Synthesis of *N*-heterocycles as anti-asthma drugs, and compounds with antimycobacterial properties

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Declaration

I, Eleanor Danielle Lamming confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in my thesis.

Signed……..

Date…….

Abstract

Part A: Synthesis of *N*-heterocycles with dual pharmacology for the treatment of asthma

The most common and effective anti-inflammatory asthma treatment is carried out through use of steroids but these can have significant side effects. An alternative non-steroidal oral treatment is montelukast which targets the leukotriene inflammatory pathway, but is less effective at controlling asthma symptoms. The asthma inflammation pathway is complex involving many inflammatory mediators, and it was anticipated that a compound with dual pharmacology which impacted both leukotriene and prostaglandin pathways simultaneously would yield compounds with an enhanced ability to treat asthma. An attractive novel dual target strategy was the inhibition of the 5-lipoxygenase activating protein (FLAP) and antagonism of the prostaglandin D_2 receptor CRTh2. A combination of GlaxoSmithKline and literature SAR studies were elaborated in the design of the target compounds, incorporating known pharmacophores for FLAP inhibitors and CRTh2 antagonists. Synthetic routes towards the target compounds were developed and their biological activity against the intended targets determined.

Part B: Synthesis of tetrahydroisoquinolines, tetrahydrobenzazepines and profens and their antimycobacterial properties

Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* pathogen. The increasing prevalence of drug resistant strains of *M. tuberculosis* means there is an urgent need to develop new anti-TB drugs with novel modes of action. Aporphine alkaloid natural products and synthetic tetrahydroisoquinolines have demonstrated a specific antimycobacterial effect, as well as *M. tuberculosis* MurE inhibitory activity. The tetrahydroisoquinoline skeleton therefore provides a unique template for the development of new anti-TB drugs. Recently we developed biomimetic reaction conditions for the Pictet-Spengler condensation of aldehydes and amines into tetrahydroisoquinolines. The reaction is mediated by phosphate and proceeds under mild reaction conditions. The scope of the phosphate mediated Pictet-

Spengler reaction was investigated in order to access novel alkaloid structures and identify new leads for mycobacterial growth inhibitors. Studies into asymmetric versions of the reaction using chiral phosphates and extending the reaction for the construction of larger ring sizes were explored.

Another interesting class of compounds recently identified as active against mycobacterial growth were non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen. Analogues of profen compounds were synthesised for evaluation as mycobacterial growth inhibitors.

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Firstly I would like to thank my supervisor Professor Helen Hailes. I am really grateful for all the help and guidance she has given me throughout this PhD project. It was great to be able to work with someone who is always so positive and enthusiastic, and who provided many interesting ideas for taking the project forward.

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Abbreviations

$[\alpha]_D$	Specific rotation
Ac	Acetyl
Ac_2O	Acetic anhydride
AcOH	Acetic acid
AIBN	Azo(bis)isobutyronitrile
aq.	Aqueous
Ar	Aryl
BEI	Binding efficiency index
BINOL	1,1'-Bi-2-naphthol
Boc	*tert*-Butyloxycarbonyl
^{t}Bu	*tert*-Butyl
calcd	Calculated
calc LogP (ACD)	Calculated octanol-water partition coefficient by ACD software
CI	Chemical ionisation
conc.	Concentration
CRTh2	Chemoattractant receptor-homologous molecule expressed on Th2 cells
de	Diastereomeric excess
DIPEA	*N*,*N*-Diisopropylethylamine
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dppf	(Diphenylphosphino)ferrocene
ee	Enantiomeric excess
EI	Electron impact
ESI	Electrospray ionisation
Et	Ethyl

Et_3N	Triethylamine
EtOAc	Ethyl acetate
Eq.	Equivalents
FLAP	5-Lipoxygenase activating protein
h	Hours
GIC	Growth inhibitory concentration
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IC_{50}	Half maximal inhibitory concentration
IR	Infra-red
K_i	Binding affinity
KPi	Potassium phosphate
LC-MS	Liquid Chromatography - Mass Spectrometry
Lit.	Literature
LT	Leukotriene
MDAP	Mass Directed AutoPreparative HPLC
Me	Methyl
MIC	Minimum inhibitory concentration
min	Minutes
MOM	Methoxymethyl acetal
m.p.	Melting point
Ms	Mesyl
Mwt	Molecular weight
m/*z*	Mass to charge ratio
NBS	*N*-Bromosuccinimide
NMR	Nuclear magnetic resonance
Nps	*o*-Nitrophenylsulfenyl
Petrol	Petroleum ether 40-60 ^oC
PG	Prostaglandin
Ph	Phenyl

pK_a	Acid dissociation constant
pyr	Pyridine
rt	Room temperature
r_t	Retention time
sat.	Saturated
SPOTi	Spot culture growth inhibition assay
TB	Tuberculosis
TBS	*tert*-Butyldimethylsilyl ether
TEA	Triethylammonium salt
TEAB	Triethylammonium bicarbonate
TFA	Trifluoroacetic acid
THBP	Tetrahydro-2-benzazepine
THF	Tetrahydrofuran
THIQ	Tetrahydroisoquinoline
TLC	Thin layer chromatography
TPPTS	Triphenylphosphine-3,3’,3’’-trisulfonic acid sodium salt
Ts	Tosyl

Contents

Part A: Synthesis of *N*-heterocycles with dual pharmacology for the treatment of asthma

1. Introduction

1.1 Asthma - definition

Asthma is a chronic inflammatory disorder of the airways. It is typically characterised by airflow obstruction, airway hyper-responsiveness, infiltration of inflammatory cells to the airways and airway remodelling, resulting in symptoms such as coughing, wheezing, breathlessness and chest tightness.[1] The symptoms are often triggered by stimuli such as allergens, environmental exposure, exercise, chemical irritation, viral illnesses or emotional stress.

The definition of asthma is broad and describes a number of different subgroups or phenotypes of the disease. Different phenotypes can be defined by either the clinical symptoms and response to treatment, or by the underlying chemical mechanisms which are still not fully understood.[2] Recognising asthma as a heterogeneous disease is important with respect to the development of anti-asthma drugs, as drugs targeting multiple asthma pathways or tailored to specific phenotypes could be beneficial.[3]

Asthma is a global health problem which affects over 300 million people worldwide, and is expected to rise to 400 million by 2025.[4] The disease has high economic costs, both directly for healthcare systems and from indirect consequences such as lost time from work.[5] Asthma can have a serious impact on the sufferer's quality of life, and asthma attacks can be fatal, often arising from poorly controlled and undertreated asthma.[6] Despite the availability of effective asthma treatments, more than half of patients experience poor symptom control,[7] so there remains an unmet therapeutic need for many asthmatics.

1.2 Asthma treatment

There are two major classes of asthma treatment; drugs to relieve the symptoms of asthma and drugs which control the symptoms (Table 1.1).[8] Reliever drugs are

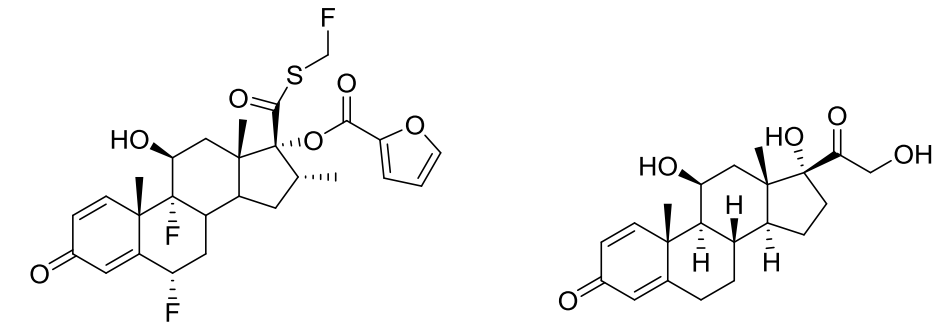
bronchodilators which provide rapid relief from asthma attacks by stimulating widening of the airways. The most widely used bronchodilators are β_2-adrenoceptor agonists, anticholinergics and methylxanthines. Controller drugs are designed to prevent asthma attacks and reduce symptoms by supressing the underlying inflammatory component. The most common controller drugs are inhaled corticosteroids, which are seen as the gold standard of asthma treatment. Alternatives include chromones and two newer classes of drugs introduced in the last 30 years, the anti-IgE monoclonal antibody omalizumab and the anti-leukotrienes.

Classification	**Drug type (*mode of action*)**	**Examples**
Reliever drugs	Short acting inhaled β_2-adrenoceptor agonists (*bronchodilator*)	Salbutamol Terbutaline
	Methylxanthines (*bronchodilator*)	Theophylline
	Inhaled anticholinergics (*bronchodilator*)	Tiotropium bromide Ipratropium bromide
Controller drugs	Corticosteroids (*anti-inflammatory*)	Fluticasone propionate Fluticasone furoate Prednisolone
	Long acting inhaled β_2-adrenoceptor agonists (*bronchodilator*)	Salmeterol Formoterol
	Chromones (*anti-inflammatory*)	Sodium cromoglycate Nedocromil sodium
	Anti-immunoglobin E (Anti-IgE) (*anti-inflammatory*)	Omalizumab
	Anti-leukotrienes (*anti-inflammatory*)	Montelukast Zafirlukast Zileuton

Table 1.1. Reliever and controller drugs used for the treatment of asthma.

Inhaled corticosteroids provide the most effective anti-inflammatory asthma treatment, helping to reduce symptoms and the risk of asthma attacks and improve lung function.[9] For example, fluticasone furoate (Figure 1.1) acts as a glucocorticoid receptor agonist and was approved by the FDA for clinical use in 2007 for allergic rhinitis and in 2014 for once-daily asthma treatment.[10] Combination inhalers of corticosteroids and long

acting β_2-adrenoceptor agonists (e.g. salmeterol/fluticasone) can also provide highly effective asthma control.[11] Inhaled corticosteroids are relatively safe for patients with mild to moderate asthma, as the drug is delivered directly into the lungs so lower doses can be used. Local side effects such as oral candidiasis and dysphonia can be problematic but rare.[12] For patients with more severe asthma, the long term use of high doses of inhaled corticosteroids can lead to side effects such as cataracts, glaucoma and osteoporosis, and another problem is poor compliance with inhaled corticosteroids, leading to the poor control of symptoms.[13] Oral treatments are generally preferred, but oral corticosteroids such as prednisolone (Figure 1.1) are only used to treat severe asthma for short periods of time due to serious systemic side effects or adverse reactions, and are not suitable as daily controller drugs.



Fluticasone furoate (GlaxoSmithKline)
Mwt = 538.6
calc LogP (ACD) = 4.0

Prednisolone
Mwt = 360.4
calc LogP (ACD) = 1.5

Figure 1.1. Anti-asthma drugs - corticosteroids.

Anti-leukotriene drugs such as montelukast, zafirlukast and zileuton (Figure 1.2) are alternatives to inhaled corticosteroids. The anti-leukotrienes target a specific inflammatory pathway or mediator and block part of the inflammatory response. Montelukast was one of the first non-steroid oral treatments available to asthma patients. With its good safety profile and few side effects it is now widely used as a daily controller drug. However when compared with inhaled corticosteroids, anti-leukotrienes are less effective in controlling asthma symptoms and are generally used in combination with other therapies.[14]

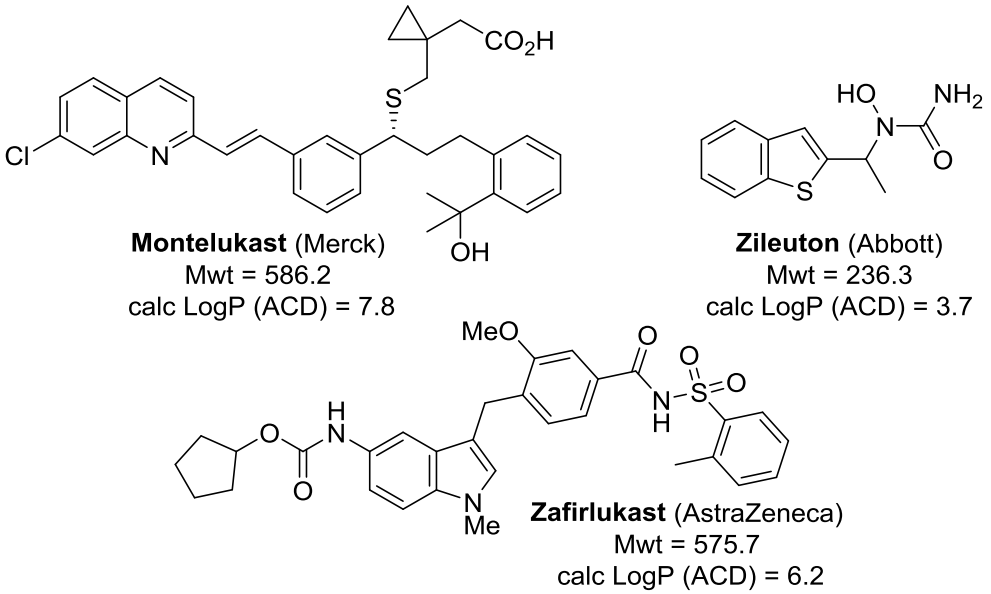


Figure 1.2. Anti-asthma drugs - anti-leukotrienes.

The monoclonal IgE antibody omalizumab was approved for asthma treatment by the FDA in 2003 and the EU in 2009. Omalizumab is used specifically for the treatment of severe allergic asthma, and works by complexing with free immunoglobin E (IgE, a key mediator of inflammation), which prevents the activation of high affinity IgE receptors on inflammatory cells. Since the approval of omalizumab there has been significant development of biologic asthma therapies, in particular those targeting pro-inflammatory cytokines such as mepolizumab (developed by GSK), which is an anti-interleukin 5 monoclonal antibody for the treatment of severe asthma.[15]

Even though there are effective asthma drugs available, it has been reported that 5-10% of patients have inadequately controlled symptoms despite taking controller medications.[16] There is still a need to develop new anti-inflammatory therapies, particularly oral treatments which are safe and effective at controlling symptoms.

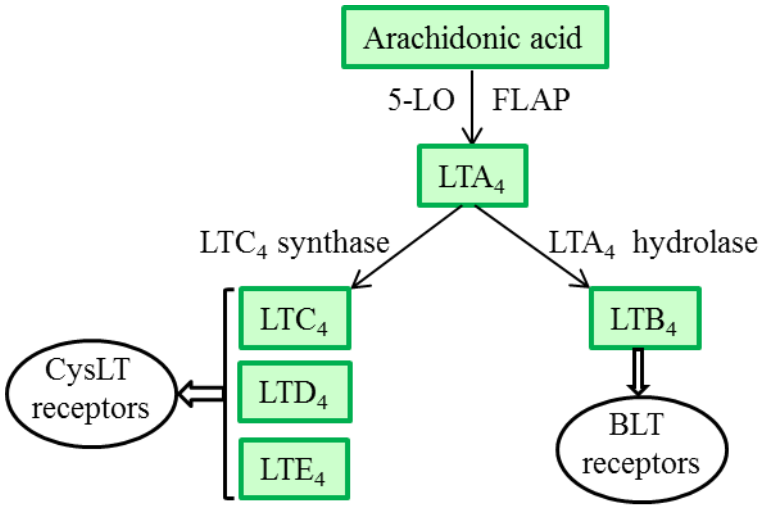
1.3 Asthma inflammation pathways

1.3.1 Biosynthesis of leukotrienes

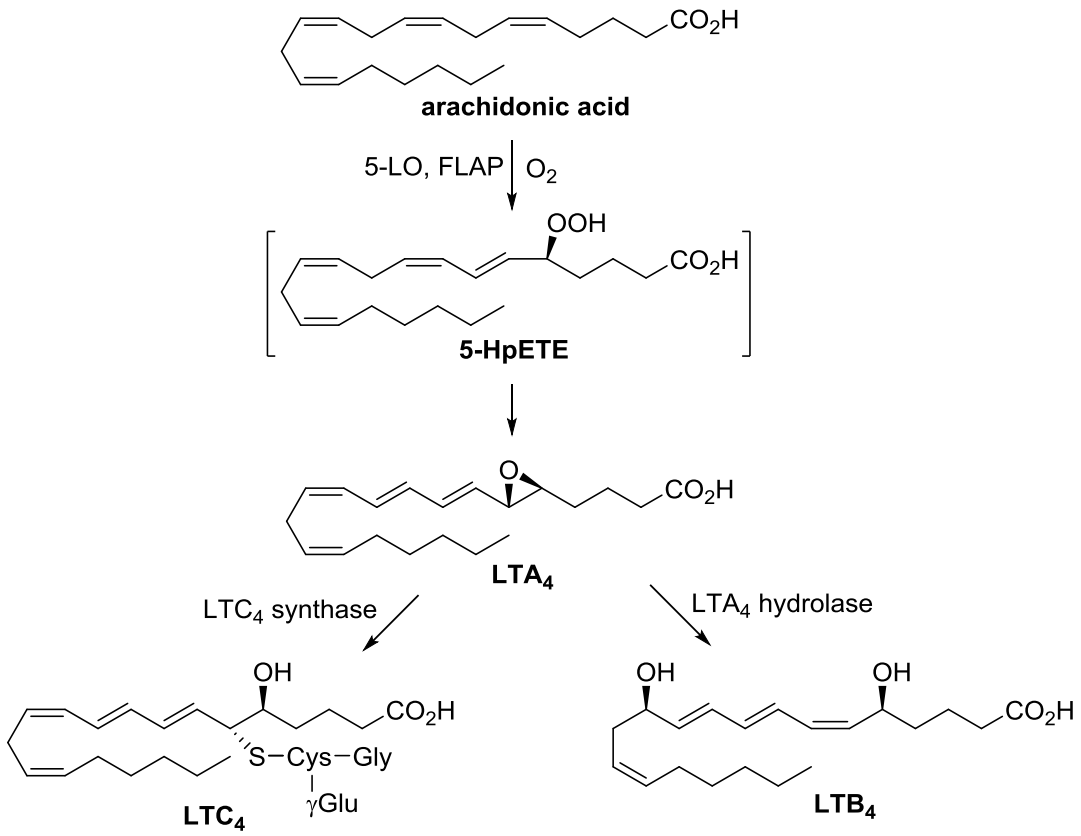
Asthma inflammatory pathways are complex, and there are a number of compounds involved in mediating the inflammatory response. These include amines, lipids,

peptides, cytokines, chemokines, proteases and other small molecules. Each have their own specific receptors through which they exert their biological effects. Eicosanoids are a class of lipid mediator inflammatory compounds and are bioactive oxygenated metabolites of fatty acids containing a 20 carbon chain length. Leukotrienes (LTs) and prostaglandins (PGs) are part of the eicosanoid family, and play a key role in inflammatory and allergic diseases such as asthma.[17] Both LTs and PGs are derived from arachidonic acid, which is released from membrane phospholipids by phospholipases in response to allergic or other cellular stimuli.

The biosynthesis of leukotrienes (Schemes 1.1 and 1.2) begins with the conversion of arachidonic acid to the unstable epoxide leukotriene A_4 (LTA_4) in a two-step oxidation/hydrolysis process catalysed by 5-lipoxygenase (5-LO).[18] The activity of the 5-LO enzyme requires the integral membrane protein, 5-lipoxygenase activating protein (FLAP), which plays a role in the transfer of arachidonic acid to 5-LO. LTA_4 is rapidly converted to either leukotriene B_4 (LTB_4) by enzymatic hydrolysis, or to leukotriene C_4 (LTC_4) by opening of the epoxide by the thiol anion of glutathione. LTC_4 is further converted to LTD_4 and LTE_4 through amide bond cleavage of the peptide side chain. Collectively, LTC_4, LTD_4 and LTE_4 are known as cysteinyl leukotrienes (CysLTs) and all contain a thioether linkage.



Scheme 1.1. Biosynthesis of leukotrienes. LT = leukotriene, 5-LO = 5-lipoxygenase, FLAP = 5-lipoxygenase activating protein.

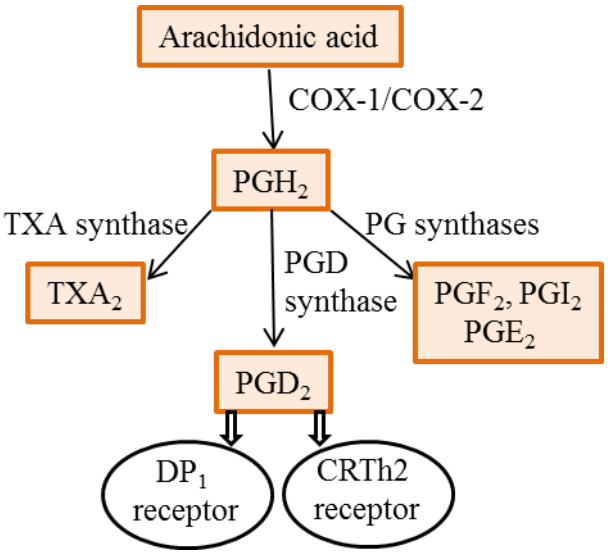


Scheme 1.2. Biosynthesis of leukotrienes (LTs) from arachidonic acid.

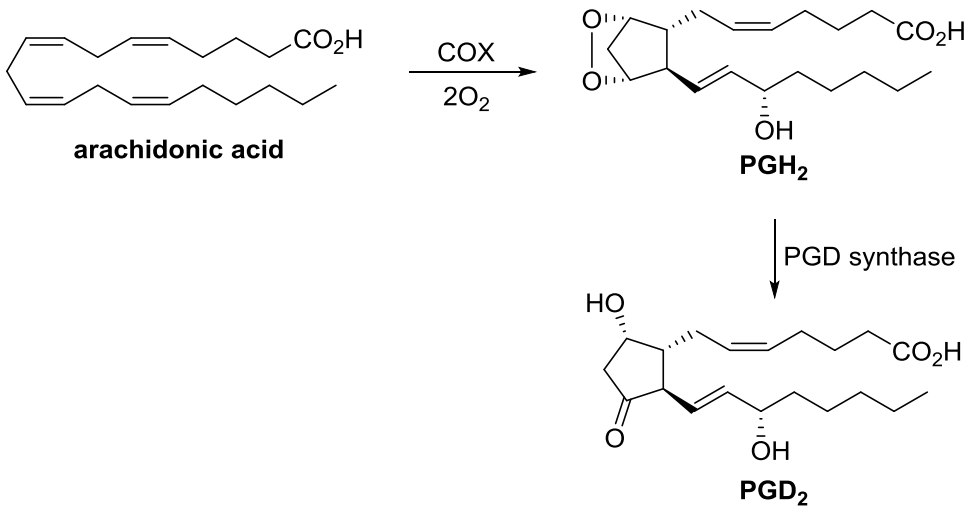
Leukotrienes are recognised by specific G protein coupled receptors (GPCRs).[19] The CysLTs bind to $CysLT_1$ and $CysLT_2$ receptors, which are involved in mediating bronchoconstriction, increasing vascular permeability, airway edema and mucus secretion. LTB_4 binds to BLT_1 and BLT_2 receptors, with a particularly high specificity for BLT_1. BLT_1 is expressed in inflammatory cells and its activation mediates chemoattractant and pro-inflammatory actions.

1.3.2 Biosynthesis of prostaglandins

The biosynthesis of prostaglandins (Schemes 1.3 and 1.4) begins with the conversion of arachidonic acid to the unstable endoperoxide intermediate prostaglandin H_2 (PGH_2), catalysed by a cyclooxygenase enzyme. PGH_2 is further metabolised to a number of biologically active mediators including prostaglandin D_2 (PGD_2), catalysed by PGD synthase.



Scheme 1.3. Biosynthesis of prostaglandins. PG = prostaglandin, TXA_2 = Thromboxane A_2, COX = cyclooxygenase.



Scheme 1.4. Biosynthesis of prostaglandin D_2 (PGD_2).

PGD_2 is of particular importance because it is the major prostaglandin produced by mast cells, is generated in high concentrations at sites of allergic inflammation and promotes an inflammatory response associated with asthma and allergy.[20] PGD_2 is a ligand for two G protein coupled receptors, DP_1 and CRTh2 (chemoattractant receptor-homologous molecule expressed on Th2 cells, also known as DP_2). The CRTh2 receptor is expressed on Th2 cells, eosinophils and basophils, and *in vitro* studies have shown PGD_2 activation of this receptor stimulates chemotaxis of these inflammatory

cells and the release of pro-inflammatory Th2 cytokines.[21] The DP_1 receptor is also activated through binding PGD_2 and mediates vasodilation and bronchodilation. A number of metabolites of PGD_2 retain the ability to activate CRTh2 but only have weak DP_1 activity, suggesting the effects of CRTh2 dominate, and that CRTh2 is able to bind a more structurally diverse range of ligands.[20,22]

1.4 Drugs targeting the arachidonic acid pathway

1.4.1 Anti-leukotriene drugs

There are two classes of anti-leukotriene drugs: leukotriene receptor antagonists (LTRAs) and inhibitors of leukotriene biosynthesis. The LTRA montelukast (Figure 1.2) targets the $CysLT_1$ receptor and is widely used for the treatment of asthma. However, montelukast is not always effective in asthmatic patients, and the lack of efficacy often associated with LTRAs may arise from their mode of action to only block the biological effect of specific leukotrienes.

Alternatively, anti-leukotriene drugs that target 5-LO or FLAP early on in the pathway inhibit the formation of all CysLTs and LTB_4, so could offer better efficacy compared to LTRAs. The 5-LO enzyme inhibitor zileuton (Figure 1.2) is currently the only leukotriene synthesis inhibitor approved clinically for asthma treatment, but is not widely used due to its poor safety profile and high dose requirements.[23] FLAP is a protein that is essential for the cellular biosynthesis of leukotrienes and hence represents an attractive target for novel anti-asthma drugs.[24] FLAP is a membrane embedded protein that selectively binds arachidonic acid and transfers it to the 5-LO enzyme. It has also been suggested that FLAP plays a role in enhancing the activity of 5-LO in the oxidation/reduction sequence to convert arachidonic acid to LTA_4.[25]

There are currently no FLAP inhibitors approved for therapeutic use, but several compounds have advanced into clinical trials for inflammatory diseases including asthma.[26] Early FLAP inhibitors include the quinoline based compound BAYX1005 **1**, indole based compound MK886 **2** and a combination of these structures MK591 **3** (Figure 1.3).[27] In 2007 the crystal structure of MK591 **3** bound to human FLAP was determined, revealing key binding interactions, and showed the inhibitor binding site was located in the membrane embedded pockets and prevented the binding of

arachidonic acid to FLAP.[28] The crystal structure of FLAP has enabled improved FLAP inhibitor design, and several compounds with different pharmacophores such as benzimidazoles, oxadiazoles and biaryls have been published.[29]

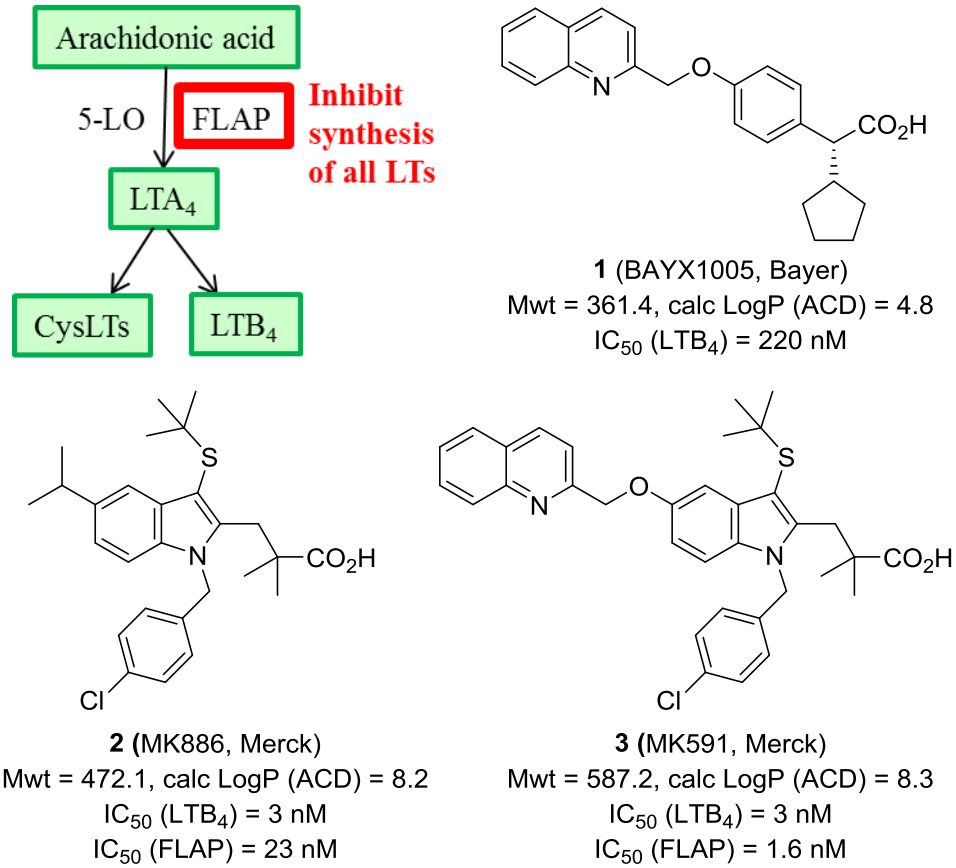


Figure 1.3. FLAP inhibitors (IC_{50} (FLAP) refers to a FLAP binding assay. IC_{50} (LTB_4) refers to the LTB_4 inhibition in human leukocytes assay).

A series of selective FLAP inhibitors containing a core indole structure were recently developed by Amira and GlaxoSmithKline (Figure 1.4). The compounds AM103 **4** and AM803 **5** had superior potency and pharmacokinetic and safety profiles compared to the indole based FLAP inhibitor MK591 **3**.[30] The key structural changes from MK591 **3** were replacement of the quinoline pharmacophore with pyridine derivatives and incorporation of heteroaromatic biaryls in place of the 4-chlorobenzyl group. AM803 **5** successfully completed phase I clinical studies and was advanced to phase II trials in asthmatic patients as an orally bioavailable FLAP inhibitor.[31]

Stock *et al.* reported an extensive structure-activity relationship study for this series of FLAP inhibitors,[32] focusing on cyclic moieties appended to the indole core, in order to replace the quinoline group present in MK591 **3** which has been implicated as being susceptible to CYP-mediated bioactivation and covalent labelling of protein. The compound AM679 **6** (Figure 1.4) containing a novel *N*-acylated-(*S*)-indoline group showed excellent potency at inhibiting LTB_4 synthesis in human blood (IC_{50} = 154 nM) as well as the human leukocyte assay (IC_{50} = 0.6 nM) and LT inhibition in several *in vivo* models. In addition, AM679 **6** displayed better solubility and an improved CYP inhibition profile compared to MK591 **3**, through replacement of the chlorobenzyl group with a heteroaromatic biaryl group.

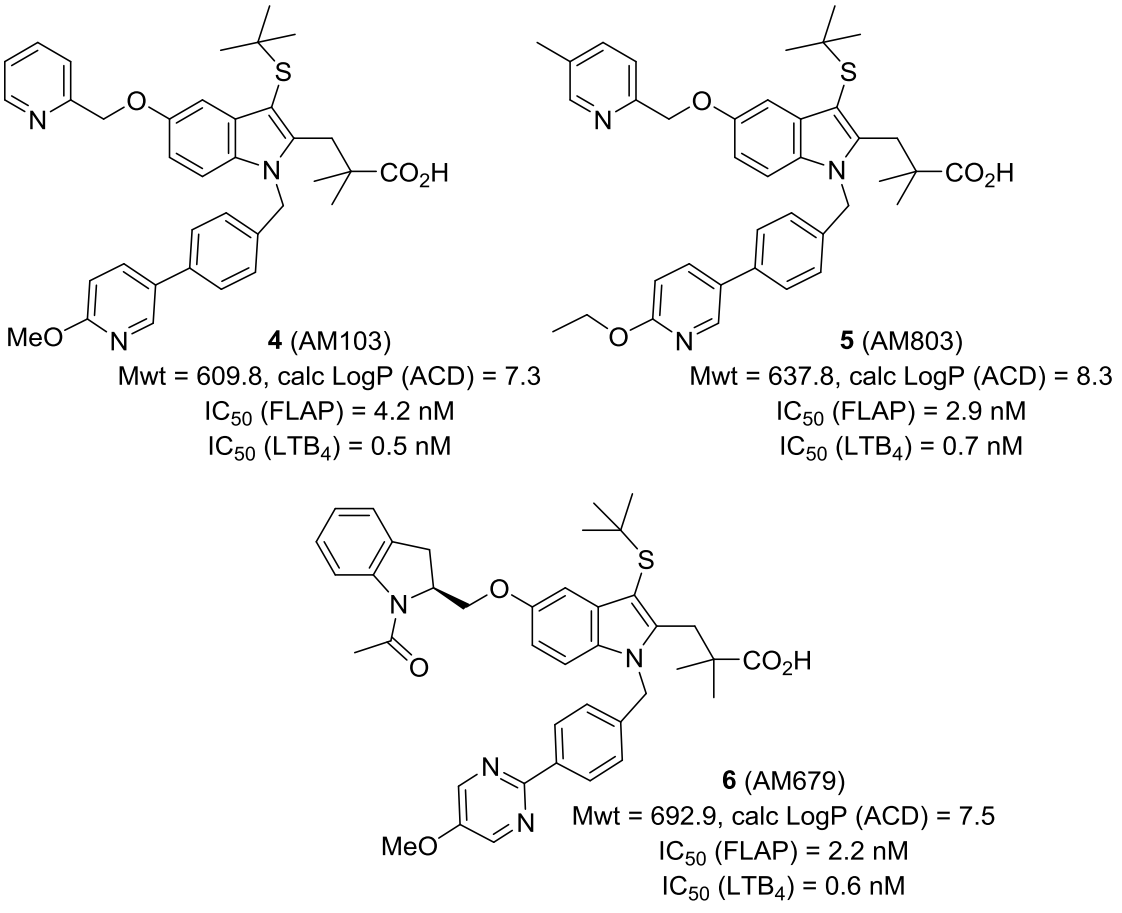


Figure 1.4. Indole based FLAP inhibitors developed by GlaxoSmithKline and Amira (IC_{50} (FLAP) refers to FLAP binding assay. IC_{50} (LTB_4) refers to the LTB_4 inhibition in human leukocytes assay).

1.4.2 Prostaglandin inhibitors and receptor antagonists

Inhibition of the synthesis of pro-inflammatory mediator PGD_2 (Scheme 1.3) could be achieved by targeting enzymes COX1 and COX2 using COX inhibitors (non-steroidal anti-inflammatory drugs). However COX inhibitors have been found to be generally ineffective as anti-asthma drugs because inhibition of COX also inhibits the biosynthesis of PGE_2, a prostaglandin with bronchodilator properties. Therefore, to achieve a therapeutic benefit in asthma treatment, selective inhibition of the biological actions of PGD_2 is needed.[20]

There has been much interest in the last 10 years in designing small molecule antagonists of the PGD_2 receptor CRTh2, as a target for anti-asthma drugs. In various biological studies, the pro-inflammatory effects from activation of the CRTh2 receptor by PGD_2 have been completely blocked with a CRTh2 antagonist. In addition, a number of CRTh2 antagonists have entered clinical trials for the treatment of asthma, COPD and allergic rhinitis, with promising proof of concept results, and hence CRTh2 is a validated drug target.[33]

The first CRTh2 antagonist discovered was ramatroban **7** (Figure 1.5). Ramatroban was marketed for allergic rhinitis in Japan as an orally bioavailable thromboxane receptor antagonist. It was subsequently found to also be a potent CRTh2 receptor antagonist and antagonises PGD_2 induced bronchoconstriction.[34]

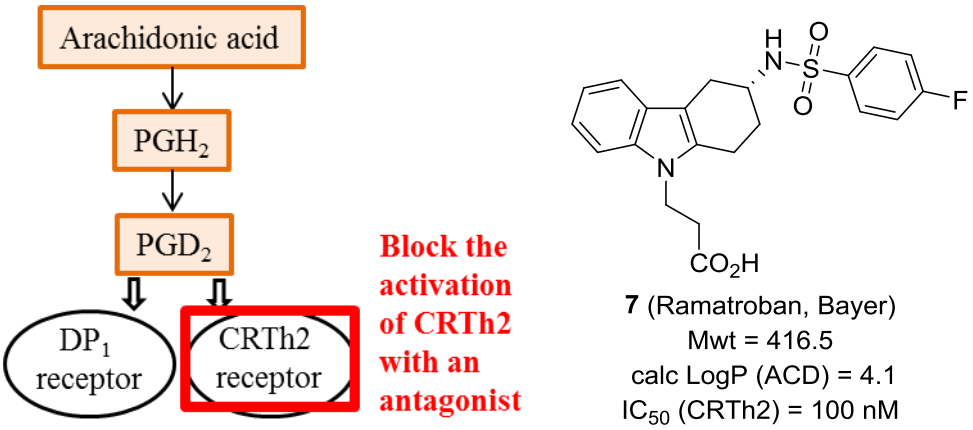
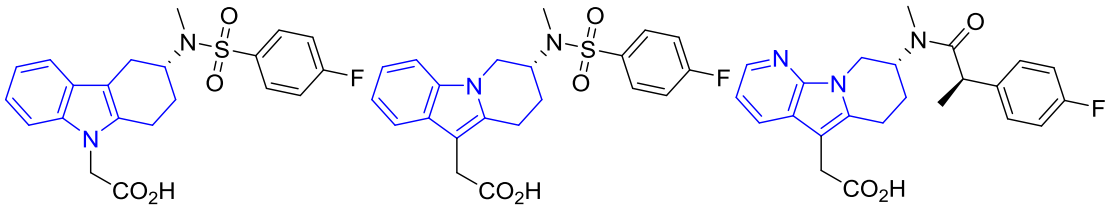


Figure 1.5. CRTh2 antagonist ramatoban (IC_{50} value refers to a CRTh2 binding assay).

The tricyclic core of ramatroban **7** has served as a starting point for the design of more potent and selective CRTh2 antagonists. 7TM Pharma demonstrated with compound **8** (TM30089, Figure 1.6) that shortening the propionic acid side chain to an acetic acid and *N*-methylation of ramatroban **7** gave a highly potent and selective CRTh2 antagonist.[35] Changes to the core indole structure of ramatroban **7** such as the reverse indole **9** (MK7246), the aza-indole **10**, and replacement of the sulfonamide side chain were also tolerated (Figure 1.6).[36]



8 (TM30089, 7TM Pharma)
Mwt = 416.5
calc LogP (ACD) = 4.3
K_i (CRTh2) = 0.6 nM

9 (MK7246, Merck)
Mwt = 416.5
calc LogP (ACD) = 3.8
K_i (CRTh2) = 2.5 nM

10 (Merck)
Mwt = 409.5
calc LogP (ACD) = 3.1
K_i (CRTh2) = 3.4 nM

Figure 1.6. CRTh2 antagonists with a tricyclic core (K_i values refer to a CRTh2 binding assay).

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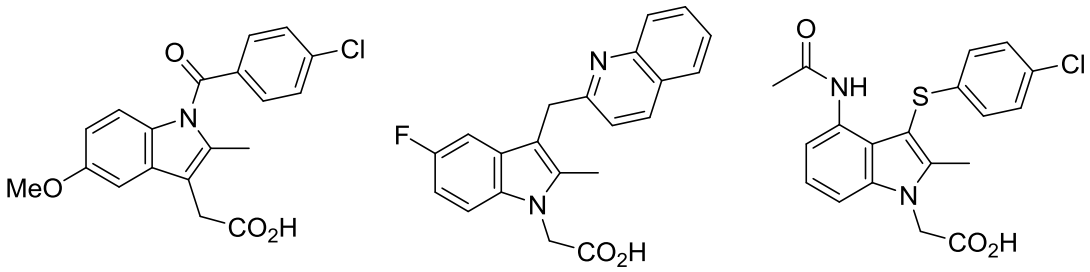
Another major structural class of CRTh2 antagonists are indole acetic acids. Interestingly this core structure was originally inspired by indomethacin **11** (Figure 1.7), which is a non-steroidal anti-inflammatory drug (NSAID) that was discovered to be a CRTh2 receptor agonist.[37] Oxagen developed a potent orally bioavailable CRTh2 antagonist OC459 **12** (Figure 1.7), which is a reverse indole analogue of **11** and with a quinoline substituent replacing the aryl ketone. Compound **12** progressed into phase II clinical trials and has been found to improve lung function and asthma control in allergic asthmatics.[38] A similar indole acetic acid scaffold was present in AstraZenecas lead CRTh2 antagonist AZD1981 **13** (Figure 1.7), which progressed into phase II clinical studies for the treatment of asthma and COPD, but lacked efficacy and has been discontinued.[39]



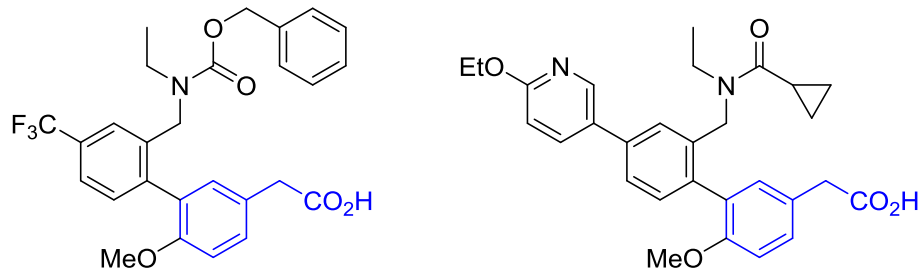
11 (Indomethacin)
Mwt = 357.8
calc LogP (ACD) = 3.1
EC_{50} (CRTh2 agonism) = 389 nM

12 (OC459, Oxagen)
Mwt = 348.4
calc LogP (ACD) = 4.4
IC_{50} (CRTh2) = 6 nM

13 (AZD1981, AstraZeneca)
Mwt = 388.9
calc LogP (ACD) = 4.3
IC_{50} (CRTh2) = 4.3 nM

Figure 1.7. Indole acetic acid series of CRTh2 antagonists (IC_{50} values refer to a CRTh2 binding assay).

Phenoxy and phenyl acetic acids are other common motifs for CRTh2 antagonists. Amira have optimised a series of biphenylacetic acid CRTh2 selective antagonists (Figure 1.8). Compounds such as **14** and **15** were highly potent in both binding and functional assays, and demonstrated good *in vivo* activity in mouse models of inflammatory disease after oral dosing.[40]



14 (Amira)
Mwt = 501.5, calc LogP (ACD) = 6.2
IC_{50} (CRTh2) = 4 nM

15 (AM432, Amira)
Mwt = 488.6, calc LogP (ACD) = 4.0
IC_{50} (CRTh2) = 6 nM

Figure 1.8. Phenyl acetic acid based CRTh2 antagonists (IC_{50} values refer to a CRTh2 binding assay).

7TM Pharma identified the first phenoxyacetic acid CRTh2 antagonists such as compound **16** (Figure 1.9) from virtual screening.[41] A series of phenoxyacetic acids **17a-c** (Figure 1.9) were reported by AstraZeneca, where changes to the acetic acid side chain were investigated.[42] Isosteres of the carboxylic acid were not tolerated, and

increasing steric bulk next to the carboxylic acid was also unfavourable with respect to CRTh2 binding affinity. In general, the majority of CRTh2 antagonists in development contain a carboxylic acid group. It is likely that this group mimics the carboxylic acid present in PGD_2, the natural ligand for the CRTh2 receptor.

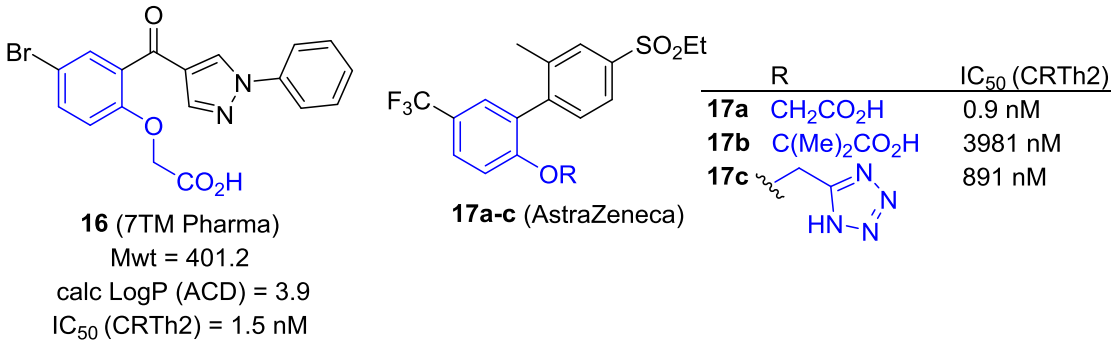


Figure 1.9. Phenoxy acetic acid based CRTh2 antagonists (IC_{50} values refer to a CRTh2 binding assay).

A recent review of CRTh2 antagonist clinical candidates has suggested increased potency is required for CRTh2 antagonists, as recently evaluated clinical candidates have failed to produce the efficacy required for them to be clinically useful for the treatment of asthma or allergic diseases.[43]

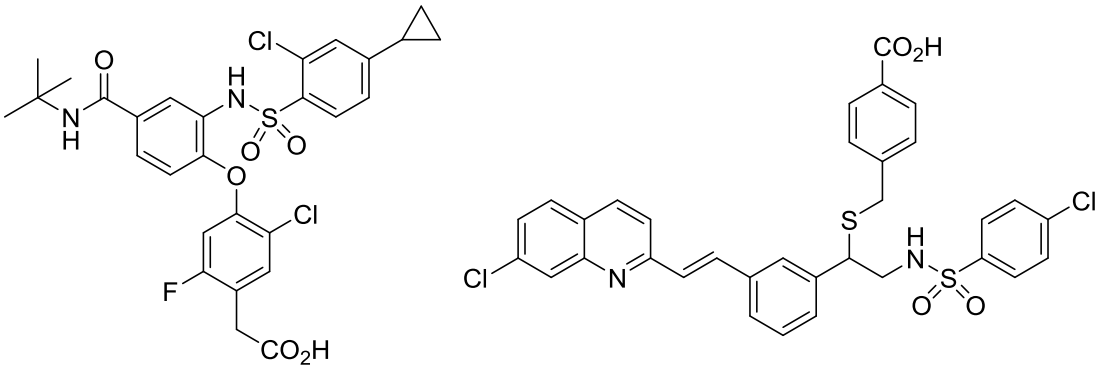
1.4.3 Dual inhibitors

Due to the complexity of asthma, compounds that inhibit more than one target have the potential to be more effective treatments than those targeting a single mediator. Also, several single targeting compounds that have advanced to clinical development for the treatment of asthma have been discontinued due to lack of efficacy.

Amgen have recently described the optimisation of a series of phenylacetic acid derivatives as potent dual antagonists of both the PGD_2 receptors, CRTh2 and DP_1.[44] Their clinical candidate AMG853 **18** (Figure 1.10) was evaluated in phase II clinical studies for the treatment of asthma but failed to improve asthma symptoms in patients with poorly controlled asthma and has since been discontinued. Dual CRTh2/DP_1 antagonists are yet to show any anti-asthma benefits over selective CRTh2 antagonists.

Studies of a selective DP_1 antagonist (laropiprant) have indicated that antagonism of the DP_1 receptor does not have a therapeutic benefit for the treatment of asthma and actually DP_1 activation plays a role in some anti-inflammatory responses.[45]

Another approach to dual inhibition is the development of compounds that target both the leukotriene and prostaglandin pathways. Single inhibition along one of the pathways can cause a shift in arachidonic acid metabolism to the other uninhibited pathway, resulting in increased biosynthesis of either LT or PG mediators.[46] Compounds exhibiting dual antagonism of $CysLT_1$ (LTD_4 receptor in the leukotriene pathway) and TP (TXA_2 receptor in the prostaglandin pathway) have been under development as anti-asthma drugs. Initially Astellas Pharmaceutical reported compound YM158 was a selective and potent dual antagonist of LTD_4/TXA_2 receptors, and *in vitro* functional assays demonstrated inhibition of asthmatic responses.[47] They have more recently reported a quinoline derivative **19** (Figure 1.10) as an orally active dual LTD_4/TXA_2 receptor antagonist.[48] The design of compound **19** was based on combining the lipophilic chloroquinolylvinyl moiety of montelukast ($CysLT_1$ antagonist, Figure 1.2) with a chlorobenzenesulfonamide group present in daltroban (a TXA_2 receptor antagonist). However, its activity is almost 1000 fold lower at the TXA_2 receptor, so the anti-inflammatory effects may result mainly from antagonism of the $CysLT_1$ receptor.



18 (AMG853, Amgen)
Mwt = 609.5, calc LogP (ACD) = 6.2
IC_{50} (CRTh2) = 3 nM
IC_{50} (DP_1) = 4 nM

19 (Astellas)
Mwt = 649.6, calc LogP (ACD) = 9.2
IC_{50} (LTD_4) = 0.4 nM
IC_{50} (TXA_2) = 340 nM

Figure 1.10. Compounds with dual activity along the asthma inflammatory pathways.

1.5 Aims of the project

Leukotriene and prostaglandin lipid mediators play important roles in the pathogenesis of asthma and it is anticipated that a compound with dual pharmacology, which impacts both the leukotriene and prostaglandin inflammatory pathways simultaneously, would yield compounds with an enhanced ability to treat asthma.

An attractive novel dual target combination is the inhibition of FLAP to block the synthesis of all leukotrienes, and antagonism of the prostaglandin receptor CRTh2 to block PGD_2 mediated asthma responses. Dual FLAP/CRTh2 inhibitors have excellent potential as anti-asthma drugs because currently both single targeting FLAP inhibitors and CRTh2 antagonists are already in clinical development, but efficacy has often been a problem. Therefore a dual inhibitor targeting both LT and PG pathways could overcome this. In addition, many structurally diverse compounds bind to FLAP or CRTh2. To date, many literature CRTh2 antagonists are lipophilic carboxylic acid containing compounds, features also often found in FLAP inhibitors.

The aim of this part of the project was to identify compounds that inhibit both leukotriene synthesis and antagonise CRTh2 in order to discover compounds with dual pharmacology. The target compounds **20** and **21** (Figure 1.11) were designed based on a combination of previous GlaxoSmithKline and literature SAR studies. As well as featuring a phenylacetic acid group which is a known pharmacophore for CRTh2 antagonism, the target compounds also incorporated either the polar aliphatic acyl indoline group present in the FLAP inhibitor AM679 (**6**, Figure 1.4), or the smaller acyl pyrrolidine group also identified by Stock *et al*. as enhancing potency of FLAP inhibition (Figure 1.12).[32]

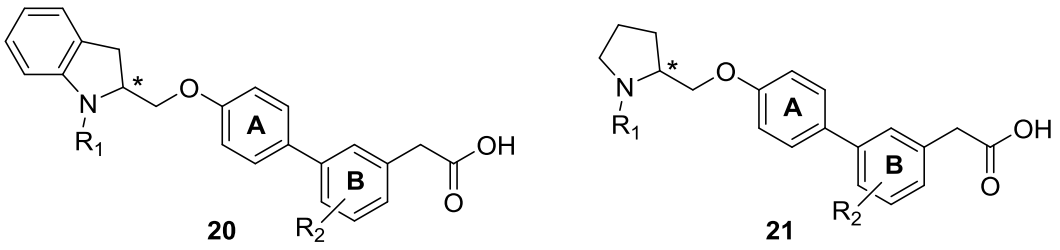


Figure 1.11. Target compounds: potential dual FLAP inhibitors and CRTh2 antagonists.

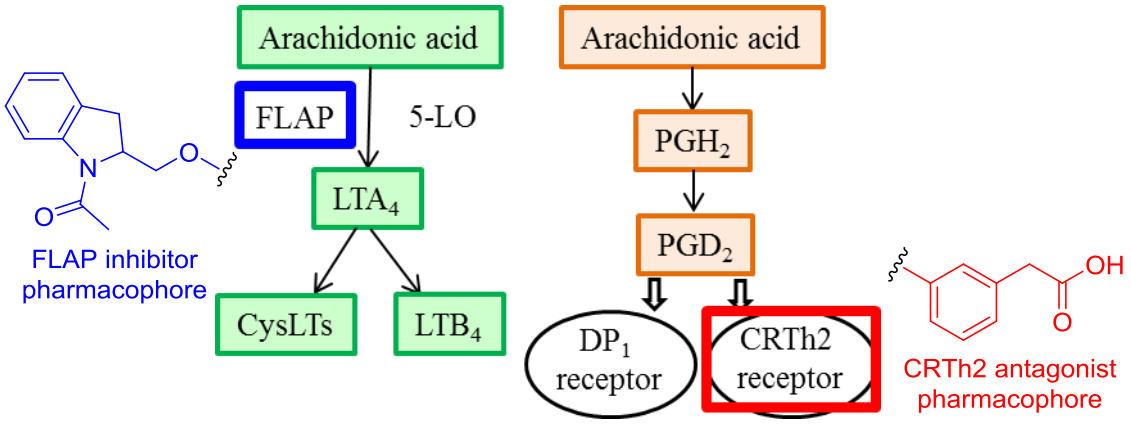


Figure 1.12. Known pharmacophores for FLAP inhibitors and CRTh2 antagonists.

The first part of the project was to investigate a synthetic route towards the target compounds via initial synthesis of the arylboronic ester intermediates **22** (Figure 1.13), which would facilitate attachment of ring B by a Suzuki coupling.

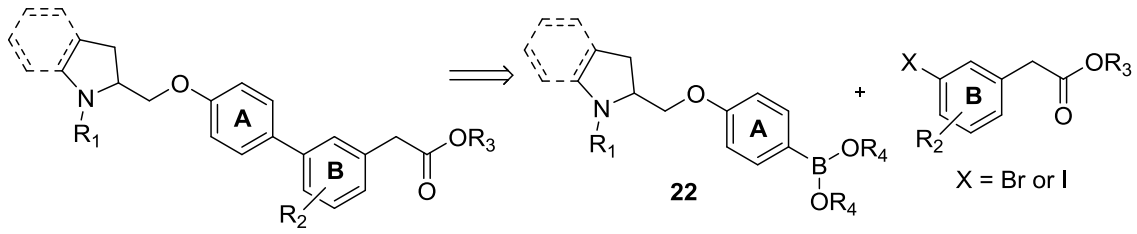


Figure 1.13. Proposed synthetic route towards target compounds.

Compounds of type **20** and **21** would then be evaluated though biological assays for their activity against leukotriene synthesis inhibition and antagonism of the CRTh2 receptor. The assays would determine if the compounds showed potential FLAP inhibitory activity by inhibiting LTB_4 production and if the compounds inhibited the binding of PGD_2 to the CRTh2 receptor. Compounds showing dual pharmacology could serve as an excellent starting point for further lead optimisation for novel anti-asthma drugs.

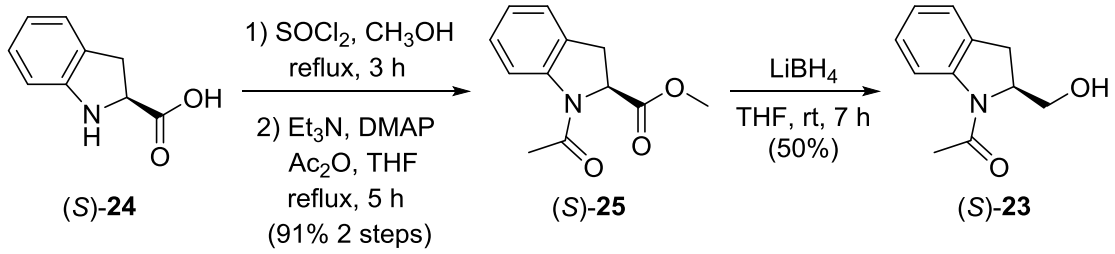
2. Results and discussion

2.1 Synthesis of boronic ester intermediates (ring A)

2.1.1 Attempted synthesis by direct acetylation

The first approach to the preparation of the boronic ester intermediates **22** (Figure 1.13) was via an initial *N*-acetylation. This would allow the synthesis of the target compounds **20** and **21** with $R^1 = COCH_3$ (Figure 1.11), which was chosen because Stock *et al*. have reported SAR studies of *N*-acyl indoline groups which led to their discovery of the potent FLAP inhibitor **6** (Figure 1.4).[32]

Following a procedure reported by Kuwano *et al*.,[49] the *N*-acetyl indoline alcohol (*S*)-**23** was synthesised over 3 steps from commercially available (*S*)-indoline-2-carboxylic acid (*S*)-**24** (Scheme 2.1). Treatment of the carboxylic acid (*S*)-**24** with thionyl chloride in methanol gave the intermediate methyl ester as the hydrochloride salt. Subsequent acetylation with acetic anhydride, triethylamine and the addition of DMAP (to enable a shorter reaction time) afforded the amide (*S*)-**25** in 91% yield over 2 steps, which was similar to the yield reported in the literature of 86%.[49]

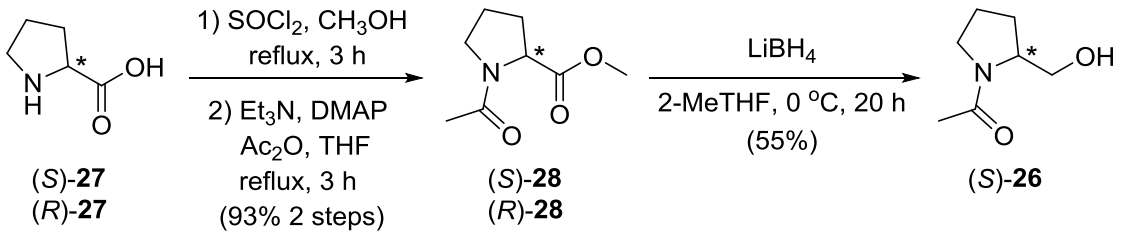


Scheme 2.1. Synthesis of *N*-acetyl indoline alcohol (*S*)-**23**.

Reduction of the methyl ester moiety in compound (*S*)-**25** (Scheme 2.1) was achieved using lithium borohydride in THF at room temperature, which gave the *N*-acetyl indoline alcohol (*S*)-**23** in 50% yield after purification by column chromatography. This was a lower yield than that reported in the literature, where the same product was isolated in 74% yield.[49] Lithium borohydride was used rather than lithium aluminium

hydride because it enables the selective reduction of esters to alcohols without affecting amides, however a side product isolated from the reaction was indolin-2-ylmethanol[50] in 19% yield, where the *N*-acetyl group had been converted to the *N*-H group. This side product may have formed from the *N*-acetyl indoline alcohol (*S*)-**23** through an initial *N*-*O* intramolecular acyl transfer, resulting in the *O*-acetyl compound, which would then be reduced to the alcohol by lithium borohydride.

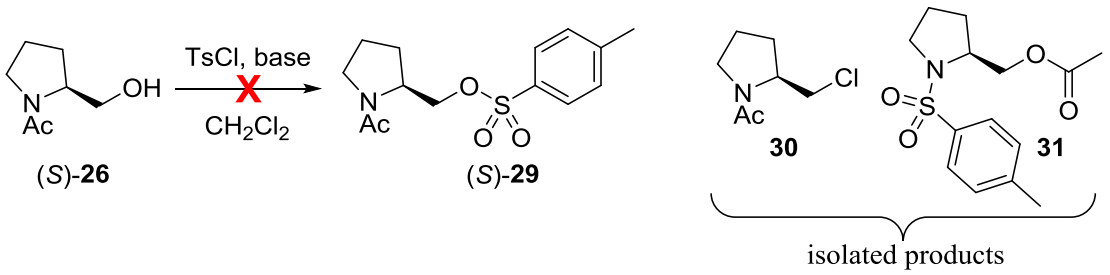
The *N*-acetyl pyrrolidine analogue (*S*)-**26** was prepared via the same route over 3 steps from readily available (*S*)-proline (*S*)-**27** (Scheme 2.2). Esterification and subsequent acetylation of (*S*)-**27** gave the compound (*S*)-**28**[51] in 93% yield over two steps. The same synthesis was applied with the opposite enantiomer, (*R*)-proline (*R*)-**27**, where esterification followed by acetylation afforded the *N*-acetyl compound (*R*)-**28**.[52] Reduction of the methyl ester moiety in compound (*S*)-**28** was carried out using lithium borohydride at a low temperature in order to supress the formation of side products, and this afforded the *N*-acetyl pyrrolidine alcohol (*S*)-**26**[53] in 55% yield after purification by column chromatography. It was likely that the moderate yield was due to the instability of (*S*)-**26**, with possible *N*-*O* acyl transfer occurring. The use of 2-methyl THF as a solvent instead of THF was found to give a better separation of the aqueous and organic phases during the work-up and improve the yield of the product isolated.



Scheme 2.2. Synthesis of *N*-acetyl pyrrolidine alcohols.

The next step in the synthesis of the boronic ester intermediates was to activate the alcohol as a tosylate to provide a good leaving group for the subsequent coupling to a phenol boronic acid derivative. Tosylation of the pyrrolidine alcohol (*S*)-**26** to form the tosylate (*S*)-**29** was not as straightforward as anticipated (Scheme 2.3). Under standard tosylation conditions with equimolar amounts of pyridine and tosyl chloride (Table 2.1,

entry 1), unreacted tosyl chloride was recovered (42%) as well as a complex mixture of products. The reaction was repeated using an excess of pyridine (Table 2.1, entry 2). The crude mixture of products formed from this reaction were separated using column chromatography, resulting in the isolation of the chloride derivative **30**[53] in 45% yield and the unexpected *N*-tosylate **31** in 7% yield (Scheme 2.3).



Scheme 2.3. Attempted tosylation of alcohol (*S*)-**26**.

Entry	Base (eq)	TsCl (eq)	Conditions	Results (yield after column chromatography)
1	pyridine (1.1)	1.1	rt, 30 h	Mixture[a] Recovered TsCl (42%)
2	pyridine (5.0)	1.2	0 °C to rt, 20 h	Chloride **31** (45%) *N*-tosylate **32** (7%)
3	pyridine (5.0)	2.0	0 °C, 4 h	Mixture[a] containing chloride **31** and *N*-tosylate **32** (ratio 43:57)
4	2,6-lutidine (5.0)	1.2	0 °C to rt, 15 h	*N*-tosylate **32** (10%)
5	2,6-lutidine (5.0) Et_3N (1.5)	1.2	0 °C to rt, 15 h	*N*-tosylate **32** (19%)

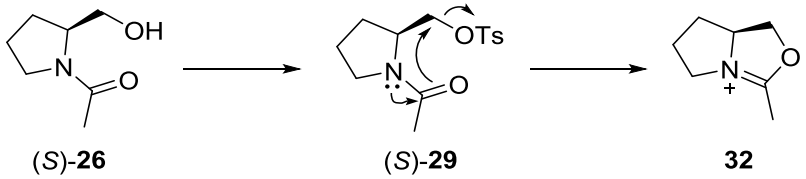
Table 2.1. Attempted tosylation reactions. [a]Crude material contained a complex mixture of compounds.

It has been established in the literature that tosylation reactions using tosyl chloride and pyridine as a base, can lead to the formation of undesired side products.[54] These include pyridinium salts (where reactive tosylate products react with pyridine hydrochloride formed during the reaction) resulting in a loss of the desired tosylate with the

pyridinium salt lost during the work-up. Also, the conversion of tosylate into the corresponding chloride product can be a problem, where the pyridine hydrochloride formed during the reaction acts as the chloride nucleophile. These side products have often been formed at high temperatures or when a large excess of pyridine was used.[54]

Attempts to suppress the formation of the chloride **30** by lowering the reaction temperature to 0 ^{o}C were unsuccessful (Table 2.1, entry 3), and formation of both the chloride **30** and the *N*-tosylate **31** was detected by ^{1}H-NMR spectroscopy of the crude product. The tosylation reaction was repeated with 2,6-lutidine as an alternative bulkier base compared to pyridine (Table 2.1, entry 4), that can suppress pyridinium salt formation.[54] Under these conditions, the chloride **30** was not formed, however *N*-tosylate **31** was still isolated in 10% yield after purification by column chromatography. Addition of triethylamine to the reaction (which could react with HCl to give the corresponding salt), also only yielded *N*-tosylate **31** but in a 19% yield (Table 2.1, entry 5). Since the reactions were carried out, the synthesis of tosylate (*S*)-**29** was reported in a patent, from alcohol (*S*)-**26** using tosyl chloride, triethylamine and DMAP.[55] However limited experimental details were provided and the yield of the product was not given.

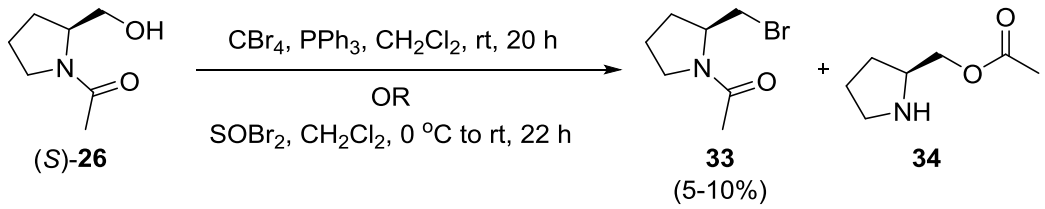
It was possible that during the reaction the desired tosylate (*S*)-**29** formed readily but was unstable, and neighbouring group participation of the *N*-acetyl group could lead to the formation of cyclic intermediate **32** (Scheme 2.4). A chloride nucleophile could then attack to form the chloride **30**. It was also possible that compound **26** underwent an *N*-*O* acyl transfer followed by sulfonation of the amine to yield the *N*-tosylate **31**.



Scheme 2.4. Proposed mechanism of neighbouring group participation.

An alternative strategy, still retaining the *N*-acetyl group (which would be useful for the direct preparation of the boronic ester intermediates **22** with R^1 = $COCH_3$, Figure 1.13), was to convert the alcohol (*S*)-**26** to the alkyl bromide **33** (Scheme 2.5). The alcohol (*S*)-**26** was treated with carbon tetrabromide and triphenylphosphine at room

temperature, which afforded the alkyl bromide **33** in a low yield of 5% after purification by column chromatography. The triphenylphosphine oxide impurities were difficult to separate from the product **33** by column chromatography. After further purification by filtration, it was observed by ^{1}H-NMR spectroscopy that bromide **33** had rearranged to the acetate **34**.[56] Bromination of alcohol (*S*)-**26** with thionyl bromide resulted in the bromide product **33** being isolated in a low yield (10%), and **33** was also found to be unstable over time, rearranging to the acetate **34** (Scheme 2.5).



Scheme 2.5. Attempted conversion of alcohol to alkyl bromide.

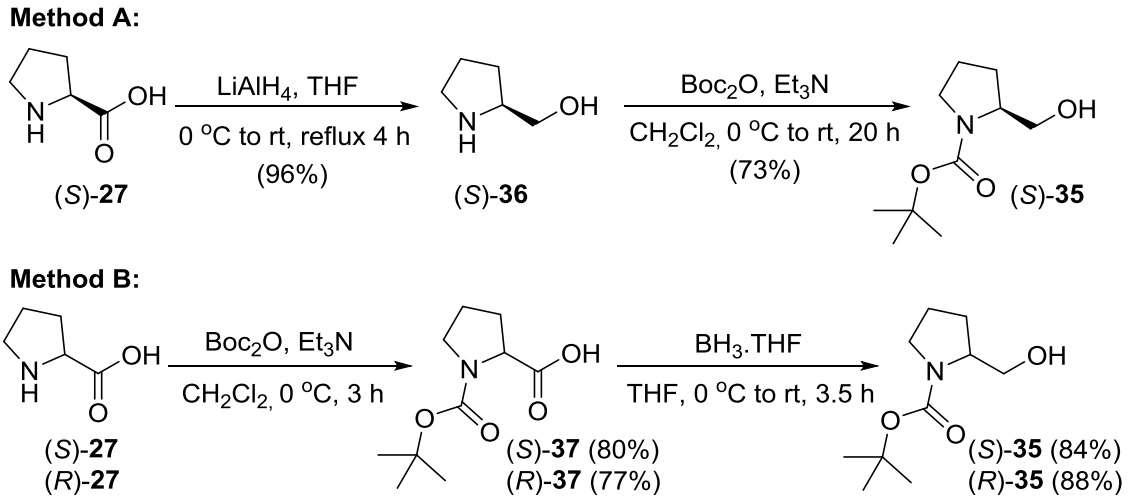
2.1.2 Synthesis via *N*-Boc protection route

An alternative synthetic approach was to prepare the boronic ester intermediates **22** (Figure 1.13) with R^1 = Boc. Substitution of the acetyl group of compound **26** with a Boc group, which has a lower reactivity, was expected to alleviate the problem of *N* to *O*-acyl transfer and neighbouring group participation. In addition, Bartoli *et al.* have reported a procedure for tosylation of an *N*-Boc pyrrolidine alcohol derivative which afforded the tosylate product in an excellent yield.[57] Following this strategy, once the target compounds had been synthesised, the Boc group could then be deprotected and subsequent *N*-acetylation would yield the target compounds with the desired *N*-acetyl group.

N-Boc-prolinol **35** was synthesised by two different methods (methods A and B, Scheme 2.6). Following literature procedures, method A began with reduction of optically pure proline (*S*)-**27** using lithium aluminium hydride to furnish the alcohol (*S*)-**36** in 96% yield, which was consistent with the yield of 94% reported in the literature.[58] Subsequent Boc protection using Boc anhydride and triethylamine,[59] afforded *N*-Boc-prolinol (*S*)-**35** in 73% yield after purification by column chromatography. Several

methods have been reported in the literature for the reduction of optically pure α-amino acids to optically pure β-amino alcohols. These include reduction with $LiBH_4$ and Me_3SiCl,[60] $NaBH_4$ with iodine or H_2SO_4,[61] and boron reagents.[62] The most routinely used reagent for the reduction of α-amino acids is lithium aluminium hydride which has been shown to proceed without racemisation at the α-carbon[63] and successfully gave the alcohol (*S*)-**36** here.

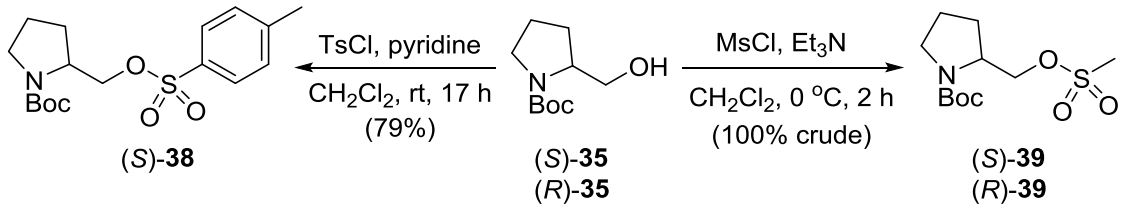
Difficulties were encountered when removing impurities from the reduction with lithium aluminium hydride, particularly when the reaction was scaled up to 5 g of the starting material (*S*)-proline. An alternative synthetic route was therefore used to synthesise *N*-Boc-prolinol **35** following literature procedures (method B, Scheme 2.6).[57] First, (*S*)-proline (*S*)-**27** was Boc protected using Boc anhydride and triethylamine to give the compound (*S*)-**37** in 80% yield after purification by recrystallisation. Selective reduction of the carboxylic acid moiety in (*S*)-**37** with borane tetrahydrofuran complex, afforded the desired alcohol (*S*)-**35** in 84% yield, which was comparable to the yield reported in the literature of 82%.[57] Synthesis of the enantiomeric alcohol (*R*)-**35**[64] was prepared using the same procedure from optically pure proline (*R*)-**27** (Scheme 2.6).



Scheme 2.6. Synthesis of *N*-Boc-prolinol using either method A or method B.

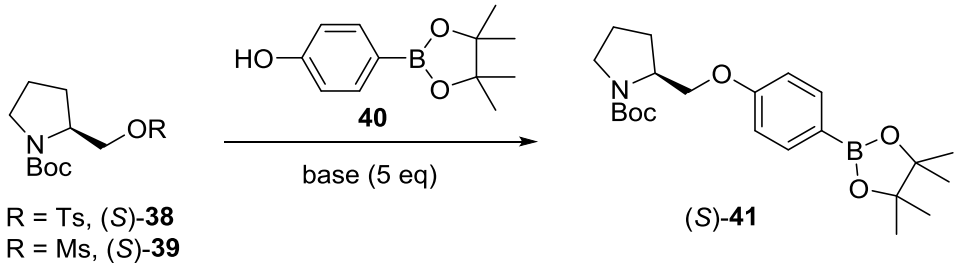
Tosylate (*S*)-**38** was next prepared from the alcohol (*S*)-**35** using a modified version of a literature procedure using tosyl chloride and pyridine (Scheme 2.7).[57] Tosylate (*S*)-**38** was found to be unstable if stored over long periods of time under argon at 4 °C.

Therefore the mesylates (*S*)-**39** and (*R*)-**39** were prepared from alcohols (*S*)-**35** and (*R*)-**35** in quantitative yield, using mesyl chloride and triethylamine.[65] Mesylates (*S*)-**39** and (*R*)-**39** were noted to be stable when stored under argon at 4 °C for extended periods.



Scheme 2.7. Preparation of tosylate and mesylate prolinol derivatives.

The final step in the preparation of the boronic ester intermediates was a nucleophilic displacement of either the tosyl group in **38**, or the mesyl group in **39**, with the phenol boronic ester **40** (Scheme 2.8). Using similar conditions to those reported by Stock *et al.*,[32] the tosylate (*S*)-**38** and phenol **40** were reacted with a carbonate base overnight at 50 °C (Table 2.2, entries 1-2). No reaction occurred and only starting materials were recovered. Repeating the reaction at a higher temperature, either in reluxing acetonitrile or in DMF at 110 °C (Table 2.2, entries 3-4) resulted in the formation of a complex mixture of products. Shortening the reaction time to 4 hours and using acetonitrile as the solvent, resulted in a low yield of 12% of the desired product (*S*)-**41** isolated after purification by column chromatography (Table 2.2, entry 5). The other fractions eluted from the column contained mostly starting materials. In an attempt to optimise the yield of the product (*S*)-**41**, the same reaction conditions using a carbonate base were attempted with the less bulky and more stable mesylate analogue (*S*)-**39** (Table 2.2, entry 6), however the yield of the product (*S*)-**41** achieved was low (9%).

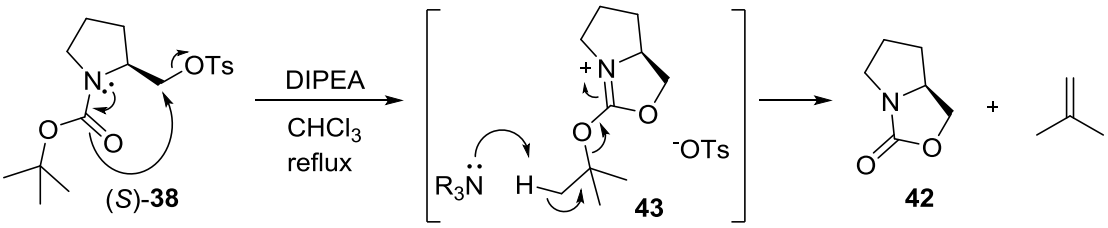


Scheme 2.8. Coupling the tosylate or mesylate with a phenol derivative.

Entry	R group	Base (5 eq)	Solvent	Conditions	Yield of (*S*)-41
1	Ts	K_2CO_3	CH_3CN	50 ^oC, 18 h	0% [a]
2	Ts	Cs_2CO_3	CH_3CN	50 ^oC, 18 h	0% [a]
3	Ts	Cs_2CO_3	CH_3CN	Reflux, 18 h	0% [b]
4	Ts	Cs_2CO_3	DMF	110 ^oC, 18 h	0% [b]
5	Ts	Cs_2CO_3	CH_3CN	Reflux, 4 h	12% [c]
6	Ms	Cs_2CO_3	CH_3CN	Reflux, 18 h	9% [c]

Table 2.2. Reaction conditions for synthesis of boronic ester intermediates. [a]Only starting materials recovered. [b]No starting materials but a mixture of products isolated. [c]Yield after column chromatography.

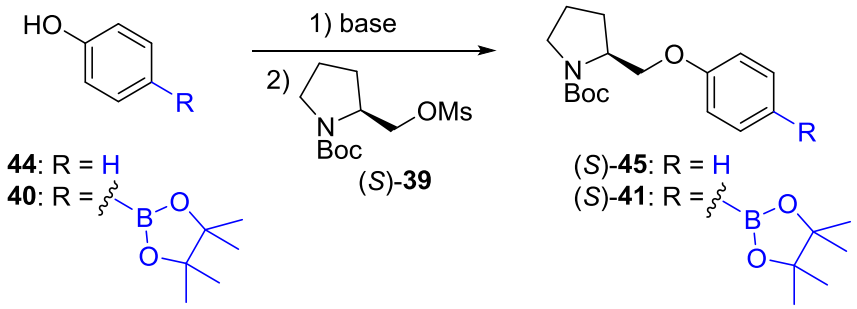
One of the side products identified that was formed during the phenol coupling reactions was oxazolidone **42** (Scheme 2.9). This product had also previously been reported as being formed from a base catalysed rearrangement of *N*-Boc pyrrolidine tosylate **38**.[66] The Boc group carbonyl can attack the electrophilic carbon and displace the tosylate to give the cationic intermediate **43**. Then abstraction of a proton from one of the *tert*-butyl methyl groups can lead to the loss of 2-methylpropene and the formation of oxazolidone **42**.



Scheme 2.9. Proposed mechanism of the base catalysed rearrangement of tosylate **38** by Curran *at al.*[66]

In order to suppress the base catalysed rearrangement, the order of addition of reagents used in the phenol coupling reaction was investigated. The base was first added to the phenol derivative to generate the phenolate, followed by the addition of mesylate (*S*)-**39** (Scheme 2.10). Optimal reaction conditions were explored using the less expensive phenol **44** as a model substrate for the phenol boronic ester **40**. Following a literature procedure, the reactions were initially carried out using sodium hydride in THF (Table

2.3, entries 1-2).[67] The yield of product (*S*)-**45** obtained after 24 hours was 30%, and the yield increased only to 36% if the reaction was carried out for 48 hours, which was much lower than the yield reported in the literature of 78%.[67] Changing the solvent to DMF and repeating the reaction at 95 ^{o}C for 4 hours, resulted in the product (*S*)-**45** which was isolated in 37% yield (Table 2.3, entry 3). Using the same conditions but increasing the amount of sodium hydride from 1.5 equivalents to 2 equivalents resulted in an increase of the product yield to 69%, but using 3 equivalents of sodium hydride resulted in a slightly lower yield of 62% of the product (Table 2.3, entries 4-5). Applying the optimal reaction conditions using 2 equivalents of sodium hydride in DMF with the phenol boronic ester derivative **40**, resulted in isolation of the desired boronic ester product (*S*)-**41** in a disappointing 31% yield (Table 2.3, entry 6).

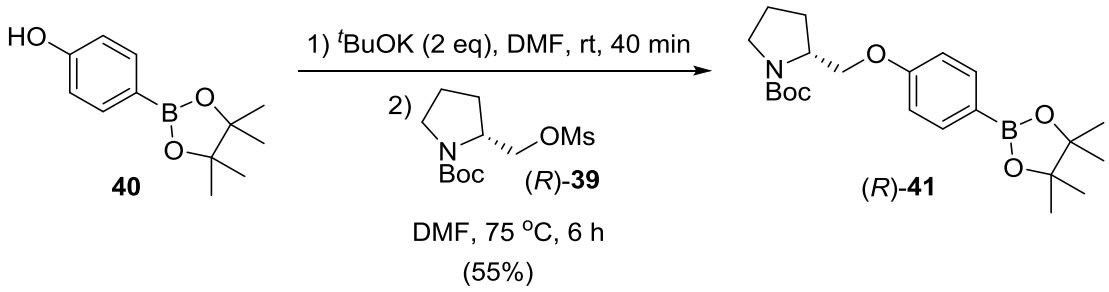


Scheme 2.10. Optimisation of the synthesis of boronic ester compounds.

Entry	Phenol	Base	Solvent	Conditions	Yield (%)[a]
1	**44**	NaH (1.5 eq)	THF	reflux, 22 h	30
2	**44**	NaH (1.5 eq)	THF	reflux, 48 h	36
3	**44**	NaH (1.5 eq)	DMF	95 ^{o}C, 4 h	37
4	**44**	NaH (2.0 eq)	DMF	95 ^{o}C, 3.5 h	69
5	**44**	NaH (3.0 eq)	DMF	95 ^{o}C, 3.5 h	62
6	**40**	NaH (2.0 eq)	DMF	95 ^{o}C, 3.5 h	31
7	**44**	tBuOK (1.1 eq)	THF	reflux, 27 h	30
8	**44**	^{t}BuOK (1.1 eq)	DMF	75 ^{o}C, 6 h	70
9	**40**	^{t}BuOK (1.1 eq)	DMF	75 ^{o}C, 6 h	25
10	**40**	^{t}BuOK (2.0 eq)	DMF	75 ^{o}C, 6 h	46

Table 2.3. Coupling reactions of phenols with mesylate (*S*)-**39**. [a]Isolated yield after purification by column chromatography.

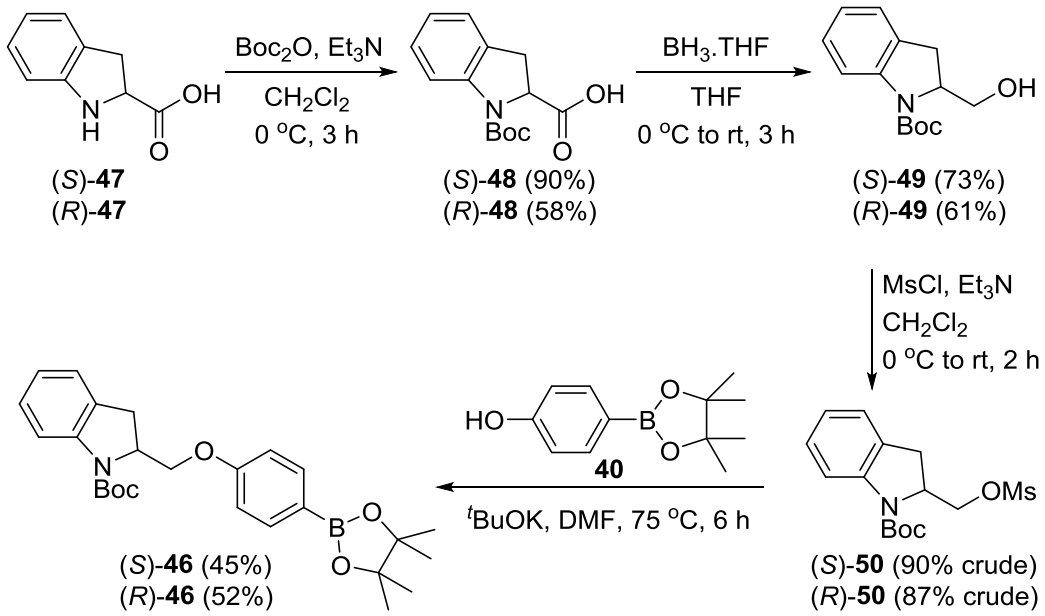
The coupling reaction was repeated with a different base, potassium *tert*-butoxide (Table 2.3, entries 7-10). It has been reported in the literature that using potassium *tert*-butoxide rather than sodium hydride in DMF allows lower reaction temperatures to be used.[57] Therefore the reaction temperatures were lowered to 75 ^{o}C. Reaction of the model phenol **44** with mesylate (*S*)-**39** in DMF at 75 ^{o}C for 6 hours, gave the corresponding product (*S*)-**45** in 70% yield after purification by column chromatography (Table 2.3, entry 8). Applying the same reaction conditions to the phenol boronic ester **40** afforded the desired boronic ester product (*S*)-**41** in a low yield of 25% (Table 2.3, entry 9). Repeating the reaction but increasing the amount of potassium *tert*-butoxide from 1.1 equivalents to 2 equivalents resulted in the isolation of the desired product (*S*)-**41** in 46% yield (Table 2.3, entry 10). The lower yields achieved with the boronic ester substrate may be due to coordination of ^{t}BuOK to form a boronate complex. The same procedure using the optimal conditions was repeated for the synthesis of the opposite enantiomer of the boronic ester intermediate (*R*)-**41**, which was isolated in 55% yield (Scheme 2.11).



Scheme 2.11. Synthesis of *N*-Boc protected pyrrolidine boronic ester intermediate.

The same synthetic route was followed to access the novel indoline *N*-Boc protected boronic ester analogues (*S*)-**46** and (*R*)-**46** (Scheme 2.12). Treatment of commercially available indoline-2-carboxylic acid **47** with Boc anhydride and triethylamine gave the Boc protected compound **48** in 90% yield for the (*S*)-enantiomer[68] and 58% yield for the (*R*)-enantiomer.[69] Subsequent reduction of the carboxylic acid moiety with borane.THF complex resulted in the isolation of alcohol **49** in 73% yield for the (*S*)-enantiomer[70] and 61% yield for the (*R*)-enantiomer.[71] Conversion of the alcohol **49** to mesylate **50** was achieved using mesyl chloride and triethylamine. Finally, displacement of the

mesyl group with phenol boronic ester **40** using potassium *tert*-butoxide in DMF at 75 ^{o}C, resulted in isolation of the indoline boronic ester intermediate **46**, in 45% yield for the (*S*)-enantiomer and 52% yield for the (*R*)-enantiomer.



