Design, Synthesis and Evaluation of Fluorescent CB_2 Cannabinoid Receptor Ligands

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Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

July 2009

Abstract

Cannabis has been used as a medicinal and natural product for thousands of years. Whether it has been used to make rope or paper, or been used to treat pain or depression, cannabis has always had a place in human civilisation.

With the isolation of the psychoactive compounds responsible for cannabis' effects, the discovery of two human cannabinoid receptors and an expanding knowledge of the therapeutic uses of cannabis, interest in the development of novel cannabinoids grew. The CB_2 cannabinoid receptor has gained particular attention, as the often unwanted central and psychoactive effects of cannabinoids has been attributed to the CB_1 cannabinoid receptor. Development of CB_2 receptor selective ligands offers treatment opportunities in many areas, but most especially for pain, multiple sclerosis and immunomodulation.

The preparation of fluorescently labelled ligands for a variety of receptors has improved compound screening techniques, as well as allowing use as biomolecular probes for aiding our understanding of the receptor *in situ*. The aim of this work is to design, synthesise and evaluate novel fluorescently labelled cannabinoids, with a particular interest in CB_2 selective compounds.

Focusing on the CB_2 receptor selective alkylindole JWH-015, targeted substitutions were made to its naphthyl ring to identify sites that might be suitable for fluorophore attachment. With a site chosen, a series of fluorescent JWH-015 analogues was synthesised and evaluated for their CB_2 receptor binding affinities. Though none of the evaluated compounds showed sufficient binding affinity for them to be used as biomolecular tools, the structure activity relationships gained suggested that improved design of fluorescent JWH-015 analogues in future could lead to the first ever active fluorescent cannabinoid.

Acknowledgements

I am very grateful for the opportunity that my supervisors Barrie Kellam and David Kendall have given me in researching for this doctorate, and for the support and funding from the University of Nottingham and BBSRC that made it possible. I also thank my supervisors for their time and advice throughout my PhD, as well as supporting me in my submission.

I would also like to acknowledge all the help, support and friendship given by all of my colleagues on C floor of the Centre for Biomolecular Sciences and E floor of the Medical School. In particular I want to thank Rich for helping me get started in a chemistry laboratory after a break of over two years, and for Dave for guiding me through my pharmacology work.

I thank Claire for helping to proof read and Sara for being there when I needed it most. I apologise to my family and Claire over having to put up with me while I was writing this thesis, and thank them for their belief in me. I hope that my work makes all my family, living or sadly passed away, proud.

This thesis is dedicated to my mum.

Abbreviations

AcOH	acetic acid
aq	aqueous
BBr_3	boron tribromide
Boc	*t*butoxycarbonyl
Br_2	bromine
°C	degrees centigrade
calcd	calculated
CCl_4	tetrachloromethane
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
CHO	chinese hamster ovary
conc.	concentrated
COSY	correlation spectroscopy
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIBAL	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
EDTA	ethylenediaminetetraacetic acid
Et_2AlCl	diethylaluminium chloride
Et_3N	triethylamine
FT	fourier transform
FTIR	fourier transform infrared spectroscopy
g	gram
GDP	guanosine diphosphate
GPCR	G-protein coupled receptor
GTP	guanosine triphosphate
h	hour
H_2	hydrogen

HCl	hydrochloric acid
HNO_3	nitric acid
H_2O	water
HOBt	N-hydroxybenzotriazole
HPLC	high performance liquid chromatography
H_2SO_4	sulfuric acid
HTS	high-throughput screening
Hz	hertz
I_2	iodine
iPrOH	isopropanol
KBr	potassium bromide
K_2CO_3	potassium carbonate
KH	potassium hydride
KI	potassium iodide
KOH	potassium hydroxide
$LiAlH_4$	lithium aluminium hydride
MeBr	methyl bromide
MeCN	methyl cyanide
MeI	methyl iodide
MeOH	methanol
Mg	magnesium
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulfate
MHz	megahertz
min	minute
mL	millilitre
mm	millimetre
mmol	millimole
m.p.	melting point
NaCl	sodium chloride
$NaClO_2$	sodium chlorite
$NaHCO_3$	sodium bicarbonate
NaH	sodium hydride
NaH_2PO_4	sodium dihydrogen phosphate

NaN_3	sodium azide
$NaNH_2$	sodium amide
$NaNO_2$	sodium nitrite
NBD	7-nitrobenzofurazan
NaOH	sodium hydroxide
NH_3	ammonia
nm	nanometre
NMR	nuclear magnetic resonance
ppm	parts per million
Pd/C	palladium on activated charcoal
Ph_3P	triphenylphosphine
RP-HPLC	reverse-phase high performance liquid chromatography
R_t	retention time
PTSA	para-toluene sulfonic acid
sat.	saturated
$SnCl_2$	tin (II) chloride
$SOCl_2$	thionyl chloride
tBuOH	tert-butanol
tBuOK	potassium tert-butoxide
THC	(-)-Δ^9-tetrahydrocannabinol
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TOF-ES	time of flight electroscopy
Tris	tris(hydroxymethyl)aminomethane
µL	microlitre
UV	ultra violet

Table of Contents

1 Introduction

1.1 Cannabis sativa L.

Cannabis sativa L., hereafter referred to as cannabis, is a species of the hemp family Cannabaceae, which also contains the genus *Humulus* L., or hops, used in the brewing of beer[1]. There are currently two subspecies of cannabis, sativa and indica, as well as a number of varieties, though there is some discussion whether these subspecies may actually be separate species or not. It is thought that these two subspecies both originate from the plateaus of Central Asia, with sativa having been predominately found in Europe and indica in Indochina[2].

1.1.1 Brief history of the uses of cannabis

Extensive cultivation of cannabis, since the development of agriculture over 10,000 years ago, has lead to its ubiquitous presence throughout the inhabited world, both as a cultivated and feral plant[2, 3]. Cannabis is a versatile crop providing a source of food, medicine, fuel, oil, paper, building materials and textiles[3].

Hemp, the common name for cannabis used when referring to the whole plant, was, and still is, cultivated for its fibres and seeds and not for its psychoactive content, which is indeed very low when grown for industrial uses. There is evidence that ancient cultures used hemp as a textile for clothing, the seeds as a food source, and as an incense in funeral rites[4]. The first paper, possibly invented in China, was produced from hemp[5]. In Tudor England, hemp was so essential for the production of rope, needed in large supplies for the success of the navy, that Henry VIII issued a royal edict that all farmers should grow the plant or pay a fine[5, 6]. During the twentieth century, due to the introduction of national and international legislation controlling the use of cannabis and the introduction of synthetic materials, hemp was decreasingly cultivated for its practical uses. However, with the general growth of interest in natural and

organic products, hemp is increasingly being used for a wide variety of applications including in cosmetics, textiles and food, to name but a few[7].

Cannabis has also been used for a vast array of medicinal purposes for thousands of years. Though almost certainly used before, the first documented evidence of the medicinal use of cannabis was in the famous Chinese pharmacopoeia, the *Shen Nong Ben Cao Jing* (The Divine Farmer's Materia Medica), written in the first or second century AD, which advocated its use to allay senility and as a surgical anaesthetic[4]. The Egyptians are thought to have used cannabis as a suppository to stimulate vaginal contractions, it was used by the Persians as an analgesic[4] and by the Romans as a method of contraception[6]. In the last millennium Europeans and Americans have continued to use cannabis for applications described by the classical civilisations, and have also used it for the treatment of other conditions including jaundice, gout and as an anti-anorexic.

During the twentieth century, legislation quickly turned against the use of cannabis, for reasons described in Section 1.2.3. Doctors in the UK could legally prescribe cannabis up until when it was reclassified under the Misuse of Drugs Act 1971, which effectively implied that cannabis had no medicinal value, contrary to thousands of years of experience[4]. Cannabis is still used illegally by many individuals to treat such conditions as neuropathic pain and glaucoma, despite the risk of prosecution in many countries across the world[8, 9]. For a detailed insight into the evidence for the extensive history of the medicinal uses of cannabis see the work of Russo[2, 4].

1.1.2 Identification of the natural cannabinoids

There are over 60 terpenoid compounds known as the natural cannabinoids, found exclusively in cannabis, the major source being found in the leaves and the female flower. Why the plant produces such an array of cannabinoids is not clear, but there are suggestions that they act as antioxidants and as antimicrobials. Cannabis also contains many other natural products thought to

be of biological and medicinal interest including terpenes, flavonoids and carotenoids[3].

(-)-Δ^9-tetrahydrocannabinol (THC) **1** (Figure 1.1) was first identified as the major active component of cannabis, and in particular of hashish (the active resin of the female flowering top of cannabis), in 1964[10]. Its absolute stereochemistry was determined in 1967[11]. Other important cannabinoids include Δ^8- tetrahydrocannabinol **2** and cannabinol **3**[12], and it is the mixture of these natural cannabinoids and other natural products that gives cannabis its physiological and psychological effects[13].

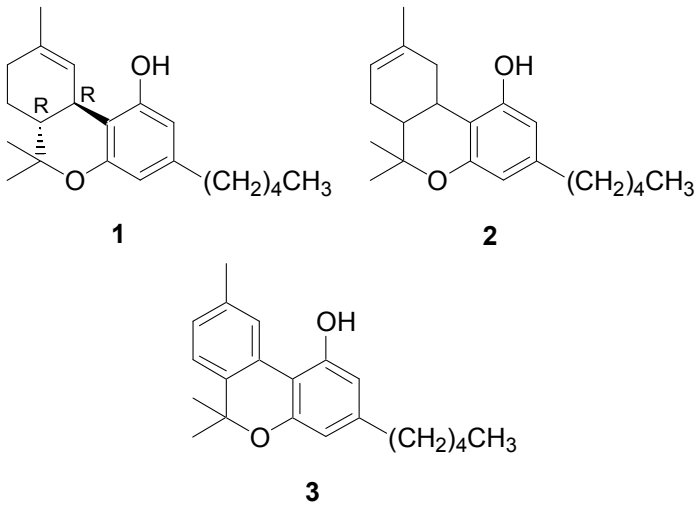


Figure 1.1 Structures of some natural cannabinoids.

With the isolation of the natural cannabinoids, and an interest in their therapeutic effects, a significant amount of research was attempted in trying to improve these molecules. This has led to a vast array of structurally similar compounds which has helped to develop our knowledge on the important cannabinoid structure activity relationships[14]. It was not until the discovery of the cannabinoid receptors (Section 1.2), their endogenous ligands (Section 1.4.1), and the development of novel cannabinoid ligands and pharmacophores (Section 1.4) that our understanding of the cannabinoid system really advanced.

1.2 Cannabinoid receptors

Before the discovery of the cannabinoid receptors, all research into the activity of natural and synthetic cannabinoids involved use of *in vivo* animal models, with all their associated disadvantages. The identification of the membrane bound G-protein coupled receptors or guanosine binding protein coupled receptors (GPCRs) CB_1 in 1988[15] and CB_2 in 1993[16] was a major breakthrough in cannabinoid research. This section will offer a brief overview of the characterisation and pharmacology of the two known cannabinoid receptors. For an excellent and detailed recent review on this area see work by Howlett AC *et al*[17].

The two receptors, both with the characteristic seven transmembrane α–helix domain structure of all GPCRs (Figure 1.2), are distinguished by their differences in amino acid sequence, tissue distribution and function[17]. CB_1 and CB_2 receptors have an overall amino acid sequence homology of 44% and in the transmembrane α–helix domains this rises to 68%[16, 18]. CB_1 rat, mouse and human receptors have been cloned and show a higher cross species sequence homology (97-99%) than that of cloned CB_2 mouse and human receptors (82%)[17, 19, 20].

1.2.1 G-protein coupled receptors

The GPCR super family are generally found imbedded in the cellular phospholipid membrane. These proteins comprise of seven α-helixes that span the phospholipid membrane in a cylinder like conformation, and are connected by a total of three intra-cellular and three extra-cellular loops. The GPCR position within the phospholipid membrane provides both opportunities and difficulties for their investigation. As the receptors are designed to interact with ligands at the extracellular side of the membrane, investigation of ligand affinity and efficacy can be assessed in whole cell populations. If the receptor was internalised, as found with steroid receptors, any investigation into receptor-ligand binding would need to overcome the problems of introducing the ligand into the intracellular compartment. On the other hand, direct

investigation of the GPCR structure itself is made very difficult because its structure is designed to reside within the phospholipid membrane bilayer, with therefore a varying hydrophobic and hydrophilic nature across the macromolecule. GPCRs therefore have poor solubility in both organic and aqueous solvents, making their isolation, purification and crystallisation difficult. Standard methods of whole protein analysis such as X-crystallography and nuclear magnetic resonance are extremely difficult to achieve with the cannabinoids receptors, as with all GPCRs.

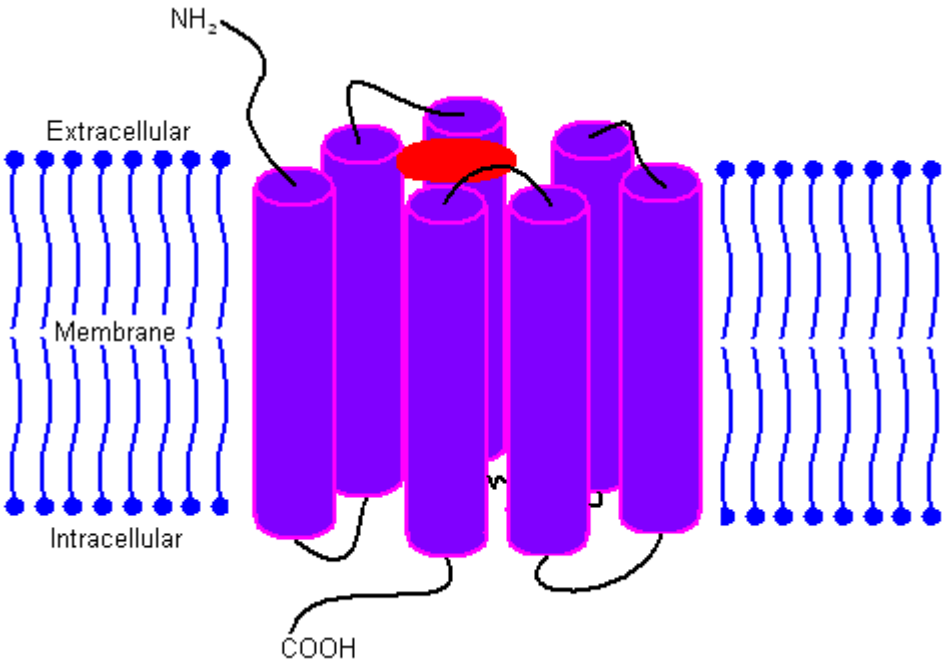


Figure 1.2 A cartoon description of a GPCR receptor. Purple cylinders represent α–helix transmembrane domains; black lines represent intra and extracellular loops; red ellipse represents bound ligand.

Until recently all GPCR modelling studies were based on homology modelling from rhodopsin, a ubiquitous protein important for the detection of light. Homology modelling involves using a known crystal structure of a GPCR, aligning the desired sequence in the transmembrane α-helix domains using such methods as FASTA[21] or BLAST[22], mutating residues, addition of extracellular and intracellular loops from structural databases and energy minimisation using molecular mechanics leading to the ultimate validation of

the designed model. For detail on this process see Leach's in depth work[23] and the step by step guide by Oliveira *et al*[24].

Such modelling began using the crystal structure of bacteriorhodopsin[25, 26], which is not considered a true GPCR as it is not associated with a G-protein, and so the subsequent determination of the crystal structure of bovine rhodopsin was a major breakthrough[27, 28]. It has been commented that although bacteriorhodopsin homology modelling leads to good general GPCR conformation, it is not so suitable for rational drug design, and hence has been superseded by bovine rhodopsin homology models[29].

Recently the publication of a high resolution crystal structure of a ligand bound, antibody tagged, β_2-adrenoceptor, grown in a lipid medium has been elucidated[30-32]. In detail, the β_2-adrenoceptor was expressed in Sf9 insect cells, whose membrane was suitably solublised and purified by a range of processes including antibody chromatography. Once purified a β_2-adrenoceptor Fab (a fragment of the antigen binding region that was cloned that could bind to the third intracellular loop of the receptor) was formed and isolated. Crystals were then grown from a mix of the β_2-adrenoceptor Fab complex, bound to the inverse agonist carazolol, in a bicelle medium over seven to ten days. Diffraction images obtained by microcrystallography were of high resolution (3.4Å within plane and 3.7Å perpendicular to plane of membrane) allowing residue identification of the complex, even with the Fab component present. This work, as well as the possibility of using solid state nuclear magnetic resonance techniques to analyse isotopically labelled proteins in their membranes[33], leads to hopes that similar methods may be utilised for other GPCRs, helping to validate existing models and lead to the development of improved receptor understanding, though significant hurdles still need to be overcome[34].

The main reason for interest in the GPCR structure is because minor changes in structure can greatly affect how a GPCR ligand interacts with its receptor. GPCR ligands bind in a receptor active site somewhere within the cylinder like structure, formed by the seven transmembrane spanning α-helixes, at varying

depths depending on the GPCR. Though the active site is generally composed of amino acid residues from the α-helixes, the three extracellular loops can play a role in both ligand binding and in restricting access to the receptor active site[35].

When a ligand binds to a GPCR it causes a cascade of events, whereby the conformation of the GPCR structure is altered, which affects various interactions with intracellular bound proteins. The G-protein coupled receptors are named so, as their intracellular bound protein is a heterotrimeric G-protein, made up of three different subunits (α, β, and γ). On the binding of a GPCR agonist, the resulting conformational change leads to the release of the G-protein. Once released from the GPCR, the G-protein α subunit changes conformation allowing binding of guanosine triphosphate (GTP) and displacement of guanosine diphosphate (GDP). This allows the βγ complex and α subunit to separate, and leads to various effects depending on their type.

There are a number of different α, β, and γ subunits available, with different cell types having different distributions, and the effects of the GPCR are related to what G-proteins are available in the cell, as well as which G-proteins it can couple with. In the case of both cannabinoid receptors, they interact with the $G_{i/o}$ protein[17], and on activation leads to the release of both the $\alpha_{i/o}$ subunit and less variable βγ complex. Due to the intrinsic GTPase activity of the α subunit, it has a limited activated life span before dephosphorylation reforms the deactivated α subunit – GDP complex and a complete G-protein is reformed with the βγ complex. Activation of the cannabinoid receptor leads the activated $\alpha_{i/o}$ subunit, via inhibition of adenylate cyclase, and βγ complex, via activation of mitogen-activated protein and phospholipase C, resulting in signal transduction (Figure 1.3). In the case of the CB_1 receptor, the $\alpha_{i/o}$ subunit is also involved in various ion channels e.g. positively to inwardly rectifying potassium channels and negatively to calcium channels[17, 36]. Generally the effect of the activated $G_{i/o}$ protein is to induce a chemical brake to the cell. This is particularly due to inhibition of adenylate cyclase, which decreases the production of cyclic adenylate monophophate which is essential for direct or indirect activation of numerous cellular proteins. Depending on

the location and role of the specific cell, its effects may be inhibitory or excitatory on the physiology of interest.

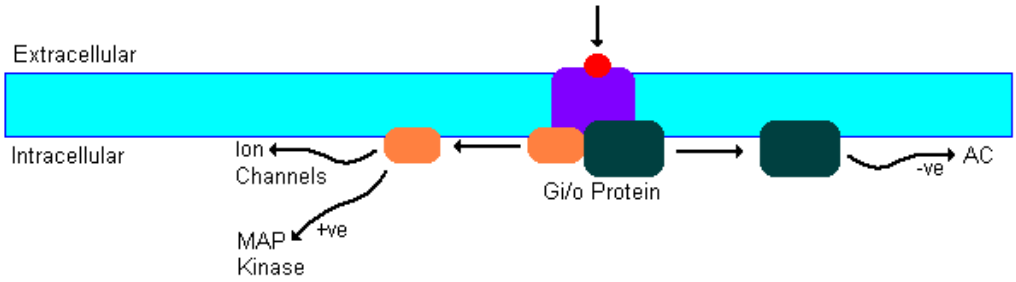


Figure 1.3 A cartoon description of the cascade effect on activation of a GPCR receptor coupled to a $G_{i/o}$ protein. Agonist (red circle) binding to the GPCR receptor (purple), causes dissociation and activation of the activated $\alpha_{i/o}$-subunit (green) from $\beta\gamma$-subunit (orange), leading to signal transduction.

Other factors affecting signal transduction, are that activation of a GPCR can lead to activation of more than one G-protein, contributing to signal amplification, but excess activation of GPCRs can lead to their cellular internalisation and desensitisation[37]. Furthermore, some GPCRs, including the cannabinoid receptors, are likely to have a degree of constitutive activity even without the presence of ligands, as some antagonists can actually act as inverse agonists reducing cellular responses below basal levels[17]. GPCR effects, like most physiological systems, are sensitive to numerous variables and are controlled by multiple negative feedback processes.

1.2.2 Receptor distribution

The CB_1 receptor is mainly distributed in the central nervous system e.g. brain and spinal cord, as well as some peripheral tissues e.g. reproductive and gastrointestinal systems. Its central distribution is not homogenous and is most densely located in the cerebellum, basal ganglia, hippocampus, and the cerebral cortex[17]. Its pharmacological action causes alteration of neurotransmission by inhibition of the release of excitatory and inhibitory neurotransmitters e.g. acetylcholine, noradrenaline, serotonin, glutamate, γ-aminobutyric acid and dopamine[17, 20]. The CB_2 receptor is mainly distributed in the immune system e.g. spleen and lymph glands, as well as in some parts of

the nervous system[38]. Its pharmacological action results in regulation of cytokine release in both physiological and pathological states, and affects immune cells, either directly or indirectly, e.g. macrophages, natural killer cells and other T-lymphocytes[17, 20].

1.2.3 Cannabinoid receptors as therapeutic targets

The cannabinoid receptors offer a range of therapeutic opportunities. Cannabis has shown itself to be potentially one of the most versatile herbal medicinal products available to mankind, with applications in pain and inflammation, nausea and vomiting, anorexia, anxiety, depression, psychosis, substance dependence, muscle spasticity, epilepsy, glaucoma and neuroprotection[39-41]. Although cannabis is generally well tolerated, and overdose is unlikely to be associated with toxicity, this herbal product is harshly controlled when compared with another psychoactive medicinal product, alcohol, or with tobacco which is significantly more toxic than cannabis in the long term. It is due to its place in history that cannabis is somewhat of an oddity as an illegal drug.

Throughout civilisation, attempts have been made to restrict or ban cannabis as a recreational drug, but such attempts failed up until the 20th century[4]. During this time of industrialised labour, the smoking of cannabis was said to be a social evil as it decreased productivity of the workers. Another herbal medicine at the time that was also causing concern was opium. In the western world its overuse as a pain killer resulted in middle class addicts. Though, restriction of opium eventually occurred, due to cannabis being used predominately by the working class, and the relevant authorities seeing little to no medicinal use for it, a rush of legislation, led by the U.S., saw the criminalisation of cannabis across the world. This criminalisation of cannabis, which still applies to the vast majority of developed countries, is not only relevant to its recreational use, but to all medicinal uses as well[42].

Ironically, since its criminalisation, interest and research in the medicinal use of cannabis has steadily grown. In the U.K. this led a Royal College of

Physicians working party in 2005 to support the development of oral or other alternative delivery system cannabis products for a number of therapeutic uses[43], and the establishment of GW Pharmaceuticals in 1998, which is currently in extensive human trials of a cannabis oral spray Sativex® for the treatment of chronic pain in multiple sclerosis[44]. It is hoped that these developments will soon lead to declassification of cannabis Schedule 1 Controlled Drug in the U.K., allowing doctors to again prescribe cannabis for medicinal purposes[42].

The main hurdle to the therapeutic use of cannabis is the public and authorities concern of its psychoactive effects. This is particularly the case, as a systematic review of the literature shows that cannabis use does increase the chance of developing psychoses[45]. With the identification of the two cannabinoid receptors, it became possible to isolate which receptor is responsible for which effects. It was unsurprising that the activation of the centrally located CB_1 receptors was responsible for the undesired central effects. Of course some of CB_1 receptor activated central affects have therapeutic potential themselves e.g. in anxiety, anorexia, depression and nausea and vomiting[17]. Nevertheless, the targeting of the CB_2 receptor and preparation of CB_2 receptor selective ligands, would remove the largest hurdle to the use of cannabinoids – the removal of the CB_1 receptor mediated psychoactive effects that mean it can both be abused and lead to long term psychiatric problems.

1.3 Therapeutic applications of CB_2 receptor ligands

The CB_2 receptor is implicated in the therapy of numerous disease states, including most notably in pain, multiple sclerosis, and for its use as an immunosuppressant and anti-inflammatory agent.

1.3.1. Pain

It was previously thought that the analgesic effects of cannabis was mediated by the CB_1 receptor, but evidence that a selective CB_2 receptor agonist provides analgesia in a neuropathic pain model in mice[46, 47], and that a selective CB_2 receptor antagonist blocks the analgesia[47], meant that the CB_2 receptor was a potential therapeutic target for peripheral and neuropathic pain. It is still unknown as to the mechanism of this analgesia, though the possible presence of CB_2 receptors on peripheral neurons may suggest a locally mediated effect. It is generally accepted that the CB_2 receptor is likely to be modulating pain as a local level, possibly involving inhibition of the immune system. For a comprehensive review of the possible mechanism, as well as CB_2 receptor agonist evaluated in pain models, see the work of Whiteside *et al*[48]. With neuropathic pain and injury often resistant to treatment, CB_2 receptor selective agonists offer another valuable therapeutic option[48].

1.3.2 Multiple Sclerosis

Cannabis is thought to have a number of applications in treatment of multiple sclerosis, especially for the control of spasticity, pain, tremor and bladder function. Multiple sclerosis is a complicated disease, but at a simple level, all these problems are associated with impaired neural transmission and control. The cannabinoid system is thought be in important in the inhibition of synaptic transmission, and therefore its activation improves neural control[49]. As commented upon in Section 1.2.3, GW pharmaceuticals are currently have their cannabis preparation Sativa® in human trials for the treatment of multiple sclerosis.

With respect to the CB_2 receptor, muscle spasticity in multiple sclerosis models is decreased with use of a selective CB_2 receptor agonist and increased with the selective CB_2 receptor antagonist SR144528 **21**[50]. In another study the CB_2 receptor selective agonist JWH-015 **7** significantly improved neurological defects[51]. Multiple sclerosis is a disease that cannabis shows great promise for, with at least some of its effects appearing to be mediated by the CB_2 receptor.

1.3.3 Immunomodulation

Due to their location, CB_2 receptors are thought to be important in immunomodulation and there is evidence that the cannabinoid receptor agonist CP55-940 **5** causes immunosuppression, and increasing susceptibility to the herpes simplex virus in mice[52]. An aggressive glioma tumour has been shown to have a positive correlation with CB_2 receptor expression and its severity, and inhibition of the tumour can be achieved with a selective CB_2 receptor agonist[53]. Furthermore administration of the selective CB_2 receptor agonist JWH-015 **7** has been shown to induce apoptosis *in vivo* of thymocytes, leading to decrease T-cell response to mitogens. This suggests that CB_2 receptor selective ligands may be used as immunosuppressive therapy e.g. in rheumatoid arthritis, Crohn's disease, organ or bone marrow transplant patients, or at lower doses as an anti-inflammatory.

1.4 Cannabinoid ligands

A vast array of natural cannabinoids have been identified and synthetic cannabinoids designed and evaluated. Khanolkar *et al*[54] divided the cannabinoids into six main classes and each which will discussed in more detail below (Figure 1.4). Over the last decade there has been the development of numerous other cannabinoid structures both similar and quite different from the six main classes discussed below, see the review of Padgett for more details[55].

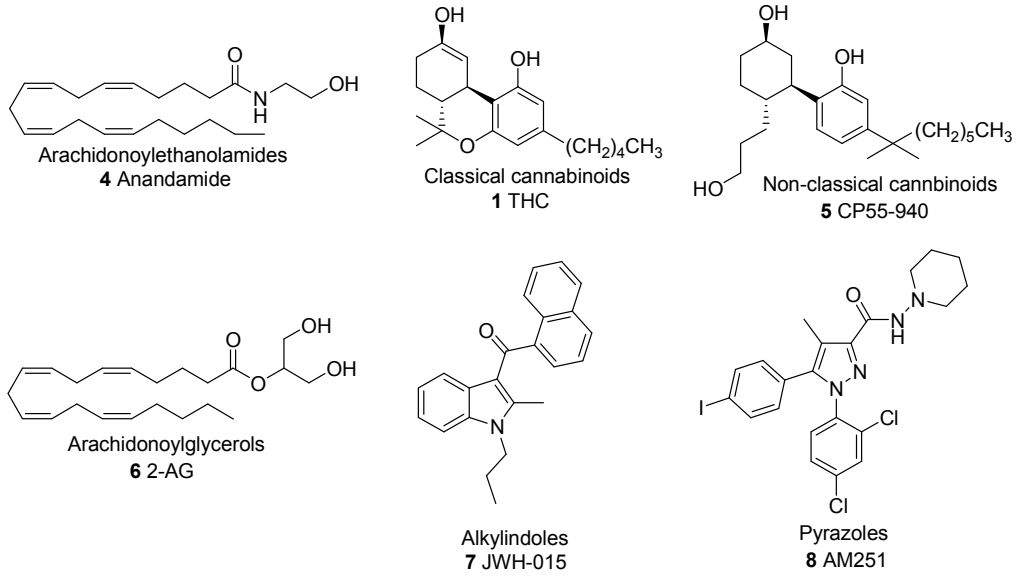
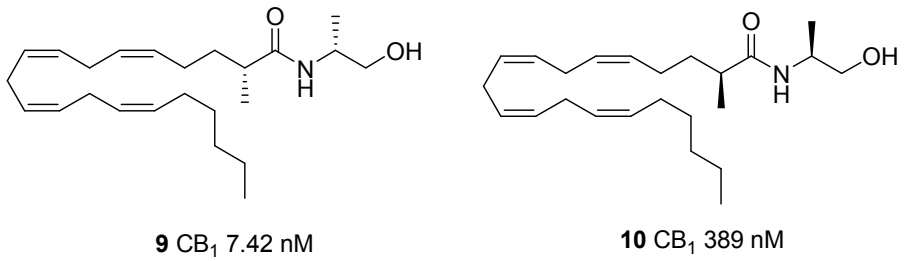


Figure 1.4 Examples of the main six cannabinoid ligand types.

1.4.1 Endogenous ligands

The discovery of the endogenous ligands for the cannabinoid receptors identified two new structurally similar types of cannabinoid ligand with the prototypical anandamide **4** of the arachidonoylethanolamides[56, 57] and 2-arachidonyl glycerol (2-AG) **6** of the arachidonoylglycerols[58], amongst others[17], having helped to give a greater understanding of the physiological role of the cannabinoid receptors in the body. Their biosynthetic routes have been extensively investigated[59] and their metabolism by the enzymes fatty acid amide hydrolase and monoacylglycerol lipase, as well as proposed membrane transportation protein, anandamide transporter, have been described[54, 59, 60].

All of the endocannabinoids ligands have affinity for both cannabinoid receptors with a degree of selectivity for the CB_1 receptor[17]. Work has focused on increasing this selectivity for CB_1 receptor, and the synthesis of several chiral methylated arachidonoylethanolamides **9** and **10** potentially highlighting a CB_1 receptor hydrophobic sub-site (Figure 1.5)[61].



9 CB_1 7.42 nM **10** CB_1 389 nM

Figure 1.5 Examples of synthetic arachidonoylethanolamide cannabinoids. Receptor binding affinities (Ki) quoted[61].

With an increasing knowledge of this endocannabinoid system, further studies might be able to elucidate the roles of this system and the cannabinoid receptors in physiological and pathological states. The development of fatty acid amide hydrolase enzyme inhibitors as well as anandamide transporter inhibitors will aid this study[62].

1.4.2 Classical cannabinoids

Classical cannabinoids are based upon the structure of natural cannabinoids detailed in Section 1.1.2, and in particular of THC **1**. They retain the tricyclic dibenzopyran structure, with investigation limited to alteration of functional groups and side chains. For example, the introduction of the northern aliphatic hydroxyl at the C_9 position, which leads to increased activity, and removal of the C_9 double bond, which is not essential for activity, led to 9-nor-9-hydroxyhexahydrocannabinol **11**[63] (Figure 1.6). Optimisation of the alkyl side chain to the 1,1-dimethylheptyl group has led to dimthylhepyl-HHC **12**[62] and HU-210 **13**[17, 36], the latter having binding at both the CB_1 and CB_2 receptors in the sub-nanomolar range.

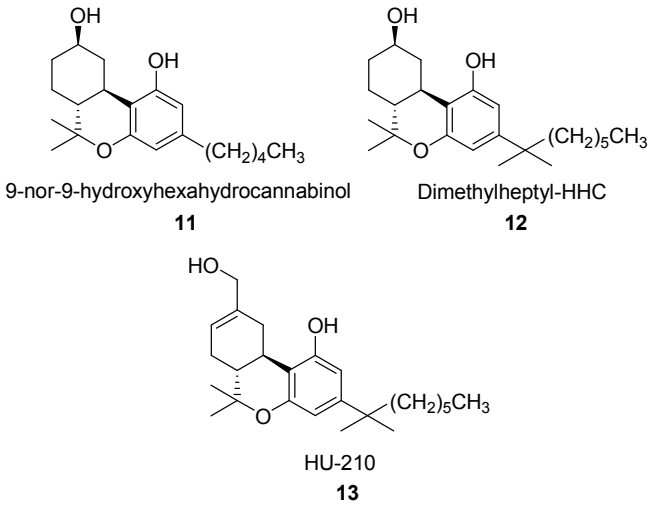


Figure 1.6 Examples of synthetic classical cannabinoids.

1.4.3 Non-classical cannabinoids

The discovery that the pyran ring of classical cannabinoids was not required for cannabinoid activity was first seen with the synthesis of CP47-497 **14**[64] leading to the development of non-classical cannabinoids by Pfizer (Figure 1.7). Further refinement led to the introduction of an additional pharmacophore to that of classical cannabinoids, the C_{12} southern aliphatic hydroxyl e.g. CP55-940 **5** with binding at the CB_1 and CB_2 receptors in the sub-nanomolar range[65]. Tricyclic derivatives have also been developed e.g. CP55-244 **15**[66], whereby the C_{12} southern aliphatic hydroxyl is locked into a set conformation, theoretically decreasing the entropy of binding.

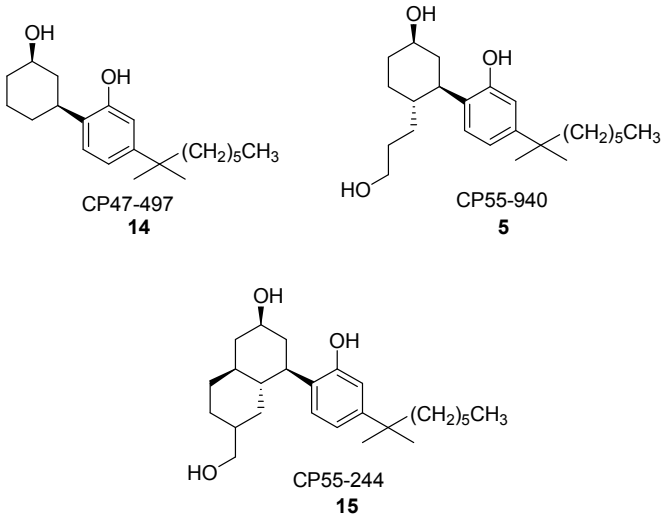


Figure 1.7 Examples of non-classical cannabinoids.

To take advantage of this new C_{12} southern aliphatic hydroxyl pharmacophore in the tricyclic classical cannabinoids, a number hybrids were developed e.g. AM919 **16**[67] (Figure 1.8). Further development of the C_{12} southern aliphatic side chain has led to some degree of CB_1 receptor selectivity with AM938 **17** and CB_2 receptor selectivity with **18**[68, 69].

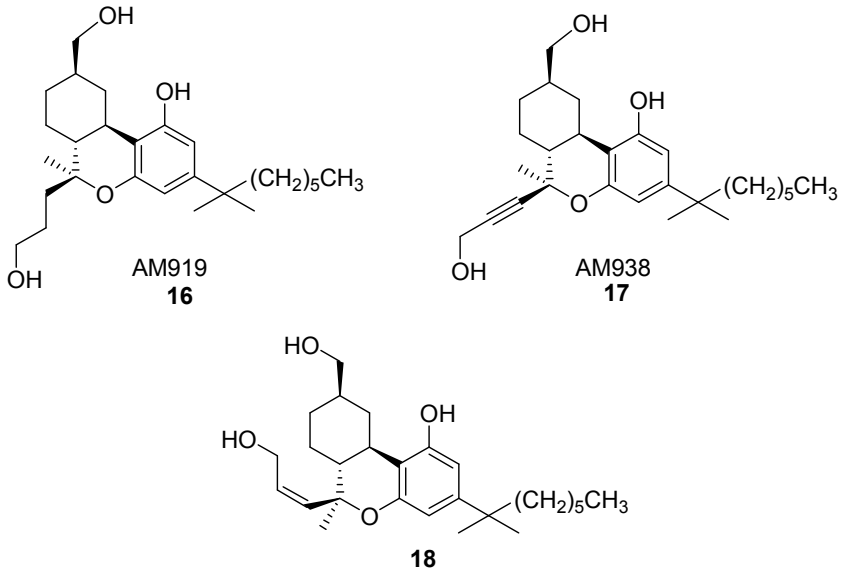


Figure 1.8 Examples of classical / non-classical cannabinoid hybrids.

A more detailed review of the important structure activity relationship for the classical and non-classical cannabinoid pharmacophores is presented in Chapter 4, as well as the more detail on alterations that lead to CB_2 receptor selectivity.

1.4.4 Alkylindole cannabinoids

The alkylindole cannabinoids were developed by a Sterling Winthrop group during research into new non-steroidal anti-inflammatory agents[70], and they are not based on the any previously known cannabinoid structures. They include both the prototypical WIN-55212 **19**[71], which has a small degree of CB_2 receptor selectivity, and other more selective CB_2 receptor agonists e.g. JWH-015 **7** (Figure 1.9)[72]. Evidence suggests that the alkylindoles bind at a sub-site lower in the cannabinoid receptor active site but overlapping with the classical cannabinoid sub-site, allowing mutual displacement[36].

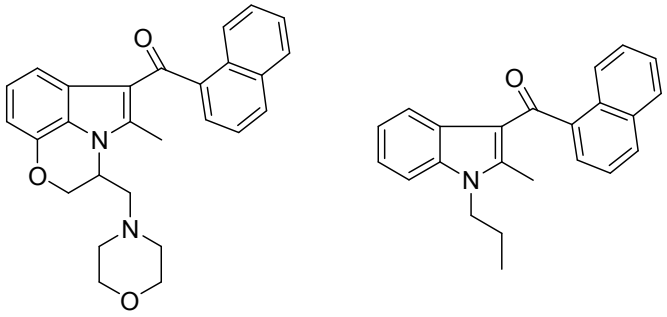


Figure 1.9 Examples of alkylindole cannabinoids.

As with the classical and non-classical cannabinoids, a more detailed review of the important structure activity relationships and alterations that lead to CB_2 receptor selectivity are presented in Chapter 2.

1.4.5 Diarylpyrazole cannabinoids

The first cannabinoid receptor antagonists were developed by Sanofi and are based on a diarylpyrazole structure. They include both the highly selective CB_1 receptor antagonists e.g. SR-141716A **20**[73, 74], as well as highly selective CB_2 receptor antagonists e.g. SR-144528 **21**[75], and there is great interest in their development both as biomolecular tools and potential therapeutic agents (Figure 1.10)[36, 76]. It is currently thought that the diarylpyrazoles act at the same binding site as the cannabinoid agonists discussed above, though interacting with different amino acid residues, and not at an allosteric binding site[36].

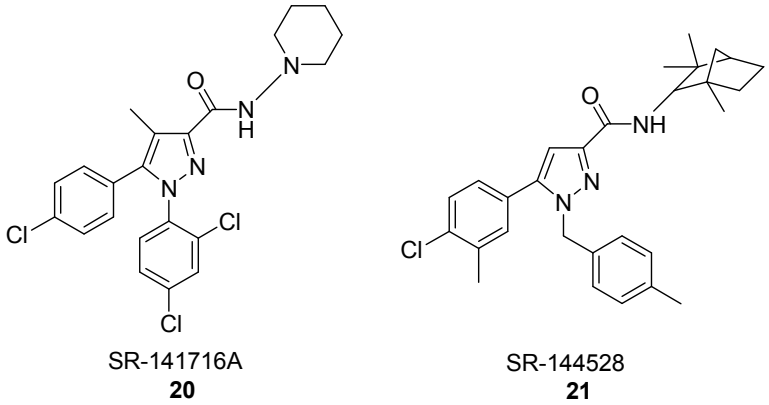


Figure 1.10 Examples of diarylpyrazole cannabinoids.

1.5 Fluorescence

Fluorescence is the property of certain molecules to absorb electromagnetic radiation, normally ultraviolet (UV) or visible light, at a discrete wavelength and then emit radiation of a longer wavelength (lower energy), with the fluorescence persisting only as long as the stimulating radiation is continued[77]. For example the green fluorescent protein, that is used extensively for visualisation of expressed protein in molecular biology, absorbs UV light at 398 nm and emits green light at 508 nm[78].

Fluorescence occurs in three steps (Figure 1.11). First, absorption of a photon raises an electron from its ground state (S_0) to an excited singlet state (S_1) with a variety of vibrational levels. This may be followed by small loses of energy by non-radiation pathways, moving the electron to a lower vibrational level. Finally the electron emits its energy in the form of electromagnetic radiation, as it returns to its ground state (S_0), the whole process occurring over 10^{-8} s. The loss of energy between excitation of the electron and emission of radiation, leads to a longer wavelength of radiation being emitted than is absorbed, and this change in wavelength is called the red or Stokes shift.

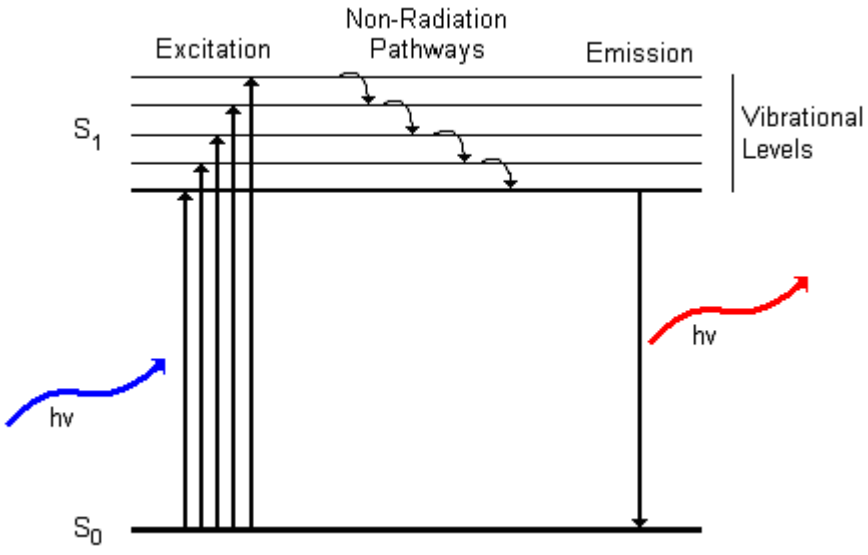


Figure 1.11 Excitation and emission of fluorescent molecules.

Competing with this fluorescence are a number of other processes that can interfere, causing quenching of the desired fluorescence[79]. Internal conversion occurs when energy is transferred from the S_1 level of one molecule to the S_1 level of another molecule of the same compound. This process can take place over relatively long distances and so can occur when the fluorescent compound is in solution. Intersystem crossing involves the formation of a triplet state, where there is an alteration of the direction of spin of the S_1 electron. This spin state has a much longer life time than normal, and means that its energy will likely be lost through non-radiative pathways and lead to fluorescence quenching. Finally photobleaching can occur if a high energy excitation light source such as a laser is used, which can initiate a free radical mediated chemical reaction. This not only quenches the fluorescence but more importantly degrades the fluorophore. The fluorescent efficiency of a compound is measured as quantum yield i.e. the proportion of fluorescence photon emissions compared to the number of photons absorbed by the system. The greater the fluorescence quenching the lower the quantum yield. Knowledge of the importance of these competing processes and the quantum yields in various environments for different fluorophores, can be important for choosing the right fluorophore for a specific application.

1.5.1 Uses of fluorescent GPCR ligands

Fluorescent labelled small molecules, specifically GPCR ligands, have three main uses; high-throughput screening, fluorescent confocal microscopy and small molecule detection techniques. Potentially they could also be used in bioassays to determine the presence of a particular receptor. If the presence of that receptor was a marker of a specific pathophysiology, then such a technique would have use as a diagnostic tool.

1.5.1.1 High-throughput screening

Fluorescently labelled GPCR ligands are a valuable tool for drug discovery, especially when used in high-throughput screening (HTS). Generally HTS are performed with radiolabelled ligands, which require both the use of significant volumes of scintillation fluid for processing and additional costs for the

disposal of radioactive waste. Any fluorescent-based HTS assays would enable screening of new lead compounds from libraries of hundreds to millions of compounds, which would be faster, cheaper and avoid the use of radioactive materials. Whereas radioligand assays requires filtration of the GPCR bound ligand and then addition of scintillation fluid to allow counting of radiation present, use of fluorescent ligands only need to be filtered before a measure of the remaining fluorescence need be taken. Fluorescent-based HTS assays can be combined with confocal and small molecule techniques, detailed below, to allow miniaturisation of the assay and allow for quick analysis at small volumes[80].

1.5.1.2 Confocal microscopy

Confocal microscopy with fluorescent labelled ligands allows visualisation *in vitro* of receptor binding and can be used to identify receptor localisation, density and internalization in three dimensions, as well as following its progress over time. Confocal microscopy offers the advantage over conventional light microscopy, in that it can allow identification within a single cell of the location of a fluorescent ligand receptor complex, and may change how we approach experimental pharmacology[81]. When designing fluorescent ligands for confocal applications the large excitation energies that must be used, can increase photobleaching and therefore fluorescence quenching, with not all fluorophores suitable[79].

1.5.1.3 Single molecule detection

Single molecule detection covers a number of techniques that detect bursts of fluorescence, meaning that they are capable of monitoring systems with a small volume and hence small concentrations of fluorescent ligand. Such techniques reduce the use of potentially expensive and difficult to synthesise materials.

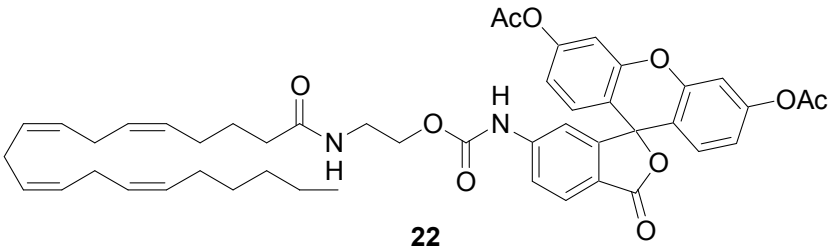
One of the most important small molecule detection techniques is fluorescence correlation spectroscopy. It is minimally invasive and allows with high temporal and spatial resolution the study of dynamic processes in biological systems, both in vitro and in vivo. For a ligand GPCR interaction, it would

give a highly accurate picture of their location and reaction to binding. Fluorescence correlation spectroscopy is an ever growing field, for an overview of current developments see Haustein and Schwille's review[82].

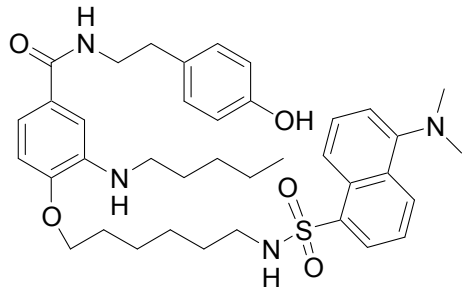
1.5.2 Fluorescent CB_2 receptor ligands

A number of fluorescent ligands have been prepared for a variety of GPCRs, including for the α and β-adrenoceptors, nicotinic, opiod, and histamine receptors[77, 81]. Our research group has also been developing fluorescent ligands for example for the A_1-adenosine receptor[83]. If a fluorescent GPCR ligand with suitable affinity can be prepared, then it is an extremely valuable biomolecular tool, not only for the use in HTS but also in other techniques mentioned above to help improve our knowledge of the location and properties of the GPCR itself.

There have been two previous attempts to prepare a fluorescent cannabinoid receptor ligand. The first was by fluorescein labelling anandamide **22** (Figure 1.12), which had no detectable CB_1 receptor binding affinity, though it is taken up by anandamide transporter system and can be used to track the cellular re-uptake of anandamide **4**[84]. The other work was by our group using homology modelling led design. A fluorescently labelled aminoalkyl analogue **24** was found to have a very small amount CB_2 binding affinity[85], detailed in Section 2.1. Other fluorescent cannabinoids were based on a novel cannabinoid structures known as Japan Tobacco ligands. A dansyl attached analogue **23** is shown, with all of the series having no affinity for the CB_2 receptor[86]. This means there is still an interest in developing a fluorescently labelled CB_2 receptor ligand.



22



23

Figure 1.12 Structures of previously synthesised fluorescent cannabinoids.

1.6 Aims and Objectives of PhD Project

1.6.1 Aims of PhD Research

The ultimate aim of this research is to design, synthesise and evaluate a fluorescent CB_2 receptor ligand, primarily for use as a molecular tool in HTS or single molecule detection techniques.

A suitable starting ligand needs to be chosen and sites for fluorophore ligation investigated. Such a starting ligand will have high affinity for the CB_2 receptor and well understood structure activity relationships. Choosing a site for fluorophore ligation will be aided by published structure activity relationships, homology modelling and mutagenesis studies, as well as our own site specific investigations on whether a fluorophore could be accommodated at that site.

