NOVEL BICYCLIC AND TRICYCLIC CANNABINERGIC LIGANDS

AS THERAPEUTIC AGENTS

A dissertation presented

by

Shama Bajaj

to

The Department of Chemistry and Chemical Biology
In partial fulfillment of the requirements for the degree of
Doctor of Philosophy
In the field of Chemistry

Northeastern University
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September, 2012
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ABSTRACT OF DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of Northeastern University

September, 2012
Abstract

The endocannabinoid system (ECS) encompasses cannabinoid receptors CB1 and CB2, their endogenous ligands (endocannabinoids), enzymes, proteins and FAAH-like anandamide transporter (FLAT), involved in endocannabinoid formation, transportation and bio-inactivation. The ECS is shown to be involved in an ever increasing number of pathological conditions including pain, immune-suppression and appetite disorders and has become an important target for the development of novel medications. ∆9-THC, the major psychoactive ingredient of marijuana, produces its physiological response through interaction with two well-characterized cannabinoid receptors (CB1 and CB2). Extensive structure-activity relationship (SAR) studies on tricyclic classical cannabinoids have established that the C-3 side chain plays a pivotal role in the ligand's affinity, selectivity, as well as potency towards these two receptors. Earlier our laboratory reported that AM411, a tricyclic cannabinoid bearing a pendant 1-adamantyl group at the C-3 position exhibits improved affinity and selectivity for CB1 as compared to ∆9-THC. Our present work involves optimizing other structural features within the tricyclic template to further improve CB1 affinity, selectivity, potency and in vivo profile. Variations in the Northern Aliphatic Hydroxyl (NAH) region and the Southern Aliphatic Hydroxyl (SAH) region of the optimized template were carried out to optimize molecule’s interaction with CB1 and CB2 receptors. This work has identified AM8607 as very potent and highly efficacious CB1-selective ligand. It is also functionally biased ligand with high potency in cAMP assay with no activity in β-arrestin assay. Also, AM8607 is much more potent in in vivo studies such as edema and allodynia experiments when compared with THC. This compound showed minimum tolerance development as compared to THC in tetrad experiments. When compared with diclofenac, a well-known painkiller, AM8607 was found to be about 50-fold
more potent as analgesic in an anti-allodynia experiment. With this potent and selective ligand in hand, we further explored SAR in the southern binding pocket. Also, to advance AM8607 for further in vivo studies, this compound was needed in larger quantities. With the original 23-steps synthesis it was not amenable to explore further SAR and synthesize AM8607 on large scale to enhance the project further. A shorter and more efficient synthetic route was optimized and developed to make AM8607 in good yield. Overall yield was improved from 1.8% in the earlier route to 2.2% in the new synthetic route. This synthetic route yielded a mixture of two enantiomers which can be fully separated on chiral HPLC to give the desired SAH enantiomer.

Another chapter of this thesis focuses on cannabidiol analogs (CBD). Naturally occurring (-)-CBD does not bind to cannabinoid receptors whereas (+)-CBD and its analogs exhibit significant affinity (nanomolar range) at both CB1/CB2 receptors.\textsuperscript{7,8} The lack of a versatile synthetic route has limited the SAR around the (+)-CBD template. Synthetic elaboration of intermediates synthesized during hybrid adamantyl cannabinoids led to the development of novel hexahydro-cannabidiols (HH-CBDs). With the objective of improving potency at the CB receptors and to understand the structural requirements, various HH-CBD analogs were synthesized with variation in C-3 side chain, northern functionality, southern functionality and stereochemistry. These CBD compounds led to some interesting findings. All the adamantyl compounds showed high affinity for mCB2 but with species subtype selectivity for hCB2. This issue was resolved by replacing adamantyl group with a dimethylheptyl (DMH) side chain. Addition of southern aldehyde and hydroxyl groups on DMH analogs improved the affinity and functional potency. Interestingly, 9-morpholine group in the northern pharmacophore imparted CB2 selectivity in both affinity and functional potency. Entire SAR of the HH-CBD analogs has been explained in Chapter 5.
The last part of this thesis focuses on the development of CB2 selective ligands. CB2 receptors have minimal presence in brain (neuronal CB2 role not known). Our laboratory previously showed that CB2 agonists can be used for the treatment of neuropathic pain without having CB1 mediated side effects. Key benzophenone analog AM4052, previously developed in our laboratory, was shown to possess high mCB2 affinity and selectivity over rCB1. However, it exhibits significant species subtype selectivity. Some enantiomeric as well as side-chain variation analogs of AM4052 were synthesized to resolve species subtype selectivity issue. Successfully, new compounds were synthesized with improved hCB2 affinity and no species subtype selectivity.
Dedicated to my Parents

Mother: Nirmal Kumari

&

Father: Surender Kumar
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<tr>
<td>2-AG</td>
<td>2-Arachidonylglycerol</td>
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<tr>
<td>AEA</td>
<td>Arachidonylethanolamide</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
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<td>CBD</td>
<td>Cannabidiol</td>
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<tr>
<td>CB</td>
<td>Cannabinoid</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>EtOAc</td>
<td>Ethyl acetate</td>
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<td>ECS</td>
<td>Endocannabinoid system</td>
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<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
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<td>Δ⁹-THC</td>
<td>Δ⁹-tetrahydrocannabinol</td>
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<td>CBN</td>
<td>Cannabinol</td>
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<td>Cannabinodiol</td>
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<td>CBG</td>
<td>Cannabigerol</td>
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<td>GPCR</td>
<td>G-protein coupled receptor</td>
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<tr>
<td>TRPV1</td>
<td>Transient receptor potential cation channel subfamily V member 1</td>
</tr>
<tr>
<td>hCB</td>
<td>Human cannabinoid receptor</td>
</tr>
<tr>
<td>rCB</td>
<td>Rat cannabinoid receptor</td>
</tr>
<tr>
<td>mCB</td>
<td>Mouse cannabinoid receptor</td>
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<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>Gi/o</td>
<td>Guanine nucleotide binding protein, alpha inhibiting activity polypeptide</td>
</tr>
<tr>
<td>GRKs</td>
<td>GPCR kinases</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>FAAH</td>
<td>Fatty acid amide hydrolase</td>
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<td>FLAT</td>
<td>FAAH-1 like anandamide transporter</td>
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<tr>
<td>MGL</td>
<td>Monoacyl glycerol lipase</td>
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<td>CCs</td>
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<td>AAIs</td>
<td>Aminoalkyl indoles</td>
</tr>
<tr>
<td>NAH</td>
<td>Northern aliphatic hydroxyl</td>
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<tr>
<td>PMHS</td>
<td>Polymethylhydrosiloxane</td>
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<tr>
<td>SAH</td>
<td>Southern aliphatic hydroxyl</td>
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<tr>
<td>SAR</td>
<td>Structure activity relationship</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
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<td>$^1$H</td>
<td>Proton</td>
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Chapter One

Introduction to Cannabinoids

1.1 Background

Since the discovery of cannabinoid receptors and their endogenous ligands in early 1990s, the endocannabinoid system (ECS) has been shown to play a vital role in several pathophysiological conditions. It has been targeted for the treatment of several diseases including neurodegenerative diseases (Parkinson’s, Alzheimer’s, Huntington’s and multiple sclerosis), cancer, obesity, inflammatory bowel disease, neuropathic and inflammatory pain. The last decade has witnessed remarkable advances in the development of cannabinergic ligands displaying high affinity, selectivity and potency towards two subtypes of cannabinoid receptors, namely CB1 and CB2. The objective of this research work was to retain or enhance the desired effects of earlier known cannabinergic ligands while minimizing the central side effects by limiting the blood-brain barrier penetrability and thus, leading to peripherally restricted CB1/CB2 ligands. Also, efforts were focused on developing functionally selective ligands, with high potency for G-proteins recruitment, but low or no activity for β-arrestin recruitment. A biased ligand approach with limited β-arrestin activity could potentially be an important factor in reducing the tolerance development, an undesirable effect associated with cannabinoids.

1.2 History of Cannabis and Cannabinoids

Cannabinoids are the chemicals which activate cannabinoid receptors. Among all of the cannabinoids, including endocannabinoids, phytocannabinoids (from plant) and synthetic cannabinoids, the most important are the phytocannabinoids of the marijuana plant (Cannabis
Marijuana has been used for thousands of years for medicinal and recreational purposes in Central Asia. In China, in 2737 BC, cannabis was used for therapeutic purposes, but the first documented evidence for its therapeutic application was found in the 19th century, with the pioneering work of Dr. William O'Shaughnessy who introduced marijuana to western medicine. He used cannabis extracts as analgesics for rheumatoid arthritis and as an anticonvulsant while working as a surgeon. This attracted researchers in England, North America and other European countries to explore its therapeutic potential. Use of marijuana became illegal in USA after the 1937 Marijuana Tax Act because of its abuse potential. The research in the cannabinoid field advanced with the discovery of the main pharmacologically active constituents of Cannabis sativa which are Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and (-)-cannabidiol (CBD) (Figure 1.1). Δ⁹-THC the main psychoactive component of the marijuana, was isolated, synthesized and characterized by Prof. Rafael Mechoulam in 1964. Δ⁹-THC along with its high therapeutic potential also has some side effects like mood alteration and adverse central nervous system (CNS) effect. In 1990, the cloning of the cannabinoid receptors which are involved in the CNS side effects produced by Δ⁹-THC, enhanced the quest for endogenous ligands for these receptors.

1.3 Chemical Constituents of Cannabis

The main chemical constituents of cannabis include cannabinoids, terpenoids and flavonoids. Among the known phytocannabinoids, THC, CBD and cannabiol (CBN) are the most important for pharmacological usage (Fig. 1.1). There are many other cannabinoid classes, some of which include cannabidiol (CBND), and cannabigerol (CBG). To date, about 500 compounds have been isolated from cannabis, of which 66 cannabinoids have been identified and are divided into 10 subclasses. CBD was first isolated in 1940, but its correct structure was not identified until
1963 by Mechoulam and his co-workers.\textsuperscript{15,16} CBD constitutes approximately 40\% of the cannabinoids in cannabis and has been shown to have anti-inflammatory, anti-anxiety and antipsychotic effects. THC was first isolated in 1942 and the correct structure and stereochemistry was identified in 1964 by Mechoulam.\textsuperscript{17,18} $\Delta^9$-THC binds to both CB1 and CB2 receptors in the body enhances hunger and increases the food intake in animals and humans.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{cannabinoids.png}
\caption{Main Components of \textit{Cannabis sativa}}
\end{figure}

\textbf{1.4 Discovery of Cannabinoid Receptors}

The membrane-bound cannabinoid receptors belong to G-protein coupled receptors (GPCRs). To date, two cannabinoid receptors, (CB1 and CB2), have been isolated, cloned and expressed.\textsuperscript{4,5} In 1990, the cloning of CB1 receptors and expression of a complementary DNA that encodes a GPCR involved in CNS effects enhanced the quest for endogenous ligands for these receptors.\textsuperscript{19} Later on another cannabinoid receptor was identified from human pro-myelocytic leukemic cell lines and named as CB2 receptor.\textsuperscript{5} This receptor was not expressed in the brain but rather in macrophages and spleen. With the further development in the cannabinoid field, nuclear receptor protein PPARs (peroxisome proliferator-activated receptors),\textsuperscript{20} ion channels such as TRPV1 and GPCRs as GPR55 were also considered to be novel cannabinoid receptors. Recently, Ryberg et al presented convincing \textit{in vitro} evidence that the orphan GPCR, GPR55, is a cannabinoid receptor.\textsuperscript{21} GPR55 binds to endocannabinoids and activates GTP\gammaS binding with high potency.
and is activated by endocannabinoids 2-AG and AEA as well as cannabinergic ligands such as Δ⁹-THC, CP-55,940 etc. However, this interaction has been shown to be cell-type and tissue-dependent. The TRPV1 channel is activated by anandamide, an endogenous ligand. To date, classification of any known channel or GPR55 as a CB3 receptor is debatable. It is because a novel CB3 receptor should be activated with significant potency by endogenous and synthetic CB ligands. Additionally, it should display some amino acid sequence homology with CB1 or CB2 or rhodopsin GPCRs. Low overall sequence homology of GPR55 with CB1 and CB2 as well as its distinct pharmacology has prevented it from getting considered as a cannabinoid receptor.

1.5 Structure of Cannabinoid Receptors

CB1 and CB2 cannabinoid receptors are G protein-coupled receptors and belong to the rhodopsin-like subfamily. CB receptors have seven hydrophobic domains which span the membrane seven times. The human CB1 (hCB1) receptor is a large protein with 472 amino acids whereas rat (rCB1) and mouse (mCB1) cannabinoid receptors have 473 amino acids. The hCB1 receptors share 97.3% homology with rat/mouse CB₁ receptors at the amino acid level and 100% homology within the trans-membrane regions. Due to this high similarity, rCB1 and hCB1 receptors can be used interchangeably for in vitro studies.

CB1 and CB2 receptors share approximately 45% sequence homology in the entire amino acid sequence and 68% homology in the trans-membrane domains. The hCB2 receptors are comprised of 360 amino acids and share less homology between species than CB1 receptors. Human (hCB2) and mouse (mCB2) receptors have 82% sequence homology, and rCB2 and hCB2 receptors have 81% homology, whereas mCB2 and rCB2 share 93% homology. Due to
this significant difference in trans-membrane domains, various cannabnergic ligands show species selectivity between mCB2 and hCB2. The rCB2 amino acid sequence is longer than hCB2, whereas mCB2 amino acid sequence is shorter than human CB2.

1.6 Binding and Signal Transduction of Cannabinoid Receptors

The ECS, through its CB receptors, can activate various signaling pathways depending on the demand, in response to stress, pathogen attack or stimuli, and can enhance tissue repair and cell survival (Fig. 1.2). These receptors get coupled with G_{i/o} proteins to inhibit adenylyl cyclase activity and thus inhibit the intracellular cyclic AMP (cAMP). cAMP inhibition starts mitogen-activated protein kinase (MAPK) activation which initiates the extracellular signal regulated kinase (ERK) cascade. CB1 receptors are also coupled to G-proteins via β, γ-mediated inhibition of calcium ion through inhibition of N- and P/Q-type voltage gated Ca^{2+} channels. Also, through activation of G-protein-activated inwardly rectifying K^+ channels (GIRKs), CB1 receptors are coupled to inhibition of cAMP/protein kinase A (Fig. 1.2). The CB1 cannabinoid receptor is constitutively active even in the absence of ligand. The CB1 receptor can also prevent other G_{i/o}-coupled receptors from signaling such as GABA, norepinephrine, etc. Under certain conditions, CB1 receptors can also signal through G_s proteins by enhancing the cAMP levels, CB2 receptors on the other hand, work only through G_{i/o} pathway. The positive effects of activation of the cannabinoid receptors lead to modulation of neurotransmission and to the regulation of pain, emotion, and endocrine functions in mammals. Work by Hungund and co-workers provided evidence that endocannabinoid signaling might be altered in specific brain regions and peripheral tissues during depression or negative affective states. Indeed, endogenous CB1 agonists probably serve as retrograde synaptic messengers.
Figure 1.2: Mechanism of signal transduction of CB1 receptors

1.7 Functionally Biased Cannabinoid Ligands

Earlier it was thought that GPCR signaling occurs only through G-proteins as shown in Figure 1.3a. When an agonist binds to the receptor, it activates G-proteins which through various secondary messengers produce different cell responses. This signaling is terminated by phosphorylation of the receptor caused by GPCR kinases (GRKs). These kinases bind to β-arrestin protein and lead to subsequent desensitization and internalization of the receptors. So, it was thought to be a simple process of signal transduction through G-proteins and desensitization and internalization through β-arrestin.\textsuperscript{45} However, it was found by Violin and co-workers that agonists binding to GPCRs are not always activating only G-proteins and GRKs but they can be biased for one of the pathways.\textsuperscript{46} Recently it was shown that protein β-arrestin aain addition to receptor desensitization is also capable of recruiting intracellular signaling.\textsuperscript{47} Biased agonism can
be an important therapeutic approach because signal transduction through both G-proteins and \(\beta\)-arrestin results in different cellular responses. Thus, ability to selectively manipulate physiological functions, through activation of a particular signaling pathway may be helpful in drug discovery for the development of safe and efficacious cannabinoid based drugs.\(^{48,49}\) Bohn’s lab found that tolerance development in opioids is due to \(\beta\)-arrestin recruitment and can be controlled with \(\beta\)-arrestin knockout.\(^{50}\) So, a biased ligand for one pathway could be helpful in targeting particular responses. As shown in Figure 1.3b, a G-protein biased ligand will undergo normal signal transduction but will have very low desensitization and internalization. On the other hand, a \(\beta\)-arrestin biased ligand will have signaling through activation of kinases, trans-activation and desensitization of the receptor.

Selective signaling of the cannabinoid receptors can result in various physiological responses and may result in drug molecules lacking unwanted side-effects. Long-term treatment with a GPCR agonist produces a number of physiological changes at cellular levels. For example, prolonged use of morphine results in tolerance\(^{51}\) which leads to desensitization at the \(\mu\)-opioid receptors.\(^{52}\) A functionally selective ligand lacking \(\beta\)-arrestin recruitment produces desired physiological responses with no tolerance development. Mackie’s lab worked on identification of functionally selective CB2 agonists and emphasized the different physiological effects produced by functionally biased ligands.\(^{53}\) They have found a difference in the functional selectivity of CP-55,940 and WIN55,212-2, both potent CB agonists. CP-55,940 inhibited voltage-gated calcium channels via CB2 receptor activation, whereas WIN55,212-2 was ineffective and antagonized the effects of CP-55,940. This can be the reason for internalization by CP-55,940 which was competitively antagonized by WIN55,212-2. The above example explains the effects of a
functionally biased ligand on physiological response and changes on cellular level.\textsuperscript{53}

**Figure 1.3:** a) Classical model for GPCR activation, signaling by G proteins and desensitization by $\beta$-arrestins. b) Current model for activation, signaling by G proteins and $\beta$-arrestins, as well as desensitization and internalization by $\beta$-arrestins (GFR, epidermal growth factor receptor; GRK, G-protein coupled receptor kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase).\textsuperscript{49}

### 1.8 Endocannabinoid System (ECS)

The first endocannabinoid arachidonylethanolamide (AEA) or anandamide was isolated in 1992 by Mechoulam.\textsuperscript{54} Its name was derived from a Sanskrit word “ananda” meaning “bliss”. The second endocannabinoid called 2-arachidonylglycerol (2-AG) was also discovered in Mechoulam's lab 1995.\textsuperscript{55} Our body utilizes its own enzymes fatty acid amide hydrolase (FAAH-1) and monoacylglycerol lipase (MGL) to degrade AEA and 2-AG. These two ligands are synthesized by, and released from, neurons on demand in Ca\textsuperscript{2+}-dependent pathways.\textsuperscript{56} They are produced “on demand” and are not stored in the vesicles like other neurotransmitters such as dopamine and norepinephrine.
AEA is a partial agonist at hCB1 receptor with modest affinity of 240 nM and a weak hCB2 agonist with affinity 1930 nM and has very short half-life as it is hydrolyzed by FAAH.\textsuperscript{33,57}

Another endocannabinoid, 2-AG is a full agonist at both CB1 and CB2 receptors, with lower affinity (K\textsubscript{i} = 472 nM and 1400 nM, respectively) and greater efficacy relative to AEA.\textsuperscript{57} Concentration of 2-AG is about 800 times more than that of anandamide in the brain.\textsuperscript{58} Evidence indicates that 2-AG is the most efficacious endocannabinoid identified to date. 2-AG can be synthesized from arachidonic-acid rich phospholipids, such as inositol, through hydrolysis with phospholipase C and diacylglycerol lipase (Fig. 1.4). Also, 2-AG can be synthesized from phosphatidylcholine through hydrolysis by phospholipase A1 and lysophospholipase C.\textsuperscript{59,60} 2-AG is hydrolyzed by MGL into arachidonic acid and glycerol which may be recycled back into phosphatidylinositol pool.\textsuperscript{58} The cannabinoid system proteins, including the CB1 and CB2 receptors, FAAH-1, intracellular membrane-bound amidases FAAH-1 and FAAH-2, FAAH like anandamide transporter (FLAT) as well as MGL have been shown to be excellent targets for the development of novel medications for various conditions, including pain, immunosuppression, peripheral vascular disease, appetite enhancement or suppression, and motor disorders.\textsuperscript{3,61} Inhibitors targeting these enzymes specifically could provide a novel therapeutic approach for the treatment of pain and movement disorders.\textsuperscript{62} This anandamide transporter “FLAT” was shown to lack amidase activity and found to be involved in translocation of AEA to reach CB1 receptors to mediate some of the cannabinerigic effects.\textsuperscript{3,63}
Figure 1.4: Biosynthesis of 2-AG.

1.9 Cannabinergic Ligands

Natural products or synthetic compounds which bind to cannabinoid receptors are termed as cannabinergic ligands. They can be divided into different classes based upon their structure, function, receptor selectivity and binding site.

1.9.1 Classification Based on Ligand’s Effect

1.9.1.1 Selective Cannabinoid Agonists

Those ligands which inhibit the adenylate cyclase activity and thereby decrease production of cAMP in functional assays, behave as CB agonists. These ligands increase the response produced by CB1 and CB2 receptors like hunger, immune response etc. These are also classified as CB1 agonists and CB2 agonists depending on the receptor subtype selectivity. Some of the important and selective CB1 or CB2 agonists include O-1812, R-(+)

methanandamide, JWH-133, HU-308 and GW-405833 as shown in Figure 1.5.
1.9.1.2 Selective Cannabinoid Antagonists or Inverse Agonists

Cannabinoid antagonists are those ligands that antagonize the agonist effects but have no effect of their own, whereas, inverse agonists are ligands which on their own have effects opposite of agonists. The first CB1 inverse agonist discovered was SR141716A (rimonabant).\(^2\) AM251 and NESS-0327\(^68\) are selective CB1 antagonists. JTE-907\(^69\) is a highly selective CB2 inverse agonist as shown in Figure 1.6.\(^70\) O-2050 is a silent/neutral CB1 antagonist.\(^71\)

1.9.1.3 Partial Agonists

Cannabinoid ligands which activate the cannabinoid receptor like agonists but with low efficacy are termed as partial agonists. In the presence of a full agonist these ligands behave as competitive antagonists. The best example of a partial agonist is THC, the naturally acting cannabinoid. THC acts as a partial agonist at both CB1 and CB2 receptors. Some of the important synthetic partial agonists at CB1 include O-1238 and O-584 (Figure 1.7).\(^72,73\)
1.9.2 Classification Based on Binding Site

1.9.2.1 Orthosteric Ligands

Ligands which interact with the receptor orthosteric site to shift the equilibrium to the receptor being in an active or inactive conformation are called orthosteric ligands. All the CB1 and CB2 ligands discussed so far are orthosteric ligands. They can be agonists, antagonists or partial agonists.

1.9.2.2 Allosteric Ligands

Those ligands which bind to the sites on the receptor distinct from the orthosteric binding site but alter the properties of orthosteric ligands by inducing conformational changes in the receptor are called allosteric modulators (Fig. 1.8). These ligands include negative allosteric modulators (NAMs) which inhibit the action of cannabinoid agonists or endocannabinoids and positive allosteric modulators (PAMs) which enhance the action of cannabinoid agonists or endocannabinoids. While orthosteric ligands compete with endogenous cannabinoid agonists, allosteric ligands do not. PSNCBAM-1 and Org 27759 are two of the important CB1 NAMs and RTI-371 is an example of a PAM.

Figure 1.7: Synthetic CB1 partial agonists
1.9.3 Classification Based on Structure

Based upon the structural templates, cannabinergic ligands mainly divided into five major classes, classical cannabinoids (CCs), non-classical cannabinoids (NCCs), diarylpyrazoles, aminoalkyl indoles (AAIs) and eicosanoids. However, in last decade a variety of new chemical classes of cannabinergic have been discovered.

1.9.3.1 Classical Cannabinoids

The ligands in this class include ABC- tricyclic benzopyran ring derivatives. These ligands are either natural or synthetic THC analogs or derivatives (Fig. 1.9). The most studied examples in this class are $\Delta^9$-THC, $\Delta^8$-THC and 11-hydroxy-tetrahydrocannabinol (HU-210).

The first SAR determinations based on the THC structure were summarized by Edery and his co-workers in 1971 and numerous reviews on this topic have since appeared. SAR studies have explored some important pharmacophores. A hydroxyl group at the C-1 aromatic position...
and an alkyl group on the C-3 aromatic position are necessary as well. The aromatic hydroxyl group must be free for high CB1 binding. Protecting the C-1 hydroxyl group as ether, completely removing it or converting into other functional group reduces the CB1 activity. C-3 chain length is the most explored pharmacophore so far in the tricyclic template. Chain length plays an important role in deciding the affinity, sub-type selectivity for CB1 or CB2 receptor and functional potency. CB1 receptor affinity increases with increases in the side chain length. A 7-carbon chain length is the most optimized chain length. Also, many functional groups like cyano, morpholino, bromo are tolerated at the end of the side chain and influence the affinity and potency. The northern pharmacophore includes 11-hydroxy THC-like analogs as potent cannabinoid ligands at both CB1 and CB2 receptors. Further oxidation of the C-11 hydroxyl group to a carboxyl group results in loss of CB binding activity. Hydroxyl, alkyne, propargyl alcohol and other smaller groups are well tolerated in the southern pocket. Small polar groups in the southern region enhance binding affinity of the ligand. The contribution of these groups towards functional potency, functional selectivity as well as the in vivo profile of resulting hybrid compounds has been discussed in Chapter 3.

1.9.3.2 Diarylpyrazoles

Discovery and earlier SAR work of this class of compounds has been reported by Sanofi pharmaceuticals. These compounds act as antagonists or inverse agonists. One drug molecule by Sanofi, SR141716A (rimonabant) is a potent CB1-selective inverse agonist (Fig. 1.6). Another lead compound SR144528 is a potent CB2 inverse agonist. These compounds prevent or reverse the effects of CB agonists. They behave as inverse agonists to reduce the constitutive activity of signal transduction pathways of cannabinoid receptors. AM251 is an analog of SR141716A, where chlorine group is replaced with iodine (Fig. 1.10), is more potent and
selective than rimonabant. SAR around the rimonabant template has developed many cannabinoid antagonists. A new series of 3,4-diarylpyrazolines was developed that was different than rimonabant’s 1,5-diarylpyrazole chemotype. One of the examples of this series is SLV-319, also called ibipinabant. It is a 1000-fold more selective CB1 antagonist over CB2. From the SAR studies on diarylpyrazoles, it is clear that replacement of the amide function by ketone, ether or alcohol decreases CB1 binding affinity. Replacement of chlorine with different substituents at the para-position is well tolerated. Replacement of the pyrazole ring with azitidine, imidazole and pyrimidine rings either improved or retained the affinity and selectivity.

![Figure 1.10: Important diarylpyrazole analogs](image)

**1.9.3.3 Aminoalkyl Indoles**

The discovery of pravadoline (Fig. 1.11) and its analogs in early 1990s led to the development of a new class of cannabimimetics called aminoalkyl indoles. These compounds are structurally different from the THC-like template. R-(+)-WIN55212-2 (Figure 1.11) is the most highly studied, high affinity cannabinoid ligand. It has moderate selectivity for the CB2 receptor and produces pharmacological effects similar to that of THC in *in vivo* studies. It’s S-(-)-enantiomer, is inactive in both *in vitro* and *in vivo* experiments. Radiolabelled [³H]-R-(+)-WIN55212-2 is commercially available and has been used to characterize cannabinoid receptors.
in rat brain. SAR around this template has developed many selective and high affinity ligands. Important examples include JWH-015 and AM630. Replacement of the bulky morpholine group of WIN55212-2 with alkyl chain retained the CB2 selectivity and affinity. AM630, a 6-iodo analog of pravadoline, has 10-fold higher affinity and much higher selectivity for CB2 than pravadoline.

![Chemical structures](image)

**Figure 1.11:** Aminoalkyl indole analogs

### 1.9.3.4 Non-classical Cannabinoids

This class of compounds lacks the typical ABC ring template and has either AC-bicyclic or ACD-tricyclic template. This class was first discovered by researchers at Pfizer. Their bicyclic compound CP-55,940 (Fig. 1.12) was lacking dihydropyran ring of THC. CP-55,940 binds to both CB1 and CB2 receptors with similar affinity and is more potent than THC in *in vivo* tetrad model. To date this is the most used radiolabeled ligand and standard to test the affinity of new cannabinoid agonists. Tritiated-[³H]-CP-55,940 was instrumental in the discovery and characterization of the CB1 cannabinoid receptor. An example of ACD-tricyclic ring non-classical cannabinoid is CP-55,244 (Fig. 1.12), which also has high affinity for CB1 receptors. Another very important example of non-classical cannabinoids with AC-bicyclic template is cannabidiol (CBD). (-)-CBD (Figure 1.1) is one of the active ingredients of the cannabis plant and has no CB affinity, but has been found to reduce THC-linked psychotic side-effects.
Synthetic cannabidiol ((+)-CBD), also called abnormal CBD (Fig. 1.12), binds to both CB1 and CB2 receptors. Both cannabidiols have various pharmacological effects which will be discussed later in Chapter 5.

**Figure 1.12:** Non-classical cannabinoids

1.9.3.5 Eicosanoids

This group of compounds include endocannabinoids i.e. anandamide, 2-arachidonoylglycerol and their synthetic derivatives (Fig. 1.13). Anandamide resembles THC by behaving as a partial agonist at CB1 and CB2 receptors. Methylation and other variations of the anandamide have improved affinity as well as metabolic stability. One of the important synthetic analogs is O-1812 (Fig. 1.5) which has higher CB1 affinity, selectivity, very low susceptibility to FAAH hydrolysis and higher in vivo potency as a CB1 receptor agonist as compared to anandamide.

Many structural changes are well tolerated at the head and tail part of the anandamide scaffold. 2-AG, unlike anandamide, is a full agonist at both CB1 and CB2 receptors and behaves as partial agonist at TRPV1 receptors.

**Figure 1.13:** Eicosanoids analogs
1.10 Cannabinoid based drugs on the market

In the nineteenth century, marijuana was used to treat wide range of health problems but its abuse potential limited its use. In the last 3 decades, research on the cannabinoids has once again revealed the pharmacological potential of marijuana. Various studies in animals and humans with marijuana based drugs have produced promising results for the treatment of cancer, obesity, spasticity and many other disorders. Marijuana ingredients, THC and CBD also have immunosuppressive and anti-inflammatory properties; which supports the basis of their therapeutic potential in chronic inflammatory diseases. There are only a few drugs on the market which have THC and/or CBD as active ingredients.

Dronabinol (Marinol®) is synthetic Δ⁹-THC (Fig. 1.1), the first marijuana-based drug approved by the United States Food and Drug Administration (FDA) in 1985 for the treatment of nausea and vomiting in cancer patients undergoing chemotherapy and as an appetite stimulant in AIDS patients. Later on, in 1992, it was approved as an appetite stimulant. Dronabinol acts as partial agonist at both CB receptors. It has rapid onset of action but has poor oral bioavailability. To improve its bioavailability it is administered via an inhaler and is available with the name Marinol.

Nabilone® is a synthetic cannabinoid developed by Lilly Pharma and currently marketed by Valeant Pharmaceuticals. It was approved in 1985 by FDA as antiemetic agent for treatment of chemotherapy induced nausea and vomiting. Although drug was approved in 1985 by FDA but this drug came in US market in 2006 for cancer patients. This synthetic compound is a racemic mixture of (S,S) and the (R,R) isomers (trans-isomers). It is also approved for the treatment of anorexia and weight loss in AIDS patients.
Sativex® also called nabiximols is a drug developed by GW Pharmaceuticals. It is 1:1 mixture of THC and CBD in the form of oromucosal spray. It was approved in the UK and Canada in 2010 for the treatment of neuropathic pain and spasticity in Multiple Sclerosis (MS) patients and as an analgesic for severe pain in cancer patients.

1.11 Need for the Novel Cannabinoid Ligands as Drugs

To date all of the cannabinoid drugs on the market are either natural cannabinoids or their synthetic analogs. Even after so many years of research there is not much success with regards to the synthesis and availability of new cannabinoid based drugs. There are many side effects including dizziness, tiredness, drowsiness, nausea, blurred vision and hallucinations when using THC as a drug. Decades of research in cannabinoid field emphasized the importance of cannabinoids in various physiological functions of the body. Preclinical data supports their use in the treatment of multiple sclerosis, spinal cord injury, pain, inflammatory disorders, bronchial asthma, cirrhosis, and cancer. There are many more complex conditions where cannabinoid receptors play an important role. Some of them are mentioned below:

**Alzheimer's disease** is the most common form of dementia. Alzheimer's disease is a chronic and progressive neurodegenerative disorder which accounts for 50 to 80 percent of dementia cases. This disease is mainly found in elderly people above the age of 60 but can be found in any age group. There is much evidence in the literature which supports the role of the CB2 receptor and its agonists for the treatment of Alzheimer's disease. The mechanism through which CB2 receptors treat neurological disorders is still under investigation.

**Glaucoma** is a leading cause of blindness in the world. There are diverse therapeutic opportunities available, but better treatments are highly desirable. For many years marijuana smoking was found to be beneficial. Cannabinoids are known to effectively lower the intraocular
pressure (IOP) and have neuroprotective actions. Researchers have proved that ocular CB1 receptors play an important role in this condition.

**Pain and Inflammatory Disorders:** Pain is an unpleasant experience associated with actual or potential tissue damage. Pain is mainly of two types, nociceptive and neuropathic. Nociceptive pain involves direct pain which means receptors detect mechanical, thermal or chemical changes and carry signals to brain. On the other hand, neuropathic pain is a damage to the central or peripheral nervous system due to disease or trauma. Currently available opioid analgesics have various side effects like constipation, sedation and nausea. Natural cannabinoids have analgesic properties with no such side effects which enhance their potential as targets for pain and inflammation management. Cannabinoid ligands have the potential to reduce nociception, inflammation, reduce the development of allodynia and hyperalgesia and protect secondary tissue damage in traumatic head injury. Activation of the CB2 receptor appears to be involved more in the down regulation of the inflammatory response. CB2 receptors are also involved in indirect stimulation of opioid receptors. Development of CB agonists with no psychoactive side effects will be a step towards pain treatment.

Because of the vast pharmacological profile there is a need to get potent CB1 and/or CB2 agonists that are devoid of side effects such as dependence and tolerance. These side effects can be potentially overcome by developing biased ligands that activate only one or limited pathways and produce pharmacologically beneficial effects. Also, to prevent psychotic side effects, a CB ligand with limited brain permeability and reduced off target activity can be an important lead.

**1.12 Objectives and Aims**

The need for novel CB1 and/or CB2 agonists as potential therapeutic agents has been discussed above. So, the objective of the work described in this thesis proposal is to explore the SAR
around tricyclic and bicyclic templates of THC and CBD to produce potent and efficacious ligands with a favorable pharmacological profile. With this objective in mind the following specific aims have been accomplished:

1. SAR studies on the earlier explored northern aliphatic hydroxyl (NAH) pharmacophore were pursued and the most potent compound was used for the *in vivo* studies and to explore other pharmacophores.

2. SAR studies on the SAH pharmacophoric site were pursued and effect of SAH groups on functional potency and efficacy was determined.

3. The pharmacologically important SAH analogs were synthesized through a concise, more economical and reproducible route.

4. A novel hexahydrocannabidiol (HH-CBD) template was explored for the first time and compounds were evaluated for their functional potencies.

5. SAR on the earlier explored (in our lab) bicyclic benzophenone template was pursued which led to CB2 selective ligands.
Chapter 2

Northern Modified Adamantyl (NAH) Cannabinoids

2.1 Introduction

G-protein coupled receptors (GPCRs) are the most abundant class of central nervous system (CNS) receptors in mammals, and are targets of many therapeutic medications. (-)-Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the main psychoactive ingredient of cannabis¹¹⁴ produces its biochemical and pharmacological effects by interacting with two well-characterized GPCRs, CB1 and CB2. During the last decade, numerous ligands with high affinities and selectivity profiles for cannabinoid receptors (CB₁ and CB₂) evolved from rigorously pursued structure-activity relationship (SAR) studies.⁷⁸,¹¹⁵,¹¹⁶ Δ⁹-THC exhibits no receptor subtype CB₁/CB₂ selectivity. Also, SAR studies with a number of synthetic cannabinoids structurally related to Δ⁹-THC have identified some key pharmacophores associated with cannabimimetic activity including; a) a phenolic hydroxyl group (PH) at C-1; b) a C-3 side chain (SC); c) 9-OH or 11-OH northern aliphatic hydroxyl group (NAH); d) a southern aliphatic hydroxyl group (SAH).¹¹⁵

![Figure 2.1: Important pharmacophores of THC template required for CB binding.](image)

Modify the phenolic hydroxyl in cannabinoids into a methoxy group or completely removing it leads to analogs with severely reduced CB₁ affinities. However, such modifications produce
only marginal effects on the compounds’ affinities for CB2. Additionally, analogs in which C-1 phenolic OH group is absent have been shown to exhibit CB2 selectivity and this observation has served as the basis for the synthesis of CB2 selective compounds. The C-3 aliphatic side chain is the most studied pharmacophore and was shown to have the most drastic effects on CB1/CB2 affinity, potency and selectivity. For example, compounds with shorter side chain such as those carrying C-3 butyl group exhibit enhanced CB2 selectivity whereas analogs with longer seven or eight carbon side chains were shown to have preference for CB1. Optimal activity is obtained with 1′,1′-dimethylheptyl chain which imparts about 100-fold increase in potency compared to the n-pentyl side chain of ∆⁹-THC. Cannabivarlin, a 3-propyl ∆⁹-THC analog exhibits poor binding to both CB receptors but behaves as a functional CB1 antagonist in tissue preparations. Incorporation of cyclic or aryl moieties at the 3-position are well tolerated. In earlier work, research from our laboratory has shown that an analog of (-)∆⁸-THC, an equiactive isomer of the ∆⁹-prototype, carrying a 1-adamantyl side chain (AM11) exhibits substantial CB1 affinity and selectivity. The 2-adamantyl analog, on the other hand, shows preference for CB2. The adamantyl group in AM411 (Figure 2.1) exhibits very slow desensitization of receptor and a longer duration of action. Research from our group has also explored other cyclic side chains such as 3-bornyl and 3-isobornyl analogs, aromatic groups, cycloalkyl chain or chains incorporating cycloalkyl groups. The other two pharmacophores NAH and SAH appear to play substantial roles in modulating CB1 and CB2 affinities, potencies and selectivities. The pharmacological evaluation of these compounds has shown us that the NAH substituents play an important role in affecting CB1 affinity, potency as well as efficacy. With the target of producing more potent and selective CB1 ligands with 1-adamantyl side chain as CB1 preferring pharmacophore, a new generation of tricyclic analogs with northern
modifications were synthesized in our lab. 11-β-OH adamantyl compound (AM4054) was found to be very potent; however, tolerance of longer chain in the northern region was not explored. With the objective of exploring deeper in the northern pocket newer. NAH analogs were synthesized. Also, the C-ring aromatized analog AM4089, with hydroxymethyl group, synthesized previously in our research group showed good affinity at both CB receptors with partial agonist activity at CB1.

![Diagram of adamantyl analogs](image)

**Figure 2.2:** Key adamantyl analogs developed earlier in our laboratory AM411, AM4054 and AM4089 which led to development of new analogs.

To understand the role of hydroxymethyl group in the affinity and potency, couple of aromatic analogs has been synthesized by replacing it with other functionalities. The synthesis and pharmacological evaluation of these compounds have shown the role of NAH group and C-ring aromaticity for CB1 affinity, and potency
2.2 Chemistry

Scheme 2.1: Synthesis of tricyclic NAH compounds

Adamantyl resorcinol 1 was synthesized from 2, 6-dimethoxyphenol in 4-steps by following a procedure we previously reported.6,127 The mixture of chiral terpene diacetates 2, which was used earlier in the stereospecific synthesis of 9-oxo-cannabinoids with (6aR, 10aR) absolute configuration, was obtained from commercially available (+)-(1R)-nopinone utilizing our earlier reported reaction conditions.85,128,129 Coupling of resorcinol 1 with 2 in the presence of catalytic p-toluenesulfonic acid led to norpinanone 3 (Scheme 2.1) in 78% yield. The structure of 3 was established by 1D NMR, COSY, NOESY correlations, HSQC and HMBC. TMSOTf promoted rearrangement-cyclization on 3 proceeded smoothly to give 4 in 61% yield. Introduction of the
C-9 aldehyde group was accomplished by treating 4 with (methoxymethylene)triphenylphosphorane. Unlike our earlier report \textsuperscript{130}, we found that this reaction did not require the previous protection of the phenolic OH groups. The 9-aldehyde diastereomeric aldehydes 6 were obtained in 98\% yield as a 2:1 \( \beta \) versus \( \alpha \) from the hydrolysis of its precursor vinyl ether \textit{cis} : \textit{trans} (1:4) mixture of isomers 5 (Scheme 2.1). Epimerization of the diastereomeric mixture of aldehydes produced the \( \beta \)-equatorial isomer 6 in 88\% isolated yield. One carbon Wittig homologation was performed again on the \( \beta \)-aldehyde 6 to give one carbon extended enol ethers mixture 7 (AM8616) in 90\% yield. Enol ethers were further hydrolyzed to give 8 (AM8617) in 86\% yield and then aldehyde was reduced to give alcohol 9 (AM8618) in 91\% yield. Analogs 7, 8 and 9 were evaluated for their affinity for CB1 and CB2 receptors as well as their functional potency using cAMP assay.

For the synthesis of the cannabinol analogs carrying 9-OH (15) or 9-OCH\(_3\) (16) group, an earlier published approach was adopted.\textsuperscript{131} Suzuki coupling of the boronic acid 10 with bromo adamantyl derivative 11 yielded the biphenyl derivative 12 in 72\% yield as shown in Scheme 2.2. Selective demethylation of biphenyl 12 with 9-I-BBN, followed by acetic acid catalyzed intramolecular cyclization gave cannibilactone 13 in a combined 63\% isolated yield. Demethylation of cannibilactone 13 to obtain the bis-phenolic lactone 14 (68\% yield) which was then converted to the desired 6,6-dimethyl analog 15 by treatment with excess methyl magnesium iodide followed by cyclization in the presence of \( p \)-toluenesulfonic acid in a 77\% combined yield. The other cannabinol analog 16 was obtained in 74\% yield by gem-dimethylation and cyclization.
Scheme 2.2: Synthesis of cannabinol compounds

Reagents and conditions: (a) Pd(PPh₃)₄, Ba(OH)₂, DME, H₂O, MW, 30min, 72%; (b) 9-Iodo-9-BBN, CH₂Cl₂ then acetic acid, reflux, 8h, 63%; (c) BBr₃, CH₂Cl₂, reflux, overnight, 68%; (d) CH₃MgI, THF, rt→reflux, 2h (e) p-TSA.H₂O, CHCl₃, rt, 77% & 74% (2 steps).