Scheme 2.12. Synthesis of indoline *N*-Boc protected boronic ester intermediates.

2.2 Synthesis of aryl iodides (ring B)

In order to synthesise target compounds **20** and **21** (Figure 1.11), aryl halides were required for Suzuki coupling to the boronic ester intermediates **42** and **46**. Initially, aryl iodides **51** and **52** (Figure 2.1), with an iodide group *meta* to the acetic acid side chain and a methyl substituent (R_2 = CH_3), were chosen as the ring B structure. These structural features were chosen based on literature phenylacetic acid CRTh2 antagonists developed by Amira (Figure 1.8) and previous GlaxoSmithKline SAR studies.[40]

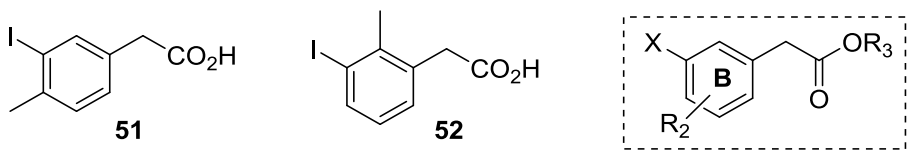
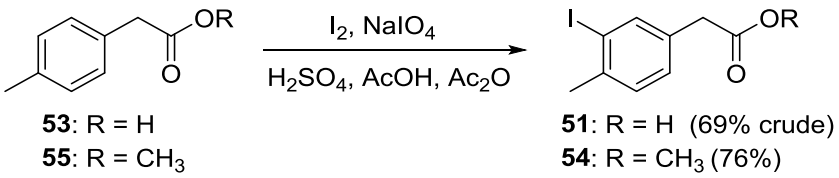


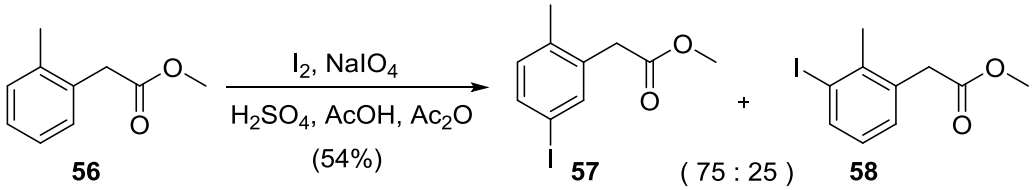
Figure 2.1. Target compounds for use in Suzuki reactions - aryl iodides.

Aryl iodide **51** was prepared by aromatic oxidative iodination according to a procedure by Skulski *et al.*, where arenes were mono-iodinated with strong electrophilic I^+ reagents.[72] *p*-Tolylacetic acid **53** was reacted with iodine and sodium periodate as the oxidant, in a strongly acidic solution (Scheme 2.13) to afford the crude aryl iodide **51** in 69% yield. The crude product contained a small amount of a di-iodinated product as confirmed by mass spectrometry. The same procedure was followed to synthesise the corresponding methyl ester analogue **54** in 76% yield, from methyl 2-(*p*-tolyl)acetate **55** (Scheme 2.13). Purification of the crude aryl iodide **51** by recrystallisation and the crude aryl iodide methyl ester **54** by column chromatography resulted in approximately 3-5% of the di-iodinated compound impurity remaining. It was hoped that this could be removed after the next coupling step.



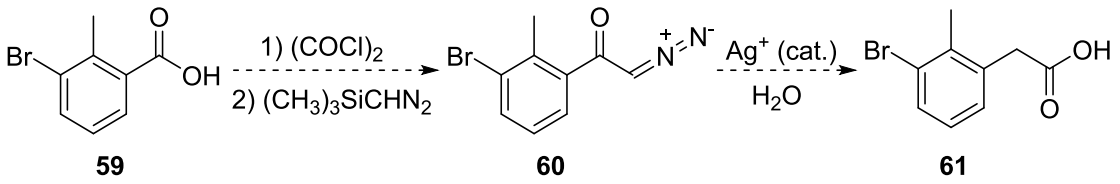
Scheme 2.13. Aromatic iodination of *p*-tolylacetic acid and its methyl ester analogue.

The synthesis of the aryl iodide with a methyl group *ortho* to the acetic acid side chain was problematic due to the *ortho* and *para* directing methyl group (Scheme 2.14). Using the same iodination procedure as above with methyl 2-(*o*-tolyl)acetate **56** resulted in a mixture of aryl iodide regioisomers formed in 54% yield. The major regioisomer **57** had the iodide group *para* to the methyl group, whilst the minor regioisomer was the desired *ortho* iodinated product **58**. The regioisomers were inseparable by column chromatography.



Scheme 2.14. Aromatic iodination of methyl 2-(*o*-tolyl)acetate.

An alternative proposed synthetic route to aryl halides of type **52** and **58** was the Arndt-Eistert synthesis (Scheme 2.15).[73] Commercially available 3-bromo-2-methylbenzoic acid **59** could be converted to the acid chloride and reacted with diazomethane to give diazoketone **60**. A Wolff rearrangement in the presence of water should allow for the formation of the homologated carboxylic acid **61**. This would yield the bromide analogue of compound **52**, which should still be effective for the Suzuki coupling required in the next stage of the synthesis of the target compounds. However it was decided to continue the synthesis of the target compounds with aryl iodides **51** and **54**, and obtain the series of compounds with the methyl substituent *para* to the acetic acid side chain first.



59 **60** **61**

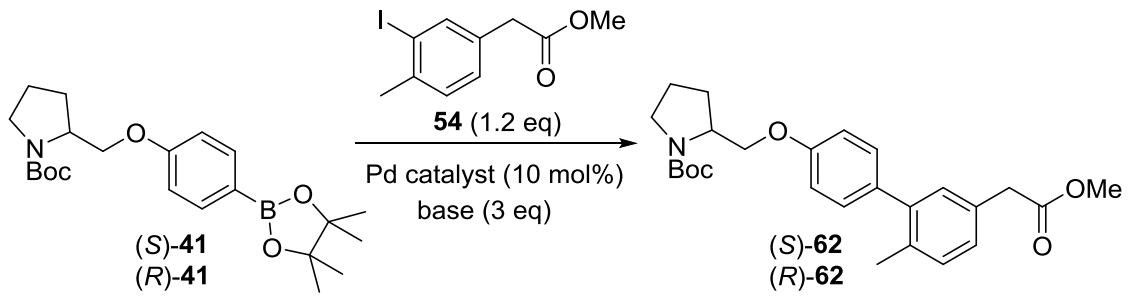
Scheme 2.15. Possible synthetic route to aryl bromides via an Arndt-Eistert synthesis.

2.3 Suzuki coupling

The boronic ester intermediates were coupled to the aryl iodides via a Suzuki reaction[74] to generate the biaryl products. Initial Suzuki reactions were carried out with aryl iodide **51** (Figure 2.1) containing the carboxylic acid moiety. This would have been useful, as it directly generated the target products with the desired carboxylic acid attached. Unfortunately, using aryl iodide **51** in the Suzuki reactions resulted in very low yields of biaryl products. Therefore the methyl ester aryl iodide **54** was used, which made the purification of the products easier and allowed higher yields of products to be generated. However, this also added an additional ester hydrolysis step at the end of the synthesis in order to generate the desired carboxylic acids.

The Suzuki coupling to form the biaryl compounds involved heating the boronic ester, aryl iodide, a palladium catalyst and a base in a suitable solvent (Scheme 2.16). Reaction of the *N*-Boc pyrrolidine boronic ester (*R*)-**41** and aryl iodide **54**, under

standard Suzuki conditions with potassium carbonate and $Pd(PPh_3)_4$ in a mixture of DME/H_2O at 85 ^oC,[32] afforded the novel biaryl product (*R*)-**62** in 20% yield after purification by column chromatography (Table 2.4, entry 1). The other fractions collected from the column contained the starting materials, indicating that the reaction had not gone to completion.



Scheme 2.16. Suzuki coupling reaction of *N*-Boc proline boronic esters and aryl iodide **54**.

Entry	Catalyst	Base	Solvent	Conditions	Yield[a] [enantiomer]
1	$Pd(PPh_3)_4$	K_2CO_3	DME/H_2O (2:1)	85 ^oC, 22 h	20% [(*R*)-**62**]
2	$Pd(dppf)Cl_2.CH_2Cl_2$	K_3PO_4	Dioxane/H_2O (4:1)	85 ^oC, 22 h	17% crude [(*R*)-**62**]
3	$Pd(OAc)_2$ + PPh_3	K_3PO_4	Dioxane/H_2O (4:1)	85 ^oC, 48 h	37% [(*R*)-**62**]
4	$Pd(PPh_3)_4$	K_3PO_4	DMF	85 ^oC, 24 h	82% [(*R*)-**62**]
5	$Pd(PPh_3)_4$	K_3PO_4	DMF	85 ^oC, 24 h	74% [(*S*)-**62**]

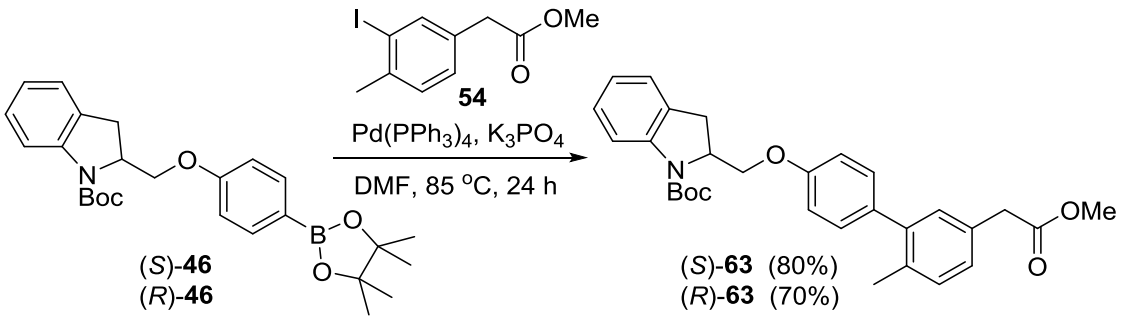
Table 2.4. Suzuki coupling reaction conditions. [a]Isolated yield after purification by column chromatography.

To optimise the yield of the biaryl product, the use of other palladium catalysts and bases was investigated. A wide range of Pd(0) catalysts can be used for cross couplings, or the catalyst is often added as a Pd(II) complex which forms the active catalytic Pd(0) species *in situ*. $Pd(PPh_3)_4$ is a commonly used catalyst, but others such as $PdCl_2(PPh_3)_2$, $Pd(dppf)Cl_2$ and $Pd(OAc)_2$ plus phosphine ligands are also efficient as they are air stable.[74] Changing the catalyst to the less air sensitive $Pd(dppf)Cl_2$ complex and repeating the reaction using potassium phosphate as the base in a mixture of

dioxane/H_2O did not improve the yield of the product (*R*)-**62**, which was obtained in 17% crude yield (Table 2.4, entry 2). Repeating the reaction with $Pd(OAc)_2$ and PPh_3 (Table 2.4, entry 3) did result in a slightly improved yield of the cross-coupled product (*R*)-**62** to 37% but this was only achieved after the reaction was carried out over 2 days.

In the literature, poor product yields have previously been reported for Suzuki couplings using aqueous basic conditions and if the aryl boronic ester is sterically hindered or contains electron withdrawing substituents.[75] This has been attributed to steric hinderance or hydrolytic deboronation and has been overcome by using an anhydrous base such as K_3PO_4 in DMF.[75] Repeating the coupling reaction of boronic ester (*R*)-**41** with aryl iodide **54** using these reaction conditions, with $Pd(PPh_3)_4$ as the catalyst and K_3PO_4 in DMF at 85 ^oC for 24 hours, greatly improved the yield of the biaryl product (*R*)-**62** to 82% (Table 2.4, entry 4). The same conditions were applied to synthesise the opposite biaryl enantiomer (*S*)-**62** in 74% yield (Table 2.4, entry 5).

The optimised Suzuki conditions with K_3PO_4 and $Pd(PPh_3)_4$ in DMF were also applied to the synthesis of the biaryl *N*-Boc indoline analogues (*S*)-**63** and (*R*)-**63** which were isolated in 80% and 70% yield respectively (Scheme 2.17).

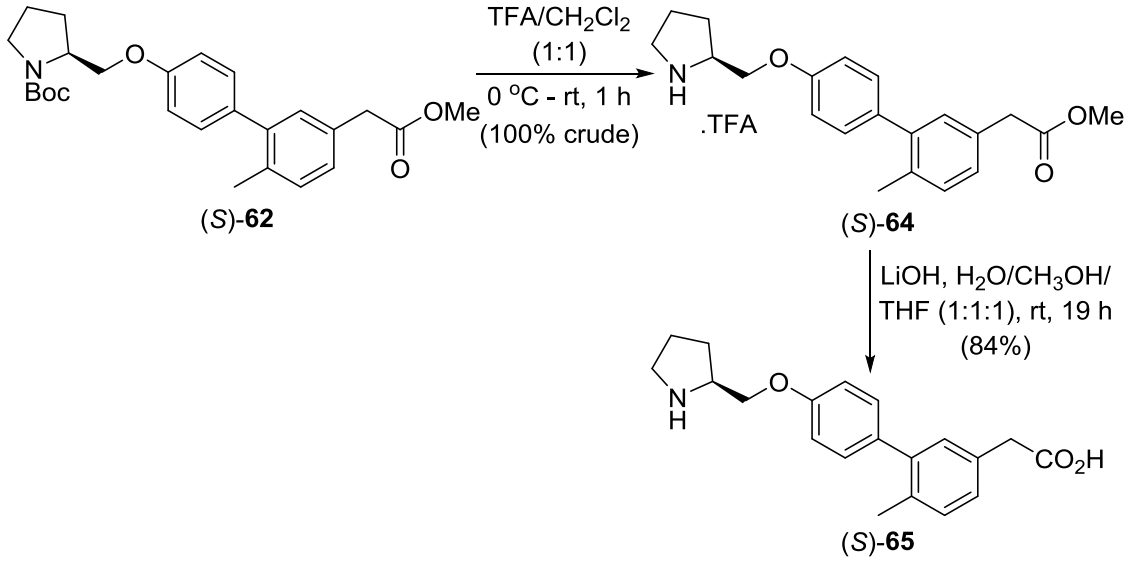


Scheme 2.17. Suzuki coupling reaction of *N*-Boc indoline boronic esters and aryl iodide **54**.

2.4 Final deprotection and acetylation steps

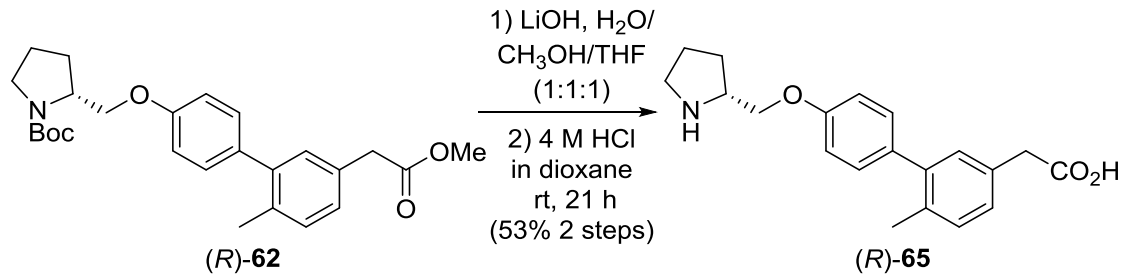
The final target compounds of type **20** and **21** with the nitrogen substituent R_1 = H (Figure 1.11) were prepared using a series of deprotection steps of the *N*-Boc pyrrolidine compounds (*S*)-**62** and (*R*)-**62** and the *N*-Boc indoline compounds (*S*)-**63**

and (*R*)-**63**. Removal of the Boc group from pyrrolidine derivative (*S*)-**62** under acidic conditions with TFA in dichloromethane yielded the amine (*S*)-**64** in quantitative yield (Scheme 2.18). Subsequent ester hydrolysis of compound (*S*)-**64** with lithium hydroxide at room temperature afforded the target compound (*S*)-**65** in 84% yield.



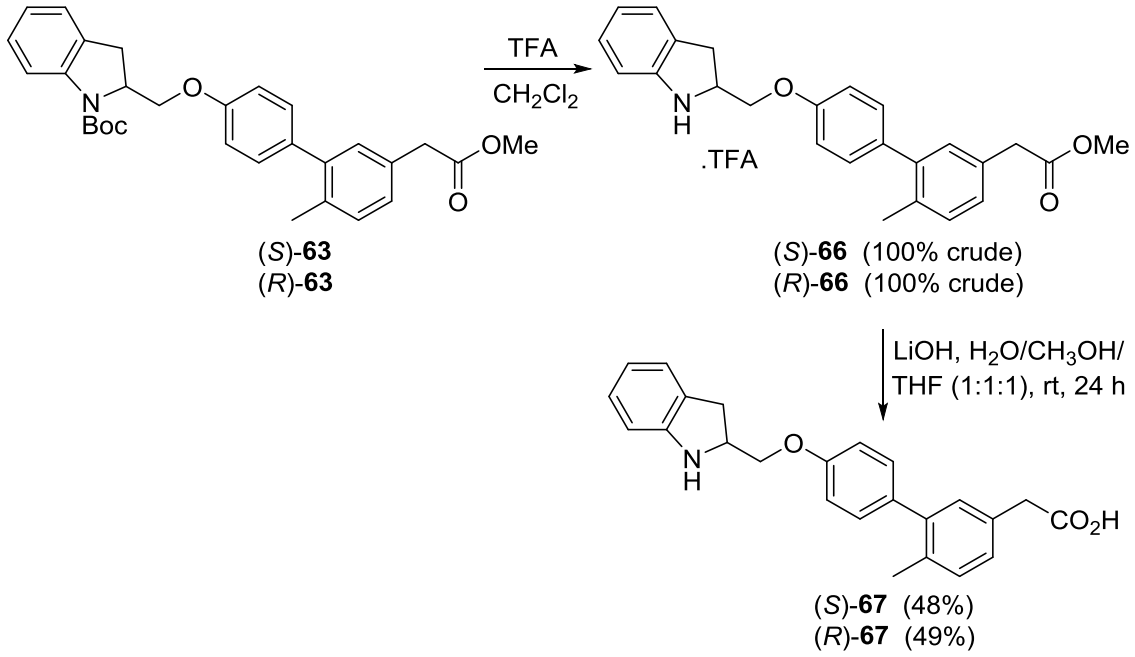
Scheme 2.18. Synthesis of target compound via Boc deprotection then ester hydrolysis.

An alternative method was used for the synthesis of the opposite enantiomer (*R*)-**65** (Scheme 2.19). First, ester hydrolysis of compound (*R*)-**62** was accomplished with lithium hydroxide, which was then followed by Boc deprotection using 4 M HCl in dioxane, to afford the target compound (*R*)-**65** in 53% yield over two steps.



Scheme 2.19. Synthesis of target compound via ester hydrolysis then Boc deprotection.

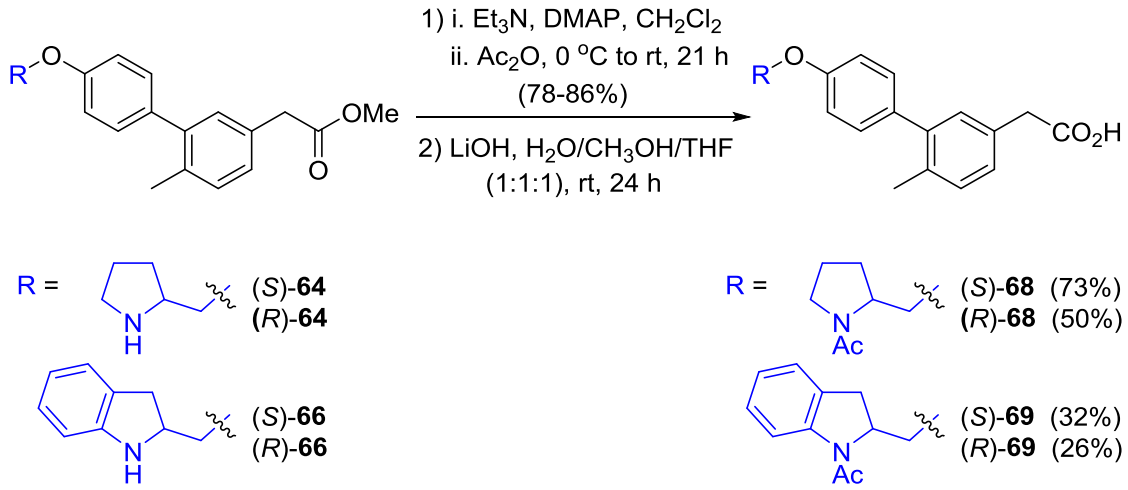
It was decided to apply the route which involved Boc deprotection first, followed by the ester hydrolysis, to the synthesis of the target indoline analogues. This was because the intermediate Boc deprotected compounds would be useful in the preparation of other target compounds featuring an *N*-acetyl group. Deprotection of the Boc group from the indoline derivatives (*S*)-**63** and (*R*)-**63** with TFA yielded amines (*S*)-**66** and (*R*)-**66** in quantitative yields (Scheme 2.20). This was followed by ester hydrolysis with lithium hydroxide to afford the target compounds (*S*)-**67** in 48% yield, and (*R*)-**67** in 49% yield after purification by column chromatography.



Scheme 2.20. Synthesis of indoline target compound via Boc deprotection and ester hydrolysis.

The final target compounds of type **20** and **21** with the nitrogen substituent $R_1 = COCH_3$ (Figure 1.11) were prepared by acetylation and ester hydrolysis of the pyrrolidine compounds (*S*)-**64** and (*R*)-**64** and indoline compounds (*S*)-**66** and (*R*)-**66** (Scheme 2.21). Acetylation was achieved using acetic anhydride, triethylamine and DMAP, which resulted in the isolation of the *N*-acetyl intermediates in yields between 78-86%. Subsequent hydrolysis of the methyl ester resulted in the formation of the *N*-acetyl pyrrolidine target compounds (*S*)-**68** and (*R*)-**68** in 73% and 50% yields respectively, and the *N*-acetyl indoline target compounds (*S*)-**69** and (*R*)-**69** in 32% and 26% yields

after purification by column chromatography. The low isolated yields of the indoline derivatives could have been improved by optimisation of the purification procedure. During the purification by column chromatography of indoline compounds (*S*)-**69** and (*R*)-**69**, a fraction was collected that contained a mixture of the desired product and an impurity, but this fraction was not re-purified because enough of the clean desired product had already been isolated for analysis in the biological assays.



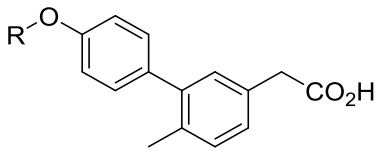
Scheme 2.21. Synthesis of *N*-acetyl pyrrolidine and indoline target compounds.

2.5 Leukotriene B_4 synthesis inhibition

2.5.1 LTB_4 synthesis inhibition assay

Compounds **65**, **67**, **68** and **69** were tested by GVK Bio in a cell based assay using human neutrophils to measure the inhibition of LTB_4 synthesis, in order to determine the potency of the compounds in inhibiting leukotriene synthesis inhibition.[i] All of the compounds tested had IC_{50} values in the micromolar range and were inhibitors of LTB_4 biosynthesis (Table 2.5).

[i] The LTB_4 synthesis inhibition assay was carried out by GVK Bio (GVK Biosciences, Private Limited, Plot No. 28A, IDA Nacharam, Hyderabad – 500076, India). The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.



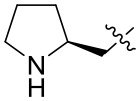
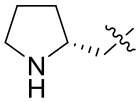
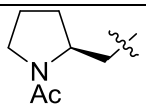
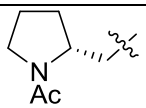
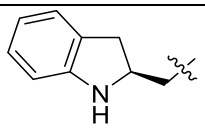
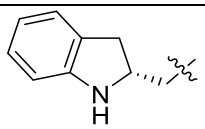
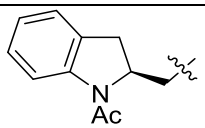
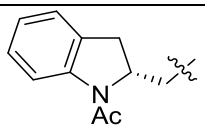
No.	R	Mwt	calc LogP (ACD)	IC_{50} (μM)[a]	pIC_{50}[b]	BEI[c]
(*S*)-**65**		325.4	3.4	1.29	5.9	18.1
(*R*)-**65**		325.4	3.4	0.58	6.2	19.1
(*S*)-**68**		367.4	3.2	6.64	5.2	14.2
(*R*)-**68**		367.4	3.2	3.49	5.5	15.0
(*S*)-**67**		373.4	4.6	3.48	5.5	14.7
(*R*)-**67**		373.4	4.6	1.84	5.7	15.3
(*S*)-**69**		415.5	4.9	0.46	6.3	15.2
(*R*)-**69**		415.5	4.9	0.50	6.3	15.2

Table 2.5. LTB_4 synthesis inhibitory activity. [a]Inhibition of LTB_4 synthesis in human neutrophils by Enzyme Linked Immunosorbent Assay (ELISA) carried out by GVK Bio. The FLAP inhibitor MK886 was used as a reference compound and had IC_{50} = 0.88-0.99 nM, pIC_{50} = 9.1. [b]pIC_{50} = -log(IC_{50}(M)). [c]BEI = Binding Efficiency Index = (pIC_{50}/Mwt)*1000

Of the compounds tested, those with the (*R*)-stereochemistry were slightly more active than those with the corresponding (*S*)-stereochemistry, with the exception of the *N*-acetyl indoline compounds (*S*)-**69** and (*R*)-**69**, which had the same pIC_{50} value of 6.3. Analysis of the free amine compounds (**65** and **67**) showed that the pyrrolidine moiety gave rise to a better potency than the corresponding indoline analogues, for example pyrrolidine (*R*)-**65** (pIC_{50} = 6.2) was 3 times more potent than the indoline analogue (*R*)-

67 (pIC_{50} = 5.7). The opposite trend was observed for the *N*-acetyl compound set (**68** and **69**), where the indoline analogues were more potent LTB_4 synthesis inhibitors than the pyrrolidines, for example indoline (*R*)-**69** (pIC_{50} = 6.3) had a 7 fold better potency than its pyrrolidine analogue (*R*)-**68** (pIC_{50} = 5.5).

The X-ray crystal structure of FLAP bound to inhibitor MK591 **3** (Figure 1.3) indicated that the binding pocket for the quinoline moiety was restricted and positioned deep within the FLAP trimer.[28] Hence it was possible some of the differences in potency observed between the various pyrrolidine and indoline groups were caused by limited space in the binding pocket. The most potent LTB_4 synthesis inhibitors were the *N*-acetyl indolines (*S*)-**69** and (*R*)-**69** (pIC_{50} = 6.3). These results corresponded well to the SAR reported for the FLAP inhibitor AM679 **6** (Figure 1.4), where the *N*-acetyl indoline moiety enhanced the potency of LTB_4 synthesis inhibition.[32]

The ligand efficiency related to the efficiency per mass unit (BEI = binding efficiency index)[76] was calculated for each compound. One of the most potent compounds, pyrrolidine (*R*)-**65**, was a reasonably efficient inhibitor of LTB_4 synthesis, with BEI = 19.1. This compared favourably to the efficiency of literature FLAP inhibitors in their capacity to inhibit LTB_4 synthesis, such as MK886 **2** (Figure 1.3) with BEI = 18.1, and AM679 **6** (Figure 1.4) with BEI = 13.3.[27,32]

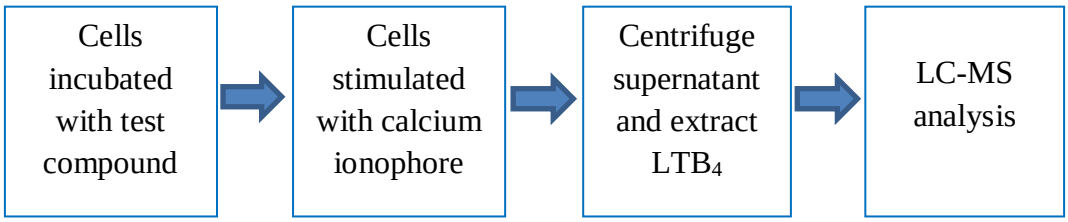
Based on structural similarities with literature FLAP inhibitors, it was reasonable to hypothesise that the compounds found to be LTB_4 synthesis inhibitors were targeting the protein FLAP. However, in order to confirm the target, additional assays would need to be carried out such as a FLAP binding assay.

2.5.2 Development of an in-house assay

An investigation into the development of an in-house LTB_4 inhibition assay using mass spectrometry for the quantification of LTB_4 was undertaken.[ii] This would have allowed for screening a larger number of compounds at a lower cost compared to those required for outsourcing. Development of the assay was carried out in collaboration with Dr

[ii] The LTB_4 inhibition assay was carried out in collaboration with Dr Dean Willis at the UCL pharmacy department. The development of the MS method and analysis was carried out in collaboration with Dr Lisa D. Haigh in the UCL chemistry mass spectrometry facility.

Willis at UCL pharmacy department. The LTB_4 inhibition assay was carried out following published procedures.[77] The assay involved the addition of the test compound to the cells (mouse leukocytes or human neutrophils were used) (Scheme 2.22). Then the cells were stimulated with a calcium ionophore A23187 which liberates arachidonic acid and activates the biosynthesis of leukotrienes. To terminate the reaction, the cell supernatant was centrifuged and the supernatant extracted with a mixture of dichloromethane and methanol containing 1% formic acid. After evaporation of the organic solvents, the resultant residue was reconstituted in methanol for LC-MS analysis.



Scheme 2.22. Summary of the LTB_4 inhibition assay.

Key to the success of the method was development of a mass spectrometry method for quantitative LTB_4 analysis. This proved to be very problematic. A calibration curve using LTB_4 purchased from Sigma-Aldrich was established, which was based on LC-MS methods reported in the literature (Figure 2.2).[78,79] From the calibration curve, the LTB_4 quantification range was 0.08-10 μg/mL, however this was not sufficiently sensitive. Literature methods have analysed LTB_4 in the ng/mL and pg/mL concentration ranges.[79]

The mass spectrometer was operated in the ESI negative mode so that LTB_4 was detected as the deprotonated species $[M - H]^-$. Selected ion monitoring (SIM) was used over the mass range *m*/*z* 334-337, so only compounds with a mass corresponding to the LTB_4 $[M - H]^-$ ion at *m*/*z* 335 would be detected. LC-MS ion chromatograms of the standard LTB_4 solutions containing a known concentration of LTB_4 in methanol, showed a single peak at r_t = 10 min (Figure 2.3).

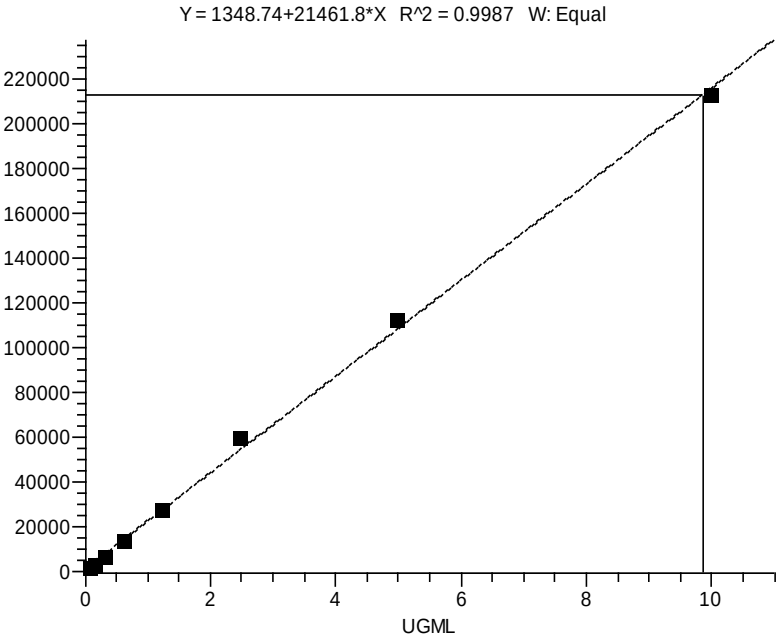


Figure 2.2. Calibration curve (range 0.08-10 μg/mL) of LTB_4.

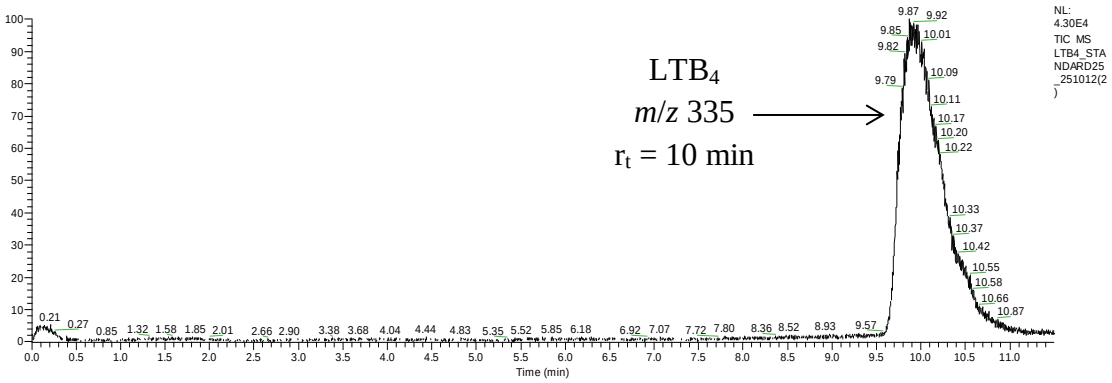


Figure 2.3. LC-MS ion chromatogram of a standard LTB_4 solution.

To test the LTB_4 inhibition assay and confirm that the cells were producing LTB_4 once stimulated with the calcium ionophore, the assay was carried out with no test compound added to the cells. It was expected that the LC-MS ion chromatogram would indicate the presence of LTB_4 with a peak at retention time = 10 min, however this was not observed and peaks with retention times at 2 and 7 minutes were noted (Figure 2.4).

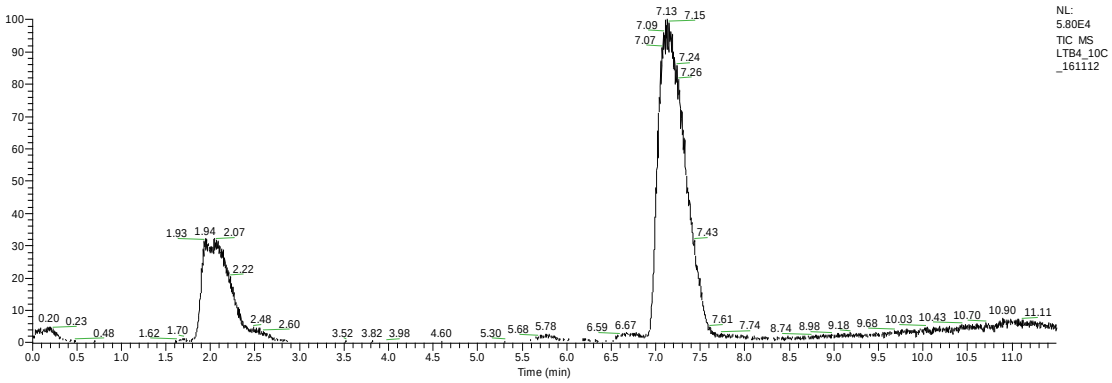
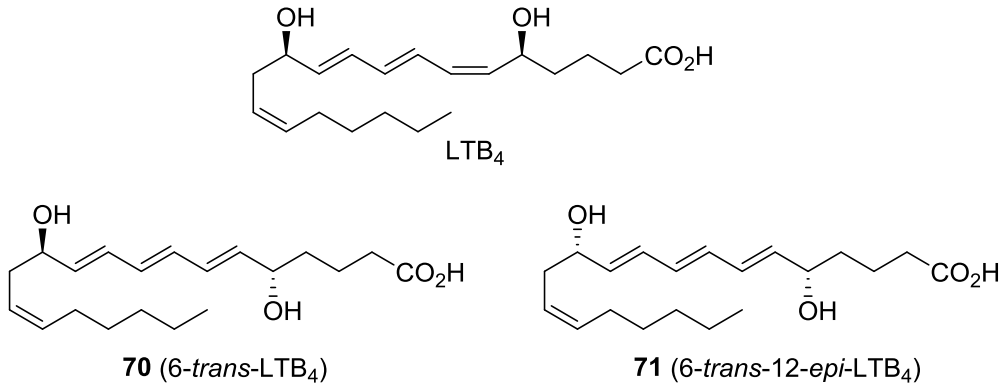


Figure 2.4. LC-MS ion chromatogram of extracts from calcium ionophore stimulated cells.

The stability of LTB_4 in the samples could have been an issue. Two isomers of LTB_4 include 6-*trans*-LTB_4 **70** and 6-*trans*-12-*epi*-LTB_4 **71** (Figure 2.5), which are non-enzymatic hydrolysis products of LTA_4, but can also form from isomerisation of LTB_4 at low pH and high temperatures.[80] LTB_4 and its isomers have the same $[M - H]^-$ ion at *m*/*z* 335 and so the specificity of the LC-MS detection method was likely to have been a problem. The LTB_4 inhibition assay was repeated with the addition of zileuton (a known LTB_4 synthesis inhibitor, Figure 1.2) to the cells. If the extra peaks seen in the LC-MS ion chromatogram disappeared then it would have been likely these peaks corresponded to isomers of LTB_4. However the chromatograms obtained still showed multiple peaks. The data from these experiments proved to be very unreliable and further development of the assay was not continued.

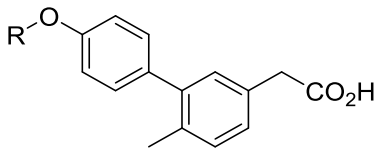


70 (6-*trans*-LTB_4) **71** (6-*trans*-12-*epi*-LTB_4)

Figure 2.5. Structures of LTB_4 and two isomers 6-*trans*-LTB_4 and 6-*trans*-12-*epi*-LTB_4.

2.6 CRTh2 antagonist activity assay

Compounds **65**, **67**, **68** and **69** were tested in a CRTh2 functional assay by Euroscreen FAST, for antagonist activity on recombinant human CRTh2 (Table 2.6).[iii] Each compound was tested at a single concentration of 10 μM. The percentage inhibition refers to the inhibition of CRTh2 receptor activation measured by radioligand GTPγ^{35}S binding.



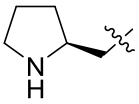
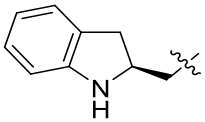
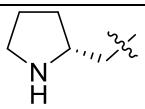
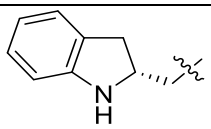
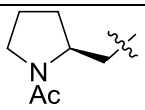
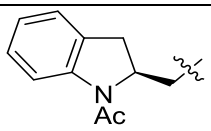
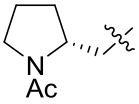
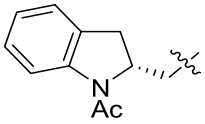
No.	R	Percentage inhibition[a]	No.	R	Percentage inhibition[a]
(*S*)-**65**		14%	(*S*)-**67**		64%
(*R*)-**65**		-3%	(*R*)-**67**		22%
(*S*)-**68**		92%	(*S*)-**69**		132%
(*R*)-**68**		27%	(*R*)-**69**		72%

Table 2.6. CRTh2 antagonist activity assay results. [a]Average percentage inhibition of CRTh2 activation (antagonist activity) on human recombinant CRTh2 receptor, the compounds were tested at a single concentration of 10 μM in duplicate (n = 2).

The carboxylic acid functionality was already known to be important for CRTh2 binding because the majority of literature CRTh2 antagonists contain this moiety. Therefore, having retained the phenylacetic acid group, the SAR for this set of compounds focused on the modifications of the indole or pyrrolidine group. The assay results revealed three clear trends. The first was the (*S*)-stereochemistry gave rise to

[iii] The CRTh2 assay was conducted by Euroscreen (Euroscreen FAST Business Unit, Euroscreen SA, 47 Rue Adrienne Bolland, 6041 Gosselies, Belgium). The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

more potent antagonism than the corresponding (*R*)-stereochemistry analogues. The second trend highlighted the indoline ring was a more potent pharmacophore than the pyrrolidine ring analogues, for example, replacement of the pyrrolidine ring in (*R*)-**68** with the indoline ring (*R*)-**69**, improved the percentage inhibition by 45%. The final trend was that an *N*-acetyl group significantly improved CRTh2 antagonism compared to the free amines, for example the *N*-acetyl pyrrolidine compound (*S*)-**68** had a 78% higher percentage inhibition compared to the *N*-H pyrrolidine compound (*S*)-**65**.

With the current data it was only possible to conclude that the compounds showing antagonistic activity at CRTh2 at 10 μM were (*S*)-**67**, (*S*)-**68**, (*S*)-**69** and (*R*)-**69**. The most potent CRTh2 antagonist was the *N*-acetyl indoline (*S*)-**69**. Testing these four active compounds in a dose response screen would provide more information about the effects of the compounds on the CRTh2 receptor at different concentrations, which would then allow comparisons to other CRTh2 antagonists reported in the literature.

2.7 Conclusions

A novel series of pyrrolidine and indoline biphenylacetic acids were synthesised as dual inhibitors of the leukotriene and prostaglandin inflammatory pathways, by targeting FLAP and the CRTh2 receptor. The compounds were designed based on a combination of known pharmacophores for potent FLAP inhibitors and CRTh2 antagonists. The compounds evaluated in the biological assays inhibited the synthesis of LTB_4, with some also active CRTh2 antagonists. The compound that exhibited the most promising dual pharmacology was indoline (*S*)-**69**, which had a pIC_{50} value of 6.3 for LTB_4 synthesis inhibition, and was an antagonist of CRTh2 at 10 μM. Compound (*S*)-**69** could provide a good starting point for lead optimisation of dual inhibitors of leukotriene synthesis and the CRTh2 receptor, as potential anti-inflammatory drugs for diseases such as asthma.

Future biological testing should include a FLAP binding assay to determine if this is the target of the compounds which leads to their LTB_4 synthesis inhibitory activity. In addition, dose response curves should be generated for the compounds that were active against the CRTh2 receptor.

Future medicinal chemistry studies could focus on further SAR based on the most promising dual inhibitor (*S*)-**69**. Modifying aryl ring functionalities could be explored, as well as the positioning of the acetic acid group. Modification of the indoline ring to either the indole or reverse indole (which are common pharmacophores for CRTh2 receptor antagonists) could also be explored.

Unfortunately the project was not able to be continued further because the required assays to determine the bioactivities of the compounds was very expensive to outsource. It would not have been possible to obtain the biological data for anymore compounds that were synthesised.

Part B: Synthesis of tetrahydroisoquinolines, tetrahydrobenzazepines and profens and their antimycobacterial properties

3. Introduction

3.1 Antimycobacterial drug discovery

3.1.1 Tuberculosis: drugs and resistance

Tuberculosis (TB) is an infectious disease caused by the pathogen *Mycobacterium tuberculosis* that most commonly affects the lungs. It is a serious global health problem that is particularly widespread in developing countries, and is the second leading cause of death from a single infectious agent. In 2013 it was estimated that 9 million people developed TB and 1.5 million died from the disease.[81] About one third of the world's population is estimated to be infected with asymptomatic latent *M. tuberculosis* where the mycobacteria are viable but apparently non-replicating, and the risk of reactivation and developing infectious TB is approximately 5%.[82] This is even higher in patients with compromised immune systems such as those with HIV infection, diabetes, malnutrition and cancer.[83]

The current treatment for drug-susceptible TB (TB that is not drug resistant) relies on drugs developed over 50 years ago, and is at least a 6 month regimen of a combination of the four first-line anti-TB drugs: isoniazid **72**, rifampicin **73**, pyrazinamide **74** and ethambutol **75** (Figure 3.1). A combination of drugs is used because they have different targets and mechanisms of action (Table 3.1), which allows for the shortest treatment time and reduces the risk of resistance emerging. Successful cure rates of over 90% for drug-susceptible TB are achieved following the first-line drugs treatment.[81] Despite this, patient adherence to the long treatment time and multiple drug combinations can be a problem and can result in relapse of the *M. tuberculosis* infection, the continuous spread of the disease and the emergence of drug resistance.

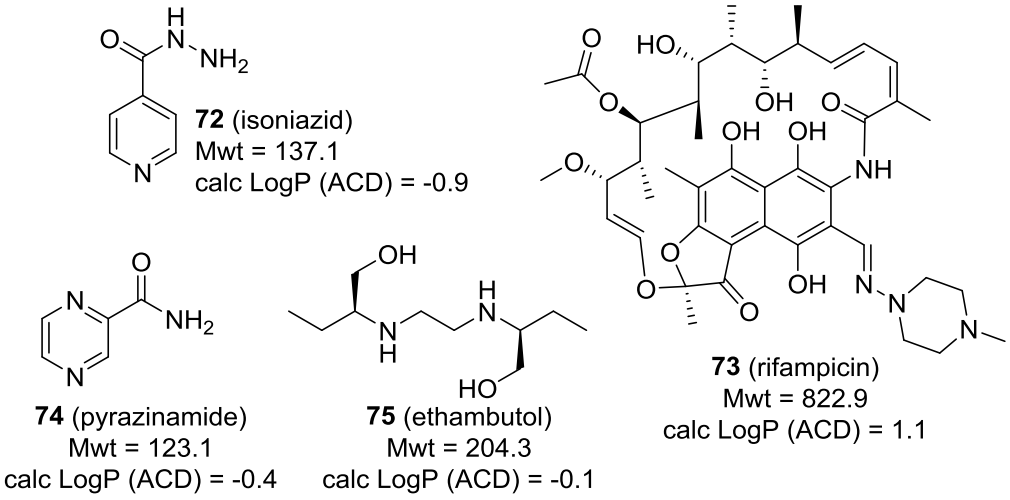


Figure 3.1 First-line anti-TB drugs.

First-line drug (year discovered)	**Primary target**	**Mechanism of action**
Isoniazid (1952)	Enoyl-acyl-carrier-protein reductase	Inhibits mycolic acid synthesis
Rifampicin (1963)	RNA polymerase	Inhibits transcription
Pyrazinamide (1954)	30S ribosomal subunit	Inhibits translation, acidifies cytoplasm
Ethambutol (1961)	Arabinosyl transferases	Inhibits arabinogalactan synthesis

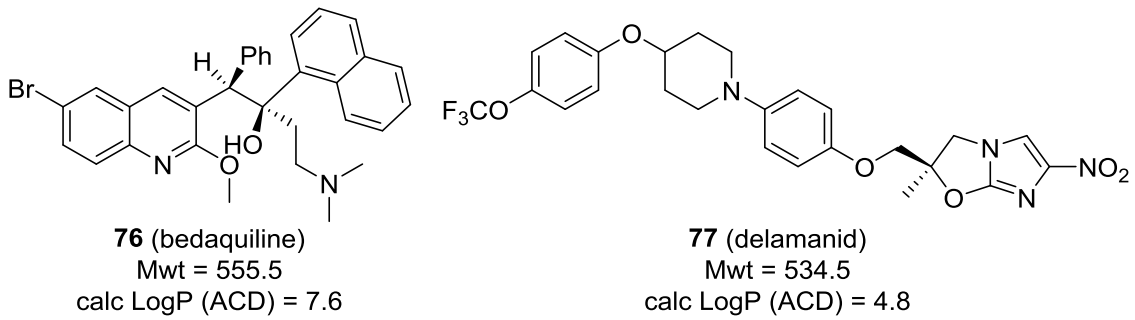
Table 3.1. First-line anti-TB drugs and their targets and mechanism of action.

Multi-drug resistant TB (MDR-TB) is caused by mycobacteria that are resistant to the two most potent first-line drugs isoniazid **72** and rifampicin **73**. In 2013 an estimated 3.5% of new and 20.5% of previously treated TB cases were MDR-TB.[81] The current treatment for MDR-TB requires a minimum of 20 months using a combination of second-line anti-TB drugs (Table 3.2). These are either less effective than first-line drugs or have serious side effects, and include aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine and *p*-aminosalicylic acid.[84] Recently two new drugs were approved for use against MDR-TB. In 2012 a diarylquinoline compound bedaquiline **76** (Figure 3.2) was approved by the FDA, but for use only when no other drugs are available due to safety concerns.[85] In 2014 a nitroimidazole delamanid **77** (Figure 3.2) was approved by the European Medicines Agency and is

undergoing clinical studies.[86] Chemical scaffolds such as benzothiazinones and oxazolidinones have also been shown to have promising potency against mycobacteria and are in preclinical or clinical development as potential anti-TB drugs.

Second-line drug (year discovered)	**Primary target**	**Mechanism of action**
Aminoglycosides: • Streptomycin (1944) • Kanamycin (1957)	30S ribosomal subunit	Inhibits protein synthesis
Polypeptides: • Capreomycin (1960s)	Ribosomal subunit interface	Inhibits protein synthesis
Fluoroquinolones: • Levofloxacin • Moxifloxacin	DNA gyrase and DNA topoisomerase	Inhibits DNA supercoiling
Thioamides: • Ethionamide (1961)	Enoyl-acyl-carrier-protein reductase	Inhibits mycolic acid synthesis
Cycloserine (1955)	D-alanine racemase and ligase	Inhibits peptidoglycan synthesis
p-Aminosalicylic acid (1948)	Dihydropteroate synthase	Inhibits folate biosynthesis
Bedaquiline (2005)	ATP synthase	Inhibits energy metabolism
Delamanid (2006)	Unknown	Inhibits mycolic acid synthesis

Table 3.2. Second-line drugs used for the treatment of tuberculosis.



76 (bedaquiline)
Mwt = 555.5
calc LogP (ACD) = 7.6

77 (delamanid)
Mwt = 534.5
calc LogP (ACD) = 4.8

Figure 3.2. Recently approved compounds for the treatment of MDR-TB.

The emergence of extremely drug resistant TB (XDR-TB) has made the disease even more difficult to treat. XDR-TB is caused by mycobacteria resistant to the first-line

drugs isoniazid **72** and rifampicin **73**, and the second-line drugs (a fluoroquinolone and at least one of the injectable aminoglycosides or polypeptides). The emergence of MDR- and XDR-TB highlights the urgent need for new anti-TB drugs that shorten the duration of treatment and have good efficacy and safety profiles.

TB drugs occupy a diverse chemical space and often have physiochemical properties that are not typical of drug-like compounds or do not conform to Lipinski's rules.[87] For example, the first-line drugs include small polar molecules such as isoniazid **72** and ethambutol **75**, but also large and complex molecules such as rifampicin **73** (Figure 3.1). The second-line drug bedaquiline **76** (Figure 3.2) is highly lipophilic (calc logP (ACD) = 7.6) with a fairly high molecular weight of 555.5. The nitroimidazole group present in delamanid **77** (Figure 3.2) is essential for its antimycobacterial activity but not typically associated with drug-like molecules. These examples highlight the diversity in physiochemical properties of some of the most potent antimycobacterial compounds, and it has been proposed that new lead compounds should be sought from a more chemically diverse space such as natural products and their derivatives.[88]

3.1.2 Determining antimycobacterial potency

Whole-cell (or phenotypic) screening involves testing series of compounds for their ability to inhibit the growth of mycobacteria and gives a measure of a compounds antimycobacterial potency. It has proven to be a successful strategy for discovering new antimycobacterial compounds, such as in the discovery of bedaquiline **76** as well as other compounds currently in clinical trials for the treatment of tuberculosis.[89] The major advantage of whole-cell screening is the ability to directly determine if a compound inhibits the growth of mycobacteria at a specific concentration. In comparison, the alternative method of target based screening (which involves testing compounds for their ability to inhibit a specific target) can be limited by the potency of a compound for a specific target not translating into whole cell antimycobacterial activity. Whole-cell screening also offers the advantage of being able to discover novel antimycobacterial structures with new mechanisms of action.

Tuberculosis drug discovery is challenging because of the slow growth rate and highly infectious nature of *M. tuberculosis* which can only be handled in specialist

laboratories. A way to avoid these limitations is to use different mycobacterial species as surrogates, such as *M. aurum*, *M. smegmatis* or *M bovis* BCG. *M. aurum* is relatively fast growing and non-pathogenic and has been shown to have an antibiotic susceptibility profile similar to that of *M. tuberculosis*.[90] This makes it an excellent surrogate model for the early stage screening of compounds antimycobacterial activity.

A whole-cell screening method developed by the Bhakta group known as SPOTi (spot culture growth inhibition assay), is a rapid and convenient high throughput assay for determining antimycobacterial activity of large libraries of compounds, and has been used in combination with *M. tuberculosis* surrogates such as *M. aurum*.[90] The SPOTi method allows for the determination of the MIC (minimum inhibitory concentration) of a compound. This is defined as the minimum concentration of a compound required to completely inhibit the growth of the mycobacteria. The MIC is important for assessing antimycobacterial potency of compounds in early stage drug discovery.

3.1.3 Repurposing drugs

The repurposing of drugs describes the discovery of new pharmacological activities for existing or old drugs. Existing drugs already have known pharmacological, toxicity and safety profiles, so if they are repurposed for a different disease the time and expense of clinical development is reduced.[91] This would be a major advantage for the development of new drugs for the treatment of infectious diseases such as TB.[92]

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain and inflammation but have recently been shown to also possess interesting antimycobacterial properties. In a study by Gold *et al*., the NSAID oxyphenbutazone was shown to be selectively mycobactericidal to non-replicating TB.[93] In another study by the Bhakta group, NSAIDs with a 2-arylpropanoic acid structure (profens) were evaluated as potential anti-TB drugs.[94] Ibuprofen **78**, loxoprofen **79** and carprofen **80** displayed growth inhibitory properties against *M. tuberculosis* and had MICs between 40-75 μg/mL (Figure 3.3). Synthetic derivatives of ibuprofen **78** with substitution of the carboxylic acid moiety for either the methyl ester or an amide derivative resulted in loss of activity or increased cytotoxicity (Figure 3.3). This suggested a free carboxylic acid was required for antimycobacterial activity. Modifying the aromatic ring of

ibuprofen **78** to the 3,5-dinitro analogue resulted in an improvement of activity with MIC = 30 μg/mL (Figure 3.3).

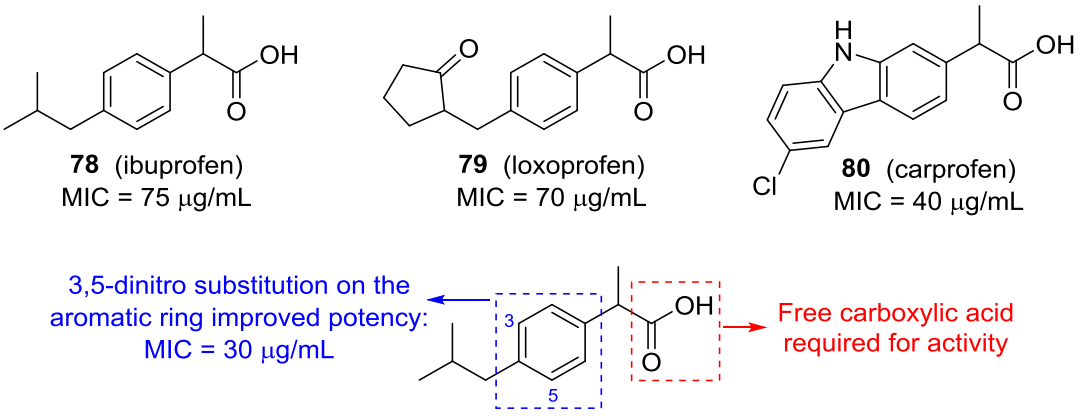


Figure 3.3. Antimycobacterial activity of NSAID 2-arylpropanoic acid racemates (MIC refers to the minimum inhibitory concentration against *M. tuberculosis* H_{37}Rv).

Ibuprofen **78**, carprofen **80** and the dinitro analogue of ibuprofen were also shown to have activity against the growth of MDR-TB strains and could have a potential novel mechanism of action compared to current TB treatments. Further SAR of the 2-arylpropanoic acids would be beneficial in the development of more potent antimycobacterial compounds and could help with target elucidation.

3.1.4 Mycobacteria efflux pumps

Mycobacteria such as *M. tuberculosis* can acquire mutations in target genes which are responsible for resistance to individual drugs.[95] However, mutations are not the only cause of drug resistance in TB. Other key factors play a role, including intrinsic (or natural) resistance from the permeability barrier of the cell wall and the active efflux of drugs out of the cell. Bacterial efflux pumps are membrane proteins that can transport a range of substrates out of the cell, and can contribute to drug resistance by either preventing a drug from reaching the intended target or by decreasing the concentration of drug inside the cell to a sub-inhibitory level.[96] A sub-inhibitory level of an anti-TB drug over the long treatment period represents a particular problem as it increases the chances of genetic mutations occurring.[97]

Using compounds that are efflux pump inhibitors (EPIs) in combination with anti-TB drugs is a possible strategy for preventing the emergence of drug resistant TB, as well as improving efficacy of anti-TB drugs that are subject to efflux.[98] EPIs include verapamil **81**, chlorpromazine **82**, the plant alkaloid reserpine **83** and spectinomycin analogues **84** (Figure 3.4).[99] These compounds are often used experimentally but there are currently no mycobacterial efflux pump inhibitors in clinical use.

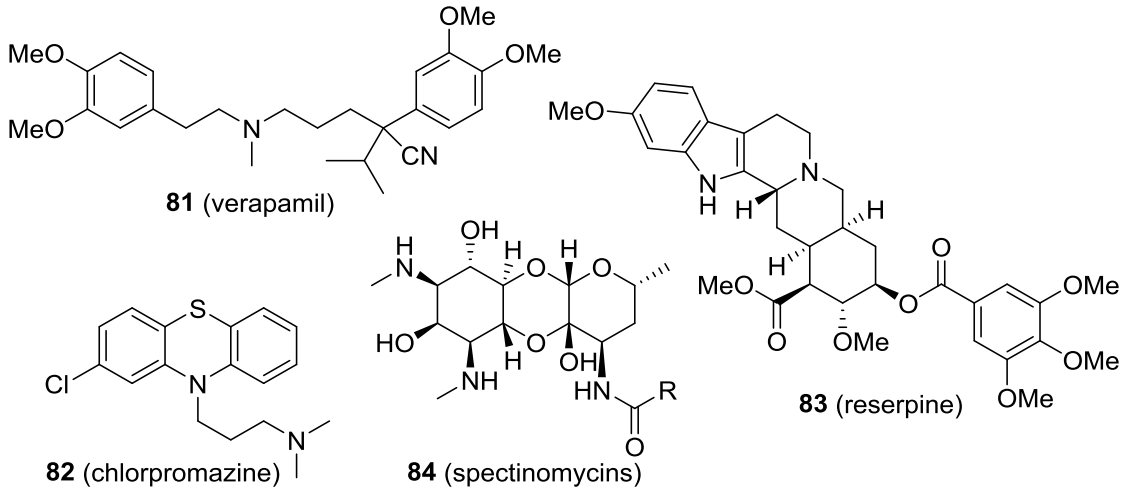


Figure 3.4. Efflux pump inhibitors of mycobacteria.

Verapamil **81**, when used in combination with the first-line anti-TB drug rifampicin **73**, has been shown to reduce the MIC 4-fold against *M. tuberulosis in vitro* compared to using rifampicin **73** alone.[100] When verapamil **81** was used in combination with the second-line drug bedaquiline **76**, an 8-fold reduction in the MIC was reported, with equally promising results observed in *in vivo* studies.[101] These studies highlight the important role EPIs could have in anti-TB drug discovery.

3.2 Tetrahydroisoquinolines

3.2.1 Structure and biological activity

1,2,3,4-Tetrahydroisoquinoline (THIQ) derivatives are important nitrogen containing heterocyclic compounds in medicinal chemistry. These compounds are abundant in

nature, with the THIQ ring system present in many alkaloid natural products which display a wide range of biological and pharmaceutical activities. Alkaloid THIQ derivatives such as morphine, emetine, berberine, noscapine and tubocurarine have been used for various pharmaceutical applications (Figure 3.5). The 1-substituted THIQ scaffold has also been used in synthetic drugs such as solifenacin (Figure 3.5).

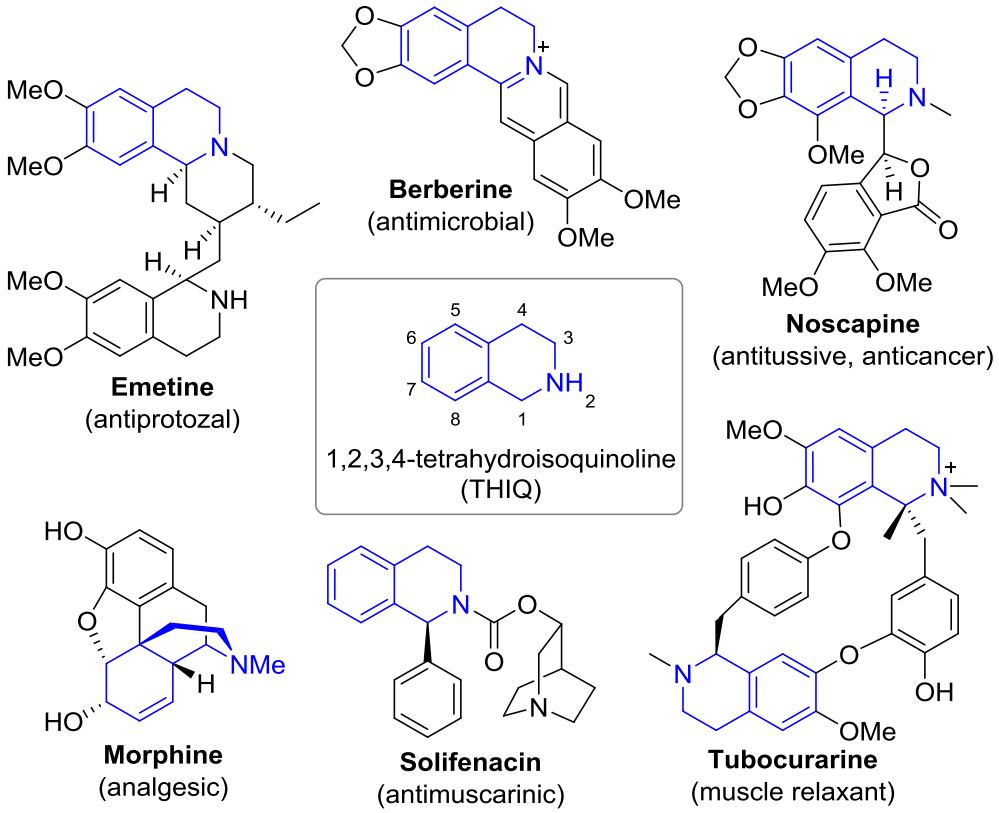
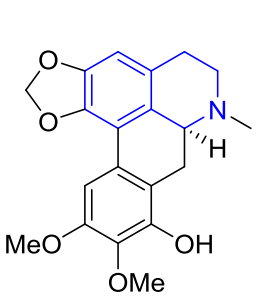


Figure 3.5. Tetrahydroisoquinoline derivatives and their pharmaceutical properties.

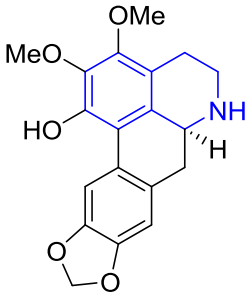
THIQ derivatives are structurally diverse, ranging from the more simple 1-substituted THIQs to more complex structures like morphine. Simple THIQs have a broad range of bioactivities which makes them attractive targets in drug discovery. They can also be useful precursors for the synthesis of more complex structures and therefore the development of efficient strategies for their synthesis is important.

3.2.2 Antimycobacterial properties

Antimycobacterial properties have been reported for both natural THIQ containing alkaloids[102] and synthetic THIQs.[103] The Bhakta group recently identified aporphine alkaloids (*S*)-leucoxine **85** and 3-methoxynordomesticine **86** (that both contain a THIQ moiety) that inhibited the growth of mycobacterial species including *M. bovis* BCG and *M. tuberculosis* (Figure 3.6).[104]



85 ((*S*)-leucoxine)
Isolated from *Ocotea macrophylla*
MIC *M. bovis* BCG = 25 μg/mL



86 (3-methoxynordosmesticine)
Isolated from *Rhodostemonodaphne crenaticupula*
MIC *M. bovis* BCG = 25 μg/mL
MIC *M. tb* $H_{37}Rv$ = 64 μg/mL

Figure 3.6. THIQ containing aporphine alkaloids with antimycobacterial properties.

To further examine the THIQ scaffold, a series of 1-substituted THIQ analogues were synthesised in the Hailes group using either a Bischler-Napieralski reaction (section 3.3.1) or a biomimetic Pictet-Spengler reaction (section 3.3.4) which required a phosphate catalyst and was suitable for a variety of aldehyde and amine substrates.[105] These compounds were evaluated for their antimycobacterial properties by the Bhakta group.[105] THIQs **87-90** with a 6,7-dihydroxy or a 6-hydroxy substitution inhibited the growth of *M. bovis* BCG with MIC = 80-100 μg/mL (Figure 3.7). Compound **87** (MIC = 100 μg/mL) was the most structurally similar to the aporphine **86** which suggested that the methylenedioxybenzyl substituent at the C-1 position was contributing to the antimycobacterial activity. The presence of a lipophilic chain at the C-1 position also conferred antimycobacterial activity in compounds **89** (MIC = 80 μg/mL) and **90** (MIC = 100 μg/mL).

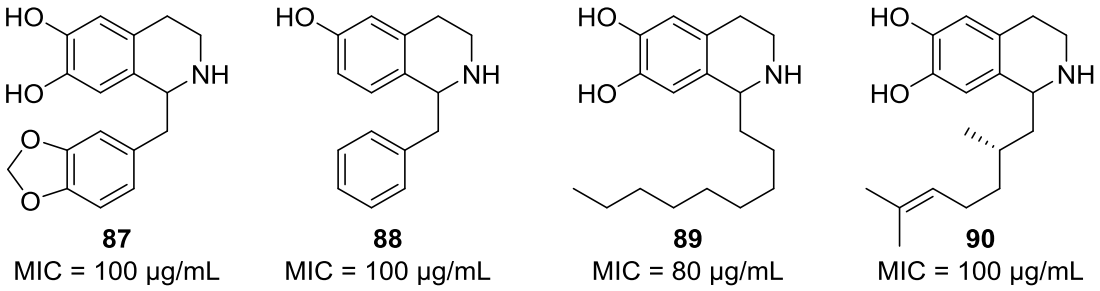


Figure 3.7. 1-Substituted THIQs that inhibit the growth of mycobacterial species. MIC values refer to the concentration of compound required to inhibit the growth of *M. bovis* BCG in a SPOTi assay.

In the same study another series of THIQ compounds with a halogen at the C-5 position, a hydroxy or methoxy group at the C-8 position, and a benzylic or phenyl substituent at the C-1 position were shown to inhibit the growth of *M. bovis* BCG (Figure 3.8).[105] THIQs **91** and **92** with a 5-bromo-8-hydroxy substitution conferred marked antimycobacterial activity, and the benzylic derivative **92** (MIC = 20 µg/mL) was three times more potent than the phenyl derivative **91** (MIC = 60 µg/mL). If the hydroxy group was replaced with a methoxy group, such as in compound **93**, the antimycobacterial activity was retained (MIC = 20 µg/mL) but the cytotoxicity of the compound towards murine macrophage cells (RAW264.7) increased. It was also observed that the 5-bromo analogue **93** conferred more potency than either the 5-chloro analogue **94** (MIC = 40 µg/mL) or the 5-iodo analogue **95** (MIC = 50 µg/mL).

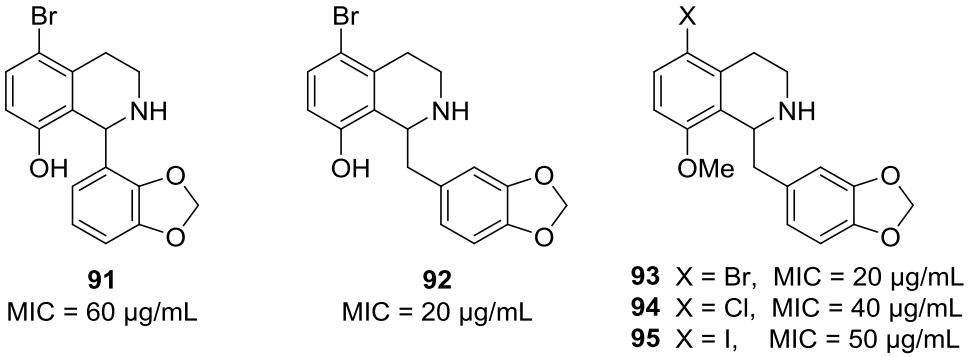


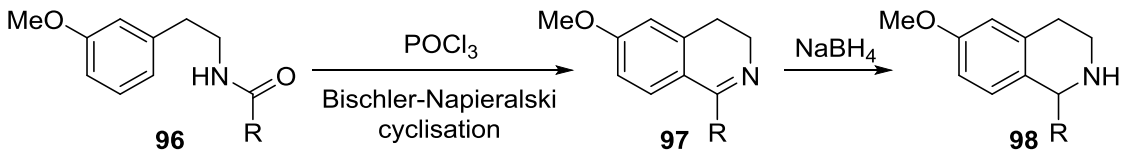
Figure 3.8. Halogenated 1-substituted THIQs that inhibit the growth of mycobacteria. MIC values refer to the concentration of compound required to inhibit the growth of *M. bovis* BCG in a SPOTi assay.

The THIQs **87**, **89** and **94** were found to be specific inhibitors of mycobacteria. They were active against other species of mycobacteria such as *M. smegmatis* and *M. aurum*, but they were not growth inhibitors of Gram-negative bacteria such as *E. coli* and *P. putida*, or acid-fast *Rhodococcus equi* RHA1. This suggests these compounds target a mechanism that is specific to mycobacteria. A number of the THIQ compounds including **91** and **92** were shown to inhibit the MurE ligase of *M. tuberculosis* (IC_{50} <111 μM), a key enzyme involved in the early stage of mycobacterial cell wall peptidoglycan biosynthesis. There was a positive trend observed for the correlation between the growth inhibition of *M. tuberculosis* (MIC) and the inhibition of MurE (IC_{50}) but it was not particularly strong. Therefore it is probable that other targets are also involved. The results from this study highlighted the excellent potential of the THIQ scaffold for the development of future anti-TB drugs.

3.3 Synthesis of tetrahydroisoquinolines

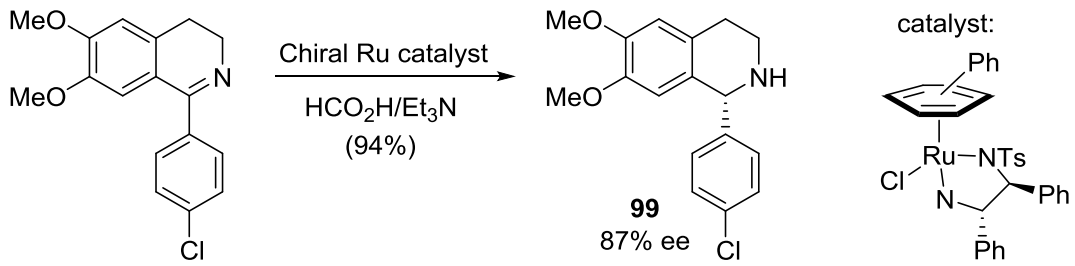
3.3.1 Bischler-Napieralski reaction

The Bischler-Napieralski reaction is a frequently used method for the synthesis of 1-substituted THIQs.[106] It involves the cyclisation of a phenylethylamide such as **96** in the presence of a dehydrating agent to form a 1-substituted 3,4-dihydroisoquinoline **97**, and in a second step this is reduced to the 1-substituted THIQ **98** (Scheme 3.1). Common dehydrating agents include phosphorus oxychloride ($POCl_3$) and phosphorus pentoxide (P_2O_5), and the cyclisation reaction is typically carried out in refluxing toluene or xylene. Many functional groups do not tolerate the harsh conditions needed for the reaction and so protecting groups are often required which can make this synthetic approach step-intensive.



Scheme 3.1. Bischler-Napieralski cyclisation/reduction reaction for 1-substituted THIQ synthesis.

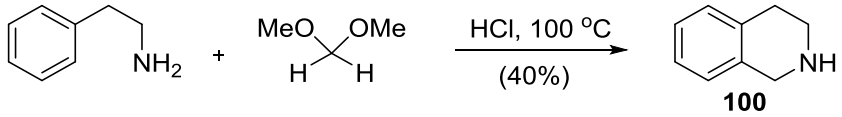
The intermediate 3,4-dihydroisoquinoline contains the C=N bond which has the potential to be reduced asymmetrically, yielding THIQs with a single enantiomeric configuration at the C-1 position. Metal catalysed asymmetric hydrogenation of 3,4-dihydroisoquinoline intermediates has been used for the preparation of chiral THIQs. For example in a recent synthesis towards an antiepileptic compound, a chiral ruthenium catalyst was used to generate the THIQ **99** in 87% ee (Scheme 3.2).[107] Whilst this enantioselectivity was good, it would need to be higher for pharmaceutical compound synthesis. Metal catalysts used in the asymmetric synthesis of 1-substituted THIQs can be expensive, have limited occurrence and are often toxic. Therefore sustainable synthetic approaches are more desirable.



Scheme 3.2. Ruthenium catalysed asymmetric hydrogenation of Bischler-Napieralski intermediates.

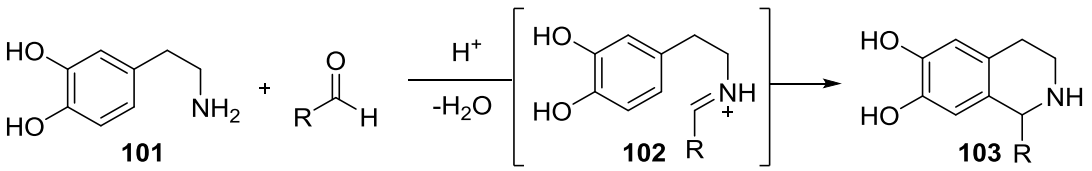
3.3.2 Classical Pictet-Spengler reaction

The Pictet-Spengler reaction is a widely used method for the synthesis of 1-substituted THIQs. It was discovered in 1911 by Amé Pictet and Theodor Spengler who synthesised 1,2,3,4-tetrahydroisoquinoline **100** by heating phenylethylamine and dimethoxymethane (a formaldehyde equivalent) in hydrochloric acid (Scheme 3.3).[108]



Scheme 3.3. The first discovered Pictet-Spengler reaction.

The Pictet-Spengler reaction typically involves the condensation of a phenylethylamine such as dopamine **101** and an aldehyde under acidic conditions to form an intermediate iminium ion **102** (Scheme 3.4). This is followed by cyclisation through an intramolecular electrophilic aromatic substitution to form the THIQ product **103**.

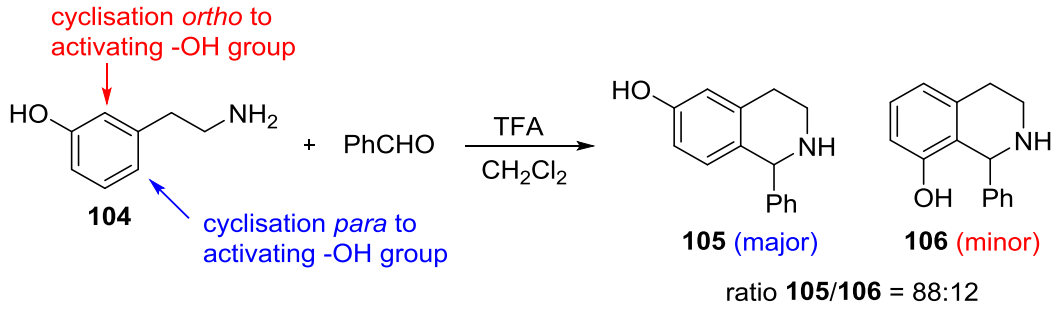


Scheme 3.4. Mechanism of the acid catalysed Pictet-Spengler reaction for the synthesis of THIQs.

One particular challenge in synthesising THIQs using the Pictet-Spengler method can be the low reactivity of the imine intermediate. To overcome this, high temperatures and strong acidic conditions or even superacidic conditions have been used to promote the reaction, but this limits the functional group compatability.[109] Strong Bronsted acid catalysts are commonly used including acetic acid, trifluoroacetic acid and *p*-toluenesulfonic acid. More recently examples of Lewis acid catalysed Pictet-Spengler reactions have been reported including calcium complexes and $Yb(OTf)_3$.[110,111] Another strategy used to enhance the electrophilicity of the intermediate imine has been to generate *N*-acyliminium ions, commonly called acyl Pictet-Spengler reactions.[112]

The Pictet-Spengler reaction is promoted by phenylethylamines with electron rich aromatic rings. Electron donating groups on the aromatic ring (such as hydroxy or methoxy substituents) that are *para* or *ortho* to the cyclisation position facilitate the reaction by increasing the nucleophilicity at the position of cyclisation.[113] The regioselectivity of a Pictet-Spengler reaction also depends on the electron donating substituents on the aromatic ring. For example, for phenylethylamine **104** (Scheme 3.5), cyclisation of the iminium intermediate can occur either at the position *para* to the hydroxy group to yield THIQ **105** or the position *ortho* to the hydroxy group to yield THIQ **106**.[114] Generally the least sterically hindered position is the predominant site for cyclisation (unless this position is blocked by a substituent) resulting in THIQ **105** being the major regioisomer formed. The ratio of regioisomers can vary depending on

reaction conditions and complete regioselectivity for the THIQ product where cyclisation occurs *para* to the electron donating group is often observed.



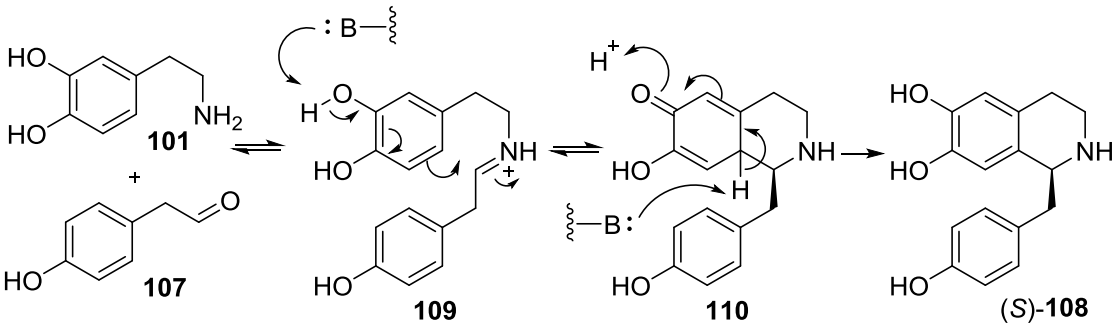
Scheme 3.5. Regioselectivity of the Pictet-Spengler reaction.

Despite some of the requirements for Pictet-Spengler reactions, it has remained a useful method for the synthesis of many THIQ derived natural products and biologically active compounds.

3.3.3 Pictet-Spengler reaction in nature

In nature the Pictet-Spengler reaction is used to generate THIQ alkaloids. The enzymes that catalyse this biosynthetic reaction are called Pictet-Spenglerases. One of these enzymes is norcoclaurine synthase (NCS) which catalyses the stereospecific condensation of dopamine **101** and 4-hydroxyphenylacetaldehyde (4-HPAA) **107** to give the THIQ product (*S*)-norcoclaurine **108** (Scheme 3.6).[115] This is an important reaction because it is the first step in the pathway to all the benzylisoquinoline alkaloids, which consist of more than 2500 known structures and are all synthesised from the central precursor (*S*)-norcoclaurine **108**.[116]

The proposed mechanism of the NCS catalysed Pictet-Spengler reaction (Scheme 3.6) begins with the initial formation of an iminium ion **109**, followed by deprotonation of the 3-hydroxy group which promotes cyclisation onto the iminum moiety.[117] The resulting quinone **110** is deprotonated irreversibly to form (*S*)-norcoclaurine **108**.

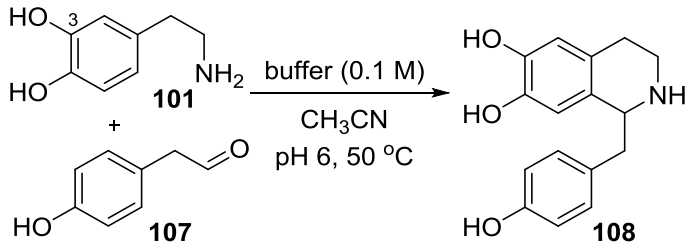


Scheme 3.6. Proposed mechanism of the Pictet-Spengler reaction catalysed by NCS.

In plants NCS catalyses the stereospecific formation of (*S*)-norcoclaurine **108**, with the newly created chiral centre at position C-1 formed exclusively in the (*S*)-configuration. This excellent stereoselectivity has been utilised in NCS enzyme catalysis for the synthesis of structurally diverse (*S*)-THIQs, and this approach also offers a sustainable alternative compared to classical organic synthesis.[118,119,120] A limitation of enzyme catalysis can be substrate specificity. Several aldehydes are accepted as substrates by wild type NCS but dopamine tolerates less functionalisation which can limit its synthetic applications. However, evaluation of the substrate scope of NCS enzymes remains an active area of research, with more detailed studies of the mechanism and kinetics of the Pictet-Spenglerase NCS being reported.[121]

3.3.4 Biomimetic Pictet-Spengler reaction

A biomimetic version of the Pictet-Spengler reaction mediated by phosphate catalysis in water was reported by the Hailes group.[122] The reaction mimics the naturally occurring reaction in plants for the synthesis of norcoclaurine **108**. It was established that dopamine **101** undergoes a Pictet-Spengler condensation with 4-HPAA **107** in the presence of a phosphate buffer under mild reaction conditions (pH 6, 50 ^{o}C) to yield the THIQ norcoclaurine **108** (Scheme 3.7). Phosphates were shown to have an important role in the reaction and act as catalysts, since when the reaction was carried out in water with no phosphate present, less than 1% of norcoclaurine **108** was formed. In addition, carbonate, sulfate, borate and vanadate yielded the product **108** with less than 4% conversion. Phosphates shown to catalyse the reaction included inorganic phosphates as well as uridine 5'-monophosphate (UMP) and glucose-1-phosphate.

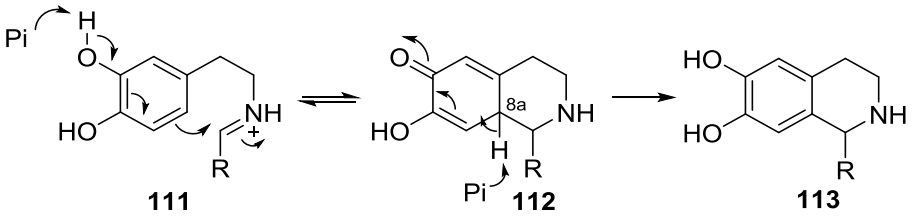


Buffer	Conversion to **108**
KH_2PO_4	77%
NaH_2PO_4	75%
UMP	75%
Glu-1-phosphate	74%
water	<1%

Scheme 3.7. Biomimetic phosphate mediated Pictet-Spengler reaction.

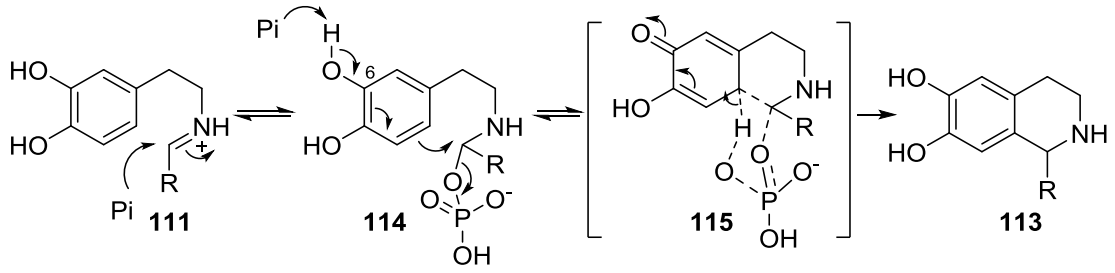
The mild conditions used for the biomimetic reaction makes it suitable for a range of less stable amine and aldehyde substrates, and in contrast to the Bischler-Napieralski reaction does not require the use of protecting groups. However, the phenylethylamines require an electron donating –OH or -NH_2 group at the C-3 position in order sufficiently activate cyclisation of the imine intermediate. A wide range of aldehydes were tolerated in the biomimetic Pictet-Spengler reaction with dopamine **101** including aliphatic, aromatic and benzylic aldehydes. This simple one step method is suitable for the synthesis of structurally diverse sets of THIQ alkaloids, as demonstrated by the synthesis of a library of compounds for screening in antimycobacterial assays.[105]

A proposed mechanism of the phosphate mediated Pictet-Spengler reaction involves two possible roles of the phosphate (Schemes 3.8 and 3.9). Phosphate could act as a base and abstract a proton from the C-6 hydroxy group of the imine **111**, activating the ring for addition to the electrophilic imine (Scheme 3.8). Abstraction of the proton at position C-8a of **112** by phosphate could then facilitate rearomatisation to THIQ **113**.



