New SPECT Imaging Agents for the Translocator Protein

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A thesis submitted in part fulfilment of the requirements of the degree of Doctor of Philosophy

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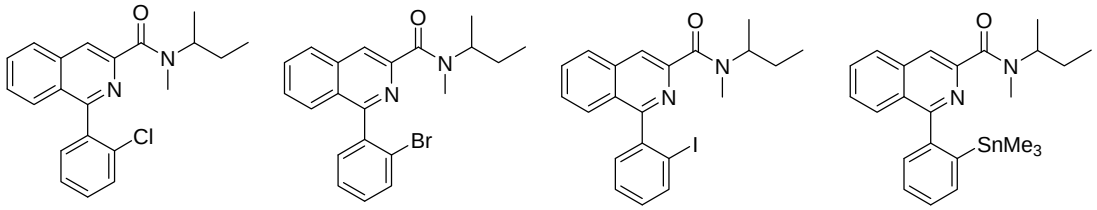
June 2010

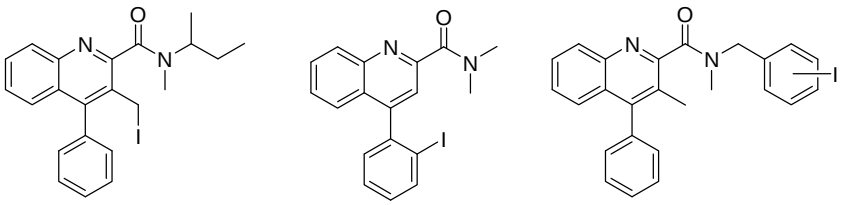
Abstract

During the course of the studies outlined in this thesis, a new short and efficient synthesis of PK11195 was developed using Pd(0)-mediated reactions to effect the key carbon-carbon bond forming reactions. PK11195 is a ligand that interacts with the translocator protein (TSPO) in the brain. During brain injury the concentration of TSPO increases, making TSPO an ideal and sensitive biomarker. Using single photon emission computed tomography along with [^{123}I]PK11195, medical practitioners are able to visualize the receptor. In addition to the synthesis of PK11195, three other analogues were devised using the same methodology as for PK11195. Firstly, a Heck reaction was used to form the isoquinoline core, followed by a Suzuki reaction to couple the correct phenyl ring.



PK11195

However, PK11195 has some limitations regarding biodistrubution, on crossing the blood brain barrier the ligand is not particularly selective and interacts with other receptors. Therefore a new class of TSPO ligands, quinoline-2-carboxamides were developed and tested for their binding affinity to TSPO. Two successful routes were developed to synthesise new iodinated quinoline-2-carboxamides. The first route involved a two step condensation reaction to form the quinoline ring system and the second a chlorotrimethylsilane-mediated Friëdlander condensation reaction.



The final project of this thesis involved developments towards the total synthesis of crispine C.

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Acknowledgements

Firstly, I would like to say a huge thank you to my supervisor, Dr Andrew Sutherland for giving me the opportunity to study in his research group and for his help and support throughout the course of my PhD.

Thank you also goes to our collaborators, Adriana Tavares and Dr Deborah Dewar from the Division of Clinical Neurosciences and Dr Sally Pimlott from the West of Scotland Radionuclide Dispensary.

I would like to thank all the technical staff for their kind assistance throughout: Dr David Adam (NMR spectroscopy), Jim Tweedie (Mass Spectrometry), Stuart Mackay and Ted Easdon.

Thank you also to the past and present members of the Sutherland group who helped make the group a great place to work. In addition, thank you to the past and present members of the Hartley group for making my time in the lab so enjoyable.

Finally I would like to say a big thank you to my husband, Nikolay, my Mum and Dad and all my family for their encouragement and support throughout my time at Glasgow University.

Financial support from the Biotechnology and Biological Sciences Research Council (BBRSC) is also gratefully acknowledged.

Author's Declaration

This thesis represents the original work of Louise Stevenson unless explicitly stated otherwise in the text. The research was carried out at the University of Glasgow in the Loudon Laboratory under the supervision of Dr Andrew Sutherland during the period of October 2006 to March 2010. Portions of the work described herein have been published elsewhere as listed below.

L. Stevenson, S. L. Pimlott and A. Sutherland, *Tetrahedron Lett*, 2007, **48**, 7137.

S. L. Pimlott, L. Stevenson, D. J. Wyper and A. Sutherland, *Nucl. Med. Biol*, 2008, **35**, 537.

L. Stevenson, A. A. S. Tavares, A. Brunet, F. I. McGonagle, D. Dewar, S. L. Pimlott and A. Sutherland, *Bioorg. Med. Chem. Lett*, 2010, **20**, 954.

Abbreviations

aq.	aqueous
Boc	*tert*-butoxycarbonyl
br	broad
°C	degrees Celsius
cat.	catalytic
$CDCl_3$	deuterated chloroform
CI	chemical ionisation
Ci	curie
d	doublet
dd	doublet of doublets
DMSO	dimethyl sulfoxide
dq	doublet of quartets
dt	doublet of triplets
e.e.	enantiomeric excess
E.I.	electron impact
equiv	equivalents
FAB	fast atom bombardment
g	gram(s)
GBq	gigabecquerel
h	hour(s)
HCl	hydrochloric acid
HPLC	High performance liquid chromatography
Hz	hertz
IC_{50}	half maximal inhibitory concentration
IR	infrared
J	NMR spectra coupling constant
KDa	kilodaltons
K_i	inhibition constant
µL	microlitre
L	litre or ligand
lit.	literature
mCi	millicurie
µM	micromolar
M	molar

m	multiplet
mg	milligram
MHz	megahertz
mL	millilitre
mM	millimolar
mol	mole(s)
mp	melting point
NMR	nuclear magnetic resonance
q	quartet
RT	room temperature
s	singlet
SEM	standard error of mean
t	triplet

1 Introduction

1.1 Translocator Protein

The translocator protein 18 kDa (TSPO, formerly known as the peripheral benzodiazepine receptor) was identified in 1977 when investigators were searching for binding sites for the benzodiazepine, diazepam in the rat.[1,2] Benzodiazepines bind with high affinity to receptors concentrated in the central nervous system and these receptors were named $GABA_a$ receptors .[3] During the same investigation Braestrup and Squires discovered that [^{3}H]diazepam bound to sites other than in the central nervous system, and that [^{3}H]diazepam had a high affinity to the peripheral tissues in the body including the brain, leading to the "peripheral benzodiazepine receptors".[4,5]

TSPO is found at high levels on the outer mitochondrial membrane of cells in the peripheral organs such as kidney, heart, lung, adrenal cortex, salivary gland, testis or ovaries[6] as well as at low levels in the brain, primarily associated with the glial cells.[7,8]

1.1.1 Structure and Function

TSPO is an 18 kDa protein consisting of 169 amino-acids.[9] It is highly hydrophobic and rich in tryptophan.[10] The TSPO appears to be a multimeric 140-200 kDa complex[1] with an isoquinoline binding subunit (18 kDa), a 32 kDa voltage-dependent anion channel (VDAC) also called mitochondrial porin and an 30 kDa adenine nucleotide (ANT) carrier in the outer mitochondrial membrane (Figure 1).[9,11]

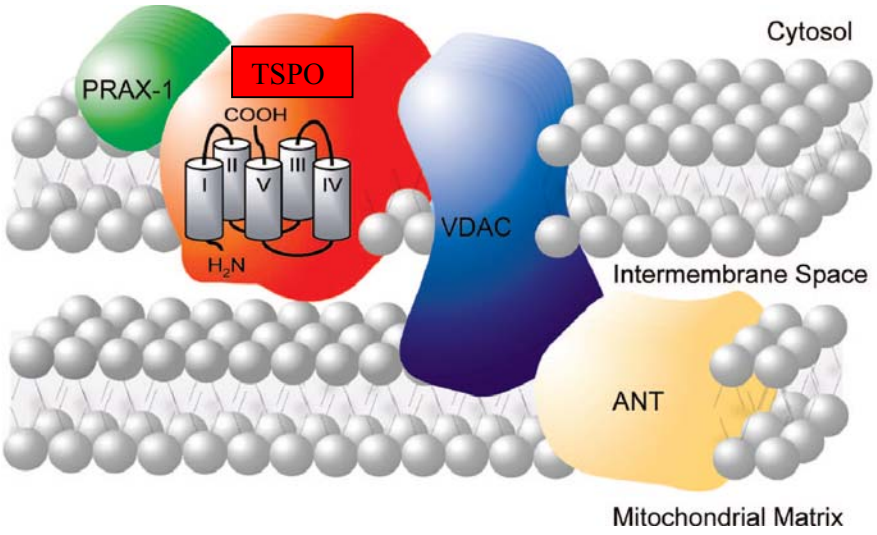


Figure 1: Multimeric Complex[4] Reprinted with permission from *J. Med. Chem* **2009**, 52, 581. Copyright 2009 American Chemical Society.

Decades of study of mammalian TSPO has showed that this protein is involved in many physiological functions, including cholesterol transport,[7,12] steroid hormone synthesis, [7,12,13] cellular proliferation, apoptosis, mitochondrial respiration and chemotaxis.[7] It is also implicated in a number of nervous system disorders such as cerebral ischaemia, epilepsy, nerve injury and neurodegenerative diseases as well as immune system diseases such as cancer.[14]

1.1.2 TSPO and Neuroinflammation

Levels of TSPO are very low in the body under normal conditions and limited to glial cells (astrocytes and microglia).[9] Following brain injury, illness or infection, glial cells in the brain activate in response to the stimuli and increase TSPO receptor density.[15,16] Microglial cells respond to any pathological event, through the production and release of proinflammatory cytokines such as TNF-α, interleukin-1β and nitric oxide (Figure 2). This process in normal brain inflammation allows the brain to respond to changes in its environment and dispose of damaged tissue. However, this useful process sometimes gets out of balance and the neuroinflammatory process persists, which cause the overproduction of these cytokines, making them neurotoxic.[14,17]

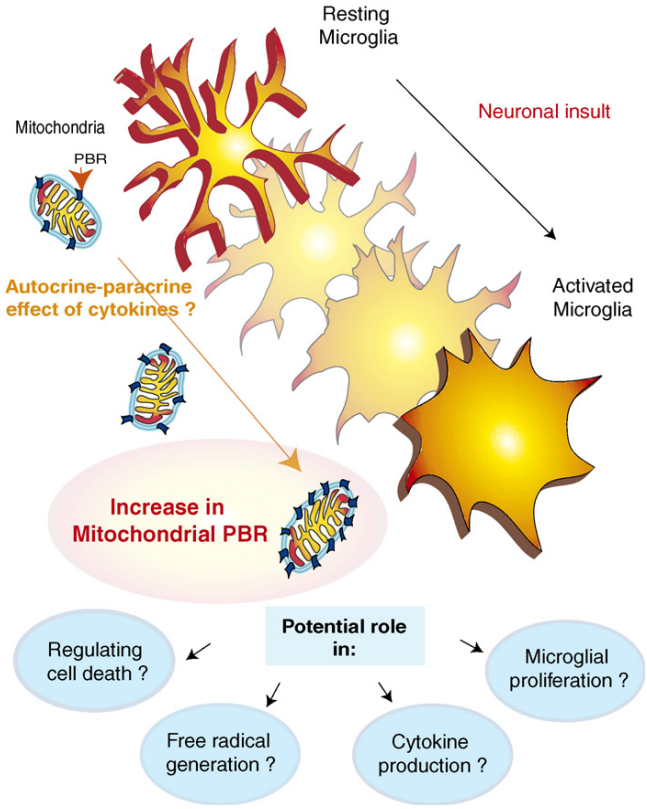


Figure 2: Function and Mechanism of TSPO[18] Reprinted with permission from *Progress in Neurobiology* **2006**, 80, 308. Copyright 2006 Elsevier.

As TSPO concentration increases during brain injury, it makes TSPO an ideal and sensitive biomarker for activated microglia.[9] This change makes it possible to determine the role of neuroinflammation in specific neurological and neurodegenerative conditions.[17] These findings have prompted researchers to develop radioligands for TSPO, which can be used to visualize this receptor.[15] One such ligand devised for interaction with TSPO is PK11195.

1.2 TSPO Ligands

Dr Leo Sternbach synthesised the first benzodiazepine, diazepam and introduced it into clinical medicine in 1961.[3,19] Benzodiazepines are a class of drugs prescribed widely for their pharmacological importance in relieving anxiety and insomnia, in addition to having a sedative and anticonvulsant effect (Figure 3).[20] After discovering that [^{3}H]diazepam binds with relatively high affinity (3 nM) to both $GABA_a$ and TSPO in rat brain, other benzodiazepine derivatives were used to determine the difference between the two receptors. It was found that the 4-chloro derivative of diazepam, Ro5-4864 binds with

high affinity to TSPO in rodents and low affinity to $GABA_a$ and clonazepam binds with high affinity to $GABA_a$ and shows low affinity to TSPO.[4,21]

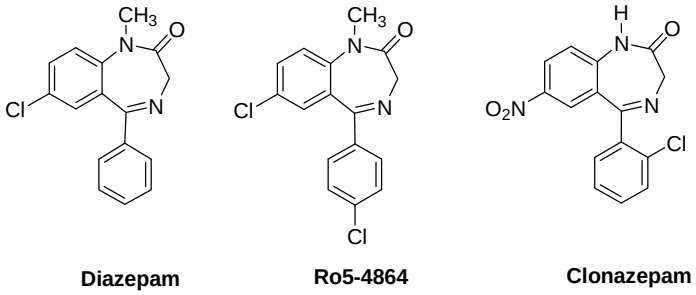


Figure 3: Benzodiazepines

In addition to benzodiazepines, LeFur and co-workers in 1983 detailed a new class of compounds called isoquinoline carboxamides (Figure 4).[21] They are structurally different from benzodiazepines and with a higher selectivity for the TSPO than for $GABA_a$. PK11195, 1-(2-chlorophenyl)isoquinoline-3-*N*-methyl-*N*-*sec*-butylcarboxamide, was the first non-benzodiazepine ligand found to bind to TSPO with nanomolar affinity, and for the last 20 years it is the most commonly used radioligand for this receptor.[22] They distinguished between PK11195 and Ro5-4864, and classified PK11195 as an antagonist and Ro5-4864 as agonist or partial agonist for TSPO.[23]

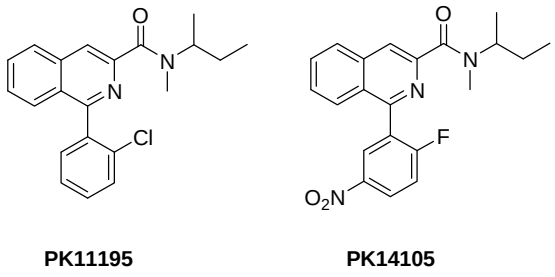


Figure 4: Isoquinoline Carboxamides

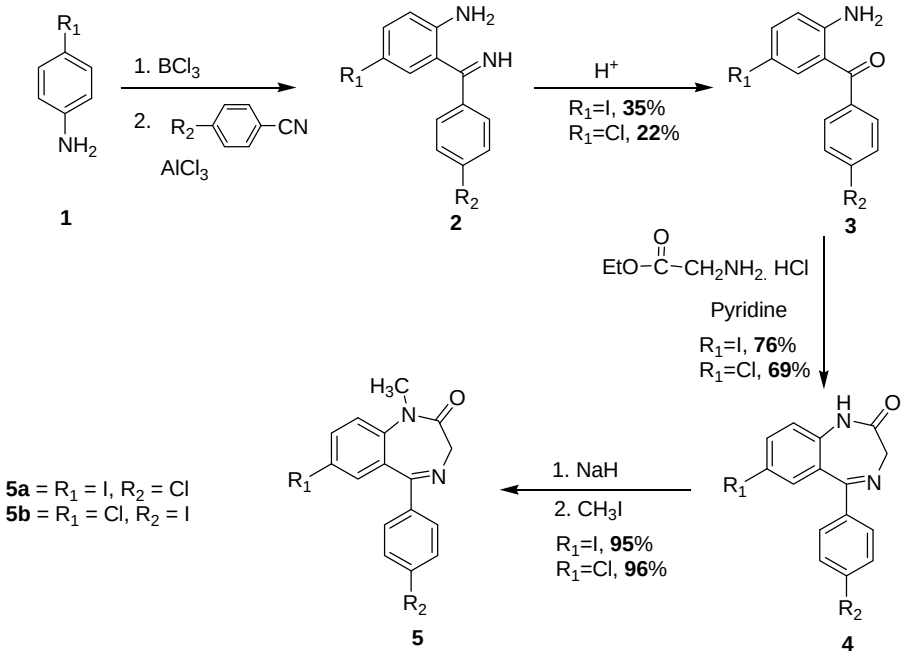
There has been a wide range of ligands developed over the years that all show high affinity to the TSPO binding site. These ligands all share some common features that allow researchers to understand the TSPO binding domain better. The common features include a fused bicyclic aromatic ring system containing two electronegative boundaries and a freely rotating aromatic ring.[20] Furthermore, many ligands also contain a third electronegative group surrounded by lipophilic substituents.[20]

1.2.1 Ro5-4864

As mentioned above Ro5-4864 (7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one) binds to TSPO with micromolar affinity, unlike other benzodiazepines that bind to $GABA_a$. Ro5-4864 is dissimilar from its class in that it has no anxiolytic or anticonvulsant activity.[24] This ligand has been used for many years in various experiments, it is however, not used for *in vivo* studies due to the side effects i.e. convulsant in rodents.[25] Awad and Gavish in 1987 carried out research on the binding of [^{3}H]Ro5-4864 to cerebral cortex and peripheral tissues in various species. They concluded that the interaction of the ligand on TSPO is highly species dependant.[26] For rat kidney and cerebral cortex, [^{3}H]Ro5-4864 showed nanomolar (0.05-30 nM) affinity, but in calf cerebral cortex and kidney, binding is negligible.[20,26] Ro5-4864 binds to all the components in the receptor, VADC, ANT and IBP whereas PK11195 only binds to the IBP component.[27]

Benzodiazepines are bicyclic heterocyclic compounds, with a benzene ring fused to a seven-membered ring containing two nitrogen atoms. Van Dort and co-workers reported a synthesis of radioiodinated Ro5-4864 analogues to image TSPO receptor in tumors outlined in Scheme 1.[28]

The first step involved the condensation of aniline-boron trichloride complex with a benzonitrile analogue to form the ketimine, followed by acid hydrolysis to give 2-aminobenzophenone analogue **3**. Next the seven membered ring was formed by reaction with glycine ethyl ester hydrochloride to form an imine, then cyclisation gave the desired 1,4-benzodiazepine analogue **4**. Finally *N*-methylation using sodium hydride and methyl iodide gave **5**.



Scheme 1: Synthesis of Ro5-4864

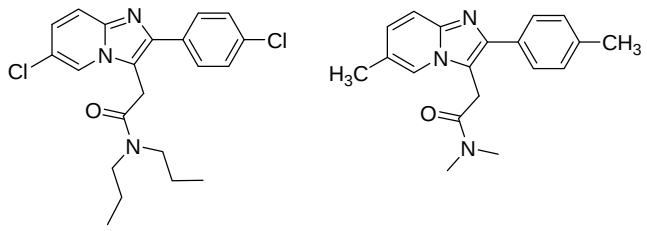
Both compounds **5a** and **5b** were tested for affinity for the $GABA_a$ and TSPO binding sites, Table 1 shows the results. From the data, both **5a** and **5b** show affinity for TSPO, therefore substitution of chlorine for iodine makes only a slight difference to the binding affinity.

Ligand	R_1	R_2	TSPO K_i (nM)	$GABA_a$ K_i (nM)
Ro5-4864	Cl	Cl	7.3 ± 2.3	>1000
5a	I	Cl	13.3 ± 4.8	>1000
5b	Cl	I	13.6 ± 4.5	>1000

Table 1: Binding affinity of Ro5-4864

1.2.2 Imidazopyridines

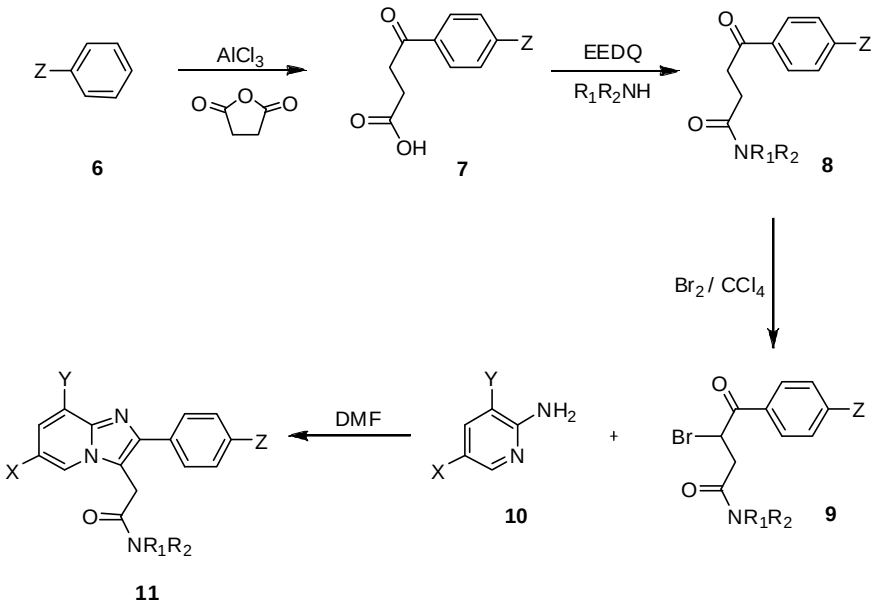
Imidazopyridines, a group of high affinity TSPO ligands, show similar pharmacological action and binding properties to benzodiazepines.[20] They have been shown to have the ability of stimulating pregnenolone formation from mitochondria and bind to both TSPO and $GABA_a$.[21,29] The derivative, alpidem, shows high binding across many species, making it a promising ligand for TSPO in humans.[20]



Alpidem **Zolpidem**

Figure 5: Imidazopyridine

Trapani and co-workers designed new analogues of alpidem, in order to improve the affinity and selectivity to TSPO (Scheme 2).[30,31] Acetamidoimidazo[1,2-*a*]pyridine compounds were prepared by Friedel-Crafts acylation of appropriate aromatic compounds with succinic anhydride to give **7**. Using ethyl 1,2-dihydro-2-ethoxy-1-quinolinecarboxylate (EEDQ) as a dehydrating agent, reaction of **7** with appropriate dialkylamines gave **8**. Bromination in carbon tetrachloride gave the desired bromoketoamides **9**. Lastly condensation of the required substituted 2-aminopyridines **10**, with the bromoketoamides afforded the required acetamidoimidazo[1,2-*a*]pyridines **11**.



Scheme 2: Synthesis of acetamidoimidazo[1,2-*a*]pyridines

Table 2 shows various analogues of alpidem and their overall yields along with the binding affinity to TSPO.

Compound	X	Y	Z	R_1	R_2	Yield (%)	IC_{50} (nM)
Alpidem	Cl	H	Cl	C_3H_7	C_3H_7	23	7.90
11a	Cl	Cl	H	C_4H_9	H	35	6.53
11b	H	H	Cl	C_3H_7	H	15	6.23
11c	H	H	H	C_6H_{13}	C_6H_{13}	15	7.72
11d	H	$NHCOCH_3$	Cl	C_3H_7	C_3H_7	42	8.00
11e	H	$NHCOCH_3$	H	C_3H_7	C_3H_7	40	6.59

Table 2: Binding affinity of alpidem analogues

Trapani and co-workers concluded that 2-phenylimidazo[1,2-*a*]-pyridine derivatives are potent and selective ligands for TSPO and stimulate steroidogenesis in both the brain and periphery.

1.2.3 Pyrazolopyrimidines

The pyrazoloprimidine, DPA-713 (*N*,*N*-diethyl-2-[2-(4-methoxyphenyl)-5,7-dimethylpyrazolo[1,5-*a*]pyrimidin-3-yl]acetamide) is structurally closely related to alpidem (Figure 6). In 2001, Selleri and co-workers decided to synthesise compounds that were related to alpidem.[32] Although, alpidem binds to TSPO (K_i 0.5-7 nM) and $GABA_a$ (K_i 1-28 nM) with nanomolar affinity it was still a good starting point even though selective TSPO binding was required. During the study of alpidem, it was shown that the tertiary acetamide moiety was important for hydrogen bond interactions to TSPO and therefore the group decided to keep this moiety. They also decided to investigate the effect of *para*-substituents (R) on the phenyl ring and the R_1, R_2, and R_3 positions. They selected the same substituents, H, OCH_3, CH_3, and Cl but alternated them around the R positions. One compound to show good binding affinity to TSPO and no affinity for $GABA_a$ was DPA-713 (K_i 4.7 nM).

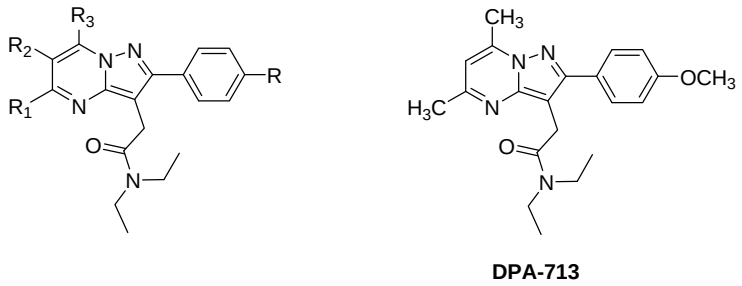
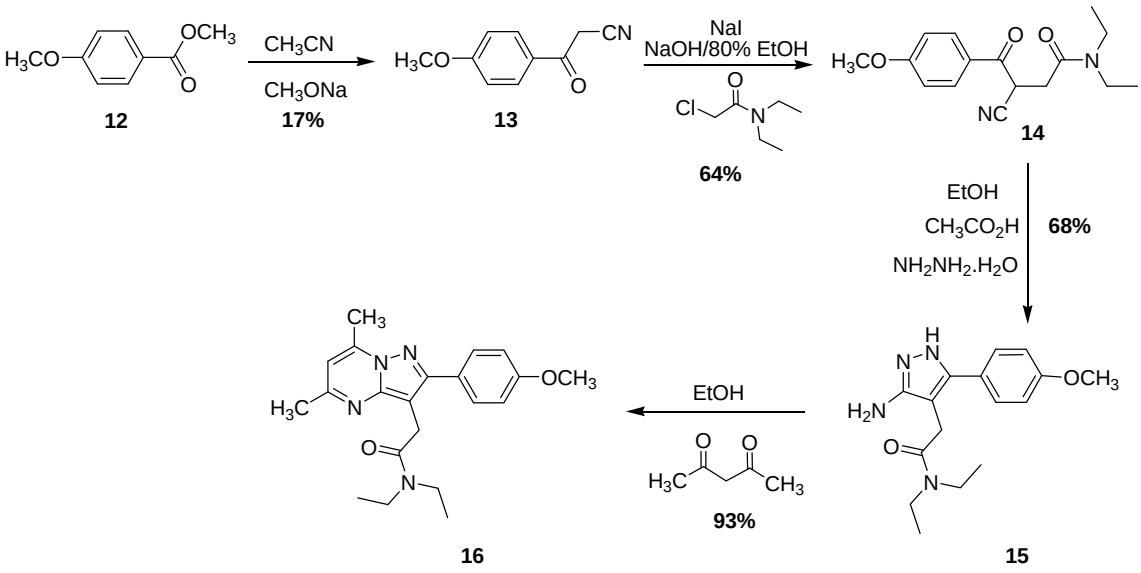


Figure 6: Pyrazolopyrimidines

James and co-workers then radiolabelled DPA-713 with carbon-11 in 2005. They performed *in vivo* studies on baboons using positron emission tomography (PET) and it proved to have potent and selective activity at TSPO.[33] This was the first analogue of its class to be radiolabelled and showed promising evidence of being a good ligand for imaging studies.

James and co-workers reported a synthesis of DPA-713 starting from commercially available methyl 4-methoxybenzoate **12** (Scheme 3).[33] Compound **12** was reacted with sodium methoxide and acetonitrile at 100 °C to give aroylacetonitrile **13** in 17% yield. Next **13** was reacted with *N*,*N*-diethylchloroacetamide and sodium iodide in an alkaline solution to afford **14** in 64% yield. This was followed by the conversion of **14** to the pyrazole **15** by heating under reflux in ethanol with hydrazine hydrate. The final step

involved the condensation of **15** with electrophilic 2,4-pentadione, then closure of the pyrimidine ring to form **16** in 93% yield.



Scheme 3: Synthesis of DPA-713

1.2.4 Phenoxyphenylacetamides

A more recent class of TSPO ligands described are the phenoxyphenylacetamide derivatives, derived by opening the diazepine ring of benzodiazepines (Figure 7).[4] DAA1106, *N*-(2,5-dimethoxybenzyl)-*N*-(4-fluoro-2-phenoxyphenyl)acetamide and DAA1097, *N*-(4-chloro-2-phenoxyphenyl)-*N*-(2-isopropoxybenzyl)acetamide, are selective and potent TSPO agonists, with high lipophilicity and anxiolytic effects.[4,34]

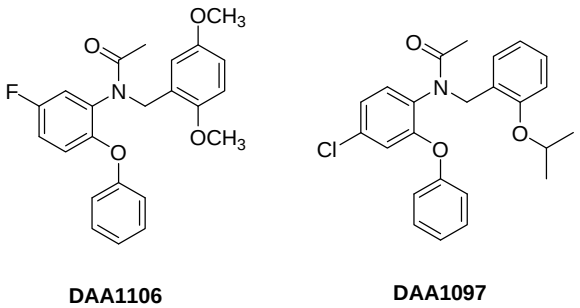


Figure 7: Phenoxyphenylacetamide

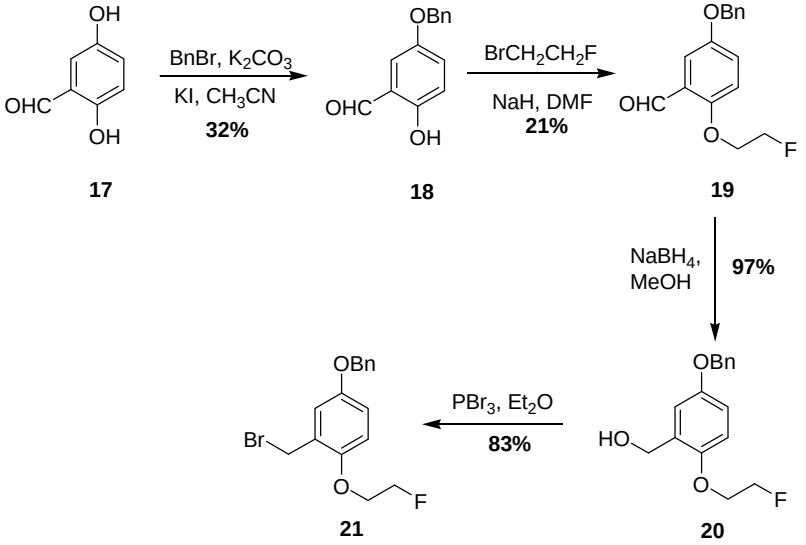
There are a number of subtle differences between DAA1106 and DAA1097, which allows an understanding of the TSPO binding site. DAA1097, like PK11195 activates steroidogenesis, whereas DAA1106 is unable to activate steroidogenesis alone. Both DAA1106 and DAA1097 have the capability to displace PK11195 at nanomolar concentrations suggesting that both ligands have the same binding domains.[4]

Although PK11195 is the oldest and most widely used radioligand for imaging, advances in this field over the years have led to DAA1106 analogues which are more effective than PK11195. DAA1106 analogues have been shown to have more potent *in vitro* binding affinity for TSPO and have significant selectivity against other receptors including the $GABA_a$.[15] PK11195 has low brain uptake, whereas DAA1106 shows higher uptake in *in vivo* studies on rodent and primate brains.[15]

In 1999, Chaki and co-workers reported their study on binding characteristics of [^{3}H]DAA1106 and demonstrated the advantages of this new ligand.[35] They performed autoradiographic and biochemical studies in the rat brain and found that [^{3}H]DAA1106 binding is localized in the olfactory bulb, and structures related to cerebrospinal fluid, followed by the cerebellum and cerebral cortex. Their results indicate the same binding for [^{3}H]PK11195. Furthermore, as DAA1106 is not in the same class of compounds as PK11195 and benzodiazepine derivatives, Chaki and co-workers postulated that DAA1106 could have its own binding domain therefore helping to identify the molecular characteristics of TSPO.[35] They also carried out their studies on rhesus monkeys as well as rats and found very little difference in affinity for mitochondrial preparations, just that the binding affinity in monkey was slightly lower. This result suggested that binding for DAA1106 is not species dependant and that DAA1106 might also have high affinity for human TSPO.

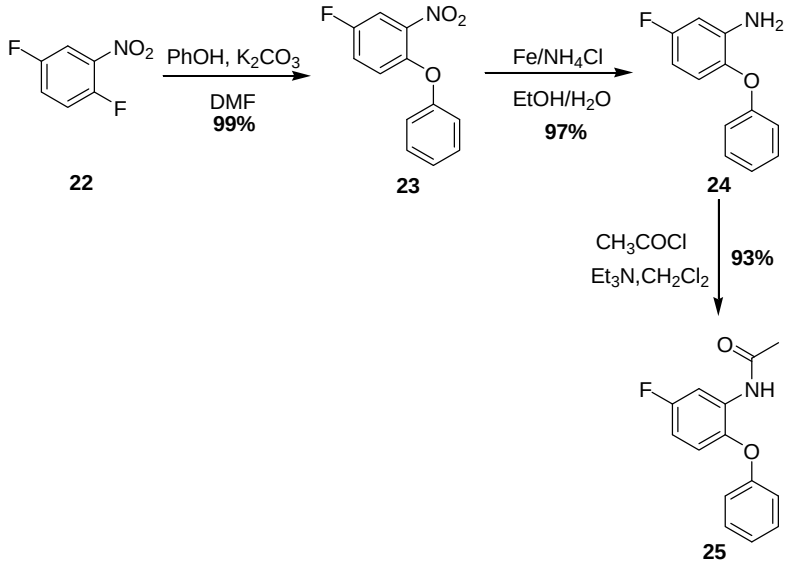
A derivative of DAA1106, FEDAA1106 *N*-(5-fluoro-2-phenoxyphenyl)-*N*-(2-(2-fluoroethoxy)-5-methoxybenzyl)acetamide shows even better TSPO binding (K_i 0.078 nM) than DAA1106 (K_i 0.16 nM). FEDAA1106 radiolabelled with fluorine-18 and DAA1106 with carbon-11 are used as radioligands for PET. Wang and co-workers from University of Indiana, synthesised [^{11}C]FEDAA1106 because of the ease of the radiosynthesis compared to fluorine-18. They successfully synthesised and radiosynthesised, FEDAA1106 encouraging the use of [^{11}C]FEDAA1106 as a potential radioligand for imaging TSPO in the brain.[14]

They reported a synthesis starting form commercially available 2,5-dihydroxybenzaldehyde **17**, which was benzylated using benzyl bromide to give **18** in 32% yield (Scheme 4). Alkylation of the hydroxyl group with 1-bromo-2-fluoroethane gave 2-fluoroethyl ether **19** in 21% yield. Reduction of the aldehyde then gave **20** and finally reaction with phosphorus tribromide gave benzylic bromide **21**.



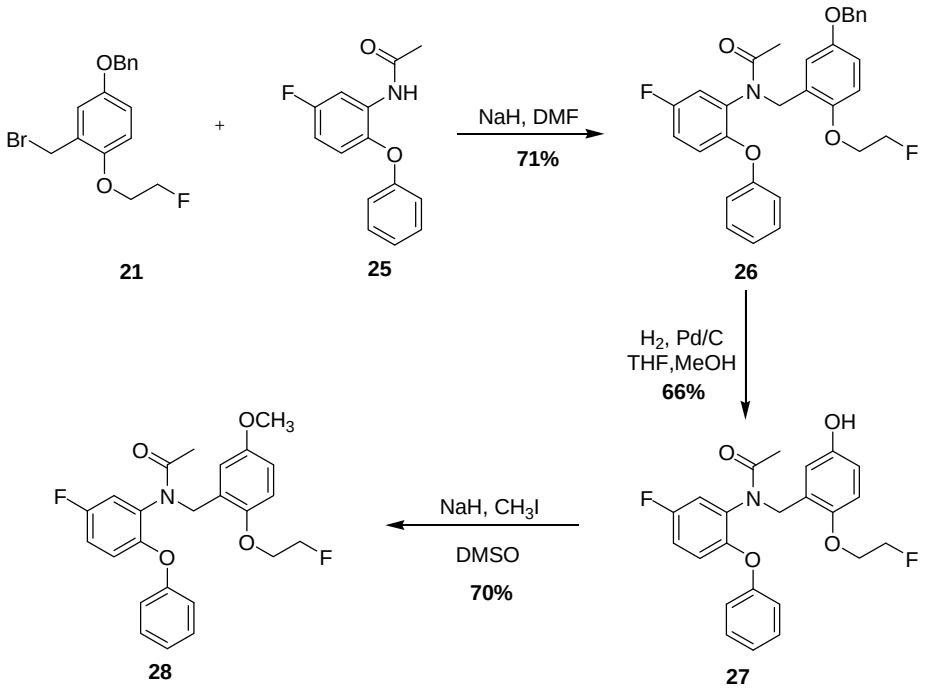
Scheme 4: Synthesis of 21

The next key intermediate formed was the acetanilide component, which was prepared from 1,4-difluoro-2-nitrobenzene **22**. Nucleophilic aromatic substitution with phenol gave **23** in 99% yield. Reduction of **23** with iron gave aniline **24** in 97% yield, which was then acetylated with acetyl chloride to give **25** in 93% yield.



Scheme 5: Synthesis of 25

To conclude the synthesis, Wang and co-workers, reacted **21** with acetanilide **25** using sodium hydride to give compound **26** in 71% yield. The benzyl group was then removed by catalytic hydrogenation with 10% Pd/C to give **27** in 66% yield. Finally *O*-methylation of **27** with methyl iodide and sodium hydride gave FEDAA1106 **28** in 70% yield.



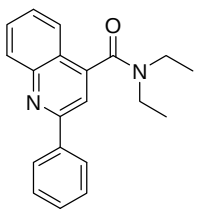
Scheme 6: Synthesis of FEDAA1106

1.3 PK11195

PK11195 is the mostly widely used ligand used to image the TSPO in the brain. It is involved in the inhibition of cell proliferation, induction of apoptosis, stimulates steroid synthesis and inhibition of immune response and inflammation.[4] PK11195 does not show species variation and binding affinity remains high (K_d < 20 nM).[36] It is also not affected by temperature (4 °C–25 °C) change unlike Ro5-4864, and the affinity of [^{3}H]PK11195 in binding to brain cortex and olfactory bulb membranes is constant.[37]

1.3.1 Structure-Affinity Relationships

PK11195 dates back to the early 1980's from a company in France called Pharmuka Laboratories. The first set of analogous, the PK series started with 2-phenylquinolines. The first analogue **29**, showed fairly good affinity to the $GABA_a$ but not to the TSPO (Table 3). The *N*,*N*-diethylamide side chain was changed to a more lipophilic group, *N*-methyl-*N*-*iso*butyl, which led to dramatic loss in $GABA_a$ affinity but improved TSPO binding. Changing the nitrogen to a different position also improved TSPO binding, to 45 nM.[38]



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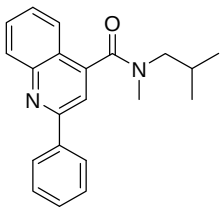

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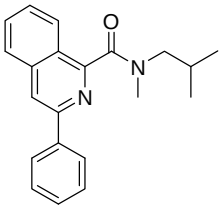

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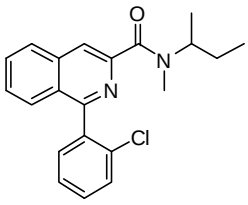
$GABA_a$ IC_{50} (nM)	50	1000	
TSPO IC_{50} (nM)	360	100	45

Table 3: Binding affinity of 2-phenylquinolines

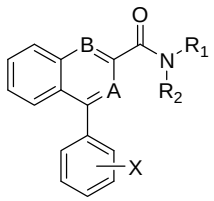
Subsequent studies led to the isoquinoline series, where the benzene-fused ring is moved towards the freely rotating aromatic ring, greatly improving selectivity.[38]



32 (9 nM for TSPO)

It has been well documented that *o*-chlorophenyl derivative PK11195 is a selective ligand for TSPO, from work carried out in 1983 by Le Fur and co-workers.[39,40] A review article by Bourguignon describes the SAR for PK11195.[38] As shown in table **4** the binding affinity varies, referring to **33** as the reference compound, if chlorine is placed on the

phenyl ring the affinity increases significantly. Also changing *N*,*N*-diethyl group to a more lipophilic group *N*,*N*-di-*n*-butyl **33b**, the affinity is more favourable and even better for the *N*-methyl-*N*-isobutyl carboxamide **33c**. By replacing the *sec*-butyl group on PK11195, to a benzyl group **33f** the affinity decreases considerably.

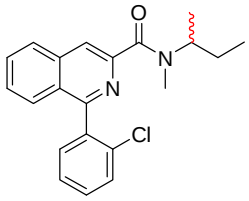


33

Compound	A	B	X	R_1	R_2	K_i (nM)
33	N	CH	H	Et	Et	150
33b	N	CH	H	*n*-Bu	*n*-Bu	21
33c	N	CH	H	Me	*i*-Bu	2
33d	N	CH	2-Cl	Et	Et	17
33e	N	CH	2-Cl	Pr	Pr	18
PK11195	N	CH	2-Cl	Me	*sec*-Bu	9
33f	N	CH	2-Cl	Me	Bn	117
33g	N	CH	3-Cl	Et	Et	9
33h	CH	N	H	Et	Et	27
33i	CH	N	2-F	Me	i-Bu	2
33j	N	N	H	Et	Et	27

Table 4: Binding affinity of PK11195 analogues

While PK11195 includes a stereogenic centre in its structure, the racemate has the same binding affinity for TSPO as the more potent *R*-enantiomer.

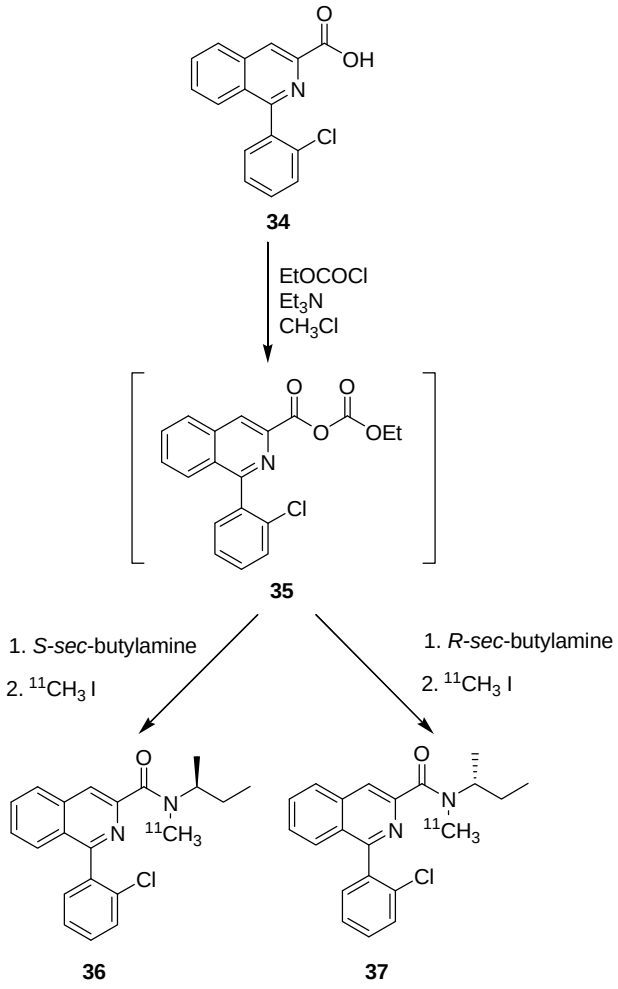


32

Stereoisomer	IC_{50} (nM)
R,*S*	9
S	19
R	9

Table 5: Stereoisomers of PK11195

Shah and co-workers synthesised both enantiomers of [*N*-methyl-^{11}C]PK11195 (Scheme 7) and compared their behaviours for TSPO binding in rats.[8] Previously, work with radiolabelled PK11195 for *in vivo* studies used the racemate, however, it is know that enantiomers can behave differently. They synthesised both enantiomers from 1-(2-chlorophenyl)isoquinoline-3-carboxylic acid, firstly by reacting the acid with ethyl chloroformate, followed by addition of either *R*-(-)-*sec*-butylamine or *S*-(+)-*sec*-butylamine to form both the *R* and *S* enantiomers, respectively. Lastly, radiosynthesis with [^{11}C]iodomethane gave the desired radioligands **36** and **37**.



Scheme 7: Synthesis of PK11195

The group concluded that both *R* and *S* enantiomers bind *in vivo* to TSPO but discovered that more radioactivity is retained in the tissue after intravenous injection of the *R*-enantiomer, indicating that it binds with higher affinity to the binding site than the *S*-enantiomer. They postulated that the two enantiomers bind differently due to the interactions of the chiral *sec*-butyl groups with a lipophilic domain in the binding site. The methyl and ethyl groups on the *R*-enantiomer would interact with the lipophilic domain, whereas the methyl group on the *S*-enantiomer would be further away from the lipophilic domain (Figure 8).

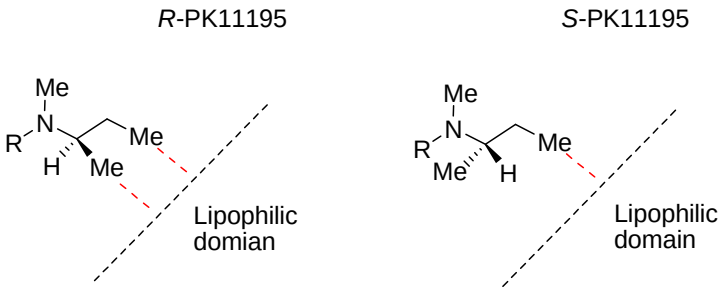


Figure 8: Binding of *R* and *S* enantiomers of PK11195

1.3.2 X-Ray Crystal Structure of PK11195

Knowing the conformation of PK11195 helps in the understanding of the binding to the receptor. Kubicki and Codding carried out X-ray crystallographic studies of PK11195 and found that the crystal structure is highly disordered (Figure 9).[41] The methylpropyl substituent was found in two different positions and the chloride substituent on the chlorophenyl fragment was found in two positions, C2' and C6'.