1.6.2 Objectives of PhD Research

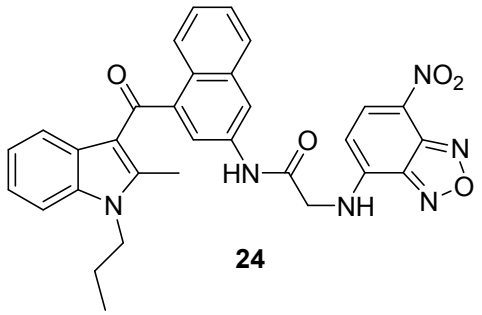
- To determine suitable starting cannabinoids for fluorophore ligation.
- To review the published structure activity relationships, homology modelling and mutagenesis studies to identify suitable sites for fluorophore ligation.
- To design, synthesise and evaluate novel cannabinoid ligands to help confirm a site's suitability for fluorophore ligation.
- To design, synthesise and evaluate novel fluorescently labelled cannabinoids at the CB_2 receptor.
- To use the developed structure activity relationship to develop a second generation of novel fluorescently labelled cannabinoids.
- To evaluate the most promising fluorescently labelled cannabinoids in fluorescent based *in vitro* assays and small molecule detection techniques.

2. Investigation of structure activity relationships of JWH-015 analogues for the CB_2 receptor

2.1 Aims

This chapter describes the design, synthesis and pharmacology of indole cannabinoid analogues for the CB_2 receptor. The main aim of this chapter is to study various substitutions to the indole cannabinoid scaffold and to identify possible sites for fluorophore ligation.

Previous work by our group using homology modelling led design resulted in the synthesis and evaluation of 3-(3-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-ylglycylamino naphthoyl)-2-methyl-1-*N*-propylindole **24** (Figure 2.1) based on a 3-naphthyl substitution to JWH-015 **7**. This work was only partially successful with **24** displacing 25% of [^{3}H]CP55-940 **5** at 10 μM[85, 86].



24

Figure 2.1 Fluorescent indole cannabinoid **24**.

In this chapter we continue the investigation of JWH-015 **7** substitutions to help determine suitable sites for fluorophore ligation. CB_2 receptor affinity and not activity is the primary endpoint, as the desired fluorescent cannabinoid will have use as a molecular tool to further our knowledge of the CB_2 receptor and in high throughput screening assays to identify new cannabinoids.

2.2 JWH-015

An ideal ligand for the development of a selective CB_2 fluorescent ligand will possess the following:

- High affinity for the CB_2 receptor.
- High CB_2 to CB_1 selectivity or potential for modifications leading to CB_2 selectivity.
- Good understanding of the ligand's pharmacophore and structure activity relationships.
- Suitable sites for fluorophore ligation, as well as the length and type of linker required.

JWH-015 **7** meets three of these four criteria. It has the desired pharmacology with a CB_2 receptor K_i = 13.8 nM, and a CB_2/CB_1 selectivity of 24.3[87], with a wealth of knowledge on substitutions that increase or decrease this selectivity[88, 89]. There is also an excellent understanding of the important pharmacophores and structure activity relationships for JWH-015 **7**, which is detailed in Section 2.2.1. What is currently lacking are detailed studies into suitable sites for fluorophore ligation, with our own group's work being the sole attempt in such coupling to indole cannabinoids[85, 86].

Furthermore, while dealing with cannabinoids in the UK, the legal requirements as laid out in the Misuse of Drugs Act 1971 need to be followed as only cannabis, its extracts and natural cannabinoids, in particular THC **1** and cannabinol **2** and their derivatives are currently subject to the act; indole cannabinoids can be synthesised without license[90].

2.2.1 Indole cannabinoid structure activity relationships

The indole class of cannabinoids were first discovered by the Sterling-Winthrop group with pravadoline **25** (Figure 2.2), while trying to create a new non-steroidal anti-inflammatory drug[70]. Conformationally restrained analogues of pravadoline led to aminoalkylindole WIN51522-2 **19**, with decreased non-steroidal anti-inflammatory but increased cannabinoid activity[71]. It was soon

realised that a morpholino or other cyclic groups were not needed for activity[91], and that a simple unbranched alkyl chain produced good CB_1 and CB_2 receptor affinities with JWH-015 (n=2) **7**[87] and JWH-007 (n=4) **26** respectively[91].

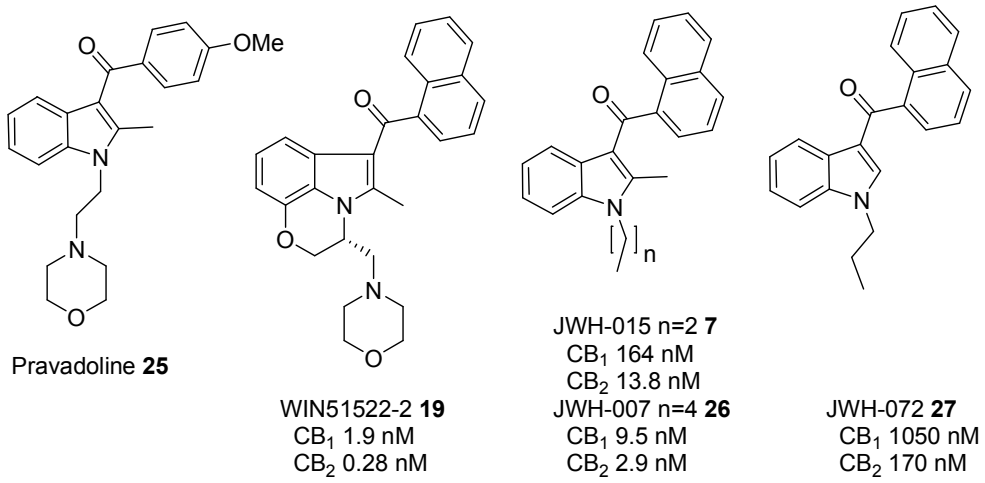


Figure 2.2 Examples of indole cannabinoids. Receptor binding affinities (K_i) quoted[92].

An aminoalkyl chain is required for activity at both the CB_1 and CB_2 receptors, with *N*-methyl and *N*-ethyl chain analogues showing very poor affinity. *N*-Propyl to *N*-hexyl chains lead to good affinity at both receptors, with the *N*-pentyl chain giving greatest affinity[87]. Extension beyond a *N*-hexyl chain leads to loss of affinity[87], which draws comparison to the C_3 alkyl chain side extension in classical and non-classical cannabinoids, whereby beyond a certain point affinity is gradually lost[93]. Though CB_2 receptor affinity is greatest in *N*-pentyl analogues, CB_2 receptor selectivity is found to be greatest in *N*-propyl analogues whilst still retaining good affinity[87].

2-Methylindole analogues with no substitution on the naphthyl ring e.g. JWH-015 **7**, generally have increased affinity for both receptors compared to unmethylated analogues e.g. JWH-072 **27**, and also induce a modest degree of CB_2 receptor selectivity. Extending the 2-alkylindole chain further leads to loss of CB_2 receptor selectivity, as well as significant loss of activity at both receptors[87]. Greatest CB_2 selectivity is found with the 2-methyl *N*-propyl analogues e.g. JWH-015 **7**[94].

Studies have also investigated whether the indole could be reduced to a pyrrole ring. Initially it was found that such alterations led to significant loss of activity and the complete indole structure was thought to be required for cannabinoid binding[91]. However, both with the development of the pyrazole cannabinoid antagonists SR-141716A **20**[95] and SR-144528 **22**[75] (Section 1.4.5), as well as investigation by Huffman *et al* of various *N*-pentyl-4-substituted pyrroles **28** (Figure 2.3), good affinity for both the CB_1 and CB_2 receptors is not reliant on the indole ring[96]. It has been suggested that these aromatic substitutions investigated by Huffman *et al*, and hence also the complete indole, improve receptor binding through aromatic stacking interactions and are important for high affinity for both the CB_1 and CB_2 receptors.

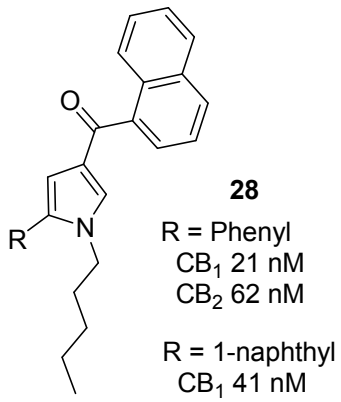
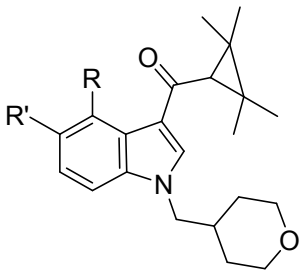


Figure 2.3 Examples of pyrrole cannabinoids. Receptor binding affinities (K_i) quoted[96].

A number of groups have studied the effect of varying the 3-indole substituent, and have found that a variety of aromatic groups are tolerated including the 3-para-anisoyl[97], 3-phenylacetyl[92] and morpholinylacetyl ring systems[98]. Unlike the indole ring system, the 3-indole substituent does not have to be aromatic in nature, with the recently developed 3-yl-tetramethylcyclopropylketones **29** having good affinity for the CB_1 and CB_2 receptors if the indole ring is unsubstituted[99] (Figure 2.4), suggesting that the 3-indole may not be interacting by an aromatic stacking mechanism.



29

R = H R' = H
CB_1 12 nM
CB_2 0.21 nM

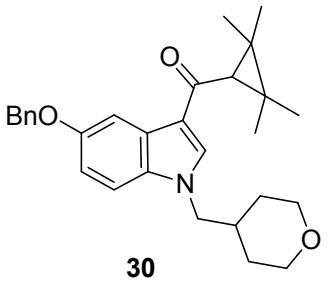
R = Br R' = H
CB_1 6.6 nM
CB_2 1930 nM

R = F R' = H
CB_1 1210 nM
CB_2 1.6 nM

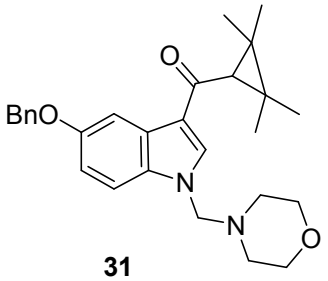
R = H R' = OBn
CB_1 9.3 nM
CB_2 > 10000 nM

Figure 2.4 Examples of 3-yl-tetramethylcyclopropylketone indole cannabinoids. Receptor binding affinities (K_i) quoted[99].

Finally, a few studies have looked at whether substitution at the 4, 5, 6 and 7-indole positions can modify cannabinoid receptor selectivity[98]. In particular, the series of 3-yl-tetramethylcyclopropyl ketones **29** showed that hydrophobic substitutions at the 4 and 5-indole position can induce significant CB_2 receptor selectivity without a large loss of CB_2 receptor binding[99]. The same research shows though that it is difficult to predict which substitutions produce these effects, especially with concurrent alteration of the *N*-alklyl and 2-indole positions. For example, a 5-benzyloxy substitution of the indole **30** would appear to be ideal for high CB_2 affinity and selectivity, but the same substitution in the conservative *N*-morpholino-ethyl analogue **31** leads to a drastic loss of affinity for both receptors (Figure 2.5). There is certainly potential for significant modification of the indole ring to increase CB_2 receptor selectivity without undue loss of affinity, but currently it is undetermined which substitutions will reliably achieve this goal.



30
CB_1 238 nM
CB_2 1.3 nM



31
CB_1 >10000 nM
CB_2 >1000 nM

Figure 2.5 3-yl-tetramethylcyclopropylketone indole cannabinoids with alteration of the *N*-alkyl group. Receptor binding affinities (K_i) quoted[99].

In summary this research shows; the importance of choosing the correct length *N*-alkyl chain on cannabinoid receptor affinity and CB_2 receptor selectivity; that a 2-methyl substitution can improve CB_2 receptor selectivity; that an indole ring system is not required and can be replaced by a pyrrole with appropriate aromatic substitution; that only a hydrophobic group and not an aromatic one needs to be attached to the 3-indole position; and that various substitutions to the 4 and 5-indole positions can help increase CB_2 receptor selectivity.

In summary, JWH-015 and related indole cannabinoids interact with the CB_2 receptor mostly through the hydrophobic interactions of the indole, naphthyl and *N*-alkyl and 2-indole side chains (Figure 2.6), offering limited possibilities for extensive substitution. Although it is thought that a number of these receptor interactions are via aromatic stacking (see Section 2.2.2), both the naphthyl and indole structures can be replaced with non-aromatic ring systems or substituted pyrroles respectively and still retain excellent binding affinities.

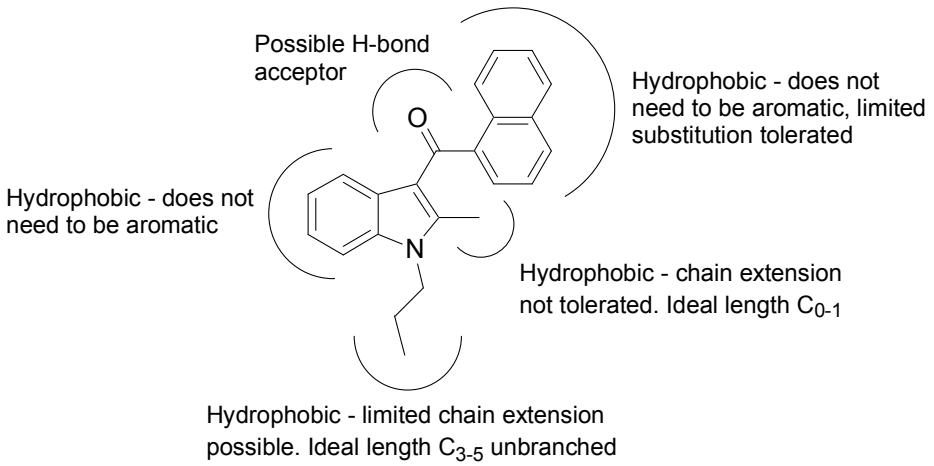


Figure 2.6 Main pharmacophores of JWH-015 and related indole cannabinoids.

Since their development, the indole cannabinoids have been greatly studied and it is beyond the scope of this work to cover this in further detail. For an up to date review of the large number of different indole cannabinoids and their variants that have been evaluated please see the work of Manera *et al*[100].

2.2.2 Cannabinoid receptor homology modelling

A number of homology modelling studies have been carried out for both the CB_1 and CB_2 receptors, with WIN55212-2 **19** being used as the archetypical indole for such research. Due to a 44% homology between the CB_1 and CB_2 receptors, raising to 68% if only the seven transmembrane α–helix domains are taken into account[16, 18], CB_1 homology models are not automatically relevant to the CB_2 receptor. Furthermore, as noted above, homology modelling based on bacteriorhodopsin has been superseded, and therefore only CB_2 models derived from the bovine rhodopsin crystal structure will be explored here.

Amino acid residues are identified by transmembrane α-helix placement, with CB_1 and CB_2 sequences aligned at the most highly conserved residue in each α-helix, with this residue given a value of x.50 (where x is the helix number), and all other residues numbered accordingly, as devised by Ballesteros and Weinstein[101]. CB_2 receptor amino acid residues are also identified by a sequence number as catalogued by Universal Protein Resource, UniProt Knowledgebase for the human CB_2 receptor[102].

CB_1 receptor models have consistently shown that the aromatic groups of WIN55212-2 **19** are embedded in the receptor, with the indole and naphthyl ring systems interacting with the aromatic residues F3.36 and W5.43, and that the aromatic residues W6.48 and Y5.39 are also likely to be important[36, 100]. The CB_2 receptor has a slightly different active site, and a translation of these results might not be appropriate, hence a number of groups have developed CB_2 receptor homology directly from the bovine rhodopsin crystal structure.

Xie *et al* and Montero *et al* have both published extensive work on the development of their model, but have as yet not published any results concerning the positioning of indole cannabinoids in the receptor binding site[103-105].

Salo *et al*, who have developed both CB_1 and CB_2 receptor models, used the CB_2 receptor model for virtual screening for novel cannabinoids, using the

classical cannabinoid HU-210 **13** as a reference ligand[106]. Again no alkylindole cannabinoid docking data is presented, and as the classical cannabinoids bind higher in the receptor than alkylindole the results are not useful[36].

Tuccinardi's group and our own group's work present the only published research for the docking of an alkylindole into a CB_2 receptor homology model based directly on the structure on bovine rhodopsin. Tuccinardi's group found that WIN55212-2 **19** had important interactions with the conserved F3.36(117), W5.43(194), W6.48(258) as found with the CB_1 receptor, but that the unconserved F5.46(197) of the CB_2 receptor is also likely to interact with WIN55212-2 **19** aromatic systems, possibly explaining its CB_2 selectivity[107]. These results were obtained by orientating the WIN55212-2 **19** with the naphthyl imbedded deep within the active site, and with a hydrophilic interaction between WIN55212-2 **19** morpholinic group and the unconserved S3.31(112) residue. Our own group's work similarly found that the lowest energy minimisation was found with WIN55212-2 **19** naphthyl embedded within the active site[85, 86]. JWH-015 **7** and its analogues do not have this hydrophilic side chain, but instead similar sized straight chain alkyl chains and whether this one result is applicable to all alkylindoles is undetermined. It would suggest that if the naphthyl ring system was so embedded, that any substitution to the naphthyl ring would lead to rapid loss of binding, yet a number of analogues have proven this not to be the case (Section 2.2.4).

2.2.3 Cannabinoid receptor mutagenesis studies

Extensive single-point mutation and chimeric studies have been carried out for both the CB_1 and CB_2 receptors. Like the cannabinoid receptor homology modelling detailed above, WIN55212-2 **19** is used as the archetypical indole cannabinoid for such studies, and due to a modest 68% homology between the two receptors in the transmembrane α–helix domains[16, 18], CB_1 receptor mutagenesis studies are not automatically relevant to the CB_2 receptor. For a comprehensive list of both CB_1 and CB_2 receptor mutagenesis studies see the reviews by Manera *et al*[100] and Ortega-Gutierrez *et al*[108].

Mutagenesis studies have identified a number of amino acid residues, including D3.49(130)[109], W4.50(158)[110], W4.64(172)[110], C174 (extra-cellular loop 2)[111], C179 (extra-cellular loop 2)[111] and Y7.53(299)[112], that either maintain the correct conformation for binding of WIN55212-2 **19** or allow signal transduction to the CB_2 receptor. These amino acids are thought to be too far away from the active site of the receptor for their mutation to directly affect binding, especially as they are essential for the binding of a number of cannabinoid ligand types and mutation will often abolish all binding.

It has been found that the conserved aromatic amino acid residues F3.36, W5.43 and W6.48 are important for WIN55212-2 **19** CB_1 binding with the F3.36A, W5.43A and W6.48A mutations leading to significant loss of activity[113]. The replacement of the aromatic phenylalanine or tryptophans with non-aromatic alanine backs up the modelling studies detailed above, that these residues are involved in aromatic stacking interactions in the cannabinoid active site, and that indole cannabinoid binding is in a higher pocket of the active site compared with classical cannabinoid ligands. Chimera studies have shown that the same 4^{th} transmembrane helix to 5^{th} transmembrane helix region is essential for WIN55212-2 **19** activity at the CB_2 receptor[114], and that as the F3.36(117), W5.43(194) and W6.48(258) are conserved in both receptors, they are also the site for indole cannabinoid binding in the CB_2 receptor[100, 113, 115].

Another residue that has been identified as important for WIN55212-2 **19** CB_2 binding is Y5.39(191). A conservative mutation of Y5.39F produced only modest changes in CB_1 and CB_2 receptor activities for a number of ligands, whereas Y5.39I mutation led to complete loss of binding for WIN55212-2 **19** as well as non-classical cannabinoid CP55-940 **5**[115]. This leads to the possibility that this residue may also be important for indole cannabinoid binding via aromatic interactions, especially due to its proximity to the known active site[115].

Finally, as WIN55212-2 **19** has been reported to be slightly selective for the CB_2 (K_i 0.3 nM) over the CB_1 receptor (K_i 1.9 nM)[116], a number of mutagenesis studies have investigated the amino acid residues that may be responsible for this selectivity. In particular it has been found that the CB_2 receptor residues S3.31(112) and F5.46(197) compared with the CB_1 receptor residues G3.31 and V5.46 may be responsible for this selectivity. F5.46V mutation of the CB_2 receptor led to a 12 fold loss of WIN55212-2 **19** binding compared to wild type, while the opposite V5.46F mutation at the CB_1 receptor led to a 14 fold gain in binding, with neither mutation having any significant effect on binding of the classical cannabinoid HU-210 **13**, the non-classical cannabinoid CP55-940 **19** or the endogenous cannabinoid anandamide **4**[117]. This suggests that F5.46 is part of the CB_2 receptor active site for indole cannabinoids and probably important for its aromatic stacking interactions.

S3.31G mutation of the CB_2 receptor led to a three fold loss of WIN55212-2 **19** binding[118], while the opposite G3.31S mutation at the CB_1 receptor led to a 4 fold gain in binding[119]. The CB_2 receptor mutagenesis study involved the double-point mutation K3.28A/S3.31G, which also led to the complete loss of binding of the classical cannabinoids THC **1** and cannabinol **2**, the non-classical cannabinoid CP55-940 **5** and the endogenous cannabinoid anandamide **4**, as well as its more minor effect on the indole cannabinoids WIN-55212-2 **19** and JWH-015 **7**. It is thought that this serine of the CB_2 receptor, though by no means essential, may improve indole cannabinoid binding through a hydrogen bonding interaction, helping to explain WIN-55212-2 **6** selectivity for the CB_2 receptor[119]. Further evidence has been found in the 3D QSAR study which identified a possible H-bonding interaction in the CB_2 receptor, absent in the CB_1 receptor, between the carbonyl of the indole cannabinoid and a H-donor in the active site[120].

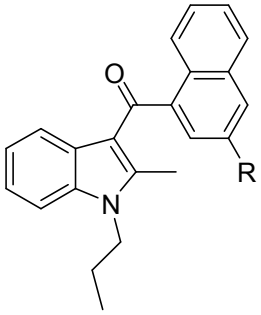
In similar vein to the homology modelling studies detailed above, mutagenesis studies have not offered any conclusive insight into the orientation of the indole cannabinoids within the CB_2 receptor active site, and therefore can not help in identifying suitable sites for fluorophore ligation. It is thought that a H-

bonding interaction available at S3.31(112) allows a degree of specificity for the CB_2 receptor, though the likely H-bond donor, the carbonyl linker between e.g. the indole and naphthyl groups of JWH-015 **7**, is present in the vast majority of indole cannabinoids, and does not offer an obvious method to improve selectivity. It might be that only the *N*-morpholinic alkylindoles such as WIN55212-2 **19** can utilise this sub-site, and that S3.31(112) has no role in CB_2 receptor selectivity for other alkylindoles.

2.2.4 Selection of JWH-015 site for investigation

In Section 2.2.1 we noted that the *N*-alkyl chain and 2-indole position will not tolerate excessive extension before complete loss of CB_2 receptor binding. An indole or other aromatic structure is required for aromatic stacking interactions needed for cannabinoid binding, and currently the limited studies of substitutions of the 4, 5, 6, and 7-indole positions suggest a role for substitution at these positions in enhancing CB_2 selectivity but not for fluorophore attachment. Furthermore, homology modelling and mutagenesis studies have been inconclusive as to the orientation of the indole cannabinoids within the active site. Therefore the primary candidate for fluorophore attachment is the naphthyl ring, and this is especially the case due to the substantial amount of work already completed on the effect of various substitutions. Here we review that work, and prioritise the naphthyl positions most suitable for study.

Previous work by our group, and in particular Yates[85, 86], identified by way of CB_2 receptor homology modelling the 3-naphthyl position of JWH-015 **7** as a site where a small fluorophore could be attached via a short linker glycine. The final fluorescent compound **24,** as well as the intermediates in its synthesis, were investigated for their CB_2 receptor affinities (Table 2.1).



R substituent	CB_2 receptor K_i (nM)
H (JWH-015) **7**	36 (lit. 13.8[87])
NO_2	143
NH_2	191
NH-Gly	420
NH-Gly-NBD	25% displacement at 10 μM

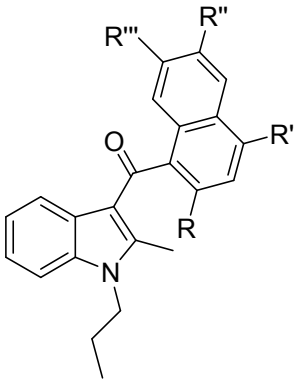
Table 2.1 3-naphthyl substitutions to JWH-015 **7**[85, 86].

These results show that increasing the steric bulk of the 3-naphthyl substitution leads to a loss of CB_2 receptor affinity. Even so, the relatively bulky and hydrophilic R = NH-Gly analogue retains a degree of binding. If there was no room in the active site for any substitution at the 3-naphthyl position, one as relatively bulky as a glycine group would abolish binding affinity. Furthermore the original fluorescent ligand identified during Yates's work as a potential compound that could fit into the active site, did not have a glycine linker. This linker had to be added to prevent quenching of the NBD fluorophore by the naphthyl ring system, and might be responsible for increasing the substitution size beyond what the CB_2 receptor can easily accept[85, 86]. It could also be the case that the linker length is not sufficient to allow removal of the attached fluorophore from interfering with cannabinoid binding, where as a longer linker might. Therefore the 3-naphthyl is still a promising candidate for investigation, especially as no other published research has been carried out on substitution at this position.

One other group has looked at the effect on naphthyl substitution on cannabinoid receptor binding, detailed in the extensive work by Huffman *et*

al[92], and of particular interest is the *N*-propyl naphthyl substituted series (Table 2.2).

Looking at the naphthyl substitutions in order, the 2-naphthyl position appears poorly suited for investigation. Though the 2-methoxy substitution is tolerated, it is likely to be competing with the 2-methylindole substituent which, if removed, improves 2-methoxy substituted analogues greatly (data not shown)[92]. As extension of the 2-indole substituent leads to loss of binding affinity for both receptors, it is likely that extension of the 2-naphthyl position will do the same. Hence the 2-naphthyl position is not an ideal candidate for investigation.



R	R´	R´´	R´´´	CB_2 receptor K_i (nM)
H	H	H	H	13.8 (JWH-015 **7**)
OCH_3	H	H	H	455
H	CH_3	H	H	14
H	C_2H_5	H	H	12
H	C_3H_7	H	H	12
H	C_4H_9	H	H	49
H	OCH_3	H	H	97
H	OC_2H_5	H	H	221
H	H	OCH_3	H	30
H	H	H	CH_3	16
H	H	H	C_2H_5	240
H	H	H	OCH_3	441

Table 2.2 2, 4, 6 and 7-naphthyl substitutions to JWH-015 **7**[94].

The 4-naphthyl position tolerates both alkyl and alkoxy substitutions, with alkoxy substitutions leading to a greater loss of CB_2 receptor binding. The fact that a 4-butyl substitution leads to only a modest loss of affinity compared to JWH-015 **7** is very encouraging and, with the 4-methyl, -ethyl and -propyl analogues having very similar binding to JWH-015 **7**, shows that there is at the very least room for a certain amount of 4-naphthyl substitution. There has also been a number of longer 4-alkoxy naphthyl substitutions made, but unfortunately their cannabinoid receptor affinities have not been published[121]. Even so, the 4-naphthyl position, like the 3-naphthyl position of our group's work detailed above, is a good prospect for investigation.

The 6 and 7-naphthyl positions will be considered together, as although 6-methoxy and 7-methyl substitutions are well tolerated, further extension of the 7-naphthyl substitution to a 7-ethyl or 7-methoxy leads to a large loss of CB_2 receptor binding affinity. With no further extension of the 6-naphthyl itself, the poor results for 7-naphthyl may also translate to similar results for the 6-naphthyl substitution extension, as the two occupy a close location. Therefore, neither offer a good prospect for investigation, especially as the 4-naphthyl position has shown positive results with a number of larger substitutions.

These two pieces of research have shown that the 3 and in particular the 4-naphthyl position may be the most suitable for substitution, but that substitution at either site will likely lead to some loss of CB_2 receptor affinity. To offset this, the use of a *N*-pentyl side chain, i.e. an analogue of JWH-007 **26**, may improve binding at both cannabinoid receptors, while decreasing any CB_2 receptor selectivity. Therefore investigation of *N*-propyl and *N*-pentyl indole cannabinoids with substitution at the 4-naphthyl position will be the primary objective and investigation of substitution at the 3-naphthyl position a secondary objective of this current work.

2.3 Synthetic strategy

In order to make various analogues of JWH-015 **7** and JWH-007 **26**, with substitution at the 3 and 4-naphthyl positions, a strategy was required that allowed efficient analogue production with the fewest synthetic steps.

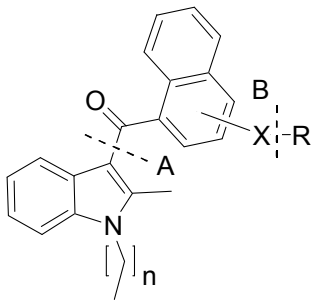
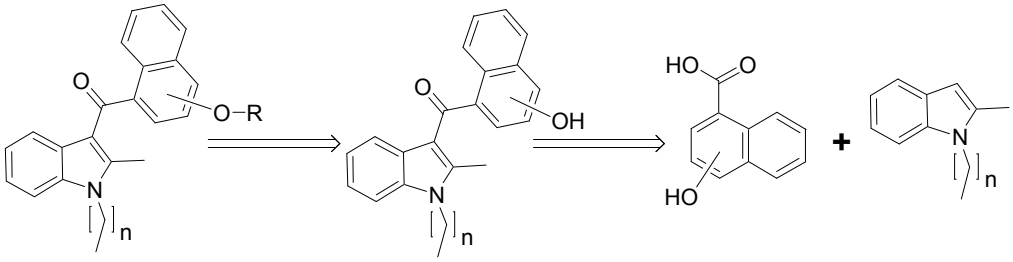


Figure 2.7 Disconnections (A and B) for the synthesis of JWH-015 analogues. X = heteroatom, R = desired substitutions.

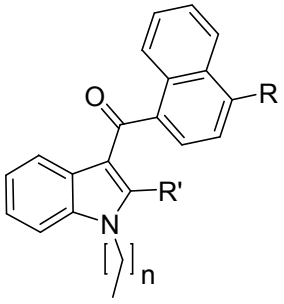
There are two important disconnections shown in Figure 2.7: A and B. Disconnection A is between the indole and naphthyl rings, and disconnection B is between the R substituent and the naphthyl ring. Coupling of the two ring systems before the addition of the R substituent allows bulk production of the completed ring system as well as addition of R substituents that may interfere with the coupling reaction, leading to the retrosynthesis depicted in Scheme 2.1.



Scheme 2.1 Retrosynthetic analysis of JWH-015 (n = 2) and JWH-007 (n = 4) analogues with substitution at the 3 and 4-naphthyl position.

Due to the difficulties in controlling aromatic addition chemistry, a heteroatom linker to the R substituents was used to facilitate analogue synthesis. Oxygen was chosen as this heteroatom (X = O in Figure 2.6) as previous research had

shown that alkoxy compared with alkyl substituents at the 2 and 4-naphthyl position can be tolerated, leading to a modest lost of affinity at the CB_2 receptor (see Table 2.3 for a comparison of 4-methoxy versus similarly sized 4-ethyl naphthyl substituted analogues)[88].

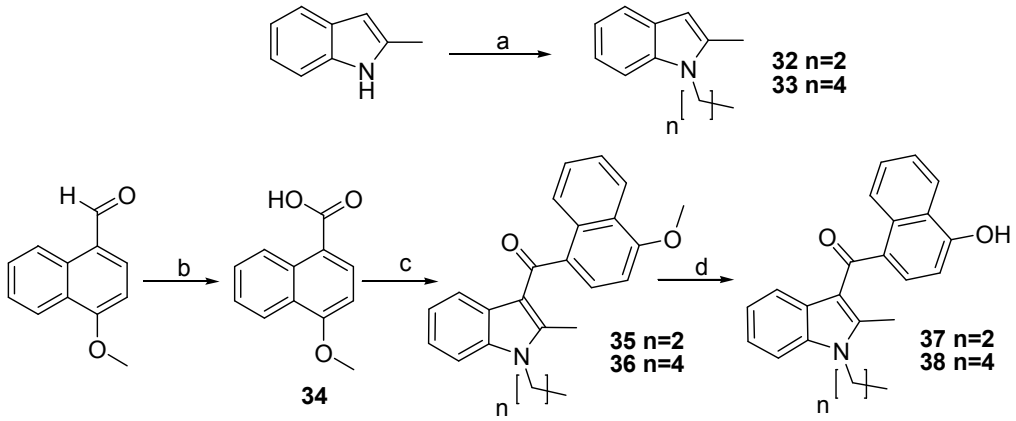


R	R´	N	CB_2 receptor K_i (nM)
CH_2CH_3	H	2	10
OCH_3	H	2	32
CH_2CH_3	CH_3	2	12
OCH_3	CH_3	2	97
CH_2CH_3	H	4	0.69
OCH_3	H	4	12.4
CH_2CH_3	CH_3	4	0.42
OCH_3	CH_3	4	1.9

Table 2.3 Comparison of JWH-015 **7** (n = 2) and JWH-007 **26** (n = 4) analogues with 4-ethyl and 4-methoxy naphthyl substitutions, from Huffman *et al*[88].

2.4 Synthesis of 4-naphthyl modified analogues

The 4-hydroxy JWH-015/JWH-007 analogues **37/38** were synthesised from the commercially available 2-methylindole and 4-methoxy-1-naphthaldehyde as shown in Scheme 2.2.



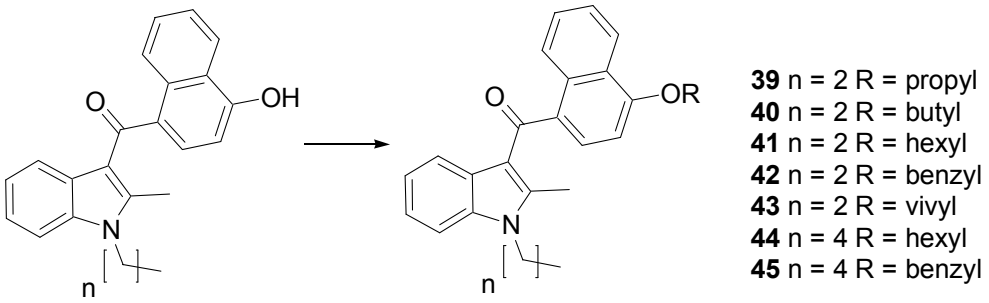
Scheme 2.2 Synthesis of 4-hydroxy JWH-015/JWH-007 analogues **37/38** (a) NaH, DMF, n=2: 1-bromopropane (89 %); n=4: 1-bromopentane (98 %); (b) $NaClO_2$, NaH_2PO_4, H_2O, *t*BuOH, 2-methyl-2-butene (34 %); (c) (i) $SOCl_2$, reflux; (ii) **32** or **33**, Et_2AlCl, DCM (n=2 50 %, n=4 71 %); (d) BBr_3, DCM (n=2 58%, n=4 67 %).

2-Methylindole was N-alkylated using sodium hydride as the deprotonating base and the corresponding straight chained 1-bromoalkene to produce 2-methyl-*N*-propylindole **32** and 2-methyl-*N*-pentylindole **33**. Purification by flash column chromatography gave almost quantitative yields, but both indoles were found to partially degrade from a colourless to brown oil with orange precipitate, even whilst protected from light and stored at below 0 °C. Therefore for all subsequent reactions, these alkylated indoles were freshly purified by flash column chromatography.

Oxidation of the 4-methoxy-1-naphthaldehyde to the corresponding naphthoic acid **34** was performed using sodium chlorite, in the presence of the chlorine scavenger 2-methyl-2-butene, which gave modest but repeatable yields with multiple recrystallisations from methanol[122-124]. An attempt to use Jones Reagent for this oxidation failed to produce the desired carboxylic acid.

For the acylation of indoles it has been found that traditional Friedel Crafts coupling with aluminium chloride, even with protected *N*-alkylindoles, leads to poor or non-existent conversion[88, 125, 126]. Okauchi *et al* found that use of the milder alkylaluminium chlorides, in particular dimethyl or diethylaluminium chloride, led to much improved yields of 50-90%[125]. Therefore the acylation between 2-methyl-*N*-aklylindole **32/33** and the acyl chloride formed when 4-methoxynaphthalene-1-carboxylic acid **34** was heated at reflux with thionyl chloride, was catalysed with diethylaluninium chloride producing the 2-methyl-*N*-alkyl-3-(4-methoxy-1-naphthoyl)-indoles **35/36** in good yields of 50-71% after flash column chromatography purification.

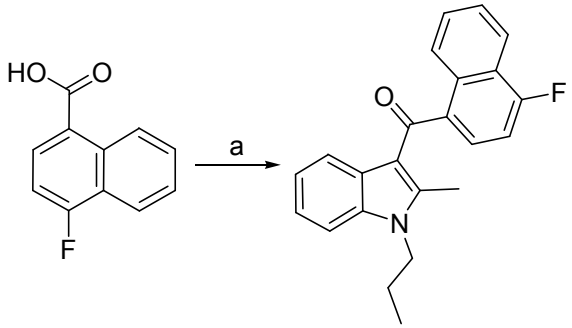
Demethylation with boron tribromide of **35/36** afforded the 2-methyl-*N*-alkyl-3-(4-hydroxy-1-naphthoyl)-indoles **37/38**, which when carefully purified by flash column chromatography produced reasonable yields of 58-67%[127-129]. These 4-hydroxyl analogues were produced in sufficient quantity to allow the synthesis of a variety of 4-alkoxynaphthoyl substituted analogues of JWH-015 **7** and JWH-007 **26** (Scheme 2.3).



Scheme 2.3 Synthesis of 4-alkoxynaphthoyl substituted analogues of JWH-015 and JWH-007 (a) NaH, DMF, 1-BrR (17-32 %).

The *N*-propyl-4-propoxy, butoxy, hexyloxy, benzyloxy and vinyloxynaphthoyl analogues (**39-43** respectively) of JWH-015 **7** and the *N*-pentyl-4-hexyloxy and benzyloxynaphthoyl analogues (**44-45** respectively) of JWH-007 **7** were produced by alkylation of the relevant 2-methyl-*N*-alkyl-3-(4-hydroxy-1-naphthoyl)-indoles **37**/**38** using sodium hydride base with the corresponding straight chained 1-bromoalkane, benzyl bromide or allyl bromide (Scheme 2.3) and purified by preparative layer chromatography. Low purified yields for

these alkylations were recorded (17-32%); this was due to the isolation of only the purist part of the compound's chromatographic band and that some compounds (e.g. **42** and **45**) required multiple purifications to remove side products with similar rates of flow. It was found impossible to suitably purify the n=4 benzyloxy analogue **45** to allow its pharmacological evaluation due to these side products, even when a variety of different mobile phases were used.

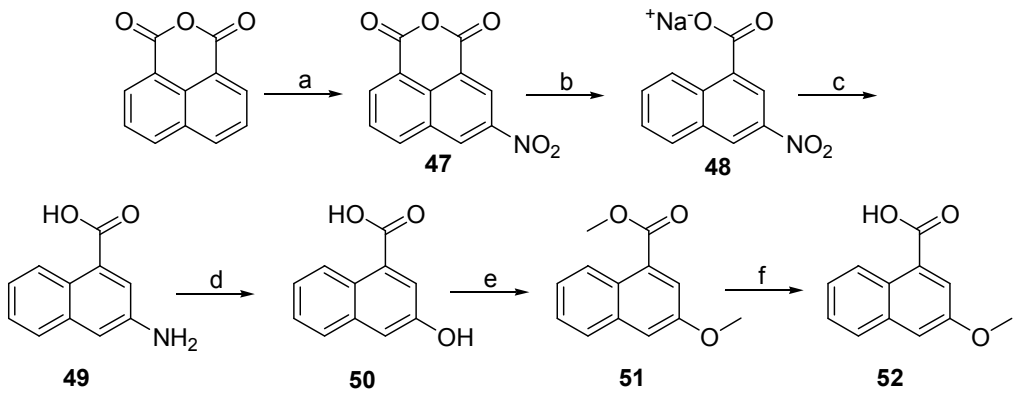


Scheme 2.4 Synthesis of 4-fluoronaphthoyl JWH-015 analogue **46** (a) (i) $SOCl_2$, reflux; (ii) **32**, Et_2AlCl, DCM (43 %).

Finally the *N*-propyl-4-fluoronaphthoyl analogue **46** was synthesised by coupling the commercially available 4-fluoronaphthalene-1-carboxylic acid, with 2-methyl-*N*-propylindole **32** and, as with the synthesis of **35/36,** a variation of Friedel Crafts acylation utilising diethylaluminium chloride was used (Scheme 2.4)[125]. Purification was by preparative layer chromatography, produced a slightly lower yield of 43% than the previous Friedel Crafts acylations. This was due to the desire for a purer compound appropriate for pharmacogical evaluation and the generally lower yields achieved with preparative layer compared to flash column chromatography.

2.5 Synthesis of 3-naphthyl modified analogues

To synthesise the 3-methoxynaphthoyl JWH-015 analogue **53**, and thereafter other desired 3-ethernaphthoyl analogues, 3-methoxy-1-naphthoic acid **52** had to be first synthesised as shown in Scheme 2.5.

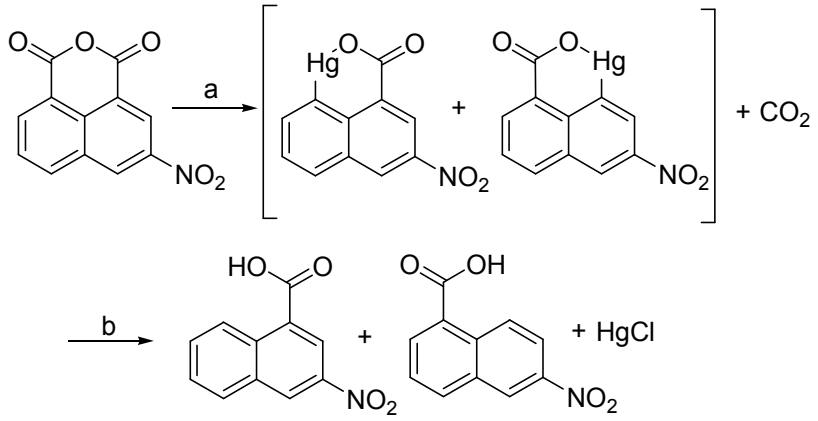


Scheme 2.5 Synthesis of 3-methoxy-1-naphthoic acid **52** (a) HNO_3, H_2SO_4 (69 %); (b) (i) HgO, $NaOH_{(aq)}$, microwave 200 °C; (ii) $HCl_{(aq)}$, reflux (35 %); (c) H_2, Pd/C, EtOH, $NaOH_{(aq)}$ (98 %); (d) $NaNO_2$, $H_2SO_{4(aq)}$, (63 %); (e) MeI, NaH, DMF (70 %); (f) KOH, MeOH, reflux (57 %).

Though commercially available 3-nitro-1,8-naphthoic anhydride **47** was simply prepared from 1,8-naphthoic anhydride by a single *meta* nitration using one equivalent of nitric acid in concentrated sulfuric acid, and purified by recrystallisation from acetic acid[130, 131]. Though crude yields were quantitative, difficulties with the filtration of insoluble particulates from the hot acetic acid crystallisation solution lead to only a modest purified yield of 69%.

Selective decarboxylation of 3-nitro-1,8-naphthoic anhydride **47** to form the 3-nitro-1-naphthoic acid geometric isomer **48** was performed using yellow mercury (II) oxide. Previous studies of this reaction have suggested that although the mechanism is not clear, mercury (II) acetate is formed and that this mercury species initially adds between the carboxyl groups, and then onto the naphthyl ring releasing one carboxyl group as carbon dioxide (Scheme 2.6). The organo-mercury intermediates are stable but undergo acid hydrolysis to release the mercury salt and the desired mono acids[132]. The presence of an

electron withdrawing group such as the 3-nitro group leads predominantly to the 3-nitro-1-naphthoic acid geometric isomer **48** in good yields[132, 133].



Scheme 2.6 Selective decarboxylation of 3-nitro-1,8-naphthoic anhydride **47** (a) (i) $NaOH_{(aq)}$; (ii) HgO, 3:1 AcOH:H_2O, reflux (b) HCl, reflux.

The initial insertion of the mercury species into the anhydride is thermally driven and even at reflux the reaction may take 2-4 days to complete[133-136]. A previous study[85] and trial reactions followed this procedure, but after recrystallisation, it was necessary to remove any undesired geometric isomer, hence yields on the trial reactions were found to be less than 20%. A way to improve both yield and reduce reaction time had been demonstrated by Moseley and Gilday using microwave irradiation with temperatures up to 200 °C, for 15-30 min[132]. Replication of this method, with microwave irradiation for 28 min at 200 °C, followed by acid hydrolysis and recrystallisation from acetone led to an improved yield of 35%, with the whole reaction taking less than 24 h to reach completion. To prevent excessive pressure build up from carbon dioxide release in the microwave reactor, initial heating was gradual. Once at 200 °C, the pressure was stable and the carbon dioxide was safely contained within the microwave tube.

Isolation of the single geometric isomer 3-nitro-1-naphthoic acid **48** was confirmed by proton and COSY nuclear magnetic resonance. Analysis of COSY NMR spectrum with H^1 NMR overlaid, shown in Figure 2.8, identifies six protons in the aromatic region, two of which are sharp doublets with a coupling constant of J = 2.4-2.5 Hz. The COSY spectrum shows one set of

coupling between the two sharp doublets and another set of coupling between the four one proton multiplets. Both of these observations are consistent with the *meta* substitution pattern of 3-nitro-1-naphthoic acid, confirming this isomer's purity and the absence of 6-nitro-1-naphthoic acid.

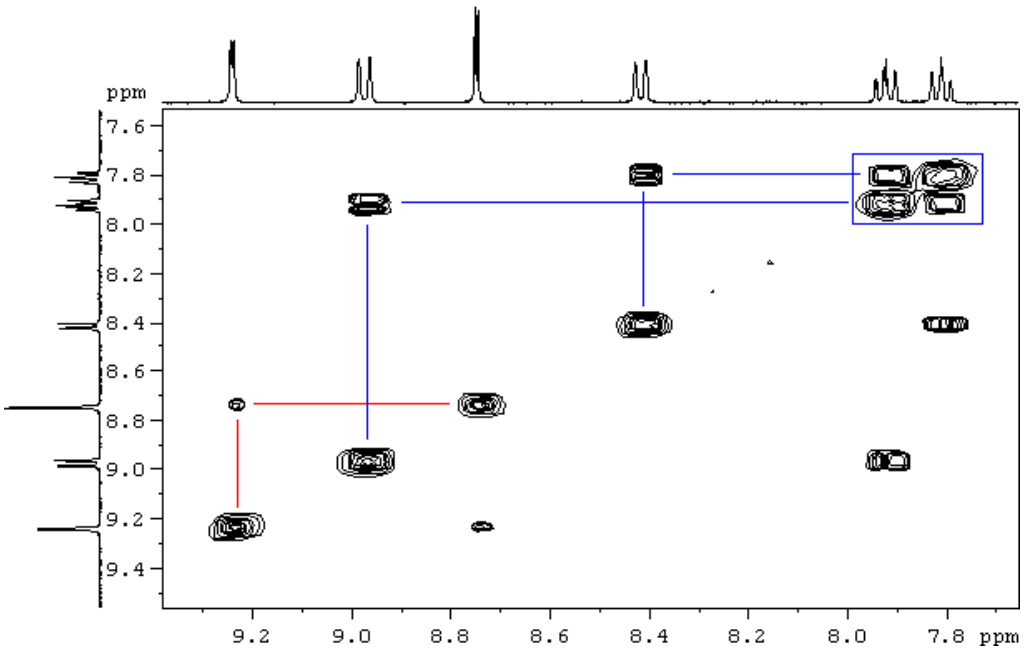


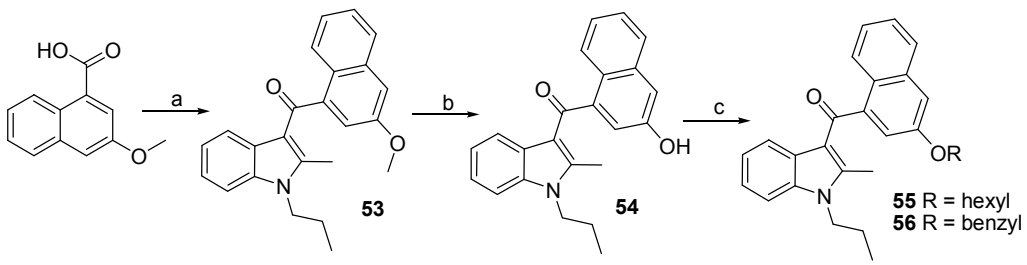
Figure 2.8 COSY NMR of 3-nitro-1-naphthoic acid **48**, with H^1 NMR on left and top axis. Red lines show coupling between 2-H and 4-H, and blue lines show coupling between 5-H, 6-H, 7-H and 8-H.

3-Nitro-1-naphthoic acid **48** was reduced with hydrogen and palladium catalysis over activated charcoal, to produce sodium 3-amino-1-naphthate **49** in quantitative yields[133]. 3-Amino-1-naphthoic acid was converted to 3-azido-1-naphthoic acid, by precipitating the free acid and then oxidising with sodium nitrite. Immediate treatment with refluxing 40 % sulphuric $acid_{(aq)}$, hot filtration, and precipitation produced 3-hydroxy-1-naphthoic acid **50** in a good yield of 63% considering the harshness of the conditions used[133, 137].

To obtain the desired 3-methoxy-1-naphthoic acid **52**, 3-hydroxy-1-naphthoic acid **50** was methylated at both the phenol and carboxylic acid using methyl iodide and sodium hydride base in excess to form methyl 3-methoxy-naphthalene-1-carboxylate **51**, with lower yields than expected obtained due to loss of some material during workup. Base hydrolysis of the ester with

potassium hydroxide and recrystallisation from ethanol:water (1:1) produced the 3-methoxy-1-naphthoic acid **52** required for the synthesis of 3-ethernaphthoyl JWH-015 analogues. A modest yield of 57% was obtained due to the need for multiple recrystallisations to ensure sufficient purity for the Friedel Crafts acylations.

The *N*-propyl-3-methoxynaphthoyl analogue **53** was synthesised by coupling the synthesised 3-methoxy-1-naphthoic acid **52**, with 2-methyl-*N*-propylindole **32,** and as with the synthesis of 4-methoxy analogues in section 2.4**,** a variation of Friedel Crafts acylation utilising diethylaluminium chloride was used, again producing an excellent yield of 90% after flash column chromatography purification (Scheme 2.7)[125].