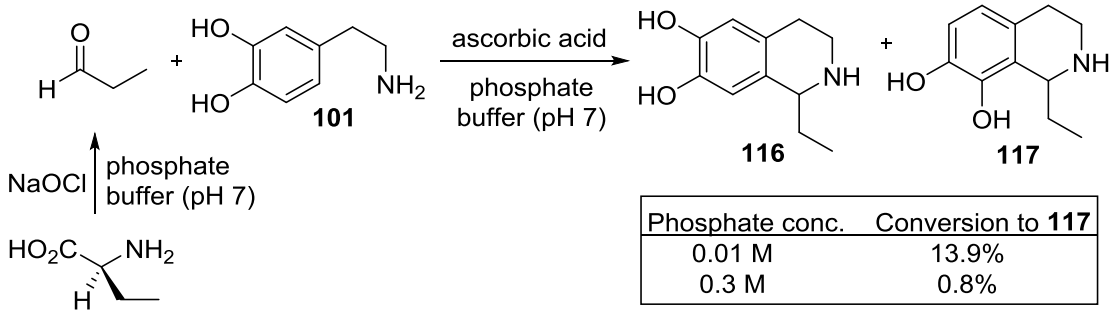
Scheme 3.8. Proposed role of phosphate in the biomimetic Pictet-Spengler reaction (Pi is inorganic phosphate).

Phosphate could also act as a nucleophile (Scheme 3.9) and attack the imine **111** to form aminophosphate **114**. Subsequent deprotonation of the C-6 hydroxy group and rearomatisation could be facilitated by the phosphate group through the formation of a 6-membered ring intermediate **115**.



Scheme 3.9. Proposed role of phosphate in the biomimetic Pictet-Spengler reaction.

A recent study by the Maresh group has shown that the concentration of phosphate used in the biomimetic Pictet-Spengler reaction can affect the regioselectivity of the reaction.[119] In their one pot procedure for the synthesis of THIQs from amino acids, they monitored the Pictet-Spengler reaction between propanal and dopamine **101** by HPLC for the formation of THIQ products **116** and **117** (Scheme 3.10).



Phosphate conc.	Conversion to **117**
0.01 M	13.9%
0.3 M	0.8%

Scheme 3.10. One-pot biomimetic Pictet-Spengler reaction from amino acids.

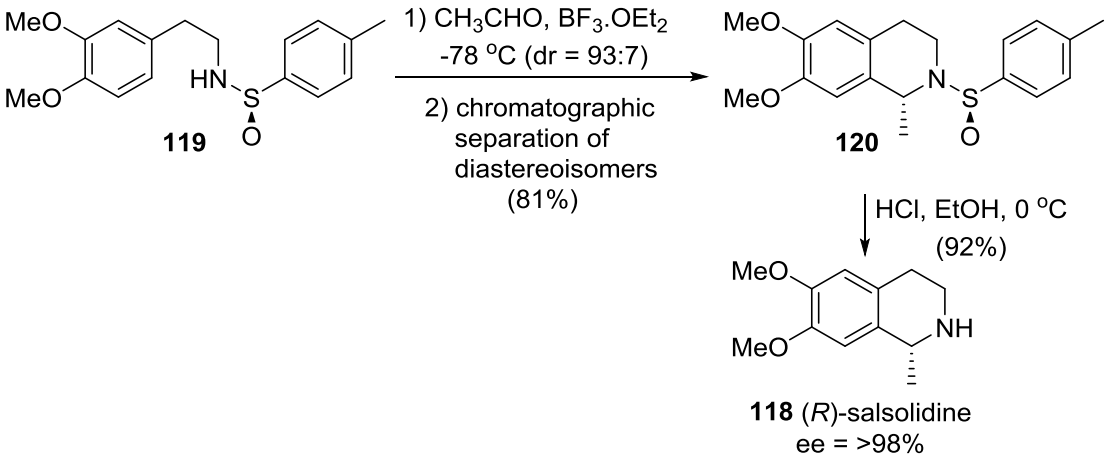
When a high concentration of phosphate was used (0.3 M), the THIQ **116** was formed as the major product where cyclisation occurred *para* to the electron donating hydroxy group. Less than 1% of the regioisomer **117** (formed through cyclisation *ortho* to the

electron donating hydroxy group) was detected by HPLC. When the reaction was repeated at a lower phosphate concentration of 0.01 M, the formation of the minor THIQ regioisomer **117** became more significant, with approximately 14% conversion.

3.3.5 Asymmetric Pictet-Spengler reaction

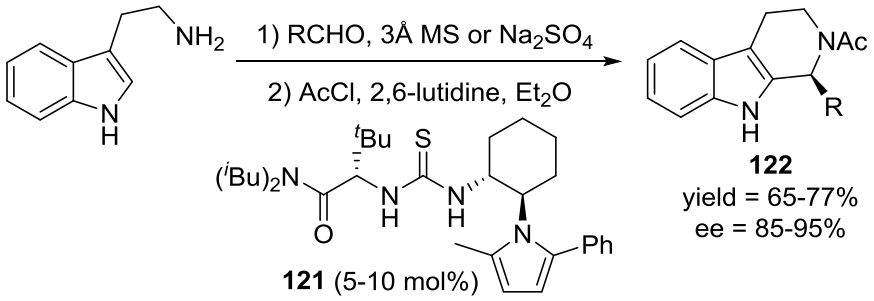
The cyclisation step in the Pictet-Spengler reaction creates a new chiral centre at the C-1 position of the THIQ product. The biological or pharmacological activity of 1-substituted THIQs is often displayed by a specific enantiomer and therefore research into asymmetric methods for their synthesis is important for drug development.

There are a number of diastereoselective Pictet-Spengler reactions for the synthesis of 1-substituted THIQs using substrates containing chiral auxiliaries.[123] For example, in a synthesis of (*R*)-salsolidine **118**, a *p*-tolylsulfinyl chiral auxiliary was used to control the stereochemistry (Scheme 3.11).[124] The Pictet-Spengler reaction of *N*-sulfinyl phenylethylamine **119** with acetaldehyde yielded THIQ **120** in 86% de, and the chiral auxiliary was removed in the presence of hydrochloric acid without racemisation. Although diastereoselective reactions allow for the synthesis of a single 1-substituted THIQ enantiomer, they can be step intensive requiring the installation and removal of chiral auxiliaries.



Scheme 3.11. Asymmetric synthesis of a 1-substituted THIQ using a chiral auxiliary approach.

Asymmetric organocatalysis is the use of chiral organic molecules to catalyse enantioselective transformations. The first enantioselective organocatalytic Pictet-Spengler reaction was reported by Taylor and Jacobsen in 2004 but it was not for the preparation of the THIQ scaffold, instead for the synthesis of tetrahydro-β-carbolines (THBCs).[125] Their strategy involved using a chiral thiourea catalyst **121** (a hydrogen bond donor catalyst) and enhancing the reactivity of the imine intermediate by generating the *N*-acyliminium ion, which afforded the *N*-acyl THBC products **122** in 85-95% ee (Scheme 3.12).

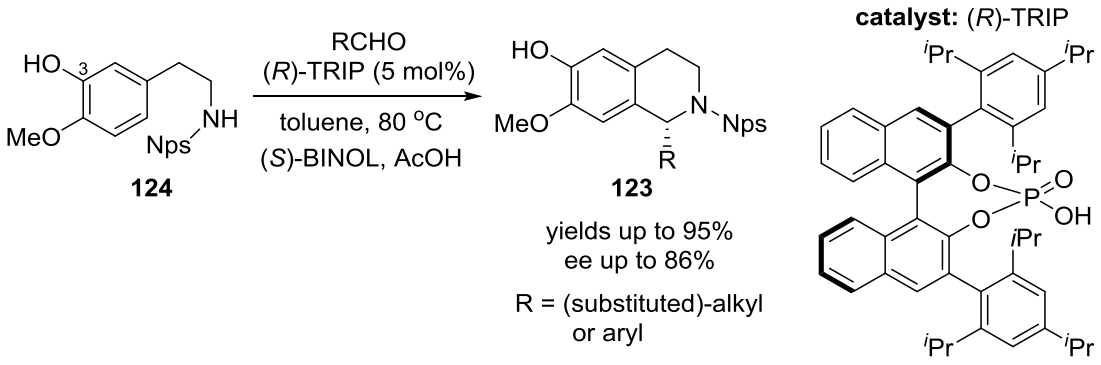


Scheme 3.12. Asymmetric acyl Pictet-Spengler reaction (R = alkyl group).

A number of chiral thiourea catalysts and chiral Bronsted phosphoric acid catalysts have been used in the enantioselective organocatalytic Pictet-Spengler cyclisation, but most of these have been restricted to tryptamine derivatives.[126] The success of these reactions can be attributed to the high nucleophilicity of the indole system, and the indole NH can hydrogen bond to the Bronsted acid catalyst enabling greater stereoselective control. In comparison, the benzene ring in phenylethylamines is less nucleophilic than the indole in tryptamine. Therefore the Pictet-Spengler cyclisation of phenylethylamines to THIQs often requires higher temperatures and stronger acidic conditions which makes the development of asymmetric versions more challenging.

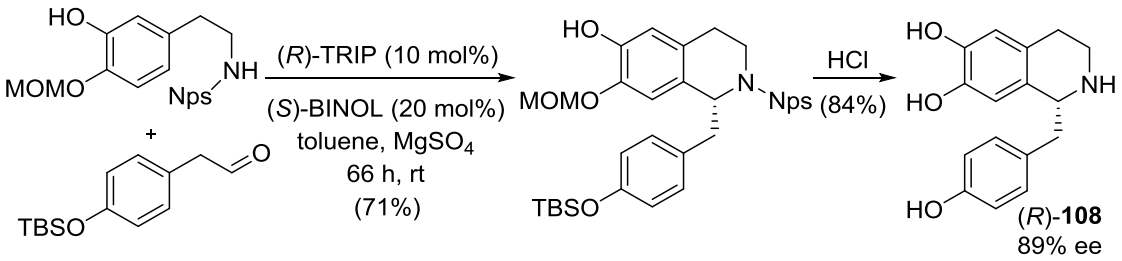
Only recently the enantioselective organocatalytic approach was extended to the synthesis of THIQs by Hiemstra and coworkers.[127] A series of 1-substituted THIQs **123** (Scheme 3.13) with ee up to 86% were prepared from *N*-(*o*-nitrophenylsulfenyl)-phenylethylamines **124** using BINOL phosphoric acid catalysed Pictet-Spengler reactions. The phenylethylamine required a 3-hydroxy substituent in order to enhance

the reactivity of the ring. The sulfenyl moiety was crucial for enantioselectivity and to increase reactivity of the iminium ion intermediate. A range of aliphatic aldehydes were used in the reaction resulting in the chiral THIQ products **123** in yields of up to 95%.



Scheme 3.13. Enantioselective organocatalytic Pictet-Spengler reaction. Nps = *o*-nitrophenylsulfenyl.

The Hiemstra group also used the enantioselective phosphoric acid catalysed Pictet-Spengler reaction to prepare 1-benzyl THIQs from *N*-sulfenyl phenylethylamines and phenylacetaldehydes (Scheme 3.14).[128] They achieved good yields (70-85%) by carrying out the reactions at room temperature in the presence of a drying agent. Higher temperatures caused decomposition and side products of the phenylacetaldehyde substrates. Using (*R*)-TRIP as the catalyst led to the formation of 1-benzyl THIQs in the (*R*)-configuration with ee between 85-92%. After protecting group manipulations the alkaloid (*R*)-norcoclaurine (*R*)-**108** was isolated in 89% ee. This is opposite to the configuration produced by the NCS Pictet-Spenglerase enzyme in plants, which produce the (*S*)-configuration stereoselectively.



Scheme 3.14. Enantioselective organocatalytic Pictet-Spengler reaction for 1-benzyl THIQs.

3.4 Aqueous phosphate mediated catalysis

Water is an attractive reaction medium for organic synthesis and has a number of advantages over traditional organic solvents including lower costs, a reduced environmental impact and it is safe, non-toxic and non-flammable.[129] In nature, biosynthetic reactions take place in water, and the synthesis of chiral biological molecules via enzymatic reactions are highly stereospecific. For synthetic chemists, water compatible asymmetric catalysts are attractive as a sustainable approach for the preparation of enantiomerically pure compounds.[130] However, water is a strong hydrogen bond donor/acceptor which can disrupt interactions between a chiral catalyst and a substrate and significantly influence the enantioselectivity that can be achieved. This is particularly a problem for catalysts that rely on non-covalent interactions such as hydrogen bonding or coulombic attraction between ion pairs. Therefore asymmetric catalysis in aqueous media is challenging.

Aqueous phosphate mediated catalysis has been demonstrated for a number of reactions, including Pictet-Spengler reactions that are promoted by a phosphate buffer[122] (section 3.3.4) and reactions catalysed by heterogeneous apatite catalysts featuring a phosphate functionalised surface.[131] In aqueous solution phosphate exists in different ionisation states dependent on the pH of the solution (Figure 3.9), which can influence the catalytic activity of the phosphate. In strongly acidic solution phosphoric acid is the predominant form. In strong basic conditions the fully deprotonated phosphate ion (PO_4^{3-}) predominates. Under neutral, weakly acidic or weakly basic conditions, mostly hydrogen and dihydrogen phosphate forms are present.

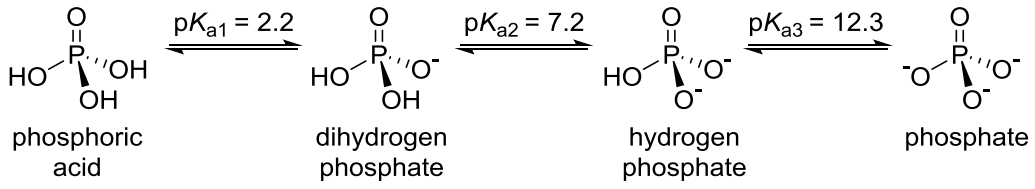


Figure 3.9. Ionisation states of phosphate (pK_a values at 25 ^oC are from the CRC Handbook of Chemistry and Physics).

Chiral BINOL-derived phosphoric acids (Figure 3.10) have been established as an efficient and versatile class of organocatalysts for asymmetric reactions.[132] The axially chiral biaryl (BINOL) moiety gives the catalyst its source of chirality, and the addition of bulky substituents at the 3,3'-positions of the biaryl improves enantioselectivity by providing a chiral environment where the substrate is activated. Although many BINOL-derived phosphoric acid catalysts have been developed and used for the asymmetric synthesis of pharmaceutically useful structures, generally strictly anhydrous conditions are required to achieve high enantioselectivities.

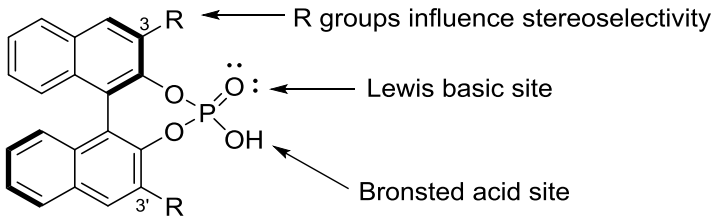
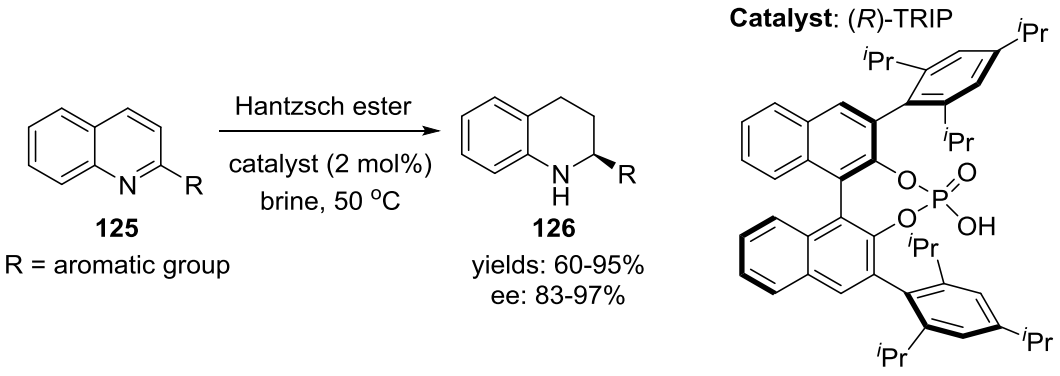


Figure 3.10. General structure of chiral BINOL-derived phosphoric acids.

In 2010 the Rueping group reported the first example of a BINOL-derived phosphoric acid catalysed asymmetric reaction in water (Scheme 3.15).[133] They performed an enantioselective hydrogenation of quinolines **125** using a Hantzsch ester as the hydride source, to prepare 2-substituted tetrahydroquinolines **126**. The phosphoric acid catalyst (*R*)-TRIP was proposed to induce selectivity through formation of a chiral ion pair with the quinoline imine and direct the hydride donor to a particular face. High enantioselectivities were observed when the reaction was carried out in water, and even higher selectivity (ee up to 97%) was achieved in a saturated NaCl solution, despite the fact that water is a strong hydrogen bond donor and could disrupt interactions between the catalyst and substrate. This was attributed to 'hydrophobic hydration' where non polar molecules align so that the contact surface between these molecules and water is minimised. This principle plays an important role in biological processes such as enzyme-substrate interactions. In addition, the bulky isopropyl substituents on the catalyst created a hydrophobic pocket for the substrate to interact with the active catalytic phosphate centre.

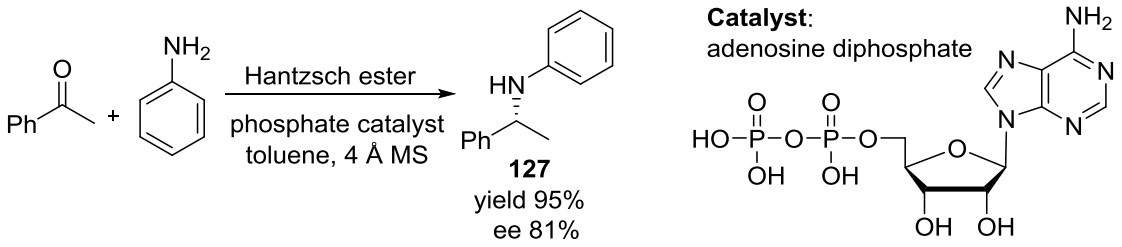


Scheme 3.15. Enantioselective hydrogenation of quinolines catalysed by a BINOL-derived phosphoric acid in water.

An emerging class of water compatible asymmetric catalysts are chiral biological phosphates. Biological molecules are an excellent starting point for the development of water compatible catalysts because they are typically highly soluble in water. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) have been shown to successfully catalyse a number of carbon-carbon bond forming reactions such as Diels-Alder reactions, Friedel-Crafts reactions and Michael additions with high enantioselectivity.[134] DNA-based asymmetric catalysts involve the binding of a catalytically active metal complex to the DNA through either covalent bonding or supramolecular interactions.[135] Chiral catalysts lower the energy of one diastereomeric transition state to result in the preferential formation of a single diastereoisomer. The right handed double helix structure of DNA provides the source of chirality, but the mechanism of how it lowers the energy of one diastereomeric transition state is not fully understood, and possible interactions between the DNA and the substrate include groove binding, intercalation or interactions with the phosphate backbone.

Natural unmodified DNA as a catalyst has also been applied to aldol reactions, Henry (nitroaldol) reactions and Michael additions in water.[136] However, currently no enantioselectivity has been reported for these reactions. DNA is composed of nucleotides that each contain a phosphate group, a deoxyribose sugar and a nucleobase. These groups are capable of various interactions that could be responsible for the catalytic activity. For example, Wang *et al*., found that the catalytic activity of DNA in the synthesis of dithioacetals from carbonyl compounds could be associated with the phosphate group.[137] The Kumar group have reported the use of a single nucleotide as a

catalyst for asymmetric reductive amination of ketones (Scheme 3.16).[138] Adenosine mono-, di- and triphosphate nucleotides promoted the reaction to give the amine product **127** in excellent yields, but for good enantioselectivity to be achieved the reactions required anhydrous conditions. It was proposed that the phosphate group of the nucleotides played a key role in the catalytic activity, through hydrogen bonding with the imine intermediate.



Scheme 3.16. Enantioselective reductive amination catalysed by a single nucleotide.

The advantages of using chiral biological phosphates such as DNA or nucleotides as catalysts are their commercial availability and their compatibility with aqueous media. They are emerging as excellent catalysts for a number of organic transformations and show promising ability to control the stereoselectivity of reactions.

3.5 Tetrahydro-2-benzazepines

3.5.1 Structure and biological activity

2,3,4,5-Tetrahydro-2-benzazepines (THBPs) are 7-membered ring analogues of tetrahydroisoquinoline. The THBP ring system is present in both alkaloid natural products and synthetic drugs that possess a wide range of biological activities (Figure 3.11). For example, a number of *Amaryllidaceae* alkaloids contain the THBP structure such as galanthamine which is an acetylcholinesterase inhibitor used for the treatment of Alzheimer's disease and haemanthidine which was recently shown to have antimalarial properties.[139] Synthetic THBP derivatives include capsazepine which is a vanilloid receptor antagonist and BMS791325 which is undergoing clinical studies as an inhibitor of the hepatitis C virus.[140]

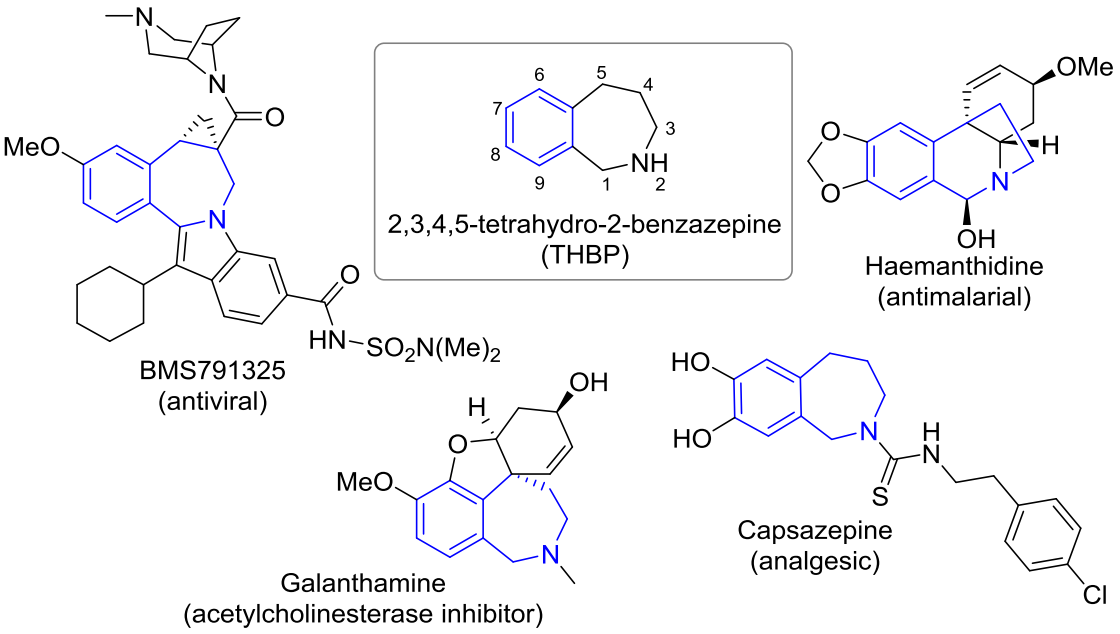


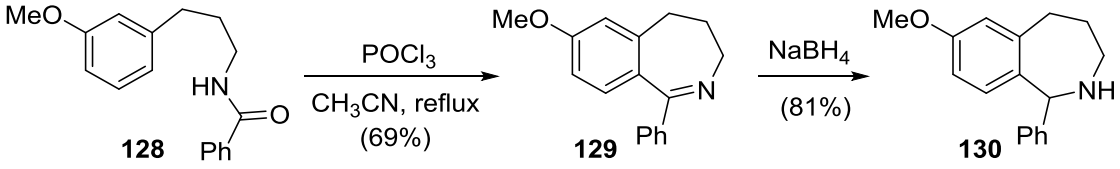
Figure 3.11. Examples of bioactive tetrahydro-2-benzazepine (THBP) derivatives.

1-Substituted THBPs are interesting as potential drug molecules as they are closely related to the 1-substituted THIQs, of which there are many biologically active compounds. For SAR studies, changing the ring size from a 6-membered ring (THIQ) to a 7-membered ring (THBP) can be useful because conformational changes in the ring can alter the position of the substituents and influence the potency against a target.[141] In addition, 7-membered ring THBPs may have different metabolic profiles compared to the corresponding 6-membered ring THIQs and may offer improved pharmacokinetic properties.

3.5.2 Synthesis

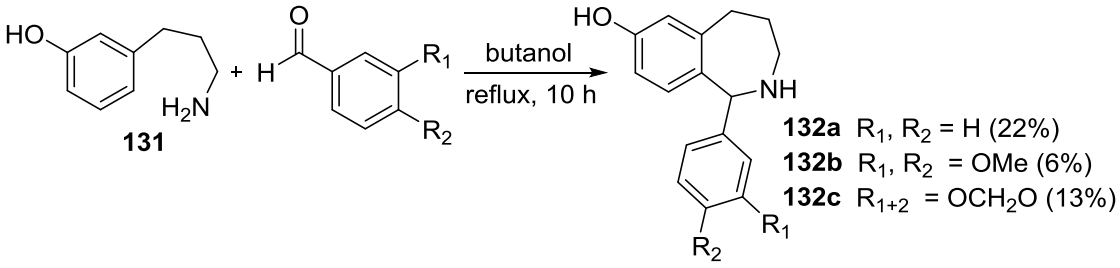
Strategies reported for the synthesis of 1-substituted THBPs are fairly limited. Ring expansions have been used such as the Schmidt ring expansion followed by further steps to incorporate a C-1 substitutent onto the benzazepine ring.[142] More common routes involve a cyclisation to form the 7-membered ring system. Reactions such as ring closing metathesis and a gold catalysed [2 + 5] cycloaddition have been used to prepare 1-substituted THBPs, but these involved multiple steps to prepare the required compounds for cyclisation. The Bischler-Napieralski reaction (described in section

3.3.1) has been used for cyclisation to form 7-membered rings. For example, the activated phenylpropylamide **128** was converted to the cyclic imine **129** in the presence of phosphorus oxychloride at high temperature (Scheme 3.17). The imine was subsequently reduced to yield the 1-substituted THBP **130**.[143]



Scheme 3.17. Synthesis of a 1-substituted THBP by a Bischler-Napieralski/reduction method.

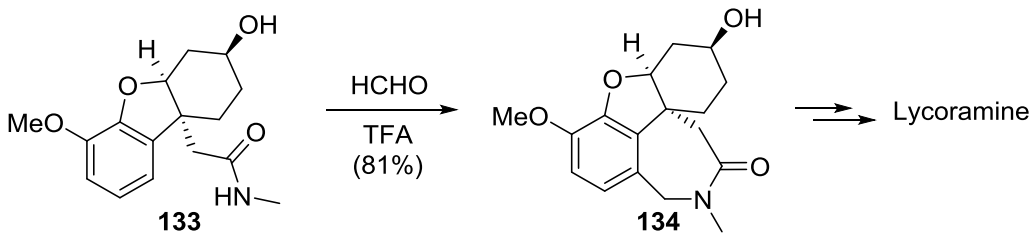
The Pictet-Spengler cyclisation (described in section 3.3.2) has also been used for the synthesis of THBPs. The cyclisation between phenylpropylamine **131** and benzaldehyde derivatives to generate 1-substituted THBPs **132a-c** has been reported, but the reactions required high temperatures and the yields of the THBP products were low (6-22%) (Scheme 3.18).[144] It was essential for the phenylpropylamine **131** to have an electron donating hydroxy substituent to activate the ring at the position *para* to the cyclisation site.



Scheme 3.18. Synthesis of 1-substituted THBPs by a Pictet-Spengler cyclisation.

Although 7-endo trig cyclisations are favoured by Baldwin's rules, the Pictet-Spengler cyclisation to yield 7-membered rings is challenging. Reported methods often rely on using modified Pictet-Spengler conditions involving either *N*-acyl or *N*-sulfonyl groups (to increase the reactivity of the imine intermediate) and are often limited to the use of formaldehyde. For example in the total synthesis of lycoramine, an *Amaryllidaceae*

alkaloid, the cyclisation of compound **133** under acidic conditions yielded THBP **134**, proceeding via an *N*-acyliminium intermediate (Scheme 3.19).[145]



Scheme 3.19. Modified Pictet-Spengler cyclisation in the total synthesis of lycoramine.

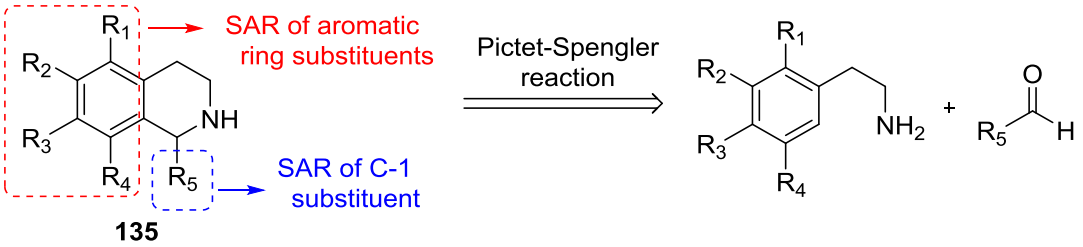
Cyclisation to form 7-membered rings is challenging with strategies often involving numerous steps that are not generally applicable to a wide range of substrates. Cyclisation methods that are compatible with unprotected substrates, operate under mild conditions and do not involve step-intensive procedures would be useful, since compounds containing the 1-substituted tetrahydro-2-benzazepine structure show wide ranging bioactivities and are interesting structures for medicinal chemistry.

3.6 Aims of the project

The aim of this part of the project was to synthesise a series of 1-substituted THIQs and establish their antimycobacterial properties, in order to further previous SAR studies where the THIQ structure was identified as a promising new pharmacophore for anti-TB drugs.[105] The target compounds **135** (Scheme 3.20) were designed based on THIQs that were previously found to show antimycobacterial activity within the Hailes and Bhakta groups. The substituents on the aromatic ring (R_1-R_4) included hydroxy groups (which were previously found to influence antimycobacterial activity but the optimal position was not established) and a bromide substituent (which was present in THIQs **91**-**93** (Figure 3.8) and conferred excellent antimycobacterial activity). The aim was to also investigate substituents at the C-1 position (R_5) such as alkyl chains, benzylic groups or functionalised groups that could allow for further manipulation of the THIQs to generate new structures. The phosphate mediated Pictet-Spengler reaction was chosen

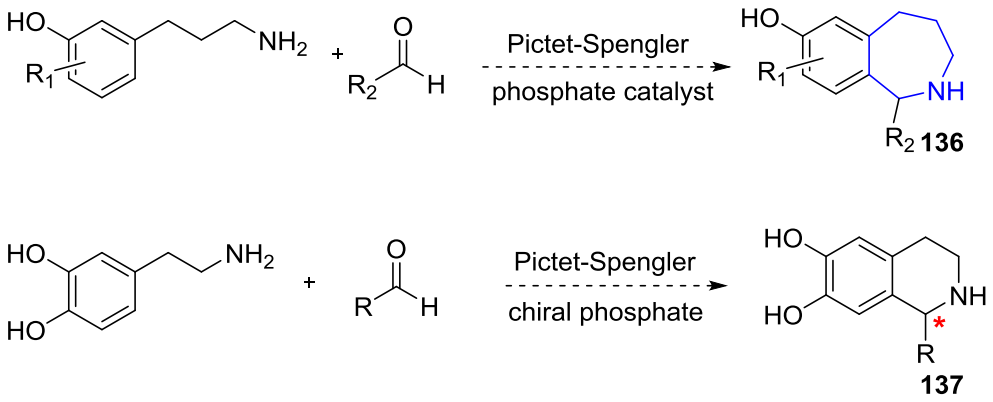
for the preparation of the target compounds because this method readily allows for variations of the substituents at C-1 (R_5) and the aromatic ring (R_1-R_4) through the choice of amine and aldehyde starting materials (Scheme 3.20).

To determine the antimycobacterial activity of the THIQs, the aim was to evaluate the compounds in a number of assays by biological collaborators, including SPOTi and cytotoxicity assays. Compounds showing promising antimycobacterial activity could then undergo further analysis for possible modes of action including an efflux pump inhibition assay which has been established by the Bhakta group. In addition to the SAR studies, two 1-substituted THIQs **91** and **92** (Figure 3.8) that were previously found to be excellent inhibitors of mycobacterial growth, required synthesis for pharmacokinetic analysis.



Scheme 3.20. Target THIQs to be synthesised by the phosphate mediated Pictet-Spengler reaction.

The aim of the project was also to extend the use of the phosphate mediated Pictet-Spengler reaction. Previously the cyclisation reaction had only been used for the construction of racemic 6-membered ring THIQs. Extending the reaction to generate 7-membered ring analogues from readily accessible starting materials would generate a new range of 1-substituted THBPs **136** (Scheme 3.21) which may also possess interesting antimycobacterial activities. In addition, extending the phosphate mediated Pictet-Spengler reaction to generate optically active THIQs **137** (Scheme 3.21) in aqueous media would be attractive as a synthetic tool. Therefore, the aim was to screen chiral biological phosphates and BINOL-derived phosphoric acids as chiral catalysts, and to determine the enantiomeric excess of the THIQ products generated.



Scheme 3.21. Phosphate mediated Pictet-Spengler reaction to prepare 7-membered ring THBPs and optically active THIQs.

The final part of this project was to prepare a small set of profens (2-arylpropionic acids) for antimycobacterial screening. The aim was to improve potency compared to the profens reported to date.[94] The target compounds **138** and **139** (Figure 3.12) were derivatives of ibuprofen and loxoprofen. These were chosen for derivatisation because the Bhakta group found that they were good inhibitors of the growth of *M. tuberculosis*.[94] Also, ibuprofen was readily available, and loxoprofen could be prepared following literature procedures from cheap commercially available starting materials.

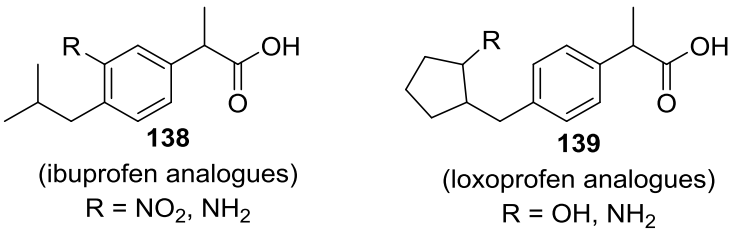


Figure 3.12. Target derivatives of ibuprofen and loxoprofen.

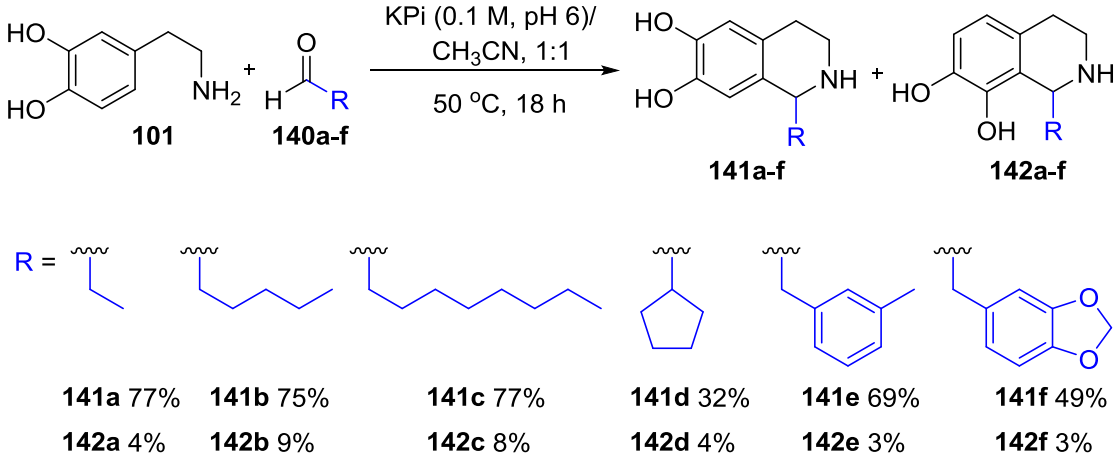
4. Tetrahydroisoquinolines

4.1 SAR of C-1 and aromatic ring substituents

4.1.1 Synthesis of first generation THIQs

The first generation of 1-substituted THIQs incorporated various groups at the C-1 position, with the aim of identifying substituents that conferred antimycobacterial activity. The substituents included alkyl chains of varying length, a cyclic alkyl group and benzylic groups (Scheme 4.1). These were selected based on the good antimycobacterial activities previously reported for THIQs that featured a methylenedioxybenzyl substituent and a nonyl substituent (section 3.2.2, Figures 3.7 and 3.8).[105]

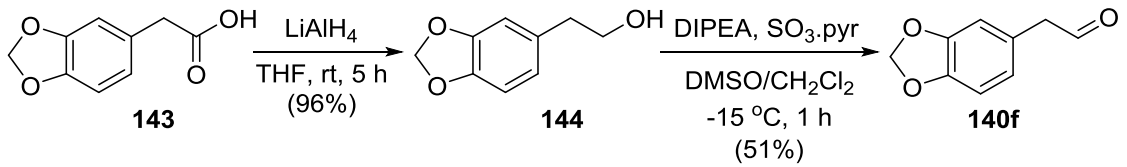
The phosphate mediated Pictet-Spengler reaction (described in section 3.3.4)[122] was used to synthesise the THIQs (Scheme 4.1). Dopamine **101** was chosen as the phenylethylamine starting material in order to prepare THIQ compounds with a 6,7-dihydroxy substitution pattern on the aromatic ring. Compounds featuring this moiety have previously shown antimycobacterial activity.[105] In addition, dopamine **101** was readily available and favours Pictet-Spengler cyclisations due to the electron rich aromatic ring. The aldehydes used included aliphatic aldehydes **140a-d** and phenylacetaldehyde derivatives **140e-f**.



Scheme 4.1. Synthesis of first generation THIQs (Yields = isolated yield after purification by HPLC).

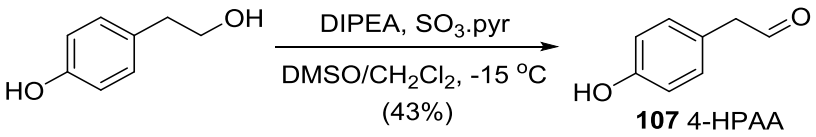
The Pictet-Spengler reactions were carried out in a 1:1 mixture of potassium phosphate buffer (pH 6) and acetonitrile at 50 °C. The crude products were purified using reverse-phase prep-HPLC, eluting with water and acetonitrile containing 0.1% TFA. The THIQ products collected from the HPLC purification were subsequently washed with methanol to remove the TFA. The major THIQ products **141a-f** were isolated in yields between 32-77%. A small amount of the regioisomers **142a-f** were formed as a minor product in each reaction (isolated yields 3-9%). The regioisomers were separated during the reverse-phase HPLC purification, but due to their structural similarity the compounds eluted close together. A good separation was achieved by running the HPLC method for an extended period of time (methods described in Chapter 8). Therefore, in addition to the desired 6,7-dihydroxy THIQs **141a-f**, the 7,8-dihydroxy THIQs **142a-f** were available for evaluation for antimycobacterial activity.

The phenylacetaldehydes used in the synthesis of the first generation THIQs were prepared using a Parikh-Doering oxidation.[146] Phenylacetaldehydes can be difficult to prepare because they are prone to polymerisation and aldol condensations. The Parikh-Doering oxidation involves the activation of DMSO for the oxidation of alcohols using sulphur trioxide, and the reaction proceeds under mild basic conditions and has been used by Pesnot *et al*. as a general route for the synthesis of phenylacetaldehydes.[118a] Aldehyde **140f** was prepared in two steps from commercially available phenylacetic acid **143** (Scheme 4.2). The phenylacetic acid **143** underwent reduction using lithium aluminium hydride to afford the alcohol **144**[147] in 96% yield, followed by oxidation using SO_3.pyridine in DMSO and dichloromethane to give the aldehyde **140f** in 51% yield. For optimal yields the reaction temperature needed to be kept at -15 °C, hence dichloromethane was required as a co-solvent to avoid freezing of the DMSO solution. The same oxidation method was used to prepare 2-(*m*-tolyl)acetaldehyde **140e**[118b] from commercially available 3-methylphenethyl alcohol in 60% yield.



Scheme 4.2. Synthesis of 3,4-(methylenedioxy)phenylacetaldehyde **140f**.

The aldehyde 4-HPAA **107**[122] was also synthesised following the Parikh-Doering oxidation method (Scheme 4.3) in 43% yield. 4-HPAA **107** was difficult to isolate in good yields because the phenol moiety enhances the ability of the aldehyde to undergo aldol condensations. It was found that optimal yields were achieved when purification of the crude product by column chromatography was carried out quickly, and evaporation of the solvents *in vacuo* was performed at room temperature. The resulting product **107** was stable when stored under argon at -80 ^{o}C in acetonitrile solution. The prepared 4-HPAA **107** was subsequently used in a study of the mechanism and kinetics of the NCS catalysed Pictet-Spengler biosynthetic pathway (section 3.3.3) by Lichman *et al.*[121]

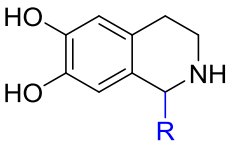


Scheme 4.3. Synthesis of 4-HPAA.

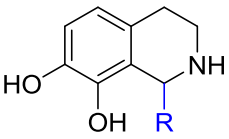
4.1.2 Antimycobacterial activity of first generation THIQs

The compounds were tested for their growth inhibition of *M. aurum* using SPOTi (spot culture growth inhibition assay, described in section 3.1.2) (Table 4.1).[iv] The MIC (minimum inhibitory concentration) values were determined as the concentration at which no growth of mycobacteria was observed. The compounds were also evaluated for their effect on mammalian cells using a eukaryotic cell cytotoxicity resazurin assay with murine macrophage cells RAW 264.7. The GIC (growth inhibitory concentration) values were determined as the concentration at which more than 90% inhibition of cell viability was observed (Table 4.1). Both of these assays were carried out by biological collaborators in the Bhakta group at Birkbeck, University of London.

[iv] The SPOTi and cytotoxicity assays described in this chapter were carried out by Arundhati Maitra and Dr Parisa N. Mortazavi in the Bhakta group in the Mycobacterial Research Laboratory at Birkbeck, University of London.



141a-f



142a-f

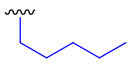
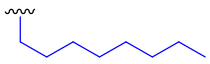
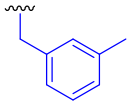
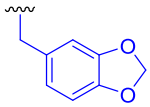
R group	THIQ	Mwt	calc LogP (ACD)	MIC *M. aurum* (μg/mL)	GIC RAW 264.7 (μg/mL)
	**141a**	193.3	1.72	no inhibition[a]	15.6
	142a	193.3	1.72	250	15.6
	**141b**	235.3	3.09	500	31.3
	142b	235.3	3.09	15.6	15.6
	**141c**	277.4	4.45	125	7.8
	142c	277.4	4.45	250	15.6
	**141d**	233.3	2.27	no inhibition[a]	62.5
	142d	233.3	2.27	250	7.8
	**141e**	269.3	3.05	500	31.3
	142e	269.3	3.05	250	15.6
	**141f**	299.3	2.82	500	62.5
	142f	299.3	2.82	250	31.3

Table 4.1. Antimycobacterial properties of the first generation THIQs. MIC = lowest concentration at which no growth of mycobacteria was observed. GIC = concentration at which more than 90% inhibition of cell viability (RAW 264.7) was observed. [a]Highest concentration tested was 500 μg/mL.

The data indicated that the 6,7-dihydroxy-THIQs with alkyl chains at the C-1 position (**141a-c**) became increasingly potent against *M. aurum* as the length of the alkyl chain increased. For example, the 1-ethyl substituted compound **141a** did not inhibit the growth of *M. aurum* at a concentration of 500 μg/mL, whereas the 1-octyl substituted compound **141c** had a MIC = 125 μg/mL. The 6,7-dihydroxy-THIQ **141d** with a cyclopentyl C-1 substituent did not inhibit *M. aurum* growth at the maximium concentration tested (500 μg/mL), suggesting a more flexible alkyl chain was required for potency. The 6,7-dihydroxy-THIQs **141e** and **141f** featuring a benzylic group at the C-1 position, both had MIC values of 500 μg/mL, so a high concentration of the compound was required in order to inhibit mycobacterial growth.

Previously within the Hailes and Bhakta groups the 6,7-dihydroxy-THIQ **141f** had been evaluated for its activity against the growth of *M. bovis* BCG using SPOTi, and had a MIC of 100 μg/mL.[105] In comparison with this study, the MIC of the same compound **141f** against *M. aurum* was significantly higher (MIC = 500 μg/mL). The difference in activities was likely to arise from differences between *M. bovis* BCG[148] and *M. aurum*.[149] *M. aurum* is a relatively fast growing species whereas *M. bovis* BCG is slow growing and more closely related to the slow growing *M. tuberculosis*.

The 7,8-dihydroxy-THIQs **142** exhibited better *M. aurum* growth inhibition compared to the corresponding 6,7-dihydroxy-THIQ **141** regioisomers. For example, compounds **142e-f** had MIC values of 250 μg/mL and the regioisomers **141e-f** had MICs of 500 μg/mL. An exception to this trend was seen for the compounds with an octyl substituent at the C-1 position, where the trend was reversed and the 6,7-dihydroxy derivative **141c** (MIC = 125 μg/mL) was more active than the 7,8-dihydroxy derivative **142c** (MIC = 250 μg/mL). The 7,8-dihydroxy-THIQ **142b** with a pentyl chain at the C-1 position was the most potent compound of the series with a MIC of 15.6 μg/mL.

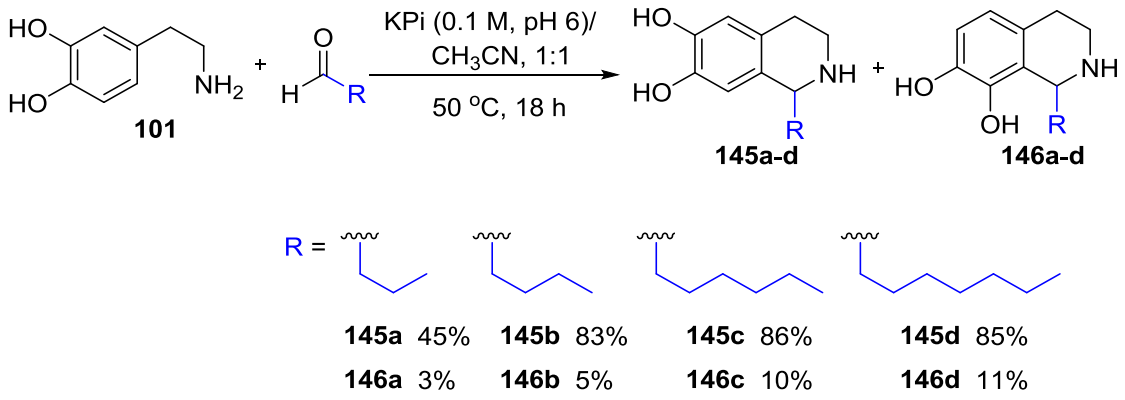
The MICs of the first generation THIQs did not show a correlation with the calculated LogP values (Table 4.1). The LogP values (octanol/water partition coefficient) express the compounds lipophilicity, and according to Lipinski's rules, drug absorption and permeability is more likely when LogP is less than 5.[87] TB drugs often do not conform to this rule, and a number of studies have shown the tendency for LogP to increase during early drug discovery. For example, in a series of indolecarboxamide anti-TB compounds, the calculated LogP of the initial hit compound was 4.5, and this increased to 6.1 for the optimised compound.[89] This may be linked to more lipophilic compounds having improved permeability across the highly waxy mycobacterial cell wall.[150] However this was not observed in this SAR study where there were significant differences in activity between the 6,7-dihydroxy-THIQ and 7,8-dihydroxy-THIQ regioisomers which had the same calculated LogP values. This suggested the position of the hydroxy groups on the aromatic ring influenced the potency, possibly through interactions with an unknown target.

Analysis of the GIC data showed that the THIQs displayed cytotoxicity against mammalian cells (Table 4.1). For all of the compounds the concentration that was required to inhibit mycobacterial growth would also be toxic to mammalian cells. The

compounds that exhibited the lowest cytotoxicity against mammalian cells (GIC = 62.5 μg/mL) were the 6,7-dihydroxy-THIQs **141d** and **141f**. In order to be useful as potential drug like compounds, the MICs would need to be much lower than the GIC values so that selective inhibition for mycobacteria growth could be achieved without the compounds being toxic to mammalian cells.

4.1.3 Synthesis of second generation THIQs

The most potent compound from the first generation THIQs was **142b** with a pentyl chain at position C-1. In order to determine if this was the optimal chain length for antimycobacterial activity, an investigation into the propyl, butyl, hexyl and heptyl chain lengths was carried out. The second generation THIQs with varying length alkyl chains at the C-1 position were prepared using the phosphate mediated Pictet-Spengler reaction as described for the first generation THIQs (Scheme 4.4). The reaction between dopamine **101** and the aliphatic aldehydes yielded a mixture of 6,7-dihydroxy-THIQs **145a-d** as the major products and 7,8-dihydroxy-THIQs **146a-d** as the minor products.



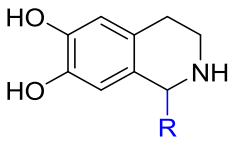
Scheme 4.4. Synthesis of second generation THIQs (Yields = isolated yields after purification by HPLC).

The major regioisomers with a butyl **145b**, hexyl **145c** and heptyl **145d** chain at the C-1 position were isolated after reverse phase prep-HPLC in good yields of between 83-86%. The THIQ **145a** with a shorter propyl chain was isolated in a lower yield of 45%. This was because some of the product was lost at the purification stage in order to

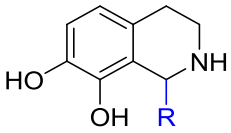
ensure all of the product **146a** was completely separated from its regioisomer. The corresponding minor regioisomers **146a-d** were isolated in yields of between 3-11% and the yields increased as the length of the alkyl chain increased.

4.1.4 Antimycobacterial activity of second generation THIQs

The second generation THIQs were screened for their growth inhibition of *M. aurum* and cytotoxicity against mammalian cells (Table 4.2).



145a-d



146a-d and **142b**

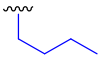
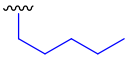
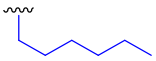
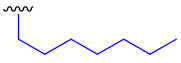
R group	THIQ	Mwt	calc LogP (ACD)	MIC *M. aurum* (µg/mL)	GIC RAW 264.7 (µg/mL)
	**145a**	207.3	2.18	>500[a]	31.3
	146a	207.3	2.18	250	15.6
	**145b**	221.3	2.63	>500[a]	125
	146b	221.3	2.63	250	15.6
	**142b**	235.3	3.09	125	15.6
	**145c**	249.4	3.54	250	62.5
	146c	249.4	3.54	250	15.6
	**145d**	263.4	3.99	250	7.8
	146d	263.4	3.99	500	15.6

Table 4.2. Antimycobacterial properties of the second generation THIQs. [a]The highest concentration of THIQ tested was 500 µg/mL. MIC = lowest concentration at which no growth of mycobacteria was observed. GIC = concentration at which more than 90% inhibition of cell viability was observed.

The MIC values of the 6,7-dihydroxy-THIQs **145a-d** decreased as the length of the alkyl chain was increased, as previously observed for the first generation compounds. Compounds **145a-b** with the propyl and butyl chains at position C-1 did not completely inhibit the growth of *M. aurum* at the highest concentration tested (500 µg/mL).

Compounds **145c-d** with a hexyl or heptyl chain at the C-1 postion had lower MIC values of 250 μg/mL. This suggested that increasing the lipophilicity of the 6,7-dihydroxy-THIQ compounds resulted in increased antimycobacterial activity.

The 7,8-dihydroxy-THIQs **146a-d** and **142b** showed a different pattern of antimycobacterial activity compared to the corresponding 6,7-dihydroxy regioisomers. Compounds with a propyl **146a** or butyl **146b** substituent at the C-1 position had a MIC value of 250 μg/mL. Increasing the chain length to the pentyl substituent **142b** resulted in a more potent compound with a decrease in the MIC to 125 μg/mL. Increasing the chain further to the hexyl **146c** and heptyl **146d** substituents resulted in the MICs increasing (MIC = 250 and 500 μg/mL respectively).

When compound **142b** was screened in the first generation compounds the MIC was determined as 15.6 μg/mL against *M. aurum* (Table 4.1). However, when screened again with the second generation compounds the MIC value against *M. aurum* was 125 μg/mL (Table 4.2). To confirm the actual MIC, compound **142b** was synthesised again and purified, and re-tested in the same assay where its MIC was found to be 125 μg/mL. Hence this was determined as the true MIC value, and although it was not as potent as it originally seemed, THIQ **142b** was still the most potent compound of the series with the lowest MIC value.

The GIC data (Table 4.2) showed that the least cytotoxic compound to mammalian cells was the 6,7-dihydroxy-THIQ **145b** with a butyl C-1 substituent (GIC = 125 μg/mL). All of the 7,8-dihydroxy-THIQs **146a-d** had a GIC of 15.6 μg/mL. As also seen for the first generation THIQs, generally the GIC values were lower than the MIC values. Therefore the concentration of THIQ required to inhibit mycobacterial growth would also be toxic to mammalian cells. To optimise these compounds, the mammalian cell toxicity would need to be reduced whilst enhancing or at least maintaining the level of antimycobacterial activities.

4.1.5 Synthesis of third generation THIQs

The significant trend observed for the first and second generation THIQs was that the 7,8-dihydroxy-THIQs were generally more potent for *M. aurum* growth inhibition than

the 6,7-dihydroxy-THIQs. Therefore the third generation compounds were designed to focus on the activity of the aromatic ring substituents and to determine if the increase in potency arose from the presence of a hydroxy group at position C-8 or the removal of a hydroxy group at position C-6.

The target compounds (Figure 4.1) included THIQs with a single hydroxy group at positions C-6, C-7 and C-8, or a 6,8-dihydroxy substitution, to identify which hydroxy substituents increased the antimycobacterial activity of the compounds. Also, THIQs with a 5-bromo-8-hydroxy substitution were synthesised because this motif was previously found within the group to confer good antimycobacterial properties (described in section 3.2.2).[105] All of the target compounds were designed to retain the pentyl substituent at position C-1, which was found to confer the highest antimycobacterial activity in the SAR studies on the C-1 substituent.

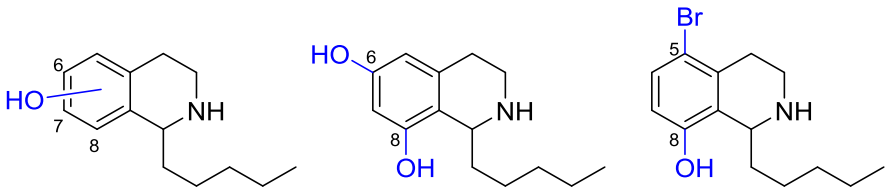


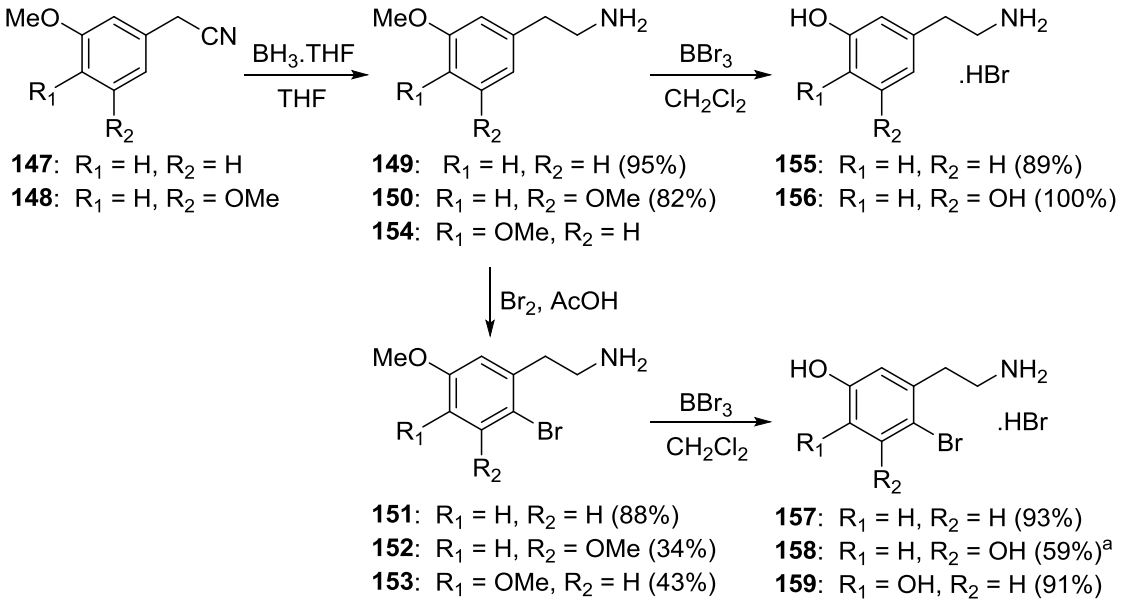
Figure 4.1. Target compounds for SAR of aromatic ring substituents.

Most of the target compounds were synthesised using the phosphate mediated Pictet-Spengler reaction.[122] Initial preparation of the hydroxyphenylethylamine starting materials was required (Scheme 4.5). These were prepared as previously reported via the reduction of commercially available methoxyphenylacetonitriles to the corresponding amines, followed by demethylation.[105,118a] Reduction of 3-methoxyphenylacetonitriles **147** and **148** was accomplished using borane (as the THF complex) in anhydrous tetrahydrofuran at room temperature for 24 hours. The crude products were purified by column chromatography using triethylamine as an additive to ensure the product remained as the uncharged free amine, to give the phenylethylamines **149**[118a] and **150**[151] in good yields (95% and 82% respectively).

Bromination of 3-methoxyphenylethylamine **149** was carried out based on a literature procedure[152] and involved the slow addition of bromine in acetic acid to the phenylethylamine at 15 ^{o}C (Scheme 4.5). After purification of the crude product by column chromatography, the mono-brominated compound **151** was obtained in 88%

yield. The same bromination method was followed to prepare compounds **152**[153] and **153**[154] from amines **150** and **154** (amine **154** was commercially available). Bromination of 3,5-dimethoxyphenylethylamine **150** yielded a mixture of the mono- and di-brominated products (under a carefully controlled reaction temperature and using 1 equivalent of bromine) that were formed due to the activation of the ring at the positions *para* to each methoxy group. These products were separated using column chromatography and the mono-brominated product **152** was isolated in 34% yield.

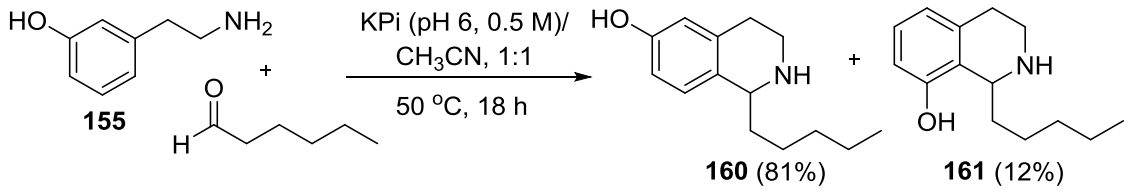
The final step was demethylation of the methoxy groups of the 3-methoxyphenylethylamine derivatives using boron tribromide in anhydrous dichloromethane (Scheme 4.5).[105,118a] When the reactions were complete they were quenched with methanol, and subsequent evaporation of the volatiles led to isolation of the 3-hydroxyphenylethylamine products **155-159** as the hydrobromide salt in good yields (89-100%). This work-up procedure proved to be more facile than quenching the reactions with water, as otherwise it was difficult to isolate the desired products from the aqueous phase. The dihydroxyphenylethylamine **158** was further purified by reverse phase prep-HPLC to remove any oxidised impurities. The phenylethylamine **155** prepared for this SAR study was also used by biological collaborators in a one-pot synthesis of an (*S*)-benzylisoquinoline alkaloid, using the enzymes transaminase (TAm) and norcoclaurine synthase (NCS) in a 'triangular' cascade.[120]



147: R_1 = H, R_2 = H
148: R_1 = H, R_2 = OMe
149: R_1 = H, R_2 = H (95%)
150: R_1 = H, R_2 = OMe (82%)
154: R_1 = OMe, R_2 = H
155: R_1 = H, R_2 = H (89%)
156: R_1 = H, R_2 = OH (100%)
151: R_1 = H, R_2 = H (88%)
152: R_1 = H, R_2 = OMe (34%)
153: R_1 = OMe, R_2 = H (43%)
157: R_1 = H, R_2 = H (93%)
158: R_1 = H, R_2 = OH (59%)[a]
159: R_1 = OH, R_2 = H (91%)

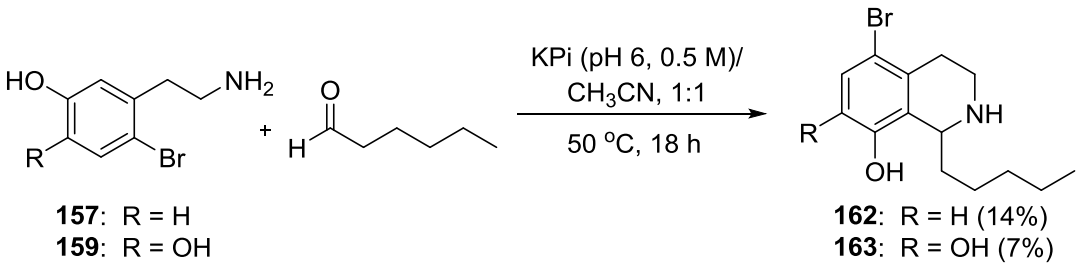
Scheme 4.5. Synthesis of hydroxyphenylethylamines. [a]Isolated yield of the TFA salt after HPLC.

The target THIQ compounds **160** and **161** were prepared from the phosphate mediated Pictet-Spengler reaction between phenylethylamine **155** and hexanal (Scheme 4.6). The reaction was carried out in a mixture of potassium phosphate buffer (pH 6, 0.5 M) and acetonitrile at 50 ^{o}C. Two regioisomers were generated and were separated by reverse phase prep-HPLC. The major product 6-hydroxy-THIQ **160** was isolated in 81% yield and the minor product 8-hydroxy-THIQ **161** in 12% yield.



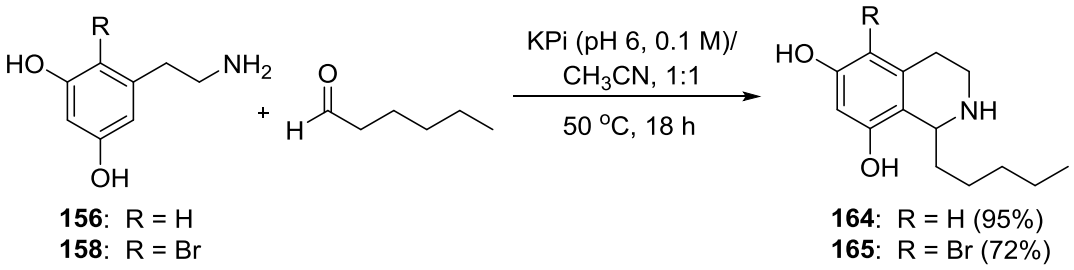
Scheme 4.6. Synthesis of target compounds **160** and **161** with a single hydroxy group.

The target THIQ compounds **162** and **163** with a 5-bromo-8-hydroxy substitution were also synthesised using the phosphate mediated Pictet-Spengler reaction (Scheme 4.7). The reaction between phenylethylamines **157** and **159** with hexanal yielded the desired products **162** and **163** in low yields of 14% and 7%. The low yields were most likely to result from a combination of sterics and electronics of the intermediate imine. Cyclisation of the imine proceeds at the more sterically hindered position *ortho* to the hydroxy group, and the aromatic ring may not be sufficiently electron rich to promote the cyclisation to achieve good yields. Usually the position *para* to the activating hydroxy group would be favoured for Pictet-Spengler reactions but this position was blocked by the bromine substituent. Despite the low yields, sufficient quantities of the products were isolated for the antimycobacterial activity assays.



Scheme 4.7. Synthesis of target compounds with a 5-bromo-8-hydroxy substitution.

The 3,5-dihydroxyphenylethylamines **156** and **158** were excellent substrates for the phosphate mediated Pictet-Spengler reaction (Scheme 4.8). The advantage of using amines with a 3,5-dihydroxy substitution was that cyclisation of the intermediate imine yielded one product and avoided a mixture of regioisomers. In addition, both the hydroxy substituents of **156** and **158** activated the aromatic ring for cyclisation to the same position, so although cyclisation occurred at a position which was sterically hindered (*ortho* to a hydroxy group), the ring was sufficiently activated to achieve good yields of the cyclised products. The target compound **164** was isolated after reverse phase HPLC in an excellent yield of 95%, and the bromo analogue **165** isolated in 72%.



156: R = H
158: R = Br

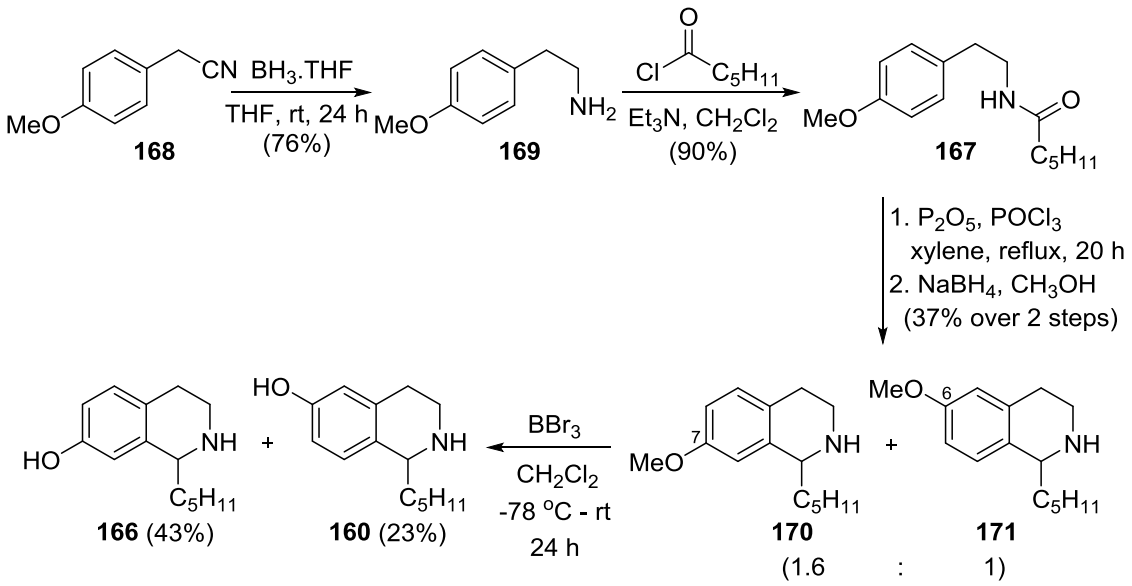
164: R = H (95%)
165: R = Br (72%)

Scheme 4.8. Synthesis of target compounds with a 6,8-dihydroxy substitution.

The final target compound was THIQ **166** with a single hydroxy group at the C-7 position (Scheme 4.9). This compound lacked an electron donating hydroxy group in a position which would activate the aromatic ring to cyclise in a Pictet-Spengler reaction (see sections 3.3.2 and 3.3.4).[113,122] Therefore a Bischler-Napieralski reaction (described in section 3.3.1) was employed to prepare THIQ **166**.[155] The synthesis began with the preparation of phenylethylamide **167** (Scheme 4.9). The commercially available phenylacetonitrile **168** was reduced to amine **169**[156] in 76% yield and then reacted with hexanoyl chloride to form the desired amide **167** in 90% yield.

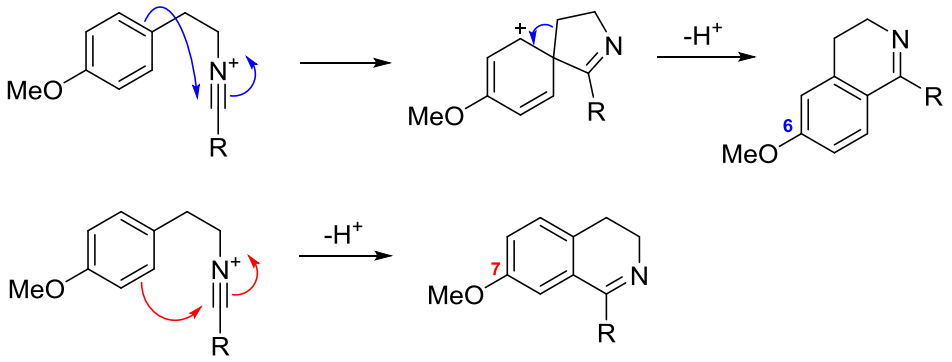
The Bischler-Napieralski cyclisation of phenylethylamide **167** required forcing conditions (Scheme 4.9). Initially phosphorus oxychloride was employed as the dehydrating agent and used in acetonitrile at reflux. These conditions did not produce any of the cyclised product. The solvent was changed to toluene to enable a higher reaction temperature but also using these conditions did not yield any cyclised product. Next, a procedure reported for the synthesis of similar 7-methoxy-THIQs was

followed.[155] A mixture of phosphorus oxychloride and phosphorus pentoxide in refluxing xylene, followed by reduction of the cyclic imine intermediate with sodium borohydride produced a mixture of the 7-methoxy-THIQ **170** and the unexpected regioisomer 6-methoxy-THIQ **171**. The regioisomers could not be separated by HPLC and were isolated as a mixture in 37% yield (ratio **170**/**171**, 1.6:1). The desired 7-methoxy-THIQ **170** was assigned as the major product as determined by ^{1}H-NMR spectroscopy. The final step was the demethylation of the methoxy groups using boron tribromide to yield the hydroxy-THIQs **166** and **160**. These regioisomers could be separated by reverse phase prep-HPLC and the target compound **166** was isolated in 43% yield.



Scheme 4.9. Synthesis of the target compound with a 7-hydroxy substitution.

A mechanism for the formation of the unexpected regioisomer **171** generated in the Bischler-Napieralski reaction has been reported.[157] It was proposed that the use of phosphorus pentoxide would generate a nitrile intermediate (Scheme 4.10). Attack of the aromatic ipso carbon onto the nitrile in a 5-endo dig cyclisation forms a spirocyclic carbocation which rearranges to the cyclic imine with the methoxy substituent at the C-6 position.



Scheme 4.10. Proposed mechanism by S. Doi *et al*. for the formation of 6-methoxy- and 7-methoxy-THIQs under Bischler-Napieralski reaction conditions.

4.1.6 Antimycobacterial activity of third generation THIQs

The third generation THIQs were screened for their growth inhibition of *M. aurum* and cytotoxicity against mammalian cells as previously described for the first and second generation compounds (Table 4.3). Analysis of the MIC values revealed three key trends. The first trend was that a hydroxy group at the C-8 position (THIQ **161**, MIC = 250 μg/mL) enhanced the antimycobacterial activity more than a hydroxy group at position C-6 or C-7 (THIQs **160** and **166**, MICs = 500 μg/mL). The second important SAR was that despite the C-8 hydroxy group conferring optimal mycobacteria growth inhibition, the presence of a hydroxy group at the C-6 position was tolerated without loss of activity. This was demonstrated by the comparison of inhibition data for the 8-hydroxy-THIQ **161** with 6,8-dihydroxy-THIQ **164** which both had a MIC of 250 μg/mL. The 6,8-dihydroxy-THIQ derivatives could be synthesised using the phosphate mediated Pictet-Spengler reaction in excellent yields and further series of compounds containing this pharmacophore could easily be prepared for further SAR studies.

The third key trend was that the presence of a bromine substituent at the C-5 position on the THIQs improved the mycobacteria growth inhibition activity. This was consistent with previous studies in the group for 5-bromo-8-hydroxy analogues of phenyl or benzyl C-1 substituted THIQs (described in section 3.2.2).[105] For example, when comparing 8-hydroxy-THIQ **161** to 5-bromo-8-hydroxy-THIQ **162**, a decrease in MIC from 250 μg/mL to 62.5 μg/mL was observed. Similarly, when comparing 6,8-dihydroxy-THIQ **164** to 5-bromo-6,8-dihydroxy-THIQ **165**, a decrease in the MIC from 250 μg/mL to 125 μg/mL was observed. The most potent compound was THIQ **162**

(MIC = 62.5 μg/mL) and was also the most lipophilic compound of the series with a calculated LogP (ACD) of 4.16. It was possible that the higher lipophilicity of THIQ **162** improved its permeability across the highly waxy mycobacterial cell wall.[150]

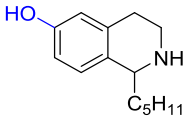
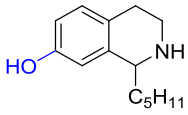
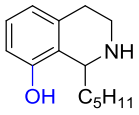
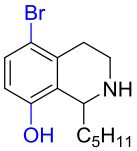
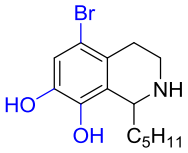
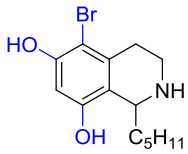
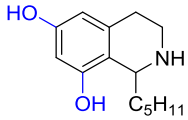
THIQ	Mwt	calc LogP (ACD)	MIC *M. aurum* (μg/mL)	GIC RAW 264.7 (μg/mL)
160 	219.3	3.42	500	62.5
166 	219.3	3.42	500	125
161 	219.3	3.42	250	31.3
162 	298.2	4.16	62.5	15.6
163 	314.2	3.81	250	31.3
165 	314.2	3.81	125	31.3
164 	235.3	3.09	250	62.5

Table 4.3. Antimycobacterial properties of the third generation THIQs. MIC = lowest concentration at which no growth of mycobacteria was observed. GIC = concentration at which more than 90% inhibition of cell viability (RAW 264.7) was observed.

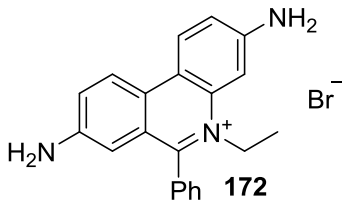
The GIC data showed that the THIQs displayed some cytotoxicity towards mammalian cells (Table 4.3), and were therefore not selectively toxic towards mycobacterial cells. The least cytotoxic compound was the 7-hydroxy-THIQ **166** with a GIC of 125 μg/mL. The THIQs containing a bromo substituent (**162**, **163** and **165**) that were the most active

against the growth of *M. aurum* also displayed the most cytotoxicity towards mammalian cells (GIC = 15.6-31.3 μg/mL). In order to be useful as potential anti-mycobacterial drugs, the concentration of the THIQs required for antimycobacterial activity would need to be at least two fold lower than the GIC values.

4.2 Efflux pump inhibition

The first, second and third generation THIQ compounds were screened in a mycobacterial efflux pump inhibition assay, to probe whether efflux pump inhibition (described in section 3.1.4) was a possible mode of action. The screening was carried out by biological collaborators in the Bhakta group at Birkbeck.[v] A real-time fluorometric method based on a protocol reported by Rodrigues *et al*. was employed to evaluate the efflux pump inhibition ability of the THIQs against *M. aurum*.[158]

The assay involved the use of ethidium bromide **172** (Figure 4.2), a commonly used efflux pump substrate.[158] Ethidium bromide **172** has a low fluorescent signal when outside the mycobacterial cell, but becomes strongly fluorescent once inside the cell in a concentration dependent manner. When the test compound and ethidium bromide **172** were added to *M. aurum*, an increase in fluorescence over time indicated that ethidium bromide was accumulating inside the *M. aurum* cells, suggesting that efflux pump activity was inhibited. Two known potent efflux pump inhibitors (verapamil **81** and chlorpromazine **82**, Figure 3.4) were used as positive controls. Experiments containing only the viable mycobacterial cells and ethidium bromide **172** served as negative controls. In order to ensure mycobacterial cell viability were unaffected, a concentration of quarter the MIC of the THIQ compounds was used in the assay.



172

Figure 4.2. Efflux pump substrate ethidium bromide **172**.

[v] The efflux pump inhibition assays were carried out by Arundhati Maitra and Dr Parisa N. Mortazavi in the Bhakta group in the Mycobacterial Research Laboratory at Birkbeck, University of London.

A number of the first and second generation THIQs showed mycobacterial efflux pump inhibitory activity (Figure 4.3 shows an example of the data obtained from the efflux inhibition assay. For the complete set of results and graphs see Appendix chapter 9). To compare the efflux inhibitory activity of the THIQ compounds, an efflux value was assigned using a scale of 0 to 4 (Table 4.4). The efflux value reflected the amount of accumulation of ethidium bromide **172** in *M. aurum* after 60 minutes (Figure 4.3). The positive control containing the efflux pump inhibitor verapamil **81** had the highest fluorescence intensity in the experiments and was assigned an efflux value of 4 (efflux value 4 = accumulation of ethidium bromide comparable with verapamil, representing compounds with potent efflux pump inhibitory activity). The negative control containing no efflux pump inhibitor was assigned a value of 0 (efflux value 0 = accumulation of ethidium bromide was not above the levels of the negative control, representing compounds with no efflux pump inhibitory activity).

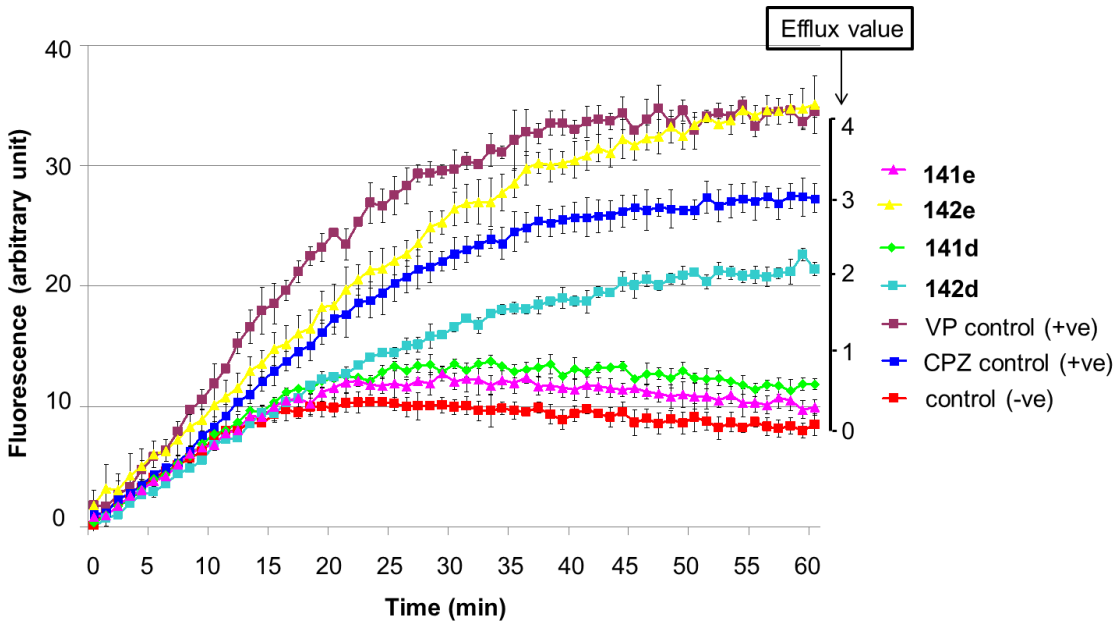
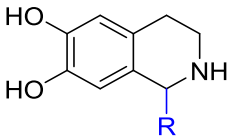
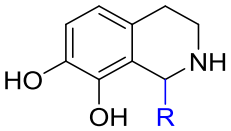


Figure 4.3. Effect of THIQs on the accumulation of ethidium bromide in *M. aurum* (VP = verapamil **81** and CPZ = chlorpromazine **82**)





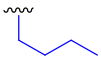
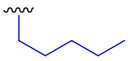
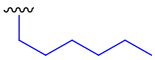
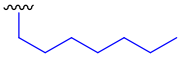
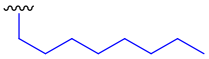
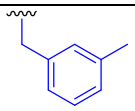
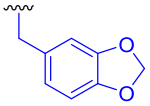
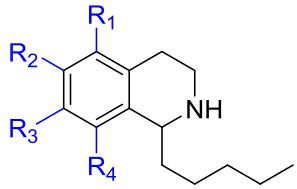
R	THIQ	Efflux value	THIQ	Efflux value
	**141a**	0	**142a**	2
	**145a**	0	**146a**	2
	**145b**	0	**146b**	2
	**141b**	0	**142b**	2
	**145c**	1	**146c**	3
	**145d**	1	**146d**	0
	**141c**	1	**142c**	3
	**141d**	0	**142d**	2
	**141e**	0	**142e**	4
	**141f**	0	**142f**	3

Table 4.4. *M. aurum* efflux pump inhibitory activity of the first and second generation THIQs. Efflux values were assigned using a scale of 0-4, where 0 = accumulation of ethidium bromide not above the levels of the negative control, and 4 = accumulation of ethidium bromide comparable with the efflux pump inhibitor verapamil.

The 6,7-dihydroxy-THIQs **141a-f** and **145a-d** were either poor efflux pump inhibitors or displayed no efflux pump inhibition with efflux values = 0-1 (Table 4.4). In contrast, the data indicated that the 7,8-dihydroxy-THIQs were efflux pump inhibitors. The 7,8-dihydroxy-THIQs **142a-d** and **146a-d** with aliphatic substituents at the C-1 position had efflux values between 2-3 and promoted the accumulation of ethidium bromide in *M. aurum*. An exception was compound **146d** which did not show any efflux pump inhibitory activity (efflux value = 0). The 7,8-dihydroxy-THIQs **142e-f** containing a benzylic C-1 substituent also showed an increase in the fluorescence intensity, and

compound **142f** promoted the highest accumulation of ethidium bromide (efflux value = 4) which was comparable to verapamil **81**. A correlation between efflux pump activity and the MICs of the first and second generation THIQs was not observed. The results strongly suggested that the 7,8-dihydroxy substitution on the THIQs was contributing to the whole-cell efflux pump inhibitory activity.

The third generation THIQs were also screened in the efflux pump inhibition assay (Table 4.5, Figure 4.4 and for the complete set of results and graphs see Appendix Chapter 9). The data for THIQs **160** (efflux value = 1), **166** (efflux value = 4) and **161** (efflux value = 0) containing a single hydroxy group clearly indicated that a hydroxy group at the C-7 position promoted the highest accumulation of ethidium bromide in *M. aurum*. The 7-hydroxy-THIQ **166** was also able to inhibit efflux slightly better than the known efflux pump inhibitor verapamil (Figure 4.4). Interestingly, THIQ **161** with a single hydroxy group at the C-8 position (which was found to enhance the MIC values) showed no efflux pump inhibitory activity. The 6,8-dihydroxy-THIQs **164** and **165** with no C-7 hydroxy group also showed no efflux pump inhibitory activity. The data indicated that the brominated 7,8-dihydroxy-THIQ **163** (efflux value = 4) inhibited efflux in *M. aurum* and suggested that the presence of an 8-hydroxy group was tolerated without loss of efflux inhibitory activity. In comparison, a 6,7-dihydroxy substitution pattern generally resulted in THIQs with little or no efflux inhibitory activity (Table 4.4), even though a C-7 hydroxy substituent was present.



THIQ	R_1	R_2	R_3	R_4	Efflux value
160	H	OH	H	H	1
166	H	H	OH	H	4
161	H	H	H	OH	0
162	Br	H	H	OH	2
163	Br	H	OH	OH	4
165	Br	OH	H	OH	0
164	H	OH	H	OH	0

Table 4.5. *M. aurum* efflux pump inhibitory activity of the third generation THIQs.