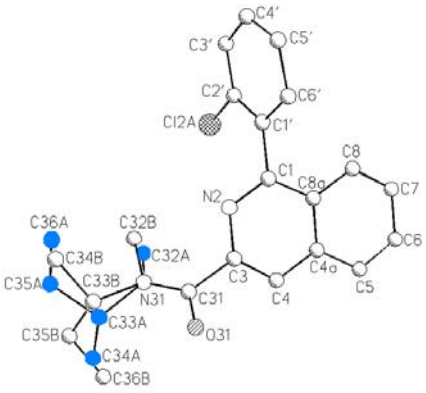
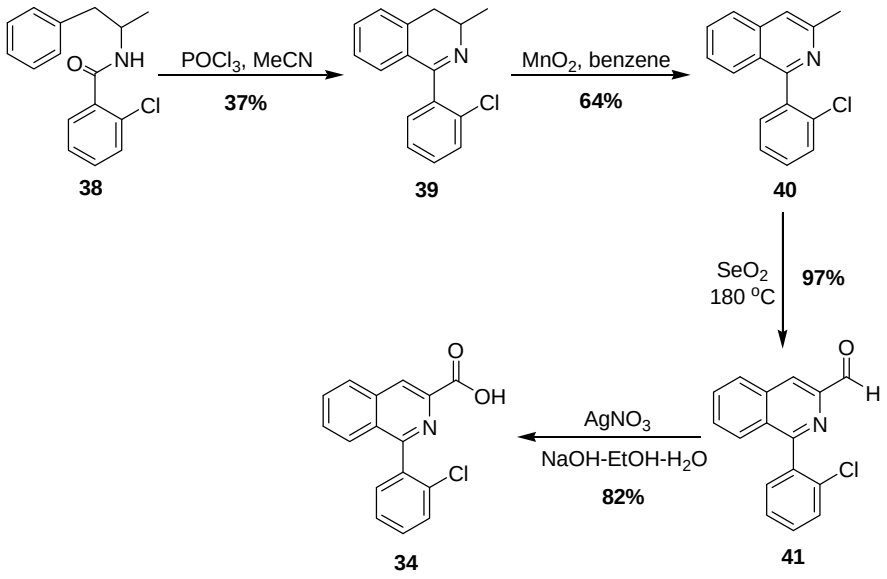


Figure 9: X-ray crystal structure of PK11195

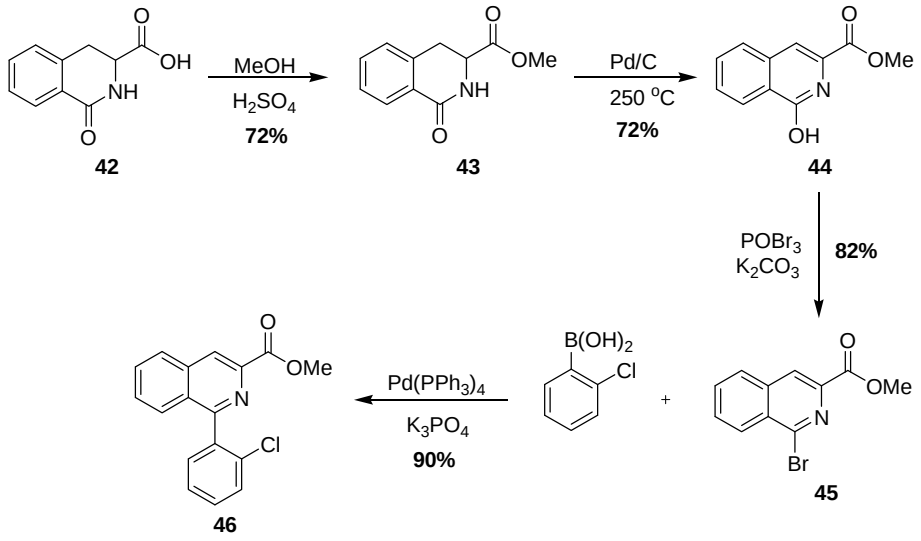
1.3.3 Syntheses of PK11195

In 2002, Janin and co-workers published their synthesis of 1-(2-chlorophenyl)isoquinoline as their contribution towards the synthesis of PK11195 as no synthesis had yet been published.[42] They started from commercially available 2-chlorobenzoyl chloride and reacted it with amphetamine in acetonitrile with triethylamine to form *ortho*-chlorobenzamide **38**. As shown in Scheme 8, Bischler-Napieralski synthesis was used to form the isoquinoline core followed by dehydrogenation to the aromatised product **40**. Firstly, sulfur in decalin was used for the aromatization but only achieved a 25% yield. Therefore, excess manganese oxide in benzene, was used and gave **40** in 64% yield. The methyl group was then oxidised to aldehyde **41** using selenium oxide in 1,2 dichlorobenzene in 97% yield. Further oxidation of the aldehyde gave the carboxylic acid target **34**.



Scheme 8: Synthesis of 1-(2-chlorophenyl)isoquinoline

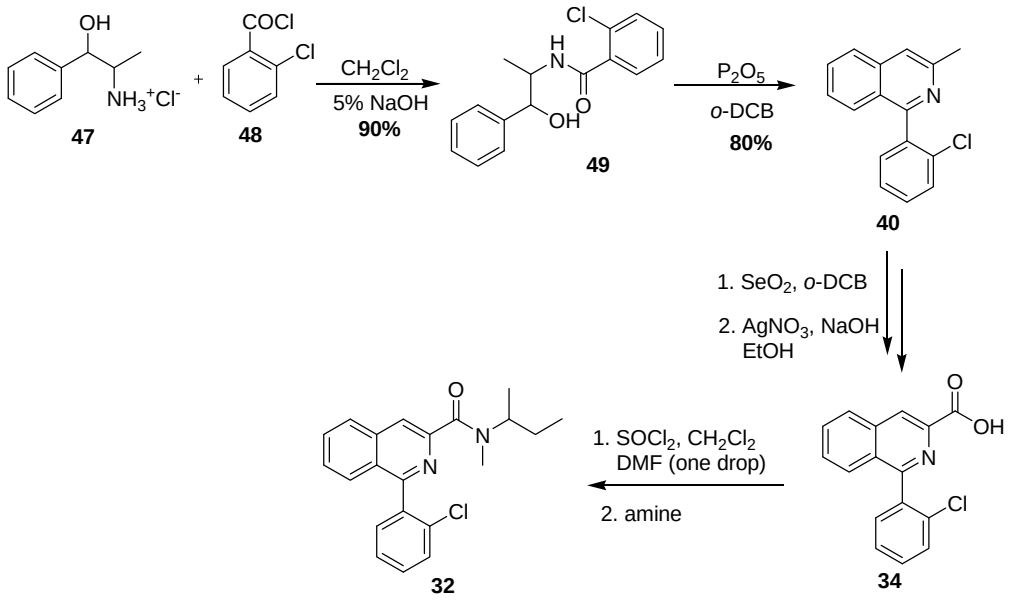
The same group reported another synthesis for the 1-(2-chlorophenyl)isoquinoline-3-carboxylic acid core for PK11195 (Scheme 9),[42] this time starting from commercially available dihydroisoquinoline carboxylic acid **42**. The acid was converted to the methyl ester **43**, followed by aromatization using palladium over charcoal in diphenyl ether to give isoquinoline **44**. Treatment of the isoquinoline core with phosphorus oxybromide afforded the brominated product in 82% yield. Bromide **45** underwent a Suzuki reaction with 2-chlorophenylboronic acid in dimethylformamide, which gave the final product **46** in 90% yield.



Scheme 9: Second synthesis of 1-(2-chlorophenyl)isoquinoline

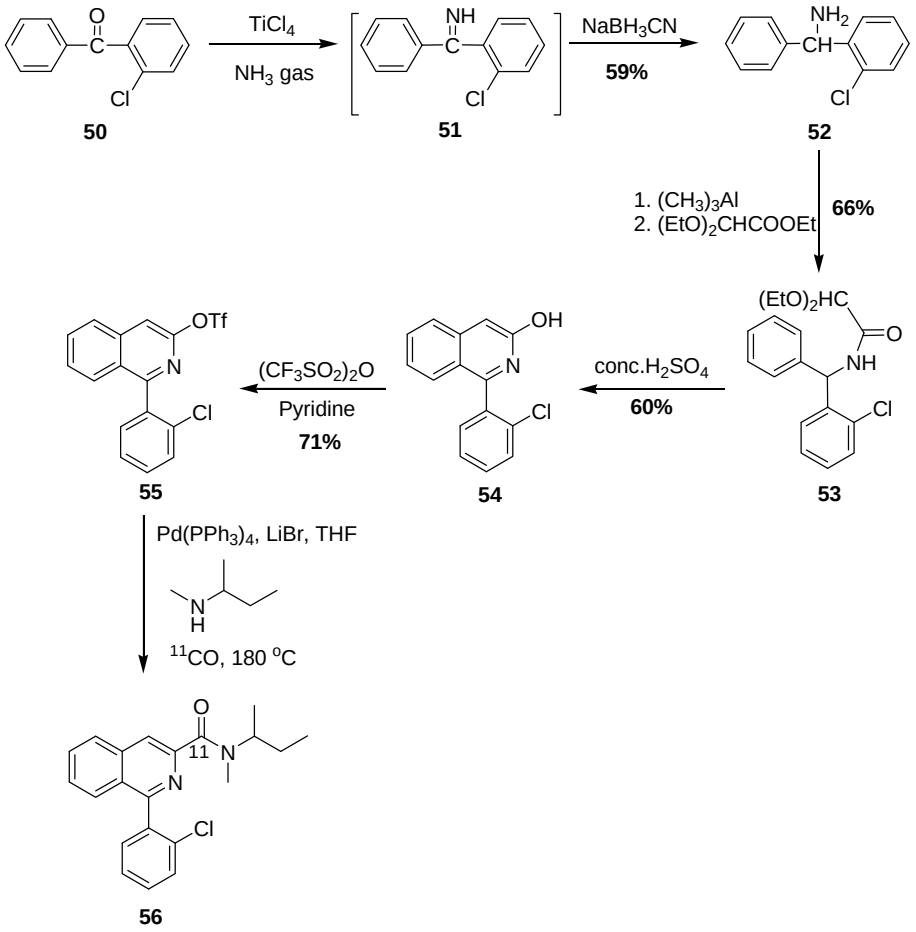
Janin and co-workers reported two different approaches for the synthesis of the isoquinoline core, which would facilitate the preparation of new TSPO ligands. Their first synthesis was achieved in 4 steps with an overall yield of 19% and the second synthesis was also achieved in 4 steps but with a better overall yield of 42%.[42]

Another approach to the synthesis of PK11195 was reported by Jaing and co-workers, as shown in Scheme 10.[43] The first half of the synthesis was developed by Manning and co-workers[44] and the second half uses the same chemistry developed by Janin and co-workers (Scheme 8).[42] The first intermediate **49** was achieved via base catalysed coupling of norephedrine with 2-chlorobenzoyl chloride. Next, condensation with phosphorus pentoxide in *o*-dichlorobenzene formed the isoquinoline ring **40**. Subsequent steps follow the same chemistry as Janin and co-workers to form the carboxylic acid derivative. The amide coupling was achieved via the acid chloride followed by reaction with appropriate amines. This approach for the synthesis of the isoquinoline core is significantly more efficient than the approach described by Janin and co-workers.[43]



Scheme 10: Synthesis of PK11195

Later in 2002, Rahman and co-workers published their full synthesis of PK11195 and analogues (Scheme 11).[45] The first step started with commercially available 2-chlorobenzophenone **50**, which underwent a one-pot reductive amination of the ketone to give **52** via imine **51**. Amine **52** was converted to amide **53** by reaction with ethyl diethoxyacetate. Firstly the amine was activated by conversion to the aluminium amide by treatment with trimethylaluminium, followed by the reaction of the aluminium amide with ethyl diethoxyacetate to give **53** in 66% yield. Cyclisation of **53** using conc H_2SO_4 gave **54**. The resulting alcohol was converted to triflate **55** by reaction of alcohol with trifluoromethanesulfonic anhydride at ambient temperature. Finally, [^{11}C]PK11195 was prepared by reaction with *N*-methyl-*sec*-butylamine and [^{11}C]carbon monoxide in a Pd(0) mediated reaction.



Scheme 11: Synthesis of [^{11}C]PK11195

Rahman and co-workers synthesised [^{11}C]PK11195 with the carbonyl carbon instead of the *N*-methyl group labelled because using their approach they were able to produce a broad range of ^{11}C-labelled analogues of PK11195 by using different amines. Labelling at the *N*-methyl group can sometimes prove difficult depending on the amine groups used whereas Rahman's way prevents this difficulty. They also found that using their way; the specific radioactivity is high (393 GBq μmol^{-1}) compared to labelling at the methyl group (20–96 GBq μmol^{-1}).

1.4 Molecular Imaging

Molecular imaging is a non-invasive imaging technique used to visualise and characterise biochemical pathways, molecular interaction, drug pharmacokinetic and pharmacodynamics.[46,47] A radiotracer is used along with *in vivo* imaging techniques such as Positron Emission Tomograpy (PET) and Single Photon Emission Computed Tomography (SPECT). A radiotracer contains a radionuclide and an organic ligand that is selective and has high binding affinity for the required binding site. The ligand that is radiolabelled should also have low non-specific binding, good blood-brain barrier penetration, antagonist action and slow metabolism.[48]

Choosing the right radionuclide to use to label the ligand is important. The half-life of the radionuclide is a major factor in choosing the correct isotope; half-life is defined in two parts, physical half-life and biological half-life. The physical half-life is the time taken for half the atoms to disintegrate and biological half-life depends on the excretion from the body. If the half-life is short then it needs to be produced onsite and administered straight away. Similarly, if the half-life is too long, then it can still be in the patient after the scan is finished, thereby affecting the patient and other people around them.[49]

1.4.1 PET

A PET scanner is used to detect the gamma rays leaving the body after a radioactive tracer has been injected, providing 3D information of the binding site. When the patient is injected with the radioactive tracer it travels to and binds to the required receptor. The radioisotope starts to decay and emits a positron (Figure 10). The positron interacts after a short distance with an electron from the surrounding tissue resulting in annihilation producing two gamma photons of 511 KeV, emitted at 180^{o} to each other.[50] A ring of detectors surrounding the patient detects the photons and constructs a tomographic image of the required site, resulting in 3D image of the tissue.[46] PET uses isotopes with short half-lives, e.g. carbon-11 (20.3 min), nitrogen-13 (10 min), oxygen-15 (~ 2 min) and fluorine-18 (109.8 min),[7] these isotopes are relatively small and are already present in the compound. They are useful for monitoring processes that are finished within a short time but are less useful at imaging at later times as the radioactive isotope has significantly decade resulting in poor analysis.[47] Due to the short half-lives of the isotopes used there needs to be a cyclotron on site to generate these isotopes, which can be expensive.

However, the PET has a great advantage with respect to sensitivity (pico- to nanomolar) and resolution to measure receptor density and interactions *in vivo*. It has gained clinical popularity over the years, with PET- based studies reaching 3.2 million.[47,50]

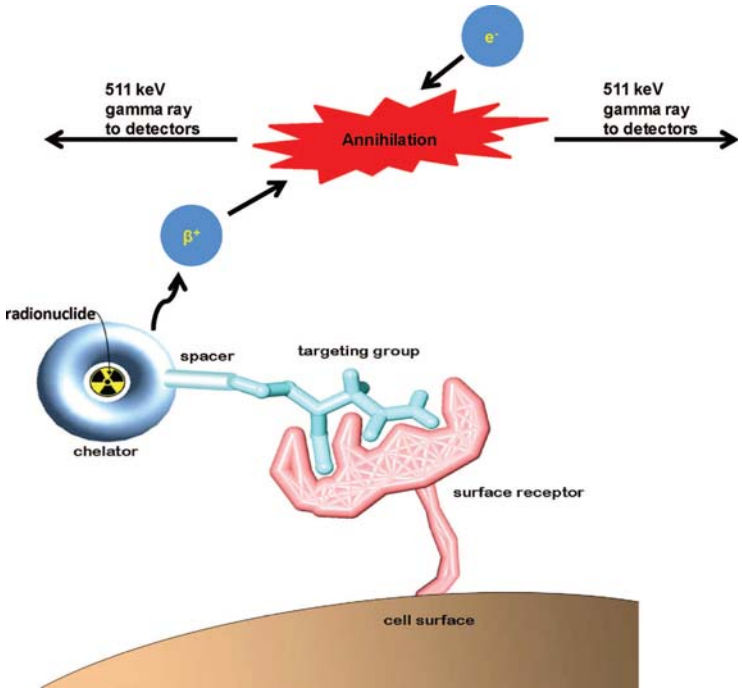


Figure 10: Fundamental principle of PET[47] Reprinted with permission from *Chemical Reviews* **2010**, 110, 2858. Copyright 2010 American Chemical Society.

1.4.2 SPECT

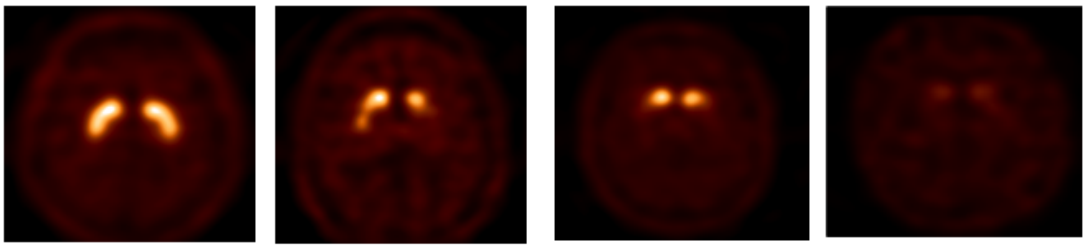
Although SPECT imaging has much lower detector efficiency and less quantitative accuracy than PET, it is more practical and has wider clinical applications.[51] The isotopes that are used have longer half-lives e.g. iodine-123 (13 h) and technetium-99 (6 h), therefore this allows the radioisotopes to be produced away from the site of use and transported when required. Due to the longer half-live, the observation time frame can be widened, allowing observation of biological processes *in vivo* over several hours or days after the labelled ligand has be injected.[50] A disadvantage of SPECT is the use of a large iodine atom that affects the binding and lipophilicity of the ligand. SPECT scanners use radionuclides that decay emitting single photons (gamma rays) at a range of angles. The cameras rotate 360^{o} around the patient and image reconstruction techniques are used to form a 3D image.[49] An example of a SPECT scanner is shown in Figure 11.

Figure 11: SPECT Scanner

1.4.3 Clinical Use

Previously, the only way for medical practitioners to detect neurological and neurodegenerative damage to the nervous system was to carry out biopsy or post-mortems. However, due to advances in technology, brain damage and inflammation in neurodegenerative diseases can be visualised in living humans through the use of PET or SPECT with suitable ligands. These techniques have the potential to clarify the relationship between changes in the receptor with progress of certain diseases.[48] The information would allow more effective diagnosis, analysis of progression and determine different approaches to take in the treatment of each individual disease.[4]

Parkinson's disease is one neurodegenerative disease diagnosed using a SPECT scanner. The images shown in Figure 12 shows the progression of Parkinson's disease in the brain, the left image is that of a normal brain, image B and C shows mild Parkinson's symptoms and D shows a patient with chronic Parkinson's disease.[52]



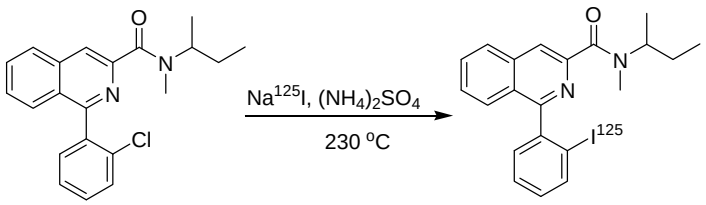
A **B** **C** **D**

Figure 12: Brain images of the progression of Parkinson's disease

1.4.4 Radiotracers for TSPO

Both PET and SPECT have been used widely along with the ligand, PK11195 to image the TSPO receptor. PK11195 has been labelled with tritium-3, carbon-11 and iodine-123 for *in vitro* and *in vivo* studies. PK11195 was first labelled with carbon-11 by Camsonne and co-workers in 1984, for use with PET to investigate the use of PK11195 as a new radiopharmaceutical.[53] They methylated the *N*-desmethyl analogue with [^{11}C]iodomethane in 45 minutes and obtained a high specific activity of 769 Ci/mmol. Radiolabelling of PK11195 led to noninvasive investigation of cardiac receptors which before was otherwise impossible.[54] Charbonneau and co-workers were the first to successfully perform *in vivo* studies to investigate the TSPO binding sites in human and dog hearts, using Camsonne's method of radiolabelling PK11195.[54]

A few years later and after the success of [^{11}C]-PK11195, Gildersleeve decided that a radioiodine labelled analogue of PK11195 might be beneficial for the use with SPECT imaging of TSPO.[55] They synthesised [^{125}I]PK11195 via solid-state interhalogen exchange (Scheme 12). Using a vial containing 3 mm glass beads, PK11195, ammonium sulfate, [^{125}I]sodium iodide and sodium hydroxide in 1:2 ethanol/water was heated for 20 minutes. They achieved their synthesis in a short time and the product was obtained in 50-76% radiochemical yield, >94% radiochemical purity and a good specific activity of 15-17 Ci/mmol.

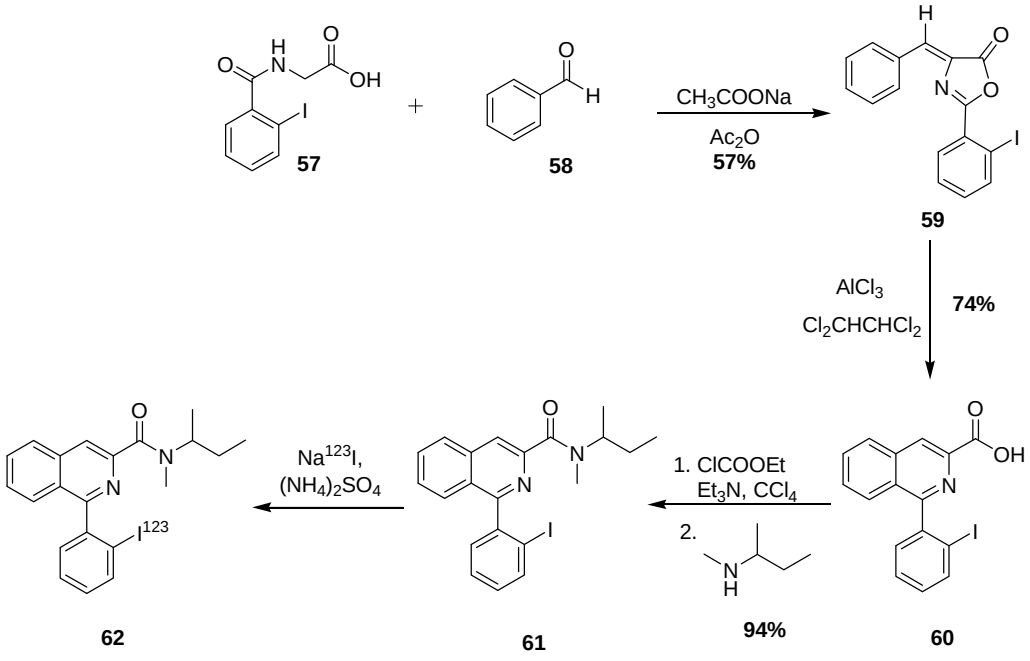


32

Scheme 12: Synthesis of [^{125}I]PK11195

As previously mentioned chlorine and fluorine in the *ortho* position exhibit high affinity binding for TSPO but there have been no studies on the use with bromine or iodine. Furthermore, the use of iodine would allow the use of SPECT imaging. Using iodine/SPECT would not require an on-site cyclotron. Gildersleeve reported that [^{125}I]-PK11195 in rat glioma had the same high specificity and affinity as PK11195 and would be a good tool for *in vivo* studies in the future.[55]

In 1996 Gildersleeve and co-workers attempted to radioiodinate PK11195 again but using iodine-123, however this was ineffective because of its low yield and the trouble to remove unreacted chlorinated material.[56] Therefore, I-PK11195 and the [^{123}I]-PK11195 were synthesised (Scheme 13). The synthesis of I-PK11195 was achieved in an overall yield of 40%. The first step was the condensation of 2-iodohippuric acid **57** with benzaldehyde **58** to give **59** in 57% yield. Next, aluminium trichloride-mediated rearrangement of **59** gave isoquinoline-3-carboxylic acid **60**, followed by reaction of the acid with ethyl chloroformate to form the activated ester. Finally, reaction with *N*-methyl-*sec*-butylamine gave **61** in 94% yield. The radiosynthesis was carried out as before with a >60% isolated radiochemical yield, >99% radiochemical purity and specific activity of 233-348 mCi/mmol.



Scheme 13: Synthesis of [^{123}I]PK11195

1.5 Proposed research

Although [^{123}I]PK11195 is being used successfully for SPECT imaging, the syntheses of PK11195 are long and low yielding and not very flexible. The radiosynthesis is also not very effective and previous synthesis uses solid state interhalogen isotope exchange which suffers from low activity. This project aims to develop a novel, more efficient synthesis of PK11195 and will investigate the different methods of radioiodination, in order to obtain a more rapid and efficient method that will give a high radiochemical yield, a high radiochemical purity and a high specific activity.

The second part of this research is to develop a new class of TSPO ligands, quinoline-2-carboxamides. Despite PK11195 being the most widely used isoquinoline ligand, there are some limitations regarding biodistribution. On crossing the blood brain barrier the ligand is not particularly selective and interacts with other receptors.

The aims of the PhD project were:

- To develop a short efficient synthesis of PK11195 analogues (Figure 13).
- Investigate new reactions for the radiosynthesis of [^{123}I]PK11195.
- Generate a small library of iodinated quinoline-2-carboxamides as new SPECT ligands for TSPO.
- Perform binding assays to check compounds for affinity with TSPO.

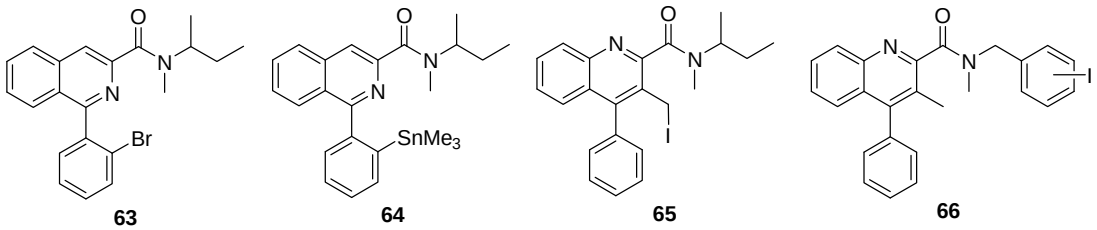


Figure 13: Analogues of PK11195 and quinoline-2-carboxamides

2 Results and Discussion

2.1 The Synthesis of PK11195 and Analogues

Early syntheses of PK11195 are relatively long, inefficient and limited to the number of analogues that can be prepared. The first aim of this project was to devise a synthesis that is short and efficient but also flexible enough to prepare various analogues from a common intermediate. Including PK11195, three other derivatives were synthesised that could be radioiodinated for SPECT imaging (Figure 14).

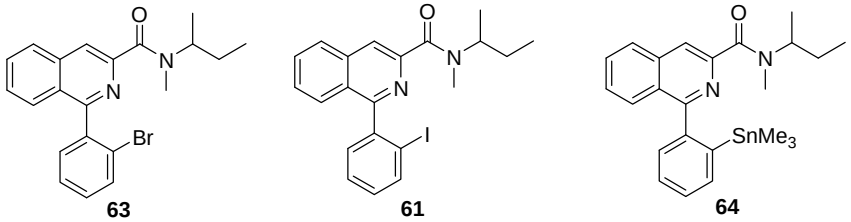


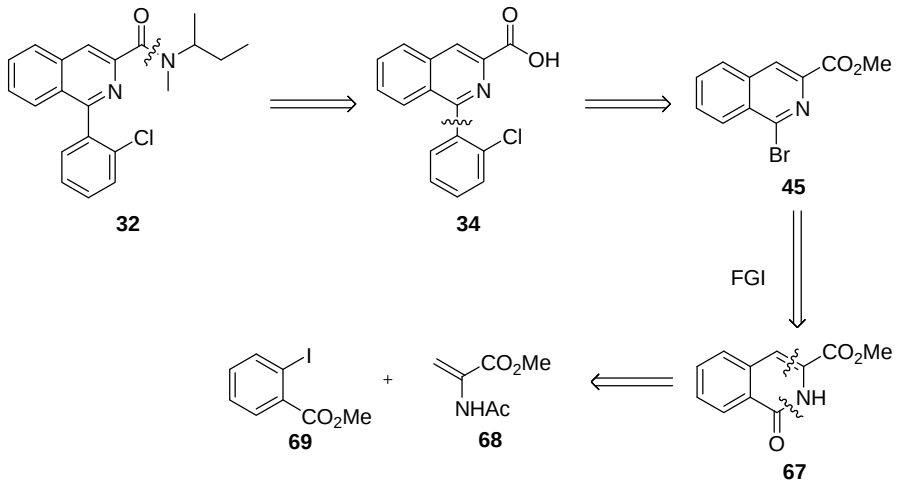
Figure 14: Analogues of PK11195

The same synthetic route for PK11195 was adapted to form bromo-analogue **63**, which was then used to synthesis iodo-analogue **61** using a copper catalysed, aromatic halogen exchange reaction. The last analogue that was synthesised was organostannane derivative **64**, again synthesised from the bromo-analogue.

The next aim of the project was to synthesise [^{123}I]PK11195 from the bromo and organostannane derivatives and investigate the best method of radioiodination. Previous methods are not very efficient, therefore the aim was to obtain a rapid and efficient synthesis of [^{123}I]PK11195 with a high specific activity and a high isolated radiochemical yield. I-PK11195 was synthesised as a standard for HPLC to check if [^{123}I]PK11195 had been made successfully.

2.1.1 Retrosynthetic Analysis of PK11195

The proposed retrosynthetic analysis of PK11195 is shown in Scheme 14. Disconnection of the amide side chain leads back to carboxylic acid **34**. Further disconnection of the 2-chlorophenyl ring gives **45,** followed by functional group interconversion of the halide to a carbonyl group, **67**. Isoquinolin-1-one **67** can be prepared from commercially available methyl 2-iodobenzoate **69** and methyl 2-acetamidoacrylate **68**.

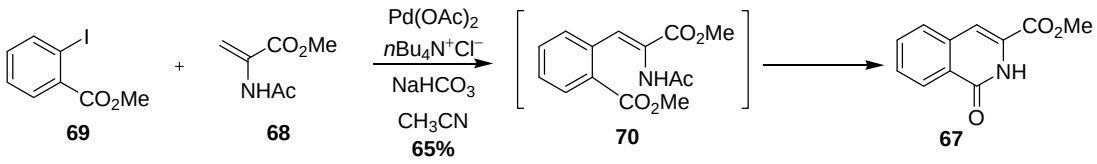


Scheme 14: Retrosynthesis of PK11195

There are two key reactions in the formation of PK11195, firstly a Heck reaction to form the isoquinoline core and a Suzuki reaction to couple the 2-chlorophenyl ring.

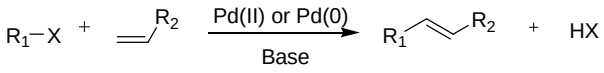
2.1.2 Synthesis of PK11195

As shown in Scheme 15, the synthesis of PK11195 started from commercially available methyl 2-iodobenzoate **69.** A one-pot procedure developed by Chattopadhyay and co-workers,[57] was used to couple **68** and **69** to give methyl isoquinolin-1-one-3-carboxylate **67** in 65% yield. Initially the reaction was carried out in *N*,*N*-dimethylformamide (DMF) but gave a poor yield of 23%. Acetonitrile was then used improving the yield to 65%. The reaction proceeds *via* a palladium(0) catalysed Heck type arylation of **68** and **69**, to give intermediate **70**, followed by cyclisation through nucleophilic attack of the acetamide on the ester carbonyl and loss of *N*-acetyl group to give **67**.[58] For the Heck reaction, conditions developed by Jeffery were used, involving tetrabutylammonium chloride as the phase transfer agent, sodium hydrogen carbonate as a base and catalytic palladium acetate.[59]



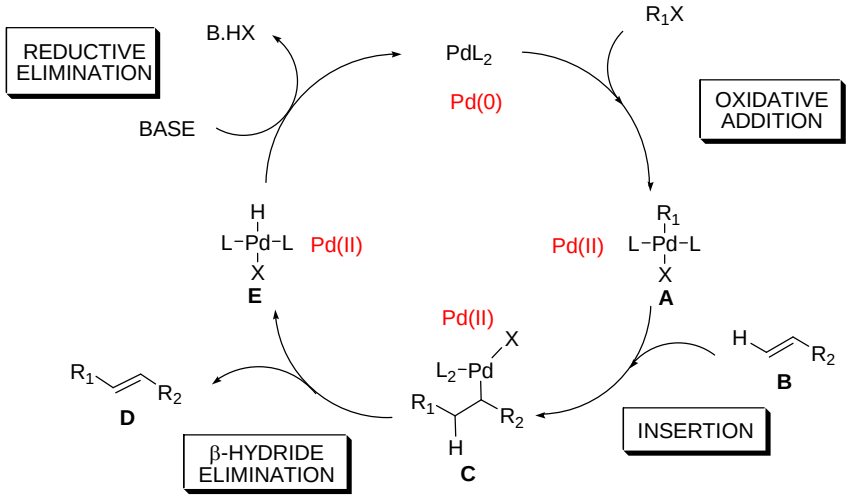
Scheme 15: Synthesis of methyl isoquinolin-1-one-3-carboxylate 67

Palladium-catalysed coupling reaction of an alkene with an organic halide was first reported by Mizoroki and Heck in the 1970's.[60] The reaction is very versatile and a wide range of olefins and aryl species have been reported to form a new alkene bond. Scheme 16 shows the general transformation of a Heck reaction, where R_1 can be aryl, vinyl or allyl and X can be a halide or triflate.



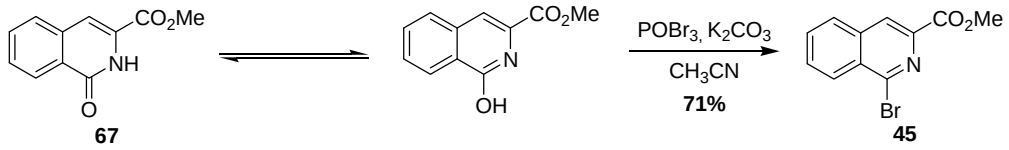
Scheme 16: General Heck reaction

The reaction occurs *via* a palladium-catalysed cycle, as shown in Scheme 17.[60] Firstly oxidative addition takes place in which the palladium(0) inserts between the aryl halide bond to form complex **A**, followed by insertion of the alkene into Pd-R_1 bond to give **C**. Subsequent β-hydride elimination yields the desired double bond **D**, and removes the palladium species. Finally the base is used to convert the palladium(II) complex back to palladium(0).



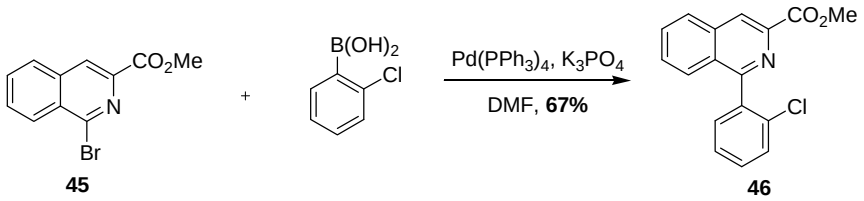
Scheme 17: Palladium-catalysed Heck reaction

The next key stage of the synthesis involves the use of the Suzuki cross-coupling reaction. A Suzuki reaction is used to couple various boronic acids to aryl or vinyl halides using a palladium catalyst.[61] In order to carry out this reaction, oxoisoquinoline **67** was initially converted using phosphorus(V) oxybromide to the required halide **45** in a 71% yield (Scheme 18). The reaction proceeds *via* the enol tautomer. The hydroxyl group attacks the phosphorus reagent to make an O-P bond at the same time as displacing a bromine atom. The nucleophilic bromine then attacks the carbon and displaces the phosphorus species generating a P=O bond thereby driving the reaction to completion.



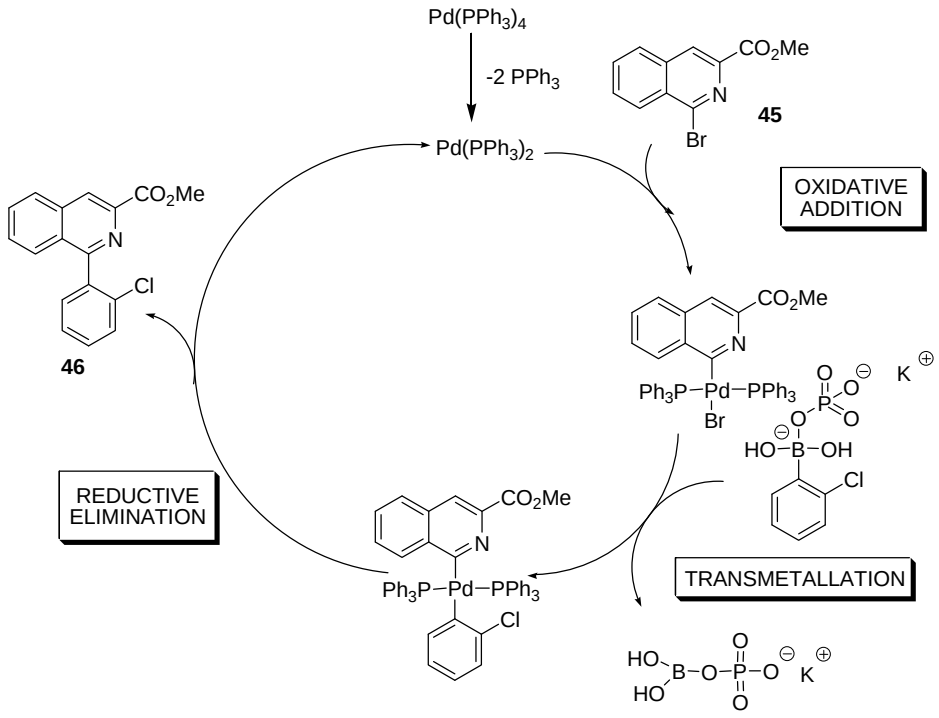
Scheme 18: Synthesis of 45

Having formed the bromide intermediate **45**, a Suzuki reaction was then used to couple 2-chlorophenylboronic acid to compound **45** using tetrakis(triphenylphosphine)palladium(0) as the catalyst and potassium phosphate as the base in DMF (Scheme 19). The reaction was initially attempted using a co-solvent system of toluene/ethanol and sodium carbonate in water but was unsuccessful with a very poor yield of 9%. Therefore, the conditions shown in Scheme 19 proved to be a better alternative to form 1-(2-chlorophenyl)isoquinoline-3-carboxylate **46** in 67% yield.



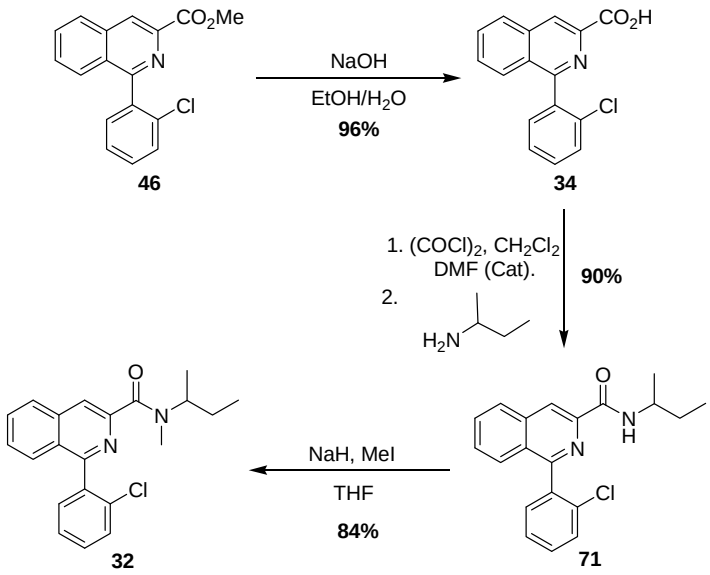
Scheme 19: Synthesis of 1-(2-chlorophenyl)isoquinoline-3-carboxylate 46

The reaction mechanism for the Suzuki reaction proceeds *via* a catalytic cycle similar to the Heck reaction but with another key step, transmetallation. Shown in Scheme 20 is the catalytic cycle for the formation of 1-(2-chlorophenyl)isoquinoline-3-carboxylate **46**. Initially, oxidative addition of palladium(0) complex into the aryl halide bond of **45** takes place to form a palladium(II) species. The additional key step, transmetallation is slow, which allows the coupling of 2-chlorophenylboronic acid to **45**, followed by reductive elimination to give required product **46** and regeneration of palladium(0).



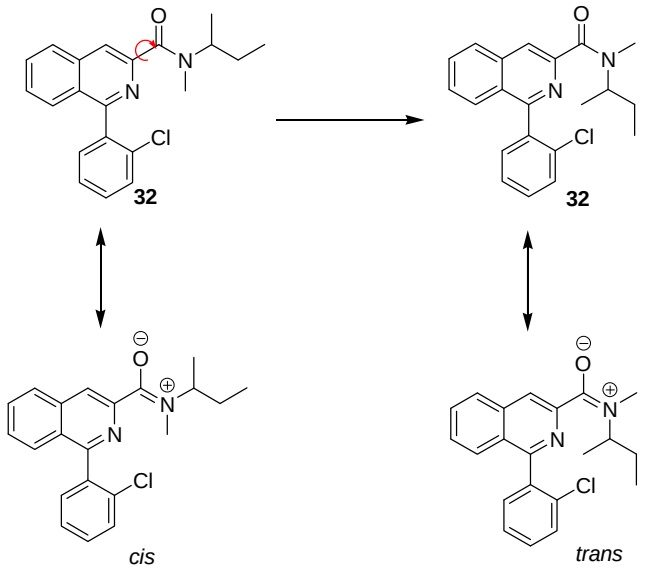
Scheme 20: Suzuki reaction

The second half of the synthesis required the coupling of an appropriate amine side chain. Hydrolysis of methyl ester **46** gave carboxylic acid **34** in excellent yield of 96% (Scheme 21). Coupling of the carboxylic acid **34** and an amine was initially attempted using *N*-methyl-*sec*-butylamine using the standard coupling reagents, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP). Coupling of *N*-methyl-*sec*-butylamine was also attempted using the acid chloride from **34**. However, these reactions gave either the desired product in very low yield (10%) or just starting material. An alternative approach used by Cappelli and co-workers proved very efficient.[62] Reaction with oxalyl chloride to form the acid chloride and *in situ* reaction with *sec*-butylamine gave **71** in 90% yield. To complete the synthesis, the secondary amide was converted into the required tertiary amide by *N*-methylation using sodium hydride and methyl iodide to give **32** in 84% yield.



Scheme 21: Completion of PK11195 32

After the methylation of secondary amide **71** the NMR spectra showed the presence of rotamers in both proton and carbon spectra. This occurs because the amide bond is able to rotate and the lone pair of electrons on the nitrogen is delocalized into the carbonyl group generating *cis* and *trans* isomers (Scheme 22). The NMR spectrum shows signals for both the *cis* and *trans* conformations resulting in more than one peak for each proton and carbon. In general, rotation of the amide is so fast that there should only be one conformation but because rotation is slow on the NMR timescale there are two conformations. Shown in Figure 15 is the ^{1}H NMR spectrum for PK11195, showing the presence of two rotamers. The final step completed the six step synthesis of PK11195 to give the target compound in a 22% overall yield.



