University of Southampton

Faculty of Engineering and Physical Sciences

Chemistry

The Synthesis of Bioactive Fluorinated Bile Acid Analogues

By

David Evans

Thesis for the degree of Doctor of Philosophy

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Abstract

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Bile acids are naturally occurring steroidal compounds found within the bile of humans and other mammals. They have key physiological functions including the absorption of dietary lipids and hydrophobic drug molecules and they also are responsible for the regulation of cholesterol levels.

Bile acids have recently emerged also as signalling molecules and are agonists at the FXR nuclear receptor and TGR5 membrane-bound receptor, both of which are found in a multitude of human tissue types. Because of this, natural and semi-synthetic bile acids have steadily been gaining traction over recent decades as drug candidates. Pharmaceutical companies are investigating selective bile acid receptor agonists for a broad range of diseases including cancer, Parkinson's disease, diabetes, metabolic disorder and liver disease, with some progressing well through clinical trials.

Fluorine is widely used during drug discovery to selectively modify a myriad of physiochemical properties including acidity, lipophilicity and hydrogen bond donating capacity. Therefore, the introduction of fluorine to potential bile acid drug candidates is of great interest. This thesis describes the synthesis of a number of fluorinated bile acid analogues, with a particular focus on targeting non-alcoholic steatohepatitis (NASH), a prominent condition underlying liver disease. A brief summary of preliminary biological data is also presented.

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Research Thesis: Declaration of Authorship

Print name: DAVID EVANS

Title of thesis: THE SYNTHESIS OF BIOACTIVE FLUORIANTED BILE ACID ANALOGUES

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

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2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
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Definitions and Abbreviations

AD	Alzheimer's disease
AIBN	Azobisisobutyronitrile
ATP	Adenosine triphosphate
BA	Bile acid
BBB	Blood-brain barrier
BDE	Bond dissociation enthalpy
BP	Byproduct
BPO	Dibenzoyl peroxide
BSEP	Bile salt export pump
CA	Cholic acid
CAM	Cerium ammonium molybdate
cAMP	Cyclic adenosine monophosphate
CDCA	Chenodeoxycholic acid
CDI	1,1'-dicarbonyldiimidazole
COSY	Correlation spectroscopy
CSDS	Cambridge Structural Database System
d	Doublet
DAST	Dimethylaminosulfurtrifluoride
DBU	1,8-Diazabiyclo(5.4.0)undec-7-ene
DCA	Deoxycholic acid
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DIAD	Diisopropyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethylsulfoxide
DMPU	1,3-Dimethyl-2-oxahexahydropyrimidine
DPPA	Diphenylphosphoryl azide

EA	Ethyl acetate
EC	Effective concentration
ESI	Electrospray ionisation
FTIR	Fourier-transform infrared
FXR	Farnesoid X receptor
Gen.	Generation
GPCR	G protein-coupled receptor
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HDCA	Hyodeoxycholic acid
HIS	Histidine
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single-quantum correlation spectroscopy
IMHB	Intramolecular hydrogen bond
IMDP	Intramolecular deprotonation
IR	Infrared
IUPAC	International Union of Pure and Applied Chemists
LCA	Lithocholic acid
LDA	Lithium diisopropyl amine
LDL	Low density lipoprotein
LRMS	Low resolution mass spectrometry
Ltd.	Limited (context: private limited company)
m	Multiplet
*m*CPBA	*meta*-chloro perbenzoic acid
MDR	Multi-drug resistant
m.p.	Melting point
MS	Mass spectrometry
NASH	Non-alcoholic steatohepatitis
NBS	*N*-bromosuccinimide

