O$_3$ [M+H]$^+$: 205.0859, found: 205.0857.

Representative procedure for trimethylsilyl triflate catalyzed cyclization:

Trimethylsilyl triflate (0.85 mL of a 0.11 M solution in CH$_2$Cl$_2$, 0.094 mmol) was added to solution of 154 (0.098 g, 0.48 mmol) in CH$_2$Cl$_2$ (1.0 mL) at 0 °C. After 2h, when thin layer chromatographic analysis indicated complete consumption of 154, water was added and the reaction mixture was extracted with CH$_2$Cl$_2$. The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated to 1 mL. Flash chromatography (gradient, hexanes:ethyl acetate 10:1 to 4:1) afforded 0.060 g of a mixture of 155 and 156 in an 80:20 ratio (60 % yield) and 0.007 g of 157 (7%). Data for 155: $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.43-7.54 (m, 4H), 6.04 (d, 1H, $J$ = 7.2 Hz), 4.72 (s, 1H), 3.44 (s, 3H), 3.32 (m, 1H), 2.94 (dd, 1H, $J$ = 18.2, 10.6 Hz), 2.35 (dd, 1H, $J$ = 18.2, 6.9 Hz); $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 176.1, 141.1, 139.6, 130.1, 130.1, 126.5, 126.3, 88.3, 85.9, 56.6, 44.4, 32.9; IR (neat) 1773 cm$^{-1}$, 1172 cm$^{-1}$, 1092 cm$^{-1}$, 1024 cm$^{-1}$; HRMS (DART) calculated for C$_{12}$H$_{13}$O$_3$ [M+H]$^+$: 205.0859, found: 205.0857.
Data for **156**: $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.35-7.49 (m, 4H), 5.72 (d, 1H, $J = 7.2$ Hz), 4.82 (d, 1H, $J = 6.9$ Hz), 3.55-3.66 (m, 1H), 3.48 (s, 3H), 2.73 (dd, 1H, $J = 18.2$, 7.2 Hz), 2.58 (dd, 1H, $J = 18.2$, 10.2 Hz). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta$ 176.9, 141.9, 137.9, 130.0, 129.4, 126.0, 125.5, 83.6, 82.0, 57.7, 41.9, 28.0. IR (neat) 1773 cm$^{-1}$, 1176 cm$^{-1}$, 1112 cm$^{-1}$, 1092 cm$^{-1}$, 1025 cm$^{-1}$. HRMS (DART) calculated for C$_{12}$H$_{13}$O$_3$ [M+H]$^+$: 205.0859, found: 205.0859.

Data for **157**: $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 7.45 (d, 1H, $J = 7.5$ Hz), 7.16-7.28 (m, 3H), 6.69 (s, 1H), 5.09 (s, 1H), 3.74 (s, 3H), 3.46 (d, 1H, $J = 17.0$ Hz), 3.39 (d, 1H, $J = 17.0$ Hz), 3.06 (s, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz): $\delta$ 171.2, 142.9, 141.8, 141.2, 131.3, 128.5, 125.4, 123.9, 121.1, 83.8, 52.4, 52.0, 33.6. IR (neat) 1735 cm$^{-1}$. HRMS (DART) calculated for C$_{13}$H$_{15}$O$_3$ [M+H]$^+$: 219.1016, found: 219.1011.

**Representative procedure for triflic acid catalyzed cyclization:** Triflic acid (0.21 mL of a 0.23 M solution in CH$_2$Cl$_2$, 0.048 mmol) was added to solution of **154** (0.098 g, 0.48 mmol) in CH$_2$Cl$_2$ (4.0 mL) at -78 °C. After 1h, the mixture was warmed to 0 °C and stirred for 3 h, when thin layer chromatographic analysis indicated complete consumption of **154**. The reaction mixture was then poured onto ice and extracted with CH$_2$Cl$_2$. The combined organic extract was washed with brine, dried over magnesium sulfate, and concentrated to 1 mL. Flash chromatography (gradient, hexanes:ethyl acetate 10:1 to 4:1) afforded 0.067 g of a mixture of **155** and **156** in a 99:1 ratio (68% yield) and 0.002 g of **157** (2%). The compounds exhibited spectral data identical to those obtained above.
Benzyl (E)-4-(2-hydroxymethyl)phenyl)but-3-enoate (165):

The E alcohol methyl ester 152 (containing 4 % of Z isomer) was added to a solution of concentrated hydrochloric acid (0.36 mL) in benzyl alcohol (8 mL), and the mixture was heated at 50 °C for 2h. Hydrochloric acid and benzyl alcohol were distilled off by vacuum distillation, and the crude product (ca. 900 mg) was purified by column chromatography (hexanes:ethyl acetate 10:1) to give the product in 95 % yield (290 mg). Rf = 0.17 (hexanes:ethyl acetate, 4:1). 1H NMR (CDCl₃, 300 MHz): δ = 7.50-7.47 (m, 1H); 7.38-7.24 (m, 8H); 6.82 (d, 1H, 15.7 Hz); 6.24 (dt, 1H, 15.7 Hz, 7.3 Hz); 5.16 (s, 2H); 4.70 (s, 2H); 3.33 (dd, 2H, 7.3 Hz, 1.5 Hz); 1.73 (br s, 1H, OH).

Benzyl (E)-4-(2-formylphenyl)but-3-enoate (166):

A solution of alcohol 165 (290 mg, 1.03 mmol) in DCM (2 mL) was added to a mixture of PCC (321 mg, 1.49 mmol) and celite (320 mg) in DCM (10 mL). After stirring for 1h at room temperature, the mixture was diluted with diethyl ether, filtered through celite, and evaporated. Column chromatography (hexanes: ethyl acetate, 20:1) gave the product as a colorless oil (248 mg, 86 %). Rf = 0.50 (hexanes:ethyl acetate, 4:1). 1H NMR (CDCl₃, 300 MHz): δ = 10.22 (s, 1H); 7.79 (d, 1H, 7.4 Hz); 7.56-7.30 (m, 9H); 6.24 (dt, 1H, 15.7 Hz, 7.3 Hz); 5.16 (s, 2H); 3.38 (dd, 2H, 6.9 Hz, 1.5 Hz). 13CNMR (CDCl₃, 75 MHz): δ = 158.5 (C=O), 133.8 (C=O), 128.9 (CH), 128.1 (CH), 127.1 (CH), 126.0 (CH), 123.8 (CH), 120.0 (CH), 117.8 (CH), 114.2 (CH), 112.9 (CH), 110.3 (CH), 105.8 (CH), 103.3 (CH), 101.3 (CH), 100.0 (CH), 97.8 (CH), 96.7 (CH), 95.6 (CH), 94.5 (CH), 93.4 (CH), 92.3 (CH), 91.2 (CH), 90.1 (CH), 89.0 (CH), 87.9 (CH), 86.8 (CH), 85.7 (CH), 84.6 (CH), 83.5 (CH), 82.4 (CH), 81.3 (CH), 80.2 (CH), 79.1 (CH), 78.0 (CH), 76.9 (CH), 75.8 (CH), 74.7 (CH), 73.6 (CH), 72.5 (CH), 71.4 (CH), 70.3 (CH), 69.2 (CH), 68.1 (CH), 67.0 (CH), 65.9 (CH), 64.8 (CH), 63.7 (CH), 62.6 (CH), 61.5 (CH), 60.4 (CH), 59.3 (CH), 58.2 (CH), 57.1 (CH), 56.0 (CH), 54.9 (CH), 53.8 (CH), 52.7 (CH), 51.6 (CH), 50.5 (CH), 49.4 (CH), 48.3 (CH), 47.2 (CH), 46.1 (CH), 45.0 (CH), 43.9 (CH), 42.8 (CH), 41.7 (CH), 40.6 (CH), 39.5 (CH), 38.4 (CH), 37.3 (CH), 36.2 (CH), 35.1 (CH), 34.0 (CH), 32.9 (CH), 31.8 (CH), 30.7 (CH), 29.6 (CH), 28.5 (CH), 27.4 (CH), 26.3 (CH), 25.2 (CH), 24.1 (CH), 23.0 (CH), 21.9 (CH), 20.8 (CH), 19.7 (CH), 18.6 (CH), 17.5 (CH), 16.4 (CH), 15.3 (CH), 14.2 (CH), 13.1 (CH), 12.0 (CH), 10.9 (CH), 9.8 (CH).
MHz): $\delta = 192.3, 170.9, 139.3, 135.6, 133.6, 132.6, 131.6, 129.7, 128.5, 128.3, 128.2, 127.7, 127.6, 127.2, 66.6, 38.3$.