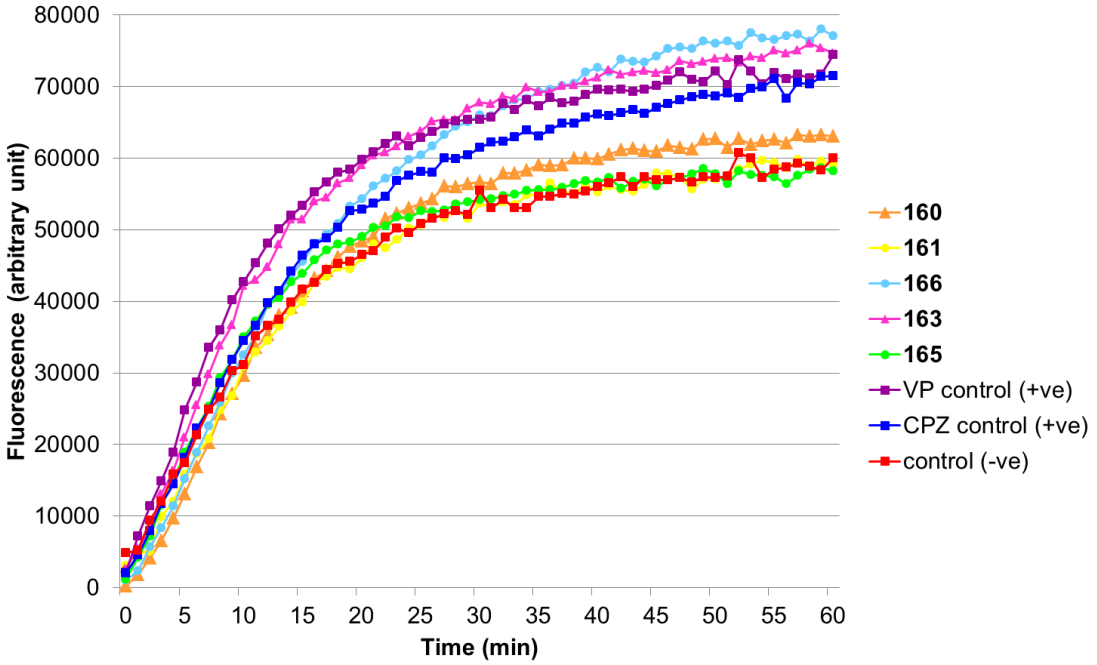


Figure 4.4. Effect of third generation THIQs on the accumulation of ethidium bromide in *M. aurum* (VP = verapamil **81** and CPZ = chlorpromazine **82**)

4.3 Synergism

Using efflux pump inhibitors in combination with existing anti-TB drugs represents a possible strategy for preventing the emergence of drug resistant TB and improving the efficacy of drugs that are subject to efflux (see section 3.1.4).[98] Several efflux pumps have been identified in various species of mycobacteria that are associated with low level drug resistance. For example, the first-line anti-TB drug rifampicin **73** (Figure 3.1) has been found to cause low level resistance in *M. smegmatis*, *M. aurum* and *M. tuberculosis*.[159] Also, efflux activity in *M. tuberculosis* has been shown to contribute to resistance to the first-line drugs isoniazid **72** and ethambutol **75**.[160]

The efflux pump inhibition assay results of the first, second and third generation THIQs (section 4.2) suggested that the 7,8-dihydroxy-THIQs and the 5-bromo-8-hydroxy-THIQ showed *M. aurum* efflux pump inhibitory activity. Six of the THIQs that showed efflux pump activity were selected for screening in a synergism assay (Figure 4.5), which was carried out by biological collaborators at Birkbeck.[vi] In addition, the 5-

[vi] The synergism assays were carried out by Arundhati Maitra in the Bhakta group in the Mycobacterial Research Laboratory at Birkbeck, University of London.

bromo-8-hydroxy-THIQ **91** (Figure 4.5) was selected for screening in the synergism assay because it was previously demonstrated to inhibit the growth of *M. bovis* BCG in the Hailes and Bhakta groups.[105]

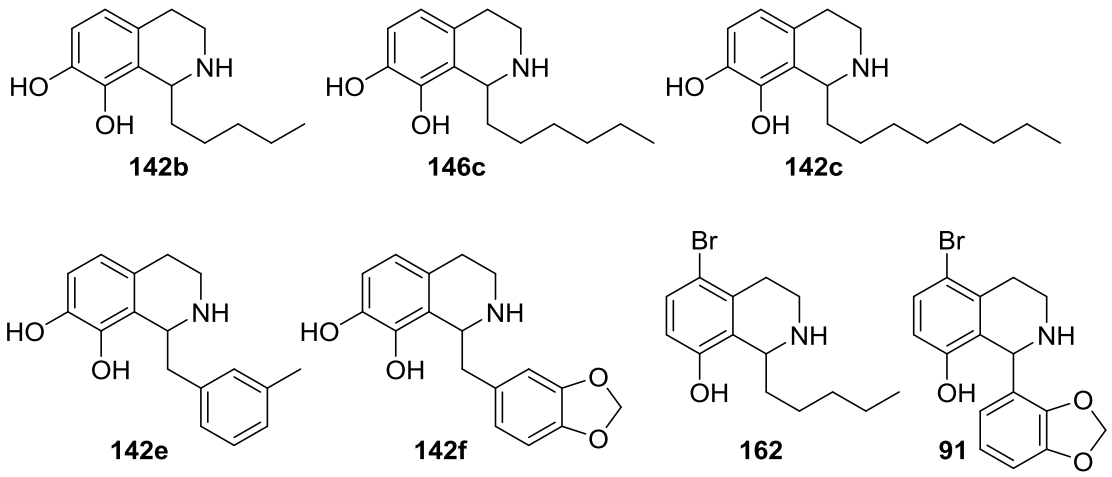


Figure 4.5. THIQ compounds selected for synergism studies.

The THIQs were evaluated in combination with the anti-TB drugs isoniazid **72**, rifampicin **73** and ethambutol **75** (Figure 3.1) for their MICs against *M. aurum* and evaluated for possible synergistic interactions (Table 4.6).

The MIC of isoniazid **72** alone was 0.938 μg/mL. In the presence of THIQ **162** at a concentration of 62.5 μg/mL the MIC of isoniazid was reduced 2-fold to 0.468 μg/mL. The other THIQs that were evaluated in combination with isoniazid **72** either did not show any reduction in the MIC or had a negative effect on the MIC (increased to 1.875 μg/mL). It was possible that the THIQs were involved in preventing isoniazid from its entry into the cell and/or any endogenous antitubercular mechanisms of actions.

The MIC of rifampicin **73** against *M. aurum* alone was 0.625 μg/mL. When rifampicin **73** was used in combination with the THIQs **142b**, **142c**, **142e**, **162** and **91** the MIC decreased 2-fold to 0.312 μg/mL. Particularly noteworthy were THIQs **142c** (featuring an octyl alkyl chain at the C-1 position) and **91** (featuring a 5-bromo-8-hydroxy substitution) because the decrease in the MIC was achieved when these compounds were used at a low concentration of 0.1 μg/mL.

Anti-TB drug (MIC/ μg/mL)	THIQ	MIC in combination/ μg/mL (THIQ conc/μg/mL)	Fold reduction of MIC
Isoniazid **72** (MIC = 0.938)	**142b**	**1.875** (62.5)	0
	146c	**1.875** (31.3)	0
	142c	**1.875** (125)	0
	142e	0.938	0
	142f	**1.875** (62.5)	0
	162	**0.468** (62.5)	2
	91	0.938	0
Rifampicin **73** (MIC = 0.625)	**142b**	**0.312** (15.6)	2
	146c	0.625	0
	142c	**0.312** (0.1)	2
	142e	**0.312** (125)	2
	142f	0.625	0
	162	**0.312** (0.97)	2
	91	**0.312** (0.1)	2
Ethambutol **75** (MIC = 0.312)	**142b**	**0.150** (125)	2
	146c	**0.150** (0.1)	2
	142c	0.312	0
	142e	0.312	0
	142f	0.312	0
	162	**0.150** (15.6)	2
	91	0.312	0

Table 4.6. Effect of selected THIQs on the MIC of first-line anti-TB drugs.

The MIC of ethambutol **75** against *M. aurum* was 0.312 μg/mL. The MIC of ethambutol **75** was reduced 2-fold when used in combination with the THIQs **142b** and **146c**, both featuring an alkyl chain at the C-1 position. THIQ **146c** contributed to lowering the MIC when used at a lower concentration of 0.1 μg/mL compared to THIQ **142b** which required a concentration of 125 μg/mL. The 7,8-dihydroxy-THIQs **142e** and **142f** featuring a benzylic C-1 substitutent did not show any reduction in the MIC of ethambutol **75**. THIQ **162** with a 5-bromo-8-hydroxy substitution reduced the MIC of ethambutol **75** 2-fold at a low concentration of 15.6 μg/mL.

Synergism has previously been defined as when the activity of a combination of two drugs against a given microorganism is greater than the sum of the individual activities of each drug.[100] A combination of two compounds is said to be synergistic when the

FICI (fractional inhibitory concentration index) is less than or equal to 0.5.[161] The combination of the THIQs with the anti-TB drugs that resulted in a 2-fold decrease in the MIC (compared to the MIC of the anti-TB drug alone) all had a FICI value of 0.5, and could be described as acting synergistically.

THIQ **162** enhanced the activity of all three of the anti-TB drugs tested. Other promising THIQs included **142b**, **142c** and **91** which were able to reduce the MIC of rifampicin **73** at a low concentration, and THIQ **146c** when used in combination with ethambutol. These preliminary investigations have shown the potential of THIQs to improve the activity of existing anti-TB drugs, possibly through whole-cell efflux pump inhibition. Evaluating these compounds in a synergism study using *M. tuberculosis* would allow comparisons to other efflux pump inhibitors reported in the literature.

4.4 Phenol bioisosteres

Phenolic compounds are often prone to metabolism via oxidative reactions of the phenolic hydroxy group by cytochrome P450 enzymes, generating reactive quinones that have been associated with toxicological issues.[162] In addition, phase II conjugation reactions are significant metabolic pathways such as glucuronidation (glucuronic acid is conjugated to the phenolic hydroxy group) and sulfation (sulfate is conjugated to the hydroxy group to form a phenyl sulfate).[163] This can reduce the oral bioavailability of the compounds.

The first, second and third generation THIQs all contained at least one hydroxy substituent on the aromatic ring. Based on the SAR results, the hydroxy group appeared to play an important role in the antimycobacterial activities of these compounds (sections 4.1.2, 4.1.4 and 4.1.6). An investigation into bioisosteres (defined as groups which have physiochemical similarities producing broadly similar biological effects) of phenols would be useful to identify compounds that may be less susceptible to metabolic inactivation and have an improved safety profile, whilst retaining antimycobacterial activity. This could lead to the development of more clinically relevant drug-like compounds.

The presence of a hydroxy group at the C-8 position of the THIQ scaffold resulted in compounds with the best antimycobacterial activities. Therefore target bioisosteric

analogues were designed by replacing the C-8 hydroxy group (Figure 4.6). THIQ **173** featured an 8-amino group which represents a classical bioisosteric replacement of a hydroxy group, but does affect acid-base properties. Alternative bioisosteres that have been reported include methanesulfonamides or ureas, and the sulfonamide N-H has been shown to closely resemble the phenol O-H in terms of acidity and hydrogen bond donor properties.[164] Replacement of phenols with heterocyclic rings such as indoles has also been used, as indoles can form hydrogen bonds in a similar manner to phenol hydroxy groups.[165] The target compounds **174-176** were designed based on these known bioisosteric replacements (Figure 4.6).

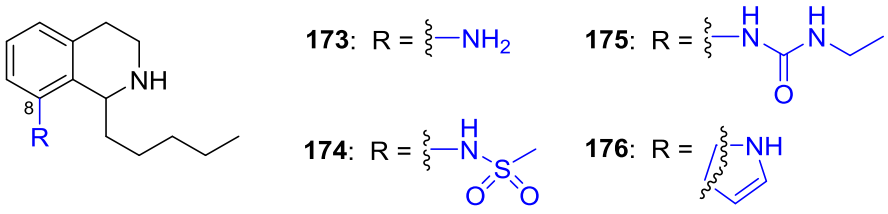
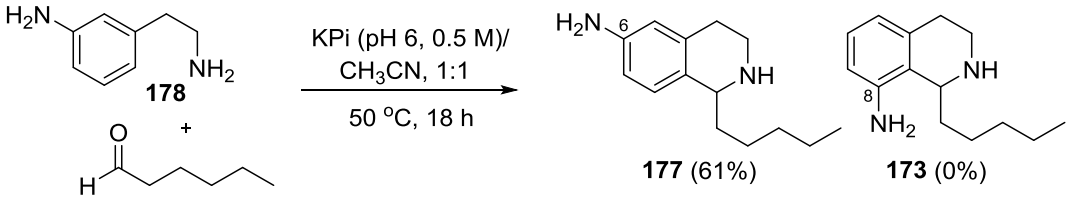


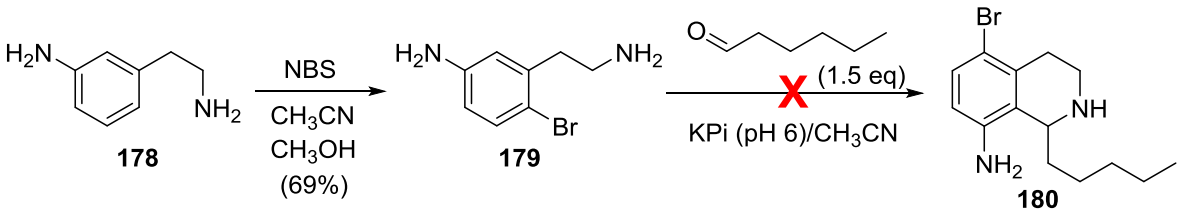
Figure 4.6. Target THIQ phenol bioisosteres.

Investigating a route to prepare the 8-amino-THIQ **173** was initially undertaken (Scheme 4.11). The phosphate mediated Pictet-Spengler reaction has been shown to extend to the use of aminophenylethylamine substrates for the preparation of amino-THIQs.[122] It was anticipated that using this method might yield a mixture of the regioisomers 6-amino-THIQ **177** and the desired 8-amino-THIQ **173**, as was observed previously for the hydroxy-THIQ series (section 4.1.1). 3-Aminophenylethylamine **178** was first prepared by hydrogenation of 3-nitrophenylethylamine using a Pd/C catalyst.[122] The Pictet-Spengler reaction between amine **178** and hexanal was then carried out under standard conditions in a 1:1 mixture of phosphate buffer (pH 6) and acetonitrile at 50 ^{o}C. After purification of the product by reverse phase prep-HPLC, the 6-amino-THIQ **177** was isolated in 61% yield, but unfortunately the regioisomer 8-amino-THIQ **173** was not detected.



Scheme 4.11. Phosphate mediated Pictet-Spengler reaction of aniline derivatives.

In an attempt to block the cyclisation occurring at the position *para* to the amino group, a bromine substituent was added at this position to ensure cyclisation occurred at the position *ortho* to the amino group. The required brominated starting material **179** was first prepared from 3-aminophenylethylamine **178** using *N*-bromosuccinimide in acetonitrile (Scheme 4.12).[166] The Pictet-Spengler reaction was carried out in a 1:1 mixture of phosphate buffer (pH 6, 0.1 M) and acetonitrile at 50 °C (Scheme 4.12, Exp. 1). After 24 hours an aliquot of the reaction mixture was analysed by mass spectrometry. The desired 8-amino-THIQ **180** was not detected. A further equivalent of hexanal was added to the reaction mixture and after 24 hours the reaction was analysed by mass spectrometry again. Only starting materials were detected, and none of the desired product. The reaction was repeated under more forcing conditions at a temperature of 80 °C and using a higher concentration of phosphate buffer (0.5 M), but again none of the desired 8-amino-THIQ **180** was formed (Scheme 4.12, Exp. 2).



Exp.	KPi conc.	Temp.	Time
1	0.1 M	50 °C	24 h
2	0.5 M	80 °C	24 h
3	0.01 M	rt	24 h
4	0.1 M	rt	24 h
5	1 M	rt	24 h

Scheme 4.12. Attempted phosphate mediated Pictet-Spengler reaction for the synthesis of THIQ **180**.

A further three experiments were carried out on a small scale to study the effect of lowering the phosphate concentration and performing the reactions at room temperature for a longer period of time (Scheme 4.12, Exp. 3-5). These conditions were investigated because the Maresh group have reported that for the phosphate mediated Pictet-Spengler synthesis of hydroxy-THIQs, lowering the phosphate concentration and carrying out the reaction at room temperature yielded higher amounts of products where cyclisation occurred *ortho* to the activating group.[119] However under these conditions no amino-THIQ **180** was detected when the reactions were monitored by mass spectrometry. Mostly the starting material **179** was detected, as well as side products which were not characterised.

In order to determine why the Pictet-Spengler reaction did not occur at the position *ortho* to the activating amino group, an analysis of the side products formed would be beneficial. It was possible that the aniline was reacting with the aldehyde and forming an imine which would increase steric hinderance at the position *ortho* to the aniline group, as well as decreasing the electrophilicity of the aromatic ring. The 8-amino-THIQs **173** and **180** would have been a good starting point for the preparation of the phenol bioisostere compounds **174** and **175** (Figure 4.6). Therefore further exploration of these bioisosteres was not continued.

4.5 Lactam/lactone synthesis from 1-substituted THIQs

Benzo[*a*]quinolizines and pyrroloisoquinolines are tricyclic THIQ derivatives. These structures are found in a number alkaloids including schulzeines A-C, (-)-trolline and crispine A as well as in pharmaceuticals such as tetrabenazine, and display a variety of pharmacological activities including antibacterial activity (Figure 4.7).

The δ-lactam and γ-lactam rings present in compounds such as schulzeines A-C and trolline (Figure 4.7) have previously been constructed via intramolecular cyclisation reactions. These include a trifluoromethanesulfonic acid mediated imide carbonyl activation and cyclisation strategy,[167] an acid catalysed acyl-Pictet-Spengler reaction[168] and intramolecular amidation reactions,[169] and often require protecting groups for phenolic hydroxy groups and multi-step procedures.

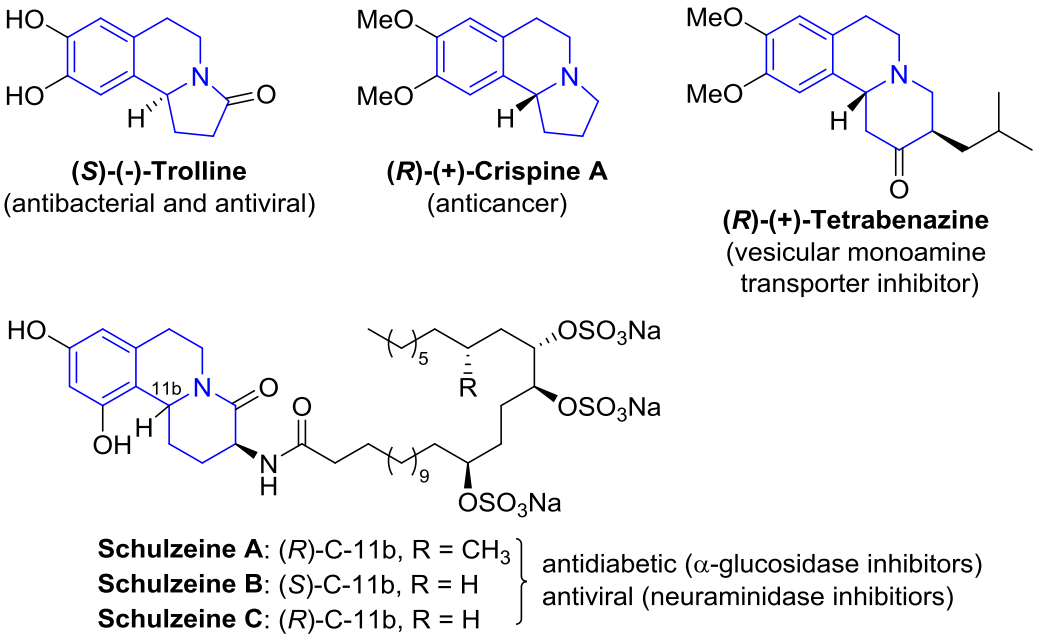
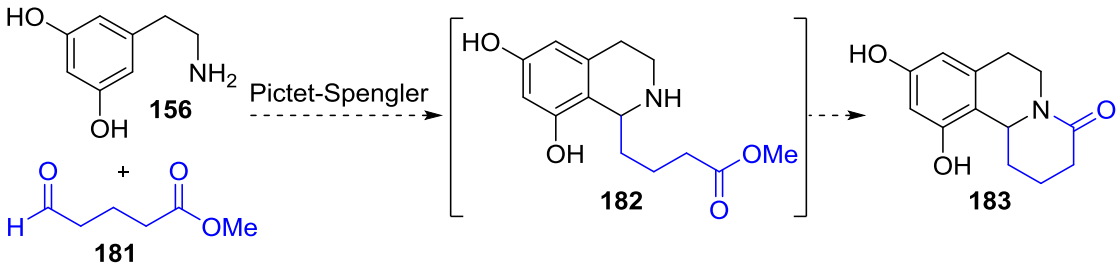


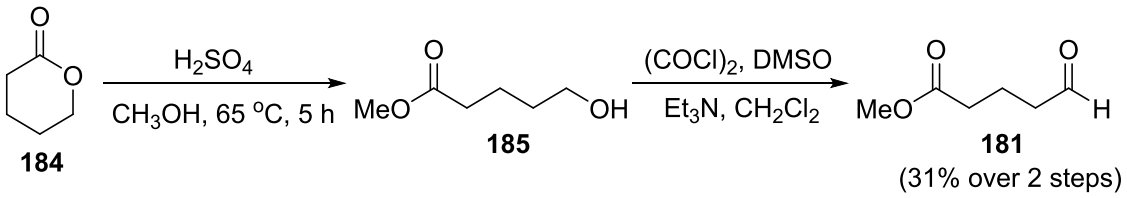
Figure 4.7. Benzo[*a*]quinolizine and pyrroloisoquinoline bioactive alkaloids.

It was envisioned that the phosphate mediated Pictet-Spengler reaction between a phenylethylamine and methyl 5-oxopentanoate **181** containing an ester functional group would yield the 1-substituted THIQ **182** (Scheme 4.13). The ester functionality could then spontaneously cyclise via an intramolecular amidation to form the δ-lactam **183**, generating the benzo[*a*]quinolizin-4-one tricyclic structure. To explore this hypothesis, the 3,5-dihydroxyphenylethylamine **156** was chosen as the starting material because it was already shown to cyclise under the phosphate mediated Pictet-Spengler conditions in excellent yields to generate a single THIQ product (see section 4.1.5).



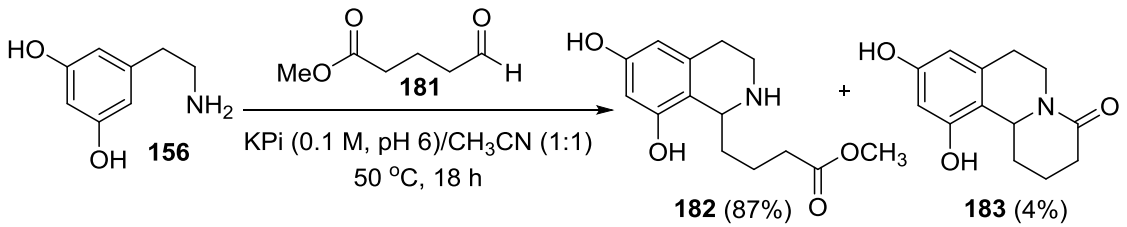
Scheme 4.13. Potential one-pot Pictet-Spengler/lactamisation reaction.

Aldehyde **181** was prepared following literature procedures (Scheme 4.14).[170] The commercially available δ-valerolactone **184** was opened to the corresponding alcohol **185**, and then a Swern oxidation afforded aldehyde **181** in 31% yield over the two steps. This yield was significantly lower than that reported in the literature. The actual yield of the aldehyde was likely to have been higher but some material was lost due to the volatility of the aldehyde and during purification by column chromatography.



Scheme 4.14. Synthesis of methyl 5-oxopentanoate.

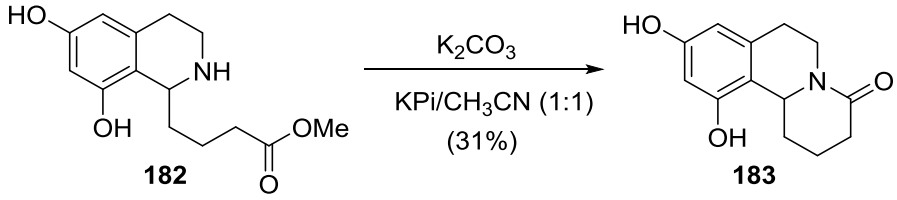
The phosphate mediated Pictet-Spengler reaction between phenylethylamine **156** and aldehyde **181** was carried out at 50 °C overnight, yielding the 1-substituted THIQ **182** as the major product and a small amount of lactam **183** (Scheme 4.15). The mixture of products was separated by reverse phase prep-HPLC, and THIQ **182** was isolated in 87% yield and lactam **183** in 4% yield. Therefore under these conditions, the cyclisation of THIQ **182** to the lactam **183** was not favoured.



Scheme 4.15. Pictet-Spengler cyclisation of amine **156** with aldehyde **181**.

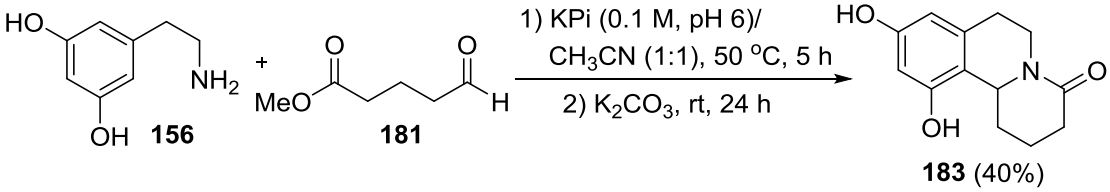
Published procedures for lactam synthesis from esters often require basic reaction conditions.[169] Taylor *et al.* have reported the cyclisation of amino esters to the corresponding lactams in excellent yields when using potassium carbonate in a mixture

of methanol and water.[171] These reaction conditions were interesting because after formation of THIQs from the phosphate mediated Pictet-Spengler reaction, the addition of potassium carbonate could then facilitate lactam formation in one pot. To investigate this, THIQ **182** was added to a mixture of phosphate buffer (pH 6, 0.1 M) and acetonitrile, then 1 equivalent of potassium carbonate was added (Scheme 4.16). After stirring at room temperature for 24 hours, lactam **183** was isolated in 31% yield.



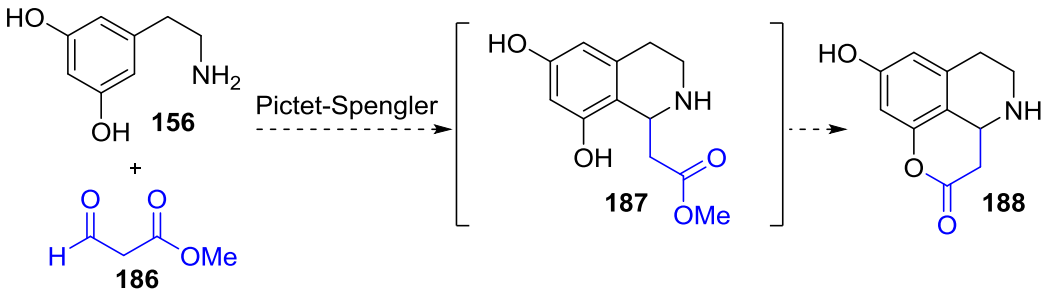
Scheme 4.16. Lactam synthesis using basic conditions.

It was probable that the conditions used for the construction of the δ-lactam would be able to be optimised by exploring various parameters such as the effect of pH, the equivalents of base and the reaction temperature. However due to only having a small amount of THIQ **182** remaining towards the end of the project, the one-pot synthesis of the lactam from phenylethylamine **156** and aldehyde **181** was attempted at this stage (Scheme 4.17). The Pictet-Spengler reaction was carried out under the standard conditions, and after 5 hours all of the phenylethylamine **156** starting material had converted to the THIQ intermediate **182** (monitored by LC-MS). Subsequent addition of potassium carbonate to the reaction mixture promoted the lactamisation to generate lactam **183** which was isolated in 40% yield. To improve the yield, optimisation of the lactamisation step would be necessary, as described above.



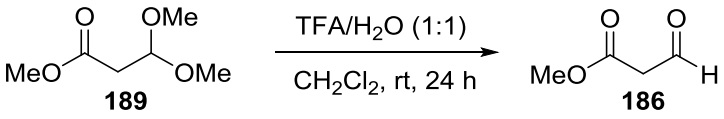
Scheme 4.17. One-pot Pictet-Spengler/lactamisation reaction.

In addition to investigating δ-lactam synthesis from 1-substituted THIQs, an intramolecular cyclisation to form lactones was also explored. It was anticipated that the phosphate mediated Pictet-Spengler reaction between a phenylethylamine and methyl 3-oxopropanoate **186** would yield the 1-substituted THIQ **187** (Scheme 4.18). The ester functionality could spontaneously cyclise onto the C-8 hydroxy group to produce lactone **188**, generating a tricyclic structure. The synthesis of tricyclic THIQ lactone structures such as **188** have not previously been reported in the literature.



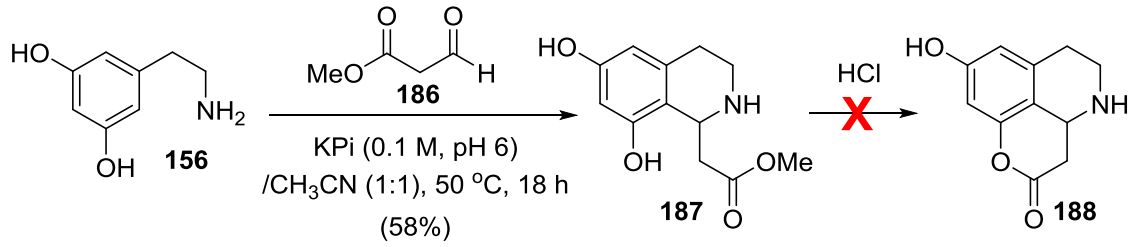
Scheme 4.18. Potential one-pot Pictet-Spengler/lactonisation reaction.

Aldehyde **186** was prepared from the commercially available dimethyl acetal **189** based on a literature procedure (Scheme 4.19).[172] The ^{1}H-NMR spectrum of the crude product confirmed the complete conversion of starting material to the product. However the purification of aldehyde **186** was not straightforward. Purification by column chromatography resulted in product decomposition and none of the aldehyde **186** was isolated. Therefore the crude product was used directly in the next step without purification.



Scheme 4.19. Synthesis of methyl 3-oxopropanoate.

The phosphate mediated Pictet-Spengler reaction between phenylethylamine **156** and aldehyde **186** yielded the 1-substituted THIQ **187** in a moderate yield of 58% (Scheme 4.20). No formation of lactone **188** was observed. Lactone formation from esters and phenolic hydroxy groups is often promoted by acidic conditions and high temperatures.[173] Therefore, THIQ **187** was stirred in dilute hydrochloric acid, but after 24 hours, analysis of the reaction mixture by LC-MS revealed that only starting material was present. The THIQ **187** was then dissolved in a mixture of TFA and dichloromethane and heated at reflux, but again only the starting material was isolated from the reaction after purification of the crude material using reverse phase prep-HPLC. It was possible that lactone **188** did form using these conditions, but was hydrolysed back to the starting materials during reverse phase HPLC purification or LC-MS analysis under the aqueous conditions. Therefore attempts to synthesise the lactone were not continued.

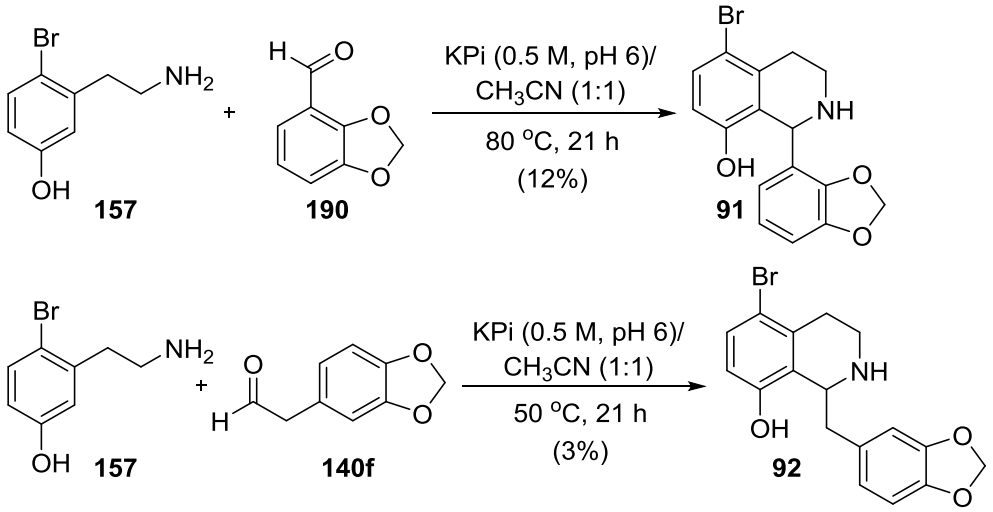


Scheme 4.20. Pictet-Spengler cyclisation of amine **156** with aldehyde **186** and attempted lactonisation.

4.6 Pharmacokinetic and pharmacodynamic properties

The THIQs **91** and **92** have previously been identified within the Hailes group as possessing potent antimycobacterial activity and moderate selectivity for mycobacterial cells (Figure 3.8, section 3.2.2).[105] These compounds were selected for pharmacokinetic analysis to determine their potential for progress into further drug development. THIQs **91** and **92** were prepared using the phosphate mediated Pictet-Spengler reaction as previously described (Scheme 4.21), but would also be possible to prepare via a Bischler-Napieralski route.[105] The reaction between amine **157** and commercially available 2,3-(methylenedioxy)benzaldehyde **190** generated the THIQ product **91** in 12% isolated yield after purification by reverse phase prep-HPLC. The reaction

between amine **157** and aldehyde **140f** generated THIQ **92** in only 3% isolated yield. The Pictet-Spengler cyclisation can only proceed *ortho* to the electron donating hydroxy group, and it was likely that the low yields resulted from steric hinderance around the newly formed carbon-carbon bond as well as competition with undesired side reactions.



Scheme 4.21. Synthesis of THIQs for pharmacokinetic analysis.

The following assays were performed by Pharmidex: aqueous solubility assay, metabolic stability assays, permeability assay and CYP450 inhibition assay.[vii] The aqueous kinetic solubility of compounds **91** and **92** was measured at a concentration of 1 mg/mL. Compound **91** had a solubility of 0.31 mg/mL and compound **92** had a higher solubility of 0.82 mg/mL. Both of the compounds showed encouraging aqueous solubility, particularly THIQ **92**.

The metabolic stability of compounds **91** and **92** were evaluated in a human microsome stability assay and a human hepatocyte stability assay. The microsomal assay gave an indication of whether the compounds were susceptible to phase I metabolism by measuring the amount of compound remaining that was exposed to microsomes over time (Figure 4.8). Compound **92** had a shorter half-life of 9.81 min compared to **91** which had a half-life of 110 min. This suggested the benzylic substituent at position C-1 of compound **92** was prone to rapid metabolism.

[vii] The solubility assay, metabolic stability assays, permeability assay and CYP450 inhibition assay were carried out by Pharmidex (Pharmidex Pharmaceuticals Ltd, 14 Hanover Street, London, W1S 1YH).

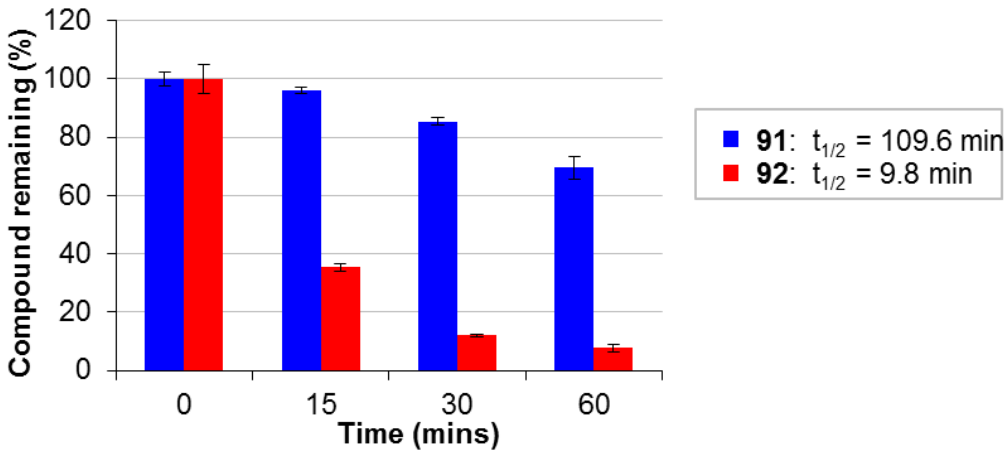


Figure 4.8. Results from the human microsome stability assay. $t_{1/2}$ = half-life.

The hepatocyte stability assay gave an indication of whether the compounds were susceptible to phase I and II metabolism (Figure 4.9). The same trend as seen in the microsome assay was also observed in the hepatocyte assay. Compound **92** had a shorter half-life of 23.2 min in comparison to compound **91** (half-life = 78.6 min), and therefore **92** had a higher metabolic instability. The hepatic extraction ratio was also evaluated which gave an indication of the amount of drug removed when passing through the liver, and refers to the ratio of the hepatic clearance to the blood flow. A high hepatic extraction ratio was defined as greater than 0.35. Both compounds **91** and **92** had a high extraction ratio of greater than 0.6, indicating these compounds would be rapidly metabolised by the liver.

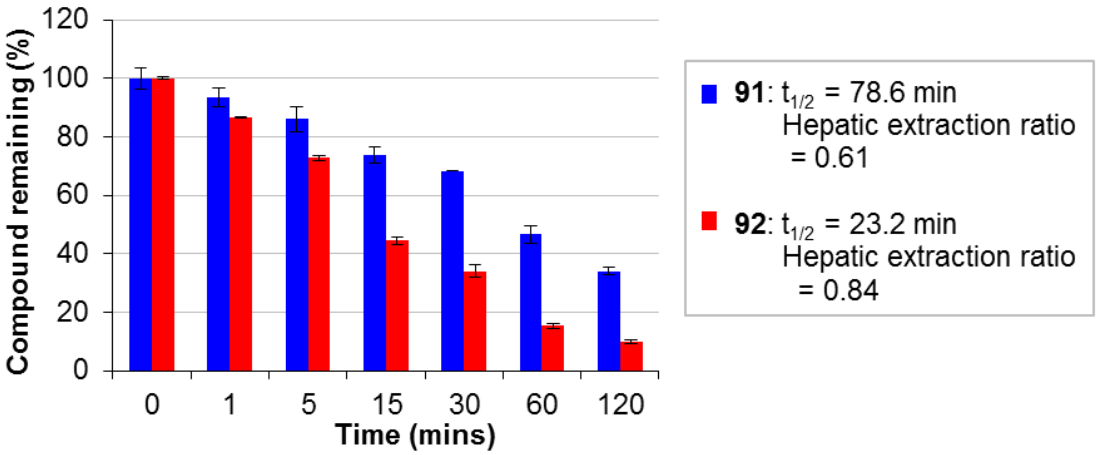


Figure 4.9. Results from the human hepatocyte stability assay. $t_{1/2}$ = half-life.

The CYP450 (cytochrome P450) inhibition assay showed that compound **92** inhibited the activity of CYP450 enzymes to a greater extent than compound **91** (Table 4.7). The high inhibition of these enzymes by compound **92** (greater than 80% inhibition) could be a problem, as drug-drug interactions would be more likely because CYP450 oxidase enzymes are involved in the metabolism of many commonly prescribed drugs. Compound **91** also displayed a high inhibition of CYP2C19, CYP2D6 and CYP3A4, but a lower inhibition of CYP1A2 and CYP2C (inhibition less than 50%).

THIQ	**% Inhibition**				
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
91	37%	45%	98%	81%	80%
92	81%	82%	97%	93%	93%

Table 4.7. Results from the CYP450 inhibition assay.

The Madin Darby Canine Kidney (MDCK) cell line transfected with the human multidrug resistant-1 gene (MDR1) was used to study the transcellular permeability of the compounds and to assess the effect of efflux by P-glycoprotein (Pgp) on compound permeability (Table 4.8). Compound **91** was moderately permeable in the MDCK monolayer assay (3.2 x 10^{-6} cm/s), while compound **92** had a lower permeability (1.7 x 10^{-6} cm/s). The high aqueous solubility of **92** and more probable hydrophilic nature would explain its lower cellular permeability compared to compound **91**. The efflux permeability ratios classified both compounds as Pgp efflux substrates. Therefore drug efflux was occurring, which could lead to poor exposure for oral drug development.

THIQ	**P_{app} (x 10^{-6} cm/s)**[a]		**Efflux ratio (B-A/A-B)**	**Pgp substrate**[b]
	A-B	**B-A**		
91	3.2 (medium)	38.0	11.9	Yes
92	1.7 (low)	18.8	11.3	Yes

Table 4.8. Results from the MDR1-MDCK permeability assay. [a]P_{app} = apparent permeability of a compound for apical to basal (A-B) and basal to apical (B-A) permeation. Permeability (A-B): $P_{app} < 2$ (low), $P_{app} > 20$ (high). [b]Compounds with efflux ratio > 1.5 were classed as Pgp substrates.

To evaluate any off-target activities of compounds **91** and **92**, a hit profiling screen was contracted to and carried out by Eurofins Panlabs.[viii] The hit profiling screen indicated any adverse activity of the compounds against 35 targets including ion channels, adrenergic receptors, GPCRs and CYP450 enzymes (Table 4.9 highlights the targets where greater than 50% inhibition was observed. For the complete list of targets see Chapter 8 experimental). The compounds were tested at a single concentration of 10 μM (IC_{50} data was not determined) and results were for ligand binding data (agonist/antagonist mode of action was not determined). The responses were considered significant if greater than 50% inhibition of a target was observed.

Assay	**Species**	**THIQ 92 % inhibition**	**THIQ 91 % inhibition**
CYP450, 1A2	human	60	-
CYP450, 2C19	human	99	73
CYP450, 2C9	human	63	-
CYP450, 2D6	human	96	-
CYP450, 3A4	human	94	-
Adrenergic α_{1A}	rat	83	67
Adrenergic α_{1B}	rat	78	55
Adrenergic α_{2A}	human	93	65
Adrenergic β_1	human	93	85
Adrenergic β_2	human	91	75
Dopamine D_1	human	57	-
Imidazoline I_2, central	rat	69	-
Opiate μ (OP3, MOP)	human	70	-
Serotonin 5-HT_{2B}	human	99	-
Sigma σ_1	human	86	52
Sodium channel, site 2	rat	88	88
Transporter, norepinephrine (NET)	human	72	-

Table 4.9. Results from Eurofins Panlabs hit profiling screen, showing >50% inhibition.

Both compounds displayed a number of off-target activities, but compound **92** displayed significantly greater off-target activity compared to compound **91**. The

[viii] The hit profiling screen was carried out by Eurofins Panlabs (Eurofins Panlabs Taiwan Ltd, Pharmacology Laboratories, 158 Li-The Road, Peitou, Taipei, Taiwan 112, Taiwan R.O.C.).

results showed compound **92** displayed a significant response in 17 of the 35 assays, whereas compound **91** showed a significant response for 8 out of the 35 assays (Table 4.9). The widespread off-target activities on adrenergic receptors and other off-target activities would need to be reduced in order to develop a promising lead compound whilst still retaining potency against mycobacteria.

In conclusion the results from the pharmacokinetic analysis of THIQ compounds **91** and **92** have highlighted compound **91** as a more promising structure to develop further as an anti-TB scaffold.

5. Tetrahydrobenzazepines and profens

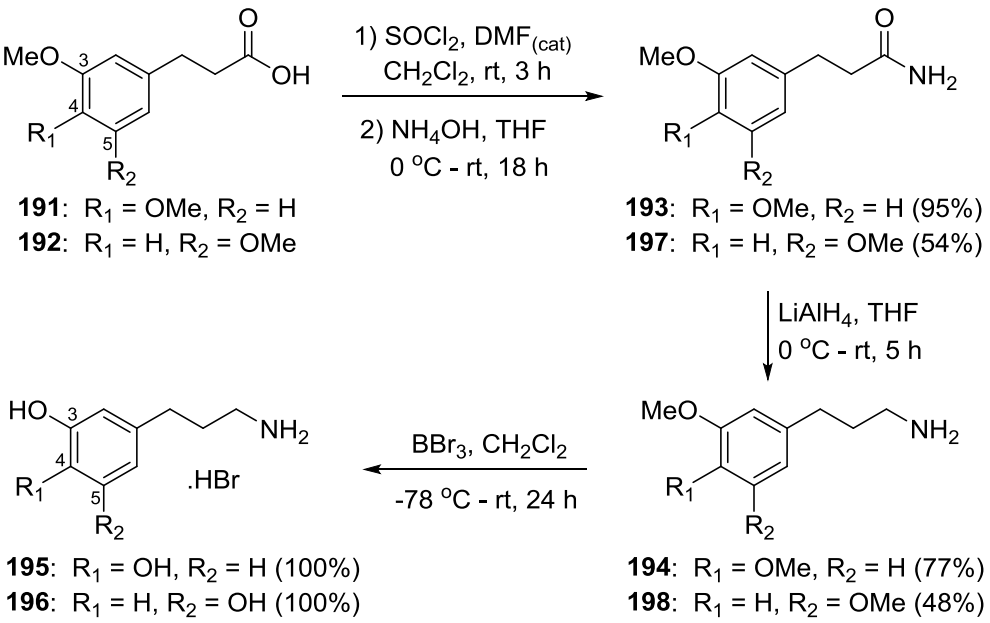
5.1 Tetrahydrobenzazepine synthesis

5.1.1 Cyclisation of 3,4-dihydroxyphenylpropylamines

An investigation into applying the phosphate mediated Pictet-Spengler reaction (described in section 3.3.4) to the synthesis of 7-membered ring tetrahydro-2-benzazepines (THBPs) was conducted. The aim was to prepare novel 1-substituted THBPs that were analogues of the 6-membered ring THIQs that have previously been shown to possess interesting antimycobacterial activity.[105]

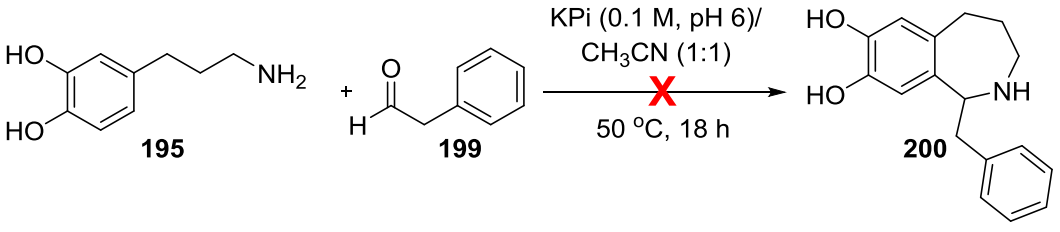
First, a synthetic route for the preparation of 3-hydroxyphenylpropylamine derivatives was required. A number of 3-methoxyphenylpropanoic acids were commercially available including compounds **191** and **192**, and so these were used as the starting materials (Scheme 5.1). Based on a literature procedure,[174] the carboxylic acid **191** was treated with thionyl chloride to produce an acyl chloride intermediate. This was then dissolved in anhydrous THF and added dropwise to an excess of 28% ammonium hydroxide solution (to ensure the acyl chloride did not hydrolyse back to the acid) at 0 ^{o}C to give the amide **193** in 95% yield. Amide **193** was reduced to the amine **194**[175] using lithium aluminium hydride in THF, and subsequent demethylation with boron tribromide yielded the desired 3,4-dihydroxyphenylpropylamine **195** in quantitative yield as the hydrobromide salt (Scheme 5.1). The demethylation step was initially attempted using hydrobromic acid and acetic acid which had previously been described for the preparation of compound **195**.[176] However it was found that it was difficult to purify the product using this method due to excess acetic acid in the crude material.

The same synthetic route was also applied to the synthesis of the analogue 3,5-dihydroxyphenylpropylamine **196** (Scheme 5.1). Commercially available carboxylic acid **192** was converted to the amide **197**[177] in 54% yield. Reduction of amide **197** to the amine **198** followed by the demethylation step yielded the product **196** as the hydrobromide salt in quantitative yield.



Scheme 5.1. Synthesis of hydroxyphenylpropylamines.

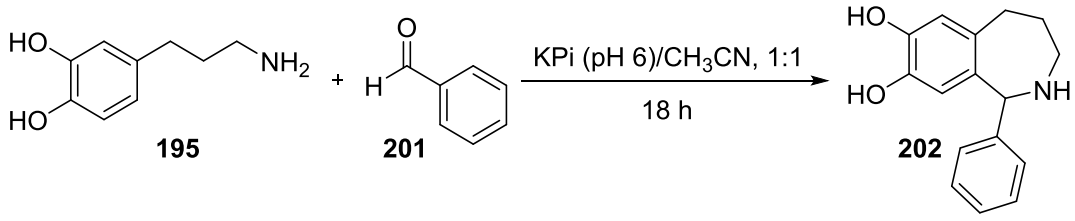
The phosphate mediated Pictet-Spengler reaction was initially attempted using 3,4-dihydroxyphenylpropylamine **195** and phenylacetaldehyde **199** (Scheme 5.2). These starting materials were chosen because they were close analogues of dopamine and 4-HPAA, that are the natural substrates for the enzymatic Pictet-Spengler reaction to generate the 6-membered ring THIQ norcoclaurine (described in section 3.3.3). The Pictet-Spengler reaction was carried out in a 1:1 mixture of 0.1 M potassium phosphate buffer at pH 6 and acetonitrile at 50 °C. Analysis of the reaction mixture by LC-MS after 18 hours revealed the presence of mainly unreacted amine starting material **195** and a small amount of side products. The desired 7-membered ring THBP **200** was not detected. The reaction was repeated using a higher concentration of phosphate but the same result was observed.



Scheme 5.2. Attempted Pictet-Spengler reaction for the synthesis of THBP **200**.

These initial results suggested that the formation of a 7-membered ring via the phosphate mediated Pictet-Spengler reaction would be significantly more challenging than for the analogous 6-membered ring formation. In general, 7-membered rings are more difficult to construct than the smaller 5- or 6-membered ring analogues because of entropic costs of the ring closure, transannular interactions and increased ring strain in the cyclic product.[178] Only a limited number of Pictet-Spengler type cyclisations to form 7-membered rings have previously been reported (examples in section 3.5.2).

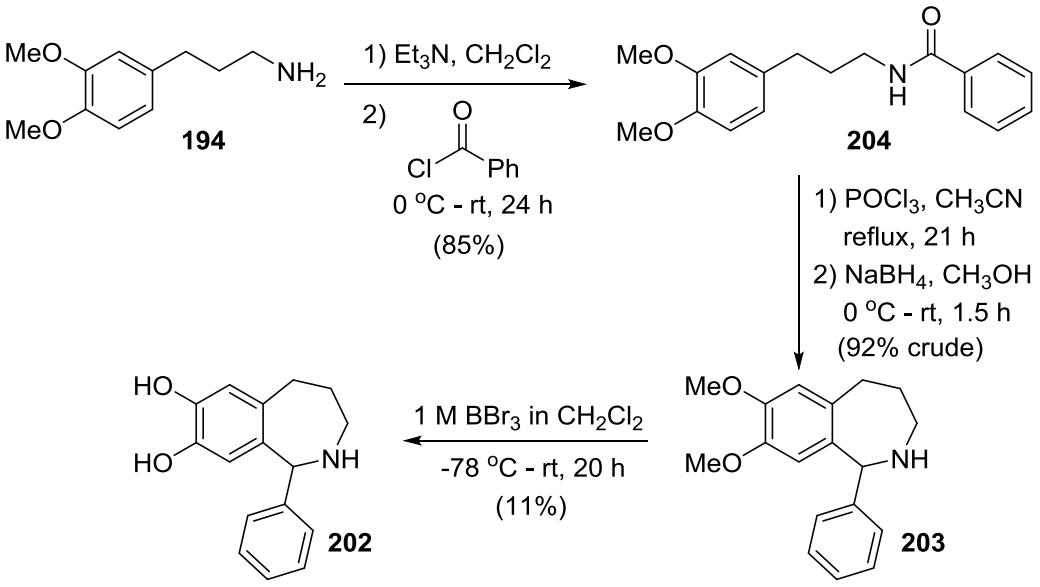
Due to the instability of phenylacetaldehyde and its potential to form unwanted side products, further investigations into Pictet-Spengler reactions were carried out using either benzaldehyde or an aliphatic aldehyde. Phenylpropylamine **195** and benzaldehyde **201** were reacted at 50 ^{o}C overnight in a 1:1 solution of potassium phosphate buffer (pH 6) and acetonitrile, and the effect of changing the concentration of phosphate buffer from 0.1 to 0.5 M was explored (Table 5.1, entries 1-3). In all three experiments analysis of the crude products by LC-MS showed that mostly starting materials were present. When the reaction was repeated at a higher temperature of 80 ^{o}C the mass of the desired THBP **202** was detected in the crude product (Table 5.1, entry 4). However, very little product was formed and the majority of the crude mixture consisted of the unreacted starting materials. It was not possible to isolate any of the THBP **102** from this reaction.



Entry	Phosphate conc.	Temperature	LC-MS of crude product
1	0.1 M	50 ^{o}C	Mixture of starting materials
2	0.25 M	50 ^{o}C	Mixture of starting materials
3	0.5 M	50 ^{o}C	Mixture of starting materials
4	0.1 M	80 ^{o}C	Mixture of starting materials and a mass corresponding to that of THBP **202** was detected.

Table 5.1. Attempted phosphate mediated Pictet-Spengler reactions for the synthesis of THBP **202**.

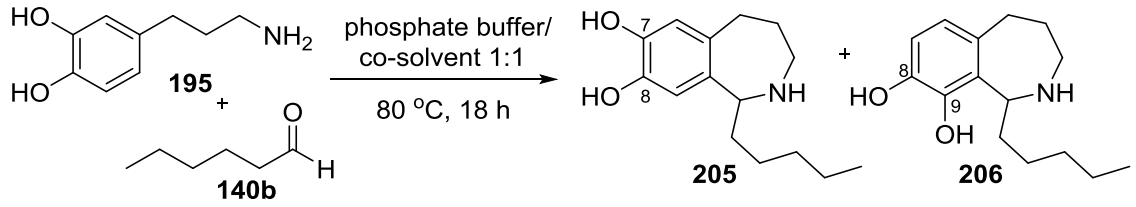
In parallel to investigating the Pictet-Spengler reactions, the alternative Bischler-Napieralski reaction (described in section 3.3.1) was used to prepare THBP **202** (Scheme 5.3). The dimethoxy analogue **203** of the desired THBP **202** was first prepared according to a literature procedure.[143] Amidation of phenylpropylamine **194** with benzoyl chloride gave the amide **204** in 85% yield. The Bischler-Napieralski cyclisation of amide **204** was achieved using phosphorus oxychloride in refluxing acetonitrile, which was followed by reduction of the intermediate imine with sodium borohydride, producing the dimethoxy-THBP **203** in 92% yield. This was similar to the yield reported in the literature of 90%.[143] The dimethoxy-THBP **203** was treated with boron tribromide in dichloromethane and once the demethylation reaction had gone to completion water was added, however it proved difficult to extract the product from the aqueous phase. The small amount of product which was extracted was purified by reverse phase prep-HPLC to remove impurities, and the THBP **202** was isolated in a low yield of 11%. It is likely that a higher yield could be achieved using an alternative work-up involving quenching the boron tribromide with methanol and evaporating the volatiles generated.



Scheme 5.3. Bischler-Napieralski route to prepare THBP **202**.

The preparation of the novel 1-phenyl-THBP **202** via the Bischler-Napieralski route indicated that compounds with this type of structure were accessible, and therefore

investigations into the phosphate mediated Pictet-Spengler synthesis of 1-substituted THBPs was continued. The next Pictet-Spengler reactions were attempted using the more reactive and less sterically demanding aliphatic aldehyde hexanal **140b** (Table 5.2). This aldehyde was selected because it would result in THBP products with a pentyl substituent at the C-1 position, and the 6-membered ring THIQ analogues with this substitution displayed good antimycobacterial properties (Chapter 4).



Entry	Phosphate buffer	Co-solvent	Yield of 205[a]	Yield of 206[a]
1	water (no phosphate)	CH_3CN	0%[b]	0%[b]
2	0.5 M, pH 6	CH_3CN	5%	3%
3	0.5 M, pH 6	CH_3OH	2%	1%

Table 5.2. Pictet-Spengler reactions for the synthesis of 1-pentyl-THBPs. [a]Isolated yield after HPLC. [b]The mass of the THBP product was not seen in the MS analysis of the crude mixture.

Phenylpropylamine **195** and hexanal **140b** were reacted at 80 °C in a mixture of water and acetonitrile with no phosphate present (Table 5.2, entry 1). Analysis of the mass spectrum of the crude product showed that there was no mass corresponding to the THBP product, but mostly starting materials present. When the reaction was repeated using the same conditions but with phosphate buffer (pH 6, 0.5 M) instead of water, the THBP **205** was detected in the mass spectrum of the crude reaction product (Table 5.2, entry 2). The crude product was purified by reverse phase prep-HPLC and two products were isolated. The first eluted product was the 7,8-dihydroxy-THBP **205**, formed from the cyclisation occurring *para* to the activating hydroxy group, and was isolated in 5% yield. The second eluted product was the 8,9-dihydroxy-THBP **206**, formed from the cyclisation occurring *ortho* to the activating hydroxy group, and was isolated in 3% yield. The reaction was also repeated using methanol as the co-solvent (Table 5.2, entry 3), but after separation of the THBPs **205** and **206** using reverse phase-HPLC an even

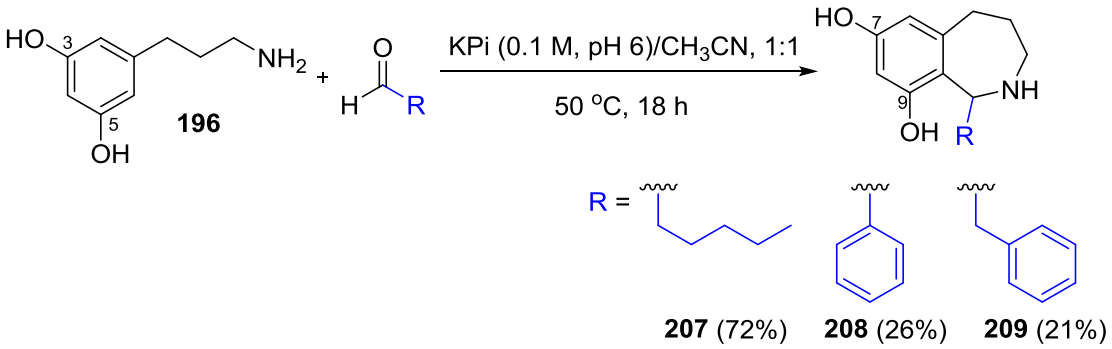
lower yield of 1-2% was obtained. Therefore acetonitrile was considered to be a better co-solvent than methanol.

In general, for the synthesis of the analogous 6-membered ring THIQs via the Pictet-Spengler reaction, the *para* cyclised THIQs (6,7-dihydroxy-THIQs) were formed as the major product and the *ortho* cyclised THIQs (7,8-dihydroxy-THIQs) were only formed as a minor product (discussed in section 3.3.2 and 4.1.1). It was therefore interesting that for the 7-membered rings, the 8,9-dihydroxy-THBP **206** was formed in a similar quantity to the 7,8-dihydroxy-THBP **205**, even though the yields of both the regioisomers were very low. The 8,9-dihydroxy-THBP structure was novel and exploring biological activities including antimycobacterial activity would be interesting.

5.1.2 Cyclisation of 3,5-dihydroxyphenylpropylamines

It was found for the synthesis of 6-membered ring THIQs that using 3,5-dihydroxyphenylethylamine substrates with a highly activated electron rich ring, resulted in excellent yields of the cyclised products and no regioisomer formation (section 4.1.5). It was therefore anticipated that for 7-membered ring synthesis, using 3,5-dihydroxyphenylpropylamine substrates would result in higher yields of THBP products.

The phosphate mediated Pictet-Spengler reaction between amine **196** and hexanal was carried out in a 1:1 solution of phosphate buffer at pH 6 and acetonitrile at 50 ^{o}C (Scheme 5.4). The 7,9-dihydroxy-THBP **207** was isolated from this reaction in 72% yield after purification by reverse-phase prep-HPLC. The reaction was then repeated with different aldehydes. Using benzaldehyde provided the THBP **208** in 26% yield, and using phenylacetaldehyde resulted in the formation of THBP **209** which was isolated in 21% yield (Scheme 5.4). These results were a significant improvement in comparison to the reactions carried out with 3,4-dihydroxyphenylpropylamine **195**, where less than 5% of THBP products were isolated. This indicated that for the cyclisation to form a 7-membered ring to occur, a highly activated aromatic ring was needed. The 3,5-dihydroxy substitution on the aromatic ring of **196** would activate position C-6 towards cyclisation (by increasing the nucleophilicity at this position) more than the 3,4-dihydroxy substitution on amine **195**.



Scheme 5.4. Pictet-Spengler reaction for the synthesis of 7,9-dihydroxy-THBPs. Yields refer to the isolated yield of THBP after purification by HPLC.

The aldehyde used also affected the yield of the 7,9-dihydroxy-THBP products (Scheme 5.4). Higher yields were obtained with the aliphatic aldehyde hexanal. The lower yield of THBP **208** when benzaldehyde was used, was possibly because the intermediate imine was more sterically hindered by the bulky phenyl group. The lower yield of THBP **209** when phenylacetaldehyde was used was attributed to unwanted side product formation which lowered the amount of aldehyde available for the phosphate mediated Pictet-Spengler reaction.

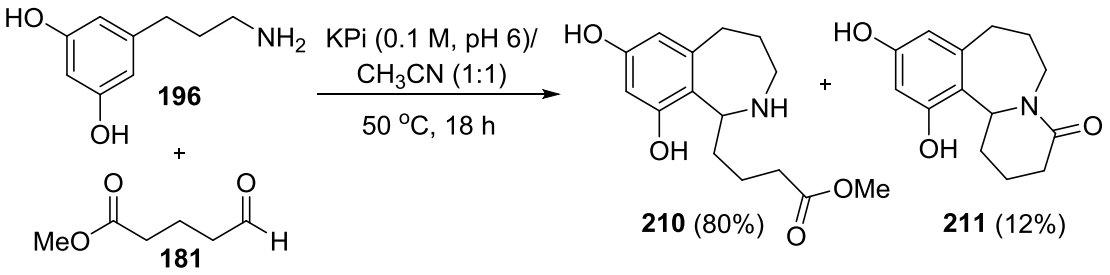
The novel 7,9-dihydroxy-THBPs with various 1-substituents would be good candidates for antimycobacterial screening and work is ongoing to test the activities of these 7-membered ring compounds and compare them to the corresponding 6-membered ring THIQ analogues.

5.1.3 Lactam synthesis from 1-substituted THBPs

It was previously found for the 6-membered ring THIQs that if the substituent at position C-1 contained an appropriate ester functionality, cyclisation onto the amine (N-2) produced lactam tricyclic derivatives (section 4.5). It was proposed that a similar reaction could occur with the 7-membered ring THBP analogues.

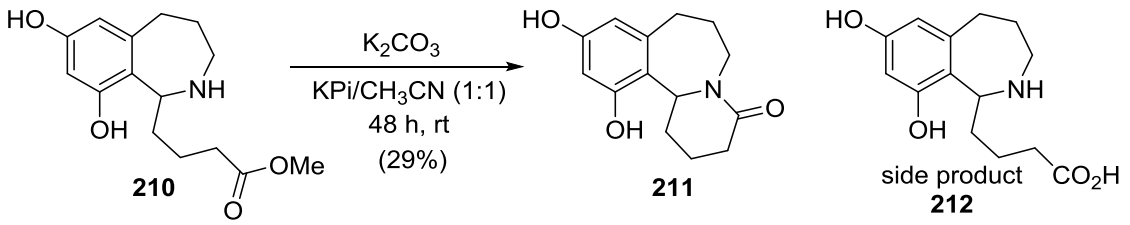
The phosphate mediated Pictet-Spengler reaction between phenylpropylamine **196** and aldehyde **181** at 50 °C yielded THBP **210** as the major product and lactam **211** as the minor product (Scheme 5.5). The products were separated using reverse phase HPLC, and THBP **210** was isolated in 80% yield and lactam **211** in 12% yield. In comparison,

the Pictet-Spengler reaction to prepare the 6-membered ring THIQ analogues yielded the 1-substituted THIQ **182** in 87% yield and lactam **183** in 4% yield (Scheme 4.15). Similar to the THIQ reaction, under the Pictet-Spengler reaction conditions cyclisation of THBP **210** to generate the lactam **211** was not favoured. However, a higher yield of lactam **211** (12%) was formed compared to the THIQ analogue lactam **183** (4% yield).



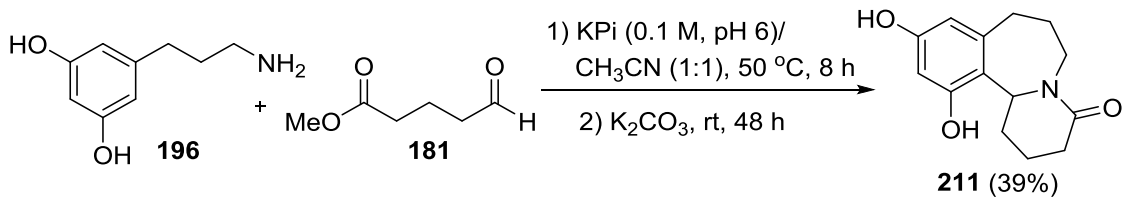
Scheme 5.5. Pictet-Spengler cyclisation of amine **196** with aldehyde **181**.

To establish whether THBP **210** could be cyclised to the lactam **211** under aqueous basic conditions, **210** was dissolved in a phosphate buffer and acetonitrile solution and treated with potassium carbonate (Scheme 5.6). The reaction was stirred at room temperature and analysed by LC-MS to monitor the disappearance of THBP **210**. After 36 hours most of the THBP **210** had been converted to product. After 48 hours the LC-MS trace appeared the same as when run at 36 hours, so the reaction was stopped at this time. Purification of the crude product by prep-HPLC resulted in isolation of lactam **211** in only 29% yield. A side product which was detected in the LC-MS analysis of the reaction mixture corresponded to the hydrolysed product **212**.



Scheme 5.6. Lactam synthesis under basic conditions.

The cyclisation to form the δ-lactam from THBP **210** was not optimised due to only having a small quantity of the THBP **210** and the amine **196** remaining towards the end of the PhD. However, a one-pot synthesis of lactam **211** from phenylpropylamine **196** and aldehyde **181** was attempted (Scheme 5.7). The Pictet-Spengler reaction was carried out under the standard conditions and after 8 hours all of the phenylpropylamine **196** had converted to the THBP intermediate **210** (monitored by LC-MS). Subsequent addition of potassium carbonate to the reaction mixture promoted the cyclisation to form lactam **211**, which was isolated in 39% yield.



Scheme 5.7. One-pot Pictet-Spengler/lactamisation reaction.

It was likely that the reaction conditions (such as pH, equivalents of base and temperature) used for the construction of the δ-lactam ring in the second step of the one-pot synthesis would be able to be optimised in order to improve the yield of the product. The tricyclic THBP derivative **211** represents a novel structure and testing of its antimycobacterial properties would be interesting for future studies.

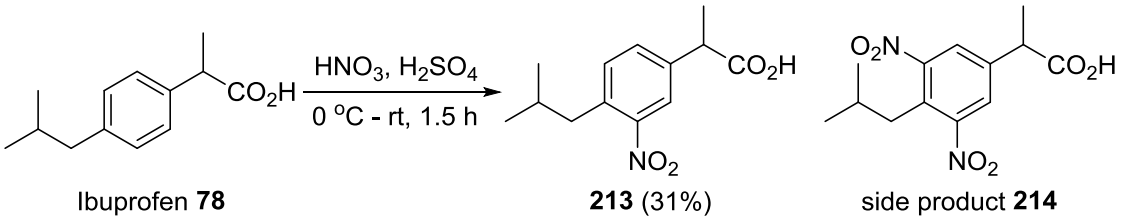
5.2 Profen synthesis

5.2.1 Ibuprofen analogues

A set of analogues of the non-steroidal anti-inflammatory drugs ibuprofen **78** and loxoprofen **79** were prepared for antimycobacterial screening. The Bhakta group have previously shown profens (2-arylpropionic acids) including **78** and **79** to be good inhibitors of the growth of *M. tuberculosis* as well as MDR-TB strains (described in section 3.1.3).[94] The target compounds included nitro- and amino-ibuprofen derivatives **138** (Figure 3.12). These were selected because a dinitro-ibuprofen derivative was

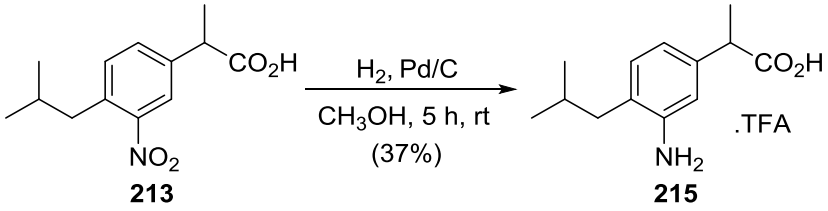
previously shown to improve the antimycobacterial potency against the growth of *M. tuberculosis* compared to ibuprofen.[94]

The nitration of ibuprofen **78** was carried out according to a literature procedure,[179] using 70% nitric acid and concentrated sulfuric acid (Scheme 5.8). This afforded a mixture of the starting material **78**, the mono-nitrated product **213** and the di-nitrated product **214**, in the ratio 3:75:22 as determined by ^{1}H-NMR spectroscopy. The di-nitrated compound **214** was removed from the mixture using column chromatography. This left a mixture of the mono-nitrated product **213** containing approximately 5% of the starting material **78**. Recrystallisation from ethyl acetate/hexane successfully removed the starting material to yield the mono-nitrated product **213** in 31% yield. The nitro group added *ortho* to the activating *iso*-butyl group and this regioisomer was confirmed by NMR spectroscopy.



Scheme 5.8. Nitration of ibuprofen.

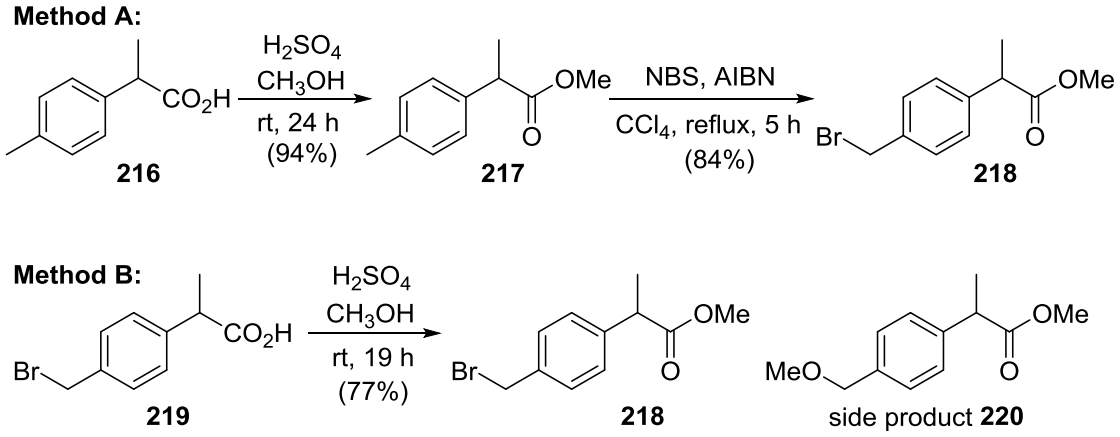
The nitro compound **213** was reduced to the aniline **215** via catalytic hydrogenation with a palladium on carbon catalyst in methanol (Scheme 5.9).[180] After purification by reverse phase prep-HPLC, the aniline **215** was isolated as the TFA salt in 37% yield. The ibuprofen derivatives **213** and **215** will be screened for their antimycobacterial activity by biological collaborators (Bhakta group) to determine the influence of the nitro and amino aromatic ring substituents on the antimycobacterial properties.



Scheme 5.9. Reduction of nitro-ibuprofen.

5.2.2 Loxoprofen analogues

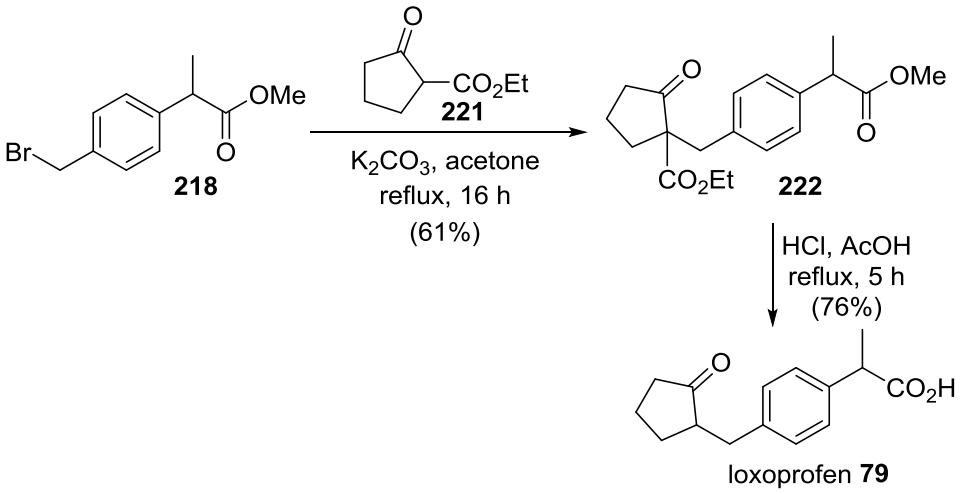
In order to prepare the loxoprofen derivative target compounds (**139**, Figure 3.12), first loxoprofen **79** was synthesised following a similar route to that reported by Yamakawa *et al*.[181] Methyl esterification of commercially available 2-(*p*-tolyl)propanoic acid **216** under acidic conditions gave the methyl ester **217** in 94% yield (Scheme 5.10, Method A). Compound **217** was subsequently treated with *N*-bromosuccinimide (NBS) and azo(bis)isobutyronitrile (AIBN) in carbon tetrachloride. Heating the mixture at reflux for 5 hours gave the bromo derivative **218** in 84% yield after filtration of the reaction mixture and purification of the product by column chromatography. The bromo derivative **218** was also synthesised by an alternative route starting from 2-(4-(bromomethyl)phenyl)propanoic acid **219** (Scheme 5.10, Method B). Methyl esterification of compound **219** by stirring at room temperature in methanol with sulfuric acid as a catalyst yielded the bromo derivative **218** in 77% yield after purification by column chromatography. A minor side product from the reaction was characterised as the methoxy derivative **220**,[182] which eluted as a second fraction from the column.



Scheme 5.10. Synthesis of the bromo intermediate **218** for the preparation of loxoprofen.

The bromo derivative **218** was next added to a solution of ethyl 2-oxocyclopentane carboxylate **221** in acetone in the presence of potassium carbonate (Scheme 5.11). After heating at reflux for 16 hours, the diester **222** was isolated in 61% yield. Finally, decarboxylation and hydrolysis of the diester **222** under strong acidic conditions

afforded loxoprofen **79** in 76% yield as a mixture of stereoisomers with NMR data consistent with literature reports.[183]



Scheme 5.11. Synthesis of loxoprofen.

Loxoprofen **79** contains two chiral centres and is used clinically as an anti-inflammatory drug as a racemic mixture of all four stereoisomers (Figure 5.1). It is a prodrug, and in humans is metabolised through reduction of the ketone moiety to the corresponding alcohol metabolites, of which there are 8 possible stereoisomers.[184] The most pharmacologically active metabolite, the (2*S*,1'*R*,2'*S*)-*trans*-alcohol **223** (Figure 5.1), is formed in larger amounts than the other stereoisomers and is also derived from the metabolic inversion of the *cis*-alcohol metabolites.[185] Therefore, interesting target loxoprofen derivatives to prepare for antimycobacterial screening included compounds accessible via reduction of the ketone.

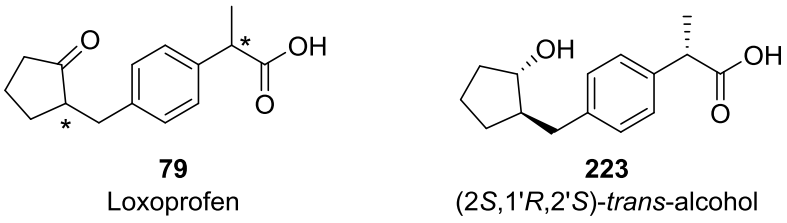
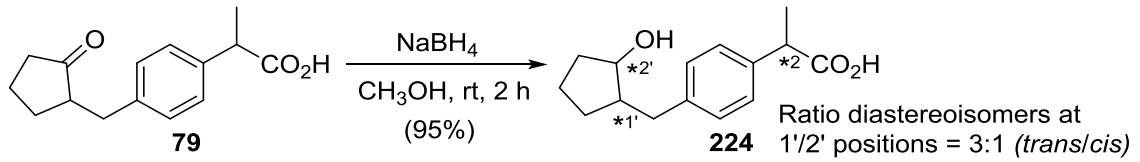


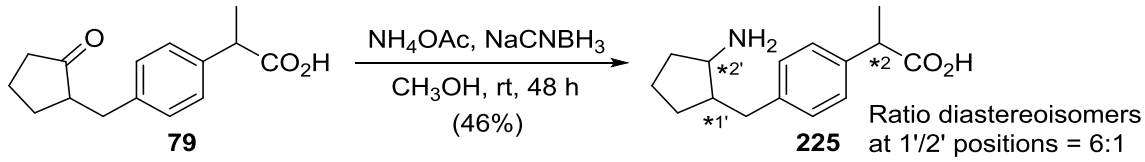
Figure 5.1. Prodrug loxoprofen and its bioactive alcohol metabolite.

The ketone moiety of loxoprofen **79** was reduced with sodium borohydride in methanol, which afforded the alcohol derivative **224** in 95% yield (Scheme 5.12). Alcohol **224** was isolated as a 3:1 mixture of the *trans*/*cis* stereoisomers as determined from ^{1}H-NMR spectroscopy data.[183] Depending on the results of the antimycobacterial evaluation of compound **224**, future investigations into the preparation of single stereoisomers may be useful. Asymmetric syntheses of single enantiomers of alcohol **224** have been reported using either asymmetric hydrogenation[186] or chemoenzymatic routes involving the reduction of the ketone with a ketoreductase.[187]



Scheme 5.12. Reduction of loxoprofen to the alcohol analogue.

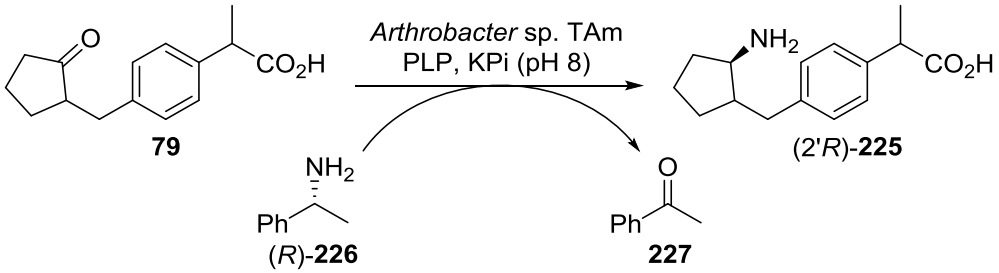
The second analogue of loxoprofen **79** that was prepared was the novel amino compound **225** (Scheme 5.13). Reductive amination was carried out by treatment of loxoprofen **79** in methanol with ammonium acetate and sodium cyanoborohydride at room temperature.[188] Purification of the crude product by reverse phase prep-HPLC, followed by recrystallisation afforded the amino compound **225** in 46% yield. Similar to the alcohol derivative **224**, the amino compound **225** was isolated as a mixture of stereoisomers in a 6:1 ratio at the 1'/2' positions, determined by ^{1}H-NMR spectroscopy.



Scheme 5.13. Reductive amination of loxoprofen.

A method for the asymmetric reductive amination of loxoprofen **79** was also desirable as it would enable the preparation of either the (2'*R*)- or (2'*S*)-chiral amine **225**, which

would then be able to be evaluated separately in the antimycobacterial assays. Transaminases (TAms) have been used in the asymmetric synthesis of chiral amines for pharmaceutically relevant compounds. They catalyse the transfer of an amino group (from an amine donor substrate) to a carbonyl such as a ketone, generating a chiral amine. The *Arthrobacter* sp. transaminase is (*R*)-selective and has previously been reported for the amination of ketones for compounds including steroids and 1,3-ketoamides.[189] The potential of using *Arthrobacter* TAm for the asymmetric amination of loxoprofen **79** was tested using (*R*)-methylbenzylamine **226** as the amine donor and the cofactor pyridoxal-5'-phosphate (PLP) (Scheme 5.14). The production of acetophenone **227** was monitored by analytical HPLC. When the transaminase reaction was carried out, only a small amount of acetophenone **227** was detected. This suggested that an alternative amine donor such as isopropylamine should be used, or that loxoprofen **79** was not accepted by the enzyme as a substrate.



Scheme 5.14. Attempted (*R*)-selective transaminase catalysed amination of loxoprofen

The transaminase *Chromobacterium violaceum* (CV-TAm) has previously been shown to accept a wide range of ketones and is an (*S*)-selective transaminase.[190] The amination reaction of loxoprofen **79** was carried out using CV-TAm, however problems were encountered with the HPLC monitoring of the production of acetophenone **227**. The results were not reliable and would need to be repeated, however due to the limited time available at the end of the PhD this was not further investigated.

An evaluation of the antimycobacterial properties of the ibuprofen analogues **213** and **215** and the loxoprofen derivatives **224** and **225** is currently ongoing by biological collaborators (Bhakta group).

6. Asymmetric phosphate mediated Pictet-Spengler reaction

6.1 Chiral sugar phosphates

Water is an attractive reaction medium for organic synthesis, but asymmetric catalysis in water is challenging because of its strong hydrogen bond donor/acceptor properties, which can disrupt interactions between a chiral catalyst and a substrate. An emerging class of water compatible asymmetric catalysts are chiral biological phosphates such as DNA, RNA and nucleotides (described in section 3.4). Previous work published by the Hailes group demonstrated that a range of phosphates including the chiral uridine 5'-monophosphate (UMP) **228** and glucose-1-phosphate **229** (Figure 6.1), acted as efficient catalysts in the biomimetic phosphate mediated Pictet-Spengler reaction for the synthesis of norcoclaurine **108** (Section 3.3.4, Scheme 3.7).[122] The stereoselectivity of the reaction was not analysed, but UMP **228** and glucose-1-phosphate **229** both contain multiple chiral centres within the single isomer molecule which could potentially influence the stereoselectivity. Asymmetric versions of the Pictet-Spengler reaction have been reported for the synthesis of THIQs with a chiral centre at the C-1 position (described in section 3.3.5), but to date there are no examples where the reactions have been performed in aqueous media.

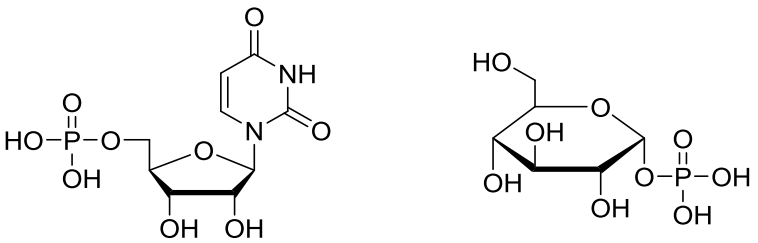
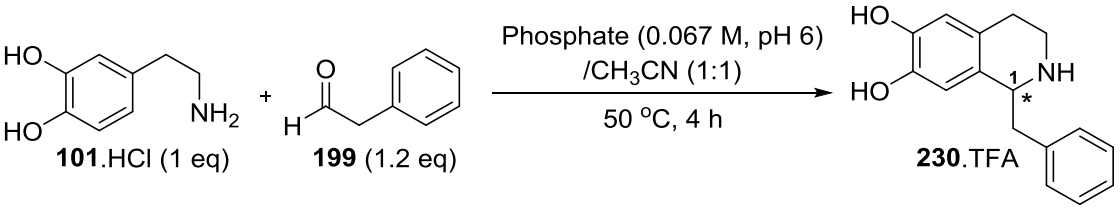


Figure 6.1. Structure of uridine 5'-monophosphate and glucose-1-phosphate.