NCS	*N*-chlorosuccinimide
NFOBS	*N*-fluorobenzenesulfonamide
NFSI	*N*-fluorobenzene
NMP	*N*-methylpyrolidinone
NMR	Nuclear magnetic resonance
OCA	Obeticholic acid
O/N	Overnight
PBC	Primary biliary cirrhosis
PCC	Pyridinium chlorochromate
PD	Parkinson's Disease
PE	petroleum ether
ppm	Parts per million
*p*TSA	*para*-toluenesulfonic acid
PXR	Pregnane X receptor
q	Quartet
R_f	Retention factor
ROS	Reactive oxygen species
rt	Room temperature
s	Singlet
SER	Smooth endoplasmic reticulum
SET	Single-electron transfer
t	Triplet
TBAF	tetra-*N*-butylammonium fluoride
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TGR5	Takedea G protein-coupled
THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TLC	Thin layer chromatography
TMSCl	Chlorotrimethylsilane
TMS	Trimethylsilyl
TRP	Tryptophan

TUDCA	Tauroursodeoxycholic acid
UDCA	Ursodeoxycholic acid
UV	Ultraviolet
VDW	Van der Waals
WRT	With respect to

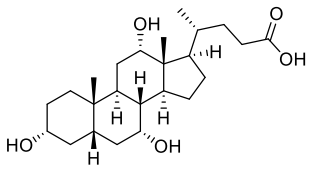
Chapter 1 Introduction

1.1 General introduction to bile acids

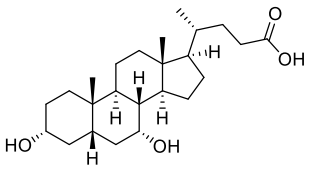
1.1.1 Endogenous bile acids and their biosynthesis

Bile acids (BAs) are a class of acidic steroid and a product of cholesterol metabolism.[1 2] They are classified as either primary, secondary or tertiary, based on their biosynthetic origin. Bile acids have key functions such as the absorption and digestion of orally ingested fats, lipophilic vitamins, drugs and steroids from the small intestine.[3] They also signal for the excretion of cholesterol, thereby importantly regulating its concentration within the body.[4] Typically, 90-95% of bile acids are found in the body as their anionic sodium salts from conjugation to either taurine or glycine to provide a greater aqueous solubility across the physiological pH range.[2, 5] These conjugates are then released into the duodenum of the gastrointestinal tract through the bile ducts as they are unable to pass through the epithelium of the small intestine.[2]

Primary BAs in humans are produced exclusively by the liver and are cholic acid (CA) (**1.1**) and chenodeoxycholic acid (CDCA) (**1.2**) (*Figure 1.1*) These endogenous primary BAs are the main components of human bile and originate from cholesterol.



1.1 Cholic acid



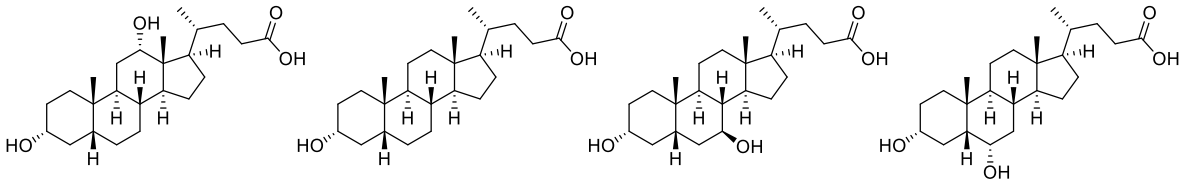
1.2 Chenodeoxycholic acid

*Figure 1.1. Primary bile acids cholic acid (**1.1**) and chenodeoxycholic acid (**1.2**).*

Almost 95% of primary bile acids synthesised are reabsorbed into the ileum and colon and actively transported back to the liver.[6] Known as enterohepatic circulation, this recycling mechanism is key in the homeostasis of cholesterol.[7]

Secondary and tertiary bile acids are biosynthetic products of their primary counterparts and are a result of enzymatic transformations at gut flora in the ileum.[5] Shown in *Figure 1.2* are deoxycholic acid (DCA) (**1.3**), lithocholic acid (LCA) (**1.4**), ursodeoxycholic acid (UDCA) (**1.5**) and hyodeoxycholic acid (HDCA) (**1.6**).[8 9] UDCA is present in the bile acid pool at around 1-3% and is formed by the epimerisation of a hydroxyl group of CDCA.[5, 10] Deoxygenation of the same hydroxyl motif of CDCA yields LCA and this BA is present in trace amounts, but, is notably the most toxic of all endogenous bile acids, causing liver damage at high concentrations.[11, 12] Applying the same deoxygenation

reaction to cholic acid yields DCA. HDCA is a primary bile acid in pigs but is biosynthesised in humans by the hydroxylation of LCA.[8] The toxicity of LCA and other bile acids is attenuated by their presence as the corresponding sodium salts of glycine and taurine conjugates where they are typically bound tightly to intracellular or plasma proteins.[5, 10]