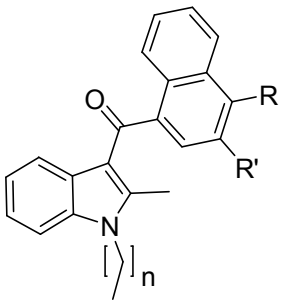


Scheme 2.7 Synthesis of 2-methyl-*N*-propyl-3-(3-alkyloxy-1-naphthoyl)-indoles (a) (i) $SOCl_2$, reflux; (ii) **32**, Et_2AlCl, DCM (90 %); (b) BBr_3, DCM (71 %).(c) NaH, DMF, 1-BrR, (16-32 %).

Demethylation with boron tribromide of **53** produced 2-methyl-*N*-propyl-3-(3-hydroxy-1-naphthoyl)-indole **54** in good yields after flash column chromatography purification[127-129]. The *N*-propyl-3-hexyloxy **55** and benzyloxy **56** analogues of JWH-015 **7** were produced by alkylation of **54** using sodium hydride with either straight chained 1-bromohexane or benzyl bromide respectively, and were purified by preparative layer chromatography. Similar yields were obtained as with the 4-naphthyl analogues, with the benzyloxy analogue **56** again being difficult to purify due to side products with similar rates of flow.

2.6 Pharmacology

All synthesised 3 and 4-naphthyl analogues, except 2-methyl-*N*-pentyl-3-(4-benzyloxy-1-naphthoyl)-indole **45** were assessed for their CB_2 binding affinity (Table 2.4).



Compound	N	R	R´	CB_2 K_i (nM)
JWH-015 **7**	2	H	H	lit. 13.8[87]
46	2	F	H	32.3 (± 8.93)
37	2	OH	H	418 (± 159)
JWH-094 **35**	2	OMethyl	H	31.4 ± 10.3 lit. 97.3[87]
JWH-261	2	OEthyl	H	lit. 221[92]
39	2	OPropyl	H	91.8 (± 4.12)
40	2	OButyl	H	271 (± 94.3)
41	2	OHexyl	H	843 (± 81.1)
42	2	OBenzyl	H	560 (± 70.1)
43	2	OAllyl	H	87.1 (± 10.3)
JWH-007 **26**	4	H	H	lit. 2.94[87]
38	4	OH	H	6.86 ± (2.50)
JWH-098 **36**	4	OMethyl	H	2.71 (± 0.918) lit. 1.88[87]
JWH-260	4	OEthyl	H	lit. 25[92]
44	4	OHexyl	H	143 (± 23.0)
45	4	OBenzyl	H	not tested
54	2	H	OH	77.3 (± 22.4)
53	2	H	OMethyl	92.8 (± 18.9)
55	2	H	OHexyl	809 (± 230)
56	2	H	OBenzyl	166 (± 67.4)

Table 2.4 CB_2 receptor K_i were determined from radioligand binding assays with [^{3}H]CP55-940 **5**. The results in parentheses are standard error of the mean for three individual experiments run in triplicate.

To ensure appropriate purity for pharmacological testing, all compounds were analysed using two different HPLC methods to confirm greater than 98 % purity. Compounds that were not sufficiently pure were further purified by

preparative layer chromatography until they were. In the case of the n=4 benzyloxy analogue **45**, it was not found possible to separate this compound from a side product with a very similar rate of flow in a range of different systems. Microanalysis was not used to assess purity due to the majority of compounds being oils, and the inherent difficulties in ensuring complete solvent removal necessary for the accuracy of this technique.

The *N*-propyl-4-naphthyl series demonstrates that increasing the bulk of the substitution at this position leads to a gradual loss of CB_2 binding affinity. This loss of affinity is in line with results of other 4-naphthyl analogues developed by Huffman *et al*[94], with a gradual reduction of affinity with the 4-methoxy **35**, -ethoxy, -propoxy **39**, -butyloxy **40** and -hexyloxy **41** series. Although the 4-ethoxy, -propoxy and -butyloxy analogues still have modest affinity, the 4-hexyloxy analogue has poor affinity. The unsaturated substitutions of 4-vinyloxy **43** and -benzyloxy **42** also result in a loss of CB_2 receptor binding affinity. This loss is greatest with the bulkier 4-benzyloxy analogue but it still has a higher affinity than the 4-hexyloxy analogue. The 4-vinyloxy analogue has comparable affinity to the similar sized 4-propoxy analogue. The 4-hydroxyl **37** analogue led to a large drop in CB_2 receptor binding affinity, which cannot be explained with respect to its size, and is likely due to this region of the active site being intolerant of hydrophilic groups. This backs up previous research that showed 4-alkoxy substituents cause a greater reduction in binding than similar sized 4-alkyl substitutions[92]. Lastly the 4-fluoro **46** substitution appeared to have little effect on CB_2 receptor binding affinity as this compound had only a slight loss of affinity compared to JWH-015 **7**.

These results suggest that there may be a limit to the 4-naphthyl substitution size that can be accommodated, and that hydrophilic substitutions such as a 4-hydroxyl hinder binding greatly. It is also likely that there is not a hydrophobic sub-site near the 4-naphthyl available to interact with saturated or unsaturated hydrophobic groups.

The *N*-pentyl-4-naphthyl series demonstrate an increase in CB_2 receptor binding compared with the same substitutions in the *N*-propyl-4-naphthyl

series, as found in previous work[92]. With no CB_1 receptor binding affinities available it is not possible to say whether the *N*-pentyl series has lost a degree of CB_2 receptor selectivity compared to the *N*-propyl series. However, it is very interesting that the *N*-pentyl-4-hydroxyl **38** and -4-hexyl **44** analogues have sixty and six fold increases respectively in CB_2 binding affinity compared to the same substitutions in the *N*-propyl series.

These results add weight to previous research that *N*-pentyl analogues have greater CB_2 receptor binding affinity when compared to the similarly substituted *N*-propyl analogues. Furthermore, the large increases in binding affinity of the *N*-pentyl-4-hydroxyl and -4-hexyl analogues compared with *N*-propyl analogues, suggest that though these groups may not be well suited for *N*-propyl analogues, the extra affinity of the *N*-pentyl means a wider range of both hydrophilic groups and larger hydrophobic groups may be tolerated. These results, therefore, cast into doubt the suggestion from the *N*-propyl analogues results that there may be a limit to the 4-naphthyl substitution size that can be accommodated.

Finally the *N*-propyl-3-naphthyl series show a similar profile to the *N*-propyl-4-naphthyl series. The 3-methoxy **53** analogue retains modest affinity for the CB_2 receptor, whilst the 3-hydroxyl **54** analogue also retains modest affinity with over four fold greater affinity than experienced by the *N*-propyl-4-hydroxyl analogue. The 3-hexyloxy **55** analogue has a similarly poor affinity for the CB_2 receptor as the *N*-propyl-4-hexyloxy analogue, where as the 3-benzyloxy **56** analogue retains modest affinity with over three fold greater affinity than experienced by the *N*-propyl-4-benzyloxy analogue.

These results suggest that as with the *N*-propyl-4-naphthyl series, increasing chain length and bulk leads to loss of affinity, with the potential that, beyond a certain point, chain extension could not be accommodated. Considering the results with the *N*-pentyl-4-naphthyl series, this suggestion should be judged with care, especially as the *N*-propyl-3-hydroxyl and -3-benzyl analogues have significantly greater CB_2 receptor binding than their comparable *N*-propyl- 4-

naphthyl analogues. Also, it may be the case that the 3-naphthyl position offers a more suitable substitution site for hydrophilic and unsaturated groups.

Both the 3 and 4-naphthyl positions show the ability to accommodate relatively bulky groups, although with a moderate degree of CB_2 receptor binding affinity loss, though not complete abolition. In particular the *N*-propyl and *N*-pentyl-4-naphthyl series have included substitutions much larger, and of a greater variety than have been investigated before.

2.7 Summary

We have successfully synthesised a series of seventeen *N*-alkyl-3/4-naphthyl substituted JWH-015 analogues for the CB_2 receptor. These have shown a mixture of results, but generally confirm that, although larger or hydrophilic substitutions are tolerated to a degree, they do lead to a significant reduction in CB_2 receptor binding affinity. Furthermore, unsaturated substitutions do not appear to be interacting with any aromatic stacking receptor sub-site, and instead lead to a loss of CB_2 receptor binding at a rate comparable to their size. However, the *N*-pentyl-4-naphthyl substituted series showed that although larger substitutions may lead to significant loss of CB_2 receptor binding, this effect can be reduced with other modifications to the indole cannabinoid scaffold. It also showed that the 4-naphthyl position can tolerate large substitutions, suggesting that there is significant space in, or outside of, the receptor active site to accommodate such substitutions.

These results provide sufficient promise that fluorophore substitution at the 3 and especially the 4-naphthyl position may indeed be tolerated. In Chapter 3 we utilise these results to continue our work towards an understanding of the structure activity relationships of 4-naphthyl substitutions, as well as synthesising fluorescently labelled CB_2 receptor ligands based on the JWH-015 **7** indole cannabinoid scaffold.

3. Investigation of JWH-015 fluorescent analogues for the CB_2 receptor

3.1 Aims

This chapter describes the design, synthesis and pharmacology of fluorescent indole cannabinoid analogues for the CB_2 receptor. The main aim of this chapter is to study various linker lengths and types from the indole cannabinoid scaffold, as well as the effect of attaching various fluorophores to that linker.

In this chapter we utilise the efforts detailed in Chapter 2, as well as the work by Yates on the synthesis and evaluation of the fluorescently labelled JWH-015 analogue 3-(3-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-ylglycylamino napthoyl)-2-methyl-1-*N*-propylindole **24**[85, 86], to help in the design and synthesis of a series of new fluorescent compounds. We also investigate the effect of linker length on CB_2 receptor binding as well as if a more hydrophilic linker affects CB_2 receptor binding affinity.

3.2 Design of fluorescent JWH-015 analogues

A number of variables need to be decided upon when designing any fluorescent JWH-015 analogues for synthesis. Firstly, where on the JWH-015 **5** scaffold should substitution take place? The aim of Chapter 2 was to help identify if the 3 and 4-naphthyl positions would be suitable choices for fluorophore attachment. Secondly, to both prevent quenching of the fluorophore by the naphthyl ring system and to remove the fluorophore far enough away from the cannabinoid ligand to decrease interference on its CB_2 receptor binding, what is a suitable length and type of linker for these purposes? Finally, with numerous fluorophores available on the market, with varying size and fluorescent properties, which should be chosen for attachment?

3.2.1 Selection of JWH-015 site for fluorophore ligation

The research in Chapter 2, helped to confirm that both the 3 and 4-naphthyl positions of JWH-015 **5** are good prospects for investigation. Considering the greater number of results for the 4-naphthyl substitutions that show a large range of additions can be accommodated, and that a previous attempt by Yates to synthesise a 3-naphthyl substituted fluorophore with very limited affinity **22**[85, 86], efforts for fluorophore ligation will be focused on the 4-naphthyl position.

3.2.2 Selection of length and nature of fluorophore linker

The results presented in Section 2.6, as well as the previous work by Yates[85, 86] and Huffman *et al*[94] detailed in Section 2.2.6, show that hydrophobic substitutions as the 4-naphthyl position is favoured over smaller hydrophilic substitutions. This suggests that any linker between the indole cannabinoid scaffold and fluorophore should be generally hydrophobic in nature. As such linkers have, for practical synthetic purposes, terminal hydrophilic functional groups to allow coupling, the rest of the linker should probably be of a straight chain saturated nature.

The optimum length that the hydrophobic linkers should be, is unclear. Yates' 3-(3-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-ylglycylamino napthoyl)-2-methyl-1-*N*-propylindole **24**[85, 86] contained a glycine linker which may be either too large for the active site when added to the bulk of the NBD fluorophore, or too short and therefore not removing the NBD fluorophore far enough away from the active site. The *N*-alkyl-4-naphthyl analogues, detailed in Section 2.6 and by Huffman *et al*[92], show that there is certainly space for 4-butyl or 4-ethoxy substitutions, and that even larger groups such as 4-benzyloxy and 4-hexyloxy can be tolerated with moderate loss of CB_2 receptor binding. This suggests that there may either be a sub-site pocket within the active sight that can accommodate some steric bulk, or that substitutions at the 4-naphthyl are directed towards the exit of the receptor active site. The latter option is of particular interest, because if true, the loss of affinity on increasing chain length may partly be due to an entropic effect, and the 4-naphthyl may be ideal for attaching a long linker to remove any fluorophore from the active site. Obviously with increasing linker length, entropy costs increase and so decreases CB_2 receptor binding affinity. If though an allosteric binding site could be found that interacted with either the fluorophore's functional groups or aromatic nature, then binding affinity may be restored. With the prospect of both room in the active site for a small fluorophore substitutents, or access to an exit from it, a range of different length hydrophobic linkers should be investigated to determine their effects. Therefore preparation of ethyl, butyl, hexyl and octyl linker lengths will be the synthetic target.

Although the priority should be in using hydrophobic linkers, preparing similar sized hydrophilic analogues might allow useful comparisons. For example, an analogue of a straight chained octyl linker could be (2-(2-ethoxy)ethoxy)ethyl, which has two regularly placed oxygens instead of carbons in the chain, and where the main difference is in hydrophilicity not size. As the results in section 2.6 confirm that hydrophilic groups near the naphthyl ring system e.g. 4-hydroxyl, are not well tolerated, any investigation using hydrophilic linkers should be of sufficient length that they may interact with favourable amino acid residues outside of the immediate indole cannabinoid active site.

Therefore preparation of the partially oxygen substituted octyl linker analogue e.g. (2-(2-ethoxy)ethoxy)ethyl will also be part of the synthetic strategy.

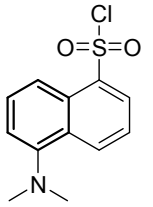
3.2.3 Selection of fluorophores for ligation

The selection of the fluorophore for ligation is essential in creating suitable fluorescent cannabinoids. Generally small amine reactive fluorophores e.g. dansyl chloride **57** and 4-fluoro 7-nitrobenzofurazan (NBD-F) **58** (Figure 3.1) are used, with a number of GPCR ligands being successfully labelled with these fluorophores[138]. Small fluorophores may have a number of advantages over larger fluorophores in that they are generally cheap and are less likely to interfere with ligand binding. The larger the substitution, the greater the entropic costs of binding, as well as the increased risk of the substituent directly preventing the ligand receptor interaction. The dansyl and NBD fluorophores do suffer from relatively poor fluorescent properties, especially that their fluorescence is attenuated in aqueous environments[139], meaning that increased radiation intensity may be required for confocal microscopy or small molecule detection techniques, that may damage cells.

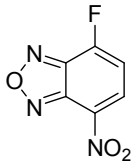
At the other end of the scale are the much larger BODIPY® and Cyanine fluorophores which have also been successfully used to produce fluorescent GPCR ligands[138]. With longer excitation wavelengths, hence they are termed red absorbing dyes, and good fluorescence in aqueous environments, these fluorophores are more suited for small molecule detection techniques. However BODIPY® compounds are only commercially available from Molecular Probes, Invitrogen, as are Cyanine compounds from GE Healthcare, both are expensive and come with a pre-attached activated linker .e.g. Bodipy 630/650-X Osuccimide **60** and Cy5-Osuccimide **61**. This is ideal for fluorescently labelling proteins, nucleotides and other macro molecules where a pre-attached linker removes the need for an extra synthetic step. For small molecule work, whereby the length and nature of the linker may be crucial, fluorophores with pre-attached linkers reduce the choice of both length and nature of linker. As we wish to assess the linker intermediates for their CB_2

receptor binding and so help develop structure activity relationships, use of BODIPY® and Cyanine fluorophores would prevent this.

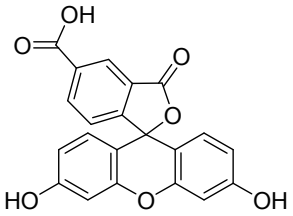
In between these two fluorophore extremes are moderately sized fluorophores such as 5-carboxyfluorescein **59**. Fluorescein has similar fluorescence properties to dansyl and NBD, absorbing at a slightly longer wavelength, but is considerably larger in size, comparable to BODIPY® and Cyanine. 5-carboxyfluorescein has no pre-attached linker, and offers a cheap alternative to BODIPY® and Cyanine. If 5-carboxyfluorescein attachment is tolerated then investigation of BODIPY® and Cyanine may be warranted.



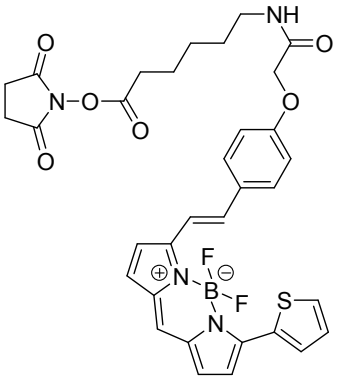
57 dansyl chloride
Absorbs 340-350 nm
Emits 510-560 nm



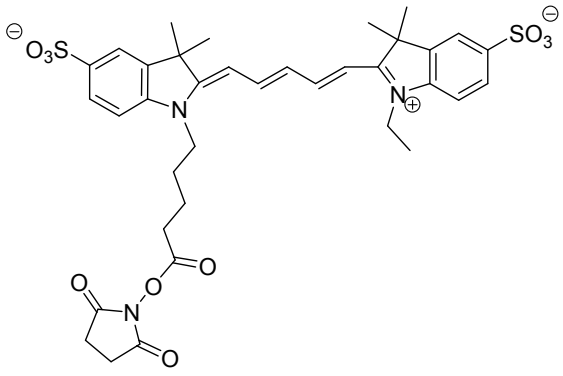
58 4-fluoro-7-nitrobenzofurazan (NBD-F)
Absorbs 470 nm
Emits 540 nm



59 5-carboxyfluorescein
Absorbs 492 nm
Emits 517 nm



60 BODIPY 630/650-X Osuccimide
Absorbs 629 nm
Emits 624 nm



61 Cy5-Osuccimide
Absorbs 647 nm
Emits 663 nm

Figure 3.1 Structure and spectral data for amine reactive fluorophores. λ_{max} absorbances and emission are quoted. Dansyl chloride and NBD-F data taken from Lakowicz[139], and, as dansyl chloride alone is non-fluorescent, a range of values of its conjugates are given. 5-carboxyfluorescien data is taken from product specifications of Sigma-Aldrich[140], and Bodipy 630/650-X Osuccimide and Cy5-Osuccimide data taken by work from Buschmann *et al*[141].

As this investigation of fluorophore ligation at the 4-naphthyl position of JWH-015 **5** requires preparation of a number of varying linker length and types, the use of fluorophores such as BODIPY® and Cyanine would be both expensive and inconvenient. If the synthesis and evaluation is successful in producing a fluorescently labelled indole cannabinoid, then BODIPY® and Cyanine analogues could be developed to try and improve fluorescent properties.

Though the dansyl and NBD fluorophores do not have ideal fluorescent properties, their smaller size is less likely to interfere with binding of indole cannabinoid scaffold, which is especially important as the results of Chapter 2 show that increasing steric bulk leads to loss of CB_2 receptor binding affinity. Furthermore, the dansyl and NBD fluorophores are a good choice for investigation, as they do not have an integrated linker and so allow the use of a greater variety of linkers. This also allows pharmacological evaluation of the initial 4-naphthyl JWH-015 linker coupling products, before the fluorophore attachment. To investigate what effect a larger sized fluorophore might have on CB_2 receptor binding affinity in comparison to the smaller dansyl and NBD molecules, 5-carboxyfluorescein is also a fluorophore for investigation.

3.3 Synthetic strategy

During the previous Chapter's synthesis of various JWH-015 analogues, sufficient quantities of the starting material for this synthetic strategy, 2-methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37**, were produced to allow for preparation of a number of fluorescent analogues. To allow efficient analogue production, a synthetic strategy with the fewest number of steps as well as the opportunity to investigate any promising intermediates' structure activity relationships was sort.

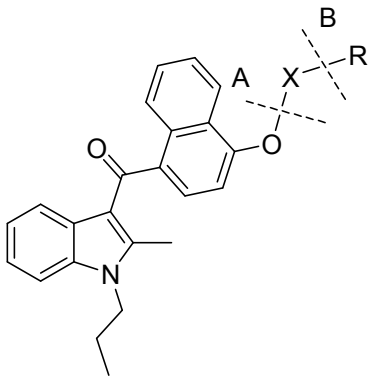
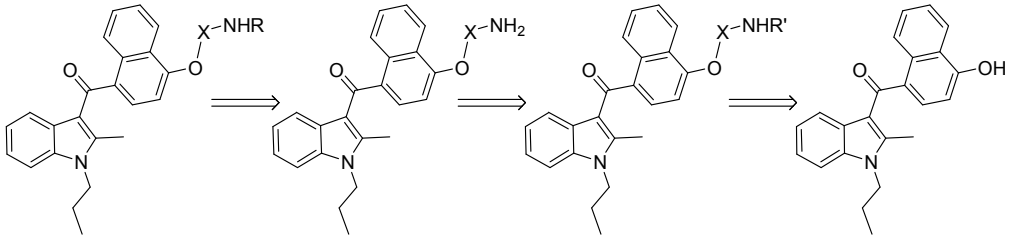


Figure 3.2 Disconnections (A and B) for the synthesis of fluorescent JWH-015 analogues. X = linker, R = fluorophore.

There are two important disconnections shown in Figure 3.2, A and B. Disconnection A is between the hydroxyl and the desired linker, and disconnection B is between the linker and the desired fluorophore. Linker attachment to the naphthyl ring before coupling to any fluorophore prevents loss of potentially expensive fluorophore starting material. Any pre-attachment of the fluorophore to the linker would likely involve protection of the linking functional group, which would need to be deprotected before any coupling to the naphthyl ring, which itself will be unlikely to be a high yielding reaction. As the JWH-015 **5** scaffold contains no functional groups requiring protection during the addition of the linker or fluorophore, leaving the fluorophore attachment to the final step removes two extra steps involving the fluorophore. Furthermore, the preparation of a number of JWH-015 analogues with various linker lengths and types allows them to be isolated and their CB_2 binding affinities evaluated, hence adding to our understanding of structure activity

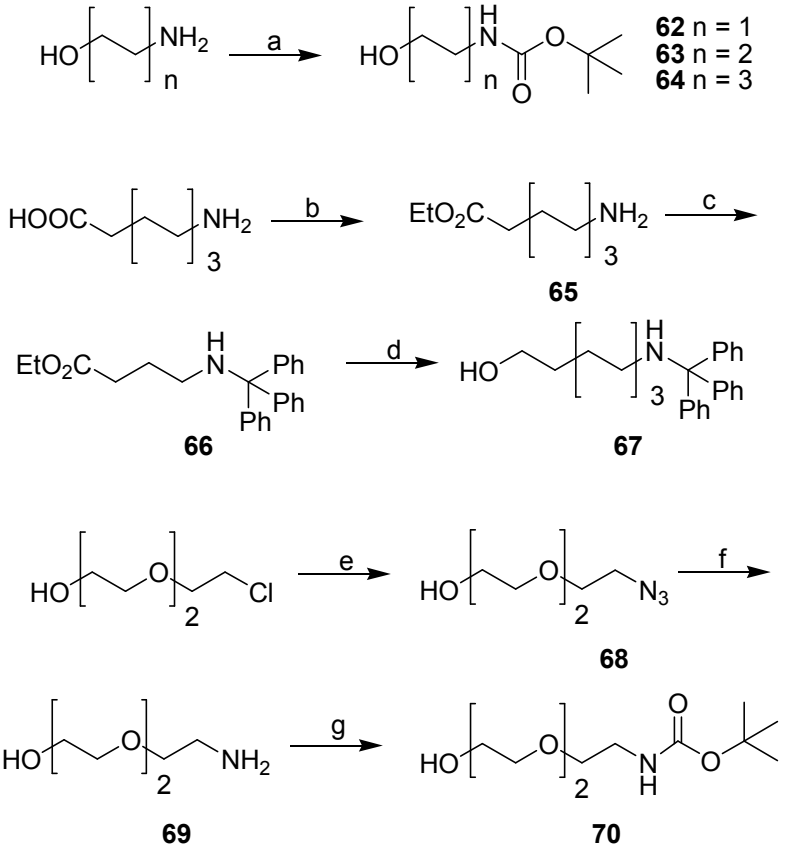
relationships on the effect of substitution at the 4-naphthyl position of JWH-015 **5**. This rationale leads to the retrosynthesis depicted in Scheme 3.1.



Scheme 3.1 Retrosynthetic analysis of fluorescent JWH-015 analogues. X = linker, R = fluorophore and R´ = amine protecting group.

3.4 Synthesis of protected linkers

Before attachment of the desired linkers to 2-methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** the linkers needed to be suitably protected as detailed in Scheme 3.2.



Scheme 3.2 Synthesis of *N*-*t*butoxycarbonyl or *N*-trityl protected linkers (a) *t*butylphenyl carbonate, TEA, EtOH n=1 (76 %), n=2 (65 %), n=3 (96 %); (b) PTSA, EtOH, reflux, (96 %); (c) trityl chloride, TEA, $CHCl_3$, (89 %); (d) $LiAlH_4$, THF, (65 %); (e) NaN_3, DMF, (99 %); (f) $SnCl_2$, EtOH, (54 %), or Ph_3P, H_2O, ether, reflux (31 %); (g) di-*t*butyl dicarbonate, $KOH_{(aq)}$, (68 %).

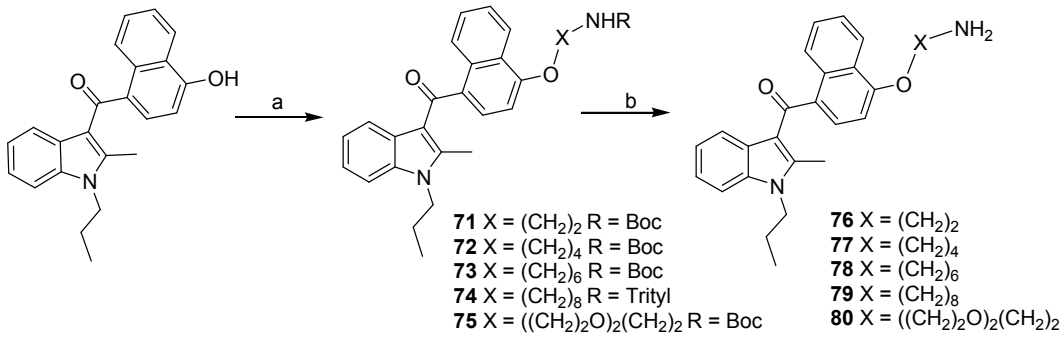
The ethyl, butyl and hexyl *t*butylhydroxyalkylcarbamates **62**-**64** were prepared from the corresponding aminoalcohols with standard amine boc protection with *t*butylphenyl carbonate, all purified by flash column chromatography to produce good yields. 8-Aminooctan-1-ol and 2-(2-(2-aminoethoxy)ethoxy)ethanol were not commercially available and a different strategy was required for the production of their amine protected counter parts.

8-Aminooctanoic acid was the starting material used for the octyl linker, following a synthetic pathway devised by Ammann *et al*[142]. Before any amine protection could take place the carboxylic acid needed to be protected and this was achieved by acid catalysed ethyl esterification and flash column chromatography purification to form ethyl 8-aminooctanoate **65** in near quantitative yields. Esterification also improved handling of the compound, as the hydrophilic nature of 8-aminoctanioc acid made it insoluble in most organic solvents. This ester was now amine protected by treatment with trityl chloride, to form the trityl protected ethyl 8-(tritylamino)octanoate **66** in good yield after purification by flash column chromatography. A trityl protection was chosen as it is resistant to the strong reducing conditions required for conversion of the ester to an alcohol, unlike other amine protecting groups such as boc. Lithium aluminium hydride reduction yielded the desired amine protected aminoalcohol 8-(tritylamino)octan-1-ol **67**, again purified by flash column chromatography to a modest yield expected with this type of reduction, which was then ready for coupling to 2-methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37**.

Finally, a method was found to synthesise the desired 2-(2-(2-aminoethoxy)ethoxy)ethanol **69** from the starting material 2-(2-(2-chloroethoxy)ethoxy)ethanol. Nucleophilic substitution of the terminal chloro functional group using sodium azide gave 2-(2-(2-azidoethoxy)ethoxy)ethanol **68** in quantitative yields, which it was envisaged could be reduced to the terminal amine analogue. Previous research had performed this azide reduction with use of hydrogen with a palladium on activated carbon catalyst[143], but attempts to follow this method were unsuccessful, and therefore two alternative methods were investigated for this reduction. Both of these methods were successful, but after flash column purification a method utilising tin (II) chloride heated at reflux in ethanol[144], provided a greater yield of 2-(2-(2-aminoethoxy)ethoxy)ethanol **69**, than a method utilising triphenylphosphine heated at reflux in water[145]. This aminoalcohol then underwent standard amine boc protection with di-*t*butyl dicarbonate and purification yielded the desired *t*butyl 2-(2-(2-hydroxyethoxy)ethoxy)ethylcarbamate **70** in good yields.

3.4 Synthesis of fluorescent JWH-015 analogues

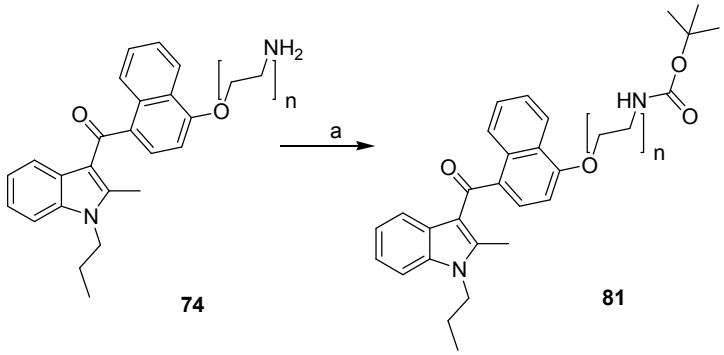
With the five desired linkers prepared in Section 3.3, they could now be coupled to 2-methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** as detailed in Scheme 3.3.



Scheme 3.3 Coupling of protected linkers and deprotection (a) **62-64, 67, 70**, DEAD, Ph_3P, THF, (37-91 %); (b) TFA, H_2O, DCM, (29-62 %).

All coupling was via the mild Mitsunobu reaction[146] between the 4-hydroxyl of the indole cannabinoid and the unprotected terminal alcohol of the desired linker. Standard conditions were used, with an excess of diethyl azodicarboxylate and triphenylphosphine, to form the protected 4-naphthyl linker attached indole cannabinoid analogues **71-75**. Good yields of the crude products were obtained for all reactions, but due to the presence of the reduction product diethyl hydrazine-1,2-dicarboxylate, which had a very similar rate of flow to some of the desired products, flash column chromatography was needed to be repeated for a number of compounds resulting in a lower purified yield of between 37-91%, with the lowest yields resulting from the need for multiple purifications. Standard removal of the boc and trityl amine protecting groups under acidic conditions, and purification by preparative layer chromatography yielded the desired amines **76-80**, ready for fluorophore ligation. Though high crude yields were obtained for these simple deprotections, purification greatly lowered the yields, as only the purist part of the compound's chromatographic band was isolated and some compounds required multiple purifications to remove side products with similar rates of flow that were identified by HPLC.

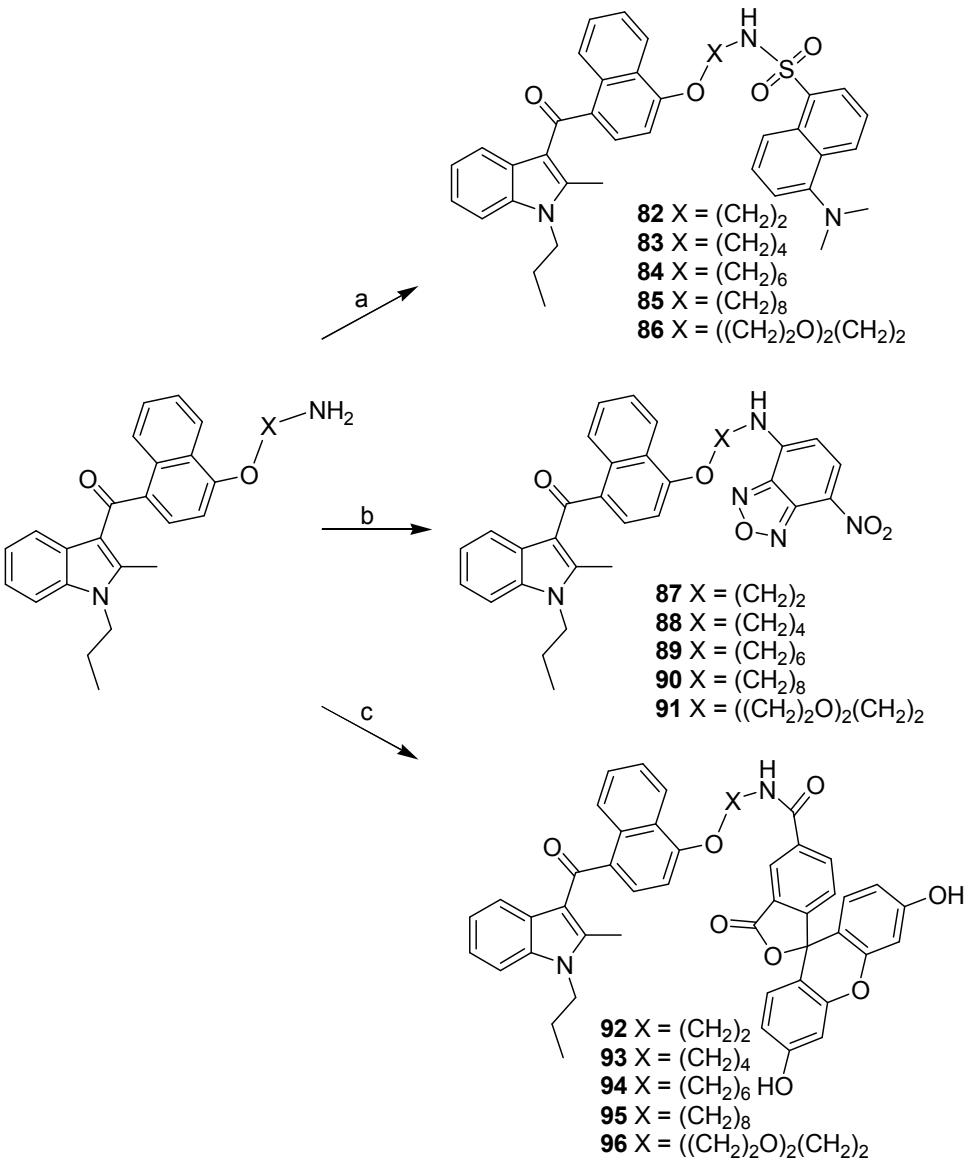
As well as investigating the effect of fluorophore attachment on CB_2 receptor binding, the protected and free amine linker analogues would also be assessed for their affinity. As the octyl linker analogue utilised a trityl protection, a boc protected analogue was synthesised so that it could be compared (Scheme 3.4). Boc protection was achieved using *t*butylphenyl carbonate in alkaline aqueous conditions, and purification by preparative layer chromatography gave in modest yields, the desired amine protected analogue **81**.



Scheme 3.4 Preparation of boc protected X = octyl linker analogue **81** (a) *t*butylphenyl carbonate, $NaHCO_{3(aq)}$, (37 %).

The final fluorophore coupling could now take place, as detailed in Scheme 3.5. All three fluorophores readily undergo nucleophilic substitution by the primary amine of the 4-naphthyl linker analogues **76-80**. After preparative layer chromatography purification, dansyl **82-86** and NDB **87-91** analogues were isolated in modest yields, partly due to incomplete coupling and partly due to some of these analogues not being found to be suitably pure on HPLC analysis, and were repurified as required. In some cases this meant that insufficient compound was left remaining to allow either further HPLC analysis or use in CB_2 radioligand binding assays.

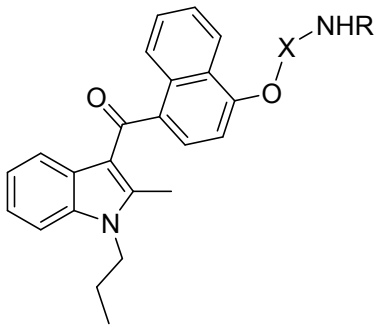
In the case of the 5-carboxyfluorescein analogues, initial attempts to use the unactivated 5-carboxyfluorescein for coupling failed to produce the desired compounds. The activated 5-carboxyfluorescein-*N*-succinimdyl ester did produce the desired results, with very high yields of **92-96** after purification by preparative layer chromatography.



Scheme 3.5 Preparation of fluorescent JWH-015 analogues (a) dansyl chloride, TEA, DCM, (29-46 %); (b) 4-fluoro-7-nitrobenzofurazan TEA, DCM, (15-29 %); 5-carboxyfluorescein-*N*-succinimdyl ester, TEA, DCM, (80-96 %).

3.5 Pharmacology

All synthesised JWH-015 4-naphthyl linker (boc protected or free amine) analogues, as well as the majority of JWH-015 4-naphthyl fluorophore analogues were assessed for their CB_2 binding affinity (Table 3.1).



Compound	X	R	CB_2 K_i (nM) or % displacement at 10 μM
71	$(CH_2)_2$	Boc	25%
72	$(CH_2)_4$	Boc	44%
73	$(CH_2)_6$	Boc	566 (± 229)
81	$(CH_2)_8$	Boc	52%
75	$((CH_2)_2O)_2(CH_2)_2$	Boc	1810 (± 162)
76	$(CH_2)_2$	H	1260 (± 194)
77	$(CH_2)_4$	H	1270 (± 117)
78	$(CH_2)_6$	H	762 (± 372)
79	$(CH_2)_8$	H	1580 (± 212)
80	$((CH_2)_2O)_2(CH_2)_2$	H	1210 (± 311)
82	$(CH_2)_2$	Dansyl	48%
83	$(CH_2)_4$	Dansyl	34%
84	$(CH_2)_6$	Dansyl	not tested
85	$(CH_2)_8$	Dansyl	not tested
86	$((CH_2)_2O)_2(CH_2)_2$	Dansyl	not tested
87	$(CH_2)_2$	NBD	71%
88	$(CH_2)_4$	NBD	61%
89	$(CH_2)_6$	NBD	34%
90	$(CH_2)_8$	NBD	not tested
91	$((CH_2)_2O)_2(CH_2)_2$	NBD	57%
92	$(CH_2)_2$	5-Carboxyfluorescein	25%
93	$(CH_2)_4$	5-Carboxyfluorescein	41%
94	$(CH_2)_6$	5-Carboxyfluorescein	41%
95	$(CH_2)_8$	5-Carboxyfluorescein	33%
96	$((CH_2)_2O)_2(CH_2)_2$	5-Carboxyfluorescein	21%

Table 3.1. CB_2 receptor K_i or % specific binding displacement at 10 μM were determined from radioligand binding assays with [^{3}H]CP55-940 **5**. Displacement results are from two individual experiments run in triplicate and K_i results are the mean from three individual experiments run in triplicate with the standard error of the mean in parentheses.

To ensure appropriate purity for pharmacological testing, all compounds were analysed using two different HPLC methods to confirm greater than 98 % purity. Compounds that were not sufficiently pure were further purified by preparative layer chromatography until they were. In the case of one NBD (**90**) and three dansyl (**84-86**) analogues, it was not found possible to separate these compounds from trace impurities without rendering the quantity of compound insufficient for testing. Microanalysis was not used to assess purity due to all compounds being oils and the majority of purified fluorescent compounds being isolated in small quantities, where such analysis would consume a significant portion.

With regard to the ten 4-naphthyl linker analogues **71-73**, **75-80** and **81**, the general effect of the attachment of all linkers is a substantial drop in CB_2 receptor binding affinity when compared with JWH-015 **7**. Apart from the hexyl analogues **73** and **78**, the free amine linker analogues have greater CB_2 receptor affinity than their boc protected precursors. This effect is difficult to quantify as the majority of boc protected analogues, in particular the ethyl, butyl and octyl linkers, have insufficient CB_2 receptor binding to allow calculation of a K_i value. All compounds for pharmacological testing were prepared as a stock solution by dissolving in a suitable organic solvent, e.g. DMSO or ethanol. Beyond a certain concentration of compound, in this case 10 μM, these organic solvents would be at a concentration that significantly interferes with the binding assay. Therefore, for compounds with a very high K_i, their CB_2 receptor binding affinity is measured as the percentage of specific [^{3}H]CP55-940 **5** binding displaced at 10 μM. In the case of the (2-(2-ethoxy)ethoxy)ethyl analogues, K_i values were obtainable for both the boc protected **75** and free amine **80** analogues, with the free amine analogue having 33% greater affinity.

In the case of the hexyl analogues, both have moderately poor CB_2 receptor binding affinity, with the free amine analogue **78** having slightly lower K_i than its boc protected precursor **73**, but both have relatively large error margins that makes conclusive interpretation difficult. These two hexyl analogues do stand out from the other 4-naphthyl linker analogues with the two lowest K_i of the

series. Finally a comparison between the octyl **81** and **79** and (2-(2-ethoxy)ethoxy)ethyl **75** and **80** analogues shows that there is a slightly greater affinity for the hydrophilic rather than the similar sized hydrophobic chain.

These results suggest that, whatever their length or nature, linkers with a terminal free amine or boc-protected amine lead to a large loss of CB_2 receptor binding affinity. Whether the free amine analogues are better tolerated than the boc protected analogues because of reduced steric bulk or interaction with a hydrophilic residue is unknown. The fact that increasing linker length does not gradually lead to a loss of affinity across the series, with the octyl and (2-(2-ethoxy)ethoxy)ethyl analogues having similar or greater affinity than the ethyl and butyl, suggests that the 4-naphthyl position is not embedded deep within the active site. If it was, and there was not a large sub-site for the 4-naphthyl substitutions to be accommodated, then increasing chain length should rapidly lead to abolishment of CB_2 receptor binding affinity. These results also suggest that a more hydrophilic linker such as (2-(2-ethoxy)ethoxy)ethyl may improve affinity compared to hydrophobic analogues, but whether this effect may be due to interaction with hydrophilic amino acid residues is unknown. Finally the hexyl linker analogues, showing the greatest CB_2 receptor binding affinity of the series, suggest that at that linker length, and hence position of the primary or secondary amine, there may be an interaction with amino acid residues that is helping to improve affinity.

Of the fifteen synthesised fluorescently labelled JWH-015 analogues, four were not tested due to difficulties in purifying to the required standard, and this unfortunately leaves the results table missing important data. With the eleven evaluated fluorescently labelled analogues, all substituents were found to reduce CB_2 receptor binding affinity to the point where a K_i could not be calculated.

The highest CB_2 receptor binding affinity was found with use of the smallest fluorophore of NBD. In particular, the ethyl **87**, butyl **88** and (2-(2-ethoxy)ethoxy)ethyl **91** NBD analogues were the only fluorescent compounds to displace over 50% of [^{3}H]CP55-940 **5** specific binding, suggesting that the

NBD fluorophore may be tolerated both close to the naphthyl ring and further away at the end of a longer linker. It is also surprising that all eleven fluorescently labelled analogues displaced [^{3}H]CP55-940 **5** to a noticeable degree, from the small dansyl fluorophore with an ethyl linker to the large fluorescein fluorophore with octyl linker. This adds weight to the results of the 4-naphthyl linker series above, which suggested that substitution at the 4-naphthyl position is not embedded within the active site and may give access to an exit of the receptor site.

The fluorescently labelled JWH-015 analogues show no discernable pattern except that the NBD fluorophore is better tolerated than either the dansyl or 5-carboxyfluorescein fluorophore. This might be due to interactions between the heteroatoms of the NBD molecule and various receptor amino acid residues, as might be the case with primary amine 4-naphthyl linker analogues. The NBD fluorophore, as found with Yates work[85, 86], seems to be a good fluorophore candidate for substitution of the indole cannabinoids.

3.6 Summary

We have successfully synthesised and evaluated a series of JWH-015 4-naphthyl linker analogues and 4-naphthyl fluorescently labelled JWH-015 analogues, with varying linker length and nature, coupled to three different fluorophores.

For the JWH-015 4-naphthyl linker analogues, a number of aminoalcohols were amine boc protected to facilitate coupling to 2-methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** using a mild Mitsunobu reaction. In the case of the amine boc protected 2-(2-(2-aminoethoxy)ethoxy)ethanol **70**, a synthetic pathway from the commercially available 2-(2-(2-chloroethoxy)ethoxy)ethanol was employed, whereas for the aminooctanol linker, a synthetic pathway from the commercially available 8-aminooctanoic acid was utilised, requiring amine trityl protection.

All five Mitsunubo couplings proceeded smoothly, with simply acid cleavage of the boc or trityl protecting group yielding the desired free amines for analysis and conjugation. For comparative purposes the boc protected octyl linker analogue **81** was also synthesised. Simple nucleophilic substitution at the free amine by either dansyl chloride or 4-fluoro-7-nitrobenzofurazan yielded the desired dansyl or NBD fluorescent JWH-015 analogues respectively. In the case of the fluorescein JWH-015 analogues, 5-carboxyfluorescein alone was not sufficiently reactive to allow conjugation, hence the activated 5-carboxyfluorescein-*N*-succinimdyl ester was used to achieve high yields of the desired products..

Pharmacological analysis of the series of JWH-015 4-naphthyl linker analogues showed a general loss of affinity with increasing linker length as expected. None of these analogues led to complete loss of affinity though, suggesting that an area, around the 4-naphthyl position of JWH-015 when bound to the CB_2 receptor, can tolerate a reasonable degree of steric bulk. The fact that the free amine analogues were generally better tolerated than their corresponding boc protected analogues, and that the 2-(2-(2-aminoethoxy)ethoxy)ethoxy linker was better tolerated than the similarly sized aminooctyl linker, suggests that this area of the CB_2 receptor binding site may have a degree of hydrophilic character. Finally this series showed most promise with the boc protected **73** and free amine **78** hexyl linker analogues, which against the general trend of lost affinity with increasing linker length, led to modest improvements suggesting possible positive interactions with receptor amino acid residues.

The series of fluorescently labelled JWH-015 analogues all gave such low binding affinities that calculation of their binding affinities was not appropriate. There was no pattern found between the length of linker and binding affinity, suggesting that the fluorophore bulk is a greater factor in losing affinity than any affect of the linker, and that the linker position or length used was not sufficient to remove the fluorophore from interfering with receptor binding. Of the fluorophores used the NBD analogues showed most promise, consistently displacing a greater percentage of radioligand than the

other two fluorophores, possible because its smaller size is interfering less with receptor binding.

It is disappointing that none of the fluorescently labelled indole cannabinoids showed significant CB_2 receptor binding affinity. The results do however help to increase our understanding of the structure/activity relationship around 4-naphthyl substitution. In particular, the hexyl linker analogues suggest that, at this position, the amine may be interacting with receptor amino acid residues, and that the NBD is the fluorophore of choice for future investigation of naphthyl-substituted indole cannabinoids.

4. Investigation of structure activity relationships of CP55-940 analogues for the CB_2 receptor

4.1 Aims

This chapter describes the design and synthesis of non-classical cannabinoid analogues for the CB_2 receptor. The main aim of this chapter is to study various substitutions to the non-classical cannabinoid scaffold and to identify possible sites for fluorophore attachment.

There has previously been no published work investigating fluorophore ligation to either the classical or non-classical cannabinoid scaffold. Before any such molecules can be synthesised an investigation of the literature, as well as in house synthesis and evaluation of various substitutions to the non-classical cannabinoid bicyclic structure is required. Ideally, substitutions that are both large and hydrophobic need to be investigated, due to the nature of the fluorophore ligation. The fluorophores likely to be used are large and generally hydrophobic in nature, and may even be larger than the original cannabinoid molecule. Furthermore the fluorophore needs to separate from the main cannabinoid ligand by a suitable length linker, to help prevent both interference with cannabinoid binding and any possible fluorescent quenching by the cannabinoid molecule.

In this chapter we aim to investigate substitutions to the non-classical cannabinoid CP55-940 **5** to help determine suitable sites for fluorophore ligation. In a similar way to with the investigation of substitutions to the indole cannabinoid JWH-015 **7** in Section 2, CB_2 receptor affinity and not activity is the primary endpoint, as the desired fluorescent cannabinoid will have use as a molecular tool to further our knowledge of the CB_2 receptor and in high throughput screening assays to identify new cannabinoids.

4.2 CP55-940

An ideal ligand for the development of a selective CB_2 fluorescent ligand will possess the following:

- High affinity for the CB_2 receptor.
- High CB_2 to CB_1 selectivity or potential for modifications leading to CB_2 selectivity.
- Good understanding of the ligand's pharmacophores and structure activity relationships.
- Suitable sites for fluorophore ligation, and the length/type of linker required.

Unlike JWH-015 **7** in Section 2.2, CP55-940 **2** (Figure 4.1) meets only two of these four criteria. It has the desired pharmacology, with a very high affinity for the CB_2 receptor with a K_i = 0.7 nM, but is not selective for either the CB_1 or CB_2 receptors[69]. There are a number of possibilities for modification to CP55-940 that may induce CB_2 receptor selectivity detailed in Section 4.2.1.3. Though CB_2 selectivity is desired, in the case of CP55-940 a high degree of selectivity may be difficult to achieve without excessive modification. As any fluorescent cannabinoid will have primarily a use as a molecular tool, CB_2 selectivity in any assay could be induced by the addition of a CB_1 receptor antagonist such as SR-141716A **20**.

We also have an excellent understanding of the important pharmacophores and the structure activity relationships for CP55-940 **5** that may allow identification of suitable sites for investigation, see Sections 4.2.1.2 and 4.2.4 respectively. Unfortunately, like JWH-015 **7**, there is a current lack of detailed studies into suitable sites for fluorophore ligation, with no attempts to make a fluorescent cannabinoid based on CP55-940. This means that an investigation into suitable fluorophore ligation sites needs to take place before the work and expense of synthesising fluorescent compounds. Finally, CP55-940 and its derivatives are not currently subject to the UK's Misuse of Drugs Act, and hence can be synthesised without license[90].

4.2.1 Non-classical cannabinoid structure activity relationships

4.2.1.1 CP55-940 numbering

CP55-940 **5** is a bicyclic non-classical cannabinoid, that by definition lacks the pyran ring found in the classical cannabinoids. Both groups contain four important pharmacophores: the C_3 alkyl side chain, optimised in CP55,940 **5** to the 1,1-dimethylheptyl group; the C_1 phenol, the C_9 northern aliphatic hydroxyl and the C_{12} southern aliphatic side chain and hydroxyl. When referring to the numbering of CP55-940 **5** and its analogues, the classical cannabinoid numbering used for THC **1** and other classical cannabinoids will be used, and is detailed in Figure 4.1[147].