Racemic (3aS,4S,8bS)-4-(benzyloxy)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (167):

The product was made from benzyl (E)-4-(2-formylphenyl)but-3-enoate (166) (117 mg, 0.42 mmol) (containing 4 % of Z isomer), using a representative procedure for triflic acid catalyzed cyclization (0.1 equivalent of TfOH). The product was obtained in 66 % yield as mixture of 167 and 168 in a 95:5 ratio. The compound 169, resulting from single cyclization was also isolated in 3 % yield. $R_f = 0.41$ (hexanes:ethyl acetate, 2:1). $^1$H NMR (CDCl$_3$, 500 MHz): $\delta = 7.53-7.31$ (m, 9H); 6.05 (d, 1H, 7.0 Hz); 4.90 (d, 1H, 1.5 Hz); 4.66 (s, 2H); 3.56-3.19 (m, 1H); 2.88 (dd, 1H, 18.2 Hz, 10.7 Hz); 2.29 (dd, 1H, 18.2 Hz, 6.7 Hz). $^{13}$CNMR (CDCl$_3$, 125 MHz): $\delta = 176.1, 141.5, 139.5, 137.5, 130.1, 130.0, 128.5, 127.9, 127.7, 126.4, 126.2, 86.4, 85.9, 71.3, 44.9, 32.8$.

Benzyl 2-(1-(benzyloxy)-1H-inden-2-yl)acetate (169):

$^1$H NMR (CDCl$_3$, 300 MHz): $\delta = 7.47$ (d, 1H, 7.1 Hz); 7.37-7.21 (m, 13H); 6.70 (s, 1H); 5.25 (s, 1H); 5.15 (s, 2H); 4.24 (s, 2H); 3.51-3.49 (m, 2H).
**Allyl 4-(2-formylphenyl)but-3-enoate (170):**

A solution of alcohol 139 (280 mg, 1.21 mmol) in dichloromethane (3 mL) was added to a mixture of PCC (364 mg, 1.69 mmol) and Celite (364 mg) in dichloromethane (9 mL). After 1h of stirring at room temperature, the mixture was diluted with diethyl ether, filtered through celite, and the solvents were evaporated. Purification by column chromatography (hexanes: ethyl acetate 10:1) gave the product as a colorless oil (277 mg, 86 % yield). Rf = 0.74 (hexanes:ethyl acetate, 2:1). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta=$ 10.27 (s, 1H); 10.23 (s, 0.6 H); 7.92 (dd, 0.6 H, 7.6 Hz, 1.4 Hz); 7.82 (s, 1H, 7.4 Hz); 7.64-7.53 (m, 2.6H); 7.48-7.41 (m, 2H); 7.36-7.30 (m, 1.2H); 7.09 (d, 1H, 11.3 Hz); 6.29 (dt, 1H, 15.8 Hz, 7.1 Hz); 6.15 (dt, 1H, 11.3 Hz, 7.6 Hz); 6.02-5.85 (m, 1.6H); 5.39-5.22 (m, 3.2H); 4.65 (dt, 2H, 6.0 Hz, 1.1 Hz); 4.60 (dt, 1.2 H, 6.0 Hz, 1.1 Hz); 3.38 (dd, 2H, 7.1 Hz, 1.4 Hz); 3.16 (dd, 1.2H, 7.6 Hz, 1.6 Hz). $^{13}$C NMR (CDCl$_3$, 75 MHz): $\delta=$ 192.4, 191.9, 170.8, 170.7, 139.4, 139.1, 133.7, 132.6, 131.9, 131.8, 131.7, 130.2, 129.7, 129.6, 129.3, 127.8, 127.7, 127.6, 127.2, 126.2, 118.5, 65.5, 38.3, 34.0.
Racemic (3aS,4S,8bS)-4-(allyloxy)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (171) and racemic (3aS,4R,8bS)-4-(allyloxy)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (172):

The product was made from allyl 4-(2-formylphenyl)but-3-enoate (170), using a representative procedure for triflic acid catalyzed cyclization (3x 0.15 equivalent of TfOH). Rf = 0.51 (hexanes:ethyl acetate, 2:1). ^1H NMR (CDCl₃, 300 MHz): δ 7.55-7.37 (m, 171 4H, 172 3.2H); 6.05 (d, 171 1H, 6.9 Hz); 6.03-5.89 (m, 171 1H, 172 0.8H); 5.73 (d, 172 0.8H, 7.4 Hz); 5.41-5.22 (m, 171 2H, 172 1.6H); 5.00 (d, 172 0.8H, 6.8 Hz); 4.86 (d, 171 1H, 1.7 Hz); 4.18-4.11 (m, 171 2H, 172 1.6H); 3.67-3.56 (m, 172, 0.8 Hz); 3.33 (dtd, 171 1H, 10.6 Hz, 6.9 Hz, 1.7 Hz); 2.93 (dd, 171 1H, 18.3 Hz, 10.6 Hz); 2.78 (dd, 172 0.8H, 18.4 Hz, 7.4 Hz); 2.58 (dd, 172 0.8H, 18.4 Hz, 10.3 Hz); 2.36 (dd, 171 1H, 18.3 Hz, 6.9 Hz). ^13C NMR (CDCl₃, 75 MHz): δ 176.9, 176.1, 142.0, 141.2, 139.6, 137.9, 134.2, 134.1, 130.1, 130.0, 129.4, 126.5, 126.2, 126.0, 125.5, 117.7, 117.5, 86.3, 85.9, 83.6, 79.7, 71.0, 70.2, 45.0, 42.3, 32.9, 28.5. HRMS (DART): calculated for C₁₄H₁₄O₃ [M+NH₄]⁺: 248.1281, found: 248.1283.
Racemic (3aS,4S,8bS)-4-hydroxy-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (173) and racemic (3aS,4R,8bS)-4-hydroxy-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (174):