A range of commercially available single isomer sugar phosphates were selected to screen in the phosphate mediated Pictet-Spengler reaction between dopamine **101** and phenylacetaldehyde **199**, generating the THIQ product **230** (Table 6.1).

Phenylacetaldehyde **199** was selected because it was a close analogue of 4-HPAA **107**, the natural substrate for the NCS enzyme catalysed Pictet-Spengler reaction (Scheme 3.7, section 3.3.4). In contrast to 4-HPAA **107** (which was unstable and required synthesis from the corresponding alcohol, Scheme 4.3), phenylacetaldehyde **199** was commercially available and more chemically stable. The Pictet-Spengler reactions were carried out using standard conditions in a 1:1 mixture of aqueous phosphate solution (pH 6) and acetonitrile at 50 ^{o}C. After 4 hours the crude product was purified by reverse phase prep-HPLC to obtain the THIQ product **230** (Table 6.1).



Entry	Phosphate	Yield[a] (%)	ee[c] (%)	1*S*/1*R* ratio
1	No phosphate added	0[b]	-	-
2	Potassium phosphate	64	2	51/49
3	α-D-Glucose-1-phosphate	71	2	49/51
4	D-Glucose-6-phosphate	69	0	50/50
5	D-Fructose-6-phosphate	64	2	51/49
6	Uridine 5'-monophosphate (UMP)	67	2	49/51
7	Cytidine 5'-monophosphate (CMP)	19	0	50/50
8	2'-Deoxyuridine 5'-monophosphate (dUMP)	45	2	49/51
9	2'-Deoxythymidine 5'-monophosphate (dTMP)	69	2	49/51
10	Inosine 5'-monophosphate (IMP)	41	6	47/53
11	Guanosine 5'-monophosphate (GMP)	34	8	46/54
12	2'-Deoxyguanosine 5'-monophosphate (dGMP)	57	6	47/53
13	Adenosine 5'-monophosphate (5'-AMP)	25	6	47/53
14	Adenosine 3'-monophosphate (3'-AMP)	43	6	53/47
15	Cyclic adenosine monophosphate (cAMP)	17	0	50/50

Table 6.1. Screening sugar phosphates in the biomimetic Pictet-Spengler reaction. [a]Isolated yield after purification by reverse phase HPLC. [b]LC-MS of the reaction mixture showed a very small amount of THIQ product had formed which was not isolated. [c]Determined by chiral HPLC.

A chiral analytical HPLC method for the separation of THIQs (1*R*)-**230** and (1*S*)-**230** was developed at GSK by Dr Eric Hortense, which was then also adopted at UCL.[ix] The assignment of enantiomers was made by comparison of a racemic sample of THIQ **230** with a sample of the enantiopure (1*S*)-**230**, which was synthesised by collaborators via an enzymatic route.[120,121] The enantiomeric excess (ee) of **230** was determined from the chiral HPLC analysis.

All of the phosphates used successfully promoted the Pictet-Spengler cyclisation to yield THIQ **230** (Table 6.1), highlighting the key role that the phosphate has in mediating the reaction. When the reaction was performed without the addition of phosphate (Table 6.1, entry 1), only negligible amounts of the product **230** were detected by LC-MS and therefore it was not isolated or purified. As a positive control, the reaction was carried out using potassium phosphate as the catalyst to generate the racemic THIQ **230** in 64% yield (Table 1, entry 2). The glucose and fructose mono-phosphorylated sugars did not induce stereoselectivity in the product (Table 6.1, entries 3-5), but were as effective in mediating the reactions as potassium phosphate, generating THIQ **230** in 64-71% yields.

A set of commercially available pyrimidine nucleotides were screened in the Pictet-Spengler reaction, including uridine, cytidine and thymidine 5'-monophosphates (Table 6.1, entries 6-9). These phosphates did not induce stereoselectivity in the product **230**. The isolated yields of THIQ **230** were between 45-69% when uridine or thymidine nucleotides were used as the phosphate source, but reduced to 19% yield when cytidine monophosphate was used. A set of purine nucleotides were also screened in the Pictet-Spengler reaction (Table 6.1, entries 10-15). For these reactions the product **230** was isolated in lower yields ranging from 25-57%, but a small stereoselectivity was observed (6-8% ee). An exception was when cyclic adenosine monophosphate was used, where no stereoselectivity was observed (0% ee). The purine nucleotides that were mono-phosphorylated at the 5'-position (IMP, GMP, dGMP and 5'-AMP, Table 6.1, entries 10-13) induced the same stereoselectivity in the product, with (1*R*)-**230** as the predominant configuration. This was opposite to the stereoselectivity of the enzymatic reaction, which gives rise to THIQs with a (1*S*)-configuration.[115] When the

[ix] Chiral HPLC analysis for reaction entries 1-9 and 11-14 in table 6.1 were performed by Dr. Eric Hortense at GSK, Stevenage. All other chiral HPLC analysis described in chapter 6 were carried out at UCL.

purine nucleotide was mono-phosphorylated at the 3'-position (3'-AMP), the (1*S*)-stereoisomer was favoured with a small enantiomeric excess of 6% (Table 1, entry 14). This was opposite to that observed with adenosine 5'-monophosphate, suggesting that the stereoselectivity may be influenced by the position of attachment of the phosphate group on the furanose ring.

The role of phosphate in mediating the Pictet-Spengler reaction has previously been proposed as either acting as a general base (abstracting a proton from the C-6 hydroxy group of the intermediate imine) or forming an intermediate aminophosphate (section 3.3.4, Schemes 3.8 and 3.9). The phosphate group in 5'-AMP **231** (Figure 6.2) lies on the same face of the furanose ring as the adenine group. In 3'-AMP **232** (Figure 6.2), the phosphate group lies on the opposite side of the furanose ring to the adenine group. Therefore, the intermediate imine/aminophosphate group could be held either above or below the furanose ring which may influence stereoselectivity, as well as possible π-π interactions between the phenyl groups of the substrate and the adenine ring of the nucleotide. Interactions between catecholamines and adenine nucleotides in aqueous solution have previously been demonstrated in ^{1}H-NMR studies by Granot *et al.*, through stacking interactions between the catechol and the adenine ring.[191]

The phosphate group in cAMP **233** (Figure 6.2) is held in a ring in a rigid position, which may have not allowed the intermediate imine/aminophosphate group to form any stabilising interactions with the adenine ring. It was also possible the ionisation properties of the phosphate moiety played a role. cAMP **233** is a phosphodiester and at pH 6 exists as the monoanion. In comparison, the other purine nucleotides screened including 5'-AMP **231** and 3'-AMP **232** would have existed as a mixture of the mono- and di-anion species.

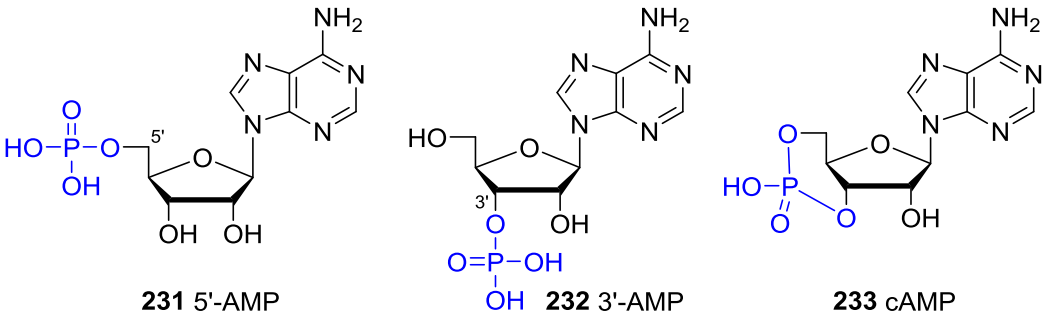
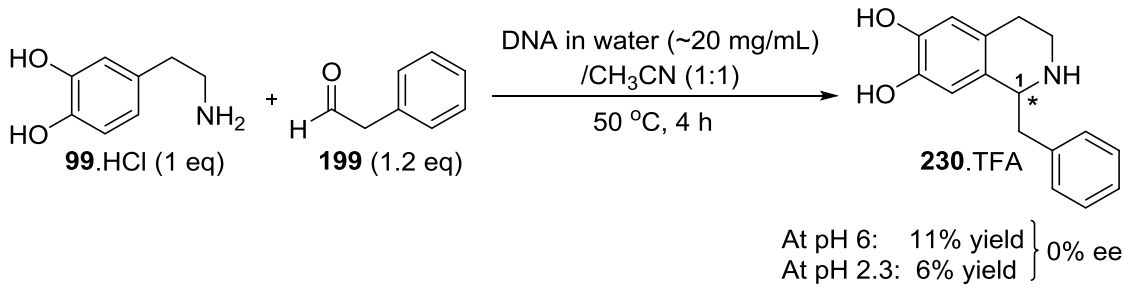


Figure 6.2. Structure of adenosine monophosphates.

DNA was also screened in the phosphate mediated Pictet-Spengler reaction (Scheme 6.1). The reaction was carried out at pH 6 or pH 2.3 (the pH of the DNA when dissolved in water). The yield of THIQ **230** from these reactions after 4 hours was low (6-11%). The chiral environment of the DNA helix did not induce stereoselectivity in the product **230** (0% ee determined by chiral HPLC analysis). DNA is a polynucleotide, with the 5'-phosphate group of one nucleotide linked to the 3'-hydroxy group of an adjacent nucleotide. Similar to cAMP **233** (Figure 6.2) the phosphate group is part of a more rigid structure than the phosphate groups present in single nucleotides and may be less accessible to promote the Pictet-Spengler cyclisation.



Scheme 6.1. DNA (herring sperm) as the phosphate source in the biomimetic Pictet-Spengler reaction.

6.2 Modified adenosine monophosphates

The general trend found from screening chiral single isomer nucleotides as mediators for the biomimetic Pictet-Spengler reaction, was that purine 5'-monophosphates induced a small enantiomeric excess in the THIQ product, which was not observed with pyrimidine nucleotides. This suggested the stereoselectivity could be influenced by the presence of the purine moiety.

Purine heterocycles are larger than pyrimidines and exhibit restricted rotation about the *N*-glycosidic bond, giving rise to the *syn*- and *anti*-conformations (Figure 6.3). Purine nucleotides generally favour the *anti*-conformation, but the *syn*/*anti* equilibrium can be altered by the addition of substituents at the C-8 position of the purine ring. For example, adenosine 5'-monophosphate with a bromo substituent at the C-8 position has been shown to favour the *syn*-conformation in water, due to increased steric hinderance between the bromo group and the sugar (Figure 6.3).[192]

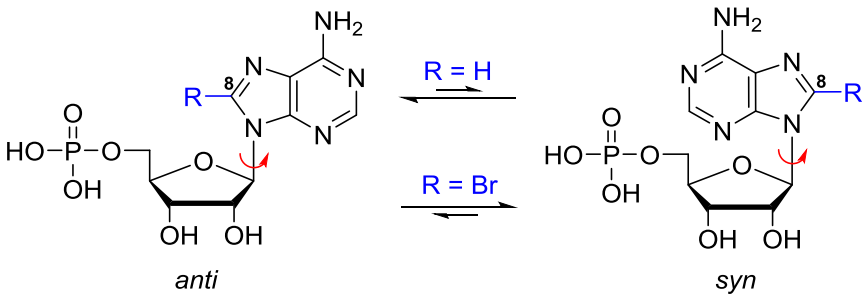
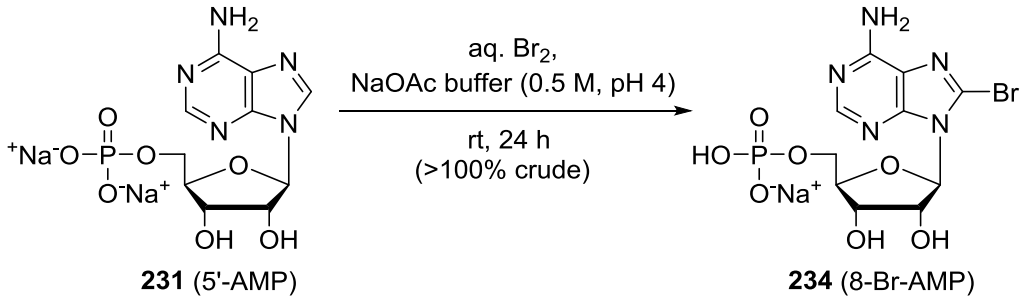


Figure 6.3. *Syn*- and *anti*-conformations of purine nucleotides.

In order to investigate the steric effects of the nucleotide purine ring on the stereoselectivity of the phosphate mediated Pictet-Spengler reaction, it was decided to prepare analogues of 5'-AMP **231** with various substituents at the C-8 position following literature procedures. First, 8-bromo adenosine 5'-monophosphate (8-Br-AMP) **234** was prepared according to a procedure by the Wagner group, where the unprotected nucleotide 5'-AMP **231** in sodium acetate buffer was directly modified by the addition of a saturated aqueous bromine solution (Scheme 6.2).[193] The crude product contained a small amount of residual sodium acetate, and could be used directly in the next step without further purification.

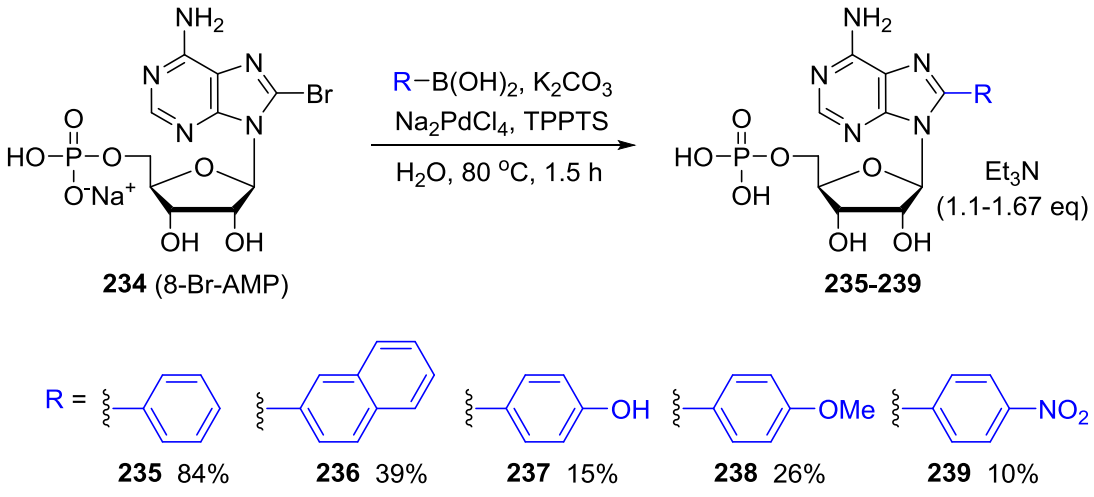


Scheme 6.2. Synthesis of 8-Br-AMP.

8-Br-AMP **234** was next used in Pd-catalysed cross couplings to introduce aryl groups at position C-8 (Scheme 6.3). According to a procedure described by the Wagner group, the cross-coupling reactions between 8-Br-AMP **234** and aryl-boronic acids were carried out in water, using a catalytic system involving the water soluble ligand TPPTS

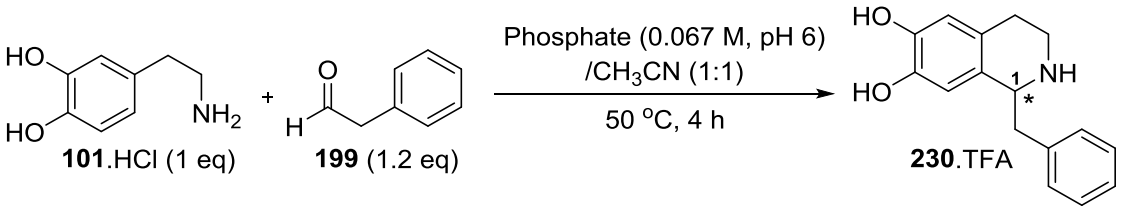
(triphenylphosphine-3,3',3''-trisulfonic acid sodium salt) and the Pd source, sodium tetrachloropalladate(II).[193] Purification of the crude 8-phenyl-AMP **235** product was initially attempted by passing the material through a short column of reverse phase silica, and eluting with ^{i}PrOH/H_2O/NH_4OH. Unfortunately this purification method was unsuccessful, and the isolated product **235** contained impurities. In the literature, ion pair chromatography was used to afford 8-phenyl-AMP **235** in 82% yield.[193] Therefore for the purification of 8-phenyl-AMP **235**, reverse phase prep-HPLC was carried out using a gradient of 0.05 M TEAB (triethylammonium bicarbonate) against acetonitrile as the mobile phase. It was very difficult to optimise the HPLC conditions to separate the compounds in the mixture, and minor impurities were still present in the product as observed by ^{1}H-NMR spectroscopy. The isolated yield of compound **235** was 84% but this contained impurities.

The cross coupling method[193] was also applied to the synthesis of four novel 8-aryl-AMP analogues **236-239** (Scheme 6.3). The isolated yields ranged from 10-39%, which were low due to the loss of material when purifying the nucleotides by reverse phase HPLC. The 8-naphthyl-AMP analogue **236** was prepared so that the effects of sterics on the stereoselectivity of the phosphate mediated Pictet-Spengler reaction could be studied. The 8-aryl-AMP analogues **237-239** featured either an electron donating hydroxy or methoxy group or an electron withdrawing nitro group on the aryl ring, so that electronic effects could be explored.



Scheme 6.3. Synthesis of 8-aryl-AMP compounds.

The modified adenosine 5'-monophosphate nucleotides were used as the phosphate source in the phosphate mediated Pictet-Spengler reaction between dopamine **101** and phenylacetaldehyde **199** (Table 6.2). The reactions were performed using standard conditions of a 1:1 mixture of the aqueous phosphate solution (0.067 M, pH 6) and acetonitrile at 50 ^{o}C. After 4 hours the crude product was purified by reverse phase prep-HPLC to obtain the THIQ **230**, which was analysed by chiral HPLC to determine the enantiomeric excess.



Entry	Phosphate	Yield[a] (%)	ee[b] (%)	1*S*/1*R* ratio
1	8-Bromo-AMP **234**	49	10	45/55
2	8-Phenyl-AMP **235**	31	10	45/55
3	8-Naphthyl-AMP **236**	59	8	46/54
4	8-Hydroxyphenyl-AMP **237**	49	10	45/55
5	8-Methoxyphenyl-AMP **238**	43	12	44/56
6	8-Nitrophenyl-AMP **239**	45	14	43/57

Table 6.2. Screening modified adenosine 5'-monophosphates in the biomimetic Pictet-Spengler reaction. [a]Isolated yield after purification by reverse phase HPLC. [b]Determined by chiral HPLC.

The modified adenosine 5'-monophosphates **234**-**239** mediated the Pictet-Spengler reaction to generate the THIQ product **230** in isolated yields between 31-59% (Table 6.2). The enantiomeric excess of product **230** for these reactions was between 8-14%, with (1*R*)-**230** as the predominant configuration. These were slightly higher than the stereoselectivity induced by the unmodified adenosine 5'-monophosphate (6% ee, Table 6.1). Sterics appeared to have little effect on the stereoselectivity. For example, the 8-phenyl-AMP analogue **235** and the 8-naphthyl-AMP analogue **236** gave rise to a similar enantiomeric excess of the product **230** (Table 6.2, entries 2-3). Comparing AMP derivatives **237**, **238** and **239** (Table 6.2, entries 4-6) showed that the electron donating

or electron withdrawing substituent attached to the phenyl ring at the C-8 position also had little effect on the stereoselectivity.

Overall the modified adenosine 5'-monophosphates did induce a small stereoselectivity in the phosphate mediated Pictet-Spengler reaction between dopamine **101** and phenylacetaldehyde **199**. Although the enantiomeric excess values of the product **230** were too small to make the reaction useful, it was still interesting that only the purine 5'-monophosphate nucleotides were able to induce this stereoselectivity for the (1*R*)-stereoisomer. It may be possible to increase the stereoselectivity in future investigations by exploring the reaction conditions, such as the effects of temperature, reaction time and the amount of nucleotide added to the reaction.

6.3 BINOL phosphoric acids

BINOL-derived phosphoric acids are a versatile class of organocatalysts for asymmetric reactions, and have been used in the Pictet-Spengler reaction but only under strictly anhydrous conditions (section 3.3.5). The phosphoric acid (*R*)-TRIP (Figure 6.4) has successfully been utilised as an asymmetric catalyst in aqueous media for the enantioselective hydrogenation of quinolines (section 3.4). A structurally simpler analogue of (*R*)-TRIP, was compound **240** (Figure 6.4), which was screened in the phosphate mediated Pictet-Spengler reaction in order to determine if BINOL phosphoric acids were able to promote the reaction. Phosphoric acid **240** was selected because it was readily available, whereas (*R*)-TRIP was expensive from commercial sources or would have required a multistep synthesis.

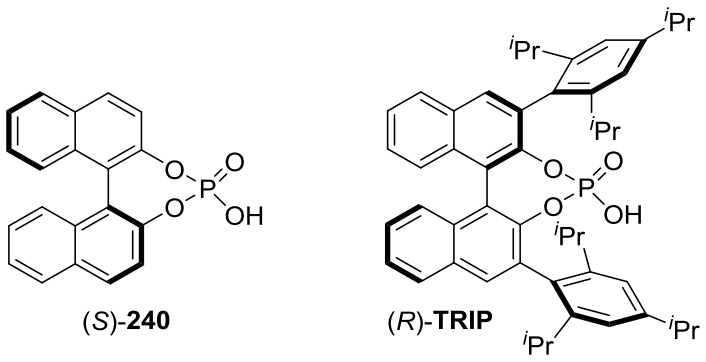
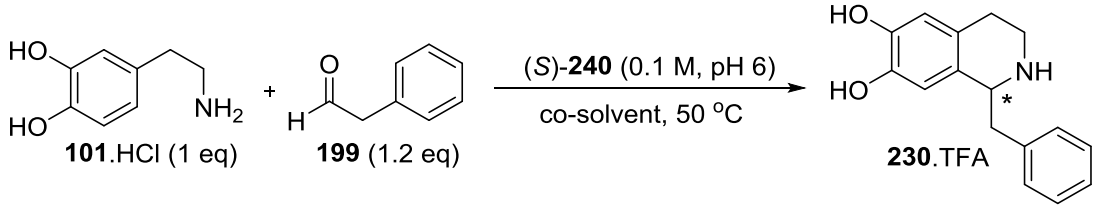


Figure 6.4. Structure of BINOL-derived phosphoric acids.

The Pictet-Spengler reaction was initially performed using a 1:1 mixture of an aqueous solution of phosphoric acid (*S*)-**240** (0.1 M, pH 6) and acetonitrile (Table 6.3, entry 1). After 30 hours the crude product was purified by reverse phase HPLC, generating the THIQ **230** in 33% isolated yield. A problem that was encountered when carrying out the reaction was that phosphoric acid (*S*)-**240** was not completely soluble in water, and the accurate preparation of the 0.1 M solution at pH 6 was difficult. It was encouraging however that the THIQ product **230** was isolated from the reaction.



Entry	Aq. phosphoric acid ((*S*)-**240**)/ co-solvent, ratio	Time (h)	Yield (%)
1	(*S*)-**240**/acetonitrile, 1:1	30	33[a]
2	(*S*)-**240** (no co-solvent)	4	0
3	(*S*)-**240**/acetonitrile, 2:1	4	0
4	(*S*)-**240**/acetonitrile, 1:1	4	0
5	(*S*)-**240**/acetonitrile, 1:2	4	0
6	(*S*)-**240**/methanol, 2:1	4	0
7	(*S*)-**240**/methanol, 1:1	4	0
8	(*S*)-**240**/methanol, 1:2	4	0
9	(*S*)-**240**/DMF, 2:1	4	0
10	(*S*)-**240**/DMF, 1:1	4	0
11	(*S*)-**240**/DMF, 1:2	4	0

Table 6.3. Screening BINOL-derived phosphoric acid (*S*)-**240** in the biomimetic Pictet-Spengler reaction. [a]Isolated yield after reverse phase HPLC.

A number of reactions were next carried out in parallel (Table 6.3, entries 2-11). As the solubility of phosphoric acid (*S*)-**240** was a problem, the co-solvent was investigated including acetonitrile, methanol and DMF, which have previously been shown to result in high conversions when used in the biomimetic Pictet-Spengler reaction.[122] The reactions were carried out for 4 hours (the same length of time as previously used when

investigating the stereoselectivity of the sugar phosphate mediators), with the aim of isolating the THIQ product **230** and analysing the enantiomeric excess. However, in all of the reactions none of the desired product **230** was isolated. This suggested that the reactions required a longer reaction time for conversion to THIQ **230** to be achieved.

The BINOL phosphoric acid (*S*)-**240** was not effective at promoting the Pictet-Spengler reaction and therefore was not investigated further during this project.

7. Conclusions and future work

A series of 1-substituted THIQs were synthesised and their antimycobacterial properties evaluated against *M. aurum*. Most of the 1-substituted THIQs displayed antimycobacterial activity, but also exhibited high cytotoxicities. Structure activity relationships (regarding the compounds ability to inhibit the growth of *M. aurum*) revealed that THIQs featuring an 8-hydroxy substitution were generally more potent inhibitors than THIQs without this group. This included compounds with a 6,8- and 7,8-dihydroxy substitution. The addition of a bromine substituent at the C-5 position also increased the potency of the compounds, consistent with results from previous studies.[105] The 5-bromo-8-hydroxy-THIQ **162** (Figure 7.1) which had a 1-pentyl chain was identified as the most compound of the series (MIC = 62.5 μg/mL).

Structure activity relationships regarding *M. aurum* efflux pump inhibition showed that most of the THIQs with a 7,8-dihydroxy substitution displayed efflux pump inhibitory activity, whereas the 6,7-dihydroxy-THIQs did not. Among the THIQs showing efflux pump inhibitory activity, some also showed synergistic activity when used in combination with current anti-TB drugs. In particular, THIQ **162** was very promising as a synergistic drug and enhanced the activity of all three first-line anti-TB drugs tested (isoniazid, rifampicin and ethambutol). THIQs **142b**, **146c**, **142c** and **91** (Figure 7.1) also displayed synergistic activity when used in combination with rifampicin or ethambutol at a low concentration of 0.1 μg/mL. These preliminary studies highlight the potential of the THIQ scaffold as a novel class of mycobacterial efflux pump inhibitors, and provide a good starting point for further exploration of these compounds.

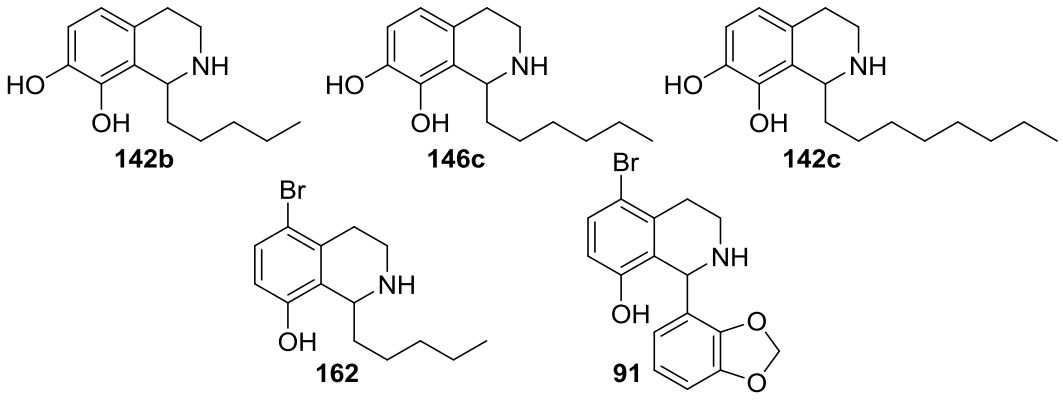
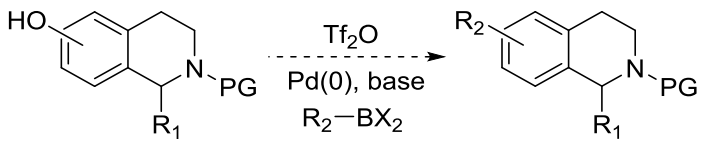


Figure 7.1. Structures of promising antimycobacterial THIQ compounds.

The phosphate mediated Pictet-Spengler reaction was an efficient method for the preparation of the 1-substituted THIQs. The scope of this reaction was extended to the preparation of THIQs with 1-substituents containing an ester functional group, which could be further cyclised to form a tricyclic structure through a second intramolecular lactamisation reaction. The phosphate mediated Pictet-Spengler reaction was also used to prepare a series of novel 7-membered ring THBPs. Cyclisation to generate 7-membered rings was more difficult than cyclisation to form the 6-membered ring THIQs, and the substrate scope was limited to phenylpropylamines with a highly activated ring (3,5-dihydroxy substitution). Finally it was demonstrated that chiral single isomer sugar phosphates promoted the phosphate mediated Pictet-Spengler reaction for the preparation of 1-benzyl-THIQs. A study into the stereoselectivity of these reactions demonstrated that generally the sugar phosphates did not induce stereoselectivity. However purine nucleotides were able to induce a very small stereoselectivity, with the enantiomeric excess of the product THIQ between 6-14%.

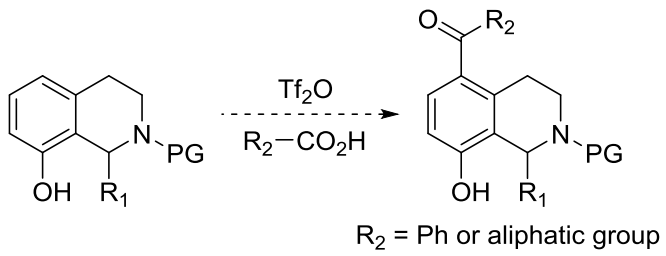
Evaluation of the synthesised 7-membered ring THBPs and profen analogues as potential anti-TB compounds is ongoing. Future antimycobacterial testing should also include screening the most active THIQs such as compound **162** against *M. tuberculosis*, as currently the compounds have only been screened against *M aurum*. Future Medicinal Chemistry studies could focus on further SAR based around the THIQ structure, including functionalisation of aromatic ring substituents. For example, hydroxy substituents at either the C-6 or C-8 position could be converted to the triflate to enable Suzuki cross coupling reactions to be performed with a range of commercially available boronic acids (Scheme 7.1).[194]



Scheme 7.1. Functionalisation of THIQs via a Suzuki cross coupling reaction. PG = protecting group.

Also, 8-hydroxy-THIQs could be further functionalised with various groups at the C-5 position (*para* to the phenol hydroxy group), such as the introduction of an acyl group

via a triflic anhydride mediated *para* acylation reaction (Scheme 7.2).[195] The antimycobacterial properties of these compounds could then be compared to the 5-bromo-8-hydroxy-THIQ **162**.



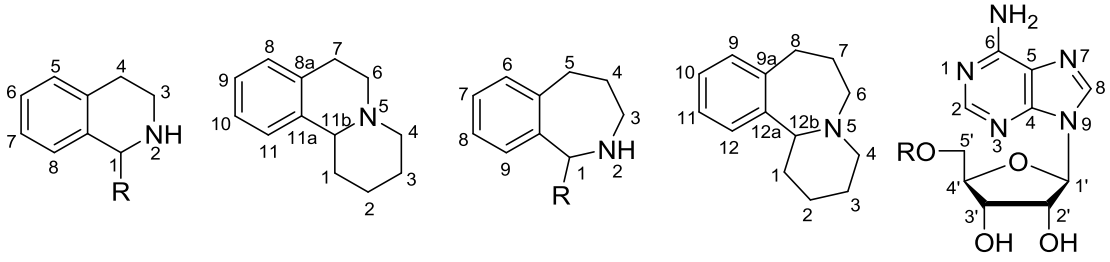
Scheme 7.2. Functionalisation of THIQs via a triflic anhydride mediated acylation. PG = protecting group.

8. Experimental

8.1 General experimental

All reagents and solvents were purchased from Sigma-Aldrich, Alfa Aesar, Acros and Fisher scientific, and were used as provided unless otherwise indicated. Dichloromethane, THF, diethyl ether and toluene were obtained from an anhydrous solvent system providing high quality, oxygen free solvents. Petrol refers to petroleum ether (40-60 ^{o}C fraction). For non-aqueous chemistry, reactions were carried out in oven-dried glassware under a nitrogen or argon atmosphere unless otherwise indicated. Thin layer chromatography (TLC) was carried out using Macherey-Nagel Polygram® silica 60 polyester sheets, 0.2 mm layer thickness, UV_{254} indicator, 40 x 80 mm plate size. TLC plates were visualised under UV or stained with a potassium permanganate solution. Flash column chromatography was performed using Merck silica gel 60, 40-63 µm as the stationary phase.

^{1}H and ^{13}C NMR spectra were obtained using either a Bruker AMX300, Bruker Avance 400, Bruker Avance 500 or Bruker Avance 600 spectrometer. Chemical shifts (δ) were quoted in parts per million (ppm) relative to residual solvent peaks, $CDCl_3$ (^{1}H NMR δ 7.26 and ^{13}C NMR δ 77.2) and CD_3OD (^{1}H NMR δ 3.31 and ^{13}C NMR δ 49.0), using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. The coupling constants (*J*) were measured in Hertz (Hz). Where appropriate, COSY HMQC and HMBC experiments were carried out to aid assignment. The numbering systems used for assigning NMR spectra for THIQ, THBP and nucleotide structures were as follows:



Melting points were determined using the Stuart Scientific SMP11 analogue apparatus and are uncorrected. Infra-red spectra were obtained using a Perkin Elmer Spectrum

100 FT-IR spectrometer or a Thermo Scientific Nicolet 6700 FT-IR spectrometer. Optical rotations were determined using a Perkin Elmer 343 polarimeter and units for specific rotation are 10^{-3} deg cm^2 g^{-1}. Mass spectra were obtained using either a Fisons VG70-SE, a Thermo Finnigan MAT 900XP, or a Waters LCT Premier XE spectrometer. The pH meter used was a Mettler-Toledo model MP225.

Mass Directed AutoPreparative HPLC (MDAP): MDAP was conducted on a Sunfire C18 column (150 mm length x 30 mm i.d., 5 μm packing diameter) at ambient temperature, eluting with solvents A/B (A: 0.1% v/v solution of trifluoroacetic acid in water, B: 0.1% v/v solution of trifluoroacetic acid in acetonitrile), according to one of the following gradients. Gradient A: 5-30% B in A. Gradient B: 15-55% B in A. Gradient C: 30-85% B in A. Gradient D: 50-99% B in A. The flow rate was 40 mL/min. The run time was 20 min unless otherwise stated. The UV detection was a summed/averaged signal from wavelength of 210 nm to 350 nm. The mass spectra were recorded using positive mode electrospray ionisation.

Preparative HPLC Method 1: HPLC was conducted on a Varian Prostar or Dionex instrument equipped with a UV-visible detector, using a Phenomenex Onyx Monolithic semi-prep C18 column (100 mm length x 10 mm i.d.) at room temperature, eluting with solvents A/B (A: 0.1% v/v solution of trifluoroacetic acid in water, B: 0.1% v/v solution of trifluoroacetic acid in acetonitrile). The gradient employed was 5-40% B in A. The run time was 20 min and the flow rate was 8 mL/min unless otherwise stated. The UV detection was at wavelength 280 nm and 254 nm.

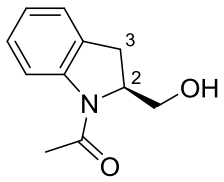
Preparative HPLC Method 2: HPLC was conducted on a Varian Prostar or Dionex instrument equipped with a UV-visible detector, using an Acentis C18 column (150 mm length x 21.2 mm i.d., 5 μm) at room temperature, eluting with solvents A/B (A: 0.1% v/v solution of trifluoroacetic acid in water, B: 0.1% v/v solution of trifluoroacetic acid in acetonitrile), according to one of the following gradients. Gradient A: 5-15% B in A, Gradient B: 5-20% B in A, Gradient C: 5-30% B in A, Gradient D: 10-15% B in A, Gradient E: 10-25% B in A, Gradient F: 15% B in A. The run time was 40 min and the flow rate was 7 mL/min unless otherwise stated. The UV detection was at wavelength 280 nm and 254 nm.

Preparative HPLC Method 3: HPLC was conducted on a Varian Prostar instrument equipped with a UV-visible detector, using a Supelco Discovery Bio Wide Pore C18 column (250 mm length x 21.1 mm i.d., 10 μm) at room temperature, eluting with solvents A/B (A: 0.05 M triethylammonium bicarbonate buffer, B: acetonitrile), according to one of the following gradients. Gradient A: 0-15% B in A, Gradient B: 0-25% B in A, Gradient C: 0-30% B in A, Gradient D: 0-60% B in A, Gradient E: 0-98% B in A. The flow rate was 8 mL/min. The run time was 25 min unless otherwise stated. The UV detection was at wavelength 254 nm and 214 nm.

Analytical chiral HPLC: Chiral HPLC analysis was performed on a Varian Prostar instrument equipped with an autosampler and a UV-visible detector, using a Supelco Astec Chirobiotic T2 column (250 mm length x 4.6 mm i.d., 5 μm packing diameter) at ambient temperature, eluting with an isocratic mobile phase of methanol (0.1% acetic acid, 0.2% triethylamine). The flow rate was 1 mL/min. The injection volume was 10 μL. The run time was 40 min. Compounds were detected by UV absorbance at wavelength 230 nm.

LC-MS: UPLC analysis was performed using a Waters Acquity UPLC-MS system, using a Zorbax SB-C18 column (2.1 x 2.0 mm i.d., 1.8 μm packing diameter), eluting with solvents A/B (A: 0.1% v/v solution of formic acid in water, B: 0.1% v/v solution of formic acid in acetonitrile). The flow rate was 1 mL/min, and the gradient employed was: 0 min (A: 100%, B: 0%), 4 min (A: 5%, B: 95%), 4.5 min (A: 5%, B: 95%), 5.0 min (A: 100%, B: 0%). The UV detection was at wavelength 254 nm. The mass spectra were recorded on a single quadrupole (SQD) mass spectrometer using positive ion electrospray ionisation mode.

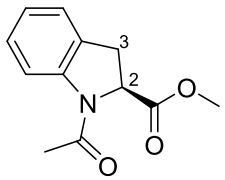
8.2 (*S*)-1-(2-(Hydroxymethyl)indolin-1-yl)ethan-1-one ((*S*)-**23**)[49]



The title compound was prepared following a literature procedure.[49] Lithium borohydride (46.8 mg, 2.15 mmol) was added portionwise to a stirred solution of (*S*)-**25**

(254 mg, 1.16 mmol) in THF (2 mL) at 0 °C and stirred at rt for 7 h. The reaction was quenched by addition of 10% HCl and extracted with EtOAc (3 x 15 mL). The combined organic extracts were washed with sat. Na_2CO_3, brine, dried (Na_2SO_4), and solvent removed *in vacuo* to give a yellow oil, which was purified by column chromatography (EtOAc/hexane, 2:1) to afford the alcohol (*S*)-**23** as a pale brown solid (111 mg, 50%), as a mixture of amide rotamers (2:1 in $CDCl_3$). R_f 0.19 (EtOAc/hexane, 2:1); m.p. 99-100 °C; $[\alpha]_D^{25}$ -50.3 (c 0.89, EtOH); δ_H (500 MHz; $CDCl_3$) 8.18-6.92 (4H, m, 4 x Ar-H), 4.97-4.40 (1H, m, 2-CH), 3.75-3.50 (2H, m, CH_2O), 3.35-2.57 (2H, m, 3-CH_2), 2.50-2.25 (3H, m, CH_3); *m*/*z* (CI) 192 ($[M + H]^+$, 100%); HRMS $C_{11}H_{14}O_2N$, calcd 192.1025, found 192.1028.

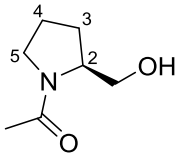
8.3 Methyl (*S*)-1-acetylindoline-2-carboxylate ((*S*)-**25**)[49]



The title compound was prepared using a modified version of a literature procedure.[49] Thionyl chloride (4 mL, 54.8 mmol) was added dropwise to a stirred solution of (*S*)-indoline-2-carboxylic acid (3.01 g, 18.4 mmol) in methanol (35 mL) at 0 °C. The solution was stirred at rt for 1 h and heated at reflux for 3 h. The mixture was concentrated *in vacuo* to a brown residue, which was washed with methanol (3 x 35 mL) and after each methanol addition concentrated *in vacuo* to remove residual thionyl chloride to give the intermediate methyl (*S*)-indoline-2-carboxylate hydrochloride as a brown solid (4.37 g) which was then dissolved in THF (30 mL) and DMAP (244 mg, 1.99 mmol) and triethylamine (10 mL, 71.7 mmol) were added. After 30 min, acetic anhydride (4.30 mL, 45.5 mmol) was added to the reaction mixture at 0 °C, and stirred at rt for 10 min, then heated at reflux for 5 h. The mixture was diluted with water (100 mL) and extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with 1 M HCl (2 x 50 mL), sat. Na_2CO_3 (2 x 50 mL), brine (50 mL), passed through a hydrophobic frit and solvent removed *in vacuo* to give the product (*S*)-**25** as a brown solid (3.68 g, 91%), as a mixture of amide rotamers (1.2:1 in $CDCl_3$). R_f 0.37 (hexane/EtOAc, 1:1); m.p. 75-77 °C; $[\alpha]_D^{25}$ -115.6 (c 0.90, EtOH), lit[196] $[\alpha]_D^{26}$ -48.0 (c 0.80, $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 8.23-7.14 (3H, m, 3 x Ar-H), 7.02 (1H, t, *J* = 7.0

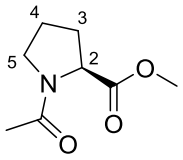
Hz, Ar-H), 5.16-4.91 (1H, m, 2-CH), 3.76-3.73 (3H, m, OCH_3), 3.62-3.10 (2H, m, 3-CH_2), 2.48-2.17 (3H, m, $COCH_3$); δ_C (125 MHz; $CDCl_3$) 174.6 (CO_2CH_3), 171.7 and 171.3 ($COCH_3$), 145.4 and 144.1 (Ar-C), 133.6 and 131.2 (Ar-C), 130.8 and 130.6 (Ar-CH), 128.5 and 127.0 and 126.8 and 126.2 (2 x Ar-CH), 120.2 and 116.6 (Ar-CH), 64.2 and 63.0 (2-CH), 55.8 and 55.3 (OCH_3), 36.4 and 34.3 (3-CH_2), 27.3 and 26.5 ($COCH_3$); *m/z* (CI) 220 ($[M + H]^+$, 100%); HRMS $C_{12}H_{14}O_3N$, calcd 220.0974, found 220.0965.

8.4 (*S*)-1-(2-(Hydroxymethyl)pyrrolidin-1-yl)ethan-1-one ((*S*)-**26**)[53]



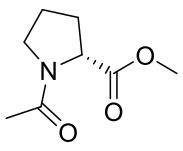
Lithium borohydride (760 mg, 34.9 mmol) was added portionwise to a stirred solution of (*S*)-**28** (3.78 g, 22.1 mmol) in 2-MeTHF (38 mL) at 0 ^oC and stirred at 0 ^oC for 20 h. The reaction was quenched by addition of 1 M HCl, then brine (40 mL), and extracted with CH_2Cl_2 (2 x 50 mL). The aqueous phase was adjusted to pH 11 by addition of sat. Na_2CO_3, then extracted with CH_2Cl_2 (2 x 50 mL). Combined organic extracts were washed with sat. Na_2CO_3, brine, passed through a hydrophobic frit, and solvent removed *in vacuo* to give a yellow oil, which was purified by column chromatography (EtOAc/CH_3OH, 95:5) to afford the alcohol (*S*)-**26** as a pale yellow oil (1.73 g, 55%), as a mixture of amide rotamers (16:1 in $CDCl_3$). R_f 0.14 (EtOAc/CH_3OH, 95:5); $[\alpha]_D^{25}$ -61.0 (c 0.90, EtOH), lit[197] $[\alpha]_D$ -69.5 (c 1.07, EtOH); δ_H (500 MHz; $CDCl_3$) 4.65 (1H, br s, OH), 4.21-4.14 (1H, m, 2-CH), 3.63 (1H, dd, *J* = 11.0 and 3.0 Hz, CHHO), 3.56 (1H, dd, *J* = 11.0 and 8.0 Hz, CHHO), 3.53-3.43 (2H, m, 5-CH_2), 2.08 (3H, s, CH_3), 2.06-1.98 (1H, m, 3-CHH), 1.98-1.81 (2H, m, 4-CH_2), 1.63-1.55 (1H, m, 3-CHH); δ_C (125 MHz; $CDCl_3$) major amide rotamer, 172.1 ($COCH_3$), 67.4 (CH_2O), 61.2 (2-CH), 49.1 (5-CH_2), 28.5 (3-CH_2), 24.4 (4-CH_2), 23.0 (CH_3); *m/z* (CI) 144 ($[M + H]^+$, 100%); HRMS $C_7H_{14}O_2N$, calcd 144.1025, found 144.1022.

8.5 Methyl acetyl-(*S*)-prolinate ((*S*)-**28**)[51]



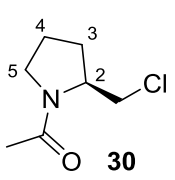
Thionyl chloride (9.50 mL, 130 mmol) was added dropwise to a stirred solution of (*S*)-proline (5.08 g, 44.1 mmol) in methanol (50 mL) at 0 ^{o}C. The solution was stirred at rt for 1 h and heated at reflux for 3 h. The mixture was concentrated *in vacuo* to give a yellow oil, which was washed with methanol (3 x 20 mL) and after each methanol addition concentrated *in vacuo* to remove residual thionyl chloride, to yield the intermediate methyl (*S*)-prolinate hydrochloride as a yellow oil (8.09 g), which was then dissolved in THF (60 mL) and DMAP (500 mg, 4.09 mmol) and triethylamine (25.0 mL, 179 mmol) were added. After 30 min, acetic anhydride (11.5 mL, 122 mmol) was added to the reaction mixture at 0 ^{o}C, which was stirred at rt for 10 min, then heated at reflux for 3 h. The mixture was diluted with brine (130 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were washed with brine (50 mL), passed through a hydrophobic frit, and solvent removed *in vacuo* to give a yellow oil, which was purified by column chromatography (EtOAc) to yield the product (*S*)-**28** as a pale yellow oil (7.06 g, 93%), as a mixture of amide rotamers (4:1 in $CDCl_3$). Published data assigns the major rotamer as the one with the acetamide carbonyl pointing towards the ester (*trans*).[198] R_f 0.31 (EtOAc); $[\alpha]_D^{25}$ -93.4 (c 0.88, EtOH), lit[199] $[\alpha]_D^{21}$ –101.7 (c 1.00, EtOH); δ_H (500 MHz; $CDCl_3$) 4.48-4.33 (1H, m, 2-C<u>H</u>), 3.74-3.70 (3H, m, OC<u>H</u>$_3$), 3.66-3.44 (2H, m, 5-C<u>H</u>$_2$), 2.31-2.01 (2H, m, 3-C<u>H</u>$_2$), 2.07-1.85 (5H, m, 4-C<u>H</u>$_2$, COC<u>H</u>$_3$); δ_C (125 MHz; $CDCl_3$) 173.0 and 172.7 (<u>C</u>O$_2$CH$_3$), 169.7 and 169.5 (<u>C</u>OCH$_3$), 60.2 and 58.5 (2-<u>C</u>H), 52.6 and 52.3 (O<u>C</u>H$_3$), 47.8 and 46.4 (5-<u>C</u>H$_2$), 31.5 and 29.5 (3-<u>C</u>H$_2$), 24.8 and 22.9 (4-<u>C</u>H$_2$), 22.3 (CO<u>C</u>H$_3$); *m*/*z* (EI) 171 (M^+, 22%), 112 (M^+ - CO_2CH_3, 100%); HRMS $C_8H_{13}O_3N$, calcd 171.0890, found 171.0883.

8.6 Methyl acetyl-(*R*)-prolinate ((*R*)-**28**)[52]

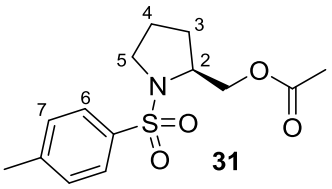


The title compound was prepared according to the procedure described in section 8.5, from (*R*)-proline (2.51 g, 21.8 mmol). The crude product was purified by column chromatography (EtOAc) to yield (*R*)-**28** as an orange oil (3.49 g, 93%). Characterisation data was identical to that of (*S*)-**28** except $[\alpha]_D^{25}$ +80.5 (c 0.96, EtOH).

8.7 (*S*)-1-(2-(Chloromethyl)pyrrolidin-1-yl)ethan-1-one (**30**)[53] **and (*S*)-(1-tosylpyrrolidin-2-yl)methyl acetate** (**31**).



30

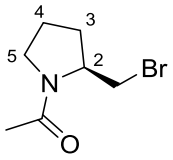


31

Compounds **30** and **31** were isolated as side products from an attempted tosylation reaction. Tosyl chloride (620 mg, 3.25 mmol) was added portionwise to a stirred solution of alcohol (*S*)-**26** (356 mg, 2.49 mmol) and anhydrous pyridine (1.0 mL, 12.4 mmol) in CH_2Cl_2 (5 mL) at 0 ^oC. The reaction mixture was stirred at rt for 20 h, then diluted with CH_2Cl_2 (5 mL) and washed with water, 1 M HCl, sat. Na_2CO_3 and brine, passed through a hydrophobic frit, and solvent removed *in vacuo* to give a mixture of products as a bright yellow-orange oil, which were separated by column chromatography (EtOAc). The first product eluted was the *N*-tosyl product **31** as a pale yellow oil (52.0 mg, 7%). R_f 0.91 (EtOAc); m.p. 53-54 ^oC; υ_{max}/cm^{-1} (KBr, $CDCl_3$ cast) 2986, 2971, 2886, 1741, 1600; δ_H (500 MHz; $CDCl_3$) 7.72 (2H, d, *J* = 8.0 Hz, 2 x 6-Ar-H), 7.31 (2H, d, *J* = 8.0 Hz, 2 x 7-Ar-H), 4.19 (1H, dd, *J* = 11.0 and 5.0 Hz, CHHO), 4.10 (1H, dd, *J* = 11.0 and 7.0 Hz, CHHO), 3.90-3.84 (1H, m, 2-CH), 3.46-3.40 (1H, m, 5-CHH), 3.19-3.12 (1H, m, 5-CHH), 2.42 (3H, s, Ar-CH_3), 2.06 (3H, s, $COCH_3$), 1.89-1.78 (1H, m, 4-CHH), 1.74-1.66 (1H, m, 3-CHH), 1.64-1.53 (2H, m, 4-CHH, 3-CHH); δ_C (125 MHz; $CDCl_3$) 170.9 ($COCH_3$), 143.7 (Ar-C), 134.5 (Ar-C), 129.9 (2 x 7-Ar-CH), 127.7 (2 x 6-Ar-CH), 66.3 (CH_2O), 57.9 (2-CH), 49.2 (5-CH_2), 28.7 (3-CH_2), 24.0 (4-CH_2), 21.6 (Ar-CH_3), 21.0 ($COCH_3$); *m*/*z* (CI) 298 ($[M + H]^+$, 100%); HRMS $C_{14}H_{20}O_4NS$, calcd 298.1113, found 298.1118. The second product eluted was the chloride product **30** as a pale yellow oil (181 mg, 45%). R_f 0.23 (EtOAc); δ_H (300 MHz; $CDCl_3$) 4.35-4.24 (1H, m, 2-CH), 3.78-3.60 (2H, m, CH_2Cl), 3.55-3.38 (2H, m, 5-CH_2), 2.06 (3H, s, CH_3), 2.06-1.80 (4H, m, 3-CH_2, 4-CH_2); *m*/*z*

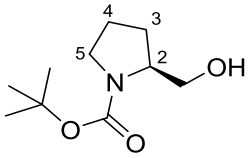
(EI) 161 ((^{35}Cl)M^+, 15%), 112 (M^+ - CH_2Cl, 100%); HRMS $C_7H_{12}ON^{35}Cl$, calcd 161.0602, found 161.0604.

8.8 (*S*)-1-(2-(Bromomethyl)pyrrolidin-1-yl)ethan-1-one (**33**)



Thionyl bromide (0.300 mL, 3.87 mmol) was added dropwise to a stirred solution of alcohol (*S*)-**26** (212 mg, 1.48 mmol) in CH_2Cl_2 (10 mL) at 0 ^oC. The reaction mixture was stirred at rt for 22 h, then concentrated to give a bright orange solid which was dissolved in ethyl acetate (25 mL). The mixture was filtered to remove the precipitate. The solvent was removed *in vacuo* and the resulting orange oil was purified by column chromatography (EtOAc) to yield the bromide **33** as a yellow oil (29.3 mg, 10%), as a mixture of amide rotamers (5:1 in $CDCl_3$). R_f 0.40 (EtOAc); δ_H (500 MHz; $CDCl_3$) major rotamer, 4.31-4.26 (1H, m, 2-C<u>H</u>), 3.63 (1H, dd, *J* = 9.5 and 3.0 Hz, C<u>H</u>HBr), 3.55 (1H, dd, *J* = 9.5 and 8.0 Hz, CH<u>H</u>Br), 3.47 (2H, m, 5-C<u>H</u>$_2$), 2.06 (3H, s, C<u>H</u>$_3$), 2.05-1.84 (4H, m, 3-C<u>H</u>$_2$, 4-C<u>H</u>$_2$); δ_C (125 MHz; $CDCl_3$) major rotamer, 170.0 (<u>C</u>OCH$_3$), 57.5 (2-<u>C</u>H), 48.7 (5-<u>C</u>H$_2$), 34.5 (<u>C</u>H$_2$Br), 29.0 (<u>C</u>H$_2$), 24.0 (<u>C</u>H$_2$), 23.0 (<u>C</u>H$_3$); *m/z* (CI) 208 ([(^{81}Br)M + H]$^+$, 15%), 206 ([(^{79}Br)M + H]$^+$, 15%); HRMS $C_7H_{13}ON^{79}Br$, calcd 206.0190, found 206.0185. The bromide **33** was unstable and rearranged to the acetate **34** (pyrrolidin-2-ylmethyl acetate) at room temperature as observed by ^{1}H-NMR spectroscopy with the appearance of signals consistent with those reported in the literature.[56]

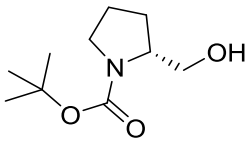
8.9 *tert*-Butyl (*S*)-2-(hydroxymethyl)pyrrolidine-1-carboxylate ((*S*)-**35**)[57]



The title compound was prepared following a literature procedure.[57] Borane tetrahydrofuran complex (1 M, 38.0 mL, 38.0 mmol) was added dropwise to a solution of (*S*)-**37** (4.01 g, 18.6 mmol) in THF (30 mL) at 0 ^oC. The solution was stirred at 0 ^oC

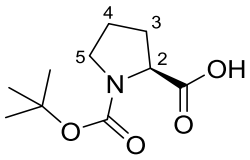
for 2 h, then rt for 1.5 h. The reaction mixture was cooled in an ice bath and cold water (70 mL) was added. The aqueous phase was extracted with EtOAc and the organic extracts were washed with brine, sat. $NaHCO_3$ and water, dried (Na_2SO_4) and solvent removed *in vacuo* to give a yellow oil which was purified by column chromatography (hexane/EtOAc, 3:2) to yield the product (*S*)-**35** as a white solid (3.16 g, 84%). R_f 0.43 (EtOAc/hexane, 2:3); m.p. 54-55 ^oC (lit[200] 58-59 ^oC, EtOAc); $[\alpha]_D^{25}$ -43.8 (c 1.00, $CHCl_3$), lit[57] $[\alpha]_D$ -47.3 (c 1.00, $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 4.73 (1H, br s, O<u>H</u>), 3.98-3.91 (1H, m, 2-C<u>H</u>), 3.65-3.54 (2H, m, C<u>H</u>$_2$O), 3.47-3.27 (2H, m, 5-C<u>H</u>$_2$), 2.03-1.96 (1H, m, 3-C<u>H</u>H), 1.85-1.75 (2H, m, 4-C<u>H</u>$_2$), 1.55-1.50 (1H, m, 3-CH<u>H</u>), 1.46 (9H, s, C(C<u>H</u>$_3$)$_3$); δ_C (125 MHz; $CDCl_3$) 157.3 (<u>C</u>O$_2$^{t}Bu), 80.4 (<u>C</u>(CH$_3$)$_3$), 67.9 (<u>C</u>H$_2$O), 60.3 (2-<u>C</u>H), 47.7 (5-<u>C</u>H$_2$), 28.8 (3-<u>C</u>H$_2$), 28.6 (C(<u>C</u>H$_3$)$_3$), 24.2 (4-<u>C</u>H$_2$); *m/z* (CI) 202 ([M + H]$^+$, 100%); HRMS $C_{10}H_{20}O_3N$, calcd 202.1443, found 202.1445.

8.10 *tert*-Butyl (*R*)-2-(hydroxymethyl)pyrrolidine-1-carboxylate ((*R*)-35)[64]



The title compound was prepared according to the procedure described in section 8.9, from (*R*)-**37** (3.99 g, 18.5 mmol). The crude product was purified by column chromatography (hexane/EtOAc, 3:2) to yield the product (*R*)-**35** as white crystals (3.29 g, 88%). M.p. 53-55 ^oC (lit[64] 59-60 ^oC, hexane). NMR and MS data identical to that of (*S*)-**35**.

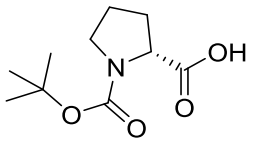
8.11 (*tert*-Butoxycarbonyl)-(*S*)-proline ((*S*)-37)[57]



The title compound (*S*)-**37** was prepared following a literature procedure.[57] Triethylamine (9.40 mL, 67.4 mmol) was added to a mixture of (*S*)-proline (6.43 g, 55.8 mmol) in CH_2Cl_2 (130 mL) at 0 ^oC. Boc anhydride (15.7 g, 71.9 mmol) in CH_2Cl_2 (6.5 mL) was added to the reaction mixture over 10 min at 0 ^oC, and stirred for 3 h at 0 ^oC.

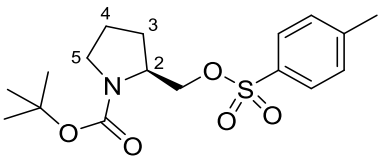
The reaction was quenched by adding saturated aq. citric acid (35 mL) and the organic phase washed with brine, dried (Na_2SO_4) and solvent removed *in vacuo*. The crude product was dissolved in hot EtOAc, followed by addition of hexane (300 mL). The crystallised product was collected by filtration to yield (*S*)-**37** as white crystals (9.58 g, 80%), as a mixture of amide rotamers (55:45 in $CDCl_3$). M.p. 129-131 ^oC (lit[57] 135-137 ^oC, EtOAc/hexane); $[\alpha]_D^{25}$ -89.1 (c 1.00, $CHCl_3$) lit[201] $[\alpha]_D^{20}$ -92.0 (c 1.10, $CHCl_3$); δ_H (500 MHz; $CDCl_3$) 7.35 (1H, br s, O<u>H</u>), 4.40-4.20 (1H, m, 2-C<u>H</u>), 3.60-3.30 (2H, m, 5-C<u>H</u>$_2$), 2.36-2.00 (2H, m, 3-C<u>H</u>$_2$), 1.99-1.81 (2H, m, 4-C<u>H</u>$_2$), 1.53-1.36 (9H, m, C(C<u>H</u>$_3$)$_3$); δ_C (125 MHz; $CDCl_3$) 178.8 and 175.0 (<u>C</u>O$_2$H), 156.6 and 154.0 (<u>C</u>O$_2{}^t$Bu), 81.5 and 80.4 (<u>C</u>(CH$_3$)$_3$), 59.2 and 59.0 (2-<u>C</u>H), 47.1 and 46.4 (5-<u>C</u>H$_2$), 30.9 and 28.6 (3-<u>C</u>H$_2$), 28.5 and 28.3 (C(<u>C</u>H$_3$)$_3$), 24.4 and 23.7 (4-<u>C</u>H$_2$); *m*/*z* (CI) 216 ($[M + H]^+$, 5%), 114 ($[M + H]^+$ - $C_5H_{10}O_2$, 100%); HRMS $C_{10}H_{18}O_4N$, calcd 216.1236, found 216.1233.

8.12 (*tert*-Butoxycarbonyl)-(*R*)-proline ((*R*)-37)[202]



The title compound was prepared according to the procedure described in section 8.11, from (*R*)-proline (3.02 g, 26.2 mmol). The crude product was purified by recrystallisation (EtOAc/hexane) to yield the product (*R*)-**37** as white crystals (4.34 g, 77%). Characterisation data was identical to that of (*S*)-**37** except $[\alpha]_D^{25}$ +88.0 (c 1.00, $CHCl_3$), lit[202] $[\alpha]_D^{25}$ +66.5 (c 7.29, $CHCl_3$).

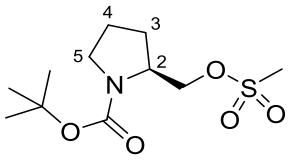
8.13 *tert*-Butyl (*S*)-2-((tosyloxy)methyl)pyrrolidine-1-carboxylate ((*S*)-38)[57]



Tosyl chloride (412 mg, 2.16 mmol) was added portionwise to a solution of (*S*)-**35** (344 mg, 1.71 mmol) in CH_2Cl_2 (3.5 mL) and anhydrous pyridine (0.80 mL) at 0 ^oC. The solution was stirred at rt for 17 h after which time a precipitate formed. The solution

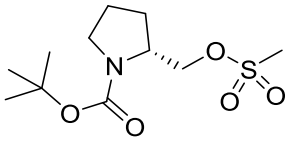
was diluted with CH_2Cl_2 (5 mL), washed with water, 0.5 M HCl, and brine, dried (Na_2SO_4) and solvent removed *in vacuo* to give a yellow oil, which was purified by column chromatography (hexane/EtOAc, 4:1) to yield the tosylate (*S*)-**38** as a colourless oil (478 mg, 79%). R_f 0.38 (hexane/EtOAc, 3:1); δ_H (500 MHz; $CDCl_3$) broadening of signals due to amide rotamers, 7.77 (2H, d, *J* = 8.0 Hz, 2 x Ar-H), 7.33 (2H, d, *J* = 8.0 Hz, 2 x Ar-H), 4.18-3.78 (3H, m, 2-CH, CH_2O), 3.40-3.18 (2H, m, 5-CH_2), 2.48 (3H, s, Ar-CH_3), 2.01-1.69 (4H, m, 3-CH_2, 4-CH_2), 1.37 (9H, s, $C(CH_3)_3$); *m*/*z* (ESI) 378 ([M + Na]$^+$, 100%); HRMS $C_{17}H_{25}O_5NSNa$, calcd 378.1351, found 378.1361.

8.14 *tert*-Butyl (*S*)-2-(((methylsulfonyl)oxy)methyl)pyrrolidine-1-carboxylate ((*S*)-**39**)[65]



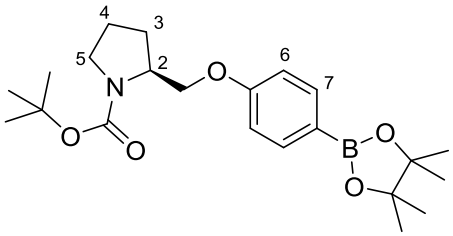
Triethylamine (3.20 mL, 23.0 mmol) followed by methanesulfonyl chloride (1.30 mL, 16.8 mmol) were added dropwise to a solution of the alcohol (*S*)-**35** (3.00 g, 14.9 mmol) in CH_2Cl_2 (35 mL) at 0 ^oC and stirred at 0 ^oC for 1 h and rt for 1 h, after which time a precipitate had formed. The reaction mixture was diluted with CH_2Cl_2 (40 mL), washed with sat. $NaHCO_3$ (2 x 40 mL) and brine (2 x 40 mL), dried (Na_2SO_4) and solvent removed *in vacuo* to yield the mesylate (*S*)-**39** as a yellow oil (4.17 g, 100% crude) as a mixture of amide rotamers, which was used without further purification. R_f 0.40 (EtOAc/hexane, 2:3); $[\alpha]_D^{25}$ -51.9 (c 1.00, $CHCl_3$); δ_H (500 MHz; $CDCl_3$) broadening of signals due to amide rotamers, 4.37-3.91 (3H, m, 2-CH, CH_2O), 3.47-3.25 (2H, m, 5-CH_2), 3.00 (3H, s, SCH_3), 2.10-1.76 (4H, m, 3-CH_2, 4-CH_2), 1.45 (9H, s, $C(CH_3)_3$); δ_C (125 MHz; $CDCl_3$) 154.7 and 154.2 ($CO_2{}^tBu$), 80.3 and 79.9 ($C(CH_3)_3$), 69.8 and 69.5 (CH_2O), 55.8 (2-CH), 47.1 and 46.7 (5-CH_2), 37.5 and 37.0 (SCH_3), 28.7 and 27.9 (3-CH_2), 28.5 ($C(CH_3)_3$), 23.8 and 23.0 (4-CH_2); *m*/*z* (CI) 280 ([M + H]$^+$, 85%), 224 ([M + H]$^+$ - C_4H_8, 100%), 180 ([M + H]$^+$ - $C_5H_8O_2$, 80%); HRMS $C_{11}H_{22}O_5NS$, calcd 280.1219, found 280.1212.

8.15 *tert*-Butyl (*R*)-(((methylsulfonyl)oxy)methyl)pyrrolidine-1-carboxylate ((*R*)-**39**)[65]



The title compound was prepared according to the procedure described in section 8.14, from (*R*)-**35** (3.23 g, 16.0 mmol). The crude product (*R*)-**39** was isolated as a yellow oil (4.68 g, 100% crude) and used without further purification. NMR and MS data was identical to that of (*S*)-**39**.

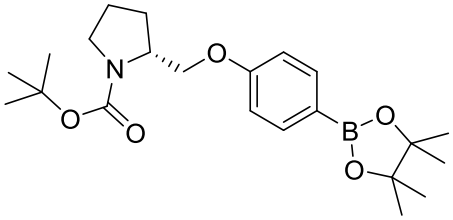
8.16 *tert*-Butyl (*S*)-2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methyl) pyrrolidine-1-carboxylate ((*S*)-**41**)[203]



To a solution of 4-hydroxyphenylboronic acid pinacol ester (411 mg, 1.87 mmol) in DMF (2.5 mL) with molecular sieves, was added ^{t}BuOK (300 mg, 2.67 mmol) portionwise at rt, and stirred for 40 min. A solution of mesylate (*S*)-**39** (347 mg, 1.24 mmol) in DMF (2.5 mL) was added dropwise and heated at 75 ^{o}C for 6 h. The resulting dark brown mixture was diluted with EtOAc (75 mL) and water (25 mL). The aqueous phase was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with water (4 x 20 mL), 1 M NaOH (3 x 15 mL), brine (15 mL), passed through a hydrophobic frit, and solvent removed *in vacuo* to give an orange oil, which was purified by column chromatography (petrol/EtOAc, 9:1) to yield the boronic ester (*S*)-**41** as a yellow oil (231 mg, 46%). R_f 0.40 (hexane/EtOAc, 4:1); $[\alpha]_D^{25}$ -35.1 (c 0.70, $CHCl_3$); δ_H (500 MHz; $CDCl_3$) broadening/splitting of signals due to amide rotamers, 7.73 (2H, d, *J* = 8.0 Hz, 2 x 7-Ar-H), 6.91 (2H, d, *J* = 8.0 Hz, 2 x 6-Ar-H), 4.23-3.73 (3H, m, 2-CH, CH_2O), 3.50-3.26 (2H, m, 5-CH_2), 2.11-1.80 (4H, m, 3-CH_2, 4-CH_2), 1.47 (9H, s, $C(CH_3)_3$), 1.33 (12H, s, 2 x $C(CH_3)_2$); δ_C (125 MHz; $CDCl_3$) 161.4 (Ar-CO), 154.6 ($CO_2{}^{t}$Bu), 136.6 (2 x 7-Ar-CH), 120.7 (Ar-C), 114.0 (2 x 6-Ar-CH), 83.6 (2 x $C(CH_3)_2$), 79.8 and 79.4 ($C(CH_3)_3$), 68.0 (CH_2O), 56.2 and 55.8 (2-CH), 47.0 and 46.6 (5-CH_2), 28.6 ($C(CH_3)_3$), 28.1 (3-CH_2), 24.9 (2 x $C(CH_3)_2$), 24.0 and 22.9 (4-CH_2); *m*/*z*

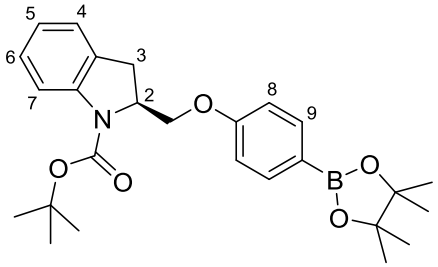
(EI) 403 (M^+, 5%), 114 (M^+ - C_4H_8 – $C_{13}H_{18}O_3B$, 100%); HRMS $C_{22}H_{34}O_5NB$, calcd 403.2525, found 403.2528.

8.17 *tert*-Butyl (*R*)-2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methyl) pyrrolidine-1-carboxylate ((*R*)-**41**)[203]



The title compound was prepared according to the procedure described in section 8.16, from mesylate (*R*)-**39** (809 mg, 2.90 mmol). The crude product was purified by column chromatography (hexane/EtOAc, 9:1) to yield the product (*R*)-**41** as a pale yellow oil (644 mg, 55%). NMR and MS data was identical to that of (*S*)-**41**.

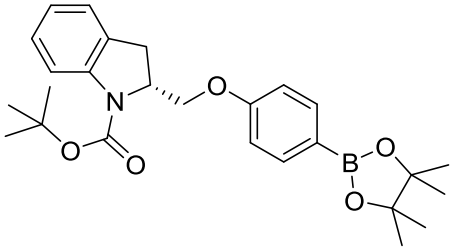
8.18 *tert*-Butyl (*S*)-2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methyl) indoline-1-carboxylate ((*S*)-**46**)



The title compound was prepared according to the procedure described in section 8.16, from mesylate (*S*)-**50** (798 mg, 2.44 mmol). The crude product was purified by column chromatography (hexane/EtOAc, 95:5) to yield the product (*S*)-**46** as a pale yellow solid (499 mg, 45%). R_f 0.25 (hexane/EtOAc, 9:1); m.p. 122-124 ^oC; υ_{max}/cm^{-1} ($CDCl_3$ cast) 2977, 2932, 1701, 1604, 1571, 1484, 1391, 1361; δ_H (500 MHz; $CDCl_3$) 8.10-7.34 (1H, br m, 7-Ar-H), 7.74 (2H, d, *J* = 8.0 Hz, 2 x 9-Ar-H), 7.23-7.12 (2H, m, 4-Ar-H, 6-Ar-H), 6.97 (1H, t, *J* = 7.5 Hz, 5-Ar-H), 6.90 (2H, d, *J* = 8.0 Hz, 2 x 8-Ar-H), 4.91-4.70 (1H, m, 2-CH), 4.28 (1H, dd, *J* = 9.0 and 3.5 Hz, CHHO), 3.98-3.81 (1H, m, CHHO), 3.34 (1H, dd, *J* = 16.5 and 10.0 Hz, 3-CHH), 3.12 (1H, dd, *J* = 16.5 and 1.5 Hz, 3-CHH), 1.59 (9H, s, $C(CH_3)_3$), 1.34 (12H, s, 2 x $C(CH_3)_2$); δ_C (125 MHz; $CDCl_3$) 161.3 (Ar-C), 152.3 ($CO_2{}^t$Bu), 142.2 (Ar-C), 136.6 (2 x 9-Ar-CH), 130.0 (Ar-C), 127.5 (4-Ar-

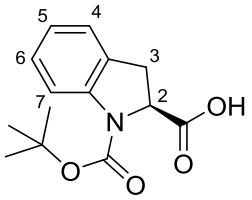
CH), 125.1 (6-Ar-CH), 122.8 (5-Ar-CH), 121.2 (Ar-C), 115.5 (7-Ar-CH), 114.1 (2 x 8-Ar-CH), 83.6 (2 x C(CH_3)$_2$), 81.5 (C(CH_3)$_3$), 67.7 (CH_2O), 57.8 (2-CH), 31.4 (3-CH_2), 28.6 (C(CH_3)$_3$), 24.9 (2 x C(CH_3)$_2$); *m*/*z* (ESI) 474 ([M + Na]$^+$, 80%), 352 (M^+ - $C_5H_8O_2$, 100%); HRMS $C_{26}H_{34}BNO_5Na$, calcd 474.2428, found 474.2420.

8.19 *tert*-Butyl (*R*)-2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)methyl) indoline-1-carboxylate ((*R*)-**46**)



The title compound was prepared according to the procedure described in section 8.16, from mesylate (*R*)-**50** (814 mg, 2.49 mmol). The crude product was purified by column chromatography (hexane/EtOAc, 95:5) to yield the product (*R*)-**46** as a white solid (587 mg, 52%). Characterisation data was identical to that of (*S*)-**46**.

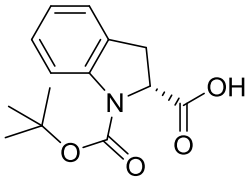
8.20 (*S*)-1-(*tert*-Butoxycarbonyl)indoline-2-carboxylic acid ((*S*)-**48**)[68]



The title compound was prepared according to the procedure described in section 8.11, from (*S*)-indoline-2-carboxylic acid (2.00 g, 12.3 mmol). The crude product was recrystallised (EtOAc/hexane) to yield compound (*S*)-**48** as a pale brown solid (2.90 g, 90%) as a mixture of amide rotamers (7:4 in $CDCl_3$). M.p. 124-126 ^oC (lit[68] 124-125 ^oC, EtOAc/hexane); δ_H (500 MHz; $CDCl_3$) 10.60 (1H, br s, OH), 8.00-7.37 (1H, m, 7-Ar-H), 7.24-7.06 (2H, m, 4-Ar-H, 6-Ar-H), 7.00-6.91 (1H, m, 5-Ar-H), 5.07-4.78 (1H, m, 2-CH), 3.63-3.09 (2H, m, 3-CH_2), 1.68-1.50 (9H, m, C(CH_3)$_3$); δ_C (150 MHz; $CDCl_3$) 178.4 and 177.0 (CO_2H), 151.6 and 153.4 ($CO_2{}^t$Bu), 142.5 and 141.3 (Ar-C), 128.2 and 128.0 (Ar-CH), 128.9 and 127.7 (Ar-C), 125.0 and 124.5 (Ar-CH), 122.9 (Ar-CH), 114.9 and 114.7 (Ar-CH), 83.2 and 81.8 (C(CH_3)$_3$), 60.1 (2-CH), 32.7 and 31.6 (3-

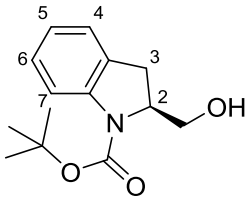
$\underline{C}H_2$), 28.5 and 28.3 ($C(\underline{C}H_3)_3$); *m*/*z* (ESI) 262 ($[M - H]^-$, 100%); HRMS $C_{14}H_{16}NO_4$, calcd 262.1079, found 262.1092.

8.21 (*R*)-1-(*tert*-Butoxycarbonyl)indoline-2-carboxylic acid ((*R*)-**48**)[69]



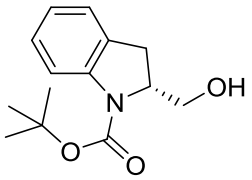
The title compound was prepared according to the procedure described in section 8.11, from (*R*)-indoline-2-carboxylic acid (1.94 g, 11.9 mmol). The crude product was recrystallised (EtOAc/hexane) to yield compound (*R*)-**48** as a pale brown solid (1.81 g, 58%). Characterisation data was identical to that of (*S*)-**48**.

8.22 *tert*-Butyl (*S*)-2-(hydroxymethyl)indoline-1-carboxylate ((*S*)-**49**)[70]



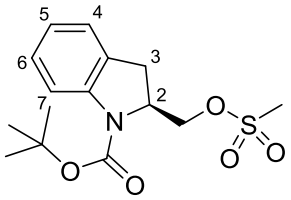
The title compound was prepared according to the procedure described in section 8.9, from (*S*)-**48** (1.80 g, 6.84 mmol). The crude product was purified by column chromatography (hexane/EtOAc, 4:1) to yield the product (*S*)-**49** as a yellow oil (1.24 g, 73%). R_f 0.43 (hexane/EtOAc, 2:1); δ_H (500 MHz; $CDCl_3$) 7.55 (1H, br s, 7-Ar-$\underline{H}$), 7.19-7.11 (2H, m, 4-Ar-$\underline{H}$, 6-Ar-$\underline{H}$), 6.95 (1H, t, *J* = 7.0 Hz, 5-Ar-$\underline{H}$), 4.69-4.51 (1H, m, 2-C$\underline{H}$), 3.76 (1H, dd, *J* = 11.0 and 6.5 Hz, C$\underline{H}$HO), 3.69 (1H, dd, *J* = 11.0 and 4.5 Hz, CH$\underline{H}$O), 3.33 (1H, dd, *J* = 16.0 Hz and 10.5 Hz, 3-C$\underline{H}$H), 2.95-2.60 (2H, m, 3-CH$\underline{H}$, O$\underline{H}$), 1.59 (9H, s, $C(C\underline{H}_3)_3$); δ_C (150 MHz; $CDCl_3$) 154.6 ($\underline{C}O_2{}^t$Bu), 141.8 (Ar-$\underline{C}$), 130.0 (Ar-$\underline{C}$), 127.5 (Ar-$\underline{C}$H), 125.0 (Ar-$\underline{C}$H), 122.9 (Ar-$\underline{C}$H), 115.7 (Ar-$\underline{C}$H), 82.5 ($\underline{C}(CH_3)_3$), 66.9 ($\underline{C}H_2O$), 61.2 (2-$\underline{C}$H), 31.2 (3-$\underline{C}H_2$), 28.5 ($C(\underline{C}H_3)_3$); *m*/*z* (EI) 249 (M^+, 10%), 118 ($M^+ - C_6H_{11}O_3$, 100%); HRMS $C_{14}H_{19}O_3N$, calcd 249.1360, found 249.1362.

8.23 *tert*-Butyl (*R*)-2-(hydroxymethyl)indoline-1-carboxylate ((*R*)-**49**)[71]



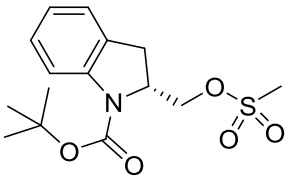
The title compound was prepared according to the procedure described in section 8.9, from (*R*)-**48** (1.70 g, 6.46 mmol). The crude product was purified by column chromatography (hexane/EtOAc, 4:1) to yield the alcohol (*R*)-**49** as a yellow oil (0.987 g, 61%). Characterisation data was identical to that of (*S*)-**49**.

8.24 *tert*-Butyl (*S*)-2-(((methylsulfonyl)oxy)methyl)indoline-1-carboxylate ((*S*)-**50**)



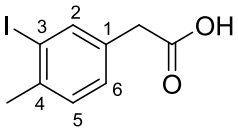
The title compound was prepared according to the procedure described in section 8.14, from (*S*)-**49** (1.12 g, 4.49 mmol). The crude product (*S*)-**50** was isolated as a brown solid (1.33 g, 90% crude), and used without further purification. M.p. 50-55 ^{o}C; υ_{max}/cm^{-1} ($CDCl_3$ cast) 2976, 2938, 1698, 1603, 1484; δ_H (500 MHz; $CDCl_3$) 7.98-7.36 (1H, br m, 7-Ar-H), 7.20-7.14 (2H, m, 4-Ar-H, 6-Ar-H), 6.96 (1H, t, *J* = 7.5 Hz, 5-Ar-H), 4.73-4.66 (1H, m, 2-CH), 4.38 (1H, dd, *J* = 10.0 and 3.5 Hz, CHHO), 4.35-4.30 (1H, m, CHHO), 3.37 (1H, dd, *J* = 16.5 and 10.0 Hz, 3-CHH), 3.03 (1H, dd, *J* = 16.5 and 2.5 Hz, 3-CHH), 2.89 (3H, s, SO_2CH_3), 1.58 (9H, s, $C(CH_3)_3$); δ_C (150 MHz; $CDCl_3$) 152.1 ($CO_2{}^t$Bu), 142.1 (Ar-C), 129.6 (Ar-C), 127.8 (4-Ar-CH), 125.1 (6-Ar-CH), 123.1 (5-Ar-CH), 115.3 (7-Ar-CH), 81.9 ($C(CH_3)_3$), 69.3 (CH_2O), 57.6 (2-CH), 37.5 (SO_2CH_3), 31.2 (3-CH_2), 28.5 ($C(CH_3)_3$); *m*/*z* (EI) 327 (M^+, 20%), 272 (M^+ - C_4H_7, 60%), 228 (M^+ - $C_5H_7O_2$, 85%), 176 (M^+ - $C_5H_{11}O_3S$, 100%); HRMS $C_{15}H_{21}O_5NS$, calcd 327.1135, found 327.1134.

8.25 *tert*-Butyl (*R*)-2-(((methylsulfonyl)oxy)methyl)indoline-1-carboxylate ((*R*)-**50**)



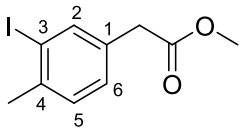
The title compound was prepared according to the procedure described in section 8.14, from (*R*)-**49** (891 mg, 3.57 mmol). The crude product was isolated as a brown solid (1.02 g, 87% crude) and used without further purification. Characterisation data was identical to that of (*S*)-**50**.

8.26 2-(3-Iodo-4-methylphenyl)acetic acid (**51**)[204]



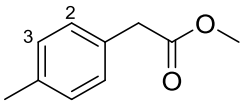
The title compound was prepared based on a literature procedure.[72] Sodium periodate (281 mg, 1.31 mmol) and powdered iodine (224 mg, 0.883 mmol) were added to a stirred solution of glacial acetic acid (2 mL) and acetic anhydride (1 mL) at 5 ^{o}C under argon. Concentrated H_2SO_4 (0.90 mL, 17.7 mmol) was added slowly dropwise to the vigorously stirred reaction mixture at 5 ^{o}C. *p*-Tolylacetic acid **53** (408 mg, 2.72 mmol) was added portionwise to the reaction mixture at 5 ^{o}C and stirred for 1 h at rt, then heated at 40 ^{o}C for 3 h, during which time the dark brown solution turned yellow. The reaction mixture was slowly poured into an ice cold solution of Na_2SO_3 (2.21 g, 17.6 mmol) in water (30 mL) and stirred for 15 min. The solid was collected by filtration, yielding the crude product **51** as a pale yellow solid (516 mg, 69% crude). A portion of the crude product was purified by recrystallisation (hexane) to give **51** as a white solid. δ_H (600 MHz; $CDCl_3$) 11.0 (1H, br s, O<u>H</u>), 7.73 (1H, s, 2-Ar-<u>H</u>), 7.20-7.15 (2H, m, 5-Ar-<u>H</u>, 6-Ar-<u>H</u>), 3.57 (2H, s, C<u>H</u>$_2$CO$_2$H), 2.40 (3H, s, C<u>H</u>$_3$); δ_C (150 MHz; $CDCl_3$) 176.9 (<u>C</u>O$_2$H), 140.7 (4-Ar-<u>C</u>), 139.7 (2-Ar-<u>C</u>H), 132.5 (1-Ar-<u>C</u>), 129.9 (Ar-<u>C</u>H), 129.4 (Ar-<u>C</u>H), 101.3 (3-Ar-<u>C</u>), 39.8 (<u>C</u>H$_2$CO$_2$H), 27.8 (<u>C</u>H$_3$); *m*/*z* (EI) 276 (M^+, 100%), 231 (M^+ - CO_2H, 100%); HRMS $C_9H_9O_2I$, calcd 275.9642, found 275.9646.

8.27 Methyl 2-(3-iodo-4-methylphenyl)acetate (**54**)



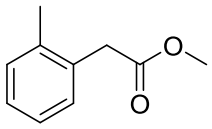
The title compound was prepared according to the procedure described in section 8.26 from methyl ester **55** (4.55 g, 27.7 mmol). The crude yellow-brown oil was purified by column chromatography (petrol/EtOAc, 95:5) to yield the product **54** as a pale yellow oil (6.07 g, 76%). R_f 0.27 (petrol/EtOAc, 95:5); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2950, 1737, 1601, 1557, 1484; δ_H (500 MHz; $CDCl_3$) 7.73 (1H, s, 2-Ar-H), 7.20-7.14 (2H, m, 5-Ar-H, 6-Ar-H), 3.69 (3H, s, OCH_3), 3.54 (2H, s, $CH_2CO_2CH_3$), 2.40 (3H, s, Ar-CH_3); δ_C (125 MHz; $CDCl_3$) 171.7 (CO_2CH_3), 140.3 (4-Ar-C), 139.6 (2-Ar-CH), 133.2 (1-Ar-C), 129.8, (Ar-CH), 129.2 (Ar-CH), 101.2 (3-Ar-C), 52.2 (OCH_3), 40.0 ($CH_2CO_2CH_3$), 27.7 (Ar-CH_3); *m*/*z* (EI) 290 (M^+, 80%), 231 (M^+ - CO_2CH_3, 100%); HRMS $C_{10}H_{11}O_2I$ calcd 289.9798, found 289.9799.