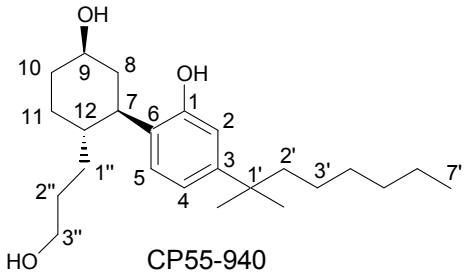


Figure 4.1 CP55-940 **5** numbering.

4.2.1.2 Optimisation for affinity and efficacy

The majority of work on improving cannabinoid receptor binding of classical cannabinoids was done before the discovery of the two cannabinoid receptors. For an excellent overview of this work see Razdan's and Makriyannis' in depth reviews on the subject[14, 148]. Hence, as only the centrally mediated effects of cannabinoids such as THC **1** were directly observed, measures of cannabinoid efficacy were based on CB_1 receptor binding alone. It was not until the discovery of the CB_2 receptor in 1993[16] that a differentiation and eventual selectivity of binding at the two receptors could be investigated.

Initially there were only two main pharmacophores of interest in the classical cannabinoids: The C_3 alkyl side chain and the C_9 northern aliphatic hydroxyl. It had been determined early on that any unsaturation on the C_7 to C_{12} ring was

not required for activity[14], and indeed with the C_7 to C_{12} benzene ring of Cannabinol **2** CB_1 and CB_2, receptor binding is substantially reduced.

It was found that extension of the C_3 alkyl side chain beyond the pentyl of THC **1** and other natural cannabinoids, to a straight chained heptyl side chain greatly improved CB_1 and CB_2 receptor binding and efficacy[14]. It was later discovered that a dimethyl substitution at the C_1 position of this alkyl side chain provided access to a hydrophobic sub-site that further improved binding at both receptors[149-151]. A number of investigations of this hydrophobic sub-site show that it can also accommodate small hydrophobic cyclic substitutions[152, 153], as well as unsaturation e.g. to a Z or E alkene, or alkyne[154, 155], but not hydrophilic groups such as alcohols[156]. The de facto C_3 alkyl side chain now used is the 1,1-dimethylheptyl group for both classical and non-classical cannabinoids.

The development of the C_9 northern aliphatic and C_{12} southern aliphatic groups have helped to boost both CB_1 and CB_2 receptor binding, leading to the knowledge and understanding of the four main pharmacophores detailed in Figure 2.2 [36]. C_9 hydroxyl and methylhydroxyl substitutions are now commonly used in synthetic cannabinoid as they are thought to give access to a hydrophilic sub-site[149, 157].

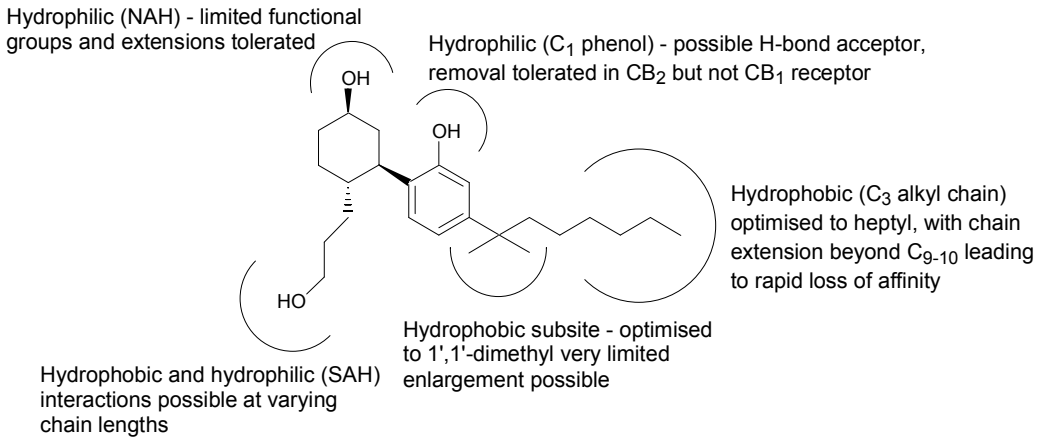


Figure 4.2 Main pharmacophores of CP55-940 and related non-classical cannabinoids.

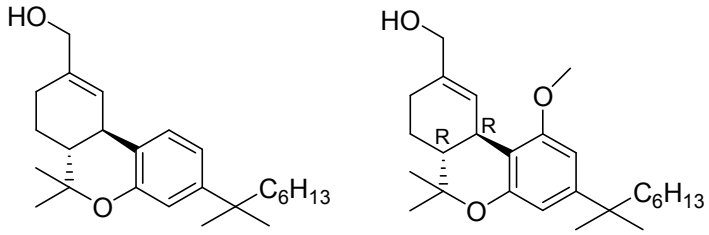
The invention of the non-classical cannabinoids discovered the C_{12} southern aliphatic pharmacophore and it is thought that it can interact with a hydrophilic

or hydrophobic sub-site[67]. Fixing the orientation of the side chain can differentiate between these receptor sub-sites[158].

4.2.1.3 CB_2 receptor selectivity

Unlike the alkylindoles, the classical cannabinoids offer limited possibilities for modification to allow CB_2 receptor selectivity. The C_1 phenol and the $C_{1'}$ of the C_3 alkyl side chain are the main options for the development of CB_2 selectivity in the non-classical cannabinoids. Of these positions, alteration of the C_1 phenol is most useful, as it can be alkylated if the CP55-940's other functional groups are protected.

The C_1 phenol was considered essential for cannabinoid activity until the discovery of the CB_2 receptor, whereby modification at the C_1 phenol led to selectivity for the CB_2 receptor. 1-Deoxy e.g. **97** and 1-methoxy e.g. **98** analogues in particular were found to give a degree of CB_2 selectivity (Figure 4.3)[149, 159].



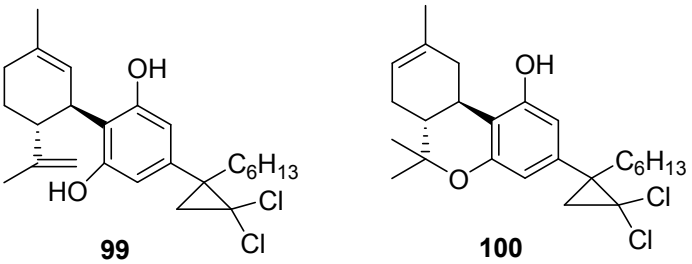
97
CB_1 1.2 nM
CB_2 0.03 nM

98
CB_1 22.8 nM
CB_2 2.9 nM

Figure 4.3 CB_2 receptor selective modifications at C_1 Phenol. Receptor binding affinities (Ki) quoted.

Incorporation of larger substitutions at the $C_{1'}$ position of the C_3 alkyl side chain, than the traditional 1,1-dimethyl, may be better tolerated at the CB_1 receptor than at the CB_2 receptor, leading to a degree of selectivity. Papahatjis' group has investigated a series of analogues with varying bulky substitutions at the $C_{1'}$ position, and found evidence for a difference in the size of between the CB_1 and CB_2 receptor sub-site pocket there[153, 156, 160]. Though this mostly offers opportunities for CB_1 receptor selectivity, a few analogues showed CB_2

selectivity instead e.g. **99** and **100** (Figure 4.4). Though of interest, these modifications are a not priority when designing a CB_2 receptor select ligand.



99

CB_1 665 nM
CB_2 33 nM

100

CB_1 1.27 nM
CB_2 0.29 nM

Figure 4.4 CB_2 receptor selective modifications at $C_{1'}$ position. Receptor binding affinities (Ki) quoted.

4.2.3 Selection of CP55-940 sites for investigation

CP55-940 **5** has four synthetically practical sites for fluorophore ligation: the C_3 alkyl side chain; the C_1 phenol, the C_9 northern aliphatic hydroxyl and the C_{12} southern aliphatic hydroxyl. These are also the sites where most structure activity relationship studies have taken place, though the majority of these have looked at the CB_1 receptor only and may not always be applicable to the CB_2 receptor due to differences in their binding sites[36]. Also many of these studies involved investigation of substitutions to the classical cannabinoid tricyclic scaffold, though in this case they are likely to be also relevant to the bicyclic non-classical cannabinoids due to large similarities between the two groups. Other parts of the CP55-940 **5** molecule may still be suitable for fluorophore ligation but limited investigation at these sites makes them less desirable as an initial choice to investigate. Of note though, is that substitution at the C_2 and C_4 positions of the phenyl ring[157, 161] and movement of the northern aliphatic hydroxyl side chain to the C_{10} position[14, 162] is tolerated and may provide alternative possibilities for fluorophore ligation.

4.2.3.1 C_3 Alkyl side chain

The C_3 alkyl side chain is unlikely to be a good site for fluorophore ligation or substitution, even though it would seem like an obvious choice for attachment as it is already quite a large hydrophobic alkyl chain. This is due to the alkyl

side chain being essential for the efficacy of the cannabinoid, as it is thought to be responsible for activating the cannabinoid receptor[93]. The alkyl side chain has been optimised for maximum efficacy to a 1,1-dimethylheptyl group[150], and some modifications at the $C_{1'}$ position have been utilised for CB_1 or CB_2 selectivity (Section 4.2.1.3).

Investigation at the CB_1 receptor has found that a heptyl in the 1,1-dimethylalkyl side chain analogues has greatest efficacy and, although some binding is maintained with chain extension, it leads to rapid loss of efficacy[93]. This, as well as results from quantitative structure activity relationship studies, suggests that the alkyl chain needs to be flexible and of appropriate length to bend around in the cannabinoid receptor active site to form the desired receptor ligand interactions for receptor activation[93, 161].

Introduction at the $C_{1'}$ position of functional groups that could be used for fluorophore ligation, such as a ketone or an alcohol, led to a hundred fold reduction in binding affinity[156]. Furthermore, research suggests that the 1,1-dimethyl moiety fits into a hydrophobic pocket of limited size which could not support a larger substitution such as a fluorophore[153, 160]. Therefore fluorophore ligation or substitution at either the $C_{1'}$ position, at the alkyl chain terminus or somewhere along the C_3 alkyl side chain is likely to cause complete loss of efficacy as well as binding affinity.

4.2.3.2 C_1 Phenol

The C_1 phenol was considered essential for cannabinoid activity until the discovery of the CB_2 receptor whereby modification at the C_1 phenol led to selectivity for the CB_2 receptor (Section 4.2.1.3)[149, 159, 163]. Interest has mainly stayed focused on modifications to promote CB_2 receptor selectivity and there is little information on whether a large hydrophobic molecule would be appropriate for attachment at the C_1 phenol.

Work done before the discovery of the cannabinoid receptors, when ligands were evaluated with CB_1 receptor *in vivo* assays, suggests that esterification but not etherification could be tolerated e.g. L-Naboctate **101** retains

cannabinoid activity (Figure 4.5)[14]. Care is needed with this result though, as the ester may be hydrolysed back to the phenol *in vivo*, and it may be this metabolised phenol that is responsible for the cannabinoid activity.

More recent work suggests there is some room for extension around the C_1 phenol as cyclisation and etherification of the phenol and the C_2 carbon **102** led to a modest ten fold loss of CB_2 receptor binding[157]. However, other work with modification of the C_1 phenol to a diethyl phosphate group **103** led to a thousand fold loss of CB_2 receptor binding[163].

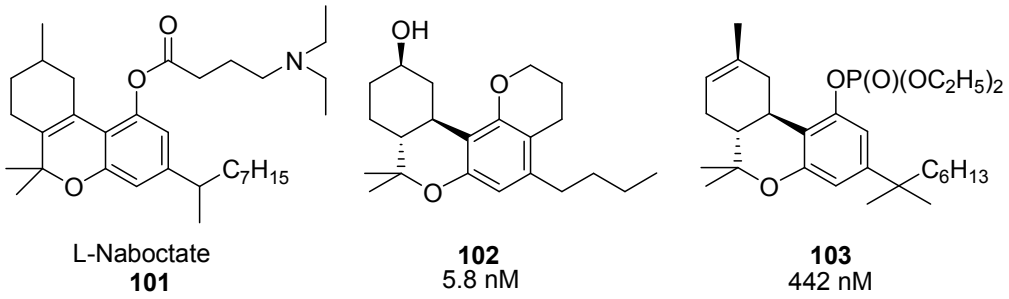


Figure 4.5 Modification at C_1 Phenol. CB_2 receptor binding affinities (Ki) quoted.

Unlike the C_3 alkyl side chain, there is no significant evidence suggesting that fluorophore ligation at the C_1 phenol would be inappropriate for CB_2 receptor binding. However, due to no significant evidence suggesting that fluorophore ligation at the C_1 phenol would be appropriate, it may be a useful target in developing SAR, but not first choice for fluorophore ligation.

4.2.3.3 C_9 Northern aliphatic hydroxyl

The northern aliphatic hydroxyl (NAH) is a versatile pharmacophore that can be extended, without loss of binding affinity, to a methyl alcohol e.g. AM919 **104** [67], or substituted with modest loss of binding to a ketone[149, 163], methylene[149] or methyl ester **105** (Figure 4.6)[164]. This is encouraging, as any fluorophore ligation would obviously require modification of the northern aliphatic hydroxyl functional group.

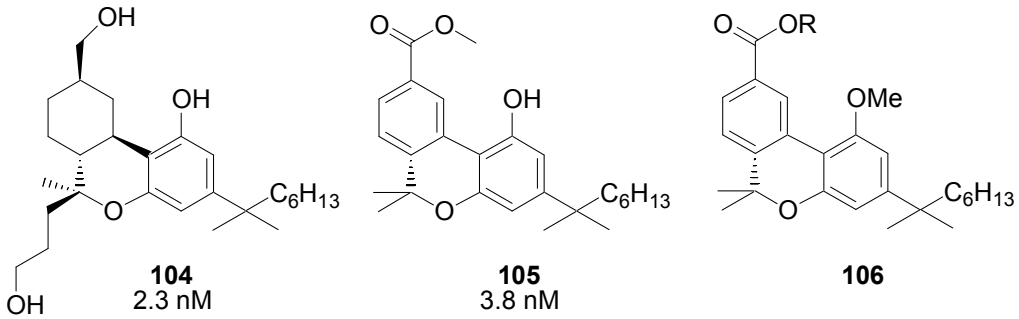


Figure 4.6 Modification at C9 northern aliphatic hydroxyl. CB2 receptor binding affinities (Ki) quoted.

Mahadevan *et al* went on to investigate larger esterifications with the series **106** R = methyl, isopropyl, *n*-propyl, *n*-hexyl, benzyl and naphthyl, but this time with a C_1 methoxy substitution instead of the phenol found in **105**[164]. This series had very poor binding affinities e.g. with the R = methyl analogue having a CB_2 receptor K_i of 529 nM, and most others having a $K_i > 10$ μM. This suggests that loss of a hydroxyl at both the C_1 and C_9 is highly detrimental to cannabinoid binding. However, the R = *n*propyl analogue had the greatest binding affinity of the series with a Ki of 214 nM, suggesting that there may be room for extension of a C_9 side chain, and even the existence of an extra hydrophobic binding site within the CB_2 receptor active site.

The northern aliphatic hydroxyl is certainly a promising site for fluorophore ligation, both with the ability to accommodate different functional groups and with the potential to allow attachment of a large hydrophobic molecule. A continuation of the work of Mahadevan *et al*[164] would be especially interesting with a series of C_9 esters with a conservative C_1 phenol.

4.2.3.4 C_{12} Southern aliphatic hydroxyl

There has so far been only limited research into substitution or extension of the southern aliphatic hydroxyl (SAH). One investigation into the length and nature of the southern aliphatic side chain of classical cannabinoids found that either a hydrophobic e.g. 3-iodopropyl **107** or a hydrophilic e.g. 3-hydroxypropyl **108** character can be given to the side chain without greatly affecting the CB_2 receptor binding affinity (Figure 4.7)[67]. These and earlier results suggest that there is both a smaller hydrophobic receptor sub-site and a

larger hydrophilic receptor sub-site for interaction with the southern aliphatic side chain, and that extension beyond a certain chain length begins to cause deterioration of binding affinity, but does not abolish it[67, 165]. Furthermore, changing the hydrophilic nature of the southern aliphatic hydroxyl to an amine **109**, carboxylic acid **110** or aldehyde **111** leads to greater than one hundred fold loss of affinity for the CB_1 receptor, suggesting that this pharamcophore is very sensitive to the functional group present[165].

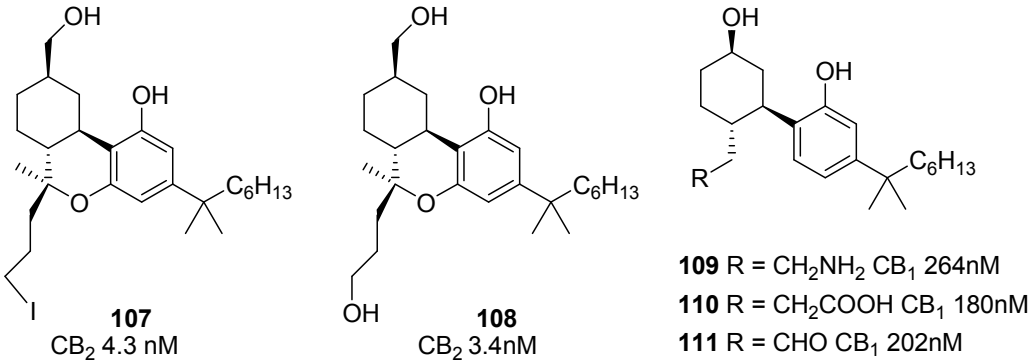


Figure 4.7 Modification of southern aliphatic hydroxyl. Receptor binding affinities (Ki) quoted.

The southern aliphatic hydroxyl might not be the ideal site for fluorophore ligation, but is likely to be better choice than the C_3 alkyl side chain or C_1 phenol, especially as it is not crucial for cannabinoid efficacy. There is also no evidence that further extension of this side chain will lead to total loss of binding affinity and this is worth investigating. Any substitution or extension will have to take into account that certain functional groups may lead to a large loss of affinity.

4.2.4 Prioritisation of CP55-940 sites for investigation

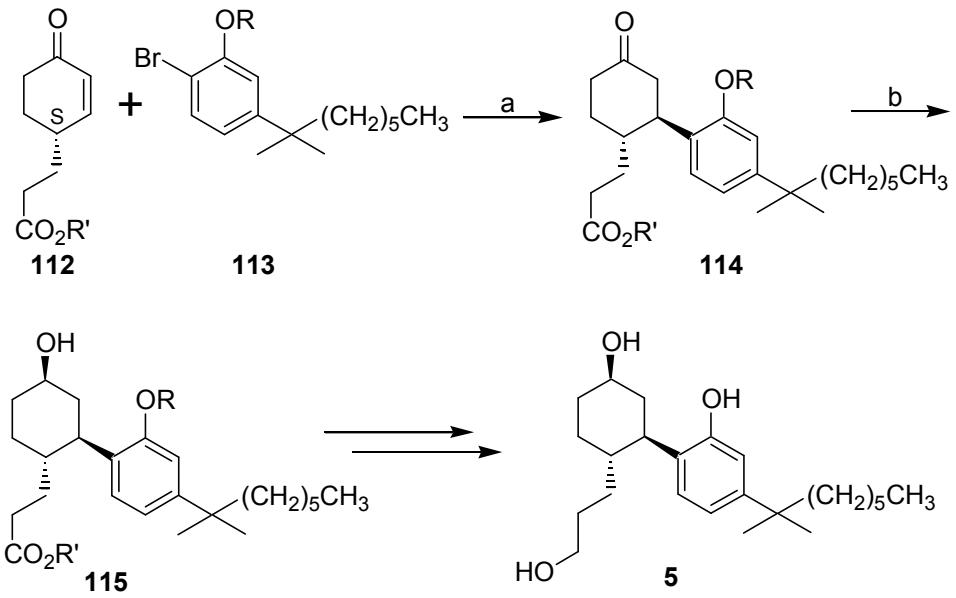
Investigation of the literature has shown that primarily the C_9 northern aliphatic hydroxyl and then the C_{12} southern aliphatic hydroxyl sites should be the ones investigated, although the C_1 phenol may also be of interest. Mutagenesis and modelling studies have been inconclusive, mostly due to difficulties in identifying which hydrophilic groups of the classical and non-classical cannabinoids interact with which amino acid residues of the

cannabinoid receptors. This is further complicated by the innate flexibility of the non-classical cannabinoids, making it difficult to predict which part of the molecule is nearest the exit of the receptor binding site.

Therefore, both substitutions to the northern aliphatic hydroxyl and southern aliphatic hydroxyl should be prioritised for investigation. Though substitution to the C_1 phenol may still be possible within any synthetic strategy (Section 4.3), initial investigations should limit its alteration to a C_1 methoxy which should improve CB_2 receptor selectivity. In particular it would be interesting to compare the C_1 phenol and methoxy analogues with substitutions at the northern aliphatic hydroxyl.

4.3 Synthetic strategy for CP55-940 Analogues

Synthetic design of CP55-940 analogues was based on the work of a Pfizer research group who first developed CP55-940 **5** (Scheme 4.1). A Grignard reaction between a chirally pure (S)-alkyl 3-[4-(2-cyclohexenone)] propionate **112**[166-168] and a suitably phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene **113**[65, 167] forms the conjugate product **114**, with 1,4 addition encouraged over 1,2 addition via transmetalation with a cuprous salt such as cuprous iodide[65, 167]. Thereafter, mild hydride reduction, e.g. with sodium hydride, of cyclohexanone **114** led to cyclohexanol **115**[65], which if reduced further to the hydroxypropyl side chain and the phenol protecting group removed, yields CP55-940 **5**[65]. For our purposes of investigating various substitutions on the northern aliphatic, southern aliphatic and phenol pharmacophores, these protecting groups could be manipulated to allow various alkylations at the desired site before deprotection.



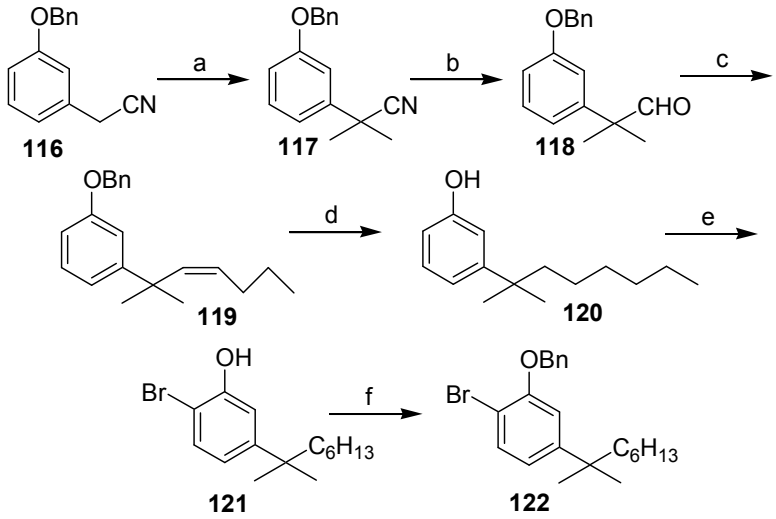
Scheme 4.1 Synthetic design of CP55-940 analogues (a) (i) **113**, Mg, CuI, THF; (ii) **112**; (b) $NaBH_4$, THF, MeOH.

Before such analogues could be made, the chirally pure cyclohexenone **112** and protected 2-bromophenol **113** needed to be synthesised. Cyclohexenone **112** synthesis has been documented in great detail by the Pfizer research group

that designed CP55-940 **5**[166-168], with our synthetic strategy following their work and is detailed in Section 4.4.

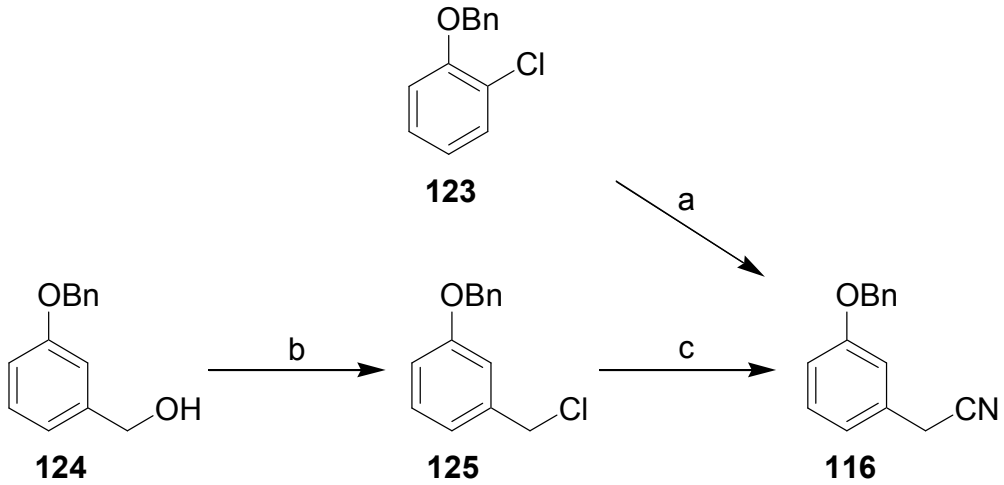
4.3.1 Synthetic strategy for protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene

For the phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene **112** the same Pfizer group devised the synthetic strategy detailed in Scheme 4.2[65]. This synthesis involves the double methylation of (3-benzyloxyphenyl)acetonitrile **116** with methyl bromide to 2-(3-benzyloxyphenyl)-2-methylpropionitrile **117**, and then reduction with DIBAL to the carbonyl 2-(3-benzyloxyphenyl)-2-methyl-propionaldehyde **118**. Wittig condensation with pentyltriphenylphosphonium bromide to the olefin l-(benzyloxy)-3-(l,l-dimethyl-2-heptenyl)benzene **119** and then catalytic hydrogenation reduces both the alkene and removes the benzyl protecting group of the phenol to provide 3-(l,l-dimethylheptyl)phenol **120**. Subsequent bromination is regioselective due to *ortho*/*para* activation by the phenol, and steric hindrance by the dimethylheptyl side chain, helping to protect the C_2 and C_4 positions. Protection with a suitable phenol protecting agent, in this case benzyloxy, yields 1-bromo-4-(1,1-dimethylheptyl)-2-benzyloxybenzene **122**.



Scheme 4.2 Synthetic strategy for protected 2-bromophenol **113** (a) MeBr, NaOH, DMSO; (b) DIBAL, THF; (c) pentyltriphenylphosphonium bromide, NaH, DMSO; (d) Pd/C, H_2, EtOH; (e) Br_2, CCl_4; (f) benzyl bromide, KH, DMF.

A number of problems were found with this proposed synthesis including the expense of the starting nitrile **116**, and the difficulties of handling methyl bromide required for the double alkylation of **116**. To investigate how the starting nitrile **116** could be synthesised the literature was searched for possible strategies (Scheme 4.3).

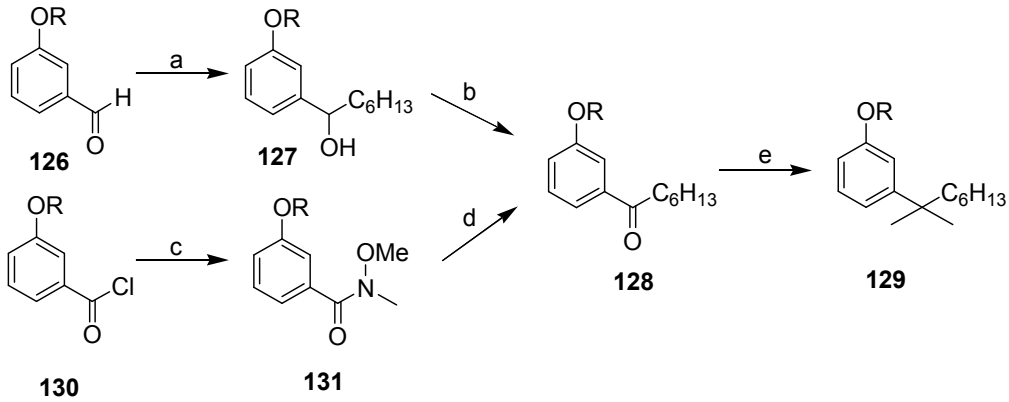


Scheme 4.3 Possible synthesis of (3-benzyloxyphenyl)acetonitrile **116** (a) $NaNH_2$, NH_3, MeCN; (b) $SOCl_2$, dioxane; (c) tetraethylammonium cyanide, MeCN.

The first published method involves a cyanomethyl substitution *ortho* to the chloro group of 2-benzyloxy-1-chlorobenzene **123** to form the desired nitrile **116**. It is a violent reaction, with poor yields and the required starting material is not commercially available[169, 170]. The second published method involves two steps; first chlorination of 3-(benzyloxy)benzyl alcohol **124** to afford 3-(benzyloxy)benzyl chloride **125**, and then reaction with the toxic hazardous tetraethylammonium cyanide salt to yield the desired nitrile **116**[171]. Although alcohol **124** is commercially available it is expensive and comparative in price to nitrile **116**. Due to the expense and difficulty in synthesising the required starting material for the synthesis detailed in Scheme 4.2, as well as the associated difficulties and hazards involved with the alkylations involving methyl bromide, an alternative synthesis for a protected 2-bromo-5-(1,l-dimethylheptyl) phenol **113** was sought.

One alternative strategy is to synthesise the phenol protected 1-(3-hydroxyphenyl)heptan-1-one **128**, which can then be dimethylated to afford the required dimethylheptyl side chain **129**. Two strategies were found for the

synthesis of this ketone **128** and are detailed in Scheme 4.4. The first strategy involves a Grignard reaction between the phenol protected 3-hydroxybenzaldehyde **126** and 1-bromohexane to form the protected 3-(1-hydroxyheptyl)phenol **127**, and then suitable oxidation to form the desired ketone **128**[156]. The second strategy, developed by the Makriyannis group, utilises a Grignard reaction between a protected Weinreb amide **131**[172], formed by the substitution of a phenol protected 3-hydroxybenzoyl chloride **130** with N,O-dimethylhydroxylamine, with 1-bromohexane to form the desired ketone **128**[68, 173, 174].



Scheme 4.4 Alternative synthesis of the dimethylheptyl side chain (a) (i) 1-bromohexane, Mg, THF; (ii) **126** added, THF; (b) Jone's reagent, acetone; (c) N,O-dimethylhydroxylamine, Et_3N, DCM; (d) (i) 1-bromohexane, Mg, THF (ii) **131** added, THF Scheme 4.4 (e) $TiCl_4$, $ZnMe_2$ or $AlMe_3$, DCM.

Unlike the disubstitution of ordinary carbonyls, a Weinreb amide only undergoes monosubstitution during a Grignard reaction, and so retains the carbonyl oxidation state needed for subsequent dimethylation. Monosubsitution forms a stable metal-chelated 5-membered cyclic intermediate, which protects the carbonyl carbon from further substitution by the Grignard reagent but will readily break down on acid workup (Figure 4.8)[172]. This strategy avoids the need for oxidizing agents and an extra step to reform the carbonyl.

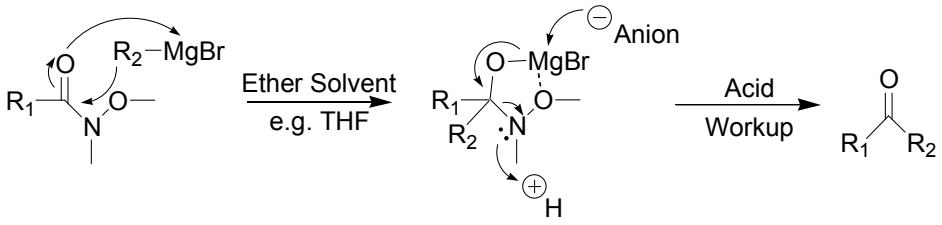


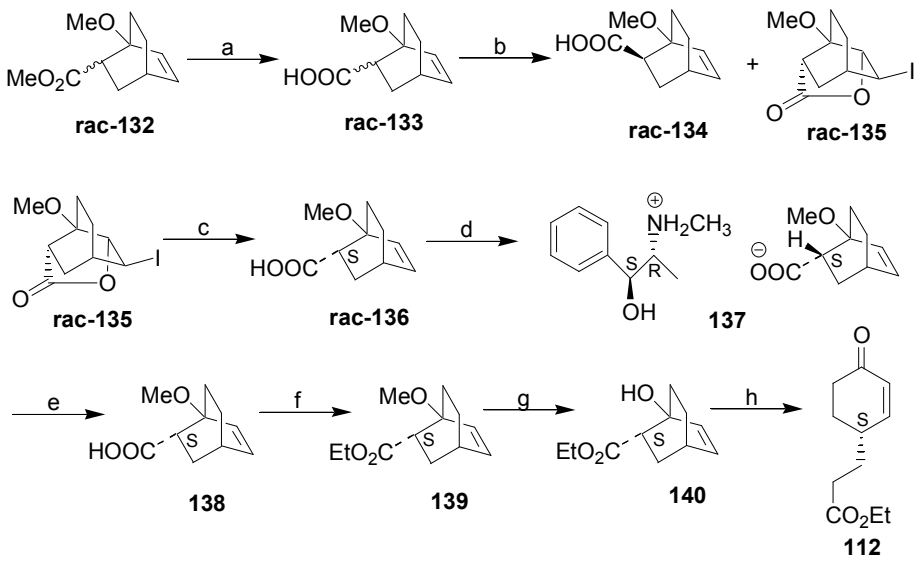
Figure 4.8 Grignard reaction mechanism with a Weinreb amide

The final dimethylation to form the required dimethylheptyl side chain **129** can be achieved with either use of dimethyl zinc[173, 174] or trimethyl aluminium[68] in the presence of titanium chloride[175]. Dimethyl zinc is a better choice for this reaction as it produces superior yields, 84% c.f. 59%[68, 173, 174].

Hence it was decided that synthesis of the protected bromophenol **113** should utilise the methods of the Makriyannis group, with use of a Weinreb amide to allow conversion to the desired dimethylheptyl side chain, and thereafter follow Pfizer's methods for bromination. This not only allows use of cheaper starting materials but also the use of more controlled and safer reaction conditions. The full synthetic pathway is detailed in Section 4.5.

4.4 Synthesis of (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate

Synthesis of the (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112** followed an established route as devised by the Pfizer research group (Scheme 4.5)[166, 168].



Scheme 4.5 (a) KOH, MeOH, reflux (87 %); (b) I_2, KI, $NaOH_{(aq)}$, $NaHCO_{3(aq)}$ (**rac-135** 89 %* and **rac-134** 58 %*); (c) Zn, EtOH, reflux (69 %); (d) (+)-ephedrine, EtOAc, recrystallisation; (60 %); (e) 2M $HCl_{(aq)}$, DCM (95 %); (f) PTSA, EtOH, reflux (57 %); (g) BBr_3, DCM, -30°C (61 %); (h) *t*BuOK, *t*BuOH, 35°C (78 %). Rac denotes a racemic mixture. * Theoretical yield calculated from the endo:exo ratio of 23:10.

The starting material for this synthesis is a commercially available racemic, endo/exo mixture of methyl 1-methoxybicyclo[2.2.2]oct-5-ene-2-carboxylate **rac-132**. This is prepared through the Diels-Alder reaction between 1-methoxycyclohexa-1,3-diene and methyl acrylate (Figure 4.9), whereby the conjugated diene can be attacked at either face by the activated dienophile forming the racemic mixture **rac-132**.

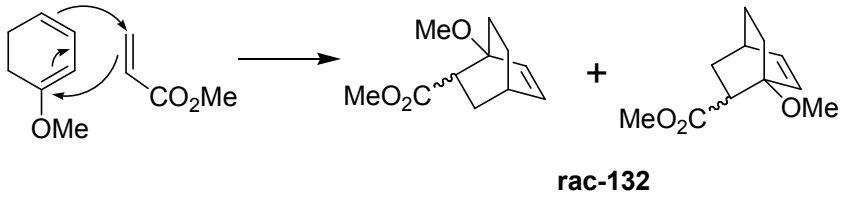


Figure 4.9 Diels-Alder reaction to form of rac-**132**.

During the Diels-Alder reaction formation of the endo isomer, with substituents in the axial position, is kinetically favoured over formation of the exo isomer, with the substituents in the equatorial position. This is due to the preferred interactions that occur during endo isomer formation. As this is an irreversible Diels-Alder reaction, kinetic control favours endo isomer formation and a ratio of 2-3:1 endo to exo isomers would be expected.

The initial aim of the synthesis described in Scheme 4.5 was to isolate the enantiomer (2S,4S)-(-)-1-methoxy-bicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid **138** with high enantiomeric purity, and then to convert to the desired cyclohexanone **112**. Cyclohexanone **112** can be derived from both this endo enantiomer **138** as well as from the exo enantiomer (2R,4R)-(+)-1-methoxy-bicyclo[2.2.2]-oct-5-ene-2-[exo]carboxylic acid, but work was focused on the endo enantiomer due to its predominance over the exo enantiomer.

Before separation of the enantiomers could be achieved, the endo and exo isomers of starting material **rac-132** needed to be isolated. This was achieved by the base hydrolysis of the ester to the acid **rac-133**, which was purified by recrystallisation from 2,2,4-trimethylpentane to produce good yields of off white crystals. This unusual solvent was used after attempts with the standard range of solvents used for recrystallisation failed to produce good yields, with 2,2,4-trimethylpentane been identified as a potential suitable candidate due to its similarity of structure to the solvent used by previous researchers[167]. **Rac-133** then underwent selective iodolactonisation under basic conditions. Only the endo iodolactone **rac-135** can be formed during this reaction, as the equatorial position of the exo acid prevents cyclisation even though the iodonium ion is still formed (Figure 4.10), and hence the exo acid **rac-134** is left unreacted. The iodolactone **rac-135** was isolated from the exo acid **rac-134** during workup by organic extraction from the basic aqueous reaction mixture, and then purified by recrystallisation from isopropanol to give excellent a yield of white crystals. The ratio of isolated endo iodolactone to exo acid was found to be 2.5:1, as expected with such a Diels-Alder product.

The endo acid **rac-136** was then reformed by refluxing with zinc, and purified by recrystallisation from petroleum ether 100/120 to give pure white crystals in modest yield.

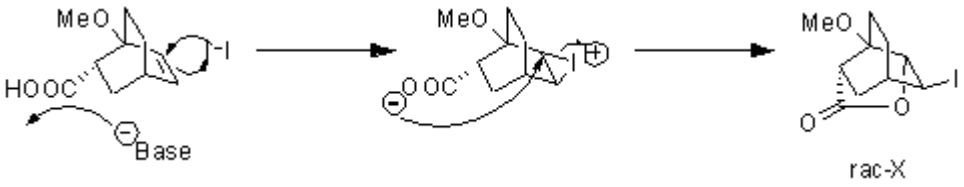
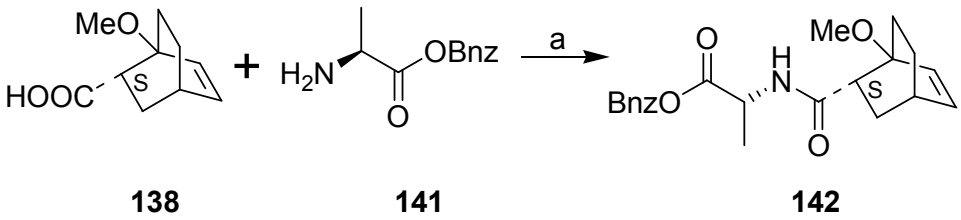


Figure 4.10 Selective endo iodolactonisation to form rac-**135**

Chiral resolution of endo acid **rac-136** was achieved by preparation of the dextrorotatory (+)-ephedrine salt **137**, crystallised from hot ethyl acetate, with multiple recrystallisations to remove the undesired enantiomer[176]. Acidic breakdown of the (+)-ephedrine salt **137** quantitatively yielded the (2S,4S)-(-)-1-methoxy-bicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid **138**. To determine how many recrystallisations of the (+)-ephedrine salt **137** were required for a high enantiomeric purity, formation of a diastereoisomeric complex is required so that physical methods can identify the quantity of each enantiomer. Some published methods include the use of NMR techniques whereby short lived diastereoisomeric complexes can be formed *in situ*, e.g. chiral lanthanide shift reagents, or long lived diastereoisomeric complexes are formed before analysis e.g. chiral derivatising agents[177, 178].

A simple method chosen here was to synthesise a long lived diastereoisomeric complex of (2S,4S)-(-)-endo acid **138**, by the formation of an amide **142** with L-alanine benzyl ester **141**, using dicyclohexylcarbodiimide and N-hydroxybenzotriazole catalysts (Scheme 4.6)[179]. This amide **142** is a mixture of two diastereoisomers, and by use of H^1 NMR spectroscopic analysis their relative amounts can be calculated. In this case, the integration of the C_1 methoxy group was used to compare the enantiomeric purity of products from different numbers of recrystallisations. The C_1 methoxy is ideal, as it is both in an area of the spectrum devoid of other proton signals, and its relative position to the chiral carbon of interest means that each diastereoisomer has significantly different C_1 methoxy signal positions. It was found that both two

and three recrystallisations of ephedrine salt **137** gave moderate enantiomeric purity of **142**, with 64% and 88% enantiomeric excess. Therefore, three recrystallisations of the dextrorotatory (+)-ephedrine salt **137** were chosen as, although each recrystallisation is associated with some loss of product, the greater enantiomeric purity was desirable.



138 **141** **142**

Scheme 4.6 Formation of a long lived diastereoisomeric complex between (2S,4S)-(-)-1-methoxy-bicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid and L-alanine benzyl ester (a) DCC, HOBt, DIPEA.

The (2S,4S)-(-)-endo acid **138** was esterified to form (2S,4S)-(-)-ethyl 1-methoxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylate **139**, purified by flash column chromatography to modest yields, with an ethyl ester being chosen over methyl or propyl analogues as it has been demonstrated to be more stable to the boron tribromide conditions then required for the cleavage of the methyl ether to form (+)-(2S,4S)-(-)-ethyl 1-hydroxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylate **140**, again giving modest yields[166]. This cyclic alcohol **140** then underwent base catalysed reverse Claisen rearrangement (Figure 4.11) and was purified by preparatory layer chromatography to give good yields of the desired (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112**.

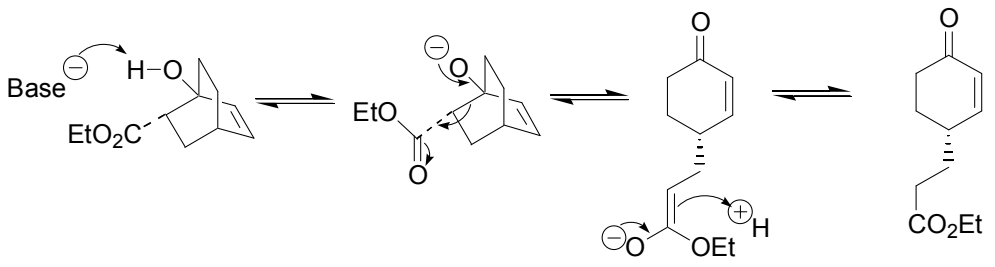


Figure 4.11 Base catalysed reverse Claisen rearrangement to form cyclohexanone **112**.

Care was needed during this synthetic strategy to avoid certain extreme physical conditions. Once the endo acid **rac-136** was isolated from the exo

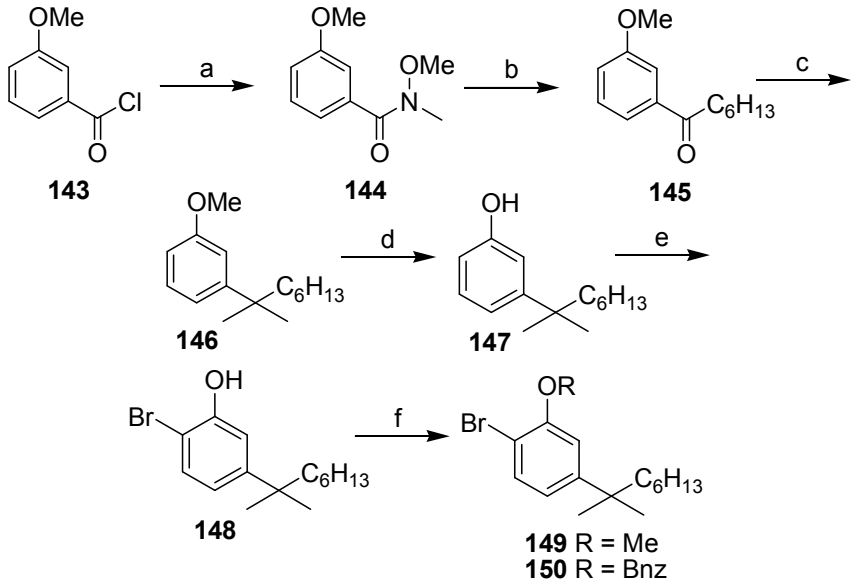
diastereoisomers, very high temperatures were avoided that could cause isomerisation. Furthermore once the purified (+)-endo acid **138** was prepared, extremes of pH were also avoided as these could cause base or acid catalysed racemisation at the α-carbon to the carboxylic acid.

The synthesis of the ephedrine salt **137** showed a comparative optical rotation to that of the literature[166, 168], and helps to confirm that the three recrystallisations of this salt provide a high enantiomeric purity, as determined with the synthesis of the diastereoisomeric complexes investigation discussed above. On further modification, although the degree of optical rotation was found to be of a lower magnitude than that found in the literature, e.g. (2S,4S)-(-)-1-methoxy-bicyclo[2.2.2]-oct-5-ene-2-[endo],carboxylic acid **138** had an $[\alpha]_D$ in dichloromethane of -20.7˚ compared to the literature values of -26.3˚ and -25.0˚, and (2S,4S)-(-)-ethyl 1-methoxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylate **139** had an $[\alpha]_D$ in chloroform of -3.08˚ compared to the literature value of -5.1˚[166, 168]. This suggests that a degree of racemisation occurred lowering the enantiomeric purity of the compounds, which will lead to a lower enantiomeric purity with any final compounds made.

During this successful synthesis of (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112**, most compounds were either purified by crystallisation or chromatography, which had not been undertaken in the previously published work[166-168]. With the aid of more powerful analytical methods, there has been improved characterisation of these compounds, which is detailed in Section 6.1.

4.5 Synthesis of phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene

Synthesis of 1-bromo-4-(1,1-dimethylheptyl)-2-benzyloxybenzene **150** and 1-bromo-4-(1,1-dimethylheptyl)-2-methoxybenzene **149** followed the devised route as discussed in Section 4.3.1, and described in Scheme 4.7, where all compounds were purified by flash column chromatography or preparative layer chromatography.



Scheme 4.7 Synthesis of the 1-bromo-4-(1,1-dimethylheptyl)-2-benzyloxybenzene **150** and 1-bromo-4-(1,1-dimethylheptyl)-2-methoxybenzene **149** (a) *N*,*O*-dimethylhydroxylamine, Et_3N, DCM (65 %); (b) 1-bromohexane, Mg, cat I_2, THF; (ii) **144** added, THF (92 %); (c) $TiCl_4$, $ZnMe_2$, DCM (98 %); (d) BBr_3, DCM (77 %); (e) Br_2, DCM (83 %);(f) K_2CO_3, DMF, methyl iodide (R = Me 61 %); NaH, DMF, benzyl bromide (R = Bn 91 %).

Formation of the Weinreb amide (3-methoxyphenyl)-*N*-methoxy-*N*-methylcarboxamide **144** in good yields was by nucleophilic substitution of the commercially available 3-methoxybenzoyl chloride **143** with *N*,*O*-dimethylhydroxylamine using triethylamine base. The following Grignard reaction between this Weinreb amide **144** and 1-bromohexane led to the desired monosubstitution product 1-(3-methoxyphenyl)heptan-1-one **145** in excellent yields, and then dimethyl zinc with titanium (IV) chloride catalyst dimethylation proceeded smoothly to form the desired dimethylheptyl side

chain of 1-(1,1-dimethylheptyl)-3-methoxybenzene **146**, again in excellent yields.

Demethylation with boron tribromide gave 1-(1,1-dimethylheptyl)-3-hydroxybenzene **147**, in modest yields associated with this deprotection, which could then undergo aromatic bromination with precisely one equivalent of bromine[180, 181]. The desired C_6 bromination was achieved and yielded 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene **148**, with minimal di and tribromination products that were easily removed during chromatographic purification while retaining good yields. C_6 bromination of 1-(1,1-dimethylheptyl)-3-hydroxybenzene **148** was confirmed over other aromatic monosubstitutions through the use of H^1 and COSY NMR spectroscopic analysis. The COSY NMR spectra with proton NMR overlaid, shown in Figure 4.12, has only three protons in the aromatic region, confirming a single purified product. Of these three protons, one is a doublet at 7.37 ppm with a coupling constant of 8.5 Hz, one is a doublet at 7.02 ppm with a coupling constant of 2.3 Hz and the last is a doublet of doublets at 6.80 ppm with coupling constants of 8.5 and 2.3 Hz. The 8.5 Hz coupling constant is suggestive of ortho coupling with the 2.3 Hz being suggestive of meta coupling. The COSY spectra confirms ortho coupling between the 8.5 Hz doublet and the doublet of doublets proton, as well as meta coupling between the 2.3 Hz doublet and the doublet of doublets proton. Both of these observations are consistent with either a C_6 or C_4 monobromination of 1-(1,1-dimethylheptyl)-3-hydroxybenzene **137**. C_4 substitution can be ruled out though due to the relatively upfield position of 6.80 ppm of doublets proton. This indicates that this proton is unlikely to be ortho to the phenol group which would be found with a C_4 substitution, and hence confirms the sole product as 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene **138**.