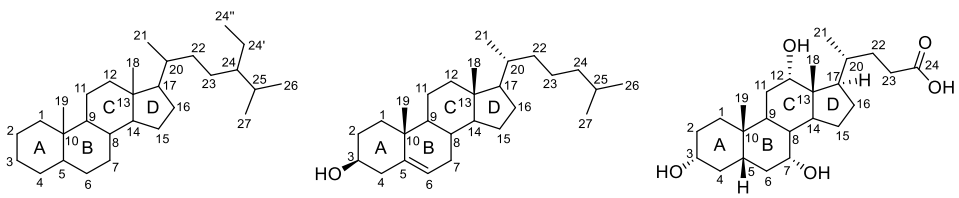
1.3 Deoxycholic acid **1.4** Lithocholic acid **1.5** Ursodeoxycholic acid **1.6** Hyodeoxycholic acid

*Figure 1.2. Secondary and tertiary bile acids DCA (**1.3**), LCA (**1.4**), UDCA (**1.5**) and HDCA (**1.6**).*

Commercially, primary bile acids and the secondary bile acids DCA and LCA are sourced from bovine bile, the only current economically viable source of bile acids. By contrast, UDCA is unsustainably available only from the bile of bears. Several syntheses, some exclusively chemical and some merging the merits of chemical and biological approaches, have been described to produce UDCA from cholic acid.[13] Recent literature also exists to describe the synthesis of bile acids from non-animal sources.[14, 15]

1.1.2 Steroid numbering

The IUPAC recommendations for systematic steroid nomenclature will be used herein.[16] *Figure 1.3* shows cholesterol **1.7** and cholic acid **1.1** and ring assignment. Conventionally, the A-ring of the steroid is positioned to the lower left corner. Bile acids exhibit a *cis*-A,B ring juncture that is essential to the chemical behaviour and biological functions of all bile acids, leading to the characteristic three-dimensional structure (see: *Section 1.1.3*). The stereochemistry of the C-H bonds at carbons 8, 9 and 14 (as shown in Figures 1.1 and 1.2) will be implied throughout the remainder of this thesis but omitted for concision.

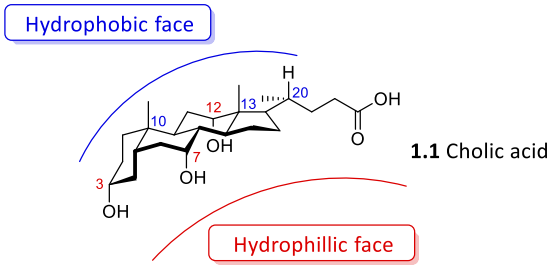


General steroid **1.7** Cholesterol **1.1** Cholic acid

Figure 1.3. Steroid numbering according to IUPAC recommendations.

1.1.3 Bile acid amphipathic structure

There is a clear difference in the hydrophobicity of the bile acid faces. Apolar and β-methyl residues at carbons ten, thirteen and twenty give the β-face of the steroid a distinctly hydrophobic character while the polar and α-alcohol groups at carbons three, seven (and, in primary bile acids, carbon twelve) give the α-face a hydrophilic character (*Figure 1.4*). The resultant polarity gradient across the steroid backbone classifies BAs as 'amphipathic' and explains their role as biological detergents; the formation of mixed micelles with dietary fats is synonymous with bile acid behaviour.[1, 5]