2.3 Result and Discussion

The SAR of novel adamantyl cannabinoids was examined by measuring their affinities for CB1 and CB2 receptors (Table 2.1). The 1-adamantyl cannabinergic analogs included in this study exhibited binding properties that were distinct from those of their Δ⁸- or C-3 alkyl counterparts. One carbon extension of AM4054 gave 9 (AM8618) which also has high affinity but low potency in cAMP assays (Table 2.2). Also, enol ether and aldehyde analogs 7 (AM8616) and 8 (AM8617) showed good binding affinity but had very low functional potencies. This shows that extension in the northern pocket is hindering the activation of the receptor and hence shows low functional potency. Then converting the hexahydro ring into aromatic ring generated the compounds 15 (AM8601) and 16 (AM8602) (Table 2.1). Both these compounds did not bind well in CB1 and CB2 binding pocket. This shows that OH and OMe groups attached to the aromatic ring are not tolerated. But one carbon extension synthesized earlier, which was benzyl alcohol (AM4089) was very well tolerated in the binding pocket. Although there was not much
difference in the hexahydro, tetrahydro and aromatic ring analogs (synthesized earlier) in the binding affinities but there is substantial difference in their functional profile (Table 2.2). Among all the screened analogs synthesized now and earlier in the lab, AM4054 was the most efficacious and potent in binding, functional and in vivo studies. It was shown not to have off-target activity when screened against a panel of 30 GPCRs and was more potent and efficacious than its parent compound AM411.

Table 2.1: Binding data of tricyclic adamantyl cannabinoids with variations in the C-ring
(*= Synthesized by Dr. Ganesh Thakur and N.A. = Not Applicable)

<table>
<thead>
<tr>
<th>Compound</th>
<th>AM No.</th>
<th>C-ring variation</th>
<th>Ki (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>rCB1</td>
</tr>
<tr>
<td>A</td>
<td>AM411*</td>
<td><img src="image" alt="C-ring variation" /></td>
<td>6.8 ± 0.04</td>
</tr>
<tr>
<td>B</td>
<td>AM4054*</td>
<td><img src="image" alt="C-ring variation" /></td>
<td>4.9 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>----</td>
<td>----</td>
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<td>-----</td>
</tr>
<tr>
<td></td>
<td>AM8616</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>31 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>AM8617</td>
<td></td>
<td>13.2 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>AM8618</td>
<td></td>
<td>8.6 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AM4089*</td>
<td></td>
<td>2.1 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>AM8601</td>
<td></td>
<td>88.6 ± 1.8</td>
</tr>
<tr>
<td>16</td>
<td>AM8602</td>
<td></td>
<td>507 ± 2.1</td>
</tr>
</tbody>
</table>
Table 2.2: Functional potencies (cAMP) of cannabinol analogs at CB1 and CB2 receptors

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>AM No.</th>
<th>Functional Data (cAMP)</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>hCB1 (% inhibition)</td>
<td>hCB2 (% inhibition)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10nM</td>
<td>1uM</td>
<td>10nM</td>
</tr>
<tr>
<td>A</td>
<td>AM411</td>
<td>--</td>
<td>95%</td>
<td>--</td>
</tr>
<tr>
<td>B</td>
<td>AM4054</td>
<td>42%</td>
<td>95%</td>
<td>37%</td>
</tr>
<tr>
<td>C</td>
<td>AM4089</td>
<td>--</td>
<td>102%</td>
<td>22%</td>
</tr>
<tr>
<td>8</td>
<td>AM8617</td>
<td>1%</td>
<td>100%</td>
<td>24%</td>
</tr>
<tr>
<td>9</td>
<td>AM8618</td>
<td>8%</td>
<td>100%</td>
<td>18%</td>
</tr>
</tbody>
</table>

-- = No activity

2.4 Conclusion:

Further SAR on AM4054 and AM4089 template has been developed. Variations on AM4054 template are well tolerated in the binding pocket but not active in the functional assays as suggested by the data for 8 and 9 in Table 2.2. Functionality on the AM4089 template, directly attached to the aromatic ring is not well tolerated. This shows that among the so far explored SAR AM4054 is the most potent agonist.
2.5 Experimental

(4R)-4-[4-(1-Adamantyl)-2,6-dihydroxyphenyl]-6,6-dimethyl-2-norpinanone (3). To a degassed solution of 1 (6.50 g, 26.60 mmol) and diacetates 2 (8.86 g, 37.20 mmol; 10.19 g, 87% pure diacetates 2 were used) in CHCl₃ (267 mL) at 0 °C, under an argon atmosphere was added p-toluenesulfonic acid monohydrate (7.085 g, 37.25 mmol). The reaction mixture was warmed to room temperature and stirred for 3.5 days. The reaction mixture was diluted with ether and washed sequentially with water, saturated aqueous NaHCO₃, and brine. The organic phase was dried (MgSO₄) and the solvent was removed under reduced pressure to give crude product as a brown oil. Recrystallization from CH₂Cl₂ and hexane (2:3) gave 3 as a white crystalline solid (7.95 g, 80.5% yield). Mp = 284-286°C. Rf = 0.4 (ethyl acetate : hexanes = 30/70) ¹H NMR (CDCl₃ + 2 drops DMSO) δ: 6.50 (br s, 2H, ArOH), 6.38 (s, 2H), 4.00 (t, J = 8.0 Hz, 1H), 3.63 (dd, J = 19.0 Hz, J = 8.0 Hz), 2.60-2.52 (m, 3H), 2.50-2.44 (m, 1H), 2.28 (t, J = 5.0 Hz, 1H), 2.06 (br s, 3H), 1.82 (d, J = 2.0 Hz, 6H), 1.77 (d, J = 12.5 Hz, 3H), 1.70 (d, J = 12.5 Hz, 3H), 1.35 (s, 3H), 0.99 (s, 3H). HRMS (ESI) calculated for C₂₅H₃₃O₃: calculated 381.2430; found 381.2433; [α]D²² = +62.6° (c = 0.40, CHCl₃).
(6aR,10aR)-6,6a,7,8,10a-Hexahydro-1-hydroxy-6,6-dimethyl-3-(1-adamantyl)-9H-dibenzo[b,d]pyran-9-one (4). To a solution of 3 (3.95 g, 10.38 mmol) in anhydrous CH₂Cl₂/CH₃NO₂ (3:1, 260 mL) at 0 °C, under an argon atmosphere was added trimethylsilyl trifluoromethanesulfonate (13.84 mL, 0.3 M solution in CH₃NO₂, 4.152 mmol) and the resulting mixture was stirred at 10 °C for 8 h. The reaction was quenched with saturated aqueous NaHCO₃/brine (1:1), and diethyl ether was added. The organic phase was separated, the aqueous phase was extracted with diethyl ether, and the combined organic phase was washed with brine and dried over MgSO₄. Solvent evaporation and purification by flash column chromatography on silica gel (acetone/hexane = 20/80) afforded 4 as white crystalline solid (2.41 g, 61% yield). M.P. = 263-264 °C. \( R_f = 0.16 \) (diethyl ether/hexanes = 20/80), \( R_f = 0.55 \) (ethyl acetate/hexanes = 40/60). \(^1\)H NMR (500 MHz, CDCl₃) \( \delta \): 6.74 (br s, 1H, OH), 6.41 (d, \( J = 2.0 \) Hz, 1H, ArH), 6.37 (d, \( J = 2.0 \) Hz, 1H, ArH), 4.07 (ddd, \( J = 15.0 \) Hz, \( J = 3.5 \) Hz, \( J = 2.5 \) Hz, 1H, 10eq-H), 2.90 (ddd, \( J = 13.5 \) Hz, \( J = 12.5 \) Hz, \( J = 4.0 \) Hz, 1H, 10a-H), 2.66-2.59 (m, 1H, 8eq-H), 2.51-2.42 (m, 1H, 8ax-H), 2.20-2.12 (m, 2H, 10ax-H, 7eq-H), 2.05 (br s, 3H), 1.97 (dt, \( J = 12.0 \) Hz, \( J = 2.5 \) Hz, 1H), 1.85 (d, \( J = 3.0 \) Hz, 6H), 1.76 (d, \( J = 12.5 \) Hz, 3H), 1.71 (d, \( J = 12.5 \) Hz, 3H), 1.53 (dq, \( J = 12.5 \) Hz, \( J = 5.0 \) Hz, 1H), 1.47 (s, 3H), 1.13 (s, 3H); HRMS (ESI) calculated for C₂₅H₃₃O₃: calculated 381.2430; found 381.2433; \([\alpha]^{20}_{D} = -57.8^\circ \) (\( c = 0.52, \text{CH}_2\text{Cl}_2 \)).
(6aR,10aR)-3-(Adamantan-1-yl)-9-(methoxymethylene)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol (5): To a suspension of (methoxymethyl)-triphenylphosphonium chloride (8.12 g, 23.7 mmol) in 90 mL anhydrous THF at -30°C was added a solution of n-BuLi in THF (9.21 mL, 23.02 mmol, 2.5M in hexane). The resulting blood red colored solution was warmed to 0°C over a period of 15 min. A solution of ketone 4 (1.288 g, 3.38 mmol) in anhydrous THF (60 mL) was then added through cannula keeping the reaction temperature at 0°C. The resulting solution was stirred for 15 min and then quenched by addition of water and stirred for 30 min till the solution turns colorless. Reaction mixture was diluted with ether, organic phase separated and the aqueous phase extracted with ether (2x). Combined organic extracts was washed with brine and dried (MgSO₄). Purification by flash chromatography on silica gel (2-20% ethyl acetate : hexanes) gave 5 as a white foam (1.33 g, 96%, 1:4 mixture of geometric isomers). \(Rf = 0.50 \) (Ethyl acetate/hexane = 20/80). \(^1\)H NMR (500 MHz, CDCl₃) δ: 6.41 (d, \(J = 2.0 \text{ Hz} \)), 6.40 (d, \(J = 2.0 \text{ Hz} \)), 6.26 (d, \(J = 2.0 \text{ Hz} \)), 6.24 (d, \(J = 2.0 \text{ Hz} \)), 5.94-5.92 (m), 5.86-5.85 (m), 4.79 (s, OH), 4.65 (s, OH), 4.18-4.12 (m), 3.59 (s, OCH₃), 3.58 (s, OCH₃), 3.47-3.40 (m), 2.96-2.90 (m), 2.44-2.37 (m), 2.08-2.02 (m), 1.93-1.86 (m), 1.85 (br s), 1.79-1.67 (m, especially 1.76, d, \(J = 12.5 \text{ Hz} \)) and 1.70, d, \(J = 12.0 \text{ Hz} \)), 1.65-1.58 (m), 1.39 (s, 6β-CH₃), 1.38 (s, 6β-CH₃), 1.06 (6α-CH₃). HRMS (ESI) calculated for C₂₇H₃₇O₃: calculated 409.2743; found 409.2735; \([\alpha]^{20}_{D} = -22.5^\circ \) (c = 0.3, CH₂Cl₂).
Aldehyde mixture: To a solution of enol ether 5 (1.21 g, 2.96 mmol) in 70 mL CH₂Cl₂ at room temperature was added wet trichloroacetic acid (4.84 g, 29.6 mmol in 5 mL water). The resulting solution was stirred at room temperature for 45 min, quenched with saturated NaHCO₃, and diluted with water. The organic layer separated and aqueous phase was extracted with CH₂Cl₂ (2x). The combined organic layer was washed with water, brine (1x), dried (MgSO₄) and concentrated to give crude aldehyde. Purification by flash chromatography on silica gel (7-15% ethyl acetate : hexanes) gave 6 as a white foam (1.15 g, 98% yield; ratio of α:β epimers = 1:2).

β-isomer: Rf = 0.49 (ethyl acetate/hexanes = 30/70). ¹H NMR (500 MHz, CDCl₃) δ: 9.65 (s, 1H, CHO), 6.40 (d, J = 1.5 Hz, 1H, ArH), 6.26 (d, J = 1.5 Hz, 1H, ArH), 5.55 (br s, 1H, OH), 3.52 (m as d, J = 13.0 Hz, 1H), 2.56-2.45 (m, 2H), 2.15-2.08 (m, 1H), 2.06-1.96 (m, 4H, especially 2.03, br s, 3H), 1.81 (br s, 6H), 1.74 (d, J = 12.5 Hz, 3H), 1.68 (d, J = 11.5 Hz, 3H), 1.52-1.35 (m, 5H, especially 1.39, s, 3H, 6β-CH₃), 1.16 (dq, J = 13.0 Hz, J = 4.0 Hz, 1H), 1.09 (s, 3H, 6α-CH₃), 1.06 (q, J = 12.0 Hz, 1H); HRMS (ESI) calculated for C₂₆H₃₅O₃: calculated 395.2586; found 395.2582.

α-isomer: Rf = 0.35 (ethyl acetate/hexanes = 20/80) ¹H NMR (500 MHz, CDCl₃) δ: 9.86 (s, 1H, CHO), 6.40 (d, J = 2.0 Hz, 1H, ArH), 6.31 (d, J = 2.0 Hz, 1H, ArH), 4.95 (s, 1H, OH), 3.56 (m as dd, J = 14.0 Hz, J = 2.5 Hz, 1H), 2.64 (br s, 1H), 2.41 (m as dd, J = 14.0 Hz, J = 2.0 Hz, 1H), 2.31 (dt, J = 11.5 Hz, J = 3.0 Hz, 1H), 2.05 (br s, 3H), 1.84 (d, J = 2.5 Hz, 6H), 1.82-1.64 (m, 8H), 1.55-1.48 (m, 1H), 1.41 (ddd, J = 13.5 Hz, J = 11.5 Hz, J = 5.0 Hz, 1H),
1.36 (s, 3H, 6β-CH₃), 1.08 (dq, J = 13.0 Hz, J = 4.0 Hz, 1H), 0.99 (s, 3H, 6α-CH₃). HRMS (ESI) calculated for C₂₆H₃₅O₃: calculated 395.2586; found 395.2585.

(6aR,9R,10aR)-3-(Adamantan-1-yl)-1-hydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromene-9-carbaldehyde (6): The aldehyde mixture (1.0 g, 2.53 mmol) was dissolved in 70 mL methanol and added via cannula to powdered K₂CO₃ (1.75 g, 12.67 mmol). After stirring the reaction mixture for 4 h at room temperature, methanol was removed under reduced pressure, diluted with water and extracted with ether (3x30 mL). Combined organic extracts washed with brine and dried (MgSO₄). Evaporation of volatiles under reduced pressure gave crude that was purified by column chromatography (7%-15% ethyl acetate : hexanes) to give β-aldehyde (0.88 g, 88%) as a white foam. HRMS (ESI) calculated for C₂₆H₃₅O₃: calculated 395.2586; found 395.2585; [α]⁻²⁰ = -93.7° (c = 0.85, CH₂Cl₂).
(6aR,9R,10aR)-3-(Adamantan-1-yl)-9-(2-methoxyvinyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol (7): To a suspension of (methoxymethyl)-triphenylphosphonium chloride (5.21 g, 15.21 mmol) in 90 mL anhydrous THF at -30 °C was added a solution of n-BuLi in THF (5.88 mL, 14.70 mmol, 2.5M in hexane). The resulting blood red colored solution was warmed to 0 °C over a period of 15 min. A solution of aldehyde 6 (1.0 gm, 2.53 mmol) in anhydrous THF (60 mL) was then added through cannula keeping the reaction temperature at 0 °C. The resulting solution was stirred for 15 min and then quenched by addition of water and stirred for 30 min till the solution turned colorless. Reaction mixture was diluted with diethyl ether, organic phase separated and the aqueous phase extracted with ether (2x). Combined organic extracts was washed with brine and then dried (MgSO₄). Purification by flash chromatography on silica gel (2%-20% ethyl acetate : hexanes ) gave mixture of enol ethers 7 (0.92 g, 86%, mixture of geometric isomers) as a white foam. ¹H NMR (500 MHz, CDCl₃) δ:
6.39 (d, J = 2.0 Hz, 1H), 6.24 (d, J = 2.0 Hz, 1H), 5.82 (d, J = 6.5 Hz, 1H), 4.78 (s, 1H, OH), 4.22 (dd, J = 9.0 Hz, J = 6.5 Hz, 1H), 3.60 (s, 3H, OCH₃), 3.05 (br d, J = 12.5 Hz, 1H), 2.72-2.62 (m, 1H), 2.51 (dt, J = 12.0 Hz, J = 2.5 Hz, 1H), 2.04 (br s, 3H), 1.92-1.80 (m, 8H, especially 1.84, s, 6H), 1.78-1.66 (m, 7H, especially 1.75, d, J = 12.5 Hz, 3H and 1.69, d, J = 11.5 Hz, 3H), 1.48 (t, J = 11.0 H, 1H), 1.37 (s, 3H, 6β-CH₃), 1.22-1.11 (m, 1H), 1.07 (s, 3H, 6α-CH₃), 0.88-
0.83 (m, 1H). HRMS (ESI) calculated for C$_{28}$H$_{39}$O$_3$: calculated 423.2899 found 423.2896; [$\alpha$]$^{20}_D$ = 9.3° (c = 1.4, CH$_2$Cl$_2$).

2-((6aR,9R,10aR)-3-(-Adamantan-1-yl)-1-hydroxy-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-9-yl)acetaldehyde (8). To a solution of enol ether mixture 7 (800 mg, 1.893 mmol) in 40 mL CH$_2$Cl$_2$ at room temperature was added wet trichloroacetic acid (1.55 g, 9.47 mmol in 5 mL water). The resulting solution was stirred at room temperature for 45 min, quenched with saturated NaHCO$_3$, and diluted with water. The aqueous phase was extracted with CH$_2$Cl$_2$ (2x), and the combined organic extracts were washed with water, brine (1x), dried (MgSO$_4$) and concentrated to give crude aldehyde. Purification by flash chromatography on silica gel (7%-15% ethyl acetate : hexanes) gave 8 as a white foam. $R_f$ = 0.24 (ethyl acetate/hexanes = 20/80). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 9.79 (s, 1H, CHO), 6.40 (d, $J = 1.5$ Hz, 1H, ArH), 6.24 (d, $J = 1.5$ Hz, 1H, ArH), 4.64 (br s, 1H, OH), 3.18 (br d, $J = 12.5$ Hz, 1H), 2.52 (dt, $J = 11.5$ Hz, $J = 3.0$ Hz, 1H), 2.44-2.32 (m, 2H), 2.22-1.92 (m, 1H), 2.04 (br s, 3H), 1.97-1.86 (m, 2H), 1.82 (s, 3H), 1.81 (s, 3H), 1.78-1.64 (m, 7H, especially 1.75, d, $J = 12.0$ Hz, 3H and 1 69, d, $J = 12.0$ Hz, 3H), 1.48 (dt, $J = 11.5$ Hz, $J = 2.5$ Hz, 1H), 1.37 (s, 3H, 6β-CH$_3$), 1.29-124 (m, 1H), 1.17 (br t, $J = 10$ Hz, 1H), 1.08 (s, 3H, 6α-CH$_3$), 0.92-0.82 (m, 1H); HRMS (ESI) calculated for C$_{27}$H$_{37}$O$_3$: calculated 409.2743; found 409.2740; [$\alpha$]$^{22}_D$ = -38.5° (c = 1.22, CHCl$_3$).
(6aR,9R,10aR)-3-(Adamantan-1-yl)-9-(2-hydroxyethyl)-6,6-dimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol (9). To a solution of aldehyde 8 (100 mg, 0.245 mmol) in 10 mL methanol at room temperature was added NaBH₄ (46.3 mg, 1.224 mmol) portion wise. The reaction mixture was stirred for 30 mins and quenched with 10% acetic acid, and the mixture diluted with ether. The aqueous phase was extracted twice with ethyl acetate, and the combined organic extract was washed with brine and dried (MgSO₄). The solvent was evaporated and the crude was chromatographed (ethyl acetate/hexanes = 40/60→50/50) to produce pure β-alcohol 9 (91 mg, 91%). ¹H NMR (500 MHz, CDCl₃) δ: 6.43 (d, J = 2.0 Hz, 1H), 6.27 (d, J = 2.0 Hz, 1H), 4.92 (s, 1H, OH), 3.82-3.70 (m, 2H), 3.17 (br d, J = 13.5 Hz, 1H), 2.46 (dt, J = 11.5 Hz, J = 2.5 Hz, 1H), 2.05 (br s, 3H), 1.96-1.82 (m, 8H especially 1.83, s, 3H, and 1.82, s, 3H), 1.79-1.66 (m, 7H, especially 1.75, d, J = 12.5 Hz, 3H, and 1.70, d, J = 11.5 Hz, 3H), 1.62-1.44 (m, 4H), 1.25 (br s, 1H, OH), 1.16-1.05 (m, 5H, especially 1.07, s, 3H, 6α-CH₃), 0.82 (q, J = 12.5 Hz, 1H). HRMS (ESI) calculated for C₂₇H₃₉O₃: calculated 411.2899; found 411.2904; [α]²²_D = -53.7° (c = 0.06, CHCl₃).
5-(1-Adamantyl)-2-bromo-1,3-dimethoxybenzene (11): Bromine (0.38mL, 7.34mmol) was added drop-wise to a stirred solution of 1 [5-(1-adamantyl)-1,3-dimethoxybenzene] (2g, 7.34mmol) and 18-crown-6 (0.194g, 0.734mmol) in 74 mL of anhydrous CH₂Cl₂ at 0 °C. The reaction mixture was stirred at rt for 30 mins and quenched by addition of saturated aq. sodium bisulfite solution. Organic layer separated and washed with water, brine and then dried (MgSO₄). Evaporation of volatiles under reduced pressure gave a crude solid product 11 (2.58g) which was >98% pure by NMR and used for the next reaction without further purification. ¹H NMR (500 MHz, CDCl₃) δ: 6.59 (s, 2H), 3.91 (s, 6H), 2.11 (br s, 3H), 1.94-1.91 (m, 3H), 1.82 (d, J = 12.0 Hz, 3H), 1.77 (d, J = 12.0 Hz, 3H). HRMS (ESI) for C₁₈H₂₄BrO₂ : calculated 351.0960; found, 351.0959.

4′-(1-Adamantyl)-N, N-diisopropyl-2′, 5, 6′-trimethoxy biphenyl-2-carboxamide (12): Argon was bubbled through a mixture of boronic acid 10 (0.715g, 2.562mmol), bromo compound 11 (0.750g, 2.135mmol), Ba(OH)₂·8H₂O (1.01g, 3.203mmol), 2.5mL of water, and 16 mL of dimethoxyethane for 10 mins. The Pd(PPh₃)₄ (0.247g, 0.213mmol) catalyst was added to
the mixture while argon bubbling was maintained through the mixture and degassing was continued for an additional 5 min. The reaction mixture was microwaved for 25 min at 160 °C in a CEM Discover apparatus. Then the mixture was cooled to room temperature and filtered through a short Celite pad. The filtrate was concentrated and Et₂O was added. The ether solution was washed with water, brine and dried (MgSO₄). Evaporation of solvent under reduced pressure gave crude which was chromatographed (ethyl acetate/hexanes: 30/70 → 40/60) on silica gel to afford 0.776g of biphenyl 12 (72% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ: 7.25(d, J = 9.0Hz, 1H), 6.86 (dd, J = 9.0 Hz, J = 3.0 Hz, 1H), 6.80 (d, J = 3.0 Hz, 1H), 6.58 (s, 1H), 6.56 (s, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.72 (s, 3H), 3.68 (sept, J = 6.5 Hz, 1H), 3.17 (sept, J = 6.5 Hz, 1H), 2.12 (bs, 3H), 1.94 (br s, 6H), 1.84-1.74 (m, 6H), 1.45 (d, J = 6.5 Hz, 3H), 1.07 (d, J = 6.5 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.55 (d, J = 6.5 Hz, 3H). HRMS (ESI) for C₃₂H₄₄NO₄: calculated 506.3270; found 506.3268

3-(1-Adamantyl)-1-hydroxy-9-methoxy-6H-benzo[c]-chromene-6-one (13): A solution of 12 (1.20 g, 2.37 mmol) in 25 mL of anhydrous CH₂Cl₂ was cooled to 0 °C and 9.50 mL of 9-Iodo-9-BBN (1.0M solution in hexane, 9.50 mmol) was added drop wise. The reaction mixture was stirred at 0 °C for 4h. The CH₂Cl₂ was removed and the residue was dissolved in anhydrous Et₂O (50 mL). To this mixture was added 10 mL of ethanolamine solution (1.0M in ether). The reaction mixture was stirred for 40 min and then filtered through a short celite column. The
filtrate was concentrated and dissolved in 10 mL glacial acetic acid. The reaction mixture was refluxed for 5h and then cooled to room temperature, water was added cautiously to the mixture at 0 °C followed by addition of ether (50 mL). The organic layer was separated and washed with water, 15% aqueous NaHCO₃, water, brine and then dried (MgSO₄). Evaporation of volatiles under reduced pressure gave crude product that was chromatographed (ethyl acetate/hexanes : 40/60 → 60/40) on silica gel to give 0.60g (63% yield) of 13 as a white solid. \( R_f = 0.42 \) (Ethyl acetate/Hexane = 30/70). ^1H NMR (CDCl₃ + 2 drops of DMSO, 500 MHz) δ: 8.57 (d, \( J = 2.5 \) Hz, 1H), 8.34 (d, \( J = 8.5 \) Hz, 1H), 7.55 (s, 1H), 7.05 (dd, \( J = 9.0 \) Hz, \( J = 2.5 \) Hz, 1H), 6.94 (d, \( J = 2.0 \) Hz, 1H), 6.80 (d, \( J = 2.0 \) Hz, 1H), 6.50 (s, 1H), 3.96 (s, 3H), 2.12 (br s, 3H), 1.92-1.88 (m, 6H), 1.81 (d, \( J = 12.5 \) Hz, 3H), 1.75 (d, \( J = 12.5 \) Hz, 3H). HRMS (ESI) for C₂₄H₂₅O₄: calculated 377.1753; found 377.1751.

![3-(1-Adamantyl)-1, 9-dihydroxy-6H-benzo[c]chromen-6-one (14):](image)

3-(1-Adamantyl)-1, 9-dihydroxy-6H-benzo[c]chromen-6-one (14): To a suspension of 13 (105 mg; 0.279 mmol) in anhydrous CH₂Cl₂ (15 mL) was added a solution of boron tribromide (0.56 mL, 1.0M in CH₂Cl₂, 0.56 mmol) at room temperature under an argon atmosphere. The reaction mixture was stirred at same temperature for 30 min and then refluxed for 24 h. Reaction was then cooled and quenched by addition of ice-water and diluted with Et₂O. Aqueous layer was extracted with ether (2x20 mL) and the combined organic layer was washed with 15% aq. NaHCO₃, water, brine and then dried (MgSO₄). Evaporation of volatiles under reduced pressure
gave crude product that was chromatographed (ethyl acetate/hexanes = 30/70 → 80/20) to give 78 mg (68% yield) of 14 as a white solid. $RF = 0.45$ (Ethyl acetate/Hexane = 50/50). $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 10.76 (br s, 2H, ArOH), 8.49 (d, $J = 2.0$ Hz), 8.10 (d, $J = 9.0$ Hz, 1H), 6.97 (dd, $J = 9.0$ Hz, $J = 2.5$ Hz, 1H), 6.88 (d, $J = 2.0$ Hz, 1H), 6.80 (d, $J = 2.0$ Hz, 1H), 2.07 (br s, 3H), 1.86 (br s, 6H), 1.80-1.70 (m, 6H). HRMS (ESI) for C$_{23}$H$_{23}$O$_4$: calculated 363.1596; found 363.1598.

3-(1-Adamantyl)-6,6-dimethyl-6H-benzo[c]chromene-1,9-diol (15): To a solution of 14 (0.3 g, 0.828 mmol) in anhydrous THF (16 mL) was added methyl magnesium iodide (1.38 mL, 3.0M solution in ether, 4.14 mmol) at room temperature under an argon atmosphere. The reaction mixture was stirred at room temperature for 30 min and then refluxed for 2 h. The reaction was cooled to room temperature and quenched by addition by saturated aqueous NH$_4$Cl (30 mL). THF was removed and the residue was dissolved in Et$_2$O (50 mL). The organic phase separated and washed with water, brine and dried (MgSO$_4$). Evaporation of volatiles under reduced pressure gave the crude intermediate that was used without further purification in the subsequent cyclization reaction. The crude was dissolved in CHCl$_3$ (15 mL) and $p$-toluenesulfonic acid monohydrate (50 mg; 0.262 mmol) was added under argon atmosphere. The reaction was stirred at room temperature for 6 h and then treated with 10 mL water. The organic phase was separated and washed with saturated aqueous NaHCO$_3$, water, brine and then dried (MgSO$_4$). Solvent
removal under reduced pressure gave the crude product that was chromatographed (ethyl acetate/hexanes = 10/90→20/80) to give 200 mg (77% yield) of 15 as a white solid. \( R_f = 0.42 \) (Ethyl acetate/Hexane =30/70). \(^1\)H NMR (CDCl\(_3\), 500 MHz) \( \delta \): 7.92 (d, \( J = 2.5 \) Hz, 1H), 7.09 (d, \( J = 9.0 \) Hz, 1H), 6.73 (dd, \( J = 9.0 \) Hz, \( J = 2.5 \) Hz, 1H), 6.58 (d, \( J = 1.5 \) Hz, 1H), 6.40 (d, \( J = 1.5 \) Hz, 1H), 5.58 (br s, 1H, OH), 5.30 (br s, 1H, OH),  2.05 (br s, 3H), 1.85-1.82 (m, 6H), 1.80-1.68 (m, 6H), 1.58 (s, 6H). HRMS (ESI) for C\(_{25}\)H\(_{29}\)O\(_3\): calculated 377.2117; found 377.2114.

\[ \text{3-(1-Adamantyl)-9-methoxy-6,6-dimethyl-6H-benzo[c]chromen-1-ol (16):} \] This compound was prepared analogously to 15, starting from 13 (0.400 gm, 1.06 mmol) in THF (22 mL), methyl magnesium iodide (1.77 mL, 3.0M solution in ether, 5.30 mmol) and cyclization using p-TSA.H\(_2\)O (50 mg, 0.262 mmol). Yield = 72% (0.301mg) of 16 as a white crystalline solid. \( R_f = 0.39 \) (ethyl acetate/hexanes = 20/80). \(^1\)H NMR (CDCl\(_3\), 500 MHz) \( \delta \): 7.99 (d, \( J = 3.0 \) Hz), 7.16 (d, \( J = 9.0 \) Hz), 6.79 (dd, \( J = 9.0 \) Hz, \( J = 3.0 \) Hz), 6.60 (d, \( J = 2.0 \) Hz), 6.43 (d, \( J = 2.0 \) Hz), 5.18 (br s, 1H, OH), 3.84 (s, 3H), 2.08 (br s, 3H), 1.89-1.86 (m, 6H), 1.78 (d, \( J = 12.0 \) Hz, 3h), 1.72 (d, \( J = 12.0 \) Hz, 3H), 1.60 (s, 6H). HRMS (ESI) for C\(_{26}\)H\(_{31}\)O\(_3\): calculated 391.2273; found 391.2279.
Detailed description of methods used for in vitro studies:

In vitro assays (Biochemistry group in CDD):

1. Membrane Preparations from Tissue Culture Sources: HEK293 cells expressing the mCB2 or hCB2 receptor are used for membrane preparations according to the method described by Abadji et al.\textsuperscript{132} The resulting pellet is resuspended in 10 mM Tris-chloride, pH 7.4 with 5 mM MgCl\textsubscript{2} and 2 mM EDTA (TME) and stored at -80 °C for no longer than two months. Protein content is assayed by using the Bio-Rad protein assay according to the manufacturer’s protocol.

2. Membrane Preparations from Tissue Sources: Frozen rat brains (CB1 source) are obtained from Pel-Freeze Biologicals (Rogers, AK) and stored at -80 °C until use. Membranes were prepared according to the method described by Dodd et al. and adapted for use in our laboratory as previously reported.\textsuperscript{133-135}

3. rCB1, hCB2, and mCB2 Binding Assays: All compounds synthesized for this grant are tested for their ability to bind to CB1 and CB2 receptors using rat brain or HEK293 cell membranes expressing hCB2 membrane preparations, respectively, as previously described via competition-equilibrium binding using [\textsuperscript{3}H]CP-55,940.\textsuperscript{135,136} The results were analyzed using nonlinear regression to determine the actual IC\textsubscript{50} of the ligand (Prizm by GraphPad Software, Inc.) and the K\textsubscript{i} values are calculated from the IC\textsubscript{50}.\textsuperscript{137}

4. Signal Transduction Assays (cAMP assay): HEK-293 cells transfected with rCB1, mCB2, or hCB2 receptor are used with the PerkinElmer’s Lance ultra cAMP kit following the protocol of the manufacturer. Briefly, the assays were carried out in 384-well format using 1000 cells/well. Test compounds were added to wells containing stimulation buffer and 2 μM forskolin followed by cell suspension. After 30 minutes stimulation, the Eu-cAMP tracer and
Ulight-anti-cAMP are added to the plate and incubated at room temperature for 1h prior to detection via PerkinElmer Envision; data are analyzed using GraphPad Prism software.

5. **Preliminary distribution in brain/plasma and drug oral bioavailability:** Mice (CD-1, weighing 25-30 g) are injected intravenously or orally with 0.1-2 mg/kg of the compound mixture. Fifteen minutes post-IV injection, or 30 and 60 minutes post-oral administration, the animals are sacrificed by decapitation followed by blood collection (~500 µL) and tissue dissection; samples are flash frozen with liquid nitrogen to prevent post-mortem degradation of the compounds. Tissues (plasma or brain) are extracted following published procedures and analyzed using a Thermo-Finnigan Quantum Ultra triple quadrupole mass spectrometer in SRM mode with an Agilent 1100 HPLC front-end with internal standards used for quantitation.
Chapter 3

Southern Modified Adamantyl (SAH) Cannabinoids

3.1 Introduction

The role of THC and other cannabinoids from cannabis in producing broad spectrum of pharmacological effects is very well established.\textsuperscript{139,140} Since the last 30 years extensive SAR has been explored on THC template and a vast set of cannbinergic agonists have been developed.\textsuperscript{116,141,142} Earlier SAR studies have revealed some important pharmacophores of the THC template.\textsuperscript{6,143,144} Most explored pharmacophoric site is aliphatic chain region.\textsuperscript{145} In the search of potent and highly efficacious ligands this lab has also explored the aliphatic side chain and developed tricyclic 1-adamantyl chain analog AM411 (Fig. 2.1) as the CB1 selective analog among the series, more potent than THC.\textsuperscript{6} Other important pharmacophores are phenolic hydroxyl, northern aliphatic hydroxyl (NAH) and southern aliphatic hydroxyl (SAH) groups.\textsuperscript{146,147} With the further efforts in this lab using AM411 as lead compound because of the unique 1-adamantyl pendant, the northern pharmacophore was explored. The SAR in the northern binding pocket resulted in AM4054 (Fig. 2.1) as the most potent compound in the series. AM4054 was studied extensively \textit{in vivo} and displayed a profile of efficacious and potent CB1 agonist with shorter duration of action as compared to the parent AM411 with no off target activity in multiple receptor screening with a panel of 30 GPCRs.

The SAH pharmacophore was identified firstly by Pfizer in 1974 in non-classical cannabinoid CP-55,940. First work on SAH tricyclic cannabinoids also called as hybrid cannabinoids was accomplished in Tius/Makriyannis labs\textsuperscript{85} (Fig. 3.1). They identified the required stereochemistry for CB binding affinity.\textsuperscript{148} The 6-\(\beta\)-stereochemistry for the southern functionality imparted high affinity ligands at both CB receptors.\textsuperscript{118} Different approaches were utilized to synthesize
optically pure hybrid compounds and limited SAR was developed.\textsuperscript{85,149} The contribution of the SAH pharmacophore towards functional potency and \textit{in vivo} activity of cannabinergic ligands was not explored earlier. Utilizing the key SAH groups from the earlier explored SAR and combining it with our lab finding of 1-adamantyl analog AM4054, a series of analogs were designed and synthesized. The SAH pharmacophore is now being explored for its role in functional potency, selectivity and \textit{in vivo} efficacy.

\textbf{Figure 3.1:} Development of earlier synthesized SAH hybrid cannabinoids

After decades of research in THC and cannabinoids, the most important problems associated with THC are tolerance, poor bioavailability and lipophilicity.\textsuperscript{150-152} Research in GPCRs has established that tolerance or dependence is linked to desensitization of the receptor which is controlled by $\beta$-arrestin.\textsuperscript{45} It was shown in opioids that reduction of $\beta$-arrestin interaction with opioid receptors may of a therapeutic interest in reducing opioid tolerance.\textsuperscript{153} Similar to other GPCRs, reducing the tolerance can be achieved by modifying the interaction with $\beta$-arrestin or getting a biased ligand with minimum or no $\beta$-arrestin activity.

All the synthesized hybrid SAH analogs were tested for their binding affinities and functional potencies at both CB1 and CB2 receptors. Potent analogs were also tested for $\beta$-arrestin recruitment and, interestingly, biased agonists were identified. Some of the analogs showed high potency in the cAMP assay but no $\beta$-arrestin recruitment. This can be of great therapeutic
importance in drug discovery because most of GPCRs function through the activation of more than one signaling pathway. cAMP and β-arrestin are also result of the activation of two different signaling pathways. So, if a ligand activates only one signaling pathway then it can target limited physiological functions effected by activation of that particular pathway and hence minimizing side effects through biased G-protein ligands. The most interesting analog AM8607 (15, Scheme 3.2) with southern hydroxymethyl group was studied extensively for in vivo studies. This analog displayed good affinity, potency in cAMP assay, functional selectivity for cAMP against β-arrestin, high in vivo potency as an analgesic. It was shown to develop much less tolerance when compared to THC.

3.2 Rational Design

The earlier work done in our research group and present work suggested that AM4054 which has 11-β-OH group is much more potent and efficacious than the other known THC type ligands (Figure 3.2).

Figure 3.2: Rational for designing SAH analogs
Also, the significance of southern pocket is not well established in the literature. So, hybrid compounds were designed to understand and evaluate their functional and *in vivo* profile. Also, the terminal carbon of the side chain was functionalized with various polar groups to modulate the compound’s polarity and CNS permeability.

### 3.3 Chemistry

Hybrid tricyclic analogs were synthesized starting from adamantyl resorcinol 1 and diacetates mixture 2 which served as starting point to get bicyclic intermediate (Scheme 3.1). Both resorcinol and (+)-*R*-nopinone diacetate mixture were synthesized following the earlier reported procedures.\(^{85,130}\) Coupling of resorcinol and diacetates in the presence of pTSA, gave bicyclic keto intermediate 3 in 78% yield. Protection of the phenolic hydroxyl groups as tert-butyldimethylsilyl (TBDMS) ethers using TBDMSCl and imidazole yielded 4 in 70% yield.

**Scheme 3.1: Synthesis of bicyclic intermediate for SAH synthesis**

![Reagents and conditions:](a) p-TSA.H\(_2\)O, CHCl\(_3\), rt, 3.5 days, 78.5%; (b) TBDMSCl, Imidazole, DMAP, DMF, rt, 12h, 70%]

The strained cyclobutyl ring was opened with TMSI in the presence of catalytic *t*-butanol to give a tertiary iodide intermediate 5a (Scheme 3.2). The earlier reported dehydrohalogenation using DBU were not reproducible after many efforts.\(^{85}\) So the reaction conditions were optimized and dehydrohalogenation of the iodide 5a under mild conditions using sodium acetate in acetic acid gave the less substituted terminal alkene 5 in 52% yield (Scheme 3.2).\(^{154}\) To synthesize 11-β-hydroxyl group on the northern position, one carbon Wittig homologation of the keto gave the
mixture of enol ethers 6 in 70% yield, which without further separation were hydrolyzed with wet trichloroacetic acid to give diastereomeric aldehyde mixture 7 in 78% yield. Epimerization of aldehydes 7 with potassium carbonate led to the exclusive formation of β-equatorial aldehyde 8 in 82% yield. Sodium borohydride reduction of aldehyde 8 led to pure β-equatorial alcohol 9 in 88% yield. Protection of the primary OH group as TBDMS using the earlier used conditions gave 10 in 80% yield.

To introduce SAH functionality, allylic oxidation was performed on the isopropenyl group of 10 to give 11 in 55% yield.\textsuperscript{155} Reduction of the allylic aldehyde 11 with sodium borohydride led to the formation of intermediate 12 with southern hydroxyl group in 82% yield. Selective deprotection of one aromatic TBS group of intermediate 12 with 1 equivalent of tetrabutylammonium fluoride (TBAF) led to 13 in 70% yield.\textsuperscript{156}

This open ring derivative 13 was cyclized via oxymercuration-demercuration reaction\textsuperscript{85} using mercuric acetate and sodium borohydride to give the key first tricyclic intermediate 14 in 48% yield. TBDMS groups were deprotected using TBAF conditions\textsuperscript{156} to give 15 (AM8607) in 93% yield. Relative stereochemistry at all the chiral centers including the C-6 positon in 15 was determined with $^1$H NMR, HSQC, HMBC, COSEY and NOE correlations between C-6α methyl, C-12 methylene, C-10a benzylic and C-9 axial protons. Strong enhancement of benzylic proton at 2.53 ppm and 9-axial proton between 1.94-1.89 ppm was observed when C-6α methyl protons at 1.0 ppm were irradiated (Fig. 3.3). Since the stereochemistry at the junction must be trans as seen from large coupling constant between C-10a and C-6a protons in 1H NMR, the stereochemistry of 15 (AM8607) will be as shown in the Scheme 3.2. In case of opposite steroisomer at 6 positon, irradiation of C-10a benzylic proton should have enhanced the C-12 methylene proton at 3.67 ppm and 9-axial proton which was not observed here (Fig. 3.4).
Scheme 3.2: Synthesis of tricyclic SAH compound

Reagents and conditions: a) i) TMSI, t-BuOH, CCl₄, 5 °C, 16h, ii) NaOAc, CH₃COOH, 90 °C, 2h, 52%; b) (C₆H₅)₃P(Cl)CH₂OCH₃, n-BuLi, THF, -30 °C, 1h, 70%; c) CCl₃COOH, DCM, H₂O, rt, 5h, 78%; d) K₂CO₃, MeOH, rt, 12h, 82%; e) NaBH₄, MeOH, 0 °C, 1h, 88%; f) TBDMSCl, Imidazole, DMAP, DMS, rt, 12h, 80%; g) SeO₂, Salicylic acid, t-BuOOH, DCM, rt, 12h, 55%; h) NaBH₄, MeOH, 0 °C, 1h, 82%; i) TBAF 1 eq., THF, 0 °C, 1h, 70%; j) Hg(OAc)₂, MeOH, NaBH₄, rt, 16h, 48%; k) TBAF, THF, 5h, rt, 93%;
Figure 3.3: 1D-NOESY studies of AM8607 (15). Irradiation of C-6α methyl is showing enhancement of 9-axial, 7-axial, 10a-benzylic and 12-β-methylene protons.
Figure 3.4: Further 1D-NOESY studies on AM8607 (15). Irradiation of C-10a benzylic, C-10-equatorial, C-10-axial protons showing β-stereochemistry at C-9 and C-6 junction.
Figure 3.5: 2D-HSQC studies on AM8607 (15).
Figure 3.6: Further 2D-ROESY studies on AM8607 (15).