Scheme 22: *Cis* and *trans* isomers of PK11195

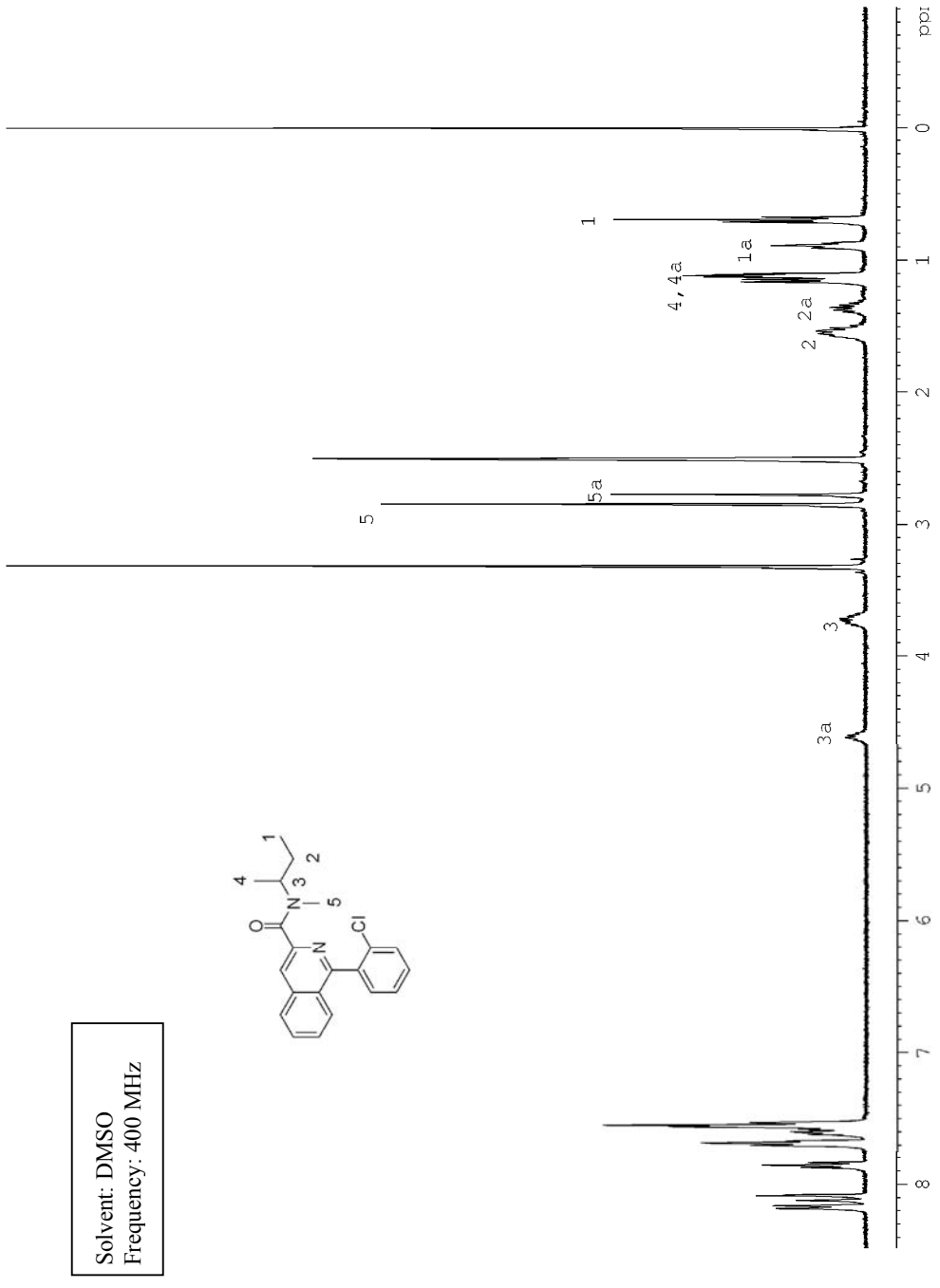
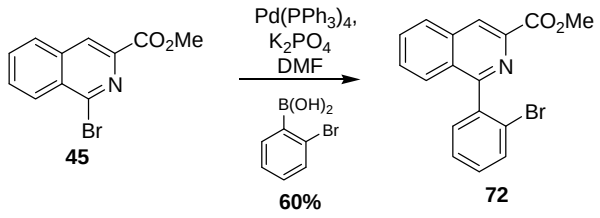


Figure 15: NMR of PK11195

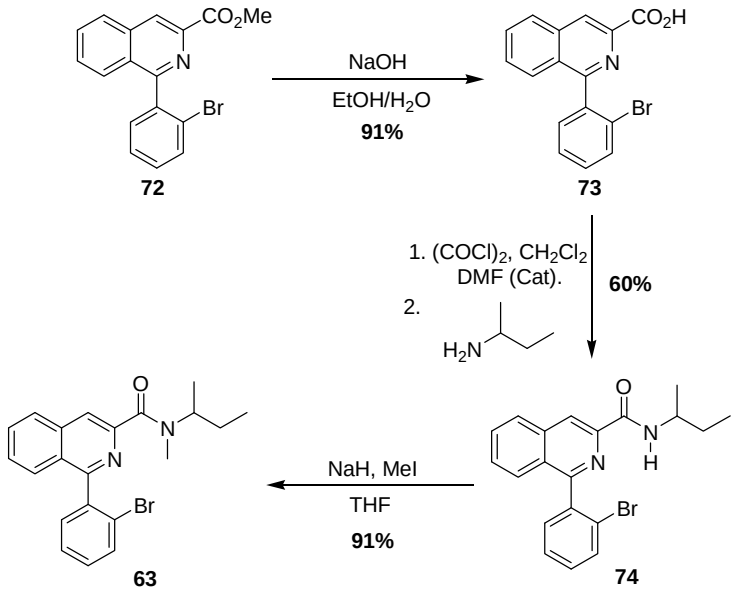
2.1.3 Synthesis of Bromo-Analogue 63

Having established the route, the next aim was to prepare other analogues using the chemistry already developed. To prepare the iodo-analogue, the bromo-derivative was prepared first. This was achieved exactly the same way as PK11195, using 2-bromophenylboronic acid to yield **72** in 60% (Scheme 23).



Scheme 23: Synthesis of 72

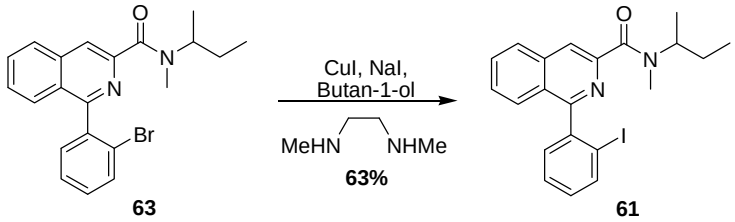
Hydrolysis of the methyl ester **72**, gave carboxylic acid **73** in 91% yield. Reaction of carboxylic acid **73** with oxalyl chloride followed by *in situ* reaction with *sec*-butylamine gave **74** in a 60% yield. Again, completion of the synthetic route involved *N*-methylation using prior conditions to form bromo-analogue **63** in an excellent 91% yield.



Scheme 24: Synthesis of Br-PK11195

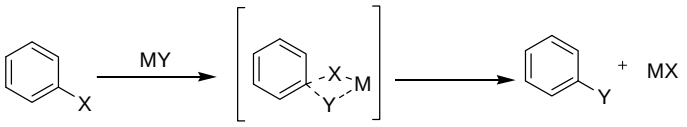
2.1.4 Iodo-Analogue 61

As shown in Scheme 25, the iodo-analogue was prepared using a copper-catalysed aromatic Finkelstein reaction, using conditions originally published by Klapars and Buchwald.[63] Bromide **63** was reacted with sodium iodide and catalytic amounts of copper iodide and the diamine ligand *N*,*N*-dimethylethylenediamine at high temperature. This gave iodide **61** in 63% yield.

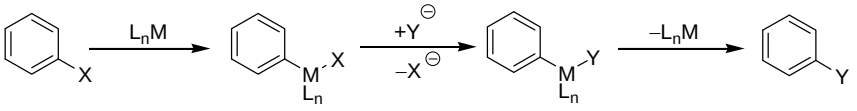


Scheme 25: Synthesis of 61

The reaction mechanism for the halogen exchange is not fully understood. One proposed mechanism as shown in Scheme 26, path A, occurs *via* a 4-centred transition state.[64] Another plausible mechanism is a cross coupling pathway (path B). Oxidative addition of the metal between the aryl halide bond, followed by ligand exchange to introduce the new halide. Finally reductive elimination to give the required product.



Path A

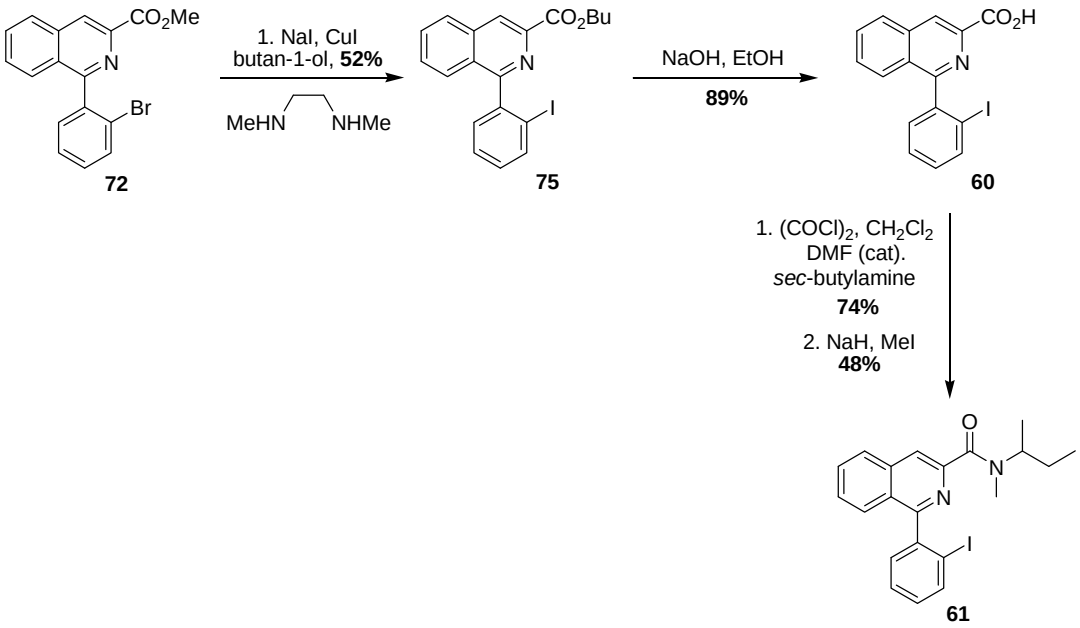


Path B

Scheme 26: Halogen exchange reaction

Another approach to incorporate the iodide was successfully achieved by addition of the iodide before the coupling of the amide side chain (Scheme 27). The copper-catalysed halogen exchange reaction involving bromide **72** gave the iodinated butyl ester analogue **75** in 52% yield. Ester hydrolysis gave **60**. Reaction with *sec*-butyl amine followed by *N*-

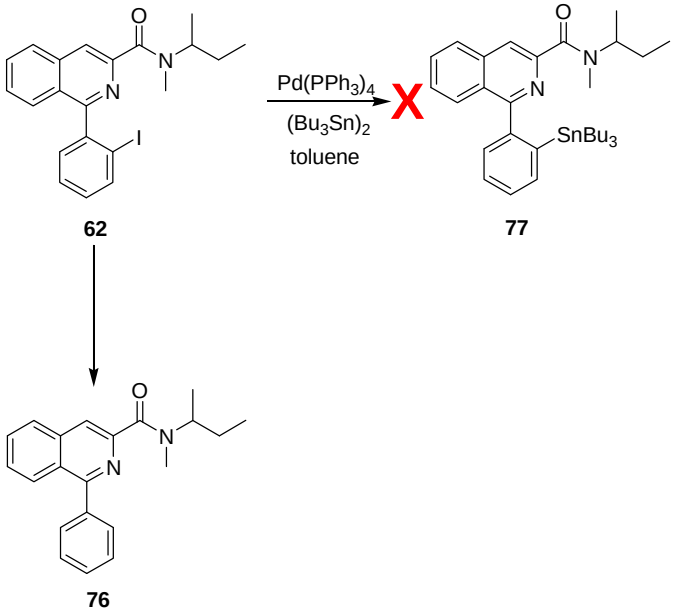
methylation gave the required iodo-analogue **61**. This alternative route was investigated as analysis of the reaction described in Scheme 25 by NMR spectroscopy proved difficult to follow due to the presence of rotomers. Thus, incorporation of the iodine atom at the ester stage allowed clearer analysis as to when the reaction was complete and may be useful in generating other amide analogues of PK11195.



Scheme 27: Synthesis of Iodo-PK11195

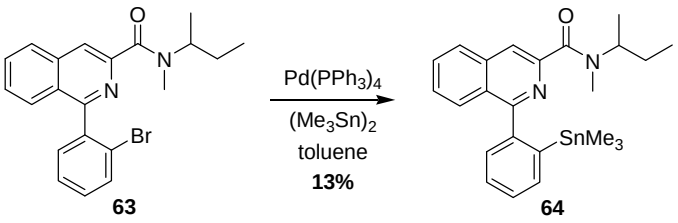
2.1.5 Synthesis of Organostannane-Analogue 64

The last target from the PK11195 series was the organostannane derivative. Initial attempts using a palladium(0)-catalysed procedure developed by Katsifis and co-workers,[6] with hexabutylditin and iodo-analogue **61** were unsuccessful (Scheme 28). Instead of forming the required product **77**, the reduced product **76** was recovered. It was proposed that failure of this reaction was probably due to steric hindrance associated with the *ortho*-iodo position and the bulky hexabutylditin reagent.



Scheme 28: First attempt of the synthesis of 64

With the failure of the reaction above, another method was adopted. Homes and co-workers were able to convert an aromatic bromide to the trimethylstannyl derivative using the same reaction conditions as Katsifis.[65] So, instead of using the iodo-analogue, the bromo-analogue was used and the less bulky hexamethylditin reagent (Scheme 29). The reaction gave organostannane **64** in a low 13% yield. The reaction was very low yielding and not optimised at the time as the amount of **64** prepared by this method was sufficient for the radiosynthesis study.

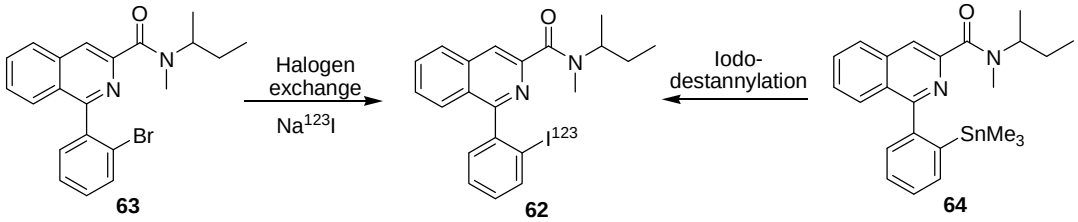


Scheme 29: Synthesis of organostanne-analogue 64

2.1.6 Radiosynthesis of [^{123}I]PK11195

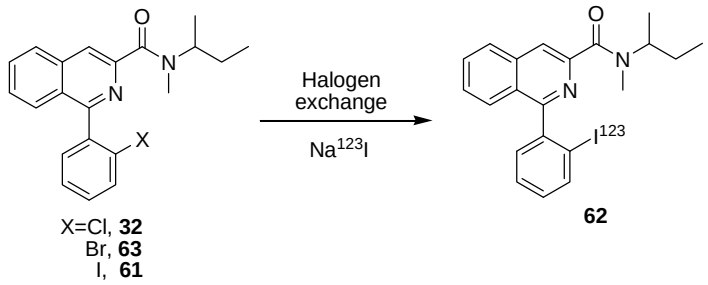
Our collaborator, Dr S Pimlott at the Department of Clinical Physics, carried out the radiosynthesis of the PK11195 analogues. The aim was to develop the radiosynthesis of [^{123}I]PK11195 in a rapid and efficient method with high specific activity and radiochemical yield.

The Br-PK11195 was radiolabelled using a solid-state interhalogen exchange reaction, a modification of the technique previously called solid-state isotopic exchange developed by Gildersleeve and co-workers.[17,56] Organostannane analogue **64** was radiolabelled *via* electrophilic iododestannylation using peracetic acid or chloramine-T as an oxidant (Scheme 30).



Scheme 30: Radiosynthesis of [^{123}I]PK11195

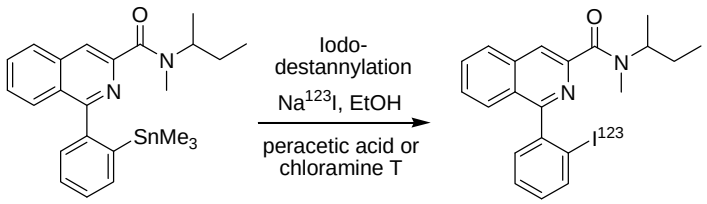
With reference to table 6,[17] **63** was radiolabelled to give [^{123}I]PK11195 with a slightly improved radiochemical yield, radiochemical purity and specific activity than using PK11195. However, the reaction time is also important and the reaction with Br-PK11195 **63** took more than double the time of that with PK11195. Compared to I-PK11195 **61**, Br-PK11195 **63** was labelled with a reduced isolated radiochemical yield and higher specific activity and again the reaction time was longer.



	Radiochemical yield (%)	Isolated radiochemical yield (%)	Radiochemical purity (%)	Specific activity (Ci/µmol)	Rxn time (min)
Br	66.0±4.0	41.3±11.2	>99	1.9±0.7	45
Cl	50-76	10-20	>94	0.26-0.91	20
I	-	>60	>99	0.23-0.35	30

Table 6: Halogen exchange reaction

The electrophilic iododestannylation technique using organostannane **64** proved to be very efficient (Table 7). There was a significant increase in the radiochemical yield and isolated radiochemical yield compared to the halogen exchange method. The use of chloramine-T as the oxidant was more beneficial as the reaction time dropped dramatically to 5 minutes producing [^{123}I]PK11195 **62** with an excellent specific activity of 25.5 Ci/μmol. One of the most important aspects in radiolabelling a ligand is to gain high specific activity. The higher the specific activity the less dosage is needed to be injected into the patient.



64 **62**

Reagent	Radiochemical yield (%)	Isolated radiochemical yield (%)	Radiochemical purity (%)	Specific activity (Ci/μmol)	Rxn time (min)
Peracetic acid	92.3±5.1	72.4±9.8	>99	8.3±0.3	25
Chloramine T	97.6±0.3	83.9±4.1	>99	25.5±7.4	5

Table 7: Electrophilic iododestannylation

2.2 Second Generation Quinolinecarboxamides

As mentioned before, PK11195 is the mostly widely used ligand for SPECT imaging but has a number of limitations regarding biodistribution. The high lipophilicity and low bioavailability means that on crossing the blood brain barrier the ligand is not particularly selective and interacts with a number of other receptors.[66] In 1997, the Cappelli group synthesised a wide range of PK11195 derivatives and tested them for their affinity with TSPO.[62] They discovered that the derivative quinoline-2-carboxamides had better affinity for TSPO and showed potential in biodistribution (i.e. they cross the blood brain barrier). The group then radiolabelled various analogues of quinoline-2-carboxamides with carbon-11 for PET imaging.[66] However there has been no reported synthesis of quinoline-2-carboxamides for SPECT imaging. Therefore, the aim of this stage of the project was to develop a small library of iodinated quinoline-2-carboxamides (Figure 16) and test them for affinity with TSPO.

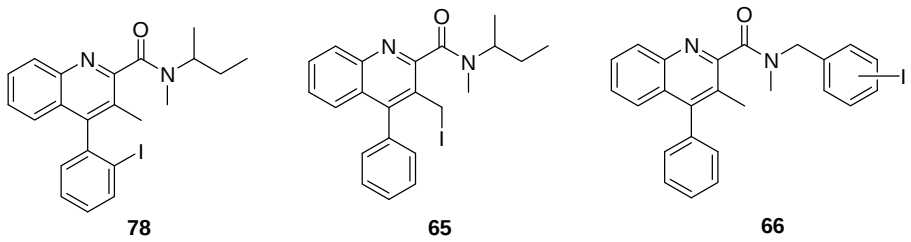
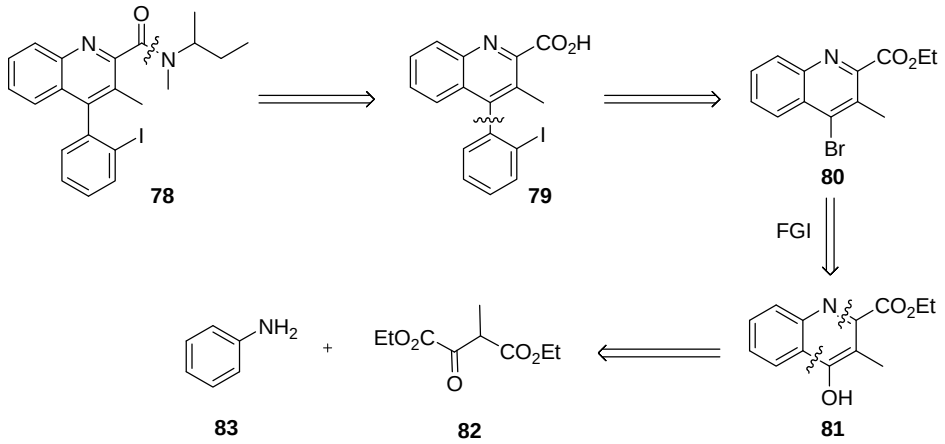


Figure 16: Second generation quinolinecarboxamides

The second generation ligands retain some of the important features that are needed to bind to the receptor. The quinoline and phenyl moiety are needed to bind into the lipophillic binding pockets in TSPO and the amide is essential for binding in the hydrophobic region of TSPO. The addition of the 3-methyl substituent was to increase the rigidity of the compound, preventing the phenyl ring from rotating and improving binding affinity. It was proposed that compounds **78** and **65** could be further modified by replacing *sec*-butylamine with other amines to try and improve binding affinity. Compound **66**, with the iodide attached to the amide side chain was devised to probe the binding pocket of the amide side chain. Also the iodide would be easier to radiosynthesise as it is not hindered by any other part of the compound.

2.2.1 Retrosynthetic Route of Quinolinecarboxamide 78

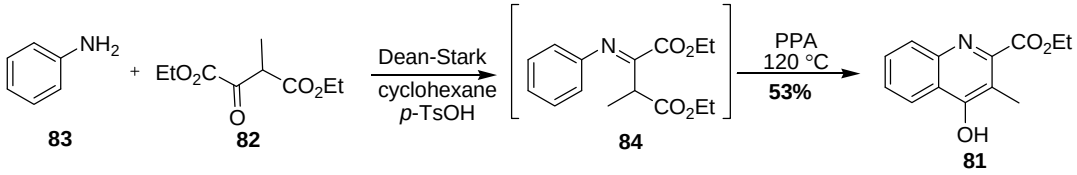
The proposed route to synthesis compound **78** utilises some of the chemistry used to synthesis PK11195. The retrosynthetic analysis of **78** is shown in Scheme 31. Firstly disconnection of the amide bond leads back to carboxylic acid intermediate **79**, followed by further disconnection of the phenyl ring to give **80**. Next functional group interconversion of the halide **80** gives hydroxyl compound, **81**. Formation of the quinoline carboxamide core can be prepared from commercially available aniline and diethyl oxalpropionate.



Scheme 31: Reterosynthesis of 78

2.2.2 Synthesis of Quinolinecarboxamide 78

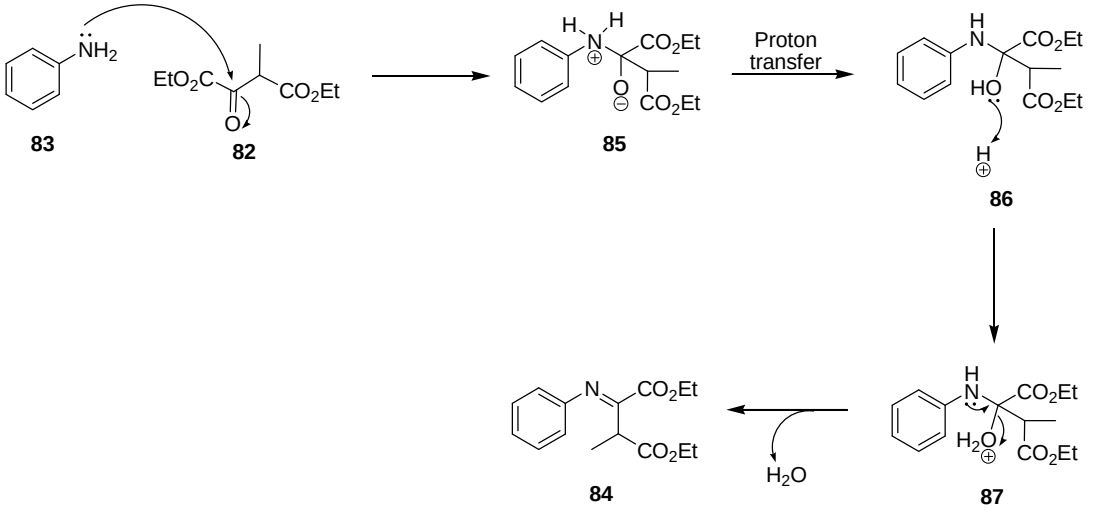
The first step in the synthesis of quinolinecarboxamide **78** was to prepare the quinoline carboxamide core. This was achieved in two steps by condensation of aniline with diethyl oxalpropionate followed by acid-mediated cyclisation of the imine to form **81** in 53% yield (Scheme 32).[67,68] This transformation is known as Combes quinoline synthesis (cf. Conrad-Limpach reaction).[67]



Scheme 32: Synthesis of 81

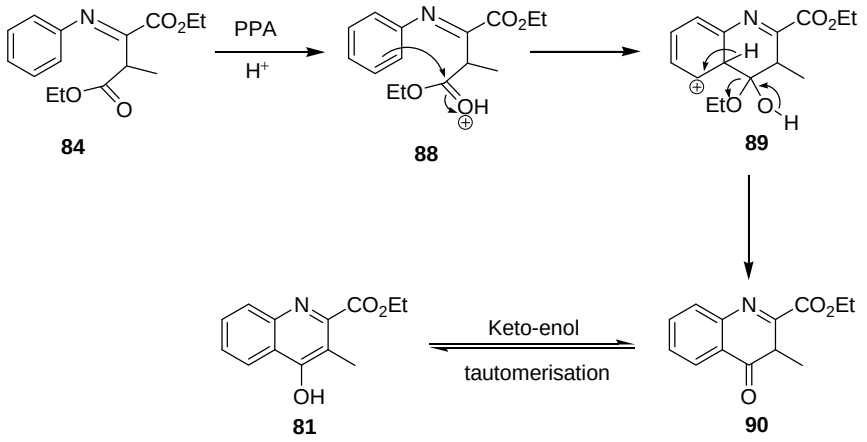
The reaction was carried out using Dean-Stark apparatus to remove water that was produced from the condensation of aniline and diethyl oxalpropionate. Initially, the reaction was attempted using benzene as the solvent but this did not go to completion. The reaction was repeated with increased temperature, but still did not go to completion. Next toluene was used with molecular sieves, to help remove water but still no improvement. A procedure reported by Bradbury and co-workers allowed the synthesis of imine **84**, this was achieved using cyclohexane and catalytic amount of *p*-toluenesulfonic acid.[67] Although imines are normally too unstable to purify on a silica column, imine **84** was thought to be stable enough to column due to the conjugation from the aryl and ester groups. However, after completion of the column, the NMR spectrum showed only starting material indicating that the imine had hydrolysed on the silica column. To overcome this, the imine was taken onto the next step without purification. The next step occurs *via* an acid catalysed cyclisation using polyphosphoric acid to form quinoline compound **81** in 53% over two steps.[68]

Formation of the imine **84** as shown in Scheme 33, occurs *via* nucleophilic attack of the carbonyl to form intermediate **85**. Proton transfer and dehydration then gives imine **84**.



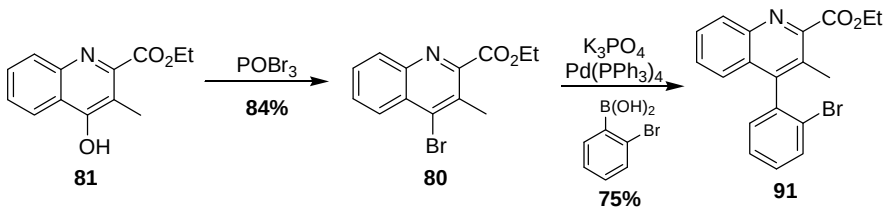
Scheme 33: Mechanism of the formation of imine 84

To conclude the formation of the quinoline ring system, the strong polyphosphoric acid aids the cyclisation by protonating the carbonyl group, followed by electrophilic aromatic substitution to form the ring (Scheme 34). Next, regeneration of aromaticity, elimination of ethanol and finally keto-enol tautomerisation, formed product **81**.



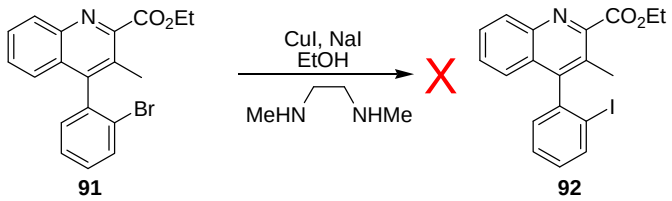
Scheme 34: Cyclisation of 84 to form 81

The next stage in the synthesis uses the same chemistry already used for the formation of the isoquinolines. Conversion of **81** to the bromide using phosphorus oxybromide gave **80** in 84% yield. A Suzuki cross coupling reaction was used to couple 2-bromophenylboronic acid to compound **80**, using tetrakis(triphenylphosphine)palladium(0). The reaction was successful and gave **91** in 75% yield.



Scheme 35: Synthesis of 91

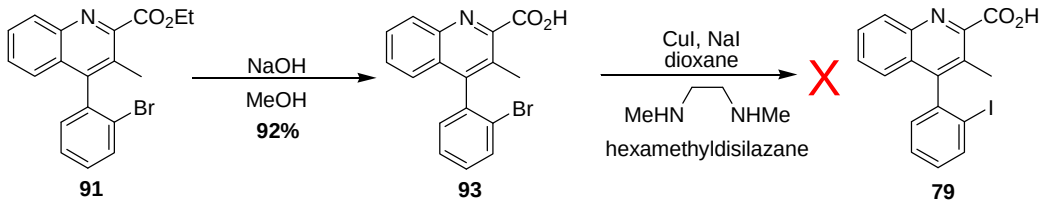
Completion of the synthesis of **78**, involved coupling of the appropriate amine side chain and a copper-catalysed halogen exchange reaction to introduce the iodide moiety. Based on previous work during the synthesis of PK11195 **32**, the iodide was introduced before the coupling of the amine. Therefore, to complete the quinoline carboxamide **78**, the copper-halogen exchange reaction was undertaken first (Scheme 36). The reaction was carried out using the same conditions developed for the iodination of PK11195. The only modification was a change of solvent of butanol to ethanol. This was because previously, the reaction at the ester stage underwent transesterification to produce the butyl ester. This is not an issue but can be avoided with the use of ethanol. Unfortunately, the reaction was ineffective and only starting material was recovered after five days.



91 **92**

Scheme 36: Iodination of 91

Another approach was attempted but using the carboxylic acid derivative instead (Scheme 37). The ethanol was exchanged for dioxane and hexamethyldisilazane was added to the reaction mixture. Since the carboxylic acid derivative was used, hexamethyldisilazane was included to convert the carboxylic acid to a more soluble trimethylsilyl ester, which could be cleaved during the work-up.[63] Again, the reaction did not work and starting material was recovered. Another attempt involved using butanol as the solvent in the reaction. However, this also showed no reaction and thus, this synthetic approach was abandoned.



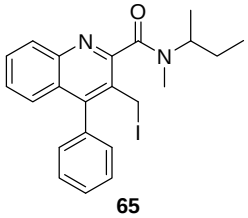
91 **93** **79**

Scheme 37: Iodination of 93

One possible explanation for the reaction not working could be due to the steric hindrance of the methyl substituent at position 3 of the quinoline ring which is in close proximity to the bromine atom.

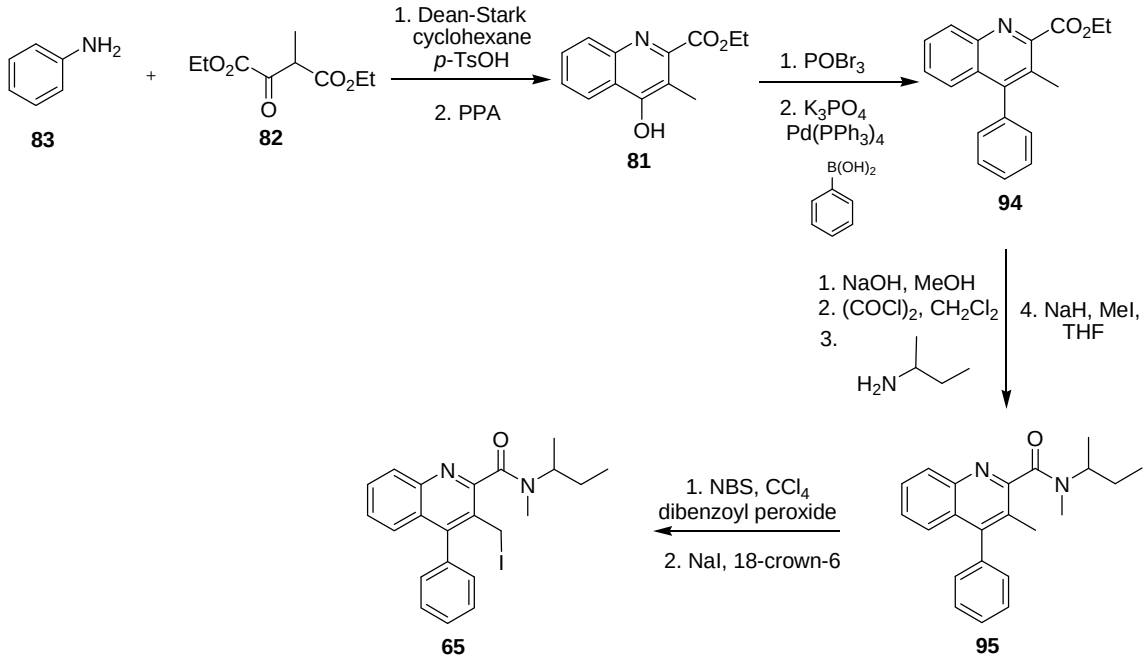
2.2.3 3-Iodomethylquinoline-2-carboxamides

With the unsuccessful completion of quinoline carboxamide **78**, the next aim was to synthesise other analogues, with the incorporation of the iodide atom at the 3-methyl position of the quinoline ring (e.g. **65**).



65

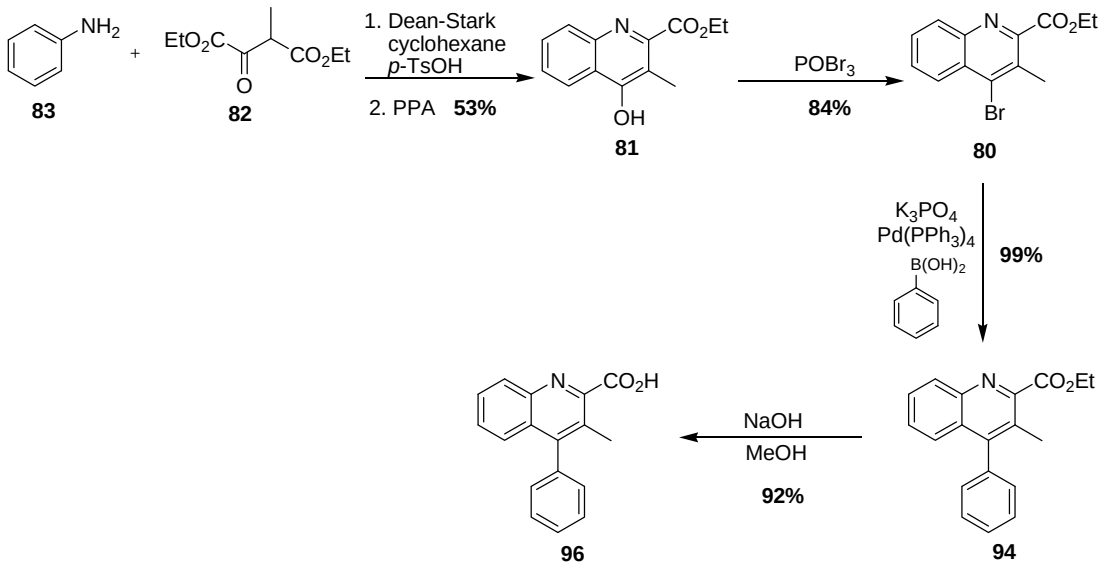
Utilising the same chemistry as before the synthesis of quinoline carboxamide **65** starts from condensation of aniline and diethyl oxalpropionate to form **81** (Scheme 38). Bromination followed by a Suzuki cross coupling reaction would give intermediate **94**. Next coupling of the amide side chain will give compound **95** and finally bromination at the methyl substituent, followed by conversion to iodide would give **65**.



83 **82** **81** **94** **95** **65**

Scheme 38: Proposed synthesis of 3-iodomethylquinoline-2-carboxamides

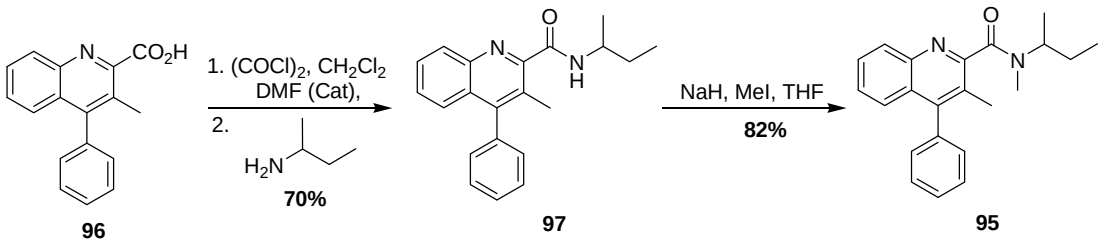
The formation of quinoline carboxamide **65**, started from commercially available aniline and diethyl oxalpropionate as described in Scheme 32. Condensation of aniline and diethyl oxalpropionate formed an imine, followed by acid mediated cyclisation which gave the quinoline product **81**. Bromination using phosphorus oxybromide gave **80** in 84% yield. Suzuki reaction using phenylboronic acid then gave **94** in an excellent 99% yield. Hydrolysis of the ethyl ester **94** using standard conditions afforded **96** in 92% yield.



83 **82** **81** **80** **94** **96**

Scheme 39: Synthesis of 96

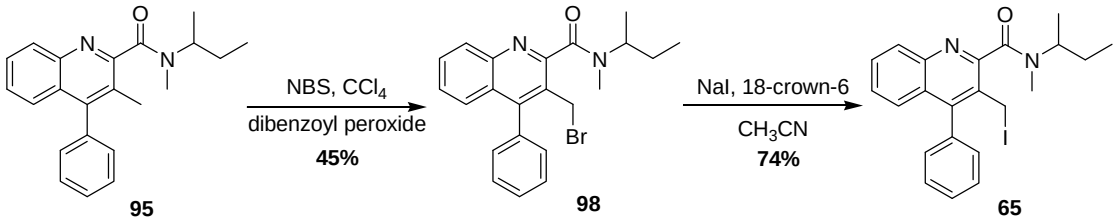
The first analogue of the 3-iodomethylquinoline-2-carboxamide series to be prepared, contained the same side chain as PK11195, *N*-methyl-*sec*-butyl amine. The series of transformations involved the reaction of carboxylic acid **96** with oxalyl chloride to form the acid chloride and *in situ* reaction with *sec*-butyl amine which gave **97** in 70% yield. Methylation of **97** using methyl iodide and sodium hydride gave **95** in 82% yield.



96 **97** **95**

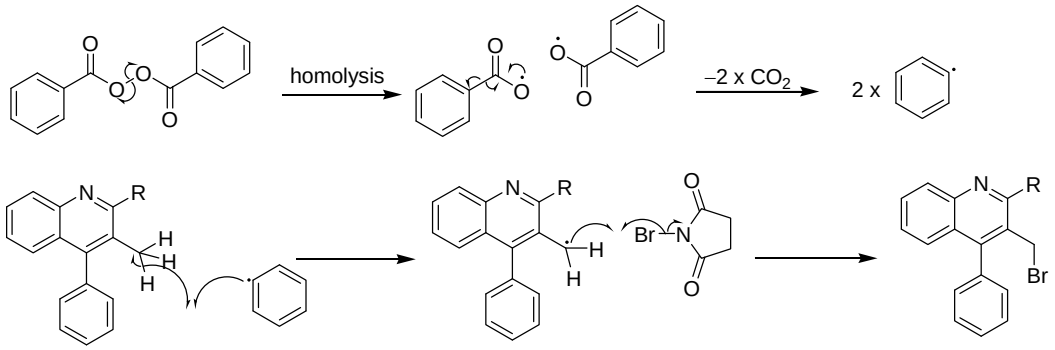
Scheme 40: Synthesis of 95

The final stages involved bromination of **95** *via* a radical reaction using *N*-bromosuccinimide (NBS) as the bromine source and dibenzoyl peroxide as the initiator. This gave bromide **98** in 45% yield. Nucleophilic substitution with sodium iodide and 18-crown-6 gave 3-iodomethylquinoline-2-carboxamide **65** in 74% yield.[5]



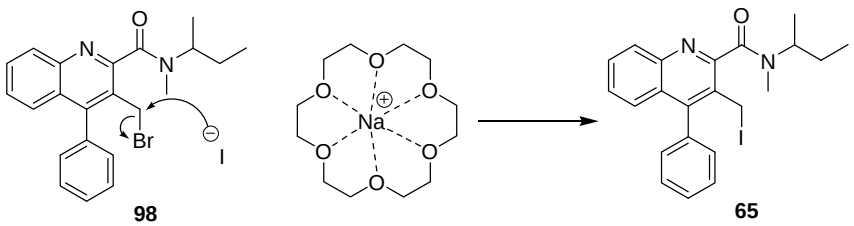
Scheme 41: Completion of 3-iodomethylquinoline-2-carboxamide 65

As shown in Scheme 42, the bromination occurs *via* a radical chain mechanism. Firstly, homolytic cleavage of the weak oxygen-oxygen bond in dibenzoyl peroxide forms free radicals. The initiator then selectively abstracts a hydrogen atom from the methyl to give a carbon centred radical, which can then abstract a bromide from NBS to form the required product.



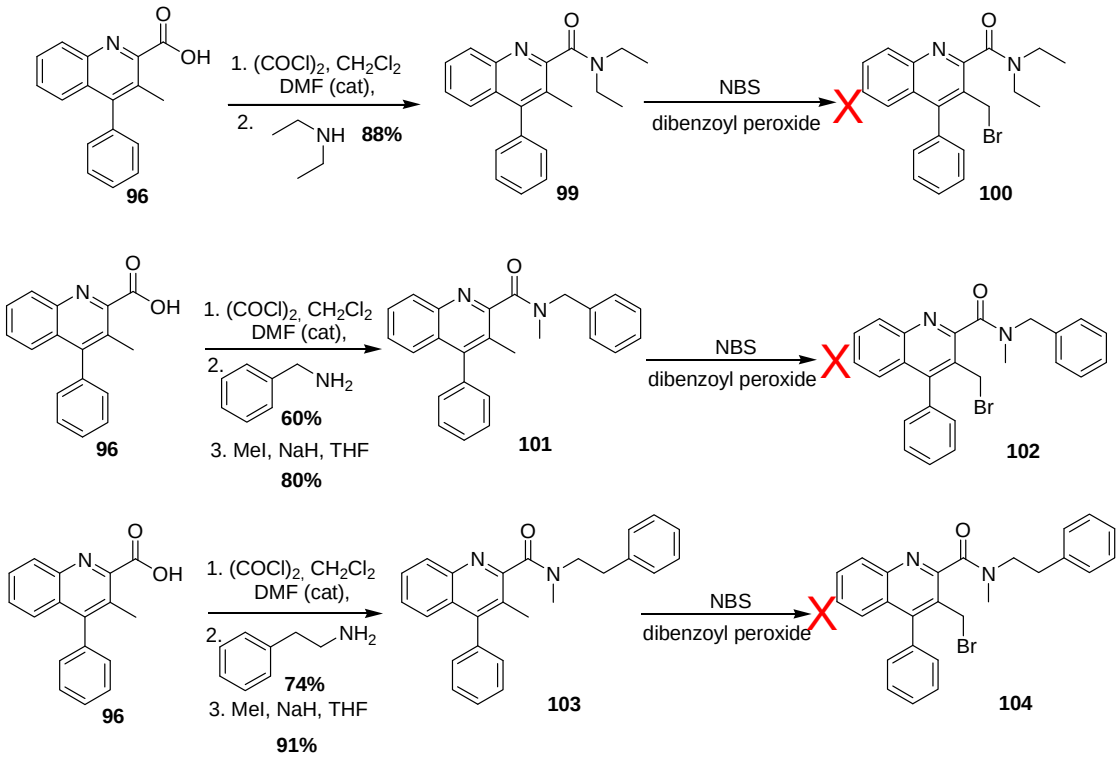
Scheme 42: Radical chain mechanism

Introduction of the iodide occurs *via* a Finkelstein-like reaction, with the 18-crown-6 having the effect of co-ordinating the sodium cation and leaving the naked iodide anion to carry out an S_N2 reaction with the bromide to give **65** in 74% yield.[5]



Scheme 43: Finkelstein-like reaction to give 65

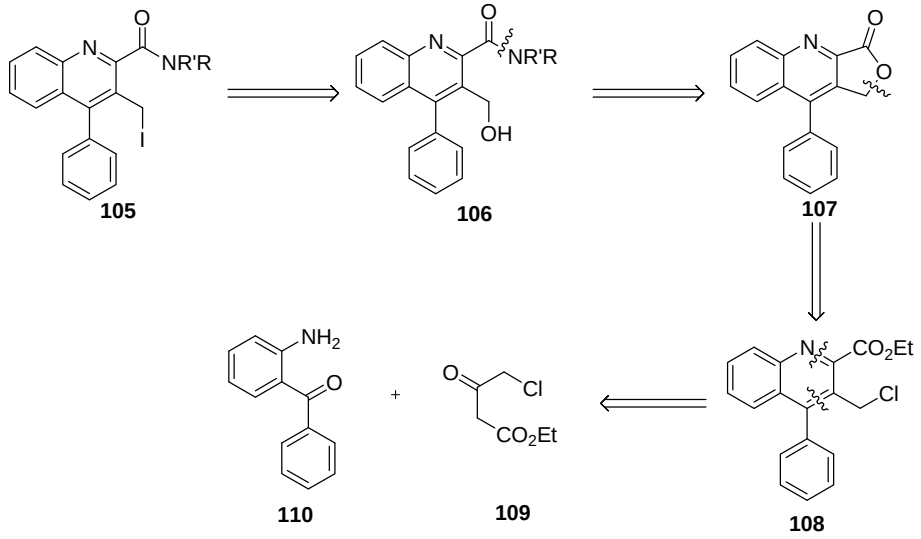
With the successful completion of the synthesis of **65**, the next stage was to introduce other amine side chains. Diethylamine, benzylamine and phenylethylamine were used to replace *sec*-butylamine. As before, the carboxylic acid **96** intermediate was reacted with oxalyl chloride to form the acid chloride and *in situ* reaction with diethylamine, benzylamine or phenylethylamine gave **99**, **101** and **103** in good yields. However problems were encountered during the NBS bromination stage, resulting in very low yields or no reaction. This could be due to introduction of bulkier amide side chains, preventing the bromide from reacting with the 3-methyl group. Instead of using NBS as the bromine source, 1,3-dibromo-5,5-dimethyl hydantoin (DBDMH) was tried as an alternative. However, low yields or no reaction was observed.