1.1 Cholic acid

*Figure 1.4. Cholic acid (**1.1**) shown in three-dimensions.*

1.2 Bile acids as therapeutic targets

1.2.1 Historical uses of bile acids as therapeutics

1.2.1.1 UDCA in Chinese Medicine

Traditional Chinese medicines have used ursodeoxycholic for centuries in the treatment of biliary stone disease.[17] The nomenclature of UDCA derives from the term Ursidae, meaning bears, where UDCA is uniquely found as a primary bile acid.[18] In the early 1900's the structure of UDCA was first determined, yet it took almost another 80 years for UDCA to become recognised as a clinical therapeutic in the western world.[19]

1.2.1.2 UDCA in the treatment of primary biliary cirrhosis

Death by liver failure is likely in the event of untreated primary biliary cirrhosis (PBC), an autoimmune disease characterised by the destruction of bile ducts leading to liver cirrhosis.[20] For a number of years UDCA has been used to treat PBC and is the only known treatment. The therapeutic mechanism is described to be the displacement of the hepatotoxic bile acids DCA and LCA by UDCA from the bile acid pool, during extensive enterohepatic circulation.[21, 22] Despite several and ongoing questions as to the efficacy of UDCA in the treatment of PBC, extensive data has shown a general decrease in mortality upon administration.[23]

1.2.1.3 Sequestering of bile acids

Bile acid sequestrants prevent the reabsorption of BAs into the gut lumen. They are positively charged polymers, orally absorbed and bind to bile acids and their corresponding bile salts in the gastrointestinal tract, ultimately resulting in their excretion.[24] With BAs thereby deficient in the cycle of enterohepatic circulation, their synthesis is then promoted, giving a direct reduction in cholesterol levels (for the biosynthetic pathway from cholesterol, see: *Section 1.1.1)*. In males with hypercholesteremia and evaluated over three decades, an overall 20% reduction in LDL-cholesterol was described giving a 19% reduction in the relative risk of myocardial infarction. Following this prolonged success, a second generation of bile acid sequestrant emerged to market in 2008 (*Figure 1.5*).[24]

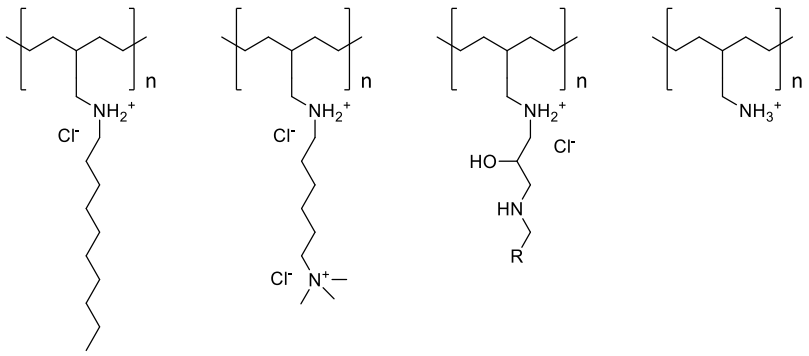


Figure 1.5. Key components of second-gen bile sequestrant Colesevelam hydrochloride.[24]

1.2.2 Introduction to non-alcohol steatohepatitis (NASH)

Non-alcoholic steatohepatitis (NASH) is a disease of rapidly increasingly prevalence and is characterised by hepatocellular injury and lobular inflammation.[25] Often accompanied by liver steatosis (lipid accumulation), NASH is a condition that stems from non-alcoholic fatty liver disease (NAFLD), arguably the contemporary indicator for liver transplantation and a hepatic manifestation of metabolic syndrome.[26] NAFLD is one of the major diseases underlying all chronic liver disease cases worldwide and affects almost 30% of the UK population and similar figures are found for other developed countries globally.[27] Of concern is that NAFLD cases are mirroring the increasing trends in obesity and diagnoses of type 2 diabetes and so contribute to a proliferating public health burden. Furthermore, those suffering NASH are inherently predisposed to hepatocellular carcinoma (HCC), liver fibrosis, cirrhosis and cardiovascular disease.[28]