A mixture of racemic (3aS,4S,8bS)-4-(allyloxy)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (171) and racemic (3aS,4R,8bS)-4-(allyloxy)-3,3a,4,8b-tetrahydro-2H-indeno[1,2-b]furan-2-one (172) in a 55:45 ratio (30 mg, 0.13 mmol) was dissolved in THF (2 mL). Anhydrous zinc chloride (23 mg, 0.17 mmol) was added, the mixture was stirred for 15 min, then triphenylphosphine palladium tetrakis was added (7 mg, 6.5 µmol, 5mol%), the mixture was stirred for 10 min, and tributyltin hydride (69 µL, 0.26 mmol) was added. After stirring for 1h at room temperature, the mixture was diluted with ethyl acetate (4 mL) and water (1 mL), acidified to pH4 with 1M HCl, extracted with ethyl acetate, washed with brine, dried over MgSO4, and evaporated. Purification by column chromatography (gradient of hexanes and ethyl acetate 6:1 to 2:1) afforded 173 in 41 % yield (10.2 mg) and 174 in 35 % yield (8.8 mg). Data for 173: Rf= 0.17 (hexanes:ethyl acetate, 1:1). 1H NMR (CDCl3, 300 MHz): δ= 7.53-7.41 (m, 4H); 6.02 (d, 1H, 6.9 Hz); 5.13 (d, 1H, 1.8 Hz); 3.27-3.19 (m, 1H); 2.93 (dd, 1H, 18.3 Hz, 10.4 Hz); 2.44 (dd, 1H, 18.3 Hz, 5.9 Hz). 13CNMR (CDCl3, 75 MHz): δ= 176.3, 143.9, 138.8, 130.7, 130.1, 126.6, 125.4, 85.8, 80.4, 47.9, 33.0.

Data for 174: Rf= 0.22 (hexanes:ethyl acetate, 1:1). 1H NMR (CDCl3, 300 MHz): δ= 7.54-7.39 (m, 4H); 5.72 (d, 1H, 7.0 Hz); 5.29 (d, 1H, 7.0 Hz); 3.54-3.44 (m, 1H); 2.86
(dd, 1H, 18.5 Hz, 5.9 Hz); 2.66 (dd, 1H, 18.5 Hz, 10.3 Hz). $^{13}$CNMR (CDCl$_3$, 75 MHz):

\[ \delta = 177.2, 144.1, 138.2, 130.5, 129.6, 126.3, 125.2, 84.3, 73.4, 43.4, 28.8. \]

![Chemical structure](image)

2-hydroxy-3,4-dimethylbenzaldehyde (182):

Anhydrous magnesium chloride (23.4 g, 246 mmol) and dry triethylamine (84.0 mL, 603 mmol) were added to a solution of 2,3-dimethylphenol (19.5 g, 160 mmol) in acetonitrile (650 mL), and the mixture was stirred for 15 min. Dry (P$_2$O$_5$) paraformaldehyde (28.4 g, 946 mmol) was then added and the reaction mixture was heated under reflux for 45 min. The mixture was cooled to room temperature, 1M HCl (200 mL) was added, and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were dried over magnesium sulfate, filtered through celite, evaporated, and the residue was purified by flash chromatography (hexane:ethyl acetate 25:1), yielding 17.9 g of 182 as a white solid (74 % yield). $R_f = 0.54$ (hexanes: ethyl acetate, 10:1). $^1$H NMR (CDCl$_3$, 300 MHz): \[ \delta = 11.36 \text{ (s, 1H)}, 9.80 \text{ (s, 1H)}, 7.28 \text{ (d, 1H, J= 7.8 Hz)}, 6.82 \text{ (d, 1H, J= 7.8 Hz)}, 2.33 \text{ (s, 3H)}, 2.18 \text{ (s, 3H)}. \]

$^{13}$CNMR (CDCl$_3$, 75 MHz): \[ \delta = 196.1, 159.8, 147.1, 130.7, 124.8, 121.5, 118.4, 20.9, 10.7. \]

IR (neat): \( \nu \) 3056 (broad), 2841, 2745, 1644, 1622, 1499, 1311, 1277, 770, 752, 735 cm$^{-1}$. 

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6-formyl-2,3-dimethylphenyl trifluoromethanesulfonate (180):

A solution of triflic anhydride (17.0 mL, 101 mmol) in DCM (60 mL) was added at −78 °C to a solution of phenol 182 (9.5 g, 63 mmol) and triethylamine (30.0 mL, 215 mmol) in DCM (240 mL). The mixture was stirred at −78 °C for 3 h and then at 0 °C for 40 min. Water (20 mL) was added, the layers were separated, and the organic layer was washed with water (20 mL), dried over magnesium sulfate and evaporated. The residue was purified by column chromatography (hexane:ethyl acetate 25:1) to give 16.8 g (95 % yield) of the pure product as colorless oil. Rf = 0.50 (hexanes: ethyl acetate, 10:1). ¹H NMR (CDCl₃, 300 MHz): δ 10.19 (s, 1H), 7.76 (d, 1H, J= 7.8 Hz), 7.35 (d, 1H, J= 7.8 Hz), 2.43 (s, 3H), 2.35 (s, 3H). ¹³CNMR (CDCl₃, 75 MHz): δ 186.8, 147.4, 147.2, 131.2, 129.9, 127.5, 127.4, 118.5 (q, J= 1277 Hz), 20.7, 13.0. IR: ν 2959, 2889, 2754, 1696, 1612, 1567, 1427, 1407, 1254, 1215, 1137, 1055, 953, 895, 809, 770 cm⁻¹.

2-(4-hydroxybut-1-yn-1-yl)-3,4-dimethylbenzaldehyde (186):

Diethylamine (2.43 mL, 23 mmol) was added to a mixture of palladium tetrakis (136 mg, 0.12 mmol) and copper iodide (52 mg, 0.27 mmol) in tetrahydrofuran (10 mL). A solution of triflate 180 (1.040 g, 3.91 mmol) in tetrahydrofuran (10 mL) was then added, followed by a solution of 3-butylnol (356 mg, 5.08 mmol) in tetrahydrofuran (5 mL). The mixture was heated to reflux for 3 h, cooled down to room temperature, diluted with diethyl ether
(80 mL), washed with 1M HCl, washed with brine, dried over magnesium sulfate, and evaporated. The residue was purified by chromatography column (hexane:ethyl acetate gradient 10:1 to 3:1) to yield 186 as a white solid (790 mg, 78 % yield). Rf= 0.23 (hexanes: ethyl acetate, 2:1). 1H NMR (CDCl₃, 300 MHz): δ 10.38 (s, 1H), 7.59 (d, 1H, J= 7.8 Hz), 7.15 (d, 1H, J= 7.8 Hz), 3.88 (t, 2H, J= 6.3 Hz), 2.90 (br s, 1H), 2.79 (t, 2H, J= 6.3 Hz), 2.39 (s, 3H), 2.31 (s, 3H). 13CNMR (CDCl₃, 75 MHz): δ 192.6, 143.5, 139.7, 134.3, 129.3, 126.1, 125.8, 98.2, 77.4, 61.0, 24.1, 20.9, 17.0. IR: ν 3429, 2945, 2882, 2745, 2227, 1687, 1584, 1250, 1048, 821, 779 cm⁻¹.