Scheme 44: Synthesis of 100, 102 and 104

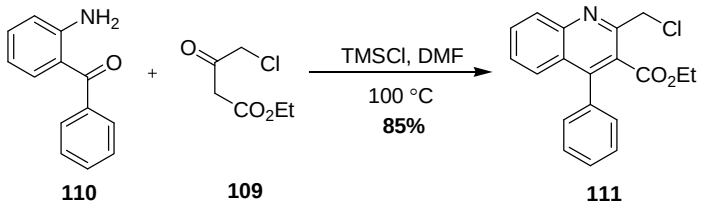
2.2.4 New Synthetic Route

Since the initial attempt failed to brominate derivatives **99, 101** and **103,** another route was developed to synthesise the target compounds. As shown in Scheme 45, the retrosynthetic analysis of **105** begins by functional group interconversion of the iodide to alcohol **106**, followed by formation of the lactone ring to give intermediate **107.** Next cleavage of the carbon oxygen bond to give **108** and further disconnection of the quinoline ring gave 2-aminobenzophenone **110** and ethyl 4-chloroacetoacetate **109** as the two starting materials.



Scheme 45: Retrosynthesis of 106

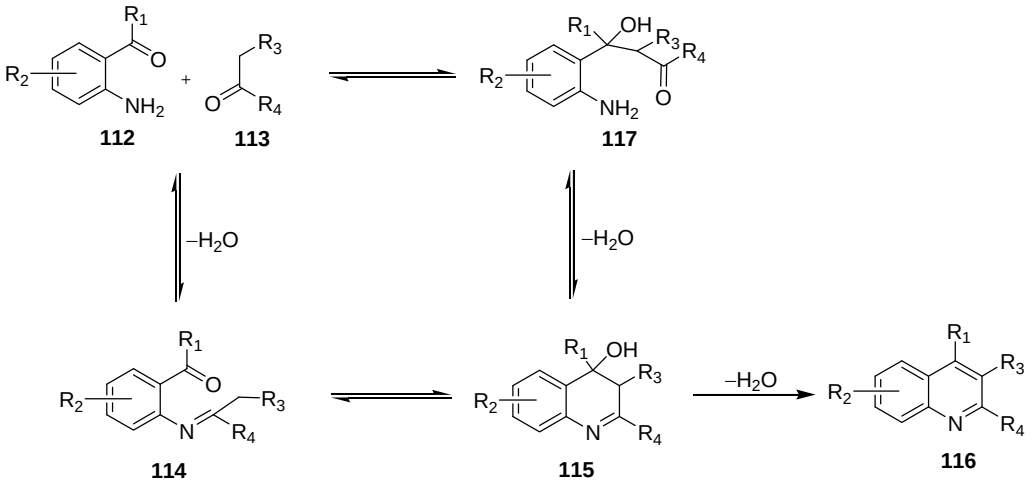
A chlorotrimethylsilane-mediated Friëdlander condensation was used to synthesise quinoline **111**.[69] Starting from commercially available 2-aminobenzophenone and ethyl 4-chloroacetoacetate the reaction was carried out in a Schlenk tube in DMF to give **111** in 85% yield.



Scheme 46: Synthesis of 111

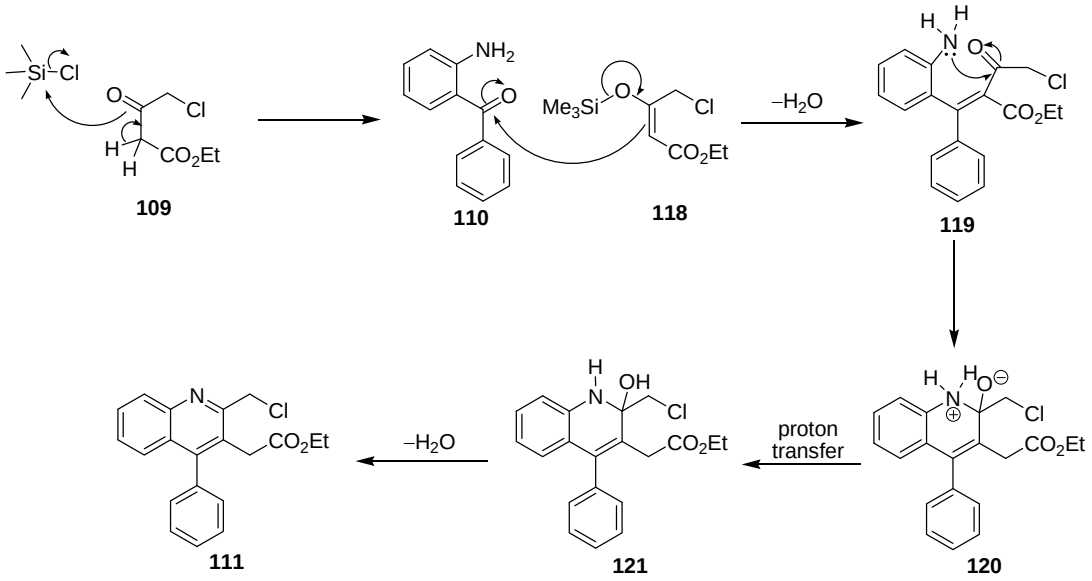
Although there are various methods for quinoline synthesis, the Friëdlander condensation reaction is able to produce polysubstituted quinolines in a staightforward approach.[69] Previous reaction conditions involved the use of basic or acidic catalysts, the latter being more effective. More recently iodine, Lewis acids and a combination of acidic catalysts and microwave irradiation have been useful. However, the use of catalysts can be very expensive and the use of specialised equipment limits the availability of the reaction.

Shown in Scheme 47 is the general mechanism for two possible ways in which the Friëdlander reaction can proceed. Starting from 2-amino substituted aromatic carbonyl compound **112** and ketone **113**, the first step involves the formation of Schiff base **114** as an intermediate. Next an intramolecular aldol reaction forms the hydroxy imine **115**, followed by dehydration to give quinoline **116**. The second route occurs *via* an intermolecular aldol reaction to give **117**, followed by loss of water to afford quinoline **116**.[70]



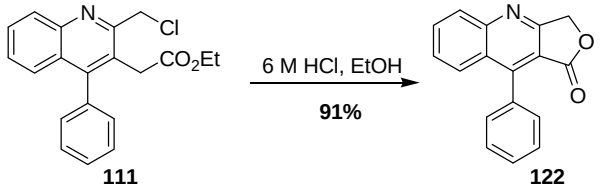
Scheme 47: General mechanism of the Friëdlander reaction

Rayabukhin and co-workers reported a procedure for the Friëdlander synthesis of polysubstiuted quinolines using chlorotrimethylsilane (TMSCl).[69] With the successful completion of a number of quinolines with high yields using TMSCl, the same reaction conditions were adopted for the synthesis of **111**. Shown in Scheme 48 below is the reaction mechanism in the presence of TMSCl. Reaction of TMSCl generates a silyl enol ether **118** which attacks the electrophilic carbonyl of 2-aminobenzophenone **110** to form intermediate **119**. Cyclocondensation closes the ring to form an amine **121**, followed by dehydration to give quinoline **111**.



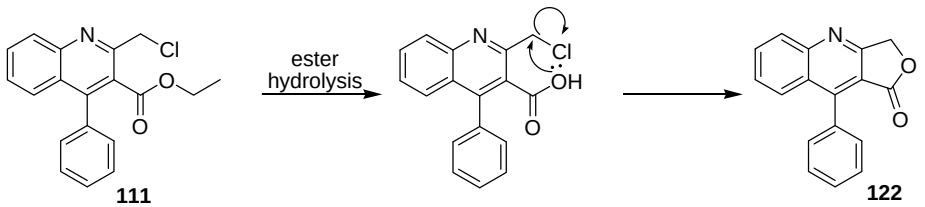
Scheme 48: Friëdlander synthesis

The next stage was to couple the amine side chain however before this could be completed there were a number of reactions to be performed in order for the ester to be in position 2. Firstly, formation of a lactone ring using 6 M hydrochloric acid in ethanol gave 9-phenylfuro[3,4-*b*]quinolin-1(3*H*)-one **122** in an excellent 91% yield (Scheme 49).[71]



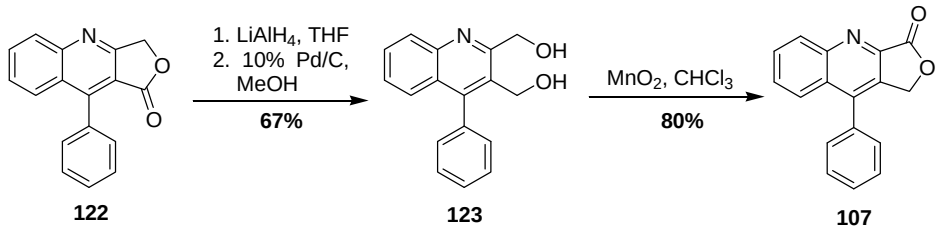
Scheme 49: Formation of lactone 122

One possible way in which the lactone ring could be formed, would be ester hydrolysis to form a carboxylic acid followed by attack of the lone pair on the oxygen to the carbon chloride bond to form the lactone ring (Scheme 50).



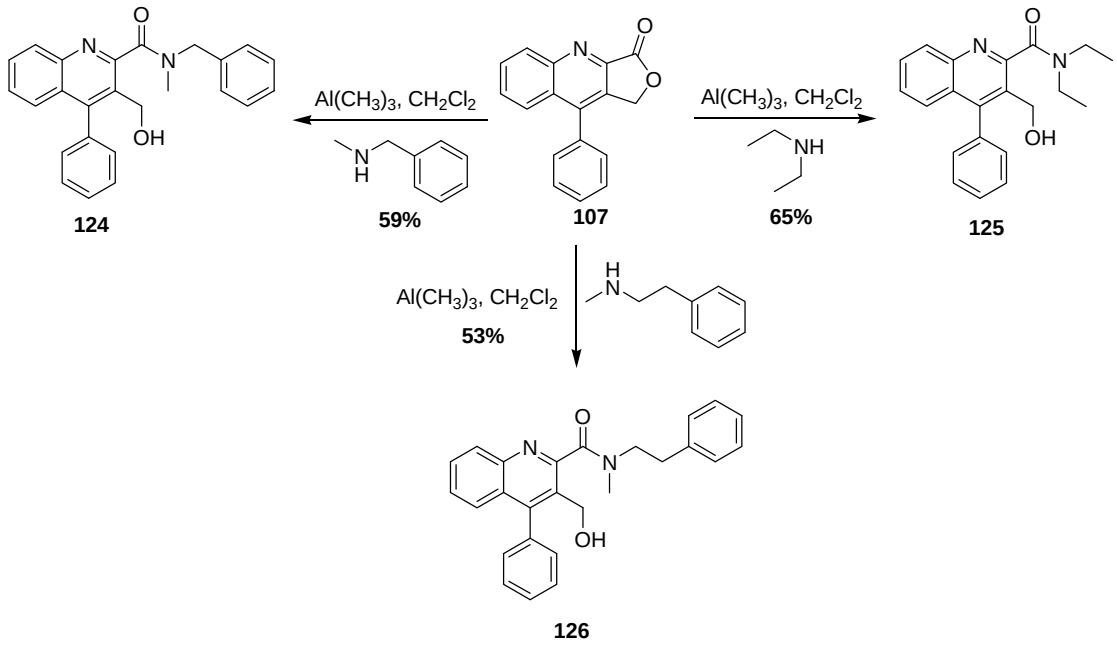
Scheme 50: Mechanism for lactone formation

Next the lactone was reduced using lithium aluminium hydride to achieve diol **123** in a moderate 67% yield. During the reduction, small quantities of the dihydroquinoline are also formed. The quinoline was reformed by treatment of the reaction mixture with palladium on carbon. Regioselective oxidation with manganese dioxide gave the key isomeric lactone intermediate **107** in 80% yield.[71]



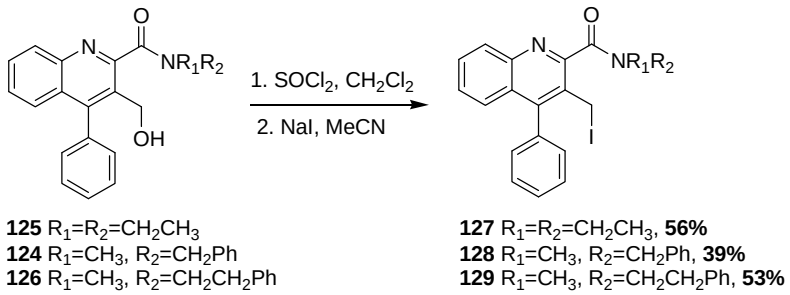
Scheme 51: Synthesis of 107

To incorporate the desired amide side-chains, lactone **107** underwent aminolysis in the presence of trimethylaluminum with a series of secondary amines to afford 3-hydroxymethylquinoline-2-carboxamides **124, 125** and **126** in reasonable yields (Scheme 52). The trimethylaluminium reacts with the amine to form a complex, which makes the amine more nucleophilic allowing the amine to attack the carbonyl forming a new C-N bond.



Scheme 52: Synthesis of 124,125 and 126

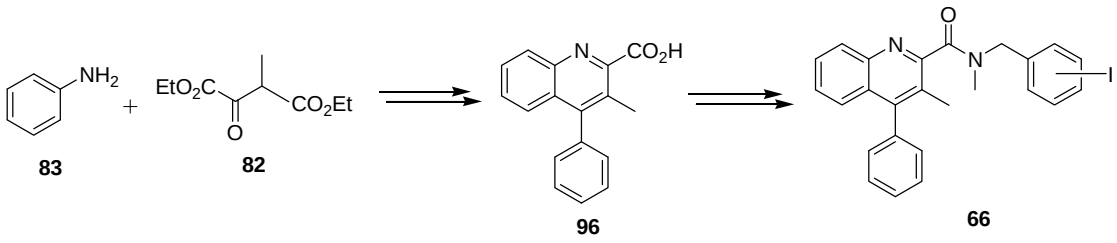
Completion of the synthesis was accomplished by chlorination of the hydroxymethyl group with thionyl chloride followed by a halogen exchange reaction with sodium iodide which gave 3-iodomethylquinoline-2-carboxamides in moderate yields (Scheme 53). Formation of the iodide was achieved using sodium iodide as the iodide source in acetonitrile. The reactions went to completion within two hours to afford **127, 128** and **129**. During the development of this reaction, it was noted that a non-aqueous work-up was required to provide the iodides cleanly.



Scheme 53: Synthesis of 127, 128 and 129

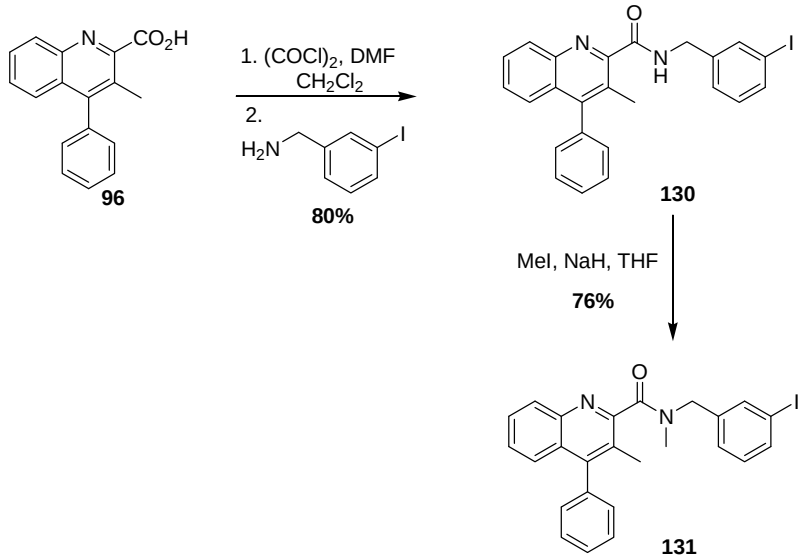
2.2.5 3- and 4-Iodobenzyl Analogues

The proposed synthesis of 3- and 4-iodobenzyl analogues involves the same chemistry used to synthesis compound **65**. Starting from aniline **83** and diethyl oxalpropionate **82,** a number of key reactions were used to form carboxylic acid **96**, followed by acid chloride formation and finally reaction with 3- or 4-iodobenzylamine to form **66**.



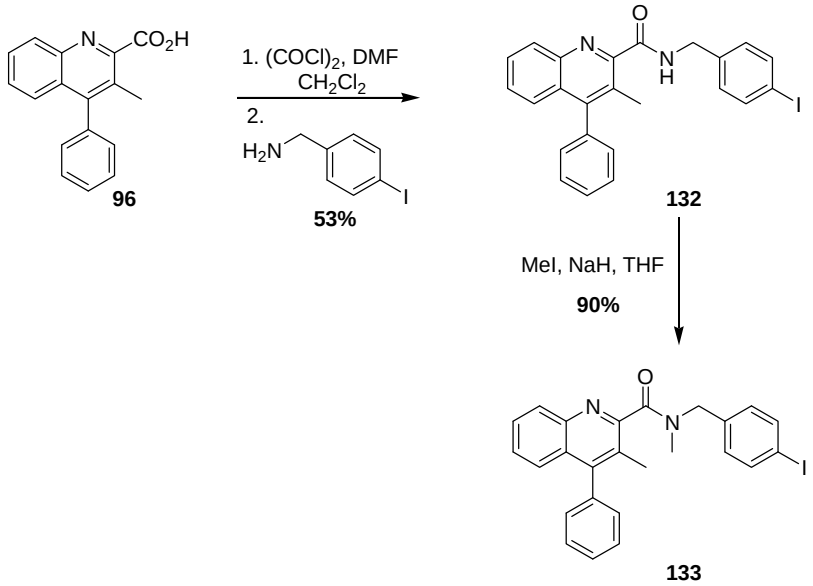
Scheme 54: Synthesis of 3- and 4-Iodobenzyl analogues

Starting from previously prepared quinoline carboxylate **96**, the acid chloride was formed, followed by reaction with 3-iodobenzylamine to give **130** in a good 80% yield (Scheme 55). The final step involved methylation using standard conditions, iodomethane and sodium hydride to give **131** in 76% yield.



Scheme 55: Synthesis of 131

The next analogue that was prepared involved the coupling of 4-iodobenzylamine. The reaction procedure was the same as the 3-iodobenzyl analogue **131**, firstly conversion to the acid chloride, followed by coupling with 4-iodobenzylamine which gave **132** in 53% yield (Scheme 56). Finally methylation gave desired analogue **133** in 90% yield.

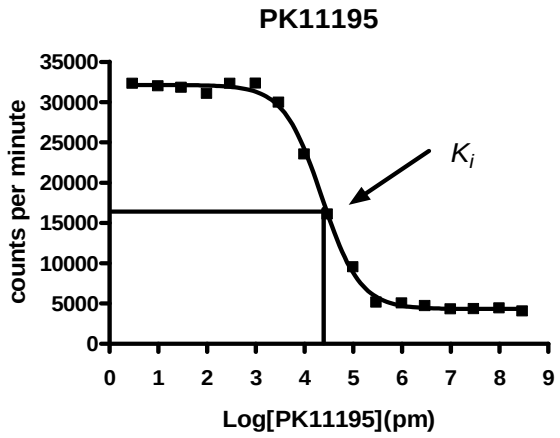


Scheme 56: Synthesis of 133

2.2.6 Biological evaluations

In discovering a new molecular imaging agent, it is important to identify direct binding interactions of the ligand to the required receptor. The interaction between the ligand and receptor can be quantified in ligand binding assays. With the successful completion of a new library of compounds, the next aim was to investigate the binding affinity, with the intent of finding an analogue that proved to have higher binding affinity for TSPO than PK11195. A competition binding assay technique was used to evaluate *in vitro* binding using rat brain homogenates. Competition binding assays test the binding affinity of new compound versus a labelled ligand. Once the assay was complete, competition curves were obtained by plotting ligand binding (counts per minute) against the log concentration of the competing ligand. From the curve, the K_i of the receptor for the competing compound was determined. The K_i is the equilibrium dissociation constant; it is the concentration of the unlabelled compound that would bind to half the binding sites at equilibrium.

Firstly, the binding affinity of PK11195 was determined, in order to use it as a direct comparison to the new compounds. Shown below in Figure 17, is the competition binding curve of PK11195. The top of the curve is a plateau at a value equal to radioligand binding in the presence of lower concentration of the competing unlabelled compound (i.e Totals). The bottom of the curve is a plateau equal to nonspecific binding i.e. binding of high concentrations of unlabelled ligand.[72] The equilibrium point in the curve represents the K_i value corresponding to the concentration when half the binding sites are occupied by the unlabelled ligand. The K_i value was determined from nonlinear regression analysis using GraphPad Prism Version 4 (GraphPad Software Inc). The K_i can be calculated from the equation below developed by Cheng and Prussoff.[73]



$$K_i = \frac{EC_{50}}{1 + \frac{[ligand]}{K_d}}$$

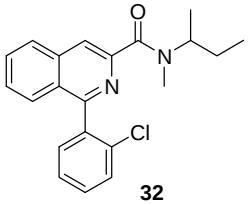
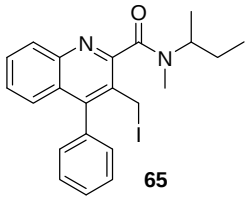
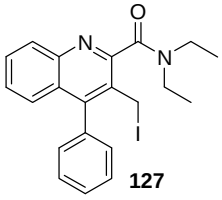
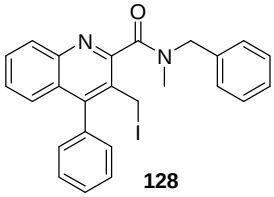
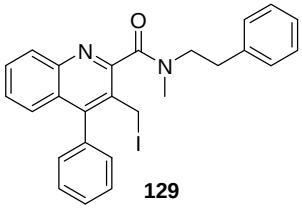
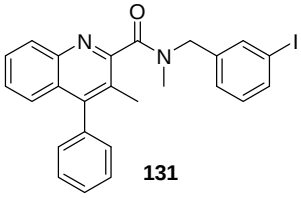
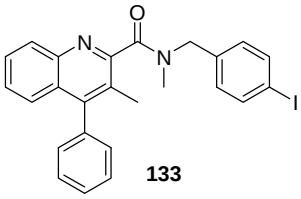
Figure 17: Competition binding curve of PK11195

After determining the K_i for PK11195 (9.8 ± 1.6 nM), the same conditions were used to determine the binding affinity of the new compounds. For each compound, three independent experiments were carried out to determine the final K_i value. From Table 8, it can be seen that two compounds from the 3-iodomethylquinoline-2-carboxamide series have excellent binding affinity for TSPO. Compound **128** with the benzyl side chain was found to have a K_i value of 26.1 nM whilst, the diethyl analogue **127** has an even better binding affinity of 12.0 nM, similar to that of PK11195, the most widely used imaging agent.

Compound **65**, with the same amide side chain as PK11195 **32** has low binding affinity to TSPO with a K_i value of 173 nM. The last compound in the 3-iodomethylquinoline-2-carboxamide series, compound **129** with an extra carbon in the amide position compared to compound **128,** again has a lower binding affinity of 411 nM.

The second group of analogues with the iodine incorporated into the amide side chain, compounds **131** and **133,** show poor affinity for TSPO. Compound **131** has a K_i value of 455 nM while compound **133** has a significantly lower affinity.

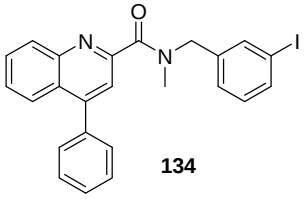
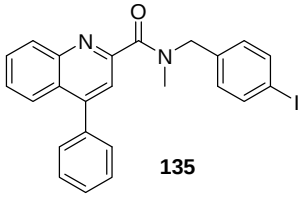
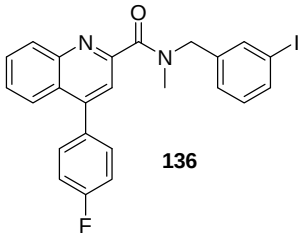
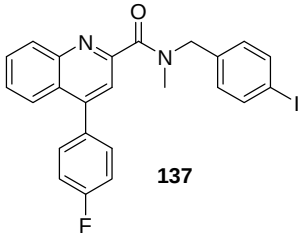
As well as determining how well the ligand binds it is also important to determine the ligands distribution i.e. how easily the ligand can reach its intended target in the body, and how long it will remain in the body in an active form once it reaches the target. A high log *P* value means that the ligand is too lipophilic and not able to cross cell walls. A low log *P* value indicates that the ligand will be too hydrophilic and will probably not cross the blood brain barrier and will be eliminated from the body quickly. The log *P* values for these compounds were determined by our collaborator Adriana A. S. Tavares from the Division of Clinical Neuroscience. The log *P* was determined using an HPLC method involving C18 chromatography. The compounds all have relatively high log *P* values which are not ideal for imaging agents. The diethyl analogue **127** still has a high log *P* of 4.76 but is comparable to other SPECT imaging agents known to cross the blood brain barrier.

Compound	K_i [a] (nM)	Log *P*
 **32**	9.8 ± 1.6	3.85
 **65**	173 ± 35	5.17
 **127**	12.0 ± 1.3	4.76
 **128**	26.1 ± 4.7	5.39
 **129**	411 ± 62	5.17
 **131**	455 ± 51	5.03
 **133**	>1000	5.12

[a] Calculated from three independent experiments.

Table 8: Binding affinity of quinoline-2-carboxamide analogues

Another set of compounds similar to **131** and **133** without the methyl substituent on the quinoline ring were synthesised by A. Brunet from the group and tested for binding affinity against TSPO. These also showed very poor affinity for TSPO (Table 9). Compounds **134** and **135** have K_i values greater than 1000 nM and with high log *P* values rendering them unsuitable as imaging agents. The final group of analogues to be tested incorporated a fluoride at the *para*-position of the phenyl ring. Incorporation of a fluoride and iodide atom allows the compound to be used for both PET and SPECT imaging. However, the binding affinity was again high (K_i >1000 nM).

Compound	K_i (nM)	Log *P*
 **134**	>1000	5.11
 **135**	>1000	5.12
 **136**	>1000	5.16
 **137**	>1000	5.21

[a] Calculated from three independent experiments.

Table 9: Binding affinity of compounds 134, 135, 136 and 137

2.2.7 Space Filling Models

Using a software package called Spartan by Wavefunction, Inc. space filling models were constructed to give an insight into the overall molecular size and shape of these compounds. Shown in Figure 18, are the energy minimised models of various compounds. The first space filling model shows I-PK11195. The large purple atom at the back of the structure is the iodine atom and the red atom is the oxygen. This model proposes why I-PK11195 has high binding affinity to TSPO. The iodine atom does not hinder formation of the key hydrogen bonds when the amide side-chain binds to the receptor. Compound **127** has similar attributes to PK11195, the large iodine atom is situated in the cleft of the compound therefore allowing the compound to bind effectively to the receptor. Compound **133,** however, has poor affinity due to the large iodine atom possibly blocking the amide side-chain from binding.

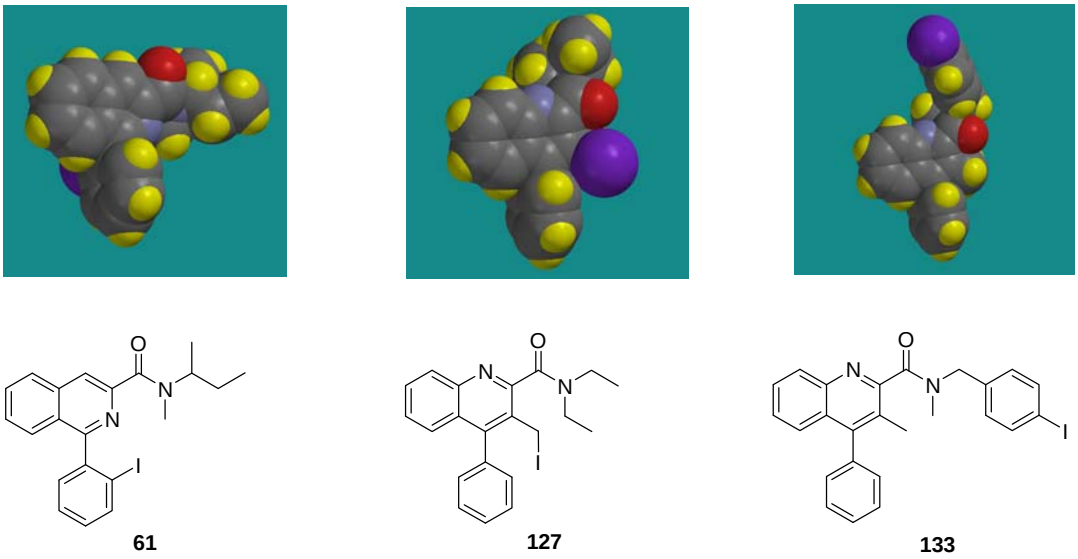


Figure 18: Space filling models of compound 61, 127 and 133

2.3 Third Generation Quinoline-2-carboxamides

Having learned that the larger the amide side chain the worse the binding affinity to TSPO was, the next step forward was to develop new compounds with shorter amide side chains. From the binding assay experiments, the diethyl compound **127** was shown to have the best affinity and therefore it was decided to use the same core structure but incorporate a shorter amine substituent. Also, a morpholine substituent was used to try and lower the log *P* value.

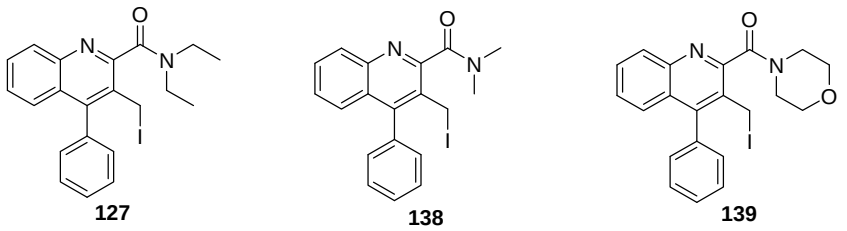
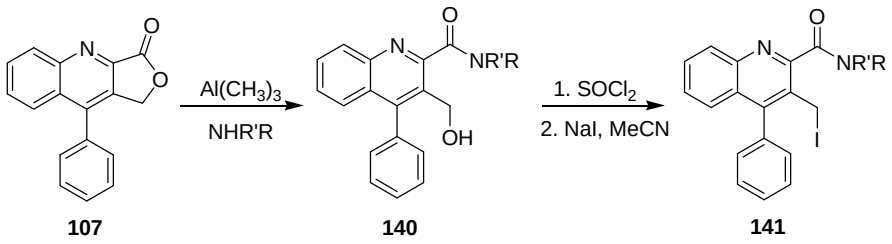


Figure 19: Third generation quinoline-2-carboxamides

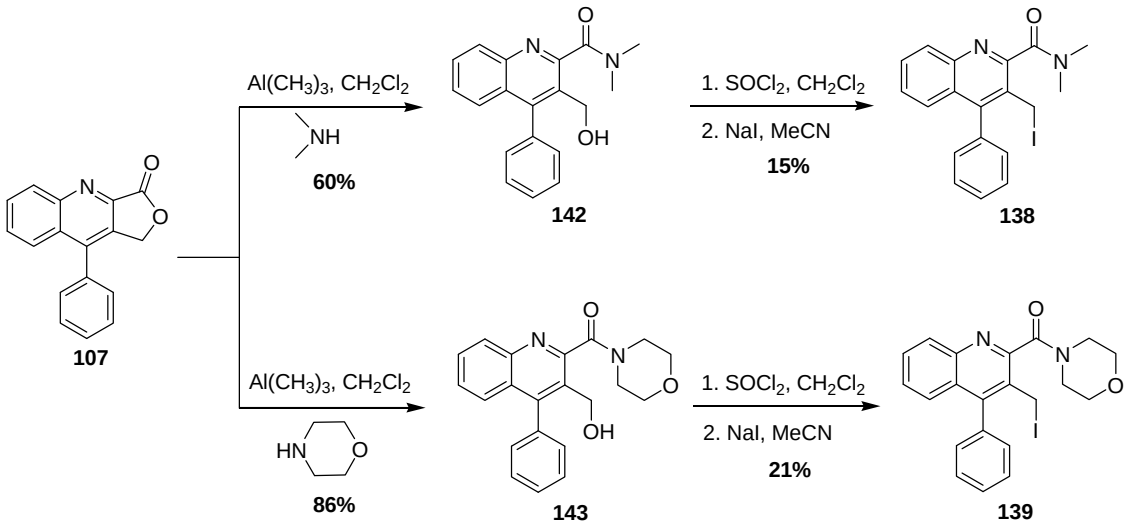
The synthesis of compounds **138** and **139** followed the same synthetic route used previously to synthesise 3-iodomethyl-4-phenylquinoline-2-*N*-diethylcarboxamide **127**. Starting from a common intermediate lactone **107**, the amine was coupled to form **140**. Introduction of the iodide was achieved by reaction of the hydroxyl group with thionyl chloride to form the chloride, followed by reaction with sodium iodide to give **141**.



Scheme 57: Synthesis of 141

As shown in Scheme 58, the coupling of the appropriate amine involved aminolysis of the lactone ring using trimethylaluminium to afford compound **142** and **143** in good yields. Purification of the morpholine analogue was problematic, whilst on the silica column the morpholine ring was removed and the lactone intermediate **107** was reformed. This was due to the fact that morpholine is a good leaving group and since the silica is acidic it helps promote the formation of the lactone ring. The next stage was formation of the chloride derivative, which was achieved by reaction of the hydroxymethyl group with thionyl

chloride. With the completion of the chloride derivative, the final step involved a halogen exchange reaction of the chloride for the iodide. This was carried out in the presence of sodium iodide in acetonitrile which gave 3-iodomethylquinoline-2-carboxamides **138** in 15% yield and **139** in 21% yield over the two steps.

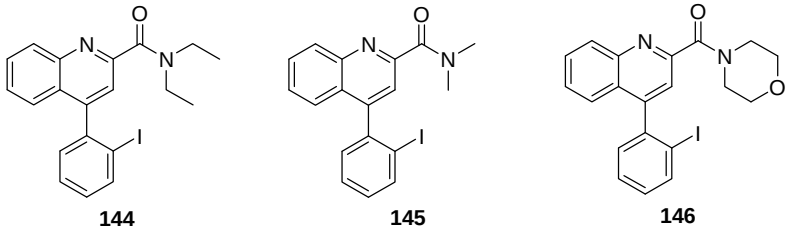


107 **142** **138** **143** **139**

Scheme 58: Synthesis of 138 and 139

2.3.1 Final Analogues

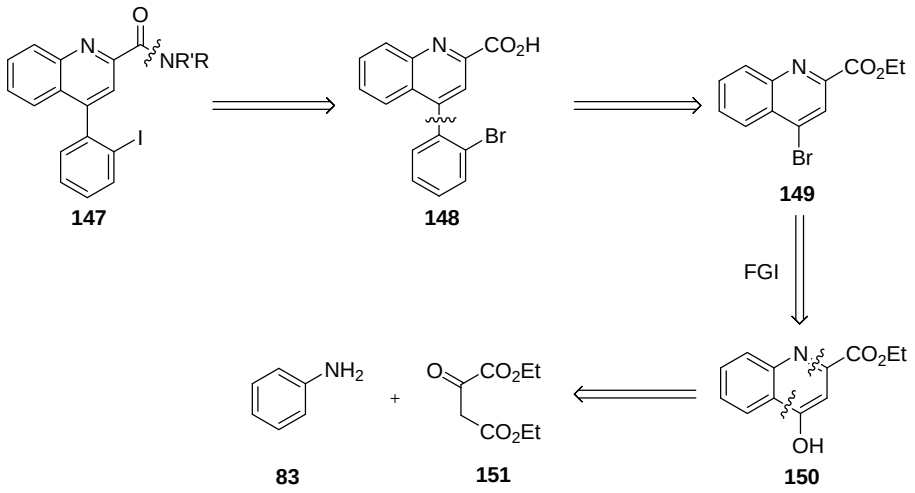
The final series of analogues that were synthesised incorporated the iodide onto the phenyl ring in a similar fashion to PK11195. These compounds were of interest as they are more likely to be more stable than the 3-iodomethylquinoline-2-carboxamide due to the stronger carbon iodine bond. The synthetic route followed the same procedures developed in the synthesis of 4-phenyl-3-iodomethylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide **65**. Shown in Figure 20, are the three new proposed compounds.



144 **145** **146**

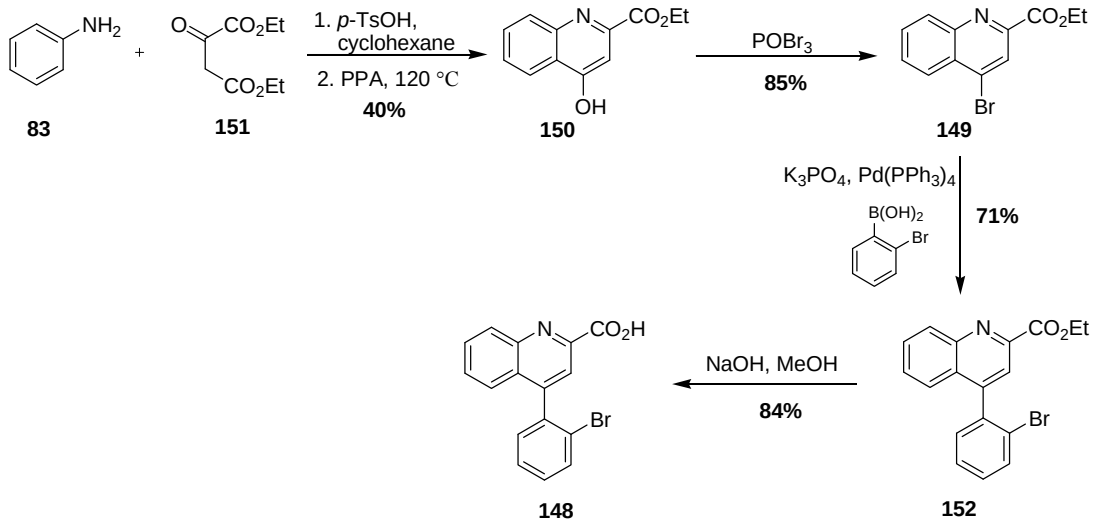
Figure 20: Final analogues

The proposed retrosynthetic analysis is shown in Scheme 59. Disconnection of the amide side chain leads back to carboxylic acid **148**. Further disconnection of the 2-bromophenyl ring gives **149,** followed by functional group interconversion of the halide to a hydroxyl group, **150**. Quinoline can be prepared from commercially available aniline **83** and diethyl oxalacetate **151**.



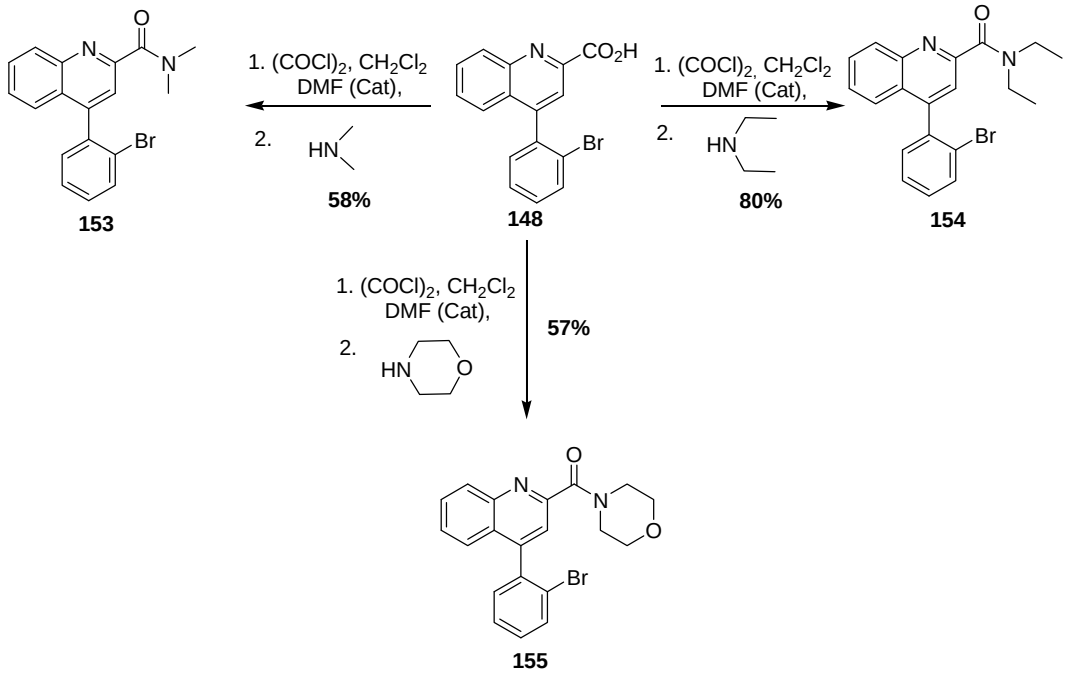
Scheme 59: Retrosynthesis of 147

The quinoline carboxamide was prepared in two steps by condensation of aniline **83** with diethyl oxalacetate **151** to form **150** in 40% yield over two steps (Scheme 60). Bromination of the hydroxyl group on **150** using phosphorus oxybromide afforded **149** in an excellent 85% yield. Next, a Suzuki reaction using 2-bromophenylboronic acid in the presence of catalytic tetrakis(triphenylphosphine) palladium(0), introduced the *ortho*-bromophenyl ring, which gave **152** in a good 71% yield. The last step before the amide coupling involved ester hydrolysis using sodium hydroxide in methanol/water to form the carboxylic acid intermediate **148** in 84% yield.



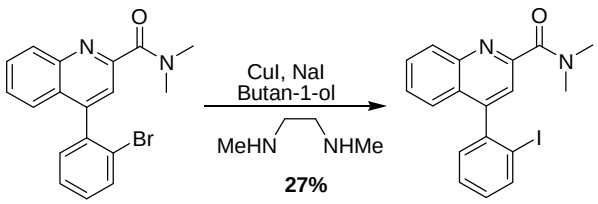
Scheme 60: Synthesis of 148

Formation of the amide side chain was carried out by incorporating diethylamine, dimethylamine or morpholine amine using the same reaction conditions as before. Firstly, formation of the acid chloride using oxalyl chloride, followed by reaction with the required amine gave target compounds **153**, **154** and **155** in good overall yields.



Scheme 61: Synthesis of 153, 154 and 155

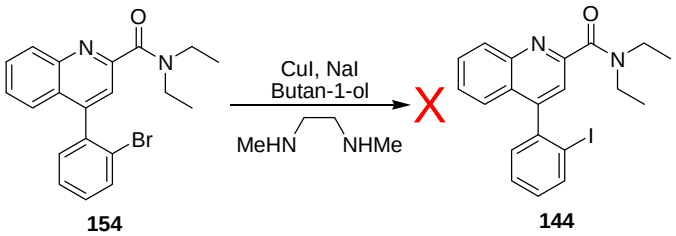
Completion of the synthesis to give the final products involved a copper-catalysed aromatic Finkelstein reaction to exchange the bromide to the iodide. The first compound to be synthesised was the dimethyl analogue **153**, using the same reaction conditions developed by Klapers and Buchwald.[63] The first attempt was unsuccessful with only recovery of starting material after four days. The reaction was repeated and after 5 days, analysis using mass spectrometry showed the presence of the desired iodide along with the bromide starting material. The reaction was attempted again and heated for 7 days. This time, the reaction went to completion and gave iododinated compound **145** in 27% yield. The yield of **145** was low but it gave sufficient amounts of **145** for binding experiments.



153 **145**

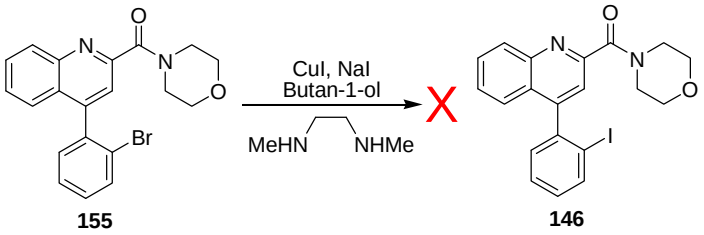
Scheme 62: Iodination of 153

The synthesis of the final two analogues, diethyl **144** and morpholine **146** were unsuccessful (Scheme 63 and 64). The reaction was attempted using the same reaction conditions as for **153** and monitored over the course of 2-3 weeks. After 3 weeks, the reaction was thought to have gone to completion but the mass spectrum for each analogue showed the presence of both bromide and iodide. Attempts to separate the bromide and iodide derivatives by flash column chromatography were unsuccessful. The reaction was not attempted again as after one week the reaction is not a viable method as it takes too long. Increasing the size of the amide side chain adds extra bulk therefore, steric hindrance probably retards halogen exchange between the bromide and iodide and slows the reaction significantly.



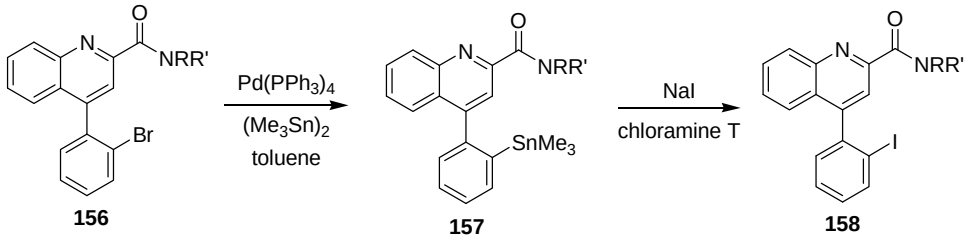
154 **144**

Scheme 63: Iodination of 154



Scheme 64: Iodination of 155

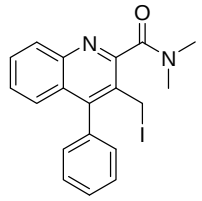
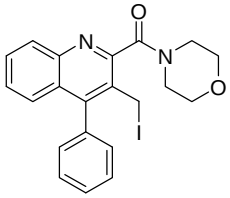
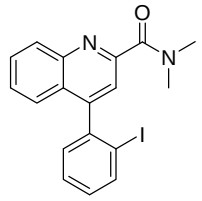
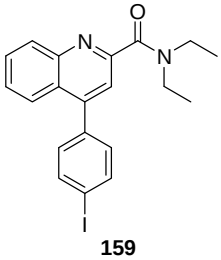
Due to time restraints the reactions were not modified. However future work on these analogues would consist of another method of iodination. One way in which the problem could be overcome would be to prepare the iodide *via* an organostannane derivative (Scheme 65). Failing that, another approach would be to use microwave conditions, to increase the rate of the halogen exchange reaction.