The development of NASH, stemming from liver steatosis, involves a pathological mechanism that is yet to be fully understood. The current consensus describes cytokine-induced cellular damage deriving from several factors including oxidative stress, endoplasmic reticulum stress and lipotoxicity, the consequences of which are further exacerbated by innate immune defence

mechanisms.[29] Another significant factor is hepatic lipogenesis and is caused by carbohydrate rich diets and/or hyperinsulinemia (insulin resistance). Lipotoxicity (in the context of the liver) occurs from the build-up of free fatty acids (FFAs) that are stored as triglycerides and the associated triglyceride-derived metabolites are what prove hepatically noxious. Additionally, such an accumulation of fat within the liver prompts counteractive oxidative mechanisms involving mitochondria and peroxisomes. Alas, these organelles eventually succumb to failure and invariably provide an excess of reactive oxygen species (ROS).

The accumulation of ROS and FFAs trigger a further harmful cascade of inflammation and apoptosis that actively sustains the progression of NASH.[30] NASH progression has also been linked to inflammasome-mediated microbial imbalance owing to an altered gut microbiota and heightened gut permeability. Altogether, it is clear that this cocktail of noxious hepatic events reflects NASH as a complex multifactorial disease. Moreover, a multitude of potential avenues by which to target NASH, are prevalent.[25]

To-date, there are no effective therapies on the market for the prevention or treatment of NASH. Typically, healthcare professionals will recommend strict lifestyle changes for weight loss and to inherently overcome hyperinsulinemia, but these require a compliant will-power from patients and, typically, dietary changes soon regress.[31] A pharmacological solution to target NASH could involve one or more nuclear hormone receptors of the subfamily NR1, such as the Farnesoid X Receptor (FXR), that are found in several of the aforementioned mechanisms that relate to the aetiology of the disease.

1.2.3 Bile acids as nuclear receptor agonists (FXR)

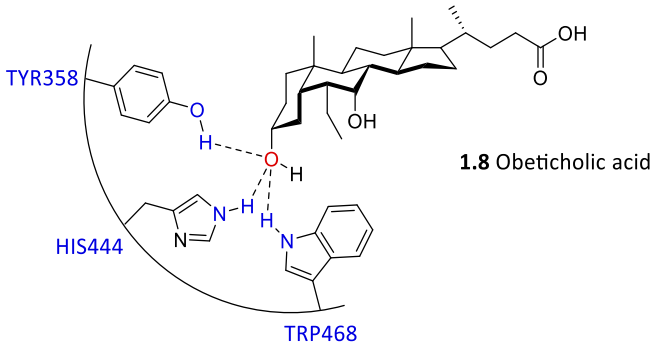
In addition to their synonymous detergent properties (see: *Section 1.1.3*), seminal work by Makishima and Parks in 1999 described BAs to play vital roles for nuclear receptors such as the FXR.[32, 33] Other nuclear receptors whereby bile acids act as physiologically active ligands include the pregnane X receptor (PXR) and vitamin D receptor,[11, 32] however these will not be discussed further in the context of this introduction.

1.2.3.1 Introduction to the FXR

The Farnesoid X receptor is an orphan nuclear receptor expressed abundantly in the liver, kidneys and intestines. The FXR acts as a ligand-activated transcription factor, as is true for all nuclear receptors, that regulates vital cellular machinery responsible for transcription. The FXR sits inactive until the binding of a ligand induces conformational change in chromatin to provide access of RNA polymerase and transcription factors to their promotors; thus, ligand binding to FXR initiates

transcription.[34] Ultimately, FXR-initiated transcription leads to the suppression of CYP7A1 in the smooth endoplasmic reticulum (SER) of hepatocytes and this cytochromic enzyme (see: *Section 1.1.1*), is responsible for the 7α-hydroxylation of cholesterol in the first, and rate limiting, step in the biosynthesis of primary bile acids.[32, 35-38] Additionally, activation of FXR by bile acids reduces the hepatic BA concentration further by initiating the synthesis of bile acid conjugation enzymes and upregulation of the bile salt export pump (BSEP).[39, 40] Therefore, FXR activation is a key handle in the homeostasis of primary BAs and, so, cholesterol. The activation of FXR also initiates the clearance of FFAs in the liver and peripheral tissues through several mechanisms including the upregulation of peroxisome proliferator-activated receptor alpha (PPARα) for FFA catabolism by mitochondrial β-oxidation.[41]