4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-yn-1-ol (187):
A mixture of aldehyde (186) (496 mg, 2.45 mmol), 1,3-propanediol (0.36 mL, 4.94 mmol), p-TsOH (40 mg), and benzene (35 mL) was heated under reflux for 1 h. Water formed during the reaction was removed by a Dean-Stark trap. The cooled reaction mixture was diluted with diethyl ether (80 mL), washed with saturated aqueous solution of sodium bicarbonate, washed with brine, dried over magnesium sulfate, and evaporated. The residue was purified by column chromatography (hexanes:ethyl acetate gradient 10:1 to 2:1) to yield 187 as a white solid (523 mg, 82 % yield). Rf= 0.20 (hexanes: ethyl acetate, 2:1). 1H NMR (CDCl₃, 300 MHz): δ 7.38 (d, 1H, J= 7.8 Hz), 7.10 (d, 1H, J= 7.8 Hz), 5.83 (s, 1H), 4.23-4.28 (m, 2H), 3.96-4.04 (m, 2H), 3.81 (t, 2H, J= 6.4 Hz), 2.75 (t, 2H, J= 6.4Hz), 2.36 (s, 3H+1H), 2.24 (m, 3H+1H), 1.42 (d, 1H, J= 13.3 Hz). 13CNMR (CDCl₃, 75 MHz): δ 138.4, 137.6, 137.0, 129.4, 122.8, 121.3, 100.7, 94.7, 79.1,
67.5, 61.2, 25.7, 24.1, 20.3, 17.4. IR: ν 3424, 2962, 2926, 2857, 2228, 1595, 1458, 1388, 1152, 1108, 1040, 992, 823 cm⁻¹.

(E)-4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-en-1-ol (188):
The solution of alkyne 187 (170 mg, 0.65 mmol) in tetrahydrofuran (4 mL) was added to a suspension of lithium aluminium hydride (37 mg, 0.98 mmol) in tetrahydrofuran (3 mL) at 0 °C. The mixture was then heated under reflux for 3 h, cooled down to 0 °C, water was added (30 µL), followed by 15 % sodium hydroxide (30 µL), and water (90 µL). The mixture was diluted with ethyl acetate, filter through a celite plug, and evaporated. The residue was purified by chromatography column (hexane:ethyl acetate gradient 3:1 to 2:1) to yield 188 as a white solid (163 mg, 95 % yield). Rf = 0.23 (hexanes: ethyl acetate, 2:1). ¹H NMR (CDCl₃, 300 MHz): δ = 7.40 (d, 1H, J= 8.1 Hz), 7.09 (d, 1H, J= 8.1 Hz), 6.59 (d, 1H, J= 16.2 Hz), 5.64 (dt, 1H, J= 16.2 Hz, 7.2 Hz), 5.56 (s, 1H), 4.18-4.24 (m, 2H), 3.86-3.95 (m, 2H), 3.77 (t, 2H, J= 6.3 Hz), 2.53 (dt, 2H, J= 7.2 Hz, 6.3 Hz, 1.5 Hz), 2.19-2.26 (m, 7H), 1.72 (br s, 1H), 1.36-1.42 (m, 1H). ¹³C NMR (CDCl₃, 75 MHz): δ 137.2, 136.2, 134.1, 133.9, 132.1, 130.0, 128.5, 123.5, 100.9, 67.3, 61.9, 36.7, 25.7, 20.6, 16.6. IR: ν 3423, 2957, 2858, 1380, 1106, 983 cm⁻¹.
Dess-Martin Periodinane (191)

The reagent (11.7 g) was prepared by oxidation of 2-iodobenzoic acid to IBX (193) with oxone, followed by acylation, using 0.5 % p-toluenesulfonic acid and acetic anhydride.

(E)-4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-enal (189):

A solution of (E)-4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-en-1-ol (188) (1.224 g, 4.66 mmol) in dichloromethane (15 mL) was added to a solution of DMP (191) (2.777g, 6.55 mmol) in dichloromethane (35 mL), and the reaction mixture was stirred at room temperature for 1h. The reaction mixture was then poured into an Erlenmeyer flask containing diethyl ether (180 mL) and sodium thiosulphate (12 g) dissolved in saturated aqueous sodium bicarbonate solution (120 mL), and the mixture was stirred for 10 min. The layers were separated, the aqueous layer was extracted with diethyl ether, the combined organic layers were washed with saturated aqueous sodium bicarbonate and brine, dried over MgSO₄, and evaporated to yield the product as yellow oil that was pure by ¹H NMR (1.116 g, 92 % yield). Rᵢ= 0.50 (hexanes: ethyl acetate, 2:1). ¹H NMR (CDCl₃, 300 MHz): δ= 9.83 (t, 1H, 1.6 Hz); 7.46 (d, 1H, 7.8 Hz); 7.11 (d, 1H, 7.8 Hz); 6.59 (d, 1H, 16.2 Hz); 5.76 (dt, 1H, 16.2 Hz, 7.2 Hz); 5.62 (s, 1H); 4.16-4.23 (m, 2H); 3.91-4.00
(m, 2H); 3.40-3.44 (m, 2H); 2.26 (s, 3H); 2.16-2.27 (m, 1H); 2.17 (s, 3H); 1.37-1.44 (m, 1H). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ = 199.4, 137.0, 135.5, 134.1, 134.0, 132.4, 128.9, 125.1, 123.4, 100.2, 67.2, 47.8, 25.7, 20.5, 16.5. IR: $\nu$ 2963, 2854, 2726, 1721, 1106, 986, 822 cm$^{-1}$.