Scheme 65: Synthesis of 158

2.3.2 Biological Evaluations

The binding affinity of the small library of compounds for TSPO was carried out *in vitro* using the same conditions as previous. Shown in Table 10 are the final binding assay results of four analogues that were tested. The dimethyl analogue **138,** a derivative of the diethyl analogue that was shown to have good affinity (K_i = 12 nM), showed poor affinity for TSPO. The best result was the morpholine derivative **139** with binding affinity of K_i = 27.8 ± 2.89 nM, however the result was still not good enough to proceed further and develop this into an imaging agent.

Compound	K_i (nM)	Log *P*
 **138**	>1000[a]	4.50
 **139**	27.8 ± 2.89[b]	3.62
 **145**	>1000[a]	2.91
 **159**	491.3 ± 154[a,*]	4.50

[a] Calculated from three independent experiments

[b] Calculated from two independent experiments

* Synthesised by Jonathan Gardiner from our group

Table 10: Binding affinity of quinoline-2carboxamide analogues

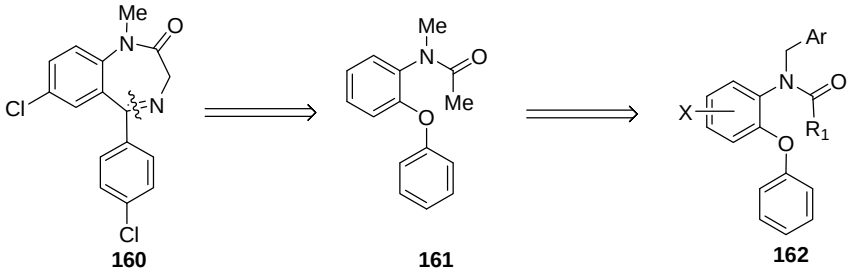
2.3.3 Conclusions

Two successful routes were developed to synthesise new iodinated quinoline-2-carboxamides. The first route involved a two step condensation reaction to form the quinoline ring system and the second a chlorotrimethylsilane-mediated Friëdlander condensation reaction. The second half of the project determined the binding affinity of these compounds for TSPO. The results showed that the diethyl analogue **127** had the best affinity and that compounds with large amide side chains, particulary containing the large iodine atom had poor affinity for TSPO. This could be easily observed from space filling models which showed the relative size of the iodine atom and how this possibly prevents binding to the TSPO active site

Whilst compound **127** has good binding affinity, the iodomethyl moiety, a well know alkylating group, could cause stability problems. This therefore, led the investigation of iodophenyl derivatives. However, due to poor affinity for TSPO, a new class of ligands need to be investigated.

2.3.4. Future Work

Future work for this project would be to synthesise a new class of ligands that would have high binding affinity to TSPO. Okubo and co-workers reported a class of compounds called phenoxyphenylacetamides, that were derived by opening the diazepine ring of Ro5-4864 (Scheme 66).[74] They exhibit high and selective affinity for TSPO with an IC_{50} in nanomolar range (0.28-0.98 nM). These could also be designed with suitable log *P* values that could generate compounds with good pharmacokinetics.



Scheme 66: Retrosynthesis of Ro5-4864

As well as designing good analogues that bind well to TSPO, it would also be beneficial to develop analogues with multi-labelled positions which could be used for either PET or SPECT imaging. This would result in more widespread use of a single ligand.

2.4 Synthetic Approaches Towards The First Total Synthesis of Crispine C

2.4.1 Introduction

Carduus Crispus L (welted thistle) is an invasive plant studied for its potential pharmacological activity (Figure 21). It is found in Asia and Europe and has been used in Chinese folk medicine for the treatment of colds, stomach ache and rheumatism.[75] Researchers have discovered that the extracts from *C. crispus* inhibit the growth of various human cancer lines *in vitro* and show significant cytotoxic activity.[75, 76] In 2002, Zhao and co-workers isolated a new class of isoquinoline alkaloids from C. *crispus,* crispine A-E. Crispine A and B have pyrrolo-[2,1-*a*]isoquinoline skeletons whereas crispine C-E are isoquinoline alkaloids with guanidinyl groups (Figure 22).[75]

Figure 21: Welted thistle

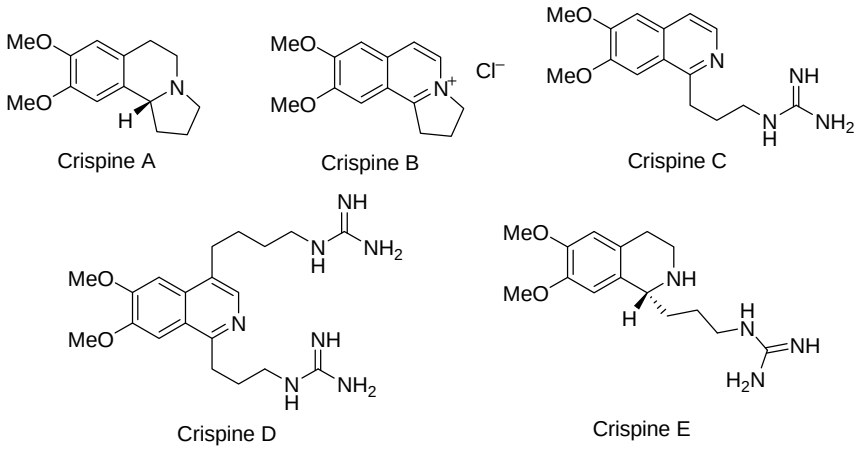
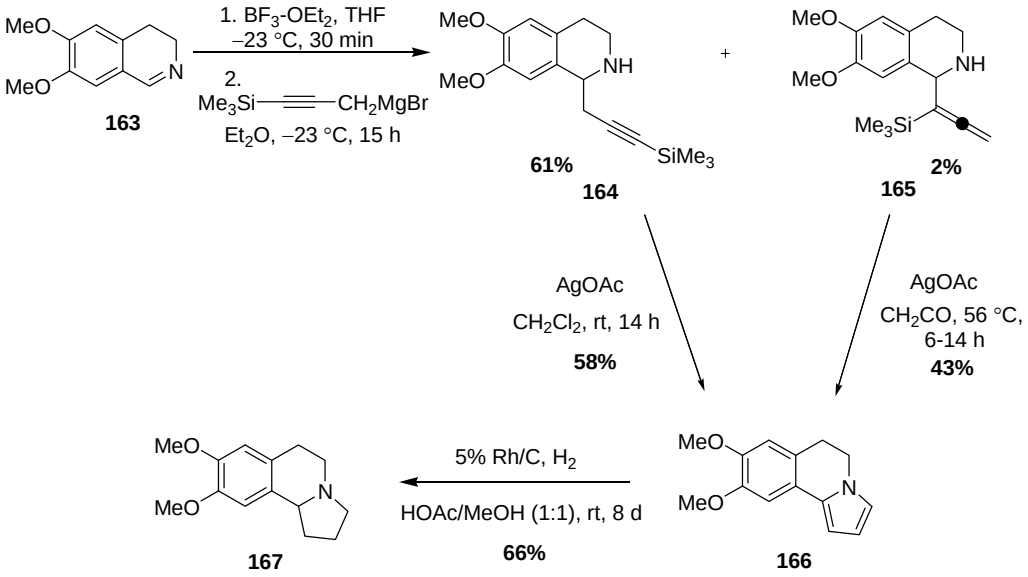


Figure 22: Crispine A-E

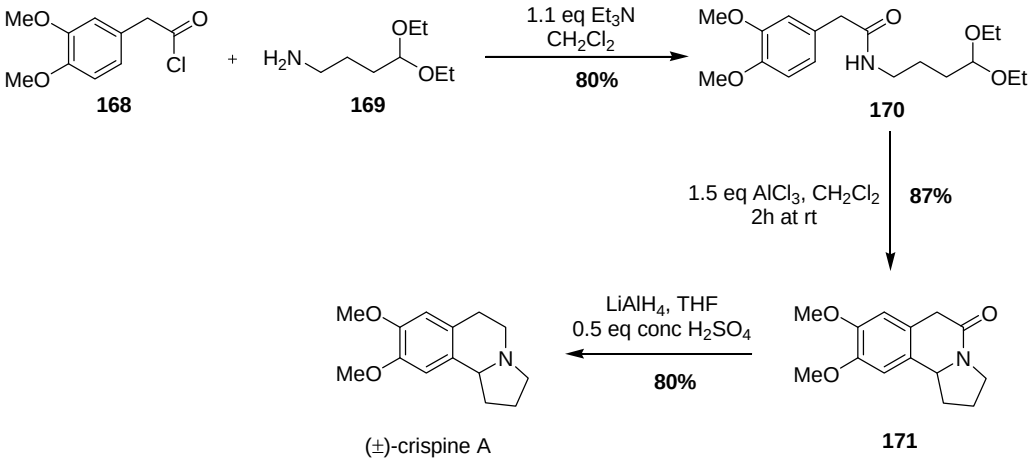
2.4.2 Synthesis of Crispine A

Pyrrolo[2,1-*a*]isoquinoline alkaloid crispine A, has been synthesised by a number of different groups. In 2005, Knölker and Agarwal synthesised (±)-crispine A in a short and direct route using a approach for the preparation of pyrrole ring that they had developed in their group.[76] Shown in Scheme 67 is their synthesis of crispine A which starts from 3,4-dihydro-6,7-dimethoxyisoquinoline **163**.[76] Compound **163** was made by the Bischler-Napierlski cyclisation of *N*-formyl-3,4-dimethoxyphenylethylamine. Next, boron trifluoride aided addition of 3-trimethylsilylpropargylmagnesium bromide to compound **163** gave **164** in 61% yield and the allene **165** in 2% yield. Oxidative cyclisation of **164** using silver acetate to pyrrole **166** occurred in 58% yield. Allene **165** also underwent oxidative cyclisation to afford pyrrole **166** in 43% yield but at 53 °C rather than at room temperature. The final step involves chemoselective hydrogenation of the pyrrole ring to give **167** in 66% yield. Knölker and Agarwal synthesised (±)-crispine A in three steps with an overall yield of 24%.



Scheme 67: Synthesis of crispine A

In 2007, King developed a three step synthesis of (±)-crispine A via an acyliminium ion cyclisation to afford the natural product in an overall 55% yield.[77] Starting from 3,4-dimethoxyphenacylchloride **168**, compound **170** was prepared in 80% yield. Cyclisation of **170** occurred using aluminium chloride in dichloromethane to afford **171** in an excellent 87% yield. The final step involved the reduction of **171** using lithium aluminium hydride as the reducing reagent to give (±)-crispine A in 80% yield. The synthesis of (±)-crispine A was successfully achieved in three steps with an excellent overall 55% yield, an improvement to the synthesis developed by Knölker and Agarwal.



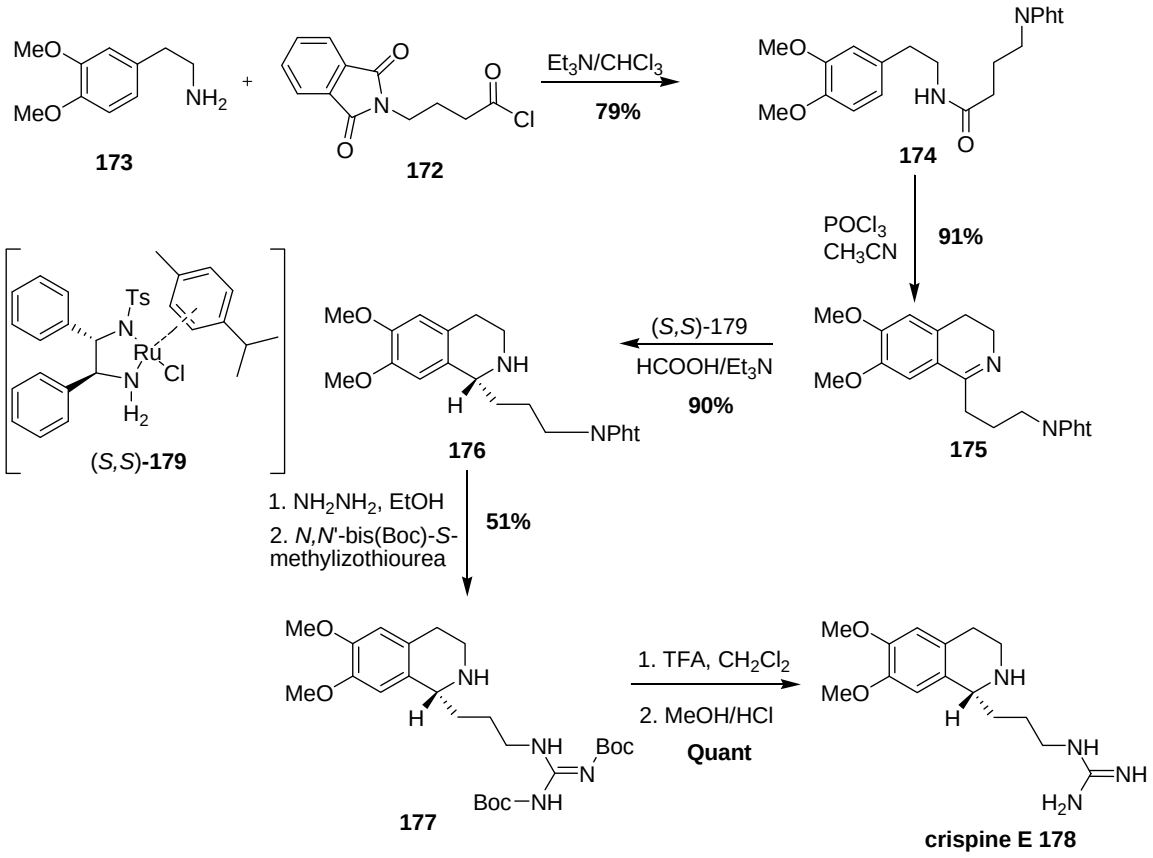
Scheme 68: Synthesis of crispine A

2.4.3 Synthesis of Crispine E

The second class of alkaloids from *C. crispus* series is crispine C-E which contain the isoquinoline group with a guanidinyl unit. Czarnocki and co-workers successfully synthesised (+)-crispine E using the same synthetic route they used to synthesise the alkaloid (+)-tryargine.[78] Compound **172** was prepared in a number of steps. Firstly the amine group of γ-aminobutyric acid was protected using phthalic anhydride to form the *N*-phthaloyl derivative. The carboxylic acid side was then reacted with thionyl chloride to give the acid chloride **172**. Next, the acid chloride was reacted with 3,4-dimethoxyphenethylamine **173** to afford amide **174** in 79% yield. Cyclisation of **174** using the Bischler-Napieralski approach gave imine **175** in 91% yield. Asymmetric transfer

hydrogenation was used to reduce **175** to compound **176**. Using the catalyst (*S*,*S*)-**179** in a solution of formic acid and triethylamine gave **176** in an excellent 90% yield and 89% ee.

The completion of the synthesis of crispine E, involved deprotection of the phthaloyl group using hydrazine in ethanol, followed by derivatisation with *N*,*N*'-bis(Boc)-*S*-methylisothiourea in DMF to give Boc-protected crispine E, **177**. Finally removal of the Boc groups with TFA and exchange of the anion by repeated evaporation with methanolic HCl gave (+)-crispine E in quantitative yield. (+)-Crispine E was completed in a good 53% overall yield.



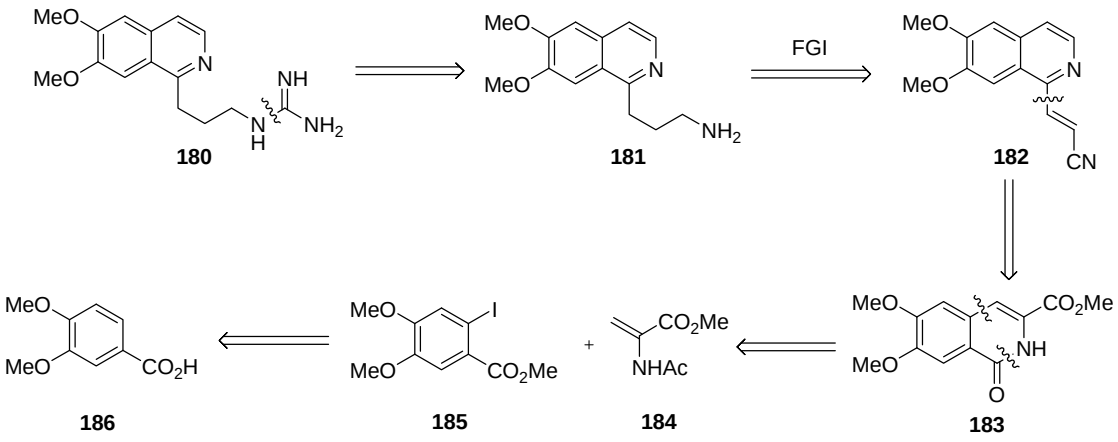
Scheme 69: Synthesis of Crispine E

2.4.4 Proposed Research

To date there has been no reported synthesis of crispine C. The aim of this project was to develop the first total synthesis of crispine C using Pd(0)-mediated reactions to effect the key carbon-carbon bond forming reactions.

2.4.5 Retrosynthetic Analysis of Crispine C

The proposed retrosynthesis of crispine C is shown in Scheme 70. Disconnection of the guanidine unit of **180** would give amine **181** followed by functional group interconversion to give α,β-unsaturated nitrile **182**. Further disconnection of the acrylonitrile side chain would give a bromide, followed by functional group interconversion to the carbonyl, **183**. Isoquinoline core **183** could be synthesised from methyl 2-iodo-4,5-dimethoxybenzoate **185** and amidoacrylate **184** via a one-pot Heck/amide cyclisation. Finally compound **185** could be synthesised from 3,4-dimethoxybenzoic acid **186**.

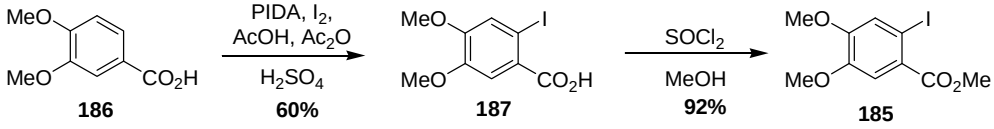


Scheme 70: Retrosynthesis of crispine C

The key transformations in the synthesis of crispine C involves Pd(0)-mediated reactions similar to those developed for the preparation of PK11195 described earlier in this thesis (see section 2.1.2).

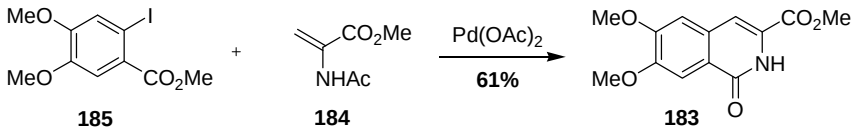
2.4.6 Synthesis of Crispine C

The first step in the synthesis was to form the isoquinoline core (Scheme 71). This was achieved by iodinating commercially available 3,4-dimethoxybenzoic acid with the use of diacetoxyiodobenzene and iodine to afford **187** in 60% yield.[79] The procedure was developed by Kryska and Skulski. They showed that a range of aromatic iodides could be prepared under mild conditions using iodine, diacetoxyiodobenzene as an oxidant in the presence of catalytic amounts of sulfuric acid. The advantage of using diacetoxyiodobenzene as the oxidant is that it is relatively cheap, stable and very accessible compared to other oxidants. The resulting compound **187** was then esterified under standard conditions using thionyl chloride in methanol, to give **185** in an excellent 92% yield.



Scheme 71: Synthesis of 185

To complete the isoquinoline core the next step involves a palladium(0) catalysed Heck reaction (Scheme 72). A one-pot procedure developed by Chattopadhyay and co-workers allowed the coupling of 2-iodo-4,5-dimethybenzoic acid **185** with amidoacrylate **184** to afford **183** in 61% yield.[57] The reaction works via a palladium-catalysed cycle and starts by oxidative addition of the palladium(0) in between the aryl halide bond of **187**, followed by insertion of amidoacrylate into the Pd-halide bond and lastly β-hydride elimination of the palladium.

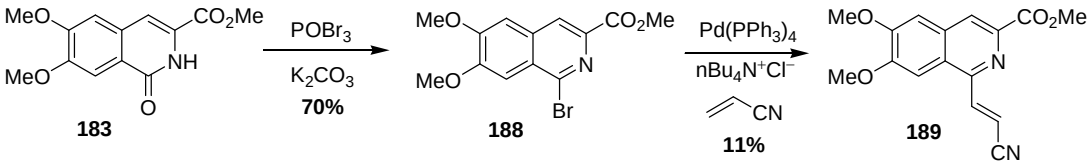


Scheme 72: Synthesis of 183

The resulting oxoquinoline **183** was converted to the bromide using phosphorus(V) oxybromide to give **188** in 70% (Scheme 73). The next stage was the introduction of the appropriate side-chain, *via* a Heck reaction using acrylonitrile as the coupling partner. The reaction was first attempted using tetrakis(triphenylphosphine)palladium(0) as the catalyst in the presence of triethylamine.[80] However, the reaction was unsuccessful and only

starting material was recovered. This was because, whilst heating the reaction, the acrylonitrile was evaporating, therefore not in the mixture for the reaction to occur. The next procedure that was attempted used a method reported by Wang and co-workers. Their method involved the use of palladium acetate, triphenylphosphine and silver carbonate as the base.[81] The reaction was carried out at room temperature and reacted for 48 h but unfortunantely after 48 h there was no sign of any product.

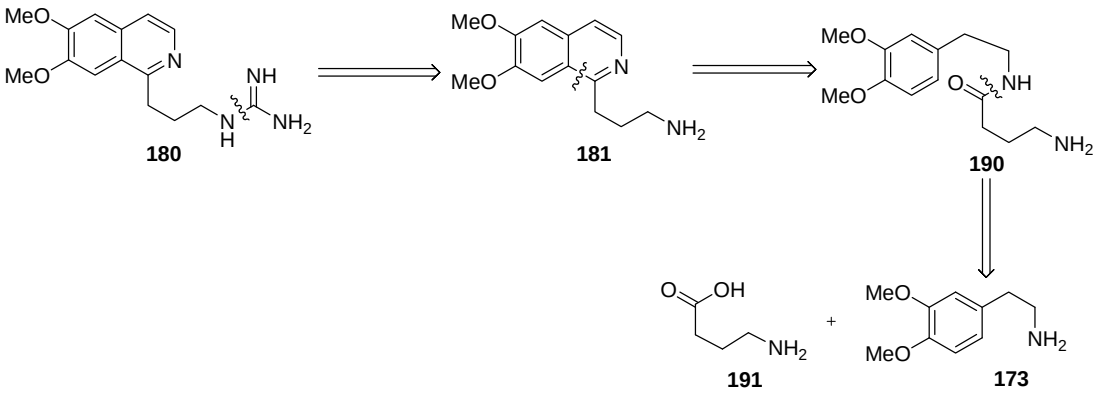
Another procedure reported by Deng and co-workers used Jeffery's conditions using palladium acetate and tetra-*n*-butylammonium bromide in DMF.[82] The reaction was carried out in a Schlenk tube in order for it to be heated to 80 °C. This time the reaction did work but the reaction took three weeks to produce **189** with a very low 11% yield. The last attempt involved using tributylphosphine and heating the reaction to 150 °C. The reaction was slightly better taking four nights to afford **189** in a poor 13% yield. At this stage it was decided more efficient route was required.



Scheme 73: Synthesis of 189

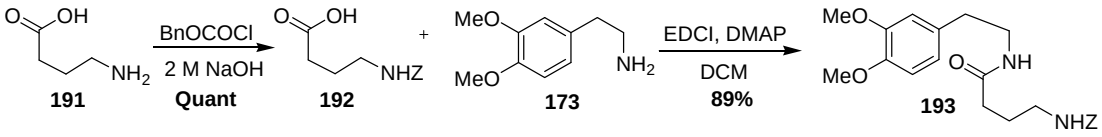
2.4.7 New Synthetic Route

A new shorter seven step approach was devised. Instead of using Pd(0) chemistry the new route involves the Bischler-Napieralski approach to form the isoquinoline ring. Shown in Scheme 74 is the retrosynthesis of crispine C. Disconnection of the guanidine unit of **180** would give amine **181**. Further disconnection of the isoquinoline ring to form amide **190**, followed by disconnection of amide bond leads back to **173** and **191.**



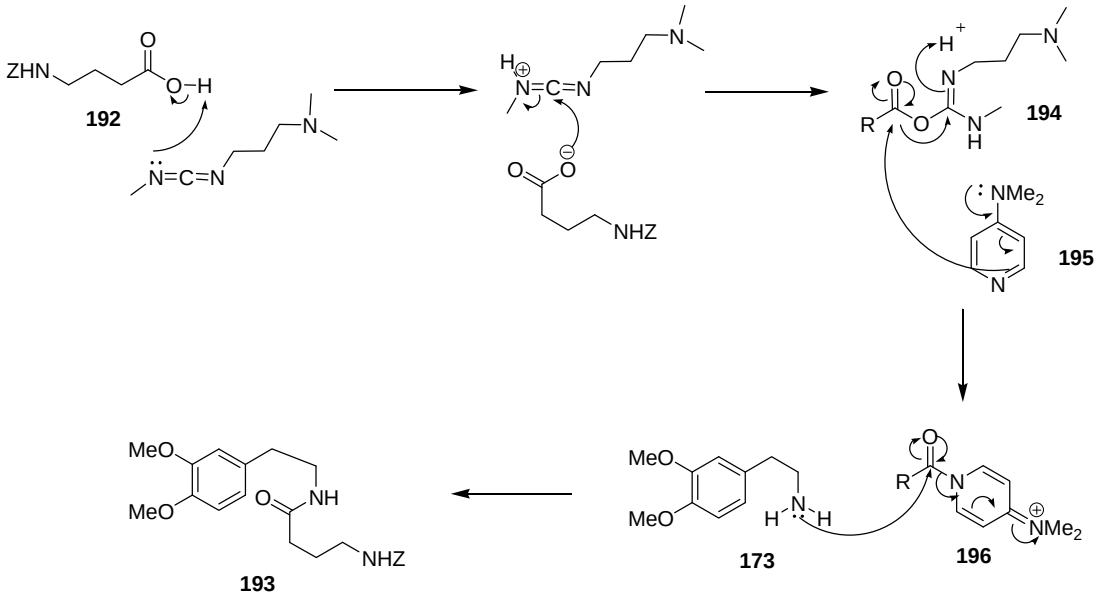
Scheme 74: Retrosynthesis of 180

The first step involves the coupling of 3,4-dimethoxyphenethylamine and γ-aminobutyric acid. Firstly, the γ-aminobutyric acid was protected using benzyl chloroformate in 2 M sodium hydroxide to afford **192** in quantitative yield. The coupling reaction of Cbz-protected γ-aminobutyric acid and 3,4 dimethoxyphenethylamine **173**, involved ethyl 3-(3-dimethylaminopropyl)carbodiimide and dimethylaminopyridine as the catalyst in dichloromethane to afford **193** in an excellent 89% yield.[83]



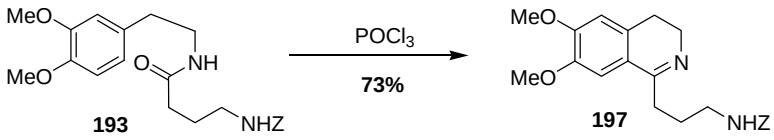
Scheme 75: Synthesis of 193

The coupling reaction forms a new amide bond between 3,4-dimethoxyphenethylamine **173** and γ-aminobutyric acid **192.** Firstly EDCl reacts with γ-aminobutyric acid **192** to form intermediate **194**. Next dimethylaminopyridine attacks the activated carbonyl to form new intermediate **196**. The amino group of 3,4-dimethoxyphenethylamine attacks the DMAP amide to give **193** in a very fast reaction.[84]



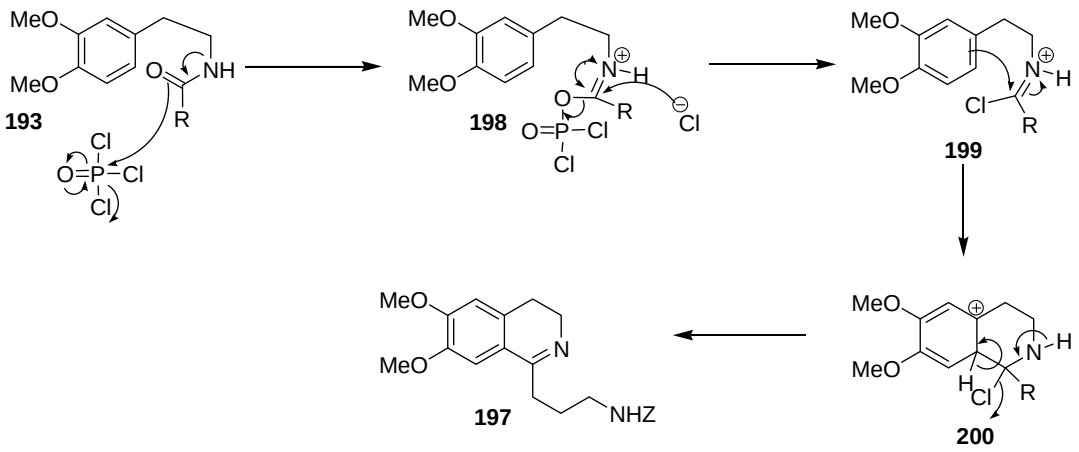
Scheme 76: Amide coupling mechanism

Cyclisation of *N*-(3,4-dimethoxyphenethyl)-4-(benzyloxycarbonylamino)butanamide **193,** was then carried out using the Bischler-Napieralski reaction (Scheme 77).[78] The reaction using phosphorus(V) oxychloride in dichloromethane was found to be unpredictable. The reaction went to completion the first time to afford **197** in 60% yield. However, when the reaction was repeated several times after, it failed. The reaction was then repeated using neat phosphorus(V) oxychloride which gave imine **197** in an improved 73% yield.[85]



Scheme 77: Cyclisation of 193

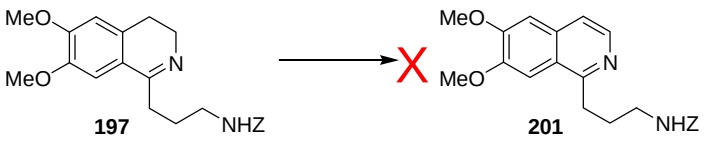
Shown in Scheme 78, is the mechanism for the Bischler-Napieralski reaction. The mechanism involves the conversion of amide **195** to protonated imidoyl chloride **202**, followed by electrophilic aromatic substitution to give **200**.



Scheme 78: Mechanism for the Bischler-Napieralski reaction

The next stage involves a dehydrogenation reaction to obtain the isoquinoline ring system. Formation of compound **201** was attempted using several different reaction conditions but all ended in failure. The first procedure involved the use palladium on carbon in diphenyl ether at 170 °C to remove two hydrogen atoms. However, the reaction failed and the starting material decomposed.[86] The second attempt to effect this transformation involved the use of selenium dioxide. A paper published by Bernstein and Littell used selenium dioxide in the presence of *t*-butyl alcohol and glacial acetic acid. The same reaction conditions were carried out on compound **197** but proved unsuccessful.[87] The reaction was repeated without glacial acetic acid and with the addition of deactivated Raney nickel catalyst.[87] After two hours heating the reaction under reflux no reaction was observed and the starting material had decomposed.

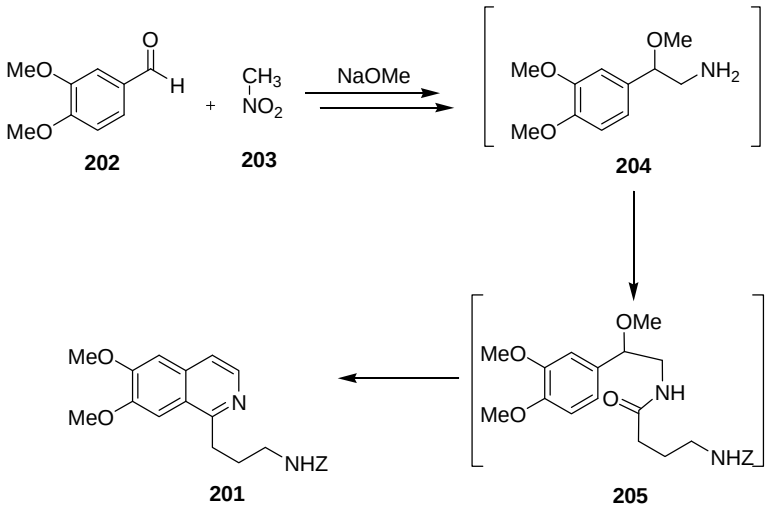
The next strategy was to use the common dehydrogenating reagent 2,3-dichloro-5,6-dicyano benzoquinone (DDQ).[88] The reaction was heated under reflux in toluene overnight. Again this gave no reaction. The last attempt to dehydrogenate **197,** was to use manganese dioxide under Dean-Stark conditions. However, the reaction reopened the ring to form compound **193.** Further research into methods for dehydrogenation led to a paper from 1983 by Walker and co-workers. They reported difficulties in the dehydrogenation of similar isoquinoline ring systems.[89] They overcame these problems by incorporating a leaving group and the use of the Pictet-Gams reaction which allowed more facile generation of the isoquinoline ring system. A route using the Pictet-Gams reaction was devised to overcome problems with the dehydrogenation step.



Scheme 79

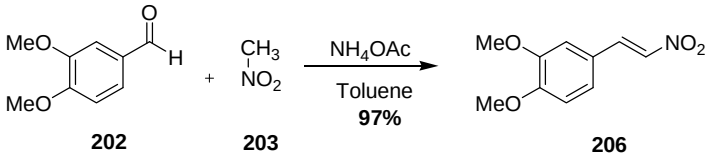
2.4.8 Third Route, Use of the Pictet-Gams Reaction

The Pictet-Gams reaction involves the addition of a good leaving group, *beta* to the amide. This allows cyclisation and aromatization in one step, by-passing the dehydrogenation reaction. Before the cyclisation stage there were a number of reactions required to prepare amine **204.** Shown in Scheme 80, is the proposed route to the synthesis of the key isoquinoline intermediate **201**. Firstly formation of amine **204**, followed by the coupling reaction to give **205** and finally the Pictet-Gams reaction to form the isoquinoline **201.**



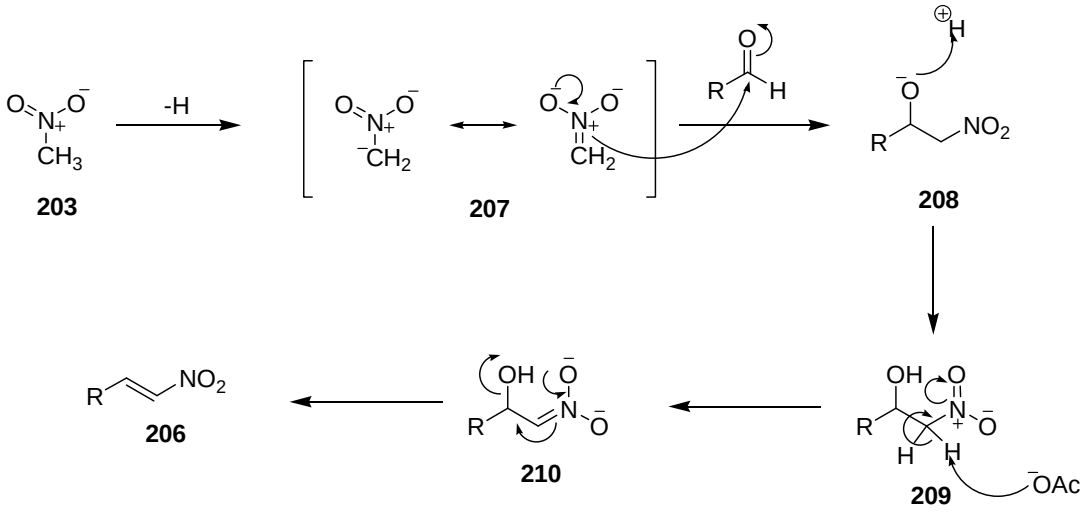
Scheme 80: Proposed route for the synthesis of crispine C

The first step of the synthesis involves condensation of veratraldehyde and nitromethane to form β-nitrostyrene **206**. The reaction is a Henry Reaction which similar to an Aldol addition reaction and can also be referred to as the nitro Aldol reaction. The reaction was heated under reflux in toluene to afford **206** in an excellent 97% yield.[89]



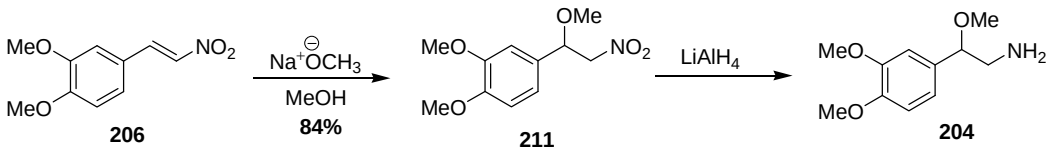
Scheme 81: Synthesis of 206

The reaction mechanism is shown below in Scheme 82. The first step in the mechanism is the deprotonation of the nitromethane by the base (ammonium acetate) at the α position to form the corresponding resonance stabilized anion **207**. Next an aldol reaction takes place, between the nitromethane and the carbonyl to form intermediate **208**, Protonation of the alkoxide to form the nitro alcohol **209**, followed by elimination of water gave compound **206**.



Scheme 82: Mechanism of the formation of 206

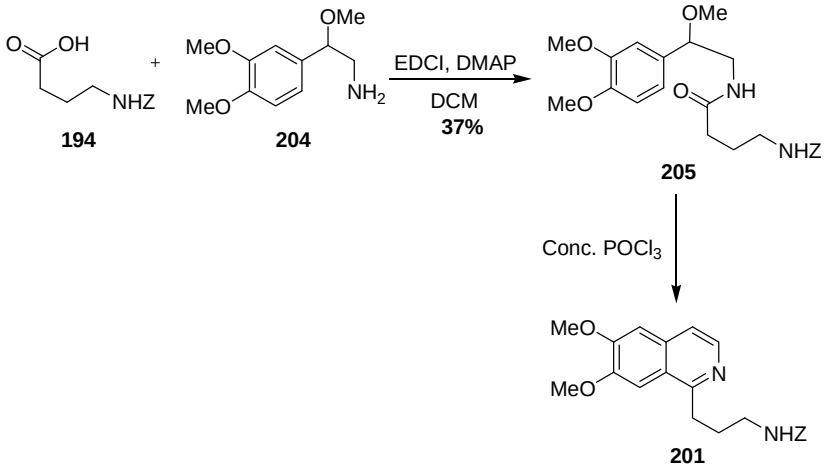
The next stage in the synthesis was the conversion of nitrostyrene **206** to the nitroethane compound **211** by the conjugate addition of the methoxide group. This gave **211** in 84% yield (Scheme 83).[89] Reduction of the nitro group to afford β-methoxyphenethylamine **204** was problematic and was attempted a number of times using various reagents. Firstly the reaction was attempted numerous times using lithium aluminium hydride in diethyl ether as the reducing agent but the reaction was very low yielding (17-34% yield). Problems arose in attempting to isolate **204** during the work-up. A second attempt using ammonium formate and palladium on carbon led to decomposition of the starting material.[90] Another, reported procedure for the reduction of nitro groups involved the use of tin chloride but again this method returned only starting material.



Scheme 83: Synthesis of 204

Due to time restraints it was thought that it would be beneficial to take the crude amine **204** produced using the $LiAlH_4$ procedure onto the next few steps to test whether the Pictet-Gams reaction would successful or not, before any more time was spend on developing the nitro reduction stage.

The next stage involved the coupling of compound **204** to Cbz-protected γ-aminobutyric acid **194**, which gave **205** in 37% yield over two steps. The Pictet-Gams reaction was then carried out neat in phosphorous oxychloride. Although the compound was not isolated, the ^1H NMR spectrum of the crude material indicated formation of the desired compound.



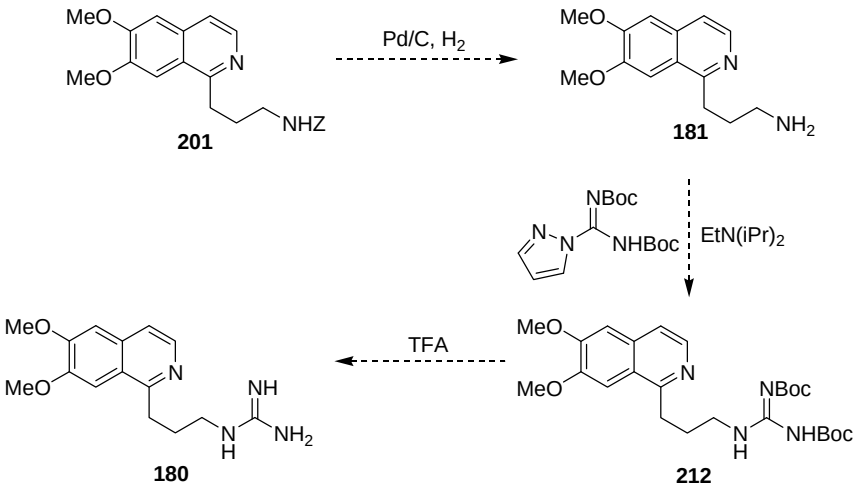
Scheme 84: Synthesis of 201

2.4.9 Conclusions

The synthesis of crispine C, was first attempted using Pd(0)-mediated reactions to effect the key carbon-carbon bond forming reactions. However, failure of the Heck reaction to couple the appropraite side-chain resulted in a new synthesis. The new route involved the Bischler-Napieralski approach to form the isoquinoline core. To complete the isoquinoline ring system, a dehydrogenation reaction was attempted but proved difficult. Therefore, a route using the Pictet-Gams reaction was devised to overcome problems with dehydrogenation, allowing the cyclisation and aromatization in one step. To date the third route is promising but still requires optimisation of nitro group reduction.

2.4.10 Future Work

Future work for this project would include the modification of the nitro reduction stage to increase the yield. Further work would be involved in improving the purification of the Pictet-Gams reaction to obtain a clean compound ready for the final steps. Shown in Scheme 85 would be the final steps to complete the synthesis of crispine C. Firstly deprotection of the amine would give **212**, followed by coupling the resulting amine with commercially available *N*,*N*-bis(*tert*-butoxycarbonyl)-1*H*-pyrazole-1-carboxamidine in the presence of Hünig's base. This will give the Boc-protected guinidine substituent to afford compound **212**. Treatment of **212** with trifluoroacetic acid will remove the Boc-protecting groups and complete the first total synthesis of crispine C.



Scheme 85: Completion of the synthesis of crispine C

3 Experimental

3.1 Experimental

General experimental

All reactions were performed under an argon atmosphere unless otherwise noted. Reagents and starting materials were obtained from commercial sources and used as received. Dry solvents were purified using a PureSolv 500 MD solvent purification system. Flash column chromatography was carried out using Fisher matrix silica 60. Macherey-Nagel aluminium backed plates pre-coated with silica gel 60 (UV_{254}) were used for thin layer chromatography and were visualised by staining with potassium permangante. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 spectrometer with chemical shift values in ppm relative to TMS (δ_H 0.00 and δ_C 0.0) or residual chloroform (δ_H 7.28 and δ_C 77.2) as standard. Proton and carbon assignments are based on two-dimensional COSY and DEPT experiments, respectively. Infrared spectra were recorded using sodium chloride or potassium chloride plates on a JASCO FTIR 410 spectrometer or neat on the SHIMADZU spectrometer and mass spectra were obtained using a JEOL JMS-700 spectrometer.

General Procedure 1: Suzuki Coupling. To a solution of the isoquinoline bromide in *N*,*N*-dimethylformamide (150 mL) was added the boronic acid (1.3 equiv), potassium phosphate (1.30 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.03 equiv) and the mixture heated under reflux for 24 h. An additional amount of the boronic acid (0.65 equiv) and potassium phosphate (0.65 equiv) was added and the mixture heated under reflux for a further 24 h. The reaction mixture was cooled to room temperature and the product extracted into dichloromethane (150 mL). The organic layer was washed with water (3 × 20 mL) and then dried ($MgSO_4$). The resulting solution was concentrated under reduced pressure to afford a dark brown oil. The product was purified by flash column chromatography using a graduated eluent of petroleum ether > petroleum ether/ethyl acetate (50:50) to give the coupled product.

General Procedure 2: Ester Hydrolysis. To a solution of the ethyl ester in 50% aqueous ethanol (150 mL) was added ground sodium hydroxide (4 equiv) and the solution stirred at 80 °C for 1.5 h. The reaction mixture was cooled to room temperature and the ethanol removed under reduced pressure leaving an aqueous solution. The solution was acidified with 1 M hydrochloric acid (20 mL) and the product extracted into dichloromethane. The

organic layer was washed with water (3 × 20 mL) and dried ($MgSO_4$). The organic solvent was removed under reduced pressure to afford the carboxylic acid which was used without further purification.