8.28 Methyl 2-(*p*-tolyl)acetate (**55**)[205]



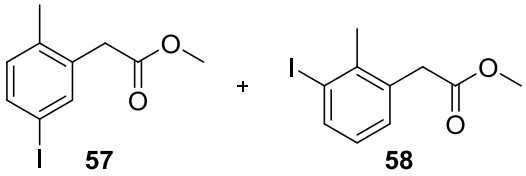
To a solution of *p*-tolylacetic acid (5.01 g, 33.4 mmol) in methanol (65 mL) was added concentrated H_2SO_4 (0.40 mL, 7.50 mmol) and heated at reflux for 6 h. The solution was concentrated *in vacuo*, and the residue dissolved in EtOAc (180 mL) and washed with $NaHCO_3$, brine, dried ($MgSO_4$) and solvent removed *in vacuo* to yield the product **55** as a pale yellow oil (5.21 g, 95%). δ_H (500 MHz; $CDCl_3$) 7.17 (2H, d, *J* = 8.0 Hz, 2 x 2-Ar-H), 7.13 (2H, d, *J* = 8.0 Hz, 2 x 3-Ar-H), 3.68 (3H, s, OCH_3), 3.59 (2H, s, $CH_2CO_2CH_3$), 2.33 (3H, s, Ar-CH_3); δ_C (125 MHz; $CDCl_3$) 172.3 (CO_2CH_3), 136.8 (Ar-C), 131.0 (Ar-C), 129.4 (2 x 2-Ar-CH), 129.2 (2 x 3-Ar-CH), 52.1 (OCH_3), 40.9 ($CH_2CO_2CH_3$), 21.2 (Ar-CH_3); *m*/*z* (EI) 164 (M^+, 30%), 105 (M^+ - CO_2CH_3, 100%); HRMS $C_{10}H_{12}O_2$, calcd 164.0832, found 164.0827.

8.29 Methyl 2-(*o*-tolyl)acetate (56)[206]



The title compound was prepared according to the procedure described in section 8.28, from *o*-tolylacetic acid (5.04 g, 33.6 mmol), to yield the product **56** as a pale yellow oil (5.27 g, 96%). δ_H (500 MHz; $CDCl_3$) 7.23-7.14 (4H, m, 4 x Ar-H), 3.69 (3H, s, OCH_3), 3.65 (2H, s, $CH_2CO_2CH_3$), 2.32 (3H, s, Ar-CH_3); δ_C (125 MHz; $CDCl_3$) 172.0 (CO_2CH_3), 136.9 (Ar-C), 132.8 (Ar-C), 130.4 (Ar-CH), 130.2 (Ar-CH), 127.5 (Ar-CH), 126.2 (Ar-CH), 52.1 (OCH_3), 39.1 ($CH_2CO_2CH_3$), 19.7 (Ar-CH_3); *m*/*z* (EI) 164 (M^+, 100%), 105 (M^+ - CO_2CH_3, 60%); HRMS $C_{10}H_{12}O_2$, calcd 164.0832, found 164.0828.

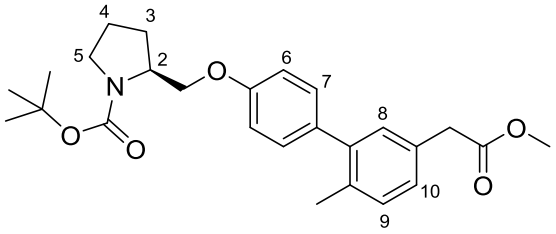
8.30 Methyl 2-(5-iodo-2-methylphenyl)acetate (57)[207] and **methyl 2-(3-iodo-2-methylphenyl)acetate (58)**[208]



57 **58**

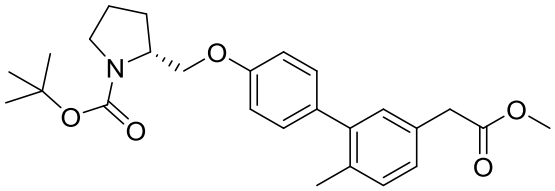
The mixture of aryl iodide regioisomers were synthesised following the procedure described in section 8.26, from methyl ester **56** (501 mg, 3.05 mmol). The crude brown oil was purified by column chromatography (hexane/EtOAc, 9:1) yielding a mixture of inseparable regioisomers **57** and **58** as a colourless oil (482 mg, 54%, ratio **57**/**58** = 3:1). R_f 0.69 (hexane/EtOAc, 4:1); δ_H (500 MHz; $CDCl_3$) major regioisomer **57**: 7.52 (1H, d, *J* = 1.5 Hz, Ar-H), 7.49 (1H, dd, *J* = 8.0 and 1.5 Hz, Ar-H), 6.91 (1H, d, *J* = 8.0 Hz, Ar-H), 3.69 (3H, s, OCH_3), 3.57 (2H, s, $CH_2CO_2CH_3$), 2.25 (3H, s, Ar-CH_3), minor regioisomer **58**: 7.77 (1H, d, *J* = 7.5 Hz, Ar-H), 7.15 (1H, d, *J* = 7.5 Hz, Ar-H), 6.84 (1H, t, *J* = 7.5 Hz, Ar-H), 3.71 (2H, s, $CH_2CO_2CH_3$), 3.69 (3H, s, OCH_3), 2.44 (3H, s, Ar-CH_3).

8.31 *tert*-Butyl (*S*)-2-(((5'-(2-methoxy-2-oxoethyl)-2'-methyl-[1,1'-biphenyl]-4-yl)oxy)methyl)pyrrolidine-1-carboxylate ((*S*)-**62**)



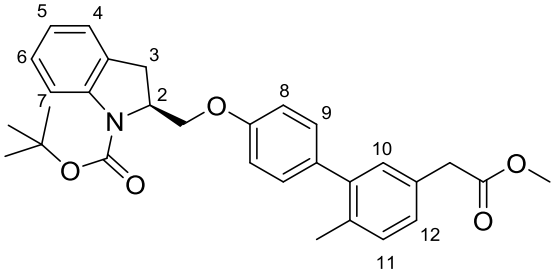
To a solution of boronic ester (*S*)-**41** (260 mg, 0.645 mmol) in DMF (11 mL) was added aryl iodide **54** (250 mg, 0862 mmol) and K_3PO_4 (414 mg, 1.95 mmol). Argon was bubbled through the solution for 10 min. Tetrakis(triphenylphosphine)palladium(0) (96.5 mg, 0.0835 mmol) was added to the reaction mixture and heated at 85 ^oC for 24 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with water (6 x 50 mL) and brine (50 mL), dried (phase separator) and solvent removed *in vacuo* to give a crude orange-brown oil, which was purified by column chromatography (hexane/EtOAc, 9:1) to afford the biaryl product (*S*)-**62** as a yellow oil (211 mg, 74%). R_f 0.31 (hexane/EtOAc, 4:1); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2973, 1740, 1691, 1609, 1516; δ_H (600 MHz; $CDCl_3$) broadening/splitting of signals due to amide rotamers, 7.25-7.19 (3H, m, 2 x 7-Ar-H, 9-Ar-H), 7.15 (1H, d, *J* = 8.0 Hz, 10-Ar-H), 7.12 (1H, s, 8-Ar-H), 6.97 (2H, d, *J* = 7.0 Hz, 2 x 6-Ar-H), 4.25-3.76 (3H, m, 2-CH, CH_2O), 3.69 (3H, s, OCH_3), 3.62 (2H, s, $CH_2CO_2CH_3$), 3.48-3.29 (2H, m, 5-CH_2), 2.25 (3H, s, Ar-CH_3), 2.12-1.82 (4H, m, 3-CH_2, 4-CH_2), 1.49 (9H, s, $C(CH_3)_3$); δ_C (150 MHz; $CDCl_3$) 172.3 (CO_2CH_3), 158.0 and 157.9 (Ar-C), 154.8 and 154.7 ($CO_2{}^tBu$), 141.9 and 141.8 (Ar-C), 134.5 (Ar-C), 134.3 and 134.1 (Ar-C), 131.4 (Ar-C), 130.9 (8-Ar-CH), 130.7 (9-Ar-CH), 130.4 (2 x 7-Ar-CH), 127.9 (10-Ar-CH), 114.2 (2 x 6-Ar-CH), 79.9 and 79.5 ($C(CH_3)_3$), 68.4 and 68.1 (CH_2O), 56.1 and 56.0 (2-CH), 52.2 (OCH_3), 47.1 and 46.7 (5-CH_2), 40.9 ($CH_2CO_2CH_3$), 28.9 and 28.1 (3-CH_2), 28.7 ($C(CH_3)_3$), 24.0 and 22.9 (4-CH_2), 20.3 (Ar-CH_3); *m/z* (EI) 439 (M^+, 15%), 114 (M^+ - C_4H_8 - $C_{17}H_{17}O_3$, 100%); HRMS $C_{26}H_{33}O_5N$, calcd 439.2353, found 439.2350.

8.32 *tert*-Butyl (*R*)-2-(((5'-(2-methoxy-2-oxoethyl)-2'-methyl-[1,1'-biphenyl]-4-yl)oxy)methyl)pyrrolidine-1-carboxylate ((*R*)-**62**)



The title compound was prepared according to the procedure described in section 8.31, from the boronic ester (*R*)-**41** (60.0 mg, 0.149 mmol). The crude brown oil was purified by column chromatography (hexane/EtOAc, 9:1) to yield the biaryl product (*R*)-**62** as a pale yellow oil (53.5 mg, 82%). Characterisation data was identical to that of (*S*)-**62**.

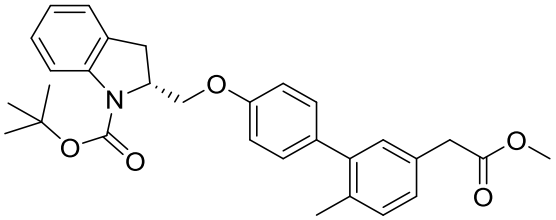
8.33 *tert*-Butyl (*S*)-2-(((5'-(2-methoxy-2-oxoethyl)-2'-methyl-[1,1'-biphenyl]-4-yl)oxy)methyl)indoline-1-carboxylate ((*S*)-**63**)



The title compound was prepared according to the procedure described in section 8.31, from the boronic ester (*S*)-**46** (0.288 g, 0.638 mmol). The crude brown oil was purified by column chromatography (hexane/EtOAc, 9:1) to yield the biaryl product (*S*)-**63** as a yellow oil (0.248 g, 80%). R_f 0.18 (hexane/EtOAc, 9:1); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2975, 2951, 1738, 1692, 1607, 1576, 1515; δ_H (500 MHz; $CDCl_3$) δ_H (500 MHz; $CDCl_3$) broadening/splitting of signals due to amide rotamers, 8.05-7.32 (1H, br m, 7-Ar-H), 7.25-7.14 (6H, m, 4-Ar-H, 6-Ar-H, 2 x 9-Ar-H, 11-Ar-H, 12-Ar-H), 7.12 (1H, d, *J* = 1.5 Hz, 10-Ar-H), 6.98 (1H, t, *J* = 7.5 Hz, 5-Ar-H), 6.95 (2H, d, *J* = 8.5 Hz, 2 x 8-Ar-H), 4.94-4.77 (1H, m, 2-CH), 4.37-4.23 (1H, m, CHHO), 4.01-3.87 (1H, m, CHHO), 3.70 (3H, s, OCH_3), 3.62 (2H, s, $CH_2CO_2CH_3$), 3.37 (1H, dd, *J* = 16.5 and 9.5 Hz, 3-CHH), 3.16 (1H, dd, *J* = 16.5 and 1.5 Hz, 3-CHH), 2.25 (3H, s, Ar-CH_3), 1.60 (9H, s, $C(CH_3)_3$); δ_C (125 MHz; $CDCl_3$) major rotamer, some Ar-C signals obscured by Ar-CH signals, 172.2 (CO_2CH_3), 157.8 (Ar-C), 152.4 ($CO_2{}^tBu$), 142.3 (Ar-C), 141.7 (Ar-C), 134.5 (Ar-C), 134.4 (Ar-C), 131.4 (Ar-C), 130.8 (10-Ar-CH), 130.7 (11-Ar-CH), 130.3 (2 x 9-Ar-CH), 127.9 (12-Ar-CH), 127.5 (4-Ar-CH), 125.1 (6-Ar-CH), 122.8 (5-Ar-

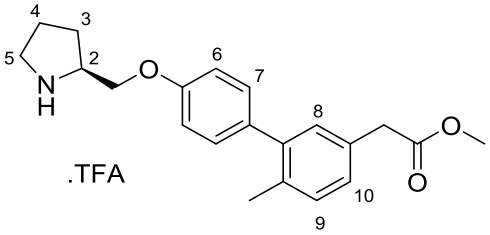
CH), 115.5 (7-Ar-CH), 114.3 (2 x 8-Ar-CH), 81.4 ($C(CH_3)_3$), 68.0 (CH_2O), 57.9 (2-CH), 52.1 (OCH_3), 40.8 ($CH_2CO_2CH_3$), 31.4 (3-CH_2), 28.6 ($C(CH_3)_3$), 20.2 (Ar-CH_3); *m*/*z* (ESI) 510 ($[M + Na]^+$, 25%), 388 (M^+ - $C_5H_7O_2$, 100%); HRMS $C_{30}H_{33}NO_5Na$, calcd 510.2256, found 510.2260.

8.34 *tert*-Butyl (*R*)-2-(((5'-(2-methoxy-2-oxoethyl)-2'-methyl-[1,1'-biphenyl]-4-yl)oxy)methyl)indoline-1-carboxylate ((*R*)-**63**)



The title compound was prepared according to the procedure described in section 8.31, from the boronic ester (*R*)-**46** (480 mg, 1.06 mmol). The crude brown oil was purified by column chromatography (hexane/EtOAc, 9:1) to yield the biaryl product (*R*)-**63** as a yellow-brown oil (360 mg, 70%). Characterisation data was identical to that of (*S*)-**63**.

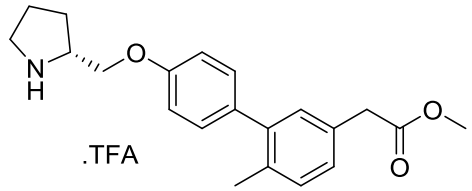
8.35 Methyl (*S*)-2-(6-methyl-4'-(pyrrolidin-2-ylmethoxy)-[1,1'-biphenyl]-3-yl)acetate ((*S*)-**64**)



Trifluoroacetic acid (1 mL) was added dropwise to a solution of (*S*)-**62** (158 mg, 0.358 mmol) in CH_2Cl_2 (1 mL) at 0 ^oC, and stirred for 1 h at rt. The solution was concentrated *in vacuo* to give the crude product as the TFA salt (165 mg, 100% crude) as an orange oil, which was used without further purification. υ_{max}/cm^{-1} ($CDCl_3$ cast) 2956, 2775, 1778, 1734, 1670, 1610, 1516, 1496; δ_H (500 MHz; $CDCl_3$) 9.69 (1H, br s, NH), 8.30 (1H, br s, NH), 7.25-7.18 (3H, m, 2 x 7-Ar-H, 9-Ar-H), 7.15 (1H, dd, *J* = 8.0 and 1.5 Hz, 10-Ar-H), 7.08 (1H, d, *J* = 1.5 Hz, 8-Ar-H), 6.90 (2H, d, *J* = 8.0 Hz, 2 x 6-Ar-H), 4.27 (1H, dd, *J* = 10.0 and 3.0 Hz, CHHO), 4.17 (1H, dd, *J* = 10.0 and 5.5 Hz, CHHO), 4.13-4.04 (1H, m, 2-CH), 3.69 (3H, s, OCH_3), 3.61 (2H, s, $CH_2CO_2CH_3$), 3.45-3.35 (2H, m, 5-CH_2), 2.21 (3H, s, Ar-CH_3), 2.30-1.99 (4H, m, 3-CH_2, 4-CH_2); δ_C (125 MHz;

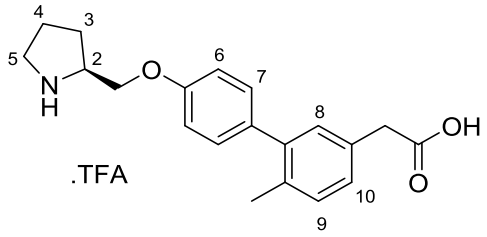
$CDCl_3$) 172.5 ($\underline{C}O_2CH_3$), 156.5 (Ar-$\underline{C}$), 141.4 (Ar-$\underline{C}$), 135.6 (Ar-$\underline{C}$), 134.4 (Ar-$\underline{C}$), 131.4 (Ar-$\underline{C}$), 130.7 and 130.5 (2 x 7-Ar-$\underline{C}$H, 8-Ar-$\underline{C}$H, 9-Ar-$\underline{C}$H), 128.0 (10-Ar-$\underline{C}$H), 114.2 (2 x 6-Ar-$\underline{C}$H), 66.1 ($\underline{C}H_2O$), 59.2 (2-$\underline{C}$H), 52.2 ($O\underline{C}H_3$), 46.2 (5-$\underline{C}H_2$), 40.8 ($\underline{C}H_2CO_2CH_3$), 26.8 ($\underline{C}H_2$), 24.0 ($\underline{C}H_2$), 20.1 (Ar-$\underline{C}H_3$); *m*/*z* (ESI) 340 ($[M + H]^+$, 100%); HRMS $C_{21}H_{26}NO_3$, calcd 340.1913, found 340.1907.

8.36 Methyl (*R*)-2-(6-methyl-4'-(pyrrolidin-2-ylmethoxy)-[1,1'-biphenyl]-3-yl)acetate ((*R*)-**64**)



The title compound was prepared according to the procedure described in section 8.35, from (*R*)-**62** (153 mg, 0.348 mmol). The crude product (*R*)-**64** was isolated as a yellow-brown oil as the TFA salt (164 mg, 100% crude), and used without further purification. Characterisation data was identical to that of (*S*)-**64**.

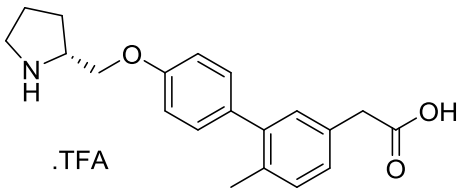
8.37 (*S*)-2-(6-Methyl-4'-(pyrrolidin-2-ylmethoxy)-[1,1'-biphenyl]-3-yl)acetic acid ((*S*)-**65**)



A solution of lithium hydroxide (3.45 mg, 0.144 mmol) in water (0.1 mL) was added to a solution of the ester (*S*)-**64** (16.3 mg, 0.0434 mmol, HCl salt) in methanol (0.1 mL) and THF (0.1 mL). The solution was stirred at rt for 19 h, then concentrated *in vacuo*. The resulting residue was dissolved in water (5 mL) and pH adjusted to 7 by addition of 0.2 M HCl. The aqueous phase was extracted with EtOAc (2 x 10 mL), CH_2Cl_2 (2 x 10 mL) and 20% methanol in CH_2Cl_2 (2 x 10 mL). The combined organic extracts were passed through a hydrophobic frit and evaporated *in vacuo*. None of the desired compound was extracted. The aqueous phase was concentrated *in vacuo* and the residue was dissolved in CH_3OH:DMSO (1:1, 1 mL), filtered and purified by MDAP (Gradient

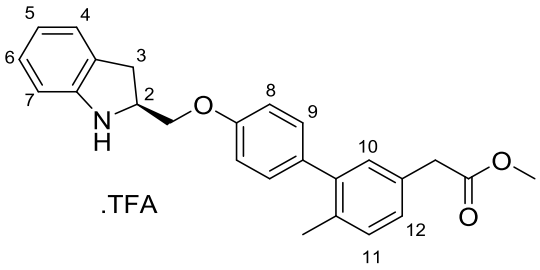
B, r_t = 9.82 min). Appropriate fractions were combined and evaporated *in vacuo* to yield (*S*)-**65** as the TFA salt as a colourless oil (16.1 mg, 84%). $[\alpha]_D^{25}$ +9.9 (c 0.81, $CHCl_3$); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2976, 1672, 1610, 1516; δ_H (600 MHz; $CDCl_3$) 10.33 (1H, br s, NH), 8.83 (1H, br s, NH), 7.18 (1H, d, *J* = 8.0 Hz, 10-Ar-H), 7.11-7.07 (3H, m, 2 x 7-Ar-H, 9-Ar-H), 6.94 (1H, s, 8-Ar-H), 6.79 (2H, d, *J* = 8.0 Hz, 2 x 6-Ar-H), 4.14 (1H, dd, *J* = 10.5 and 4.0 Hz, CHHO), 4.07 (1H, dd, *J* = 10.5 and 6.0 Hz, CHHO), 3.99-3.92 (1H, m, 2-CH), 3.50 (2H, s, CH_2CO_2H), 3.40-3.29 (2H, m, 5-CH_2), 2.18 (3H, s, Ar-CH_3), 2.20-1.91 (4H, m, 3-CH_2, 4-CH_2); δ_C (150 MHz; $CDCl_3$) 176.5 (CO_2H), 156.7 (Ar-C), 141.4 (Ar-C), 135.2 (Ar-C), 134.4 (Ar-C), 131.3 (Ar-C), 130.8 (8-Ar-CH), 130.7 (9-Ar-CH), 130.5 (2 x 7-Ar-CH), 128.2 (10-Ar-CH), 114.3 (2 x 6-Ar-CH), 66.3 (CH_2O), 58.7 (2-CH), 45.7 (5-CH_2), 40.7 (CH_2CO_2H), 26.9 (3-CH_2), 24.1 (4-CH_2), 20.2 (Ar-CH_3); *m/z* (ESI) 326 ($[M + H]^+$, 100%); HRMS $C_{20}H_{24}NO_3$, calcd 326.1756, found 326.1771.

8.38 (*R*)-2-(6-Methyl-4'-(pyrrolidin-2-ylmethoxy)-[1,1'-biphenyl]-3-yl)acetic acid ((*R*)-65)



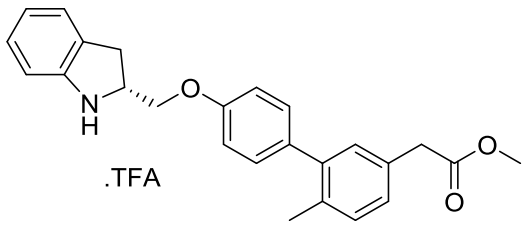
A solution of lithium hydroxide (5.86 mg, 0.132 mmol) in water (0.2 mL) was added to a stirred solution of the ester (*R*)-**62** (36.0 mg, 0.0819 mmol) in methanol (0.2 mL) and THF (0.2 mL). The solution was stirred at rt for 21 h, then concentrated *in vacuo* to give a pale yellow residue, which was dissolved in CH_3OH:DMSO (1:1, 1 mL), filtered and purified by MDAP (Gradient D, r_t = 13.9 min, *m/z* 426 $[M + H]^+$). Appropriate fractions were combined and solvent removed *in vacuo* to yield a pale yellow oil (22.0 mg), which was dissolved in 4 M HCl in 1,4-dioxane (1 mL, 4.00 mmol) and stirred at rt for 21 h. The volatiles were removed from the reaction under a stream of N_2 to give the crude product as a pale yellow oil which was dissolved in CH_3OH:DMSO (1:1, 1 mL), filtered and purified by MDAP (run time 10 min, Gradient B, r_t = 9.78 min). Appropriate fractions were combined and evaporated *in vacuo* to yield the product (*R*)-**65** as the TFA salt as a colourless oil (19.1 mg, 53%). Characterisation data was identical to that of (*S*)-**65** except $[\alpha]_D^{25}$ -6.0 (c 0.96, $CHCl_3$).

8.39 Methyl (*S*)-2-(4'-(indolin-2-ylmethoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetate ((*S*)-66)



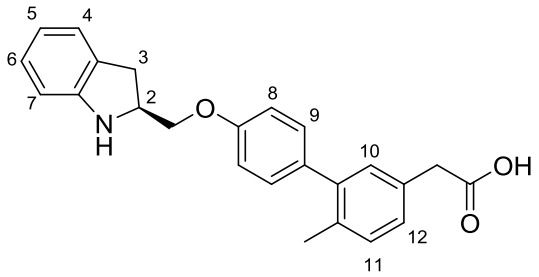
The title compound was prepared following the same procedure as described in section 8.35, from (*S*)-**63** (190 mg, 0.391 mmol). The crude product was isolated as an orange oil as the TFA salt (0.198 g, 100% crude) and used without further purification. υ_{max}/cm^{-1} ($CDCl_3$ cast) 2954, 2543, 1778, 1735, 1666, 1610, 1516; δ_H (500 MHz; $CDCl_3$) 7.41-7.35 (3H, m, 4-Ar-H, 6-Ar-H, 7-Ar-H), 7.31-7.26 (1H, m, 5-Ar-H), 7.23 (2H, d, *J* = 9.0 Hz, 2 x 9-Ar-H), 7.21 (1H, d, *J* = 8.0 Hz, 11-Ar-H), 7.15 (1H, d, *J* = 8.0 Hz, 12-Ar-H), 7.09 (1H, s, 10-Ar-H), 6.93 (2H, d, *J* = 9.0 Hz, 2 x 8-Ar-H), 4.80-4.70 (1H, m, 2-CH), 4.43-4.31 (2H, m, CH_2O), 3.69 (3H, s, OCH_3), 3.61 (2H, s, $CH_2CO_2CH_3$), 3.51 (1H, dd, *J* = 16.5 and 9.0 Hz, 3-CHH), 3.30 (1H, dd, *J* = 16.5 and 7.5 Hz, 3-CHH), 2.21 (3H, s, Ar-CH_3); δ_C (125 MHz; $CDCl_3$) 172.5 (CO_2H), 156.5 (Ar-C), 141.4 (Ar-C), 136.4 (Ar-C), 135.6 (Ar-C), 134.4 (Ar-C), 133.6 (Ar-C), 131.4 (Ar-C), 130.8 (Ar-CH), 130.7 (Ar-CH), 130.5 (2 x 9-Ar-CH), 129.4 (Ar-CH), 128.9 (Ar-CH), 128.0 (Ar-CH), 126.0 (Ar-CH), 119.0 (Ar-CH), 114.3 (2 x 8-Ar-CH), 66.5 (CH_2O), 59.6 (2-CH), 52.2 (OCH_3), 40.8 ($CH_2CO_2CH_3$), 32.1 (3-CH_2), 20.1 (Ar-CH_3); *m/z* (ESI) 388 ($[M + H]^+$, 100%); HRMS $C_{25}H_{26}NO_3$, calcd 388.1913, found 388.1921.

8.40 Methyl (*R*)-2-(4'-(indolin-2-ylmethoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetate ((*R*)-66)



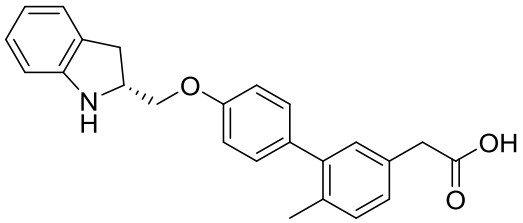
The title compound was prepared following the same procedure as described in section 8.35, from (*R*)-**63** (290 mg, 0.597 mmol). The crude product was isolated as a yellow oil as the TFA salt (0.303 g, 100% crude) and used without further purification. Characterisation data was identical to that of (*S*)-**66**.

8.41 (*S*)-2-(4'-(Indolin-2-ylmethoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetic acid ((*S*)-**67**)



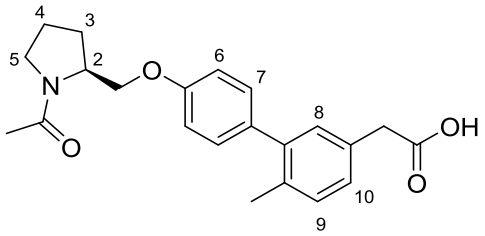
To a solution of compound (*S*)-**66** (55.0 mg, 0.110 mmol) in THF (0.3 mL) and CH_3OH (0.3 mL), was added a suspension of $LiOH.H_2O$ (20.0 mg, 0.477 mmol) in water (0.3 mL), and the mixture stirred at rt for 24 h. The reaction mixture was diluted with water (10 mL) and 1 M HCl was added dropwise until the pH ~ 3. The aqueous phase was extracted with EtOAc (3 x 15 mL). The combined organic extracts were washed with brine (15 mL), dried (Na_2SO_4) and solvent removed *in vacuo* to give a crude brown oil which was purified by column chromatography (EtOAc) to give the product (*S*)-**67** as a yellow oil (19.6 mg, 48%). R_f 0.89 (EtOAc); $[\alpha]_D^{25}$ +49.0 (c 0.80, $CHCl_3$); υ_{max}/cm^{-1} ($CDCl_3$ cast) 3035, 2924, 2858, 1708, 1608, 1515; δ_H (500 MHz; $CDCl_3$) 7.25-7.20 (3H, m, 2 x 9-Ar-H, 11-Ar-H), 7.16 (1H, d, *J* = 8.0 Hz, 12-Ar-H), 7.14-7.10 (2H, m, 4-Ar-H, 10-Ar-H), 7.05 (1H, t, *J* = 7.5 Hz, 6-Ar-H), 6.95 (2H, d, *J* = 8.5 Hz, 2 x 8-Ar-H), 6.74 (1H, t, *J* = 7.5 Hz, 5-Ar-H), 6.67 (1H, d, *J* = 7.5 Hz, 7-Ar-H), 4.36-4.27 (1H, m, 2-CH), 4.04-3.98 (2H, m, CH_2O), 3.64 (2H, s, CH_2CO_2H), 3.27 (1H, dd, *J* = 15.5 and 9.0 Hz, 3-CHH), 2.85 (1H, dd, *J* = 15.5 and 6.5 Hz, 3-CHH), 2.25 (3H, s, Ar-CH_3); δ_C (125 MHz; $CDCl_3$) 177.5 (CO_2H), 157.8 (Ar-C), 150.2 (Ar-C), 141.8 (Ar-C), 134.7 (Ar-C), 134.4 (Ar-C), 131.0 (10-Ar-CH), 130.8 (Ar-C), 130.7 (11-Ar-CH), 130.4 (2 x 9-Ar-CH), 128.0 (12-Ar-CH), 127.9 (Ar-C), 127.7 (6-Ar-CH), 125.0 (4-Ar-CH), 119.0 (5-Ar-CH), 114.2 (2 x 8-Ar-CH), 109.9 (7-Ar-CH), 71.4 (CH_2O), 58.1 (2-CH), 40.6 (CH_2CO_2H), 32.6 (3-CH_2), 20.3 (Ar-CH_3); *m*/*z* (ESI) 374 ($[M + H]^+$, 100%); HRMS $C_{24}H_{24}NO_3$, calcd 374.1756, found 374.1750.

8.42 (*R*)-2-(4'-(Indolin-2-ylmethoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetic acid ((*R*)-**67**)



The title compound was prepared following the same procedure as described in section 8.41, from (*R*)-**66** (56.0 mg, 0.112 mmol). The crude product was purified by column chromatography (EtOAc) to yield the product (*R*)-**67** as a yellow oil (20.6 mg, 49%). Characterisation data was identical to that of (*S*)-**67** except $[\alpha]_D^{25}$ -44.6 (c 0.70, $CHCl_3$).

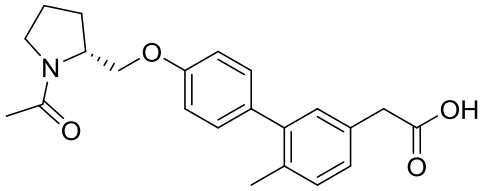
8.43 (*S*)-2-(4'-((1-Acetylpyrrolidin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetic acid ((*S*)-**68**)



DMAP (6.00 mg, 0.0491 mmol) followed by anhydrous triethylamine (0.10 mL, 0.717 mmol) were added to a solution of (*S*)-**64** (60.4 mg, 0.158 mmol) in CH_2Cl_2 (2 mL). The solution was stirred at rt for 45 min, then cooled to 0 ^oC and acetic anhydride (0.05 mL, 0.529 mmol) was added. The solution was stirred at rt for 21 h and then diluted with CH_2Cl_2 (10 mL) and brine (10 mL). The aqueous phase was extracted with CH_2Cl_2 (2 x 10 mL). The combined organic extracts were washed with brine (10 mL), dried (Na_2SO_4) and solvent removed *in vacuo* to give a yellow oil which was purified by column chromatography (EtOAc) to yield the intermediate methyl (*S*)-2-(4'-((1-acetylpyrrolidin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetate as a pale yellow oil (51.0 mg, 84%), as a mixture of amide rotamers (4:1 in $CDCl_3$). R_f 0.30 (EtOAc); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2952, 2876, 1738, 1641, 1610, 1516; δ_H (500 MHz; $CDCl_3$) 7.25-7.08 (5H, m, 2 x 7-Ar-H, 8-Ar-H, 9-Ar-H, 10-Ar-H), 7.00-6.89 (2H, m, 2 x 6-Ar-H), 4.50-3.82 (3H, m, 2-CH, CH_2O), 3.69 (3H, s, OCH_3), 3.61 (2H, s, $CH_2CO_2CH_3$), 3.60-3.40 (2H, m, 5-CH_2), 2.25-2.20 (3H, m, Ar-CH_3), 2.19-1.92 (7H, m, $COCH_3$, 3-CH_2, 4-CH_2); *m*/*z* (ESI) 404 ($[M + Na]^+$, 85%), 382 ($[M + H]^+$, 100%); HRMS

$C_{23}H_{27}NO_4Na$, calcd 404.1838, found 404.1825. Methyl (*S*)-2-(4'-((1-acetylpyrrolidin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetate (31.0 mg, 0.0813 mmol) was dissolved in THF (0.2 mL) and CH_3OH (0.2 mL), and a suspension of $LiOH.H_2O$ (10.0 mg, 0.238 mmol) in water (0.2 mL) was added. The reaction mixture was stirred at rt for 24 h, then concentrated *in vacuo* and the resulting residue diluted with water (8 mL) and pH adjusted to 3 by addition of 1 M HCl. The aqueous phase was extracted with EtOAc (4 x 10 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and solvent removed *in vacuo* to yield a crude yellow oil which was dissolved in CH_3OH/DMSO (1:1, 1 mL), filtered and purified by MDAP (Gradient C, r_t = 10.8 min). Appropriate fractions were combined and evaporated *in vacuo* to yield (*S*)-**68** as a pale yellow oil (21.9 mg, 73%), as a mixture of amide rotamers (4:1 in $CDCl_3$). $[\alpha]_D^{25}$ -56.5 (c 1.10, $CHCl_3$); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2956, 2878, 1723, 1606, 1516; δ_H (400 MHz; $CDCl_3$) 7.31-7.14 (5H, m, 2 x 7-Ar-H, 8-Ar-H, 9-Ar-H, 10-Ar-H), 7.05-6.91 (2H, m, 2 x 6-Ar-H), 4.60-4.28 (1H, m, 2-CH), 4.27-3.88 (2H, m, CH_2O), 3.67 (2H, s, CH_2CO_2H), 3.64-3.45 (2H, m, 5-CH_2), 2.28 (3H, s, Ar-CH_3), 2.26-1.97 (7H, m, $COCH_3$, 3-CH_2, 4-CH_2); δ_C (100 MHz; $CDCl_3$) 176.1 (CO_2H), 170.3 ($COCH_3$), 157.7 and 157.3 (Ar-CO), 141.7 and 141.5 (Ar-C), 134.4 (Ar-C), 134.1 (Ar-C), 131.2 (Ar-C), 130.9 (Ar-CH), 130.6 (Ar-CH), 130.4 and 130.3 (2 x 7-Ar-CH), 128.0 and 127.8 (Ar-CH), 114.1 and 114.0 (2 x 6-Ar-CH), 68.9 and 67.3 (CH_2O), 57.1 and 56.1 (2-CH), 48.3 and 45.8 (5-CH_2), 40.7 (CH_2CO_2H), 28.9 and 27.7 (CH_2), 24.3 and 22.1 (CH_2), 22.7 and 22.2 ($COCH_3$), 20.2 (Ar-CH_3); *m/z* (ESI) 390 ($[M + Na]^+$, 100%), 368 ($[M + H]^+$, 80%); HRMS $C_{22}H_{25}NO_4Na$, calcd 390.1681, found 390.1688.

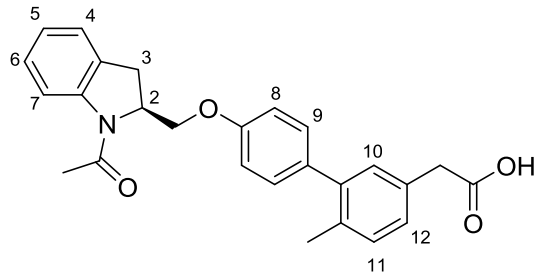
8.44 (*R*)-2-(4'-((1-Acetylpyrrolidin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetic acid ((*R*)-**68**)



The title compound was prepared according to the procedure described in section 8.43. The intermediate methyl (*R*)-2-(4'-((1-acetylpyrrolidin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetate was prepared from (*R*)-**64** (113 mg, 0.249 mmol). The crude oil was purified by column chromatography (EtOAc/CH_3OH, 99:1) to yield the

intermediate compound as a yellow oil (74.3 mg, 78%). Characterisation data of intermediate methyl (*R*)-2-(4'-((1-acetylpyrrolidin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetate was identical to the corresponding (*S*)-enantiomer described in section 8.43. The title compound was prepared from the intermediate (52.0 mg, 0.136 mmol) and purified according to the procedure described in section 8.43. The product (*R*)-**68** was a pale yellow oil (32.2 mg, 50%). Characterisation data was identical to that of (*S*)-**68** except $[\alpha]_D^{25}$ +56.5 (c 1.60, $CHCl_3$).

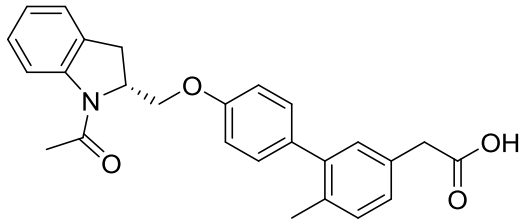
8.45 (*S*)-2-(4'-((1-Acetylindolin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetic acid ((*S*)-**69**)



The title compound was prepared according to the procedure described in section 8.43. The intermediate methyl (*S*)-2-(4'-((1-acetylindolin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl) acetate was prepared from (*S*)-**66** (0.120 g, 0.239 mmol). The crude oil was purified by column chromatography (hexane/EtOAc, 2:1) to yield the intermediate compound as a yellow oil (80.8 mg, 79%), as a mixture of amide rotamers (3:2 in $CDCl_3$). R_f 0.26 (hexane/EtOAc, 2:1); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2951, 2925, 1736, 1655, 1608, 1515, 1480; δ_H (500 MHz; $CDCl_3$) 8.22-7.17 (6H, m, 4-Ar-H, 6-Ar-H, 7-Ar-H, 2 x 9-Ar-H, 11-Ar-H), 7.15 (1H, d, *J* = 8.0 Hz, 12-Ar-H), 7.10 (1H, s, 10-Ar-H), 7.06 (1H, t, *J* = 7.5 Hz, 5-Ar-H), 6.97-6.84 (2H, m, 2 x 8-Ar-H), 5.23-4.74 (1H, m, 2-CH), 4.40-4.00 (1H, m, CHHO), 3.93 (1H, t, *J* = 7.0 Hz, CHHO), 3.69 (3H, s, OCH_3), 3.61 (2H, s, $CH_2CO_2CH_3$), 3.53-2.95 (2H, m, 3-CH_2), 2.44 (3H, s, $COCH_3$), 2.23 (3H, s, Ar-CH_3); *m*/*z* (ESI) 452 ($[M + Na]^+$, 20%), 430 ($[M + H]^+$, 50%), 429 (M^+, 55%), 388 (M^+ - C_2HO, 100%); HRMS $C_{27}H_{27}NO_4Na$, calcd 452.1838, found 452.1819. The title compound was prepared from the intermediate (56.9 mg, 0.132 mmol). The crude product was purified by column chromatography (EtOAc) to yield the product (*S*)-**69** as a yellow oil (17.6 mg, 32%), as a mixture of amide rotamers (7:4 in $CDCl_3$). R_f 0.58 (EtOAc); $[\alpha]_D^{25}$ -53.0 (c 0.72, $CHCl_3$); υ_{max}/cm^{-1} ($CDCl_3$ cast) 3020, 2962, 2850, 1715, 1657, 1611, 1590, 1515; δ_H (500 MHz; $CDCl_3$) 10.57 (1H, br s, CO_2H), 8.19-7.17 (6H,

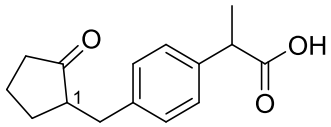
m, 4-Ar-H, 6-Ar-H, 7-Ar-H, 2 x 9-Ar-H, 11-Ar-H), 7.15 (1H, d, *J* = 8.0 Hz, 12-Ar-H), 7.10 (1H, s, 10-Ar-H), 7.06 (1H, t, *J* = 7.5 Hz, 5-Ar-H), 6.93-6.85 (2H, m, 2 x 8-Ar-H), 5.26-4.73 (1H, m, 2-CH), 4.37-4.00 (1H, m, CHHO), 3.92 (1H, t, *J* = 8.0 Hz, CHHO), 3.63 (2H, s, CH_2CO_2H), 3.53-2.96 (2H, m, 3-CH_2), 2.45 (3H, s, $COCH_3$), 2.23 (3H, s, Ar-CH_3); δ_C (150 MHz; $CDCl_3$) major rotamer, some Ar-C signals obscured by the Ar-CH signals, 176.6 (CO_2H), 169.3 ($COCH_3$), 157.3 (Ar-C), 142.1 (Ar-C), 141.6 (Ar-C), 134.9 (Ar-C), 134.7 (Ar-C), 131.0 (Ar-CH), 130.8 (Ar-CH), 130.5 (2 x 9-Ar-CH), 128.1 (Ar-CH), 127.9 (Ar-CH), 125.1 (Ar-CH), 124.4 (Ar-CH), 118.4 (Ar-CH), 114.1 (2 x 8-Ar-CH), 69.0 (CH_2O), 59.6 (2-CH), 40.5 (CH_2CO_2H), 32.3 (3-CH_2), 23.8 ($COCH_3$), 20.3 (Ar-CH_3); *m*/*z* (ESI) 438 ($[M + Na]^+$, 40%), 416 ($[M + H]^+$, 100%), 374 (M^+ - C_2HO, 50%); HRMS $C_{26}H_{26}NO_4$, calcd 416.1862, found 416.1858.

8.46 (*R*)-2-(4'-((1-Acetylindolin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl)acetic acid ((*R*)-**69**)



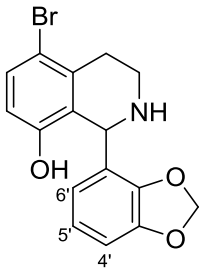
The title compound was prepared according to the procedure described in section 8.43. The intermediate methyl (*R*)-2-(4'-((1-acetylindolin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl) acetate was prepared from (*R*)-**66** (232 mg, 0.463 mmol). The crude oil was purified by column chromatography (hexane/EtOAc, 2:1) to yield the intermediate compound as a yellow oil (170 mg, 86%). Characterisation data of methyl (*S*)-2-(4'-((1-acetylindolin-2-yl)methoxy)-6-methyl-[1,1'-biphenyl]-3-yl) acetate was identical to that of the corresponding (*S*)-enantiomer described in section 8.45. The title compound was prepared from the intermediate compound (119 mg, 0.277 mmol). The crude product was purified by column chromatography (EtOAc) to yield the product (*R*)-**69** as a yellow oil (30.4 mg, 26%). Characterisation data was identical to that of (*S*)-**69** except $[\alpha]_D^{25}$ +52.3 (c 1.20, $CHCl_3$).

8.47 2-(4-((2-Oxocyclopentyl)methyl)phenyl)propanoic acid (loxoprofen) (79)[181]



Loxoprofen was prepared based on a procedure reported by Yamakawa *et al.* for similar analogues.[181] Concentrated HCl (24.0 mL) was added to a solution of diester **222** (440 mg, 1.32 mmol) in acetic acid (12.0 mL) and the mixture heated at reflux for 5 h. After cooling to rt the reaction mixture was diluted with water (150 mL) and extracted with CH_2Cl_2 (3 x 100 mL). The organic extracts were combined, washed with brine, dried (Na_2SO_4) and solvent removed *in vacuo* to give the crude product as a yellow oil which was purified by column chromatography (hexane/EtOAc, 4:1) to give the product as a pale yellow oil (249 mg, 76%). R_f 0.19 (hexane/EtOAc, 4:1); δ_H (500 MHz; $CDCl_3$) 7.22 (2H, d, *J* = 8.0 Hz, 2 x Ar-H), 7.12 (2H, d, *J* = 8.0 Hz, 2 x Ar-H), 3.70 (1H, q, *J* = 7.0 Hz, CHCO$_2$H), 3.12 (1H, dd, *J* = 14.0 and 4.0 Hz, 1-CHCHH), 2.50 (1H, dd, *J* = 14.0 and 9.5 Hz, 1-CHCHH), 2.38-1.50 (7H, m, 1-CH, 3 x CH$_2$), 1.49 (3H, d, *J* = 7.0 Hz, CH$_3$); δ_C (125 MHz; $CDCl_3$) 220.1 (CO), 180.1 (CO$_2$H), 139.3 (Ar-C), 137.7 (Ar-C), 129.3 (2 x Ar-CH), 127.7 (2 x Ar-CH), 51.1 (1-CH), 44.9 (CHCO$_2$H), 38.2 (CH$_2$), 35.3 (1-CHCH$_2$), 29.3 (CH$_2$), 20.6 (CH$_2$), 18.2 (CH$_3$); *m/z* (ESI) 269 ([M + Na]$^+$, 100%); HRMS $C_{15}H_{18}O_3Na$, calcd 269.1154, found 269.1159.

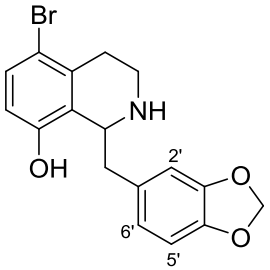
8.48 1-(Benzo[*d*][1,3]dioxol-4-yl)-5-bromo-1,2,3,4-tetrahydroisoquinolin-8-ol (91)[105]



A solution of 2,3-(methylenedioxy)benzaldehyde (36.4 mg, 0.242 mmol) in acetonitrile (3 mL) was added to a solution of amine **157** (60.0 mg, 0.202 mmol) in potassium phosphate buffer (3 mL, 0.5 M, pH 6) under argon and stirred at 80 ^oC for 21 h. The reaction mixture was concentrated *in vacuo*. CH_2Cl_2/CH_3OH (1:1, 10 mL) was added to the resultant residue and the suspension filtered to remove the solid. The filtrate was evaporated to dryness under vacuum to give the crude product as a yellow oil, which was purified by prep-HPLC (Method 1, r_t = 4.4 min). Product containing fractions were

concentrated and co-evaporated with methanol (x3) to give the product as a pale yellow oil (8.7 mg, 12%). δ_H (600 MHz; CD_3OD) 7.48 (1H, d, *J* = 8.5 Hz, 6-Ar-H), 6.89 (1H, d, *J* = 8.0 Hz, 4'-Ar-H), 6.82 (1H, t, *J* = 8.0 Hz, 5'-Ar-H), 6.68 (1H, d, *J* = 8.5 Hz, 7-Ar-H), 6.43 (1H, d, *J* = 8.0 Hz, 6'-Ar-H), 6.08 (1H, d, *J* = 1.0 Hz, OCHHO), 6.03 (1H, d, *J* = 1.0 Hz, OCHHO), 5.90 (1H, s, 1-CH), 3.51-3.28 (2H, m, 3-CH_2), 3.21-3.01 (2H, m, 4-CH_2); δ_C (150 MHz; CD_3OD) 155.2 (Ar-CO), 149.4 (Ar-CO), 147.6 (Ar-CO), 134.2 (6-Ar-CH), 133.7 (Ar-C), 123.4 (6'-Ar-CH), 123.0 (5'-Ar-CH), 120.3 (Ar-C), 118.0 (Ar-C), 115.9 (7-Ar-CH), 114.2 (Ar-C), 110.7 (4'-Ar-CH), 103.1 (OCH_2O), 50.4 (1-CH), 38.0 (3-CH_2), 27.5 (4-CH_2); *m/z* (ESI) 350 ([(^{81}Br)M + H]$^+$, 95%), 348 ([(^{79}Br)M + H]$^+$, 100%); HRMS $C_{16}H_{15}NO_3{}^{79}Br$, calcd 348.0235, found 348.0235.

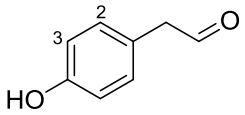
8.49 1-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-5-bromo-1,2,3,4-tetrahydroisoquinolin-8-ol (92)[105]



A solution of aldehyde **140f** (106 mg, 0.646 mmol) in acetonitrile (8 mL) was added to a solution of amine **157** (160 mg, 0.538 mmol) in potassium phosphate buffer (8 mL, 0.5 M, pH 6) under argon and stirred at 50 ^oC for 21 h. The reaction mixture was concentrated *in vacuo*. CH_2Cl_2/CH_3OH (1:1, 10 mL) was added to the resultant residue and the suspension filtered to remove the solid. The filtrate was evaporated to dryness under vacuum to give the crude product as a brown oil, which was purified by prep-HPLC (Method 1, r_t = 7.5 min). Product containing fractions were concentrated and co-evaporated with methanol (x3) to give the product as a pale yellow oil (6.2 mg, 3%). δ_H (600 MHz; CD_3OD) 7.45 (1H, d, *J* = 8.5 Hz, 6-Ar-H), 6.89-6.83 (3H, m, 2'-Ar-H, 5'-Ar-H, 6'-Ar-H), 6.76 (1H, d, *J* = 8.5 Hz, 7-Ar-H), 5.97 (2H, s, OCH_2O), 4.89-4.85 (1H, m, 1-CH), 3.66-3.59 (1H, m, 3-CHH), 3.49 (1H, dd, *J* = 15.0 and 3.5 Hz, 1-CHCHH), 3.44-3.38 (1H, m, 3-CHH), 3.13-2.93 (3H, m, 1-CHCHH, 4-CH_2); δ_C (150 MHz; CD_3OD) 154.9 (Ar-CO), 149.9 (Ar-CO), 148.8 (Ar-CO), 133.8 (Ar-CH), 132.8 (Ar-C), 130.4 (Ar-C), 123.6 (Ar-CH), 122.7 (Ar-C), 116.1 (Ar-CH), 114.6 (Ar-C), 110.2 (Ar-CH), 109.8 (Ar-CH), 102.6 (OCH_2O), 54.3 (1-CH), 37.5 (1-$CHCH_2$), 37.3 (3-CH_2),

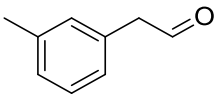
27.5 (4-$\underline{C}H_2$); *m/z* (ESI) 364 ([M(^{81}Br) + H]$^+$, 100%), 362 ([M(^{79}Br) + H]$^+$, 100%); HRMS $C_{17}H_{17}NO_3{}^{79}Br$, calcd 362.0392, found 362.0370.

8.50 2-(4-Hydroxyphenyl)acetaldehyde (107)[122]



Tyrosol (500 mg, 3.62 mmol) was dissolved in CH_2Cl_2 (10 mL) and DMSO (5 mL) and the solution was cooled to -15 ^oC. DIPEA (1.26 mL, 7.24 mmol) was added dropwise to the stirred reaction mixture at -15 ^oC. A fully dissolved solution of SO_3.pyridine (1.15 g, 7.24 mmol) in DMSO (4 mL) was added to the reaction mixture dropwise over 25 min. The reaction was stirred for 1 h 15 min at -15 ^oC and quenched by the addition of ice cold water (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were washed with ice cold water (2 x 40 mL), dried (Na_2SO_4) and solvent evaporated. The residue was co-evaporated with CH_2Cl_2 (3 x 30 mL) to give the crude product as yellow oil, which was purified by column chromatography (hexane/EtOAc, 3:1) to give the product as a pale yellow oil (211 mg, 43%). The product was stored in degassed acetonitrile (0.5 M) at -80 ^oC. R_f 0.40 (hexane/EtOAc, 2:1); δ_H (600 MHz; $CDCl_3$) 9.72 (1H, t, *J* = 2.5 Hz, C$\underline{H}$O), 7.08 (2H, d, *J* = 8.0 Hz, 2 x 2-Ar-$\underline{H}$), 6.83 (2H, d, *J* = 8.0 Hz, 2 x 3-Ar-$\underline{H}$), 5.09 (1H, br s, O$\underline{H}$), 3.62 (2H, d, *J* = 2.5 Hz, C$\underline{H}_2$CHO); δ_C (150 MHz; $CDCl_3$) 200.2 ($\underline{C}$HO), 155.2 (Ar-$\underline{C}$OH), 131.0 (2 x 2-Ar-$\underline{C}$H), 123.8 (Ar-$\underline{C}$), 116.0 (2 x 3-Ar-$\underline{C}$H), 49.8 ($\underline{C}H_2$CHO); *m/z* (CI) 137 ([M + H]$^+$, 100%); HRMS $C_8H_9O_2$, calcd 137.0603, found 137.0597.

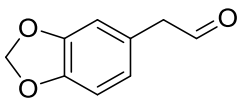
8.51 2-(*m*-Tolyl)acetaldehyde (140e)[118b]



3-Methylphenethyl alcohol (500 mg, 3.59 mmol) was dissolved in DMSO/CH_2Cl_2 1:1 (14 mL) and cooled to -15 ^oC. DIPEA (1.88 mL, 10.8 mmol) was added to the reaction mixture at -15 ^oC, followed by a solution of SO_3.pyridine (1.43 g, 8.98 mmol) in DMSO (7 mL) over 15 min and stirred for 1 h at -15 ^oC. The reaction was quenched by the addition of ice cold water (40 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x

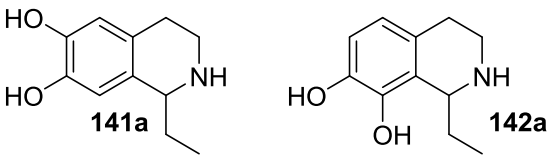
40 mL). The combined organic extracts were washed with ice cold water (5 x 25 mL), brine (25 mL), dried (Na_2SO_4) and solvent evaporated to give the crude product as a yellow oil, which was purified by column chromatography (hexane/EtOAc, 95:5) to give the product as a pale yellow oil (294 mg, 60%). R_f 0.68 (hexane/EtOAc, 2:1); δ_H (600 MHz; $CDCl_3$) 9.74 (1H, t, *J* = 2.5 Hz, C<u>H</u>O), 7.26 (1H, t, *J* = 7.5 Hz, Ar-<u>H</u>), 7.12 (1H, d, *J* = 7.5 Hz, Ar-<u>H</u>), 7.05-7.00 (2H, m, 2 x Ar-<u>H</u>), 3.65 (2H, d, *J* = 2.5 Hz, C<u>H</u>$_2$CHO), 2.36 (3H, s, C<u>H</u>$_3$); δ_C (150 MHz; $CDCl_3$) 199.8 (<u>C</u>HO), 138.9 (Ar-<u>C</u>), 131.8 (Ar-<u>C</u>), 130.8 (Ar-<u>C</u>H), 129.0 (Ar-<u>C</u>H), 128.3 (Ar-<u>C</u>H), 126.8 (Ar-<u>C</u>H), 50.7 (<u>C</u>H$_2$CHO), 21.5 (<u>C</u>H$_3$); *m/z* (CI) 135 ([M + H]$^+$, 100%); HRMS $C_9H_{11}O$, calcd 135.0810, found 135.0804.

8.52 3,4-(Methylenedioxy)phenylacetaldehyde (140f)[118a]



The title compound was prepared according to the procedure described in section 8.51, from alcohol **144** (500 mg, 3.01 mmol). The crude product was purified by column chromatography (hexane/EtOAc, 85:15) to give the product as a pale yellow oil (254 mg, 51%). R_f 0.50 (hexane/EtOAc, 2:1); δ_H (600 MHz; $CDCl_3$) 9.70 (1H, s, C<u>H</u>O), 6.80 (1H, d, *J* = 8.0 Hz, Ar-<u>H</u>), 6.69 (1H, s, Ar-<u>H</u>), 6.66 (1H, d, *J* = 8.0 Hz, Ar-<u>H</u>), 5.97 (2H, s, OC<u>H</u>$_2$O), 3.60 (2H, s, C<u>H</u>$_2$CHO); δ_C (150 MHz; $CDCl_3$) 199.5 (<u>C</u>HO), 148.3 (Ar-<u>C</u>O), 147.1 (Ar-<u>C</u>O), 125.4 (Ar-<u>C</u>), 123.0 (Ar-<u>C</u>H), 110.1 (Ar-<u>C</u>H), 109.0 (Ar-<u>C</u>H), 101.4 (O<u>C</u>H$_2$O), 50.3 (<u>C</u>H$_2$CHO); *m/z* (CI) 165 ([M + H]$^+$, 100%); HRMS $C_9H_9O_3$, calcd 165.0552, found 165.0550.

8.53 1-Ethyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (141a)[209] and 1-Ethyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (142a)[119]



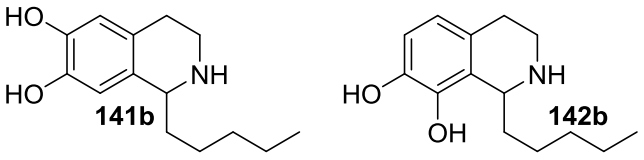
Propionaldehyde (92.0 µL, 1.27 mmol) was added to a solution of dopamine hydrochloride (200 mg, 1.05 mmol) in potassium phosphate buffer (10 mL, 0.1 M, pH

6) and acetonitrile (10 mL) under argon. The solution was stirred at 50 ^{o}C for 18 h. The reaction was concentrated under vacuum. CH_2Cl_2/CH_3OH (1:1, 20 mL) was added to the resultant residue and the suspension filtered to remove the solid. The filtrate was evaporated to dryness under vacuum to give the crude product as a brown oil. The regioisomers were separated by prep-HPLC (Method 1, run time: 10 min, flow rate: 5 mL/min) and appropriate fractions were combined, concentrated and co-evaporated with methanol (x3) to give **141a** as a pale brown oil (r_t = 1.5 min, 156 mg, 77%) and **142a** as a pale yellow oil (r_t = 2.6 min, 8.3 mg, 4%).

Major regioisomer **141a**: δ_H (500 MHz; CD_3OD) 6.67 (1H, s, 8-Ar-H), 6.63 (1H, s, 5-Ar-H), 4.33-4.29 (1H, m, 1-CH), 3.56-3.29 (2H, m, 3-CH_2), 3.05-2.87 (2H, m, 4-CH_2), 2.17-1.88 (2H, m, 1-CHCH_2), 1.12 (3H, t, *J* = 7.5 Hz, CH_3); δ_C (125 MHz; CD_3OD) 145.2 (Ar-COH), 144.4 (Ar-COH), 122.5 (Ar-C), 122.2 (Ar-C), 114.7 (5-Ar-CH), 112.4 (8-Ar-CH), 56.4 (1-CH), 39.6 (3-CH_2), 26.4 (CH_2), 24.2 (CH_2), 8.5 (CH_3); *m/z* (CI) 194 ($[M + H]^+$, 10%); HRMS $C_{11}H_{16}O_2N$, calcd 194.1181, found 194.1179.

Minor regioisomer **142a**: δ_H (500 MHz; CD_3OD) 6.73 (1H, d, *J* = 8.0 Hz, 6-Ar-H), 6.55 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 4.57-4.51 (1H, m, 1-CH), 3.51-3.27 (2H, m, 3-CH_2), 3.04-2.89 (2H, m, 4-CH_2), 2.25-1-81 (2H, m, 1-CHCH_2), 1.11 (3H, t, *J* = 7.5 Hz, CH_3); δ_C (125 MHz; CD_3OD) 144.4 (Ar-COH), 143.4 (Ar-COH), 123.3 (Ar-C), 121.1 (Ar-C), 120.4 (5-Ar-CH), 116.0 (6-Ar-CH), 54.8 (1-CH), 38.8 (3-CH_2), 26.1 (CH_2), 25.5 (CH_2), 10.7 (CH_3); *m/z* (CI) 194 ($[M + H]^+$, 30%), 164 ($[M + H]^+ - C_2H_6$, 100%); HRMS $C_{11}H_{16}O_2N$, calcd 194.1181, found 194.1180.

8.54 1-Pentyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (141b)[209] and 1-Pentyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (142b)

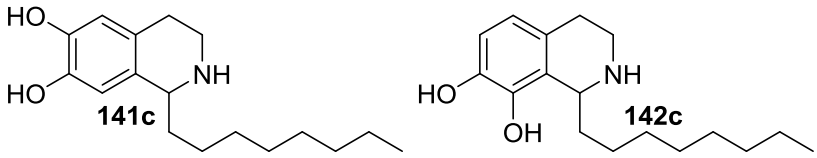


The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (100 mg, 0.527 mmol) and hexanal (77.2 µL, 0.632 mmol). The crude mixture of products were separated and purified by prep-HPLC (Method 1, run time: 30 min) to give **141b** as a white solid (r_t = 17.2 min, 92.7 mg, 75%) and **142b** as an orange oil (r_t = 22.6 min, 11.3 mg, 9%).

Major regioisomer **141b**: M.p. 166-168 °C (lit[209] 164-170 °C, Et_2O); δ_H (500 MHz; CD_3OD) 6.64 (1H, s, 8-Ar-H), 6.60 (1H, s, 5-Ar-H), 4.36-4.30 (1H, m, 1-CH), 3.51-3.26 (2H, m, 3-CH_2), 3.02-2.84 (2H, m, 4-CH_2), 2.08-1.81 (2H, m, 1-CHCH_2), 1.56-1.33 (6H, m, 3 x CH_2), 0.94 (3H, t, *J* = 6.5 Hz, CH_3); δ_C (125 MHz; CD_3OD) 146.7 (Ar-COH), 145.9 (Ar-COH), 124.3 (Ar-C), 123.6 (Ar-C), 116.2 (5-Ar-CH), 113.9 (8-Ar-CH), 56.7 (1-CH), 41.0 (3-CH_2), 35.1 (1-CHCH_2), 32.7 (CH_2), 26.1 (4-CH_2), 25.7 (CH_2), 23.5 (CH_2), 14.3 (CH_3); *m/z* (CI) 236 ($[M + H]^+$, 85%), 164 ($[M + H]^+ - C_5H_{12}$, 100%); HRMS $C_{14}H_{22}O_2N$, calcd 236.1645, found 236.1644.

Minor regioisomer **142b**: υ_{max}/cm^{-1} 3039 br, 2961, 2932, 1662, 1617, 1578, 1501; δ_H (600 MHz; CD_3OD) 6.74 (1H, d, *J* = 8.0 Hz, 6-Ar-H), 6.55 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 4.62 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.51-3.28 (2H, m, 3-CH_2), 3.04-2.92 (2H, m, 4-CH_2), 2.17-1.80 (2H, m, 1-CHCH_2), 1.62-1.47 (2H, m, 1-CHCH_2CH_2), 1.45-1.35 (4H, m, $CH_2CH_2CH_3$), 0.94 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 144.6 (Ar-COH), 143.3 (Ar-COH), 123.3 (Ar-C), 121.3 (Ar-C), 120.4 (5-Ar-CH), 116.0 (6-Ar-CH), 53.4 (1-CH), 38.7 (3-CH_2), 33.0 (1-CHCH_2), 32.5 (CH_2), 26.6 (1-CHCH_2CH_2), 25.5 (4-CH_2), 23.4 (CH_2), 14.3 (CH_3); *m/z* (CI) 236 ($[M + H]^+$, 35%), 164 ($[M + H]^+ - C_5H_{10}$, 100%); HRMS $C_{14}H_{22}O_2N$, calcd 236.1645, found 236.1644.

8.55 1-Octyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (141c) and 1-Octyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (142c)



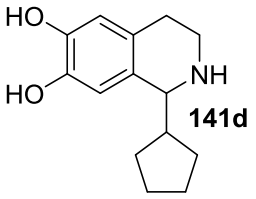
The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (200 mg, 1.05 mmol) and nonanal (218 µL, 1.27 mmol). The mixture of products in ¾ of the crude material were separated and purified by prep-HPLC (Method 2, Gradient F, flow rate: 8 mL/min) to give **141c** as a black oil (r_t = 17.3 min, 168 mg, 77%) and **142c** as a yellow oil (r_t = 23.9 min, 18.0 mg, 8%).

Major regioisomer **141c**: υ_{max}/cm^{-1} 3047 br, 2927, 2859, 1669, 1617, 1530; δ_H (500 MHz; CD_3OD) 6.64 (1H, s, 8-Ar-H), 6.60 (1H, s, 5-Ar-H), 4.35-4.29 (1H, m, 1-CH), 3.52-3.25 (2H, m, 3-CH_2), 3.01-2.83 (2H, m, 4-CH_2), 2.07-1.80 (2H, m, 1-CHCH_2), 1.55-1.22 (12H, m, 6 x CH_2), 0.89 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (125 MHz; CD_3OD)

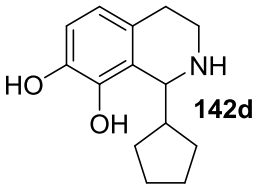
146.7 (Ar-<u>C</u>OH), 145.9 (Ar-<u>C</u>OH), 124.3 (Ar-<u>C</u>), 123.7 (Ar-<u>C</u>), 116.2 (5-Ar-<u>C</u>H), 113.9 (8-Ar-<u>C</u>H), 56.7 (1-<u>C</u>H), 41.0 (3-<u>C</u>H_2), 35.1 (<u>C</u>H_2), 33.0 (<u>C</u>H_2), 30.5 (<u>C</u>H_2), 30.4 (<u>C</u>H_2), 30.3 (<u>C</u>H_2), 26.4 (<u>C</u>H_2), 25.7 (4-<u>C</u>H_2), 23.7 (<u>C</u>H_2), 14.4 (<u>C</u>H_3); *m/z* (ESI) 278 ([M + H]$^+$, 100%), 261 ([M + H]$^+$ - OH, 60%); HRMS $C_{17}H_{28}NO_2$, calcd 278.2120, found 278.2124.

Minor regioisomer **142c**: υ_{max}/cm^{-1} 3320, 3047, 2928, 2852, 1664, 1625, 1506; δ_H (500 MHz; CD_3OD) 6.73 (1H, d, *J* = 8.0 Hz, 6-Ar-<u>H</u>), 6.54 (1H, d, *J* = 8.0 Hz, 5-Ar-<u>H</u>), 4.64-4.58 (1H, m, 1-C<u>H</u>), 3.51-3.25 (2H, m, 3-C<u>H</u>$_2$), 3.03-2.90 (2H, m, 4-C<u>H</u>$_2$), 2.17-1.77 (2H, m, 1-CHC<u>H</u>$_2$), 1.61-1.21 (12H, m, 6 x C<u>H</u>$_2$), 0.89 (3H, t, *J* = 7.0 Hz, C<u>H</u>$_3$); δ_C (125 MHz; CD_3OD) 144.6 (Ar-<u>C</u>OH), 143.3 (Ar-<u>C</u>OH), 123.4 (Ar-<u>C</u>), 121.3 (Ar-<u>C</u>), 120.5 (5-Ar-<u>C</u>H), 116.0 (6-Ar-<u>C</u>H), 53.4 (1-<u>C</u>H), 38.8 (3-<u>C</u>H_2), 33.1 (<u>C</u>H_2), 33.0 (<u>C</u>H_2), 30.4 (<u>C</u>H_2), 30.3 (2 x <u>C</u>H_2), 26.9 (<u>C</u>H_2), 25.5 (<u>C</u>H_2), 23.7 (<u>C</u>H_2), 14.4 (<u>C</u>H_3); *m/z* (ESI) 278 ([M + H]$^+$, 100%), 261 ([M + H]$^+$ - OH, 40%); HRMS $C_{17}H_{28}NO_2$, calcd 278.2120, found 278.2114.

8.56 1-Cyclopentyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (141d)[210] and 1-Cyclopentyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (142d)



141d



142d

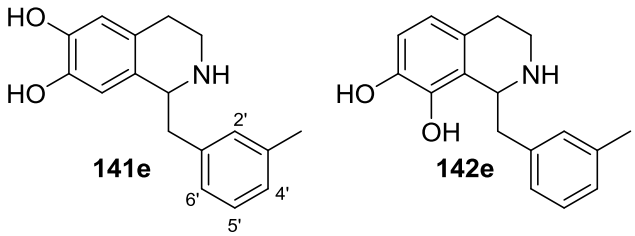
The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (150 mg, 0.791 mmol) and cyclopentanecarboxaldehyde (155 mg, 1.58 mmol). The mixture of products in the crude material were separated and purified by prep-HPLC (Method 2, Gradient F, flow rate: 8 mL/min) to give **141d** as a white solid (r_t = 10.0 min, 59.2 mg, 32%) and **142d** as a colourless oil (r_t = 26.5 min, 6.5 mg, 4%).

Major regioisomer **141d**: M.p. 207-210 ^{o}C; υ_{max}/cm^{-1} 3135 br, 2969, 1675, 1612, 1531; δ_H (600 MHz; CD_3OD) 6.69 (1H, s, 8-Ar-<u>H</u>), 6.62 (1H, s, 5-Ar-<u>H</u>), 4.22 (1H, d, *J* = 8.0 Hz, 1-C<u>H</u>), 3.56-3.32 (2H, m, 3-C<u>H</u>$_2$), 3.02-2.92 (2H, m, 4-C<u>H</u>$_2$), 2.44-2.35 (1H, m, 1-CHC<u>H</u>), 1.92-1.60 (6H, m, 3 x C<u>H</u>$_2$), 1.53-1.31 (2H, m, C<u>H</u>$_2$); δ_C (150 MHz; CD_3OD) 146.9 (Ar-<u>C</u>OH), 145.5 (Ar-<u>C</u>OH), 124.2 (Ar-<u>C</u>), 123.6 (Ar-<u>C</u>), 116.2 (5-Ar-<u>C</u>H), 114.8

(8-Ar-CH), 60.4 (1-CH), 44.6 (1-CHCH), 39.8 (3-CH_2), 31.4 (CH_2), 29.8 (CH_2), 26.0 (CH_2), 25.7 (CH_2), 25.3 (4-CH_2); *m/z* (EI) 233 (M^+, 30%), 165 (M^+ - C_5H_8, 100%); HRMS $C_{14}H_{19}O_2N$, calcd 233.1416, found 233.1419.

Minor regioisomer **142d**: υ_{max}/cm^{-1} 3063 br, 2969, 2924, 2875, 1668, 1633, 1516; δ_H (600 MHz; CD_3OD) 6.75 (1H, d, *J* = 8.0 Hz, 6-Ar-H), 6.57 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 4.70 (1H, d, *J* = 8.5 Hz, 1-CH), 3.64-3.24 (2H, m, 3-CH_2), 3.09-2.94 (2H, m, 4-CH_2), 2.51-2.41 (1H, m, 1-CHCH), 1.96-1.42 (8H, m, 4 x CH_2); δ_C (CD_3OD, 150 MHz) 144.7 (Ar-COH), 143.8 (Ar-COH), 123.8 (Ar-C), 121.3 (Ar-C), 120.4 (5-Ar-CH), 116.1 (6-Ar-CH), 55.8 (1-CH), 44.0 (1-CHCH), 38.6 (3-CH_2), 30.5 (CH_2), 30.4 (CH_2), 25.9 (CH_2), 25.6 (CH_2), 24.7 (4-CH_2); *m/z* (CI) 234 ($[M + H]^+$, 20%), 164 ($[M + H]^+$ - C_5H_{10}, 100%); HRMS $C_{14}H_{20}O_2N$, calcd 234.1494, found 234.1491.

8.57 1-(3-Methylbenzyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol (141e)[118b] and 1-(3-Methylbenzyl)-1,2,3,4-tetrahydroisoquinoline-7,8-diol (142e)



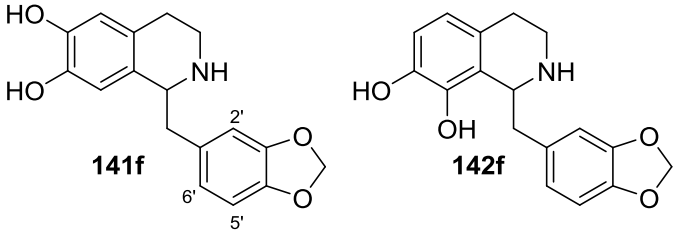
The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (150 mg, 0.791 mmol) and aldehyde **140e** (127 mg, 0.949 mmol). The mixture of products in the crude material were separated and purified by prep-HPLC (Method 2, Gradient F) to give **141e** as a yellow oil (r_t = 13.0 min, 148 mg, 69%) and **142e** as a yellow oil (r_t = 29.4 min, 6.1 mg, 3%).

Major regioisomer **141e**: M.p. 60-64 ^oC; υ_{max}/cm^{-1} 3034 br, 2859, 1667, 1617, 1530; δ_H (500 MHz; CD_3OD) 7.27 (1H, t, *J* = 7.5 Hz, 5'-Ar-H), 7.17-7.07 (3H, m, 2'-Ar-H, 4'-Ar-H, 6'-Ar-H), 6.63-6.59 (2H, m, 5-Ar-H, 8-Ar-H), 4.66-4.40 (1H, m, 1-CH), 3.50-3.20 (3H, m, 3-CH_2, 1-CHCHH), 3.06-2.85 (3H, m, 4-CH_2, 1-CHCHH), 2.35 (3H, s, CH_3); δ_C (125 MHz; CD_3OD) 146.9 (Ar-COH), 145.8 (Ar-COH), 140.1 (Ar-C), 136.5 (Ar-C), 131.2 (Ar-CH), 130.1 (Ar-CH), 129.5 (Ar-CH), 127.6 (Ar-CH), 123.7 (2 x Ar-C), 116.2 (5-Ar-CH), 114.2 (8-Ar-CH), 57.8 (1-CH), 41.2 (CH_2), 41.0 (CH_2), 25.7 (4-

$\underline{C}H_2$), 21.4 ($\underline{C}H_3$); *m/z* (CI) 270 ($[M + H]^+$, 15%), 164 ($[M + H]^+$ - C_8H_{10}, 100%); HRMS $C_{17}H_{20}O_2N$, calcd 270.1494, found 270.1485.

Minor regioisomer **142e**: υ_{max}/cm^{-1} 3063 br, 2945, 2924, 2844, 1672, 1617, 1502; δ_H (600 MHz; CD_3OD) 7.28 (1H, t, *J* = 7.5 Hz, 5'-Ar-$\underline{H}$), 7.21 (1H, s, 2'-Ar-$\underline{H}$), 7.20-7.13 (2H, m, 4'-Ar-$\underline{H}$, 6'-Ar-$\underline{H}$), 6.79 (1H, d, *J* = 8.5 Hz, 6-Ar-$\underline{H}$), 6.59 (1H, d, *J* = 8.5 Hz, 5-Ar-$\underline{H}$), 4.95.4.85 (1H, m, 1-C$\underline{H}$), 3.67-3.60 (1H, m, 1-CHC$\underline{H}$H), 3.53-3.23 (2H, m, 3-C$\underline{H}_2$), 3.05-2.93 (3H, m, 4-C$\underline{H}_2$, 1-CHCH$\underline{H}$), 2.36 (3H, s, C$\underline{H}_3$); δ_C (150 MHz; CD_3OD) 144.8 (Ar-$\underline{C}$OH), 143.4 (Ar-$\underline{C}$OH), 140.1 (Ar-$\underline{C}$), 137.3 (Ar-$\underline{C}$), 131.0 (2'-Ar-$\underline{C}$H), 130.2 (5'-Ar-$\underline{C}$H), 129.4 (Ar-$\underline{C}$H), 127.3 (Ar-$\underline{C}$H), 123.4 (Ar-$\underline{C}$), 120.6 (5-Ar-$\underline{C}$H), 120.4 (Ar-$\underline{C}$), 116.3 (6-Ar-$\underline{C}$H), 55.0 (1-$\underline{C}$H), 38.7 (3-$\underline{C}H_2$), 38.1 (1-CH$\underline{C}H_2$), 25.5 (4-$\underline{C}H_2$), 21.5 ($\underline{C}H_3$); *m/z* (CI) 270 ($[M + H]^+$, 70%), 164 ($[M + H]^+$ - C_8H_{10}, 100%); HRMS $C_{17}H_{20}O_2N$, calcd 270.1494, found 270.1488.

8.58 1-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol (**141f**)[105] and 1-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-1,2,3,4-tetrahydroisoquinoline-7,8-diol (**142f**)



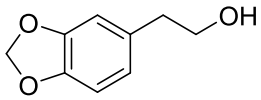
The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (145 mg, 0.765 mmol) and aldehyde **140f** (150 mg, 0.917 mmol). The mixture of products in the crude material were separated and purified by prep-HPLC (Method 2, Gradient D) to give **141f** as a yellow oil (r_t = 16.0 min, 112 mg, 49%) and **142f** as a pale yellow oil (r_t = 23.0 min, 6.2 mg, 3%).

Major regioisomer **141f**: M.p. 64-66 ^oC; υ_{max}/cm^{-1} 3018 br, 2859, 1668, 1617, 1531; δ_H (600 MHz; CD_3OD) 6.85 (1H, d, *J* = 1.5 Hz, 2'-Ar-$\underline{H}$), 6.83 (1H, d, *J* = 8.0 Hz, 5'-Ar-$\underline{H}$), 6.78 (1H, dd, *J* = 8.0 and 1.5 Hz, 6'-Ar-$\underline{H}$), 6.64-6.61 (2H, m, 5-Ar-$\underline{H}$, 8-Ar-$\underline{H}$), 5.97 (2H, s, O$\underline{C}H_2$O), 4.62-4.58 (1H, m, 1-C$\underline{H}$), 3.51-3.45 (1H, m, 3-C$\underline{H}$H), 3.40 (1H, dd, *J* = 14.5 and 5.5 Hz, 1-CHC$\underline{H}$H), 3.29-3.23 (1H, m, 3-CH$\underline{H}$), 3.04-2.88 (3H, m, 4-C$\underline{H}_2$, 1-CHCH$\underline{H}$); δ_C (150 MHz; CD_3OD) 149.9 (Ar-$\underline{C}$O), 148.8 (Ar-$\underline{C}$O), 146.9 (Ar-

CO), 145.8 (Ar-CO), 130.1 (Ar-C), 123.9 (6'-Ar-CH), 123.6 (2 x Ar-C), 116.2 (Ar-CH), 114.1 (Ar-CH), 110.5 (2'-Ar-CH), 109.7 (5'-Ar-CH), 102.6 (OCH_2O), 57.8 (1-CH), 40.9 (3-CH_2, 1-$CHCH_2$), 25.7 (4-CH_2); *m/z* (ESI) 300 ($[M + H]^+$, 100%), 283 ($[M + H]^+$ - OH, 80%), 164 ($[M + H]^+$ - $C_8H_8O_2$, 100%); HRMS $C_{17}H_{18}NO_4$, calcd 300.1236, found 300.1221.

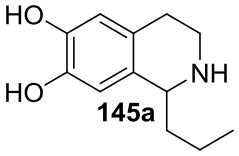
Minor regioisomer **142f**: υ_{max}/cm^{-1} 3029 br, 1667, 1625; δ_H (600 MHz; CD_3OD) 6.88-6.83 (3H, m, 2'-Ar-H, 5'-Ar-H, 6'-Ar-H), 6.79 (1H, d, *J* = 8.0 Hz, 6-Ar-H), 6.59 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 5.96 (2H, s, OCH_2O), 4.85 (1H, dd, *J* = 10.5 and 3.0 Hz, 1-CH), 3.59-3.48 (2H, m, 3-CHH, 1-CHCHH), 3.30-3.24 (1H, m, 3-CHH), 3.05-2.92 (3H, m, 4-CH_2, 1-CHCHH); δ_C (150 MHz; CD_3OD) 149.9 (Ar-CO), 148.7 (Ar-CO), 144.8 (Ar-CO), 143.3 (Ar-CO), 130.9 (Ar-C), 123.6 (Ar-CH), 123.3 (Ar-C), 120.6 (5-Ar-CH), 120.3 (Ar-C), 116.3 (6-Ar-CH), 110.3 (Ar-CH), 109.7 (Ar-CH), 102.5 (OCH_2O), 55.1 (1-CH), 38.6 (3-CH_2), 37.8 (1-$CHCH_2$), 25.5 (4-CH_2); *m/z* (ESI) 300 ($[M + H]^+$, 40%), 164 ($[M + H]^+$ - $C_8H_8O_2$, 100%); HRMS $C_{17}H_{18}NO_4$, calcd 300.1236, found 300.1236.

8.59 3,4-(Methylenedioxy)phenethyl alcohol (**144**)[147]

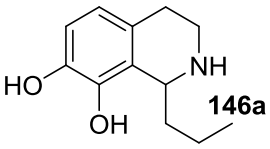


A solution of 3,4-(methylenedioxy)phenylacetic acid (2.00 g, 11.1 mmol) in THF (50 mL) was added slowly to a gently stirred solution of lithium aluminium hydride in THF (1 M, 24.4 mL, 24.4 mmol) at 0 °C and stirred at rt for 5 h. The reaction mixture was cooled to 0 °C and quenched with water (25 mL). EtOAc (100 mL) was added and the mixture was filtered to remove the solids. The solids were washed with EtOAc (3 x 50 mL). The combined organic extracts were washed with brine (40 mL), dried ($MgSO_4$) and solvent removed *in vacuo* to yield the product as a pale yellow oil (1.77 g, 96%) which was used without further purification. R_f 0.2 (hexane/EtOAc, 2:1); δ_H (300 MHz; $CDCl_3$) 6.81-6.65 (3H, m, 3 x Ar-H), 5.93 (2H, s, OCH_2O), 3.81 (2H, t, *J* = 6.5 Hz, CH_2OH), 2.78 (2H, t, *J* = 6.5 Hz, CH_2CH_2OH); *m/z* (CI) 166 (M^+, 20%), 149 (M^+ - OH, 100%); HRMS $C_9H_{10}O_3$, calcd 166.0630, found 166.0623.

8.60 1-Propyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (145a)[209] and 1-Propyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (146a)



145a



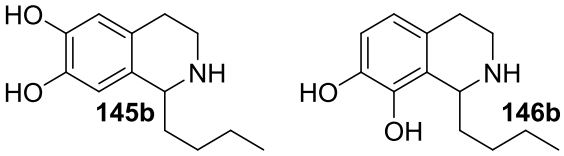
146a

The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (200 mg, 1.05 mmol) and butyraldehyde (114 µL, 1.27 mmol). The mixture of products in the crude material were separated and purified by prep-HPLC (Method 2, Gradient A) to give **145a** as a pale brown solid (r_t = 17.8 min, 99.0 mg, 45%) and **146a** as a pale yellow oil (r_t = 30.4 min, 7.65 mg, 3%).

Major regioisomer **145a**: M.p. 193-195 ^oC; δ_H (600 MHz; CD_3OD) 6.65 (1H, s, 8-Ar-H), 6.61 (1H, s, 5-Ar-H), 4.37-4.32 (1H, m, 1-CH), 3.54-3.28 (2H, m, 3-CH_2), 3.02-2.87 (2H, m, 4-CH_2), 2.05-1.81 (2H, m, 1-CHCH_2), 1.59-1.47 (2H, m, 1-CHCH_2CH_2), 1.05 (3H, t, *J* = 7.5 Hz, CH_3); δ_C (150 MHz; CD_3OD) 146.7 (Ar-COH), 145.9 (Ar-COH), 124.2 (Ar-C), 123.5 (Ar-C), 116.2 (5-Ar-CH), 113.8 (8-Ar-CH), 56.4 (1-CH), 40.9 (3-CH_2), 37.3 (1-CHCH_2), 25.7 (4-CH_2), 19.8 (1-CHCH_2CH_2), 14.1 (CH_3); *m/z* (CI) 208 ($[M + H]^+$, 50%), 131 ($[M + H]^+$ - $C_3H_9O_2$, 40%); HRMS $C_{12}H_{18}NO_2$, calcd 208.1338, found 208.1331.