(E)-4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-enoic acid (190):

A solution of sodium chlorite (80 % w/w, 437 mg, 3.86 mmol) and potassium dihydrogenphosphate (527 mg, 3.86 mmol) in water (9 mL) was added via a glass pipet over 20 min into a solution of aldehyde (189) (335 mg, 1.29 mmol) and 2-methyl-2-butene (5.5 mL, 52 mmol) in tert-butanol (27 mL). The mixture was stirred at room temperature for 3.5 h, extracted with ethyl acetate, washed with water and brine, dried (MgSO$_4$), and evaporated. The crude product was purified by column chromatography (hexanes: ethyl acetate, 10:1 to 1:1 gradient) to give a yellow solid (305 mg) that was further purified by recrystallization. The yellow solid was dissolved in ethyl acetate at room temperature, hexanes were added until the solution became milky, the mixture was kept in a $-20^\circ$C freezer overnight, and filtered to give a pure product as a white solid (249 mg, 70 %). $R_f$ = 0.10 (hexanes: ethyl acetate, 2:1). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ = 7.44 (d, 1H, 7.8 Hz); 7.11 (d, 1H, 7.8 Hz); 6.60 (d, 1H, 15.9 Hz); 5.78 (dt, 1H, 15.9 Hz, 7.2 Hz); 5.63 (s, 1H); 4.22-4.17 (m, 2H); 3.97-3.88 (m, 2H); 3.36 (dd, 2H, 7.2 Hz, 1.5 Hz); 2.26 (s, 3H); 2.28-2.16 (m, 1H); 2.17 (s, 3H); 1.41-1.36 (m, 1H). $^{13}$CNMR
(CDCl₃, 75 MHz): δ = 177.8, 137.0, 135.3, 134.2, 134.1, 131.5, 128.9, 126.5, 123.4, 100.2, 67.2, 38.3, 25.8, 20.5, 16.4. IR: ν 3500-2850, 1710, 1106, 982, 822 cm⁻¹.

(E)-4-(6-formyl-2,3-dimethylphenyl)but-3-enoic acid (176):

The acetal (190) (84 mg, 0.31 mmol) was dissolved in a solution of acetone (3 mL) and 1M HCl (0.3 mL), and the mixture was stirred at room temperature for 3 h. Water was added, the mixture was extracted with dichloromethane, washed with brine, dried (MgSO₄), and evaporated to give the product as a white solid (56 mg, 85 %). ¹H NMR (CDCl₃, 300 MHz): δ = 10.58 (br s, 1H); 10.19 (s, 1H); 7.71 (d, 1H, 7.8 Hz); 7.21 (d, 1H, 7.8 Hz); 6.79 (d, 1H, 15.9 Hz); 5.72 (dt, 1H, 15.9 Hz, 7.2 Hz); 3.43 (dd, 2H, 7.2 Hz, 0.9 Hz); 2.35 (s, 3H); 2.25 (s, 3H). ¹³CNMR (CDCl₃, 75 MHz): δ = 193.2, 176.7, 143.3, 140.8, 135.5, 132.8, 130.4, 129.4, 129.2, 125.7, 38.1, 21.0, 15.8. IR: ν 3500-2500, 1710, 1679, 1586, 1244, 977, 822, 779 cm⁻¹.

Methyl (E)-4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-enoate (196):

Pyridinium dichromate (5.100 g, 13.6 mmol) was added at 0 ºC to a solution of aldehyde (189) (600 mg, 2.31 mmol) in methanol (0.56 mL, 13.8 mmol) and DMF (14 mL). The mixture was stirred at 0 ºC in the dark for 1 h and at room temperature for 1.5 h when the TLC analysis indicated full consumption of the substrate. The mixture was diluted
with hexanes:diethyl ether (3:2, 100 mL), filtered through celite, washed with water (3 x 8 mL), and brine, dried (MgSO₄), and evaporated. The crude was purified by column chromatography using hexanes:ethyl acetate 10:1 as an eluent to give the product as a white solid (321 mg, 48 % yield). Rᵥ= 0.38 (hexanes: ethyl acetate, 2:1). ¹H NMR (CDCl₃, 300 MHz): δ = 7.46 (d, 1H, 7.9 Hz); 7.11 (d, 1H, 7.9 Hz); 6.56 (d, 1H, 15.9 Hz); 5.79 (dt, 1H, 15.9 Hz, 7.1 Hz); 5.64 (s, 1H); 4.21 (dd, 2H, 11.6 Hz, 5.1 Hz); 4.00-3.91 (m, 2H); 3.76 (s, 3H); 3.32 (dd, 2H, 7.2 Hz, 1.3 Hz); 2.31-2.21 (m, 1H); 2.27 (s, 3H); 2.18 (s, 3H); 1.43-1.39 (m, 1H). ¹³CNMR (CDCl₃, 75 MHz): δ= 172.1, 136.9, 135.5, 134.2, 134.1, 130.9, 128.8, 127.2, 123.3, 100.1, 67.2, 51.8, 38.4, 25.8, 20.5, 16.4. IR: ν 2948, 2843, 1737, 1153, 1107, 986, 822 cm⁻¹.

Methyl (E)-4-(6-formyl-2,3-dimethylphenyl)but-3-enoate (197):

The acetal (196) (321 mg, 1.11 mmol) was dissolved in a solution of 1 M HCl (60 μL), acetone (8 mL) and water (1.3 mL), and the mixture was stirred at room temperature for 2 h. The mixture was extracted with dichloromethane, washed with brine, dried (MgSO₄), and evaporated. The crude was purified by column chromatography (hexanes:ethyl acetate, 10:1) to give the product as a white solid (198 mg, 75 % yield). ¹H NMR (CDCl₃, 300 MHz): δ = 10.18 (s, 1H, ); 7.69 (d, 1H, 7.9 Hz); 7.20 (d, 1H, 7.9 Hz); 6.76 (d, 1H, 15.9 Hz); 5.71 (dt, 1H, 15.9 Hz, 7.2 Hz); 3.74 (s, 3H); 3.37 (dd, 2H, 7.2 Hz, 1.2 Hz); 2.35 (s, 3H); 2.24 (s, 3H). ¹³CNMR (CDCl₃, 75 MHz): δ=192.8, 171.4, 143.1,
140.9, 135.5, 133.0, 131.0, 129.1, 128.9, 125.5, 52.0, 38.2, 21.0, 15.8. IR: ν 2953, 1736, 1586, 1240, 1201, 1165, 973, 840, 778 cm⁻¹.

(Z)-4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-en-1-ol (200):
Alkyne (187) (346 mg, 1.33 mmol) was dissolved in methanol (6 mL), quinoline (30 μL, 0.25 mmol), and 10 % wt. Lindlar catalyst (30 mg) were added, and the mixture was purged with hydrogen from a balloon for 1.5 h. The mixture was filtered through celite, and evaporated. Purification by column chromatography (hexanes:ethyl acetate 10:1 to 2:1 gradient) gave the product as lightly yellow oil (301 mg, 85 %). Rf = 0.33 (hexanes: ethyl acetate, 2:1). ¹H NMR (CDCl₃, 300 MHz): δ = 7.39 (d, 1H, 7.8 Hz); 7.11 (d, 1H, 7.8 Hz); 6.59 (d, 1H, 11.4 Hz); 5.87 (dt, 1H, 11.4 Hz, 7.2 Hz); 5.54 (s, 1H); 4.19 (m, 2H); 3.86-3.94 (m, 2H); 3.55-3.61 (m, 2H); 2.27 (s, 3H); 2.14-2.21 (m, 1H); 2.13 (s, 3H); 2.04-2.11 (m, 2H); 1.81 (t, 1H, 5.0 Hz, OH); 1.36-1.43 (m, 1H). ¹³CNMR (CDCl₃, 75 MHz): δ = 137.3, 134.9, 134.1, 133.9, 130.5, 129.2, 128.6, 123.1, 100.5, 67.4, 61.7, 32.2, 25.7, 20.6, 16.3. IR: ν 3422, 2961, 2857, 1376, 1152, 1108, 1038, 989, 822 cm⁻¹.
(Z)-4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-enal (201):