To further explore the southern pocket, SAH hydroxyl intermediate 14 was oxidized to aldehyde 16 using Dess Martin Periodinane (DMP) in 75% yield (Scheme 3.3). Corey-Fuchs reaction was performed on aldehyde 16 to give alkyne 18 which was deprotected using TBAF to give final compound 19 in 76% yield. The alkyne intermediate 18 was lithiated and quenched with paraformaldehyde to form propargyl alcohol 20. The TBS groups of 20 were deprotected to give 21 (AM8615) in 74% yield.
Scheme 3.3: Synthesis of various SAH analogs

Reagents and conditions: a) DMP, DCM, 0 °C, 1h, 75%; b) CBr₄, PPh₃, DCM, -5 °C, 30 min., 73%; c) n-BuLi, THF, -30 °C, 1h, 69%; d) TBAF, THF, 0 °C to rt, 5h, 76% & 74%; e) formaldehyde, n-BuLi, THF, 0 °C, 2h, 72%

From the aldehyde intermediate 16, more polar analogs were synthesized (Scheme 3.4). The aldehyde intermediate was oxidized with sodium chlorite using literature conditions¹⁵⁷ to give acid derivative 22 in 72% yield which was deprotected to give final compound 24 in 73% yield. Esterification of the acid intermediate 22 with trimethylsilyl diazomethane (TMSCH₂N₂) in methanol gave methyl ester 23 in 78% yield. Deprotection of the TBDMS groups with TBAF led to the final ester analog 25 in 75% yield. Aldehyde 16 was transformed to morpholine substituted analog 26 (58% yield) by reductive amination using morpholine and sodium triacetoxyborohydride followed by TBDMS deprotection.
Scheme 3.4: Synthesis of SAH aldehyde derivatives

Reagent and Conditions: a) NaH, 2-methyl-2-butene, NaClO₂, t-BuOH, DCM, rt, 4h, 72%; b) TBAF, THF, 0 °C - rt, 5h, 73%, 75%; c) N₂CH₂TMS, Methanol, rt, 12h, 78%; d) Morpholine, NaBH(OCOCH₃), DCM, CH₃COOH, rt, 12h, 58%

3.4 Result and discussion

A set of compounds were synthesized with modifications in the southern pharmacophore to understand the role of SAH groups towards imparting functional and in vivo potency to the molecule. It is very interesting to see from the following results that different functionalities are well tolerated in the southern pocket. The following data (Table 3.1) shows the high nanomolar affinities of SAH ligands at both CB1 and CB2 receptors. Although there is no difference in the binding affinities of these compounds with different functionalities such as hydroxyl, alkyne, propargyl alcohol and ester (Table 3.1), there is significant difference in their functional potencies, with 15 (AM8607) and 21 (AM8615) being the most potent agonists for CB1 in cAMP assays. EC₅₀ values of both of these compounds are in single digit nanomolar range, as they are showing 100% cAMP inhibition at 10 nM concentration.
From this interesting cAMP data, it seems that the β-hydroxyl group of 15 (AM8607) and hydroxyl group of propargyl alcohol analog 21 (AM8615) has enhanced functional potency at both CB1 and CB2 receptors. The functional potency of 15 (AM8607) at CB1 receptor as measured by decrease in forskelin stimulated cAMP (method described in the Experimental Section) has an EC₅₀ of 2.4 nM and it behaves as a full agonist. 26 (AM8643) compound with a bulky morpholine group does not fit in the southern binding pocket and has a Kᵢ value more than 1000 nM (Table 3.1). Also, the acid analog 24 (AM8621) is not well tolerated, whereas, ester analog 25 (AM8622) has high affinity and potency. This presents a good example for retrometabolic drug design through a soft drug approach with parent ester compound being active but its metabolite acid being inactive.

All the high affinity and potent ligands were tested for arrestin recruitment (procedure mentioned in the Experimental Section) in β-arrestin assays and compared with WIN-55,212-2 as a standard. β-arrestin is a key mediator for the internalization and desensitization of the cannabinoid receptors. Now, it is well established that β-arrestin, similar to G-proteins is also capable of interacting with ligand and recruiting cell signaling. So, GPCR ligands may be biased towards G-proteins signaling or towards β-arrestin signaling and these biased ligands may result in specific pharmacological results. The results from the arrestin assay show that similar high potency SAH ligands behaved differently from each other in β-arrestin assay and showed biased agonism towards G-proteins or β-arrestin. The ester analog AM8622 (25) has low potency of β-arrestin recruitment of 1.3 µM and 0.1 µM at both CB1 and CB2 receptors. In cAMP assays, potency of 25 is in single digit nanomolar range. The SAH alkyne compound AM8619 (19) has very high potency of β-recruitment at CB1 with an EC₅₀ of 75 nM but low EC₅₀ value of 13 µM at CB2.
It showed a similar trend in cAMP assay. **19** (AM8619) is an unbiased potent CB1 ligand. AM8615 (**21**), a high potency ligand through cAMP assay, also shows very high $\beta$-arrestin recruitment at CB2 with EC$_{50}$ value of 78 nM but no recruitment at all at CB1 receptor. This shows that AM8615 (**21**) is a potent biased CB1 agonist. AM8607 (**15**) exhibited most interesting finding from this series. This ligand has highest potency in cAMP assays but is inactive (silent) in $\beta$-arrestin assay i.e. no $\beta$-arrestin recruitment at CB1 and CB2 receptors. This implies that **15** is a completely biased ligand towards G-proteins at both CB1 and CB2 receptors.

Because of these interesting results of separation of binding affinity from functional potency and functional selectivity observed here, AM8607 (**15**) was tested *in vivo* for its ability to cross blood-brain barrier and further evaluated *in vivo* assays.

**Table 3.1: Comparison of Binding and Functional Affinities of Hybrid Analogs**

<table>
<thead>
<tr>
<th>Com-pd</th>
<th>AM no.</th>
<th>Structure</th>
<th>Binding Data (K$_i$ in nM)</th>
<th>Functional Data cAMP (% inhibition)</th>
<th>Functional Data $\beta$-arrestin (EC$_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>rCB1 mCB hCB2 hCB2 hCB1 hCB2 10nM 1µM 10nM 1µM</td>
<td>hCB1 hCB2</td>
<td>Not Active</td>
</tr>
<tr>
<td><strong>15</strong></td>
<td>8607</td>
<td><img src="image1" alt="" /></td>
<td>4.9±0.1 0.4±0.03 5.2±0.2</td>
<td>94% 99% 52% 88%</td>
<td>Not Active</td>
</tr>
<tr>
<td><strong>19</strong></td>
<td>8619</td>
<td><img src="image2" alt="" /></td>
<td>1.4±0.04 8.5±0.4 6.2±0.1</td>
<td>79% 97% 16% 89%</td>
<td>75 nM 0.1 µM</td>
</tr>
<tr>
<td>21</td>
<td>8615</td>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td>5.7±0.4</td>
<td>4.2±0.09</td>
<td>7±0.2</td>
</tr>
<tr>
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N.A. = Not Applicable

### 3.4.1 Blood Brain Barrier (BBB) Studies

AM8607 (15) was given to rats with a dose of 0.25 mg/kg as intravenous injection and brain to plasma ratio was measured after sacrificing the animal. 0.03ng/g of the compound was found in the brain with 0.077ng/µl in the plasma with brain to plasma ratio of 0.4. This ratio represents low brain permeability as compared to THC and AM4054. The presence of the additional hydroxyl groups as compared to AM4054 and THC has imparted AM8607 (15) high polarity (polar surface area, tPSA = 70) and low lipophilicity (CLogP = 5.9). This suggested that
AM8607 (15), with limited brain permeability, will have lesser CNS side effects as compared to THC and other centrally acting cannabinergics.

3.4.2 *In vivo* profile of AM8607

The most interesting analog, AM8607, from binding and functional data was explored for its *in vivo* profile and potency at both CB1 and CB2 receptors both in central and peripheral tests. *In vivo* studies for AM8607 included hypothermia, antinociception, tolerance development, edema, allodynia and comparison of edema and allodynia effects with diclofenac. All these studies were done in Dr. Aron Lichtman’s laboratory. It was also tested for decreased gastric motility and diarrhea reduction in Dr. Martin Storr’s laboratory.

**Hypothermia:** Rectal temperature was measured by inserting a thermocouple probe 2 cm in the rectum. AM8607 decreased the temperature in a dose-dependent manner (Fig. 3.3). Cumulative doses of 0.1, 0.3, 1, and 3 mg/kg were given after every 40 minutes and temperature was measured after 30 minutes of every i.p.(intra peritoneal) injection. There was no temperature change at 0.1 mg/kg dose. A slight decrease of 1 °C temperature was observed at 0.3 mg/kg. At a dose of 3 mg/kg more than 6 °C fall in temperature was observed. Dose at which AM8607 showed 50% of the maximum effect (i.e. ED<sub>50</sub>) is 0.7±0.1 mg/kg.

![Graph showing hypothermia effects of AM8607](image)

**Figure 3.7:** Effects of AM8607 in hypothermia and Antinociception (Dr. Aron Lichtman)
**Antinociception:** Antinociception was measured using tail immersion test. In this test, the tip of mouse’s tail is dipped in the warm water and latency time for the mouse to flick its tail or exhibit a gross movement was observed. AM8607 increased the tail flick latency over the same dose range as in the hypothermia measurements and with the similar time course (Fig. 3.3). At a dose of 3 mg/kg AM8607 produced the 100% response. ED$_{50}$ value observed for anti-nociception is 0.8±0.2 mg/kg.

**Reduction in Edema and Allodynia:** AM8607 was tested for carrageenan induced edema by change in paw diameter to confirm its role as an anti-inflammatory agent (Fig. 3.4). Role of AM8607 in analgesia is confirmed through allodynia by measuring paw withdrawal threshold. At a dose of 0.1mg/kg it shows 50% of the maximum possible effect. When compared to THC, AM8607 was found to be about 50-fold more potent. THC produced the same effect at a dose as high as 5mg/kg.

![Graphs showing changes in paw diameter and withdrawal threshold](image)

C=Contralateral paw (non carrageenan paw), V= vehicle animal (carrageenan paw)

**Figure 3.8:** Different doses of AM8607 reduced carrageenan induced edema and allodynia (Dr. Aron Lichtman)
Figure 3.9: Diclofenac partially reduced edema and allodynia at a dose of 5mg/kg (Dr. Aron Lichtman).

Rats were given an i.p. injection of AM8607 30 minutes before carrageenan injection. Both edema and allodynia were measured after 5 hours of injection. Dose-dependent effects were observed in both edema and allodynia experiments at 0.1, 0.2, 0.4 and 0.8 mg/kg of AM8607. At a dose of 0.2 mg/kg anti-allodynic effect was almost maximum. This shows that AM8607 is a potent analgesic. These results were compared with the well-known pain killer diclofenac (Fig. 3.4). Interestingly, it was found that AM8607 is about 50 times more potent than diclofenac in anti-allodynia experiment (compare Fig. 3.4 & 3.5). A low dose of 0.1 mg/kg of AM8607 produced similar effects as produced by 5 mg/kg of diclofenac. In anti-edema experiments also, AM8607 is 25 times more potent than diclofenac. AM8607 was also evaluated in understanding the involvement of CB1 or CB2 receptors in edema and alldynia. Injecting the mice with AM8607 alone or with CB1 or CB2 antagonist and evaluating anti-edema and anti-allodynia data reveals that both CB1 and CB2 receptors are involved in anti-allodynic effects of AM8607. On the other hand only CB2 receptors mediate the anti-edematous effects.
**Tolerance or Dependence Study:** For studying tolerance to hypothermia and antinociception, mice were divided into two groups. Group 1 received repeated doses of vehicle (twice daily) as shown in Figure 3.6. Group 2 received 3mg/kg dose of AM8607 (twice daily) for five days and only the morning injection on the sixth day. On the seventh day, 24 h after the last injection, Group 1 animals received cumulative dose of AM8607 (0.3, 1, 3 mg/kg) and Group 2 animals received cumulative dose of AM8607 (0.3, 1, 3 and 10 mg/kg). Only group 2 or chronic group received the highest dose of 10mg/kg on the last day because group 1 or acute animals were showing hyper-reflexia and maximum hypothermia with the last but one dose (i.e. 3mg/kg).

**Figure 3.10:** Tolerance study in acute and chronic animal models for hypothermia and antinociception (Dr. Aron Lichtman)

The above results from the studies performed in Dr. Lichtman lab (Figure 3.6) indicate that AM8607 has produced tolerance in chronic animals at a dose of 3mg/kg in hypothermia and tail withdrawal and animals need more dose to give the same effect. But it is also important to mention that the magnitude of tolerance is substantially less than the magnitude of tolerance to an equi-effective dose of THC (i.e. 50 mg/kg)\(^\text{158}\). The relatively low tolerance following repeated AM8607 could be a result of minimal CB1 down-regulation and/or desensitization. This can be further supported through its functional data as a biased ligand with no \(\beta\)-arrestin recruitment.
**Gastric Motility and Diarrhea:** CB1 receptors are involved in reduced peristaltic activity in small intestine and reduced colonic propulsion\(^{159}\). Reduction in the colon movement results in decrease in gastric emptying.

AM8607 was tested in Dr. Storr’s laboratory for the reduced gut movement and castor-oil induced diarrhea reduction by the method explained in the Experimental Section. A very small dose of 0.1 mg/kg reduced the rate of gastric emptying and time to diarrhea by 50\% as shown in Figure 3.7 & 3.8. To confirm whether these effects are through CB1 or CB2 receptors, AM8607 was injected along with CB1 and CB2 antagonists. The results proved that the effect is antagonized only by CB1 antagonist (Figure 3.7). Hence, the effects are produced by CB1 agonist and AM8607 behaves as a potent agonist in these *in vivo* studies.

**Figure 3.11:** Gastric emptying reduction by AM8607 and effects are antagonized by CB1 antagonist (Dr. Martin Storr)
3.5 Conclusion:

In summary, the SAR study of novel hybrid analogs containing 1-adamantyl side chain at C-3 position reveals its role in the binding affinity and functional potency. Various functionalities as hydroxyl, alkyne, and ester are well tolerated at the southern region except acid and morpholine moiety.

Further, functional testing in cAMP and β-arrestin assays disclosed that southern modifications impart higher functional potency and selectivity to the ligands, with AM8607 being the most selective or biased. It has highest potency in cAMP assay but no arresin recruitment in β-arrestin assay. This biased agonism may possibly account for low tolerance development seen with AM8607, when compared to THC. Furthermore, in vivo studies of AM8607 showed that it is a high potency CB1 agonist with 50-fold higher potency than diclofenac in anti-allodynia experiment. To understand the reason for less tolerance development seen with AM8607, further in vivo studies will be required.

3.6 Future Directions:

These SAR studies show that the hydroxyl group in SAH region imparts functional potency as well as selectivity and ionizable groups e.g. acid and amine are not well tolerated in the binding
pocket. This work guides future development of more polar but possibly neutral (non-ionizable molecules at physiological pH) analogs with small ring variations e.g. triazole, tetrazole and oxazole appended directly at the C-6β.

3.7 Experimental:

(1R,4R,5R)-4-(4-Adamantan-1-yl)-2,6-dihydroxyphenyl)-6,6-dimethylbicyclo[3.1.1]heptan-2-one (3): Synthesis and NMR is presented in Chapter 2 as compound 3. \([\alpha]^{22}_D = +62.6^\circ (c = 0.40, \text{CHCl}_3)\)

(1R,4R,5R)-4-(4-Adamantan-1-yl)-2,6-bis((tert-butyldimethylsilyl)oxy)phenyl)-6,6-dimethylbicyclo [3.1.1]heptan-2-one (4): To a solution of 3 (3g, 7.88 mmol), imidazole (4.29 g, 63.1 mmol, dried at 40 °C/0.1 mmHg for 2 h) and DMAP (0.193 g, 1.577 mmol, resublimed) in anhydrous DMF (30 mL) was added TBDMSCl (7.13 g, 47.3 mmol) under positive argon pressure. The reaction mixture was allowed to stir for 36 h at room temperature. The reaction was quenched by addition of saturated aqueous NaHCO₃ and extracted with 3x200 mL diethyl
ether. The organic layer was washed with water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to give brown oil which was purified by flash chromatography (0-5% diethyl ether : hexanes) to give white solid 4 (4.2 g, 6.90 mmol, 87% yield). ¹H NMR (500 MHz, CDCl₃) δ: 6.46 (s, 2H), 4.01 (t, J = 6.5 Hz, 1H), 3.74 (dd, J = 19.0 Hz, J = 7.0 Hz, 1H), 2.60-2.42 (m, 4H), 2.53 (t, J = 7.0 Hz, 1H), 2.07 (br s, 3H), 1.86-1.74 (m, 9H, especially 1.83, s, 3H and 1.82, s, 3H), 1.72 (d, J = 12.5 Hz, 3H), 1.58 (s, 1H), 1.34 (s, 3H), 1.05-0.98 (m, 20H, especially 1.01, s, 18H), 0.31 (s, 12H). HRMS (ESI) calculated for C₃₇H₆₀O₃Si₂: calculated 608.4082; found 608.4081; [α]²²ₒ = +4.8⁰ (c = 8.60, CHCl₃)

(3R,4R)-3-(4-(Adamantan-1-yl)-2,6-bis((tert-butyldimethylsilyl)oxy)phenyl)-4-(prop-1-en-2-yl)cyclohexanone (5): To a solution of 4 (4.2g, 6.90 mmol) in CCl₄ (300 mL) at 0 °C was added iodotrimethylsilane (0.939 mL, 6.90 mmol) drop wise. To this yellow solution was added catalytic amount of t-butanol (0.020 mL, 0.207 mmol) and was allowed to stir. After 8 h, the resultant orange colored solution was cooled and again iodotrimethylsilane (2.82 mL, 20.69 mmol) was added and was stirred for another 12 h. The reaction was quenched with saturated aqueous sodium thiosulfate and diluted with 300 mL ether. The organic layer was extracted and washed sequentially with saturated NaHCO₃, water and brine. The reaction solution was dried over MgSO₄ and the solvent was evaporated to give crude tertiary iodide 5a which was used immediately for the next step without any purification. The crude iodo 5a (4.6 g, 6.24 mmol) and
sodium acetae (2.048 g, 24.97 mmol) were dissolved in acetic acid (25 mL) and stirred for 1.5 h at 90 °C. The reaction mixture was quenched by evaporating acetic acid under high pressure. The crude was dissolved in 200 mL diethyl ether and was washed with water (3x50 mL), saturated NaHCO₃ (2x30 mL) and brine. The combined organics were dried over anhydrous MgSO₄ and concentrated under reduced pressure to give black crude oil. Purification via column chromatography (5% ethyl acetae : hexanes) yielded product 5 (2.8 g, 4.60 mmol, 73% yield) as cream foam. ^1H NMR (500 MHz, CDCl₃ δ: 6.38 (s, 1H), 6.35 (s, 1H), 4.66 (d, J = 1.5 Hz, 1H), 4.51 (br s, 1H), 3.64 (ddd, J = 13.0 Hz, J = 11.5 Hz, J = 4.0 Hz, 1H), 3.43 (dt, J = 11.5 Hz, J = 3.0 Hz, 1H), 3.17 (t, J = 14.0 Hz, 1H), 2.46 (dd, J = 9.0 Hz, J = 4.5 Hz, 2H), 2.30 (dd, J = 14.0 Hz, J = 4.5 Hz, 1H), 2.07 (br s, 3H), 2.01 (qd, J = 8.0 Hz, J = 4.0 Hz, 1H), 1.86-1.68 (m, 13H, especially 1.80, s, 6H, 1.77, d, J = 12.5 Hz, 3H and 1.72, d, J = 12.5 Hz, 3H), 1.56 (s, 3H), 1.07 (s, 9H), 0.99 (s, 9H), 0.36 (s, 3H), 0.34 (s, 3H), 0.25 (s, 3H), 0.17 (s, 3H). HRMS (ESI) calculated for C₃⁷H₆₀O₃Si₂: calculated 608.4081; found 608.4081; [α]²²_D = -19.9° (c = 9.23, CHCl₃).

((5-(-Adamantan-1-yl)-2-((1R,2R)-5-(methoxymethylene)-2-(prop-1-en-2-yl)cyclohexyl)-1,3-phenylene)bis(oxy))bis(tert-butyldimethylsilane) (6): To a suspension of (methoxymethyl)triphenyl- phosphonium chloride (9.46 g, 27.6 mmol) in anhydrous THF (20 mL) at -30 °C was added n-butyllithium (10.67 mL, 26.7 mmol). The resultant red color solution
was allowed to stir at the same temperature for 30 minutes to allow complete ylide formation. To this clear red color solution was added keto 5 (2.8 g, 4.60 mmol) dissolved in THF (20 mL) and was stirred for 10 minutes while warming to room temperature. The reaction mixture was quenched by addition of water. The aqueous phase was extracted with ether (3x40 mL). The combined organics were washed with brine, dried over MgSO4 and evaporated under high pressure to give yellow crude. Purification by flash column chromatography using 0-5% ethyl acetate : hexanes gave 1:4 mixture of enol-ethers ( cis : trans) 6 (2.3 g, 3.61 mmol, 79% yield) as white solid. 1H NMR (500 MHz, CDCl3 δ: 6.34 (d, J = 2 Hz, 1H), 6.32 (d, J = 2 Hz, 1H), 5.77 (s, 0.8H), 5.71 (s, 0.2H), 4.59 (d, J = 2.5 Hz, 1H), 4.41 (d, J = 2.5 Hz, 1H), 3.53 (s, 0.6H), 3.48 (s, 2.4H), 3.22-3.14 (m, 2H), 2.83 (dd, J = 10 Hz, J = 4.5 Hz, 0.2H), 2.67 (dd, J = 13.0 Hz, J = 2.0 Hz, 1H), 2.35 (br t, J = 12.5 Hz, 0.8H), 2.12-2.0 (m, 4H, especially 2.05, br s, 3H), 1.80 (br s, 8H), 1.77 (d, J = 12.5 Hz, 3H), 1.72 (d, J = 12.0 Hz, 3H), 1.54 (s, 2.4H), 1.53 (s, 0.6H), 1.40-1.30 (m, 1H), 1.06 (s, 9H), 1.02 (s, 9H), 0.33 (s, 0.6H), 0.323 (s, 2.4H), 0.32 (s, 0.6H), 0.31 (s, 2.4H), 0.26 (s, 0.6H), 0.25 (s, 2.4H), 0.20 (s, 2.4H), 0.19 (s, 0.6H). HRMS (ESI) calculated for C39H64O3Si2: calculated 636.4391; found 636.4394; [α]D22 = 6.6° (c = 3.08, CHCl3).

(3R,4R)-3-(4-(Adamantan-1-yl)-2,6-bis((tert-butyldimethylsilyl)oxy)phenyl)-4-(prop-1-en-2-yl)cyclohexanecarbaldehyde (7): To a solution of enol ether mixture 6 (2.5 g, 3.92 mmol) in DCM (100 mL) was added trichloroacetic acid (3.21 g, 19.62 mmol) dissolved in water (10 mL)
and the resultant biphasic reaction was allowed to stir for 5h. The reaction mixture was quenched by addition of saturated NaHCO₃. The reaction solution was diluted with 100 mL of DCM and organic phase was washed with water, brine and dried over MgSO₄. Solvent was evaporated to give crude which was purified by flash column chromatography (0-7% diethyl ether : hexanes) to give a mixture of aldehydes 7 (2 g, 3.21 mmol, 82% yield) as white foam. ¹H NMR (500 MHz, CDCl₃) δ: 9.59 (d, J = 1.5 Hz, 1H), 6.35 (d, J = 1.5 Hz, 1H), 6.33 (d, J = 1.5 Hz, 1H), 4.59 (d, J = 1.5 Hz, 1H), 4.44 (d, J = 1.5 Hz, 1H), 3.29 (dt, J = 12.0 Hz, J = 3 Hz, 1H), 3.03 (dt, J = 11.5 Hz, J = 3 Hz, 1H), 2.30 (br t, J = 8.5 Hz, 1H), 2.14-1.96 (m, 5H, especially 2.06, br s, 3H), 1.90-1.66 (m, 14H), 1.55 (s, 3H), 1.50-1.40 (m, 2H), 1.05 (s, 9H), 1.02 (s, 9H), 0.32 (s, 3H), 0.31 (s, 3H), 0.26 (s, 3H), 0.18 (s, 3H). HRMS (ESI) calculated for C₃₈H₆₂O₃Si₂: calculated 622.4213; found 622.4215.

(1R,3R,4R)-3-(4-(Adamantan-1-yl)-2,6-bis((tert-butyldimethylsilyl)oxy)phenyl)-4-(prop-1-en-2-yl)cyclohexanecarbaldehyde (8): To the aldehyde mixture 7 (2.6 g, 4.17 mmol) and anhydrous K₂CO₃ (1.730 g, 12.52 mmol) was added dry MeOH (30 mL) and stirred for 12 h at 25 °C. Examination of an aliquot by ¹H NMR showed the completion of reaction. Reaction solvent was evaporated under high pressure and the crude was diluted with 200 mL ether, washed with saturated NH₄Cl solution. Combined organics were washed with water (2x50 mL), brine and dried over MgSO₄. Solvents evaporated to give crude which was purified by flash
chromatography (0-10% diethyl ether : hexanes) to give 8 (2.25 g, 3.61 mmol, 87% yield) as white foam. $^1$H NMR (500 MHz, CDCl$_3$) δ: 9.59 (d, $J = 1.5$ Hz, 1H), 6.35 (d, $J = 1.5$ Hz, 1H), 6.33 (d, $J = 1.5$ Hz, 1H), 4.59 (d, $J = 1.5$ Hz, 1H), 4.44 (d, $J = 1.5$ Hz, 1H), 3.29 (dt, $J = 12.0$ Hz, $J = 3$ Hz, 1H), 3.03 (dt, $J = 11.5$ Hz, $J = 3$ Hz, 1H), 2.30 (br t, $J = 8.5$ Hz, 1H), 2.14-1.96 (m, 5H, especially 2.06, br s, 3H), 1.90-1.66 (m, 14H), 1.55 (s, 3H), 1.50-1.40 (m, 2H), 1.05 (s, 9H), 1.02 (s, 9H), 0.32 (s, 3H), 0.31 (s, 3H), 0.26 (s, 3H), 0.18 (s, 3H). HRMS (ESI) calculated for C$_{38}$H$_{62}$O$_3$Si$_2$: calculated 622.4214; found 622.4218; [α]$^2$$_D$ = -17.9° (c = 2.91, CHCl$_3$).

((1R,3R,4R)-3-(4-(adamantan-1-yl)-2,6-bis((tert-butyldimethylsilyl)oxy)phenyl)-4-(prop-1-en-2-yl)cyclohexyl)methanol (9): To 8 (2 g, 3.21 mmol) in MeOH (30 mL) at 0 °C was added sodium borohydride (0.850 g, 22.47 mmol) and the reaction mixture was stirred for 30 minutes at the same temperature. The reaction was quenched by adding saturated aqueous NH$_4$Cl solution. Organics were extracted with 2x100 mL ethyl acetate, washed with water, brine and dried over MgSO$_4$. Solvents were evaporated to give crude which was purified with flash column chromatography using 0-20% ethyl acetate : hexanes to give 9 (1.8 g, 2.88 mmol, 90% yield) as a white solid. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.35 (s, 1H), 6.32 (s, 1H), 4.58 (s, 1H), 4.43 (s, 1H), 3.78-3.55 (m, 1H), 3.48 (dq, $J = 6.5$ Hz, $J = 1.5$ Hz, 1H), 3.28 (dt, $J = 11.5$ Hz, $J = 3$ Hz, 1H), 3.04-2.94 (m, 1H), 2.10-1.88 (m, 4H, especially 2.07, s, 3H), 1.88-1.78 (m, 7H, especially 1.80, s, 6H), 1.78-1.66 (m, 6H), 1.53 (s, 3H), 1.46-1.34 (m, 3H), 1.30-1.18 (m, 1H), 1.05 (s, 9H),
0.97 (s, 9H), 0.92-0.78 (m, 2H) 0.33 (s, 3H), 0.32 (s, 3H), 0.27 (s, 3H), 0.18 (s, 3H). HRMS (ESI) calculated for C_{38}H_{64}O_{3}Si_{2}: calculated 624.4425; found 622.44421; [α]^{22}_D = -12.5° (c = 0.57, CHCl₃).

((5-(Adamantan-1-yl)-2-((1R,2R,5R)-5-((tert-butyldimethylsilyl)oxy)methyl)-2-(prop-1-en-2-yl)cyclohexyl)-1,3-phenylene)bis(oxy))bis(tert-butyldimethylsilane) (10): To a solution of 9 (2.1 g, 3.36 mmol), imidazole (1.830 g, 26.9 mmol) and DMAP (0.082 g, 0.672 mmol) in anhydrous DMF (30 mL) was added TBDMSOCl (3.04 g, 20.16 mmol) dissolved in DMF (40 mL) under positive nitrogen pressure and stirred at room temperature for 18 h. The reaction mixture was quenched by addition of saturated aqueous NaHCO₃ and extracted with 3x100 mL of diethyl ether. The combined ethereal extracts were washed with 2x100 mL of water and brine and dried over MgSO₄. Solvent evaporation gave a pale yellow oil which was purified by flash chromatography using 0-8% diethyl ether : hexanes to produce 10 (2.1 g, 2.84 mmol, 85% yield) as white foam. \(^1\)H NMR (500 MHz, CDCl₃) δ: 6.33 (d, J = 2.0 Hz, 1H), 6.31 (d, J = 2.0 Hz, 1H), 4.57 (d, J = 2.5 Hz, 1H), 4.40 (dd, J = 2.5 Hz, J = 1.5 Hz, 1H), 3.40 (sept, 6.0 Hz, 2H), 3.22 (dt, J = 12.0 Hz, J = 3 Hz, 1H), 3.02 (dt, J = 12.0 Hz, J = 3.0 Hz, 1H), 2.07 (br s, 3H), 1.85 (br d, J = 11.5 Hz, 1H), 1.80 (s, 3H ), 1.79 (s, 3H ), 1.78-1.65 (m, 8H, especially 1.77, d, J = 12.0 Hz, 3H and 1.72, d, J = 12.0 Hz, 3H), 1.65-1.58 (m, 1H), 1.58-1.52 (m, 5H, especially 1.54, s, 3H), 1.38 (dq, J = 12.5 Hz, J = 3.5 Hz, 1H), 1.06 (s, 9H), 1.02 (s, 9H), 0.88 (s, 9H), 0.31 (s, 6H), 0.25 (s,
3H), 0.16 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H). HRMS (ESI) calculated for C_{44}H_{78}O_{3}Si_{3}: calculated 738.5329; found 738.5333; [α]^{22}_{D} = -10.3^o (c = 16.4, CHCl_{3}).