The structural basis for the binding of bile acids to the Farnesoid X receptor was described by Mi in 2003 using 6α-ethyl CDCA (obeticholic acid, OCA) (**1.8**) as a model substrate (*Figure 1.6*).[42] The findings were presented from X-ray crystallography data and the 3α-hydroxyl group of OCA was found to behave as a hydrogen bond acceptor with three HBD receptor residues, namely tyrosine-358, histidine-444 and tryptophan-468 through hydrogen bond formation and also electrostatic interactions. The shape of the binding pocket of FXR also possesses discriminatory power and permits only steroids with a *cis*-A,B ring juncture to enter; other classes of steroids such as oestrogens and progesterones are excluded.



1.8 Obeticholic acid

*Figure 1.6. Interaction of the FXR with obeticholic acid (**1.8**) at the 3α-OH group.*[42]

Clinical studies involving the semi-synthetic BA OCA (**1.8**) have described improved insulin sensitivity in NASH diagnosed obese-animal models and in human patients.[43, 44]

Activation of the FXR is possible by both 'free' bile acids (with a carboxylic acid at C_{24}) and their anionic conjugates to taurine and glycine. Lithocholic acid is the most potent activator of the FXR, closely followed by CDCA (both at micromolar concentrations) while cholic acid and UDCA were found to not activate the FXR.[5, 32, 33] Further quantitative analyses of FXR activation by BAs can be found in *Section 1.2.6*.

1.2.3.2 Targeting the FXR for the treatment of cancer

Since the pioneering work by Makishima and Parks before the turn of the century, the FXR has since been discovered populous within tumorous breast tissue and has been identified in both the cysts and plasma of postmenopausal individuals suffering with breast cancer.[45] Although still within the early stages of mechanistic elucidation, it was proven that the delivery of FXR agonists to cancerous breast cell lines prohibited tumour growth; similar studies concerning cancers in the liver and colon have described the same results.[45, 46] FXR agonism has also been found to upregulate tumour suppressor genes, further prohibiting tumour growth and further metastasis in mouse-liver models.[47, 48] These early research findings highlight the extraordinary potential of the FXR as an oncological therapeutic handle.

1.2.4 Bile acids as membrane bound receptor agonists (TGR5)

Takeda G protein-coupled receptor 5 (TGR5, also known as GPBAR1, BG-37, and M-BAR) is a G protein-coupled receptor activated directly by BAs.[49] This membrane-bound receptor is expressed in a plethora of tissues including the colon, gall bladder, ileum, liver, muscle and brown adipose tissue and its activation has been linked to the intracellular accumulation of cyclic adenosine monophosphate (cAMP).[50] This response has been shown to illicit an immunosuppressive effect in several cell types relevant to inflammation, such as monocytes and macrophages; the concentration of a range of pro-inflammatory cytokines that have a role in chronic inflammatory diseases, such as interleukin-1α, IL-1β, IL-6 and tumour necrosis factor-α, were observed to decrease.[51] Additionally, agonism of the TGR5 receptor on the surface of Kupffer cells and sinusoidal endothelial liver cells has been discovered to activate endothelial nitric oxide synthase (NOS); NO is a critical signalling molecule known to act as a vasodilator and exhibit antiatherogenic behaviour.[52] Furthermore, the stimulation of TGR5 has been proven to enhance energy expenditure in adipocytes and myocytes by the cAMP-dependant induction of type 2 iodothyronine deiodinase (D2). These observations indicate that BA-mediated activation of TGR5 has a potential therapeutic application to diseases relating to the cardiovascular system, chronically inflamed tissues, metabolic disorders and diabetes.