A solution of alcohol (200) (250 mg, 0.95 mmol) in dichloromethane (6 mL) was added to a solution of DMP (603 mg, 1.42 mmol) in dichloromethane (8 mL), and the mixture was stirred at room temperature for 50 min. The mixture was poured into an Erlenmeyer containing diethyl ether (40 mL) and a solution of sodium thiosulphate Na$_2$S$_2$O$_3$ (2.5 g) in saturated aqueous sodium bicarbonate (25 mL), and stirred for 10 min. The layers were separated, the aqueous layer was extracted with diethyl ether, the combined organic layers were washed with sodium bicarbonate and brine, dried (MgSO$_4$), and evaporated to give the product as lightly yellow oil (200 mg, 81 %). $R_f= 0.73$ (hexanes: ethyl acetate, 2:1). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta= 9.59$ (s, 1H); 7.42 (d, 1H, 8.1 Hz); 7.13 (d, 1H, 8.1 Hz); 6.67 (d, 1H, 11.4 Hz); 6.08 (dt, 1H, 11.4 Hz, 7.2 Hz); 5.45 (s, 1H), 4.10-4.21 (m, 2H); 3.78-3.93 (m, 2H); 2.89-3.11 (m, 2H); 2.26 (s, 3H); 2.12-2.25 (m, 1H); 2.10 (s, 3H); 1.34-1.43 (m, 1H). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta= 199.8$, 137.4, 134.2, 134.1, 134.0, 131.4, 129.1, 123.7, 123.1, 100.1, 67.4, 67.3, 43.6, 25.7, 20.5, 16.3. IR: $\nu$ 2964, 2926, 2855, 2725, 1726, 1375, 1237, 1152, 1107, 991, 822 cm$^{-1}$.
Methyl (Z)-4-(6-(1,3-dioxan-2-yl)-2,3-dimethylphenyl)but-3-enoate (202):  
Pyridinium dichromate (1.730 g, 4.60 mmol) was added at 0 ºC to a solution of aldehyde (201) (173 mg, 0.66 mmol) in methanol (0.19 mL, 4.69 mmol) and DMF (14 mL). The mixture was stirred at 0 ºC in the dark for 2 h and at room temperature for 2 h. When the TLC analysis indicated full consumption of the substrate, the mixture was diluted with diethyl ether, filtered through celite, washed with water (3x), and brine, dried (MgSO₄), and evaporated. The crude was purified by column chromatography using hexanes:ethyl acetate 10:1 as an eluent to give the product as colorless oil (76 mg, 39 %). Rᵣ= 0.78 (hexanes: ethyl acetate, 2:1). ¹H NMR (CDCl₃, 300 MHz): δ= 7.41 (d, 1H, 7.8 Hz); 7.11 (d, 1H, 7.8 Hz); 6.58 (d, 1H, 11.1 Hz); 6.04 (dt, 1H, 11.1 Hz, 7.2 Hz); 5.48 (s, 1H); 4.10-4.20 (m, 2H); 3.82-3.94 (m, 2H); 3.64 (s, 3H); 2.82-2.89 (m, 2H); 2.26 (s, 3H); 2.13-2.24 (m, 1H); 2.10 (s, 3H); 1.34-1.41 (m, 1H). ¹³C NMR (CDCl₃, 75 MHz): δ= 172.2, 137.1, 134.1, 134.0, 133.9, 130.0, 128.9, 125.4, 122.9, 100.1, 67.3, 67.2, 51.5, 33.5, 25.7, 20.4, 16.2. IR: ν 2954, 2851, 1736, 1153, 1107, 990, 951, 925, 822 cm⁻¹.

Methyl (Z)-4-(6-formyl-2,3-dimethylphenyl)but-3-enoate (203):  
The acetal (202) was dissolved in a solution of 1M HCl (15 μL), water (0.3 mL) and acetone (2 mL), and stirred at room temperature for 2.5 h. The mixture was diluted with
DCM, washed with water and brine, dried (MgSO₄), and evaporated. Purification by column chromatography (hexanes:ethyl acetate, 10:1) gave 48 mg of the product (80 % yield). ¹H NMR (CDCl₃, 300 MHz): δ= 10.13 (s, 1H); 7.70 (d, 1H, 7.8 Hz); 7.23 (d, 1H, 7.8 Hz); 6.77 (d, 1H, 11.4 Hz); 6.22 (dt, 1H, 11.4 Hz, 7.1 Hz); 3.63 (s, 3H); 2.84 (dd, 2H, 7.1 Hz, 1.5 Hz); 2.36 (s, 3H); 2.18 (s, 3H). ¹³CNMR (CDCl₃, 75 MHz): δ= 192.5, 171.2, 143.9, 139.3, 135.5, 132.1, 129.3, 128.3, 127.4, 125.1, 51.8, 33.8, 21.0, 15.8.

A.7 Alternative pharmacophore

![Alternative pharmacophore](image)

3-phenyl-1-(triisopropylsilyl)-1H-pyrrole (207a):

3-Bromo-1-triisopropylsilylpyrrole (205) (203 mg, 0.67 mmol, TCI America), phenylboronic acid (174 mg, 1.43 mmol), and palladium tetrakis (50 mg, 0.032 mmol, 5 mol %) were stirred in a mixture of 2 M aqueous solution of sodium carbonate (0.67 mL, 1.34 mmol), methanol (3 mL) and toluene (15 mL) at 70 ºC for 4 h. Solvents were removed on the rotovap, the remaining oil was dissolved in ethyl acetate to precipitate triphenylphosphine, and the mixture was filtered through anhydrous sodium sulphate plug. The crude was purified by column chromatography using petroleum ether as an eluent to give the product as colorless oil (121 mg, 60 % yield). Rᵣ=0.24 (petroleum ether) ¹H NMR (CDCl₃, 300 MHz): δ 7.58-7.54 (m, 2H); 7.34 (tt, 2H, 7.6 Hz, 1.5 Hz); 7.16 (tt, 1H, 7.6 Hz, 1.2 Hz); 7.08 (t, 1H, 1.8 Hz); 6.82 (t, 1H, 2.3 Hz); 6.63 (dd, 1H, 2.8 Hz, 1.6 Hz); 1.50 (septet, 3H, 7.3 Hz); 1.15 (d, 18H, 7.3 Hz).
3-(2-methoxyphenyl)-1-(triisopropylsilyl)-1H-pyrrole (207b):