2-((1R,2R,4R)-2-(4-(Adamantan-1-yl)-2,6-bis((tert-butyldimethylsilyl)oxy)phenyl)-4-(((tert-butyl dimethylsilyl)oxy)methyl)cyclohexyl)acrylaldehyde (11): To a suspension of 10 (500 mg, 0.676 mmol) and selenium dioxide (22.51 mg, 0.203 mmol) in CH_{2}Cl_{2} (20 mL) at 0 °C was added tert-butyl hydroperoxide (0.983 mL, 10.14 mmol) drop wise and the reaction mixture was stirred at room temperature overnight. The reaction solution was quenched with saturated sodium sulfite solution and diluted with 60 mL diethyl ether. Organic layer was washed with 3x50 mL water, brine and dried over MgSO_{4}. Volatiles were evaporated under reduced pressure to give yellow crude which was purified by flash column chromatography using 0-15% ethyl acetate : hexanes to give 11 (350 mg, 0.465 mmol, 69% yield) as white foam. \(^1\)H NMR (500 MHz, CDCl_{3}) δ: 9.28 (s, 1H), 6.32 (s, 1H), 6.31 (d, J = 1.5 Hz, 1H), 6.29 (d, J = 1.5 Hz, 1H), 5.73 (s, 1H), 3.53 (dt, J = 11.5 Hz, J = 3.0 Hz, 1H), 3.42 (d, J = 6.5 Hz, 2H), 3.36 (dt, J = 11.5 Hz, J = 3.0 Hz, 1H), 2.05 (br s, 3H), 1.98-1.92 (m, 1H ), 1.85-1.79 (m, 2H), 1.79-1.76 (m, 7H, especially 1.78, s, 3H and 1.77, s, 3H), 1.76-1.66 (m, 6H), 1.64-1.58 (m, 1H), 1.57 (s, 1H), 1.08-1.02 (m, 19H, especially 1.05, s, 18H), 0.88 (s, 9H), 0.88 (s, 9H), 0.31 (s, 3H), 0.30 (s, 3H), 0.27 (s, 3H), 0.18 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H). HRMS (ESI) calculated for C_{44}H_{76}O_{4}Si_{3}: calculated 752.5047; found 752.5051; [α]^{22}_{D} = 32.7^o (c = 0.23, CHCl_{3}).
2-((1R,2R,4R)-2-(4-(Adamantan-1-yl)-2,6-bis((tert-butyldimethylsilyl)oxy)phenyl)-4-((tert-butyl dimethylsilyl)oxy)methyl)cyclohexyl)prop-2-en-1-ol (12): To a solution of 11 (1.2 g, 1.593 mmol) in MeOH (30 mL) and diethyl ether (10 mL) at 0 °C was added sodium borohydride (0.422 g, 11.15 mmol) and reaction mixture was stirred for 1h at the same temperature. The reaction was quenched by adding saturated aqueous NH₄Cl solution. Organics were extracted with 2x100 mL ethyl acetate, washed with water, brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified with flash column chromatography using 0-10% ethyl acetate : hexanes to give 12 (1.1 g, 1.456 mmol, 91% yield) as white foam. ¹H NMR (500 MHz, CDCl₃) δ: 6.36 (d, J = 2 Hz, 1H), 6.32 (d, J = 1.5 Hz, 1H), 4.83 (br s, 1H), 4.79 (d, J = 1.5 Hz, 1H), 3.95 (dd, J = 14.0 Hz, J = 6.5 Hz, 1H), 3.85 (dd, J = 14 Hz, J = 6.5 Hz, 1H), 3.41 (dd, J = 6.5 Hz, J = 2.0 Hz, 2H), 3.27 (dt, J = 12.0 Hz, J = 3.5 Hz, 1H), 2.96 (dt, J = 12.0 Hz, J = 3.0 Hz, 1H), 2.05 (br s, 3H), 1.86 (d, J = 11.5 Hz, 2H), 1.80 - 1.74 (m, 10H especially 1.79, d, J = 2.5 Hz, 6H), 1.71 (d, J = 12.0 Hz, 4H), 1.65 (d, J = 13.5 Hz, 1H), 1.62 - 1.56 (m, 1H), 1.36 (t, J = 6.5 Hz, 1H), 1.06 (s, 9H), 1.03 (s, 9H), 0.88 (s, 9H), 0.32 (s, 3H), 0.31 (s, 3H), 0.24 (s, 3H), 0.17 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H). HRMS (ESI) calculated for C₄₄H₇₈O₄Si₃: calculated 754.5215; found 754.5208; [α]²²D = 32.7° (c = 0.23, CHCl₃).
5-(Adamantan-1-yl)-3-((\textit{tert}-butyldimethylsilyl)oxy)-2-((1R,2R,5R)-5-((\textit{tert}-butyldimethylsilyl)-oxy)methyl)-2-(3-hydroxyprop-1-en-2-yl)cyclohexylphenol (13): To a solution of 12 (620 mg, 0.821 mmol) in anhydrous THF (30 mL) at -30 °C was added 1 equivalent of tetrabutylammonium fluoride (TBAF) (0.821 mL, 0.821 mmol) to selectively deprotect one phenolic hydroxy group. The reaction mixture was stirred at the same temperature for 15 minutes. Reaction was quenched by addition of water and organics were extracted in 100 mL diethyl ether. Organic layer was washed with brine and dried over MgSO₄. Volatiles were evaporated to give crude which was purified with flash column chromatography using 10-50% ethyl acetate : hexanes to give white foam 13 (500 mg, 0.78 mmol, 95% yield). \(^1\)H NMR (500 MHz, CDCl₃) δ 6.33 (s, 1H), 6.24 (s, 1H), 5.32 (s, 1H), 4.88 (s, 1H), 4.82 (s, 1H), 3.95 (s, 2H), 3.42 (d, \(J = 6.0\) Hz, 2H), 3.30 (dt, \(J = 11.0\) Hz, \(J = 3.5\) Hz, 1H), 2.95 (dt, \(J = 12.0\) Hz, \(J = 2.5\) Hz, 1H), 2.05 (br s, 3H), 1.89 (dt, \(J = 12.0\) Hz, \(J = 3.0\) Hz, 2H), 1.82 - 1.58 (m, 1H especially 1.78, s, 6H), 1.40 - 1.30 (m, 1H), 1.04 (s, 9H), 0.88 (s, 9H), 0.25 (s, 3H), 0.18 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H). HRMS (ESI) calculated for C\(_{38}\)H\(_{64}\)O\(_4\)Si\(_2\): calculated 640.4338; found 640.4343; \([\alpha]^{22}_D = -14.4^\circ\) (c = 1.92, CH\(_2\)Cl\(_2\)).
((6R,6aR,9R,10aR)-3-(Adamantan-1-yl)-1-((tert-butyldimethylsilyl)oxy)-9-((tert-butyl dimethyl-silyl)oxy)methyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-6-yl)methanol (14): To a solution of 13 (400 mg, 0.624 mmol) in THF (30 mL) was added mercuric acetate (398 mg, 1.248 mmol) and the reaction solution was stirred for 24h at room temperature. The reaction mixture was cooled to -30 °C and sodium borohydride (165 mg, 4.37 mmol) was added along with a 1M solution of sodium methoxide (539 mg, 9.98 mmol)/MeOH (10 mL). The mixture was stirred at the same temperature for 30 minutes, quenched with degassed NH₄Cl solution (15 mL) and warmed to room temperature. The reaction mixture was diluted with 50 mL diethyl ether and organic phase was washed with water, brine and dried over MgSO₄. Solvents were evaporated to give crude that was purified with flash column chromatography using 2-45% ethyl acetate : hexanes to give 14 (250 mg, 0.390 mmol, 62% yield) as white foam. ¹H NMR (500 MHz, CDCl₃) δ: 6.43 (d, J = 1.5 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H), 3.68 (d, J = 2.5 Hz, 1H), 3.66 (s, 1H), 3.55 (dd, J = 10.0 Hz, J = 5.0 Hz, 1H), 3.34 (dd, J = 10.0 Hz, J = 7.5 Hz, 1H), 3.08 (d, J = 13.0 Hz, 1H), 2.41 (dt, J = 10.5 Hz, J = 2.5 Hz, 1H), 2.09 (dd, J = 8.0 Hz, J = 6.5 Hz, 1H), 2.06 (br s, 3H), 2.01 (d, J = 13.0 Hz, 1H), 1.83 (d, J = 2.5 Hz, 6H), 1.76 (d, J = 12.5 Hz, 4H), 1.71 (d, J = 13.0 Hz, 4H), 1.01 (s, 12H), 0.89 (s, 9H), 0.78 (q, J = 11.5 Hz, 1H), 0.24 (s, 3H), 0.15 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H). HRMS (ESI) calculated for C₃₈H₆₄O₄Si₂: calculated 640.4341; found 640.4343; [α]²₂D = -44.77° (c = 5.22, CHCl₃).
((6R,6aR,9R,10aR)-3-(adamantan-1-yl)-1-hydroxy-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromene-6,9-diyl)dimethanol (15): To a solution of 14 (30 mg, 0.047 mmol) in dry THF (5 mL) was added 1M TBAF (0.328 mL, 0.328 mmol) at 0 °C and stirred for 1h at the same temperature. Purple color reaction solution was quenched with saturated NH₄Cl solution. Organic layer was extracted in 30 mL diethyl ether, washed with water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to give crude, which was purified with flash column chromatography using 10-70% ethyl acetate : hexanes to give 15 (18 mg, 0.044 mmol, 93% yield) as white foam. ¹H NMR (500 MHz, CDCl₃) δ 6.40 (d, J = 2.0 Hz, 1H), 6.28 (d, J = 1.5 Hz, 1H), 5.70 (br s, 1H), 3.67 (s, 2H), 3.51 (t, J = 6.5 Hz, 2H), 3.29 (d, J = 12.5 Hz, 1H), 2.53 (dt, J = 6.0 Hz, J = 2.5 Hz, 1H), 2.05 (d, J = 1.0 Hz, 3H), 1.94-1.89 (m, 1H), 1.87 (td, J = 7.5 Hz, J = 2.0 Hz, 1H), 1.82 (d, J = 2.0 Hz, 6H), 1.76 (d, J = 12.0 Hz, 4H), 1.70 (d, J = 11.0 Hz, 4H), 1.13 (q, J = 8.0 Hz, 2H), 1.0 (s, 3H), 0.82 (q, J = 12.0 Hz, 1H). HRMS (ESI) calculated for C_{26}H_{36}O₄: calculated 412.2646; found 412.2641. ; [α]_{D}^{22} = -45.8° (c = 1.31, CHCl₃).
(6R,6aR,9R,10aR)-3-(Adamantan-1-yl)-1-((tert-butyl(dimethyl)silyl)oxy)-9-((tert-butyl-dimethylsilyl)-oxy)methyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromene-6-carbaldehyde (16): To a suspension of Dess-Martin Periodinane (1.64 g, 3.87 mmol) in DCM (20 mL) at 0 °C was added 14 (620 mg, 0.967 mmol) dissolved in DCM (20 mL) and the resultant solution was stirred for 4h at same temperature. The reaction mixture was diluted with 40 mL ether and stirred for 10 minutes in 25 mL aqueous saturated NaHCO₃ and saturated Na₂S₂O₃ (1:7) solution. The organic layer was separated, washed with water, brine and dried over MgSO₄. Solvents were evaporated to give brown oil which was purified with flash column chromatography using 2-20% ethyl acetate : hexanes to give 16 (500 mg, 0.782 mmol, 81% yield) as white foam. \(^1\H NMR (500 MHz, CDCl₃) δ: 9.60 (s, 1H), 6.52 (d, \(J = 2.0\) Hz, 1H), 6.41 (s, 1H), 3.54 (dd, \(J = 10.0\) Hz, \(J = 4.5\) Hz, 1H), 3.36 (t, \(J = 8.5\) Hz, 1H), 3.14 (d, \(J = 12.5\) Hz, 1H), 2.45 (t, \(J = 11.5\) Hz, 1H), 2.07 (br s, 3H), 1.97 (d, \(J = 7.5\) Hz, 1H), 1.84 (s, 6H), 1.77 (d, \(J = 12.0\) Hz, 4H), 1.72 (d, \(J = 12.0\) Hz, 4H), 1.65 (dd, \(J = 11.0\) Hz, \(J = 2.5\) Hz, 1H), 1.17 (s, 3H), 1.09 (t, \(J = 10.0\) Hz, 2H), 1.01 (s, 9H), 0.88 (s, 9H), 0.84 (q, \(J = 12.0\) Hz, 1H), 0.26 (s, 3H), 0.16 (s, 3H), 0.03 (s, 6H). HRMS (ESI) calculated for C₃₈H₆₂O₄Si₂: calculated 638.4185; found 638.4191; \([\alpha]^{22}_D = -13.9^\circ\) (c = 0.4, CHCl₃).
((6S,6aR,9R,10aR)-3-(Adamantan-1-yl)-1-((tert-butyldimethylsilyl)oxy)-6-(2,2-
ibromovinyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-9-yl)methoxy)(tert-
butyl)-dimethysilane (17): To a solution of carbon tetrabromide (104 mg, 0.313 mmol) in dry
DCM (5 mL) was added triphenylphosphine (123 mg, 0.469 mmol) dissolved in DCM (5 mL) at
0 °C. The yellow color solution was stirred for 10 minutes until it turns orange showing the
completion of ylide formation. This orange color solution was added to a solution of 16 (50 mg,
0.078 mmol) in DCM (5 mL) at 0 °C with the help of cannula. The reaction mixture was stirred
at the same temperature for 45 minutes. The reaction mixture was quenched by adding water and
diluting it with 30 mL ether. The organic layer was separated, washed with brine and dried over
MgSO₄. Solvents were evaporated to give brown oil which was purified with flash column
chromatography using 0-10% ethyl acetate : hexanes to give 17 (35 mg, 0.044 mmol, 56% yield,
dr = 92 : 8) as a gum. ¹H NMR (500 MHz, CDCl₃) δ 6.73 (s, 1H), 6.46 (d, J = 2.0 Hz, 1H), 6.36
(d, J = 2.0 Hz, 1H), 3.55 (dd, J = 10.0 Hz, J = 4.5 Hz, 1H), 3.35 (dd, J = 10.0 Hz, J = 7.5 Hz,
1H), 3.09 (d, J = 13.0 Hz, 1H), 2.40 (dt, J = 11.0 Hz, J = 2.0 Hz, 1H), 2.06 (br s, 3H), 1.92-1.86
(m, 1H), 1.83 (br s, 6H), 1.76 (d, J = 12.0 Hz, 4H), 1.71 (d, J = 12.5 Hz, 4H), 1.69-1.63 (m, 1H),
1.30 (s, 3H), 1.20-1.11 (m, 2H), 1.0 (s, 9H), 0.90 (s, 9H), 0.79 (q, J = 12.0 Hz, 1H), 0.24 (s, 3H),
0.15 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H). Mass spectrum m/z (relative intensity) 793.4 (M⁺, 60),
795.2 (M+2, 97), 797.1 (M+4, 75), 635.4 (95).
(((6R,6aR,9R,10aR)-3-(adamantan-1-yl)-1-((tert-butyldimethylsilyl)oxy)-6-ethynyl-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-9-yl)methoxy)(tert-butyl)dimethylsilane (18): To a solution of 17 (45 mg, 0.057 mmol) in THF (5 mL) was added $n$-butyllithium (18.19 mg, 0.170 mmol) at -30 °C. The yellow color solution was stirred for 1 h at the same temperature. The reaction mixture was quenched by addition of 1N HCl and diluted with 30 mL ether. The organic layer was separated, washed with water, brine and dried over MgSO$_4$. Solvents were evaporated to give yellow crude which was purified with flash column chromatography using 0-10% ethyl acetate : hexanes to give 18 (30 mg, 0.047 mmol, 83% yield) as white foam. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 6.52 (d, $J = 2.0$ Hz, 1H), 6.37 (d, $J = 2.0$ Hz, 1H), 3.55 (dd, $J = 10.0$ Hz, $J = 5.0$ Hz, 1H), 3.35 (dd, $J = 10.0$ Hz, $J = 7.5$ Hz, 1H), 3.07 (d, $J = 12.5$ Hz, 1H), 2.58 (s, 1H, Alkyne H), 2.38 (dt, $J = 11.0$ Hz, $J = 2.5$ Hz, 1H), 2.24-2.20 (m, 1H), 2.05 (br s, 3H), 1.84 (br s, 1H), 1.82 (d, $J = 2.5$ Hz, 6H), 1.75 (d, $J = 12.5$ Hz, 4H), 1.70 (d, $J = 11.5$ Hz, 4H), 1.37 (s, 3H), 1.20 - 1.15 (m, 2H), 1.0 (s, 9H), 0.89 (s, 9H), 0.80 (q, $J = 12.0$ Hz, 1H), 0.24 (s, 3H), 0.14 (s, 3H), 0.07 (s, 3H), 0.04 (s, 3H). HRMS (ESI) calculated for C$_{39}$H$_{62}$O$_3$Si$_2$: calculated 634.4217; found 634.4221; $[\alpha]^{22}_D = -33.7^\circ$ (c = 0.73, CHCl$_3$).
(6R,6aR,9R,10aR)-3-(Adamantan-1-yl)-6-ethynyl-9-(hydroxymethyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol (19): To a solution of 18 (10 mg, 0.016 mmol) in dry THF (5 mL) was added 1M TBAF (0.079 ml, 0.079 mmol) and stirred for 1h at 0 °C. Purple color reaction solution was quenched by 1N NH₄Cl solution. The organic layer was extracted in 30 mL diethyl ether, washed with water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to give crude, which was purified with flash column chromatography (10-70% ethyl acetate : hexanes) to give 19 [5 mg, 0.012 mmol, 78% yield, dr ratio = 80 (6β) : 20 (6α)] as white foam. ¹H NMR (500 MHz, CDCl₃) δ 6.50 (d, J = 1.5 Hz, 1H), 6.27 (d, J = 2.0 Hz, 1H), 5.15 (br s, 1H), 3.53 (d, J = 6.5 Hz, 2H), 3.26 (d, J = 12.5 Hz, 1H), 2.59 (s, 1H), 2.52 (dt, J = 11.5 Hz, J = 2.5 Hz, 1H), 2.24 (td, J = 9.0 Hz, J = 3.0 Hz, 1H), 2.05 (s, 3H), 1.99 (d, J = 11.0 Hz, 1H), 1.82 (d, J = 2.0 Hz, 6H), 1.75 (d, J = 12.5 Hz, 4H), 1.70 (d, J = 12.0 Hz, 4H), 1.36 (s, 3H), 1.30-1.24 (m, 2H), 0.83 (q, J = 12.0 Hz, 1H). HRMS (ESI) calculated for C₂₇H₃₄O₃: calculated 406.2497; found 406.2502. IR (neat, cm⁻¹): 2902 (br), 1614, 1562, 1471, 1410, 1253, 1060. [α]D
3-((6R,6aR,9R,10aR)-3-(Adamantan-1-yl)-1-((tert-butyldimethylsilyl)oxy)-9-(((tert-butyl dimethylsilyl)oxy)methyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-6-y1)prop-2-yn-1-ol (20): To a solution of 18 (25 mg, 0.039 mmol) in THF (5 mL) at -30 °C was added n-butyllithium (3.03 mg, 0.047 mmol). The yellow colored solution was stirred for 1h at the same temperature. To this was added paraformaldehyde (5.91 mg, 0.197 mmol) and reaction was gradually warmed to room temperature and allowed to stir for 12h. Reaction was quenched with saturated NH₄Cl solution and diluted with 30 mL ether. The organic layer was separated, washed with water, brine and dried over MgSO₄. Solvents were evaporated to give yellow crude which was purified with flash column chromatography (1-10% ethyl acetate : hexanes) to give 20 (18 mg, 0.027 mmol, 69% yield) as white foam. ¹H NMR (500 MHz, CDCl₃): δ 6.46 (d, J = 2.0 Hz, 1H), 6.40 (dd, J = 2.0 Hz, J = 1.0 Hz 1H), 4.16 (d, J = 6.5 Hz, 2H), 3.54 (dd, J = 10.0 Hz, J = 5.0 Hz, 1H), 3.35 (dd, J = 10.0 Hz, J = 8.0 Hz, 1H), 3.04 (d, J = 13.0 Hz, 1H), 2.59 (dt, J = 11.0 Hz, J = 2.0 Hz, 1H), 2.05 (br s, 3H), 1.93 (dd, J = 13.0 Hz, J = 2.5 Hz, 1H), 1.84 (d, J = 2.0 Hz, 6H), 1.76 (d, J = 13.0 Hz, 4H), 1.71 (d, J = 12.5 Hz, 4H), 1.62 (s, 3H), 1.47 (dt, J = 13.0 Hz, J = 3.5 Hz, 1H), 0.90 (s, 9H), 0.89 (s, 9H), 0.78 (q, J = 12.0 Hz, 1H), 0.21 (s, 3H), 0.13 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H). HRMS (ESI) calculated for C₄₀H₆₄O₄Si₂: calculated 664.4303; found 664.4298; [α]²²_D = -44.3° (c = 0.09, CHCl₃).
(6R,6aR,9R,10aR)-3-(Adamantan-1-yl)-9-(hydroxymethyl)-6-(3-hydroxyprop-1-yn-1-yl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol (21): To a solution of 20 (20 mg, 0.030 mmol) in dry THF (5 mL) was added 1M TBAF (39.3 mg, 0.150 mmol) and stirred for 1h at 0 °C. Purple color reaction solution was quenched by saturated NH₄Cl. Organic layer was extracted in 30 mL diethyl ether, washed with water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to give crude, which was purified with flash column chromatography using 10-60% ethyl acetate : hexanes to give 21 (10 mg, 0.023 mmol, 76% yield) as white solid. ¹H NMR (500 MHz, CDCl₃) δ: 6.49 (d, J = 2.0 Hz, 1H), 6.25 (d, J = 2.0 Hz, 1H), 4.71 (s, 1H), 4.38 (d, J = 5.5 Hz, 2H), 3.53 (t, J = 6.0 Hz, 2H), 3.23 (d, J = 12.0 Hz, 1H), 2.52 (dt, J = 11.0 Hz, J = 2.5 Hz, 1H), 2.22 (td, J = 9.0 Hz, J = 3.0 Hz, 1H), 2.05 (d, J = 1.5 Hz, 3H), 2.0 (d, J = 11.0 Hz, 1H), 1.82 (d, J = 2.5 Hz, 6H) 1.76 (d, J = 12.5 Hz, 4H), 1.70 (d, J = 11.5 Hz, 4H), 1.39 (s, 3H), 1.23-1.17 (m, 2H), 0.83 (q, J = 12.0 Hz, 1H). HRMS (ESI) calculated for C₂₈H₃₆O₄: calculated 436.2616; found 436.2613; [α]²²_D = -28.6° (c = 0.06, CHCl₃).
(6S,6aR,9R,10aR)-3-(Adamantan-1-yl)-1-((tert-butyldimethylsilyl)oxy)-9-(((tert-butyldimethylsilyl)-oxy)methyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromene-6-carboxylic acid (22): To 16 (50 mg, 0.078 mmol) and sodium dihydrogen phosphate (103 mg, 0.861 mmol) was added water (1.5 mL) and tert-butanol (5.61 mL, 58.7 mmol) at 0 °C. To this solution was added 2-methyl-2-butene (0.174 mL, 1.643 mmol) and the reaction mixture was stirred vigorously for 10 minutes. To this was added sodium chlorite (21.23 mg, 0.235 mmol) in water (1.5 mL) and the reaction mixture was stirred for 4 hrs. The reaction solution was quenched by diluting with DCM (20 mL). Organic layer was separated, washed with 10 mL water, brine and dried over MgSO₄ and concentrated under reduced pressure to give crude, which was purified with flash column chromatography (3-20% ethyl acetate : hexanes) to give 22 (38 mg, 0.058 mmol, 74% yield) as white solid. ¹H NMR (500 MHz, CDCl₃) δ: 6.50 (br s, 1H), 6.42 (s, 1H), 3.53 (dd, J = 10.0 Hz, J = 5.0 Hz, 1H), 3.36 (dd, J = 10.0 Hz, J = 7.5 Hz, 1H), 3.10 (d, J = 12.0 Hz, 1H), 2.45 (t, J = 11.0 Hz, 1H), 2.06 (br s, 3H), 2.03-1.88 (m, 2H), 1.82 (s, 6H), 1.76 (d, J = 12 Hz, 3H), 1.71 (d, J = 12 Hz, 3H), 1.43 (s, 1H), 1.34 (s, 3H), 1.25 (s, 2H), 1.16-1.06 (m, 1H), 1.01 (s, 9H), 0.88 (s, 9H), 0.82-0.76 (m, 1H), 0.25 (s, 3H), 0.15 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H). HRMS (ESI) calculated for C₃₈H₆₂O₅Si₂: calculated 654.4056; found 654.4061; [α]D²² = -10.7° (c = 0.38, CHCl₃).
(6S,6aR,9R,10aR)-Methyl-3-(adamantan-1-yl)-1-(((tert-butyldimethylsilyl)oxy)-9-(((tert-butyldimethylsilyl)oxy)methyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromene-6-carboxylate (23): To 22 (30 mg, 0.046 mmol) in dry toluene (2 mL) and MeOH (2 mL) was added (diazomethyl)trimethylsilane (0.458 mL, 0.458 mmol) and the reaction solution was stirred overnight at room temperature. TLC in 7% ethyl acetate : hexanes shows the completion of reaction. Solvent was evaporated under reduced pressure and crude was as such loaded in silica gel column by dissolving in ether. Crude was purified by silica gel chromatography (2-10% ethyl acetate : hexanes) to give 23 (19 mg, 0.029 mmol, 65% yield). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 6.50 (d, $J = 2.0$ Hz, 1H), 6.42 (d, $J = 1.5$ Hz, 1H), 3.83 (s, 3H), 3.54 (dd, $J = 10.5$ Hz, $J = 5.0$ Hz, 1H), 3.48 (q, $J = 6.5$ Hz, 1H), 3.34 (dd, $J = 9.5$ Hz, $J = 7.0$ Hz, 1H), 3.08 (d, $J = 9.0$ Hz, 1H), 2.41 (dt, $J = 9.0$ Hz, $J = 2.5$ Hz, 1H), 2.05 (br. s., 3H), 2.02-1.96 (m, 1H), 1.94-1.86 (m, 1H), 1.82 (s, 6H), 1.75 (d, $J = 13.0$ Hz, 3H), 1.70 (d, $J = 13.0$ Hz, 3H), 1.64-1.56 (m, 1H), 1.32 (s, 3H), 1.29-1.23 (m, 2H), 1.21 (t, $J = 6.5$ Hz, 1H), 1.01 (s, 9H), 0.88 (s, 9H), 0.25 (s, 3H), 0.14 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H). HRMS (ESI) calculated for C$_{39}$H$_{64}$O$_5$Si$_2$: calculated 668.4257; found 668.4261; [$\alpha$]$^2$$_D$ = -12.2° ($c = 0.09$, CHCl$_3$).
((6R,6aR,9R,10aR)-3-(Adamantan-1-yl)-1-hydroxy-9-(hydroxymethyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromene-6-carboxylic acid (24): To a solution of 22 (10 mg, 0.018 mmol) in dry THF (4 mL) at 0 °C was added TBAF (0.112 ml, 0.112 mmol) and the reaction mixture was stirred for 6 h at 0 °C. Purple colored reaction solution was quenched by addition of 1N NH₄Cl solution. The organic layer was extracted in 30 mL diethyl ether, washed with water, brine and dried (MgSO₄). Solvents were evaporated under reduced pressure to give crude, which was purified with flash column chromatography (5-70% ethyl acetate : hexanes) to give 24 (5 mg, 0.010 mmol, 78% yield) as yellow gum. ¹H NMR (500 MHz, CDCl₃) δ: 6.37 (d, J = 2.0 Hz, 1H), 6.32 (d, J = 2.0 Hz, 1H), 3.44 (d, J = 6.0 Hz, 1H), 3.42 (d, J = 6.0 Hz, 1H), 3.39 (d, J = 12.5 Hz, 1H), 3.38-3.36 (m, 1H), 3.36-3.34 (m, 1H), 2.52 (dt, J = 9.0 Hz, J = 2.0 Hz, 1H), 2.05 (s, 3H), 1.94 (br d, J = 8.5 Hz, 1H), 1.85 (s, 6H), 1.80 (d, J = 12.0 Hz, 3H), 1.75 (d, J = 12.0 Hz, 3H), 1.72-1.68 (m, 1H), 1.25 (t, J = 4.5 Hz, 3H), 1.22 (dq, J = 12.5 Hz, J = 3.5 Hz, 1H), 1.09 (dq, J = 12.5 Hz, J = 3.5 Hz, 1H), 0.74 (q, J = 12.0 Hz, 1H). IR (neat, cm⁻¹): 2928, 1717, 1563, 1411, 1253, 1141. HRMS (ESI) calculated for C₂₇H₃₆O₅: calculated 426.2434; found 426.2439; [α]²²D = -10.1° (c = 0.07, CHCl₃).
(6R,6aR,9R,10aR)-Methyl-3-(adamantan-1-yl)-1-hydroxy-9-(hydroxymethyl)-6-methyl-
6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromene-6-carboxylate (25): To a solution of 23
(15 mg, 0.022 mmol) in dry THF (4 mL) at 0 °C was added TBAF (0.112 mL, 0.112 mmol) and
the reaction mixture was stirred at the same temperature for 1h. The reaction solution was
quenched by addition of 1N NH₄Cl solution. The organic layer was extracted in 30 mL diethyl
ether, washed with water, brine and dried (MgSO₄). Solvents were evaporated under reduced
pressure to give crude, which was purified with flash column chromatography (5-70% ethyl
acetate : hexanes) to give 25 (8 mg, 0.018 mmol, 81% yield) as yellow gum. ¹H NMR (500
MHz, CDCl₃) δ: 6.45 (s, 1H), 6.28 (s, 1H), 5.30 (s, 1H, OH), 5.09 (br s, 1H, OH), 3.83 (s, 3H),
3.52 (d, J = 6.0 Hz, 2H), 3.27 (d, J = 12.5 Hz, 1H), 2.56 (t, J = 9.0 Hz, 1H), 2.05 (s, 3H), 1.94 (t,
J = 10.5 Hz, 1H), 1.82 (s, 6H), 1.75 (d, J = 12.0 Hz, 3H), 1.69 (d, J = 12.0 Hz, 3H), 1.35 (s, 3H),
1.24 (s, 2H), 1.11 (dq, J = 12.5 Hz, J = 3.5 Hz, 2H), 0.85 (q, J = 12.0 Hz, 2H). IR (neat, cm⁻¹):
2928, 1744, 1563, 1411, 1254, 1137, 1085. HRMS (ESI) calculated for C₂₇H₃₆O₅: calculated
440.2584; found 440.2579; [α]D²² = -11.4° (c = 0.06, CHCl₃).
(6R,6aR,9R,10aR)-3-(Adamantan-1-yl)-9-(hydroxymethyl)-6-methyl-6-(morpholinomethyl)-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol (26): To a solution of 16 (25 mg, 0.039 mmol) in THF (2 mL) and DCM (2 mL) at room temperature was added morpholine (0.02 mL, 0.196 mmol) and sodium triacetoxyborohydride (41.5 mg, 0.196 mmol). The resultant solution was stirred for 30 min. Acetic acid (2.239 µl, 0.039 mmol) was added and the reaction was stirred for 18 h. The reaction was quenched with addition of 1N NH₄Cl, diluted with diethyl ether (10 mL) and water (10 mL). Organic layer was separated, washed with saturated sodium bicarbonate, 5 mL water and brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to afford crude product. The crude was taken to the next step for TBDMS deprotection using earlier mentioned TBAF method for compound 25. Rf = 0.32 in 50% ethyl acetate : hexanes. Purification by flash chromatography on silica gel in 10-85% ethyl acetate : hexanes afforded desired product as 26 (10 mg, 0.021 mmol, 53% yield). ¹H NMR (500 MHz, CDCl₃) δ: 6.42 (d, J = 1.5 Hz, 1H), 6.25 (d, J = 2.0 Hz, 1H), 4.01 (br s, 4H, OH), 3.48 (dd, J = 10.0 Hz, J = 6.0 Hz, 1H), 3.40 (dd, J = 10.0 Hz, J = 6.0 Hz, 1H), 3.29 (d, J = 13.0 Hz, 2H), 3.22-3.14 (m, 4H), 3.13 (d, J = 12.5 Hz, 2H), 2.43 (dt, J = 10.5 Hz, J = 2.0 Hz, 1H), 2.04 (s, 3H), 1.80 (d, J = 2 Hz, 6H), 1.75 (d, J = 13.0 Hz, 3H), 1.69 (d, J = 11.5 Hz, 3H), 1.66-1.56 (m, 3H), 1.19 (s, 3H), 1.09 (q, J = 9.0 Hz, 2H), 0.73 (q, J = 12.0 Hz, 1H). HRMS (ESI) calculated for C₃₀H₄₃NO₄: calculated 481.3168; found 481.3173; [α]²²D = -24.1⁰ (c = 0.18, CHCl₃).
Detailed description of methods used for *in vitro* and *in vivo* testing:

**In vitro** studies done in Biochemistry lab in CDD:

1. **Membrane Preparations from Tissue Culture Sources:** HEK293 cells expressing the mCB2 or hCB2 receptor are used for membrane preparations according to the method described by Abadji et al.\(^{132}\) The resulting pellet is resuspended in 10 mM Tris-chloride, pH 7.4 with 5 mM \(\text{MgCl}_2\) and 2 mM EDTA (TME) and stored at -80 °C for no longer than two months. Protein content is assayed by using the Bio-Rad protein assay according to the manufacturer’s protocol.

2. **Membrane Preparations from Tissue Sources:** Frozen rat brains (CB1 source) are obtained from Pel-Freeze Biologicals (Rogers, AK) and stored at -80 °C until use. Membranes were prepared according to the method described by Dodd et al. and adapted for use in our laboratory as previously reported.\(^{133-135}\)

3. **rCB1, hCB2, and mCB2 Binding Assays:** All compounds synthesized for this grant are tested for their ability to bind to CB1 and CB2 receptors using rat brain or HEK293 cell membranes expressing hCB2 membrane preparations, respectively, as previously described via competition-equilibrium binding using \([^3]H\)CP-55,940.\(^{135,136}\) The results were analyzed using nonlinear regression to determine the actual IC\(_{50}\) of the ligand (Prizm by GraphPad Software, Inc.) and the \(K_i\) values are calculated from the IC\(_{50}\).\(^{137}\)

4. **Signal Transduction Assays (cAMP assay):** HEK-293 cells transfected with rCB1, mCB2, or hCB2 receptor are used with the PerkinElmer’s Lance ultra cAMP kit following the protocol of the manufacturer. Briefly, the assays were carried out in 384-well format using 1000 cells/well. Test compounds were added to wells containing stimulation buffer and 2 \(\mu\)M forskolin followed by cell suspension. After 30 minutes stimulation, the Eu-cAMP tracer and
Ulight-anti-cAMP are added to the plate and incubated at room temperature for 1h prior to detection via PerkinElmer Envision; data are analyzed using GraphPad Prism software.

5. Preliminary distribution in brain/plasma and drug oral bioavailability: Mice (CD-1, weighing 25-30 g) are injected intravenously or orally with 0.1-2 mg/kg of the compound mixture. Fifteen minutes post-IV injection, or 30 and 60 minutes post-oral administration, the animals are sacrificed by decapitation followed by blood collection (~500 µL) and tissue dissection; samples are flash frozen with liquid nitrogen to prevent post-mortem degradation of the compounds. Tissues (plasma or brain) are extracted following published procedures and analyzed using a Thermo-Finnigan Quantum Ultra triple quadrupole mass spectrometer in SRM mode with an Agilent 1100 HPLC front-end with internal standards used for quantitation.

β-Arrestin Assay done in Duke University: Cells were treated with a serial concentration of test compounds. Known agonists were used as positive control. Cells were fixed with 1% PFA for 40 mins after administration of compounds. Images were acquired and analyzed as described below. Data are from 2-3 independent experiments with duplicate samples.

Image-based β-arrestin2 translocation assay- U2OS cells stably expressing the CB1-E cannabinoid receptors and β-arrestin2-GFP were split into glass-bottom 384 well plates (MGB101-1-2-LG, MatriCal, Spokane, WA) at a density of 8,000 cells/30 µl media/well using a Multidrop 384 dispenser (Thermo Electron Corporation). The plates were incubated overnight at 37 °C in 5% CO2. The following day, culture medium was changed to 30 µl/well of clear minimum Eagle’s medium (MEM) with 10mM HEPES. A set of serial diluted 4x concentration of each AM compound (10 mM in DMSO) was prepared in the same medium and applied to cells at a volume of 10 µl. The cells were incubated with compound for 40 mins at 37 °C prior to
fixation with an equal volume of PBS containing 2% paraformaldehyde (sigma). Plates were stored at 4 °C until analysis. β-arrestin2-GFP aggregates were identified. Dose response curves were analyzed by nonlinear regression techniques using GraphPad Prism 5.0 software (GraphPad, San Diego, CA), and data were fitted to sigmoidal dose-response curves to obtain EC\textsubscript{50} and efficacy values.

**Methods used for in vivo studies:**

1. **For Gastrointestinal studies (Dr. Storr’s Laboratory):**

**Animals:** Male BALB/c mice (Medical University of Lodz, Poland), weighing 22-26 g, were used for all experiments. The animals were housed at a constant temperature (22°C) and maintained under a 12 h light/dark cycle in sawdust-lined plastic cages with access to laboratory chow and tap water ad libitum. Animal use for these studies was approved by the Medical University of Lodz Animal Care Committee and the experiments were performed in accordance with institutional and national animal ethics committee guidelines.

**In vivo upper gastrointestinal transit:** After an overnight fasting with free access to water, mice received 0.15 mL of a charcoal marker (10% activated charcoal and 5% gum Arabic in water), which was gavaged into the stomach using a 20-gauge animal feeding tube. Twenty minutes later mice were sacrificed and the small intestine was immediately removed. The distance from the pyloric sphincter to the front of the marker was measured and expressed as percent of the total length of the small intestine. In all experiments the animals were gavaged 15 min after i.p. administration of AM8607, or vehicle. The antagonists were administered i.p. 15 min before AM8607, or vehicle.
Gastric emptying and geometric center of upper intestinal transit: Gastric emptying (GE) and geometric center (GC) experiments were performed according to techniques described earlier. Briefly, mice were fasted overnight with free access to tap water. On the day of experiment, the animals received a gavage of 0.2 mL of a marker solution (50 mg phenol red in 100 mL 1.5% methylcellulose, constantly stirred and held at 37 °C). Mice were sacrificed 20 min after administration of the meal. The stomach and the small intestine were carefully removed. The stomach was subsequently opened and its contents transferred to a test tube containing 4 mL of distilled water. After 20 min of sedimentation, 1 mL of supernatant was transferred to another tube containing 1 mL of 1 M NaOH to develop the maximum intensity of the color. The solutions were calorimetrically assayed with a Beckman DU 65 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA) at 560 nm. Gastric emptying (%) was calculated according to the following formula:

\[ GE = 100 \times (1 - \frac{\text{amount of phenol red after 20 min}}{\text{amount of phenol red after 0 min}}) \]

In the GC studies, 20 min after the administration of a meal the entire small intestine with its content was isolated and divided into 10 segments of equal length. The intestinal contents of each bowel segment were vigorously mixed with 2 mL of distilled water. After 20 min period of sedimentation, 1 mL of supernatant was transferred to another tube containing 1 mL of 1 M NaOH to develop the maximum intensity of the color. The solutions were calorimetrically assayed with a Beckman DU 65 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA) at 560 nm. GC of small intestinal transit was calculated according to the following formula: \[ GC = \sum[\%A \text{ per segment} \times \text{segment number}] \]. GC ranged from 1 (minimal motility) to 10 (maximal motility). In all GE and GC experiments, animals were gavaged 15 min after i.p. administration of AM8607, or vehicle.
**Castor oil-induced diarrhea:** After an overnight fasting with free access to water, mice were treated with a diarrhea-inducing agent castor oil (0.2 mL/mouse), which was gavaged into the stomach using a 20-gauge animal feeding tube. Immediately after the gavage, each animal was placed in a cage with the floor lined with blotting paper and time to diarrhea was measured. In all experiments the animals were treated with castor oil 15 min after i.p. administration of AM8607 or vehicle.

**Statistics:** Statistical analyses were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The data are expressed as means ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. For the analysis of the effects of antagonists a two-way ANOVA was performed, followed by Bonferroni post hoc test. P values < 0.05 were considered statistically significant.

**Drugs:** All drugs and reagents, unless otherwise stated, were purchased from Sigma-Aldrich (Poznan, Poland). N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM 251) and 6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl[4-methoxyphenyl)methanone (AM630) were purchased from Tocris Bioscience (Warsaw, Poland). Drugs were dissolved in vehicle containing 5% dimethyl sulfoxide in saline. The vehicle in the used concentration had no effect on parameters observed.

2. **Methods used for in vivo studies (Dr. Lichtman Laboratory):**

**For tetrad test:**

Subjects consisted of 12 C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME) divided into two groups of mice, with sample sizes of 6. Group 1 received repeated doses of vehicle, which consisted of ethanol, emulphor, and saline in a ratio of 1:1:18. Group 2 received
cumulative doses of AM8607. Food and water were removed immediately before the first injection.

**Drug:** AM8607 was dissolved in a solution of ethanol:emulphor:saline (1:1:18). A cumulative dosing procedure was used to assess the dose-response relationship in which mice received i.p. injections of 0.1, 0.2, 0.7, and 0.2 mg/kg AM8607 that resulted in cumulative doses of 0.1, 0.3, 1, and 3 mg/kg. Injections were given every 40 min. The control group received a total of four i.p. injections of vehicle, with 40 min between each injection. Both groups were tested 30 min after each injection. The injection volume was 1 ml / 100 g body weight.

**Dependent Measures (Taken in this order)**

1. Catalepsy: Bar test (bar 0.7 cm in diameter that is 4.5 cm from the bench top)
   a. Two timers were used.
   b. The mouse's forepaws are placed on the bar
   c. Timer #1 and Timer #2 were turned on when the mouse first exhibited catalepsy on the bar. Timer #2 was then started/stopped to record time when the mouse was not moving, except for slight movements to breath or whisker/body twitches.
   d. If the mouse moved off the bar, it was placed back on in the original position. The assay ends when either Timer #1 reaches 60 s or when the mouse moves off the bar on attempt #4.
   e. The catalepsy time was recorded as the amount of time on Timer #2.

2. Tail withdrawal test: 52° C water bath
   a. Bag is made
   b. Mouse is gently placed head first into a bag consisting of stapled surgery sheets to form a small pocket.
c. The tip of the mouse’s tail is dipped into the warm water such that the first 1 cm is submerged

d. Time for the mouse to flick its tail or exhibit a gross movement of the was recorded as latency to tail-flick

3. Rectal temperature: thermocouple probe dipped in mineral oil was inserted 2 cm into the rectum.

**Procedure***

Obtained baseline values of catalepsy, tail withdrawal latency and rectal temperature

0 min: i.p. injection of vehicle (Group 1) or AM8607 (0.1 mg/kg) (Group 2)

30 min: assessed catalepsy, tail withdrawal latency and rectal temperature

40 min: i.p. injection of vehicle (Group 1) or AM8607 (0.3 mg/kg) (Group 2)

70 min: assessed catalepsy, tail withdrawal latency and rectal temperature

80 min: i.p. injection of vehicle (Group 1) or AM8607 (1 mg/kg) (Group 2)

110 min: assessed catalepsy, tail withdrawal latency and rectal temperature

120 min: i.p. injection of vehicle (Group 1) or AM8607 (3 mg/kg) (Group 2)

150 min: assessed catalepsy, tail withdrawal latency and rectal temperature

* The order of behavioral tests was bar test, tail immersion test, and rectal temperature (from least invasive to most invasive)

**Data Analysis**

Data were analyzed using two-way ANOVA followed by Tukey post hoc. Differences were considered significant at p < 0.05. The ED50 values with 95% confidence intervals (CI) were calculated using standard linear regression analysis of the cumulative dose–response curve.

**Method used for studying tolerance (Dr. Lichtman’s Laboratory):**
Subjects: Subjects consisted of 12 C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME) divided into two groups of mice, with each group consisting of a sample size of 6. Group 1 received repeated doses of vehicle, which consisted of ethanol, emulphor, and saline in a ratio of 1:1:18.(twice daily) Group 2 received 3 mg/kg dose of AM8607(twice daily) for five days and only the morning injection on the sixth day. This dose of AM8607 was selected because it produced maximal pharmacological effects in Experiment 1. On the seventh day, 24 h after the last injection, animals received cumulative dose AM8607.

Drug: AM8607 was dissolved in a solution of ethanol:emulphor:saline (1:1:18) in concentrations of 0.3mg/ml mg/ml (3 mg/kg). Route of administration = i.p. Volume = 1mL/100 g body weight.

For the cumulative studies AM8607 is dissolved in a solution of ethanol:emulphor:saline (1:1:18) in concentrations of 0.03 mg/ml (i.e.0.3 mg/kg), 0.07 mg/ml, (i.e. 1 mg/kg), 0.2 mg/ml (i.e. 3 mg/kg), 0.7mg/ml (10mg/kg) and and will result in cumulative doses of 0.3,1,3 10 mg/kg. Route of administration = i.p. Volume = 1ml / 100 g body weight.

Dependent Measures (Taken in this order)
1. Catalepsy: Bar test (bar 0.7 cm in diameter that is 4.5 cm from the bench top)
   a. Two timers were used. b. The mouse's forepaws are placed on the bar c. Timer #1 and Timer #2 were turned on when the mouse first exhibited catalepsy on the bar. Timer #2 was then started/stopped to record time when the mouse was not moving, except for slight movements to breath or whisker/body twitches. d. If the mouse moved off the bar, it was placed back on in the original position. The assay ends when either Timer #1 reaches 60 s or when the mouse moves off the bar on attempt #4. e. The catalepsy time was recorded as the amount of time on Timer #2.
2. Tail withdrawal test: 52° C water bath
   a. Bag is made b. Mouse is gently placed head first into a bag consisting of stapled surgery sheets
to form a small pocket. c. The tip of the mouse’s tail is dipped into the warm water such that the
first 1 cm is submerged d. Time for the mouse to flick its tail or exhibit a gross movement of the
was recorded as latency to tail-flick
3. Rectal temperature: thermocouple probe dipped in mineral oil was inserted 2 cm into the
rectum.

**Procedure**

Day1-Day5 Group 1: Veh twice daily.
Day1-Day5 Group 2: AM8607 (3mg/kg) twice daily.
On Day 6: Group 1 Veh once in the morning
On Day 6: Group 2 AM8607(3mg/kg) in the morning
On Day 7:
Obtained baseline values of catalepsy, tail withdrawal latency and rectal temperature
0 min: i.p. injection of 0.3 mg/kg (Group 1+2)
30 min: assessed catalepsy, tail withdrawal latency and rectal temperature
40 min: i.p. injection of 1 mg/kg (Group 1+2)
70 min: assessed catalepsy, tail withdrawal latency and rectal temperature
80 min: i.p. injection of 3 mg/kg (Group1+ 2)
110 min: assessed catalepsy, tail withdrawal latency and rectal temperature
120 min: i.p. injection of 10 mg/kg (Group2) (this dose was only given to the group that had
received repeated AM8607)
150 min: assessed catalepsy, tail withdrawal latency and rectal temperature
* The order of behavioral tests was bar test, tail immersion test, and rectal temperature (from least invasive to most invasive)

**Data Analysis**

Data were analyzed using two-way ANOVA followed by Bonferroni post hoc. Differences were considered significant at $p < 0.05$. The ED50 values with 95% confidence intervals (CI) were calculated using standard linear regression analysis of the cumulative dose–response curve.

**Method used for edema and allodynia tests:**

**Subjects:** Subjects consisted of 35 C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME), divided into five groups, with each group consisting of a sample size of 7.

Group 1 received vehicle, which consists of ethanol, emulphor, and saline in a ratio of 1:1:18.

Group 2 received 0.8mg/kg AM8607; Group 3 received 0.4mg/kg AM8607; Group 4 received 0.2mg/kg AM8607; Group 5 received 0.1mg/kg AM8607.

**Method used for pain model:**

**Subjects:** Subjects consisted of 12 C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME).

2 groups of mice, with each group consisting of a sample size of 6

Group 1 received vehicle, which is 0.9% saline; Group 2 received 5mg/kg Diclofenac

**Drug:** Diclofenac was dissolved in saline

**Pretreatment Time:** 30 mins. Carrageenan injected after 30 mins

Allodynia measured 5 hours after carrageenan injection

Edema measured prior to and 5 hours after carrageenan injection

**Data Analysis**

Data were analyzed using unpaired t-test. Differences were considered significant at $p < 0.05$. 
Chapter 4

Novel Short Synthetic Route to Synthesize Southern Modified Cannabinoids

4.1 Introduction:
The recent work done in Tius/Makriyannis group revealed the importance of southern aliphatic hydroxyl (SAH) group and modifications in the southern pharmacophore in functional and \textit{in vivo} studies. SAR exploration on southern pocket resulted in AM8607 (Fig. 1) with functionally biased ligand activity in \textit{in vitro} and \textit{in vivo} studies. The SAH ligand AM8607 displayed favorable pharmacological profile with high \textit{in vivo} potency. This ligand was synthesized with a tedious multiple step synthesis (22 steps) with many challenges and sub-optimal steps. With the established synthetic route, it would be difficult to access this compound in larger quantities for further \textit{in vivo} studies. To further understand the therapeutic potential and to explore more SAR in the southern pocket, a more convenient and scalable short synthetic route was required. Earlier efforts in the Tius lab to synthesize southern functionalized hybrid compounds through different approaches utilized the intramolecular Diels-Alder reaction for the cyclization in one of his approach.\textsuperscript{84,85,149} Also, the intramolecular Diels-Alder reaction on the \textit{o}-quinone methide to synthesize classical hexahydro-tricyclic cannabinoids with \textit{trans}-stereochemistry has been well established in the literature.\textsuperscript{149,155,161,162}

4.2 Rational Design:
Intramolecular Diels-Alder cyclization to get SAH hybrid cannabinoids with northern and southern functionalized pharmacophores simultaneously had not been previously reported. Using Citronellal (Fig. 4.1, first approach) as aliphatic half, tricyclic cannabinoid with 9-methyl substitution and \textit{trans} stereochemistry was synthesized.\textsuperscript{161} Later on similar approach with southern functionalization as propargyl alcohol was used but this route yielded only southern
functionalized analog before cyclization.\textsuperscript{149} Utilizing this literature knowledge, a retrosynthetic route was designed (Fig. 4.1) where both northern and southern hydroxyl groups are installed before key cyclization. The functionalized intermediate would be cyclized to give AM8607 and more SAH analogs easily in higher yields and less number of steps. Different aliphatic and aromatic halves were designed and coupled to get the advanced bicyclic intermediate for Diels-Alder cyclization.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure41.png}
\caption{Rational design for SAH analogs}
\end{figure}

\subsection*{4.3 Chemistry}

Retrosynthetic analysis of 12 resulted in two segments. Aliphatic segment 9 and aromatic segment 4. To start with the aromatic segment synthesis, ytterbium(III) triflate-catalyzed acylation of 1 resulted in the phenone 2 in 70\% yield.\textsuperscript{149} Demethylation of the aromatic methyl ethers was accomplished by treating 2 with boron tribromide to give 3 in 78\% yield. Phenolic
hydroxyl groups of 3 were protected as MOM ethers by treating with MOMBr/NaH to yield 4 in 80%. Deprotection and protection with labile group at this stage was essential to avoid complications in the later stages of synthesis.

**Scheme 4.1: 1st Attempt towards synthesis**

![Scheme 4.1: 1st Attempt towards synthesis](image)

**Reagents and Conditions:**
(a) Yb(Tf)₃, (CH₃CO)₂O, rt, 12 h, 70%; (b) BBr₃, DCM, 0 °C, 12 h, 78%; (c) MOMBr, NaH, DMF, 0 °C, 12 h, 80%; (d) SeO₂, tert-BuOOH, DCM, 0 °C, 4 h, 55%; (e) NaH, BnBr, DMF, 0 °C, 3 h, 60%; (f) TMSCl, LDA, THF, mCPBA, DCM, TBAF, THF, 58%; (g) NaH, BnBr, DMF, 0 °C, 3h, 5%; (h) LDA, THF, -30 °C, 1 h

Preparation of the aliphatic segment 9 started from the commercially available starting material 5. Allylic hydroxylation of 5 with selenium dioxide and tert-butyl hydroperoxide resulted in 6 with 55% yield. Hydroxyl group needed to be protected with a group stable to both acidic and basic conditions; it was protected as a benzyl ether using sodium hydride and benzyl bromide to give 7 in 60% yield. α-Hydroxylation of ketone 7 was accomplished by trapping kinetic enolate of 7 with LDA and TMSCl to give silyl-enol ether. Oxidation of the unstable silyl
ether with mCPBA and deprotection of the silyl ether with TBAF resulted in 8 in 58% over all yield. All attempts for the protection of α-hydroxyl of 8 as p-methoxy benzyl ether failed.\textsuperscript{166,167} Compound 9 as benzyl ether was isolated only once in only 5% yield.\textsuperscript{164} The mentioned compound 9 was taken forward but unfortunately, coupling of two segments 9 and 4 under various conditions did not yield the desired product.\textsuperscript{168} This prompted revisions to the retrosynthetic route.

4.3.1 Second generation synthetic route

The retrosynthetic route was revised with modifications in the aliphatic segment (Scheme 4.2).

**Scheme 4.2**: Redesigned retrosynthetic route to get desired aliphatic segment for coupling with aromatic segment 4

To synthesize the above proposed intermediate and final compound (Scheme 4.2) the chemistry utilized the same starting material 5 and related steps till α-hydroxyl ketone intermediate 8 as shown in Scheme 4.1. The ketone 8 was reduced with sodium borohydride to yield 11 in 78% yield (Scheme 4.3). Selective protection of less substituted terminal hydroxyl of the diol as p-
methoxy benzyl (PMB) ether was achieved by refluxing 11 with dibutyltin oxide in toluene and treating the tin intermediate with PMBCl and tetrabutylammonium iodide to give 12 in 42% yield.169 Hydroxy group was exchanged with bromo by treating 12 with dibromotriphenylphosphine to give 13 in 71% yield.170 Attempted coupling of 13 with the earlier synthesized aromatic segment 4 using the lithium halogen exchange aldol reaction and Grignard conditions did not yield the desired product.171

Scheme 4.3: Revised synthetic route

**Reagents and conditions:** (a) NaBH₄, MeOH, 0 °C, 1 h, 78%; (b) i.) DBTO, toluene, 120 °C, 12 h; ii.) PMBCl, TBAI, 90 °C, 1 h, 42%; (c) imidazole, Ph₃PBr₂, DCM, 0 °C, 24 h, 71%; (d) N-ethylidene cyclohexanamine, HMPA, THF, 0 °C, 12 h; (e) LDA, THF, -30 °C, 1 h, no product

Alternative to this route was to couple the aldehyde aliphatic fragment with the aromatic segment 14 directly. Conversion of bromo 13 to aldehyde 14 using freshly synthesized N-ethylidene cyclohexanamine failed and starting material was recovered.172 Failure of the synthetic led to the further revisions to the design of aliphatic and aromatic segments as discussed below.
4.3.2 Third generation synthetic route

Understanding of the failure of so far proposed synthetic routes can be concluded as: (i) Aldol-type coupling between two ketones is not amenable in this chemistry. (ii) Bromo intermediate (13) was not suitable for performing any further chemistry. Retrosynthetic route was analyzed and redesigned (Scheme 4.4) carefully by utilizing the literature precedence and findings from the earlier performed synthesis (Scheme 4.1 & Scheme 4.3). Aromatic segment was kept same as was reported in literature\textsuperscript{161} and the aliphatic segment was designed accordingly to synthesize the coupled product.