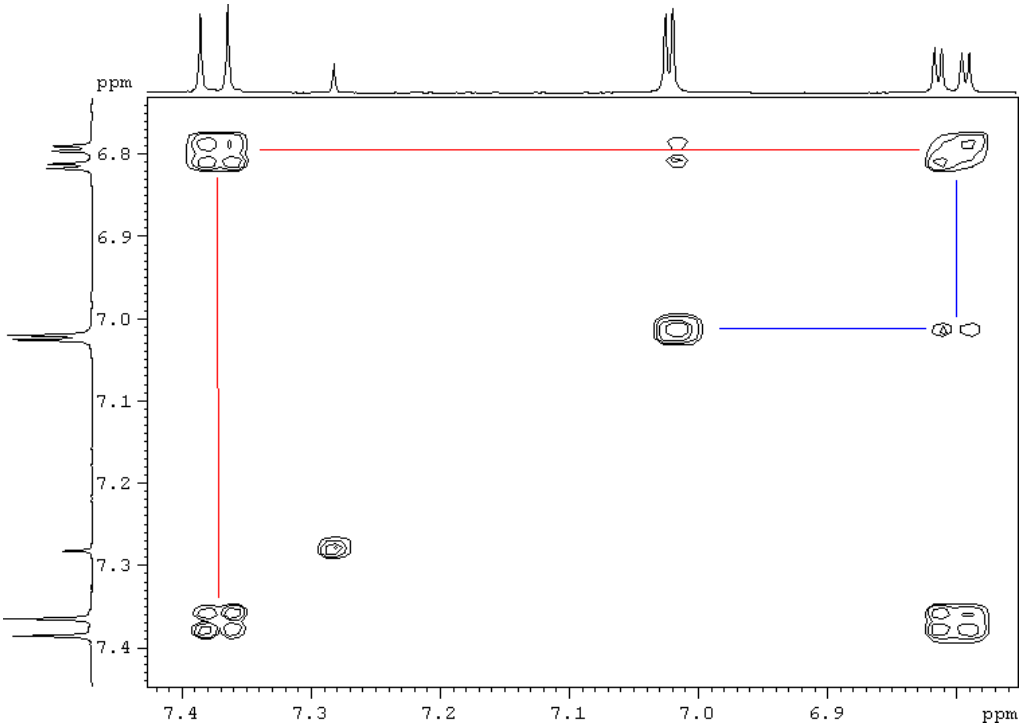
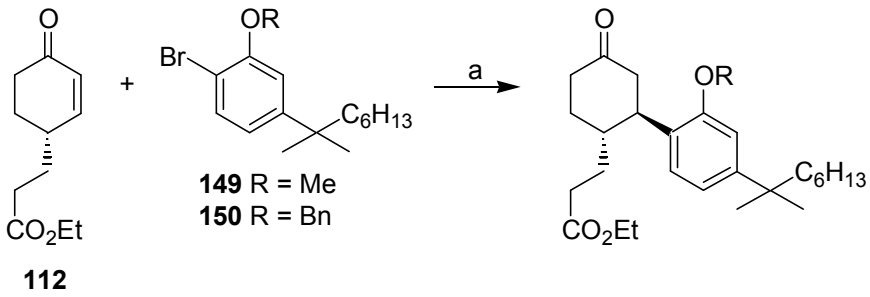


Figure 4.12 COSY NMR of 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene **138**, with proton NMR on left and top axis. The red lines show ortho coupling and the blue lines show meta coupling.

Before any Grignard reaction the phenol of the 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene **138** needs to be protected. Previous work by other groups have used a benzyloxy protection[65, 167, 168], but with an aim to improving CB_2 receptor selectivity, a methoxy protection was also chosen so that eventual analogues with substitutions at the southern and northern aliphatic groups could be assessed with and without the methoxy protection. For these protections 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene **148** was alkylated with either benzyl bromide or methyl iodide to give 1-bromo-4-(1,1-dimethylheptyl)-2-benzyloxybenzene **150** and 1-bromo-4-(1,1-dimethylheptyl)-2-methoxybenzene **149** respectively, both in good purified yields.

4.6 Attempted Grignard reactions

With the successful synthesis of (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112** and the two phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzenes **150** and **149** detailed above, a Grignard coupling between the two was attempted (Scheme 4.8). To encourage 1,4 addition at the conjugated ketone, transmetallation of the Grignard reagent with a copper salt was included in the process.



Scheme 4.8 Attempted Grignard couplings (a) (i) **149** or **150**, Mg, I_2, THF; (ii) **112** added with CuI.

Initially the reaction between the C_1 methoxy protected 1-bromo-4-(1,1-dimethylheptyl)-2-methoxybenzene **149** and (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112** was attempted. When the desired product was not yielded, the reaction using the C_2 benzyloxy protected 1-bromo-4-(1,1-dimethylheptyl)-2-benzyloxybenzene **150** was attempted, as this was the compound used by previously published research for this Grignard coupling[65, 167]. When this reaction did not succeed, a more detailed look at the compounds remaining showed that the desired Grignard product had not been formed, and that the two phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzenes **149** and **150** remained intact. If the Grignard product had been formed, but failed to react with the cyclohexenone, then on aqueous workup the debrominated products 1-(1,1-dimethylheptyl)-3-methoxybenzene and 1-(1,1-dimethylheptyl)-3-benzyloxybenzene would have been identified (Figure 4.13). Note that during these reactions the expected white-grey precipitate on formation of the Grignard reagent was not seen. At the time this was thought to be due to the small quantities being used, with the entire

Grignard reagent being dissolved in the ether solvent. In retrospect this is further evidence that the Grignard reagent did not form.

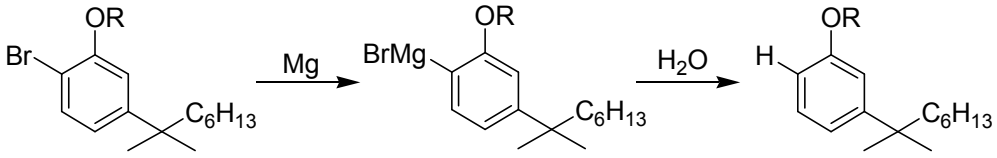
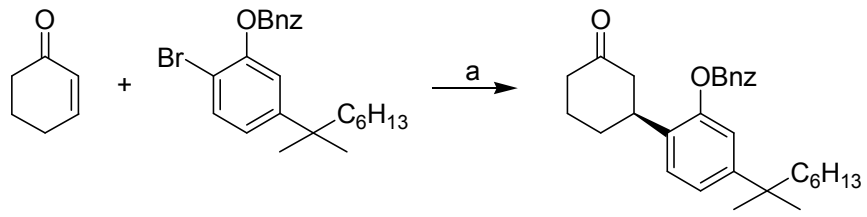


Figure 4.13 Formation of the Grignard reagent and debromination on aqueous workup.

The most likely reason for the Grignard reagent not forming is that the magnesium was not suitably active to allow initiation of the reaction. In particular, a magnesium oxide surface layer will prevent initiation, and an oily residue on the surface can increase this hindrance. To help remove any oily residue the magnesium was cleaned with hexane washings and then stored under an inert atmosphere. To help activate the magnesium, a catalytic amount of iodine, often used for this purpose, reduces some of the magnesium oxide allowing initiation of the reaction. In this case however, these methods were not sufficient.

One of the previously published work on this reaction used the hydride reducing agent sodium bis(2-methoxyethoxy)aluminium hydride instead of iodine to remove the magnesium oxide surface layer and help initiate the reaction[167]. To determine if this catalyst would allow formation and coupling of the desired Grignard reagent, a trial reaction between the C_1 benzyloxy protected 1-bromo-4-(1,1-dimethylheptyl)-2-benzyloxybenzene **150** and cyclohex-2-enone was attempted, so as not to waste any more of the chirally purified (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **149** (Scheme 4.9). This time a white-grey precipitate was seen on the formation on the Grignard reagent, although the desired product was again not yielded. Both mass spectrometry and NMR analysis showed that the debrominated 1-(1,1-dimethylheptyl)-3-benzyloxybenzene was formed, confirming the formation of the Grignard reagent *in situ*.



Scheme 4.9 Trial Grignard coupling (a) (i) **149**, Mg, sodium bis(2-methoxyethoxy)aluminium hydride (Red-Al®), THF; (ii) cyclohex-2-enone added with CuI.

The reason the Grignard reagent did not react with the cyclohex-2-enone is currently unknown. All compounds were freshly purified, the tetrahydrofuran freshly dried, all apparatus flame dried and all parts of the reaction undertaken in an inert atmosphere. There are obvious difficulties in transferring a precipitate from one vessel to another, achieved with a wide bore cannula and solvent washing, and the addition of a solid such a copper iodide to the reaction mixture, achieved using a nitrogen filled dry bag. Even if the reaction was not rigorously dry and some of the Grignard reagent was lost, at least some 1,2 or 1,4 addition products would have been expected, but no evidence was found for the formation of either of these. This reaction has been repeated, with and without the addition of copper iodide, but in all cases, even though the Grignard reagent was confirmed to have been formed, no evidence of addition to the cyclohex-2-enone has been found. Work is therefore currently ongoing on how to improve and succeed at this reaction and therefore produce the desired compounds needed before any investigation of CP55,940 substitutions on CB_2 receptor binding can take place. One simple way envisaged to improve the practicalities of this reaction, is to use an organic soluble copper transmetallation agent such as copper bromide dimethylsulfide complex thus avoiding the need to add a solid under inert conditions[182].

4.7 Summary

We have successfully synthesised (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112** and the two phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzenes **149** and **150**, but have so far been unable to perform the Grignard reaction necessary for the coupling of these two compounds that would allow synthesis of a range of substituted CP55-940 analogues and evaluate their effect on CB_2 receptor binding.

Though the synthesis of (S)-(+)-ethyl 3-[4(2-cyclohexenone)] followed an established pathway, we present more detailed characterisation of both the intermediates and final compound in Section 6. Furthermore, to evaluate the ephedrine recrystallisations that serve to separate the enantiomers of 1-methoxy-bicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid, a simple method for the production of a diastereoisomer complex and its analysis by proton NMR is demonstrated

We also present an improved synthesis of the C_2 benzyloxy protected 1-bromo-4-(1,1-dimethylheptyl)-2-benzyloxybenzene **150** and the C_2 methoxy protected 1-bromo-4-(1,1-dimethylheptyl)-2-methoxybenzene **149**, using a Weinreb amide intermediate to facilitate the desired alkylations with the minimal number of synthetic steps.

CP55-940 still presents a promising candidate for formation of a CB_2 receptor fluorescent ligand, especially as it binds nearer the exit of the active site than do indole cannabinoids, potentially allowing any fluorophore and linker less opportunity to interfere with cannabinoid binding. With (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112** and the two phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzenes **149** and **150** already prepared in sufficient amounts to allow the synthesis of numerous substituted CP55-940 analogues, it is hoped that work will be ongoing to succeed in our aims.

5. Conclusion

During this thesis we have designed, synthesised and evaluated a series of cannabinoid ligands, with an aim of producing a fluorescently labelled CB_2 receptor selective ligand for use as a biomolecular tool.

Efforts were initially focused on the alkylindoles, and in particular JWH-015 **7**. The literature was reviewed for any relevant structure activity relationships, homology modelling and mutagenesis studies that would help in the identification of sites on the alkylindole scaffold where fluorophore attachment maybe tolerated. The 3 and 4-naphthyl positions of JWH-015 **7** were chosen to determine the effect of a range of substitutions at these positions on CB_2 receptor binding affinity. A total of fifteen compounds were evaluated, providing new structure activity relationship data for alkylindole CB_2 receptor binding. The results confirmed that increasing steric bulk at these positions decreased CB_2 receptor affinity and that hydrophobic substitutions were tolerated better than hydrophilic ones of a similar size. Although the 2-methyl-*N*-propyl-3-(4-hexyloxy-1-naphthoyl)-indole **41** analogue was found to be a poor CB_2 receptor ligand, replacing the *N*-propyl with a *N*-pentyl resulted in the 2-methyl-*N*-pentyl-3-(4-hexyloxy-1-naphthoyl)-indole **44** analogue having significantly greater CB_2 binding affinity. These results suggested that bulky substitution at the 4-naphthyl position of JWH-015 **7**, could be tolerated to varying degrees, depending on optimisation of other pharmacophores.

The success of the 4-naphthyl substituted JWH-015 series, fed into the work in designing a fluorescently labelled CB_2 receptor ligand. The 4-naphthyl position was now chosen as a potential fluorophore ligation site. A variety of different linker length and types, as well as different fluorophores were chosen to aid in this development, the aim being to produce an array of non-fluorescent and fluorescent compounds for investigation. Ten non-fluorescent, linker only analogues and eleven fluorescent analogues were evaluated, all leading to large decreases in CB_2 binding affinities. Of the analogues with only a linker attached, the hexyl linker analogues of 2-methyl-*N*-propyl-3-(4-(tert-butyl 6-hexyloxycarbamate)-1-naphthoyl)-indole **73** and 2-methyl-*N*-propyl-3-

(4-(4-aminohexyloxy)-1-naphthoyl)-indole **78** were found to have the highest CB_2 receptor binding affinity of the series, suggesting that at that linker length there may be a receptor sub-site for which they can interact with. Of the fluorescent analogues, the NBD fluorophore produced the most promising compounds, but with insufficient CB_2 receptor binding affinity to obtain their K_i values. This suggests that a smaller fluorophore such as NBD may be tolerated within that region of the receptor active site. The alkylindoles, and in particular substitutions to the naphthyl ring of JWH-015 **7** still offer a good opportunity for the development of fluorescently labelled CB_2 receptor ligands.

Efforts were then turned to the non-classical cannabinoids, and in particular CP55-940 **5**. Again the literature was reviewed for any relevant structure activity relationships, homology modelling and mutagenesis studies that would help in the identification of sites on the non-classical cannabinoid scaffold where fluorophore attachment may be tolerated. The C_9 northern aliphatic group and C_{12} southern aliphatic group were chosen as candidates for investigation, with potential modification at the C_1 phenol to induce CB_2 selectivity. Though the initial synthesis of the CP55-940 **5** precursors (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112** and two phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzenes **149** and **150**, were successful, attempts to couple these compounds by the published Grignard method failed to yield the desired compound. Modification of reaction conditions and numerous trial reactions, failed to produce the desired Grignard coupling. The non-classical cannabinoids still are an interesting choice for investigating potential development of fluorescently labelled CB_2 receptor ligands.

5.1 Future Work

The breadth and depth of research into cannabis, the cannabinoid system and cannabinoid ligands continues apace and the development of fluorescent cannabinoids would offer a valuable tool for the whole field of research. With no fluorescently labelled cannabinoid for either the CB_1 or CB_2 receptor currently reported in the literature, our ongoing efforts at their design and synthesis should continue.

5.1.1 JWH-015 analogues

Although the research presented has not produced the desired fluorescent CB_2 receptor ligand, there is still more useful information to be gained from the fluorescent and non-fluorescent JWH-015 analogues developed so far. In particular, assessment of CB_1 receptor affinity as well as functional assays at both receptors to determine agonist-antagonist properties for all compounds, will add to our understanding of the structure activity relationships at both receptors, as well as highlight any modifications that induce either CB_1 or CB_2 receptor selectivity. Furthermore, application of fluorescent confocal microscopy, with the highest affinity fluorescent cannabinoids evaluated (i.e. a number of the NBD analogues), would allow spatial and temporal identification of fluorescent compounds binding to whole cell populations expressing either the CB_1 or CB_2 receptor. This will help confirm cannabinoid receptor binding for these low affinity ligands, although a complication is that as the fluorescent JWH-015 analogues are so highly hydrophobic it is envisaged that significant diffusion through the membrane into the cellular compartment will occur.

Beyond further evaluation of the compounds already synthesised, a second generation of fluorescent JWH-015 analogues should be investigated. Initially there are a number of subtle improvements or alterations to the JWH-015 scaffold that could be utilised to enhance cannabinoid receptor affinity. Most notably extension of the *N*-alkyl side chain to an *N*-pentyl group, should significantly improve both CB_1 and CB_2 receptor binding affinities, whereas as

removal of the indole 2-methyl group may improve affinities, as substitutions at the 3-naphthyl and possibly the 4- naphthyl position have been shown to be poorly tolerated in the presence of the 2-methyl group. Both such alterations would likely lead to a potential loss of any CB_2 receptor selectivity present.

If these simple alterations do not boost affinity enough, more fundamental changes in the fluorescent JWH-015 analogue should be investigated. Such changes may include;

- use of longer linker lengths to help remove the bulky fluorophore from the receptor active site,
- use of different fluorophores, though most other fluorophores are larger than the most promising NBD,
- continued investigation of 3-naphthyl position which has already been shown to tolerate a degree of substitution like the 4-naphthyl position,
- investigation of new substitution sites that have so far been neglected e.g. the 6 and 7-naphthyl positions which tolerate a degree of substitution or replacement of the indole with a pyrrole moiety where various aromatic substitution are well tolerated.

Choosing which of these routes to develop and in which direction, will greatly benefit from the frequent developments in alkylindole structure activity relationships published in the literature, as well as the development of new and improved CB_2 receptor homology models.

When a suitable fluorescently labelled alkylindole cannabinoid e.g. with a K_i of 100 nM or less is developed, then this can go on to be used as a platform for use in numerous applications including;

- high throughput screening for new cannabinoid ligand types,
- confocal microscopy and single molecule detection techniques, to help advance our knowledge and understanding of the cannabinoid receptor system,

- in house homology model refinement, where a fluorescent cannabinoid with good affinity may help solve the questions concerning orientation of the alkylindoles in the active site,
- bioassays for the identification of cannabinoid receptor over expression which could help guide diagnosis and therapy e.g. in some glioma tumours an over expression of CB_2 receptor is positively correlated with severity and prognosis.

On a more practical note, once a successful fluorescently labelled cannabinoid is developed, an assessment of both its core physiochemical properties and synthetic pathway will need to be undertaken. With respect to its physiochemical properties, any fluorescent cannabinoid is likely to be highly hydrophobic. This may cause difficulties in choosing an appropriate solvent to dissolve in, where some solvents such as DMSO are known have deleterious effects on cell membranes, and using such solvents may interfere with a number of assays. Furthermore, if a GPCR ligand is of a certain hydrophobic nature, excessive quantities may diffuse through cell membranes, meaning greatly increased quantities will be required for use in whole cell populations, as well as such cell compartment localisation complicating confocal microscopy applications. In terms of its synthesis, although a fluorescent cannabinoid is unlikely to be produced on an industrial scale, significant quantities may be required for the purposes of high throughput screening or bioassays. Therefore as well as significant scale up of synthesis, attention will need to be focused on minimising loses from relatively low yielding reactions e.g. the Fridel Crafts coupling between naphthyl and indole moieties or the final fluorophore conjugation step.

5.1.2 CP55-940 analogues

CP55-940 and other non-classical cannabinoid analogues are still very strong candidates for fluorophore ligation, especially as there have been no published attempts at producing fluorescent CP55-940 analogues. The fact that the non-classical cannabinoids bind higher in the CB_2 receptor than the alkylindoles, potentially offering more suitable placement of a bulky fluorophore, meaning

that such a fluorescent cannabinoid may be easier to achieve. Therefore efforts should continue on the development of various series of CP55-940 analogues.

Initial focus should be on successful completion of the Grignard reaction between the chirally purified (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate **112** and either of the two phenol protected 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzenes **149** and **150**. A number of suggestions on how this can be achieved have been commented on in section 4.6, but considering this reaction is the standard route employed by the industry for the production of the non-classical cannabinoids, it should be a matter of when success will be and not if it will happen.

Once the protected CP55-940 precursor is produced in sufficient quantities, and chiral purity checked, a systematic process of careful protection and deprotection of the C_1 phenol, SAH and NAH, will allow selective substitution at any of these desired sites. Though work should probably focus on the SAH and NAH positions, which are more tolerant to bulky substitutions, the knowledge gained from assessing the effect of substitutents beyond a three carbon chain length at the C_1 phenol, would add to our understanding of its structure activity relationships at the cannabinoid receptors.

Similar to the JWH-015 analogue series documented in chapter 2, investigation at the C_1 phenol, SAH and NAH, should focus on developing structure activity relationship data for a range of size and type of substitutions. In particular; bulky, hydrophobic and aromatic substitutions would be useful in determining which site, and type/length of linker, would be worth while investigating further.

Once such a site is prioritised, a series similar to the JWH-015 analogues documented in chapter 3 should be synthesised with the aim of deriving a fluorescently labelled CP55-940 analogue with appropriate affinity at the CB_2 receptor. A range of linker lengths and types should be used, as well as a variety of fluorophore compounds. If a particular linker is found to be effective, a wide range of fluorophores could be used to improve both affinity

and spectroscopic properties. If a fluorescently labelled CP55-940 analogue is successfully synthesised then it should undergo detailed evaluation and development as discussed above in section 5.2.1 for the JWH-015 analogues.

6 Experimental

6.1 Chemistry

Chemicals and solvents were purchased from standard suppliers and were used without further purification. Unless otherwise stated, reactions were carried out at ambient temperature, under atmospheric pressure and monitored by thin layer chromatography on Merck Kieselgel 60 F_{254} pre-coated aluminium backed plates. Visualisation was by examination under UV light (254nm + 366mm), with use of potassium permanganate as a general stain and ninhydrin for amines.

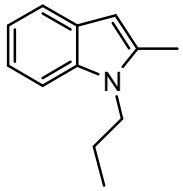
Purification by flash chromatography was carried out using Merck Kiesgel 60, 230-400 mesh silica. Purification by preparative layer chromatography was carried out using Fluka silica gel GF_{254} on glass plates (200 mm x 200 mm x 1mm).

Proton (400 MHz) nuclear magnetic resonance and carbon (100 MHz) nuclear magnetic resonance spectra were recorded on a Bruker-AV 400 spectrometer. Spectra were assigned with the aid of DEPT-135, DEPT-90, COSY and HSQC NMR. Chemical shifts (δ_H and δ_C) are quoted on the δ-scale in ppm using residual solvent or tetramethylsilane as internal standard. Coupling constants (*J*) are recorded in hertz and signal multiplicities described by: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet; app, apparent, and combinations thereof.

High resolution mass spectra TOF-ES (+/-) were recorded on a Waters 2795 Separation Module/Micromass LCT Platform. Melting points were either recorded uncorrected on a Gallerkamp 3A 3790 apparatus or on a Perkin Elmer Pyris 1 differential scanning calorimeter. FT-infrared spectra were recorded as KBr discs or as thin films between NaCl discs in the range of 4000-600 cm^{-1} using an Avatar 360 Nicolet FTIR spectrophotometer. Optical rotation was measured on a Bellingham-Stanley ADP220 polarimeter.

Analytical reverse-phase high performance liquid chromatography (HPLC) was either performed on a Waters Millenium LC system, with Waters 515 pumps and Waters 996 photodiode array detector, using a C8 (Vydac reverse phase C8 column (150 x 4.6 mm) flow rate 1mL/min) or C18 column (S.F.C.C. reverse phase C18 column (150 x 4.6 mm) flow rate 1mL/min or ACE reverse phase C18 column (250 x 4.6 mm) flow rate 1mL/min), or on a Waters automated LC system, with Water 2525 pump, Water 2767 sample manager and Waters 2487 dual λ absorbance detector using a C18 column (Onyx Monolith C18 column (100 x 4.6 mm), flow rate 3 mL/min or Waters Symmetry C18 column (75 x 4.6 mm), flow rate 1 mL/min). All solvents were degassed before use and all compounds for radioligand binding assays were purified until greater than 98 % pure with UV detection at 310 nm.

Preparation of 2-methyl-*N*-propylindole 32



Sodium hydride (60% w/w in mineral oil, 2.287 g, 57.2 mmol) was suspended in DMF (75 mL), cooled to 0 °C and 2-methylindole (5.000 g, 38.1 mmol) was added. 1-bromopropane (4.16 mL, 45.7 mmol) was added and the solution was stirred under an inert atmosphere for 3 h.

Excess hydride was reacted with ice (5 mL), stirred for 5 min and the solution concentrated *in vacuo*. The resulting solution was diluted with water (40 mL), and extracted with EtOAc (3 x 50 mL). The combined organics were washed with 2 M $HCl_{(aq)}$, water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (5:95 Ether:Petroleum Ether 60/80) to yield the desired product (5.806 g, 89 %) as a clear oil.

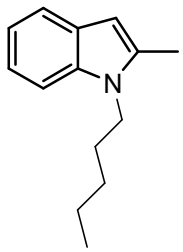
δ_H ($CDCl_3$) 7.55 (1H, d, *J* 7.8 Hz, 4-H or 7-H), 7.29 (1H, d, *J* 8.1 Hz, 4-H or 7-H), 7.15 (1H, m, 5-H or 6-H), 7.08 (1H, m, 5-H or 6-H), 6.27 (1H, s, 2-H), 4.05 (2H, t, *J* 7.4 Hz, N-CH_2), 2.45 (3H, s, 2-CH_3), 1.81 (2H, m, CH_2 β to amine), 0.98 (3H, t, *J* 7.4 Hz, CH_3 propyl).

δ_C ($CDCl_3$) 136.7, 136.5, 128.0, 120.3, 119.6, 119.1, 109.0, 99.8 (8 x aromatic) 44.8 (CH_2, N-CH_2), 23.5 (CH_2, CH_2 β to amine), 12.9 (CH_3), 11.5 (CH_3).

ES-MS MH+ calcd for $C_{12}H_{15}N_1$ 174.13 found *m/z* 174.13.

FTIR (NaCl) 3053, 2963, 2933, 2875, 1611, 1551, 1479, 1463, 1400, 1356, 1337,1310, 1223, 1172, 1138, 1104, 1013, 770, 746 cm^{-1}.

Preparation of 2-methyl-*N*-pentylindole 33



Sodium hydride (60% w/w in mineral oil, 2.287 g, 57.2 mmol) was suspended in DMF (75 mL), cooled to 0 °C and

2-methylindole (5.000 g, 38.1 mmol) was added. 1-bromopropane (5.20 mL, 41.9 mmol) was added and the solution was stirred under an inert atmosphere for 2 h.

Excess hydride was reacted with ice (5 mL), stirred for 5 min and the solution concentrated *in vacuo*. The resulting solution was diluted with water (40 mL), and extracted with EtOAc (3 x 50 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (5:95 Ether:Petroleum Ether 60/80) to yield the desired product (7.540 g, 98 %) as a clear oil.

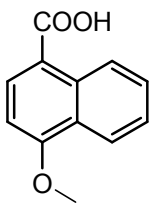
δ_H ($CDCl_3$) 7.57 (1H, d, *J* 7.8 Hz, 4-H or 7-H), 7.31 (1H, d, *J* 8.1 Hz, 4-H or 7-H), 7.18 (1H, m, 5-H o5 6-H), 7.10 (1H, m, 5-H or 6-H), 6.28 (1H, s, 2-H), 4.09 (2H, t, *J* 7.4 Hz, N-CH_2), 2.47 (3H, s, 2-CH_3), 1.79 (2H, m, CH_2 β to amine), 1-43-1.36 (4H, m, 2 x CH_2 pentyl), 0.98 (3H, t, *J* 7.4 Hz, CH_3 propyl).

δ_C ($CDCl_3$) 136.6, 136.4, 128.1, 120.3, 119.7, 119.1, 109.0, 99.8 (8 x aromatic) 43.2 (N-CH_2), 30.0 (CH_2 β to amine), 29.4, 22.5 (2 x CH_2), 14.0 (CH_3 pentyl), 12.8 (CH_3 indole).

EP-MS MH+ calcd for $C_{14}H_{19}N_1$ 202.16 found *m/z* 202.16.

FTIR (NaCl) 3054, 2956, 2930, 2859, 1611, 1551, 1479, 1460, 1401, 1382, 1356, 1338, 1311, 1232, 1168, 1140, 1014, 770, 746 cm-1.

Preparation of 4-methoxynaphthalene-1-carboxylic acid 34



4-Methoxy-1-naphthaldehyde (9.400 g, 50.5 mmol) was dissolved in tBuOH (75 mL) and 2-methyl-2-butene (35 mL), to which a solution of sodium chlorite (80% technical grade, 7.5 g, 66.4 mmol) and sodium hydrogen phosphate monohydrate (9.9 g, 71.7 mmol) in water (50 mL) was added dropwise, and the reaction mixture was stirred for 20 h.

The solution was concentrated *in vacuo*, and washed with 2M $HCl_{(aq)}$ to precipitate a light brown solid. The precipitate was washed with DCM and water, oven dried and recrystallised from methanol to yield the desired compound (3.415 g, 34 %) as cream crystals.

δ_H (DMSO-d_6) 12.75 (1H, br s, COOH), 9.03 (1H, d, *J* 8.5 Hz, 2-H), 8.26-8,22 (2H, m, aromatic), 7.66 (1H, m, aromatic), 7.57 (1H, m, aromatic), 7.06 (1H, d, *J* 8.5 Hz, 3-H), 4.05 (3H, s, CH_3).

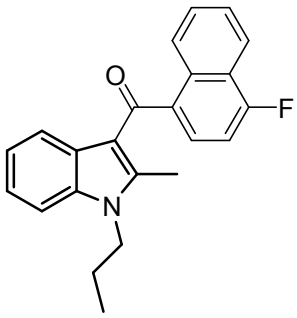
δ_C (DMSO-d_6) 168.7 (4°, COOH), 158.9 (4°), 132.9 (CH), 132.8 (4°), 128.5, 126.1, 126.0 (3 x CH), 125.4 (4°), 122.3 (CH), 119.3 (4°), 103.9 (CH), 56.5 (CH_3).

EP-MS M- calcd for $C_{12}H_{10}O_3$ 201.0552 found *m/z* 201.0560.

FTIR (KBr) 3010, 2983, 2945, 2847, 2794, 2611, 2480, 1676, 1619, 1575, 1514, 1469, 1453, 1430, 1328, 1300, 1265, 1218, 1191, 1159, 1149, 1097, 1007, 911, 827, 789, 767, 636, 609 cm^{-1}.

m.p. 244 °C lit. 236-243 °C[124, 183].

Preparation of 2-methyl-*N*-propyl-3-(4-fluoro-1-naphthoyl)-indole 46



4-Fluoronaphthalene-1-carboxylic acid (200 mg, 1.05 mmol), thionyl chloride (500 µL, 6.90 mmol), and THF (3 mL) were refluxed at 90 °C for 1 h. Solvent and excess thionyl chloride was removed *in vacuo*, DCM (5 mL) added and the resulting solution was added dropwise, at 0 °C under an inert atmosphere, to a solution of 2-methyl-1-propylindole **32** (182 mg, 1.05 mmol) and diethyl aluminium chloride (1M in hexanes, 1.58 mmol)

in DCM (5 mL). The reaction mixture was warmed to room temperature and stirred for 1 h.

The reaction was quenched with ice (50 mL), diluted with 2M $NaOH_{(aq)}$ and extracted with DCM (3 x 20 mL). The combined organics were washed with 2M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (40:60 Ether:Petroleum Ether 60/80) to yield the desired product (156 mg, 43 %) as an brown solid.

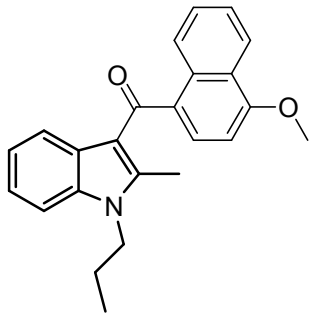
δ_H ($CDCl_3$) 8.22 (2H, m, naphthyl), 7.63-7.52 (3H, naphthyl), 7.35 (1H, m, indole), 7.23-7.14 (3H, 2 x CH indole, 1 x CH naphthyl), 7.02 (1H, m, indole), 4.11 (2H, t, *J* 7.5 Hz, N-CH_2), 4.08 (3H, s, OCH_3), 2.53 (3H, s, CH_3 indole), 1.86 (2H, m, CH_2 β to amine), 1.02 (3H, t, *J* 7.4 Hz, CH_3 propyl).

δ_F ($CDCl_3$) 119.10.

EP-MS MH+ calcd for $C_{23}H_{20}N_1O_1F$ 346.1607 found *m/z* 346.1605.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 13.94 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 3.05 min.

Preparation of 2-methyl-*N*-propyl-3-(4-methoxy-1-naphthoyl)-indole 35



4-Methoxynaphthalene-1-carboxylic acid **34** (2.246 g, 11.1 mmol) and thionyl chloride (4.0 mL, 55.5 mmol) were refluxed at 90 °C for 1 h. Excess thionyl chloride was removed *in vacuo*, DCM (20 mL) added and the resulting solution was added dropwise, at 0 °C under an inert atmosphere, to a solution of 2-methyl-1-propylindole **32** (1.920 g, 11.1 mmol) and diethyl aluminium chloride (1M in hexanes, 16.7 mmol) in

DCM (10 mL). The reaction mixture was warmed to room temperature and stirred for 4 h.

The reaction was quenched with ice (50 mL), diluted with 2M $NaOH_{(aq)}$ and extracted with DCM (3 x 50 mL). The combined organics were washed with 2M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (60:40 Ether:Petroleum Ether 60/80) to yield the desired product (1.962 g, 71 %) as an orange solid.

δ_H ($CDCl_3$) 8.39 (1H, m, naphthyl), 8.33 (1H, m, naphthyl), 7.62 (1H, d, *J* 8.0 Hz, 2-H naphthyl), 7.53 (2H, m, naphthyl), 7.34 (2H, m, indole), 7.21 (1H, m, indole), 7.05 (1H, m, indole), 6.82 (1H, d, *J* 7.8 Hz, 3-H naphthyl), 4.11 (2H, t, *J* 7.5 Hz, N-CH_2), 4.08 (3H, s, OCH_3), 2.53 (3H, s, CH_3 indole), 1.86 (2H, m, CH_2 β to amine), 1.02 (3H, t, *J* 7.4 Hz, CH_3 propyl).

δ_C ($CDCl_3$) 193.2 (C=O), 157.3, 144.9, 136.1, 132.3, 131.9 (5 x 4°), 128.5 (CH 2-naphthyl), 127.4 (CH), 127.3, 125.8 (2 x 4°), 125.7, 125.6, 122.1, 122.0, 121.6, 121.3 (6 x CH), 115.3 (4°), 109.5 (CH), 102.7 (CH 3-naphthyl), 55.7 (OCH_3), 44.9 (N-CH_2), 23.6 (CH_2 β to amine), 12.6 (indole CH_3), 11.5 (propyl CH_3).

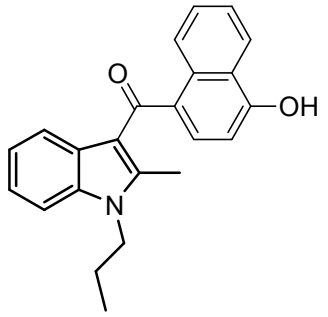
EP-MS MH+ calcd for $C_{24}H_{23}N_1O_2$ 358.1802 found *m/z* 358.1789.

FTIR (KBr) 3240, 2963, 2934, 1645, 1623, 1578, 1513, 1464, 1440, 1411, 1380, 1371, 1355, 1326, 1265, 1240, 1222, 1163, 1133, 1093, 1083, 1017, 847, 833, 793, 777, 755, 739 cm-1.

m.p. 122 °C.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 13.67 min. Monolith C18 gradient 20 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 4.89 min.

Preparation of 2-methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole 37



2-Methyl-1-propyl-3-(4-methoxy-1-naphthoyl)-indole **35** (3.890 g, 10.9 mmol) was dissolved in DCM (25 mL) and the solution brought to -78 °C under an inert atmosphere. Boron tribromide (1 M in hexanes, 12 mmol) was added, the solution slowly warmed to room temperature and stirred for 3 h.

The reaction mixture was quenched in 2 M HCl (aq, 100 mL) and stirred for 15 min. The layers were separated and the aqueous layer further extracted with DCM (2 x 50 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (25:75 Ethyl Acetate:Hexane) to yield the desired product (2.181 g, 58 %) as an orange solid.

δ_H ($CDCl_3$) 8.22 (2H, m, naphthyl), 7.45 (2H, m, naphthyl), 7.39 (1H, d, *J* 7.8 Hz, 2-H naphthyl), 7.34 (2H, m, indole), 7.20 (1H, m, indole), 7.04 (1H, m, indole), 6.69 (1H, d, *J* 7.8 Hz, 3-H naphthyl), 4.12 (2H, t, *J* 7.5 Hz, N-CH_2), 2.49 (3H, s, CH_3 indole), 1.87 (2H, m, CH_2 β to amine), 1.01 (3H, t, *J* 7.4 Hz, CH_3 propyl).

δ_C ($CDCl_3$) 194.5 (C=O), 154.6, 145.8, 136.2, 132.0, 131.7 (5 x 4°), 128.3 (CH 2-naphthyl), 127.4 (CH), 127.3 (4°), 125.3, 125.2 (2 x CH), 125.0 (4°), 122.3, 122.3, 122.0, 121.4 (4 x CH), 115.7 (4°), 109.5 (CH), 108.1 (CH 3-naphthyl), 44.9 (N-CH_2), 22.9 (CH_2 β to amine), 12.7 (CH_3 indole), 11.5 (CH_3 propyl).

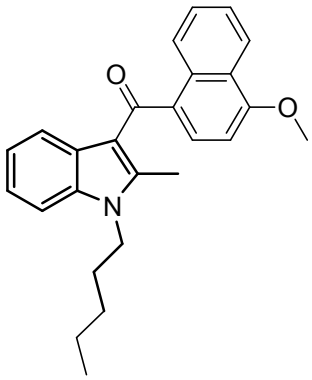
EP-MS M+ calcd for $C_{23}H_{21}N_1O_2$ 344.1651 found *m/z* 344.1646.
EP-MS M- calcd for $C_{23}H_{21}N_1O_2$ 342.1500 found *m/z* 342.1494.

FTIR (KBr) 3196, 2955, 2928, 2858, 1624, 1578.8, 1512, 1461, 1416, 1369, 1347, 1260, 1247, 1226, 1202, 1161, 1150, 1086, 1055, 828, 776, 765, 750, 740 cm^{-1}.

m.p. 212 °C.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 11.58 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 2.07 min.

Preparation of 2-methyl-*N*-pentyl-3-(4-methoxy-1-naphthoyl)-indole 36



4-Methoxynaphthalene-1-carboxylic acid **34** (1.64 g, 8.12 mmol) and thionyl chloride (3.0 mL, 40.6 mmol) were refluxed at 90 °C for 1.5 h. Excess thionyl chloride was removed *in vacuo*, DCM (30 mL) added and the resulting solution was added dropwise, at 0 °C under an inert atmosphere, to a solution of 2-methyl-1-pentyllindole **33** (1.63 g, 8.12 mmol) and diethyl aluminium chloride (1M in hexanes, 12.2 mmol) in DCM (10 mL). The reaction mixture was warmed to room temperature, and stirred for 2 h.

The reaction was quenched with ice (50 mL), diluted with sat. $NaHCO_{3(aq)}$ and extracted with DCM (3 x 100 mL). The combined organics were washed with sat. $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (40:60 Ether:Petroleum Ether 60/80) to yield the desired product (2.22 g, 71 %) as a yellow oil.

δ_H ($CDCl_3$) 8.36 (1H, m, naphthyl), 8.30 (1H, m, naphthyl), 7.61 (1H, d, *J* 8.0 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.28 (1H, m, indole), 7.20 (1H, m, indole), 7.03 (1H, m, indole), 6.82 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.15 (2H, t, *J* 7.6 Hz, N-CH_2), 4.09 (3H, s, OCH_3), 2.54 (3H, s, CH_3

indole), 1.82 (2H, m, CH_2 β to amine), 1.40 (4H, m, 2 x CH_2 pentyl) 0.94 (3H, t, *J* 7.4 Hz, CH_3 pentyl).

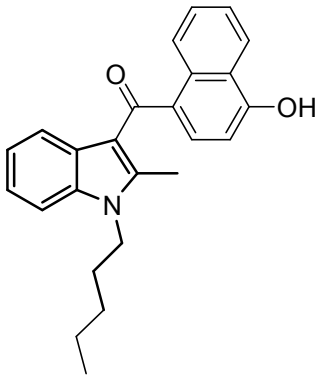
δ_C ($CDCl_3$) 193.2 (C=O), 157.2, 144.8, 136.0, 132.4, 131.9 (5 x 4°), 128.5 (CH 2-naphthyl), 127.5 (CH), 127.3, 125.8 (2 x 4°), 125.6, 125.6, 122.1, 122.0, 121.6, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 102.6 (CH 3-naphthyl), 55.7 (OCH_3), 43.4 (N-CH_2), 29.4 (CH_2 β to amine), 29.1, 22.4 (2 x CH_2 pentyl), 14.0 (CH_3 propyl), 12.5 (CH_3 indole).

EP-MS MH+ calcd for $C_{26}H_{27}N_1O_2$ 386.2115 found *m/z* 386.2128.

FTIR (KBr) 2957, 2932, 2859, 1623, 1608, 1579, 1510, 1462, 1412, 1371, 1324, 1227, 1203, 1161, 1096, 1080, 1024, 839, 819, 750, 714 cm-1.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 15.37 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 4.09 min.

Preparation of 2-methyl-*N*-pentyl-3-(4-hydroxy-1-naphthoyl)-indole 38



2-Methyl-1-pentyl-3-(4-methoxy-1-naphthoyl)-indole **36** (2.10 g, 5.45 mmol) was dissolved in DCM (20 mL) and the solution brought to -50 °C under an inert atmosphere. Boron tribromide (1 M in hexanes, 6 mmol) was added, the solution slowly warmed to room temperature and stirred for 3 h.

The reaction mixture was quenched in 2 M HCl (aq, 100 mL) and stirred for 15 min. The layers were separated and the aqueous layer further extracted with DCM (75 mL). The combined organics were washed with H_2O and brine, dried over $MgSO_4$ and reduced *in vacuo*, to yield the desired crude product (1.35 g, 67 %) as a brown solid, used without further purification. A sample of

the crude product was purified by preparatory layer chromatography (50:50 Ether:Petroleum Ether 40/60).

δ_H ($CDCl_3$) 8.21 (2H, m, naphthyl), 7.45 (2H, m, naphthyl), 7.38 (1H, d, *J* 7.8 Hz, 2-H naphthyl), 7.34 (2H, m, indole), 7.20 (1H, m, indole), 7.04 (1H, m, indole), 6.68 (1H, d, *J* 7.8 Hz, 3-H naphthyl), 4.13 (2H, t, *J* 7.6 Hz, N-CH_2), 2.48 (3H, s, CH_3 indole), 1.81 (2H, m, CH_2 β to amine), 1.43-1.35 (4H, 2 x CH_2 pentyl) 1.01 (3H, t, *J* 7.4 Hz, CH_3 pentyl).

δ_C ($CDCl_3$) 193.9 (C=O), 154.1, 145.5, 136.1, 132.3, 132.1 (5 x 4°), 128.2 (CH 2-naphthyl), 127.4 (CH), 127.3 (4°), 125.5, 125.3 (2 x CH), 124.9 (4°), 122.2, 122.1, 121.8, 121.4 (4 x CH), 115.2 (4°), 109.4 (CH), 108.0 (CH 3-naphthyl), 43.4 (N-CH_2), 29.4, 29.1 (2 x CH_2), 22.4 (CH_2 β to amine), 14.0 (CH_3 propyl), 12.6 (CH_3 indole).

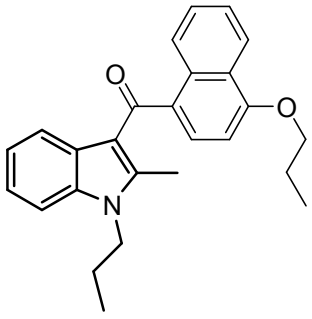
EP-MS M- calcd for $C_{25}H_{25}N_1O_1$ 370.1813 found *m/z* 370.1825.

FTIR (KBr) 3201, 2966, 2932, 2873, 1580, 1568, 1504, 1461, 1413, 1368, 1348, 1263, 1237, 1220, 1159, 1084, 1058, 1024, 1009, 994, 853, 825, 751, 715, 686, 623 cm-1.

m.p. 190 °C.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 13.30 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 3.22 min.

Preparation of 2-methyl-*N*-propyl-3-(4-propoxy-1-naphthoyl)-indole 39



Sodium hydride (60% w/w in mineral oil, 3.9 mg, 0.087 mmol) was suspended in DMF (2 mL), and added to 2-methyl-1-propyl-3-(4-hydroxy-1-

naphthoyl)-indole **37** (20 mg, 0.058 mmol). 1-Bromopropane (6.3 μL, 0.070 mmol) was added and the solution was stirred under an inert atmosphere for 24 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (4 mL), diluted with DCM (4 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with DCM (2 x 15 mL). The combined organics were washed with sat. $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 Ethyl Acetate:Hexane) to yield the desired product (6 mg, 27 %) as a yellow oil.

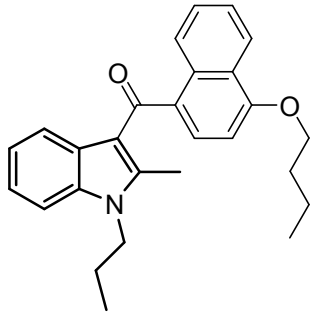
δ_H ($CDCl_3$) 8.41 (1H, m, naphthyl), 8.31 (1H, m, naphthyl), 7.59 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.32 (2H, m, indole), 7.20 (1H, m, indole), 7.03 (1H, m, indole), 6.80 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.19 (2H, t, *J* 6.4 Hz, O-CH_2), 4.13 (2H, t, *J* 7.5 Hz, N-CH_2), 2.54 (3H, s, CH_3 indole), 2.03 (2H, m, CH_2 β to ether), 1.87 (2H, m, CH_2 β to amine), 1.19 (3H, t, *J* 7.5 Hz, CH_3 O-propyl), 1.02 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.3 (C=O), 156.8, 144.8, 136.0, 132.1, 131.4 (5 x 4°), 128.7 (CH 2-naphthyl), 127.5 (CH), 127.3, 125.9 (2 x 4°), 125.6, 125.5, 122.2, 122.0, 121.6, 121.3 (6 x CH), 115.4 (4°), 109.4 (CH), 103.3 (CH 3-naphthyl), 69.8 (O-CH_2), 44.9 (N-CH_2), 23.0, 22.7 (2 x CH_2), 12.7, 11.5, 10.8 (3 x CH_3).

EP-MS MH+ calcd for $C_{26}H_{27}N_1O_2$ 386.2120 found *m/z* 386.2127.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 15.50 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 3.87 min.

Preparation of 2-methyl-*N*-propyl-3-(4-butoxy-1-naphthoyl)-indole 40



Sodium hydride (60% w/w in mineral oil, 10 mg, 0.255 mmol) was suspended in DMF (2 mL), and added to 2-methyl-1-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (60 mg, 0.175 mmol). 1-bromobutane (23 μL, 0.204 mmol) was added and the solution was stirred under an inert atmosphere for 15 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (5 mL), diluted with DCM (20 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with DCM (2 x 10 mL). The combined organics were washed with sat. $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (40:60 Ether:Petroleum Ether 60/80) to yield the desired product (15 mg, 22 %) as a yellow oil.

δ_H ($CDCl_3$) 8.40 (1H, m, naphthyl), 8.31 (1H, m, naphthyl), 7.60 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.33 (2H, m, indole), 7.20 (1H, m, indole), 7.04 (1H, m, indole), 6.81 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.23 (2H, t, *J* 6.4 Hz, O-CH_2), 4.13 (2H, t, *J* 7.6 Hz, N-CH_2), 2.54 (3H, s, CH_3 indole), 1.99 (2H, m, CH_2 β to ether), 1.89 (2H, m, CH_2 β to amine), 1.67 (2H, m, CH_2 γ to ether), 1.08 (3H, t, *J* 7.4 Hz, CH_3 butyl), 1.02 (3H, t, *J* 7.4 Hz, CH_3 propyl).

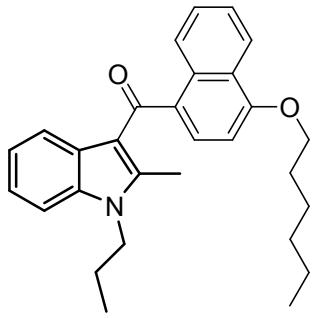
δ_C ($CDCl_3$) 193.3 (C=O), 156.8, 144.8, 136.1, 132.1, 132.0 (5 x 4°), 128.8 (CH 2-naphthyl), 127.5 (CH), 127.3, 125.9 (2 x 4°), 125.6, 125.5, 122.2, 122.0, 121.6, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 103.3 (CH 3-naphthyl), 68.1 (O-CH_2), 44.9 (N-CH_2), 31.3 (CH_2 β to ether), 23.0 (CH_2 β to amine), 19.5 (CH_2 γ to ether), 14.0 (butyl CH_3), 12.6 (CH_3 indole), 11.5 (CH_3 propyl).

EP-MS MH+ calcd for $C_{27}H_{29}N_1O_2$ 400.2277 found *m/z* 400.2308.

FTIR (NaCl) 3450, 2960, 2874, 1623, 1578, 1509, 1458, 1413, 1369, 1325. 1264, 1238, 1220, 1161, 1078, 750 cm^{-1}.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 16.37 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 4.30 min.

Preparation of 2-methyl-*N*-propyl-3-(4-hexyloxy-1-naphthoyl)-indole 41



Sodium hydride (60% w/w in mineral oil, 9 mg, 0.225 mmol) was suspended in DMF (2 mL), and added to 2-methyl-1-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (52 mg, 0.152 mmol). 1-bromohexane (20 μL, 0.180 mmol) was added and the solution was stirred under an inert atmosphere for 15 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (5 mL), diluted with DCM (20 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with DCM (2 x 10 mL). The combined organics were washed with sat. $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (40:60 Ether:Petroleum Ether 60/80) to yield the desired product (13 mg, 20 %) as a yellow oil.

δ_H ($CDCl_3$) 8.40 (1H, m, naphthyl), 8.31 (1H, m, naphthyl), 7.59 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.52 (2H, m, naphthyl), 7.32 (2H, m, indole), 7.20 (1H, m, indole), 7.03 (1H, m, indole), 6.80 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.22 (2H, t, *J* 6.4 Hz, O-CH_2), 4.13 (2H, t, *J* 7.5 Hz, N-CH_2), 2.54 (3H, s, CH_3 indole), 1.99 (2H, m, CH_2 β to ether), 1.87 (2H, m, CH_2 β to amine), 1.63 (2H, m, CH_2 γ to ether), 1.48 – 1.42 (4H, 2 x CH_2 hexyl), 1.03 (3H, t, *J* 7.4 Hz, CH_3 propyl), 0.96 (3H, m, CH_3 hexyl).