3-Bromo-1-triisopropylsilylpyrrole (205) (409 mg, 1.35 mmol, TCI America), methoxyphenylboronic acid (206, 149 mg, 0.98 mmol), and palladium tetrakis (61 mg, 0.039 mmol, 4 mol %) were stirred in a mixture of 2 M aqueous solution of sodium carbonate (1.0 mL, 2.0 mmol), methanol (2 mL) and toluene (5 mL) at 70 °C for 4 h. Solvents were removed on the rotovap, the remaining oil was dissolved in ethyl acetate to precipitate triphenylphosphine, washed with brine, dried (MgSO₄), and evaporated. The crude was purified by column chromatography using petroleum ether:ethyl acetate (10:1) as an eluent to give the product as colorless oil (97 mg, 60 % yield). Rᵣ=0.76 (petroleum ether:ethyl acetate, 10:1). ¹H NMR (CDCl₃, 300 MHz): δ 7.56 (dd, 1H, 7.5 Hz, 1.8 Hz); 7.35 (t, 1H, 1.8 Hz); 7.13 (td, 1H, 7.5 Hz, 1.8 Hz); 6.98-6.91 (m, 2H); 6.79 (t, 1H, 2.5 Hz); 6.71 (dd, 1H, 2.8 Hz, 1.5 Hz); 3.88 (s, 3H); 1.48 (septet, 3H, 7.6 Hz); 1.13 (d, 18 H, 7.6 Hz). ¹³CNMR (CDCl₃, 75 MHz): δ 156.1, 127.8, 126.0, 124.9, 124.3, 123.8, 122.1, 120.8, 111.4, 110.0, 55.4, 17.9, 11.7.

3-phenyl-1H-pyrrole (208a):³⁶₀

Tetra-n-butylammonium fluoride (1M in THF, 0.40 mL, 0.40 mmol) was added to a solution of 3-phenyl-1-(triisopropylsilyl)-1H-pyrrole (#) (113 mg, 0.38 mmol) in THF at 0 °C and the mixture was stirred at room temperature for 50 min. Water was added, the
mixture was extracted with ethyl acetate, washed with brine, dried (MgSO₄), and evaporated. The crude was purified on silica gel column using petroleum ether: ethyl acetate 100:1 to 4:1 gradient as an eluent to give the product as a white solid (46 mg, 86 %). Rᵣ=0.07 (petroleum ether). ¹H NMR (CDCl₃, 300 MHz): δ 8.31 (br s, 1H); 7.59-7.55 (m, 2H); 7.37 (t, 2H, 7.6 Hz); 7.20 (tt, 1H, 7.3 Hz, 1.5 Hz); 7.10 (dd, 1H, 4.8 Hz, 2.1 Hz); 6.84 (dd, 1H, 5.3 Hz, 2.6 Hz, 2.1 Hz); 6.58 (dd, 1H, 5.4 Hz, 2.9 Hz, 2.6 Hz). ¹³CNMR (CDCl₃, 75 MHz): δ 135.8, 128.6, 125.5, 125.2, 124.9, 118.8, 114.5, 106.5. The NMR data matched the literature data. ³⁶⁰

3-(2-methoxyphenyl)-1H-pyrrole (208b):³⁶¹

To a solution of 3-(2-methoxyphenyl)-1-(triisopropylsilyl)-1H-pyrrole (207b) (37 mg, 0.11 mmol) in THF (3 mL) was added at 0 ºC a solution of TBAF in THF (1 M, 0.12 mL, 0.12 mmol), and the mixture was stirred at room temperature for 40 min. Water was added, the mixture was extracted with ethyl acetate, washed with brine, dried (MgSO₄), and evaporated. The crude was purified by column chromatography using petroleum ether: ethyl acetate (10:1) to give 16 mg of the product (84 %). Rᵣ=0.16 (petroleum ether:ethyl acetate, 10:1). ¹H NMR (CDCl₃, 300 MHz): δ 8.24 (br s, 1H); 7.55 (dd, 1H, 7.6 Hz, 1.8 Hz); 7.35-7.33 (m, 1H); 7.16 (ddd, 1H, 8.2 Hz, 6.8 Hz, 1.8 Hz); 7.35-7.33 (m, 1H); 7.16 (ddd, 1H, 8.2 Hz, 6.8 Hz, 1.8 Hz); 7.00-6.93 (m, 2H); 6.81 (dd, 1H, 5.6 Hz, 2.7 Hz); 6.65-6.62 (td, 2.7 Hz, 1.6 Hz); 3.89 (s, 3H). ¹³CNMR (CDCl₃, 75 MHz): δ 156.1, 128.0, 124.9.
126.3, 124.6, 120.7, 120.3, 117.9, 117.6, 111.1, 108.0, 55.3. The NMR data matched the literature data.\textsuperscript{361}

\begin{equation}
\begin{array}{c}
\text{Boc} \\
\text{OH}
\end{array}
\end{equation}

(1-(\text{tert-Butoxycarbonyl})-\text{1H-pyrrol-2-yl})\text{boronic acid (213)}:\textsuperscript{353}

LDA (0.8 M in THF, 16.5 mL, 13.2 mmol, made from \text{nBuLi} and diisopropylamine) was added to a solution of N-Boc-pyrrole (Aldrich, 2.00 g, 12.0 mmol) in THF (20 mL) at \(-78^\circ\text{C}\). The reaction mixture was stirred for 40 min, then trimethylborate (6.7 mL, 60 mmol) was added, and the mixture was further stirred at \(-78^\circ\text{C}\) for 1.5 h and at 0 \({\circ\text{C}}\) for 2 h. 0.25 M aqueous hydrochloric acid was added (20 mL), the layers were separated, the aqueous layer was extracted with diethyl ether, the combined aqueous layers were washed with water (twice) and brine, dried over MgSO\textsubscript{4}, and evaporated. The crude product, dissolved in DCM, was purified by column chromatography using a mixture of hexanes and ethyl acetate (10:1 to 4:1 gradient) to give (213) as an off-white solid (1.857 g, 74 % yield). \text{R}_f = 0.16 (\text{hexanes : ethyl acetate 10:1}). \textsuperscript{1}^\text{H} \text{NMR} (\text{CDCl}_3, 300 MHz): \delta 7.45 (dd, 1H, 3.1 Hz, 1.6 Hz); 7.19 (br. s, 2H, OH); 7.10 (dd, 1H, 3.1 Hz, 1.6 Hz); 6.26 (t, 1H, 3.1 Hz); 1.62 (s, 9H). \textsuperscript{13}CNMR (\text{CDCl}_3, 75 MHz): \delta 152.2, 128.7, 127.1, 112.0, 85.5, 27.9 (The NMR data matched the reported data).\textsuperscript{362}
**tert-Butyl 2-(pyridin-3-yl)-1H-pyrrole-1-carboxylate (215):**

Tetrakis-(Triphenylphosphine)palladium (63 mg, 0.040 mmol, 4 mol %) was added to a mixture of N-(Boc)pyrrole-2-boronic acid (213, 297 mg, 1.41 mmol), 3-bromopyridine (0.10 mL, 1.04 mmol), and 2 M Na₂CO₃ (1.6 mL, 3.2 mmol) in 1,2-dimethoxyethane (7 mL) at room temperature. The mixture was stirred at reflux for 2.5 h, cooled, then water was added, and the mixture was extracted with ethyl acetate, washed with brine, dried over MgSO₄, and evaporated. The crude product was purified by column chromatography using a gradient of hexanes and ethyl acetate 20:1 to 10:1. Rᵣ = 0.12 (hexanes : ethyl acetate 10:1). ¹H NMR (CDCl₃, 300 MHz): δ 8.60 (dd, 1H, 2.3 Hz, 0.8 Hz); 8.53 (dd, 1H, 4.8 Hz, 1.7 Hz); 7.67 (ddd, 1H, 7.9 Hz, 2.3 Hz, 1.7 Hz); 7.41 (dd, 1H, 3.1 Hz, 2.0 Hz); 7.28-7.31 (ddd, 1H, 7.9 Hz, 4.8 Hz, 0.8 Hz); 6.25-6.28 (m, 2H); 1.38 (s, 9H) (The NMR data matched the reported data).