Scheme 4.4: Retrosynthetic route with redesigned aromatic and aliphatic segments

To start with the aromatic segment first, earlier synthesized compound 1 (Scheme 4.1) was treated with boron tribromide to demethylate the aromatic methyl ether to give 16 in 90 % yield (Scheme 4.5). The phenolic hydroxyl groups of 16 were protected as MOM ether by treating with sodium hydride and MOM bromide to produce aromatic segment 17 in 85% yield. The synthesis of aliphatic segment was started with the same starting material 5 as earlier (Scheme 4.1) and the same synthetic steps were used to synthesize intermediate 8. The hydroxy group of 8
was protected as TBDPS ether to give 18 in 92% yield.\textsuperscript{173} Wittig reaction was performed on 18 using sodium hydride and triethyl phosphonoacetate to produce ester 19 in 80% yield.\textsuperscript{174}

**Scheme 4.5:** Successful working synthetic route for synthesis of SAH analogs

Reagents and conditions: (a) BBr\textsubscript{3}, DCM, 0 °C, 12 h, 90%; (b) MOMBr, NaH, 0 °C, 12 h, 85%; (c) TBDPSCI, DMAP, Imidazole, DMF, 4 h, 92%; (d) Triethyl phosphonoacetate, NaH, THF, 0 °C, 4 h, 80%; (e) rac-BINAP, CuCl, NaOAc, PMHS, toluene, rt, 24 h, 75%; (f) DIBAL-H, toluene, -78 °C, 1 h, 70%; (g) n-BuLi, TMEDA, 0 °C, 4 h, 60%; (h) pTSA, Methanol, 75 °C, 6 h, 60%; (i) Pd/C, H\textsubscript{2}, rt, 12 h, 90%

Conjugate reduction of ester 19 was achieved by using Lipshutz chemistry to give 20 in 75% yield.\textsuperscript{175} DIBAL-H reduction of ester 20 to aldehyde 21 was obtained in 70% yield. Coupling of aldehyde 21 with \textit{o}-lithiated aromatic segment 17 using \textit{n}-BuLi yielded 22 in 60%.
Intramolecular Diels-Alder cycloaddition of in situ generated o-quinone methide 23a to the alkene, initiated by acidic dehydration resulted in tricyclic structure 23 in 60% yield.\textsuperscript{176} Deprotection of the benzyl ether of 23 was obtained through hydrogenolysis to give final compound 24 in 90% yield.\textsuperscript{177} The structure was confirmed by comparing the \textsuperscript{1}H NMR of 24 with the earlier synthesized chiral compound AM8607 from another route and it showed an exact match. The stereochemistry of 24 was confirmed by analyzing it over the chiral HPLC in comparison with the earlier synthesized AM8607. On chiral HPLC column (4.6 mm x 100 mm OD-H, 5-55% MeOH in CO\textsubscript{2}, 0.1 mL/min, UV detection at 220 nm) two enantiomers of 24 got separated at 2.38 min and 2.62 min. Retention time of one of the enantiomer (2.38 min) matched with the AM8607 which also appeared at 2.38 min. (4.6 mm x 100 mm OD-H, 5-55% MeOH in CO\textsubscript{2}, 0.1 mL/min, UV detection at 220 nm).

\textbf{4.4 Conclusion:} To summarize, a short and reproducible route (synthesis was done 3 times) with cheap, commercially available starting material was successfully developed. Intramolecular hetero Diels-Alder cyclization led to \textit{trans}-stereochemistry in the tricyclic ring resulting in a mixture of enantiomers which were separated by chiral HPLC to give the enantio-pure compound with desired stereochemistry. Utilizing this short-steps and amenable synthetic route, further SAR can be explored with various southern modifications. Asymmetric resolution or utilizing asymmetric synthesis earlier in the synthetic route may lead to the desired stereoisomer.
4.5 Experimental:

1-(4-(Adamantan-1-yl)-2,6-dimethoxyphenyl)ethanone (2): To a solution of earlier synthesized 1 (1.8 g, 6.61 mmol) in dry nitromethane (30 mL) was added acetic anhydride (3.12 mL, 33.0 mmol) and ytterbium(III) trifluoromethanesulfonate (0.820 g, 1.322 mmol) and stirred overnight at room temperature. The reaction was diluted with 100 mL diethyl ether and 100 mL water. The organic layer was separated, washed with water (2x50 mL), brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to afford crude product. Purification by flash chromatography on silica gel with 2-15% ethyl acetate : hexanes afforded desired product 2 (1.3 g, 4.13 mmol, 70% yield). ¹H NMR (500 MHz, CDCl₃) δ 6.55 (s, 2H), 3.81 (s, 6H), 2.47 (s, 3H), 2.11 (br s, 3H), 1.90 (d, J = 2.5 Hz, 6H), 1.80 (d, J = 12.5 Hz, 3H), 1.75 (d, J = 12.5 Hz, 3H).

1-(4-(Adamantan-1-yl)-2,6-dihydroxyphenyl)ethanone (3): To a solution of 1-(4-((1s,3s)-adamantan-1-yl)-2,6-dimethoxyphenyl)ethanone (0.7 g, 2.226 mmol) in DCM (30 mL) was added BBr₃ (0.526 mL, 5.57 mmol) at 0 °C and stirred at the same temperature overnight.
Reaction was quenched with 30 mL ice cold water. Reaction was diluted with 100 mL diethyl ether and 100 mL water. The organic layer was separated, washed with 2x50 mL water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to afford crude product. Purification by flash chromatography on silica gel with 2-15% ethyl acetate : hexanes afforded desired product 3 (0.4 g, 1.397 mmol, 78% yield). ¹H NMR (500 MHz, CDCl₃) δ 6.42 (s, 2H), 4.34 (s, 2H), 2.71 (s, 3H), 2.07 (br s, 3H), 1.85 (d, J = 3.0 Hz, 6H), 1.78 (d, J = 12.5 Hz, 3H), 1.73 (d, J = 12.5 Hz, 3H).

1-(4-(Adamantan-1-yl)-2,6-bis(methoxymethoxy)phenyl)ethanone (4): To washed sodium hydride (132 mg, 3.30 mmol) in DMF (10 mL) at 0 °C was added 3 (430 mg, 1.502 mmol) dissolved in toluene (10 mL) and DMF (3 mL). The reaction mixture was warmed to room temperature for 20 min. and then cooled to 0 °C again to add bromo(methoxy)methane (0.307 mL, 3.75 mmol) dropwise. The resultant reaction solution was stirred for 24 h at room temperature. The reaction was quenched by pouring into 50 mL 10% NaOH solution and extracted with diethyl ether. The organic layer was washed with saturated NaHCO₃, water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to afford crude product. Purification by flash chromatography on silica gel with 0-15% ethyl acetate : hexanes afforded desired product 4 (0.4 g, 1.397 mmol, 80% yield). ¹H NMR (500 MHz, CDCl₃) δ 6.81 (s, 2H), 5.16 (s, 4H), 3.47 (s, 6H), 2.51 (s, 3H), 2.09 (br s, 3H), 1.87 (d, J = 3.0 Hz, 6H), 1.78 (d, J = 12.5 Hz, 3H), 1.74 (d, J = 12.5 Hz, 3H).
(E)-7-Hydroxy-6-methylhept-5-en-2-one (6): To a suspension of selenium dioxide (3.96 g, 35.7 mmol) in anhydrous DCM (40 mL) was added tert-butyl hydroperoxide (17.83 mL, 107 mmol) at 0 °C and stirred at the same temperature for 1 h. To the resultant solution was then added 5 (9 g, 71.3 mmol) dissolved in DCM (40 mL). The reaction mixture was stirred at 0 °C for another 4 h. The reaction solution was diluted with 200 mL ethyl acetate. The organic layer was separated, washed with water, sodium bicarbonate, brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified on silica gel chromatography using 10-55% ethyl acetate : hexanes to give 6 (5.3 g, 37 mmol, 55% yield) as yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 5.31 (dt, J = 7.0 Hz, J = 1.5 Hz, 1H), 3.93 (s, 2H), 2.46 (t, J = 7.5 Hz, 2H), 2.26 (q, J = 7.0 Hz, 2H), 2.15 (s, 1H), 2.10 (s, 3H), 1.62 (s, 3H).

(E)-7-(Benzyloxy)-6-methylhept-5-en-2-one (7): To suspension of sodium hydride (1.949 g, 48.7 mmol) in dry THF (50 mL) at 0 °C was added 6 (6.6 g, 46.4 mmol) and stirred for 3 min. To this was added benzyl bromide (6.62 mL, 55.7 mmol) dissolved in 15 mL THF and the resultant solution was warmed to room temperature for 4 h. The reaction mixture was diluted with 200 mL ethyl acetate. The reaction mixture was quenched with 50 mL water and the organic layer was extracted with 200 mL ether, washed with brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified on silica gel chromatography with 2-
25% ethyl acetate : hexanes to give 7 (5.2 g, 22.38 mmol, 48% yield). $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.33-7.28 (m, 4H, especially 7.30, d, $J$ = 2Hz, 2H), 7.26-7.21 (m, 1H), 5.37 (dt, $J$ = 7.0 Hz, $J$ = 1.5 Hz, 1H), 4.41 (s, 2H), 3.86 (s, 2H), 2.45 (t, $J$ = 7.0 Hz, 2H), 2.30 (q, $J$ = 7.5 Hz, 2H), 2.08 (s, 3H), 1.67 (s, 3H).

(E)-(7-(benzylxy)-6-methylhepta-1,5-dien-2-yloxy)trimethylsilane (8): To a solution of lithium diisopropylamide (26.9 mL, 53.8 mmol) in THF (30 mL) at -78 °C was added chlorotrimethylsilane (10.89 mL, 86 mmol) slowly over a period of 5 min. Resulting solution was stirred for 10 min. and 7 (5g, 21.52 mmol) dissolved in 10 mL THF was added drop wise. The reaction mixture was stirred at the same temperature for 3h. The reaction was quenched by adding 50 mL water and diluted with 100 mL diethyl ether. The organic layer was separated, washed with 2x50 mL water, brine and dried over MgSO$_4$. Solvents were evaporated under reduced pressure to afford the crude product which was taken to the next step without further purification. To a solution of crude (6.5 g, 21.35 mmol) in dry DCM (100 mL) at 0 °C was added 3-chloroperoxybenzoic acid (4.91 g, 21.35 mmol) slowly. After 30 min. the reaction mixture was allowed to warm to room temperature and stirred for another 2 h. The reaction mixture was quenched with saturated sodium thiosulfate solution and diluted with 100 mL diethyl ether and 100 mL water. The organic layer was separated, washed with 2x50 mL water, brine and dried over MgSO$_4$. Solvents were evaporated under reduced pressure to afford the crude product which was purified by silica gel chromatography with 5-45 % ethyl acetate : hexanes to give 8 (3.9 g, 12.8 mmol) in 58% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.36-7.30 (m, 4H), 7.28-7.22 (m, 1H),
5.36 (dt, $J = 6.5$ Hz, $J = 1.0$ Hz, 1H), 4.42 (s, 2H), 4.15 (s, 2H), 3.87 (s, 2H), 3.54 (br s, 1H, OH), 2.44-2.36 (m, 2H), 2.35 (t, $J = 6.5$ Hz, 2H), 1.68 (s, 3H).

(E)-1,7-Bis(benzyloxy)-6-methylhept-5-en-2-one (9): To a suspension of sodium hydride (19.33 mg, 0.483 mmol) in dry THF (50 mL) at 0 °C was added 8 (100 mg, 0.403 mmol) and stirred for 5 min. benzyl bromide (0.057 mL, 0.483 mmol) dissolved in 10 mL THF was added and the resultant solution was stirred for 4 h at room temperature. The reaction mixture was diluted with 200 mL ethyl acetate and quenched with 50 mL water and organic layer was extracted, washed with brine and dried over MgSO$_4$. Solvents were evaporated to give crude which was purified on silica gel chromatography using 2-25% ethyl acetate : hexanes to give 9 (7 mg, 0.02 mmol) in 5% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.39-7.350 (m, 2H), 7.34 (d, $J = 4.5$ Hz, 6H), 7.32-7.26 (m, 2H), 5.43 (t, $J = 7.0$ Hz, 1H), 4.56 (s, 2H), 4.45 (s, 2H), 3.90 (s, 2H), 3.87-3.79 (m, 2H), 3.51 (dd, $J = 9.0$ Hz, $J = 2.5$ Hz, 1H), 3.34 (dd, $J = 9.0$ Hz, $J = 2.5$ Hz, 1H), 2.36 (br s, 1H, OH), 2.26-2.11 (m, 2H), 1.68 (s, 3H), 160-1.52 (m, 1H), 1.52-1.44 (m, 1H).

(E)-7-(Benzyloxy)-6-methylhept-5-ene-1,2-diol (11): To a solution of 8 (760 mg, 3.06 mmol) in MeOH (15 mL) at 0 °C was added sodium borohydride (232 mg, 6.12 mmol) and stirred for 1 h. The reaction was diluted with 100 mL diethyl ether and 100 mL water. Organic layer was
Separated, washed with water (2x50 mL), brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to afford crude product. Purification by flash chromatography on silica gel using 30-95% ethyl acetate : hexanes afforded desired product 11 (600 mg, 2.397 mmol, 78% yield). ¹H NMR (500 MHz, CDCl₃) δ: 7.33-7.29 (m, 4H), 7.28-7.22 (m, 1H), 5.41 (t, J = 7.0 Hz, 1H), 4.43 (s, 2H), 3.90 (br s, 1H), 3.87 (s, 2H), 3.68-3.59 (m, 1H), 3.53 (dd, J = 11.0 Hz, J = 2.5 Hz, 1H), 3.36 (dd, J = 11.0 Hz, J = 2.5 Hz, 1H), 2.22-2.06 (m, 2H), 1.67 (s, 3H), 1.50-1.38 (m, 2H).

(E)-7-(Benzyloxy)-1-((4-methoxybenzyl)oxy)-6-methylhept-5-en-2-ol (12): Compound 11 (400 mg, 1.598 mmol) and dibutyltin oxide (477 mg, 1.917 mmol) were dissolved in toluene (15 mL) and refluxed at 120 °C using Dean Starks apparatus. The reaction mixture was stirred for 12 h and then brought to room temperature. To this was added 4-methoxybenzyl chloride (0.305 mL, 2.237 mmol) and tetrabutylammonium iodide (885 mg, 2.397 mmol) and again refluxed for 1.5 h. The reaction mixture was poured on to water and extracted with 50 mL ethyl acetate. Organic layer was washed with water, brine and dried over MgSO₄. Solvents were evaporated to give crude which was chromatographed using 5-35% ethyl acetate : hexanes to afford 12 (250 mg, 0.675 mmol, 42% yield) as colorless oil. Rf = 0.52 in 50% ethyl acetate : hexanes. ¹H NMR (500 MHz, CDCl₃) δ: 7.36-7.31 (m, 4H), 7.29-7.22 (m, 3H), 6.89-6.85 (m, 2H), 5.42 (dt, J = 7.0 Hz, J = 1.0 Hz, 1H), 4.47 (s, 2H), 4.44 (s, 2H), 3.88 (s, 2H), 3.82-3.76 (m, 4H, especially s, 3H, 3.79), 3.46 (dd, J = 10.0 Hz, J = 3.5 Hz, 1H), 3.30 (dd, J = 9.0 Hz, J = 7.5 Hz, 1H), 2.24-2.10 (m, 2H), 1.67 (s, 3H), 1.58-1.40 (m, 2H).
(E)-1-(((7-(Benzyloxy)-2-bromo-6-methylhept-5-en-1-yl)oxy)methyl)-4-methoxybenzene (13): To a solution of 12 (300 mg, 0.810 mmol) and imidazole (221 mg, 3.24 mmol) in dry DCM (15 mL) at 0 °C was added dibromotriphenylphosphine (1025 mg, 2.429 mmol) and the reaction was warmed to room temperature and stirred for 24 h. The reaction was filtered, quenched with water and the aqueous layer was extracted with DCM. The combined organic layers were washed with water, brine filtered and dried over MgSO₄. Solvents were evaporated to give crude which was purified with silica gel chromatography using 2-15% ethyl acetate : hexanes to give 13 (250 mg, 0.577 mmol, 71% yield). \(^1\)H NMR (500 MHz, CDCl₃) δ: 7.30-7.25 (m, 4H), 7.24-7.18 (m, 3H), 6.84-6.79 (m, 2H), 5.33 (dt, \(J = 6.5\) Hz, \(J = 1.0\) Hz, 1H), 4.44 (s, 2H), 4.39 (s, 2H), 4.05-3.99 (m, 1H), 3.83 (s, 2H), 3.74 (s, 3H), 3.64 (dd, \(J = 11.0\) Hz, \(J = 6.0\) Hz, 1H), 3.57 (dd, \(J = 10.5\) Hz, \(J = 7.0\) Hz, 1H), 2.27-2.10 (m, 2H), 1.99-1.90 (m, 1H), 1.82-1.72 (m, 1H), 1.64 (s, 3H).

1-(3,5-Bis(methoxymethoxy)phenyl)adamantane (17): To a solution of earlier synthesized 16 (3.5 g, 14.32 mmol) in DCM (25 mL) was added N,N-diisopropylethylamine (7.51 mL, 43.0 mmol) at 0 °C. To this was added chloromethyl ethyl ether (4.02 mL, 43.0 mmol) and stirred
overnight and warmed to room temperature. The reaction was quenched with saturated NH₄Cl. The reaction mixture was diluted with 100 mL diethyl ether and 100 mL water. The organic layer was separated, washed with 2x50 mL water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to afford crude product. Purification by flash chromatography on silica gel using 2-30% ethyl acetate : hexanes afforded desired product 17 (3.1 g, 8.62 mmol, 65% yield) as white foam. ¹H NMR (500 MHz, CDCl₃) δ 6.70 (d, J = 2.0 Hz, 2H), 6.63 (t, J = 2.0 Hz, 1H), 5.19 (s, 4H), 3.72 (q, J = 7.0 Hz, 4H), 2.07 (s, 3H), 1.88 (d, J = 3.0 Hz, 6H), 1.77 (d, J = 12.0 Hz, 3H), 1.73 (d, J = 12.0 Hz, 3H), 1.22 (t, J = 7.0 Hz, 6H).

(E)-7-(Benzylxy)-1-((tert-butyldiphenylsilyl)oxy)-6-methylhept-5-en-2-one (18): To a stirred solution of 8 (800 mg, 3.22 mmol), imidazole (483 mg, 7.09 mmol) and DMAP (39.4 mg, 0.322 mmol) in dry DMF (20 mL) and DCM (5 mL) at 0 °C was added tert-butyldiphenylchlorosilane (0.869 mL, 3.38 mmol) and stirred at room temperature for 4 h. The reaction mixture was quenched with saturated NaHCO₃ and diluted with 100 mL diethyl ether. The organic layer was separated and washed with water, brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified by silica gel chromatography using 2-15% ethyl acetate : hexanes system to yield 18 (1.3 g, 2.67 mmol, 83% yield) as gummy material. ¹H NMR (500 MHz, CDCl₃) δ: 7.66-7.61 (m, 4H), 7.44-7.39 (m, 2H), 7.39-7.34 (m, 4H), 7.33-7.30 (m, 4H), 7.29-7.23 (m, 1H), 5.36 (dt, J = 7.0 Hz, J = 1.5 Hz, 1H), 4.42 (s, 2H), 4.17 (s, 2H), 3.86 (s, 2H), 2.58 (t, J = 7.0 Hz, 2H), 2.31 (q, J = 7.0 Hz, 2H), 1.67 (s, 3H), 1.09 (s, 9H).
(6E)-Ethyl-8-(benzyloxy)-3-(((tert-butyldiphenylsilyl)oxy)methyl)-7-methylocta-2,6-dienoate (19): To washed sodium hydride (256 mg, 6.08 mmol) (with hexanes) in dry THF (9 mL) at 0 °C was added triethyl phosphonoacetate (1.22 mL, 6.08 mmol) and the reaction mixture was stirred for 1h at the same temperature. 1mL of the clear solution was transferred to another small flask at 0 °C. To this was added 18 (260 mg, 0.55 mmol) dissolved in THF (9 mL) and warmed to room temperature for 2 h. The solution was quenched with water and the reaction mixture was extracted in 50 mL diethyl ether. The organic layer was separated and washed with water, brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified by silica gel chromatography using 2-15% ethyl acetate : hexanes system to yield 19 (180 mg, 0.36 mmol, 80% yield) as gum. ¹H NMR (500 MHz, CDCl₃) δ: 7.69-7.63 (m, 4H), 7.45-7.35 (m, 6H), 7.35-7.29 (m, 4H), 7.28-7.23 (m, 1H), 6.20 (s, 0.65H), 5.43 (t, J = 6.5 Hz, 0.35H), 5.37 (t, J = 6.5 Hz, 0.65H), 4.90 (s, 0.7H), 4.43 (s, 0.7H), 4.38 (s, 1.3H), 4.22-4.14 (m, 2.7H, especially 4.19, q, J = 7.0 Hz, 2.3H and 4.18, s, 0.3H), 3.99 (q, J = 7.0 Hz, 0.7H), 3.90 (s, 0.7H), 3.82 (s, 1.3H), 2.52 (dd, J = 17.5 Hz, J = 7.5 Hz, 2H), 2.33 (q, J = 7.5 Hz, 0.7H), 2.15 (q, J = 7.5 Hz, 1.3H), 1.67 (s, 1H), 1.61 (s, 2H), 1.29 (t, J = 7.0 Hz, 2H), 1.14 (t, J = 7.0 Hz, 1H), 1.08 (s, 9H).
(E)-Ethyl8-(benzyloxy)-3-(((tert-butylidiphenylsilyl)oxy)methyl)-7-methyloct-6-enoate (20): To degassed BINAP (83 mg, 0.133 mmol) in dry toluene (2 mL) at 25 °C was added copper(I) chloride (6.59 mg, 0.067 mmol) and sodium tert-butoxide (6.40 mg, 0.067 mmol) under argon pressure and resulting suspension was stirred for 15-20 min. To this was added PMHS (1.394 mL, 5.33 mmol) and resultant yellow suspension was stirred for 3-5 min. To this was slowly added 19 (720 mg, 1.332 mmol) dissolved in 3 mL toluene. The reaction was stirred for 16h at room temperature. Reaction was monitored by NMR. Once the reaction is complete, the two neck flask was opened and very slowly, drop by drop, ethanol was added. Frothing can be seen after adding ethanol. The resulting solution was diluted with ether, washed with water, brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified by silica gel chromatography using 0-15 % ethyl acetate : hexanes to give 20 (430 mg, 79.9 mmol, 60% yield) as gummy material with an Rᵢ of 0.52 in 10% ethyl acetate : Hexanes. ¹H NMR (500 MHz, CDCl₃) δ: 7.67-7.62 (m, 4H), 7.44-7.34 (m, 6H), 7.34-7.31 (m, 4H), 7.29-7.24 (m, 1H), 5.37 (dt, J = 7.0 Hz, J = 1.0 Hz, 1H), 4.43 (s, 2H), 4.09 (dq, J = 7.5 Hz, J = 1.5 Hz, 2H), 3.87 (s, 2H), 3.63 (dd, J = 10.5 Hz, J = 4.5 Hz, 1H), 3.56 (dd, J = 9.5 Hz, J = 5.0 Hz, 1H), 2.54 (dd, J = 15.5 Hz, J = 7.0 Hz, 1H), 2.30 (dd, J = 15.5 Hz, J = 7.0 Hz, 1H), 2.09 (septet, J = 6.0 Hz, 1H), 2.02-1.86 (m, 2H), 1.62 (s, 3H), 1.58-1.49 (m, 1H), 1.44-1.32 (m, 1H), 1.22 (t, J = 7.0 Hz, 3H), 1.05 (s, 9H).
(E)-8-(Benzyloxy)-3-(((tert-butyldiphenylsilyl)oxy)methyl)-7-methyloct-6-enal (21): To a solution of 20 (210 mg, 0.387 mmol) in dry toluene (3 mL) at -78 °C was added DIBAL-H (0.464 mL, 0.464 mmol) slowly and the reaction mixture was stirred for 45 min. To this was added 3 mL methanol and stirred at -78 °C for another 15 minutes. Then Rochelle's salt dissolved in water was added and then reaction was warmed slowly. To this was added 10 mL ether and stirred for another 10 min. Solids were separated in the bottom and the reaction mixture was passed through filter. Filtrate was washed with 1N HCl, water, brine and dried over Na$_2$SO$_4$. Solvents were evaporated to give crude which was purified by silica gel chromatography in 0-15 % ethyl acetate : hexanes system to give 21 (110 mg, 0.195 mmol, 60% yield). $R_f = 0.3$ in 10% ethyl acetate : hexanes. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 9.75 (t, $J = 2.5$ Hz, 1H), 7.67-7.62 (m, 4H), 7.43-7.34 (m, 6H), 7.34-7.28 (m, 4H), 7.26-7.21 (m, 1H), 5.35 (t, $J = 6.5$ Hz, 1H), 4.42 (s, 2H), 3.86 (s, 2H), 3.65 (dd, $J = 10.0$ Hz, $J = 4.5$ Hz, 1H), 3.52 (dd, $J = 10.0$ Hz, $J = 6.5$ Hz, 1H), 2.55 (ddd, $J = 16.5$ Hz, $J = 7.5$ Hz, $J = 2.0$ Hz, 1H), 2.38 (ddd, $J = 16.5$ Hz, $J = 6.0$ Hz, $J = 2.0$ Hz, 1H), 2.20 (septet, $J = 6.0$ Hz, 1H), 1.99 (q, $J = 7.5$ Hz, 2H), 1.61 (s, 3H), 1.47 (quintet, $J = 7.0$ Hz, 1H), 1.35 (quintet, $J = 6.5$ Hz, 1H), 1.05 (s, 9H).
(E)-1-(4-(Adamantan-1-yl)-2,6-bis(methoxymethoxy)phenyl)-8-(benzyl oxy)-3-((tert-butyldiphenylsilyl)oxy)methyl)-7-methyloct-6-en-1-ol (22): To a solution of 17 (50 mg, 0.150 mmol) in dry THF (3 mL) at 0 °C was added n-butyllithium (0.07 mL, 0.180 mmol) and the reaction mixture was warmed to room temperature. After 2 h stirring, 21 (50 mg, 0.097 mmol) dissolved in dry THF (3 mL) was added at 0 °C and the reaction was stirred for another 3 h at room temperature. The reaction mixture was quenched with water. Aqueous layer was extracted with ether. The organic layers were combined, washed with water, brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified by flash chromatography using 5-25% ethyl acetate : hexanes to give 22 (90 mg, 0.106 mmol, 71% yield) coupled product. $^1$H NMR (500 MHz, CDCl₃) δ: 7.72-7.60 (m, 4H), 7.44-7.30 (m, 10H), 7.30-7.24 (m, 1H), 6.79 (d, J = 3.5 Hz, 2H), 5.39 (t, J = 7.0 Hz, 0.4H), 5.36 (t, J = 7.0 Hz, 0.6H), 5.30-5.18 (m, 1H), 5.18-5.12 (m, 3H), 5.12-5.06 (m, 1H), 4.43 (s, 0.8H), 4.42 (s, 1.2H), 3.88 (s, 0.8H), 3.86 (s, 1.2H), 3.71 (dd, J = 9.5 Hz, J = 5.5 Hz, 0.4H), 3.66 (d, J = 4.0 Hz, 1H), 3.61 (dd, J = 9.5 Hz, J = 5.5 Hz, 0.6H), 3.55 (t, J = 11.0 Hz, 1H), 3.42 (s, 3H), 3.40 (s, 3H), 2.24-2.14 (m, 0.5H), 2.07 (br s, 3H), 2.04-1.92 (m, 2.6H), 1.86 (br s, 7H), 1.84-1.80 (m, 1H), 1.80-1.70 (m, 7H), 1.62 (s, 1.4H), 1.60 (s, 1.6H), 1.54-1.44 (m, 1H), 1.06 (s, 5H), 1.04 (s, 4H).
3-(Adamantan-1-yl)-6-((benzyloxy)methyl)-9-(hydroxymethyl)-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol (23): To a solution of 22 (40 mg, 0.047 mmol) in dry methanol (5 mL) was added dry p-toluenesulfonic acid (7.18 mg, 0.038 mmol) and refluxed for 6 h. Solvents were evaporated and the crude was dissolved in 20 mL diethyl ether and washed with saturated NaHCO₃, water, brine and dried over MgSO₄. Solvents were evaporated to give crude. Purification by silica gel column chromatography using 2-15% ethyl acetate : hexane afforded the desired product 23 (15 mg, 0.028 mmol, 60% yield) as white foam. ¹H NMR (500 MHz, CDCl₃) δ: 7.39-7.32 (m, 4H), 7.31-7.28 (m, 1H), 6.46 (d, J = 2.0 Hz, 1H), 6.24 (d, J = 2.0 Hz, 1H), 4.77 (d, J = 12.0 Hz, 1H), 4.57 (d, J = 12.5 Hz, 1H), 3.56 (q, J = 10.0 Hz, 2H), 3.52 (d, J = 3.0 Hz, 1H), 3.51-3.47 (m, 1H), 3.21 (d, J = 12.5 Hz, 1H), 2.53 (dt, J = 11.0 Hz, J = 3.0 Hz, 1H), 2.05 (br s, 3H), 1.92 (dt, J = 11.0 Hz, J = 2.0 Hz, 2H), 1.83 (d, J = 2.5 Hz, 6H), 1.76 (d, J = 12.5 Hz, 4H), 1.70 (d, J = 12.5 Hz, 4H), 1.13 (dt, J = 12.5 Hz, J = 3.0 Hz, 1H), 1.07 (dt, J = 12.5 Hz, J = 3.0 Hz, 1H), 1.01 (s, 3H), 0.86 (q, J = 12.0 Hz, 1H).
(3-((1-Adamantan-1-yl)-1-hydroxy-6-methyl-6a,7,8,9,10,10a-hexahydro-6H-benzoc[\text{c}]\text{chromene-6,9-diyl})dimethanol (24): To a solution of 23 (3 mg, 5.97 µmol) in ethanol (5 mL) at 25 °C was added palladium on carbon (6.35 mg, 0.060 mmol). The reaction mixture was degassed and left under hydrogen pressure for 12 h. Reaction was filtered through celite pad, concentrated and loaded on the silica gel column. Flash chromatographic purification with 70-95% ethyl acetate : hexanes with 1% methanol afforded desired product 24 (1.970 mg, 4.77 µmol, 80% yield) as a white foam. $^1$H NMR (500 MHz, CDCl$_3$) δ 6.40 (d, $J = 2.0$ Hz, 1H), 6.28 (d, $J = 1.5$ Hz, 1H), 5.70 (br s, 1H), 3.67 (s, 2H), 3.51 (t, $J = 6.5$ Hz, 2H), 3.29 (d, $J = 12.5$ Hz, 1H), 2.53 (dt, $J = 6.0$ Hz, $J = 2.5$ Hz, 1H), 2.05 (d, $J = 1.0$ Hz, 3H), 1.94-1.89 (m, 1H), 1.87 (td, $J = 7.5$ Hz, $J = 2.0$ Hz, 1H), 1.82 (d, $J = 2.0$ Hz, 6H), 1.76 (d, $J = 12.0$ Hz, 4H), 1.70 (d, $J = 11.0$ Hz, 4H), 1.13 (q, $J = 8.0$ Hz, 2H), 1.0 (s, 3H), 0.82 (q, $J = 12.0$ Hz, 1H).
Chapter 5

Novel Hexahydrocannabinidiols (HH-CBDs)

5.1 Introduction

Natural (-) cannabidiol (CBD) is a major nonpsychotropic constituent of Cannabis, with well recognized therapeutic potential\(^{178}\). It possesses multiple pharmacological actions, including antipsychotic, antiemetic, neuroprotective and weight loss, as demonstrated in clinical and preclinical studies\(^ {179}\). This natural CBD does not bind to CB1 and CB2 receptors but it interacts with cannabinoid signaling system. Bisogno *et al.* showed that both (-) and (+) CBD stimulate Vanilloid receptor type 1 (VR1) and increase anandamide production\(^ {180}\). Later on it was discovered that it produces various effects by interacting with serotonin, adenosine, 5-HT(1A) and TRPV receptors. It shows potent immunosuppressive and anti-inflammatory properties by activating adenosine receptors\(^ {181}\). CBD alleviates hyperphagia (obesity) by acting as serotonin receptor antagonist without producing any side effects\(^ {182}\). CBD acts as 5-HT(1A) agonist to attenuate vomiting and nausea and as antidepressant\(^ {183,184}\). It has been shown that the (+) CBD enantiomer binds to the cannabinoid receptor with weak binding affinity where as its 7-OH metabolite (Fig. 5.1) has high binding affinity, whereas 7-OH metabolite of (-) CBD has no binding at both CB1 and CB2 receptors. Extensive SAR has been explored with both the stereochemistry and functionality at the 7 position of CBD\(^ {185}\). Hydrogenation of the (-) CBD (Fig. 5.1) resulted in some interesting binding and functional data\(^ {186}\). Due to two unsaturated bonds in CBD it was difficult to selectively hydrogenate one unsaturated bond. As found in the literature, analogs of (+) CBD have shown high affinity and potency for cannabinoid receptors.
Incorporation of a longer dimethyl heptyl side chain and a hydroxy group at 7-position has significantly boosted affinities at both receptors.\(^8\)

All the SAR so far explored for CBD template is for tetrahydrocannabinols (TH-CBDs). No SAR had been explored for hexahydrocannabinols (HH-CBDs). In tricyclic template, it has been shown that a moving from tetrahydro- to hexahydro- template shows both improved affinity and potency. Do cannabidiols follow the same trend? Will hexahydrocannabinols bind to CB receptors better than tetrahydrocannabinols? Which stereochemistry is tolerated and is favorable in hexahydrocannabinols? To get the answers of all these questions, an extensive SAR was required and was performed on HH-CBDs.

![Diagram]

**Figure 5.1:** CBD compounds and their hydrogenated analogs

### 5.2 Chemistry

We explored the chemistry and biology of HH-CBDs using already available 1-adamantyl intermediates. The stereochemistry of the closed and open ring bicyclic adamantyl analogs has been described in Chapter 3. The HH-CBD adamantyl analogs were synthesized (Scheme 5.1) by deprotection of the bicyclic intermediates of the tricyclic SAH synthetic route (Scheme 3.1 and 3.2). The stereochemistry was already assigned in Chapter 3. All the intermediates in the Scheme 5.1 were deprotected using the tetrabutylammonium floride (TBAF) reaction conditions to give the final compounds from 1.2 to 4.2, 5 and 6. Stereochemistry at the ring junction of final
compounds 1.2, 2.2, 4.2, 5 and 6 is *trans* because of the large coupling constant, $J = 11.5\, \text{Hz}$ to $12.5\, \text{Hz}$, (shown in Experimental Section) between 2-axial, 3-axial and 4-allylic protons. β-stereochemistry was confirmed by HMQC, HMBC, COSY and 1DNOESY (Fig. 5.2) correlations on 4.2 establishing the powerful anisotropic effects, affecting the 2-equatorial, 1-axial and 3-axial protons.

**Figure 5.2:** 1D-NOESY studies of AM8604 (4.2). Irradiation of 1-axial, 3-axial and 4-axial protons show enhancement of *cis* and neighbouring protons confirming the stereochemistry.
Scheme 5.1: Synthesis of adamantyl HH-CBD analogs

Reagents and conditions: (a) \( \text{Ph}_3\text{PCH}_2\text{OMe}^-\text{Cl}^-, n\)-BuLi, THF, -30 °C, 1 h, 79%; (b) TBAF, THF, 0 °C, 1 h, 83% for 1,2, 86% for 2,2 and 82% for 4,2; (c) i. \( \text{CCl}_3\text{COOH} \), \( \text{H}_2\text{O} \), DCM, rt, 3 h, ii. \( \text{K}_2\text{CO}_3 \), MeOH, rt, 12 h, 82%; (d) \( \text{NaBH}_4 \), MeOH, 0 °C, 1 h, 90% for 4,1 and 85% for 6; (e) TBAF, THF, rt, 6 h, 60%

Compound 7 in Scheme 5.2 was synthesized by reduction of the keto intermediate 5.1 with sodium borohydride. The crude reaction mixture was treated with TBAF to give the 9-OH product 7 in 70% yield after 2 steps. The \( \beta \)-stereochemistry at C-1 position is confirmed from the large trans (12-18 Hz) coupling constants of 1-axial proton (3.62 (dddd, \( J = 15.0 \text{ Hz} \), \( J = 11.0 \text{ Hz} \), \( J = 4.5 \text{ Hz} \), \( J = 4.5 \text{ Hz} \), 1H, 1-axial), in \( ^1\text{H} \) NMR.

Scheme 5.2: 9-Hydroxyl adamantyl HH-CBD

Reagents and conditions: (a) \( \text{NaBH}_4 \), \( \text{CH}_3\text{OH} \), 0 °C, 1 h; (b) TBAF, THF, 0 °C, 1 h, 70% (2 steps)
Compound 8 was synthesized in 67% yield by hydrogenation of 4.1 with palladium on carbon under hydrogen atmosphere and subsequent deprotection of TBDMS groups using TBAF.

**Scheme 5.3: Hydrogenation of isopropenyl group of 11-hydroxyl adamantyl HH-CBD**

![Reaction Scheme](image)

**Reagents and conditions:** (a) Pd/C, H2, CH3OH, rt, 10 h; (b) TBAF, THF, 0°C, 1 h, 67% (2 steps)

The chemistry utilized to synthesize dimethyl heptyl (DMH) analogs (Scheme 5.4) is similar to the chemistry optimized for the adamantyl compounds (Scheme 3.1 & 3.2). A mixture (-)-(S)-nopinone diacetates 9 was synthesized following the procedures reported by the Makriyannis’ group previously. Coupling of commercial resorcinol 10 and diacetates 9 in the presence of p-toluene sulfonic acid gave bicyclic keto intermediate 11 in 60% yield (Scheme 5.4). Protection of the phenolic hydroxyl groups using TBDMSCl and imidazole gave 12 in 80% yield. With the earlier optimized reaction conditions for adamantyl compounds, the strained cyclobutyl ring was opened to produce 13.1 in 60% yield. TBDMS groups of 13.1 were deprotected with TBAF to give the final compound 13.2 in 70% yield. One carbon Wittig homologation of 13.1 gave a mixture of enol ethers 14.1 in 72% yield, which were not separated, and TBDMS groups were deprotected with TBAF to give enol ether mixtures 14.2 in 77% yield. Enol ether 14.1 was hydrolyzed to a diastereomeric aldehyde mixture with wet trichloroacetic acid and was epimerized in K2CO3 to yield exclusively β-equatorial aldehyde 15.1 in 76% yield over 2 steps. TBDMS groups of aldehyde were removed using TBAF to give aldehyde analog 15.2 in 77%
yield. Sodium borohydride reduction of aldehyde 15.2 led to 16 in 74% yield. Keto analog 13.2 was reduced with sodium borohydride to give OH analog 17 in 82% yield. Reductive amination of 9-keto intermediate 13.1, using morpholine, sodium triacetoxyborohydride and acetic acid to give 9-morpholine intermediate, which was treated with TBAF to give analog 18 in 62% yield. Aldehyde 15.2 was oxidized to acid analog 19 in 65% yield using sodium chlorite conditions.

**Scheme 5.4: Synthesis of dimethyl heptyl (DMH) HH-CBD analogs**

**Reagents and conditions:** (a) TsOH-H₂O, CHCl₃, rt, 3 days, 60%; (b) TBSCI, imidazole, DMAP, DMF, rt, 24 h, 80%; (c) i. TMSI, CCl₄, cat. t-BuOH, 0 °C, 12 h, ii. NaOAc, CH₃COOH, 90 °C, 2 h, 60%; (d) TBAF, THF, 0 °C, 1 h, 83% for 13.2, 77% for 14.2, 73% for 15.2, and 62% for 18; (e) Ph₃PCH₂OMe⁺Cl⁻, n-BuLi, THF, -30 °C, 1 h, 72%; (f) i. CCl₃COOH, H₂O, DCM, rt, 3 h, ii. K₂CO₃, MeOH, rt, 12 h, 76%; (g) NaBH₄, MeOH, 0 °C, 30 min, 74% for 16 and 83% for 17; (h) morpholine, (CH₃COO)₂BHNa, CH₃COOH, CH₂Cl₂, rt, 18 h; (i) NaH₂PO₄, 2-methyl-2-butene, NaClO₂, H₂O, t-BuOH, rt, 4 h, 65%

The stereochemistry at C-1 for all the above mentioned northern modified analogs was assigned on the basis of *trans*-stereochemistry at the ring junction, confirmed through large coupling
constants of 3-axial and 4-allylic protons. Also, large coupling constant of 1-axial proton in $^1$H NMR and its NOE correlations with 3-axial protons confirms 1,3-$cis$ and 1,4-$trans$ relations and hence, the stereochemistry of the molecules as shown in Scheme 5.4.

### Scheme 5.5: Synthesis of enantiomers of dimethyl heptyl analogs

**Reagents and conditions:** (a) TsOH, CHCl$_3$, rt, 3 days, 70%; (b) TBSCI, imidazole, DMAP, DMF, rt, 18 h, 83% for 23, 80% for 28; (c) i. TMSI, CCl$_4$, cat. $t$-BuOH, 0 °C, 12 h, ii. NaOAc, CH$_3$COOH, 90 °C, 2 h, 74%; (d) TBAF, THF, 0 °C, 1 h, 78% for 24.2, 74% for 26.2 and 82% for 27.2; (e) Ph$_3$PCH$_2$OMe$^+$$Cl^-$, $n$-BuLi, THF, -30 °C, 1 h, 79%; (f) i. CCl$_3$COOH, H$_2$O, DCM, rt, 3 h, ii. K$_2$CO$_3$, MeOH, rt, 12 h, 82%; (g) NaBH$_4$, MeOH, 0 °C, 1 h, 86% for 27.1 and 79% for 30; (h) SeO$_2$, salicylic acid, $t$-BuOOH, CH$_2$Cl$_2$, rt, 12 h, 64%; (i) TBAF, THF, rt, 6 h, 85%.

Applying the same chemistry but using a nopinone diacetate mixture with opposite ($R$)-stereochemistry, the enantiomers of the above mentioned dimethyl heptyl analogs were synthesized (Scheme 5.5). There was no difference in the $R_f$ or the NMRs of the opposite isomers obtained (Experimental Section) but they had exactly opposite specific optical rotations.
This clarified that enantiomers are being isolated. Utilizing the same reaction conditions, 9-keto intermediate 24.1 was deprotected with TBAF as mentioned earlier to give keto compound 24.2 in 78% yield. Until 11-OH analog 27.2, the same chemistry was utilized.

For the further modification in the southern binding pocket, hydroxyl group of 27.1 was protected as TBDMS using standard earlier mentioned conditions to get 28 in 80% yield. Allylic oxidation of 28 using SeO₂ yielded 29.1 in 63% yield. Deprotection of southern aldehyde 29.1 using standard TBAF conditions gave analog 29.2 in 60% yield. The aldehyde analog 29.2 was further reduced using sodium borohydride to give southern hydroxyl analog 30 in 79% yield.

Aldehyde intermediate 26.1 was oxidized using sodium chlorite, 2-methyl-2-buten conditions to give acid compound 31.1 in 63% yield (Scheme 5.6). Acid intermediate 31.1 was deprotected with standard TBAF conditions to give compound 31.2 in 69% yield.