Minor regioisomer **146a**: υ_{max}/cm^{-1} 3330 br, 3047, 2813, 1665, 1633, 1586, 1506; δ_H (600 MHz; CD_3OD) 6.74 (1H, d, *J* = 8.0 Hz, 6-Ar-H), 6.55 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 4.63 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.51-3.28 (2H, m, 3-CH_2), 3.04-2.92 (2H, m, 4-CH_2), 2.13-1.80 (2H, m, 1-CHCH_2), 1.65-1.47 (2H, m, 1-CHCH_2CH_2), 1.03 (3H, t, *J* = 7.5 Hz, CH_3); δ_C (150 MHz; CD_3OD) 144.6 (Ar-COH), 143.3 (Ar-COH), 123.2 (Ar-C), 121.2 (Ar-C), 120.4 (5-Ar-CH), 116.0 (6-Ar-CH), 53.1 (1-CH), 38.7 (3-CH_2), 35.2 (1-CHCH_2), 25.4 (4-CH_2), 20.3 (1-CHCH_2CH_2), 14.0 (CH_3); *m/z* (CI) 208 ($[M + H]^+$, 45%), 164 ($[M + H]^+$ - C_3H_8, 40%); HRMS $C_{12}H_{18}NO_2$, calcd 208.1338, found 208.1331.

8.61 1-Butyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (145b)[210] and 1-Butyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (146b)

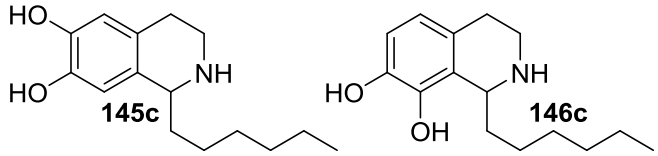


The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (200 mg, 1.05 mmol) and valeraldehyde (135 µL, 1.27 mmol). The mixture of products in the crude material were separated and purified by prep-HPLC (Method 2, Gradient A, run time: 50 min) to give **145b** as a pale brown solid (r_t = 22.5 min, 193 mg, 83%) and **146b** as a pale yellow oil (r_t = 31.7 min, 11.4 mg, 5%).

Major regioisomer **145b**: M.p. 185-186 ^oC; δ_H (600 MHz; CD_3OD) 6.65 (1H, s, 8-Ar-H), 6.61 (1H, s, 5-Ar-H), 4.36-4.32 (1H, m, 1-CH), 3.54-3.28 (2H, m, 3-CH_2), 3.03-2.87 (2H, m, 4-CH_2), 2.09-1.84 (2H, m, 1-CHCH_2), 1.53-1.39 (4H, m, $CH_2CH_2CH_3$), 0.99 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 146.7 (Ar-COH), 145.9 (Ar-COH), 124.2 (Ar-C), 123.6 (Ar-C), 116.2 (5-Ar-CH), 113.8 (8-Ar-CH), 56.6 (1-CH), 41.0 (3-CH_2), 34.8 (1-CHCH_2), 28.6 (CH_2), 25.7 (4-CH_2), 23.6 (CH_2), 14.2 (CH_3); *m/z* (CI) 222 ($[M + H]^+$, 80%), 164 ($[M + H]^+$ - C_4H_{10}, 85%), 131 ($[M + H]^+$ - $C_4H_{11}O_2$, 40%); HRMS $C_{13}H_{20}NO_2$, calcd 222.1494, found 222.1490.

Minor regioisomer **146b**: υ_{max}/cm^{-1} 3047 br, 2962, 2875, 1664, 1625, 1586, 1500; δ_H (600 MHz; CD_3OD) 6.74 (1H, d, *J* = 8.0 Hz, 6-Ar-H), 6.55 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 4.62 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.51-3.28 (2H, m, 3-CH_2), 3.04-2.92 (2H, m, 4-CH_2), 2.19-1.80 (2H, m, 1-CHCH_2), 1.59-1.37 (4H, m, $CH_2CH_2CH_3$), 0.98 (3H, t, *J* = 7.5 Hz, CH_3); δ_C (150 MHz; CD_3OD) 144.6 (Ar-COH), 143.3 (Ar-COH), 123.3 (Ar-C), 121.3 (Ar-C), 120.4 (5-Ar-CH), 116.0 (6-Ar-CH), 53.8 (1-CH), 38.7 (3-CH_2), 32.7 (1-CHCH_2), 29.1 (CH_2), 25.5 (4-CH_2), 23.3 (CH_2), 14.0 (CH_3); *m/z* (CI) 222 ($[M + H]^+$, 95%), 164 ($[M + H]^+$ - C_4H_{10}, 100%); HRMS $C_{13}H_{20}NO_2$, calcd 222.1494, found 222.1488.

8.62 1-Hexyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (145c)[118a] and 1-Hexyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (146c)[118a]

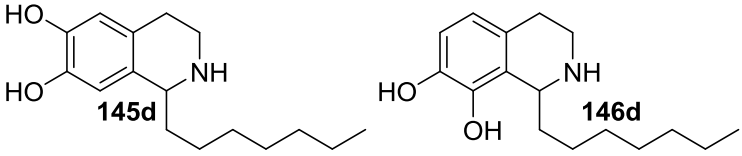


The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (200 mg, 1.05 mmol) and heptanal (177 µL, 1.27 mmol). The mixture of products in the crude material were separated and purified by prep-HPLC (Method 2, Gradient F) to give **145c** as a pale brown oil (r_t = 18.2 min, 225 mg, 86%) and **146c** as a pale yellow oil (r_t = 28.8 min, 25.1 mg, 10%).

Major regioisomer **145c**: δ_H (600 MHz; CD_3OD) 6.65 (1H, s, 8-Ar-H), 6.61 (1H, s, 5-Ar-H), 4.36-4.32 (1H, m, 1-CH), 3.54-3.28 (2H, m, 3-CH_2), 3.02-2.86 (2H, m, 4-CH_2), 2.07-1.83 (2H, m, 1-CHCH_2), 1.55-1.33 (8H, m, 4 x CH_2), 0.93 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 146.7 (Ar-COH), 145.9 (Ar-COH), 124.2 (Ar-C), 123.6 (Ar-C), 116.2 (5-Ar-CH), 113.8 (8-Ar-CH), 56.7 (1-CH), 41.0 (3-CH_2), 35.2 (1-CHCH_2), 32.8 (CH_2), 30.0 (CH_2), 26.5 (CH_2), 25.7 (4-CH_2), 23.4 (CH_2), 14.3 (CH_3); *m/z* (ESI) 250 ($[M + H]^+$, 100%); HRMS $C_{15}H_{24}NO_2$, calcd 250.1807, found 250.1809.

Minor regioisomer **146c**: δ_H (600 MHz; CD_3OD) 6.74 (1H, d, *J* = 8.0 Hz, 6-Ar-H), 6.55 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 4.61 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.51-3.28 (2H, m, 3-CH_2), 3.03-2.92 (2H, m, 4-CH_2), 2.17-1.79 (2H, m, 1-CHCH_2), 1.61-1.27 (8H, m, 4 x CH_2), 0.91 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 144.6 (Ar-COH), 143.3 (Ar-COH), 123.2 (Ar-C), 121.2 (Ar-C), 120.4 (5-Ar-CH), 116.0 (6-Ar-CH), 53.4 (1-CH), 38.7 (3-CH_2), 33.0 (1-CHCH_2), 32.7 (CH_2), 30.0 (CH_2), 27.0 (CH_2), 25.4 (4-CH_2), 23.6 (CH_2), 14.3 (CH_3); *m/z* (ESI) 250 ($[M + H]^+$, 100%), 233 ($[M + H]^+$ - OH, 90%); HRMS $C_{15}H_{24}NO_2$, calcd 250.1807, found 250.1810.

8.63 1-Heptyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (145d) and 1-Heptyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (146d)

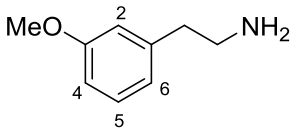


The title compounds were prepared according to the procedure described in section 8.53, from dopamine hydrochloride (200 mg, 1.05 mmol) and octanal (198 µL, 1.27 mmol). The mixture of products in the crude material were separated and purified by prep-HPLC (Method 2, Gradient D) to give **145d** as a yellow oil (r_t = 22.8 min, 238 mg, 85%) and **146d** as a yellow oil (r_t = 33.3 min, 30.9 mg, 11%).

Major regioisomer **145d**: υ_{max}/cm^{-1} 2929, 2875, 1664, 1625, 1519; δ_H (600 MHz; CD_3OD) 6.65 (1H, s, 8-Ar-H), 6.61 (1H, s, 5-Ar-H), 4.36-4.32 (1H, m, 1-CH), 3.53-3.28 (2H, m, 3-CH_2), 3.02-2.86 (2H, m, 4-CH_2), 2.97-1.83 (2H, m, 1-CHCH_2), 1.55-1.28 (10H, m, 5 x CH_2), 0.91 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 146.7 (Ar-COH), 145.9 (Ar-COH), 124.3 (Ar-C), 123.6 (Ar-C), 116.2 (5-Ar-CH), 113.8 (8-Ar-CH), 56.7 (1-CH), 41.0 (3-CH_2), 35.2 (1-CHCH_2), 32.9 (CH_2), 30.5 (CH_2), 30.2 (CH_2), 26.4 (CH_2), 25.7 (4-CH_2), 23.7 (CH_2), 14.4 (CH_3); *m/z* (CI) 264 ($[M + H]^+$, 100%), 164 ($[M + H]^+$ - C_7H_{16}, 60%); HRMS $C_{16}H_{26}NO_2$, calcd 264.1964, found 264.1967.

Minor regioisomer **146d**: υ_{max}/cm^{-1} 3031 br, 2969, 2928, 2852, 1662, 1578, 1500; δ_H (600 MHz; CD_3OD) 6.74 (1H, d, *J* = 8.0 Hz, 6-Ar-H), 6.55 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 4.61 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.51-3.28 (2H, m, 3-CH_2), 3.04-2.92 (2H, m, 4-CH_2), 2.17-1.79 (2H, m, 1-CHCH_2), 1.61-1.27 (10H, m, 5 x CH_2), 0.91 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 144.6 (Ar-COH), 143.4 (Ar-COH), 123.2 (Ar-C), 121.2 (Ar-C), 120.4 (5-Ar-CH), 116.0 (6-Ar-CH), 53.4 (1-CH), 38.7 (3-CH_2), 33.0 (1-CHCH_2), 32.9 (CH_2), 30.3 (CH_2), 30.2 (CH_2), 27.0 (CH_2), 25.5 (4-CH_2), 23.7 (CH_2), 14.4 (CH_3); *m/z* (CI) 264 ($[M + H]^+$, 100%), 164 ($[M + H]^+$ - C_7H_{16}, 50%); HRMS $C_{16}H_{26}NO_2$, calcd 264.1964, found 264.1962.

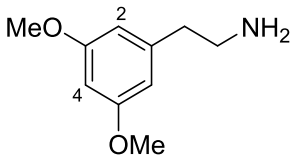
8.64 2-(3-Methoxyphenyl)ethan-1-amine (149)[118a]



To a stirred solution of 3-methoxyphenylacetonitrile (2.50 g, 17.0 mmol) in THF (35 mL) at 0 °C, was added a 1 M borane tetrahydrofuran complex solution in THF (42.5 mL, 42.5 mmol) and the reaction mixture was gradually allowed to warm to rt. After 24 h, the reaction was cooled to 0 °C and methanol (50 mL) was added dropwise. The

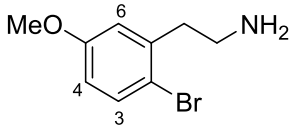
resulting clear solution was stirred at rt overnight and concentrated *in vacuo*. The residue was co-evaporated with methanol (3 x 20 mL), and the resulting crude product was purified by column chromatography (gradient of 5-15% CH_3OH (+1% Et_3N) in CH_2Cl_2) to yield the product as a pale yellow oil (2.44 g, 95%). R_f 0.16 (CH_2Cl_2/CH_3OH(+1% Et_3N), 4:1); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2937, 2836, 1582; δ_H (500 MHz; $CDCl_3$) 7.22 (1H, t, *J* = 7.5 Hz, 5-Ar-H), 6.83-6.72 (3H, m, 2-Ar-H, 4-Ar-H, 6-Ar-H), 3.80 (3H, s, OCH_3), 2.96 (2H, t, *J* = 6.5 Hz, CH_2N), 2.72 (2H, t, *J* = 6.5 Hz, CH_2CH_2N), 1.28 (2H, br s, NH_2); δ_C (125 MHz; $CDCl_3$) 159.8 (Ar-CO), 141.6 (Ar-C), 129.5 (5-Ar-CH), 121.3 (Ar-CH), 114.7 (2-Ar-CH), 111.5 (Ar-CH), 55.2 (OCH_3), 43.5 (CH_2N), 40.3 (CH_2CH_2N).

8.65 2-(3,5-Dimethoxyphenyl)ethan-1-amine (150)[151]



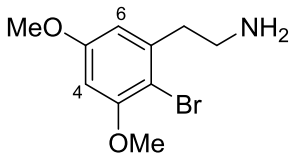
The title compound was prepared according to the procedure described in section 8.64, from (3,5-dimethoxyphenyl)acetonitrile (700 mg, 3.95 mmol) and a 1 M borane tetrahydrofuran complex solution in THF (11.9 mL, 11.9 mmol). The crude yellow oil was purified by column chromatography (CH_2Cl_2/CH_3OH(+1% Et_3N), 9:1) to give the product as a pale yellow oil (584 mg, 82%). R_f 0.24 (CH_2Cl_2/CH_3OH(+1% Et_3N), 9:1); δ_H (500 MHz; $CDCl_3$) 6.36 (2H, d, *J* = 2.0 Hz, 2 x 2-Ar-H), 6.32 (1H, t, *J* = 2.0 Hz, 4-Ar-H), 3.78 (6H, s, 2 x OCH_3), 2.96 (2H, t, *J* = 6.5 Hz, CH_2N), 2.69 (2H, t, *J* = 6.5 Hz, CH_2CH_2N), 1.51 (2H, br s, NH_2); δ_C (125 MHz; $CDCl_3$) 160.9 (2 x Ar-C), 142.2 (Ar-C), 107.0 (2 x 2-Ar-CH), 98.2 (4-Ar-CH), 55.3 (2 x OCH_3), 43.4 (CH_2N), 40.0 (CH_2CH_2N); *m/z* (CI) 182 ($[M + H]^+$, 40%), 165 ($[M + H]^+$ - NH_3, 100%); HRMS $C_{10}H_{16}O_2N$ calcd 182.1176, found 182.1167.

8.66 2-(2-Bromo-5-methoxyphenyl)ethan-1-amine (151)[152]



The title compound was prepared following a literature procedure with modifications to the work-up.[152] A solution of amine **149** (1.50 g, 9.92 mmol) in glacial acetic acid (21 mL) was treated with a solution of bromine (0.56 mL, 10.9 mmol) in acetic acid (8.50 mL) over 45 min at -15 ^{o}C. After stirring for a further 5 min, the resulting orange solution was treated with Et_2O (80 mL) and cooled to 0 ^{o}C. The product did not precipitate from the solution, so water was added (80 mL) and the aqueous phase separated. The organic phase was extracted with water (2 x 80 mL). The combined aqueous extracts were washed with Et_2O (2 x 50 mL). The aqueous phase was adjusted to pH 10 by addition of 2 M NaOH and extracted with CH_2Cl_2 (3 x 150 mL). The combined organic extracts were dried ($MgSO_4$) and solvent removed *in vacuo* to yield the product as a pale yellow oil (2.00 g, 88%), which was used without further purification. υ_{max}/cm^{-1} ($CDCl_3$ cast) 2934, 2836, 1594, 1571; δ_H (500 MHz; $CDCl_3$) 7.42 (1H, d, *J* = 8.5 Hz, 3-Ar-H), 6.78 (1H, d, *J* = 3.0 Hz, 6-Ar-H), 6.64 (1H, dd, *J* = 8.5 and 3.0 Hz, 4-Ar-H), 3.77 (3H, s, OCH_3), 2.96 (2H, t, *J* = 7.0 Hz, CH_2N), 2.84 (2H, t, *J* = 7.0 Hz, CH_2CH_2N), 1.25 (2H, br s, NH_2); δ_C (125 MHz; $CDCl_3$) 159.0 (Ar-CO), 140.2 (Ar-C), 133.5 (3-Ar-CH), 116.7 (6-Ar-CH), 115.2 (Ar-CBr), 113.5 (4-Ar-CH), 55.5 (3H, s, OCH_3), 42.2 (CH_2N), 40.6 (CH_2CH_2N).

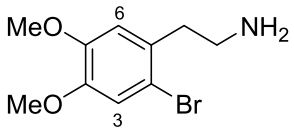
8.67 2-(2-Bromo-3,5-dimethoxyphenyl)ethan-1-amine (152)[153]



The title compound was prepared according to the procedure described in section 8.66, from amine **150** (245 mg, 1.35 mmol). The crude yellow oil was purified by column chromatography (CH_2Cl_2/CH_3OH(+1% Et_3N), 9:1). The first product eluted was the di-brominated compound 2-(2,6-dibromo-3,5-dimethoxyphenyl)ethan-1-amine[153] as a pale yellow oil (138 mg), δ_H (500 MHz; $CDCl_3$) 6.41 (1H, s, Ar-H), 3.88 (6H, s, 2 x OCH_3), 3.39-3.20 (2H, m, CH_2N), 2.98-2.88 (2H, m, CH_2CH_2N), 2.22 (2H, br s, NH_2). The second product eluted was the mono-brominated product **152** as a pale yellow oil (150 mg, 34%). R_f 0.34 (CH_2Cl_2/CH_3OH(+1% Et_3N), 4:1); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2938, 2844, 1584; δ_H (500 MHz; $CDCl_3$) 6.43 (1H, d, *J* = 2.5 Hz, Ar-H), 6.37 (1H, d, *J* = 2.5 Hz, Ar-H), 3.86 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.08-2.90 (4H, m, CH_2CH_2N), 2.01

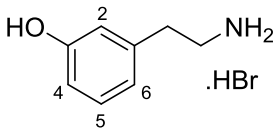
(2H, br s, N$\underline{H}_2$); δ_C (125 MHz; $CDCl_3$) 159.6 (Ar-$\underline{C}$O), 156.9 (Ar-$\underline{C}$O), 140.8 (Ar-$\underline{C}$), 107.3 (Ar-$\underline{C}$H), 105.0 (Ar-$\underline{C}$Br), 97.9 (Ar-$\underline{C}$H), 56.4 (O$\underline{C}H_3$), 55.6 (O$\underline{C}H_3$), 41.9 ($\underline{C}H_2$N), 40.4 ($\underline{C}H_2CH_2$N); *m/z* (ESI) 262 ([(^{81}Br)M + H]$^+$, 30%), 260 ([(^{79}Br)M + H]$^+$, 35%), 245 ([(^{81}Br)M + H]$^+$ - OH, 100%); HRMS $C_{10}H_{15}O_2N^{79}Br$, calcd 260.0286, found 260.0264.

8.68 2-(2-Bromo-4,5-dimethoxyphenyl)ethan-1-amine (**153**)[154]



The title compound was prepared according to the procedure described in section 8.66, from 2-(3,4-dimethoxyphenyl)ethan-1-amine (1.80 g, 9.93 mmol). The crude brown oil was purified by column chromatography (CH_2Cl_2/CH_3OH(+1% Et_3N), 9:1) to yield the product as a yellow oil (1.12 g, 43%). R_f 0.33 (CH_2Cl_2/CH_3OH(+1% Et_3N), 4:1); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2934, 2844, 1617, 1586, 1507; δ_H (500 MHz; $CDCl_3$) 6.96 (1H, s, 3-Ar-$\underline{H}$), 6.74 (1H, s, 6-Ar-$\underline{H}$), 3.85 (3H, s, OC$\underline{H}_3$), 3.84 (3H, s, OC$\underline{H}_3$), 2.95 (2H, t, *J* = 7.0 Hz, C$\underline{H}_2$N), 2.82 (2H, t, *J* = 7.0 Hz, C$\underline{H}_2$CH$_2$N), 1.43 (2H, br s, N$\underline{H}_2$); δ_C (125 MHz; $CDCl_3$) 148.4 (Ar-$\underline{C}$O), 148.2 (Ar-$\underline{C}$O), 131.1 (Ar-$\underline{C}$), 115.8 (3-Ar-$\underline{C}$H), 114.4 (Ar-$\underline{C}$Br), 113.5 (6-Ar-$\underline{C}$H), 56.2 (2 x O$\underline{C}H_3$), 42.4 ($\underline{C}H_2$N), 39.9 ($\underline{C}H_2CH_2$N); *m/z* (CI) 262 ([(^{81}Br)M + H]$^+$, 90%), 260 ([(^{79}Br)M + H]$^+$, 100%); HRMS $C_{10}H_{15}O_2N^{79}Br$, calcd 260.0286, found 260.0283.

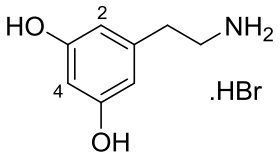
8.69 2-(3-Hydroxyphenyl)ethan-1-amine hydrobromide (**155**)[120]



A solution of 1 M boron tribromide in CH_2Cl_2 (10.2 mL, 10.2 mmol) was added to a stirred solution of amine **149** (700 mg, 4.63 mmol) in CH_2Cl_2 (20 mL) at -78 ^{o}C. The reaction was warmed to rt and stirred for 24 h. The reaction was then cooled to 0 ^{o}C and quenched by addition of methanol (40 mL). The solution was stirred at rt for 3 h and then concentrated *in vacuo* to give a brown oil. Further methanol (20 mL) was added to the oil, and solvent evaporated. This was repeated until no white fumes were

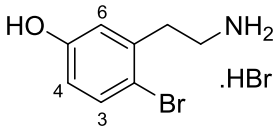
observed upon addition of methanol, to give the product as a pale brown solid as the hydrobromide salt (899 mg, 89%). M.p. 102-104 °C (lit[211] 102.5-103 °C, EtOH); δ_H (600 MHz; CD_3OD) 7.16 (1H, t, *J* = 7.5 Hz, 5-Ar-H), 6.73 (1H, d, *J* = 7.5 Hz, 6-Ar-H), 6.72-6.68 (2H, m, 2-Ar-H, 4-Ar-H), 3.15 (2H, t, *J* = 8.0 Hz, CH_2N), 2.88 (2H, t, *J* = 8.0 Hz, CH_2CH_2N); δ_C (150 MHz; CD_3OD) 159.1 (Ar-COH), 139.3 (Ar-C), 131.0 (Ar-CH), 120.7 (Ar-CH), 116.6 (Ar-CH), 115.2 (Ar-CH), 41.9 (CH_2N), 34.5 (CH_2CH_2N); *m/z* (CI) 138 ($[M + H]^+$, 70%), 121 ($[M + H]^+$ - OH/NH_3, 100%); HRMS $C_8H_{12}ON$, calcd 138.0919, found 138.0912.

8.70 2-(3,5-Dihydroxyphenyl)ethan-1-amine hydrobromide (**156**)[212]



The title compound was prepared according to the procedure described in section 8.69, from amine **150** (300 mg, 1.66 mmol) and 1 M boron tribromide in CH_2Cl_2 (4.97 mL, 4.97 mmol). The product was isolated as an orange solid as the hydrobromide salt (390 mg, 100%). M.p. 124-126 °C; δ_H (500 MHz; CD_3OD) 6.20 (2H, d, *J* = 2.0 Hz, 2 x 2-Ar-H), 6.18 (1H, t, *J* = 2.0 Hz, 4-Ar-H), 3.12 (2H, t, *J* = 7.5 Hz, CH_2N), 2.80 (2H, t, *J* = 7.5 Hz, CH_2CH_2N); δ_C (125 MHz; CD_3OD) 160.0 (2 x Ar-COH), 139.9 (Ar-C), 108.1 (2 x 2-Ar-CH), 102.4 (4-Ar-CH), 41.9 (CH_2N), 34.6 (CH_2CH_2N).

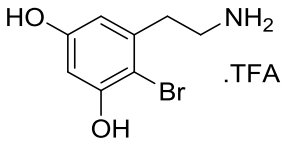
8.71 2-(2-Bromo-5-hydroxyphenyl)ethan-1-amine hydrobromide (**157**)[105]



The title compound was prepared according to the procedure described in section 8.69, from amine **151** (2.00 g, 8.69 mmol) and 1 M boron tribromide in CH_2Cl_2 (19.1 mL, 19.1 mmol). The product was isolated as a pale brown solid as the hydrobromide salt (2.40 g, 93%). M.p. 162-172 °C (decomposed); υ_{max}/cm^{-1} 3348, 2924, 1588; δ_H (500 MHz; CD_3OD) 7.36 (1H, d, *J* = 8.5 Hz, 3-Ar-H), 6.79 (1H, d, *J* = 3.0 Hz, 6-Ar-H), 6.65 (1H, dd, *J* = 8.5 and 3.0 Hz, 4-Ar-H), 3.20-3.11 (2H, m, CH_2N), 3.05-2.97 (2H, m,

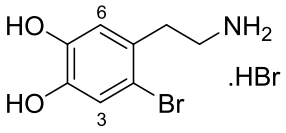
$C\underline{H}_2CH_2N$); δ_C (125 MHz; CD_3OD) 158.8 (Ar-$\underline{C}$OH), 138.0 (Ar-$\underline{C}$), 134.9 (3-Ar-$\underline{C}$H), 119.0 (6-Ar-$\underline{C}$H), 117.4 (4-Ar-$\underline{C}$H), 113.7 (Ar-$\underline{C}$Br), 40.4 ($\underline{C}H_2N$), 35.0 ($\underline{C}H_2CH_2N$).

8.72 2-(2-Bromo-4,5-dihydroxyphenyl)ethan-1-amine trifluoroacetic acid salt (**158**)



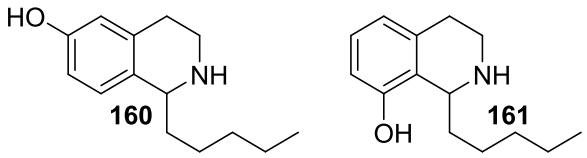
The title compound was prepared according to the procedure described in section 8.69, from amine **152** (140 mg, 0.538 mmol) and 1 M boron tribromide in CH_2Cl_2 (1.61 mL, 1.61 mmol). The crude black oil was purified by prep-HPLC (Method 2, Gradient B, run time: 30 min, r_t = 14.6 min) to yield the product as a yellow oil as the TFA salt (110 mg, 59%). δ_H (300 MHz; CD_3OD) 6.36 (1H, d, *J* = 2.5 Hz, Ar-$\underline{H}$), 6.32 (1H, d, *J* = 2.5 Hz, Ar-$\underline{H}$), 3.28-3.08 (2H, m, $C\underline{H}_2N$), 3.05-2.95 (2H, m, $C\underline{H}_2CH_2N$); δ_C (150 MHz; CD_3OD) 159.0 (Ar-$\underline{C}$OH), 156.7 (Ar-$\underline{C}$OH), 138.6 (Ar-$\underline{C}$), 110.1 (Ar-$\underline{C}$H), 103.4 (Ar-$\underline{C}$H), 102.4 (Ar-$\underline{C}$Br), 40.4 ($\underline{C}H_2N$), 35.3 ($\underline{C}H_2CH_2N$); *m/z* (CI) 234 ([(^{81}Br)M + H]$^+$, 100%), 232 ([(^{79}Br)M + H]$^+$, 95%); HRMS $C_8H_{11}NO_2{}^{79}Br$, calcd 231.9973, found 231.9973.

8.73 2-(2-Bromo-4,5-dihydroxyphenyl)ethan-1-amine hydrobromide (**159**)[213]



The title compound was prepared according to the procedure described in section 8.69, from amine **153** (655 mg, 2.52 mmol) and 1 M boron tribromide in CH_2Cl_2 (7.55 mL, 7.55 mmol). The product was isolated as a pale brown solid as the hydrobromide salt (716 mg, 91%). δ_H (600 MHz; CD_3OD) 6.97 (1H, s, 3-Ar-$\underline{H}$), 6.77 (1H, s, 6-Ar-$\underline{H}$), 3.12-3.07 (2H, m, $C\underline{H}_2N$), 2.96-2.90 (2H, m, $C\underline{H}_2CH_2N$); δ_C (150 MHz; CD_3OD) 147.0 (Ar-$\underline{C}$OH), 146.7 (Ar-$\underline{C}$OH), 127.7 (Ar-$\underline{C}$), 120.4 (3-Ar-$\underline{C}$H), 118.4 (6-Ar-$\underline{C}$H), 113.3 (Ar-$\underline{C}$Br), 40.7 ($\underline{C}H_2N$), 34.3 ($\underline{C}H_2CH_2N$); *m/z* (ESI) 234 ([(^{81}Br)M + H]$^+$, 95%), 232 ([(^{79}Br)M + H]$^+$, 100%); HRMS $C_8H_{11}NO_2{}^{79}Br$, calcd 231.9973, found 231.9978.

8.74 1-Pentyl-1,2,3,4-tetrahydroisoquinolin-6-ol (160) and 1-Pentyl-1,2,3,4-tetrahydroisoquinolin-8-ol (161)

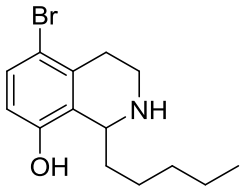


The title compounds were prepared according to the procedure described in section 8.49, from amine **155** (150 mg, 0.688 mmol) and hexanal (126 µL, 1.03 mmol). The crude mixture of products were separated and purified by prep-HPLC (Method 2, Gradient A, run time: 30 min) to give **160** as a pale yellow oil (r_t = 24.5 min, 121 mg, 81%) and **161** as a pale yellow oil (r_t = 28.8 min, 17.3 mg, 12%).

Major regioisomer **160**: υ_{max}/cm^{-1} 3031 br, 2958, 2927, 2867, 1668, 1617, 1523; δ_H (600 MHz; CD_3OD) 7.10 (1H, d, *J* = 8.5 Hz, 8-Ar-H), 6.72 (1H, dd, *J* = 8.5 and 2.5 Hz, 7-Ar-H), 6.64 (1H, d, *J* = 2.5 Hz, 5-Ar-H), 4.42-4.38 (1H, m, 1-CH), 3.55-3.31 (2H, m, 3-CH_2), 3.10-2.95 (2H, m, 4-CH_2), 2.10-1.84 (2H, m, 1-CHCH_2), 1.55-1.35 (6H, m, 3 x CH_2), 0.94 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 156.8 (Ar-COH), 132.4 (Ar-C), 127.2 (8-Ar-CH), 122.6 (Ar-C), 114.5 (5-Ar-CH), 114.2 (7-Ar-CH), 55.2 (1-CH), 39.1 (3-CH_2), 33.5 (1-CHCH_2), 31.2 (CH_2), 24.8 (4-CH_2), 24.5 (CH_2), 21.9 (CH_2), 12.7 (CH_3); *m/z* (CI) 220 ($[M + H]^+$, 80%), 148 ($[M + H]^+ - C_5H_{12}$, 20%); HRMS $C_{14}H_{22}NO$, calcd 220.1701, found 220.1706.

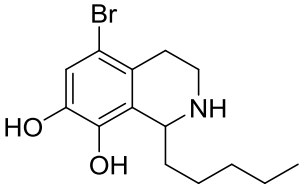
Minor regioisomer **161**: υ_{max}/cm^{-1} 3039 br, 2961, 2930, 2867, 1667, 1633, 1592; δ_H (600 MHz; CD_3OD) 7.11 (1H, t, *J* = 8.0 Hz, 6-Ar-H), 6.72-6.68 (2H, m, 5-Ar-H, 7-Ar-H), 4.60 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.55-3.32 (2H, m, 3-CH_2), 3.12-3.00 (2H, m, 4-CH_2), 2.14-1.79 (2H, m, 1-CHCH_2), 1.62-1.34 (6H, m, 3 x CH_2), 0.94 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 155.3 (Ar-COH), 133.5 (Ar-C), 129.8 (6-Ar-CH), 121.1 (Ar-C), 120.8 (Ar-CH), 114.1 (Ar-CH), 53.1 (1-CH), 38.3 (3-CH_2), 33.0 (1-CHCH_2), 32.5 (CH_2), 26.7 (CH_2), 25.9 (4-CH_2), 23.5 (CH_2), 14.3 (CH_3); *m/z* (CI) 220 ($[M + H]^+$, 30%), 148 ($[M + H]^+ - C_5H_{12}$, 100%); HRMS $C_{14}H_{22}NO$, calcd 220.1701, found 220.1698.

8.75 5-Bromo-1-pentyl-1,2,3,4-tetrahydroisoquinolin-8-ol (**162**)



Hexanal (49.0 µL, 0.404 mmol) was added to a solution of amine **157** (100 mg, 0.337 mmol) in potassium phosphate buffer (5 mL, 0.5 M, pH 6) and acetonitrile (5 mL) under argon. The solution was stirred at 50 ^{o}C for 18 h. Hexanal (21.0 µL, 0.169 mmol) was added to the reaction mixture and stirred at 50 ^{o}C for 5 h. The reaction was concentrated under vacuum. CH_2Cl_2/CH_3OH (1:1, 15 mL) was added to the resultant residue and the suspension filtered to remove the solid. The filtrate was evaporated to dryness under vacuum to give the crude product, which was purified by prep-HPLC (Method 1, r_t = 6.5 min). Appropriate fractions were combined and co-evaporated with methanol (x3) to give the product as a pale yellow oil (14.2 mg, 14%). υ_{max}/cm^{-1} 3031 br, 2953, 2928, 2859, 1666, 1641, 1581; δ_H (600 MHz; CD_3OD) 7.39 (1H, d, *J* = 8.5 Hz, 6-Ar-H), 6.70 (1H, d, *J* = 8.5 Hz, 7-Ar-H), 4.62 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.57-3.42 (2H, m, 3-CH_2), 3.09-2.95 (2H, m, 4-CH_2), 2.10-1.79 (2H, m, 1-CHCH_2), 1.64-1.47 (2H, m, 1-CHCH_2CH_2), 1.45-1.34 (4H, m, CH_2CH_2CH_3), 0.94 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 154.8 (Ar-COH), 133.4 (6-Ar-CH), 132.6 (Ar-C), 123.8 (Ar-C), 115.9 (7-Ar-CH), 114.4 (Ar-CBr), 52.7 (1-CH), 37.6 (3-CH_2), 32.6 (1-CHCH_2), 32.4 (CH_2), 27.4 (4-CH_2), 26.7 (1-CHCH_2CH_2), 23.4 (CH_2), 14.3 (CH_3); *m/z* (CI) 300 ([(^{81}Br)M + H]$^+$, 20%), 298 ([(^{79}Br)M + H]$^+$, 25%), 219 ([M + H]$^+$ - Br, 35%), 131 ([M + H]$^+$ - $C_5H_{12}BrO$, 35%); HRMS $C_{14}H_{21}NO^{79}Br$, calcd 298.0811, found 298.0807.

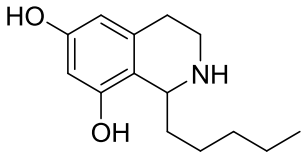
8.76 5-Bromo-1-pentyl-1,2,3,4-tetrahydroisoquinoline-7,8-diol (**163**)



The title compound was prepared according to the procedure described in section 8.53, from amine **159** (200 mg, 0.639 mmol) and hexanal (117 µL, 0.958 mmol). The crude product was purified by prep-HPLC (Method 2, Gradient C, r_t = 30.0 min) to give the

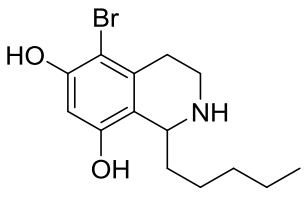
product as a yellow oil (13.2 mg, 7%). υ_{max}/cm^{-1} 3039 br, 2953, 2930, 2859, 1667, 1625; δ_H (600 MHz; CD_3OD) 7.02 (1H, s, Ar-H), 4.63 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.53-3.38 (2H, m, 3-CH_2), 3.00-2.87 (2H, m, 4-CH_2), 2.12-1.79 (2H, m, 1-CHCH_2), 1.63-1.35 (6H, m, 3 x CH_2), 0.94 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 144.2 (Ar-COH), 141.6 (Ar-COH), 121.7 (Ar-C), 120.7 (Ar-C), 117.7 (Ar-CH), 112.0 (Ar-C), 51.3 (1-CH), 36.3 (3-CH_2), 31.0 (CH_2), 30.9 (CH_2), 25.1 (4-CH_2, CH_2), 21.9 (CH_2), 13.0 (CH_3); *m/z* (ESI) 316 ([(^{81}Br)M + H]$^+$, 90%), 314 ([(^{79}Br)M + H]$^+$, 100%); HRMS $C_{14}H_{21}NO_2{}^{79}Br$, calcd 314.0756, found 314.0708.

8.77 1-Pentyl-1,2,3,4-tetrahydroisoquinoline-6,8-diol (**164**)



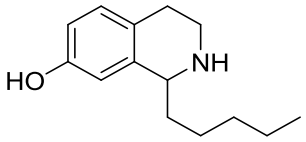
The title compound was prepared according to the procedure described in section 8.53, from amine **156** (100 mg, 0.427 mmol) and hexanal (78.0 µL, 0.641 mmol). The crude product was purified by prep-HPLC (Method 2, Gradient B, r_t = 23.1 min) to give the product as a yellow oil (95.2 mg, 95%). υ_{max}/cm^{-1} 3055 br, 2959, 2938, 2867, 1667, 1599, 1523; δ_H (600 MHz; CD_3OD) 6.22 (1H, d, *J* = 2.5 Hz, 7-Ar-H), 6.15 (1H, d, *J* = 2.5 Hz, 5-Ar-H), 4.50 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.51-3.28 (2H, m, 3-CH_2), 3.02-2.90 (2H, m, 4-CH_2), 2.10-1.75 (2H, m, 1-CHCH_2), 1.59-1.33 (6H, m, 3 x CH_2), 0.94 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 159.1 (Ar-COH), 156.5 (Ar-COH), 134.1 (Ar-C), 122.4 (Ar-C), 107.2 (5-Ar-CH), 102.0 (7-Ar-CH), 53.1 (1-CH), 38.3 (3-CH_2), 33.3 (1-CHCH_2), 32.5 (CH_2), 26.6 (CH_2), 26.1 (4-CH_2), 23.5 (CH_2), 14.4 (CH_3); *m/z* (ESI) 236 ([M + H]$^+$, 100%), 219 ([M + H]$^+$ - OH, 70%), 149 ([M + H]$^+$ - $C_5H_{11}O$, 80%); HRMS $C_{14}H_{22}NO_2$, calcd 236.1651, found 236.1660.

8.78 5-Bromo-1-pentyl-1,2,3,4-tetrahydroisoquinoline-6,8-diol (**165**)



The title compound was prepared according to the procedure described in section 8.53, from amine **158** (100 mg, 0.289 mmol) and hexanal (53.0 µL, 0.433 mmol). The crude product was purified by prep-HPLC (Method 2, Gradient E, run time: 20 min, flow rate: 8 mL/min, r_t = 7.8 min) to give the product as a yellow oil (65.0 mg, 72%). υ_{max}/cm^{-1} 3039 br, 2959, 2938, 2867, 1666, 1588; δ_H (600 MHz; CD_3OD) 6.45 (1H, s, 7-Ar-H), 4.53 (1H, dd, *J* = 9.5 and 3.5 Hz, 1-CH), 3.54-3.39 (2H, m, 3-CH_2), 3.08-2.93 (2H, m, 4-CH_2), 2.08-1.76 (2H, m, 1-CHCH_2), 1.61-1.44 (2H, m, 1-CHCH_2CH_2), 1.43-1.34 (4H, m, CH_2CH_2CH_3), 0.94 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 155.7 (Ar-COH), 155.4 (Ar-COH), 133.1 (Ar-C), 114.6 (Ar-C), 102.6 (7-Ar-CH), 102.5 (Ar-CBr), 52.6 (1-CH), 37.7 (3-CH_2), 33.0 (1-CHCH_2), 32.5 (CH_2), 27.6 (4-CH_2), 26.7 (1-CHCH_2CH_2), 23.5 (CH_2) 14.3 (CH_3); *m/z* (ESI) 316 ([(^{81}Br)M + H]$^+$, 100%), 314 ([(^{79}Br)M + H]$^+$, 100%), 229 ([(^{81}Br)M + H]$^+$ - $C_5H_{11}O$, 65%), 227 ([(^{79}Br)M + H]$^+$ - $C_5H_{11}O$, 65%); HRMS $C_{14}H_{21}NO_2{}^{79}Br$, calcd 314.0756, found 314.0801.

8.79 1-Pentyl-1,2,3,4-tetrahydroisoquinolin-7-ol (**166**)



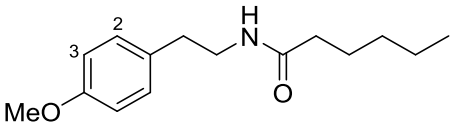
The title compound was prepared according to the procedure described in section 8.69, from the 1.6/1 mixture of THIQ regioisomers **170** and **171** (34.0 mg, 0.146 mmol) and a 1 M solution of boron tribromide in CH_2Cl_2 (0.364 mL, 0.364 mmol). The crude mixture of products were separated and purified by prep-HPLC (Method 2, Gradient A) to give **166** as a pale yellow oil (r_t = 32.6 min, 13.8 mg, 43%) and **160** as a colourless oil (r_t = 37.1 min, 7.3 mg, 23%).

Major regioisomer **166**: υ_{max}/cm^{-1} 3039 br, 2959, 2938, 2867, 1668, 1625, 1506; δ_H (600 MHz; CD_3OD) 7.06 (1H, d, *J* = 8.0 Hz, 5-Ar-H), 6.74 (1H, dd, *J* = 8.0 and 2.5 Hz, 6-Ar-H), 6.68 (1H, d, *J* = 2.5 Hz, 8-Ar-H), 4.45-4.41 (1H, m, 1-CH), 3.57-3.29 (2H, m, 3-CH_2), 3.07-2.93 (2H, m, 4-CH_2), 2.11-1.88 (2H, m, 1-CHCH_2), 1.57-1.36 (6H, m, 3 x CH_2), 0.95 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 157.7 (Ar-COH), 134.4 (Ar-C), 131.4 (5-Ar-CH), 123.0 (Ar-C), 116.7 (6-Ar-CH), 113.6 (8-Ar-CH), 56.9 (1-CH), 41.0 (3-CH_2), 35.0 (1-CHCH_2), 32.7 (CH_2), 26.2 (CH_2), 25.5 (4-CH_2), 23.5 (CH_2),

14.3 (CH_3); *m/z* (CI) 220 ([M + H]$^+$, 100%), 148 ([M + H]$^+$ - C_5H_{12}, 50%); HRMS $C_{14}H_{22}NO$, calcd 220.1701, found 220.1699.

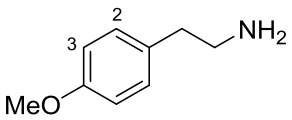
Minor regioisomer **160**: Characterisation data listed in section 8.74.

8.80 *N*-(4-Methoxyphenethyl)hexanamide (167)



Triethylamine (4.15 mL, 29.8 mmol) was added to a stirred solution of amine **169** (900 mg, 5.95 mmol) in CH_2Cl_2 (65 mL) at 0 ^oC. After 5 min, a solution of hexanoyl chloride (1 mL, 7.14 mmol) in CH_2Cl_2 (15 mL) was added to the reaction mixture dropwise at 0 ^oC. After stirring the reaction at rt for 24 h, water (100 mL) was added. The aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and solvent removed *in vacuo* to give the crude product as a white solid, which was purified by column chromatography (EtOAc/petrol, 1:1) to give the product as a white solid (1.34 g, 90%). R_f 0.34 (EtOAc/petrol, 1:1); m.p. 78-79 ^oC; υ_{max}/cm^{-1} ($CDCl_3$ cast) 3308, 2961, 2929, 2891, 1637, 1617, 1544, 1514; δ_H (500 MHz; $CDCl_3$) 7.10 (2H, d, *J* = 8.5 Hz, 2 x 2-Ar-H), 6.84 (2H, d, *J* = 8.5 Hz, 2 x 3-Ar-H), 5.41 (1H, br s, NH), 3.79 (3H, s, OCH_3), 3.52-3.45 (2H, m, CH_2N), 2.75 (2H, t, *J* = 7.0 Hz, CH_2CH_2N), 2.11 (2H, t, *J* = 7.5 Hz, COCH_2), 1.62-1.20 (6H, m, 3 x CH_2), 0.88 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (125 MHz; $CDCl_3$) 173.2 (CONH), 158.4 (Ar-CO), 131.0 (Ar-C), 129.8 (2 x 2-Ar-CH), 114.1 (2 x 3-Ar-CH), 55.3 (OCH_3), 40.7 (CH_2N), 36.9 (COCH_2), 34.9 (CH_2CH_2N), 31.5 (CH_2), 25.5 (CH_2), 22.5 (CH_2), 14.0 (CH_3); *m/z* (EI) 249 (M$^+$, 5%), 134 (M$^+$ - $C_7H_{15}O$, 100%); HRMS $C_{15}H_{23}NO_2$, calcd 249.1729, found 249.1728.

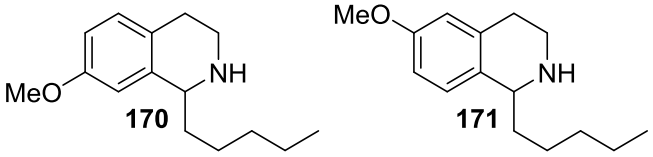
8.81 2-(4-Methoxyphenyl)ethan-1-amine (169)[156]



The title compound was prepared according to the procedure described in section 8.64, from 4-methoxyphenylacetonitrile (1.50 g, 10.2 mmol) and a 1 M borane

tetrahydrofuran complex solution in THF (25.5 mL, 25.5 mmol). The crude product was purified by column chromatography (CH_2Cl_2/CH_3OH(+1% Et_3N), 85:15) to give the product as a pale yellow oil (1.17 g, 76%). R_f 0.14 (CH_2Cl_2/CH_3OH(+1% Et_3N), 4:1); δ_H (500 MHz; $CDCl_3$) 7.11 (2H, d, *J* = 8.5 Hz, 2 x 2-Ar-H), 6.84 (2H, d, *J* = 8.5 Hz, 2 x 3-Ar-H), 3.83 (3H, s, OCH_3), 2.93 (2H, t, *J* = 7.0 Hz, CH_2N), 2.68 (2H, t, *J* = 7.0 Hz, CH_2CH_2N), 1.26 (2H, br s, NH_2); δ_C (125 MHz; $CDCl_3$) 158.1 (Ar-C), 132.0 (Ar-C), 129.8 (2 x 2-Ar-CH), 114.0 (2 x 3-Ar-CH), 55.3 (OCH_3), 43.8 (CH_2N), 39.3 (CH_2CH_2N).

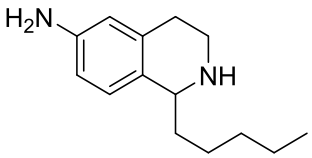
8.82 7-Methoxy-1-pentyl-1,2,3,4-tetrahydroisoquinoline (170) and 6-Methoxy-1-pentyl-1,2,3,4-tetrahydroisoquinoline (171)



To a solution of amide **167** (100 mg, 0.401 mmol) in xylene (5 mL), was added P_2O_5 (142 mg, 1.00 mmol) and $POCl_3$ (0.336 mL, 3.61 mmol), and the mixture was heated at reflux for 20 h. After cooling to rt, the reaction mixture was quenched with water (10 mL), then 2 M NaOH was added until the solution was basic. The aqueous phase was extracted with EtOAc (3 x 20 mL). The organic extracts were combined, washed with brine, dried (Na_2SO_4) and solvent removed *in vacuo* to yield the intermediate imine as a yellow oil which was used in the next step without further purification. To a solution of the imine in methanol (10 mL) at 0 ^oC, was added sodium borohydride (121 mg, 3.21 mmol) portionwise and the mixture was stirred at rt for 2 h. The mixture was concentrated under vacuum. To the resulting residue, water (15 mL) was added, and extracted with EtOAc (3 x 25 mL). The organic extracts were combined, washed with brine, dried (Na_2SO_4) and solvent evaporated *in vacuo* to give the crude product as a mixture of products **170** and **171** (56.7 mg, 37%) as a yellow oil, in a ratio 1.6:1 as determined by ^{1}H-NMR. δ_H (600 MHz; CD_3OD) 7.20 (1H, d, *J* = 8.5 Hz, minor Ar-H), 7.16 (1H, d, *J* = 8.5 Hz, major Ar-H), 6.90-6.85 (2H, m, major Ar-H, minor Ar-H), 6.81 (1H, d, *J* = 2.5 Hz, major Ar-H), 6.79 (1H, d, *J* = 2.5 Hz, minor Ar-H), 4.51-4.47 (1H, m, major 1-CH), 4.46-4.43 (1H, m, minor 1-CH), 3.79-3.78 (6H, m, major OCH_3, minor OCH_3), 3.59-3.32 (4H, m, major 3-CH_2, minor 3-CH_2), 3.16-2.97 (4H, m, major 4-CH_2, minor 4-CH_2), 2.14-1.87 (4H, m, major CH_2, minor CH_2), 1.57-1.34 (12H, m, major 3 x

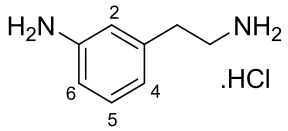
C<u>H</u>$_2$, minor 3 x C<u>H</u>$_2$), 0.98-0.91 (6H, m, major C<u>H</u>$_3$, minor C<u>H</u>$_3$); *m/z* (ESI) 234 ([M + H]$^+$, 100%).

8.83 1-Pentyl-1,2,3,4-tetrahydroisoquinolin-6-amine (**177**)



The title compound was prepared according to the procedure described in section 8.49, from amine **178** (2 eq HCl, 104 mg, 0.497 mmol) and hexanal (73.0 µL, 0.597 mmol). The crude product was purified by prep-HPLC (Method 2, Gradient A, r_t = 28.0 min) to give the product as a yellow oil (66.2 mg, 61%). υ_{max}/cm^{-1} 2960, 2934, 2864, 1656, 1523; δ_H (600 MHz; CD_3OD) 7.30 (1H, d, *J* = 8.0 Hz, 8-Ar-<u>H</u>), 7.06 (1H, d, *J* = 8.0 Hz, 7-Ar-<u>H</u>), 7.00 (1H, s, 5-Ar-<u>H</u>), 4.53-4.47 (1H, m, 1-C<u>H</u>), 3.60-3.35 (2H, m, 3-C<u>H</u>$_2$), 3.18-3.03 (2H, m, 4-C<u>H</u>$_2$), 2.13-1.87 (2H, m, 1-CHC<u>H</u>$_2$), 1.56-1.35 (6H, m, 3 x C<u>H</u>$_2$), 0.94 (3H, t, *J* = 7.0 Hz, C<u>H</u>$_3$); δ_C (150 MHz; CD_3OD) 134.6 (Ar-<u>C</u>), 129.3 (8-Ar-<u>C</u>H), 121.0 (5-Ar-<u>C</u>H), 120.0 (6-Ar-<u>C</u>H), 56.6 (1-<u>C</u>H), 40.4 (3-<u>C</u>H$_2$), 35.0 (1-CH<u>C</u>H$_2$), 32.7 (<u>C</u>H$_2$), 26.3 (4-<u>C</u>H$_2$), 26.1 (<u>C</u>H$_2$), 23.5 (<u>C</u>H$_2$), 14.3 (<u>C</u>H$_3$); *m/z* (ESI) 219 ([M + H]$^+$, 100%); HRMS $C_{14}H_{23}N_2$, calcd 219.1861, found 219.1858.

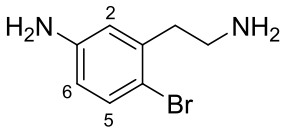
8.84 3-(2-Aminoethyl)aniline hydrochloride (**178**)[122]



The title compound was prepared based on a literature procedure.[122] To a solution of 3-nitrophenethylamine hydrochloride (100 mg, 0.493 mmol) in methanol (7 mL), was added palladium on carbon 10% (12 mg). The flask was evacuated and re-filled with H_2 using a H_2 balloon. The reaction was stirred at rt for 5 h and then filtered through Celite and washed with methanol. The filtrate was evaporated to give the product as a yellow oil (104 mg, 100%) as the hydrochloride salt. δ_H (600 MHz; CD_3OD) 7.07 (1H, t, *J* = 7.5 Hz, 5-Ar-<u>H</u>), 6.63-6.54 (3H, m, 2-Ar-<u>H</u>, 4-Ar-<u>H</u>, 6-Ar-<u>H</u>), 3.13 (2H, t, *J* = 7.5 Hz, C<u>H</u>$_2$N), 2.83 (2H, t, *J* = 7.5 Hz, C<u>H</u>$_2$CH$_2$N); δ_C (150 MHz; CD_3OD) 149.4 (Ar-<u>C</u>),

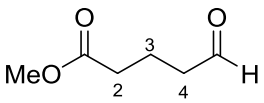
138.6 (Ar-C), 130.7 (5-Ar-CH), 119.2 (Ar-CH), 116.5 (Ar-CH), 115.4 (Ar-CH), 42.0 ($\underline{C}H_2N$), 34.7 ($\underline{C}H_2CH_2N$); *m/z* (ESI) 137 ($[M + H]^+$, 100%); HRMS $C_8H_{13}N_2$, calcd 137.1079, found 137.1079.

8.85 3-(2-Aminoethyl)-4-bromoaniline (**179**)



N-Bromosuccinimide (54.3 mg, 0.305 mmol) was added portionwise to a solution of amine **178** (1.2 eq HCl, 50 mg, 0.278 mmol) in acetonitrile (2 mL) and methanol (0.5 mL) at 0 °C. The reaction was stirred at 0 °C for 1 h, then concentrated *in vacuo*. The resulting crude product was purified by column chromatography (CH_2Cl_2/CH_3OH(+1% Et_3N), 85:15) to yield the product as a pale yellow oil (41.0 mg, 69%). R_f 0.28 (CH_2Cl_2/CH_3OH(+1% Et_3N), 4:1); υ_{max}/cm^{-1} 3331, 2945, 2875, 1597, 1578; δ_H (600 MHz; CD_3OD) 7.20 (1H, d, *J* = 8.5 Hz, 5-Ar-H), 6.63 (1H, d, *J* = 2.5 Hz, 2-Ar-H), 6.47 (1H, dd, *J* = 8.5 and 2.5 Hz, 6-Ar-H), 2.88-2.80 (2H, m, $\underline{H}_2N$), 2.78-2.74 (2H, m, $\underline{H}_2CH_2N$); δ_C (150 MHz; CD_3OD) 148.8 (Ar-C), 140.2 (Ar-C), 134.1 (5-Ar-CH), 118.6 (2-Ar-CH), 116.3 (6-Ar-CH), 112.2 (Ar-CBr), 42.6 ($\underline{C}H_2N$), 40.4 ($\underline{C}H_2CH_2N$); *m/z* (ESI) 217 ($[(^{81}Br)M + H]^+$, 100%), 215 ($[(^{79}Br)M + H]^+$, 100%); HRMS $C_8H_{12}N_2{}^{79}Br$, calcd 215.0184, found 215.0205.

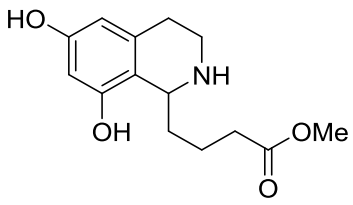
8.86 Methyl 5-oxopentanoate (**181**)[170]



The title compound was prepared according to a literature procedure, except the product was purified by column chromatography rather than distillation.[170] To a solution of δ-valerolactone (500 mg, 4.99 mmol) in methanol (10 mL) was added concentrated sulfuric acid (1 drop) and stirred at 65 °C for 5 h. The reaction mixture was cooled to 0 °C and solid $NaHCO_3$ (500 mg) was added. The mixture was filtered, and the filtrate was evaporated to give the intermediate alcohol **185** as a colourless oil (539 mg, δ_H (300 MHz; $CDCl_3$) 3.68-3.60 (5H, m, $OC\underline{H}_3$, $C\underline{H}_2OH$), 2.36 (2H, t, *J* = 7.0 Hz, $C\underline{H}_2CO_2Me$),

1.79-1.50 (4H, m, C$\underline{H}_2$C$\underline{H}_2$CH$_2$OH)), which was used immediately in the next step without further purification. To a solution of oxalylchloride (0.470 mL, 5.50 mmol) in CH_2Cl_2 (5 mL) at -78 ^oC, was added a solution of DMSO (0.780 mL, 11.0 mmol) in CH_2Cl_2 (5 mL) dropwise. The reaction mixture was stirred for 10 min, then a solution of the alcohol **185** in CH_2Cl_2 (5 mL) was added and the reaction stirred at -78 ^oC for 30 min. Triethylamine (3.69 mL, 26.5 mmol) was added and the mixture allowed to warm to rt. A saturated NH_4Cl solution (30 mL) was added to the mixture and then extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with water, brine, dried (Na_2SO_4) and the solvent evaporated under vacuum at rt to give the crude product as a yellow oil which was purified by column chromatography (hexane/Et_2O, 2:1) to give the product as a colourless oil (202 mg, 31%). R_f 0.20 (hexane/Et_2O, 2:1); δ_H (500 MHz; $CDCl_3$) 9.78 (1H, s, C$\underline{H}$O), 3.67 (3H, s, OC$\underline{H}_3$), 2.51 (2H, t, *J* = 7.0 Hz, 4-C$\underline{H}_2$), 2.37 (2H, t, *J* = 7.0 Hz, 2-C$\underline{H}_2$), 2.00-1.90 (2H, m, 3-C$\underline{H}_2$); δ_C (125 MHz; $CDCl_3$) 201.5 (C$\underline{H}$O), 173.4 (C$\underline{O}_2$CH$_3$), 51.7 (OC$\underline{H}_3$), 43.0 (4-C$\underline{H}_2$), 33.0 (2-C$\underline{H}_2$), 17.4 (3-C$\underline{H}_2$); *m/z* (CI) 131 ([M + H]$^+$, 100%); HRMS $C_6H_{11}O_3$, calcd 131.0708, found 131.0704.

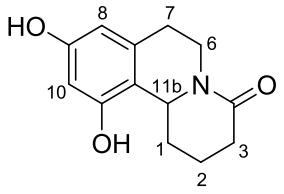
8.87 Methyl 4-(6,8-dihydroxy-1,2,3,4-tetrahydroisoquinolin-1-yl)butanoate (**182**)



The title compound was prepared according to the procedure described in section 8.53 from amine **156** (50.0 mg, 0.214 mmol) and aldehyde **181** (33.4 mg, 0.257 mmol). The crude yellow oil was purified by prep-HPLC (Method 2, Gradient B, flow rate: 8 mL/min, r_t = 15.7 min) to give the product as a yellow oil (49.1 mg, 87%). A minor product collected from the column was lactam **183** (r_t = 20.8 min, 1.9 mg, 4%). δ_H (600 MHz; CD_3OD) 6.22 (1H, d, *J* = 2.0 Hz, 7-Ar-$\underline{H}$), 6.15 (1H, d, *J* = 2.0 Hz, 5-Ar-$\underline{H}$), 4.52 (1H, dd, *J* = 9.0 and 3.5 Hz, 1-C$\underline{H}$), 3.67 (3H, s, OC$\underline{H}_3$), 3.52-3.30 (2H, m, 3-C$\underline{H}_2$), 3.04-2.89 (2H, m, 4-C$\underline{H}_2$), 2.44 (2H, t, *J* = 7.0 Hz, C$\underline{H}_2$CO$_2$CH$_3$), 2.10-1.72 (4H, m, 1-CHC$\underline{H}_2$C$\underline{H}_2$); δ_C (150 MHz; CD_3OD) 175.5 (C$\underline{O}_2$CH$_3$), 159.2 (Ar-C$\underline{O}$H), 156.4 (Ar-C$\underline{O}$H), 134.1 (Ar-C), 112.1 (Ar-C), 107.3 (5-Ar-C$\underline{H}$), 102.0 (7-Ar-C$\underline{H}$), 52.5 (1-C$\underline{H}$), 52.1 (OC$\underline{H}_3$), 38.3 (3-C$\underline{H}_2$), 34.0 (C$\underline{H}_2$CO$_2$CH$_3$), 32.7 (1-CHC$\underline{H}_2$), 26.0 (4-C$\underline{H}_2$), 22.1

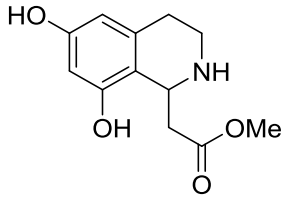
(1-CHCH$_2$C$\underline{\text{H}}_2$); *m/z* (CI) 266 ([M + H]$^+$, 100%), 235 ([M + H]$^+$ - OCH$_3$, 30%), 165 ([M + H]$^+$ - $C_5H_9O_2$, 30%); HRMS $C_{14}H_{20}NO_4$, calcd 266.1392, found 266.1390.

8.88 9,11-Dihydroxy-1,2,3,6,7,11b-hexahydro-4*H*-pyrido[2,1-*a*]isoquinolin-4-one (**183**)



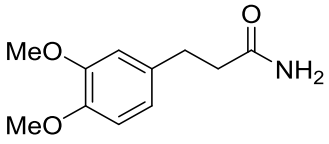
A solution of aldehyde **181** (20.0 mg, 0.154 mmol) in acetonitrile (1.5 mL) was added to a solution of amine **156** (30.0 mg, 0.128 mmol) in potassium phosphate buffer (1.5 mL, 0.1 M, pH 6) under argon, and the mixture was stirred at 50 ^{o}C for 5 h. Sat. K_2CO_3 solution (0.1 mL) was added to the reaction mixture and stirred at rt for 24 h. The pH of the mixture was adjusted to 4 by addition of dilute HCl and concentrated under vacuum. CH_2Cl_2/CH_3OH (1:1, 10 mL) was added to the resultant residue and the suspension filtered to remove the solid. The filtrate was evaporated to dryness to give the crude product as an orange oil, which was purified by prep-HPLC (Method 2, Gradient D, run time: 30 min, r_t = 22.8 min). Product containing fractions were concentrated and co-evaporated with methanol (x3), to yield the product as a yellow oil (12.1 mg, 40%). υ_{max}/cm^{-1} 3219 br, 2923, 2852, 1673, 1572; δ_H (600 MHz; CD_3OD) 6.17 (1H, d, *J* = 2.0 Hz, 10-Ar-$\underline{\text{H}}$), 6.09 (1H, d, *J* = 2.0 Hz, 8-Ar-$\underline{\text{H}}$), 4.79 (1H, d, *J* = 11.5 Hz, 6-C$\underline{\text{H}}$H), 4.72 (1H, d, *J* = 11.5 Hz, 11b-C$\underline{\text{H}}$), 2.95-2.88 (1H, m, 1-C$\underline{\text{H}}$H), 2.73-2.58 (2H, m, 6-CH$\underline{\text{H}}$, 7-C$\underline{\text{H}}$H), 2.57-2.47 (2H, m, 7-CH$\underline{\text{H}}$, 3-C$\underline{\text{H}}$H), 2.44-2.35 (1H, m, 3-CH$\underline{\text{H}}$), 1.91-1.80 (2H, m, 2-C$\underline{\text{H}}_2$), 1.35-1.20 (1H, m, 1-CH$\underline{\text{H}}$); δ_C (150 MHz; CD_3OD) 172.8 (N$\underline{\text{C}}$O), 157.7 (Ar-$\underline{\text{C}}$OH), 156.6 (Ar-$\underline{\text{C}}$OH), 138.7 (Ar-$\underline{\text{C}}$), 115.9 (Ar-$\underline{\text{C}}$), 107.5 (8-Ar-$\underline{\text{C}}$H), 102.0 (10-Ar-$\underline{\text{C}}$H), 56.1 (11b-$\underline{\text{C}}$H), 40.3 (6-$\underline{\text{C}}H_2$), 32.5 (3-$\underline{\text{C}}H_2$), 30.9 (7-$\underline{\text{C}}H_2$), 30.5 (1-$\underline{\text{C}}H_2$), 20.3 (2-$\underline{\text{C}}H_2$); *m/z* (ESI) 234 ([M + H]$^+$, 100%); HRMS $C_{13}H_{16}NO_3$, calcd 234.1130, found 234.1137.

8.89 Methyl 2-(6,8-dihydroxy-1,2,3,4-tetrahydroisoquinolin-1-yl)acetate (187)



Trifluoroacetic acid (0.5 mL) and water (0.5 mL) were added to a solution of methyl 3,3-dimethoxypropionate (400 mg, 2.70 mmol) in CH_2Cl_2 (2 mL), and the reaction was stirred at rt for 24 h. The reaction mixture was diluted with water (4 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extracts were dried (Na_2SO_4) and solvent removed *in vacuo* at rt to give crude methyl 3-oxopropanoate **186** as a colourless oil (245 mg, δ_H (300 MHz; $CDCl_3$) 9.81 (1H, t, *J* = 2.5 Hz, C<u>H</u>O), 3.79 (3H, s, OC<u>H</u>$_3$), 3.42 (2H, d, *J* = 2.5 Hz, C<u>H</u>$_2$)), which was used in the next step without further purification. The title compound was prepared according to the procedure described in section 8.53 from amine **156** (50.0 mg, 0.214 mmol) and crude aldehyde **186** (245 mg). The crude yellow oil was purified by prep-HPLC (Method 2, Gradient C, r_t = 13.5 min), to give the product as a yellow oil (29.4 mg, 58%). δ_H (600 MHz; CD_3OD) 6.23 (1H, s, 7-Ar-<u>H</u>), 6.17 (1H, s, 5-Ar-<u>H</u>), 4.90-4.87 (1H, m, 1-C<u>H</u>), 3.78 (3H, s, OC<u>H</u>$_3$), 3.49-3.34 (2H, m, 3-C<u>H</u>$_2$), 3.20-3.15 (1H, m, 1-CHC<u>H</u>H), 3.03-2.88 (3H, m, 4-C<u>H</u>$_2$, 1-CHCH<u>H</u>); δ_C (150 MHz; CD_3OD) 171.5 (<u>C</u>O$_2$CH$_3$), 158.0 (Ar-<u>C</u>OH), 154.8 (Ar-<u>C</u>OH), 133.0 (Ar-<u>C</u>), 108.4 (Ar-<u>C</u>), 105.8 (5-Ar-<u>C</u>H), 100.7 (7-Ar-<u>C</u>H), 51.2 (1-<u>C</u>H, O<u>C</u>H$_3$), 37.0 (<u>C</u>H$_2$), 34.2 (<u>C</u>H$_2$), 24.7 (4-<u>C</u>H$_2$); *m/z* (CI) 238 ([M + H]$^+$, 20%), 206 ([M + H]$^+$ - CH_4O, 100%); HRMS $C_{12}H_{16}NO_4$, calcd 238.1079, found 238.1073.

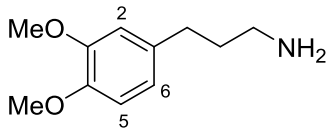
8.90 3-(3,4-Dimethoxyphenyl)propanamide (193)[174]



The title compound was prepared based on a literature procedure.[174] To a solution of 3-(3,4-dimethoxyphenyl)propanoic acid (4.00 g, 19.0 mmol) in CH_2Cl_2 (24 mL) was added thionyl chloride (4.17 mL, 57.1 mmol) and 8 drops of DMF as a catalyst. The resulting bright yellow solution was stirred at rt for 2 h. Thionyl chloride (0.70 mL, 9.50 mmol) and one drop of DMF was added to the reaction and stirred at rt for 1 h.

The reaction was concentrated *in vacuo* and and the residue co-evaporated with CH_2Cl_2 (x3) to remove excess thionyl chloride. The resultant residue was dissolved in THF (40 mL) and added dropwise to a 28% ammonium hydroxide solution (120 mL) at 0 ^oC. The solution was left overnight at rt. The solvents were removed *in vacuo* to give a white solid which was partitioned between 0.1 M aqueous NaOH (40 mL) and EtOAc (120 mL). The organic phase was separated and the aqueous phase extracted with EtOAc (2 x 100 mL). The combined organic extracts were passed through a hydrophobic frit and evaporated *in vacuo* to yield the product as a white solid (3.80 g, 95%). R_f 0.33 (EtOAc); m.p. 110-111 ^oC (lit[214] 120-121 ^oC, benzene); δ_H (500 MHz; $CDCl_3$) 6.81-6.73 (3H, m, 3 x Ar-H), 5.58-5.26 (2H, br m, $CONH_2$), 3.86 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 2.92 (2H, t, J = 7.5 Hz, CH_2), 2.51 (2H, t, J = 7.5 Hz, CH_2); δ_C (125 MHz; $CDCl_3$) 174.6 ($CONH_2$), 149.0 (Ar-C), 147.6 (Ar-C), 133.3 (Ar-C), 120.2 (Ar-CH), 111.7 (Ar-CH), 111.4 (Ar-CH), 56.0 (2 x OCH_3), 37.9 (CH_2), 31.1 (CH_2); *m/z* (EI) 209 (M^+, 30%), 151 (M^+ - CH_2CONH_2, 100%); HRMS $C_{11}H_{15}NO_3$, calcd 209.1049, found 209.1045.

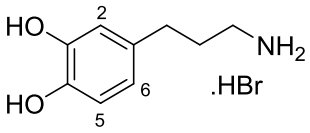
8.91 3-(3,4-Dimethoxyphenyl)propan-1-amine (194)[175]



To a stirred 1 M solution of $LiAlH_4$ in THF (28.7 mL, 28.7 mmol) at 0 ^oC was added slowly a solution of amide **193** (2.00 g, 9.56 mmol) in THF (80 mL). The solution was gently stirred at rt for 5 h, after which time a precipitate had formed. The reaction was cooled to 0 ^oC and quenched by the dropwise addition of water (20 mL). The solvents were evaporated *in vacuo*. CH_2Cl_2 (100 mL) was added to the residue and the mixture passed through a hydrophobic frit. The filtrate was concentrated *in vacuo* to give the crude product as a yellow oil which was purified by column chromatography (CH_2Cl_2/CH_3OH(+1% Et_3N), 85:15) to give the product as an off white oil (1.43 g, 77%). R_f 0.36 (CH_2Cl_2/CH_3OH(+1% Et_3N), 85:15); δ_H (500 MHz; $CDCl_3$) 6.76 (1H, d, J = 8.0 Hz, 6-Ar-H), 6.73-6.69 (2H, m, 2-Ar-H, 5-Ar-H), 3.86 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 2.74 (2H, t, J = 7.0 Hz, CH_2N), 2.60 (2H, t, J = 7.0 Hz, $CH_2(CH_2)_2N$), 2.28 (2H, br s, NH_2), 1.79 (2H, quintet, J = 7.0 Hz, CH_2CH_2N); δ_C (125 MHz; $CDCl_3$) 148.9 (Ar-CO), 147.3 (Ar-CO), 134.5 (Ar-C), 120.2 (6-Ar-CH), 111.8 (Ar-CH), 111.3

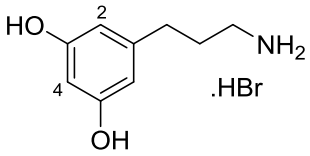
(Ar-CH), 56.0 (OCH_3), 55.9 (OCH_3), 41.5 (CH_2N), 34.8 (CH_2CH_2N), 32.8 ($CH_2(CH_2)_2N$); *m/z* (EI) 195 (M^+, 50%), 178 (M^+ - NH_3, 100%); HRMS $C_{11}H_{17}NO_2$, calcd 195.1254, found 195.1246.

8.92 3-(3,4-Dihydroxyphenyl)propan-1-amine hydrobromide (**195**)[176]



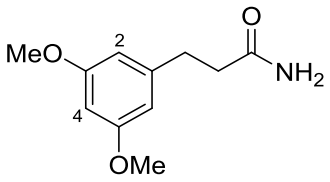
The title compound was prepared according to the procedure described in section 8.69, from amine **194** (356 mg, 1.82 mmol) and a solution of 1 M boron tribromide in CH_2Cl_2 (5.47 mL, 5.47 mmol). The product was isolated as a brown oil as the hydrobromide salt (478 mg, >100% crude). δ_H (500 MHz; CD_3OD) 6.68 (1H, d, *J* = 7.5 Hz, 5-Ar-H), 6.64 (1H, s, 2-Ar-H), 6.52 (1H, d, *J* = 7.5 Hz, 6-Ar-H), 2.89 (2H, t, *J* = 7.5 Hz, CH_2N), 2.55 (2H, t, *J* = 7.5 Hz, $CH_2(CH_2)_2N$), 1.90 (2H, quintet, *J* = 7.5 Hz, CH_2CH_2N); δ_C (125 MHz; CD_3OD) 146.4 (Ar-CO), 144.7 (Ar-CO), 133.2 (Ar-C), 120.6 (6-Ar-CH), 116.5 (Ar-CH), 116.4 (Ar-CH), 40.3 (CH_2N), 32.9 ($CH_2(CH_2)_2N$), 30.5 (CH_2CH_2N); *m/z* (ESI) 168 ($[M + H]^+$, 10%), 164 ($[M + H]^+$ - H_4, 100%).

8.93 3-(3,5-Dihydroxyphenyl)propan-1-amine hydrobromide (**196**)



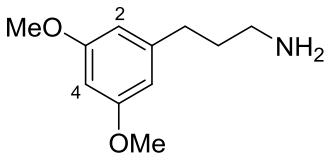
The title compound was prepared according to the procedure described in section 8.69, from amine **198** (190 mg, 0.947 mmol) and a solution of 1 M boron tribromide in CH_2Cl_2 (2.92 mL, 2.92 mmol). The product was isolated as a pale brown oil as the hydrobromide salt (242 mg, 100%). δ_H (600 MHz; CD_3OD) 6.17 (2H, s, 2 x 2-Ar-H), 6.13 (1H, s, 4-Ar-H), 2.91 (2H, t, *J* = 7.5 Hz, CH_2N), 2.56 (2H, t, *J* = 7.5 Hz, $CH_2(CH_2)_2N$), 1.93 (2H, quintet, *J* = 7.5 Hz, CH_2CH_2N); δ_C (150 MHz; CD_3OD) 159.7 (2 x Ar-C), 143.8 (Ar-C), 107.8 (2 x 2-Ar-CH), 101.5 (4-Ar-CH), 40.3 (CH_2N), 33.5 ($CH_2(CH_2)_2N$), 30.1 (CH_2CH_2N); *m/z* (ESI) 168 ($[M + H]^+$, 70%); HRMS $C_9H_{14}NO_2$, calcd 168.1025, found 168.1014.

8.94 3-(3,5-Dimethoxyphenyl)propanamide (197)[177]



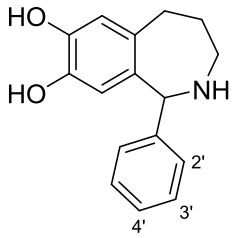
The title compound was prepared according to the procedure described in section 8.90, from 3-(3,5-dimethoxyphenyl)propanoic acid (900 mg, 4.28 mmol), to yield the product as a yellow solid (480 mg, 54%). M.p. 67-69 ^{o}C (lit[177] 80-81 ^{o}C). δ_H (500 MHz; $CDCl_3$) 6.38 (2H, s, 2 x 2-Ar-H), 6.31 (1H, s, 4-Ar-H), 5.55-5.35 (2H, m, NH_2), 3.78 (6H, s, 2 x OCH_3), 2.92 (2H, t, *J* = 7.5 Hz, CH_2CH_2CO), 2.52 (2H, t, *J* = 7.5 Hz, CH_2CO); δ_C (125 MHz; $CDCl_3$) 174.4 ($CONH_2$), 161.0 (2 x Ar-CO), 143.1 (Ar-C), 106.4 (2 x 2-Ar-CH), 98.3 (4-Ar-CH), 55.4 (2 x OCH_3), 37.4 (CH_2CO), 31.7 (CH_2CH_2CO); *m/z* (CI) 210 ($[M + H]^+$, 30%), 193 ($[M + H]^+ - NH_3$, 100%); HRMS $C_{11}H_{16}NO_3$, calcd 210.1130, found 210. 1134.

8.95 3-(3,5-Dimethoxyphenyl)propan-1-amine (198)



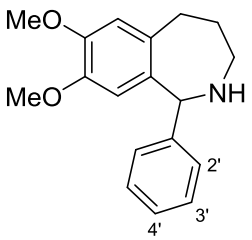
The title compound was prepared according to the procedure described in section 8.91, from amide **197** (425 mg, 2.03 mmol). The crude yellow oil was purified by column chromatography (CH_2Cl_2/CH_3OH(+1% Et_3N), 9:1) to give the product as a pale yellow oil (190 mg, 48%). R_f 0.12 (CH_2Cl_2/CH_3OH(+1% Et_3N), 9:1); υ_{max}/cm^{-1} ($CDCl_3$ cast) 2935, 2852, 1593; δ_H (600 MHz; $CDCl_3$) 6.35 (2H, d, *J* = 2.5 Hz, 2 x 2-Ar-H), 6.30 (1H, d, *J* = 2.5 Hz, 4-Ar-H), 3.77 (6H, s, 2 x OCH_3), 2.74 (2H, t, *J* = 7.5 Hz, CH_2N), 2.59 (2H, t, *J* = 7.5 Hz, $CH_2(CH_2)_2N$), 1.85-1.74 (4H, m, $CH_2CH_2NH_2$); δ_C (150 MHz; $CDCl_3$) 160.9 (2 x Ar-CO), 144.6 (Ar-C), 106.5 (2 x 2-Ar-CH), 97.8 (4-Ar-CH), 55.4 (2 x OCH_3), 41.8 (CH_2N), 34.9 (CH_2CH_2N), 33.7 ($CH_2(CH_2)_2N$); *m/z* (CI) 196 ($[M + H]^+$, 100%), 179 ($[M + H]^+ - NH_3$, 50%); HRMS $C_{11}H_{18}NO_2$, calcd 196.1338, found 196.1333.

8.96 1-Phenyl-2,3,4,5-tetrahydrobenzazepin-7,8-diol (202)



A solution of 1 M boron tribromide in heptane (0.900 mL, 0.900 mmol) was added to a stirred solution of THBP **203** (85.0 mg, 0.300 mmol) in dichloromethane (1.5 mL) at -78 ^{o}C. The reaction was warmed to rt and stirred for 20 h. The reaction was cooled to 0 ^{o}C and quenched by the addition of water (8 mL). The pH of the solution was adjusted to 7 by addition of 1 M aqueous NaOH, and the aqueous phase was extracted with EtOAc (3 x 20 mL). The aqueous phase was adjusted to pH 10 and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried by passing through a hydrophobic frit, and solvent removed *in vacuo* to give the crude product as a brown oil, which was purified by prep-HPLC (Method 1, run time: 15 min, r_t = 11.0 min). Product containing fractions were concentrated and co-evaporated with methanol (x3), to give the product as a yellow oil (8.3 mg, 11%). υ_{max}/cm^{-1} 3064 br, 1671, 1610, 1520; δ_H (500 MHz; CD_3OD) 7.59-7.44 (3H, m, 2 x 3'-Ar-H, 4'-Ar-H), 7.39 (2H, d, *J* = 7.5 Hz, 2 x 2'-Ar-H), 6.74 (1H, s, 6-Ar-H), 6.16 (1H, s, 9-Ar-H), 5.72 (1H, s, 1-CH), 3.55-3.41 (2H, m, 3-CH_2), 3.22-2.77 (2H, m, 5-CH_2), 2.21-1.75 (2H, m, 4-CH_2); δ_C (125 MHz; CD_3OD) 147.0 (Ar-CO), 144.6 (Ar-CO), 137.5 (Ar-C), 135.2 (Ar-C), 130.5 (2 x 3'-Ar-CH), 130.2 (4'-Ar-CH), 128.9 (2 x 2'-Ar-CH), 127.4 (Ar-C), 118.5 (6-Ar-CH, 9-Ar-CH), 65.5 (1-CH), 50.8 (3-CH_2), 34.2 (5-CH_2), 26.8 (4-CH_2); *m/z* (EI) 255 (M^+, 85%), 178 (M^+ - C_6H_5, 75%); HRMS $C_{16}H_{17}O_2N$, calcd 255.1259, found 255.1250.

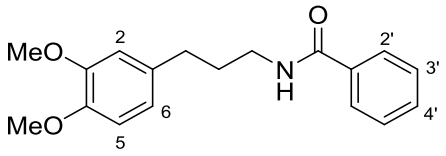
8.97 1-Phenyl-7,8-dimethoxy-2,3,4,5-tetrahydrobenzazepine (203)[143]



The title compound was prepared according to a literature procedure.[143] $POCl_3$ (0.27 mL, 2.94 mmol) was added to a solution of amide **204** (110 mg, 0.367 mmol) in

acetonitrile (3 mL) and refluxed for 21 h. The reaction mixture was concentrated *in vacuo* and the residue co-evaporated with $CHCl_3$ (2 x 3 mL) to give the intermediate imine, which was then dissolved in methanol (6 mL) and cooled to 0 ^oC. Sodium borohydride (111 mg, 2.94 mmol) was added portionwise over 15 min and the solution stirred at rt for 1.5 h. The reaction mixture was concentrated *in vacuo*. Water (10 mL) was added to the residue and extracted with $CHCl_3$ (4 x 5 mL). The combined organic extracts were dried by passing through a hydrophobic frit and solvent removed *in vacuo* to give the product as a pale yellow oil (95.7 mg, 92%). δ_H (500 MHz; $CDCl_3$) 7.41-7.24 (5H, m, 5 x Ar-H), 6.73 (1H, s, 6-Ar-H), 6.20 (1H, s, 9-Ar-H), 5.14 (1H, s, 1-CH), 3.86 (3H, s, OCH_3), 3.59 (3H, s, OCH_3), 3.38-3.11 (2H, m, 3-CH_2), 3.09-2.81 (2H, m, 5-CH_2), 1.90-1.62 (3H, m, 4-CH_2, NH); δ_C (125 MHz; $CDCl_3$) 147.1 (Ar-C), 146.4 (Ar-C), 142.8 (Ar-C), 137.2 (Ar-C), 134.8 (Ar-C), 128.5 (2 x Ar-CH), 127.9 (2 x Ar-CH), 127.0 (Ar-CH), 113.7 (6-Ar-CH), 112.6 (9-Ar-CH), 67.5 (1-CH), 56.1 (OCH_3), 55.9 (OCH_3), 50.7 (3-CH_2), 35.4 (5-CH_2), 30.3 (4-CH_2); *m/z* (EI) 283 (M^+, 60%), 253 (M^+ - C_2H_6, 100%), 206 (M^+ - C_6H_5, 100%); HRMS $C_{18}H_{21}O_2N$, calcd 283.1572, found 283.1577.

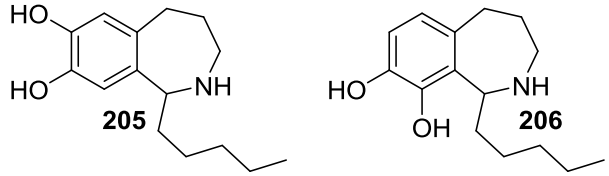
8.98 *N*-(3-(3,4-Dimethoxyphenyl)propyl)benzamide (204)[143]



The title compound was prepared according to the procedure described in section 8.80, from amine **194** (100 mg, 0.512 mmol) and benzoyl chloride (71.3 μL, 0.614 mmol). The crude yellow oil was purified by column chromatography (EtOAc/hexane, 2:3) to give the product as a white solid (130 mg, 85%). R_f 0.2 (EtOAc/hexane, 2:3); m.p. 78-80 ^oC (lit[143] 84 ^oC, EtOAc); δ_H (500 MHz; $CDCl_3$) 7.67 (2H, d, *J* = 7.5 Hz, 2 x 2'-Ar-H), 7.49 (1H, t, *J* = 7.5 Hz, 4'-Ar-H), 7.41 (2H, t, *J* = 7.5 Hz, 2 x 3'-Ar-H), 6.82-6.72 (3H, m, 2-Ar-H, 5-Ar-H, 6-Ar-H), 6.05 (1H, br s, NH), 3.86 (6H, s, 2 x OCH_3), 3.55-3.49 (2H, m, CH_2N), 2.69 (2H, t, *J* = 7.5 Hz, $CH_2(CH_2)_2N$), 1.96 (2H, quintet, *J* = 7.5 Hz, CH_2CH_2N); δ_C (125 MHz; $CDCl_3$) 167.7 (CONH), 149.1 (2 x Ar-C), 134.1 (2 x Ar-C), 131.5 (4'-Ar-CH), 128.6 (2 x Ar-CH), 126.8 (2 x Ar-CH), 120.2 (Ar-CH), 111.8 (Ar-CH), 111.4 (Ar-CH), 56.0 (2 x OCH_3), 39.9 (CH_2N), 33.3 ($CH_2(CH_2)_2N$), 31.4

($\underline{C}H_2CH_2N$); *m/z* (EI) 299 (M^+, 100%); HRMS $C_{18}H_{21}NO_3$, calcd 299.1516, found 299.1510.