The structural basis for the binding of bile acids to the TGR5 receptor has been an area of considerable research effort, especially through the work of Pellicciari and co-workers to build upon and support the earlier work of Mi at the FXR (see: *Section 1.2.3.1*1.2.3). [53] [50, 54] *In silico* studies coupled with mutagenesis experiments have pointed towards an important 3α-OH group HBA interaction between BAs and the TGR5 (*Figure 1.7*). Specifically, docking studies suggest that this hydrogen bonding interaction is to HBD residues tyrosine-89 and asparagine-93. Pellicciari also

described that a hydrophobic pocket exists on the binding site of TGR5, providing a beneficial interaction with the 6α-ethyl group of obeticholic acid (**1.8**). A high-resolution single-crystal X-ray structure of the TGR5 receptor is yet to be published.

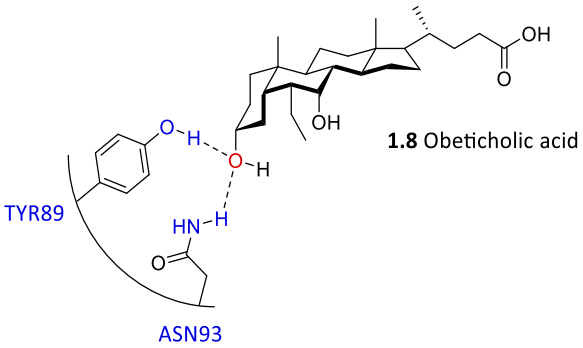


*Figure 1.7. Interaction of the TGR5 with obeticholic acid (**1.8**) at the 3α-OH group.*[50]

Similarly to the FXR, activation of the TGR5 is possible by both free bile acids and their conjugates.[9] LCA activates the TGR5 in sub-micromolar concentrations, while DCA, CDCA, CA and HDCA activate at micromolar concentrations,[49, 55, 56] and UDCA does not illicit any effect.[49] Further quantitative analyses of TGR5 activation by BAs can be found in *Section 1.2.6*.

1.2.5 Bile acid effects on apoptosis

Apoptosis, or lack thereof, plays a vital role in the progression of cancer and is key to maintaining a normal cell population. Apoptosis provides a defence mechanism for cells damaged by chemicals, disease, mutation and more.[57] Tumour suppression protein p53 is critical to restoring apoptosis, causing cell cycle arrest upon activation.[58]

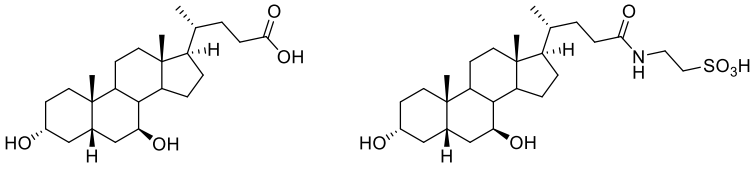
By contrast, apoptosis of healthy neuronal cells is clearly detriment and directly contributes to the progression of neurodegenerative diseases. Mitochondria have prominent roles in apoptosis mediation through the permeabilisation (and self-destruction) of their own membrane to subsequently release a cocktail of pro-apoptotic species'.[59] Interference with these mitochondrially-initiated apoptotic pathways provides neuroprotective effects.

Notably, cholic acid is observed to give no influence on apoptosis, even at high concentrations.[60] The remaining library of BAs discussed herein do however yield effects, and are presented in the following two sub-sections where most relevant.

1.2.5.1 Apoptosis inhibition for the treatment of neurodegenerative diseases

Described as a 'neurology time bomb', Parkinson's disease (PD) is a condition that currently has no known cure and the rate of diagnosis is projected to increase annually at an alarming rate.[61] Though caused by a number of well-understood mechanisms, the most notable is a mutation in the LRRK2

gene and this is the single greatest cause of late-onset PD that is monogenetically inherited.[61] The mutation of the LRRK2 gene has been attributed to poor mitochondrial function resulting in early cellular death, however this function can be rescued by an appropriate dosage of UDCA or its corresponding taurine conjugate TUDCA (*Figure 1.8*),[62, 63] providing a novel handle by-which to target PD.[64] Several complementary mechanisms exist for such rescue and provide a neuroprotective effect against neurodegenerative diseases.