Scheme 5.6: Synthesis of novel analogs of dimethyl heptyl HH-CBDs

Reagents and conditions: (a) NaH₂PO₄, 2-methyl-2-buten, NaClO₂, H₂O, t-BuOH, r.t, 4 h, 63%; (b) TBAF, THF, 0 °C, 1 h, 69%; (c) i. SOCl₂, toluene, 70 °C, 2 h, ii. NH₄OH, CH₂Cl₂, 0 °C, 12 h, 51% (2 steps); (d) i. SOCl₂, toluene, 70 °C, 2 h, ii. morpholine, CH₂Cl₂, 0 °C, 12 h, 57% (2 steps); (e) (CH₃)₃SiCHN₂, CH₃OH, toluene, 0 °C, 12 h, 71%.
Amidation of 31.1 was performed using thionyl chloride along with either ammonium hydroxide or morpholine to give amide and morpholine amide analogs, respectively, which were deprotected with TBAF to give compounds 32 in 51% yield and 33 in 57% yield. Acid compound 31.2 was esterified with TMS-diazomethane in methanol to give methyl ester 34 in 71% yield.

More polar 9-morpholine analog 35 was synthesized in an overall 60% yield from 9-keto intermediate 24.1 (Scheme 5.7) using the same conditions as mentioned for its enantiomer 18. β-stereochemistry at C-1 position and trans-stereochemistry at C-3 and C-4 positions was confirmed through ROESY (Fig. 5.3), HSQC (Fig. 5.4), HMBC (Fig. 5.5) and COSY correlations on 35.

Figure 5.3: 2D-ROESY studies of AM8641 (35) to assign stereochemistry.
Figure 5.4: 2D-HSQC study of AM8641 (35) to assign all the protons and carbons
Figure 5.5: 2D-HMBC study of AM8641 (35) to assign correlation between protons and carbons

Scheme 5.7: Synthesis of morpholine analog of DMH HH-CBD

Reagents and conditions: (a) Morpholine, (CH₃COO)₃BHNa, CH₃COOH, CH₂Cl₂, rt, 18 h; (b) TBAF, THF, 0 °C, 1 h, 60% (2 steps)

Keto compounds of both enantiomers were methylated at phenolic hydroxyl using MeI to give 36.1 [(-)-(S)] and 36.2 [(+)-(R)] in 60-65% yield (Scheme 5.8). Methoxy derivatives were
hydrogenated with palladium on carbon in hydrogen atmosphere to reduce the terminal alkene to give 37.1 and 37.2 respectively in 85-90% yield. Ketone analog 37.1 was reduced to give the hydroxyl analog 38 in 80% yield.

**Scheme 5.8: Methoxy derivatives of DMH-HH-CBDs**

**Reagents and conditions:** (a) MeI, K₂CO₃, acetone, rt, 18 h, 60-65%; (b) Pd/C, H₂, CH₃OH, rt, 10 h, 85-90%; (c) NaBH₄, MeOH, 0°C, 1 h, 80%.

**5.3 Result and Discussion**

From this work, a very interesting finding has been obtained. HH-CBD analogs with adamantyl side chain show CB2 affinity but have species subtype selectivity. As shown in Table 5.1, AM8604 (4.2) has high affinity of 16 nM at mouse CB2 (mCB2) but low affinity of 128 nM at human CB2 (hCB2). Hydrogenation of 4.2 yielded AM8608 (8) with similar binding affinity results of 22 nM (mCB2) and 106 nM (hCB2). These analogs have good binding for mCB2 but poor binding for hCB2. The same trend of species subtype selectivity continued even after functionalization of the southern position. AM8612 (5.2), a southern aldehyde analog of 4.2, has an enhanced affinity of 4 nM at mCB2 but with same species subtype selectivity issue of having lower affinity for hCB2, in this case 309 nM. This species subtype selectivity issue was resolved
by replacing adamantyl group with dimethyl heptyl side chain (based upon literature knowledge) and SAR on both the enantiomers of dimethylheptyl (DMH) was explored.

From the rational design, the northern and southern pockets of CBD were explored. The data in the Table 5.1 reveals that phenolic hydroxyl group is necessary for the binding at both CB receptors. Replacing the phenolic hydroxyl with methoxy groups in 9-keto and hydroxyl DMH analogs in both enantiomers (36.1, 36.2, 37.1, 37.2 and 38) resulted in loss of binding affinity at both CB receptors. Replacement of adamantyl group with DMH in 11-OH analog resulted in high affinity compound AM8630 (27.2) with no species subtype selectivity with Ki values 12 nM for mCB2 and 11 nM for hCB2. Its (+)-enantiomer AM8628 (16), showed lower binding affinity and functional potency, when compared with regular (-)-AM 8630 (27.2) as shown in Table 5.1 and 5.2. Similar results were obtained for the enol ether (14.2), aldehyde (15.2), morpholino (18) and acid (19) analogs with the opposite stereochemistry. It was interesting that 9-keto (13.2) and 9-hydroxy (17) analogs showed high binding affinity and selectivity for CB2 receptors whereas further modifications were not tolerated. Natural (-)-CBD has no affinity for CB receptors but enantiomeric (+)-CBD analogs bind with high affinity. In hexahydro template natural stereochemistry with DMH i.e. (-)-HH-CBD is very well tolerated in both binding and functional assays whereas the (+)-enantiomer is not well tolerated except 9-keto (13.2) & 9-OH (17) (Table 5.1). Moving forward with normal (-)-stereochemistry, SAR was explored on the HH-CBD template. The present work reveals that more polar and bulky groups such as acid (31.2), amide (32), morpholino-amide (33) and ester (34 & 34.1) are not tolerated in the northern pocket and 11-OH (27.2) is the most desired and well tolerated group. Keeping the 11-OH group, further SAR was explored on the southern pocket and southern aldehyde compound AM8650 (29.2) showed high affinity and potency (EC50 = 0.1 nM) at both CB1 and CB2 receptors with some
preference for CB2. Southern hydroxyl group was also well tolerated in the binding pocket. In search of more polar and CB2 selective cannabidiol compounds, 9-keto was converted to 9-morpholine analog AM8641 (35). It showed high binding affinity at both CB1 and CB2 receptors and high functional potency and selectivity for CB2 receptor with an EC$_{50}$ of 3.1 nM at hCB2. This compound behaves as a full potent agonist at CB2 and a weak inverse agonist (EC$_{50}$ = 809 nM) at CB1 in functional assays.

Most of the dimethylheptyl analogs showed β-arrestin recruitment at hCB2 (Table 5.3). AM8630 (27.2), a high affinity ligand, also showed high arrestin recruitment with an EC$_{50}$ value of 0.11 nM at hCB2. This shows that it is a potent unbiased ligand at CB2 receptors. AM8650 (29.2) has high functional potency of 24 nM and 1.4 nM at both CB1 and CB2 receptors in cAMP assay but has low β-arrestin recruitment of 24 µM at CB1 and 0.1 nM at CB2. This data suggests that AM8650 (29.2) is a potent CB1 biased agonist for G-proteins and potent but unbiased CB2 agonist. The 9-morpholine compound AM8641 (35) is a potent and selective CB2 agonist with high functional potency of 3.1 nM and high β-arrestin recruitment of 59 nM.

From the SAR on HH-CBD series, many high binding and functionally potent analogs were synthesized. All the high affinity CBD ligands tested for β-arrestin recruitment show no or very low arrestin recruitment for CB1 receptors. AM8630 (27.2), 11-hydroxyl analog, shows similar trend in cAMP and β-arrestin and has high potency at hCB2. Interestingly, AM8641 (35) a morpholino analog is a high potency CB2 selective agonist in both cAMP and β-arrestin assays and a very weak inverse agonist at CB1 in both the assays. AM8650 (29.2) has high potency at both CB1 and CB2 receptors in cAMP but has high potency of arrestin recruitment at CB2 only and no recruitment at CB1 receptor. This shows AM8650 (29.2) is a biased for G-proteins at
CB1 receptor. Southern group may impart biased agonism for G-proteins at CB1 receptor. This will be confirmed by testing more southern modified analogs.

Table 5.1: Binding affinities of all the synthesized CBD analogs

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</tr>
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</tr>
<tr>
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<td>105 ± 0.5</td>
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<td>Compound No.</td>
<td>AM No.</td>
<td>hCB1 (% inhibition)</td>
<td>hCB2 (% inhibition)</td>
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<td>---------------------</td>
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</tr>
<tr>
<td>16</td>
<td>8628</td>
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<td>-2% at 10nM</td>
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<td></td>
<td></td>
<td>87% at 1uM</td>
<td>93% at 1uM</td>
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<tr>
<td>27.2</td>
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<td>3% at 10nM</td>
<td>45% at 10nM</td>
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<tr>
<td></td>
<td></td>
<td>99% at 1uM</td>
<td>106% at 1uM</td>
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<tr>
<td>29.2</td>
<td>8650</td>
<td>24 nM</td>
<td>1.4 nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>8641</td>
<td>809 nM</td>
<td>3.1 nM</td>
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Table 5.3: β-arrestin recruitment of HH-CBD ligands

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<th>Compound</th>
<th>AM No.</th>
<th>hCB1 EC\textsubscript{50}</th>
<th>hCB2 EC\textsubscript{50}</th>
<th>Efficacy at hCB1</th>
<th>Efficacy at hCB2</th>
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<td>8604</td>
<td>67 µM</td>
<td>No recruitment</td>
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<td>16</td>
<td>8628</td>
<td>No recruitment</td>
<td>82 µM</td>
<td>N.A.</td>
<td>N.A.</td>
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<tr>
<td>27.2</td>
<td>8630</td>
<td>0.11 µM</td>
<td>0.11 nM</td>
<td>0.33</td>
<td>2.11</td>
</tr>
<tr>
<td>29.2</td>
<td>8650</td>
<td>24 µM</td>
<td>0.1 nM</td>
<td>1.1</td>
<td>1.8</td>
</tr>
<tr>
<td>35</td>
<td>8641</td>
<td>3.3 µM</td>
<td>59 nM</td>
<td>N.A.</td>
<td>1.95</td>
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</table>

N.A. : Not Applicable

5.4 Conclusion: In summary, SAR study of HH-CBD analogs resulted in a good understanding of a new series of cannabinergic ligands. Analogs with adamantyl and dimethylheptyl chain and with various northern and southern modifications were synthesized and tested for binding and functional assays.

Replacing the bulky adamantyl side chain with dimethylheptyl resolved the CB2 species subtype selectivity issue raised from previous \textit{in vitro} studies.

Furthermore, results of exploration of both the enantiomers in DMH side chain analogs showed that (+)-HH-CBD analogs have high binding affinity, selectivity and functional potency. An interesting 9-morpholine analog AM8641 (35) showed high CB2 agonist potency and low CB1 inverse agonist potency. \textit{In vivo} studies on this analog are in progress.

Further exploration in northern and southern binding pocket resulted in high potency southern aldehyde ligand AM8650 (29.2). These high affinity ligands also showed some selectivity for one functional assay over the other and hence displaying biased agonism.
SAR on the HH-CBD series resulted in some interesting analogs AM8630 (27.2), AM8641 (35) and AM8650 (29.2) with high affinity, selectivity and functional potency. More SAR will be required around morpholine analog to get more selective and potent peripherally acting CB2 ligands.

5.5 Experimental:

\[(3R,4R)-3-[4-(1-Adamantyl)-2,6-bis(\text{tert}-\text{butyldimethylsilyloxy})\text{phenyl}]-4-\text{isopropenylcyclohexan-1-one} \ (1.1)\]. Compound 1.1 is an intermediate of tricyclic SAH synthesis (Chapter 3; Scheme 3.2, compound 5). Its synthesis and NMR is mentioned earlier (Chapter 3; compound 5).

\[(3R,4R)-3-[4-(1-Adamantyl)-2,6-\text{dihydroxyphenyl}]-4-\text{isopropenylcyclohexan-1-one} \ (1.2)\]. To a stirred solution of 1.1 (65 mg, 0.107 mmol) in dry THF (5 mL) at 0 °C was added a solution of tetra-n-butylammonium fluoride (0.28 mL, 0.28 mmol, 1M solution in THF) and stirring was continued for 30 min. The reaction was quenched with 5 mL saturated aqueous NaHCO₃ and the mixture was extracted with Ethyl acetate (3x25 mL). The organic phase was washed with water and brine and dried (MgSO₄). Evaporation of volatiles gave crude as colorless oil which upon
purification by flash chromatography (3-30% ethyl acetate : hexanes) gave 1.2 (34 mg, 0.08 mmol, 83% yield) as a white foam. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 6.47 (d, $J$ = 1.5 Hz, 1H), 6.31 (d, $J$ = 1.5 Hz, 1H), 5.01 (s, 1H), 4.95 (s, 1H), 4.63 (s, 1H, OH), 3.61 (br s, 1H), 2.76 (s, 1H, OH), 2.36 (br s, 1H), 2.12 (dd, $J$ = 12.0 Hz, $J$ = 2.5 Hz, 1H, 3-axial), 2.07 (br s, 3H), 2.00 (dd, $J$ = 19.0 Hz, $J$ = 5.5 Hz, 1H, 4-axial), 1.95-1.90 (m, 2H), 1.89 (s, 3H), 1.85 (s, 3H), 1.84 (s, 3H), 1.77 (d, $J$ = 12.0 Hz, 3H), 1.72 (d, $J$ = 12.0 Hz, 3H), 1.68-1.60 (m, 1H), 1.30-1.24 (m, 1H). HRMS (ESI) calculated for C$_{25}$H$_{32}$O$_3$: 380.2352; found 380.2354; $[^a]_{22}^D = -6.8^o$ (c = 1.37, CHCl$_3$).

(3$R$,4$R$)-3-[4-(1-Adamantyl)-2,6-bis(tert-butyldimethylsilyloxy)phenyl]-4-isopropenyl-1-(methoxymethylene)cyclohexane (2.1). Synthesis and NMR is same as mentioned earlier (Chapter 3; compound 6)

(3$R$,4$R$)-3-[4-(1-Adamantyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-(methoxymethylene)cyclohexane (2.2). To a stirred solution of 2.1 (70 mg, 0.11 mmol) in dry THF (5 mL) at 0°C
was added a solution of tetra-\(n\)-butylammonium fluoride (0.25 mL, 0.25 mmol, 1M solution in THF) and stirring was continued for 30 min. The reaction was quenched with saturated aqueous NaHCO\(_3\) and diluted with ethyl acetate (50 mL). The organic phase was separated and aqueous layer extracted with ethyl acetate (2x25 mL). The combined organic layer was washed with water, brine and dried (MgSO\(_4\)). Evaporation of volatiles gave crude as colorless oil which on purification by flash chromatography (7-45% ethyl acetate : hexanes) gave mixture of enol ethers 2.2 (50 mg, 0.125 mmol, 86% yield) which were tested without separation of geometric isomers.

NMR (500 MHz, CDCl\(_3\)) \(\delta\): 6.29 (s, 1H), 6.22 (s, 1H), 5.79 (s, 1H), 4.70-4.60 (m, 3H), 4.49 (d, \(J = 1.5\) Hz, 1H), 3.55 (s, 3H), 3.10-2.98 (m, 2H), 2.86 (td, \(J = 13.0\) Hz, \(J = 2.0\) Hz, 1H), 2.65 (t, \(J = 12.5\) Hz, 1H), 2.05 (br s, 3H), 2.01 (s, 1H), 1.82 (s, 3H), 1.81 (s, 3H), 1.76 (d, \(J = 12.0\) Hz, 3H), 1.72 (d, \(J = 12.0\) Hz, 3H), 1.56 (s, 3H), 1.48-1.36 (m, 2H) 1.25 (s, 1H). HRMS (ESI) calculated for C\(_{27}\)H\(_{36}\)O\(_3\): 408.2665; found 408.2666; \([\alpha]^{22}_D = 30.5^o\) (\(c = 0.81\), CH\(_3\)OH)

(1\(R\),3\(R\),4\(R\))-3-[4-(1-Adamantyl)-2,6-bis(\textit{tert}-butyldimethylsilyloxy)phenyl]-4-isopropenyl-1-formylcyclohexane (3). Synthesis and NMR is same as mentioned earlier (Chapter 3; compound 7).
(1R,3R,4R)-3-[4-(1-Adamantyl)-2,6-bis(tert-butylidimethylsilyloxy)phenyl]-4-isopropenyl-1-(hydroxymethyl)cyclohexane (4.1). Synthesis and NMR is same as mentioned earlier (Chapter 3; compound 8).

(1R,3R,4R)-3-[4-(1-Adamantyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-(hydroxymethyl)cyclohexane (4.2): To a stirred solution of 4.1 (100 mg, 0.160 mmol) in dry THF (7 mL) at 0 °C was added a solution of tetra-n-butylammonium fluoride (0.48 mL, 0.48 mmol, 1M solution in THF) drop wise and the reaction mixture was stirred for 30 min. The reaction was quenched with saturated aqueous NaHCO₃ and the mixture was extracted with ethyl acetate (10 mL). The organic phase was washed with water and brine and dried (MgSO₄). Evaporation of volatiles gave colorless oil which upon purification by flash chromatography (15-65% ethyl acetate : hexanes) gave 4.2 (52 mg, 0.131 mmol, 82% yield) as white foam. ¹H NMR (500 MHz, (CD₃)₂CO) δ: 6.35 (d, J = 1.5 Hz, 1H), 6.30 (d, J = 1.0 Hz, 1H), 4.65 (d, J = 2.0 Hz, 1H), 4.37 (d, J = 2.0 Hz, 1H), 3.37 (d, J = 6.5 Hz, 2H), 3.31 (dt, J = 11.5 Hz, J = 3.0 Hz, 1H, 3-axial), 3.16
(1R,3R,4R)-3-[4-(1-Adamantyl)-2,6-dihydroxyphenyl]-4-[2′-(propenyl)]-1-(hydroxymethyl) cyclohexane (5): To a stirred solution of 11 (Scheme 3.2) (55 mg, 0.073 mmol), obtained from the earlier synthesis, in dry THF (10 mL) at 0°C was added a solution of tetra-n-butyrammonium fluoride (19.09 mg, 0.073 mmol, 1M solution in THF) drop wise and stirring was continued for 5 h at room temperature. The reaction was quenched by the addition of saturated aqueous NaHCO₃ and the mixture was extracted with ethyl acetate (2x20 mL). The combined organic layer was washed with water, brine and dried (MgSO₄). Solvent was evaporated under reduced pressure to give colorless oil which upon purification by flash chromatography (15-75% ethyl acetate : hexanes) gave 5 (18 mg, 0.044 mmol, 60% yield) as white foam. HRMS (ESI) calculated for C₂₆H₃₄O₄: 410.2457; found 410.2459. ¹H NMR (500 MHz, CD₃OD) δ: 9.22 (s, 1H), 6.35 (s, 1H), 6.18 (d, J = 1.5 Hz, 1H), 6.10 (d, J = 1.5 Hz, 1H), 5.77 (s, 1H), 4.54 (s, 2H, phenolic OH), 3.55 (dt, J = 11.5 Hz, J = 3.0 Hz, 1H), 3.39-3.28 (m,
2H), 1.95 (br s, 3H), 1.88-1.48 (m, 16H, especially 1.75, s, 3H and 1.74, s, 3H), 1.33 (s, 2H), 1.22 (br s, 1H), 1.09 (q, J = 8.5 Hz, 2H); [α]_D^{22} = 53.1° (c = 1.14, CH_3OH).

(1R,3R,4R)-3-[4-(1-Adamantyl)-2,6-dihydroxyphenyl]-4-[2′-(3′-hydroxyprop-1′-enyl)]-1-(hydroxymethyl)cyclohexane (6): To a stirred solution of 5 (40 mg, 0.053 mmol) in dry THF (10 mL) at 0°C was added a solution of tetra-n-butylammonium fluoride (0.265 mL, 0.265 mmol, 1M solution in THF) dropwise and reaction solution was stirred at room temperature for 5 h. The reaction mixture was quenched with saturated aqueous NaHCO₃ and diluted with Ethyl acetate (40 mL). Organic layer was separated and washed with water, brine and dried (MgSO₄). Evaporation of volatiles under reduced pressure gave colorless oil which upon purification by flash chromatography (20-65% ethyl acetate : hexanes) gave 6 (16 mg, 0.04 mmol, 73% yield) as white foam. HRMS (ESI) calculated for C_{26}H_{36}O₄: 412.2614; found 412.2616. ¹H NMR (500 MHz, Acetone-d₆) δ: 7.76 (s, 1H, phenolic OH), 7.74 (s, 1H, phenolic OH), 6.35 (d, J = 1.5 Hz, 1H), 6.31 (d, J = 1.5 Hz, 1H), 4.85 (d, J = 1.5 Hz, 1H), 4.80 (d, J = 1.5 Hz, 1H), 4.03 (dd, J = 14.5 Hz, J = 4.0 Hz, 1H), 3.94 (dd, J = 14.5 Hz, J = 4.0 Hz, 1H), 3.52 (t, J = 5.0 Hz, 1H), 3.42 (d, J = 6.5 Hz, 1H), 3.37 (t, J = 6.0 Hz, 2H), 3.34 (dd, J = 12.0 Hz, J = 4.0 Hz, 1H, 3-axial), 3.07 (dt, J = 12.5 Hz, J = 3.0 Hz, 1H, 4-allylic), 2.90 (s, 1H), 2.02 (br s, 3H), 1.91-1.84 (m, 1H), 1.83 (t, J = 4.0 Hz, 1H), 1.81 (s, 3H), 1.80 (s, 3H), 1.77 (d, J = 12.0 Hz, 3H), 1.72 (d, J = 12.0 Hz,
3H), 1.69-1.65 (m, 1H), 1.65-1.55 (m, 1H), 1.42 (dq, J = 12.5 Hz, J = 3.0 Hz, 1H), 1.06 (dq, J = 12.5 Hz, J = 3.0 Hz, 1H); [α]_{D}^{22} = 41.8° (c = 0.54, CH_{3}OH).

(1R,3R,4R)-3-[4-(1-Adamantyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-hydroxycyclohexane (7): To a solution of 1.1 (75 mg, 0.123 mmol) in MeOH (15 mL) at 0°C was added sodium borohydride (23.29 mg, 0.616 mmol) and the reaction mixture was stirred for 30 min. Reaction mixture was quenched by the addition of saturated aqueous NH_{4}Cl solution and partially concentrated. The mixture was extracted with ethyl acetate (2 x 60 mL) and the organic layer was washed with water and brine and dried (MgSO_{4}). Evaporation of volatiles under reduced pressure gave crude which was used into the next step without further purification. The crude was dissolved in dry THF (10 mL) at 0°C and a solution of tetra-n-butylammonium fluoride (0.62 mL, 0.62 mmol, 1M solution in THF) was added dropwise and stirred for 30 min. The reaction was quenched with saturated aqueous NaHCO_{3} and the mixture was extracted with ethyl acetate. The organic layer was washed with water, brine and dried (MgSO_{4}). Evaporation of volatiles under reduced pressure gave crude yellow oil which upon purification by flash chromatography (20-85% ethyl acetate : hexanes) gave 7 (33 mg, 0.086 mmol, 70% yield over 2 steps) as white foam. \(^1\)H NMR (500 MHz, CD_{3}OD) δ: 6.25 (d, J = 2.0 Hz, 1H), 6.22 (d, J = 2.0 Hz, 1H), 4.62 (d, J = 2.5 Hz, 1H), 4.39 (d, J = 2.5 Hz, 1H), 3.62 (dddd, J = 15.0 Hz, J = 11.0 Hz, J = 4.5 Hz, J = 4.5 Hz, 1H, 1-axial), 3.23 (dt, J = 12.0 Hz, J = 3.5 Hz, 1H, 3-axial), 3.08 (dt, J =
12.0 Hz, $J = 3.5$ Hz, 1H, 4-allylic), 2.10 (q, $J = 12.0$ Hz, 1H), 2.03 (br s, 3H), 1.99 (br d, $J = 10.0$ Hz, 1H), 1.85 (s, 3H), 1.84 (s, 3H), 1.83-1.78 (m, 4H, especially, 1.80, d, $J = 12.5$ Hz, 3H), 1.72 (d, $J = 12.5$ Hz, 3H), 1.68 (dt, $J = 6.5$ Hz, $J = 3.0$ Hz, 1H), 1.56 (s, 3H), 1.49-1.34 (m, 2H); $[\alpha]^2_D = 53.1^\circ (c = 1.14, \text{CH}_3\text{OH}); [\alpha]^2_D = -23.4^\circ (c = 0.43, \text{CH}_3\text{OH}).$

\[(1R,3R,4R)-3-[4-(1\text{-Adamantyl})-2,6\text{-dihydroxyphenyl}]4\text{-isopropyl}-1\text{-hydroxymethyl} \text{cyclohexane (8)}: \text{To a solution of} \ 4.1\ (30\ \text{mg, 0.048 mmol) in MeOH (55 mL) was added palladium on carbon (15.32 mg, 0.144 mmol) and the reaction mixture was stirred under hydrogen atmosphere for 10 h at room temperature. The reaction mixture was diluted with diethyl ether and filtered through a small celite pad to remove the catalyst. Solvents were evaporated to give the crude material which was taken to the next step without further purification. The crude was dissolved in dry THF (10 mL), cooled to 0°C and a solution of tetra-\text{-n-butylammonium fluoride solution (0.19 mL, 0.192 mmol, 1M solution in THF) dropwise and stirred for 30 min. Reaction was quenched with saturated aqueous NaHCO}_3 and the mixture was extracted with ethyl acetate (2x20 mL). The organic phase was washed with water, brine and dried (MgSO}_4). Evaporation of volatiles under reduced pressure gave crude yellow oil which upon purification by flash chromatography (20-85% ethyl acetate : hexanes) gave 8 (16 mg, 0.032 mmol, 67% yield) as white foam. HRMS (ESI) calculated for C_{26}H_{36}O_4: 398.2821; found 398.2823. \]^1\text{H NMR (500 MHz, CD}_3\text{OD) }\delta: 6.29 (d, J = 1.5 Hz, 1H), 6.24 (d, J = 1.5 Hz, 1H), 3.35 (d, J = 3.5 Hz, 1H), 3.34 (d, J = 3.5 Hz, 1H), 3.07 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 2.26
(tt, $J = 12.0$ Hz, $J = 3.0$ Hz, 1H), 2.04 (br s, 3H), 1.92-1.84 (m, 8H, especially, 1.87, s, 3H and 1.86, s, 3H), 1.81 (d, $J = 12.0$ Hz, 3H), 1.79-1.69 (m, 5H, especially 1.76, d, $J = 12.0$ Hz, 3H), 1.64-1.58 (m, 1H), 1.58-1.51 (m, 1H), 1.49 (dt, $J = 7.5$ Hz, $J = 3.0$ Hz, 1H), 1.08 (dq, $J = 12.5$ Hz, $J = 3.0$ Hz, 1H), 0.99 (dq, $J = 12.5$ Hz, $J = 3.0$ Hz, 1H), 0.81 (d, $J = 7.0$ Hz, 3H), 0.70 (d, $J = 7.0$ Hz, 3H); $\left[\alpha\right]_{D}^{22} = -14.4^\circ$ (c = 0.41, CH$_3$OH).

(1S,4S,5S)-4-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-6,6-dimethylbicyclo[3.1.1]heptan-2-one (11): To a degassed solution of resorcinol 10 (2.0 g, 8.46 mmol) and diacetates 9 (2.72 g, 11.43 mmol) in CHCl$_3$ (70 mL) under an argon atmosphere was added p-toluene sulfonic acid monohydrate (2.09 g, 11 mmol). The reaction solution was stirred in dark for 3.5 days at 25 °C. The reaction mixture was diluted with diethyl ether (100 mL) and washed sequentially with water, saturated aqueous NaHCO$_3$ and brine. The organic phase was dried over MgSO$_4$ and concentrated under reduced pressure to give brown oil. Recrystallization with CH$_2$Cl$_2$ and hexanes (2:3) gave 11 (1.9 g, 5.11 mmol, 60% yield) as white crystalline solid. $^1$H NMR (CDCl$_3$) δ: 6.29 (d, $J = 1.0$ Hz, 2H), 3.98 (t, $J = 8.5$ Hz, 1H), 3.70 (dd, $J = 19.0$ Hz, $J = 7.5$ Hz, 1H), 2.60-2.53 (m, 2H), 2.51 (d, $J = 9.0$ Hz, 1H), 2.48 (quintet, $J = 5.5$ Hz, 1H), 2.26 (t, $J = 5.5$ Hz, 1H), 1.52-1.46 (m, 2H), 1.35 (s, 3H), 1.28-1.1.14 (m, 11H, especially, s, 1.20, 6H), 1.12-1.03 (m, 2H), 0.99 (s, 3H), 0.85 (t, $J = 7.0$ Hz, 3H); $\left[\alpha\right]_{D}^{22} = -25.7^\circ$ (c = 3.31, CH$_3$OH).
(4S)-4-[4-(1′,1′-Dimethylheptyl)-2,6-bis(tert-butyldimethylsilyloxy)phenyl]-6,6-dimethyl-2-norpinanone (12): To a solution of 11 (3g, 8.05 mmol), imidazole (4.32 gm, 64 mmol) and DMAP (0.2 gm, 1.6 mmol) in anhydrous DMF (30 mL) was added tert-butyl-dimethylchlorosilane (7.4 g, 48 mmol) under positive argon pressure. The reaction mixture was allowed to stir for 36h at room temperature. The reaction was quenched by addition of saturated aqueous NaHCO$_3$ and extracted with 3x200 mL diethyl ether. The organic layer was washed with water, brine and dried over MgSO$_4$. Solvents were evaporated under reduced pressure to give brown oil which was purified by flash chromatography (0-5% diethyl ether : hexanes) to give colorless oil 12 (3.87 g, 6.44 mmol, 80% yield). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 6.40 (s, 2H), 3.99 (t, $J = 8.0$ Hz, 1H), 3.70 (dd, $J = 17.0$ Hz, $J = 7.5$ Hz, 1H), 2.55 (t, $J = 5.5$ Hz, 1H), 2.53 (q, $J = 9.0$ Hz, 1H), 2.43 (sept, $J = 5.5$ Hz, 1H), 2.39 (d, $J = 11.0$ Hz, 1H), 2.24 (t, $J = 5.5$ Hz, 1H), 1.52-1.46 (m, 2H), 1.32 (s, 3H), 1.28-1.14 (m, 12 H), 1.10-1.02 (m, 2H), 1.02-0.94 (m, 21H, especially, 0.99, s, 18H), 0.85 (t, $J = 7.0$ Hz, 3H), 0.27 (s, 12H); $[\alpha]^{22}_D = -48.3^\circ$ (c = 7.81, CHCl$_3$).

(3S,4S)-3-[4-(1′,1′-Dimethylheptyl)-2,6-bis(tert-butyldimethylsilyloxy)phenyl]-4-isopropenyl cyclohexan-1-one (13.1): To a solution of 12 (4.3 g, 7.15 mmol) in carbon tetrachloride (100
mL) at 0 °C was added iodotrimethylsilane (1.02 ml, 7.4 mmol) drop wise. To this yellow solution was added catalytic amount of tert-butanol (0.02 mL, 0.2 mmol) and the reaction mixture was gradually warmed to 5 °C and stirred for 8 h. The resultant orange colored solution was cooled to 0 °C and again iodotrimethylsilane (2.0 mL, 14.8 mmol) was added and reaction mixture was stirred at 5 °C for additional 8 h. The reaction mixture was quenched with saturated aqueous sodium thiosulfate (30 mL) and diluted with diethyl ether (300 mL). The organic layer was washed sequentially with saturated NaHCO₃, water, brine and dried (MgSO₄). Solvent evaporation gave crude iodo intermediate (4.8 g) which was used into the next step without further purification. The crude iodo intermediate (4.6 g, 6.24 mmol) and sodium acetate (2.34 g, 28.6 mmol) were dissolved in acetic acid (30 mL) and stirred for 1.5 h at 90 °C. The reaction mixture was concentrated under reduced pressure. The crude was dissolved in 200 mL ether and was washed with water, saturated NaHCO₃ and brine. The combined organics were dried (MgSO₄) and concentrated under reduced pressure to give crude as brown oil. Purification by flash chromatography (0-8% ethyl acetate : hexanes) yielded 13.1 (2.58 g, 4.29 mmol, 60% yield) as colorless oil. $^1$H NMR (500 MHz, CDCl₃) δ: 6.34 (s, 1H), 6.33 (s, 1H), 4.64 (d, $J = 1.5$ Hz, 1H), 4.49 (d, $J = 1.5$ Hz, 1H), 3.63 (ddd, $J = 13.5$ Hz, $J = 11.5$ Hz, $J = 4.0$ Hz, 1H), 3.40 (dt, $J = 12.0$ Hz, $J = 3.5$ Hz, 1H), 3.20 (t, $J = 14.5$ Hz, 1H), 2.49-2.43 (m, 2H), 2.31 (dd, $J = 14.5$ Hz, $J = 4.0$ Hz, 1H), 2.05-1.98 (m, 1H), 1.82-1.70 (m, 1H), 1.55 (s, 3H), 1.50-1.43 (m, 2H), 1.27-1.14 (m, 12H, especially 1.20, s, 3H and 1.19, s, 3H), 1.10-0.94 (m, 20H, especially 1.06, s, 9H and 0.99, s, 9H), 0.84 (t, $J = 7.5$ Hz, 3H), 0.34 (s, 3H), 0.32 (s, 3H), 0.23 (s, 3H), 0.15 (s, 3H; $[\alpha]^{22}_D = 51.2^\circ$ (c = 6.76, CHCl₃); $[\alpha]^{22}_D = 20.2^\circ$ (c = 10.28, CHCl₃).
(3S,4S)-3-[4-(1’,1’-Dimethylheptyl)-2,6-dihydroxyphenyl]-4-isopropenylcyclohexan-1-one (13.2): To a stirred solution of 13.1 (40 mg, 0.07 mmol) in dry THF (5 mL) at 0°C was added a solution of tetra-n-butylammonium fluoride (0.27 mL, 0.27 mmol, 1M solution in THF) and stirring was continued for 30 min. The reaction was quenched with saturated aqueous NaHCO₃ and diluted with Ethyl acetate (30 mL). The organic phase was separated and aqueous layer extracted with Ethyl acetate (2x25 mL). The combined organic layer was washed with water, brine and dried (MgSO₄). Evaporation of volatiles gave crude as colorless oil which on purification by flash chromatography (7-45% ethyl acetate : hexanes) gave keto analog 13.2 (21 mg, 0.06 mmol, 83% yield) as colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 6.42 (d, J = 1.5 Hz, 1H), 6.28 (d, J = 1.5 Hz, 1H), 5.01 (s, 2H), 4.94 (s, 1H), 3.61 (d, J = 1.5 Hz, 1H), 3.05 (s, 1H), 2.35 (br s, 1H), 2.12 (dd, J = 12.0 Hz, J = 2.5 Hz, 1H), 2.01 (dt, J = 13.5 Hz, J = 5.0 Hz, 1H), 1.93 (td, J = 13.5 Hz, J = 2.5 Hz, 1H), 1.88 (s, 3H), 1.84 (dd, J = 14.5 Hz, J = 3.5 Hz, 1H), 1.70-1.60 (m, 2H), 1.53-1.47 (m, 2H), 1.28-1.13 (m, 12H), 1.10-1.01 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); [α]²²D = 8.3° (c = 2.43, CHCl₃).
(3S,4S)-3-[4-(1’,1’-Dimethylheptyl)-2,6-bis(tert-butyldimethylsilyloxy)phenyl]-4-isopropenyl-1-(methoxymethylene)cyclohexane (14.1): To a suspension of (methoxymethyl) triphenylphosphonium chloride (2.85 g, 8.3 mmol) in anhydrous THF (15 mL) at -30 °C was added n-butyllithium (3.19 mL, 7.99 mmol, 2.5M solution in hexane) and stirring was continued for 30 min. To the resultant red color suspension was added 13.1 (1.0 g, 1.66 mmol) dissolved in THF (10 mL) through cannula and the reaction mixture was warmed to room temperature over a period of 15 min. The reaction mixture was quenched with water and diluted with diethyl ether (60 mL). The organic layer was separated and the aqueous phase was extracted with diethyl ether (3x20 mL). The combined organic layer was washed with brine, dried (MgSO₄) and evaporated under reduced pressure to give yellow crude. Purification by flash column chromatography (0-8% diethyl ether : hexanes) gave a mixture of enol ethers 14.1 (0.75 g, 1.2 mmol, 72% yield, 1:4 (cis : trans) mixture of geometric isomers as colorless oil. $^1$H NMR (500 MHz, CDCl₃) δ: 6.33 (s, 1H), 6.32 (s, 1H), 5.11 (t, $J = 7.5$ Hz, 0.8H), 5.07 (t, $J = 7.5$ Hz, 0.2H), 4.58 (d, $J = 2.0$ Hz, 0.2H), 4.56 (d, $J = 2.0$ Hz, 0.8H), 4.40 (br s, 1H), 3.30-3.10 (m, 2H), 2.81 (t, $J = 12.5$ Hz, 0.2H), 2.67 (d, $J = 12.5$ Hz, 0.2H), 2.60-2.46 (m, 1.6H), 2.28-2.16 (m, 1.6H), 2.10-2.01 (m, 0.4H), 1.95 (q, $J = 7.5$ Hz, 2H), 1.89-1.74 (m, 1H), 1.52 (s, 3H), 1.50-1.44 (m, 2H), 1.33 (sept, $J = 7.0$ Hz, 2H), 1.26-1.12 (m, 13H, especially 1.20, s, 3H and 1.19, s, 3H), 1.06 (s, 9H), 1.00 (s, 9H), 0.91 (t, $J = 7.5$ Hz, 1H), 0.86 (t, $J = 7.0$ Hz, 3H), 0.33 (s, 2.4H), 0.32 (s, 2.4H), 0.31 (s, 0.6H), 0.24 (s, 3H), 0.20 (s, 2.4H), 0.17 (s, 0.6H); [$\alpha$]$^{22}_D = -7.82^\circ$ (c = 0.35, CHCl₃).
(3S,4S)-3-[4-(1′,1′-Dimethylheptyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-(methoxy-methylene) cyclohexane (14.2): To a stirred solution of 14.1 (50 mg, 0.08 mmol) in dry THF (5 mL) at 0 °C was added a solution of tetra-n-butylammonium fluoride (0.33 mL, 0.33 mmol, 1M solution in THF) and stirring was continued for 30 min. The reaction was quenched with saturated aqueous NaHCO₃ and diluted with ethyl acetate (30 mL). The organic phase was separated and aqueous layer extracted with ethyl acetate (2x25 mL). The combined organic layer was washed with water, brine and dried (MgSO₄). Evaporation of volatiles gave crude as colorless oil which on purification by flash chromatography (7-45% ethyl acetate : hexanes) gave ether analog 14.2 (24 mg, 0.06 mmol, 77% yield) as colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 6.26 (s, 1H), 6.18 (s, 1H), 5.80 (s, 1H), 4.65 (s, 1H), 4.59 (s, 2H), 4.48 (s, 1H), 3.56 (s, 3H), 3.08-2.94 (m, 2H), 2.86 (br d, J = 14.0 Hz, 1H), 2.68 (t, J = 15.0 Hz, 1H), 2.04 (br d, J = 16.5 Hz, 1H), 1.88-1.74 (m, 2H), 1.58 (s, 3H), 1.55 (s, 3H), 1.50-1.38 (m, 3H) 1.28-1.12 (m, 11H especially 1.19, s, 6H), 1.06-0.94 (m, 2H), 0.84 (t, J = 8.5 Hz, 1H); [α]²²D = -27.1° (c = 1.46, CHCl₃).
(1S,3S,4S)-3-(2,6-Bis((tert-butyldimethylsilyl)oxy)-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexanecarbaldehyde (15.1): To a solution of enol ether 14.1 (3 g, 4.77 mmol) in DCM (100 mL) was added trichloroacetic acid (3.12 g, 19.07 mmol) dissolved in water (10 mL) and the resultant biphasic reaction was allowed to stir for 5 h. The reaction mixture was quenched by addition of saturated NaHCO₃. The reaction solution was diluted with 100 mL of DCM and organic phase was washed with water, brine and dried over MgSO₄. Solvent was evaporated to give crude which was epimerized by stirring with anhydrous K₂CO₃ (1.97 g, 14.3 mmol) in dry MeOH (30 mL) for 12 h at 25 °C. Examination of an aliquot by ¹H NMR showed the completion of reaction. Reaction solvent was evaporated under high pressure and the crude was diluted with 200 mL ether, washed with saturated NH₄Cl solution. Combined organics were washed with water (2x50 mL), brine and dried (MgSO₄). Solvents evaporated to give crude which was purified by flash chromatography (0-7% diethyl ether : hexanes) to give 15.1 (1.4 g, 3.62 mmol, 76% yield) as gummy material. ¹H NMR (500 MHz, CDCl₃) δ: 9.58 (s, 1H), 6.32 (s, 1H), 6.30 (d, J = 1.0 Hz, 1H), 4.57 (s, 1H), 4.43 (s, 1H), 3.28 (dt, J = 12.0 Hz, J = 3.5 Hz, 1H, 3-axial), 3.01 (td, J = 11.0 Hz, J = 3.0 Hz, 1H, 4-axial), 2.36-2.34 (m, 1H), 2.32 (t, J = 13 Hz, 1H), 2.25-2.16 (m, 1H), 2.06-1.96 (m, 1H), 1.90-1.78 (m, 1H), 1.54 (s, 3H), 1.50-1.38 (m, 4H), 1.26-1.10 (m, 12H especially 1.18, s, 6H), 1.04 (s, 9H), 1.02 (s, 9H), 1.02-0.92 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H), 0.30 (s, 6H), 0.24 (s, 3H), 0.16 (s, 3H); [α]²⁰D = 7.6° (c = 0.66, CHCl₃).
(1S,3S,4S)-3-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-ylcyclohexane carbaldehyde (15.2): To a solution of 15.1 (40 mg, 0.064 mmol) in dry THF (5 mL) was added tetra-n-butylammonium fluoride (0.254 mL, 0.254 mmol, 1M solution in THF) and stirred for 1h at 0°C. Purple color reaction solution was quenched by saturated NH₄Cl. Organic layer was extracted in 30 mL diethyl ether, washed with water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to give crude, which was purified with flash column chromatography using 10-60% ethyl acetate : hexanes to give 15.2 (18 mg, 0.046 mmol, 73% yield) as colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 9.63 (s, 1H), 6.24 (s, 1H), 6.17 (s, 1H), 4.80 (d, J = 10.0 Hz, 2H), 4.66 (s, 1H), 4.50 (s, 1H), 3.18 (dt, J = 14.5 Hz, J = 4.5 Hz, 1H), 3.08-2.88 (m, 1H), 2.44 (t, J = 14.0 Hz, 1H), 2.20-2.0 (m, 2H), 1.96 (br d, J = 17 Hz, 1H), 1.92-1.82 (m, 1H), 1.72 (s, 3H), 1.54-1.40 (m, 4H), 1.28-1.10 (m, 12H especially 1.18, s, 6H), 1.04-0.94 (m, 2H), 0.84 (t, J = 8.5 Hz, 3H). ; [α]²²D = 31.2° (c = 1.46, CHCl₃).