8.99 1-Pentyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine-7,8-diol (205) and 1-Pentyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine-8,9-diol (206)

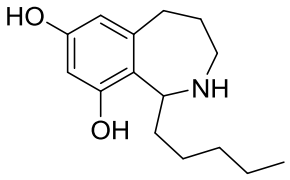


The title compounds were prepared according to the procedure described in section 8.48, from amine **195** (75.0 mg, 0.302 mmol) and hexanal (44.0 µL, 0.363 mmol). The crude mixture of products were separated and purified by prep-HPLC (Method 1, run time: 30 min) to give **205** as a yellow oil (r_t = 21.1 min, 3.4 mg, 5%) and **206** as a yellow oil (r_t = 23.7 min, 2.6 mg, 3%).

Major regioisomer **205**: δ_H (600 MHz; CD_3OD) 6.75 (1H, s, 9-Ar-$\underline{H}$), 6.67 (1H, s, 6-Ar-$\underline{H}$), 4.36-4.28 (1H, m, 1-C$\underline{H}$), 3.44-3.28 (2H, m, 3-C$\underline{H}_2$), 2.98-2.85 (2H, m, 5-C$\underline{H}_2$), 2.21-2.12 (1H, m, 4-C$\underline{H}$H), 1.99-1.86 (3H, m, 4-CH$\underline{H}$, 1-CHC$\underline{H}_2$), 1.46-1.26 (6H, m, 3 x C$\underline{H}_2$), 0.92 (3H, t, *J* = 7.0 Hz, C$\underline{H}_3$); δ_C (150 MHz; CD_3OD) 146.9 (Ar-$\underline{C}$OH), 144.8 (Ar-$\underline{C}$OH), 134.5 (Ar-$\underline{C}$), 125.9 (Ar-$\underline{C}$), 118.8 (6-Ar-$\underline{C}$H), 117.3 (9-Ar-$\underline{C}$H), 62.2 (1-$\underline{C}$H), 33.4 (5-$\underline{C}H_2$), 32.6 ($\underline{C}H_2$), 31.4 (1-CH$\underline{C}H_2$), 27.1 ($\underline{C}H_2$), 26.7 (4-$\underline{C}H_2$), 23.5 ($\underline{C}H_2$), 14.3 ($\underline{C}H_3$); *m/z* (CI) 250 ($[M + H]^+$, 15%), 178 ($[M + H]^+$ - C_5H_{12}, 25%); HRMS $C_{15}H_{24}NO_2$, calcd 250.1802, found 250.1802.

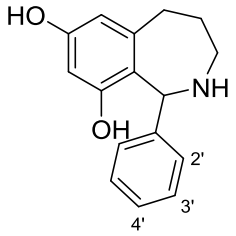
Minor regioisomer **206**: δ_H (600 MHz; CD_3OD) 6.66 (1H, d, *J* = 8.0 Hz, 7-Ar-$\underline{H}$), 6.51 (1H, d, *J* = 8.0 Hz, 6-Ar-$\underline{H}$), 5.28-5.22 (1H, m, 1-C$\underline{H}$), 3.54-3.26 (2H, m, 3-C$\underline{H}_2$), 3.17-2.73 (2H, m, 5-C$\underline{H}_2$), 2.17-2.07 (2H, m, 4-C$\underline{H}$H, 1-CHC$\underline{H}$H), 1.96-1.87 (1H, m, 1-CHCH$\underline{H}$), 1.79-1.69 (1H, m, 4-CH$\underline{H}$), 1.47-1.17 (6H, m, 3 x C$\underline{H}_2$), 0.88 (3H, t, *J* = 7.0 Hz, C$\underline{H}_3$); δ_C (150 MHz; CD_3OD) 145.6 (Ar-$\underline{C}$OH), 144.9 (Ar-$\underline{C}$OH), 122.2 (6-Ar-$\underline{C}$H), 115.6 (7-Ar-$\underline{C}$H), 54.6 (1-$\underline{C}$H), 44.3 (3-$\underline{C}H_2$), 34.1 (5-$\underline{C}H_2$), 32.5 ($\underline{C}H_2$), 30.6 (1-CH$\underline{C}H_2$), 27.2 (4-$\underline{C}H_2$), 26.6 ($\underline{C}H_2$), 23.5 ($\underline{C}H_2$), 14.3 ($\underline{C}H_3$); *m/z* (CI) 250 ($[M + H]^+$, 55%), 178 ($[M + H]^+$ - C_5H_{12}, 100%); HRMS $C_{15}H_{24}NO_2$, calcd 250.1802, found 250.1797.

8.100 1-Pentyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine-7,9-diol (**207**)



The title compound was prepared according to the procedure described in section 8.53, from amine **196** (50.0 mg, 0.202 mmol) and hexanal (37.0 µL, 0.302 mmol). The crude yellow oil was purified by prep-HPLC (Method 2, Gradient C, run time: 30 min, r_t = 17.9 min), to yield the product as a pale brown oil (36.0 mg, 72%). δ_H (600 MHz; CD_3OD) 6.23 (1H, d, *J* = 2.5 Hz, 8-Ar-H), 6.16 (1H, d, *J* = 2.5 Hz, 6-Ar-H), 5.16 (1H, dd, *J* = 10.0 and 5.5 Hz, 1-CH), 3.53-3.26 (2H, m, 3-CH_2), 3.19-3.11 (1H, m, 5-CHH), 2.71 (1H, dd, *J* = 15.5 and 5.5 Hz, 5-CHH), 2.17-2.06 (2H, m, 1-CHCHH, 4-CHH), 1.92-1.84 (1H, m, 1-CHCHH), 1.81-1.71 (1H, m, 4-CHH), 1.44-1.19 (6H, m, 3 x CH_2), 0.89 (3H, t, *J* = 7.0 Hz, CH_3); δ_C (150 MHz; CD_3OD) 159.6 (Ar-COH), 158.2 (Ar-COH), 144.6 (Ar-C), 113.6 (Ar-C), 110.4 (6-Ar-CH), 101.5 (8-Ar-CH), 54.3 (1-CH), 44.0 (3-CH_2), 34.9 (5-CH_2), 32.5 (CH_2), 30.8 (1-CHCH_2), 26.9 (4-CH_2), 26.6 (CH_2), 23.5 (CH_2), 14.3 (CH_3); *m/z* (ESI) 250 ($[M + H]^+$, 70%), 233 ($[M + H]^+$ - OH, 100%); HRMS $C_{15}H_{24}NO_2$, calcd 250.1807, found 250.1805.

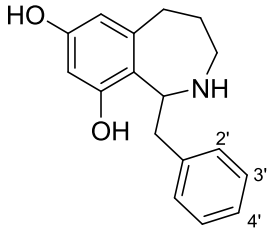
8.101 1-Phenyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine-7,9-diol (**208**)



The title compound was prepared according to the procedure described in section 8.53, from amine **196** (40.0 mg, 0.161 mmol) and benzaldehyde (24.6 µL, 0.242 mmol). The crude product was purified by prep-HPLC (Method 2, Gradient C, run time: 30 min, r_t = 22.1 min), to give the product as a pale yellow oil (10.6 mg, 26%). δ_H (600 MHz; CD_3OD) 7.46-7.37 (3H, m, 2 x 3'-Ar-H, 4'-Ar-H), 7.23 (2H, d, *J* = 8.0 Hz, 2 x 2'-Ar-H), 6.51 (1H, s, 1-CH), 6.32 (1H, d, *J* = 2.5 Hz, 8-Ar-H), 6.28 (1H, d, *J* = 2.5 Hz, 6-Ar-H), 3.32-3.16 (2H, m, 3-CH_2), 2.79-2.63 (2H, m, 5-CH_2), 2.02-1.74 (2H, m, 4-CH_2); δ_C (150 MHz; CD_3OD) 158.8 (Ar-COH), 156.9 (Ar-COH), 143.6 (Ar-C), 133.2 (Ar-C),

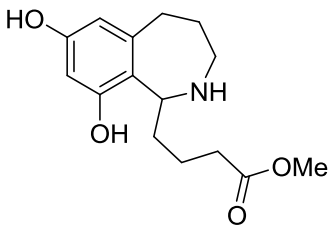
128.8 (2 x 3'-Ar-CH), 128.3 (4'-Ar-CH), 127.5 (2 x 2'-Ar-CH), 110.1 (Ar-C), 108.8 (6-Ar-CH), 100.1 (8-Ar-CH), 55.7 (1-CH), 42.8 (3-CH_2), 33.3 (5-CH_2), 24.6 (4-CH_2); *m/z* (CI) 256 ($[M + H]^+$, 100%), 239 ($[M + H]^+$ - OH, 25%); HRMS $C_{16}H_{18}NO_2$, calcd 256.1338, found 256.1338.

8.102 1-Benzyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepine-7,9-diol (**209**)



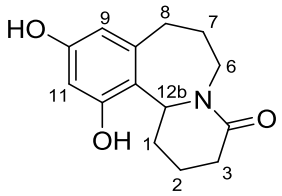
The title compound was prepared according to the procedure described in section 8.53, from amine **196** (40.0 mg, 0.161 mmol) and phenylacetaldehyde (28.3 µL, 0.242 mmol). The crude product was purified by prep-HPLC (Method 2, Gradient C, run time: 30 min, r_t = 18.9 min) to give the product as a pale yellow oil (9.0 mg, 21%). δ_H (600 MHz; CD_3OD) 7.28-7.19 (3H, m, 2 x 3'-Ar-H, 4'-Ar-H), 7.16 (2H, d, *J* = 7.5 Hz, 2 x 2'-Ar-H), 6.19 (1H, d, *J* = 2.0 Hz, 6-Ar-H), 6.09 (1H, d, *J* = 2.0 Hz, 8-Ar-H), 5.42 (1H, t, *J* = 8.0 Hz, 1-CH), 3.67-3.59 (1H, m, 3-CHH), 3.38-3.23 (4H, m, 1-CHCH_2, 3-CHH, 5-CHH), 2.80 (1H, dd, *J* = 15.5 and 5.5 Hz, 5-CHH), 2.25-1.76 (2H, m, 4-CH_2); δ_C (150 MHz; CD_3OD) 158.1 (Ar-COH), 156.5 (Ar-COH), 142.9 (Ar-C), 135.7 (Ar-C), 128.8 (2 x 2'-Ar-CH), 128.0 (2 x 3'-Ar-CH), 127.5 (4'-Ar-CH), 111.4 (Ar-C), 108.8 (6-Ar-CH), 99.8 (8-Ar-CH), 54.5 (1-CH), 42.7 (3-CH_2), 35.3 (1-CHCH_2), 33.2 (5-CH_2), 25.3 (4-CH_2); *m/z* (CI) 270 ($[M + H]^+$, 100%), 178 ($[M + H]^+$ - C_7H_8, 30%); HRMS $C_{17}H_{20}NO_2$, calcd 270.1494, found 270.1492.

8.103 Methyl 4-(7,9-dihydroxy-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-1-yl)butanoate (**210**)



The title compound was prepared according to the procedure described in section 8.53, from amine **196** (20.0 mg, 0.0806 mmol) and aldehyde **181** (12.6 mg, 0.0967 mmol). The crude product was purified by prep-HPLC (Method 2, Gradient B, r_t = 17.2 min) to give the product as a yellow oil (18.0 mg, 80%). A minor product collected from the column was lactam **211** (r_t = 19.5 min, 2.5 mg, 12%). δ_H (600 MHz; CD_3OD) 6.23 (1H, d, *J* = 2.0 Hz, 8-Ar-H), 6.17 (1H, d, *J* = 2.0 Hz, 6-Ar-H), 5.20-5.15 (1H, m, 1-CH), 3.64 (3H, s, OCH_3), 3.54-3.46 (1H, m, 3-CHH), 3.33-3.27 (1H, m, 3-CHH), 3.18-3.10 (1H, m, 5-CHH), 2.71 (1H, dd, *J* = 15.0 and 5.5 Hz, 5-CHH), 2.46-2.35 (2H, m, CH_2CO_2Me), 2.21-2.09 (2H, m, 4-CHH, 1-CHCHH), 1.96-1.88 (1H, m, 1-CHCHH), 1.81-1.74 (1H, m, 4-CHH), 1.71-1.47 (2H, $1\text{-}CHCH_2CH_2$); δ_C (150 MHz; CD_3OD) 175.4 (CO_2Me), 159.7 (Ar-CO), 158.2 (Ar-CO), 144.8 (Ar-C), 113.2 (Ar-C), 110.5 (6-Ar-CH), 101.5 (8-Ar-CH), 53.8 (1-CH), 52.1 (OCH_3), 44.1 ($3\text{-}CH_2$), 34.9 ($5\text{-}CH_2$), 33.8 (CH_2CO_2Me), 30.0 ($1\text{-}CHCH_2$), 26.8 ($4\text{-}CH_2$), 22.2 ($1\text{-}CHCH_2CH_2$); *m/z* (CI) 280 ([M + H]$^+$, 80%); HRMS $C_{15}H_{22}NO_4$, calcd 280.1549, found 280.1544.

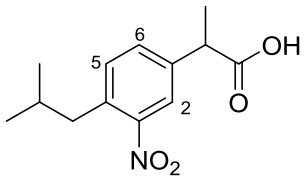
8.104 10,12-Dihydroxy-2,3,6,7,8,12b-hexahydrobenzo[*c*]pyrido[1,2-*a*]azepin-4(1*H*)-one (**211**)



A solution of aldehyde **181** (6.50 mg, 0.0498 mmol) in acetonitrile (0.5 mL) was added to a solution of amine **196** (10.3 mg, 0.0415 mmol) in potassium phosphate buffer (0.5 mL, 0.1 M, pH 6), and the mixture was stirred at 50 ^oC for 8 h. Sat. K_2CO_3 solution (0.1 mL) was added to the reaction mixture and stirred at rt for 48 h. The pH of the mixture was adjusted to 4 by addition of dilute HCl and concentrated under vacuum to give the crude product, which was purified by prep-HPLC (Method 2, Gradient D, run time: 30 min, r_t = 22.7 min). Product containing fractions were concentrated and co-evaporated with methanol (x3), to yield the product as a yellow oil (4.0 mg, 39%). δ_H (600 MHz; CD_3OD) 6.19 (1H, d, *J* = 2.5 Hz, 11-Ar-H), 6.05 (1H, d, *J* = 2.5 Hz, 9-Ar-H), 5.04 (1H, dd, *J* = 11.0 and 4.0 Hz, 12b-CH), 4.13-4.07 (1H, m, 6-CHH), 2.87-2.78 (2H, m, 6-CHH, 8-CHH), 2.49-2.43 (2H, m, $3\text{-}CH_2$), 2.36-2.29 (1H, m, 8-CHH), 2.25-2.14 (2H, m, 1-CHH, 7-CHH), 1.90-1.77 (2H, m, $2\text{-}CH_2$), 1.69-1.60 (1H, m, 1-CHH),

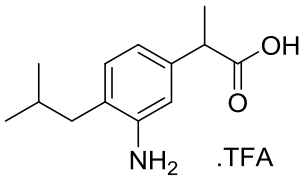
1.51-1.43 (1H, m, 7-CHH); δ_C (150 MHz; CD_3OD) 172.6 (NCO), 158.2 (Ar-CO), 156.3 (Ar-CO), 140.3 (Ar-C), 117.6 (Ar-C), 109.3 (9-Ar-CH), 101.9 (11-Ar-CH), 60.5 (12b-CH), 43.1 (6-CH_2), 32.9 (3-CH_2), 30.6 (1-CH_2, 8-CH_2), 26.0 (7-CH_2), 21.2 (2-CH_2); *m/z* (ESI) 248 ($[M + H]^+$, 100%); HRMS $C_{14}H_{18}NO_3$, calcd 248.1287, found 248.1263.

8.105 2-(4-Isobutyl-3-nitrophenyl)propanoic acid (213)[179]



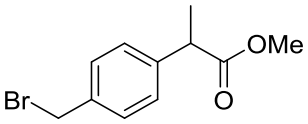
The title compound was prepared based on a literature procedure.[179] 70% Nitric acid (0.80 mL) was added dropwise to a solution of 2-(4-isobutylphenyl)propanoic acid (2.00 g, 9.70 mmol) in concentrated sulfuric acid (12 mL) at 0 ^oC. The reaction mixture was stirred at rt for 1.5 h, then poured into ice cold water (100 mL) and extracted with EtOAc (4 x 50 mL). The combined organic extracts were washed with water (30 mL), brine (30 mL), dried ($MgSO_4$) and solvent removed *in vacuo* to give the crude product as a yellow oil which was purified by column chromatography (hexane/EtOAc, 5:1) to give a mixture of the starting material and product. The starting material was removed by recrystallisation from EtOAc/hexane to give the product as a pale yellow solid (764 mg, 31%). R_f 0.65 (hexane/EtOAc, 1:1); m.p 94-95 ^oC (lit[179] 84 ^oC, methanol); δ_H (500 MHz; $CDCl_3$) 7.82 (1H, d, *J* = 2.0 Hz, 2-Ar-H), 7.46 (1H, dd, *J* = 8.0 and 2.0 Hz, 6-Ar-H), 7.28-7.24 (1H, m, 5-Ar-H), 3.79 (1H, q, *J* = 7.0 Hz, CHCO$_2$H), 2.76 (2H, d, *J* = 7.0 Hz, $CH_2CH(CH_3)_2$), 1.90-1.85 (1H, m, $CH(CH_3)_2$), 1.55 (3H, d, *J* = 7.0 Hz, $CH(CH_3)CO_2H$), 0.91 (6H, d *J* = 6.5 Hz, $CH(CH_3)_2$); δ_C (125 MHz; $CDCl_3$) 179.0 (CO_2H), 149.8 (Ar-C), 138.9 (Ar-C), 135.6 (Ar-C), 133.1 (5-Ar-CH), 131.7 (6-Ar-CH), 123.9 (2-Ar-CH), 44.5 ($CHCO_2H$), 41.5 ($CH_2CH(CH_3)_2$), 29.5 ($CH(CH_3)_2$), 22.5 ($CH(CH_3)_2$), 18.0 ($CH(CH_3)CO_2H$); *m/z* (ESI) 250 ($[M - H]^-$, 20%), 206 ($[M - H]^- - CO_2$, 100%); HRMS $C_{13}H_{16}NO_4$, calcd 250.1079, found 250.1057.

8.106 2-(3-Amino-4-isobutylphenyl)propanoic acid trifluoroacetic acid salt (215)[180]



Pd/C 10 wt. % (10 mg) was added to a solution of the nitro compound **213** (100 mg, 0.398 mmol) in methanol (6 mL). The flask was evacuated and re-filled with H_2 using a H_2 balloon. The reaction was stirred at rt for 5 h. The reaction mixture was filtered through Celite and washed with methanol (2 x 15 mL). The solvent was evaporated *in vacuo* to give the crude product which was purified by prep-HPLC (Method 2, Gradient C, run time: 20 min, flow rate: 8 mL/min, r_t = 21.0 min) to give the product as a yellow oil as the TFA salt (49.1 mg, 37%). δ_H (600 MHz; CD_3OD) 7.35 (3H, m, 3 x Ar-H), 3.75 (1H, q, *J* = 7.0 Hz, CHCO$_2$H), 2.57 (2H, d, *J* = 7.5 Hz, CH$_2$CH(CH$_3$)$_3$), 2.00-1.90 (1H, m, CH$_2$CH(CH$_3$)$_2$), 1.47 (3H, d, *J* = 7.0 Hz, CH(CH$_3$)CO$_2$H), 0.97 (6H, d, *J* = 6.5 Hz, CH(CH$_3$)$_2$); δ_C (150 MHz; CD_3OD) 177.5 (CO$_2$H), 142.4 (Ar-C), 134.3 (Ar-C), 132.9 (Ar-CH), 131.8 (Ar-C), 128.6 (Ar-CH), 123.0 (Ar-CH), 45.9 (CHCO$_2$H), 40.1 (CH$_2$CH(CH$_3$)$_2$), 29.7 (CH(CH$_3$)$_2$), 22.6 (CH(CH$_3$)$_2$), 18.9 (CH(CH$_3$)CO$_2$H); *m/z* (EI) 222 ([M + H]$^+$, 30%), 179 ([M + H]$^+$ - CO_2, 100%); HRMS $C_{13}H_{19}NO_2$, calcd 221.1416, found 221.1419.

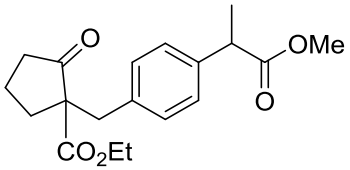
8.107 Methyl 2-(4-bromomethyl)phenyl)propanoate (218)[215]



To a solution of 2-(4-(bromomethyl)phenyl)propanoic acid (2.00 g, 8.23 mmol) in methanol (40 mL) was added concentrated H_2SO_4 (2 drops) and the solution stirred at rt for 19 h. The solvent was evaporated and the residue dissolved in $CHCl_3$ (50 mL). The organic phase was washed with sat. $NaHCO_3$ (2 x 20 mL), brine (20 mL), dried (Na_2SO_4) and solvent removed *in vacuo* to give the crude product as a yellow oil which was purified by column chromatography (hexane/EtOAc, 9:1) to yield the product as a colourless oil (1.64 g, 77%). R_f 0.29 (hexane/EtOAc, 9:1); δ_H (500 MHz; $CDCl_3$) 7.38 (2H, d, *J* = 8.0 Hz, 2 x Ar-H), 7.30-7.25 (2H, m, 2 x Ar-H), 4.52 (2H, s, CH$_2$Br), 3.72 (1H, q, *J* = 7.0 Hz, CHCO$_2$Me), 3.66 (3H, s, OCH$_3$), 1.49 (3H, d, *J* = 7.0 Hz, CH(CH$_3$)CO$_2$Me); δ_C (125 MHz; $CDCl_3$) 174.8 (CO$_2$Me), 140.9 (Ar-C), 136.7 (Ar-C),

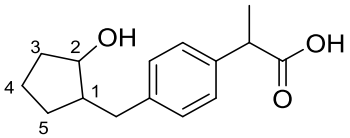
129.4 (2 x Ar-CH), 128.0 (2 x Ar-CH), 52.2 (OCH_3), 45.2 ($CHCO_2Me$), 33.3 (CH_2Br), 18.6 ($CH(CH_3)CO_2Me$); *m/z* (CI) 259 ([(^{81}Br)M + H]$^+$, 15%), 257 ([(^{79}Br)M + H]$^+$, 15%), 177 (M^+ - Br, 100%); HRMS $C_{11}H_{14}{}^{79}BrO_2$, calcd 257.0177, found 257.0173.

8.108 Ethyl 1-(4-(1-methoxy-1-oxopropan-2-yl)benzyl)-2-oxocyclopentane-1-carboxylate (222)[216]



Ethyl 2-oxocyclopentanecarboxylate (0.580 mL, 3.89 mmol) was added to a stirred suspension of K_2CO_3 (0.967 g, 7.00 mmol) in acetone (50 mL). After stirring at rt for 15 min, compound **218** (1.00 g, 3.89 mmol) was added and the resulting mixture was refluxed for 16 h. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with sat. $NaHCO_3$ (2 x 50 mL), brine (50 mL), dried (Na_2SO_4) and solvent removed *in vacuo* to give the crude product as a colourless oil, which was purified by column chromatography (hexane/EtOAc, 9:1) to give the product as a colourless oil (0.784 g, 61%). R_f 0.18 (hexane/EtOAc, 9:1); δ_H (500 MHz; $CDCl_3$) 7.17 (2H, d, *J* = 8.0 Hz, 2 x Ar-H), 7.08 (2H, d, *J* = 8.0 Hz, 2 x Ar-H), 4.16 (2H, q, *J* = 7.0 Hz, OCH_2CH_3), 3.80-3.60 (4H, m, $CHCO_2CH_3$), 3.20-3.00 (2H, m, CH_2), 2.50-1.55 (6H, m, 3 x CH_2), 1.46 (3H, d, *J* = 7.0 Hz, $CH(CH_3)CO_2Me$), 1.24 (3H, t, *J* = 7.0 Hz, OCH_2CH_3); δ_C (125 MHz; $CDCl_3$) 214.9 (CO), 175.0 (CO_2), 171.0 (CO_2), 139.1 (Ar-C), 135.6 (Ar-C), 130.5 (2 x Ar-CH), 127.5 (2 x Ar-CH), 61.7 and 61.5 ($CCO_2CH_2CH_3$), 52.1 (OCH_3), 45.1 ($CHCO_2Me$), 38.6 (CH_2), 38.3 (CH_2), 31.8 (CH_2), 19.5 (CH_2), 18.6 ($CH(CH_3)CO_2Me$), 14.1 (OCH_2CH_3); *m/z* (EI) 332 (M^+, 30%), 199 (M^+ - $C_5H_9O_4$, 100%); HRMS $C_{19}H_{24}O_5$, calcd 332.1624, found 332.1621.

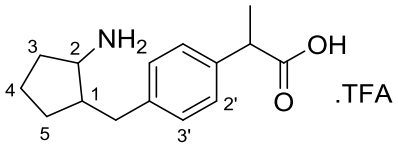
8.109 2-(4-((2-Hydroxycyclopentyl)methyl)phenyl)propanoic acid (224)[183]



Sodium borohydride (9.37 mg, 0.248 mmol) was added portionwise to a solution of loxoprofen **79** (24.4 mg, 0.0991 mmol) in methanol (1.5 mL) at 0 ^oC, and stirred at rt

for 2 h. The reaction mixture was concentrated *in vacuo.* The resulting residue was dissolved in aqueous 1 M HCl (5 mL) and extracted with $CHCl_3$ (3 x 10 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and solvent removed *in vacuo* to give the product as a white solid (23.3 mg, 95%), as a 3:1 mixture of the *trans*/*cis* diastereoisomers at C-1 and C-2 (determined by ^{1}H-NMR and based on assignments reported by Naruto *et al*.).[183] M.p. 84-108 ^{o}C (lit[183] 75-111 ^{o}C range for all 8 diastereoisomers); δ_H (600 MHz; $CDCl_3$) 7.25-7.10 (4H, m, 4 x Ar-H), 4.12-3.86 (1H, m, 2-CH), 3.71 (1H, q, *J* = 7.0 Hz, CHCO$_2$H), 2.85-2.45 (2H, m, 1-CHCH$_2$), 2.05-1.52 (6H, m, 1-CH, 3-CH$_2$, 4-CH$_2$, 5-CHH), 1.51 (3H, d, *J* = 7.0 Hz, CH$_3$), 1.30-1.18 (1H, m, 5-CHH); δ_C (150 MHz; $CDCl_3$) 179.7 (CO$_2$H), 141.1 and 140.4 (Ar-C), 137.5 and 137.3 (Ar-C), 129.3 and 129.1 (2 x Ar-CH), 127.7 (2 x Ar-CH), 78.6 and 74.5 (2-CH), 49.8 and 47.6 (1-CH), 44.9 (CHCO$_2$H), 39.4 and 35.2 (1-CHCH$_2$), 34.9 and 34.2 (3-CH$_2$), 29.9 and 28.8 (5-CH$_2$), 21.9 and 21.6 (4-CH$_2$), 18.3 (CH$_3$); *m/z* (EI) 248 (M^+, 30%), 230 (M^+ - OH_2, 85%), 185 (M^+ - CH_3O_3, 100%); HRMS $C_{15}H_{20}O_3$, calcd 248.1412, found 248.1411.

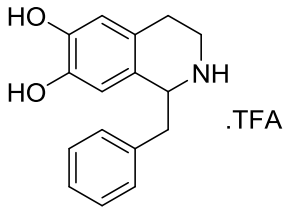
8.110 2-(4-((2-Aminocyclopentyl)methyl)phenyl)propanoic acid trifluoroacetic acid salt (**225**)



Ammonium acetate (62.6 mg, 0.812 mmol) was added to stirred solution of loxoprofen **79** (20.0 mg, 0.0812 mmol) in methanol (2.5 mL) at rt. After 5 min, sodium cyanoborohydride (6.12 mg, 0.0974 mmol) was added to the reaction mixture and stirred at rt for 48 h. The solvent was removed *in vacuo* and the resulting residue was purified by prep-HPLC (Method 2, Gradient C, run time: 25 min, flow rate: 8 mL/min, r_t = 17.8 min). Appropriate fractions were evaporated *in vacuo*, and the resulting solid was recrystallised from Et_2O/CH_3OH to give the product as a white solid as the TFA salt (13.6 mg, 46%) as a 6:1 mixture of *cis*/*trans* diastereoisomers at C-1 and C-2 (determined by ^{1}H-NMR). M.p. 38-42 ^{o}C; υ_{max}/cm^{-1} 2970, 1668, 1513; δ_H (600 MHz; CD_3OD) 7.26 (2H, d, *J* = 8.0 Hz, 2 x 2'-Ar-H), 7.18 (2H, d, *J* = 8.0 Hz, 2 x 3'-Ar-H), 3.72-3.65 (1H, m, CHCO$_2$H), 3.32-3.25 (1H, m, 2-CH), 2.97-2.89 (1H, m, 1-CHCHH), 2.53-2.39 (1H, m, 1-CHCHH), 2.20-2.11 (2H, m, 1-CH, 3-CHH), 1.88-1.61 (4H, m, 3-

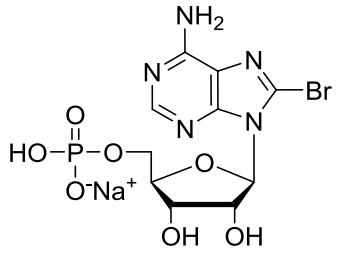
CHH, 4-CH$_2$, 5-CHH), 1.49-1.38 (4H, m, 5-CHH, CH$_3$); δ_C (150 MHz; CD$_3$OD) 178.4 (CO$_2$H), 140.6 (Ar-C), 139.9 (Ar-C), 130.1 and 129.9 (2 x 3'-Ar-CH), 128.8 (2 x 2'-Ar-CH), 57.8 and 56.2 (2-CH), 47.8 and 45.3 (1-CH), 46.2 (CHCO$_2$H), 39.6 and 35.5 (1-CHCH$_2$), 31.6 and 31.4 (3-CH$_2$), 31.2 and 29.2 (5-CH$_2$), 23.3 and 22.4 (4-CH$_2$), 19.0 (CH$_3$); *m/z* (CI) 248 ([M + H]$^+$, 100%); HRMS $C_{15}H_{22}NO_2$, calcd 248.1651, found 248.1652.

8.111 1-Benzyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol trifluoroacetic acid salt (**230**)[122]



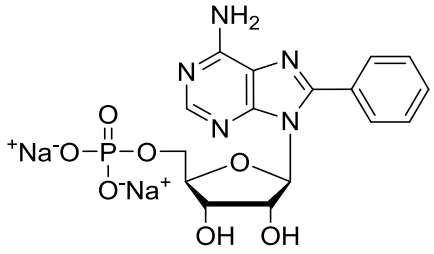
A 0.1 M stock phosphate solution was prepared by dissolving the required phosphate (0.600 mmol) in water (4 mL) and adjusting the pH to 6 by adding the appropriate amount of dilute aqueous HCl or NaOH solution. The solution was then diluted to 6 mL. Dopamine hydrochloride (60.0 mg, 0.316 mmol) and phenylacetaldehyde (44.0 μL, 0.380 mmol) were added to a mixture of water (1 mL), phosphate stock solution (2 mL, 0.1 M, pH 6) and acetonitrile (3 mL). The solution was stirred at 50 ^{o}C for 4 h. The reaction mixture was concentrated under vacuum or left in the blowdown unit overnight to remove the volatiles. CH_2Cl_2/CH_3OH 1:1 (10 mL) was added to the resultant residue and the suspension passed through a filter to remove the solid. The filtrate was evaporated to dryness and the crude product was purified by either prep-HPLC (Method 2, Gradient E, r_t = 20.4), or MDAP (Gradient A, r_t = 13.9 min). Appropriate fractions were combined and solvent removed *in vacuo* to yield the product as a yellow solid as the TFA salt. δ_H (600 MHz; CD$_3$OD) 7.45-7.28 (5H, m, 5 x Ar-H), 6.63 (1H, s, 5-Ar-H), 6.59 (1H, s, 8-Ar-H), 4.70-4.60 (1H, m, 1-CH), 3.50-3.23 (3H, m, 3-CH$_2$, 1-CHCHH), 3.11-2.87 (3H, m, 1-CHCHH, 4-CH$_2$); δ_C (150 MHz; CD$_3$OD) 149.9 (Ar-COH), 145.8 (Ar-COH), 136.7 (Ar-C), 130.6 (2 x Ar-CH), 130.2 (2 x Ar-CH), 128.8 (Ar-CH), 123.6 (Ar-C), 116.2 (5-Ar-CH), 114.2 (8-Ar-CH), 57.4 (1-CH), 41.3 (CH$_2$), 40.9 (CH$_2$), 25.7 (4-CH$_2$); *m/z* (ESI) 256 ([M + H]$^+$, 100%).

8.112 8-Bromo adenosine 5'-monophosphate (**234**)[193]



The title compound was prepared following a literature procedure.[193] To a stirred solution of adenosine monophosphate disodium salt (2.00 g, 2.56 mmol) in sodium acetate buffer (0.5 M, 80 mL, pH 4) was added saturated aqueous bromine (25 mL). After 3 h, further saturated aqueous bromine (25 mL) was added, and stirred at rt for 21 h. The aqueous solution was washed with chloroform (4 x 50 mL) and evaporated to dryness to give the crude product as an orange solid (4.49 g, >100%), which was used without further purification. A sample of the crude product was purified by prep-HPLC (Method 3, Gradient E, r_t = 8.3 min) and was used in the synthesis of nucleotides **235** and **236**. Residual sodium acetate was present in the crude and purified product (0.5 eq) as determined by ^{1}H-NMR. M.p. > 300 ^{o}C (decomposed); δ_H (600 MHz; D_2O) 8.21 (1H, s, 2-C<u>H</u>), 6.12 (1H, d, *J* = 6.0 Hz, 1'-C<u>H</u>), 5.33 (1H, t, *J* = 6.0 Hz, 2'-C<u>H</u>), 4.60 (1H, t, *J* = 6.0 Hz, 3'-C<u>H</u>), 4.30-4.25 (1H, m, 4'-C<u>H</u>), 4.12-3.97 (2H, m, 5'-C<u>H</u>$_2$); δ_C (150 MHz; D_2O) 155.0 (<u>C</u>), 153.6 (2-<u>C</u>H), 151.0 (<u>C</u>), 128.9 (<u>C</u>), 120.0 (<u>C</u>), 89.9 (1'-<u>C</u>H), 84.7 (4'-<u>C</u>H), 71.2 (2'-<u>C</u>H), 70.4 (3'-<u>C</u>H), 64.2 (5'-<u>C</u>H$_2$).

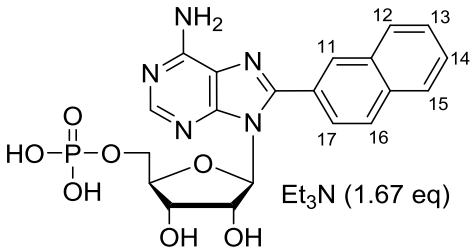
8.113 8-Phenyl adenosine 5'-monophosphate disodium salt (**235**)[193]



The title compound was prepared following a literature procedure.[193] 8-Br-AMP **234** (120 mg, 0.245 mmol), phenylboronic acid (37.3 mg, 0.306 mmol), potassium carbonate (50.9 mg, 0.368 mmol), sodium tetrachloropalladate(II) (1.80 mg, 6.13 µmol, 2.5 mol%) and triphenylphosphine-3,3',3"-trisulfonic acid trisodium salt (8.70 mg, 15.3 µmol, 2.5 eq to Pd) were added to a flask and purged with argon. Degassed water (6 mL) was added via syringe and the reaction was stirred at 80 ^{o}C for 1.5 h. The resulting

dark brown solution was filtered, and the filtrate was purified by prep-HPLC (Method 3, Gradient C, r_t = 17.5 min). Appropriate fractions were combined, concentrated and co-evaporated with methanol to remove residual TEAB. The resulting solid was dissolved in water, and pH adjusted to 8 by addition of aqueous 1 M NaOH. The solution was concentrated *in vacuo* to give the product as a brown solid (96.2 mg, 84%). M.p. > 300 ^oC (decomposed); δ_H (600 MHz; D_2O) 8.28 (1H, s, 2-C<u>H</u>), 7.77 (2H, d, *J* = 7.0 Hz, 2 x Ar-<u>H</u>), 7.70-7.61 (3H, m, 3 x Ar-<u>H</u>), 5.75 (1H, d, *J* = 6.0 Hz, 1'-C<u>H</u>), 5.06 (1H, t, *J* = 6.0 Hz, 2'-C<u>H</u>), 4.12-3.92 (4H, m, 3'-C<u>H</u>, 4'-C<u>H</u>, 5'-C<u>H</u>$_2$); δ_C (150 MHz; D_2O) 156.0 (<u>C</u>), 154.0 (2-<u>C</u>H), 153.3 (<u>C</u>), 150.9 (<u>C</u>), 131.7 (Ar-<u>C</u>), 130.2 (2 x Ar-<u>C</u>H), 129.6 (2 x Ar-<u>C</u>H), 128.8 (Ar-<u>C</u>H) 119.2 (<u>C</u>), 91.1 (1'-<u>C</u>H), 85.1 (4'-<u>C</u>H), 71.8 (2'-<u>C</u>H), 70.6 (3'-<u>C</u>H), 65.0 (5'-<u>C</u>H_2); *m/z* (ESI) 422 ([M – H]$^-$, 100%); HRMS $C_{16}H_{17}N_5O_7P$, calcd 422.0865, found 422.0853.

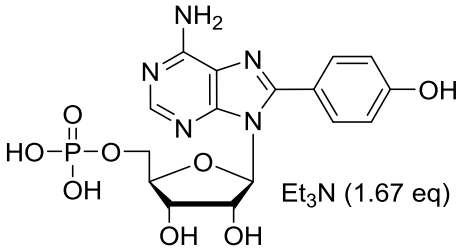
8.114 8-Naphthyl adenosine 5'-monophosphate triethylammonium salt (236)



The title compound was prepared according to the procedure described in section 8.113, from 8-Br-AMP **234** (120 mg, 0.245 mmol) and 2-naphthylboronic acid (52.6 mg, 0.306 mmol). The crude product was purified by prep-HPLC (Method 3, Gradient D, r_t = 20.0 min). Product containing fractions were concentrated *in vacuo* to give the product as a yellow solid as the triethylammonium salt (61.6 mg, 39%, 1.67 eq TEA as determined by ^{1}H-NMR). M.p. > 300 ^oC (decomposed); δ_H (600 MHz; D_2O) 8.01 (1H, s, 11-Ar-<u>H</u>), 7.93 (1H, s, 2-C<u>H</u>), 7.91-7.79 (3H, m, 12-Ar-<u>H</u>, 15-Ar-<u>H</u>, 16-Ar-<u>H</u>), 7.61 (1H, d, *J* = 8.5 Hz, 17-Ar-<u>H</u>), 7.57-7.52 (2H, m, 13-Ar-<u>H</u>, 14-Ar-<u>H</u>), 5.91 (1H, d, *J* = 6.0 Hz, 1'-C<u>H</u>), 5.18 (1H, t, *J* = 6.0 Hz, 2'-C<u>H</u>), 4.46 (1H, t, *J* = 6.0 Hz, 3'-C<u>H</u>), 4.23-4.09 (3H, m, 4'-C<u>H</u>, 5'-C<u>H</u>$_2$), 3.17 (10H, q, *J* = 7.0 Hz, C<u>H</u>$_2$ TEA), 1.26 (15H, t, *J* = 7.0 Hz, C<u>H</u>$_3$ TEA); δ_C (150 MHz; D_2O) 155.1 (<u>C</u>), 153.0 (<u>C</u>), 152.7 (2-<u>C</u>H), 150.1 (<u>C</u>), 134.0 (<u>C</u>), 132.6 (<u>C</u>), 129.9 (11-Ar-<u>C</u>H), 129.4 (Ar-<u>C</u>H), 129.1 (16-Ar-<u>C</u>H), 128.5 (Ar-<u>C</u>H), 128.2 (Ar-<u>C</u>H), 127.7 (Ar-<u>C</u>H), 126.0 (17-Ar-<u>C</u>H), 125.3 (<u>C</u>), 118.9 (<u>C</u>), 89.6 (1'-<u>C</u>H), 83.8 (4'-<u>C</u>H), 70.8 (2'-<u>C</u>H), 70.2 (3'-<u>C</u>H), 64.8 (5'-<u>C</u>H_2), 41.2 (<u>C</u>H_2 TEA), 8.8 (<u>C</u>H_3 TEA);

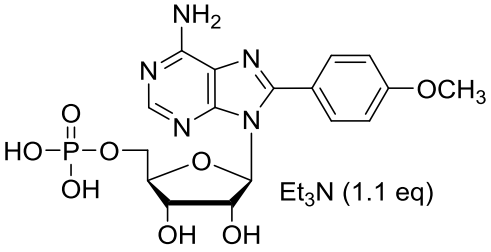
m/z (ESI) 472 ([M – H]$^-$, 100%); HRMS $C_{20}H_{19}N_5O_7P$, calcd 472.1022, found 472.1011.

8.115 8-(4-Hydroxy)phenyl adenosine 5'-monophosphate triethylammonium salt (**237**)



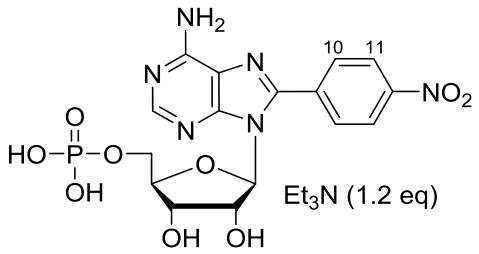
The title compound was prepared according to the procedure described in section 8.113, from 8-Br-AMP **234** (300 mg, 0.635 mmol) and 4-hydroxyphenylboronic acid (139 mg, 1.01 mmol). The crude product was purified by prep-HPLC (Method 3, Gradient A, r_t = 21.5 min). Product containing fractions were concentrated *in vacuo* to give the product as a brown solid as the triethylammonium salt (30.5 mg, 15% over 2 steps from 5'-AMP, 1.67 eq TEA as determined by ^{1}H-NMR). δ_H (600 MHz; D_2O) 8.22 (1H, s, 2-C<u>H</u>), 7.49 (2H, d, *J* = 7.0 Hz, 2 x Ar-<u>H</u>), 6.93 (2H, d, *J* = 7.0 Hz, 2 x Ar-<u>H</u>), 5.87 (1H, d, *J* = 6.0 Hz, 1'-C<u>H</u>), 5.21 (1H, t, *J* = 6.0 Hz, 2'-C<u>H</u>), 4.48-4.44 (1H, m, 3'-C<u>H</u>), 4.25-4.10 (3H, m, 4'-C<u>H</u>, 5'-C<u>H</u>$_2$), 3.21 (10H, q, *J* = 7.5 Hz, C<u>H</u>$_2$ TEA), 1.29 (15H, t, *J* = 7.5 Hz, C<u>H</u>$_3$ TEA); δ_C (150 MHz; D_2O) 158.7 (<u>C</u>), 154.8 (<u>C</u>), 153.8 (<u>C</u>), 151.9 (<u>C</u>), 150.5 (2-<u>C</u>H), 131.7 (2 x Ar-<u>C</u>H), 119.9 (<u>C</u>), 118.4 (<u>C</u>), 116.3 (2 x Ar-<u>C</u>H), 89.6 (1'-<u>C</u>H), 83.7 (4'-<u>C</u>H), 70.8 (2'-<u>C</u>H), 70.2 (3'-<u>C</u>H), 65.2 (5'-<u>C</u>H$_2$), 47.3 (<u>C</u>H$_2$ TEA), 8.9 (<u>C</u>H$_3$ TEA); *m/z* (ESI) 440 ([M + H]$^+$, 100%); HRMS $C_{16}H_{19}N_5O_8P$, calcd 440.0971, found 440.0960.

8.116 8-(4-Methoxy)phenyl adenosine 5'-monophosphate triethylammonium salt (**238**)



The title compound was prepared according to the procedure described in section 8.113, from 8-Br-AMP **234** (300 mg, 0.635 mmol) and 4-methoxyphenylboronic acid (153 mg, 1.01 mmol). The crude product was purified by prep-HPLC (Method 3, Gradient B, r_t = 24.0 min). Product containing fractions were concentrated *in vacuo* to give the product as a grey solid as the triethylammonium salt (49.1 mg, 26% over 2 steps from 5'-AMP, 1.1 eq TEA as determined by ^{1}H-NMR). δ_H (600 MHz; D_2O) 8.17 (1H, s, 2-C<u>H</u>), 7.48 (2H, d, *J* = 8.0 Hz, 2 x Ar-<u>H</u>), 6.92 (2H, d, *J* = 8.0 Hz, 2 x Ar-<u>H</u>), 5.83 (1H, d, *J* = 6.0 Hz, 1'-C<u>H</u>), 5.20 (1H, d, *J* = 6.0 Hz, 2'-C<u>H</u>), 4.50-4.45 (1H, m, 3'-C<u>H</u>), 4.25-4.12 (3H, m, 4'-C<u>H</u>, 5'-C<u>H</u>$_2$), 3.84 (3H, s, OC<u>H</u>$_3$), 3.20 (6.6H, q, *J* = 7.5 Hz, C<u>H</u>$_2$ TEA), 1.28 (9.9H, t, *J* = 7.5 Hz, C<u>H</u>$_3$ TEA); δ_C (150 MHz; D_2O) 161.3 (<u>C</u>), 154.7 (<u>C</u>), 153.3 (<u>C</u>), 151.9 (<u>C</u>), 150.3 (2-<u>C</u>H), 131.3 (2 x Ar-<u>C</u>H), 120.3 (<u>C</u>), 118.7 (<u>C</u>), 114.8 (2 x Ar-<u>C</u>H), 89.6 (1'-<u>C</u>H), 83.5 (4'-<u>C</u>H), 70.7 (2'-<u>C</u>H), 70.2 (3'-<u>C</u>H), 65.3 (5'-<u>C</u>H$_2$), 56.0 (O<u>C</u>H$_3$), 47.3 (<u>C</u>H$_2$ TEA), 8.9 (<u>C</u>H$_3$ TEA); *m/z* (ESI) 454 ([M + H]$^+$, 100%); HRMS $C_{17}H_{21}N_5O_8P$, calcd 454.1128, found 454.1119.

8.117 8-(4-Nitro)phenyl adenosine 5'-monophosphate triethylammonium salt (239)



The title compound was prepared according to the procedure described in section 8.113, from 8-Br-AMP **234** (300 mg, 0.635 mmol) and 4-nitrophenylboronic acid (169 mg, 1.01 mmol). The crude product was purified by prep-HPLC (Method 3, Gradient C, run time: 40 min, r_t = 26.0 min). Product containing fractions were concentrated *in vacuo* to give the product as a yellow solid as the triethylammonium salt (20.0 mg, 10% over 2 steps from 5'-AMP, 1.2 eq TEA as determined by ^{1}H-NMR). δ_H (600 MHz; D_2O) 8.32 (2H, d, *J* = 8.0 Hz, 2 x 11-Ar-<u>H</u>), 8.22 (1H, s, 2-C<u>H</u>), 7.89 (2H, d, *J* = 8.0 Hz, 2 x 10-Ar-<u>H</u>), 5.86 (1H, d, *J* = 6.0 Hz, 1'-C<u>H</u>), 5.27 (1H, t, *J* = 6.0 Hz, 2'-C<u>H</u>), 4.54-4.49 (1H, m, 3'-C<u>H</u>), 4.24-4.12 (3H, m, 4'-C<u>H</u>, 5'-C<u>H</u>$_2$), 3.22 (7.2H, q, *J* = 7.5 Hz, C<u>H</u>$_2$ TEA), 1.28 (10.8H, t, *J* = 7.5 Hz, C<u>H</u>$_3$ TEA); δ_C (150 MHz; D_2O) 155.0 (<u>C</u>), 153.5 (2-<u>C</u>H), 151.4 (<u>C</u>), 151.1 (<u>C</u>), 149.1 (<u>C</u>), 134.7 (<u>C</u>), 131.2 (2 x 10-Ar-<u>C</u>H), 124.6 (2 x 11-Ar-<u>C</u>H), 119.3 (<u>C</u>), 89.4 (1'-<u>C</u>H), 83.9 (4'-<u>C</u>H), 71.0 (2'-<u>C</u>H), 70.1 (3'-<u>C</u>H), 65.1 (5'-<u>C</u>H$_2$),

47.3 ($\underline{C}H_2$ TEA), 8.9 ($\underline{C}H_3$ TEA); *m/z* (ESI) 469 ($[M + H]^+$, 100%); HRMS $C_{16}H_{18}N_6O_9P$, calcd 469.0873, found 469.0874.

8.118 LTB_4 synthesis inhibition assay (GVK Bio)

The assay was conducted by GVK Bio (GVK Biosciences, Private Limited, Plot No. 28A, IDA Nacharam, Hyderabad – 500076, India). Personnel involved: Dr P. Arumugam and Dr R. Issac.

Materials: LTB_4 parameter six pack (R&D biosystems, SKGE006B), RPMI 1640 cell culture medium (Sigma-Aldrich, R6504), Hank's Balanced Salt Solution HBSS (Invitrogen, 14025076), Dimethyl sulfoxide (Sigma-Aldrich, D2650), Recombinant human complement component C5a protein (R&D biosystems, 2037-C5-025), Foetal bovine serum (Gibco, 10270-106), Pencillin-Streptomycin (Gibco, 15140-122), Ficoll Paque PLUS (GE Healthcare, 17144002), Dextran solution (Sigma-Aldrich, D8802).

Protocol: Neutrophils were isolated from human peripheral blood by ficoll-paque density gradient centrifugation and dextran sedimentation. The freshly isolated neutrophils were seeded at 100,000 numbers/well in a 96-well plate. Neutrophils were dosed with reference compound at a top concentration of 300 nM and test compound at a top concentration of 100 μM with a 1:3 step down dilution for 10 points. Neutrophils were incubated at 37 ^oC in a CO_2 incubator for 1 h, then stimulated with 50 ng/mL of recombinant human complement component C5a (1 mg/mL stock). The neutrophils were incubated at 37 ^oC in a CO_2 incubator overnight. The cell supernatant was collected from each well and diluted down to a 1:10 dilution prior to conducting the ELISA. LTB_4 release from the neutrophils was assessed by using an LTB_4 parameter kit following the manufacturer's instructions.

8.119 In-house LTB_4 synthesis inhibition assay

The assay was carried out in collaboration with Dr Dean Willis at the UCL pharmacy department. The development of the MS method and analysis was carried out in collaboration with Dr Lisa D. Haigh in the UCL chemistry mass spectrometry facility.

Materials: LTB_4 was purchased from Sigma-Aldrich as a 100 μg/mL solution in ethanol, purity ≥ 97%. The stock solution of LTB_4 was prepared by dilution of the purchased LTB_4 sample to 10 μg/mL in methanol and was stored at –20 ^{o}C. The standard solutions required for the calibration curve (concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 μg/mL) were prepared from the stock LTB_4 solution through serial dilution with methanol.

LC-MS system: LC-MS analysis was performed on an Agilent 1100 HPLC system using a C8 column at room temperature, eluting with solvents A/B (A: 10 mM ammonium acetate in water, B: 10 mM ammonium acetate in acetonitrile). The flow rate was 200 μL/min and the gradient employed was: 0 min (A: 99%, B: 1%), 11 min (A: 1%, B: 99%), 11.1 min (A: 1%, B: 99%). The sample injection volume was 5 μL. The mass spectra were recorded on a Thermo Finnigan LTQ ion-trap mass spectrometer using negative mode electrospray ionisation. Selected ion monitoring (SIM) was used over the mass range *m*/*z* 334-337. For LTB_4 concentration quantification, an eight point calibration plot was used (LTB_4 quantification range 0.08-10 μg/mL) using the peak area at r_t = 10 min.

Protocol A (using mouse leukocytes): The cells (1000 cells/mL) in Dulbecco's modified Eagle's medium (0.5 mL) were treated with the test or reference inhibitor (10 mM in DMSO). After 10 min the cells were treated with calcium ionophore A23187 (5 μM) for 4 h. The cell supernatant was collected from each well and centrifuged, and then treated with dichloromethane (2.7 mL) and a solution of methanol containing 1% formic acid (0.3 mL). The top layer was removed. The organic layer was passed through a filter (syringe filters, Klarity^{TM}, PVDF, 13 mm, 0.2 μm), and concentrated *in vacuo*. The residue was reconstituted in 50 μL methanol for LC-MS analysis. Each experiment was carried out in triplicate. As a control, each experiment was repeated with either no stimulant (calcium ionophore) added, no inhibitor added, or both stimulant and inhibitor not added.

Protocol B (using human neutrophils HL60): Neutrophils (1000 cells/mL or 2000 cells/mL) in Dulbecco's modified Eagle's medium (1 mL) were treated with calcium ionophore A23187 (10 μM) for 10 min. The cell supernatant was collected from each well and centrifuged, and then treated with dichloromethane (4.5 mL) and a solution of methanol containing 1% formic acid (0.5 mL). The top layer was removed. The

organic layer was passed through a filter (syringe filters, Klarity^{TM}, PVDF, 13 mm, 0.2 µm), and concentrated *in vacuo*. The residue was reconstituted in 75 µL methanol for LC-MS analysis. Each experiment was carried out in triplicate. As a control, each experiment was repeated where no simulant (calcium ionophore) was added.

8.120 CRTh2 antagonistic activity assay (Euroscreen FAST)

The assay was conducted by Euroscreen (Euroscreen FAST Business Unit, Euroscreen SA, 47 Rue Adrienne Bolland, 6041 Gosselies, Belgium). Personnel involved: Jerome Bernard and Sophie Brogniet.

Materials: Assay buffer – 20 mM HEPES pH 7.4, 200 mM NaCl, 10 µg/mL saponin, $MgCl_2$ at optimised concentration for the specific receptor, 0.1% BSA. Membranes – CHO-K1 cells expressing recombinant human CRTh2 receptor, membrane extracts thawed on ice, diluted in assay buffer and kept on ice. GDP – diluted in assay buffer to give optimised concentration for the specific receptor. Beads – PVT-WGA (Amersham, RPNQ001) diluted in assay buffer at 50 mg/mL. GTPγ[^{35}S] – (PerkinElmer NEG030X) diluted in assay buffer to give 0.1 nM.

Protocol: For antagonist testing, membranes were mixed with GDP (volume:volume) and incubated for 15 min on ice. In parallel, GTPγ[^{35}S] was mixed with the beads (volume:volume) just before starting the reaction. The following reagents were successively added in the wells of an Optiplate (Perkin Elmer): 50 µL of test or reference ligand, 20 µL of the membranes:GDP mix, 10 µL of reference agonist (PGD_2) at historical EC_{50} (12 nM) and 20 µL of the GTPγ[^{35}S]:beads mix. The plates were covered with a top seal, mixed on an orbital shaker for 2 min, and then incubated for 1 h at room temperature. Then the plates were centrifuged for 10 min at 3000 rpm, incubated at room temperature for 1 h and counted for 1 min/well with a PerkinElmer TopCount reader.

8.121 High throughput SPOTi assay (Bhakta group, Birkbeck)[90]

The assays were conducted by Arundhati Maitra and Dr Parisa N. Mortazavi in the Bhakta group in the Mycobacterial Research Laboratory at Birkbeck, University of London.

M. aurum was grown in Middlebrook 7H9 broth supplemented with 0.02% (v/v) glycerol, 0.05% (v/v) tween-80 and 10% oleic acid, albumin, dextrose and catalase (OADC; BD Biosciences) as a shaking culture at 150 rpm at 35 °C. The antimycobacterial activities of the compounds were tested following the SPOTi.[90] The high throughput growth inhibition assay was conducted in a semi-automated 96 well plate format. Compounds dissolved in DMSO at a final concentration of 50 mg/mL were serially diluted and dispensed in a volume of 2 µL into each well of a 96 well plate to which 200 µL of Middlebrook 7H10 agar medium kept at 55 °C supplemented with 0.05% (v/v) glycerol and 10% (v/v) OADC was added. A well with no compounds (DMSO only) and isoniazid were used as experimental controls. To all the plates, a drop (2 µL) of mycobacterial culture containing 2 x 10^6 colony-forming units (CFUs) was spotted in the middle of each well and the plates were incubated at 37 °C for 5 days. The minimum inhibitory concentrations (MICs) were determined as the lowest concentrations of the compound investigated where mycobacterial growth was completely inhibited by the presence of the compound.

8.122 Eukaryotic cell cytotoxicity testing (Bhakta group, Birkbeck)

The assays were conducted by Arundhati Maitra in the Bhakta group in the Mycobacterial Research Laboratory at Birkbeck, University of London.

The assay was performed in 96-well cell culture flat-bottom plates in duplicate. 2 µL of the compounds, dissolved in DMSO to a concentration of 50 mg/L, were placed in wells (row - A) containing 198 µL of RPMI-1640 supplemented with 2 mM L-glutamine and 10% heat inactivated fetal bovine serum. 2-fold serial dilutions were made (rows - B to H/F) and DMSO only was used as a control. To each well, 100 µL of RAW 264.7 murine macrophage cells (5×10^5 cells/mL) was added. After 48 h of incubation, the monocytes were washed twice with 1 x PBS, and fresh RPMI-1640 complete medium was added. Plates were then treated with 30 µL of freshly prepared 0.01% resazurin

solution and incubated overnight at 37 °C. A change in colour from blue to pink denoted the viable cell activity. Visual colour changes were recorded and the fluorescence intensity was measured (Fluorstar Omega, λ_{ex} 560 nm, λ_{em} 590 nm). The growth inhibitory concentration (GIC) is determined as the concentration at which > 90% inhibition of cell viability is observed.

8.123 Efflux pump inhibition assay (Bhakta group, Birkbeck)

The assays were conducted by Arundhati Maitra and Dr Parisa N. Mortazavi in the Bhakta group in the Mycobacterial Research Laboratory at Birkbeck, University of London.

The assay was modified from a previously published protocol.[158] *M. aurum* was grown until it reached early logarithmic stage of growth (OD_{600} ~ 0.8). The OD_{600} was then adjusted to 0.4 by diluting the cells with culture medium. The suspension was centrifuged at 3000 rpm for 10 min, the supernatant was discarded and the pellet was resuspended in 10 mL of sterile 1 x PBS. The test samples contained: 4-6 x 10^7 bacteria/mL in PBS, 0.4% glucose (as a source of energy for efflux pumps activity), 0.5 mg/L ethidium bromide (as a substrate for efflux pumps) and the compounds being tested at sub-MIC concentrations. Blank samples contained all of the components mentioned above except the bacterial suspension, which was replaced with 1 x PBS. The experiment was performed in a 96-well plate (Corning) which was placed in a fluorimeter (FLUOstar OPTIMA, BMG Labtech) and the instrument was programmed with the following parameters: wavelengths of 544 nm and 590 nm for excitation and detection of fluorescence, gain 2200, a temperature of 37 °C, and a cycle of measurement every min for a total period of 60 min. The accumulation or efflux of ethidium bromide was monitored for the mentioned period on a real-time basis.

8.124 Synergism assay (Bhakta group, Birkbeck)

The assays were conducted by Arundhati Maitra in the Bhakta group in the Mycobacterial Research Laboratory at Birkbeck, University of London.

The synergistic effect of the THIQs was examined in combination with isoniazid, rifampicin and ethambutol against *M aurum*. The assay was conducted in a 96-well microtitre plate using a SPOTi checkerboard distribution. The compounds were serially diluted in DMSO from sub-MIC concentrations and lower. Each column of the 48-well plate contained different concentrations of the test compound and each row contained different concentrations of the first-line drug. Row 12 and column G contained drugs and test compounds only as controls. A row with only DMSO served as an additional control. The checkerboard was constructed by adding 1 µL of each of the stock concentrations to the corresponding well and then dispensing 200 µL of warm (55 °C) Middlebrook 7H10 agar medium supplemented with 10% (v/v) OADC. The plates were then spotted with *M. aurum*. The plates were incubated at 35 °C for 5 days.

The fractional inhibitory concentration (FIC) for each compound was calculated using the following formula[217]: $FIC_{TB\text{-}drug}$ = [MIC of TB-drug in the combination with THIQ]/[MIC of TB-drug alone] and similarly, FIC_{THIQ} = [MIC of TB-drug in the combination with THIQ]/[MIC of THIQ alone]. The fractional inhibitory concentration index (FICI) was calculated using the following formula: $FICI = FIC_{TB\text{-}drug} + FIC_{THIQ}$. FICI ≤ 0.5 indicated synergism, FICI ≥ 4 indicated antagonism, and values in between corresponded to additivity or no interaction.[218]

8.125 Pharmacokinetic and Pharmacodynamic assays (Pharmidex)

The assays were contracted to and performed at Pharmidex (Pharmidex Pharmaceuticals Ltd, 3rd Floor, 14 Hanover Street, Mayfair, London, W1S 1YH). Personnel involved: Dr M. Chishty and Dr M. Yaqoob.

Aqueous kinetic solubility assay: Aqueous kinetic solubility was measured at a concentration of 1 and 0.1 mg/ml. Each experiment was carried out in triplicate, and pimozide (low aqueous solubility control) and propranolol (high aqueous solubility control) were used as reference standards. The compounds were equilibrated in 5% DMSO in phosphate buffered saline (pH 7.4) at 21°C for 24 h. The samples were centrifuged at 15000 g for 10 min. A 100 µL sample of the supernatant was carefully collected and the soluble amount of compound quantified using LC-MS/MS. A standard curve for each compound was prepared in 100% acetonitrile. The solubility

results were reported as low (less than 0.1 mg/mL), medium (0.1 – 0.5 mg/mL) and high (greater than 0.5 mg/mL).

Human microsome stability assay: Stock solutions of the test compounds were prepared in DMSO (10 mM). The stock solutions were added to PBS to give an incubation concentration of 1 µM. Microsomes (0.5 mg/mL) and NADPH were added to the solutions (total incubation volume was 100 µL) and the reactions was incubated at 37 ^{o}C for up to 60 min. Acetonitrile (300 µL) with internal standard was added to stop the incubation. The samples were centrifuged and the supernatant was analysed by HPLC-MS/MS to detect the parent compound.

Human hepatocyte stability assay: Stock solutions of the test compounds were prepared in DMSO (10 mM). The stock solutions were added to PBS so that final incubation concentration was 1 mM. Hepatocytes (70,000 per well) were added to the solution (total incubation volume was 100 µL) and the reaction was incubated at 37 ^{o}C for up to 120 min. Acetonitrile (300 µL) with internal standard was added to stop the incubation. The samples were centrifuged and the supernatant was analysed by HPLC-MS/MS to detect the parent compound.

CYP450 inhibition assay: The compounds originally dissolved in DMSO were added to PBS to give a final incubation concentration of 10 mM. To the microtitre plate was added the CYP baculosomes containing cDNA for a single human P450 isozyme, followed by the fluorogenic substrate and NADPH. The reaction was incubated at 37 ^{o}C for at least 60 min. Fluorogenic substrates are metabolised by CYP enzymes into products that are highly fluorescent in aqueous solution. The inhibition of CYP prevents this metabolism, which corresponds to a decrease in the fluorescence signal, measured on a plate reader (Instrument: Tecan Safire2).

MDR1-MDCK permeability and efflux assay: The compounds were dissolved in DMSO to provide stock solutions (10 mM) from which donor (dose) solutions were prepared in DMEM to give a final drug concentration of 10 µM. All dose solutions contained propranolol (10 µM) as an internal standard. The Maldin Darby Canine Kidney (MDCK) cells carrying the human multidrug resistant-1 gene (MDR1) were used to seed filters that were exposed to a fixed volume of the donor solution containing the compound of interest. The compounds ability to traverse the monolayer and appear in the receiver compartment was measured over 30 min. Bidirectional permeability

measurements were derived by examining the transfer of compound in both the apical to basolateral compartment and vice versa. Sample analysis was conducted using LC-MS/MS with the detection settings optimised for each test compound. The apparent permeability results (P_{app}) were reported as low ($P_{app} < 2 \times 10^{-6}$ cm/s, e.g. sucrose, mannitol, atenolol), medium (2×10^{-6} cm/s $< P_{app} < 20 \times 10^{-6}$ cm/s) and high ($P_{app} > 20 \times 10^{-6}$ cm/s, e.g. propranolol, diazepam). Compounds with efflux ratios above 1.5 were classified as Pgp substrates.

8.126 Panlabs hit profiling screen

The hit profiling screen was carried out by Eurofins Panlabs (Eurofins Panlabs Taiwan Ltd, Pharmacology Laboratories, 158 Li-The Road, Peitou, Taipei, Taiwan 112, Taiwan R.O.C.).

The hit profiling screen included 35 assays. Compounds were tested at a single concentration of 10 μM in duplicate. The assays included: CYP450 1A2 (human), CYP450 2C19 (human), CYP450 2C9 (human), CYP450 2D6 (human), CYP450 3A4 (human), Adenosine A_1 (human), Adenosine A_{2A} (human), Adrenergic α_{1A} (rat), Adrenergic α_{1B} (rat), Adrenergic α_{2A} (human), Adrenergic β_1 (human), Adrenergic β_2 (human), Calcium Channel L-Type Dihydropyridine (rat), Cannabinoid CB_1 (human), Dopamine D_1 (human), Dopamine D_{2S} (human), GABAA Flunitrazepam Central (rat), GABAA Muscimol Central (rat), Glutamate NMDA Phencyclidine (rat), Histamine H_1 (human), Imidazoline I_2 Central (rat), Muscarinic M_2 (human), Muscarinic M_3 (human), Nicotinic Acetylcholine (human), Nicotinic Acetylcholine α_1 Bungarotoxin (human), Opiate μ OP3, MOP (human), Phorbol Ester (mouse), Potassium Channel [KATP] (hamster), Potassium Channel hERG (human), Prostanoid EP_4 (human), Rolipram (rat), Serotonin 5-Hydroxytryptamine 5-HT_{2B} (human), Sigma σ_1 (human), Sodium Channel Site 2 (rat), Transporter Norepinephrine NET (human).

9. Appendix

9.1 Efflux pump inhibition assay results

Compound	MIC (μg/mL)	Concentration used for efflux assay (μg/mL)	Efflux value
141a	>500	125	0
142a	250	62.5	2
141b	500	125	0
142b	125	31.3	2
141c	125	31.3	1
142c	250 (some growth inhibition seen at 125)	31.3	3
141d	>500	125	0
142d	250	62.5	2
141e	500 (some growth inhibition seen at 250)	62.5	0
142e	250	62.5	4
141f	500 (some growth inhibition seen at 250)	62.5	0
142f	250	62.5	3
145a	>500	125	0
146a	250	62.5	2
145b	>500	125	0
146b	250	62.5	2
145c	250	62.5	1
146c	250	62.5	3
145d	250	62.5	1
146d	500	125	0
162	62.5	15.6	2
160	500	125	1
161	250	62.5	0
166	500	125	4
163	250	62.5	4
165	125	31.3	0
164	250	62.5	0
142b HCl	125	31.3	0

Table 9.1. MIC and concentrations of THIQs used in the efflux pump assay. The efflux value refers to the effect of the THIQs on the accumulation of ethidium bromide in *M. aurum*, where 4 = accumulation of ethidium bromide the same as that of known efflux pump inhibitor verapamil and 0 = no accumulation of ethidium bromide above that of normal accumulation levels when no inhibitor present. Verapamil and chlorpromazine were used as positive controls at concentrations 125 μg/mL and 10 μg/mL respectively.

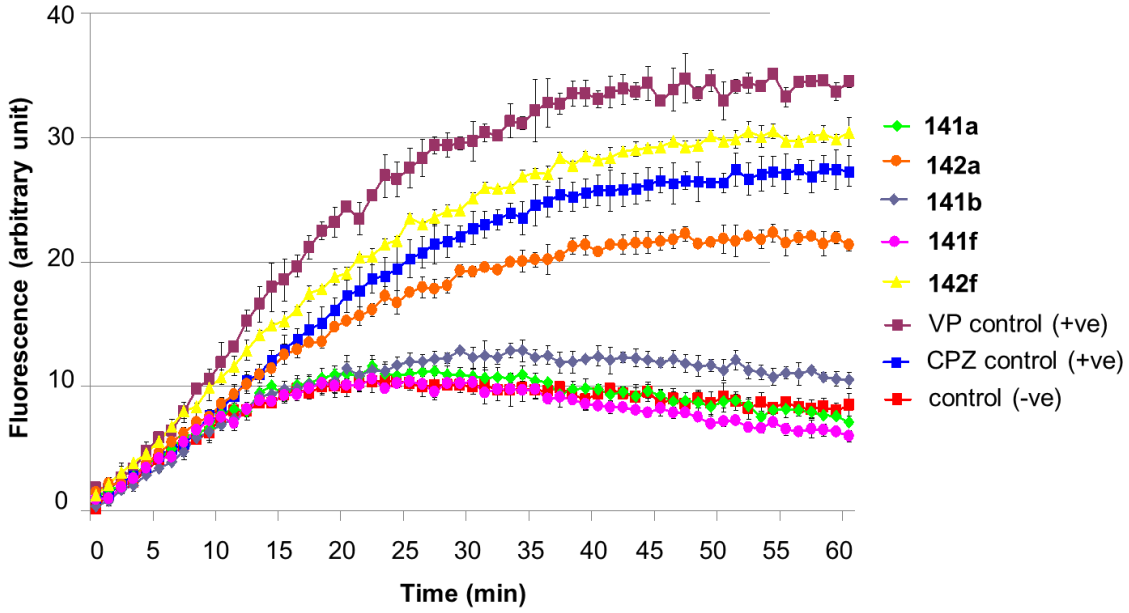


Figure 9.1. The effect of first generation THIQs on the accumulation of ethidium bromide in *M. aurum*.

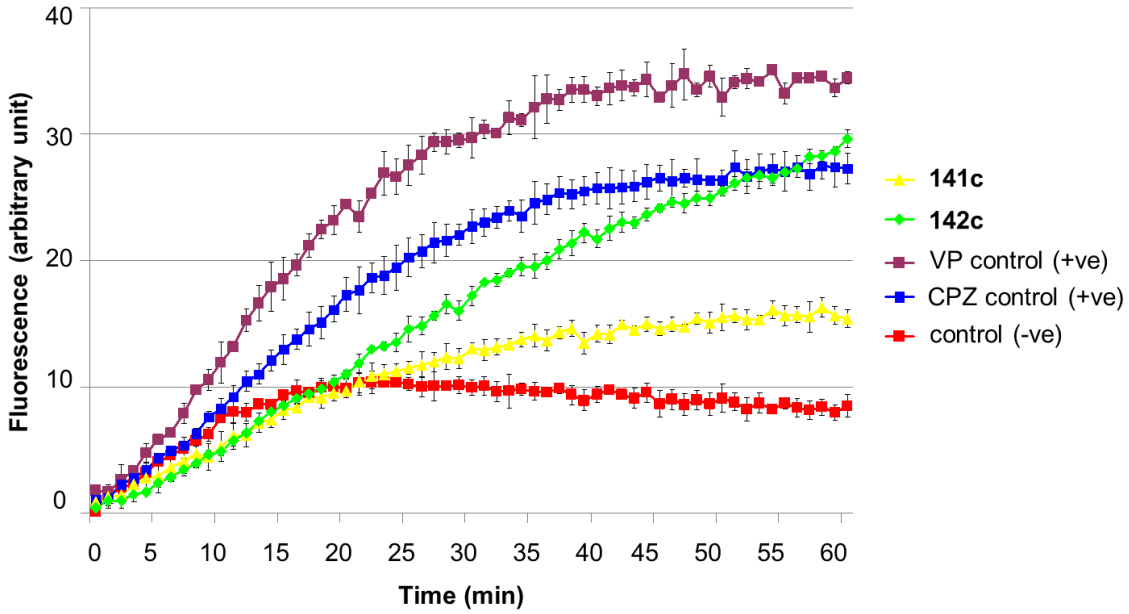


Figure 9.2. The effect of first generation THIQs on the accumulation of ethidium bromide in *M. aurum*.

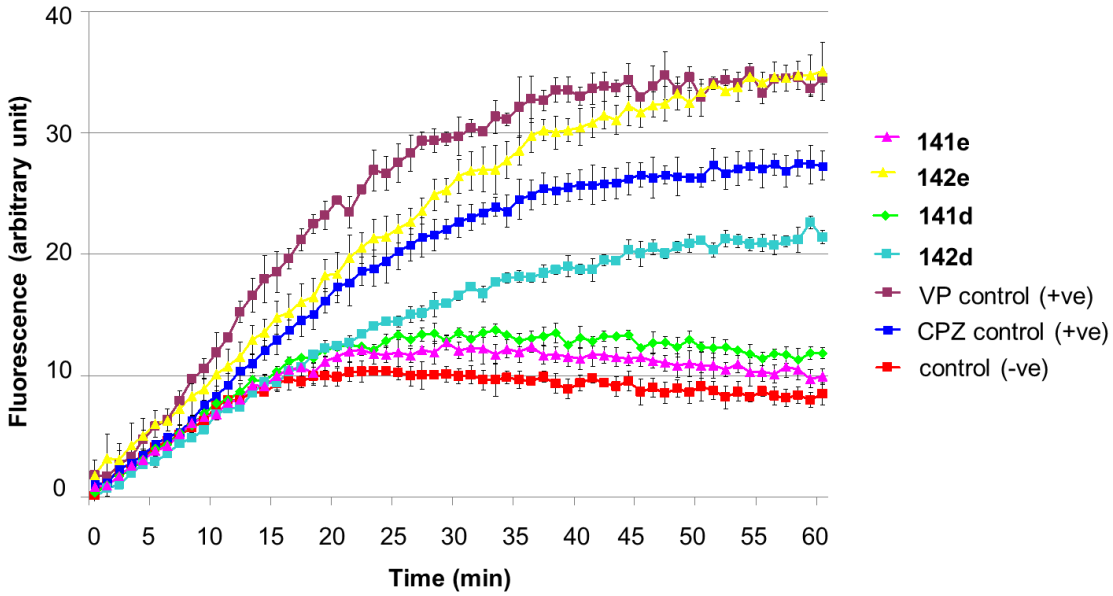


Figure 9.3. The effect of first generation THIQs on the accumulation of ethidium bromide in *M. aurum*.

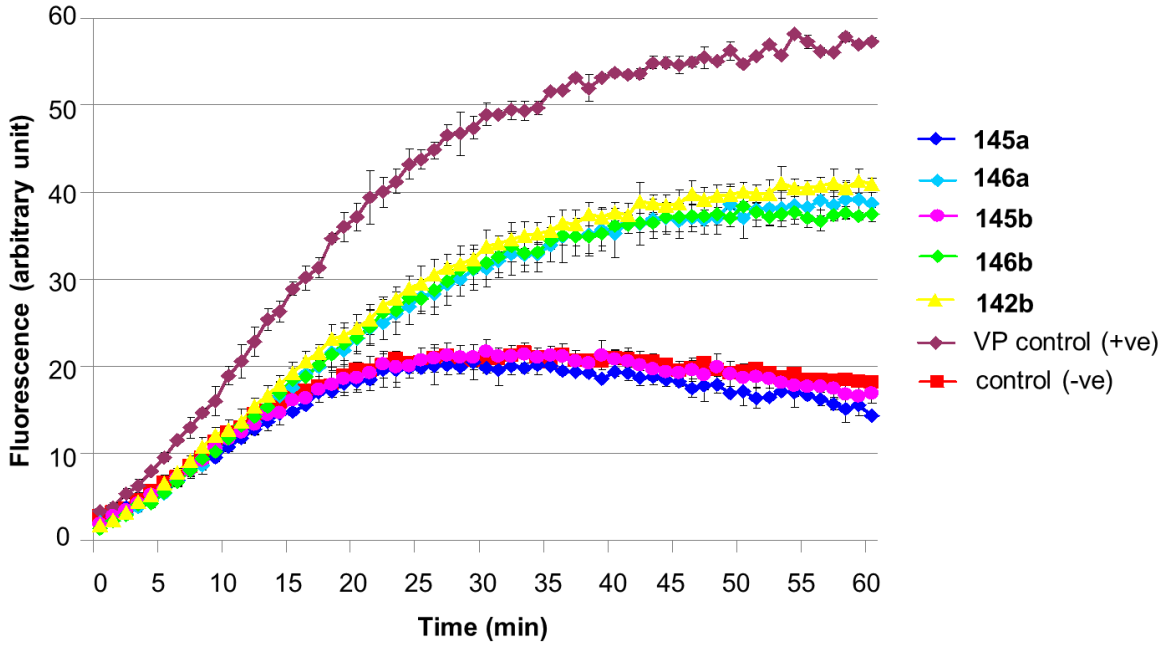


Figure 9.4. The effect of first and second generation THIQs on the accumulation of ethidium bromide in *M. aurum*.

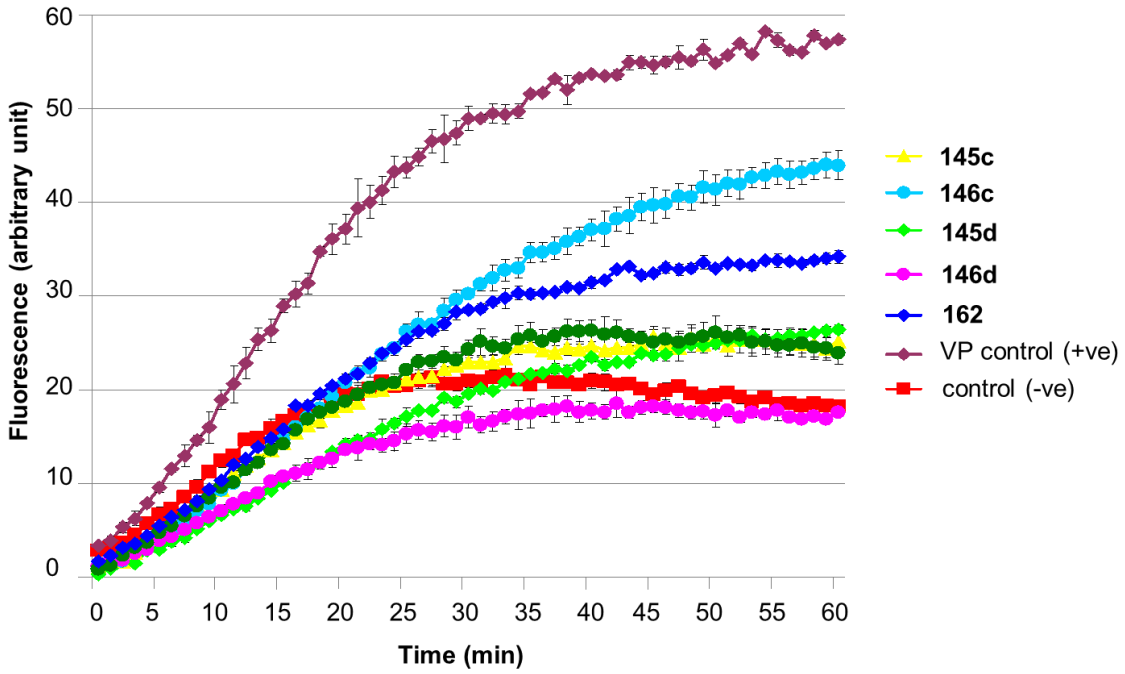


Figure 9.5. The effect of second and third generation THIQs on the accumulation of ethidium bromide in *M. aurum*.

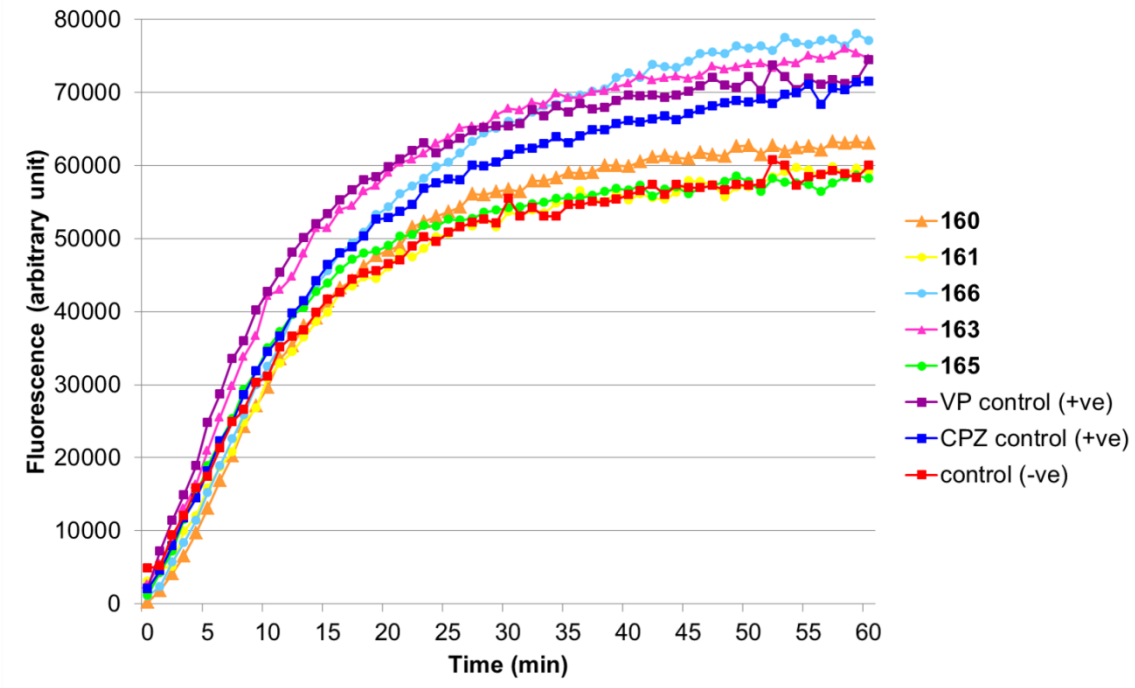


Figure 9.6. The effect of third generation THIQs on the accumulation of ethidium bromide in *M. aurum*.

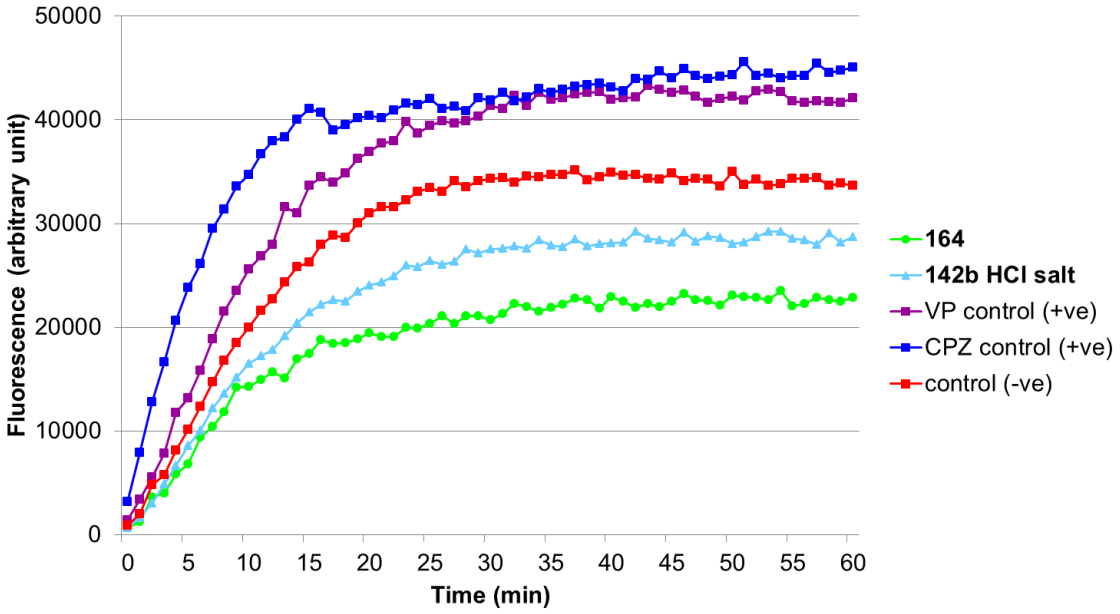


Figure 9.7. The effect of third generation THIQs on the accumulation of ethidium bromide in *M. aurum*.

9.2 Publications

1. J. D. Guzman, T. Pesnot, D. A. Barrera, H. M. Davies, E. McMahon, D. Evangelopoulos, P. N. Mortazavi, T. Munshi, A. Maitra, E. D. Lamming, R. Angell, M. C. Gershater, J. M. Redmond, D. Needham, J. M. Ward, L. E. Cuca, H. C. Hailes and S. Bhakta, *J. Antimicrob. Chemother*., 2015, **70**, 1691-1703.

2. B. R. Lichman, E. D. Lamming, T. Pesnot, J. M. Smith, H. C. Hailes and J. M. Ward, *Green Chem*., 2015, **17**, 852-855.

3. B. R. Lichman, M. C. Gershater, E. D. Lamming, T. Pesnot, A. Sula, N. H. Keep, H. C. Hailes and J. M. Ward, *FEBS J*., 2015, **282**, 1137-1151.

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