General Procedure 3: Acid Chloride Formation and Subsequent Amine Coupling. The carboxylic acid, dichloromethane (50 mL) and a few drops of *N*,*N*-dimethylformamide were cooled to 0 ^oC. Oxalyl chloride (1.2 equiv.) was then added and reaction heated to 50 ^oC for 2 h. The solvent was removed *in vacuo* and excess oxalyl chloride was removed with toluene (3 × 20 mL). The residue was re-dissolved in dichloromethane (50.0 mL), cooled to 0 ^oC and the amine (5 equiv.) was then added and heated to 50 ^oC overnight. The reaction mixture was diluted with water, extracted with ethyl acetate, dried ($MgSO_4$) and concentrated to dryness. Purification was carried out using flash column chromatography eluting with petroleum ether/ethyl acetate to give the desired compounds.

General Procedure 4: Methylation of Amides. The amide, tetrahydrofuran (50 mL) and sodium hydride (4.2 equiv.) were stirred under argon at room temperature for 0.5 h. Methyl iodide (16 equiv.) was then added to the reaction mixture and heated under reflux overnight. The reaction mixture was quenched with water (20 mL), neutralized with 1 M hydrochloric acid (pH ~1) and extracted with ethyl acetate (3 × 20 mL). Purification was carried out using flash column chromatography eluting with petroleum ether/ethyl acetate to give the desired compounds.

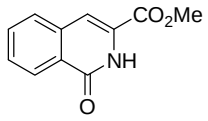
General Procedure 5: Aluminium Mediated Lactone Opening Using Secondary Amines. The amine (1.4 equiv.) was dissolved in dichloromethane (30 mL) and a 2 M solution of trimethylaluminium in toluene (1.3 equiv.) was added slowly to the reaction. After 0.25 h, 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one **108** was added to the reaction which was heated under reflux for 48 h. The reaction was quenched with 1 M hydrochloric acid (20 mL), extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and concentrated under vacuum. Purification was carried out by flash column chromatography eluting, with dichloromethane/ethyl acetate to give the desired compounds.

General Procedure 6: Chlorination and Subsequent Iodination of the Primary Alcohols

To a solution of the hydroxyl compound in dichloromethane (10 mL), thionyl chloride (22 equiv) was added. The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* and the chloro analogue used without further

purification. A solution of sodium iodide (10 equiv.) and acetonitrile (30 mL) were de-gased for 0.5 h. The hydroxyl compound was then added and the reaction mixture heated under reflux for 2.5 h. The mixture was cooled to room temperature and then concentrated to dryness. Purification was carried out by flash column chromatography eluting with dichloromethane/ethyl acetate (9:1) to give the desired compounds.

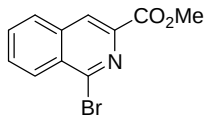
Methyl isoquinolin-1-one-3-carboxylate 67[91]



67

Methyl 2-iodobenzoate (5.00 g, 19.0 mmol) and methyl-2-acetamidoacrylate (4.10 g, 28.0 mmol) were dissolved in acetonitrile (150 mL). Palladium(II) acetate (0.40 g, 1.90 mmol), tetrabutylammonium chloride (4.20 g, 15.0 mmol) and sodium hydrogencarbonate (4.00 g, 47.0 mmol) were added to reaction mixture and heated under reflux overnight. The reaction mixture was cooled to room temperature and concentrated *in vacuo.* The reaction was diluted with water (200 mL) and subsequently extracted with ethyl acetate (200 mL). The organic phase was washed with water (3 × 200 mL), dried ($MgSO_4$) and the ethyl acetate layer was removed under reduced pressure. The product was purified by flash column chromatography using a graduated eluent of ethyl acetate/petroleum ether to give methyl isoquinoline-1-one-3-carboxylate **67** as a white solid (2.50 g, 65%). mp 146–147 °C (lit.,[91] mp 158–159 °C); δ_H (400 MHz, $CDCl_3$) 4.00 (3H, s, CH_3), 7.39 (1H, s, 4-H), 7.62–7.77 (3H, m, Ar H), 8.46 (1H, d, *J* 8.0 Hz, Ar H), 9.10 (1H, br s, NH); δ_C (100 MHz, $CDCl_3$) 53.2 (CH_3), 111.4 (CH), 127.7 (C), 128.0 (CH), 128.2 (CH), 128.4 (C), 129.5 (CH), 133.1 (CH), 136.0 (C), 161.7 (C), 162.3 (C); *m/z* (EI) 203.0578 (M^+. $C_{11}H_9NO_3$ requires 203.0582), 143 (15%), 82 (100), 46 (36).

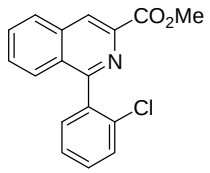
Methyl 1-bromoisoquinoline-3-carboxylate 45[42]



45

Methyl isoquinolin-1-one-3-carboxylate **67** (1.70 g, 8.30 mmol), phosphorous oxybromide (7.10 g, 2.50 mmol) and anhydrous potassium carbonate (3.50 g, 2.50 mmol) were added to acetonitrile (150 mL). The solution was heated under reflux for 1.5 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to form a oil. The residue was cautiously dispersed in water (50 mL) and the insoluble material was filtered, washed with water, dried ($MgSO_4$) and recrystallized from ethyl acetate. Methyl 1-bromoisoquinoline-3-carboxylate **45** was obtained as a light brown solid (1.60 g, 71%). mp 122–124 °C (lit.,[42] mp 125 °C); δ_H (400 MHz, $CDCl_3$) 4.10 (3H, s, CH_3), 7.83–7.87 (2H, m, Ar H), 7.97–8.01 (1H, m, Ar H), 8.38–8.41 (1H, m, Ar H), 8.56 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 53.1 (CH_3), 124.6 (CH), 128.7 (CH), 129.1 (CH), 130.4 (C), 131.1 (CH), 132.0 (CH), 136.8 (C), 141.0 (C), 145.5 (C), 165.1 (C); *m/z* (EI) 264.9742 (M^+. $C_{11}H_8{}^{79}BrNO_2$ requires 264.9738), 207 (100), 127 (83), 100 (11), 49 (37).

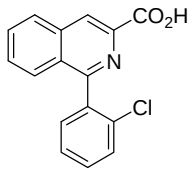
Methyl 1-(2-Chlorophenyl)isoquinoline-3-carboxylate 46[42]



46

The reaction was carried out according to general procedure 1 using methyl 1-bromoisoquinoline-3-carboxylate **45** (0.20 g, 0.75 mmol) and 2-chlorophenylboronic acid (0.15 g, 0.98 mmol) and gave methyl 1-(2-chlorophenyl)isoquinoline-3-carboxylate **46** as a yellow solid (0.15g, 67%). mp 150–152 °C; δ_H (400 MHz, $CDCl_3$) 4.10 (3H, s, CH_3), 7.34–7.47 (4H, m, Ar H), 7.56–7.62 (2H, m, Ar H), 7.70–7.74 (1H, m, Ar H), 7.98 (1H, d, *J* 8.0 Hz, Ar H), 8.61 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 53.1 (CH_3), 124.3 (CH), 127.0 (CH), 127.6 (CH), 128.4 (CH), 128.8 (C), 129.7 (2 × CH), 130.2 (CH), 131.0 (CH), 131.6 (CH) 133.5 (C), 135.9 (C), 137.8 (C), 141.0 (C), 159.3 (C), 166.5 (C); *m/z* (EI) 297.0558 (M^+. $C_{17}H_{12}{}^{35}ClNO_2$ requires 297.0557), 239 (100%), 203 (26), 43 (10).

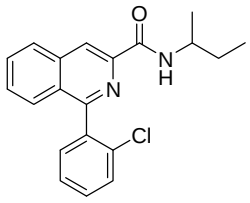
1-(2-Chlorophenyl)isoquinoline-3-carboxylic acid 34[42]



34

The reaction was carried out according to general procedure 2 using methyl 1-(2-chlorophenyl)isoquinoline-3-carboxylate **46** (1.05 g, 3.50 mmol) and sodium hydroxide (0.56 g, 14.0 mmol) and gave 1-(2-chlorophenyl)isoquinoline-3-carboxylic acid **34** as a white solid (0.95 g, 96%). mp 188–189 °C (lit.,[42] mp 191 °C); δ_H (400 MHz, $CDCl_3$) 7.45–7.56 (3H, m, Ar H), 7.60–7.62 (1H, m, Ar H), 7.70–7.79 (2H, m, Ar H), 7.84–7.88 (1H, m, Ar H), 8.12 (1H, d, *J* 8.4 Hz, Ar H), 8.74 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 122.8 (CH), 127.0 (CH), 127.8 (CH), 128.9 (CH), 129.0 (C), 130.0 (CH), 130.3 (CH), 130.6 (CH), 131.3 (CH), 131.7 (CH), 133.3 (C), 136.6 (C), 136.7 (C), 138.6 (C), 157.9 (C), 164.9 (C); *m/z* (CI) 284.0475 (MH^+. $C_{16}H_{11}{}^{35}ClNO_2$ requires 284.0478), 250 (13), 248 (8).

1-(2-Chlorophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide 71[92]

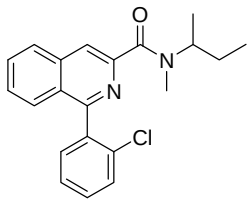


71

The reaction was carried out according to general procedure 3 using 1-(2-Chlorophenyl)isoquinoline-3-carboxylic acid **34** (0.10 g, 0.35 mmol) and *sec*-butylamine (1.1 mL, 10.5 mmol) and gave 1-(2-chlorophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide **71** as a white solid (0.11 g, 90%). mp 124–126 °C (lit.,[92] mp 124.5–125.5 °C); δ_H (400 MHz, $CDCl_3$) 0.94–1.00 (3H, m, $CHCH_2CH_3$), 1.26 (3H, d, *J* 8.0 Hz, $NCHCH_3$), 1.57–1.65 (2H, m, CH_2CH_3), 4.10–4.23 (1H, m, NC*H*), 7.46–7.53 (2H, m, Ar H), 7.57–7.61 (2H, m, Ar H), 7.67–7.70 (1H, m, Ar H), 7.75 (1H, ddd, *J* 8.0, 6.8, 1.2 Hz, Ar H), 8.05 (2H, d, *J* 8.0 Hz, Ar H), 8.67 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 10.6 (CH_3), 20.5 (CH_3), 29.8 (CH_2), 46.8 (CH), 120.2 (CH), 126.7 (CH), 127.3 (CH), 128.2 (C), 128.5 (CH), 129.8 (CH), 129.9 (CH), 130.0 (CH), 130.7 (CH) 131.5 (CH), 133.5 (C), 136.7 (C), 137.9 (C), 142.9

(C), 157.2 (C), 164.0 (C); *m/z* (CI) 339.1259 (MH^+. $C_{20}H_{20}{}^{35}ClN_2O$ requires 339.1264), 305 (10%), 113 (19), 85 (64), 69 (100).

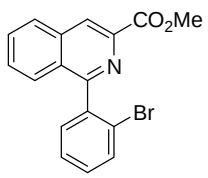
1-(2-Chlorophenyl)isoquinoline-3-*N*-methyl-*N*-*sec*-butylcarboxamide (PK11195) 32



32

The reaction was carried out according to general procedure 4 using 1-(2-chlorophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide **71** (0.08 g, 0.23 mmol) and methyl iodide (0.23 mL, 3.70 mmol) and gave 1-(2-chlorophenyl)isoquinoline-3-*N*-methyl-*N*-*sec*-butylcarboxamide (PK11195) **32** as a white solid (0.07 g, 84%). NMR spectra showed a 2:1 mixture of rotamers, only signals for the major rotamer is recorded. δ_H (400 MHz, DMSO-d_6) 0.70 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.10–1.16 (3H, m, $NCHCH_3$), 1.49–1.60 (2H, m, CH_2CH_3), 2.85 (3H, s, NCH_3), 3.67–3.83 (1H, m, $NCHCH_3$), 7.52–7.62 (4H, m, Ar H), 7.67–7.70 (2H, m, Ar H), 7.85 (1H, t, *J* 7.6 Hz, Ar H), 8.07–8.13 (1H, m, Ar H), 8.17 (1H, d, *J* 8.0 Hz Ar H); δ_C (100 MHz, DMSO-d_6) 10.8 (CH_3), 18.3 (CH_3), 26.5 (CH_2), 49.7 (CH_3), 54.9 (CH), 119.5 (CH), 126.2 (C), 126.3 (CH), 127.2 (CH), 127.7 (CH), 128.7 (CH), 129.5 (CH) 130.5 (CH), 131.0 (CH), 131.4 (CH), 132.1 (C), 136.1 (C), 137.4 (C), 147.9 (C), 157.0 (C), 168.9 (C); *m/z* (CI) 352.1336 (M^+. $C_{21}H_{21}{}^{35}ClN_2O$ requires 352.1342), 239 (95%), 203 (65), 176 (24), 86 (100).

Methyl 1-(2-Bromophenyl)isoquinoline-3-carboxylate 72

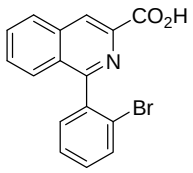


72

The reaction was carried out according to general procedure 1 using methyl 1-bromoisoquinoline-3-carboxylate **45** (1.90 g, 7.15 mmol) and 2-bromoboronic acid (1.90 g, 9.29 mmol) and gave methyl 1-(2-bromophenyl)isoquinoline-3-carboxylate **72** as a yellow

solid (1.20 g, 60%). mp 160–161 °C; ν_{max}/cm^{-1} (neat) 2939 (CH), 1715 (CO), 1562, 1450, 1292, 675; δ_H (400 MHz, $CDCl_3$) 4.05 (3H, s, CH_3), 7.35–7.40 (1H, m, Ar H), 7.48 (2H, d, *J* 4.4 Hz, Ar H) 7.65–7.66 (2H, m, Ar H), 7.71 (1H, d, *J* 7.6 Hz, Ar H), 7.77–7.79 (1H, m, Ar H), 8.04 (1H, d, *J* 8.0 Hz, Ar H), 8.67 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 53.1 (CH_3), 121.5 (C), 122.6 (CH), 125.9 (CH), 126.0 (CH), 126.8 (CH), 127.0 (C), 128.0 (CH), 128.6 (CH), 129.4 (CH), 129.9 (CH), 131.2 (CH), 134.3 (C), 138.1 (C), 139.2 (C), 158.8 (C), 164.8 (C); *m/z* (EI) 341.0058 (M^+. $C_{17}H_{12}{}^{79}BrNO_2$ requires 341.0051), 283 (53), 203 (31), 83 (100), 47 (25).

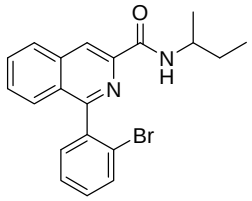
1-(2-Bromophenyl)isoquinoline-3-carboxylic acid 73



73

The reaction was carried out according to procedure 2 using methyl 1-(2-bromophenyl)isoquinoline-3-carboxylate **72** (1.50 g, 4.38 mmol) and sodium hydroxide (0.57 g, 14.2 mmol) and gave 1-(2-bromophenyl)isoquinoline-3-carboxylic acid **73** as a white solid (1.30 g, 91%); mp 178–179 °C; ν_{max}/cm^{-1} (neat) 2619 (CH), 1695 (CO), 1427, 1257, 906, 729; δ_H (400 MHz, $CDCl_3$) 7.43–7.48 (2H, m, Ar H), 7.52–7.56 (1H, m, Ar H), 7.70–7.81 (3H, m, Ar H), 7.84–7.88 (1H, m, Ar H), 8.12 (1H, d, *J* 8.4 Hz, Ar H), 8.74 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 120.9 (C), 121.0 (C), 125.7 (CH), 126.0 (CH), 127.0 (CH), 127.1 (CH), 128.5 (CH), 129.0 (CH), 129.4 (CH), 130.0 (CH), 131.4 (CH), 135.0 (C) 136.7 (C), 136.8 (C), 157.3 (C), 163.1 (C); *m/z* (CI) 326.9891 (MH^+. $C_{16}H_{10}{}^{79}BrNO_2$ requires 326.9895), 283 (100%), 204 (92), 83 (47).

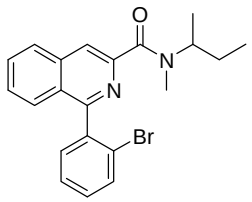
1-(2-Bromophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide 74



74

The reaction was carried out according to general procedure 3 using 1-(2-bromophenyl)isoquinoline-3-carboxylic acid **73** (0.30 g, 0.90 mmol) and *sec*-butylamine (2.76 mL, 27.4 mmol) and gave 1-(2-bromophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide **74** as a white solid (0.21 g, 60%). mp 112–114 °C; ν_{max}/cm^{-1} (KBr) 3384 (NH), 2966 (CH), 1666 (CO), 1509, 1379, 758; δ_H (400 MHz, $CDCl_3$) 0.96 (3H, t, *J* 7.4 Hz, $CHCH_2CH_3$), 1.26 (3H, d, *J* 6.6 Hz, $NCHCH_3$), 1.53–1.64 (2H, m, CH_2CH_3), 4.14–4.21 (1H, m, NC*H*), 7.40–7.54 (2H, m, Ar H), 7.57–7.61 (1H, m, Ar H), 7.67–7.70 (1H, m, Ar H), 7.73–7.80 (2H, m, Ar H), 8.05 (2H, d, *J* 8.0 Hz, Ar H), 8.67 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 10.6 (CH_3), 20.5 (CH_3), 29.9 (CH_2), 46.8 (CH), 120.2 (CH), 123.2 (C), 127.2 (CH), 127.3 (CH), 128.1 (C), 128.6 (CH), 128.8 (CH), 130.2 (CH), 130.7 (CH) 131.5 (CH), 133.2 (CH), 136.8 (C), 139.8 (C), 142.9 (C), 158.4 (C), 164.1 (C); *m/z* (CI) 383.0757 (MH^+. $C_{20}H_{20}{}^{79}BrN_2O$ requires 383.0759), 303 (18%), 196 (32), 71 (100).

1-(2-Bromophenyl)isoquinoline-3-*N*-methyl-*N*-*sec*-butylcarboxamide 63

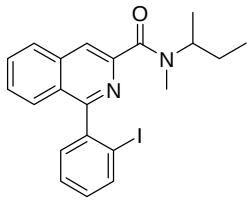


63

The reaction was carried out according to general procedure 4 using 1-(2-bromophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide **74** (0.18 g, 0.47 mmol) and methyl iodide (0.47 mL, 7.52 mmol) and gave 1-(2-bromophenyl)isoquinoline-3-*N*-methyl-*N*-*sec*-butylcarboxamide **63** as a yellow oil (0.17 g, 91%). NMR spectra showed a 3:1 mixture of rotamers, only signals for the major rotamer is recorded. ν_{max}/cm^{-1} (neat) 2968 (CH), 1622 (CO), 1472, 1091, 909; δ_H (400 MHz, $CDCl_3$) 0.97–1.00 (3H, m, $CHCH_2CH_3$), 1.17–1.28

(3H, m, NCHCH_3), 1.52–1.66 (2H, m, CH_2CH$_3$), 2.97 (3H, s, NCH_3), 3.89–3.95 (1H, m, NCHCH$_3$), 7.36–7.45 (3H, m, Ar H), 7.53–7.64 (2H, m, Ar H), 7.71–7.75 (2H, m, Ar H), 7.94–8.06 (2H, m, Ar H); δ_C (100 MHz, $CDCl_3$) 11.1 (CH_3), 18.5 (CH_3), 27.4 (CH_2), 50.4 (CH_3), 55.7 (CH), 120.0 (CH), 120.4 (CH), 120.7 (CH), 122.8 (C), 127.3 (CH), 128.2 (CH), 130.1 (CH), 130.7 (CH), 131.3 (CH), 132.8 (CH), 136.6 (C), 139.8 (C), 148.3 (C), 159.0 (C), 169.4 (C), 170.1 (C); *m/z* (CI) 397.0916 (MH^+. $C_{21}H_{21}{}^{79}BrN_2O$ requires 397.0915), 317 (87), 284 (3).

1-(2-Iodophenyl)isoquinoline-3-*N*-methyl-3-*N*-*sec*-butylcarboxamide 61

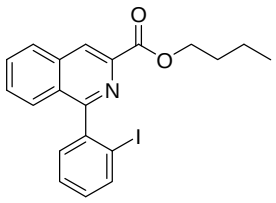


61

A Schlenk tube was charged with copper iodide (0.01 g, 0.02 mmol) and sodium iodide (0.10 g, 0.64 mmol), evacuated and backfilled with argon. *N*,*N*-Dimethylethylenediamine (0.01 g, 0.03 mmol) and 1-(2-bromophenyl)isoquinoline-3-*N*-methyl-*N*-*sec*-butylcarboxamide **63** (0.13 g, 0.32 mmol) were dissolved in butan-1-ol (6 mL) and added to the Schlenk tube. The Schlenk tube was sealed with a Teflon valve and the reaction mixture stirred at 120 °C for 24 h. The reaction had not gone to completion therefore more equivalents of copper iodide (0.01 g, 0.02 mmol), sodium iodide (0.10 g, 0.64 mmol) and *N*,*N*-dimethylethylenediamine (0.01 g, 0.03 mmol) were added and left for another 72 h. The reaction was diluted with water, extracted with ethyl acetate (3 × 20 mL), dried ($MgSO_4$) and concentrated to dryness. The product was purified by flash column chromatography, using a graduated eluent with petroleum ether/ethyl acetate to give 1-(2-Iodophenyl)isoquinoline-3-*N*-methyl-3-*N*-*sec*-butylcarboxamide **61** as a yellow oil (0.09 g, 63%). NMR spectra showed a 3:1 mixture of rotamers, only signals for the major rotamer is recorded. ν_{max}/cm^{-1} (NaCl) 2966 (CH), 1629 (CO), 1464, 1242, 754; δ_H (400 MHz, $CDCl_3$) 0.74–0.84 (3H, m, CH_2CH_3), 1.23 (3H, t, *J* 6.4 Hz, NCHCH_3), 1.49–1.68 (2H, m, CH_2CH$_3$), 2.98 (3H, s, NCH_3), 3.91–4.04 (1H, m, NCHCH$_3$), 7.18–7.22 (1H, m, Ar H), 7.33–7.42 (1H, m, Ar H), 7.47–7.57 (3H, m, Ar H), 7.71–7.74 (1H, m, Ar H), 7.94–8.06 (3H, m, Ar H); δ_C (100 MHz, $CDCl_3$) 11.1 (CH_3), 18.6 (CH_3), 26.3 (CH_3) 27.4 (CH_2), 55.8 (CH), 97.6 (C), 119.9 (CH), 120.5 (CH), 127.1 (CH), 127.4 (CH), 128.2 (CH), 128.3 (CH), 130.2 (CH) 130.8 (CH), 132.8 (C), 136.7 (C), 139.1 (CH), 142.8 (C), 148.1 (C), 161.7 (C),

170.2 (C); *m/z* (FAB) 445.0775 (MH^+. $C_{21}H_{22}IN_2O$ requires 445.0777), 397 (21%), 330 (33), 203 (19), 88 (25).

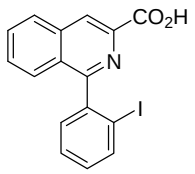
Butyl 1-(2-iodophenyl)isoquinoline-3-carboxylate 75



75

A Schlenk tube was charged with copper iodide (0.01 g, 0.03 mmol) and sodium iodide (0.18 g, 1.17 mmol), evacuated and backfilled with argon. *N*,*N*-Dimethylethylenediamine (0.01 g, 0.03 mmol) and methyl 1-(2-bromophenyl)isoquinoline-3-carboxylate **72** (0.20 g, 0.58 mmol) were dissolved in butan-1-ol (7 mL) and added to the Schlenk tube. The Schlenk tube was sealed with a Teflon valve and the reaction mixture stirred at 135 °C for 18 h. The reaction had not gone to completion therefore more equivalents of copper iodide (0.01 g, 0.03 mmol), sodium iodide (0.18 g, 1.17 mmol) and *N*,*N*-dimethylethylenediamine (0.01 g, 0.03 mmol) were added and left for another 18 h. The reaction was diluted with water, extracted with ethyl acetate (3 × 20 mL), dried ($MgSO_4$) and concentrated to dryness. The product was purified by flash column chromatography, using a graduated eluent with petroleum ether/ethyl acetate to give butyl 1-(2-iodophenyl)isoquinoline-3-carboxylate **75** as yellow oil (0.13 g, 52%). ν_{max}/cm^{-1} (NaCl) 2957 (CH), 1712 (CO), 1465, 1239, 1098, 758; δ_H (400 MHz, $CDCl_3$) 0.99 (3H, t, *J* 7.4 Hz, $CH_2CH_2CH_3$), 1.46–1.60 (2H, m, $CH_2CH_2CH_3$), 1.80–1.87 (2H, m, $CH_2CH_2CH_3$), 4.47 (2H, td, *J* 6.8, 1.6 Hz, OCH_2), 7.17–7.21 (1H, m, Ar H), 7.43–7.52 (2H, m, Ar H), 7.62–7.66 (2H, m, Ar H), 7.71–7.80 (1H, m, Ar H), 7.90 (1H, d, *J* 8.4 Hz, Ar H), 8.05 (1H, d, *J* 8.4 Hz, Ar H), 8.62 (1H, s, 4-CH); δ_C (100 MHz, $CDCl_3$) 12.4 (CH_3), 17.5 (CH_2), 29.3 (CH_2), 64.2 (CH_2), 96.7 (C), 122.4 (CH), 126.1 (CH), 126.7 (CH), 127.0 (CH), 128.1 (CH), 128.7 (CH), 129.3 (CH), 130.1 (CH), 134.5 (C), 137.7 (C), 138.2 (CH), 139.6 (C), 141.9 (C), 161.2 (C), 164.3 (C); *m/z* (EI) 431.0388 (M^+. $C_{20}H_{18}INO_2$ requires 431.0382), 358 (14), 331 (100), 203 (59).

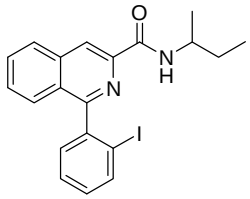
1-(2-Iodophenyl)isoquinoline-3-carboxylic acid 60



60

The reaction was carried out according to general procedure 2 using butyl 1-(2-iodophenyl)isoquinoline-3-carboxylate **75** (0.18 g, 0.41 mmol) and sodium hydroxide (0.06 g, 1.67 mmol) and gave 1-(2-iodophenyl)isoquinoline-3-carboxylic acid **60** as a yellow oil (0.14 g, 89%); mp 174–176 °C; ν_{max}/cm^{-1} (NaCl) 2923 (CH), 2902 (CH), 1719 (CO), 1379, 1101, 752; δ_H (400 MHz, $CDCl_3$) 7.26–7.29 (1H, m, Ar H), 7.41–7.45 (1H, m, Ar H), 7.51–7.58 (1H, m, Ar H), 7.71–7.80 (2H, m, Ar H), 7.86 (1H, t, *J* 6.4 Hz, Ar H), 8.07 (1H, d, *J* 8.0 Hz, Ar H), 8.13 (1H, d, *J* 8.0 Hz, Ar H), 8.75 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 97.4 (C), 122.7 (CH), 127.8 (CH), 128.1 (CH), 128.9 (CH), 130.2 (CH), 130.6 (CH), 131.7 (CH), 131.8 (CH), 133.1 (C), 136.6 (CH), 137.2 (C), 138.0 (C), 139.6 (C), 142.2 (C), 161.3 (C); *m/z* (EI) 374.9760 (M^+. $C_{16}H_{10}INO_2$ requires 374.9756), 331 (10%), 204 (19), 134 (25), 83 (100), 44 (60).

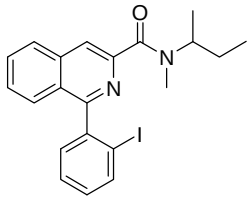
1-(2-Iodophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide



The reaction was carried out according to general procedure 3 using 1-(2-iodophenyl)isoquinoline-3-carboxylic acid **60** (0.13 g, 0.34 mmol) and *sec*-butylamine (1.05 mL, 10.4 mmol) and gave 1-(2-iodophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide as a colourless oil (0.10 g, 74%). ν_{max}/cm^{-1} (NaCl) 3377 (NH), 2964 (CH), 1669 (CO), 1619, 1515, 1017, 733; δ_H (400 MHz, $CDCl_3$) 0.87–0.92 (3H, m, $CHCH_2CH_3$), 1.17–1.20 (3H, m, $NCHCH_3$), 1.51–1.58 (2H, m, CH_2CH_3), 4.05–4.16 (1H, m, $NCHCH_3$), 7.14–7.17 (1H, m, Ar H), 7.34–7.39 (1H, m, Ar H), 7.44–7.53 (2H, m, Ar H), 7.58–7.61 (1H, m, Ar H), 7.66–7.72 (1H, m, Ar H), 7.97–8.01 (2H, m, Ar H), 8.59 (1H, s, 4-H); δ_C (100 MHz, $CDCl_3$) 8.6 (CH_3), 18.5 (CH_3), 27.7 (CH_2), 44.7 (CH), 96.1 (C), 118.1 (CH), 121.1 (CH),

125.4 (C), 125.9 (CH), 126.5 (CH), 128.1 (CH), 128.7 (C) 129.5 (CH), 131.1 (CH), 134.9 (CH), 137.7 (CH), 140.7 (C), 141.2 (C), 158.5 (C), 162.1 (C); *m/z* (FAB) 431.0623 (MH^+. $C_{20}H_{20}IN_2O$ requires 431.0620), 383 (20%), 330 (42), 203 (23), 148 (31).

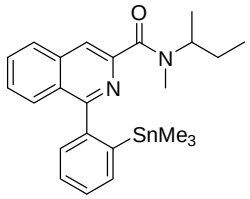
1-(2-Iodophenyl)isoquinoline-3-*N*-methyl-3-*N*-*sec*-butylcarboxamide 61



61

The reaction was carried out according to general procedure 4 using 1-(2-iodophenyl)isoquinoline-3-*N*-*sec*-butylcarboxamide (0.10 g, 0.23 mmol) and methyl iodide (0.22 mL, 3.73 mmol) and gave 1-(2-iodophenyl)isoquinoline-3-*N*-methyl-3-*N*-*sec*-butylcarboxamide **61** as an yellow oil (0.05 g, 48%). NMR spectra showed a 3:1 mixture of rotamers, only signals for the major rotamer is recorded. ν_{max}/cm^{-1} (NaCl) 2966 (CH), 1629 (CO), 1464, 1242, 754; δ_H (400 MHz, $CDCl_3$) 0.74–0.84 (3H, m, CH_2CH_3), 1.23 (3H, t, *J* 6.4 Hz, $NCHCH_3$), 1.49–1.68 (2H, m, CH_2CH_3), 2.98 (3H, s, NCH_3), 3.91–4.04 (1H, m, $NCHCH_3$), 7.18–7.22 (1H, m, Ar H), 7.33–7.42 (1H, m, Ar H), 7.47–7.57 (3H, m, Ar H), 7.71–7.74 (1H, m, Ar H), 7.94–8.06 (3H, m, Ar H); δ_C (100 MHz, $CDCl_3$) 11.1 (CH_3), 18.6 (CH_3), 26.3 (CH_3) 27.4 (CH_2), 55.8 (CH), 97.6 (C), 119.9 (CH), 120.5 (CH), 127.1 (CH), 127.4 (CH), 128.2 (CH), 128.3 (CH), 130.2 (CH) 130.8 (CH), 132.8 (C), 136.7 (C), 139.1 (CH), 142.8 (C), 148.1 (C), 161.7 (C), 170.2 (C); *m/z* (FAB) 445.0775 (MH^+. $C_{21}H_{22}IN_2O$ requires 445.0777), 397 (21%), 330 (33), 203 (19), 88 (25).

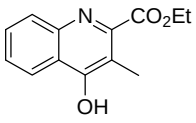
1-(2-(Trimethylstannyl)phenyl)isoquinoline-3-*N*-methyl-3-*N*-*sec*-butylcarboxamide 64



64

1-(2-Bromophenyl)isoquinoline-3-*N*-methyl-*N*-*sec*-butylcarboxamide **63** (0.10 g, 0.25 mmol) and toluene (15 mL) were de-gased for 0.5 h. Hexamethylditin (0.10 mL, 0.50 mmol) and tetrakis(triphenylphosphine)palladium (0.01 g, 0.09 mmol) were added to the reaction mixture and heated under reflux for 48 h. The black mixture was diluted with dichloromethane (15 mL), filtered and the solvent removed by rotary evaporation. The product was purified by flash column chromatography using graduated eluent with petroleum ether > petroleum ether/ethyl acetate (50:50) to give 1-(2-(trimethylstannyl)phenyl)isoquinoline-3-*N*-methyl-*N*-*sec*-butylcarboxamide **64** as a yellow oil (0.02 g, 13%). NMR spectra showed a 3:1 mixture of rotamers, only signals for the major rotamer is given here. ν_{max}/cm^{-1} (NaCl) 2967 (CH), 1636 (CO), 1459, 1090, 757; δ_H (400 MHz, $CDCl_3$) 0.04 (9H, s, $Sn(CH_3)_3$), 0.88 (3H, t, *J* 7.3 Hz, $CHCH_2CH_3$), 1.28 (3H, d, *J* 6.5 Hz, $NCHCH_3$), 1.44–1.57 (2H, m, CH_2CH_3), 3.13 (3H, s, NCH_3), 3.88–4.05 (1H, m, $NCHCH_3$), 7.60–7.65 (2H, m, Ar H), 7.70–7.74 (2H, m, Ar H), 7.86–7.91 (2H, m, Ar H), 8.06–8.10 (2H, m, Ar H), 8.17 (1H, d, *J* 8.4 Hz, 4-H); δ_C (100 MHz, $CDCl_3$) 8.1 ($3 \times CH_3$), 11.2 (CH_3), 18.4 (CH_3), 26.8 (CH_3), 27.4 (CH_2), 55.7 (CH), 119.4 (C), 119.5 (CH), 127.0 (C), 127.5 (CH), 127.6 (CH), 127.9 (CH), 129.7 (CH), 130.1 (CH), 130.5 (CH), 136.8 (CH), 136.9 (CH), 145.5 (C), 148.3 (C), 148.5 (C), 162.2 (C), 169.3 (C); *m/z* (CI) 483.1458 (MH^+. $C_{24}H_{31}N_2O^{118}Sn$ requires 483.1458), 479 (43%), 467 (32), 463 (13), 319 (6), 165 (4).

Ethyl 3-methyl-4-hydroxyquinoline-2-carboxylate 81[93]

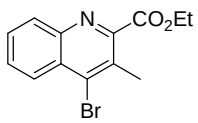


81

To a solution of aniline **83** (2.25 mL, 24.7 mmol) and diethyl oxalpropionate **82** (4.57 mL, 24.7 mmol) in cyclohexane (200 mL) was added *p*-toluenesulfonic acid (0.03 g, 0.17 mmol) and the mixture heated under reflux under an argon atmosphere at 120 °C using a

Dean-Stark condenser for 48 h. The reaction mixture was cooled to room temperature, filtered, washed with hexane and the solvent of the combined filtrate removed under reduced pressure. To this crude oil product was added polyphosphoric acid (20.0 g) and the mixture was stirred at 120 °C for 1 h to form a dark brown substance. The substance was cooled to room temperature and 2.4 M sodium carbonate solution (100 mL) was added slowly to quench the excess acid and precipitated the product as a yellow solid. The mixture was filtered, followed by dissolving in chloroform and hot filtering to remove insoluble impurities. Purification by flash column chromatography using a graduated eluent of petroleum ether > petroleum ether/ethyl acetate (50:50) afforded ethyl 3-methyl-4-hydroxyquinoline-2-carboxylate **81** as a yellow solid (3.14 g, 53%). mp 176–178 °C; ν_{max}/cm^{-1} (KBr) 3395 (OH), 2973 (CH), 1736 (CO), 1520, 1476, 1231, 763; δ_H (400 MHz, $CDCl_3$) 1.48 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 2.49 (3H, s, 3-CH_3), 4.52 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 7.31–7.37 (2H, m, Ar H), 7.62 (1H, ddd, *J* 8.4, 5.5, 1.5 Hz, Ar H), 8.35 (1H, d, *J* 8.4 Hz, Ar H), 9.18 (1H, s, OH); δ_C (100 MHz, $CDCl_3$) 11.7 (CH_3), 14.2 (CH_3), 63.3 (CH_2), 117.5 (CH), 122.8 (C), 123.7 (C), 123.8 (CH), 126.6 (CH), 132.7 (CH), 132.8 (C), 138.3 (C), 164.4 (C), 179.7 (C); *m/z* (EI) 231.0897 (M^+. $C_{13}H_{13}NO_3$ requires 231.0895), 202 (100%), 157 (98), 129 (46), 84 (41).

Ethyl 3-methyl-4-bromoquinoline-2-carboxylate 80

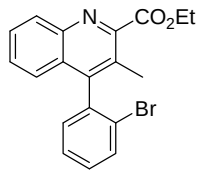


80

To a solution of ethyl 3-methyl-4-bromoquinoline-2-carboxylate **81** (4.55 g, 19.6 mmol) in anhydrous acetonitrile (250 mL) was added phosphorous oxybromide (16.9 g, 59.1 mmol) and anhydrous potassium carbonate (8.16 g, 59.1 mmol). The solution was heated under reflux for 2 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to form an oil. Water (100 mL) was added to the residue and subsequently extracted with ethyl acetate (100 mL). The ethyl acetate layer was removed under reduced pressure to form a brown oil from which ethyl 3-methyl-4-bromoquinoline-2-carboxylate precipitated as a brown solid **80** (4.85 g, 84%). mp 48–50 °C; ν_{max}/cm^{-1} (KBr) 2983 (CH), 1714 (CO), 1479, 1272, 759; δ_H (400 MHz, $CDCl_3$) 1.48 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 2.72 (3H, s, 3-CH_3), 4.55 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 7.68 (1H, ddd, *J* 8.3, 6.9, 1.4 Hz, Ar H), 7.75 (1H, ddd, *J* 8.3, 6.9, 1.4 Hz, Ar H), 8.18 (1H, d, *J* 8.3 Hz, Ar H), 8.25 (1H, d, *J* 8.3 Hz Ar H); δ_C (100 MHz, $CDCl_3$) 14.2 (CH_3), 19.9 (CH_3), 62.5 (CH_2),

126.8 (CH), 128.5 (C), 129.3 (CH), 129.5 (C), 129.9 (CH), 130.1 (CH), 137.7 (C), 145.6 (C), 150.8 (C), 166.2 (C); *m/z* (EI) 293.0053 (M^+. $C_{13}H_{12}{}^{79}BrNO_2$ requires 293.0051), 264 (39%), 221 (73), 140 (100), 114 (21), 84 (81).

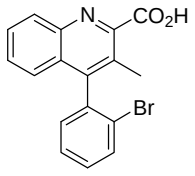
Ethyl 3-methyl-4-bromophenylquinoline-2-carboxylate 91



91

The reaction was carried out according to general procedure 1 using ethyl 3-methyl-4-bromoquinoline-2-carboxylate **80** (3.70 g, 12.5 mmol) and bromophenylboronic acid (3.28 g, 16.4 mmol) and gave ethyl 3-methyl-4-bromophenylquinoline-2-carboxylate **91** as a white solid (3.47 g, 75%). mp 88–90 °C; ν_{max}/cm^{-1} (KBr) 2987 (CH), 1719 (CO), 1468, 1370, 1244, 1072, 754; δ_H (400 MHz, $CDCl_3$) 1.49 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 2.29 (3H, s, 3-CH_3), 4.55 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 7.18–7.24 (2H, m, Ar H), 7.36–7.41 (1H, m, Ar H), 7.45–7.52 (2H, m, Ar H), 7.69 (1H, ddd, *J* 8.3, 6.7, 1.4 Hz, Ar H), 7.78 (1H, d, *J* 8.5 Hz, Ar H), 8.21 (1H, d, *J* 8.5 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 14.3 (CH_3), 16.5 (CH_3), 62.2 (CH_2), 123.4 (C), 125.4 (CH), 127.0 (C), 127.5 (C), 127.8 (CH), 128.1 (CH), 129.2 (CH), 130.0 (CH), 130.1 (CH), 130.8 (CH), 133.1 (CH), 137.7 (C), 145.7 (C), 147.4 (C), 151.4 (C), 167.7 (C); *m/z* (EI) 369.0360 (M^+. $C_{19}H_{16}{}^{79}BrO_2$ requires 369.0364), 342 (11%), 297 (70), 216 (100), 189 (31), 82 (49).

3-Methyl-4-bromophenylquinoline-2-carboxylic acid 93

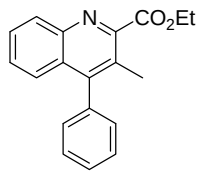


93

The reaction was carried out according to general procedure 2 using ethyl 3-methyl-4-bromophenylquinoline-2-carboxylate **91** (2.53 g, 6.84 mmol) and sodium hydroxide (0.41 g, 10.3 mmol) and gave 3-methyl-4-bromophenylquinoline-2-carboxylic acid **93** as a white solid (2.16 g, 92%). mp 133–134 °C; ν_{max}/cm^{-1} (KBr) 3479 (OH), 2932 (CH), 1747 (CO), 1470, 1375, 1230, 1023, 766; δ_H (400 MHz, $CDCl_3$) 2.62 (3H, s, 3-CH_3), 7.18–7.20 (1H, d,

J 7.5 Hz, Ar H), 7.26–7.28 (1H, m, Ar H), 7.40–7.44 (1H, m, Ar H), 7.49–7.61 (2H, m, Ar H), 7.75–7.82 (2H, m, Ar H), 7.69 (1H, d, *J* 8.4 Hz, Ar H), 7.78 (1H, d, *J* 8.5 Hz, Ar H), 8.21 (1H, d, *J* 8.5 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 17.0 (CH_3), 123.4 (C), 125.8 (CH), 126.5 (C), 127.9 (CH), 128.8 (CH), 129.0 (C), 129.2 (CH), 129.5 (CH), 130.1 (CH), 130.7 (CH), 133.2 (CH), 137.3 (C), 143.8 (C), 144.8 (C), 150.0 (C), 164.4 (C); *m/z* (EI) 341.0054 (M^+. $C_{17}H_{12}{}^{79}BrO_2$ requires 341.0051), 342 (11%), 297 (13), 262 (28), 216 (99), 189 (23), 108 (11), 63 (4).

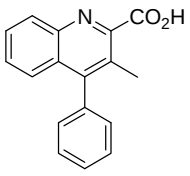
Ethyl 3-methyl-4-phenylquinoline-2-carboxylate 94[71]



94

The reaction was carried out according to general procedure 1 using ethyl 3-methyl-4-bromoquinoline-2-carboxylate **80** (3.40 g, 11.6 mmol) and phenylboronic acid (2.64 g, 21.7 mmol) and gave ethyl 3-methyl-4-phenylquinoline-2-carboxylate **94** as a white solid (3.33 g, 99%). mp 110–112 °C (lit.,[71] mp 114–115 °C); ν_{max}/cm^{-1} (KBr) 2972 (CH), 1723 (CO), 1488, 1372, 1245, 1065, 768; δ_H (400 MHz, $CDCl_3$) 1.48 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 2.32 (3H, s, 3-CH_3), 4.55 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 7.23–7.27 (2H, m, Ar H), 7.37–7.56 (5H, m, Ar H), 7.67 (1H, ddd, *J* 8.3, 6.7, 1.4 Hz, Ar H), 8.19 (1H, d, *J* 8.3 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 14.3 (CH_3), 16.8 (CH_3), 62.1 (CH_2), 126.0 (CH), 126.2 (C), 127.7 (CH), 128.1 (CH), 128.2 (C), 128.7 (2 × CH), 129.0 (CH), 129.2 (2 × CH), 129.8 (CH), 136.7 (C), 145.6 (C), 148.7 (C), 151.5 (C), 167.4 (C); *m/z* (CI) 292.1336 (MH^+. $C_{19}H_{18}NO_2$ requires 292.1338), 220 (48%), 137 (3), 113 (9), 85 (19).

3-Methyl-4-phenylquinoline-2-carboxylic acid 96

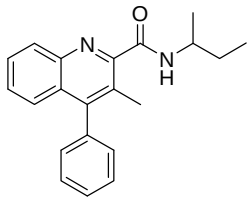


96

The reaction was carried out according to general procedure 2 using ethyl 3-methyl-4-phenylquinoline-2-carboxylate **94** (1.97 g, 6.77 mmol) and sodium hydroxide (0.62 g, 15.4

mmol) and gave 4-phenyl-3-methylquinoline-2-carboxylic acid **96** as a white solid (1.66 g, 92%). mp 130–132 °C; ν_{max}/cm^{-1} (KBr) 3437 (OH), 2931 (CH), 1722 (CO), 1649 (C=C), 1590, 1364, 1317, 768; δ_H (400 MHz, $CDCl_3$) 2.65 (3H, s, 3-CH_3), 7.23–7.25 (2H, m, Ar H), 7.43 (1H, d, *J* 8.3 Hz, Ar H), 7.50–7.60 (4H, m, Ar H), 7.75 (1H, ddd, *J* 8.3, 6.9, 1.2 Hz, Ar H), 8.14 (1H, d, *J* 8.3 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 17.4 (CH_3), 126.5 (CH), 128.4 (CH), 128.8 (2 × CH), 129.0 (CH), 129.1 (CH), 129.2 (2 × CH), 129.7 (C), 129.8 (CH), 130.2 (C), 136.4 (C), 143.6 (C), 144.2 (C), 151.4 (C), 164.4 (C); *m/z* (CI) 264.1027 (MH^+. $C_{17}H_{14}NO_2$ requires 264.1025), 220 (19%), 188 (3), 85 (27).