2-((1S,2S,5S)-5-(hydroxymethyl)-2-(prop-1-en-2-yl)cyclohexyl)-5-(2-methyloctan-2-yl)-benzene-1,3-diol (16): To 15.2 (80 mg, 0.21 mmol) in MeOH (10 mL) at 0 °C was added sodium borohydride (31 mg, 0.83 mmol) and the reaction mixture was left for 30 minutes at the same temperature. The reaction was quenched by adding saturated aqueous NH₄Cl solution.
Organics were extracted with ethyl acetate (2x30 mL), washed with water, brine and dried (MgSO₄). Solvents were evaporated to give crude which was purified with flash column chromatography (0-20% ethyl acetate : hexanes) to give 16 (59.5 mg, 0.15 mmol, 74% yield) as colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 6.3-6.1 (m, 2H), 4.60 (s, 1H), 4.30 (s, 2H), 4.66 (s, 1H), 3.73 (d, J = 7.5 Hz, 1H), 3.38 (dd, J = 7.0 Hz, J = 3.5 Hz, 1H), 3.28-3.10 (m, 3H), 1.96-1.80 (m, 2H), 1.68-1.54 (m, 5H), 1.52-1.42 (m, 4H), 1.36-1.28 (m, 1H), 1.26-1.12 (m, 14H especially 1.18, s, 6H), 1.08-0.98 (m, 3H), 0.85 (t, J = 7.0 Hz, 3H); [α]²²D = 8.8° (c = 0.27, CH₃OH).

2-((1S,2S)-5-Hydroxy-2-(prop-1-en-2-yl)cyclohexyl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (17): To a solution of 13.2 (30 mg, 0.08 mmol) in MeOH (15 mL) at 0 °C was added sodium borohydride (9.14 mg, 0.24 mmol) and the reaction mixture was stirred for 30 min. Reaction mixture was quenched by the addition of saturated aqueous NH₄Cl solution and partially concentrated. The mixture was extracted with ethyl acetate (2x60 mL) and the organic layer was washed with water and brine and dried (MgSO₄). Evaporation of volatiles under reduced pressure gave crude yellow oil which upon purification by flash chromatography (20-85% ethyl acetate : hexanes) gave 17 (25 mg, 0.07 mmol, 83% yield) as a 90 : 10 (α : β) diastereomeric mixture as colorless oil. ¹H NMR (500 MHz, CDCl₃) of α-isomer δ: 6.23 (s, 1H), 6.17 (s, 1H), 5.32 (br s, 1H, phenolic OH), 5.05 (br s, 1H, phenolic OH), 4.64 (s, 1H), 4.48 (s, 1H), 3.79 (septet, J = 4.5 Hz, 1H), 3.15 (dt, J = 12.0 Hz, J = 3.0 Hz, 1H), 2.92 (dt, J = 12.0 Hz, J = 3.0 Hz, 1H), 2.18 (q, J = 12.0, 1H), 2.07 (br s, 1H), 1.97 (br d, J = 12.5 Hz, 1H), 1.92-1.79 (m, 1H),
1.79-1.66 (m, 1H), 1.55 (s, 3H), 1.50-1.41 (m, 4H), 1.24-1.10 (m, 12H, especially, s, 1.17, 6H), 1.04-0.94 (m, 2H), 0.84 (t, J = 6.5 Hz, 3H); [α]$_D^{22}$ = 11.1° (c = 6.68, CHCl$_3$).

(IS,3S,4S)-3-[(1′,1′-Dimethylheptyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-morpholinyl cyclohexane (18): To a solution of 13.1 (200 mg, 0.333 mmol) and morpholine (0.07 mL, 0.832 mmol) in DCM (10 mL) was added sodium triacetoxyborohydride (176 mg, 0.832 mmol) at room temperature and reaction mixture was stirred for 1 h. Acetic acid (0.02 mL, 0.33 mmol) was added to the reaction mixture and stirring was continued for additional 18 h. The reaction mixture was quenched with saturated NaHCO$_3$ (10 mL) and diluted with diethyl ether (50 mL). The organic phase was separated and the aqueous layer was extracted with diethyl ether (2x25 mL). The combined organic layer was washed with water and brine and dried (MgSO$_4$). Solvent was evaporated under reduced pressure to afford the crude product which was used for the next step without further purification. To this crude in dry THF (3 mL) was added a solution of tetra-$n$-butylammonium fluoride (0.3 mL, 0.298 mmol, 1.0 M solution in THF) at 0°C and stirred for 30 min. The reaction mixture was quenched with saturated aqueous NaHCO$_3$ and extracted with ethyl acetate (2x25 mL). The organic phase was washed with water and brine and dried (MgSO$_4$). Solvent was evaporated to give yellow oil which upon purification by flash chromatography (10-70% ethyl acetate : hexanes) gave 18 (61 mg, 0.138 mmol, 62% yield) as yellow oil. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.21 (s, 2H), 4.68 (s, 1H, Ph-OH), 4.51 (s, 1H, Ph-OH), 3.78 (s, 4H), 3.52 (t, J = 11.5 Hz, 1H, 3-axial), 2.97 (dt, J = 12.0 Hz, J = 3.0 Hz, 1H, 4-
axial), 2.64-2.38 (m, 4H), 2.31 (s, 1H), 2.16 (t, \( J = 13 \text{ Hz}, 1\text{H}, 1\)-axial), 2.07 (br d, \( J = 17.0 \text{ Hz}, 1\text{H}), 1.93 (d, \( J = 14.0 \text{ Hz}, 1\text{H}), 1.86 (q, \( J = 12.5 \text{ Hz}, 1\text{H}), 1.58 (s, 3\text{H}), 1.46 (t, \( J = 3.5 \text{ Hz}, 1\text{H}), 1.43 (s, 3\text{H}), 1.28-1.14 (m, 12\text{H}), 1.04-0.97 (m, 2\text{H}), 0.84 (t, \( J = 7.0 \text{ Hz}, 3\text{H}); [\alpha]^{22}_D = 10.3^\circ (c = 0.35, \text{CHCl}_3).

\[ \text{COOH} \]

(1S,3S,4S)-3-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexane carboxylic acid (19): To aldehyde 15.1 (100 mg, 0.16 mmol) and sodium dihydrogen phosphate (0.2 g, 1.7 mmol) was added water (1.5 mL), \( t \)-BuOH (1.05 mL, 1.10 mmol) and 2-methyl-2-butene (0.6 mL, 6.7 mmol) at room temperature and the reaction was stirred vigorously for 10 minutes. Sodium chlorite solution (40 mg, 0.41 mmol) in water was added and the reaction mixture was stirred for 4 h. Reaction mixture was diluted with diethyl ether (10 mL). The organic layer was separated, washed with water (2x10 mL), brine and dried (MgSO\(_4\)). Solvents were evaporated under reduced pressure to afford crude which was utilized as such for the deprotection step without any purification. To the crude in dry THF (3 mL) at 0 °C was added a solution of tetra-\( n \)-butylammonium fluoride (0.16 mL, 0.158 mmol, 1M solution in THF) drop wise and stirred for 30 min. Reaction was quenched with saturated aqueous NaHCO\(_3\) (5 mL) and the mixture was extracted with ethyl acetate. The organic layer was washed with water, brine and dried (MgSO\(_4\)). Evaporation of volatiles under reduced pressure gave crude which upon purification by flash chromatography (0-7% methanol : DCM) afforded desired product 19 (40 mg, 0.1 mmol, 61% yield) as colorless oil. \(^1\text{H NMR} (500 \text{ MHz}, \text{CDCl}_3) \delta: 6.23 (s, 1\text{H}), 6.16 (s,
1H), 4.65 (s, 1H), 4.49 (s, 1H), 3.13 (dt, J = 12.0 Hz, J = 3.0 Hz, 1H, C-3), 2.95 (dt, J = 12.0 Hz, J = 3.0 Hz, 1H, C-4), 2.52 (tt, J = 12.5 Hz, J = 1.5 Hz, 1H, C-1), 2.27 (q, J = 12.5 Hz, 1H, C-2), 2.14-2.06 (m, 1H), 1.99 (br d, J = 14.0 Hz, 1H), 1.84 (dd, J = 13.0 Hz, J = 3.0 Hz, 1H), 1.66-1.54 (m, 4H, especially 1.57, s, 3H), 1.48-1.42 (m, 3H), 1.36-1.11 (m, 12H, especially s, 1.18, 6H), 1.04-0.94 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); [α]$_{22}^D$ = 22.7° (c = 0.61, CHCl$_3$).

(1R,4R,5R)-4-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-6,6-dimethylbicyclo[3.1.1]
heptan-2-one (22): $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.40 (s, 2H), 3.99 (t, J = 8.0 Hz, 1H), 3.70 (dd, J = 17.0 Hz, J = 7.5 Hz, 1H), 2.55 (t, J = 5.5 Hz, 1H), 2.53 (q, J = 9.0 Hz, 1H), 2.47-2.41 (m, 1H), 2.39 (d, J = 11.0 Hz, 1H), 2.24 (t, J = 5.5 Hz, 1H), 1.52-1.46 (m, 2H), 1.32 (s, 3H), 1.28-1.14 (m, 12 H), 1.10-1.02 (m, 2H), 1.02-0.94 (m, 21H, especially, 0.99, s, 18H), 0.85 (t, J = 7.0 Hz, 3H), 0.27 (s, 12H); [α]$_{22}^D$ = 63.3° (c = 1.74, CH$_3$OH).

(1R,4R,5R)-4-(2,6-Bis((tert-butyldimethylsilyl)oxy)-4-(2-methyloctan-2-yl)phenyl)-6,6-
dimethylbicyclo[3.1.1]heptan-2-one (23): $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.40 (s, 2H), 3.99 (t, J = 8.0 Hz, 1H), 3.70 (dd, J = 17.0 Hz, J = 7.5 Hz, 1H), 2.55 (t, J = 5.5 Hz, 1H), 2.53 (q, J = 9.0 Hz, 1H), 2.47-2.41 (m, 1H), 2.39 (d, J = 11.0 Hz, 1H), 2.24 (t, J = 5.5 Hz, 1H), 1.52-1.46 (m,
2H), 1.32 (s, 3H), 1.28-1.14 (m, 12H), 1.10-1.02 (m, 2H), 1.02-0.94 (m, 21H, especially, 0.99, s, 18H), 0.85 (t, J = 7.0 Hz, 3H), 0.27 (s, 12H). [α]_{D}^{22} = 46.2^\circ (c = 1.71, \text{CHCl}_3). 

(3R,4R)-3-(2,6-Bis((tert-butyldimethylsilyl)oxy)-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexanone (24.1): $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.34 (s, 2H), 4.64 (d, J = 2.0 Hz, 1H), 4.49 (d, J = 1.5 Hz, 1H), 3.63 (ddd, J = 13.5 Hz, J = 11.5 Hz, J = 4.0 Hz, 1H), 3.40 (dt, J = 12.0 Hz, J = 3.5 Hz, 1H), 3.20 (t, J = 14.5 Hz, 1H), 2.49-2.43 (m, 2H), 2.31 (dd, J = 14.5 Hz, J = 4.0 Hz, 1H), 2.05-1.98 (m, 1H), 1.82-1.70 (m, 1H), 1.55 (s, 3H), 1.50-1.43 (m, 2H), 1.27-1.14 (m, 12H, especially 1.20, s, 3H and 1.19, s, 3H), 1.10-0.94 (m, 20H, especially 1.06, br s, 9H and 0.99, br s, 9H), 0.84 (t, J = 7.5 Hz, 3H), 0.34 (s, 3H), 0.32 (s, 3H), 0.23 (s, 3H), 0.15 (s, 3H); [α]_{D}^{22} = -16.7^\circ (c = 9.51, \text{CHCl}_3). 

(3R,4R)-3-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexanone (24.2): Deprotection reaction was carried out as described in the preparation of analog 13.2 to give 24.2 as colorless oil in 78% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.42 (d, J = 1.5 Hz, 1H), 6.28 (d, J = 1.5 Hz, 1H), 5.01 (d, J = 1.5 Hz, 1H), 4.99 (s, 1H), 4.94 (s, 1H), 3.62 (d, J = 2.0 Hz, 1H), 3.02 (s, 1H), 2.36 (br s, 1H), 2.12 (dd, J = 12.0 Hz, J = 2.0 Hz, 1H), 2.01 (dt, J = 13.5 Hz, J = 5.0 Hz, 1H), 1.93 (td, J = 13.5 Hz, J = 2.5 Hz, 1H), 1.88 (s, 3H), 1.85 (dd, J = 14.5 Hz, J = 3.5 Hz, 1H), 1.78 (s, 3H).
183 Hz, 1H), 1.70-1.60 (m, 2H), 1.54-1.46 (m, 2H), 1.28-1.14 (m, 12H), 1.10-1.00 (m, 2H), 0.84 (t, $J = 6.5$ Hz, 3H); $[\alpha]_{D}^{22} = -9.2^\circ$ ($c = 0.78$, CHCl$_3$).

$$\text{(2-((1R,2R)-5-(Methoxymethylene)-2-(prop-1-en-2-yl)cyclohexyl)-5-(2-methyloctan-2-yl)-1,3-phenylene)bis(oxy))bis(tert-butyldimethylsilane) (25.1): } \text{H NMR (500 MHz, CDCl}_3\text{)} \delta:$$

6.33 (s, 1H), 6.32 (s, 1H), 5.11 (t, $J = 7.5$ Hz, 0.8H), 5.07 (t, $J = 7.5$ Hz, 0.2H), 4.58 (d, $J = 2.0$ Hz, 0.2H), 4.56 (d, $J = 2.0$ Hz, 0.8H), 4.40 (br s, 1H), 3.30-3.10 (m, 1H), 2.81 (t, $J = 12.5$ Hz, 0.2H), 2.67 (d, $J = 12.5$ Hz, 0.2H), 2.60-2.46 (m, 1H), 2.28-2.16 (m, 1H), 2.10-2.01 (m, 0.4H), 1.95 (q, $J = 7.5$ Hz, 2H), 1.89-1.74 (m, 1H), 1.52 (s, 3H), 1.50-1.44 (m, 2H), 1.33 (sept, $J = 7.0$ Hz, 2H), 1.26-1.12 (m, 13H, especially 1.20, s, 3H and 1.19, s, 3H), 1.06 (s, 9H), 1.00 (s, 9H), 0.91 (t, $J = 7.5$ Hz, 1H), 0.86 (t, $J = 7.0$ Hz, 3H), 0.33 (s, 2.4H), 0.32 (s, 2.4H), 0.31 (s, 0.6H), 0.24 (s, 3H), 0.20 (s, 2.4H), 0.17 (s, 0.6H); $[\alpha]_{D}^{22} = 7.82^\circ$ ($c = 0.45$, CHCl$_3$).

$$\text{(1R,3R,4R)-3-(2,6-Bis((tert-butyldimethylsilyl)oxy)-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexanecarbaldehyde (26.1): } \text{H NMR (500 MHz, CDCl}_3\text{)} \delta:$$

9.58 (s, 1H), 6.32 (s, 1H), 6.30 (d, $J = 1.0$ Hz, 1H), 4.57 (s, 1H), 4.43 (s, 1H), 3.28 (dt, $J = 12.0$ Hz, $J = 3.5$ Hz, 1H, 3-xial), 3.01 (td, $J = 11.0$ Hz, $J = 3.0$ Hz, 1H, 4-axial), 2.36-2.34 (m, 1H), 2.36-2.26 (m, 1H), 2.06-1.96 (m, 1H), 1.90-1.78 (m, 2H), 1.54 (s, 3H), 1.50-1.38 (m, 4H), 1.26-1.10 (m, 12H).
especially 1.18 (s, 6H), 1.04 (s, 9H), 1.02-0.92 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H), 0.30 (s, 6H), 0.24 (s, 3H), 0.16 (s, 3H); [α]^{22}_D = -48.3° (c = 7.81, CHCl_3); [α]^{22}_D = -0.6° (c = 2.56, CHCl_3).

(1R,3R,4R)-3-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexanecarbaldehyde (26.2): ^1H NMR (500 MHz, CDCl_3) δ: 9.62 (s, 1H), 6.24 (d, J = 1.5 Hz, 1H), 6.17 (d, J = 1.5 Hz, 1H), 4.70 (d, J = 5.5 Hz, 1H), 4.66 (d, J = 2.0 Hz, 1H), 4.50 (d, J = 2.0 Hz, 1H), 3.18 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 2.95 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 2.41 (dt, J = 11.0 Hz, J = 2.5 Hz, 1H), 2.10 (q, J = 13.0 Hz, 1H), 2.06-2.0 (m, 1H), 1.96 (dd, J = 11.5 Hz, J = 2.0 Hz, 1H), 1.92-1.84 (m, 1H), 1.58 (s, 3H), 1.52-1.40 (m, 4H), 1.28-1.24 (m, 1H), 1.22 (t, J = 7.0 Hz, 1H) 1.19-1.12 (m, 10H), 1.04-0.94 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); [α]^{22}_D = -31.8° (c = 1.10, CHCl_3).

(1R,3R,4R)-3-(2,6-Bis((tert-butyldimethylsilyl)oxy)-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexyl)methanol (27.1): To a solution of 26.1 (430 mg, 0.81 mmol) in MeOH (10 mL) at 0 °C was added sodium borohydride (123 mg, 3.25 mmol) and the reaction mixture was stirred for 30 minutes at the same temperature. The reaction was quenched by adding saturated aqueous NH_4Cl solution. Organics were extracted with ethyl acetate (2x100 mL),
washed with water, brine and dried (MgSO₄). Solvents were evaporated to give crude which was purified with flash column chromatography 0-20% ethyl acetate : hexanes to give 27.1 (430 mg, 0.70 mmol, 86% yield) as colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 6.30 (s, 2H), 4.53 (d, J = 2.5 Hz, 1H), 4.38 (d, J = 2.0 Hz, 1H), 3.76 (dd, J = 10.5 Hz, J = 7.0 Hz, 1H), 3.52-3.42 (m, 1H), 3.26 (dq, J = 12.0 Hz, J = 3.5 Hz, 1H), 3.08-2.98 (m, 1H), 2.02-1.94 (m, 1H), 1.92-1.84 (m, 1H), 1.82-1.74 (m, 1H), 1.68-1.55 (m, 2H), 1.52 (s, 3H), 1.49-1.41 (m, 2H), 1.26-1.10 (m, 14H, especially 1.18, s, 6H), 1.05 (s, 9H), 1.04 (s, 9H), 1.01-0.94 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H), 0.30 (s, 3H), 0.27 (s, 3H), 0.23 (s, 3H), 0.15 (s, 3H); [α]²²_D = -36.0° (c = 0.10, CHCl₃).

2-((1R,2R,5R)-5-(Hydroxymethyl)-2-(prop-1-en-2-yl)cyclohexyl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (27.2): ¹H NMR (500 MHz, CDCl₃) δ: 6.25 (d, J = 1.5 Hz, 1H), 6.16 (d, J = 1.5 Hz, 1H), 4.95 (s, 1H), 4.89 (s, 1H), 4.66 (d, J = 2.0 Hz, 1H), 4.48 (s, 1H), 3.51 (d, J = 7.0 Hz, J = 2.0 Hz, 2H), 3.14-3.09 (m, 1H), 2.91 (dt, J = 11.5 Hz, J = 3.0 Hz, 1H), 1.87 (dd, J = 8.0 Hz, J = 2.5 Hz, 1H), 1.81 (dd, J = 8.0 Hz, J = 3.0 Hz, 1H), 1.77 (d, J = 8.5 Hz, 2H), 1.74-1.66 (m, 1H), 1.57 (s, 3H), 1.49 (dt, J = 13.0 Hz, J = 3.0 Hz, 1H) 1.47-1.42 (m, 2H), 1.22 (t, J = 7.0 Hz, 1H), 1.19-1.12 (m, 12H), 1.03-0.95 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); [α]²²_D = -18.6° (c = 0.07, CHCl₃).
(2-((1R,2R,5R)-5-(((tert-Butyldimethylsilyl)oxy)methyl)-2-(prop-1-en-2-yl)cyclohexyl)-5-(2-methyloctan-2-yl)-1,3-phenylene)bis(oxy))bis(tert-butylidimethylsilane) (28): To a solution of 27.1 (0.4 g, 0.65 mmol), imidazole (177 mg, 2.59 mmol) and DMAP (16 mg, 0.13 mmol) in anhydrous DMF (10 mL) was added tert-butyldimethylchlorosilane (293 mg, 1.95 mmol) dissolved in DMF (10 mL) under positive nitrogen pressure and stirred at room temperature for 18 h. The reaction mixture was quenched by addition of saturated aqueous NaHCO₃ and extracted with 3x100 mL of diethyl ether. The combined ethereal extracts were washed with 2x100 mL of water and brine and dried over MgSO₄. Solvent evaporation gave a pale yellow oil which was purified by flash chromatography using 0-8% diethyl ether : hexane to produce 28 (0.38 g, 0.52 mmol, 80% yield) as colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 6.31 (d, J = 2.0 Hz, 1H), 6.30 (d, J = 2.0 Hz, 1H), 4.56 (d, J = 1.5 Hz, 1H), 4.40 (dd, J = 2.5 Hz, J = 1.5 Hz, 1H), 3.42 (dd, J = 6.5 Hz, J = 3.5 Hz, 2H), 3.23 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 3.01 (dt, J = 12.0 Hz, J = 3.5 Hz, 1H), 1.90-1.84 (m, 1H), 1.79-1.75 (m, 1H), 1.73 (q, J = 12.5 Hz, 1H), 1.68-1.62 (m, 2H), 1.55 (s, 3H), 1.49-1.44 (m, 2H), 1.43-1.36 (m, 1H), 1.24-1.14 (m, 13H, 1.20, s, 3H, 1.19, s, 3H), 1.07 (s, 9H), 1.04 (s, 9H), 1.02-0.96 (m, 2H), 0.89 (s, 9H), 0.84 (t, J = 7.5 Hz, 3H), 0.31 (s, 6H), 0.24 (s, 3H), 0.15 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H) ; [α] D²² = -3.6° (c = 9.15, CHCl₃).
2-((1R,2R,4R)-2-(2,6-Bis((tert-butyldimethylsilyl)oxy)-4-(2-methyloctan-2-yl)phenyl)-4-(((tert-butyldimethylsilyl)oxy)methyl)cyclohexyl)acrylaldehyde (29.1): To a suspension of 28 (100 mg, 0.13 mmol) and selenium dioxide (4.5 mg, 0.04 mmol) in CH₂Cl₂ (7 mL) at 0 °C was added tert-butyl hydroperoxide (0.2 mL, 2.35 mmol) drop wise and the reaction mixture was stirred at room temperature overnight. The reaction solution was quenched with saturated sodium sulfite solution and diluted with 60 mL diethyl ether. Organic layer was washed with water (3x50 mL), brine and dried (MgSO₄). Volatiles were evaporated under reduced pressure to give yellow crude which was purified by flash column chromatography (0-15% ethyl acetate : hexanes) to give 29.1 (65 mg, 0.087 mmol, 64% yield) as yellow oil. ¹H NMR (500 MHz, CDCl₃) δ: 9.27 (s, 1H), 6.31 (s, 1H), 6.28 (d, J = 1.5 Hz, 1H), 6.27 (d, J = 2.0 Hz, 1H), 5.72 (s, 1H), 3.52 (dt, J = 7.0 Hz, J = 3.0 Hz, 1H), 3.43 (d, J = 6.0 Hz, 2H), 3.35 (dt, J = 7.0 Hz, J = 3.5 Hz, 1H), 1.98-1.90 (m, 1H), 1.86-1.78 (m, 2H), 1.70 (dd, J = 13.5 Hz, J = 1.5 Hz, 1H), 1.66-1.56 (m, 1H), 1.48-1.40 (m, 2H), 1.22-1.08 (m, 14H), 1.05 (s, 9H), 1.04 (s, 9H), 1.0-0.93 (m, 2H), 0.88 (s, 9H), 0.83 (t, J = 7.0 Hz, 3H), 0.30 (s, 3H), 0.28 (s, 3H), 0.26 (s, 3H), 0.16 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H); [α]²² D = 25.8° (c = 1.19, CHCl₃).
2-((1R,2R,4R)-2-(2,6-Dihydroxy-4-(2-methyloctan-2-yl)phenyl)-4-(hydroxymethyl)cyclohexyl)acrylaldehyde (29.2): To a solution of 29.1 (65 mg, 0.087 mmol) in dry THF (5 mL) was added TBAF (0.44 mL, 0.44 mmol) and stirred for 1h at 0°C. Purple colored reaction solution was quenched by saturated NH₄Cl. Organic layer was extracted in 30 mL diethyl ether, washed with water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to give crude, which was purified with flash column chromatography (10-60% ethyl acetate : hexanes) to give 29.2 (30 mg, 0.023 mmol, 85% yield) as colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 9.31 (s, 1H), 6.35 (s, 1H), 6.24 (s, 1H), 6.15 (s, 1H), 5.81 (s, 1H), 5.03-4.94 (m, 2H), 3.52 (d, J= 6.5 Hz, 2H), 3.48 (dt, J= 7.0 Hz, J= 2.5 Hz, 1H), 3.34 (dt, J= 11.5 Hz, J= 3.5 Hz, 1H), 1.88 (dd, J= 13.0 Hz, J= 3.5 Hz, 2H), 1.80-1.70 (m, 1H), 1.67-1.53 (m, 1H), 1.46-1.36 (m, 2H), 1.28-1.19 (m, 3H), 1.18-1.08 (m, 10H, especially 1.15, s, 6H), 1.02-0.92 (m, 2H), 0.84 (t, J= 7.0 Hz, 3H); [α]²²_D = -36.0° (c = 0.10, CHCl₃).

2-((1R,2R,5R)-5-(Hydroxymethyl)-2-(3-hydroxyprop-1-en-2-yl)cyclohexyl)-5-(2-methyloctan-2-yl)benzene-1,3-diol (30): To 29.2 (10 mg, 0.025 mmol) in MeOH (5 mL) at 0 °C was added sodium borohydride (4.7 mg, 0.124 mmol) and the reaction mixture was left for 30 minutes at the same temperature. The reaction was quenched by adding saturated aqueous NH₄Cl.
solution. Organics were extracted with 2x20 mL ethyl acetate, washed with water, brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified with flash column chromatography 40-90% ethyl acetate : hexanes to give 30 (8 mg, 0.02 mmol, 80% yield) as gummy material. ¹H NMR (500 MHz, CDCl₃) δ: 6.31 (d, J = 2.0 Hz, 1H), 6.18 (d, J = 1.5 Hz, 1H), 4.81 (d, J = 4.0 Hz, 2H), 4.71 (br s, 1H, OH), 4.13 (d, J = 12.5 Hz, 1H), 3.96 (d, J = 12.5 Hz, 1H), 3.50 (d, J = 6.5 Hz, 2H), 3.16 (dt, J = 11.5 Hz, J = 3.0 Hz, 1H), 2.94 (dt, J = 12.0 Hz, J = 3.0 Hz, 1H), 1.95-1.87 (m, 2H), 1.80-1.74 (m, 1H), 1.74-1.66 (m, 2H), 1.49-1.42 (m, 2H), 1.25 (s, 3H), 1.23-1.12 (m, 11H), 1.04-0.96 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); [α]²²_D = -32.4° (c = 0.10, CHCl₃).

(1R,3R,4R)-3-(2,6-Bis((tert-butyldimethylsilyl)oxy)-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexanecarboxylic acid (31.1): To aldehyde 26.1 (900 mg, 1.463 mmol) and sodium dihydrogen phosphate (1.93 g, 16.10 mmol) was added water (15 mL), t-BuOH (10.50 mL, 110 mmol) and 2-methyl-2-butene (3.26 mL, 30.7 mmol) at room temperature and the reaction was stirred vigorously for 10 minutes. Sodium chlorite solution (397 mg, 4.39 mmol) in water was added and the reaction mixture was stirred for 4 h. Reaction mixture was diluted with diethyl ether (100 mL). Organic layer was separated, washed with water (2 x 50 mL), brine and dried (MgSO₄). Solvent was evaporated under reduced pressure to afford crude which upon purification by flash chromatography (5-45% ethyl acetate : hexane) afforded desired product 31.1 (580 mg, 0.919 mmol, 63% yield) as colorless oil. ¹H NMR (500 MHz, CD₃OD) δ: 6.37 (d, J = 2.0 Hz, 1H), 6.34 (d, J = 2.0 Hz, 1H), 4.58 (d, J = 2.5 Hz, 1H), 4.39 (d, J = 2.5 Hz, 1H), 3.08
(dt, $J = 12.0$ Hz, $J = 3.0$ Hz, 1H), 2.37-2.23 (m, 2H), 2.02 (br d, $J = 12.5$ Hz, 1H), 1.82-1.74 (m, 2H), 1.59 (dq, $J = 12.5$ Hz, $J = 3.0$ Hz, 1H), 1.54 (s, 3H), 1.52-1.46 (m, 2H), 1.44 (dd, $J = 12.0$ Hz, $J = 3.0$ Hz, 1H), 1.24-1.14 (m, 13H, especially 1.20, s, 3H and 1.19, s, 3H), 1.08 (s, 9H), 1.06 (s, 9H), 1.03-0.95 (m, 2H), 0.84 (t, $J = 7.0$ Hz, 3H), 0.34 (s, 6H), 0.27 (s, 3H), 0.17 (s, 3H); $[\alpha]^22_D = -11.8^\circ$ (c = 5.83, CHCl$_3$).

$\textbf{1H NMR}$ (500 MHz, CDCl$_3$) $\delta$: 6.23 (s, 1H), 6.15 (s, 1H), 4.66 (s, 1H), 4.49 (s, 1H), 3.15 (dt, $J = 12.0$ Hz, $J = 3.0$ Hz, 1H), 2.96 (dt, $J = 12.0$ Hz, $J = 3.0$ Hz, 1H), 2.51 (t, $J = 12.5$ Hz, 1H), 2.27 (q, $J = 12.5$ Hz, 1H), 2.08 (br d, $J = 11.5$ Hz, 1H), 1.98 (br d, $J = 11.5$ Hz, 1H), 1.83 (br d, $J = 13.0$ Hz, 1H), 1.68-1.54 (m, 4H, especially 1.54, s, 3H), 1.53-1.40 (m, 3H), 1.36-1.11 (m, 12H), 0.99 (br s, 2H), 0.84 (t, $J = 7.0$ Hz, 3H); $[\alpha]^22_D = -10.2^\circ$ (c = 0.11, CHCl$_3$).
(1R,3R,4R)-3-[4-(1',1'-Dimethylheptyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-
carboxamido-cyclohexane (32): To a solution of 31.1 (40 mg, 0.063 mmol) in toluene (3 mL)
was added thionyl chloride (0.014 mL, 0.190 mmol) in DMF (1 mL) and the reaction mixture
was stirred for 2 h at 70 °C. Volatiles were evaporated under reduced pressure to obtain yellow
oil. To this yellow oil in DCM (3 mL) at -5 °C was added ammonium hydroxide (0.63 mL, 0.634
mmol, 1M solution in water). Following the addition the reaction mixture was gradually warmed
to the room temperature and stirring was continued until the completion of the reaction (12 h).
The reaction mixture was diluted with diethyl ether (20 mL) and the organic layer was washed
with water and brine and dried (MgSO₄) to give crude which was used as such for the next step.
The crude was dissolved in dry THF (3 mL) cooled to 0 °C and a solution of tetra-n-
butylammonium fluoride (0.19 mL, 0.190 mmol) was added. The reaction mixture was stirred
for 30 min. The reaction mixture was quenched by the addition of saturated aqueous NaHCO₃
and the mixture was extracted with ethyl acetate (25 mL). The organic phase was washed with
water, brine and dried (MgSO₄). Solvents were evaporated to give crude which upon purification
by flash chromatography (10-65% ethyl acetate : hexane) afforded desired product 32 (13 mg,
0.032 mmol, 51% yield, over 2 steps) as colorless oil. ³¹H NMR (500 MHz, CDCl₃) δ: 6.23 (d, J
= 2.0 Hz, 1H), 6.20 (d, J = 2.0 Hz, 1H), 5.51 (br s, 1H), 5.39 (br s, 1H), 4.66 (s, 1H), 4.49 (s,
1H), 3.15 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 3.01 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 2.42-2.28
(m, 2H), 2.08-1.98 (m, 1H), 1.94-1.82 (m, 2H), 1.57 (s, 3H), 1.52-1.42 (m, 3H), 1.26-1.12 (m,
13H, especially 1.18, s, 6H), 1.03-0.94 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); [α]$_{D}^{22}$ = -88.2° (c = 0.01, CHCl$_3$).

(1$R$,3$R$,4$R$)-3-[4-(1’,1’-Dimethylheptyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-morpholino-carboxamidocyclohexane (33): The synthesis was carried out analogous to the preparation of 32 using 31.1 (35 mg, 0.055 mmol), toluene (3 mL), thionyl chloride (0.01 mL, 0.166 mmol), DMF (1 mL), DCM (3 mL), morpholine (0.02 mL, 0.277 mmol), tetra-$n$-butylammonium fluoride (0.22 mL, 0.22 mmol, 1M solution in THF). Purification by flash chromatography (20-90% ethyl acetate : hexanes) gave 33 (15 mg, 0.032 mmol, 57% yield, over 2 steps) as colorless oil. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.23 (d, J = 2.0 Hz, 1H), 6.21 (d, J = 2.0 Hz, 1H), 4.64 (d, J = 2.0 Hz, 1H), 4.44 (br s, 1H), 3.72-3.62 (m, 4H), 3.62-3.53 (m, 4H), 3.18 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 3.11 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 2.71-2.60 (m, 1H), 2.37 (q, J = 12.5 Hz, 1H), 1.86-1.72 (m, 3H), 1.68 (br d, J = 13.0 Hz, 1H), 1.59 (s, 3H), 1.52-1.40 (m, 3H), 1.32-1.12 (m, 12H, especially 1.18, s, 6H), 1.06-0.96 (m, 2H), 0.84 (t, J = 7.5 Hz, 3H); [α]$_{D}^{22}$ = 26.5° (c = 0.07, CHCl$_3$).
(1R,3R,4R)-3-[4-(1′,1′-Dimethylheptyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-
methoxycarbonyl-cyclohexane (34): To a solution of acid 31.2 (10 mg, 0.025 mmol) in methanol (3 mL) and toluene (3 mL) was added diazo(trimethylsilyl)methane (28.4 mg, 0.248 mmol) at 0 °C and the reaction mixture was warmed to room temperature and stirred overnight. The solvent was evaporated and the residue was purified by flash chromatography on silica gel (5-50% ethyl acetate : hexane) to afford product 34 (6 mg, 9.60 μmol, 71% yield) as yellow oil.

$^1$H NMR (500 MHz, CDCl$_3$) δ: 6.23 (d, $J = 1.5$ Hz, 1H), 6.17 (d, $J = 1.5$ Hz, 1H), 4.67 (s, 1H), 4.66 (d, $J = 2.0$ Hz, 1H), 4.54 (s, 1H), 4.50 (d, $J = 2.0$ Hz, 1H), 3.65 (s, 3H), 3.12 (dt, $J = 11.5$ Hz, $J = 3.0$ Hz, 1H), 2.96 (dt, $J = 11.5$ Hz, $J = 3.0$ Hz, 1H), 2.49 (ddt, $J = 12.0$ Hz, $J = 3.5$ Hz, 1H), 2.25 (q, $J = 12.5$ Hz, 1H), 2.05 (td, $J = 12.0$ Hz, $J = 2.0$ Hz, 1H), 1.96 (qdt, $J = 12.0$ Hz, $J = 2.0$ Hz, 1H), 1.57 (s, 3H), 1.49-1.40 (m, 3H), 1.28-1.12 (m, 12H, especially 1.18, s, 6H), 1.04-0.94 (m, 2H), 0.84 (t, $J = 7.0$ Hz, 3H); [α]$^{22}_D = -27.7^o$ (c = 0.50, CHCl$_3$).
(1R,3R,4R)-3-[4-(1',1'-Dimethylheptyl)-2,6-dihydroxyphenyl]-4-isopropenyl-1-morpholinyl-cyclohexane (35): To a solution of 24.1 (200 mg, 0.33 mmol) and morpholine (0.07 mL, 0.832 mmol) in DCM (10 mL) was added sodium triacetoxyborohydride (176 mg, 0.832 mmol) at room temperature and reaction mixture was stirred for 1 h. Acetic acid (0.02 mL, 0.33 mmol) was added to the reaction mixture and stirring was continued for additional 18 h. The reaction mixture was quenched with saturated NaHCO₃ (10 mL) and diluted with diethyl ether (50 mL). The organic phase was separated and the aqueous layer was extracted with diethyl ether (2x25 mL). The combined organic layer was washed with water and brine and dried (MgSO₄). Solvent was evaporated under reduced pressure to afford the crude product which was used for the next step without further purification. To this crude in dry THF (3 mL) was added a solution of tetra-n-butylammonium fluoride (0.3 mL, 0.298 mmol, 1.0 M solution in THF) at 0 °C and stirred for 30 min. The reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with ethyl acetate (2x25 mL). The organic phase was washed with water, brine and dried (MgSO₄). Solvent was evaporated to give yellow oil which upon purification by flash chromatography (10-70% ethyl acetate : hexanes) gave 35 (20 mg, 0.045 mmol, 60% yield) as colorless oil. ¹H NMR (500 MHz, CDCl₃) δ: 6.22 (s, 2H), 5.30 (br s, 1H), 4.68 (d, J = 2.0 Hz, 1H), 4.50 (d, J = 2.0 Hz, 1H), 3.81 (t, J = 4.5 Hz, 4H), 3.52 (dt, J = 12.0 Hz, J = 3.5 Hz, 1H), 3.0 (dt, J = 12.0 Hz, J = 3.5 Hz, 1H), 2.62 (br s, 2H), 2.56 (br s, 2H), 2.38 (t, J = 2.5 Hz, 1H), 2.25-2.16 (m, 1H), 2.09 (td, J
= 14.0 Hz, J = 3.0 Hz, 1H), 1.95 (qd, J = 14.5 Hz, J = 3.0 Hz, 1H), 1.88 (dq, J = 12.5 Hz, J = 3.0 Hz, 1H), 1.62-1.50 (m, 4H especially 1.57, s, 3H), 1.50-1.40 (m, 3H), 1.30-1.06 (m, 12H, especially 1.17, s, 6H), 1.06-0.94 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); [α]_{22}^{D} = -8.4^{o} (c = 3.28, CHCl_{3}).

(3R,4R)-3-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexanone (36.2): To 24.2 (100 mg, 0.268 mmol) in acetone (5 mL) was added potassium carbonate (148 mg, 1.074 mmol) and iodomethane (0.168 mL, 2.68 mmol) and resultant solution was stirred for 24 h. The reaction mixture was quenched by addition of saturated aqueous NH_{4}Cl and sodium thiosulfate solution and extracted with 3x20 mL of ethyl acetate. The combined organic layer was washed with 2x30 mL of water and brine and dried over MgSO_{4}. Solvent evaporation gave a yellow crude which was purified by flash chromatography using 0-8% diethyl ether : hexanes to produce 36.2 (83 mg, 0.207 mmol, 77% yield) as yellow oil. ^1H NMR (500 MHz, CDCl_{3}) δ: 6.43 (s, 2H), 4.55 (d, J = 2.0 Hz, 1H), 4.45 (t, J = 1.5 Hz, 1H), 3.81 (br s, 3H), 3.74 (br s, 3H), 3.71 (dt, J = 11.0 Hz, J = 4.5 Hz, 1H), 3.30 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 3.10 (t, J = 13.5 Hz, 1H), 2.50 (dt, J = 13.0 Hz, J = 10.5 Hz, 1H), 2.49-2.42 (m, 1H), 2.34 (ddd, J = 15.0 Hz, J = 4.5 Hz, J = 2.0 Hz, 1H), 2.02-1.92 (m, 1H), 1.82 (dq, J = 13.0 Hz, J = 6.0 Hz, 1H), 1.56-1.50 (m, 5H, especially 1.51, s, 3H), 1.26 (s, 6H), 1.24-1.15 (m, 6H), 1.06-0.96 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); [α]_{22}^{D} = -24.3^{o} (c = 1.06, CHCl_{3}).
(3S,4S)-3-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-(prop-1-en-2-yl)cyclohexanone (36.1): Using the same procedure as above for 36.2 yielded 36.1 (100 mg, 0.25 mmol, 74% yield) as yellow oil. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.43 (s, 2H), 4.55 (d, $J = 2.0$ Hz, 1H), 4.45 (t, $J = 1.5$ Hz, 1H), 3.81 (br s, 3H), 3.74 (br s, 3H), 3.71 (dt, $J = 11.0$ Hz, $J = 4.5$ Hz, 1H), 3.30 (dt, $J = 11.5$ Hz, $J = 3.5$ Hz, 1H), 3.10 (t, $J = 13.5$ Hz, 1H), 2.50 (dt, $J = 13.0$ Hz, $J = 10.5$ Hz, 1H), 2.49-2.42 (m, 1H), 2.34 (ddd, $J = 15.0$ Hz, $J = 4.5$ Hz, $J = 2.0$ Hz, 1H), 2.02-1.92 (m, 1H), 1.82 (dq, $J = 13.0$ Hz, $J = 6.0$ Hz, 1H), 1.56-1.50 (m, 5H, especially 1.51, s, 3H), 1.26 (s, 6H), 1.24-1.15 (m, 6H), 1.06-0.96 (m, 2H), 0.84 (t, $J = 7.0$ Hz, 3H); [α]$^2$$_D$ = 22.2° ($c = 4.66$, CHCl$_3$).

(3R,4S)-3-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-isopropylcyclohexanone (37.2): To a solution of 36.2 (30 mg, 0.075 mmol) in MeOH (3 mL) was added palladium on carbon (15 mg, 0.144 mmol) and the reaction mixture was stirred under hydrogen atmosphere for 10 h at room temperature. The reaction mixture was diluted with diethyl ether and filtered through a small celite pad to remove the catalyst. Solvents were evaporated to give the crude material which upon purification by flash chromatography (20-60% ethyl acetate : hexanes) gave 37.2 (25 mg, 0.062 mmol, 83% yield) as oil. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.46 (s, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.60 (dt, $J = 12.5$ Hz, $J = 5.0$ Hz, 1H), 3.02 (dd, $J = 15.0$ Hz, $J = 12.5$ Hz, 1H),
2.50-2.36 (m, 3H), 2.30 (ddd, $J = 14.5$ Hz, $J = 4.5$ Hz, $J = 2.0$ Hz, 1H), 1.99-1.92 (m, 1H), 1.58-1.53 (m, 2H), 1.27 (s, 6H), 1.25-1.17 (m, 6H), 1.10-1.03 (m, 2H), 0.88 (d, $J = 7.0$ Hz, 3H), 0.84 (t, $J = 7.0$ Hz, 3H); $[\alpha]^{22}_D = -28.6^\circ$ ($c = 0.11$, CHCl$_3$).

(3S,4R)-3-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-isopropylcyclohexanone (37.1): Using the same conditions as above to give 37.2 produced 37.1 (20 mg, 0.058 mmol, 80% yield) as oil. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 6.46 (s, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.60 (dt, $J = 12.5$ Hz, $J = 5.0$ Hz, 1H), 3.02 (dd, $J = 15.0$ Hz, J = 12.5 Hz, 1H), 2.50-2.36 (m, 3H), 2.30 (ddd, $J = 14.5$ Hz, $J = 4.5$ Hz, $J = 2.0$ Hz, 1H), 1.99-1.92 (m, 1H), 1.58-1.53 (m, 2H), 1.27 (s, 6H), 1.25-1.17 (m, 6H), 1.10-1.03 (m, 2H), 0.88 (d, $J = 7.0$ Hz, 3H), 0.84 (t, $J = 7.0$ Hz, 3H), 0.68 (d, $J = 7.0$ Hz, 3H); $[\alpha]^{22}_D = 23.1^\circ$ ($c = 0.74$, CHCl$_3$).