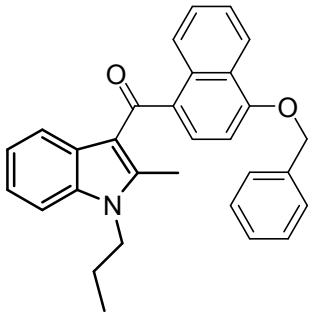
δ_C ($CDCl_3$) 193.3 (C=O), 156.8, 147.8, 136.0, 132.0, 132.0 (5 x 4°), 128.8 (CH 2- naphthyl), 127.5 (CH), 127.3, 125.9 (2 x 4°), 125.6, 125.5, 122.2, 122.0, 121.5, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 103.3 (CH 3-naphthyl), 68.4 (O-CH_2), 44.9 (N-CH_2), 31.7 (CH_2 hexyl), 29.2 (CH_2 β to ether), 26.0 (CH_2 γ to ether), 23.0 (CH_2 β to amine), 22.7 (CH_2 hexyl), 14.1 (CH_3 hexyl) 12.6 (CH_3 indole), 11.5 (CH_3 propyl).

EP-MS MH+ calcd for $C_{29}H_{33}N_1O_2$ 428.2584 found *m/z* 428.2613.

FTIR (NaCl) 3432, 2930, 2855, 1617, 1578, 1509, 1459, 1413, 1368, 1326, 1238, 1220, 1161, 1078, 750 cm^{-1}.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 17.89 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min then 95 % $MeOH_{(aq)}$ for 1 min, R_t = 5.10 min.

Preparation of 2-methyl-*N*-propyl-3-(4-benzyloxy-1-naphthoyl)-indole 42



Sodium hydride (60% w/w in mineral oil, 8 mg, 0.210 mmol) was suspended in DMF (5 mL), and added to 2-methyl-1-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (50 mg, 0.146 mmol). Benzylbromide (20 μL, 0.168 mmol) was added and the solution was stirred under an inert atmosphere for 20 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (5 mL), diluted with DCM (20 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with DCM (2 x 10 mL). The combined organics were washed with sat. $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography

(30:70 Ethyl Acetate:Hexane) to yield the desired product (21 mg, 32 %) as a yellow oil.

δ_H ($CDCl_3$) 8.47 (1H, m, naphthyl), 8.31 (1H, m, naphthyl), 7.67 – 7.37 (8H, 5 x CH benzyl, 3 x CH naphthyl), 7.35 (2H, m, indole), 7.21 (1H, m, indole), 7.04 (1H, m, indole), 6.91 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 5.35 (2H, s, O-CH_2), 4.14 (2H, t, *J* 7.5 Hz, N-CH_2), 2.55 (3H, s, CH_3 indole), 1.87 (2H, m, CH_2 β to amine) 1.03 (3H, t, *J* 7.4 Hz, CH_3 propyl).

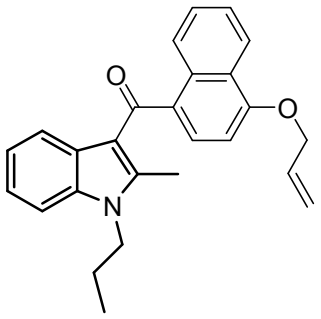
δ_C ($CDCl_3$) 193.2 (C=O), 156.2, 144.9, 136.7, 136.1, 132.7, 132.0 (6 x 4°), 128.7, 128.4, 128.1, 127.6, 127.5 (5 x CH), 127.3, 125.7 (2 x 4°), 125.6, 125.6, 122.3, 122.0, 121.6, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 104.0 (CH 3-naphthyl), 70.3 (O-CH_2), 44.9 (N-CH_2), 23.0 (CH_2 β to amine), 12.6 (CH_3 indole), 11.5 (CH_3 propyl).

EP-MS MH+ calcd for $C_{30}H_{27}N_1O_2$ 434.2120 found *m/z* 434.2129.

FTIR (NaCl) 3428, 2958, 2930, 2871, 1607, 1579, 1509, 1461, 1427, 1413, 1370, 1325, 1125, 1161, 1074, 1025, 870, 838, 818, 751, 696, 665 cm^{-1}

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 15.63 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 4.14 min.

Preparation of 2-methyl-*N*-propyl-3-(4-vinyloxy-1-naphthoyl)-indole 43



Sodium hydride (60% w/w in mineral oil, 9 mg, 0.225 mmol) was suspended in DMF (2 mL), and added to 2-methyl-1-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (52 mg, 0.152 mmol). Allyl bromide (16 μL, 0.180 mmol) was added and the solution was stirred under an inert atmosphere for 15 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (5 mL), diluted with DCM (20 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with DCM (2 x 10 mL). The combined organics were washed with sat. $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (30:70 Ethyl Acetate:Hexane) to yield the desired product (10 mg, 17 %) as a yellow oil.

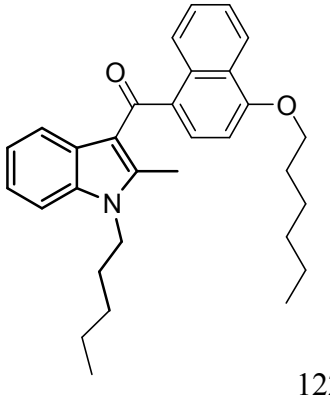
δ_H ($CDCl_3$) 8.43 (1H, m, naphthyl), 8.28 (1H, m, naphthyl), 7.59 (1H, d, *J* 8.0 Hz, 2-H naphthyl), 7.52 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.27 (1H, m, indole) 7.20 (1H, m, indole), 7.03 (1H, m, indole), 6.82 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 6.23 (1H, m, allyl), 5.58 (1H, m, allyl), 5.46 (1H, m, allyl), 4.81 (2H, m, O-CH_2), 4.13 (2H, t, *J* 7.5 Hz, N-CH_2), 2.54 (3H, s, CH_3 indole), 1.87 (2H, m, CH_2 β to amine), 1.03 (3H, t, *J* 7.4 Hz, CH_3 propyl).

δ_C ($CDCl_3$) 193.2 (C=O), 156.1, 144.9, 136.1 (3 x 4°), 132.9 (CH allyl), 132.6, 132.0 (2 x 4°), 128.4 (CH 2-naphthyl), 127.3 (CH), 127.3, 125.9 (2 x 4°), 125.6, 125.6, 122.2, 122.0, 121.6, 121.3 (6 x CH), 117.8 (CH_2 allyl), 115.3 (4°), 109.5 (CH), 103.8 (CH 3-naphthyl), 69.1 (O-CH_2), 44.8 (N-CH_2), 23.0 (CH_2 β to amine), 12.6 (CH_3 indole), 11.5 (CH_3 propyl).

EP-MS MH+ calcd for $C_{26}H_{26}N_1O_2$ 384.1964 found *m/z* 384.1971.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 14.78 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 3.50 min.

Preparation of 2-methyl-*N*-pentyl-3-(4-hexyloxy-1-naphthoyl)-indole 44



Sodium hydride (60% w/w in mineral oil, 8 mg, 0.209 mmol) was suspended in DMF (2 mL), and

added to 2-methyl-1-pentyl-3-(4-hydroxy-1-naphthoyl)-indole **38** (50 mg, 0.136 mmol). 1-bromohexane (18 μL, 0.161 mmol) was added and the solution was stirred under an inert atmosphere for 18 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (5 mL), diluted with DCM (10 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with DCM (15 mL). The combined organics were washed with 2 M $HCl_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (20:80 Ethyl Acetate:Hexane) to yield the desired product (12 mg, 19 %) as a yellow oil.

δ_H ($CDCl_3$) 8.39 (1H, m, naphthyl), 8.30 (1H, m, naphthyl), 7.59 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.35 (2H, m, indole), 7.19 (1H, m, indole), 7.03 (1H, m, indole), 6.80 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.22 (2H, t, *J* 6.4 Hz, O-CH_2), 4.15 (2H, t, *J* 7.6 Hz, N-CH_2), 2.53 (3H, s, CH_3 indole), 1.98 (2H, m, CH_2 β to ether), 1.82 (2H, m, CH_2 β to amine), 1.62 (2H, m, CH_2 γ to ether), 1.48 – 1.34 (8H, 2 x CH_2 pentyl, 2 x CH_2 hexyl) 0.98 – 0.86 (6H, CH_3 pentyl, CH_3 hexyl).

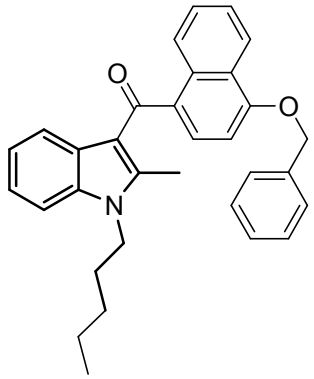
δ_C ($CDCl_3$) 193.2 (C=O), 156.8, 144.7, 136.0, 132.1, 132.0 (5 x 4°), 128.7 (CH 2-naphthyl), 127.5 (CH), 127.3, 125.9 (2 x 4°), 125.6, 125.5, 122.2, 122.0, 121.5, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 103.3 (CH 3-naphthyl), 68.4 (O-CH_2), 43.4 (N-CH_2), 31.7, 29.4, 29.2, 29.1, 28.0, 22.7, 22.4 (7 x CH_2), 14.1, 14.0 (2 x CH_3), 12.5 (CH_3 indole).

EP-MS MH+ calcd for $C_{31}H_{37}N_1O_2$ 456.2897 found *m/z* 456.2921.

FTIR (NaCl) 2958, 2922, 2851, 1726, 1607, 1580, 1510, 1462, 1412, 1371, 1324, 1261, 1227, 1160, 1096, 1081, 1019, 797, 775, 749 cm^{-1}.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 19.05 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min then 95 % $MeOH_{(aq)}$ for 1 min, R_t = 5.62 min.

Preparation of 2-methyl-*N*-pentyl-3-(4-benzyloxy-1-naphthoyl)-indole 45



Sodium hydride (60% w/w in mineral oil, 8 mg, 0.209 mmol) was suspended in DMF (5 mL), and added to 2-methyl-1-pentyl-3-(4-hydroxy-1-naphthoyl)-indole **38** (50 mg, 0.136 mmol). Benzylbromide (19 μL, 0.160 mmol) was added and the solution was stirred under an inert atmosphere for 22 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (5 mL), diluted with DCM (10 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with DCM (2 x 15 mL). The combined organics were washed with 2 M $HCl_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 Ethyl Acetate:Hexane) to yield the desired product (15 mg, 24 %) as a yellow oil.

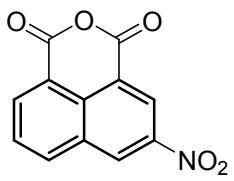
δ_H ($CDCl_3$) 8.47 (1H, m, naphthyl), 8.32 (1H, m, naphthyl), 7.63 – 7.28 (8H, 5 x CH benzyl, 3 x CH naphthyl and 1 x CH indole), 7.21 (1H, m, indole), 7.04 (1H, m, indole), 6.91 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 5.35 (2H, s, O-CH_2), 4.15 (2H, t, *J* 7.6 Hz, N-CH_2), 2.55 (3H, s, CH_3 indole), 1.82 (2H, m, CH_2 β to amine), 1.42 (4H, m, 2 x CH_2 pentyl) 0.96 (3H, t, *J* 6.5 Hz, CH_3 pentyl).

δ_C ($CDCl_3$) 193.1 (C=O), 156.3, 144.8, 136.7, 136.0, 132.8, 132.0 (6 x 4°), 128.7, 128.3, 128.1, 127.6, 127.5 (5 x CH), 127.3, 126.0 (2 x 4°), 125.6, 125.6, 122.3, 122.0, 121.6, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 104.1 (CH 3-naphthyl), 70.3 (O-CH_2), 43.4 (N-CH_2), 29.4 (CH_2 β to amine), 29.1, 22.4 (2 x CH_2 pentyl), 14.0 (CH_3 propyl), 12.5 (CH_3 indole).

EP-MS MH+ calcd for $C_{32}H_{31}N_1O_2$ 462.2428 found *m/z* 462.2427.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 17.31 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 4.74 min.

Preparation of 3-nitro-1,8-naphthoic anhydride 47



1,8-Naphthoic anhydride (20.0 g, 101 mmol) was dissolved in conc. sulphuric acid (80 mL), cooled to 0 °C and a solution of conc. nitric acid (4.19 mL, 101 mmol) in conc. sulphuric acid (20 mL) was added dropwise, and the solution was stirred for 1.5 h.

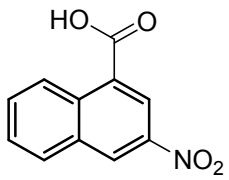
The reaction was quenched with ice water, and the precipitate was washed with water and ether, oven dried and recrystallised from acetic acid to produce the desired product (16.9 g, 69 %) as light brown crystals.

δ_H ($CDCl_3$) 9.38 (1H, d, *J* = 2.2 Hz, naphthyl), 9.26 (1H, d, *J* = 2.2 Hz, naphthyl), 8.85 (1H, dd, *J* = 1.1 + 7.3 Hz, naphthyl), 8.57 (1H, dd, *J* = 1.1 + 7.3 Hz, naphthyl), 8.05 (1H, dd, *J* = 7.4 + 8.2 Hz, naphthyl).

FTIR (KBr) 3404, 3373, 3101, 3039, 1780, 1736, 1632, 1596, 1543, 1508, 1456, 1420, 1344, 1363, 1293, 1234, 1197, 1181, 1142, 1128, 1082, 1043, 1018, 937, 799, 791, 757 721, 704, 675 cm^{-1}.

m.p. 225 °C lit. 252-253 °C[134].

Preparation of 3-nitro-1-naphthoic acid 48



Method A

3-Nitro-1,8-naphthoic anhydride **47** (5.00 g, 20.6 mmol) was suspended in a solution of NaOH (3.29 g, 82.3 mmol) in water (200 mL), and a solution of yellow mercury (II) oxide (5.80 g, 26.8 mmol) dissolved in 3:1 water:acetic acid (20 mL) was added. The reaction was then stirred at reflux for 72 h, then cooled, filtered and left to air dry.

The solid was suspended in 2 M $HCl_{(aq)}$ (200 mL) and conc. HCl (50 mL), and refluxed for 4 h. The solution was cooled, the crude product filtered, dried and recrystallised from acetone to from the desired product (0.81 g, 18 %) as light brown crystals.

Method B

3-Nitro-1,8-naphthoic anhydride **47** (10.7 g, 44.0 mmol) was split into 11 equal portions, each suspended in 1 M $NaOH_{(aq)}$ (10 mL) and stirred for 15 min at 60 °C. To each was added a solution of yellow mercury (II) oxide (998 mg, 4.6 mmol) dissolved in 3:1 water:acetic acid (4 mL) with stirring for 10 min at 60 °C. Each solution was sealed in a microwave tube (20 mL) and using a Biotage Initiator 2.0 the temperature was increased to 200 °C gradually over 2 min and then microwaved at 200 °C for 28 min.

The solutions were combined, acidified to pH 1 with conc. HCl and heated at 100 °C for 18 h with stirring. The solution was cooled, the crude product filtered, dried and recrystallised from acetone to yield the desired product (3.32 g, 35 %) as light brown crystals.

δ_H (DMSO-d_6) 13.85 (1H, br s, COOH) 9.24 (1H, d, J = 2.4 Hz, naphthyl), 8.97 (1H, app d, J = 8.2 Hz, naphthyl), 8.75 (1H, d, J = 2.5 Hz, naphthyl), 8.42

(1H, app d, J = 8.2 Hz, naphthyl), 7.92 (1H, m, naphthyl), 7.81 (1H, m, naphthyl).

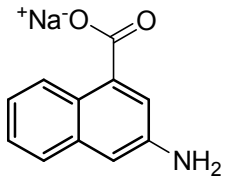
δ_C (DMSO-d_6) 167.6 (C=O), 144.3, 133.4, 133.1 (3 x 4°), 132.1, 131.7 (2 x CH), 130.1 (4°), 129.3, 128.7, 126.3, 122.9 (4 x CH).

EP-MS M- calcd for $C_{11}H_7N_1O_4$ 216.0341 found *m/z* 216.0285.

FTIR (KBr) 3067, 2643, 2643, 2604, 2360, 2342, 1700, 1653, 1617, 1597, 1539, 1528, 1507, 1455, 1412, 1337, 1287, 1254, 1203, 1163, 1146, 1102, 927, 915, 870, 796, 760, 688, 668, 625 cm^{-1}.

m.p. 264 °C lit. 266-272 °C[134, 184].

Preparation of sodium 3-amino-1- naphthalene-1-carboxylate 49



3-Nitro-1-naphthoic acid **48** (2.80 g, 12.9 mmol) and 10 % palladium on activated charcoal (0.48 g) was dispersed in a mixture of EtOH (40 mL), 2 M $NaOH_{(aq)}$ (7.5 mL) and water (15 mL), and stirred for 18 h under a hydrogen atmosphere.

The solution was filtered through a Celite 521 plug and reduced *in vacuo* to the desired product (2.55 g, 98 %) as an orange oil. The crude product was used without any further purification.

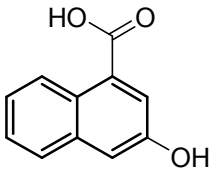
δ_H (DMSO d_6) 8.67 (1H, app d, J = 8.4 Hz, naphthyl), 7.43 (1H, app d, J = 8.0 Hz, naphthyl), 7.25 (1H, d, J = 2.4 Hz, naphthyl), 7.19 (1H, m, naphthyl), 7.01 (1H, m, naphthyl), 6.71 (1H, d, J = 2.0 Hz, naphthyl).

δ_C (DMSO d_6) 173.0 (C=O), 146.1, 141.6, 135.8 (3 x 4°), 128.8, 125.4, 125.3 (3 x CH), 125.1 (4°), 120.5, 119.0, 106.4 (3 x CH).

EP-MS MH+ calcd for $C_{11}H_9N_1O_2$ 188.0712 found *m/z* 188.0696.

FTIR (KBr) 3404, 2258, 2130, 1658, 1290, 1152, 1048, 1026, 1000, 826, 765 cm^{-1}.

Preparation of 3-hydroxy-1-naphthoic acid 50



Sodium 3-amino-1- naphthalene-1-carboxylate **49** (730 mg, 3.47 mmol) was dissolved in water (25 mL), and 2 M sulphuric $acid_{(aq)}$ (5 mL) was added to release the free acid as a fine precipitate. The suspension was cooled to 5 °C and a solution of sodium nitrite (255 mg, 3.70 mmol) in water (5 mL) was added dropwise and stirred for 45 min. This solution was added dropwise to a refluxing solution of 40% sulphuric $acid_{(aq)}$ (80 mL) over 50 min and stirred for a further 15 min.

The hot solution was filtered through glass wool and the filtrate cooled to precipitate the desired product (463 mg, 63 %) as a brown solid. The crude product was used without any further purification.

δ_H (DMSO d_6)13.15 (1H, br s, COOH) 10.01 (1H, br s, OH), 8.70 (1H, app d, *J* = 8.4 Hz, naphthyl), 7.78 (1H, app d, *J* = 8.0 Hz, naphthyl), 7.72 (1H, d, *J* = 2.6 Hz, naphthyl), 7.46 (1H, m, naphthyl), 7.40 – 7.35 (2H, 2 x naphthyl).

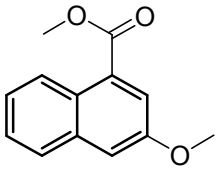
δ_C (DMSO d_6) 168.8 (C=O), 154.3, 135.8, 129.6 (3 x 4°), 127.4, 126.8 (2 x CH), 125.9 (4°), 125.9, 124.7, 122.4, 114.3 (4 x CH).

EP-MS M- calcd for $C_{11}H_8O_3$ 187.04 found *m/z* 187.03.

FTIR (KBr) 3075, 2952. 2850, 2360, 2342, 1717, 1705, 1699, 1684, 1558, 1540, 1507, 1457, 1436, 1339, 1243, 1195, 1155, 1021, 911, 795, 760, 668 cm^{-1}.

m.p. 238-240 °C lit. 239-246 °C[134, 184].

Preparation of methyl 3-methoxy-naphthalene-1-carboxylate 51



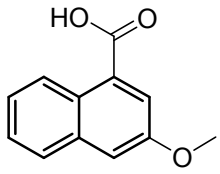
Sodium hydride (60% w/w in mineral oil, 516 mg, 12.9 mmol) was suspended in DMF (40 mL), and 3-hydroxy-1-naphthoic acid **50** (800 mg, 4.26 mmol) added. Methyl iodide (640 µL, 10.3 mmol) was added and the solution was stirred under an inert atmosphere for 18 h.

The solution was reduced *in vacuo*, excess hydride reacted with 2 M $HCl_{(aq)}$ (50 mL), diluted with DCM (50 mL), and stirred for 10 min. The resulting solution was separated and the aqueous layer extracted with DCM (2 x 50 mL). The combined organics were washed with sat. $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo* to yield the crude product (640 mg, 70 %) as an orange oil. The crude product was used without any further purification.

δ_H ($CDCl_3$) 8.81 (1H, m, naphthyl), 7.87 (1H, J = 2.4 Hz, naphthyl), 7.80 (1H, m, naphthyl), 7.50 (2H, m, naphthyl), 7.35 (1H, J = 2.8 Hz, naphthyl), 4.02 (3H, s, CH_3), 3.97 (3H, s, CH_3).

δ_C ($CDCl_3$) 167.6 (C=O), 156.1, 135.3, 128.6 (3 x 4°), 127.4(CH), 126.8 (4°), 126.7, 125.7, 125.3, 122.7, 111.3 (5 x CH), 55.6, 52.3 (2 x CH_3).

Preparation of 3-methoxy-1-naphthoic acid 52



Potassium hydroxide (768 mg, 14.0 mmol) was dissolved in MeOH (50 mL), methyl 3-methoxynaphthalene-1-carboxylate **51** (600 mg, 2.78 mmol) added, and the solution refluxed for 20 h.

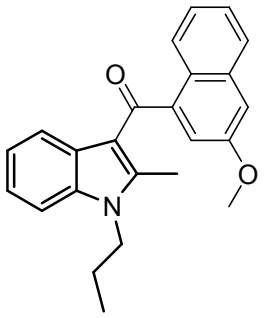
The solution was reduced *in vacuo* to an oil, which was dissolved in 1.5 M $NaOH_{(aq)}$ (50 mL) and washed with DCM (2 x 20 mL). The aqueous phase was acidified to pH 2 and extracted with DCM (3 x 20 mL). The combined organics were washed with 2 M $HCl_{(aq)}$ and brine, dried over $MgSO_4$ and

reduced *in vacuo*. The crude product was purified by recrystallisation from ethanol:water (1:1) to yield the desired product (320 mg, 57 %) as brown crystals.

δ_H ($CDCl_3$) 9.95 (1H, m, naphthyl), 8.05 (1H, *J* = 2.7 Hz, naphthyl), 7.82 (1H, m, naphthyl), 7.52 (2H, m, naphthyl), 7.40 (1H, *J* = 2.7 Hz, naphthyl), 3.99 (3H, s, CH_3).

m.p. 156 °C lit. 156-157 °C[185].

Preparation of 2-methyl-*N*-propyl-3-(3-methoxy-1-naphthoyl)-indole 53



3-Methoxynaphthalene-1-carboxylic acid **52** (320 mg, 1.58 mmol) and thionyl chloride (575 μL, 8.00 mmol) were refluxed at 90 °C for 2 h. Excess thionyl chloride was removed *in vacuo*, DCM (5 mL) added and the resulting solution was added dropwise, at 0 °C under an inert atmosphere, to a solution of 2-methyl-1-propylindole **32** (277 mg, 1.60 mmol) and diethyl aluminium chloride (1M in hexanes, 2.40 mmol) in DCM (5 mL). The reaction mixture was warmed to room temperature, and stirred for 3 h.

The reaction was quenched with ice (20 mL), diluted with 2M $NaOH_{(aq)}$ and extracted with DCM (3 x 20 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (60:40 Ether:Petroleum Ether 40/60) to yield the desired product (515 mg, 90 %) as an orange foam.

δ_H ($CDCl_3$) 7.98 (1H, d, *J* 8.5 Hz, naphthyl), 7.82 (1H, d, *J* 8.3 Hz, naphthyl), 7.47 (1H, m, naphthyl), 7.34-7.25 (5H, 3 x naphthyl, 2 x indole), 7.20 (1H, m, indole), 7.04 (1H, m, indole), 4.11 (2H, t, *J* 7.5 Hz, N-CH_2), 3.98 (3H, s, OCH_3), 2.49 (3H, s, CH_3 indole), 1.85 (2H, m, CH_2 β to amine), 1.02 (3H, t, *J* 7.4 Hz, CH_3 propyl).

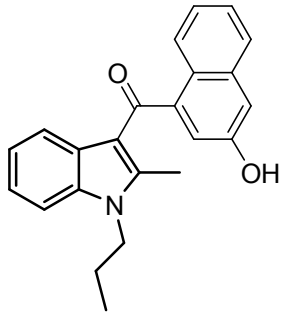
δ_C ($CDCl_3$) 192.6 (C=O), 156.8, 145.7, 142.3, 136.2, 135.2 (5 x 4°), 127.1 (CH), 127.0 (4°), 126.8 (CH). 125.9 (4°), 125.5, 124.4, 122.3, 122.1, 121.3, 117.9 (6 x CH), 114.7 (4°), 109.5 (CH), 107.8 (CH), 55.5 (OCH_3), 44.9 (N-CH_2), 22.9 (CH_2 β to amine), 12.6 (indole CH_3), 11.4 (propyl CH_3).

EP-MS MH+ calcd for $C_{24}H_{23}N_1O_2$ 358.1802 found *m/z* 358.1808.

FTIR (KBr) 2695, 2360, 1617, 1558, 1507, 1458, 1412, 1290, 1226, 1169, 1091, 1041, 949, 813, 747cm-1.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 13.62 min. Monolith C18 gradient 20 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 2.85 min.

Preparation of 2-methyl-*N*-propyl-3-(3-hydroxy-1-naphthoyl)-indole 54



2-Methyl-1-propyl-3-(3-methoxy-1-naphthoyl)-indole **53** (440 mg, 1.23 mmol) was dissolved in DCM (10 mL) and the solution brought to -50 °C under an inert atmosphere. Boron tribromide (1 M in hexanes, 1.40 mmol) was added, the solution slowly warmed to room temperature and stirred for 3 h.

The reaction mixture was quenched in 1 M HCl (aq, 20 mL) and stirred for 15 min. The layers were separated and the aqueous layer further extracted with DCM (3 x 50 mL). The combined organics were washed with H_2O and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (50:50 Ether:Petroleum Ether 40/60 to yield the desired product (302 g, 71 %) as an orange solid.

δ_H ($CDCl_3$) 7.87 (1H, d, *J* 8.4 Hz, naphthyl), 7.82 (1H, d, *J* 8.3 Hz, naphthyl), 7.38 (2H, m, indole), 7.32 (1H, m, naphthyl), 7.28-7.18 (5H, 3 x naphthyl, 2 x indole), 7.05 (1H, m, indole), 4.08 (2H, t, *J* 7.5 Hz, N-CH_2), 2.38 (3H, s, CH_3 indole), 1.81 (2H, m, CH_2 β to amine), 0.99 (3H, t, *J* 7.4 Hz, CH_3 propyl).

δ_C ($CDCl_3$) 193.4 (C=O), 153.4, 146.4, 142.0, 136.2, 135.3, 131.7 (6 x 4°), 126.8, 126.6 (2 x CH), 125.5 (4°), 125.2, 124.1, 122.5, 122.4, 121.4, 117.8, (6 x CH), 114.7 (4°), 111.8, 109.5 (2 x CH), 44.9 (N-CH_2), 22.9 (CH_2 β to amine), 12.6 (CH_3 indole), 11.4 (CH_3 propyl).

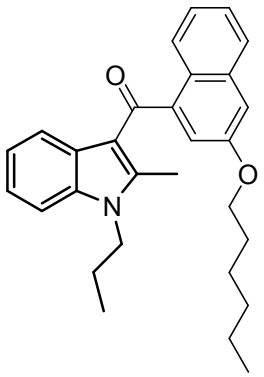
EP-MS M- calcd for $C_{23}H_{21}N_1O_2$ 342.1500 found *m/z* 342.1483.

FTIR (KBr) 3160, 2967, 2931, 2877, 1620, 1587, 1571, 1504, 1460, 1414, 1380, 1347, 1223, 1209, 1173, 1132, 1091, 836, 747, 681, 559 cm^{-1}.

m.p. 196 °C.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 18.53 min. Monolith C18 gradient 20 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 2.09 min.

Preparation of 2-methyl-*N*-propyl-3-3-hexyloxy-1-naphthoyl)-indole 55



Sodium hydride (60% w/w in mineral oil, 4 mg, 0.105 mmol) was suspended in DMF (2 mL), and added to 2-methyl-1-propyl-3-(3-hydroxy-1-naphthoyl)-indole **54** (25 mg, 0.073 mmol). 1-bromohexane (9.4 μL, 0.084 mmol) was added and the solution was stirred under an inert atmosphere for 24 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (5 mL), diluted with DCM (10 mL), and stirred for 5 min. The resulting solution was separated and the

aqueous layer extracted with DCM (2 x 10 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (70:30 Ether:Petroleum Ether 40/60) to yield the desired product (5 mg, 16 %) as a yellow oil.

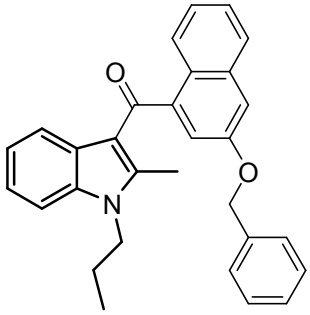
δ_H ($CDCl_3$) 7.97 (1H, d, *J* 8.4 Hz, naphthyl), 7.79 (1H, d, *J* 8.2 Hz, naphthyl), 7.45 (1H, m, naphthyl), 7.36-7.25 (5H, 3 x naphthyl, 2 x indole), 7.19 (1H, m, indole), 7.04 (1H, m, indole), 4.17-4.08 (4H, O-CH_2 and N-CH_2), 2.49 (3H, s, CH_3 indole), 1.90-1.79 (4H, CH_2 β to ether and CH_2 β to amine), 1.49 (2H, m, CH_2 γ to ether), 1.41 – 1.32 (4H, 2 x CH_2 hexyl), 1.02 (3H, t, *J* 7.4 Hz, CH_3 propyl), 0.92 (3H, m, CH_3 hexyl).

δ_C ($CDCl_3$) 192.7 (C=O), 156.3, 145.6, 142.2, 136.2, 135.3 (5 x 4°), 127.0 (CH), 127.0 (4°), 126.7 (CH). 125.9 (4°), 125.5, 124.3, 122.3, 122.1, 121.3, 118.3 (6 x CH), 114.7 (4°), 109.5, 108.8 (2 x CH), 68.3 (O-CH_2), 44.9 (N-CH_2), 31.6 (CH_2 hexyl), 29.2 (CH_2 β to ether), 25.7 (CH_2 γ to ether), 22.9 (CH_2 β to amine), 22.6 (CH_2 hexyl), 14.0 (CH_3 hexyl) 12.6 (CH_3 indole), 11.5 (CH_3 propyl).

EP-MS MH+ calcd for $C_{29}H_{33}N_1O_2$ 428.2584 found *m/z* 428.2601.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 18.86 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min then 95 % $MeOH_{(aq)}$ for 1 min, R_t = 5.03 min.

Preparation of 2-methyl-*N*-propyl-3-(3-benzyloxy-1-naphthoyl)-indole 56



Sodium hydride (60% w/w in mineral oil, 4 mg, 0.105 mmol) was suspended in DMF (2 mL), and added to 2-methyl-1-propyl-3-(3-hydroxy-1-naphthoyl)-indole **54** (25 mg, 0.073 mmol).

Benzylbromide (10.0 μL, 0.084 mmol) was added and the solution was stirred under an inert atmosphere for 24 h.

Excess hydride was reacted with 2 M $HCl_{(aq)}$ (5 mL), diluted with DCM (10 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with DCM (2 x 10 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (40:60 Ether:Petroleum Ether 40/60) to yield the desired product (10 mg, 32 %) as a yellow oil.

δ_H ($CDCl_3$) 7.99 (1H, d, *J* 8.4 Hz, naphthyl), 7.81 (1H, d, *J* 8.2 Hz, naphthyl), 7.54 – 7.28 (10H, 5 x CH benzyl, 4 x CH naphthyl, 1 x CH indole), 7.20 (1H, m, indole), 7.04 (1H, m, indole), 7.04 (1H, m, indole), 5.23 (2H, s, O-CH_2), 4.11 (2H, t, *J* 7.5 Hz, N-CH_2), 2.47 (3H, s, CH_3 indole), 1.85 (2H, m, CH_2 β to amine) 1.05 (3H, t, *J* 7.4 Hz, CH_3 propyl).

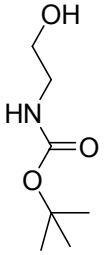
δ_C ($CDCl_3$) 192.6 (C=O), 155.9, 145.7, 142.3, 136.7, 136.2, 135.2 (6 x 4°), 128.6, 128.0, 127.5, 127.1, (4 x CH), 127.0 (4°), 126.8 (CH), 126.1 (4°), 125.5, 124.6, 122.3, 122.1, 121.3, 118.4 (6 x CH), 114.7 (4°), 109.5, 109.4 (2 x CH), 70.2 (O-CH_2), 44.9 (N-CH_2), 22.9 (CH_2 β to amine), 12.6 (CH_3 indole), 11.5 (CH_3 propyl).

EP-MS MH+ calcd for $C_{30}H_{27}N_1O_2$ 434.2120 found *m/z* 434.2093.

FTIR (NaCl) 3062, 2964, 2931, 2876, 2361, 2342, 1772, 1734, 1717, 1699, 1684, 1653, 1616, 1558, 1540, 1507, 1457, 1418, 1288, 1224, 1172, 1091, 1019, 747, 668 cm^{-1}.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 15.68 min. Monolith C18 gradient 60 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 4.09 min.

Preparation of *tert*-butyl 2-hydroxyethylcarbamate 62



Ethanolamine (13.000 g, 16.4 mmol), *t*butylphenyl carbonate (3.80 g, 19.6 mmol) and triethylamine (2.30 mL, 16.5 mmol) were dissolved in EtOH (15 mL), and stirred for 20 h.

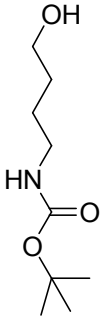
The solution was reduced *in vacuo*, suspended in water (20 mL), neutralised with 1 M $HCl_{(aq)}$ and extracted with DCM (4 x 20 mL). The combined organics were washed with 1 M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (50:50 EtOAc:Hexane) to yield the desired product (1.99 g, 76 %) as a clear oil.

δ_H ($CDCl_3$) 5.14 (1H, br s, NH), 3.68 (2H, t, *J* 4.8 Hz, OCH_2), 3.28 (2H, m, NCH_2), 2.06 (1H, s, OH), 1.44 (9H, s, 3 x CH_3).

δ_C ($CDCl_3$) 156.9 (C=O), 79.7 (4° tert-butyl), 62.4 (OCH_2), 43.1 (NCH_2), 28.4 (3 x CH_3).

ES-MS MeCN + MNa+ calcd for $C_7H_{15}N_{1O3}$ 225.1215 found *m/z* 225.1217.

Preparation of *tert*-butyl 4-hydroxybutylcarbamate 63



4-Aminobutan-1-ol (700 mg, 7.86 mmol), *t*butylphenyl carbonate (1.80 g, 9.27 mmol) and triethylamine (1.10 mL, 7.91 mmol) were dissolved in EtOH (15 mL), and stirred for 20 h.

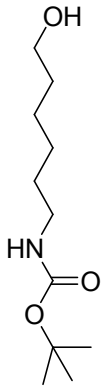
The solution was reduced *in vacuo*, suspended in water (20 mL), neutralised with 1 M $HCl_{(aq)}$ and extracted with DCM (4 x 20 mL). The combined organics were washed with 1 M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (50:50 EtOAc:Hexane) to yield the desired product (946 mg, 65 %) as a clear oil.

δ_H ($CDCl_3$) 4.67 (1H, br s, NH), 3.68 (2H, t, *J* 6.0 Hz, OCH_2), 3.16 (2H, m, NCH_2), 2.02 (1H, s, OH) 1.62-1.55 (4H, 2 x CH_2), 1.44 (9H, s, 3 x CH_3).

δ_C ($CDCl_3$) 156.3 (C=O), 79.2 (4° tert-butyl), 62.0 (OCH_2), 40.3 (NCH_2), 29.6 (CH_2), 28.4 (3 x CH_3), 26.5 (CH_2).

ES-MS MH+ calcd for $C_9H_{19}N_1O_3$ 190.1 found *m/z* 190.1.

Preparation of *tert*-butyl 6-hydroxyhexylcarbamate 64



6-Aminohexan-1-ol (1.00 g, 8.53 mmol), *t*butylphenyl carbonate (2.00 g, 10.3 mmol) and triethylamine (1.30 mL, 9.35 mmol) were dissolved in EtOH (15 mL), and stirred for 20 h.

The solution was reduced *in vacuo*, suspended in water (20 mL), neutralised with 1 M $HCl_{(aq)}$ and extracted with DCM (4 x 20 mL). The combined organics were washed with 1 M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (2:98 MeOH:DCM) to yield the desired product (1.83 g, 96 %) as a clear oil.

δ_H ($CDCl_3$) 4.53 (1H, br s, NH), 3.66 (2H, m, OCH_2), 3.15 (2H, m, NCH_2), 2.19 (1H, s, OH) 1.62-1.30 (8H, 4 x CH_2), 1.46 (9H, s, 3 x CH_3).

ES-MS MNa+ calcd for $C_{11}H_{23}N_1O_3$ 240.1565 found *m/z* 240.1568.

Preparation of ethyl 8-aminooctanoate 65

8-Aminoctanioc acid (600 mg, 3.77 mmol) and p-toluenesulphonic acid monohydrate (1.45 g, 7.63 mmol) were dissolved in ethanol (25 mL) and refluxed for 20 h.

The solution was reduced *in vacuo*, dissolved in $NaOH_{(aq)}$ (20 mL) and DCM (20 mL), layers separated and the aqueous phase extracted with DCM (2 x 20 mL). The combined organics were washed with brine, dried over $MgSO_4$ and reduced *in vacuo*, to yield the desired product (593 mg, 84 %) as a foamy white solid. The crude product was used without any further purification.

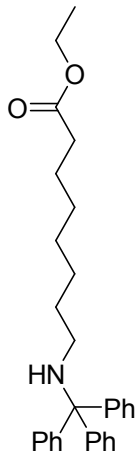
δ_H ($CDCl_3$) 4.15 (2H, q, *J* 7.2 Hz, OCH_2), 2.69 (2H, t, *J* 7.0 Hz, NCH_2), 2.30 (2H, t, *J* 7.5 Hz, $CH_2C{=}O$), 1.63 (2H, m, CH_2 β to ester), 1.45 (2H, m, CH_2 β to amine), 1.37-1.28 (6H, 3 x CH_2), 1.27 (3H, t, *J* 7.1 Hz, CH_3).

δ_C ($CDCl_3$) 173.9 (C=O), 60.2 (OCH_2), 42.2 (NCH_2), 34.4, 33.8, 29.1, 29.1, 26.7, 24.9 (6 xCH_2), 14.3 (CH_3).

ES-MS MH+ calcd for $C_{10}H_{21}N_{1O2}$ 188.1645 found *m/z* 188.1638.

FTIR (KBr) 2928, 2858, 1729, 1616, 1567, 1490, 1475, 1419, 1380, 1321, 1261, 1222, 1184 cm^{-1}.

Preparation of ethyl 8-(tritylamino)octanoate 66



To a stirred solution of ethyl 8-aminooctanoate **65** (355 mg, 1.90 mmol) in chloroform (10 mL) was added trityl chloride (560 mg, 2.01 mmol) and triethylamine (265 µL, 1.90 mmol), and the solution stirred for 20 h.

The solution was washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (10:90 EtOAc:Hexane) to yield the desired product (728 mg, 89 %) as a clear oil.

δ_H ($CDCl_3$) 7.51-7.48 (6H, 6 x CH trityl), 7.36-7.27 (6H, 6 x CH trityl), 7.22-7.18 (3H, 3 x CH trityl), 4.14 (2H, q, *J* 7.1 Hz, OCH_2), 2.29 (2H, t, *J* 7.6 Hz, $CH_2C{=}O$), 2.12 (2H, t, *J* 7.0 Hz, NCH_2), 1.59 (2H, m, CH_2 β to ester), 1.48

(2H, m, CH_2 β to amine), 1.35-1.25 (6H, 3 x CH_2), 1.27 (3H, t, *J* 7.1 Hz, CH_3).

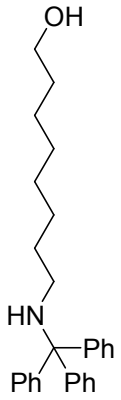
δ_C ($CDCl_3$) 173.9 (C=O), 146.4 (4º), 128.7, 128.0, 127.9, 127.7, 127.3, 126.1 (6 x CH trityl*), 70.9 (4º), 60.2 (OCH_2), 43.5 (NCH_2), 34.4, 30.8, 29.3, 29.1, 27.2, 25.0 (6 xCH_2), 14.3 (CH_3).

*Note: the trityl group appears as six chemically identifiable CH groups, three of which are twice the magnitude of the other three.

ES-MS MH+ calcd for $C_{29}H_{35}N_{1O2}$ 430.2741 found *m/z* 430.2725.

FTIR (NaCl) 3431, 2928, 2853, 2096, 1733, 1646, 1489, 1447, 1181, 1109, 1032, 706 cm^{-1}.

Preparation of 8-(tritylamino)octan-1-ol 67



To a stirred solution of ethyl 8-(tritylamino)octanoate **66** (700 mg, 1.63 mmol) in THF was added lithium aluminium hydride (124mg, 3.26 mmol), the solution stirred at 0 ºC for 1 h and then refluxed for 1.5 h.

The reaction was quenched in 1:1 MeOH:H_2O (10 mL), the solution filtered with THF and chloroform washing, and organic solvents removed *in vacuo*. The solution was extracted with DCM (3 x 10 mL), and the combined organics were washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (2:98 MeOH:DCM) to yield the desired product (410 mg, 65 %) as a clear oil.

δ_H ($CDCl_3$) 7.54-7.48 (6H, 6 x CH trityl), 7.38-7.27 (6H, 6 x CH trityl), 7.22-7.18 (3H, 3 x CH trityl), 3.65 (2H, t, *J* 6.5 Hz, OCH_2), 2.12 (2H, t, *J* 7.0 Hz, NCH_2), 1.56 (2H, m, CH_2 β to alcohol), 1.49 (2H, m, CH_2 β to amine), 1.38-1.27 (8H, 4 x CH_2).

δ_C ($CDCl_3$) 146.4 (4º), 128.7, 128.0, 127.9, 127.7, 127.3, 126.1 (6 x CH trityl*), 70.9 (4º), 63.1 (OCH_2), 43.5 (NCH_2), 32.8, 30.9, 29.6, 29.4, 27.3, 25.7 (6 xCH_2).

* see ethyl 8-(tritylamino)octanoate **66**.

ES-MS MH+ calcd for $C_{27}H_{33}N_{1O1}$ 388.2635 found *m/z* 388.2598.

FTIR (NaCl) 3421, 2927, 2853, 2095, 1645, 1489, 1448, 1032, 706 cm^{-1}.

Preparation of 2-(2-(2-azidoethoxy)ethoxy)ethanol 68

2-(2-(2-Chloroethoxy)ethoxy)ethanol (4.43 g, 26.3 mmol) and sodium azide (5.13 g, 78.9 mmol) were dissolved in DMF (50 mL), warmed to 70 ºC and stirred for 18 h.

The solution was reduced *in vacuo*, dissolved in water (100 mL) and DCM (50 mL), layers separated and the aqueous phase extracted with DCM (2 x 50 mL). The combined organics were washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (EtOAc) to yield the desired product (4.43 g, 99 %) as a yellow oil.

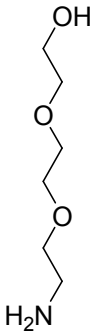
δ_H ($CDCl_3$) 3.76 (2H, m, CH_2), 3.72-3.68 (6H, 3 x CH_2), 3.63 (2H, m, CH_2), 3.42 (2H, t, *J* 5.0 Hz, CH_2), 2.31 (1H, br s, OH).

δ_C ($CDCl_3$) 72.5, 70.7, 70.4,70.1, 61.8, 50.7 (6 xCH_2).

ES-MS MNa+ calcd for $C_6H_{13}N_{3O3}$ 198.1 found *m/z* 198.1.

FTIR (NaCl) 3409, 2868, 2110, 1456, 1347, 1300, 1120 cm^{-1}.

Preparation of 2-(2-(2-aminoethoxy)ethoxy)ethanol 69



Method A

2-(2-(2-Azidoethoxy)ethoxy)ethanol **68** (1.00 g, 5.71 mmol) and tin (II) chloride (2.70 g, 14.2 mmol) were suspended in EtOH (20 mL) and stirred at reflux for 2 h.

The solution was reduced *in vacuo*, suspended in water (10 mL), basified to pH 10 with 2 M $NaOH_{(aq)}$ and extracted with EtOAc (3 x 10 mL). The combined organics were washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (3:3:1 MeOH:DCM:TEA) to yield the desired product (363 mg, 54 %) as a yellow oil.

Method B

2-(2-(2-Azidoethoxy)ethoxy)ethanol **68** (4.43 g, 26.2 mmol), triphenylphosphine (7.53 g, 28.7 mmol) and water (700 µL, 38.9 mmol) were suspended in ether (50 mL) and stirred at reflux for 5 h.

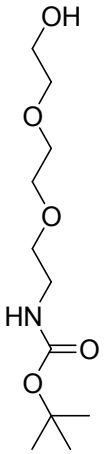
The solution was filtered and reduced *in vacuo*. The crude product was purified by flash column chromatography (3:3:1 MeOH:DCM:TEA) to yield the desired product (1.22 g, 31 %) as a yellow oil.

δ_H (DMSO-d_6) 3.57-3.54 (6H, 3 x CH_2), 3.50 (2H, m, CH_2), 3.43 (2H, m, CH_2), 2.88 (2H, t, *J* 5.5 Hz, CH_2).

δ_C (DMSO-d_6) 72.8, 70.1, 70.0, 70.0, 60.6, 39.2 (6 xCH_2).

ES-MS MH+ calcd for $C_6H_{15}N_1O_3$ 150.1125 found *m/z* 150.1133.

Preparation of *tert*-butyl 2-(2-(2-hydroxyethoxy)ethoxy)ethylcarbamate 70



2-(2-(2-Aminoethoxy)ethoxy)ethanol **69** (1.22 g, 8.19 mmol), potassium hydroxide (500 mg, 8.91 mmol) and di-*t*butyl dicarbonate (1.96 g, 8.98 mmol) were dissolved in water (20 mL) and stirred for 16 h.

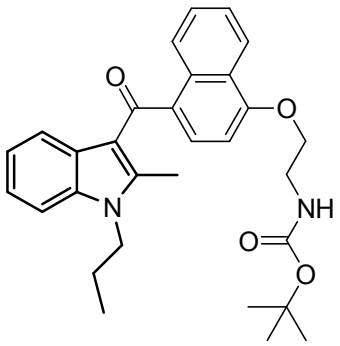
The solution was extracted with DCM (3 x 50 mL) and the combined organics were washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (80:20 EtOAc:Hexane) to yield the desired product (1.52 g, 68 %) as a clear oil.

δ_H ($CDCl_3$) 5.23 (1H, br s, NH), 3.70 (2H, m, CH_2), 3.63-3.55 (6H, 3 x CH_2), 3.52 (2H, m, CH_2), 3.27 (2H, m, CH_2), 2.96 (1H, br s, OH), 1.40 (9H, s, 3 x CH_3).

δ_C ($CDCl_3$) 156.0 (COOH), 79.2 (4º), 72.6, 70.3, 70.2, 70.2, 61.6, 40.3 (6 xCH_2), 28.4 (CH_3).

ES-MS M+ calcd for $C_{11}H_{23}N_1O_5$ 250.2 found *m/z* 250.2.

Preparation of 2-methyl-*N*-propyl-3-(4-(*tert*-butyl 2-ethoxycarbamate)-1-naphthoyl)-indole 71



2-Methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (129 mg, 0.376 mmol), *tert*-butyl 2-hydroxyethylcarbamate **62** (62 mg, 0.385 mmol) and triphenylphosphine (147 mg, 0.561 mmol) were dissolved in THF (40 mL) under an inert atmosphere. Diethyl azodicarboxylate (120 μL, 0.763 mmol) was added dropwise and the solution was stirred for 18 h.

The solution was reduced *in vacuo*, dissolved in EtOAc (50 mL) washed with 2 M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (50:50 EtOAc:Hexane) to yield the desired product (70 mg, 37 %) as a yellow oil.

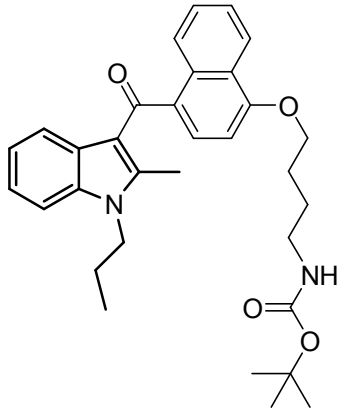
δ_H ($CDCl_3$) 8.37 (1H, m, naphthyl), 8.29 (1H, m, naphthyl), 7.58 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.52 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.26 (1H, m, indole), 7.19 (1H, m, indole), 7.02 (1H, m, indole), 6.80 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 5.12 (1H, m, NH), 4.28 (2H, t, *J* 5.1 Hz, O-CH_2), 4.13 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 3.76 (2H, m, N-CH_2 carbamate) 2.54 (3H, s, CH_3 indole), 1.87 (2H, m, CH_2 β to propyl amine), 1.50 (9H, s, 3 x CH_3 *t*Bu), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.1 (C=O), 156.0, 145.0, 136.1, 132.8, 131.9 (5 x 4°), 128.3 (CH 2-naphthyl), 127.6 (CH), 127.4, 127.3 (2 x 4°), 125.7, 125.7 (2 x CH), 125.6 (4°), 122.0, 122.0, 121.6, 121.2 (4 x CH), 115.2 (4°), 109.5 (CH), 103.5 (CH 3-naphthyl), 79.7 (4° *t*Bu), 67.7 (O-CH_2), 44.9 (N-CH_2 propyl), 40.2 (N-CH_2 carbamate), 28.4 (3 x CH_3 *t*Bu), 23.0, (CH_2 β to propyl amine), 12.6 (CH_3 indole), 11.5 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{30}H_{34}N_2O_4$ 487,2591 found *m/z* 487.2595.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 14.36 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 3.35 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(*tert*-butyl 4-butoxycarbamate)-1-naphthoyl)-indole 72



2-Methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (135 mg, 0.393 mmol), *tert*-butyl 4-hydroxybutylcarbamate **63** (74 mg, 0.392 mmol) and triphenylphosphine (155 mg, 0.592 mmol) were dissolved in THF (25 mL) under an inert atmosphere. Diethyl azodicarboxylate (108 µL, 0.683 mmol) was added dropwise and the solution was stirred for 20 h.