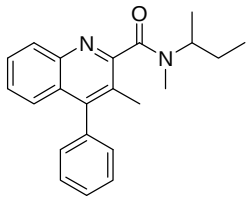
3-Methyl-4-phenylquinoline-2-*N*-*sec*-butylcarboxamide 97[62]



97

The reaction was carried out according to general procedure 3 using 3-methyl-4-phenylquinoline-2-carboxylic acid **96** (1.21 g, 4.61 mmol) and *sec*-butylamine (6.90 mL, 68.4 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-*sec*-butylcarboxamide **97** as a white solid (1.02 g, 70%). mp 152–154 °C (lit.,[62] mp 157–158 °C); ν_{max}/cm^{-1} (KBr) 3282 (NH), 2967 (CH), 1639 (CO), 1535, 1443, 1156, 761; δ_H (400 MHz, $CDCl_3$) 1.03 (3H, t, *J* 7.4 Hz, $CHCH_2CH_3$), 1.33 (3H, d, *J* 6.6 Hz, $CHCH_3$), 1.61–1.75 (2H, m, CH_2CH_3), 2.56 (3H, s, 3-CH_3), 4.08–4.20 (1H, m, $CHCH_3$), 7.21–7.25 (2H, m, Ar H), 7.35 (1H, d, *J* 8.3 Hz, Ar H), 7.40–7.55 (3H, m, Ar H), 7.65 (1H, ddd, *J* 8.3, 6.8, 1.4 Hz, Ar H), 7.90 (1H, d, *J* 8.3 Hz, Ar H), 8.09 (1H, d, *J* 8.3 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 10.6 (CH_3), 17.6 (CH_3), 20.5 (CH_3), 29.9 (CH_2), 46.8 (CH), 126.1 (CH), 127.5 (CH), 127.9 (CH), 128.5 (C), 128.6 (2 × CH), 128.7 (CH and C), 129.3 (2 × CH), 129.4 (CH), 137.2 (C), 144.6 (C), 149.4 (C), 150.1 (C), 166.2 (C); *m/z* (CI) 319.1809 (MH^+. $C_{21}H_{23}N_2O$ requires 319.1810), 220 (19%), 202 (5), 148 (6), 113 (16), 85 (77).

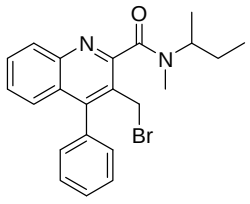
3-Methyl-4-phenylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide 95[62]



95

The reaction was carried out according to general procedure 4 using 3-methyl-4-phenylquinoline-2-*N*-*sec*-butyl-carboxamide **97** (0.90 g, 2.84 mmol) and methyl iodide (1.53 mL, 24.6 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide **95** as a dark orange solid (0.77 g, 82%). NMR spectra showed a 3:1 mixture of rotamers, only signals for the major rotamer is recorded. mp 114–117 °C (lit.,[62] mp 117–118 °C); ν_{max}/cm^{-1} (KBr) 2963 (CH), 1628 (CO), 1465, 1072, 771; δ_H (400 MHz, $CDCl_3$) 0.86 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.24 (3H, d, *J* 6.6 Hz, $CHCH_3$), 1.55–1.70 (2H, m, CH_2CH_3), 2.23 (3H, s, 3-CH_3), 3.05 (3H, s, NCH_3), 3.42–3.53 (1H, m, $CHCH_3$), 7.25–7.31 (2H, m, Ar H), 7.38–7.44 (2H, m, Ar H), 7.47–7.57 (3H, m, Ar H), 7.62–7.67 (1H, m, Ar H), 8.10 (1H, d, *J* 8.3 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 11.3 (CH_3), 16.4 (CH_3), 18.6 (CH_3), 25.5 (CH_3), 27.2 (CH_2) 49.5 (CH) 125.3 (C), 125.9 (CH), 126.8 (2 × CH), 127.4 (C), 128.0 (CH), 128.6 (CH), 128.7 (CH), 129.2 (2 × CH), 129.4 (CH), 136.8 (C), 145.8 (C), 148.0 (C), 156.1 (C), 169.7 (C); *m/z* (EI) 332.1888 (M^+. $C_{22}H_{24}N_2O$ requires 332.1889), 303 (35%), 275 (22), 218 (89), 189 (25), 86 (100).

3-Bromomethyl-4-phenylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide 98

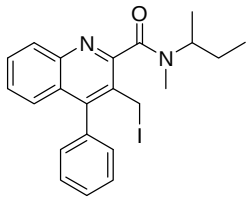


98

To a stirred, degassed solution of 3-methyl-4-phenylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide **95** (0.10 g, 0.30 mmol) in carbon tetrachloride (20 mL), was added *N*-bromosuccinimide (0.06 g, 0.34 mmol) and dibenzoyl peroxide (0.01 g, 0.03 mmol) and the orange solution heated under reflux for 24 h. The reaction mixture was cooled to room temperature and a second portion of *N*-bromosuccinimide (0.06 g, 0.34 mmol) and dibenzoyl peroxide (0.01 g, 0.03 mmol) were added. The solution was heated under reflux

for a further 48 h. A third quota of *N*-bromosuccinimide (0.06 g, 0.34 mmol) and dibenzoyl peroxide (0.01 g, 0.03 mmol) was added after 48 h and the reaction heated under reflux for a final 24 h. The reaction mixture was cooled to room temperature, filtered and the solvent removed under reduced pressure. The crude product (orange oil) was purified by flash column chromatography using an graduated eluent of dichloromethane > dichloromethane/ethyl acetate (95:5) and afforded 3-bromomethyl-4-phenylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide **98** as a white powder (0.06 g, 45%). NMR spectra showed a 2:1 mixture of rotamers, only signals for the major rotamer is recorded. mp 160–164 °C; ν_{max}/cm^{-1} (KBr) 2966 (CH), 1630 (CO), 1483, 1396, 1045, 760; δ_H (400 MHz, $CDCl_3$) 1.08 (3H, t, *J* 7.4 Hz, CH_2CH_3), 1.32 (3H, d, *J* 6.8 Hz, $CHCH_3$), 1.52–1.67 (2H, m, CH_2CH_3), 2.85 (3H, s, NCH_3), 4.60 (1H, d, *J* 10.2 Hz, 3-C*H*HBr), 4.67 (1H, d, *J* 10.2 Hz, 3-CH*H*Br), 4.83 (1H, sextet, *J* 6.8 Hz, C*H*CH_3), 7.38–7.48 (4H, m, Ar H), 7.52–7.60 (3H, m, Ar H), 7.72 (1H, ddd, *J* 8.3, 6.7, 1.5 Hz, Ar H), 8.07–8.14 (1H, m, Ar H); δ_C (100 MHz, $CDCl_3$) 11.2 (CH_3), 17.1 (CH_3), 26.6 (CH_2), 27.8 (CH_2), 30.5 (CH_3), 50.2 (CH), 126.3 (C), 126.8 (2 × CH), 127.5 (CH), 128.6 (2 × CH), 128.7 (CH), 129.1 (CH), 129.2 (CH), 129.5 (CH), 134.9 (C), 135.0 (C), 146.4 (C), 149.4 (C), 156.0 (C), 168.4 (C); *m/z* (EI) 410.0995 (M^+. $C_{22}H_{23}{}^{79}BrN_2O$ requires 410.0994), 330 (27%), 217 (57), 189 (28), 86 (100).

3-Iodomethyl-4-phenylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide 65

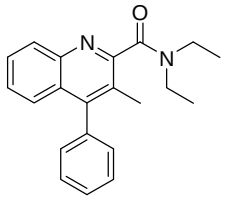


65

To a stirred solution of 18-crown-6 (0.02 g, 0.06 mmol) in acetonitrile (40 mL) was added sodium iodide (0.47 g, 3.16 mmol) and the colourless solution stirred for 1 h at room temperature. To this, a solution of 3-bromomethyl-4-phenylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide **98** (0.13 g, 0.32 mmol) in acetonitrile (10 mL) was added and the resulting cloudy mixture stirred at room temperature for 24 h. Water (10 mL) was added and the mixture was extracted using chloroform and the organic layer was dried ($MgSO_4$). The chloroform was removed under reduced pressure to form a colourless oil which was subsequently purified by flash column chromatography using an graduated eluent of dichloromethane > dichloromethane/ethyl acetate (70:30). This afforded 3-iodomethyl-4-phenylquinoline-2-*N*-methyl-*N*-*sec*-butylcarboxamide **65** as a brown solid (0.11 g, 74%). NMR spectra showed a 2:1 mixture of rotamers, only signals for the major rotamer is

recorded. mp 150–152 °C; ν_{max}/cm^{-1} (KBr) 2963 (CH), 1628 (CO), 1483, 1091, 1044, 759; δ_H (400 MHz, $CDCl_3$) 1.10 (3H, t, *J* 7.4 Hz, $CHCH_2CH_3$), 1.34 (3H, d, *J* 6.8 Hz, $CHCH_3$), 1.56–1.68 (2H, m, CH_2CH_3), 2.89 (3H, s, NCH_3), 4.53 (1H, d, *J* 9.1 Hz, 3-C*H*HI), 4.67 (1H, d, *J* 9.1 Hz, 3-CH*H*I), 4.87 (1H, sextet, *J* 6.8 Hz, $CHCH_3$), 7.32–7.37 (1H, m, Ar H), 7.39–7.47 (3H, m, Ar H), 7.52–7.60 (3H, m, Ar H), 7.67–7.73 (1H, m, Ar H), 8.09 (1H, t, *J* 8.8 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 10.9 (CH_3), 16.7 (CH_3), 26.3 (CH_2), 27.5 (CH_2), 30.3 (CH_3), 49.8 (CH), 126.2 (CH), 127.0 (CH), 127.1 (CH), 127.5 (C), 128.0 (C), 128.2 (CH), 128.4 (2 × CH and C), 129.2 (2 × CH), 129.5 (CH), 129.6 (C) 145.6 (C), 148.2 (C), 168.0 (C); *m/z* (EI) 458.0854 (M^+. $C_{22}H_{23}IN_2O$ requires 458.0855), 331 (55%), 301 (10), 216 (86), 189 (22), 151 (13), 86 (100).

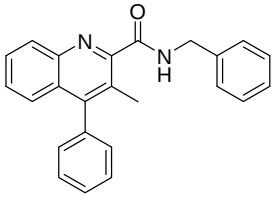
3-Methyl-4-phenylquinoline-2-*N*-diethylcarboxamide 99



99

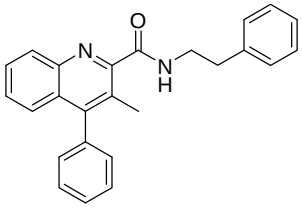
The reaction was carried out according to general procedure 3 using 3-methyl-4-phenylquinoline-2-carboxylic acid **96** (0.50 g, 1.90 mmol) and diethylamine (5.9 mL, 57.0 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-diethylcarboxamide **99** as a colourless oil (0.51 g 88%); ν_{max}/cm^{-1} (NaCl) 2970 (CH), 1624 (CO), 1442, 1265, 763; δ_H (400 MHz, $CDCl_3$) 1.17 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.34 (3H, t, *J* 7.2 Hz, CH_2CH_3), 2.22 (3H, s, 3-CH_3), 3.24 (2H, q, *J* 7.2 Hz, CH_2CH_3), 3.67 (2H, q, *J* 7.2 Hz, CH_2CH_3), 7.27 (2H, d, *J* 6.8 Hz, Ar H), 7.39–7.40 (2H, m, Ar H), 7.46–7.56 (3H, m, Ar H), 7.62–7.66 (1H, m, Ar H), 8.11 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 12.9 (CH_3), 14.0 (CH_3), 16.0 (CH_3), 39.3 (CH_2), 42.9 (CH_2), 125.0 (C), 126.0 (CH), 126.8 (CH), 128.1 (CH), 128.6 (2 × CH), 128.8 (CH), 129.0 (C), 129.2 (2 × CH), 129.4 (CH), 136.6 (C), 145.8 (C), 147.9 (C), 156.1 (C), 168.8 (C); *m/z* (EI) 318.1736 (M^+. $C_{21}H_{22}N_2O$ requires 318.1732), 247 (56%), 219 (100), 189 (12), 72 (73), 44 (25).

3-Methyl-4-phenylquinoline-2-*N*-benzylcarboxamide



The reaction was carried out according to general procedure 3 using 3-methyl-4-phenylquinoline-2-carboxylic acid **96** (0.50 g, 1.90 mmol) and benzylamine (6.24 mL, 57.0 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-benzylcarboxamide as a yellow oil (0.40 g, 60%); ν_{max}/cm^{-1} (NaCl) 3392 (NH), 2925 (CH), 1671 (CO), 1454, 1253, 766; δ_H (400 MHz, $CDCl_3$) 2.58 (3H, s, 3-C*H*$_3$), 3.64 (1H, s, NH), 4.71 (2H, s, NHC*H*$_2$), 7.19–7.23 (2H, m, Ar H), 7.25–7.30 (2H, m, Ar H) 7.33–7.42 (5H, m, Ar H), 7.44–7.54 (3H, m, Ar H), 7.61 (1H, ddd, *J* 8.4, 6.8, 1.6 Hz, Ar H), 8.03 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 17.5 (CH_3), 43.5 (CH_2), 126.1 (CH), 126.9 (C), 127.4 (CH), 127.7 (CH), 128.0 (CH), 128.6 (4 × CH), 128.8 (CH), 128.9 (CH), 129.5 (4 × CH), 137.1 (C), 138.4 (C), 138.5 (C), 144.6 (C), 149.6 (C), 149.7(C), 166.6 (C); *m/z* (EI) 352.1578 (M^+. $C_{24}H_{20}N_2O$ requires 352.1576), 309 (25%), 219 (100), 91 (35).

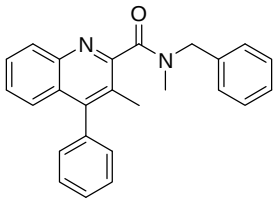
3-Methyl-4-phenylquinoline-2-*N*-phenethylcarboxamide



The reaction was carried out according to general procedure 3 using 3-methyl-4-phenylquinoline-2-carboxylic acid **96** (0.50 g, 1.90 mmol) and phenethylamine (7.10 mL, 57.0 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-phenethylcarboxamide as a yellow oil (0.51 g, 74%); ν_{max}/cm^{-1} (NaCl) 3392 (NH), 2929 (CH), 1670 (CO), 1486, 1213, 765; δ_H (400 MHz, $CDCl_3$) 2.41 (3H, s, 3-CH_3), 2.87 (2H, t, *J* 7.1 Hz, PhCH_2), 3.61–3.69 (2H, m, NHC*H*$_2$), 7.04 (2H, d, *J* 8.0 Hz, Ar H), 7.10–7.13 (1H, m, Ar H) 7.19–7.27 (5H, m, Ar H), 7.32–7.39 (3H, m, Ar H), 7.46 (1H, t, *J* 8.0 Hz, Ar H), 7.84 (1H, d, *J* 8.0 Hz, Ar H), 8.17 (1H, br t, *J* 5.2 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 16.9 (CH_3), 35.2 (CH_2), 40.3 (CH_2), 123.1 (CH), 125.0 (CH), 125.4 (CH), 126.5 (CH), 126.9 (CH), 127.0 (CH), 127.3 (4 × CH),

127.7 (2 × CH), 128.6 (2 × CH), 128.9 (C), 136.3 (C), 138.3 (C), 143.8 (C), 148.6 (C), 149.3 (C), 165.1 (C), 166.1(C); *m/z* (EI) 366.1736 (M^+. $C_{25}H_{22}N_2O$ requires 366.1732), 275 (78%), 218 (100), 140 (14), 82 (52).

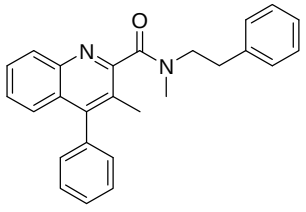
3-Methyl-4-phenylquinoline-2-*N*-methyl-*N*-benzylcarboxamide 101



101

The reaction was carried out according to general procedure 4 using 3-methyl-4-phenylquinoline-2-*N*-benzylcarboxamide (0.30 g, 0.85 mmol) and methyl iodide (0.85 mL, 13.6 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-methyl-*N*-benzylcarboxamide **101** as a yellow oil (0.25 g, 80%). NMR spectra showed a 1:1 mixture of rotamers, only signals for one rotamer is recorded; ν_{max}/cm^{-1}(NaCl) 2925 (CH), 1643 (CO), 1454, 1263, 765; δ_H (400 MHz, $CDCl_3$) 2.24 (3H, s, 3-CH_3), 2.83 (3H, s, NCH_3), 4.48 (2H, s, NHCH_2), 7.22–7.31 (4H, m, Ar H), 7.34–7.41 (4H, m, Ar H) 7.44–7.55 (4H, m, Ar H), 7.62–7.67 (1H, m, Ar H), 8.12 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 15.0 (CH_3), 34.3 (CH_3), 53.5 (CH_2), 123.9 (C), 124.9 (CH), 125.9 (CH), 126.6 (CH), 126.8 (CH), 127.0 (2 × CH), 127.4 (4 × CH), 127.8 (2 × CH), 127.9 (C), 128.1 (CH), 128.3 (CH), 135.2 (C), 135.6 (C), 144.7 (C), 147.1 (C), 154.5 (C), 168.3 (C); *m/z* (EI) 366.1733 (M^+. $C_{25}H_{22}N_2O$ requires 366.1732), 219 (14%), 120 (12), 82 (100).

3-Methyl-4-phenylquinoline-2-*N*-methyl-*N*-phenethylcarboxamide 103

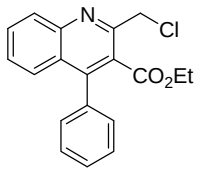


103

The reaction was carried out according to general procedure 4 using 3-methyl-4-phenylquinoline-2-*N*-benzylcarboxamide (0.30 g, 0.85 mmol) and methyl iodide (1.03 mL, 16.6 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-methyl-*N*-phenethylcarboxamide

103 as a yellow oil (0.36 g, 91%). NMR spectra showed a 1:1 mixture of rotamers, only signals for one rotamer is recorded. ν_{max}/cm^{-1} (NaCl) 2928 (CH), 1643 (CO), 1486, 1054, 765; δ_H (400 MHz, $CDCl_3$) 2.84 (3H, s, 3-CH_3), 3.10 (2H, t, *J* 7.6 Hz, PhCH_2), 3.23 (3H, s, NCH_3), 3.48 (2H, t, *J* 7.6 Hz, NCH_2), 6.94 (1H, d, *J* 7.2 Hz, Ar H) 7.10–7.17 (2H, m, Ar H), 7.18–7.25 (2H, m, Ar H), 7.28–7.35 (2H, m, Ar H), 7.39–7.41 (2H, m, Ar H), 7.45–7.54 (3H, m, Ar H), 7.61–7.68 (1H, m, Ar H), 8.12 (1H, t, *J* 9.2 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 16.1 (CH_3), 32.7 (CH_3), 33.4 (CH_2), 52.7 (CH_2), 124.9 (C), 125.6 (C), 126.1 (CH), 126.5 (CH), 126.6 (CH), 126.9 (CH), 127.1 (CH), 128.1 (CH), 128.6 (4 × CH), 128.9 (2 × CH), 129.3 (2 × CH), 136.6 (C), 138.7 (C), 145.8 (C), 148.1 (C), 155.9 (C), 169.2 (C); *m/z* (EI) 380.1887 (M^+. $C_{26}H_{24}N_2O$ requires 380.1889), 323 (28%), 289 (59), 218 (100), 134 (29), 91 (20).

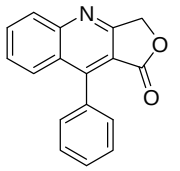
Ethyl 2-chloromethyl-4-phenylquinoline-3-carboxylate 111[69]



111

2-Aminobenzophenone **110** (4.00 g, 20.3 mmol) and ethyl 4-chloroacetoacetate **109** (3.34 g, 20.3 mmol) were placed in a sealed tube with *N*,*N*-dimethylformamide (40 mL). Chlorotrimethylsilane (10.3 mL, 81.1 mmol) was then added dropwise, the tube was sealed and heated to 100 ^oC overnight. After cooling, the reaction mixture was diluted with water, extracted with dichloronethane, dried ($MgSO_4$) and concentrated to dryness. Purification was carried out using flash column chromatography eluting with petroleum ether/ethyl acetate to give ethyl 2-chloromethyl-4-phenylquinoline-3-carboxylate **111** as yellow crystals (5.67 g, 85%). mp 109–111 °C (lit.,[69] mp 111–112 °C); ν_{max}/cm^{-1} (KBr) 2980 (CH), 1720 (CO), 1566, 1487, 1404, 1301, 1232, 768; δ_H (400 MHz, $CDCl_3$) 0.92 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 4.04 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 5.04 (2H, s, CH_2Cl), 7.35–7.37 (2H, m, Ar H), 7.48–7.52 (4H, m, Ar H), 7.63 (1H, d, *J* 8.3 Hz, Ar H), 7.77 (1H, t, *J* 7.2 Hz, Ar H), 8.14 (1H, d, *J* 8.3 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 13.5 (CH_3), 45.9 (CH_2), 61.6 (CH_2), 126.1 (C), 126.3 (C), 126.7 (CH), 127.8 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 129.3 (CH), 129.6 (CH), 130.8 (CH), 135.7 (C), 147.4 (C), 148.2 (C), 153.1 (C), 167.7 (C); *m/z* (EI) 325.0868 (M^+. $C_{19}H_{16}{}^{35}ClNO_2$ requires 325.0870), 280 (67%), 262 (61), 217 (63), 176 (22), 151(11), 84 (100).

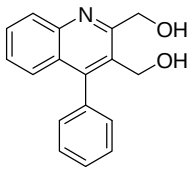
9-Phenylfuro[3,4-*b*]quinolin-1(3*H*)-one 122[71]



122

Ethyl 2-chloromethyl-4-phenylquinoline-3-carboxylate **111** (7.00 g, 21.5 mmol) was dissolved in 6 M hydrochloric acid (100 mL) and ethanol (100 mL) and heated under reflux for 9 days. The reaction mixture was concentrated to dryness and diluted with water (100 mL), made alkaline with solid sodium carbonate (pH ~ 8–9) and extracted with chloroform (100 mL). The organic layer was dried ($MgSO_4$) and concentrated under vacuum. Purification was carried out by flash column chromatography eluting, with petroleum ether/ethyl acetate to give 9-phenylfuro[3,4-*b*]quinolin-1(3*H*)-one **122** as a yellow solid (5.15 g, 91%). mp 197–199 °C (lit.,[71] mp 203–204 °C); ν_{max}/cm^{-1} (neat) 3043 (CH), 1764 (CO), 1604, 1582, 1495,1443, 1136, 1030, 777; δ_H (400 MHz, $CDCl_3$) 5.46 (2H, s, CH_2), 7.45–7.47 (2H, m, Ar H) 7.58–7.61 (4H, m, Ar H), 7.89–7.93 (2H, m, Ar H), 8.21 (1H, d, *J* 8.3 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 68.9 (CH_2), 112.8 (C), 126.4 (C), 126.7 (C), 127.4 (CH), 127.6 (2 × CH), 128.7 (CH), 128.8 (CH), 129.1 (2 × CH), 130.9 (CH), 131.9 (CH), 150.6 (C), 150.8 (C), 162.9 (C), 167.2 (C); *m/z* (FAB) 262.0867 (MH^+. $C_{17}H_{12}NO_2$ requires 262.0868), 232 (11%), 204 (18), 147 (14), 109 (14), 69 (57), 57 (100).

2,3-Bis(hydroxymethyl)-4-phenylquinoline 123[71]

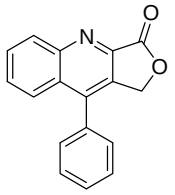


123

9-Phenylfuro[3,4-*b*]quinolin-1(3*H*)-one **122** (1.95 g, 7.47 mmol) was added to tetrahydrofuran (100 mL) and cooled to 0 °C. Lithium aluminium hydride (30.0 mL, 1.0 M in tetrahydrofuran) was then added slowly and the reaction mixture stirred at room temperature. After 1 h, the reaction was quenched with 1 M hydrochloric acid (pH~2) and the mixture extracted with ethyl acetate (2 × 50 mL), dried ($MgSO_4$) and concentrated under vacuum. The residue was then dissolved in methanol (100 mL) and 10% palladium on carbon (1.00 g) was added. The reaction mixture was stirred at room temperature

overnight. The catalyst was removed by filtration, washed with hot methanol and the filtrate was concentrated to dryness under vacuum to give 2,3-bis(hydroxymethyl)-4-phenylquinoline **123** as a white solid (1.33 g, 67%). mp 166–169 °C (lit.,[71] mp 175–177 °C); ν_{max}/cm^{-1} (neat) 3420 (OH), 2991 (CH), 1570, 1441, 1022, 1005, 765; δ_H (400 MHz, CD_3OD) 4.62 (2H, s, CH_2), 5.12 (2H, s, CH_2) 7.33–7.36 (2H, m, Ar H), 7.39–7.47 (2H, m, Ar H), 7.53–7.58 (3H, m, Ar H), 7.72 (1H, ddd, *J* 8.4, 6.8, 1.6 Hz, Ar H), 8.10 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, CD_3OD) 59.1 (CH_2), 64.7 (CH_2), 127.8 (CH), 127.9 (CH), 128.6 (C), 129.4 (CH), 129.5 (2 × CH), 129.6 (CH), 130.1 (CH), 130.8 (2 × CH), 130.9 (C), 137.3 (C), 147.4 (C), 150.4 (C), 160.9 (C); *m/z* (CI) 266.1180 (MH^+. $C_{17}H_{16}NO_2$ requires 266.1181), 264 (30%), 246 (23), 218 (6), 116 (3), 85 (6).

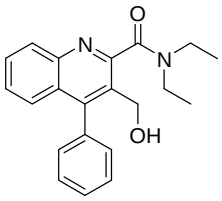
9-Phenylfuro[3,4-*b*]quinolin-3(1*H*)-one 107[71]



107

2,3-Bis(hydroxymethyl)-4-phenylquinoline **123** (0.10 g, 0.38 mmol) was dissolved in chloroform (20 mL). Activated manganese dioxide (1.02 g, 11.7 mmol) was then added and the reaction mixture stirred at room temperature for 1 h. The reaction mixture was filtered through Celite® and washed with chloroform (100 mL) to give 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one **107** as a white solid (0.08 g, 80%). mp 174–176 °C; ν_{max}/cm^{-1} (neat) 3061 (CH), 1768 (CO), 1581, 1369, 1344, 1153, 1051, 1009, 767; δ_H (400 MHz, $CDCl_3$) 5.41 (2H, s, CH_2), 7.45–7.48 (2H, m, Ar H), 7.57–7.69 (4H, m, Ar H), 7.85 (1H, ddd, *J* 8.4, 6.8, 1.2 Hz, Ar H), 7.91 (1H, d, *J* 9.0 Hz, Ar H), 8.43 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 67.9 (CH_2), 125.9 (CH), 127.9 (C), 128.9 (CH), 129.4 (2 × CH), 129.5 (2 × CH), 129.6 (CH), 130.7 (CH), 131.4 (CH), 132.3 (C), 133.6 (C), 143.9 (C), 144.3 (C), 150.7 (C), 168.8 (C); *m/z* (EI) 261.0788 (M^+. $C_{17}H_{11}NO_2$ requires 261.0790), 217 (23%), 204 (100), 176 (14), 151 (9), 95 (9), 84 (18).

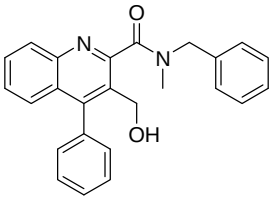
3-Hydroxymethyl-4-phenylquinoline-2-*N*-diethylcarboxamide 125[29]



125

The reaction was carried out according to general procedure 5 using 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one **107** (0.30 g, 1.15 mmol), diethylamine (0.12 g, 1.61 mmol) and trimethylaluminum (0.75 mL, 1.61 mmol) and gave 3-hydroxymethyl-4-phenylquinoline-2-*N*-diethylcarboxamide **125** as yellow oil (0.25 g, 65%). ν_{max}/cm^{-1} (neat) 3414 (OH), 2976 (CH), 1614 (CO), 1485, 1269, 1068, 765; δ_H (400 MHz, $CDCl_3$) 1.19 (3H, t, *J* 7.1 Hz, NCH_2CH_3), 1.28 (3H, t, *J* 7.1 Hz, NCH_2CH_3), 3.34 (2H, q, *J* 7.1 Hz, NCH_2CH_3), 3.60 (2H, q, *J* 7.1 Hz, NCH_2CH_3), 4.03 (1H, t, *J* 6.1 Hz, CH_2OH), 4.32 (2H, d, *J* 6.1 Hz, CH_2OH), 7.36–7.49 (7H, m, Ar H), 7.64 (1H, ddd, *J* 8.4, 6.8, 1.6 Hz, Ar H), 8.04 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 11.2 (CH_3), 12.5 (CH_3), 38.7 (CH_2), 42.1 (CH_2), 58.1 (CH_2), 124.1 (CH), 125.3 (C), 125.8 (C), 126.8 (2 × CH), 127.2 (C), 127.7 (CH), 128.1 (CH), 128.2 (CH), 129.1 (2 × CH), 133.6 (CH), 144.5 (C), 147.6 (C), 153.4 (C), 168.1 (C); *m/z* (CI) 335.1759 (MH^+. $C_{21}H_{23}N_2O_2$ requires 335.1760), 334 (54%), 317 (26), 262 (32), 234 (6), 209 (4), 74 (26).

3-Hydroxymethyl-4-phenylquinoline-2-*N*-methyl-*N*-benzylcarboxamide 124[94]

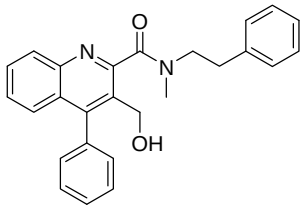


124

The reaction was carried out according to general procedure 5 using 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one **107** (0.30 g, 1.15 mmol), *N*-benzylmethylamine (0.20 g, 1.60 mmol) and trimethylaluminium (0.37 mL, 0.74 mmol) and gave 3-hydroxymethyl-4-phenylquinoline-2-*N*-methyl-*N*-benzylcarboxamide **124** as a yellow oil (0.26 g, 59%). NMR spectra showed a 1:1 mixture of rotamers, only signals for one rotamer is recorded. ν_{max}/cm^{-1} (neat) 3408 (OH), 2933 (CH), 1620 (CO), 1485, 1397, 1152, 1057, 729; δ_H (400 MHz, $CDCl_3$), 3.19 (3H, s, NCH_3), 4.10 (1H, t, *J* 7.1 Hz, CH_2OH), 4.44 (2H, d, *J* 7.1 Hz,

CH_2OH), 4.73 (2H, s, NCH_2Ph), 7.28–7.33 (4H, m, Ar H), 7.38–7.55 (8H, m, Ar H), 7.72–7.76 (1H, m, Ar H), 8.09 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 28.4 (CH_3), 53.8 (CH_2), 58.5 (CH_2), 125.5 (C), 126.3 (CH), 126.5 (CH), 126.6 (CH), 127.0 (CH), 127.2 (4 × CH), 127.4 (CH), 128.2 (CH), 128.5 (4 × CH), 129.4 (C), 133.9 (C), 135.1 (C), 144.9 (C), 148.2 (C), 153.3 (C), 169.1 (C); *m/z* (CI) 383.1758 (MH^+. $C_{25}H_{23}N_2O_2$ requires 383.1760), 367 (19%), 336 (48), 262 (82), 219 (31), 178 (21), 122 (100), 91 (24).

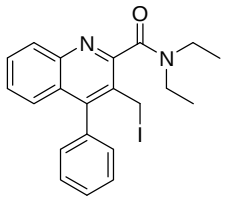
3-Hydroxymethyl-4-phenylquinoline-2-*N*-methyl-*N*-phenethylcarboxamide 126



126

The reaction was carried out according to general procedure 5 using 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one **107** (0.30 g, 1.15 mmol), *N*-methylphenethylamine (0.20 g, 1.49 mmol) and trimethylaluminium (0.37 mL, 0.74 mmol) and gave 3-hydroxymethyl-4-phenylquinoline-2-*N*-methyl-*N*-phenethyl carboxamide **126** as yellow oil (0.24 g, 53%). NMR spectra showed a 1:1 mixture of rotamers, only signals for one rotamer is recorded. ν_{max}/cm^{-1} (KBr) 3422 (OH), 2927 (CH), 1617 (CO), 1487, 1120, 1054, 1010, 701; δ_H (400 MHz, $CDCl_3$) 3.08 (2H, t, *J* 7.4 Hz, NCH_2CH_2Ph), 3.27 (3H, s, NCH_3), 3.80 (1H, t, *J* 7.4 Hz, NCH_2CH_2Ph), 3.93–3.97 (3H, m, CH_2OH), 4.08 (1H, br s, CH_2OH), 6.94 (1H, dd, *J* 7.6, 2.0 Hz, Ar H), 7.07–7.15 (2H, m, Ar H), 7.32–7.37 (2H, m, Ar H), 7.40–7.43 (2H, m, Ar H), 7.47–7.56 (5H, m, Ar H), 7.72–7.77 (1H, m, Ar H), 8.15 (1H, d, *J* 14.4, 8.0 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 33.4 (CH_3), 34.6 (CH_2), 52.8 (CH_2), 59.3 (CH_2), 126.6 (CH), 126.7 (CH), 127.0 (CH), 127.5 (CH), 127.6 (CH), 127.7 (C), 128.4 (2 × CH), 128.6 (4 × CH), 129.0 (C), 129.4 (CH), 129.8 (2 × CH), 135.2 (C), 138.0 (C), 145.8 (C), 149.3 (C), 154.4 (C), 170.0 (C); *m/z* (CI) 397.1917 (MH^+. $C_{26}H_{25}N_2O_2$ requires 397.1916), 381 (49%), 367 (42), 290 (94), 264 (92), 220 (42), 164 (22), 136 (100).

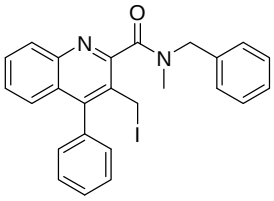
3-Iodomethyl-4-phenylquinoline-2-*N*-diethylcarboxamide 127



127

The reaction was carried out according to general procedure 6 using 3-hydroxymethyl-4-phenylquinoline-2-*N*-diethylcarboxamide **125** (0.16 g, 0.48 mmol) and oxalyl chloride (0.77 mL, 10.5 mmol), followed by sodium iodide (0.43 g, 2.83 mmol) and gave 3-iodomethyl-4-phenylquinoline-2-*N*-diethylcarboxamide **127** as yellow oil (0.12 g, 56%). ν_{max}/cm^{-1} (neat) 2978 (CH), 1626 (CO), 1560, 1481, 1267, 1067, 702; δ_H (400 MHz, $CDCl_3$) 1.33–1.43 (6H, m, 2 × NCH_2CH_3), 3.30 (2H, q, *J* 7.2 Hz, NCH_2CH_3), 3.69 (2H, q, *J* 7.2 Hz, NCH_2CH_3), 4.61 (2H, s, CH_2I), 7.33–7.36 (1H, m, Ar H), 7.40–7.47 (3H, m, Ar H), 7.51–7.60 (3H, m, Ar H), 7.69 (1H, ddd, *J* 8.4, 6.8, 1.2 Hz, Ar H), 8.09 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 0.0 (CH_2), 12.4 (CH_3), 13.4 (CH_3), 39.3 (CH_2), 43.9 (CH_2), 126.6 (CH), 127.4 (CH), 127.7 (C), 128.5 (2 × CH and C), 128.6 (2 × CH), 128.7 (CH), 129.6 (CH), 129.9 (CH), 135.1 (C), 145.8 (C), 148.4 (C), 155.3 (C), 167.9 (C); *m/z* (FAB) 445.0782 (MH^+. $C_{21}H_{22}IN_2O$ requires 445.0777), 401 (11%), 327 (22), 317 (98), 281 (56), 217 (38), 148 (5), 85 (8).

3-Iodomethyl-4-phenylquinoline-2-*N*-methyl-*N*-benzylcarboxamide 128[5]

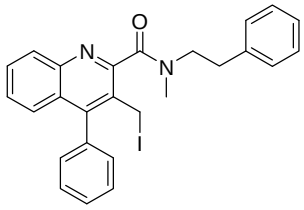


128

The reaction was carried out according to general procedure 6 using 3-hydroxymethyl-4-phenylquinoline-2-*N*-methyl-*N*-benzylcarboxamide **124** (0.10 g, 0.26 mmol) and oxalyl chloride (0.40 mL, 5.76 mmol), followed by sodium iodide (0.08 g, 0.5 mmol) and gave 3-iodomethyl-4-phenylquinoline-2-*N*-methyl-*N*-benzylcarboxamide **128** as a yellow oil (0.05 g, 39%). NMR spectra showed a 2:1 mixture of rotamers, only signals for the major rotamer is recorded. ν_{max}/cm^{-1} (neat) 2962 (CH), 1631 (CO), 1485, 1260, 1052, 699; δ_H (400 MHz, $CDCl_3$) 2.98 (3H, s, NCH_3), 4.65 (2H, s, CH_2I) 4.96 (2H, s, NCH_2Ph), 7.30–

7.37 (2H, m, Ar H), 7.39–7.48 (5H, m, Ar H), 7.53–7.60 (4H, m, Ar H), 7.64–7.73 (2H m, Ar H), 8.12 (1H, d, *J* 8.3 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 0.3 (CH_2), 36.6 (CH_3), 55.7 (CH_2), 126.7 (CH), 127.9 (CH), 128.2 (C), 128.4 (C), 128.6 (2 × CH), 128.7 (4 × CH), 128.8 (2 × CH), 129.0 (CH), 129.6 (CH), 129.7 (CH), 130.2 (CH), 135.2 (C), 136.5 (C), 146.0 (C), 148.7 (C), 155.0 (C), 168.6 (C); *m/z* (FAB) 493.0765 (MH^+. $C_{25}H_{22}IN_2O$ requires 493.0777), 365 (8%), 327 (7), 281 (28), 222 (14), 208 (19), 148 (38), 75 (100).

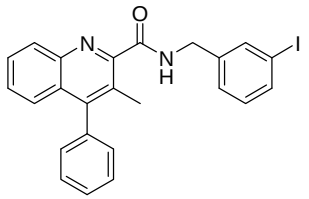
3-Iodomethyl-4-phenylquinoline-2-*N*-methyl-*N*-phenethylcarboxamide 129



129

The reaction was carried out according to general procedure 6 using 3-hydroxymethyl-4-phenylquinoline-2-*N*-methyl-*N*-phenethylcarboxamide **126** (0.04 g, 0.12 mmol) and oxalyl chloride (0.18 mL, 2.38 mmol), followed by sodium iodide (0.11 g, 0.70 mmol) and gave 3-iodomethyl-4-phenylquinoline-2-*N*-methyl-*N*-phenethylcarboxamide **129** as yellow oil (0.02 g, 53%). NMR spectra showed a 1:1 mixture of rotamers, only signals for one rotamer is recorded. ν_{max}/cm^{-1} (neat) 2962 (CH), 1636 (CO), 1561, 1486, 1394, 1261, 1055, 701; δ_H (400 MHz, $CDCl_3$) 3.08 (3H, s, NCH_3), 3.15 (2H, t, *J* 8.8 Hz, NCH_2CH_2Ph), 3.87-3.91 (2H, m, NCH_2CH_2Ph), 4.61 (2H, s, CH_2I), 7.09–7.11 (1H, m, Ar H), 7.14–7.27 (2H, m, Ar H), 7.33–7.49 (6H, m, Ar H), 7.52–7.61 (3H, m, Ar H), 7.71–7.77 (1H, m, Ar H), 8.10 (1H, dd, *J* 14.4, 8.0 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 0.1 (CH_2), 34.6 (CH_2), 37.4 (CH_3), 49.5 (CH_2), 126.3 (CH), 126.6 (CH), 127.5 (CH), 127.8 (C), 128.5 (4 × CH), 128.6 (2 × CH), 128.7 (2 × CH), 128.9 (CH), 129.3 (CH), 129.9 (CH), 130.0 (C), 134.9 (C), 138.5 (C), 145.6 (C), 148.3 (C), 154.8 (C), 168.1 (C); *m/z* (FAB) 507.0927 (MH^+. $C_{26}H_{24}IN_2O$ requires 507.0933), 415 (6%), 397 (83), 323 (6), 287 (12), 217 (53), 135 (35), 107 (28), 75 (17).

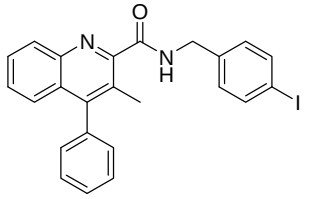
3-Methyl-4-phenylquinoline-2-*N*-(3-iodobenzyl)carboxamide 130



130

The reaction was carried out according to general procedure 3 using 3-methyl-4-phenylquinoline-2-carboxylic acid **96** (0.25 g, 0.95 mmol) and 3-iodobenzylamine (0.63 mL, 4.75 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-(3-iodobenzyl)carboxamide **130** as a yellow oil (0.36 g, 80%); ν_{max}/cm^{-1} (NaCl) 3380 (NH), 2927 (CH), 1670 (CO), 1485, 1257, 764; δ_H (400 MHz, $CDCl_3$) 2.61 (3H, s, 3-CH_3), 4.66 (2H, d, *J* 6.0 Hz, NCH_2), 7.09 (1H, t, *J* 8.0 Hz, Ar H), 7.22–7.26 (2H, m, Ar H), 7.35–7.45 (3H, m, Ar H), 7.49–7.56 (3H, m, Ar H), 7.61–7.67 (2H, m, Ar H), 7.78 (1H, s, Ar H), 8.06 (1H, d, *J* 8.0 Hz, Ar H), 8.58 (1H, br t, *J* 6.0 Hz, NH); δ_C (100 MHz, $CDCl_3$) 17.6 (CH_3), 42.8 (CH_2), 94.7 (C), 126.2 (CH), 127.1 (CH), 127.8 (CH), 128.1 (CH), 128.7 (2 × CH), 128.8 (C), 129.0 (CH), 129.3 (2 × CH), 129.4 (CH and C), 130.4 (CH), 136.5 (CH) 136.7 (CH), 137.1 (C), 141.1 (C), 144.6 (C), 149.2 (C), 149.8 (C), 166.6 (C); *m/z* (CI) 479.0617 (MH^+. $C_{24}H_{20}IN_2O$ requires 479.0620), 391 (8%), 353 (100), 263 (4), 220 (14), 136 (5), 79 (14).

3-Methyl-4-phenylquinoline-2-*N*-(4-iodobenzyl)carboxamide 132

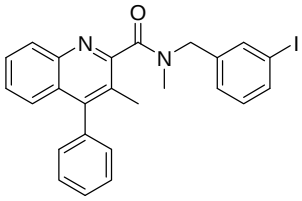


132

The reaction was carried out according to general procedure 3 using 3-methyl-4-phenylquinoline-2-carboxylic acid **96** (0.25 g, 0.95 mmol) and 4-iodobenzylamine (0.63 mL, 4.75 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-(4-iodobenzyl)carboxamide **132** as a yellow oil (0.24 g, 53%). ν_{max}/cm^{-1} (NaCl) 3378 (NH), 2927 (CH), 1670 (CO), 1513, 1484, 1006, 761; δ_H (400 MHz, $CDCl_3$) 2.46 (3H, s, 3-CH_3), 4.51 (2H, d, *J* 6.0 Hz, NCH_2), 7.04 (2H, d, *J* 8.0 Hz, Ar H), 7.09 (2H, d, *J* 8.0 Hz, Ar H), 7.22–7.24 (1H, m, Ar H) 7.28–7.32 (1H, m, Ar H), 7.36–7.49 (3H, m, Ar H), 7.50–7.54 (3H, m, Ar H), 7.91 (1H, d, *J* 8.0 Hz, Ar H), 8.53 (1H, br t, *J* 6.0 Hz, NH); δ_C (100 MHz, $CDCl_3$) 17.7 (CH_3), 43.1

(CH_2), 92.8 (C), 126.3 (CH), 128.0 (CH), 128.7 (2 × CH), 128.9 (CH and C), 129.3 (CH), 129.5 (CH), 129.8 (2 × CH), 130.9 (C), 131.8 (C), 137.2 (C), 137.8 (4 × CH), 138.4 (C), 144.6 (C), 149.7 (C), 167.0 (C); *m/z* (EI) 478.0545 (M^+. $C_{24}H_{19}IN_2O$ requires 478.0542), 435 (8%), 351 (3), 308 (3), 232 (10), 219 (100), 189 (6), 91 (13).

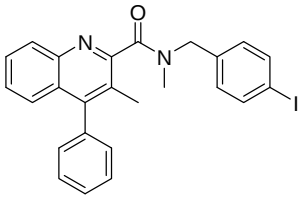
3-Methyl-4-phenylquinoline-2-*N*-methyl-*N*-(3-iodobenzyl)carboxamide 131



131

The reaction was carried out according to general procedure 4 using 3-methyl-4-phenylquinoline-2-*N*-(3-iodobenzyl)carboxamide **130** (0.22 g, 0.46 mmol) and methyl iodide (0.46 mL, 7.36 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-methyl-*N*-(3-iodobenzyl)carboxamide **131** as a yellow oil (0.17 g, 76%). NMR spectra showed a 1:1 mixture of rotamers, only signals for one rotamer is recorded. ν_{max}/cm^{-1} (NaCl) 2963 (CH), 1640 (CO), 1485, 1260, 1019, 799; δ_H (400 MHz, $CDCl_3$) 2.16 (3H, s, 3-CH_3), 2.75 (3H, s, NCH_3), 4.73 (2H, s, NCH_2), 7.06 (1H, t, *J* 7.8 Hz, Ar H), 7.17–7.20 (2H, m, Ar H), 7.24 (1H, d, *J* 7.8 Hz, Ar H), 7.30–7.37 (2H, m, Ar H), 7.41–7.48 (3H, m, Ar H), 7.53–7.63 (2H, m, Ar H), 7.69–7.74 (1H, m, ArH), 8.05 (1H, d, *J* 7.8 Hz Ar H); δ_C (100 MHz, $CDCl_3$) 16.1 (CH_3), 35.6 (CH_3), 49.7 (CH_2), 94.6 (C), 125.1 (C), 125.6 (CH), 126.1 (CH), 127.2 (CH), 127.7 (CH), 128.1 (2 × CH), 128.7 (CH), 129.0 (2 × CH), 129.3 (CH), 129.4 (CH), 130.5 (C), 136.6 (C), 136.7 (CH), 136.9 (C), 137.2 (CH), 145.9 (C), 148.4 (C), 155.4 (C), 169.5 (C); *m/z* (EI) 492.0700 (M^+. $C_{25}H_{21}IN_2O$ requires 492.0699), 435 (18%), 266 (3), 275 (3), 246 (22), 219 (100), 189 (6), 119 (7), 90 (7).