(1S,3S,4R)-3-(2,6-Dimethoxy-4-(2-methyloctan-2-yl)phenyl)-4-isopropylcyclohexanol (38): To 36.1 (20 mg, 0.05 mmol) in MeOH (5 mL) at 0 $^\circ$C was added sodium borohydride (10 mg, 0.26 mmol) and the reaction mixture was left for 30 minutes at the same temperature. The reaction was quenched by adding saturated aqueous NH$_4$Cl solution. Organics were extracted with 2x10 mL ethyl acetate, washed with water, brine and dried over MgSO$_4$. Solvents were evaporated to give crude which was purified using flash column chromatography 5-30% ethyl
acetate: hexanes to give 38 (16 mg, 0.04 mmol, 80% yield) as colorless oil. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.42 (s, 1H), 6.41 (s, 1H), 4.46 (d, J = 2.0 Hz, 1H), 4.36 (dd, J = 2.5 Hz, J = 1.5 Hz, 1H), 3.78 (s, 3H), 3.75 (s, 3H), 3.30 (dt, J = 11.5 Hz, J = 3.5 Hz, 1H), 2.94 (dt, J = 11.0 Hz, J = 4.5 Hz, 1H), 2.10-2.0 (m, 2H), 1.92-1.84 (m, 1H), 1.76-1.69 (m, 1H), 1.60-1.49 (m, 4H), 1.47 (s, 3H), 1.43 (br d, J = 11.5 Hz, 2H), 1.25 (s, 6H), 1.23-1.12 (m, 5H), 1.06-0.96 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); $[\alpha]^{22}_D = 13.3^\circ$ (c = 0.06, CH$_3$OH).

**Detailed description of methods used for in vitro testing:**

**Studies done in Biochemistry lab in CDD:**

1. **Membrane Preparations from Tissue Culture Sources:** HEK293 cells expressing the mCB2 or hCB2 receptor are used for membrane preparations according to the method described by Abadji et al.$^{132}$ The resulting pellet is resuspended in 10 mM Tris-chloride, pH 7.4 with 5 mM MgCl$_2$ and 2 mM EDTA (TME) and stored at -80 °C for no longer than two months. Protein content is assayed by using the Bio-Rad protein assay according to the manufacturer’s protocol.

2. **Membrane Preparations from Tissue Sources:** Frozen rat brains (CB1 source) are obtained from Pel-Freeze Biologicals (Rogers, AK) and stored at -80 °C until use. Membranes were prepared according to the method described by Dodd et al. and adapted for use in our laboratory as previously reported.$^{133-135}$

3. **rCB1, hCB2, and mCB2 Binding Assays:** All compounds synthesized for this grant are tested for their ability to bind to CB1 and CB2 receptors using rat brain or HEK293 cell membranes expressing hCB2 membrane preparations, respectively, as previously described via competition-equilibrium binding using $[^3]$H]CP-55,940.$^{135,136}$ The results were analyzed using
nonlinear regression to determine the actual IC$_{50}$ of the ligand (Prizm by GraphPad Software, Inc.) and the $K_i$ values are calculated from the IC$_{50}$.

4. **Signal Transduction Assays (cAMP assay):** HEK-293 cells transfected with rCB1, mCB2, or hCB2 receptor are used with the PerkinElmer’s Lance ultra cAMP kit following the protocol of the manufacturer. Briefly, the assays were carried out in 384-well format using 1000 cells/well. Test compounds were added to wells containing stimulation buffer and 2 $\mu$M forskolin followed by cell suspension. After 30 minutes stimulation, the Eu-cAMP tracer and Ulight-anti-cAMP are added to the plate and incubated at room temperature for 1h prior to detection via PerkinElmer Envision; data are analyzed using GraphPad Prism software.

5. **Preliminary distribution in brain/plasma and drug oral bioavailability:** Mice (CD-1, weighing 25-30 g) are injected intravenously or orally with 0.1-2 mg/kg of the compound mixture. Fifteen minutes post-IV injection, or 30 and 60 minutes post-oral administration, the animals are sacrificed by decapitation followed by blood collection (~500 µL) and tissue dissection; samples are flash frozen with liquid nitrogen to prevent post-mortem degradation of the compounds. Tissues (plasma or brain) are extracted following published procedures and analyzed using a Thermo-Finnigan Quantum Ultra triple quadrupole mass spectrometer in SRM mode with an Agilent 1100 HPLC front-end with internal standards used for quantitation.

**β-Arrestin Assay done in Duke University:** Cells were treated with a serial concentration of test compounds. Known agonists were used as positive control. Cells were fixed with 1% PFA for 40 mins after administration of compounds. Images were acquired and analyzed as described below. Data are from 2-3 independent experiments with duplicate samples.
**Image-based β-arrestin2 translocation assay** - U2OS cells stably expressing the CB1-E cannabinoid receptors and β-arrestin2-GFP were split into glass-bottom 384 well plates (MGB101-1-2-LG, MatriCal, Spokane, WA) at a density of 8,000 cells/30 μl media/well using a Multidrop 384 dispenser (Thermo Electron Corporation). The plates were incubated overnight at 37 °C in 5% CO2. The following day, culture medium was changed to 30 μl/well of clear minimum Eagle’s medium (MEM) with 10mM HEPES. A set of serial diluted 4x concentration of each AM compound (10 mM in DMSO) was prepared in the same medium and applied to cells at a volume of 10 μl. The cells were incubated with compound for 40 mins at 37 °C prior to fixation with an equal volume of PBS containing 2% paraformaldehyde (sigma). Plates were stored at 4 °C until analysis. β-arrestin2-GFP aggregates were identified. Dose response curves were analyzed by nonlinear regression techniques using GraphPad Prism 5.0 software (GraphPad, San Diego, CA), and data were fitted to sigmoidal dose-response curves to obtain EC$_{50}$ and efficacy values.
Chapter 6

Bicyclic Benzophenone Analogs

6.1 Introduction

The therapeutic potential of CB2 agonists is very well known. The activation of the CB2 receptor affects a large number of immune responses from inflammation to neuroprotection and shows analgesic effects in pain models. In order to get CB2 selective ligands, many templates have been explored including CBD template (Chapter 5). Moore’s group has synthesized classical tricyclic cannabinoid analogs with aromatic group in the side chain. Substitution of dimethyl heptyl chain with keto aryl, dimethyl aryl or dithiolane aryl group enhanced CB2 binding affinity and selectivity. The Makriyannis group has also explored different templates including bipenyl bicyclic, bicyclic and tricyclic keto template with side chain variations to developed potent CB2 selective ligands. Later on, hybrid potent CB2 selective compounds were synthesized by merging tricyclic Moore’s template with the biaryl template from Makriyannis group to give biaryl benzophenone type compounds.

Figure 6.1: Earlier templates from Morre’s and Makriyannis’ Groups which lead towards the current template design and synthesis.

Utilizing literature knowledge of CB2 selective ligands, our group previously developed a series of novel bicyclic biaryl cannabinoid ligands (Fig. 6.2). SAR on the bicyclic template was
explored by varying the size of cyclic rings and aromatic groups. All earlier synthesized analogs presented in Figure 6.2 provide an understanding of the groups well tolerated for CB2 binding affinity. Analogs with phenyl ring (1 & 2) show high mCB2 affinity; replacing the phenyl ring with ethylphenyl (3) or large cyclohexyl ring (4) drastically decreases the affinity at mCB2 receptors. Also, replacement of phenyl group with benzyl group (5) improves CB1 affinity but decreases mCB2 affinity. Two key analogs 1 and 2 showed high affinity for mCB2 but very low affinity at hCB2. There was a need to extend this project further, to resolve the above species subtype selectivity issue. Utilizing the similar approach, of designing enantiomers as used in Chapter 5, compounds with opposite stereochemistry and with CB2 favoring groups were designed and synthesized.

**Figure 6.2:** Some important bicyclic cannabinoids synthesized earlier in our research group
6.2 Chemistry

Ultimately, bicyclic keto analogs with (S)-stereochemistry and variable side chains were synthesized by coupling the aromatic fragment 9 with the (-)-(S)-nopinone diacetate mixture 10 (Scheme 6.1). Aromatic fragment synthesis began with the coupling of commercially available dimethoxy benzonitrile 6 with different Grignards reagents under copper bromide mediated coupling conditions, to give side chain keto intermediate 7 in 70-74% yield. The keto group was protected as its dithiolane under lewis acid conditions to give 8 in 71-78% yield. Methoxy groups of 8 were demethylated using BBr3 to give free phenolic compound 9 in 71-82% yield.

Coupling of 9 with (-)-(S)-diacetates 10 in acidic conditions using pTSA gave 11 in 20-25% yield. Yield of the reaction was low and starting material was recovered. Dithiolane group was deprotected with silver nitrate to give ketone compound 12 in 70-73% yield. Using analogous chemistry as above the enantiomeric opposite terpene analogs 14 and 15 with (R)-stereochemistry were synthesized in 21% and 67% yield respectively.

Analogs with methoxy protected phenols and (R)-stereochemistry were synthesized by methylation of 14 using MeI under basic conditions to give 16 in 51% yield. Compound 16 was treated with silver nitrate to give the deprotected keto compound 17 in 73-80% yield.
Scheme 6.1: Synthesis of bicyclic keto compounds

Analogs with (S)-stereochemistry

\[
\text{MgBr}_2 + \begin{array}{c} \text{MeO} \\ \text{CN} \end{array} \xrightarrow{\text{CuBr}} \text{Reflux, 2h} \xrightarrow{70-74\%} \begin{array}{c} \text{OMe} \\ \text{MeO} \end{array} \xrightarrow{\text{Ethanedithiol}} \text{BF}_3 \cdot \text{Et}_2\text{O}, 16\text{h} \xrightarrow{71-78\%} \begin{array}{c} \text{OMe} \\ \text{MeO} \end{array}
\]

\( R = \text{cyclopentyl} \)
\( R = \text{phenyl} \)
\( R = \text{benzyl} \)

\( \text{BBr}_3 \xrightarrow{0^\circ C, 12\text{h} \atop 71-82\%} \begin{array}{c} \text{OH} \\ \text{S} \end{array} \xrightarrow{\text{pTSA, 3 days} \atop 20-25\%} \begin{array}{c} \text{OH} \\ \text{S} \end{array} \xrightarrow{\text{AgNO}_3} \text{12h, 70-73\%} \begin{array}{c} \text{OH} \\ \text{S} \end{array}
\)

\( R = \text{cyclopentyl (11.1)} \)
\( R = \text{phenyl (11.2)} \)
\( R = \text{benzyl (11.3)} \)

Enantiomeric Analogs with (R)-stereochemistry

\( \text{AcO} \xrightarrow{0^\circ C, 12\text{h} \atop 71-82\%} \begin{array}{c} \text{OH} \\ \text{S} \end{array} \xrightarrow{\text{pTSA, 3 days} \atop 21\%} \begin{array}{c} \text{OH} \\ \text{S} \end{array} \xrightarrow{\text{AgNO}_3} \text{12h, 67\%} \begin{array}{c} \text{OH} \\ \text{S} \end{array}
\)

\( R = \text{cyclopentyl (12.1)} \)
\( R = \text{phenyl (12.2)} \)
\( R = \text{benzyl (12.3)} \)

Methylation of the phenolic hydroxy

\( \text{MeI, K}_2\text{CO}_3 \xrightarrow{12\text{h}, 51.5\%} \begin{array}{c} \text{OH} \\ \text{S} \end{array} \xrightarrow{\text{MeO} \atop 73-80\%} \begin{array}{c} \text{MeO} \\ \text{S} \end{array}
\)

\( R = \text{cyclopentyl (16.1)} \)
\( R = \text{cyclopentyl (17.1)} \)
\( R = \text{phenyl (17.2)} \)
6.3 Results and Discussion

Firstly, to understand the SAR and trend of other bicyclic keto compounds synthesized earlier in our lab, the bicyclic keto compound with (R)-stereochemistry and smaller cyclopentyl side chain, AM8644 (15), was synthesized. Compound 15 showed high binding affinity of 19 nM at mCB2 but very low affinity at both rCB1 (7425 nM) and hCB2 (2927 nM) similar to the earlier explored SAR. Masking the phenolic hydroxyl groups as methoxy or removing them has imparted significant CB2 affinity and selectivity in the various cannabinoid templates. To understand the role of phenolic hydroxyl groups in this template, hydroxyl group were protected as methoxy groups with different side chain substitutions with (R)-stereochemistry and the resultant compounds AM8647 (16), AM8648 (17.1) and AM8649 (17.2) showed no binding affinities for CB receptors. This confirmed that hydroxyl group is required for this template for binding to CB receptors. To understand and resolve the issue of species subtype selectivity, analogs with opposite stereochemistry were designed and synthesized. Enantiomers with (S)-stereochemistry enhanced the binding affinities at hCB2 and resolved the species subtype issue to some extent. Dithiane analog AM8645 (11.2) with phenyl side chain displayed moderate affinity at both CB1 and CB2 receptors and no selectivity for CB2 receptor, whereas, its keto analog AM8646 (12.2) showed high binding affinity at CB2 receptors with not much difference in the mCB2 (20 nM) and hCB2 (100nM) affinities and 54-fold selectivity over rCB1 (Table 6.1). Although analog AM8651 (12.1) showed good binding affinities of 1 nM and 100 nM at both mCB2 and hCB2 respectively still there was species subtype selectivity issue.

Interestingly, dithiane compound with benzyl side chain, AM8652 (11.3) showed high binding affinities of 18 nM and 9 nM at mCB2 and hCB2 receptors respectively with no species subtype selectivity and it also has moderate affinity of 188 nM at CB1 receptor. On the other hand,
deprotected keto analog AM8653 (12.3) showed moderate binding affinities at mCB2 (65 nM) and hCB2 (159 nM) receptors with 15-fold selectivity over rCB1 receptor. The analogs exhibiting high affinity such as 11.3, 12.2 and 15 when tested for the functional potency in cAMP assay; behaved as weak CB2 antagonists (Table 6.2). Benzylic compound AM8652 (11.3), also behaved as potent CB2 antagonist in β-arrestin recruitment assay (Fig. 6.3). Thus, by reversing the stereochemistry and varying the side chain with different aromatic groups, high affinity CB2 cannabinoid ligands with no species subtype selectivity have been synthesized.

Table 6.1: Bicyclic keto analogs, structures and binding data

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>AM No.</th>
<th>Comp. Structure</th>
<th>Kᵢ (nM) rCB1</th>
<th>Kᵢ (nM) mCB2</th>
<th>Kᵢ (nM) hCB2</th>
<th>CB1/hCB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>8644</td>
<td><img src="https://example.com/structure15.png" alt="Structure" /></td>
<td>7425 ± 4.2</td>
<td>19 ± 0.8</td>
<td>2927 ± 3.6</td>
<td>2.5</td>
</tr>
<tr>
<td>16</td>
<td>8647</td>
<td><img src="https://example.com/structure16.png" alt="Structure" /></td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>N.A</td>
<td>N.A.</td>
</tr>
<tr>
<td>17.1</td>
<td>8648</td>
<td><img src="https://example.com/structure17.1.png" alt="Structure" /></td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>N.A</td>
<td>N.A.</td>
</tr>
<tr>
<td>17.2</td>
<td>8649</td>
<td><img src="https://example.com/structure17.2.png" alt="Structure" /></td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>N.A</td>
<td>N.A.</td>
</tr>
<tr>
<td>Section</td>
<td>Code</td>
<td>N.A.</td>
<td>Value 1 ± Error 1</td>
<td>Value 2 ± Error 2</td>
<td>Value 3 ± Error 3</td>
<td>Value 4 ± Error 4</td>
</tr>
<tr>
<td>---------</td>
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<td>-----</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>11.2</td>
<td>8645</td>
<td></td>
<td>458 ± 1.2</td>
<td>145 ± 0.9</td>
<td>200 ± 1.4</td>
<td>2.3</td>
</tr>
<tr>
<td>12.2</td>
<td>8646</td>
<td></td>
<td>5400 ± 4.8</td>
<td>20 ± 0.2</td>
<td>100 ± 0.8</td>
<td>54</td>
</tr>
<tr>
<td>12.1</td>
<td>8651</td>
<td></td>
<td>2330 ± 3.2</td>
<td>1 ± 0.02</td>
<td>203 ± 1.3</td>
<td>12</td>
</tr>
<tr>
<td>11.3</td>
<td>8652</td>
<td></td>
<td>188 ± 0.6</td>
<td>18 ± 0.1</td>
<td>9 ± 0.07</td>
<td>21</td>
</tr>
<tr>
<td>12.3</td>
<td>8653</td>
<td></td>
<td>2431 ± 2.2</td>
<td>65 ± 1.1</td>
<td>159 ± 1.7</td>
<td>15</td>
</tr>
</tbody>
</table>

N.A. = Not Applicable
Table 6.2: cAMP data of two high affinity analogs

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>AM No.</th>
<th>hCB1</th>
<th>hCB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>8644</td>
<td>-20% at 10nM</td>
<td>-11% at 10nM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-18% at 1uM</td>
<td>-34% at 1uM</td>
</tr>
<tr>
<td>12.2</td>
<td>8646</td>
<td>-13% at 10nM</td>
<td>-17% at 10nM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-25% at 1uM</td>
<td>-80% at 1uM</td>
</tr>
</tbody>
</table>

Figure 6.3: β-arrestin recruitment by AM8652 as CB1 weak antagonist (left) and CB2 potent agonist (right).

6.4 Conclusion:

The species subtype selectivity issue has been addressed by synthesizing compounds with opposite stereochemistry. Opposite stereochemistry analogs with phenyl (12.2) and benzyl substitutions (11.3) showed some selectivity towards hCB2 receptor as compared to CB1 receptor. More SAR needs to be done on this bicyclic template to improve affinity, functional potency and selectivity.
6.5 Experimental:

Cyclopentyl(3,5-dimethoxyphenyl)methanone (7.1): To a solution of 3,5-dimethoxybenzonitrile (5 g, 30.6 mmol) and catalytic copper (I) bromide (0.132 g, 0.919 mmol) in THF (30 mL) was added cyclopentyl magnesium bromide (6.11 g, 35.2 mmol) under positive nitrogen pressure. The resultant dark brown color solution was refluxed for 2h. Reaction mixture was quenched by adding 20 mL water after cooling to the room temperature. After 5 minutes, 50 mL sulfuric acid (15%) was added and the reaction was again refluxed for 45 minutes. Organics were extracted in 100 mL ether, washed with water, brine and dried over MgSO$_4$. Solvents were evaporated under reduced pressure to give brown oil which was purified by flash column chromatography in 2-20% ethyl acetate : hexanes system to give 7.1 (5.3g, 22.7 mmol) in 74% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.13-7.09 (m, 2H), 6.64-6.61 (m, 1H), 3.82 (s, 6H), 3.65 (quintet, $J = 7.5$ Hz, 1H), 1.96-1.84 (m, 4H), 1.78-1.58 (m, 4H).

(3,5-Dimethoxyphenyl)(phenyl)methanone (7.2): Using the same procedure as above to give 7.2 in 70% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.62-7.55 (m, 2H), 7.22 (t, $J = 7.0$ Hz, 2H), 7.24-7.18 (m, 1H), 6.56 (s, 2H), 6.61 (t, $J = 3.0$ Hz, 1H), 3.82 (s, 6H).
1-(3,5-Dimethoxyphenyl)-2-phenylethanone (7.3): Using the same procedure as above to give 7.3 in 72% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.20-7.14 (m, 3H), 7.0 (dd, $J$ = 5 Hz, 2H), 6.77 (d, $J$ = 3.5 Hz, 2H), 6.35 (t, $J$ = 3.5 Hz, 1H), 3.74 (s, 6H), 3.60 (s, 2H)

2-Cyclopentyl-2-(3,5-dimethoxyphenyl)-1,3-dithiolane (8.1): To a solution of 7.1 (5 g, 21.34 mmol) in DCM (30 mL) was added ethane-1,2-dithiol (1.969 mL, 23.48 mmol) and boron trifluoride etherate (1.082 ml, 8.54 mmol) under argon atmosphere. The resultant solution was stirred for 16h at 25 °C. The reaction was quenched by adding 20 mL NaOH (10%) and stirring for 10 minutes. Organics were extracted in 100 mL ether (3x), washed with water, brine and dried over MgSO$_4$. Solvents were evaporated under reduced pressure to give crude which was purified by flash column chromatography using 3-25% ethyl acetate : hexanes to give 8.1 (5g, 16.10 mmol, 75 % yield) as yellow oil. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.90 (d, $J$ = 2.0 Hz, 2H), 6.26 (s, 1H), 3.75 (s, 6H), 3.30-3.20 (m, 2H), 3.14-3.06 (m, 2H), 2.76-2.66 (m, 1H), 1.72-1.62 (m, 2H), 1.62-1.52 (m, 2H), 1.52-1.38 (m, 4H).
2-(3,5-Dimethoxyphenyl)-2-phenyl-1,3-dithiolane (8.2): Using the same procedure as above to give 8.2 in 78% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.60-7.53 (m, 2H), 7.24 (t, $J = 7.5$ Hz, 2H), 7.20-7.14 (m, 1H), 6.56 (s, 2H), 6.33 (t, $J = 3.0$ Hz, 1H), 3.72 (s, 6H), 3.34-3.28 (m, 2H), 3.26-3.20 (m, 2H)

2-Benzyl-2-(3,5-dimethoxyphenyl)-1,3-dithiolane (8.3): Using the same procedure as above to give 8.3 in 71% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.20-7.14 (m, 3H), 7.0 (dd, $J = 5$ Hz, 2H), 6.77 (d, $J = 3.5$ Hz, 2H), 6.35 (t, $J = 3.5$ Hz, 1H), 3.74 (s, 6H), 3.60 (s, 2H), 3.30-3.26 (m, 2H), 3.22-3.18 (m, 2H).

5-(2-Cyclopentyl-1,3-dithiolan-2-yl)benzene-1,3-diol (9.1): To a solution of 2-cyclopentyl-2-(3,5-dimethoxyphenyl)-1,3-dithiolane (6 g, 19.33 mmol) in DCM (30 mL) at 0 °C was added boron tribromide (4.57 mL, 48.3 mmol) slowly and the reaction was stirred for 24 h. The reaction was cooled to 0 °C and ice-cold water was added slowly. The reaction mixture was diluted with 100 mL ethyl acetate and organics were extracted, washed with brine and dried over MgSO$_4$. Solvents were evaporated under reduced pressure to give crude which was purified by
flash column chromatography using 10-55% ethyl acetate : hexanes to give 9.1 (4.5 g, 15.93 mmol, 82 % yield) as yellow gum. $^1$H NMR (500 MHz, CDCl$_3$) δ: 6.90 (d, $J = 2.0$ Hz, 2H), 6.26 (s, 1H), 3.30-3.20 (m, 2H), 3.14-3.06 (m, 2H), 3.14-3.06 (m, 2H), 2.76-2.66 (m, 1H), 1.72-1.62 (m, 2H), 1.62-1.52 (m, 2H), 1.52-1.38 (m, 4H).

5-(2-Phenyl-1,3-dithiolan-2-yl)benzene-1,3-diol (9.2): Using the same procedure as above to give 9.2 in 71% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.60-7.53 (m, 2H), 7.24 (t, $J = 7.5$ Hz, 2H), 7.20-7.14 (m, 1H), 6.56 (s, 2H), 6.68 (d, $J = 1.5$ Hz, 2H), 6.25 (d, $J = 2.0$ Hz, 1H), 3.20-3.05 (m, 4H).

5-(2-Benzyl-1,3-dithiolan-2-yl)benzene-1,3-diol (9.3): Using the same procedure as above to give 9.3 in 71% yield. $^1$H NMR (500 MHz, CDCl$_3$) δ: 7.14-7.06 (m, 3H), 6.96 (dd, $J = 7.0$ Hz, $J = 2.0$ Hz, 2H), 6.83 (br s, 2H), 6.68 (d, $J = 1.5$ Hz, 2H), 6.25 (d, $J = 2.0$ Hz, 1H), 3.52 (s, 2H), 3.20-3.05 (m, 4H).
(1S,4S,5S)-4-(4-(2-Cyclopentyl-1,3-dithiolan-2-yl)-2,6-dihydroxyphenyl)-6,6-
dimethylbicyclo[3.1.1]heptan-2-one (11.1): Using the same procedure as for the opposite enantiomer to give 11.1 in 25% yield. $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$: 6.80 (s, 2H), 4.03 (d, $J = 9.0$ Hz, 1H), 3.36-3.26 (m, 2H), 3.20-3.10 (m, 2H), 2.84-2.74 (m, 1H), 2.63 (d, $J = 9.5$ Hz, 1H), 2.52 (d, $J = 6.0$ Hz, 1H), 2.50 (d, $J = 5.5$ Hz, 1H), 2.42 (d, $J = 9.0$ Hz, 1H), 2.20 (dt, $J = 5.0$ Hz, $J = 1.5$ Hz, 1H), 1.76-1.66 (m, 2H), 1.65-1.58 (m, 2H), 1.58-1.48 (m, 5H), 1.39 (s, 3H), 0.98 (s, 3H); $[\alpha]^{22}_D = -29.5^\circ$ (c = 0.81, CH$_3$OH).

(1S,4S,5S)-4-(2,6-Dihydroxy-4-(2-phenyl-1,3-dithiolan-2-yl)phenyl)-6,6-
dimethylbicyclo[3.1.1]heptan-2-one (11.2): Using the same procedure as above to give 11.2 in 22% yield. $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$: 7.60-7.53 (m, 2H), 7.24 (t, $J = 7.5$ Hz, 2H), 7.20-7.14 (m, 1H), 6.56 (s, 2H), 4.01 (t, $J = 8.0$ Hz, 1H), 3.71 (dd, $J = 19.5$ Hz, $J = 8.0$ Hz, 1H), 3.42-3.32 (m, 4H), 2.59 (d, $J = 10.0$ Hz, 1H), 2.52-2.44 (m, 2H), 2.42 (dd, $J = 19.0$ Hz, $J = 9.0$ Hz, 1H), 2.18 (t, $J = 5.5$ Hz, 1H), 1.36 (s, 3H), 0.95 (s, 3H); $[\alpha]^{22}_D = -31.5^\circ$ (c = 0.13, CHCl$_3$).
(1S,4S,5S)-4-(4-(2-Benzyl-1,3-dithiolan-2-yl)-2,6-dihydroxyphenyl)-6,6-dimethylbicyclo[3.1.1]hept-2-one (11.3): Using the same procedure as above to give 11.3 in 20% yield. $^1$H NMR (500 MHz, Methanol-$d_4$) δ: 7.68 (br s, 1H), 7.16-7.08 (m, 3H), 6.97 (dd, $J = 8.0$ Hz, $J = 2.0$ Hz, 2H), 6.61 (s, 2H), 4.04 (t, $J = 8.0$ Hz, 1H), 3.64 (dd, $J = 19.0$ Hz, $J = 3.0$ Hz, 1H), 3.25-3.14 (m, 4H), 2.58 (d, $J = 10.5$ Hz, 1H), 2.54 (dd, $J = 7.5$ Hz, $J = 3.0$ Hz, 2H), 2.49 (quintet, $J = 5.0$ Hz, 1H), 2.28 (t, $J = 5.0$ Hz, 1H), 1.37 (s, 3H), 0.99 (s, 3H); $[\alpha]_{22}^D = -59.2^\circ$ (c = 0.66, CHCl$_3$).

(1S,4S,5S)-4-(4-(Cyclopentanecarbonyl)-2,6-dihydroxyphenyl)-6,6-dimethylbicyclo[3.1.1]hept-2-one (12.1): Using the same procedure as for the opposite enantiomer to give 12.1 in 73% yield. $^1$H NMR (400 MHz, Methanol-$d_4$) δ: 6.9 (s, 2H), 4.09 (t, $J = 8.0$ Hz, 1H), 3.76-3.62 (m, 2H), 2.59 (d, $J = 10.5$ Hz, 1H), 2.53 (q, $J = 5.0$ Hz, 1H), 2.50 (d, $J = 5.0$ Hz, 1H), 2.45 (dd, $J = 18.5$ Hz, $J = 9.0$ Hz, 1H), 2.20 (dt, $J = 5.0$ Hz, $J = 1.5$ Hz, 1H), 1.96-1.87 (m, 2H), 1.86-1.76 (m, 2H), 1.74-1.62 (m, 4H), 1.38 (s, 3H), 0.98 (s, 3H); $[\alpha]_{22}^D = -34.3^\circ$ (c = 0.06, CHCl$_3$).
(1S,4S,5S)-4-(4-Benzoyl-2,6-dihydroxyphenyl)-6,6-dimethylbicyclo[3.1.1]heptan-2-one

(12.2): Using the same procedure as above to give 12.2 in 70% yield. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.75 (dd, $J = 8.0$ Hz, $J = 1.0$ Hz, 2H), 7.64-7.58 (m, 1H), 7.51 (t, $J = 8.0$ Hz, 2H), 6.74 (s, 2H), 4.13 (t, $J = 8.0$ Hz, 1H), 3.73 (dd, $J = 19.5$ Hz, $J = 8.0$ Hz, 1H), 2.61 (d, $J = 10.5$ Hz, 1H), 2.55 (dd, $J = 10.0$ Hz, $J = 5.0$ Hz, 1H), 2.53-2.48 (m, 1H), 2.46 (d, $J = 8.5$ Hz, 1H), 2.24 (t, $J = 5.0$ Hz, 1H), 1.39 (s, 3H), 0.99 (s, 3H); $[\alpha]^{22}_D = -25.3.3^\circ$ (c = 0.12, CH$_3$OH).

(1S,4S,5S)-4-(2,6-Dihydroxy-4-(2-phenylacetyl)phenyl)-6,6-dimethylbicyclo[3.1.1]heptan-2-one (12.3): Using the same procedure as above to give 12.3 in 70% yield. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.33-7.26 (m, 2H), 7.26-7.17 (m, 3H), 7.02 (s, 2H), 4.16 (s, 2H), 4.02 (t, $J = 8.5$ Hz, 1H), 3.45 (dd, $J = 9.0$ Hz, $J = 3.0$ Hz, 1H), 2.66 (d, $J = 9.0$ Hz, 1H), 2.65-2.58 (m, 1H), 2.53 (quintet, $J = 5.5$ Hz, 1H), 2.42 (d, $J = 10.5$ Hz, 1H), 2.31 (t, $J = 5.0$ Hz, 1H), 1.38 (s, 3H), 1.02 (s, 3H); $[\alpha]^{22}_D = -31.0^\circ$ (c = 1.28, CH$_3$OH).
(1R,4R,5R)-4-(4-(2-Cyclopentyl-1,3-dithiolan-2-yl)-2,6-dihydroxyphenyl)-6,6-dimethylbicyclo [3.1.1]heptan-2-one (14): To a degassed solution of diacetates (1.974 g, 8.29 mmol) and 9.1 (1.8 g, 6.37 mmol) in CHCl₃ (30 mL) was added p-toluenesulfonic acid monohydrate (1.576 g, 8.29 mmol). The reaction was stirred in dark for 3.5 days. Reaction mixture was quenched with saturated NaHCO₃ solution and diluted with 100 mL ethyl acetate. Organics were extracted, washed with brine and dried over MgSO₄. Solvents were evaporated to give crude which was purified by flash column chromatography using 10-60% ethyl acetate:hexanes to give 14 (560 mg, 1.338 mmol, 21% yield) as yellow gum. ¹H NMR (400 MHz, Methanol-d₄) δ: 6.80 (s, 2H), 4.03 (d, J = 9.0 Hz, 1H), 3.36-3.26 (m, 2H), 3.20-3.10 (m, 2H), 2.84-2.74 (m, 1H), 2.63 (d, J = 9.5 Hz, 1H), 2.52 (d, J = 6.0 Hz, 1H), 2.50 (d, J = 5.5 Hz, 1H), 2.42 (d, J = 9.0 Hz, 1H), 2.20 (dt, J = 5.0 Hz, J = 1.5 Hz, 1H), 1.76-1.66 (m, 2H), 1.65-1.58 (m, 2H), 1.58-1.48 (m, 5H), 1.39 (s, 3H), 0.98 (s, 3H); [α]²²D = 46.8° (c = 1.78, CH₃OH).

(1R,4R,5R)-4-(4-(Cyclopentanecarbonyl)-2,6-dihydroxyphenyl)-6,6-dimethylbicyclo [3.1.1]heptan-2-one (15): To 14 (100 mg, 0.239 mmol) in ethanol (10 mL) and water (1 mL) was added silver nitrate (162 mg, 0.956 mmol) and the resulting solution was stirred at 25 °C for
3 hrs. TLC in 30% ethyl acetate: hexanes showed completion of reaction. The reaction solution was filtered through a small pad of celite. The reaction mixture was diluted with 20 mL diethyl ether and 10 mL water. The organic layer was separated, washed with water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to afford crude product. Purification by flash chromatography on silica gel using 10-65% ethyl acetate: hexanes afforded desired product as 15 (55 mg, 0.161 mmol, 67% yield). ¹H NMR (400 MHz, Methanol-d₄) δ 6.93 (s, 2H), 4.10 (t, J = 8.0 Hz, 1H), 3.50 - 3.79 (m, 2H), 2.33 - 2.68 (m, 4H), 2.21 (t, J = 5.0 Hz, 1H), 1.90 (d, J = 7.5 Hz, 2H), 1.82 (dd, J = 7.5 Hz, J = 5.5 Hz, 2H), 1.67 (d, J = 3.0 Hz, 4H), 1.38 (br s, 3H), 0.98 (br s, 3H); [α]²²_D = 36.1° (c = 0.82, CH₃OH).

(1R,4R,5R)-4-(4-(2-Cyclopentyl-1,3-dithiolan-2-yl)-2,6-dimethoxyphenyl)-6,6-dimethylbicyclo [3.1.1] heptan-2-one (16): To a solution of 14 (100 mg, 0.239 mmol) in DMF (15 mL) was added K₂CO₃ (660 mg, 4.78 mmol) and methyl iodide (0.3 mL, 4.78 mmol). The reaction mixture was stirred overnight at 25 °C. Reaction suspension was evaporated under pressure to get crude and crude was diluted with 100 mL diethyl ether and 100 mL water. Organic layer was separated, washed with water, brine and dried over MgSO₄. Solvents were evaporated under reduced pressure to give crude which upon purification by flash chromatography on silica gel in 2-15% ethyl acetate: hexanes afforded desired product 16 (55 mg, 0.123 mmol, 51% yield) as yellow oil. ¹H NMR (500 MHz, CDCl₃) δ: 7.06 (s, 2H), 4.04 (t, J = 8.0 Hz, 1H), 3.82 (s, 6H), 3.36 - 3.27 (m, 2H), 3.19-3.14 (m, 2H), 2.78 (quintet, J = 5.0 Hz,
1H), 2.59-2.54 (m, 2H), 2.59-2.54 (m, 2H), 2.54-2.48 (m, 2H), 2.38 (d, $J = 10.5$ Hz, 1H), 2.20 (t, $J = 5.0$ Hz, 1H), 1.77-1.68 (m, 2H), 1.68-1.59 (m, 2H), 1.58-1.46 (m, 4H), 1.36 (s, 3H), 0.99 (s, 3H); $[\alpha]^{22}_D = 37.5^\circ$ ($c = 1.52$, CHCl$_3$).

![Chemical structure](image)

$(1R,4R,5R)$-4-(4-(Cyclopentanecarbonyl)-2,6-dimethoxyphenyl)-6,6-dimethylbicyclo

[3.1.1]heptan-2-one (17.1): To a solution of 16 (30 mg, 0.067 mmol) in ethanol (5 mL) and water (0.5 mL) was added silver nitrate (22.8 mg, 0.134 mmol) and the resulting solution was stirred at 25 °C for 12 h. TLC in 30% ethyl acetate : hexanes showed completion of reaction. The reaction solution was filtered through a small pad of celite and diluted with diethyl ether. The organic layer was washed with brine and dried over MgSO$_4$. Solvents were evaporated to give the desired keto crude which was purified with silica gel chromatography to give 17.1 (20 mg, 0.054 mmol, 80% yield). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.18 (s, 2H), 4.06 (t, $J = 8.5$ Hz, 1H), 3.86-3.70 (M, 7H, especially s, 3.78, 6H), 2.50 (quintet, $J = 5.5$ Hz, 1H), 2.41 (t, $J = 5.0$ Hz, 1H), 2.37 (dd, $J = 18.5$ Hz, $J = 8.5$ Hz, 1H), 2.31 (d, $J = 11.0$ Hz, 1H), 2.09 (t, $J = 5.5$ Hz, 1H), 1.94-1.82 (m, 2H), 1.80-1.70 (m, 2H), 1.68-1.54 (m, 4H), 1.29 (s, 3H), 1.19 (s, 1H), 0.87 (s, 3H); $[\alpha]^{22}_D = 40.3^\circ$ ($c = 0.16$, CHCl$_3$).
(1R,4R,5R)-4-(4-Benzoyl-2,6-dimethoxyphenyl)-6,6-dimethylbicyclo[3.1.1]heptan-2-one (17.2): Using the same procedure as above to afford 17.2 in 73% yield. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.81 (d, $J = 1.5$ Hz, 1H), 7.79 (d, $J = 1.5$ Hz, 1H), 7.68-7.63 (m, 1H), 7.54 (t, $J = 7.5$Hz, 2H), 7.04 (s, 2H), 4.17 (t, $J = 8.5$Hz, 1H), 3.83 (s, 6H), 3.33 (dd, $J = 18.5$ Hz, $J = 8.5$ Hz, 1H), 2.62 (quintet, $J = 5.5$ Hz, 1H), 2.53 (t, $J = 5.5$ Hz, 1H), 2.50 (dd, $J = 18.0$ Hz, $J = 8.5$ Hz, 1H), 2.43 (d, $J = 11.0$ Hz, 1H), 2.23 (t, $J = 5.5$ Hz, 1H), 1.40 (s, 3H), 0.99 (s, 3H); $[\alpha]^{22}_D = 44.1^\circ$ (c = 1.91, CHCl$_3$).

**Detailed description of methods used for in vitro testing:**

**Studies done in Biochemistry lab in CDD:**

1. **Membrane Preparations from Tissue Culture Sources:** HEK293 cells expressing the mCB2 or hCB2 receptor are used for membrane preparations according to the method described by Abadji et al.$^{132}$ The resulting pellet is resuspended in 10 mM Tris-chloride, pH 7.4 with 5 mM MgCl$_2$ and 2 mM EDTA (TME) and stored at -80 °C for no longer than two months. Protein content is assayed by using the Bio-Rad protein assay according to the manufacturer’s protocol.

2. **Membrane Preparations from Tissue Sources:** Frozen rat brains (CB1 source) are obtained from Pel-Freeze Biologicals (Rogers, AK) and stored at -80 °C until use. Membranes were prepared according to the method described by Dodd et al. and adapted for use in our laboratory as previously reported.$^{133-135}$
3. **rCB1, hCB2, and mCB2 Binding Assays:** All compounds synthesized for this grant are tested for their ability to bind to CB1 and CB2 receptors using rat brain or HEK293 cell membranes expressing hCB2 membrane preparations, respectively, as previously described via competition-equilibrium binding using \[^{3}H\]CP-55,940.\textsuperscript{135,136} The results were analyzed using nonlinear regression to determine the actual IC\textsubscript{50} of the ligand (Prizm by GraphPad Software, Inc.) and the K\textsubscript{i} values are calculated from the IC\textsubscript{50}.

4. **Signal Transduction Assays (cAMP assay):** HEK-293 cells transfected with rCB1, mCB2, or hCB2 receptor are used with the PerkinElmer’s Lance ultra cAMP kit following the protocol of the manufacturer. Briefly, the assays were carried out in 384-well format using 1000 cells/well. Test compounds were added to wells containing stimulation buffer and 2 \(\mu\)M forskolin followed by cell suspension. After 30 minutes stimulation, the Eu-cAMP tracer and Ulight-anti-cAMP are added to the plate and incubated at room temperature for 1h prior to detection via PerkinElmer Envision; data are analyzed using GraphPad Prism software.

5. **Preliminary distribution in brain/plasma and drug oral bioavailability:** Mice (CD-1, weighing 25-30 g) are injected intravenously or orally with 0.1-2 mg/kg of the compound mixture. Fifteen minutes post-IV injection, or 30 and 60 minutes post-oral administration, the animals are sacrificed by decapitation followed by blood collection (~500 \(\mu\)L) and tissue dissection; samples are flash frozen with liquid nitrogen to prevent post-mortem degradation of the compounds. Tissues (plasma or brain) are extracted following published procedures\textsuperscript{138} and analyzed using a Thermo-Finnigan Quantum Ultra triple quadrupole mass spectrometer in SRM mode with an Agilent 1100 HPLC front-end with internal standards used for quantitation.
β-Arrestin Assay done in Duke University: Cells were treated with a serial concentration of test compounds. Known agonists were used as positive control. Cells were fixed with 1% PFA for 40 mins after administration of compounds. Images were acquired and analyzed as described below. Data are from 2-3 independent experiments with duplicate samples.

Image-based β-arrestin2 translocation assay- U2OS cells stably expressing the CB1-E cannabinoid receptors and β-arrestin2-GFP were split into glass-bottom 384 well plates (MGB101-1-2-LG, MatriCal, Spokane, WA) at a density of 8,000 cells/30 μl media/well using a Multidrop 384 dispenser (Thermo Electron Corporation). The plates were incubated overnight at 37 °C in 5% CO2. The following day, culture medium was changed to 30 μl/well of clear minimum Eagle’s medium (MEM) with 10mM HEPES. A set of serial diluted 4x concentration of each AM compound (10 mM in DMSO) was prepared in the same medium and applied to cells at a volume of 10 μl. The cells were incubated with compound for 40 mins at 37 °C prior to fixation with an equal volume of PBS containing 2% paraformaldehyde (sigma). Plates were stored at 4 °C until analysis. β-arrestin2-GFP aggregates were identified. Dose response curves were analyzed by nonlinear regression techniques using GraphPad Prism 5.0 software (GraphPad, San Diego, CA), and data were fitted to sigmoidal dose-response curves to obtain EC50 and efficacy values.
References:


(44) Hungund, B. L.; Vinod, K. Y.; Kassir, S. A.; Basavarajappa, B. S.; Yalamanchili, R.; Cooper, T. B.; Mann, J. J.; Arango, V. *Molecular psychiatry* 2004, 9, 184.


(50) Raehal, K. M.; Bohn, L. M. *Neuropharmacology* 2011, 60, 58.


(74) Haack, K. K. V.; McCarty, N. A. *Pharmaceuticals* 2011, 4, 509.


(88) Pertwee, R. G. *Current medicinal chemistry* 1999, 6, 635.


(104) Pertwee, R. G. Molecular neurobiology 2007, 36, 45.

(105) Singh, J.; Budhiraja, S. Methods and findings in experimental and clinical pharmacology 2006, 28, 177.


(193) Moore, B. M.; Ferreira, A. M.; Krishnamurthy, M.; *US20040242593*, University of Tennessee Research Foundation, USA. **2004**.