The solution was reduced *in vacuo*, dissolved in EtOAc (50 mL) washed with 2 M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (70:30 Ether:Petroleum Ether 40:60) to yield the desired product (152 mg, 75 %) as a yellow oil.

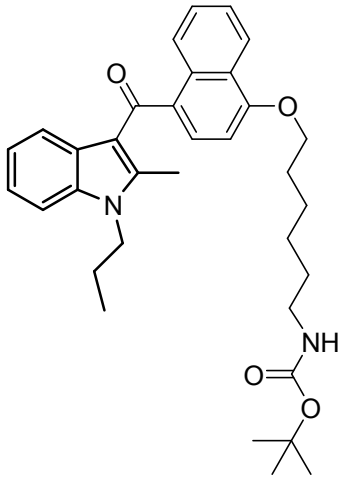
δ_H ($CDCl_3$) 8.37 (1H, m, naphthyl), 8.30 (1H, m, naphthyl), 7.58 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.30 (1H, m, indole), 7.19 (1H, m, indole), 7.03 (1H, m, indole), 6.79 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.68 (1H, m, NH), 4.23 (2H, t, *J* 5.1 Hz, O-CH_2), 4.12 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 3.29 (2H, m, N-CH_2 carbamate) 2.53 (3H, s, CH_3 indole), 2.02 (2H, m, CH_2 β to ether), 1.91-1.79 (4H, CH_2 β to propyl amine and CH_2 β to carbamate), 1.48 (9H, s, 3 x CH_3 *t*Bu), 1.02 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.2 (C=O), 156.5, 156.1, 144.9, 136.1, 132.3, 132.0 (6 x 4°), 128.6 (CH 2-naphthyl), 127.5 (CH), 127.3, 125.8 (2 x 4°), 125.6, 125.5, 122.1, 122.0, 121.6, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 103.4 (CH 3-naphthyl), 79.3 (4° *t*Bu), 67.8 (O-CH_2), 44.9 (N-CH_2 propyl), 40.4 (N-CH_2 carbamate), 28.5 (3 x CH_3 *t*Bu), 27.1 (CH_2 β to ether), 26.6 (CH_2 β to propyl amine), 23.0, (CH_2 β to carbamate amine), 12.6 (CH_3 indole), 11.5 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{32}H_{38}N_2O_4$ 515.2904 found *m/z* 515.2875.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 15.24 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ +0.06% TFA over 5 min, R_t = 3.68 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(*tert*-butyl 6-hexyloxycarbamate)-1-naphthoyl)-indole 73



2-Methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (100 mg, 0.292 mmol), *tert*-butyl 2-hydroxyhexylcarbamate **64** (55 mg, 0.291 mmol) and triphenylphosphine (152 mg, 0.580 mmol) were dissolved in DCM (25 mL) under an inert atmosphere. Diethyl azodicarboxylate (91 μL, 0.580 mmol) was added dropwise and the solution was stirred for 20 h.

The solution was washed with 2 M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (5:95 MeOH:DCM) to yield the desired product (138 mg, 87 %) as a yellow oil.

δ_H ($CDCl_3$) 8.38 (1H, m, naphthyl), 8.29 (1H, m, naphthyl), 7.58 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.33 (1H, m, indole), 7.29 (1H, m, indole), 7.19 (1H, m, indole), 7.02 (1H, m, indole), 6.79 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.67 (1H, m, NH), 4.21 (2H, t, *J* 6.3 Hz, O-CH_2), 4.13 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 3.17 (2H, m, N-CH_2 carbamate), 2.52 (3H, s, CH_3 indole), 1.99 (2H, m, CH_2 β to ether), 1.86 (2H, m, CH_2 β to propyl amine), 1.65-1.45 (6H, 3x CH_2), 1.47 (9H, s, 3 x CH_3 *t*Bu), 1.02 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

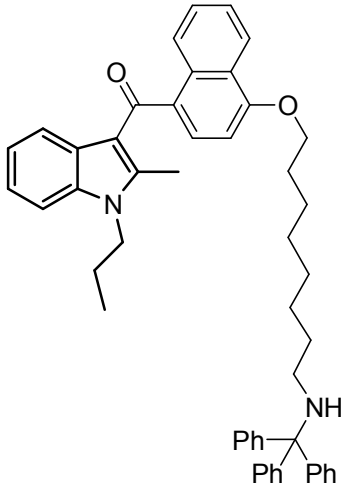
δ_C ($CDCl_3$) 193.3 (C=O), 156.7, 156.0, 144.8, 136.0, 132.2, 132.0 (6 x 4°), 128.7 (CH 2-naphthyl), 127.5 (CH), 127.3, 125.9 (2 x 4°), 125.6, 125.5, 122.1, 122.0, 121.6, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 103.3 (CH 3-naphthyl),

68.2 (O-CH_2), 44.9 (N-CH_2 propyl), 40.6 (N-CH_2 carbamate), 30.3 (4° *t*Bu), 30.1 (CH_2 β to ether), 29.2 (CH_2), 28.4 (3 x CH_3 *t*Bu), 26.8 (CH_2), 26.0 (CH_2 β to propyl amine), 12.5 (CH_3 indole), 11.5 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{34}H_{42}N_2O_4$ 543.3217 found *m/z* 543.3185.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 16.35 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ +0.06% TFA over 5 min, R_t = 4.40 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(8-(tritylamino)octyloxy)-1-naphthoyl)-indole 74



2-Methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (200 mg, 0.583 mmol), 8-(tritylamino)octan-1-ol **67** (226 mg, 0.584 mmol) and triphenylphosphine (229 mg, 0.875 mmol) were dissolved in THF (20 mL) under an inert atmosphere. Diethyl azodicarboxylate (239 mg, 1.04 mmol) was added dropwise and the solution was stirred for 20 h.

The solution was reduced *in vacuo*, dissolved in EtOAc (50 mL) washed with 2 M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (25:75 EtOAc:Hexane) to yield the desired product (230 mg, 55 %) as a yellow oil.

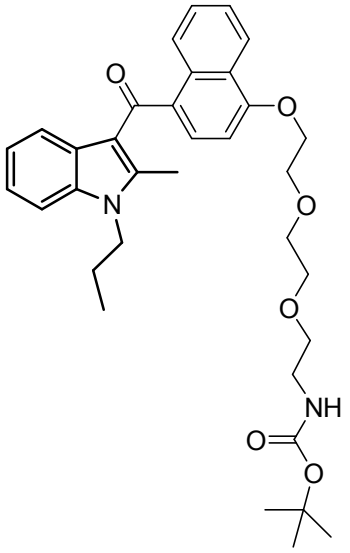
δ_H ($CDCl_3$) 8.38 (1H, m, naphthyl), 8.30 (1H, m, naphthyl), 7.58 (1H, d, *J* 8.0 Hz, 2-H naphthyl), 7.54-7.48 (8H, 2 x CH naphthyl and 6 x CH trityl), 7.34 (1H, m, indole), 7.32-7.24 (7H, 1 x CH indole and 6 x CH trityl), 7.21-7.17 (4H, 1 x CH indole and 3 x CH trityl), 7.02 (1H, m, indole), 6.79 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.21 (2H, t, *J* 6.4 Hz, O-CH_2), 4.13 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 2.54 (3H, s, CH_3 indole), 2.15 (2H, m, N-CH_2 trityl amine), 1.97 (2H,

m, CH_2 β to ether), 1.86 (2H, m, CH_2 β to propyl amine), 1.75-1.26 (10H, 5 x CH_2), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{50}H_{52}N_2O_2$ 713.4102 found *m/z* 713.4069.

FTIR (NaCl) 3419, 3054, 2986, 2929, 2855, 2685, 2410, 2305, 1608, 1510, 1421, 1264, 1160, 1078, 909, 896, 750 cm^{-1}.

Preparation of 2-methyl-*N*-propyl-3-(4-(*tert*-butyl 2-(2-(2-ethoxy)ethoxy)ethylcarbamate)-1-naphthoyl)-indole 75



2-Methyl-*N*-propyl-3-(4-hydroxy-1-naphthoyl)-indole **37** (100 mg, 0.292 mmol), *tert*-butyl 2-(2-(2-hydroxyethoxy)ethoxy)ethylcarbamate **70** (79 mg, 0.292 mmol) and triphenylphosphine (152 mg, 0.580 mmol) were dissolved in DCM (25 mL) under an inert atmosphere. Diethyl azodicarboxylate (91 μL, 0.580 mmol) was added dropwise and the solution was stirred for 20 h.

The solution was washed with 2 M $NaOH_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (5:195 MeOH:DCM) to yield the desired product (152 mg, 91 %) as a yellow oil.

δ_H ($CDCl_3$) 8.39 (1H, m, naphthyl), 8.27 (1H, m, naphthyl), 7.57 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.33 (1H, m, indole), 7.26 (1H, m, indole), 7.19 (1H, m, indole), 7.02 (1H, m, indole), 6.82 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 5.06 (1H, m, NH), 4.41 (2H, t, *J* 4.8 Hz, CH_2), 4.13 (2H, t, *J* 7.5 Hz, O-CH_2), 3.84 (2H, m, CH_2), 3.72 (2H, m, CH_2), 3.60 (2H, t, *J* 5.1 Hz, CH_2 β carbamate amine), 3.35 (2H, m, N-CH_2 carbamate), 2.53 (3H, s, CH_3 indole),

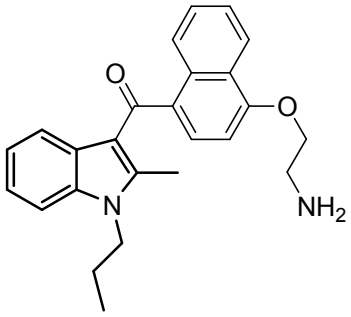
1.86 (2H, m, CH_2 β to propyl amine), 1.45 (9H, s, 3 x CH_3 *t*Bu), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.2 (C=O), 156.3, 144.9, 136.1, 133.1, 132.8, 131.9 (6 x 4°), 128.2 (CH 2-naphthyl), 127.5 (CH), 127.3, 125.8 (2 x 4°), 125.6, 125.6, 122.2, 122.0, 121.6, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 103.6 (CH 3-naphthyl), 79.2 (4º *t*Bu), 71.0, 70.4, 70.4, 69.8, 67.9 (5 x CH_2), 44.9 (N-CH_2 propyl), 40.4 (N-CH_2 carbamate), 28.4 (4° *t*Bu), 23.0 (CH_2 β to propyl amine), 12.5 (CH_3 indole), 11.5 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{34}H_{42}N_2O_6$ 575.3116 found *m/z* 575.3080.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 14.99 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ +0.06% TFA over 5 min, R_t = 3.27 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(2-aminoethoxy)-1-naphthoyl)-indole 76



2-Methyl-*N*-propyl-3-(4-(*tert*-butyl 2-ethoxycarbamate)-1-naphthoyl)-indole **71** (46 mg, 0.095 mmol) was dissolved in a mixture of DCM:TFA:H_2O (50:45:5 3 mL) and stirred for 3 h.

The solution was reduced *in vacuo*, dissolved in EtOAc (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (64:35:1 Ether:Petroleum Ether 40/60:TEA) to yield the desired product (16 mg, 44 %) as a yellow oil.

δ_H ($CDCl_3$) 8.39 (1H, m, naphthyl), 8.10 (1H, m, naphthyl), 7.47-7.30 (5H, 3 x CH naphthyl and 2 x CH indole), 7.17 (1H, m, indole), 7.00 (1H, m, indole),

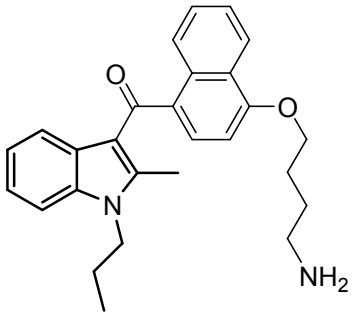
6.68 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.28 (2H, m, O-CH_2), 4.07 (2H, t, *J* 7.4 Hz, N-CH_2 propyl), 3.35 (2H, m, N-CH_2 primary amine) 2.39 (3H, s, CH_3 indole), 1.81 (2H, m, CH_2 β to propyl amine), 0.98 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.4 (C=O), 155.2, 145.6, 136.1, 133.3, 131.5 (5 x 4°), 127.7 (CH 2-naphthyl), 127.4 (CH), 127.2 (4°), 126.0 (CH), 125.4 (2 x 4°), 125.2, 122.5, 122.3, 121.9, 121.2 (5 x CH), 115.0 (4°), 109.6 (CH), 103.7 (CH 3-naphthyl), 64.3 (O-CH_2), 44.9 (N-CH_2 propyl), 39.1 (N-CH_2 primary amine), 22.9, (CH_2 β to propyl amine), 12.6 (CH_3 indole), 11.4 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{25}H_{26}N_2O_2$ 387.2067 found *m/z* 387.2072.

Analytical RP-HPLC Vydac C8 gradient 5 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 16.88 min. Symmetry C18 gradient 5 - 95% $MeOH_{(aq)}$ +0.1% formic acid over 20 min, R_t = 12.20 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(4-aminobutoxy)-1-naphthoyl)-indole 77



2-Methyl-*N*-propyl-3-(4-(*tert*-butyl 4-butoxycarbamate)-1-naphthoyl)-indole **72** (100 mg, 0.195 mmol) was dissolved in a mixture of DCM:TFA:H_2O (50:45:5 5 mL) and stirred for 2 h.

The solution was reduced *in vacuo*, dissolved in EtOAc (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (98:2 Ether:TEA) to yield the desired product (35 mg, 43 %) as a yellow oil.

δ_H ($CDCl_3$) 8.29 (1H, m, naphthyl), 8.16 (1H, m, naphthyl), 7.50-7.29 (5H, 3 x CH naphthyl and 2 x CH indole), 7.17 (1H, m, indole), 7.01 (1H, m, indole),

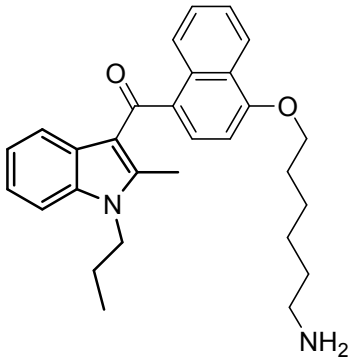
6.69 (1H, d, *J* 8.1 Hz, 3-H naphthyl), 4.08-4.03 (4H, O-CH_2 and N-CH_2 propyl), 2.95 (2H, m, CH_2) 2.41 (3H, s, CH_3 indole), 1.88-1.84 (4H, m, 2 x CH_2), 1.79 (2H, m, CH_2 β to propyl amine), 0.98 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.6 (C=O), 155.2, 145.5, 136.1, 132.3, 131.7 (5 x 4°), 128.1 (CH 2-naphthyl), 127.7 (CH), 127.2 (4°), 125.8 (CH), 125.7 (4°), 125.3, 122.2, 122.2, 121.9, 121.2 (5 x CH), 115.1 (4°), 109.5 (CH), 103.5 (CH 3-naphthyl), 67.3 (O-CH_2), 44.9 (N-CH_2 propyl), 39.7 (N-CH_2 primary amine), 26.1, 24.7, 22.9 (3 x CH_2), 12.6 (CH_3 indole), 11.4 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{27}H_{30}N_2O_2$ 415.2380 found *m/z* 415.2405.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 9.73 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ +0.06% TFA over 5 min, R_t = 0.69 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(4-aminohexyloxy)-1-naphthoyl)-indole 78



2-Methyl-*N*-propyl-3-(4-(*tert*-butyl 6-hexyloxycarbamate)-1-naphthoyl)-indole **73** (200 mg, 0.369 mmol) was dissolved in a mixture of DCM:TFA:H_2O (50:45:5 20 mL) and stirred for 3 h.

The solution was reduced *in vacuo*, dissolved in EtOAc (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (5:94:1 MeOH:DCM:TEA) to yield the desired product (30 mg, 18 %) as a yellow oil.

δ_H ($CDCl_3$) 8.35 (1H, m, naphthyl), 8.17 (1H, m, naphthyl), 7.51 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.44 (2H, m, naphthyl), 7.34-7.28 (2H, indole), 7.16 (1H,

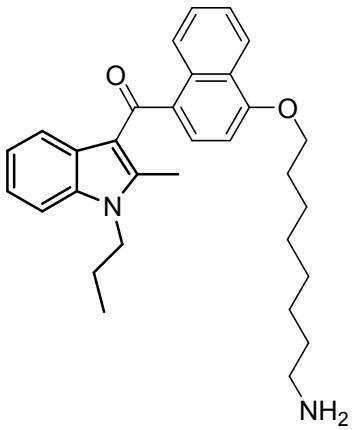
m, indole), 7.01 (1H, m, indole), 6.73 (1H, d, *J* 8.1 Hz, 3-H naphthyl), 4.11 (2H, m, O-CH_2), 4.06 (2H, t, *J* 7.4 Hz, N-CH_2 propyl), 2.83 (2H, m, N-CH_2 primary amine) 2.43 (3H, s, CH_3 indole), 1.88 (2H, m, CH_2 β to ether), 1.81 (2H, m, CH_2 β to propyl amine), 1.63 (2H, m, CH_2 β to primary amine), 1.54 (2H, m, CH_2 γ to ether), 1.39 (2H, m, CH_2 γ to primary amine), 0.97 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.5 (C=O), 156.6, 145.2, 136.1, 132.1, 131.8 (5 x 4°), 128.4 (CH 2-naphthyl), 127.5 (CH), 127.2, 125.8 (4°), 125.6, 125.4, 122.2, 122.2, 121.8, 121.2 (6 x CH), 115.2 (4°), 109.5 (CH), 103.5 (CH 3-naphthyl), 67.9 (O-CH_2), 44.9 (N-CH_2 propyl), 39.8 (N-CH_2 primary amine),28.8, 27.4, 26.1, 25.6, 22.9 (5 x CH_2), 12.5 (CH_3 indole), 11.4 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{29}H_{34}N_2O_2$ 443.2699 found *m/z* 443.2688.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 10.89 min. Symmetry C18 gradient 5 - 95% $MeOH_{(aq)}$ +0.1% formic acid over 20 min, R_t = 14.60 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(4-amino-octyloxy)-1-naphthoyl)-indole 79



2-Methyl-*N*-propyl-3-(4-(8-(tritylamino)octyloxy)-1-naphthoyl)-indole **74** (230 mg, 0.323 mmol) was dissolved in a mixture of DCM:TFA:H_2O (90:9:1 20 mL) and stirred for 1 h.

The solution was reduced *in vacuo*, dissolved in ether (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (18:90:2 MeOH:DCM:TEA) to yield the desired product (94 mg, 62 %) as a yellow oil.

δ_H ($CDCl_3$) 8.37 (1H, m, naphthyl), 8.21 (1H, m, naphthyl), 7.84 (2H, br s, NH_2), 7.54 (1H, d, *J* 8.0 Hz, 2-H naphthyl), 7.48 (2H, m, naphthyl), 7.35-7.30 (2H, indole), 7.18 (1H, m, indole), 7.01 (1H, m, indole), 6.76 (1H, d, *J* 8.1 Hz, 3-H naphthyl), 4.17 (2H, t, *J* 6.2 Hz, O-CH_2), 4.08 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 2.78 (2H, m, N-CH_2 primary amine) 2.46 (3H, s, CH_3 indole), 1.92 (2H, m, CH_2 β to ether), 1.82 (2H, m, CH_2 β to propyl amine), 1.59-1.51 (4H, 2 x CH_2), 1.40-1.23 (6H, 3 x CH_2), 0.99 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

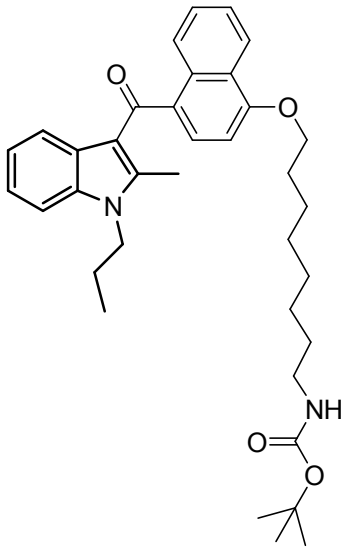
δ_C ($CDCl_3$) 193.6 (C=O), 156.7, 145.3, 136.1, 132.0, 131.8 (5 x 4°), 128.4 (CH 2-naphthyl), 127.6 (CH), 127.3, 125.9 (4°), 125.6, 125.4, 122.3, 122.1, 121.8, 121.2 (6 x CH), 115.2 (4°), 109.5 (CH), 103.5 (CH 3-naphthyl), 68.1 (O-CH_2), 44.9 (N-CH_2 propyl), 39.9 (N-CH_2 primary amine), 28.9, 28.8, 28.7, 27.3, 26.1, 25.9, 22.9 (7 x CH_2), 12.6 (CH_3 indole), 11.4 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{31}H_{38}N_2O_2$ 471.3006 found *m/z* 471.3003.

FTIR (NaCl) 3426, 2932, 2855, 1683, 1623, 1580, 1510, 1462, 1415, 1371, 1326, 1265, 1239, 1204, 1135, 1077, 840, 799, 779, 751, 722 cm-1.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 11.76 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ +0.06% TFA over 5 min, R_t = 0.69 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(4-(*tert*-butyl 8-octyloxycarbamate)-1-naphthoyl)-indole 81



2-Methyl-*N*-propyl-3-(4-(tert-butyl 2-(2-(2-ethoxy)ethoxy)ethylcarbamate)-1-naphthoyl)-indole **79** (100 mg, 0.0236 mmol), sodium bicarbonate (2.0 mg, 0.0238 mmol) and di-*t*butyl dicarbonate (12.4 mg, 0.0568 mmol) were dissolved in water (5 mL) and THF (0.2 mL)

and stirred for 24 h.

The solution was reduced *in vacuo*, dissolved in ether (20 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (1:2 EtOAc:Hexane) to yield the desired product (5 mg, 37 %) as a yellow oil.

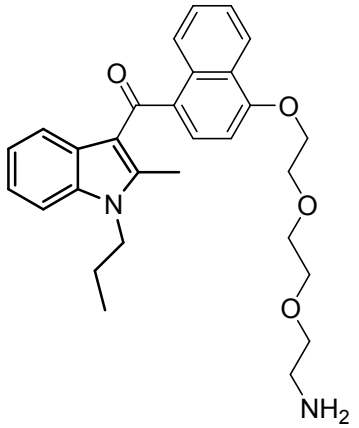
δ_H ($CDCl_3$) 8.38 (1H, m, naphthyl), 8.30 (1H, m, naphthyl), 7.59 (1H, d, *J* 8.0 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.35-7.28 (2H, indole), 7.19 (1H, m, indole), 7.02 (1H, m, indole), 6.89 (1H, d, *J* 8.1 Hz, 3-H naphthyl), 4.52 (1H, br s, NH), 4.22 (2H, t, *J* 6.4 Hz, O-CH_2), 4.13 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 3.14 (2H, m, N-CH_2 carbamate), 2.54 (3H, s, CH_3 indole), 2.01 (2H, m, CH_2 β to ether), 1.87 (2H, m, CH_2 β to propyl amine), 1.64-1.43 (10H, 5 x CH_2), 1.47 (9H, s, 3 x CH_3 *t*Bu), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.2 (C=O), 156.8, 156.0, 144.8, 136.0, 132.1, 132.0 (6 x 4°), 128.7 (CH 2-naphthyl), 127.5 (CH), 127.3, 125.9 (2 x 4°), 125.6, 125.5, 122.2, 122.0, 121.5, 121.3 (6 x CH), 115.3 (4°), 109.4 (CH), 103.3 (CH 3-naphthyl), 79.0 (4° *t*Bu), 68.3 (O-CH_2), 44.9 (N-CH_2 propyl), 40.6 (N-CH_2 carbamate), 30.1, 29.4, 29.3, 29.2 (4 x CH_2), 28.4 (3 x CH_3 *t*Bu), 26.8, 26.2, 23.0 (3 x CH_2), 12.5 (CH_3 indole), 11.5 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{36}H_{46}N_2O_4$ 571.3530 found *m/z* 571.3552.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ over 20 min, R_t = 17.61 min. Monolith C18 gradient 5 – 95 % $MeOH_{(aq)}$ +0.1 % formic acid over 5 min then 95 % $MeOH_{(aq)}$ for 1 min, R_t = 5.65 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(2-(2-(2-ethoxy)ethoxy)ethylamine)-1-naphthoyl)-indole 80



2-Methyl-*N*-propyl-3-(4-(*tert*-butyl 2-(2-(2-ethoxy)ethoxy)ethylcarbamate)-1-naphthoyl)-indole **75** (100 mg, 0.174 mmol) was dissolved in a mixture of DCM:TFA:H_2O (50:45:5 10 mL) and stirred for 2 h.

The solution was reduced *in vacuo*, dissolved in EtOAc (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (5:94:1 MeOH:DCM:TEA) to yield the desired product (24 mg, 29 %) as a yellow oil.

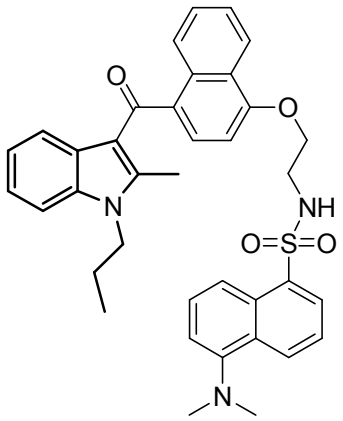
δ_H ($CDCl_3$) 8.37 (1H, m, naphthyl), 8.24 (1H, m, naphthyl), 7.54 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.50 (2H, m, naphthyl), 7.35-7.27 (2H, m, indole), 7.19 (1H, m, indole), 7.02 (1H, m, indole), 6.81 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 4.39 (2H, m, CH_2), 4.08 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 4.04 (2H, m, CH_2), 3.83 (2H, m, CH_2), 3.70 (2H, m, CH_2), 3.59 (2H, t, *J* 5.1 Hz, CH_2), 2.93 (2H, t, *J* 5.1 Hz, CH_2), 2.50 (3H, s, CH_3 indole), 1.85 (2H, m, CH_2 β to propyl amine), 1.01 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

δ_C ($CDCl_3$) 193.3 (C=O), 156.2, 145.1, 136.1, 132.7, 131.8 (5 x 4°), 128.1 (CH 2-naphthyl), 127.6 (CH), 127.3, 125.8 (4°), 125.7, 125.5, 122.2, 122.1, 121.7, 121.2 (6 x CH), 115.2 (4°), 109.5 (CH), 103.8 (CH 3-naphthyl), 70.9, 70.7, 70.2, 69.7, 67.9 (5 x CH_2), 44.9 (N-CH_2 propyl), 40.7 (CH_2), 23.0 (CH_2 β to propyl amine), 12.5 (CH_3 indole), 11.5 ($CH_{3\ propyl}$).

EP-MS MH+ calcd for $C_{29}H_{34}N_2O_4$ 475.2591 found *m/z* 475.2585.

Analytical RP-HPLC Vydac C8 gradient 20 - 100% $MeCN_{(aq)}$ +0.06% TFA over 20 min, R_t = 9.60 min. Monolith C18 gradient 60 - 95% $MeOH_{(aq)}$ over 5 min, R_t = 0.54 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(2-(5-(dimethylamino)naphthalene-1-sulfonamide)ethoxy)-1-naphthoyl)-indole 82



2-Methyl-*N*-propyl-3-(4-(4-aminoethoxy)-1-naphthoyl)-indole **76** (2.2 mg, 5.87 μmol), dansyl chloride (1.7 mg, 6.30 μmol) and triethylamine (0.6 mg, 5.94 μmol) were dissolved in DCM (2 mL) and the solution stirred for 20 h protected from light.

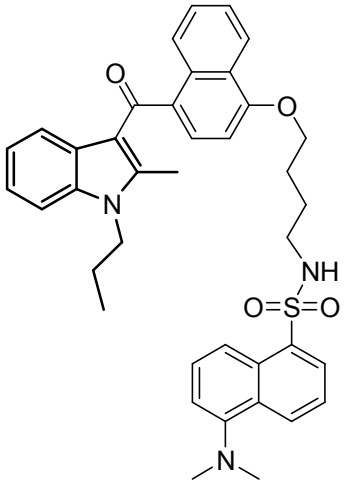
The solution was diluted in DCM (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (40:60 EtOAc:Hexane) to yield the desired product (1.6 mg, 44 %) as a yellow oil.

δ_H ($CDCl_3$) 8.37 (2H, m, dansyl), 8.25 (1H, m, naphthyl), 8.00 (1H, m, naphthyl), 7.61 (1H, m, dansyl), 7.55-7.41 (5H, 2 x CH naphthyl, 2 x CH indole, 1 x CH dansyl), 7.33 (1H, m, naphthyl), 7.22-7.17 (3H, 2 x CH dansyl and 1 x CH indole), 7.01 (1H, m, indole), 6.48 (1H, m, naphthyl), 5.35 (1H, m, NH), 4.13 (2H, t, *J* 7.4 Hz, N-CH_2 propyl), 4.04 (2H, m, O-CH_2), 3.53 (2H, m, N-CH_2 sulphonamide), 2.96 (6H, br s, $N(CH_3)_2$), 2.52 (3H, s, CH_3 indole), 1.86 (2H, m, CH_2 β to propyl amine), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{37}H_{37}N_3O_4S$ 620.2578 found *m/z* 620.2587.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 13.00 min. Monolith C18 gradient 80 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 1.13 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(4-(5-(dimethylamino)naphthalene-1-sulfonamide)butoxy)-1-naphthoyl)-indole 83



2-Methyl-*N*-propyl-3-(4-(4-aminobutoxy)-1-naphthoyl)-indole **77** (2.2 mg, 5.30 μmol), dansyl chloride (1.2 mg, 4.44 μmol) and triethylamine (0.9 mg, 8.91 μmol) were dissolved in DCM (2 mL) and the solution stirred for 20 h protected from light.

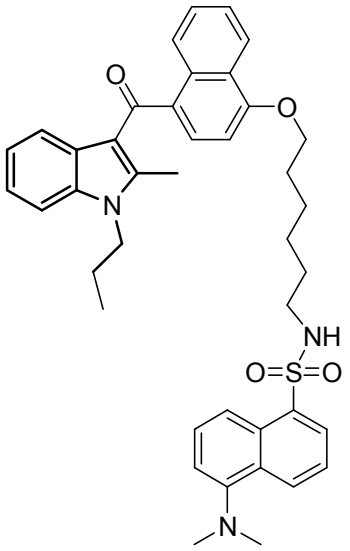
The solution was diluted in DCM (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (25:75 Ether:Petroleum Ether 40/60) to yield the desired product (1.0 mg, 29 %) as a yellow oil.

δ_H ($CDCl_3$) 8.56 (1H, m, naphthyl), 8.35-8.23 (4H, 3 x CH dansyl and 1 x CH naphthyl), 7.61-7.47 (5H, 2 x CH naphthyl, 2 x CH indole, 1 x CH dansyl), 7.33 (1H, m, naphthyl), 7.29-7.17 (3H, 2 x CH dansyl and 1 x CH indole), 7.03 (1H, m, indole), 6.69 (1H, m, naphthyl), 4.70 (1H, m, NH), 4.13 (2H, t, *J* 7.4 Hz, N-CH_2 propyl), 4.07 (2H, m, O-CH_2), 3.07 (2H, m, N-CH_2 sulphonamide), 2.90 (6H, br s, N(CH_3)$_2$), 2.53 (3H, s, CH_3 indole), 1.92-1.82 (4H, 2 x CH_2), 1.74 (2H, m, CH_2), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{39}H_{41}N_3O_4S$ 648.2891 found *m/z* 648.2905.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 14.06 min. Monolith C18 gradient 80 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 1.38 min. Found not to be > 98 % pure.

Preparation of 2-methyl-*N*-propyl-3-(4-(6-(5-(dimethylamino)naphthalene-1-sulfonamide)hexyloxy)-1-naphthoyl)-indole 84



2-Methyl-*N*-propyl-3-(4-(4-aminohexyloxy)-1-naphthoyl)-indole **78** (11.7 mg, 26.5 μmol), dansyl chloride (8.0 mg, 29.1 μmol) and triethylamine (5.3 mg, 52.5 μmol) were dissolved in DCM (2 mL) and the solution stirred for 3 h protected from light.

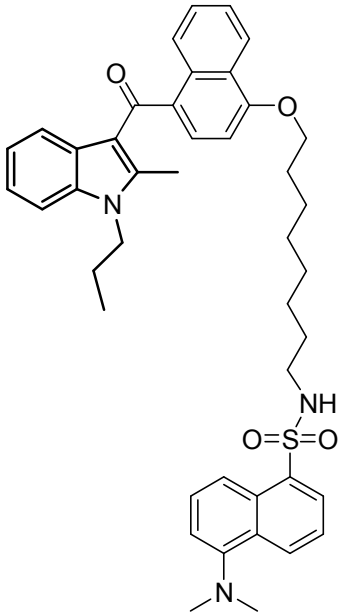
The solution was diluted in DCM (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (25:75 Ether:Petroleum Ether 40/60) to yield the desired product (8.2 mg, 46 %) as a yellow oil.

δ_H ($CDCl_3$) 8.58 (1H, m, naphthyl), 8.34-8.29 (4H, 3 x CH dansyl and 1 x CH naphthyl), 7.61-7.48 (5H, 2 x CH naphthyl, 2 x CH indole, 1 x CH dansyl), 7.34 (1H, m, naphthyl), 7.28-7.16 (3H, 2 x CH dansyl and 1 x CH indole), 7.03 (1H, m, indole), 6.69 (1H, m, naphthyl), 4.56 (1H, m, NH), 4.15-4.08 (4H, N-CH_2 propyl and O-CH_2), 2.93 (2H, m, N-CH_2 sulphonamide), 2.90 (6H, br s, $N(CH_3)_2$), 2.54 (3H, s, CH_3 indole), 1.89-1.78 (4H, 2 x CH_2), 1.51-1.40 (4H, 2 x CH_2), 1.31 (2H, m, CH_2), 1.02 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{41}H_{45}N_3O_4S$ 676.3204 found *m/z* 676.3243.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 15.29 min. Monolith C18 gradient 80 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 1.87 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(8-(5-(dimethylamino)naphthalene-1-sulfonamide)octyloxy)-1-naphthoyl)-indole 85



2-Methyl-*N*-propyl-3-(4-(4-aminoctyloxy)-1-naphthoyl)-indole **79** (2.1 mg, 4.50 μmol), dansyl chloride (1.4 mg, 5.19 μmol) and triethylamine (0.9 mg, 8.91 μmol) were dissolved in DCM (1 mL) and the solution stirred for 16 h protected from light.

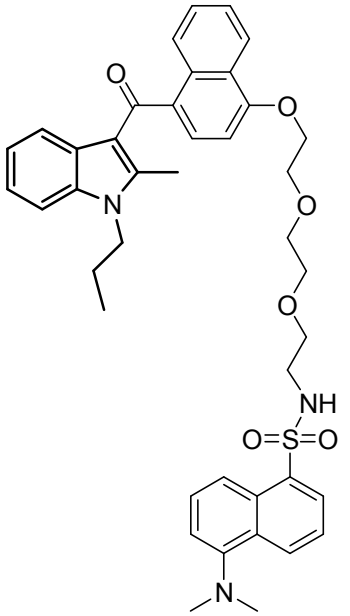
The solution was diluted in EtOAc (10 mL) and washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 EtOAc:Hexane) to yield the desired product (1.3 mg, 41 %) as a yellow oil.

δ_H ($CDCl_3$) 8.56 (1H, m, naphthyl), 8.35-8.27 (4H, 3 x CH dansyl and 1 x CH naphthyl), 7.62-7.48 (5H, 2 x CH naphthyl, 2 x CH indole, 1 x CH dansyl), 7.37-7.28 (3H, 2 x CH dansyl and 1 x CH naphthyl), 7.21(1H, m, indole), 7.03 (1H, m, indole), 6.78 (1H, d, *J* 8.0 Hz, naphthyl), 4.54 (1H, m, NH), 4.18 (2H, m, N-CH_2 propyl), 4.14 (2H, m, O-CH_2), 1.91 (2H, m, N-CH_2 sulphonamide), 2.90 (6H, br s, N(CH_3)$_2$), 2.55 (3H, s, CH_3 indole), 1.95-1.84 (4H, 2 x CH_2), 1.51 (2H, m, CH_2), 1.39 (2H, m, CH_2), 1.29 (2H, m, CH_2), 1.22-1.18 (4H, 2 x CH_2) 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{43}H_{49}N_3O_4S$ 704.3517 found *m/z* 704.3492.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 16.54 min. Monolith C18 gradient 80 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 2.57 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(2-(2-(2-ethoxy)ethoxy)ethyl(5-(dimethylamino)naphthalene-1-sulfonamide))-1-naphthoyl)-indole 86



2-Methyl-*N*-propyl-3-(4-(2-(2-(2-ethoxy)ethoxy)ethylamine)-1-naphthoyl)-indole **80** (2.2 mg, 4.67 μmol), dansyl chloride (1.4 mg, 5.19 μmol) and triethylamine (0.5 mg, 4.95 μmol) were dissolved in DCM (2 mL) and the solution stirred for 20 h protected from light.

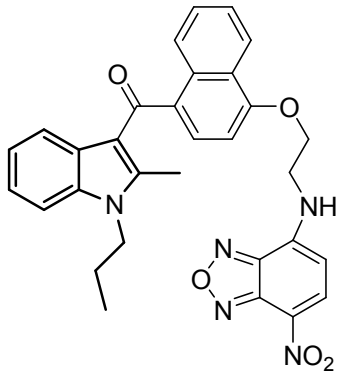
The solution was diluted in DCM (10 mL) and washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 EtOAc:Hexane) to yield the desired product (1.4 mg, 42 %) as a yellow oil.

δ_H ($CDCl_3$) 8.38-8.36 (2H, 1 x CH naphthyl and 1 x CH dansyl), 8.31-8.25 (3H, 3 x CH dansyl and 1 x CH naphthyl), 7.58-7.48 (5H, 2 x CH naphthyl, 2 x CH indole, 1 x CH dansyl), 7.34 (1H, m, 1 x CH naphthyl), 7.28-7.16 (3H, 2 x CH dansyl and 1 x CH indole), 7.00 (1H, m, indole), 6.82 (1H, m, naphthyl), 5.51 (1H, m, NH), 4.40 (2H, m, CH_2), 4.14 (2H, *J* 7.4 Hz, N-CH_2 propyl), 4.07 (2H, m, CH_2), 3.72 (2H, m, CH_2), 3.51 (2H, m, CH_2), 3.46 (2H, m, CH_2), 3.07 (6H, br s, $N(CH_3)_2$), 2.54 (3H, s, CH_3 indole), 1.86 (2H, m, CH_2 β to propyl amine) 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{41}H_{45}N_3O_6S$ 708.3102 found *m/z* 708.3109.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 13.62 min. Monolith C18 gradient 80 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 1.13 min. Found not to be > 98 % pure.

Preparation of 2-methyl-*N*-propyl-3-(4-(2-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine-ethoxy)-1-naphthoyl)-indole 87



2-Methyl-*N*-propyl-3-(4-(4-aminoethoxy)-1-naphthoyl)-indole **76** (1.5 mg, 4.00 μmol) 4-fluoro-7-nitrobenzofurazan (0.7 mg, 3.82 μmol) and triethylamine (0.4 mg, 3.96 μmol) were dissolved in DCM (2 mL) and the solution stirred for 18 h protected from light.

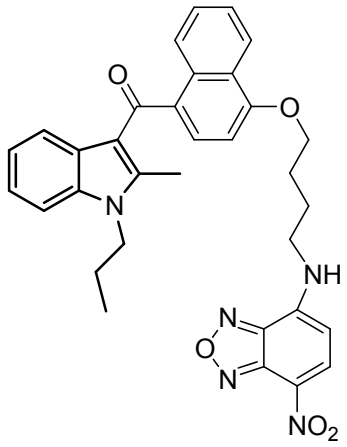
The solution was diluted in DCM (10 mL) and washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 EtOAc:Hexane) to yield the desired product (0.4 mg, 19 %) as an orange oil.

δ_H ($CDCl_3$) 8.57 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 8.26 (2H, m, naphthyl), 7.56 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.52 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.23-7.18 (2H, indole), 7.01 (1H, m, indole), 6.84 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 6.82 (1H, m, NH), 6.41 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 4.57 (2H, m, O-CH_2), 4.16-4.07 (4H, 2 x CH_2), 2.56 (3H, s, CH_3 indole), 1.87 (2H, m, CH_2 β to propyl amine), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{31}H_{27}N_5O_5$ 550.2085 found *m/z* 550.2100.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 14.26 min. Monolith C18 gradient 60 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 3.35 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(4-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine-butoxy)-1-naphthoyl)-indole 88



2-Methyl-*N*-propyl-3-(4-(4-aminobutoxy)-1-naphthoyl)-indole **77** (1.5 mg, 3.65 μmol) 4-fluoro-7-nitrobenzofurazan (0.7 mg, 3.82 μmol) and triethylamine (0.7 mg, 6.93 μmol) were dissolved in DCM (2 mL) and the solution stirred for 20 h protected from light.

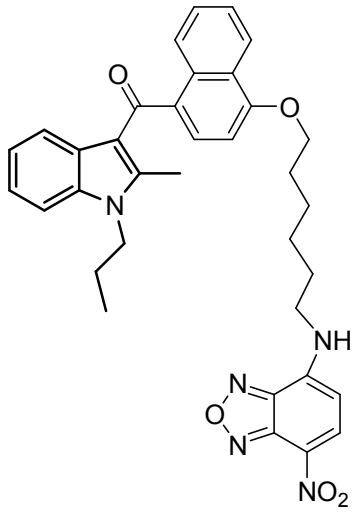
The solution was diluted in EtOAc (10 mL) and washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (60:40 EtOAc:Hexane) to yield the desired product (0.6 mg, 29 %) as an orange oil.

δ_H ($CDCl_3$) 8.49 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 8.33 (1H, m, naphthyl), 8.28 (1H, m, naphthyl), 7.59 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.51 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.24-7.17 (2H, indole), 7.01 (1H, m, indole), 6.81 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 6.39 (1H, m, NH), 6.22 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 4.33 (2H, m, O-CH_2), 4.14 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 3.35 (2H, m, N-CH_2 secondary amine), 2.57 (3H, s, CH_3 indole), 2.22-2.18 (4H, 2 x CH_2), 1.88 (2H, m, CH_2 β to propyl amine), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{33}H_{31}N_5O_5$ 578.2398 found *m/z* 578.2428.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 14.75 min. Monolith C18 gradient 60 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 3.37 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(6-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine-hexyloxy)-1-naphthoyl)-indole 89



2-Methyl-*N*-propyl-3-(4-(4-aminohexyloxy)-1-naphthoyl)-indole **78** (2.1 mg, 4.87 μmol) 4-fluoro-7-nitrobenzofurazan (0.8 mg, 4.37 μmol) and triethylamine (0.5 mg, 4.95 μmol) were dissolved in DCM (2 mL) and the solution stirred for 18 h protected from light.

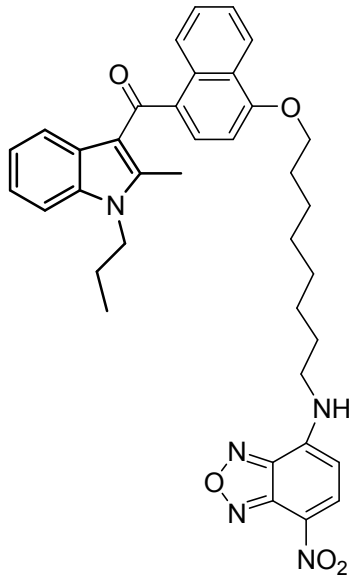
The solution was diluted in DCM (10 mL) and washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 EtOAc:Hexane) to yield the desired product (0.5 mg, 17 %) as an orange oil.

δ_H ($CDCl_3$) 8.51 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 8.37 (1H, m, naphthyl), 8.28 (1H, m, naphthyl), 7.58 (1H, d, *J* 7.9 Hz, 2-H naphthyl), 7.50 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.26-7.17 (2H, indole), 7.01 (1H, m, indole), 6.80 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 6.22 (1H, m, NH), 6.19 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 4.27 (2H, m, O-CH_2), 4.14 (2H, t, *J* 7.5 Hz, N-CH_2 propyl), 3.55 (2H, m, N-CH_2 secondary amine), 2.57 (3H, s, CH_3 indole), 2.03 (2H, m, CH_2), 1.98-1.71 (4H, 2 x CH_2), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{35}H_{35}N_5O_5$ 606.2716 found *m/z* 606.2734.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 16.08 min. Monolith C18 gradient 60 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 3.95 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(8-(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine-octyloxy)-1-naphthoyl)-indole 90



2-Methyl-*N*-propyl-3-(4-(4-aminooctyloxy)-1-naphthoyl)-indole **79** (1.8 mg, 3.85 μmol) 4-fluoro-7-nitrobenzofurazan (0.7 mg, 3.82 μmol) and triethylamine (0.8 mg, 7.92 μmol) were dissolved in DCM (2 mL) and the solution stirred for 20 h protected from light.

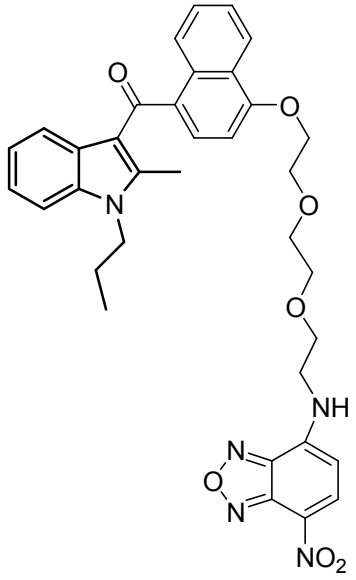
The solution was diluted in EtOAc (20 mL) and washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 EtOAc:Hexane) to yield the desired product (0.5 mg, 21 %) as an orange oil.

δ_H ($CDCl_3$) 8.51 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 8.38 (1H, m, naphthyl), 8.29 (1H, m, naphthyl), 7.59 (1H, d, *J* 8.0 Hz, 2-H naphthyl), 7.52 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.25-7.16 (2H, indole), 7.01 (1H, m, indole), 6.80 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 6.32 (1H, m, NH), 6.18 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 4.25 (2H, m, O-CH_2), 4.15 (2H, m, N-CH_2 propyl), 3.47 (2H, m, N-CH_2 secondary amine), 2.57 (3H, s, CH_3 indole), 2.02 (2H, m, CH_2), 1.86 (2H, m, CH_2), 1.80 (2H, m, CH_2), 1.70-1.46 (8H, 4 x CH_2), 1.03 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{37}H_{39}N_5O_5$ 634.3029 found *m/z* 634.3013.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 17.13 min. Found not to be > 98 % pure.

Preparation of 2-methyl-*N*-propyl-3-(4-(2-(2-(2-ethoxy)ethoxy)ethyl(7-nitro-benzo[1,2,5]oxadiazol-4-yl)-amine)-1-naphthoyl)-indole 91



2-Methyl-*N*-propyl-3-(4-(2-(2-(2-ethoxy)ethoxy)ethylamine)-1-naphthoyl)-indole **80** (2.5 mg, 5.40 μmol) 4-fluoro-7-nitrobenzofurazan (1.0 mg, 5.46 μmol) and triethylamine (0.6 mg, 5.94 μmol) were dissolved in DCM (2 mL) and the solution stirred for 18 h protected from light.

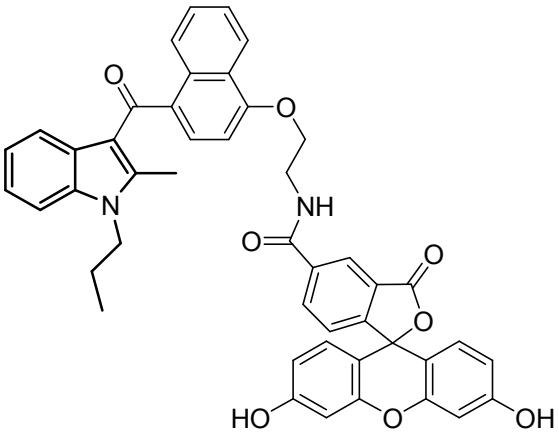
The solution was diluted in DCM (10 mL) and washed with brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 EtOAc:Hexane) to yield the desired product (0.5 mg, 15 %) as an orange oil.

δ_H ($CDCl_3$) 8.43 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 8.35 (1H, m, naphthyl), 8.26 (1H, m, naphthyl), 7.52 (1H, d, *J* 8.0 Hz, 2-H naphthyl), 7.47 (2H, m, naphthyl), 7.34 (1H, m, indole), 7.19 (2H, m, indole), 6.99 (1H, m, indole), 6.88 (1H, m, NH), 6.80 (1H, d, *J* 8.0 Hz, 3-H naphthyl), 6.04 (1H, d, *J* 8.6 Hz, nitrobenzofurazan), 4.48 (2H, m, CH_2), 4.19-4.10 (4H, 2 x CH_2), 3.88 (2H, m, CH_2), 3.85-3.76 (4H, 2 x CH_2), 3.52 (2H, m, CH_2), 2.56 (3H, s, CH_3 indole), 1.87 (2H, m, CH_2 β to propyl amine), 1.01 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{35}H_{35}N_5O_7$ 638.2615 found *m/z* 638.2577.

Analytical RP-HPLC Vydac C8 gradient 20 – 100 % $MeCN_{(aq)}$ + 0.06 % TFA over 20 min, R_t = 14.31 min. Monolith C18 gradient 60 – 95 % $MeOH_{(aq)}$ over 5 min, R_t = 2.88 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(2-(5-carboxamidofluorescein) ethoxy)-1-naphthoyl)-indole 92



2-Methyl-*N*-propyl-3-(4-(4-aminoethoxy)-1-naphthoyl)-indole **76** (1.3 mg, 3.36 μmol), 5-carboxyfluorescein-*N*-succinimdyl ester (1.0 mg, 2.10 μmol) and triethylamine (0.5 mg, 4.95 μmol) were dissolved in DCM (1 mL) and the solution stirred for 3 h protected from light.