**3-(1H-pyrrol-2-yl)pyridine (216):**

N-Boc protected 3-pyridylpyrrole (215) (144 mg, 0.59 mmol) was dissolved in THF (3 mL) and 25 % wt sodium methoxide in methanol (0.64 mL, 2.96 mmol) was added. The mixture was stirred at room temperature for 20 min, water was added, and the mixture was extracted with dichloromethane, washed with brine, dried (Na₂SO₄), and evaporated. The crude was purified by column chromatography pretreated with triethylamine, using a gradient of hexanes: ethyl acetate 10:1 to 2:1 to yield the product.
as a white solid (79 mg, 93 % yield). $R_f = 0.06$ (hexanes: ethyl acetate 4:1). $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 9.87 (br. s, 1H), 8.82 (dd, 1H, 2.3 Hz, 0.6 Hz); 8.40 (dd, 1H, 4.8 Hz, 1.7 Hz); 7.79 (ddd, 1H, 7.9 Hz, 2.3 Hz, 1.7 Hz); 7.25 (ddd, 1H, 7.9 Hz, 4.8 Hz, 0.6 Hz); 6.90-6.93 (m, 1H); 6.59-6.62 (m, 1H); 6.31-6.34 (m, 1H). $^{13}$CNMR (CDCl$_3$, 75 MHz): $\delta$ 146.5, 144.9, 131.2, 129.1, 128.6, 123.8, 120.2, 110.2, 107.2 (The NMR data matched the reported data).

3-(1H-pyrrol-2-yl)pyridin-1-ium chloride (217):

3-pyridylpyrrole (216) was dissolved in ethanol, a solution of concentrated HCl in ethanol was added, and the solvent was evaporated to yield the product in a quantitative yield. $^1$H NMR (D$_2$O, 500 MHz): $\delta$ 8.47 (d, 1H, 2.1 Hz); 8.30 (d, 1H, 5.7 Hz); 8.25 (dt, 1H, 8.4 Hz, 2.1 Hz); 7.79 (dd, 1H, 8.4 Hz, 5.7 Hz); 6.93 (dd, 1H, 2.7 Hz, 1.2 Hz); 6.58 (dd, 1H, 3.6 Hz, 1.2 Hz); 6.21 (dd, 3.6 Hz, 2.7 Hz). $^{13}$CNMR (D$_2$O, 125 MHz, acetone-d$_6$ standard): $\delta$ 139.4, 137.1, 135.7, 133.0, 127.9, 124.8, 123.9, 111.2, 110.7. HRMS (ESI): [M+H]$^+$ calculated: 145.0760, found: 145.0754.
10c

Chemical Shift (ppm)

Absolute Intensity

268
Chemical Shift (ppm)

Absolute Intensity

H$_2$O

TMS

2.13
2.84
3.32
3.41
4.18
6.69
6.97
7.08
7.27
7.38
12.98

CDCl$_3$

24.32
31.18
46.74
53.59
118.68
126.72
127.65
127.72
129.09
137.93

137.93
129.09
127.72
127.72
118.68
53.59
-46.74
-31.18
24.32
(Z)-2-TQN (70a) NOE
(E)-2-TQN (70b) NOE
68a
Chemical Shift (ppm)

Absolute Intensity

2.12 1.54 7.40 2.74 1.10 1.02 0.60 1.55 5.03 0.99 0.67

Z / E 1 : 0.6
Chemical Shift (ppm)

Absolute Intensity
STANDARD PROTON PARAMETERS
Pulse Sequence: gDOSY
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
INUVA-600 "Inova"
Relax delay 1.900 sec
Acq. time 0.162 sec
WIDTH 3628.8 Hz
20 Width 3628.8 Hz
2 repetitions
256 increments
OBSERVE H1, 600.7935162 MHz
DATA PROCESSING
Sqi; sine-bell 0.971 sec
F1 DATA PROCESSING
Sqi; sine-bell 0.335 sec
F1 q1d 1024 x 1024
Total time 18 min, 27 sec

\( \text{RCO}_{2} \cdot \text{H} \)

\( \text{(1)} \)
STANDARD PROTON PARAMETERS
Pulse Sequence: gCOSY
Solvent: CDCl3
Temp. 25.6 C / 298.1 K
INOVA-500 "Inova"
Relax. delay 1.048 sec
Acq. time 0.145 sec
Width 3523.9 Hz
29 Width 3523.9 Hz
2 repetitions
256 increments
RESOLVE HS, 599.79250441 MHz
DATA PROCESSING
Dq. sine bell 0.873 sec
Dq. sine bell 0.873 sec
FT size 1024 x 1024
Total time 10 min, 38 sec
(+ 5 % cis diastereomer)
(55:45)
Chemical Shift (ppm)

Absolute Intensity

KC-6-76C13

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

Chemical Shift (ppm)

0

0.1

0.2

0.3

0.4

0.5

0.6

0.7

0.8

0.9

1.0

1.1

1.2

1.3

1.4

1.5

1.6

1.7

1.8

1.9
(2'-F)-AB
(5'-F)-AB

Chemical Shift (ppm)

Absolute Intensity
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BIOGRAPHICAL SKETCH

Kinga Chojnacka was born in 1983 in Poznan (Poland) where she grew up. In 2003, Kinga started her studies at the Adam Mickiewicz University (Poznan), where she received her master's degree in chemistry in 2008 under supervision of Dr. Lech Celewicz. Her master's research focused on synthesis of analogs of 5-fluoro-2'-deoxyuridine 5'-phosphate with potential cytostatic activity. During her studies at the Adam Mickiewicz University, Kinga spent the 2006/2007 academic year at the University of Strasbourg (Louis Pasteur Université at the time, Strasbourg, France) within the Erasmus program (European Union student exchange program), taking classes in organic chemistry, biochemistry, medicinal chemistry, and pharmacology. At the end of that year, Kinga worked in the summer on synthesis of new fluorescent thiophene-substituted bodipys under direction of Dr. Antoinette De Nicola in Dr. Raymond Ziessel Laboratory. In the fall of 2008, Kinga started her doctoral research at the University of Florida under guidance of Dr. Nigel Richards in collaboration with Dr. Aaron Aponick, working on biosynthesis of plant hormones strigolactones. Finally, in the fall of 2011, Kinga began working with Dr. Nicole Horenstein and Dr. Roger Papke on the design and synthesis of molecules that modulate the functions of the alpha 7 nicotinic acetylcholine receptor, and received her Ph.D. at the end of 2013.