3-Methyl-4-phenylquinoline-2-*N*-methyl-*N*-(4-iodobenzyl)carboxamide 133

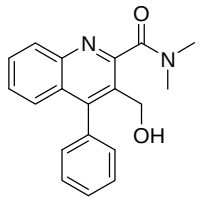


133

The reaction was carried out according to general procedure 4 using 3-methyl-4-phenylquinoline-2-*N*-(4-iodobenzyl)carboxamide **132** (0.14 g, 0.29 mmol) and methyl

iodide (0.30 mL, 4.69 mmol) and gave 3-methyl-4-phenylquinoline-2-*N*-methyl-*N*-(4-iodobenzyl)carboxamide **133** as a colourless oil (0.13 g, 90%). NMR spectra showed a 1:1 mixture of rotamers, only signals for one rotamer is recorded. ν_{max}/cm^{-1} (neat) 2926 (CH), 1644 (CO), 1485, 1399, 1052, 765; δ_H (400 MHz, $CDCl_3$) 2.14 (3H, s, 3-CH_3), 2.73 (3H, s, NCH_3), 4.71 (2H, s, NCH_2), 7.00 (1H, d, *J* 8.1 Hz, Ar H), 7.11–7.17 (3H, m, Ar H) 7.30–7.33 (2H, m, Ar H), 7.39–7.46 (3H, m, Ar H), 7.54–7.61 (3H, m, Ar H), 8.03 (1H, d, *J* 8.1 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 15.0 (CH_3), 34.4 (CH_3), 48.8 (CH_2), 92.2 (C), 123.9 (C), 126.1 (CH), 127.0 (CH), 127.1 (2 × CH), 128.1 (2 × CH), 128.3 (C), 128.8 (CH), 129.4 (2 × CH), 130.7 (CH), 130.8 (CH), 135.0 (C), 135.5 (C), 136.9 (2 × CH), 144.9 (C), 147.3 (C), 154.4 (C), 168.5 (C); *m/z* (CI) 493.0778 (MH^+. $C_{25}H_{22}IN_2O$ requires 493.0777), 445 (6%), 409 (6), 367 (100), 309 (7), 277 (4), 220 (8).

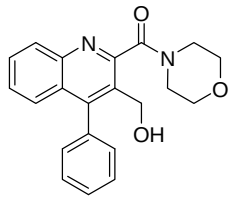
3-Hydroxymethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide 142



142

The reaction was carried out according to general procedure 5 using 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one **107** (0.15 g, 0.57 mmol) and dimethylamine (0.38 mL, 0.75 mmol) and trimethylaluminium (0.38 mL, 0.75 mmol) and gave 3-hydroxymethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide **142** as yellow oil (0.11 g, 60%). δ_H (400 MHz, $CDCl_3$) 3.05 (3H, s, NC*H*$_3$), 3.17 (3H, s, NC*H*$_3$), 4.00-4.06 (1H, m, CH_2O*H*), 4.34 (2H, s, C*H*$_2$OH), 7.33–7.48 (7H, m, Ar H), 7.62–7.66 (1H, m, Ar H), 8.03 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 37.5 (CH_3), 39.2 (CH_3), 67.9 (CH_2), 125.8 (CH), 127.6 (CH), 128.5 (2 × CH), 128.9 (CH), 129.4 (2 × CH), 130.7 (CH), 131.5 (CH), 133.5 (C), 135.2 (C), 144.3 (C), 146.1 (C), 150.7 (C), 154.7 (C), 170.0 (C); *m/z* (CI) 307.1444 (MH^+. $C_{19}H_{19}N_2O_2$ requires 307.1447), 262 (100%), 220 (19), 137 (4), 71 (25)

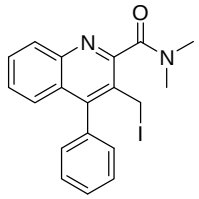
3-Hydroxymethyl-4-phenylquinoline-2-*N*-morpholincarboxamide 143



143

The reaction was carried out according to general procedure 5 using 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one **107** (0.07 g, 0.27 mmol) and morpholine (0.03 mL, 0.75 mmol) and trimethylaluminium (0.03 mL, 0.34 mmol) and gave 3-hydroxymethyl-4-phenylquinoline-2-*N*-morpholincarboxamide **143** as yellow oil (0.08 g, 86%). δ_H (400 MHz, $CDCl_3$) 3.55–3.57 (2H, m, NCH_2CH_2O), 3.66–3.69 (2H, m, NCH_2CH_2O), 3.78–3.83 (2H, m, NCH_2CH_2O), 3.87–3.90 (2H, m, NCH_2CH_2O), 4.03 (2H, d, *J* 6.5 Hz, CH_2OH), 7.32–7.37 (2H, m, Ar H), 7.42–7.51 (5H, m, Ar H),7.62–7.69 (1H, m, Ar H), 8.04 (1H, d, *J* 8.4 Hz, Ar H).

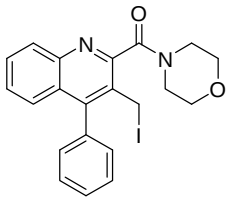
3-Iodomethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide 138



138

The reaction was carried out according to general procedure 6 using 3-hydroxymethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide **142** (0.10 g, 0.31 mmol) and oxalyl chloride (0.52 mL, 7.18 mmol), followed by sodium iodide (0.20 g, 1.4 mmol) and gave 3-iodomethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide **138** as yellow oil (0.01 g, 15%). ν_{max}/cm^{-1} (neat) 2927 (CH), 1625 (CO), 1485, 1390, 1168, 1058, 757; δ_H (400 MHz, $CDCl_3$) 3.09 (3H, s, NCH_3), 3.26 (3H, s, NCH_3) 4.61 (2H, s, CH_2I), 7.36–7.48 (4H, m, Ar H), 7.55–7.61 (3H, m, Ar H), 7.69–7.74 (1H, m, Ar H), 8.09 (1H, d, *J* 8.4, Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 0.0 (CH_2), 35.0 (CH_3), 39.2 (CH_3), 126.5 (CH), 127.5 (CH), 128.1 (C), 128.4 (2 × CH and C), 128.5 (2 × CH), 128.6 (CH), 129.2 (CH), 130.0 (CH), 134.9 (C), 145.6 (C), 148.4 (C), 155.0 (C), 168.2 (C); *m/z* (CI) 417.0459 (MH^+. $C_{19}H_{18}IN_2O$ requires 417.0464), 291 (100%), 220 (5), 137 (10).

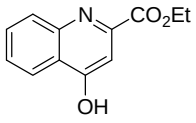
3-Iodomethyl-4-phenylquinoline-2-*N*-morpholinecarboxamide 139



139

The reaction was carried out according to general procedure 6 using 3-hydroxymethyl-4-phenylquinoline-2-*N*-morpholinecarboxamide **143** (0.10 g, 0.31 mmol) and oxalyl chloride (0.30 mL, 4.17 mmol), followed by sodium iodide (0.12 g, 0.82 mmol) and gave 3-iodomethyl-4-phenylquinoline-2-*N*-morpholinecarboxamide **139** as yellow oil (0.01 g, 21%). ν_{max}/cm^{-1} (neat) 2847 (CH), 1628 (CO), 1617, 1468, 1246, 1011, 765; δ_H (400 MHz, $CDCl_3$) 3.57 (2H, t, *J* 4.8 Hz, NC*H*$_2$CH$_2$O), 3.85 (2H, t, *J* 4.8 Hz, NC*H*$_2$CH$_2$O), 3.90–4.00 (4H, m, 2 × NCH$_2$C*H*$_2$O), 4.58 (2H, s, CH$_2$I), 7.35–7.41 (3H, m, Ar H), 7.44–7.48 (1H, m, Ar H), 7.53–7.61 (3H, m, Ar H), 7.72 (1H, ddd, *J* 8.2, 5.6, 1.2 Hz, Ar H), 8.09 (1H, d, *J* 8.2 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 0.0 (CH_2), 42.0 (CH_2), 47.4 (CH_2), 66.1 (CH_2), 66.3 (CH_2), 126.5 (CH), 127.6 (CH), 128.3 (CH), 128.5 (C), 128.5 (2 × CH and C), 128.6 (2 × CH), 129.3 (CH), 130.0 (CH), 134.7 (C), 145.0 (C), 148.2 (C), 153.8 (C), 166.6 (C); *m/z* (FAB) 459.0571 (MH^+. $C_{21}H_{20}IN_2O_2$ requires 459.0570), 410 (75%), 365 (95), 331 (100), 262 (42), 126 (25).

Ethyl 4-hydroxylquinoline-2-carboxylate 150[95]

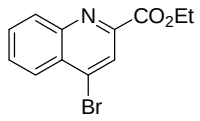


150

Aniline **83** (4.30 mL, 47.5 mmol), diethyl oxalacetate **151** (10.0 g, 47.5 mmol), and *p*-toluenesulfonic acid (9.05 g, 47.5 mmol) were dissolved in cyclohexane (300 mL) and the solution heated under Dean-Stark conditions for 40 h. The reaction mixture was cooled to room temperature and the solvent removed under reduced pressure. To the residual crude oil, polyphosphoric acid (21.0 g, 214 mmol) was added and the mixture heated at 120 °C for 1 h, forming a dark brown oil. This substance was cooled to room temperature and a 20% aqueous sodium carbonate solution (800 mL) was added slowly to quench the excess acid and precipitate the product as a brown solid. The mixture was filtered and the solid dissolved in dichloromethane (50 mL). Water (50 mL) was added and the organic layer

extracted with dichloromethane (3 × 40 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The resulting dark brown solid was recrystallised from ethyl acetate and hexane to give ethyl 4-hydroxylquinoline-2-carboxylate **150** as a light brown solid (3.90 g, 40%). mp 198–200 °C (lit.,[95] mp 213 °C); ν_{max}/cm^{-1} (neat) 2924 (CH), 1739 (CO), 1562, 1519, 1268; δ_H (400 MHz, $CDCl_3$) 1.42 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 4.46 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 7.01 (1H, s, Ar H), 7.38 (1H, t, *J* 7.4 Hz, Ar H), 7.50 (1H, d, *J* 8.3 Hz, Ar H), 7.66 (1H, ddd, *J* 8.3, 6.8, 1.2 Hz, Ar H), 8.35 (1H, d, *J* 8.3 Hz, Ar H), 9.43 (1H, br s, OH); δ_C (100 MHz, $CDCl_3$) 14.1 (CH_3), 63.3 (CH_2), 111.6 (CH), 118.4 (CH), 124.5 (CH), 126.3 (CH), 126.4 (C), 133.1 (CH), 136.6 (C), 139.1 (C), 163.0 (C), 179.7 (C); *m/z* (EI) 217.0741 (M^+. $C_{12}H_{11}NO_3$ requires 217.0739), 189 (6%), 171 (19), 143 (100), 115 (24), 89 (20).

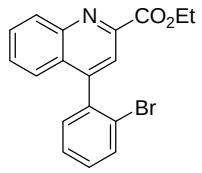
Ethyl 4-bromoquinoline-2-carboxylate 149[96]



149

Ethyl 4-hydroxylquinoline-2-carboxylate **150** (1.50 g, 6.90 mmol), phosphorous oxybromide (5.94 g, 20.7 mmol) and anhydrous potassium carbonate (2.87 g, 20.7 mmol) were dissolved in anhydrous acetonitrile (100 mL). The mixture was then heated to 92 °C under argon for 2 h, followed by cooling to room temperature and removal of the solvent under reduced pressure to form a brown solid. Water (200 mL) was added and the mixture extracted with ethyl acetate (100 mL). The organic layer was washed with water (3 × 150 mL) and then dried ($MgSO_4$). The solvent was removed under reduced pressure to give ethyl 4-bromoquinoline-2-carboxylate **149** as a dark brown solid (1.64 g, 85%). mp 91–92 °C (lit.,[96] mp 91–92 °C); ν_{max}/cm^{-1} (neat) 2993 (CH), 1712 (CO), 1365, 1195, 756; δ_H (400 MHz, $CDCl_3$) 1.50 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 4.57 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 7.76 (1H, ddd, *J* 8.4, 7.0, 1.4 Hz, Ar H), 7.85 (1H, ddd, *J* 8.4, 7.0, 1.4 Hz, Ar H), 8.25 (1H, d, *J* 8.4 Hz, Ar H), 8.32 (1H, d, *J* 8.4 Hz, Ar H), 8.48 (1H, s, Ar H); δ_C (100 MHz, $CDCl_3$) 14.4 (CH_3), 62.6 (CH_2), 125.0 (CH), 126.6 (CH), 128.8 (C), 129.9 (CH), 131.1 (CH), 131.3 (CH), 135.2 (C), 147.9 (C), 148.0 (C), 164.3 (C); *m/z* (EI) 280.9873 (M^+. $C_{12}H_{10}{}^{81}BrNO_2$ requires 280.9875), 281 (12%), 234 (13), 207 (100), 127 (60), 100 (14).

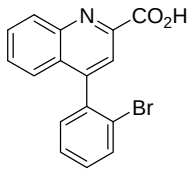
Ethyl 4-bromophenylquinoline-2-carboxylate 152



152

The reaction was carried out according to general procedure 1 using ethyl 4-bromoquinoline-2-carboxylate **149** (1.50 g, 5.37 mmol) and 2-bromophenylboronic acid (1.40 g, 6.98 mmol) and gave ethyl 4-bromophenylquinoline-2-carboxylate **152** as a light yellow solid (1.35 g, 71%). mp 77–79 °C; ν_{max}/cm^{-1} (neat) 2980 (CH), 1739 (CO), 1468, 1266, 1140, 795, 736; δ_H (400 MHz, $CDCl_3$) 1.37 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 4.42–4.49 (2H, m, OCH_2CH_3), 7.19–7.25 (2H, m, Ar H), 7.35 (1H, t, *J* 7.2 Hz Ar H), 7.42–7.48 (2H, m, Ar H), 7.63–7.70 (2H, m, Ar H), 7.99 (1H, s, Ar H), 8.28 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 14.4 (CH_3), 62.3 (CH_2), 121.6 (CH), 123.0 (C), 125.7 (CH), 127.5 (CH), 127.7 (CH), 128.7 (C), 129.8 (CH), 131.1 (2 × CH), 131.4 (CH), 133.1 (CH), 138.3 (C), 147.8 (C), 147.9 (C), 148.7 (C), 165.5 (C); *m/z* (CI) 358.0264 (MH^+. $C_{18}H_{15}{}^{81}BrNO_2$ requires 358.0268), 356 (92%), 276 (100), 263 (13).

4-Bromophenylquinoline-2-carboxylic acid 148

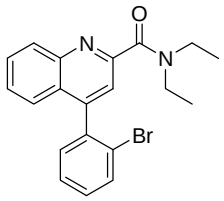


148

The reaction was carried out according to general procedure 2 using ethyl 4-bromophenylquinoline-2-carboxylate **152** (1.35 g, 3.79 mmol) and sodium hydroxide (0.23 g, 5.69 mmol) and gave 4-bromophenylquinoline-2-carboxylic acid **148** as a light yellow solid (1.05 g, 84%). mp 68–70 °C; ν_{max}/cm^{-1} (neat) 2899 (CH), 1725 (CO), 1592, 1232, 760, 728; δ_H (400 MHz, $CDCl_3$) 7.33 (1H, dd, *J* 7.2, 1.6 Hz Ar H), 7.39–7.43 (1H, m, Ar H), 7.48–7.52 (1H, m, Ar H), 7.60–7.67 (2H, m, Ar H), 7.78 (1H, dd, *J* 8.0, 1.4 Hz, Ar H), 7.86 (1H, ddd, *J* 8.4, 6.4, 1.4 Hz, Ar H), 8.20 (1H, s, Ar H), 8.24 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 119.9 (CH), 122.8 (C), 126.2 (CH), 127.6 (CH), 128.6 (C), 129.3 (CH), 129.8 (CH), 130.5 (CH), 130.9 (CH), 131.0 (CH), 133.2 (CH), 137.8 (C), 145.4 (C),

146.1 (C), 150.5 (C), 164.1 (C); *m/z* (EI) 326.9899 (M^+. $C_{16}H_{10}{}^{79}BrNO_2$ requires 326.9895), 283 (100%), 204 (95), 176 (45), 101 (20), 44 (21).

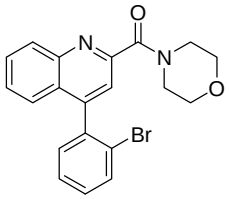
4-Bromophenylquinoline-2-*N*-diethylcarboxamide 154



154

The reaction was carried out according to general procedure 3 using 4-bromophenylquinoline-2-carboxylic acid **148** (0.30 g, 0.90 mmol) and diethylamine (0.50 mL, 4.50 mmol) and gave 4-bromphenylquinoline-2-*N*-diethylcarboxamide **154** as a light yellow solid (0.28 g, 80%). mp 111–113 °C; ν_{max}/cm^{-1} (neat) 2943 (CH), 1620 (CO), 1550, 1489, 1273, 771; δ_H (400 MHz, $CDCl_3$) 1.25 (3H, t, *J* 7.2 Hz, NCH_2CH_3), 1.33 (3H, t, *J* 7.2 Hz, NCH_2CH_3), 3.41–3.57 (2H, m, NCH_2), 3.66 (2H, qd, *J* 6.8, 1.6 Hz, NCH_2), 7.34–7.39 (2H, m, Ar H), 7.44–7.48 (1H, m, Ar H), 7.51–7.52 (2H, m, Ar H), 7.55 (1H, s, Ar H), 7.73–7.77 (2H, m, Ar H), 8.18 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 13.0 (CH_3), 14.1 (CH_3), 40.5 (CH_2), 43.5 (CH_2), 120.8 (CH), 123.0 (C), 125.8 (CH), 126.5 (C), 127.5 (2 × CH), 129.9 (2 × CH), 130.1 (CH), 131.3 (CH), 133.0 (CH), 138.4 (C), 147.0 (C), 148.3 (C), 154.4 (C), 168.6 (C); *m/z* (EI) 383.0761 (MH^+. $C_{20}H_{30}{}^{79}BrN_2O$ requires 383.0759), 303 (100%), 265 (39), 204 (5), 69 (33).

4-Bromophenylquinoline-2-*N*-morpholincarboxamide 155

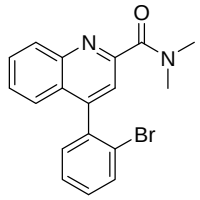


155

The reaction was carried out according to general procedure 3 using 4-bromphenylquinoline-2-carboxylic acid **148** (0.30 g, 0.90 mmol) and morpholine (0.43 mL, 4.50 mmol) and gave 4-bromphenylquinoline-2-*N*-morpholincarboxamide **155** as a yellow oil (0.21 g, 57%). ν_{max}/cm^{-1} (neat) 2970 (CH), 1620 (CO), 1473, 1249, 1111, 771; δ_H (400 MHz, $CDCl_3$) 3.74–3.91 (8H, m, NCH_2CH_2O), 7.36–7.41 (2H, m, Ar H), 7.46–7.50 (1H, m, Ar H), 7.54–7.57 (2H, m, Ar H), 7.65 (1H, s, Ar H), 7.76–7.80 (2H, m, Ar H),

8.17 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 42.9 (CH_2), 47.9 (CH_2), 66.9 (CH_2), 67.1 (CH_2), 121.5 (CH), 123.0 (C), 125.9 (CH), 126.7 (C), 127.5 (CH), 127.9 (CH), 120.1 (CH), 130.2 (2 × CH), 131.3 (CH), 133.0 (CH), 138.2 (C), 146.9 (C), 148.7 (C), 152.8 (C), 167.4 (C); *m/z* (EI) 396.0468 (M^+. $C_{20}H_{17}{}^{79}BrN_2O_2$ requires 396.0473), 312 (8%), 283 (92), 203 (88), 176 (24), 82 (100), 47 (19).

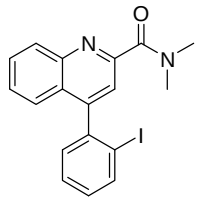
4-Bromophenylquinoline-2-*N*-dimethylcarboxamide 153



153

The reaction was carried out according to general procedure 3 using 4-bromphenylquinoline-2-carboxylic acid **148** (0.40 g, 1.20 mmol) and dimethylamine (3.00 mL, 6.00 mmol) and gave 4-bromphenylquinoline-2-*N*-dimethylcarboxamide **153** as a light yellow solid (0.25 g, 58%). mp 156–158 °C; ν_{max}/cm^{-1} (neat) 2931 (CH), 1627 (CO), 1504, 1396, 1257, 771; δ_H (400 MHz, $CDCl_3$) 3.20 (6H, s, NCH_3), 7.36–7.41 (2H, m, Ar H), 7.44–7.48 (1H, m, Ar H), 7.50–7.53 (2H, m, Ar H), 7.60 (1H, s, Ar H), 7.74–7.90 (2H, m, Ar H), 8.18 (1H, d, *J* 8.4 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 35.8 (CH_3), 39.2 (CH_3), 121.1 (CH), 123.0 (C), 125.8 (CH), 126.5 (C), 127.5 (CH), 127.7 (CH), 129.5 (CH), 130.0 (CH), 130.1 (CH), 131.3 (CH), 133.0 (CH), 138.0 (C), 146.9 (C), 148.5 (C), 153.8 (C), 168.9 (C); *m/z* (EI) 354.0366 (M^+. $C_{18}H_{15}{}^{79}BrN_2O$ requires 354.0368), 283 (72%), 203 (68), 176 (27), 82 (100).

4-Iodophenylquinoline-2-*N*-dimethylcarboxamide 145

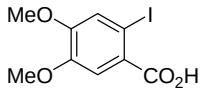


145

A Schlenk tube was charged with copper iodide (0.01 g, 0.03 mmol) and sodium iodide (0.17 g, 1.13 mmol), evacuated and backfilled with argon. *N,N*-dimethylethylenediamine (6.00 μL, 0.06 mmol) and 4-bromophenylquinoline-2-*N*-dimethylcarboxamide **153** (0.20 g, 0.56 mmol) were dissolved in butan-1-ol (6 mL) and added to the Schlenk tube. The

Schlenk tube was sealed with a Teflon valve and the reaction mixture stirred at 135 °C for 72 h. The reaction had not gone to completion therefore more equivalents of copper iodide (0.01 g, 0.03 mmol), sodium iodide (0.17 g, 1.13 mmol) and *N,N*-dimethylethylenediamine (6.00 μL, 0.06 mmol) were added and left for another 96 h. The reaction was diluted with water, extracted with ethyl acetate (3 × 20 mL), dried ($MgSO_4$) and concentrated to dryness. Purification was carried out by flash column chromatography, eluting with petroleum ether/ethyl acetate to give 4-iodophenylquinoline-2-*N*-dimethylcarboxamide **145** as light yellow solid (0.06 g, 27%). mp 137–139 °C; ν_{max}/cm^{-1} (neat) 2927 (CH), 1628 (CO), 1505, 1397, 1087, 761; δ_H (400 MHz, $CDCl_3$) 3.21 (6H, s, NCH_3), 7.34–7.39 (2H, m, Ar H), 7.45–7.49 (1H, m, Ar H), 7.51–7.56 (2H, m, Ar H), 7.60 (1H, m, Ar H), 7.75–7.80 (2H, m, Ar H), 7.62–7.60 (1H, dt, *J* 8.4, 1.0 Hz, Ar H); δ_C (100 MHz, $CDCl_3$) 36.2 (CH_3), 39.3 (CH_3), 121.1 (CH), 123.0 (C), 125.8 (CH), 126.5 (C), 127.5 (CH), 127.7 (CH), 129.8 (2 × CH), 130.0 (CH), 131.2 (CH), 133.0 (CH), 138.2 (C), 146.8 (C), 148.4 (C), 153.8 (C), 168.9 (C); *m/z* (FAB) 403.0311 (MH^+. $C_{18}H_{16}IN_2O$ requires 403.0307), 335 (100%), 284 (22), 203 (43), 162 (22), 75 (26).

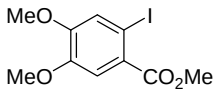
2-Iodo-4,5-dimethoxybenzoic acid 187[97]



187

3,4-Dimethoxybenzoic acid **186** (5.00 g, 27.4 mmol), diacetoxyiodobenzene (0.90 g, 3.00 mmol) and diiodine (0.90 g, 3.57 mmol) were added to a stirred solution of acetic acid (40.0 mL) and acetic anhydride (40.0 mL). Sulfuric acid (5.00 mL) was then added slowly and the reaction mixture stirred at room temperature for 2.5 h. Aqueous sodium sulfite (5%, 200 mL) was added to destroy excess diacetoxyiodobenzene and the unreacted diiodine was buffered with ammonium carbonate to neutralise the sulfuric acid. The reaction mixture was filtered, the precipitate washed with water, air-dried and re-crystallized using ethyl acetate. 2-Iodo-4,5-dimethoxybenzoic acid **187** was obtained as a light yellow solid (5.02 g, 60%). mp 193–195 °C (lit.,[97] mp 204–205 °C); ν_{max}/cm^{-1} (neat) 2990 (CH), 1688 (CO), 1508, 1269, 554; δ_H (400 MHz, $CDCl_3$) 3.92 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 7.45 (1H, s, Ar H), 7.64 (1H, s, Ar H); δ_C (100 MHz, $CDCl_3$) 56.1 (CH_3), 56.4 (CH_3), 85.8 (C), 114.7 (CH), 124.2 (CH), 124.3 (C), 148.7 (C), 152.7 (C), 170.4 (C); *m/z* (EI) 307.9550 (M^+. $C_9H_9IO_4$ requires 307.9546), 292 (29%), 236 (5), 138 (11), 82 (68).

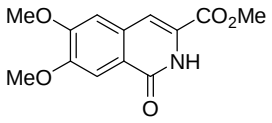
Methyl 2-iodo-4,5-dimethoxybenzoate 185[98]



185

2-Iodo-4,5-dimethoxybenzoic acid **187** (4.50 g, 14.6 mmol) and thionyl chloride (2.6 mL, 36.5 mmol) was added to methanol (60 mL) and heated under reflux for 18 h. Next, the solvent was then removed and a solution of sodium hydrogen carbonate (100 mL) added to quench the reaction. The product was extracted with ethyl acetate, dried ($MgSO_4$) and concentrated under vacuum. Purification was carried out by flash column chromatography eluting, with petroleum ether/ethyl acetate to give methyl 2-iodo-4,5-dimethoxybenzoate **185** as a white solid (4.31 g, 92%). mp: 98–100 °C (lit.,[98] mp 105–107 °C); ν_{max} /cm^{-1} (neat) 2955 (CH), 1718 (CO), 1506, 1107, 1024, 545; δ_H (400 MHz, $CDCl_3$) 3.90 (3H, s, OC*H*$_3$), 3.91 (3H, s, OC*H*$_3$), 3.92 (3H, s, OC*H*$_3$), 7.39 (1H, s, Ar H), 7.45 (1H, s, Ar H); δ_C (100 MHz, $CDCl_3$) 51.7 (CH_3), 55.5 (CH_3), 55.7 (CH_3), 84.1 (C), 113.3 (CH), 123.1 (CH), 125.4 (C), 148.1 (C), 151.3 (C), 165.3 (C); *m/z* (CI) 322.9779 (MH^+. $C_{10}H_{12}IO_4$ requires 322.9780), 279 (5%), 197 (100), 183 (28), 113 (15), 85 (65).

Methyl 1,2-dihydro-6,7-dimethoxyisoquinoline-3-carboxylate 183

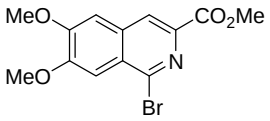


183

Methyl 2-iodo-4,5-dimethoxybenzoate **185** (0.20 g, 0.60 mmol) and methyl-2-acetamidoacrylate (0.13 g, 0.90 mmol) in acetonitrile (20 mL) was heated for 5 mins. Then palladium(II) acetate (0.02 g, 0.06 mmol), tetrabutylammonium chloride (0.14 g, 0.50 mmol) and sodium hydrogen carbonate (0.13 g, 1.55 mmol) were added to the reaction mixture and heated under reflux overnight. After 48 h, more palladium(II) acetate (0.02 g, 0.06 mmol) was added and heated under reflux for a further 48 h. The reaction mixture was cooled to room temperature, diluted with water and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. Purification was carried out by flash column chromatography eluting with ethyl acetate/petroleum ether to give methyl 1,2-dihydro-6,7-dimethoxyisoquinoline-3-carboxylate **183** as a brown solid (0.10 g, 61%). mp 223–224 °C; ν_{max} /cm^{-1} (neat) 2956 (CH), 1732 (CO), 1512, 1259, 742; δ_H (400 MHz, $CDCl_3$) 3.98 (3H, s, OCH_3), 4.02 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 7.09

(1H, s, Ar H), 7.39 (1H, s, Ar H), 7.82 (1H, s, Ar H), 9.14 (1H, br s, NH); δ_C (100 MHz, $CDCl_3$) 53.1 (CH_3), 56.2 (CH_3), 56.4 (CH_3), 107.8 (CH), 108.0 (CH), 111.2 (CH), 122.7 (C), 126.3 (C), 131.2 (C), 151.2 (C), 153.7 (C), 161.1 (C), 162.3 (C); *m/z* (EI) 263.0798 (M^+. $C_{13}H_{13}NO_5$ requires 263.0794), 248 (15%), 203 (17), 160 (22), 91 (16), 83 (35).

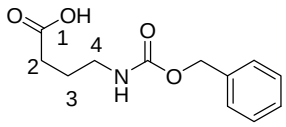
Methyl 1-bromo-6,7-dimethoxyisoquinoline-3-carboxylate 188



188

Methyl 1,2-dihydro-6,7-dimethoxyisoquinoline-3-carboxylate **183** (0.15 g, 0.57 mmol), potassium carbonate (0.63 g, 4.56 mmol), phosphorous oxybromide (1.30 g, 0.56 mmol) in dry acetonitrile (20 mL) were heated under reflux for 18 h. The reaction mixture was cooled to room temperature, and then concentrated *in vacuo.* The residue was cautiously dispersed in water (20 mL) and the insoluble material was filtered, washed with water, dried and recrystallized using ethyl acetate. Methyl 1-bromo-6,7-dimethoxyisoquinoline-3-carboxylate **188** was obtained as a light brown solid (0.13 g, 70%). mp: 192–194 °C; ν_{max} /cm^{-1} (neat) 2953 (CH), 1712 (CO), 1508, 1209, 713; δ_H (400 MHz, $CDCl_3$) 4.03 (3H, s, OCH_3), 4.07 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 7.14 (1H, s, Ar H), 7.57 (1H, s, Ar H), 8.39 (1H, s, Ar H); δ_C (100 MHz, $CDCl_3$) 52.9 (CH_3), 56.4 (CH_3), 56.5 (CH_3), 106.4 (CH), 107.1 (CH), 123.1 (CH), 126.6 (C), 133.3 (C), 139.8 (C), 142.7 (C), 153.0 (C), 154.0 (C), 165.3 (C); *m/z* (CI) 326.0021 (MH^+. $C_{13}H_{13}{}^{79}BrNO_4$ requires 326.0028), 300 (6), 248 (100), 182 (7), 97 (31), 71 (91).

4-(Benzyloxycarbonylamino)butanoic acid 192[99]

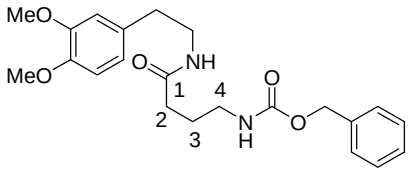


192

γ-Aminobutyric acid **191** (3.00 g, 29.1 mmol) and 2 M sodium hydroxide (15.0 mL, 30.3 mmol) were stirred in an ice bath. Further, 2 M sodium hydroxide (15.0 mL, 30.3 mmol) and benzyl chloroformate (4.40 mL, 30.8 mmol) were added simultaneously to the stirred mixture. The reaction mixture was stirred in an ice bath for 1 h and then at room temperature for an additional 1 h. The reaction mixture was extracted with diethyl ether (3

× 50 mL), the aqueous layer acidified with 6 M hydrochloric acid (pH~3) and cooled in an ice bath. The crystals formed were then filtered under suction, washed with 0.1 M hydrochloric acid (5.00 mL) and air dried to give 4-(benzyloxycarbonylamino)butanoic acid **192** as a white solid (7.06 g, 100%). mp 60–62 °C (lit.,[99] mp 65–67 °C); δ_H (400 MHz, $CDCl_3$) 1.71–1.77 (2H, m, 3-H_2), 2.30 (2H, t, *J* 7.2 Hz, 2-H_2), 3.15 (2H, q, *J* 6.4 Hz, 4-H_2), 5.00 (2H, s, CH_2Ph), 6.12 (1H, br s, NH), 7.21-7.30 (5H, m, Ar H); δ_C (100 MHz, $CDCl_3$) 25.0 (CH_2), 31.2 (CH_2), 40.3 (CH_2), 66.9 (CH_2), 128.1 (CH), 128.2 (2 × CH), 128.6 (2 × CH), 136.5 (C), 156.6 (C), 178.4 (C); *m/z* (EI) 237.1001 (M^+. $C_{12}H_{15}NO_4$ requires 237.0999), 198 (3%), 165 (2), 108 (75), 91 (100), 79 (22).

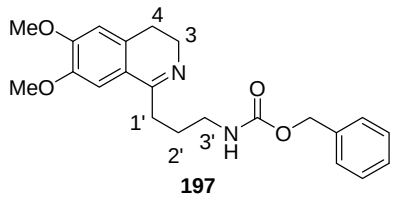
***N*-(3,4-Dimethoxyphenethyl)-4-(benzyloxycarbonylamino)butanamide 193**



193

4-(Benzyloxycarbonylamino)butanoic acid **192** (5.00 g, 21.1 mmol), 2-(3-4-dimethoxyphenyl)ethylamine **173** (3.85 mL, 23.2 mmol) and 4-(dimethylamino)pyridine (0.25 g, 2.10 mmol) were stirred in dichloromethane (100 mL) at 0 °C. Ethyl 3-(3-dimethylaminopropyl)carbodiimide hydrochloride (4.45 g, 23.2 mmol) was added and the reaction stirred for 2 h and then at room temperature overnight. The mixture was concentrated *in vacuo* and the residue dissolved in ethyl acetate (100 mL) and water (100 mL). The organic layer was washed with water (3 × 100 mL), dried ($MgSO_4$) and the filtrate concentrated *in vacuo* to give *N*-(3,4-dimethoxyphenethyl)-4-(benzyloxycarbonylamino)butanamide **193** as a white solid (7.47 g, 89%). mp 84–86 °C; ν_{max}/cm^{-1}(neat) 3304 (NH), 2931 (CH), 1681 (CO), 1516, 1139, 669; δ_H (400 MHz, $CDCl_3$) 1.77–1.84 (2H, m, 3-H_2), 2.17 (2H, t, *J* 6.8 Hz, 2-H_2), 2.76 (2H, t, *J* 7.0 Hz, PhC$H_2$$CH_2$), 3.18–3.22 (2H, m, 4-$H_2$), 3.46–3.50 (2H, m, PhC$H_2CH_2$), 3.85 (6H, s, 2 × OCH_3), 5.08 (3H, br s, CH_2Ph, NH), 5.93 (1H, br s, NH), 6.72 (2H, m, Ar H), 6.79 (1H, d, *J* 8.4 Hz, Ar H), 7.34 (5H, s, Ar H); δ_C (100 MHz, $CDCl_3$) 26.1 (CH_2), 33.7 (CH_2), 35.3 (CH_2), 40.3 (CH_2), 40.8 (CH_2), 55.9 (CH_3), 60.0 (CH_3), 66.7 (CH_2), 111.3 (CH), 111.9 (CH), 120.7 (CH), 128.1 (CH), 128.2 (CH), 128.6 (2 × CH), 131.4 (C), 136.5 (C), 147.7 (C), 149.1 (C), 156.9 (C), 172.5 (C); *m/z* (EI) 400.2003 (M^+. $C_{22}H_{28}N_2O_5$ requires 400.1998), 292 (9%), 164 (99), 107 (24), 91 (75), 79 (25).

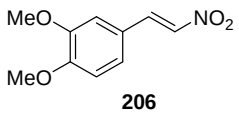
1-(Benzyloxycarbonylaminopropyl)-3-(3,4-dihydro-6,7-dimethoxyisoquinolin-1-yl) 197



197

3,4-Dimethoxyphenethyl 4-(benzyloxycarbonylamino)butanamide **193** (0.30 g, 0.75 mmol) and phosphorous oxychloride (5.00 mL) and heated under reflux for 18 h. The reaction mixture was concentrated *in vacuo* and then cold 2 M sodium hydroxide (20.0 mL) was added and subsequently extracted with dichloromethane (20.0 mL). The organic phase was washed with water (3 × 20 mL), dried ($MgSO_4$) and the dichloromethane layer was concentrated *in vacuo*. 1-(Benzyloxycarbonylaminopropyl)-3-(3,4-dihydro-6,7-dimethoxyisoquinolin-1-yl) **197** was obtained as an yellow oil (0.21 g, 73%). ν_{max} /cm^{-1} (neat) 3325 (NH), 2935 (CH), 1714 (CO), 1514, 1257, 738; δ_H (400 MHz, $CDCl_3$) 1.70–1.78 (2H, m, 2'-H_2), 2.22 (2H, t, *J* 7.6 Hz, 1'-H_2), 2.85 (2H, t, *J* 7.1 Hz, 4-H_2), 3.45 (2H, t, *J* 7.6 Hz, 3'-H_2), 3.61 (2H, t, *J* 7.1 Hz, 3-H_2), 3.73 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 5.17 (2H, s, C*H*$_2$Ph), 6.69–6.72 (2H, m, Ar H), 7.22–7.30 (3H, m, Ar H), 7.35–7.38 (2H, m, Ar H); δ_C (100 MHz, $CDCl_3$) 19.5 (CH_2), 26.7 (CH_2), 37.2 (CH_2), 47.2 (CH_2), 54.7 (CH_2), 55.8 (CH_3), 60.0 (CH_3), 67.1 (CH_2), 111.1 (CH), 112.6 (CH), 120.8 (CH), 128.1 (2 × CH), 128.1 (C), 128.5 (2 × CH) 133.3 (C), 136.3 (C), 147.3 (C), 148.6 (C), 152.0 (C), 159.1 (C); *m/z* (FAB) 383.1961 (MH^+. $C_{22}H_{27}N_2O_4$ requires 383.1971), 339 (10%), 250 (100), 243 (96), 164 (95), 92 (52).

3,4-Dimethoxy-β-nitrostyrene 206[89]

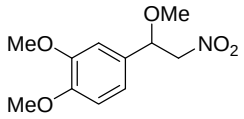


206

3,4-Dimethoxybenzaldehyde **202** (1.00 g, 6.00 mmol), nitromethane **203** (1.62 mL, 30.0 mmol) and ammonium formate (0.46 g, 6.00 mmol) in toluene (20 mL) were heated under reflux for 18 h. The resulting precipitate was separated and the filtrate was washed with water (2 × 20 mL), a saturated solution of sodium chloride (2 × 20 mL) and dried over $MgSO_4$. 3,4-Dimethoxy-β-nitrostyrene **206** was obtained as a yellow solid (1.22 g, 97%).

mp 144–146 °C (lit.,[89] mp 140–141 °C); ν_{max} /cm^{-1} (neat) 2831 (CH), 1597 (NO), 1481, 1257, 964; δ_H (400 MHz, $CDCl_3$) 3.93 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.92 (1H, d, *J* 8.4 Hz Ar H), 7.01 (1H, s, Ar H), 7.18 (1H, d, *J* 8.4 Hz Ar H), 7.53 (1H, d, *J* 13.6 Hz C*H*CH), 7.97 (1H, d, *J* 13.6 Hz CHC*H*); δ_C (100 MHz, $CDCl_3$) 56.0 (CH_3), 56.2 (CH_3), 110.2 (CH), 111.4 (CH), 122.8 (C), 124.7 (CH), 135.2 (CH), 139.4 (CH) 149.6 (C), 152.8 (C); *m/z* (EI) 209.0689 (M^+. $C_{10}H_{11}NO_4$ requires 209.0688), 178 (7%), 162 (81), 119 (39), 77 (59).

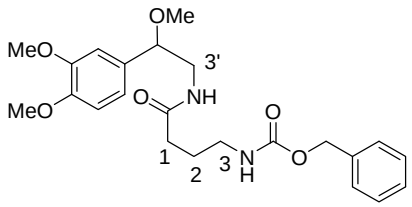
3,4-Dimethoxy-α-methoxy-β-nitrostyrene 210[89]



210

3,4-Dimethoxy-β-nitrostryene **206** (0.50 g, 4.78 mmol) was stirred in methanol (5 mL) followed by the addition of 25% sodium methoxide (5 mL) and stirred for 2.5 h. Next, glacial acetic acid (1 mL) was added and reaction stirred until a precipitate had formed. 3,4-Dimethoxy-α-methoxy-β-nitrostyrene **210** was obtained as a yellow solid (0.48 g, 84%). mp 106–108 °C (lit.,[89] mp 104–106 °C); ν_{max}/cm^{-1} (NaCl) 2937 (CH), 1593 (NO), 1551, 1261, 808; δ_H (400 MHz, $CDCl_3$) 3.27 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.91 (3H, s, OCH_3) 4.39 (1H, dd, *J* 12.8, 3.2 Hz, C*H*H), 4.60–4.65 (1H, m, C*H*OCH_3), 4.89 (1H, dd, *J* 10.4, 3.6 Hz, CH*H*), 6.88–6.94 (3H, m, Ar H); δ_C (100 MHz, $CDCl_3$) 56.0 (2 × CH_3), 57.0 (CH_3), 79.9 (CH), 80.6 (CH_2), 109.1 (CH), 111.3 (CH and C), 119.6 (CH), 128.3 (C), 149.6 (C); *m/z* (EI) 241.0945 (M^+. $C_{11}H_{15}NO_5$ requires 241.0950), 209 (56%), 181 (100), 151 (42).

N-(3,4-Dimethoxyphenethyl)-4-(benzyloxycarbonylamino)butanamide 205



205

3,4-Dimethoxy-α-methoxy-β-nitrostyrene **210** (0.12 g, 0.50 mmol) was added to lithium aluminium hydride (1.50 mL, 1.50 mmol) in diethyl ether (10.0 mL). After 2 h, the

reaction was quenched with water and the diethyl ether layer, dried ($MgSO_4$) and concentrated *in vacuo* to give 3,4-dimethoxy-β-methoxyphenethylamine **204**. Next, 4-(benzyloxycarbonylamino)butanoic acid **194** (0.10 g, 0.42 mmol), 3,4-dimethoxy-β-methoxyphenethylamine **204** (0.10 g, 0.40 mmol) and 4-(dimethylamino)pyridine (0.01 g, 0.04 mmol) were stirred in dichloromethane (5.00 mL) at 0 °C. Ethyl 3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.13 g, 0.40 mmol) was then added and the reaction was stirred for 2 h and then at room temperature overnight. The mixture was concentrated *in vacuo* and the residue dissolved in ethyl acetate (10 mL) and water (10 mL). The organic layer was washed with water (3 × 10 mL), dried ($MgSO_4$) and the filtrate concentrated *in vacuo* to give 3,4-dimethoxyphenethyl 4-(benzyloxycarbonylamino)butanamide **205** as a yellow oil (0.08 g, 37% over two steps); ν_{max}/cm^{-1} (neat) 3366 (NH), 2931 (CH), 1654 (CO), 1515, 1261, 754; δ_H (400 MHz, $CDCl_3$) 1.72–1.80 (2H, m, 2-H_2), 2.14–2.17 (2H, m, 1-H_2), 3.11–3.19 (5H, m, OC*H*$_3$ and 3-H_2), 3.55–3.62 (1H, m, 3'-*H*H), 3.80 (6H, s, 2 × OCH_3), 4.11–4.14 (1H, m, 3'-H*H*), 5.02 (2H, s, C*H*$_2$Ph), 5.11 (1H, br s, NH), 6.16 (1H, br s, NH), 6.75–6.77 (3H, m, Ar H), 7.23–7.28 (5H, m, Ar H); δ_C (100 MHz, $CDCl_3$) 24.9 (CH_2), 32.7 (CH_2), 39.4 (CH_2), 44.7 (CH_2), 54.9 (2 × CH_3), 55.6 (CH_3), 65.6 (CH_2), 81.0 (CH), 108.3 (CH), 109.9 (CH), 118.2 (CH), 127.1 (CH), 127.5 (CH), 130.5 (C), 135.5 (C), 147.9 (C), 148.2 (C), 155.7 (C), 171.4 (C); *m/z* (CI) 431.2178 (MH^+. $C_{23}H_{31}N_2O_6$ requires 431.2182), 399 (40%), 291 (100), 147 (31).

3.2 Radioligand binding methodology[100]

Whole rat brains were obtained from male adult Sprague-Dawley rats and homogenised using a Polytron in ice-cold 50 mM Tris-base, pH 7.4. Homogenates were centrifuged at 39100 g for 10 minutes at 4 °C and the resulting pellet was washed (2×) by centrifugation and resuspended. The final resuspension was stored at –50 °C until use.

For determination of K_i values, triplicate aliquots of membrane suspensions (0.6–0.4 mg of protein) were incubated for 1.5 h at 4 °C in 50 mM Tris-base, pH 7.4, in the presence of 17 concentrations of the competitor (range 3 pM–300 μM). Total incubation volume was 400 μL, with only 1% ethanol. Non-specific binding was defined in the presence of 8 μM of [^{3}H]-PK11195. Reactions were terminated by rapid filtration through Whatman GF/B glass fibre filters pre-soaked in 0.3% w/v polyethylenimine using a 24-well Brandel cell harvester. Filters received three rapid washes in ice-cold buffer and [^{3}H] counts were determined by liquid scintillation analysis. K_i values were derived from nonlinear regression analysis using GraphPad Prism Version 4 (GraphPad Software Inc).

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