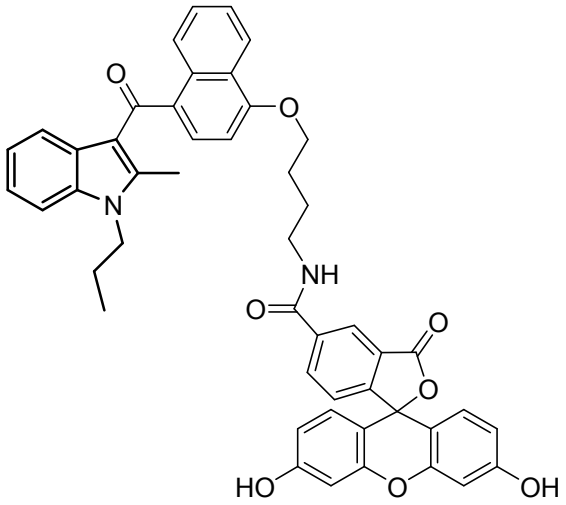
The solution was reduced *in vacuo*, and purification by preparatory layer chromatography (5:94:1 MeOH:DCM:AcOH) yields the desired product (2.3 mg, 92 %) as an orange oil.

δ_H ($CDCl_3$) 9.04 (1H, m, aromatic), 8.53 (1H, m, aromatic), 8.43 (1H, d, *J* 8.1 Hz, aromatic), 8.16 (1H, d, *J* 8.3 Hz, aromatic), 8.00 (1H, m, aromatic), 7.59-7.35 (3H, aromatic), 7.19-7.08 (4H, aromatic), 6.97 (1H, m, aromatic), 6.51 (2H, m, aromatic), 5.99 (2H, m, aromatic), 5.91 (2H, m, aromatic), 4.42 (2H, m, CH_2), 4.20 (2H, m, CH_2), 3.90 (2H, m, CH_2), 2.70 (3H, s, CH_3 indole), 2.39 (2H, CH_2), 0.90 (3H, m, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{46}H_{36}N_2O_8$ 745.2550 found *m/z* 745.2533.

Analytical RP-HPLC Vydac C8 gradient 50 – 100 % $MeCN_{(aq)}$ +0.06 % TFA over 20 min, R_t = 4.98 min. Ace C18 gradient 70 – 100 % $MeOH_{(aq)}$ +0.06 % TFA over 20 min, R_t = 11.24 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(4-(5-carboxamidofluorescein) butoxy)-1-naphthoyl)-indole 93



2-Methyl-*N*-propyl-3-(4-(4-aminobutoxy)-1-naphthoyl)-indole **77** (1.3 mg, 3.23 μmol), 5-carboxyfluorescein-*N*-succinimdyl ester (1.0 mg, 2.10 μmol) and triethylamine (0.5 mg, 4.95 μmol) were dissolved in DCM (1 mL) and the solution stirred for 3 h protected from light.

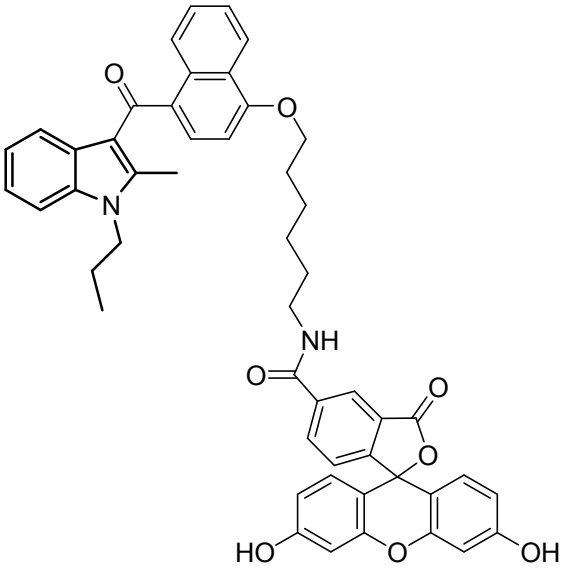
The solution was reduced *in vacuo*, and purification by preparatory layer chromatography (5:94:1 MeOH:DCM:AcOH) yields the desired product (2.4 mg, 96 %) as an orange oil.

δ_H ($CDCl_3$) 8.79 (1H, m, aromatic), 8.52 (1H, m, aromatic), 8.32 (1H, d, *J* 7.4 Hz, aromatic), 8.02 (1H, d, *J* 8.0 Hz, aromatic), 7.87 (1H, d, *J* 6.6 Hz, aromatic), 7.58-7.39 (3H, aromatic), 7.17-7.10 (3H, aromatic), 7.05 (1H, d, *J* 8.1 Hz, aromatic), 6.98 (1H, m, aromatic), 6.52 (2H, d, *J* 9.2 Hz, aromatic), 5.99 (2H, m, aromatic), 5.92 (2H, m, aromatic), 4.32 (2H, m, CH_2), 4.20 (2H, m, CH_2), 2.71 (3H, s, CH_3 indole), 2.04-1.97 (4H, 2 x CH_2), 1.91-1.84 (4H, 2 x CH_2), 0.91 (3H, t, *J* 7.4 Hz, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{48}H_{40}N_2O_8$ 773.2863 found *m/z* 773.2905.

Analytical RP-HPLC Vydac C8 gradient 50 – 100 % $MeCN_{(aq)}$ +0.06 % TFA over 20 min, R_t = 10.02 min. Ace C18 gradient 70 – 100 % $MeOH_{(aq)}$ +0.06 % TFA over 20 min, R_t = 13.04 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(6-(5-carboxamidofluorescein) hexyloxy)-1-naphthoyl)-indole 94



2-Methyl-*N*-propyl-3-(4-(4-aminohexyloxy)-1-naphthoyl)-indole **78** (1.4 mg, 2.52 μmol), 5-carboxyfluorescein-*N*-succinimdyl ester (1.0 mg, 2.10 μmol) and triethylamine (0.5 mg, 4.95 μmol) were dissolved in DCM (1 mL) and the solution stirred for 3 h protected from light.

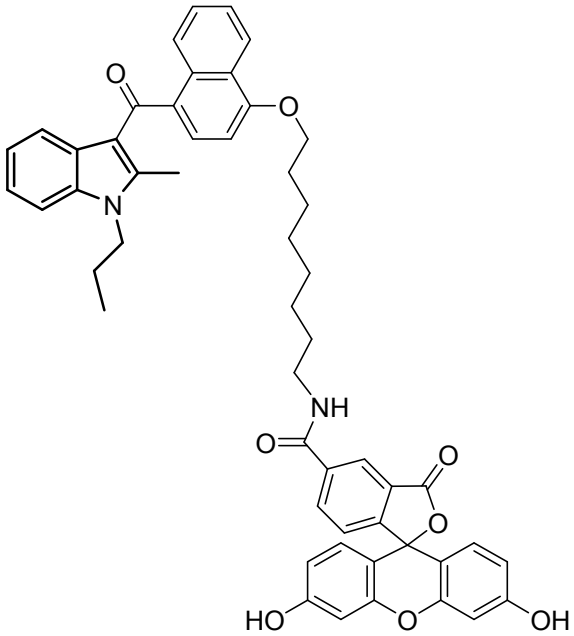
The solution was reduced *in vacuo*, and purification by preparatory layer chromatography (5:94:1 MeOH:DCM:AcOH) yields the desired product (1.9 mg, 94 %) as an orange oil.

δ_H ($CDCl_3$) 8.67 (1H, m, aromatic), 8.47 (1H, m, aromatic), 8.31 (1H, d, *J* 8.0 Hz, aromatic), 8.01 (1H, d, *J* 7.6 Hz, aromatic), 7.84 (1H, m, aromatic), 7.58-7.46 (3H, aromatic), 7.18-7.03 (4H, aromatic), 6.97 (1H, m, aromatic), 6.52 (2H, d, *J* 9.2 Hz, aromatic), 5.98 (2H, m, aromatic), 5.91 (2H, m, aromatic), 4.27 (2H, m, CH_2), 4.20 (2H, m, CH_2), 4.11 (2H, m, CH_2), 2.70 (3H, s, CH_3 indole), 2.02-1.85 (4H, 2 x CH_2), 1.80-1.69 (4H, 2 x CH_2), 1.45 (2H, CH_2) 0.92 (3H, m, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{50}H_{44}N_2O_8$ 801.3176 found *m/z* 801.3210.

Analytical RP-HPLC Vydac C8 gradient 50 – 100 % $MeCN_{(aq)}$ +0.06 % TFA over 20 min, R_t = 7.22 min. Ace C18 gradient 70 – 100 % $MeOH_{(aq)}$ +0.06 % TFA over 20 min, R_t = 15.25 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(8-(5-carboxamidofluorescein) octyloxy)-1-naphthoyl)-indole 95



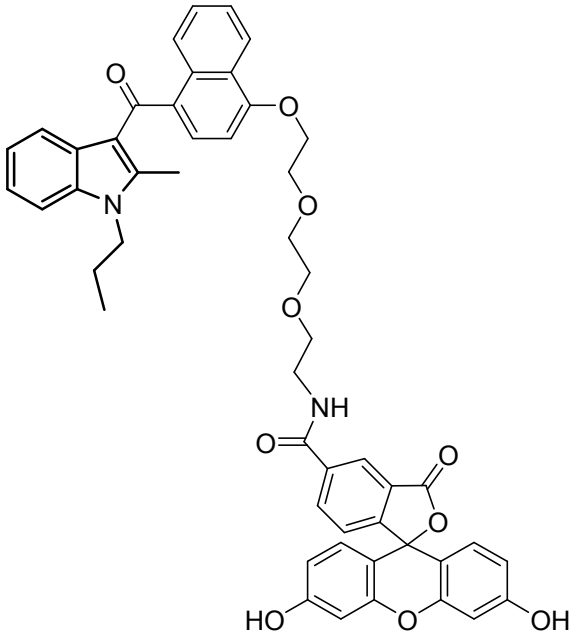
2-Methyl-*N*-propyl-3-(4-(4-aminooctyloxy)-1-naphthoyl)-indole **79** (1.5 mg, 3.18 μmol), 5-carboxyfluorescein-*N*-succinimdyl ester (1.0 mg, 2.10 μmol) and triethylamine (0.5 mg, 4.95 μmol) were dissolved in DCM (1 mL) and the solution stirred for 3 h protected from light.

The solution was reduced *in vacuo*, and purification by preparatory layer chromatography (5:94:1 MeOH:DCM:AcOH) yields the desired product (2.1 mg, 80 %) as an orange oil.

EP-MS MH+ calcd for $C_{52}H_{48}N_2O_8$ 829.3489 found *m/z* 829.3427.

Analytical RP-HPLC Vydac C8 gradient 50 – 100 % $MeCN_{(aq)}$ +0.06 % TFA over 20 min, R_t = 8.62 min. Ace C18 gradient 70 – 100 % $MeOH_{(aq)}$ +0.06 % TFA over 20 min, R_t = 17.62 min.

Preparation of 2-methyl-*N*-propyl-3-(4-(2-(2-(2-ethoxy)ethoxy)ethyl(5-carboxamidofluorescein))-1-naphthoyl)-indole 96



2-Methyl-*N*-propyl-3-(4-(2-(2-(2-ethoxy)ethoxy)ethylamine)-1-naphthoyl)-indole **80** (1.5 mg, 3.16 μmol), 5-carboxyfluorescein-*N*-succinimdyl ester (1.0 mg, 2.10 μmol) and triethylamine (0.5 mg, 4.95 μmol) were dissolved in DCM (1 mL) and the solution stirred for 3 h protected from light.

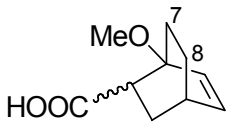
The solution was reduced *in vacuo*, and purification by preparatory layer chromatography (5:94:1 MeOH:DCM:AcOH) yields the desired product (2.4 mg, 91 %) as an orange oil.

δ_H ($CDCl_3$) 8.48 (1H, m, aromatic), 8.00(1H, m, aromatic), 8.04-7.97 (3H, aromatic), 7.49-7.40 (4H, aromatic), 7.18-7.12 (3H, aromatic), 6.98 (1H, m, aromatic), 6.93 (1H, m, aromatic), 6.61 (2H, m, aromatic), 6.67 (2H, m, aromatic), 6.54 (2H, m, aromatic), 4.42 (2H, m, CH_2), 4.20 (2H, m, CH_2), 4.11 (2H, m, CH_2), 3.98 (2H, m, CH_2), 3.79-3.75 (4H, 2 x CH_2), 3.66 (2H, m, CH_2), 2.88 (3H, s, OCH_3), 1.73 (2H, m, CH_2), 1.00 (3H, m, CH_3 N-propyl).

EP-MS MH+ calcd for $C_{50}H_{44}N_2O_{10}$ 833.3074 found *m/z* 833.3109.

Analytical RP-HPLC Vydac C8 gradient 50 – 100 % $MeCN_{(aq)}$ +0.06 % TFA over 20 min, R_t = 4.92 min. Ace C18 gradient 70 – 100 % $MeOH_{(aq)}$ +0.06 % TFA over 20 min, R_t = 11.02 min.

Preparation of 1-methoxybicyclo[2.2.2]-oct-5-ene-2-carboxylic acid rac-133



Methyl 1-methoxybicyclo[2.2.2]-oct-5-ene-2-carboxylate (18.0 g, 91.7 mmol) was added to a solution of potassium hydroxide (16.8 g, 299 mmol) in MeOH (100 mL), and the reaction mixture was refluxed for 15 h.

The reaction was quenched with water (100 mL) and reduced *in vacuo* to yield a light brown solid. This was dissolved in water (400 mL), washed with DCM, acidified with conc. HCl to pH 1, and extracted with DCM (3 x 100 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was recrystallised from 2,2,4-trimethylpentane to yield the desired product (14.6 g, 87 %) as off white crystals.

δ_H ($CDCl_3$)* 6.38-6.31 (3H, exo 5-H, exo 6-H and endo 5-H), 6.22 (1H, dd, *J* 8.9 and 0.7Hz, endo 6-H), 3.52 (3H, s, endo -OCH_3), 3.51 (3H, s, exo -OCH_3), 2.76 (1H, ddd, *J* 9.9, 4.0 and 0.5Hz, endo 2-H), 2.65-2.60 (2H, exo 2-H and endo 4-H), 2.58 (1H, m, exo 4-H), 2.22 (1H, ddd, *J* 13.0, 4.4 and 2.5Hz, exo 1 x 3-H), 2.03 (1H, app dq, *J* 13.2 and 3.6Hz, endo 1 x 3-H), 1.88-1.52 (8H, exo 1 x 3-H, exo 7-H, exo 1 x 8-H, endo 1 x 3-H, endo 7-H and endo 1 x 8-H), 1.46-1.36 (2H, exo 1 x 8-H and endo 1 x 8-H). Endo:Exo ratio found to be 23:10.

δ_C ($CDCl_3$)* 175.7 (exo COOH), 175.2 (endo COOH), 135.6 (CH, C-5), 135.5 (CH, C-5), 132.4 (CH, exo C-6), 129.9 (CH, endo C-6), 79.7 (4˚, C-1), 79.7 (4˚, C-1), 51.6 (CH_3, -OCH_3), 51.4 (CH_3, -OCH_3), 47.0 (CH, C-2), 45.9 (CH, C-2), 30.2, 29.9 (2 x CH_2), 29.3, 29.1 (2 x CH, exo C-4 and endo C-4), 27.2, 26.1, 25.2, 24.7 (4 x CH_2).

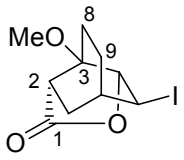
*Note: each proton and carbon signal is duplicated, as both the exo and endo diastereoisomers are present.

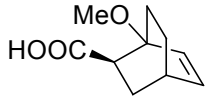
EP-MS M- calcd for $C_{10}H_{14}O_3$ 181.0870 found *m/z* 181.0874.

FTIR (KBr) 2951, 2871, 2832, 2747, 2663, 1705 (C=O), 1424, 1378, 1350, 1321, 1230, 1197, 1178, 1122, 1091, 1017, 954, 687 cm^{-1}.

m.p. 80-89 °C lit. 77-81 °C[166, 168].

Preparation of l-methoxy-5-iodo-9-keto-l0-oxatricyclo[2.2.2.2]-decane rac-135 and recovery of unreacted 1-methoxybicyclo[2.2.2]-oct-5-ene-2-[exo]carboxylic acid rac-134





1-Methoxybicyclo[2.2.2]-oct-5-ene-2-carboxylic acid **rac-133** (16.8 g, 92.3 mmol) was dissolved in a solution of sodium bicarbonte (20.0 g, 238 mmol) and sodium hydroxide (1.44 g, 36.0 mmol) in water (300 mL). This was added to a solution of iodine (28.2 g, 111 mmol) and potassium iodide (30.1 g, 181 mmol) in water (200 mL), and stirred with the exclusion of light for 18 h.

The reaction mixture was quenched with DCM (200 mL), stirred for 45 min, and 20% sodium thiosulphate solution (50 mL) was added to facilitate extraction. The layers were separated and the aqueous layer extracted with DCM (3 x 100 mL). The combined organics were washed with 20% sodium $thiosulphate_{(aq)}$ (2 x 100 mL), sat. $NaHCO_3$ solution (100 mL) and brine (150 mL), dried over $MgSO_4$ and reduced *in vacuo*. The crude product was recrystallised from *i*PrOH to yield the desired product **rac-135** (17.6 g, 89 %)* as white crystals.

δ_H ($CDCl_3$) 4.95 (1H, d, *J* 1.5Hz, 4-H), 4.44 (1H, dd, *J* 2.0 and 3.6Hz, 5-H), 3.25 (3H, s, $-OCH_3$), 2.58 (1H, app dt, *J* 10.7 and 1.5 Hz, 2-H), 2.41(1H, m, 1 x CH), 2.26 (1H, ddd, *J* 15.3, 10.8. 3.2Hz, 1 x 7-H), 2.13-2.04 (3H, 6-H, 1 x 7-H and 1 x 9-H), 1.99 (1H, m, 1 x CH), 1.83 (1H, m, 1 x CH).

δ_C ($CDCl_3$) 178.2 (4°, C-1), 86.0 (CH, C-4), 80.1 (4°, C-3), 50.6 (CH_3, -OCH_3), 40.6 (CH, C-2), 32.0 (CH, C-6), 29.1 (CH_2), 29.0 (CH, C-5), 26.6, 17.9 (2 x CH_2).

EP-MS MH+ calcd for $C_{10}H_{13}IO_3$ 309.0 found *m/z* 309.0.

FTIR (KBr) 2940, 2873, 2835, 1777 (C=O), 1450, 1339, 1362, 1314, 1195, 1145, 1102, 1083, 1018, 990, 975, 959, 926, 907, 722, 660 cm^{-1}.

m.p. 129 °C lit. 125-126 °C[166, 168].

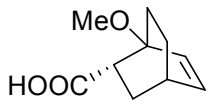
The aqueous layers from the reaction were acidified with conc. HCl to pH 1, and extracted with DCM (3 x 100 mL). The combined organics were washed with 20% aq sodium thiosulphate solution (3 x 100 mL), water (200 mL) and brine (250 mL), dried over $MgSO_4$ and reduced *in vacuo* to yield the desired product **rac-134** (4.19 g, 58 %)* as a yellow solid.

δ_H ($CDCl_3$) 6.37-6.32 (2H, 2 x CH alkene), 3.51 (3H, s, -OCH_3), 2.63 (1H, ddd, *J* 11.4, 4.6 and 3.0Hz, 2-H), 2.57 (1H, m, 4-H), 2.18 (1H, m, 1 x 3-H), 1.85-1.53 (4H, 1 x 3-H, 2 x 7-H and 1 x 8-H), 1.41 (1H, m, 1 x 8-H).

EP-MS M- calcd for $C_{10}H_{14}O_3$ 181.0870 found *m/z* 181.0874.

*Note: theoretical yield calculated from the endo:exo ratio of 23:10 as determined with the preparation of 1-methoxybicyclo[2.2.2]-oct-5-ene-2-carboxylic acid **rac-133** above.

Preparation of 1-methoxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid rac-136



Iodolactone **rac-135** (13.3 g, 43.1 mmol) and zinc dust (4.23 g, 65.1 mmol) were suspended in EtOH (250 mL) and the reaction mixture was stirred and refluxed under an inert atmosphere for 3 h.

The reaction mixture was cooled, filtered through a Celite 521 plug with EtOH washings, and reduced *in vacuo* to a solid which was dissolved in DCM (50 mL) and 2M $HCl_{(aq)}$ (50 mL). The layers were separated and aqueous layer extracted with DCM (2 x 25 mL). The combined organics were extracted with 15% $NaOH_{(aq)}$ (3 x 25 mL), and the combined aqueous layers were acidified to pH 1 with conc. HCl and extracted with DCM (3 x 40 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was recrystallised from petroleum ether 100/120 to yield the desired product (5.43 g, 69 %) as white crystals.

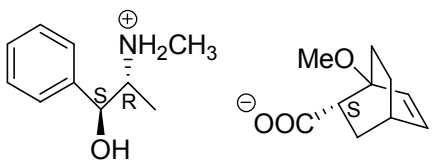
δ_H ($CDCl_3$) 6.35 (1H, dd, *J* 8.9 and 6.5Hz, 5-H), 6.22 (1H, dd, *J* 8.9 and 0.7Hz, 6-H), 3.53 (3H, s, -OCH_3), 2.75 (1H, ddd, *J* 9.9, 3.7 and 0.5Hz, 2-H), 2.63 (1H, m, 4-H), 2.07 (1H, app dq, *J* 13.2 and 3.6Hz, 1 x 3-H), 1.83 (1H, m, 1 x 3-H), 1.79-1.67 (2H, 1x 7-H and 1x 8-H), 1.56 (1H, m, 1 x 7-H), 1.41 (1H, m, 1 x 8-H).

δ_C ($CDCl_3$) 174.2 (COOH), 136.0 (CH, C-5), 129.5 (CH, C-6), 79.9 (4˚, C-1), 51.5 (CH_3, -OCH_3), 47.1 (CH, C-2), 29.4 (CH_2), 29.3 (CH, C-4), 26.9, 26.3 (2 x CH_2).

EP-MS M- calcd for $C_{10}H_{14}O_3$ 181.0870 found *m/z* 181.0850.

m.p. 82-83 °C lit. 80-82 °C[166, 168].

Preparation of (+)-ephedrine salt of (2S,4S)-(-)-1-methoxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylate 137



1-Methoxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid **rac-136** (3.20 g, 17.6 mmol) and (+)-ephedrine* (4.50 g, 27.2 mmol) were dissolved in EtOAc (25 mL) at reflux under an inert atmosphere. The solution was allowed to cool to room temperature and to crystallise at 4 ºC for 18 h.

The crystals were filtered and recrystallisation from EtOAc to yield the desired product (1.85 g, 60 %) as white crystals.

*Note: (+)-ephedrine freshly prepared from (+)-ephedrine hydrochloride.

$[\alpha]_D$ +15.3˚ [23.1 ˚C, MeOH] lit. +13.2˚ and +14.3˚ [MeOH][166, 168].*

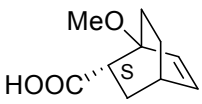
δ_H ($CDCl_3$) 7.38-7.22 (5H, aromatic), 6.83 (3H, br s, NH_2^+ and OH), 6.30-6.22 (2H, alkene), 5.12 (1H, m, 1-H ephedrine), 3.40 (3H, s, -OCH_3), 3.01 (1H, m, 2-H ephedrine), 2.77 (1H, m, 2-H acid), 2.62 (3H, s, NH_2^+-CH_3), 2.55 (1H, m, 4-H acid), 1.90 (1H, m, 1 x 3-H acid), 1.73 (1H, m, 1 x 3-H acid), 1.68-1.34 (4H, 7-H and 8-H acid), 0.98 (3H, d, *J* 6.6 Hz, 3-H ephedrine).

δ_C ($CDCl_3$) 179.3 (4˚, COO^-), 141.0 (4˚, aromatic), 133.1 (CH, alkene), 132.0 (CH, alkene), 128.2 (CH, aromatic), 127.1 (CH, aromatic), 126.0 (CH, aromatic), 79.6 (4˚, C-1 acid), 71.4, 61.1 (2 x CH, ephedrine), 51.0 (CH_3, -OCH_3), 48.7 (CH, C-2 acid), 32.5 (CH_3, N-CH_3 ephedrine), 32.2 (CH_2), 29.5 (CH, C-4 acid), 28.3, 25.3 (2 x CH_2), 11.2 (CH_3, C-3 ephedrine).

m.p. 131 °C lit. 135-136 °C[166, 168].

*Literature sources do not quote temperature for $[\alpha]_D$ results.

Preparation of (2S,4S)-(-)-1-methoxy-bicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid 138



(+)-Ephedrine salt of (-)-1-methoxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid **137** (1.75 g, 5.04 mmol) was partitioned between DCM (40 mL) and 2 M $HCl_{(aq)}$ (20 mL) and stirred for 3 h.

The layers were separated and the aqueous layer was extracted with DCM (2 x 20 mL). The combined organic layers were washed with 2M $HCl_{(aq)}$, water and

brine, dried over $MgSO_4$ and reduced *in vacuo* to yield the desired product (870 mg, 95 %) as a white solid. The crude product was used without any further purification.

$[\alpha]_D$ -7.3˚ [21.9 ˚C, MeOH]
$[\alpha]_D$ -20.7˚ [22.4 ˚C, DCM] lit. -26.3˚ and -25.0˚ [DCM][166, 168].*

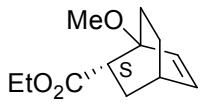
δ_H ($CDCl_3$) 6.35 (1H, dd, *J* 8.8 and 6.6Hz, 5-H), 6.22 (1H, d, *J* 8.8Hz. 6-H), 3.50 (3H, s, $-OCH_3$), 2.77 (1H, dd, *J* 9.9 and 4.1, 2-H), 2.62 (1H, m, 4-H), 1.99 (1H, m, 1 x 3-H), 1.84 (1H, m, 1 x 3-H), 1.76-1.66 (2H, 1 x 7-H and 1 x 8-H), 1.57 (1H, m, 1 x 7-H), 1.42 (1H, m, 1 x 8-H).

δ_C ($CDCl_3$) 178.0 (COOH), 135.5 (CH, C-5), 130.0 (CH, C-6), 79.7 (4˚, C-1), 51.4 (CH_3, $-OCH_3$), 47.0 (CH, C-2), 29.9 (CH_2), 29.2 (CH, C-4), 27.2 26.0 (2 x CH_2).

EP-MS M- calcd for $C_{10}H_{14}O_3$ 181.1 found *m/z* 181.1.

*Literature sources do not quote temperature for $[\alpha]_D$ results.

Preparation of (2S,4S)-(-)-ethyl 1-methoxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylate 139



(-)-1-Methoxy-bicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylic acid **138** (0.870 g, 4.77 mmol) and para-toluenesulfonic acid (95.0 mg, 0.500 mmol) were dissolved in EtOH (10 mL) and refluxed under an inert atmosphere for 21 h.

The reaction was cooled, reduced *in vacuo*, partitioned between water (15 mL) and DCM (15 mL) and separated. The aqueous layer was further extracted with DCM (2 x 15 mL) and the combined organic layers were washed with sat. $NaHCO_{3(aq)}$, 1 M $HCl_{(aq)}$ and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (10:90

EtOAc:Hexane) to yield the desired product (0.572 g, 57 %) as a colourless oil.

$[\alpha]_D$ of -3.97˚ [22.6 ˚C, MeOH]
$[\alpha]_D$ of -3.08˚ [21.8 ˚C, $CHCl_3$] lit. -5.1˚ [$CHCl_3$][166].*

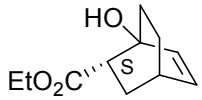
δ_H ($CDCl_3$) 6.28 (1H, dd, *J* 8.7 and 6.3Hz, 5-H), 6.23 (1H, d, *J* 8.7 Hz, 6-H), 4.11 (2H, m, -OCH_2), 3.38 (3H, s, -OCH_3), 2.89 (1H, dd, *J* 9.9 and 5.5 Hz, 2-H), 2.57 (1H, m, 4-H), 1.90 (1H, m, 1 x 3-H), 1.69-1.37 (5H, 1 x 3-H, 7-H and 8-H), 1.24 (3H, t, *J* 7.1Hz, CH_3 β to ester)

δ_C ($CDCl_3$) 174.6 (4˚, ester), 132.8 (CH, C-5), 132.4 (CH, C-6), 79.0 (4˚, C-1), 60.4 (CH_2, -OCH_2), 50.8 (CH_3, -OCH_3), 46.3 (CH, C-2), 33.0 (CH_2, C-3), 29.3 (CH, C-4), 28.5, 24.5 (2 x CH_2), 14.2 (CH_3, CH_3 β to ester)

EP-MS MH+ calcd for $C_{12}H_{18}O_3$ 211.1334 found *m/z* 211.1351.

*Literature source does not quote temperature for $[\alpha]_D$ results.

Preparation of (+)-(2S,4S)-(-)-ethyl 1-hydroxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylate 140



(-) Ethyl 1-methoxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylate **139** (1.00 g, 4.76 mmol) was dissolved in DCM (30 mL) and cooled to -25˚C. 1 M boron tribromide in DCM (5.30 mmol) was added dropwise over 10 min, and the reaction mixture was stirred at -25˚C for 1 h.

The reaction was quenched with sat. $NaHCO_{3(aq)}$ (20 mL) and stirred for 45 min. The layers were separated and the aqueous layer was extracted with DCM (2 x 20 mL). The combined organic layers were washed with water and brine, dried over $MgSO_4$, reduced *in vacuo* to yield the desired product (567 mg,

61 %) as a yellow oil. The crude product was used without any further purification.

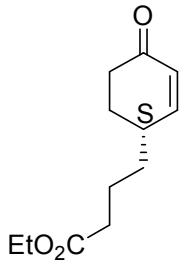
$[\alpha]_D$ of +14.9˚ [21.4 ˚C, MeOH]

δ_H ($CDCl_3$) 6.20-6.18 (2H, alkene), 4.14 (2H, m, -OCH_2), 2.69 (1H, dd, *J* 10.6 and 4.9 Hz, 2-H), 2.56 (1H, m, 4-H), 2.04 (1H, m, 1 x 3-H), 1.70-1.34 (5H, 1 x 3-H, 7-H and 8-H), 1.25 (3H, t, J 7.1Hz, CH_3 β to ester).

δ_C ($CDCl_3$) 175.4 (4˚,ester), 136.9 (CH, C-5), 132.5 (CH, C-6), 72.9 (4˚, C-1), 60.8 (CH_2, -OCH_2), 48.8 (CH, C-2), 32.1 (CH_2, C-3), 31.4 (CH, C-4), 29.6, 25.6 (2 x CH_2), 14.2 (CH_3, CH_3 β to ester).

EP-MS MH+ calcd for $C_{11}H_{16}O_3$ 197.1178 found *m/z* 197.1166.

Preparation of (S)-(+)-ethyl 3-[4(2-cyclohexenone)] propionate 112



To a solution of (+)-ethyl 1-hydroxybicyclo[2.2.2]-oct-5-ene-2-[endo]carboxylate **140** (134 mg, 0.694 mmol) in tBuOH (10 mL) at 35 ˚C, was added potassium *t*butoxide (8.0 mg, 0.071mmol) and the solution stirred at 35 ˚C for 1 h.

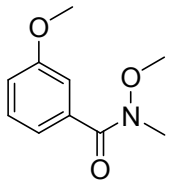
The reaction was quenched with water (10 mL) and EtOAc (10 mL), stirred for 10 min, layers separated and the aqueous layer extracted with EtOAc (15 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (50:50 Ether:Petroleum Ether 40/60) to yield the desired product (103 mg, 78 %) as a yellow oil.

δ_H ($CDCl_3$) 6.83 (1H, ddd, *J* 10.2, 2.7 and 1.4 Hz, 3-H), 6.01 (1H, ddd, J 10.7, 2.5 and 0.4 Hz, 2-H), (2H, q, *J* 7.1Hz, -OCH_2), 2.56-2.32 (5H), 2.13 (1H, m), 1.95-1.64 (3H), 1.27 (3H, t, *J* 7.1 Hz, CH_3).

δ_C ($CDCl_3$) 199.5, 173.0 (2 x 4°, 2 x C=O), 153.7 (CH, C-3), 129.5 (CH, C-2), 60.6 (CH_2, OCH_2), 36.8 (CH_2), 35.3 (CH, C-4), 31.6, 29.5, 28.3 (3 x CH_2), 14.2 (CH_3).

EP-MS MH+ calcd for $C_{11}H_{16}O_3$ 197.1178 found *m/z* 197.1163.

Preparation of (3-methoxyphenyl)-*N*-methoxy-*N*-methylcarboxamide 144



3-methoxybenzoyl chloride (4.99 g, 22,92 mmol) and N,O-dimethylhydroxylamine hydrochloride (3.43 g, 35.2 mmol) was suspended in DCM (150 mL). The solution was cooled to 0˚C, triethylamine (9.8 mL, 70.3 mmol) was added, and the solution was stirred for 20 h.

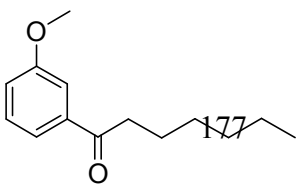
The solution was reduced *in vacuo*, and partitioned between water (100 mL) and DCM (100 mL), the layers were separated and the aqueous layer extracted with DCM (2 x 50 mL). The combined organic layers were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (2:1 Ether:Toluene) to yield the desired product (3.69 g, 65 %) as a colourless oil.

δ_H ($CDCl_3$) 7.31 (1H, app t, *J* 7.9 Hz, 5-H), 7.24 (1H, app dt, *J* 7.6 and 1.2Hz, aromatic), 7.20 (1H, dd, *J* 2.4 and 1.5Hz, 2-H), 6.98 (1H, ddd, *J* 8.2, 1.6 and 1.1Hz, aromatic), 3.83 (3H, s, $PhOCH_3$), 3.58 (3H, s,CH_3), 3.35 (3H, s, CH_3).

δ_C ($CDCl_3$) 169.7 (C=O), 159.2, 135.4 (2 x 4°, aromatic), 129.1 (CH, C-5), 120.4, 116.6 (2 x CH), 113.3 (CH, C-2), 61.1 (CH_3, $PhOCH_3$), 55.4, 33.88 (2 x CH_3).

EP-MS MH+ calcd for $C_{10}H_{13}NO_3$ 196.0974 found *m/z* 196.0985.

Preparation of 1-(3-methoxyphenyl)heptan-1-one 145



Mg turnings (620 mg, 24.6 mmol) and iodine (trace amount) were suspended in THF (25 mL) and 1-bromohexane (2.63 ml, 18.4 mmol) was added dropwise over 30 min, and stirred for 60 min. (3-methoxyphenyl)-*N*-methoxy-*N*-methylcarboxamide **144** (1.20 g, 6.15 mmol) was dissolved in THF (10 mL) under an inert atmosphere, added to the reaction mixture and the solution was stirred for 1 h.

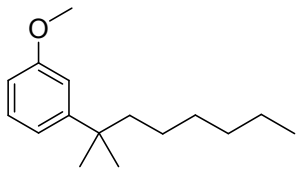
The reaction was quenched with 2 M $HCl_{(aq)}$ (10 mL), stirred for 10 min, partitioned between petroleum ether 40/60 (20 mL) and water (20 mL), the layers were separated and the aqueous layer extracted with petroleum ether 40/60 (2 x 20 mL). The combined organic layers were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (1:9 EtOAc:Hexane) to yield the desired product (1.25 g, 92 %) as a colourless oil.

δ_H ($CDCl_3$) 7.54 (1H, ddd, *J* 7.7, 1.2 and 1.2 Hz, aromatic), 7.49 (1H, dd, *J* 2.5 and 1.6 Hz, 2-H), 7.36 (1H, app t, *J* 7.9 Hz, 5-H), 7.10 (1H, ddd, *J* 8.2, 2.7 and 0.9 Hz, aromatic), 3.85 (3H, s, OCH_3), 2.95 (2H, t, *J* 7.4Hz, CH_2 α to carbonyl), 1.73 (2H, m, CH_2 β to carbonyl), 1.43-1.25 (6H, 3 x CH_2), 0.89 (3H, m, CH_3).

δ_C ($CDCl_3$) 200.4 (C=O), 159.8, 138.5 (2 x 4°, aromatic), 129.5 (CH, C-5), 120.7, 119.3 (2 x CH), 112.3 (CH, C-2), 55.4 (CH_3, OCH_3), 38.8 (CH_2, CH_2 α to carbonyl), 31.7, 29.1 (2 x CH_2), 24.4 (CH_2, CH_2 β to carbonyl), 22.6 (CH_2), 14.1 (CH_3).

EP-MS MH+ calcd for $C_{14}H_{20}O_2$ 221.1542 found *m/z* 221.1529.

Preparation of 1-(1,1-dimethylheptyl)-3-methoxybenzene 146



To a solution of 1 M titanium tetrachloride (31.5

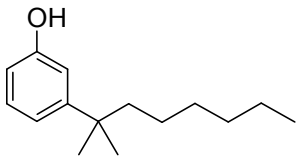
mmol) in DCM (80 mL) under an inert atmosphere at -30 ºC was added dropwise 2 M dimethyl zinc (31.5 mmol) and stirred fro 30 min. The solution was cooled to 45 ºC and a solution of 1-(3-methoxyphenyl)heptan-1-one **145** (2.89 g, 13.1 mmol) in DCM (20 mL) was added dropwise under an inert atmosphere. The solution was slowly warmed to room temperature and the reaction stirred for 24 h.

The reaction was quenched in sat $NaHCO_{3(aq)}$ (100 mL), acidified to pH 1 with 2 M $HCl_{(aq)}$, the layers separated and the aqueous layer extracted with DCM (2 x 50 mL). The combined organic layers were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (10:90 Ether:Hexane) to yield the desired product (3.00 g, 98 %) as a brown oil.

δ_H ($CDCl_3$) 7.25 (1H, app t, *J* 8.0 Hz, 4-H), 6.95 (1H, ddd, *J* 7.8, 1.7 and 0.9 Hz, aromatic), 6.90 (1H, app t, *J* 2.2 Hz, 2-H), 6.74 (1H, ddd, *J* 8.1, 2.5 and 0.7 Hz, aromatic), 3.83 (3H, s, OCH_3), 1.59 (2H, m, CH_2 α to dimethyl), 1.30 (6H, s, 2 x CH_3, 1,1-dimethyl)1.29-1.01 (6H, 3 x CH_2), 0.84 (3H, m, terminal CH_3).

δ_C ($CDCl_3$) 159.3, 151.7 (2 x 4°, aromatic), 128.8 (CH, C-5), 118.5, 112.6, 109.7 (3 x CH), 55.1 (CH_3, OCH_3), 44.6 (CH_2 α to dimethyl), 37.8 (4°), 31.7, 30.0 (2 x CH_2), 29.0 (2 x CH_3, 1,1-dimethyl), 24.4, 22.7 (2 x CH_2), 14.1 (terminal CH_3).

Preparation of 1-(1,1-dimethylheptyl)-3-hydroxybenzene 147



1-(1,1-dimethylheptyl)-3-methoxybenzene **146** (800 mg, 3.42 mmol) was dissolved in DCM (10 mL) and cooled to -30 ˚C. 1 M boron tribromide in DCM (6.84 mmol) was added dropwise over 15 min, and the reaction mixture was stirred at for 22 h.

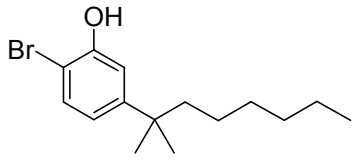
The reaction was quenched with water (20 mL) and stirred for 30 min. The layers were separated and the aqueous layer was extracted with DCM (2 x 10 mL). The combined organic layers were washed with water and brine, dried over $MgSO_4$, and reduced *in vacuo*. The crude product was purified by flash column chromatography (20:80 EtOAc:Hexane) to yield the desired product (578 mg, 77 %) as a brown oil.

δ_H ($CDCl_3$) 7.19 (1H, app t, *J* 7.9 Hz, 4-H), 6.93 (1H, ddd, *J* 7.9, 1.7 and 0.9 Hz, aromatic), 6.84 (1H, app t, *J* 2.1 Hz, 2-H), 6.67 (1H, ddd, *J* 8.0, 2.8 and 0.8 Hz, aromatic), 4.92 (1H, br s, OH) 1.59 (2H, m, CH_2 α to dimethyl), 1.30 (6H, s, 2 x CH_3, 1,1-dimethyl), 1.30-1.03 (6H, 3 x CH_2), 0.87 (3H, m, terminal CH_3).

δ_C ($CDCl_3$) 155.2, 152.1 (2 x 4°, aromatic), 129.1 (CH, C-5), 118.5, 113.1, 112.1 (3 x CH), 44.6 (CH_2 α to dimethyl), 37.7(4°), 31.8, 30.0 (2 x CH_2), 29.0 (2 x CH_3, 1,1-dimethyl), 24.7, 22.7 (2 x CH_2), 14.1 (terminal CH_3).

EP-MS M- calcd for $C_{15}H_{24}O_1$ 219.1754 found *m/z* 219.1758.

1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene 148



To a solution of 1-(1,1-dimethylheptyl)-3-hydroxybenzene **147** (922 mg, 4.51 mmol) in DCM (5 mL) was added dropwise bromine (230 μL, 4.51 mmol) and the solution stirred for 22 h.

The solution was washed with 20% sodium thiosulphate$_{(aq)}$, sat. $NaHCO_{3(aq)}$ and brine, dried over $MgSO_4$, and reduced *in vacuo*. The crude product was purified by flash column chromatography (2:98 EtOAc:Hexane) to yield the desired product (1.11 g, 83 %) as a clear oil.

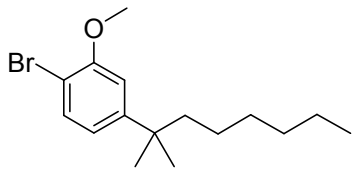
δ_H ($CDCl_3$) 7.37 (1H, d, *J* 8.5 Hz, 6-H), 7.02 (1H, d, *J* 2.3 Hz, 3-H), 6.80 (1H, dd, *J* 8.5 and 2.3 Hz, 5-H), 5.44 (1H, br s, OH), 1.57 (2H, m, CH_2 α to

dimethyl), 1.27 (6H, s, 2 x CH_3, 1,1-dimethyl), 1.26-1.03 (6H, 3 x CH_2), 0.86 (3H, m, terminal CH_3).

δ_C ($CDCl_3$) 151.9, 151.7 (2 x 4° aromatic), 131.2, 119.8, 114.0 (3 x CH), 106.7 (4°, C-1), 44.4 (CH_2 α to dimethyl), 37.7 (4º), 31.8, 30.0 (2 x CH_2), 28.9 (2 x CH_3, 1,1-dimethyl), 24.6, 22.7 (2 x CH_2), 14.1 (terminal CH_3).

EP-MS M- calcd for $C_{15}H_{23}Br_1O_1$ 297.0854 and 299. 0834 found *m/z* 297.0848 and 299.0812.

1-bromo-4-(1,1-dimethylheptyl)-2-methoxybenzene 149



To a solution of 1-bromo-4-(1,1-dimethylheptyl)-2-hydroxybenzene **148** (110 mg, 0.368 mmol) in DMF (10 mL) was added a methyl iodide (108 mg, 0.761 mmol) and potassium carbonate (158 mg, 1.14 mmol), and the solution stirred for 2 h.

The solution was partitioned between water (10 mL) and DCM (10 mL), layers separated and the aqueous layer extracted with DCM (2 x 10 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by preparatory layer chromatography (5:95 Ether:Petroleum Ether 60/80) to yield the desired product (72 mg, 61 %) as a yellow oil.

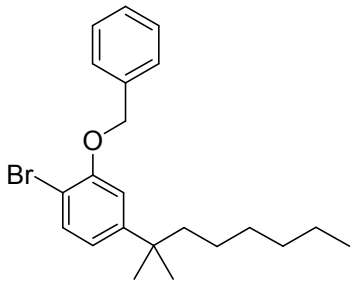
δ_H ($CDCl_3$) 7.44 (1H, d, *J* 8.3 Hz, 6-H), 6.88 (1H, d, *J* 2.1 Hz, 3-H), 6.82 (1H, dd, *J* 8.3 and 2.1 Hz, 5-H), 3.90 (3H, s ,OCH_3), 1.59 (2H, m, CH_2 α to dimethyl), 1.29-1.02 (6H, s, 2 x CH_3, 1,1-dimethyl), 1.30-1.02 (6H, 3 x CH_2), 0.87 (3H, m, terminal CH_3).

δ_C ($CDCl_3$) 155.4, 151.0 (2 x 4° aromatic), 132.5, 119.7, 110.1 (3 x CH), 108.3 (4°, C-1), 56.1 (CH_3, OCH_3) 44.5 (CH_2 α to dimethyl), 38.0 (4°), 31.8, 30.0 (2

x CH_2), 29.0 (2 x CH_3, 1,1-dimethyl), 24.6, 22.7 (2 x CH_2), 14.1 (terminal CH_3).

EP-MS MH+ calcd for $C_{16}H_{26}Br_1O_1$ 313.1167 and 315.1147 found *m/z* 313.1166 and 315.1119.

1-bromo-4-(1,1-dimethylheptyl)-2-benzyloxybenzene 150



Sodium hydride (60% w/w in mineral oil, 100 mg, 2.51 mmol) was suspended in DMF (5 mL), and added to 1-(1,1-dimethylheptyl)-3-hydroxybenzene **148** (500 mg, 1.68 mmol). Benzylbromide (400 mg, 2.34 mmol) was added and the solution was stirred under an inert atmosphere for 24 h.

Excess hydride was reacted with water, diluted with EtOAc (20 mL), and stirred for 5 min. The resulting solution was separated and the aqueous layer extracted with EtOAc (2 x 10 mL). The combined organics were washed with water and brine, dried over $MgSO_4$ and reduced *in vacuo*. The crude product was purified by flash column chromatography (5:95 Ether:Petroleum Ether 60/80) to yield the desired product (592 mg, 91 %) as a yellow oil.

δ_H ($CDCl_3$) 7.50 (2H, m, benzyl), 7.46 (1H, d, *J* 8.3 Hz, 6-H), 7.40 (2H, m, benzyl), 7.33 (1H, m, benzyl), 6.90 (1H, d, *J* 2.1 Hz, 3-H), 6.82 (1H, dd, *J* 8.3 and 2.1 Hz, 5-H), 5.19 (2H, s ,OCH_2), 1.52 (2H, m, CH_2 α to dimethyl), 1.29-0.92 (6H, 3 x CH_2), 1.25 (6H, s, 2 x CH_3, 1,1-dimethyl), 0.87 (3H, t, *J* 7.1 Hz, CH_3).

δ_C ($CDCl_3$) 155.5, 150.8, 136.79 (3 x 4° aromatic), 132.6 (CH, C-6), 128.6, 127.9, 127.2 (3 x CH benzyl), 120.1 (CH, C-5), 112.7.1 (CH, C-3), 109.3 (4°, C-1), 71.0 (CH_2, OCH_2) 44.5 (CH_2 α to dimethyl), 37.9 (4°), 31.8, 30.0 (2 x CH_2), 28.9 (2 x CH_3, 1,1-dimethyl), 24.6, 22.7 (2 x CH_2), 14.1 (terminal CH_3).

EP-MS MH+ calcd for $C_{22}H_{30}Br_1O_1$ 389.1480 and 391.1460 found *m/z* 389.1463 and 391.1451.

6.2 Pharmacology

All compounds for radioligand binding assays were purified by preparative layer chromatography, to greater than 98% purity; this was confirmed using two different analytical reverse phase high performance liquid chromatography methods and accurate mass spectrometry of the product to within 10 parts per million.

Human CB_2 receptor -transfected Chinese hamster ovary (CHO) cells (originally a kind gift from GSK Pharmaceuticals, Harlow, UK) were grown in our own laboratories in Dubelcco's modified Eagle's medium containing 5% foetal calf serum, 2 mM L-glutamine, 600 μg/mL Geneticin®, and 300 μg/mL hygromycin B, in a Sanyo CO_2 incubator (95 % O_2: 5 % CO_2) at 37 °C. Passaging of cells was every 3-4 days, using trypsin to detach the cells, which were then suspended in fresh media. Flasks were grown to confluence and the cells harvested by removing excess media and collecting cells in ice cold phosphate buffer solution. This work was kindly carried out by Marlene Groenen.

Cell disruption was performed using a Polytron hand held electric homogeniser in Tris buffer (50 mM Tris, 5 mM $MgCl_2$, 2nM EDTA, pH 7.0). The homogenate was centrifuged at 30,000g at 4 °C for 10 min, and the pellet was re-suspended in Tris buffer. Homogenisation and centrifugation were repeated twice and the final pellet was resuspended in Tris buffer to a protein concentration of 5 mg/mL. This work was kindly carried out by Professor David Kendall.

Radioligand binding assay tubes were prepared in triplicate containing 0.25 nM [^{3}H] CP55-940 (Perkin-Elmer Life Sciences), approximately 3 μL membrane protein solution and the desired concentration of compound under investigation, diluted in Tris buffer to a final volume of 1 mL (50 mM Tris, 5 mM $MgCl_2$, 2 mM EDTA, plus 0.2 mg/mL bovine serum albumin, pH7.0). Stock drugs and compounds for testing were diluted in the same buffer containing 5 mg/mL bovine serum albumin to improve solubility. Tubes were

incubated for 90 min at 30 °C and then a Brandel cell harvester was used to isolate membranes and hence separate bound from unbound radioligand. The filtered membranes [^{3}H] CP55-940 content was then quantified by liquid scintillation counting. Specific binding was calculated by subtracting non-specific binding (determined in the presence of the CB_2 receptor antagonist SR144528 1 μM), and nonlinear regression analysis was carried out using GraphPad Prism 3 (GraphPad Software). K_i values for the test compounds were determined from IC_{50} values using a [^{3}H] CP55-940 K_D value of 0.5 nM in the Cheng–Prusoff equation:

$K_i = IC_{50}/(1+ C/K_D)$, where C is the concentration of [^{3}H] CP55-940 in each assay.

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