1.5 Ursodeoxycholic acid **1.9** Tauroursodeoxycholic acid

*Figure 1.8. Effective apoptosis inhibitors UDCA (**1.5**) and taurine conjugate (TUDCA) **1.9**.*

Of greatest significance in the effort to inhibit apoptosis is the BA interference within the mitochondrial-mediated pathway that, if left untreated, initiates neurocellular death.[59, 65] TUDCA and also UDCA can also valuably stabilise and support the mitochondrial membranes in PD neuronal cells,[66, 67] maintaining energy production and preventing the loss of the mitochondrial membrane potential, [67] all of which are key to the function to support healthy mitochondria.[68] Mitochondrial stabilisation has been witnessed in neuronal cells relating to Alzheimer's and Huntington's diseases after treatment with TUDCA.[59, 65, 66, 69] Furthermore, in an AD mouse model TUDCA reduced the production of amyloid-β leading to a significant reduction in harmful amyloid-β deposits.[69] In addition, UDCA and TUDCA have been observed to reduce oxidative cell stress and suppress the formation of ROS, an important contributor to the pathogenesis of PD.[66] Finally, it is notable that cotreatment of BAs alongside chemotherapy is neuroprotective by the suppression of pro-apoptotic p53 within neuronal networks.[66, 70]

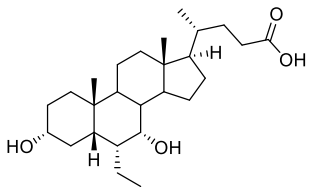
The effectiveness of therapeutic agents within neuronal networks relies on the ability of that agent to cross the blood-brain barrier (BBB) and to penetrate the cerebral spinal fluid (CSF). Studies have shown that TUDCA is effective at both, thereby reasoning its effectiveness in the treatment of PD, AD and more.[58, 63, 71]

1.2.5.2 Pro-apoptosis for the treatment of cancer

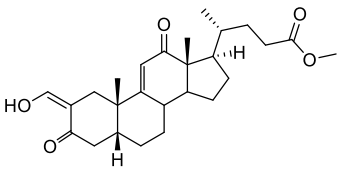
Endogenous bile acids can elicit a broad range of behaviour, favourable and unfavourable, in regard to cancer. UDCA is a potent apoptosis inhibitor,[57, 72] found to prevent cancer treatments at the colon, and CDCA and DCA even act as tumour promotors.[60, 73-75] By contrast, LCA has been found to suppress proliferation in breast cancer cell lines MCF-7 and MDA-MB-231 and promote apoptosis through increasing the expression of tumour suppression protein p53.[76]

Although cholic acid yields no effect towards cancer treatment, the corresponding glycine conjugate (glycocholic acid, GCA) strengthens the cytotoxicity of epirubicin, a traditional chemotherapy agent used in multi-drug resistant (MDR) cancer cells.[77] Other bile acid conjugates including TCA, TCDCA and GCDCA have also been observed to yield the same beneficial effect.[78]

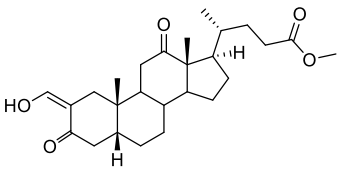
A myriad of potent semi-synthetic BAs towards cancer have emerged in the literature over recent decades following the precedence of naturally occurring bile acids. 6α-Ethyl CDCA (obeticholic acid) (**1.8**) is active in tackling cancerous liver tissue (HepG2, Huh7 and SNU-449 cell lines) at micromolar concentrations,[79] and 3-keto BA derivatives **1.10**, **1.11** and **1.12** have shown micromolar potency at breast (MCF-7), colon (HCT-116), liver (HepG2 and MH-22a), Leukaemia (K562) and duodenum (HuTu-80) cancer cell lines, comparable to the potency of 5-fluoro uracil.[80, 81] Furthermore, the relatively straightforward conjugation of the C_{24}-acid group to afford amides, sulphonamides etc has led to a vast a library of BA side-chain analogues. Notably, cholic acid derivative **1.13** displays micromolar potency at breast cancer cell line MDA-MB-231, comparable to established anticancer agents *Cis*-Platin and Doxorubicin,[82] while LCA derivative **1.14** exhibits micromolar potency towards a number of breast cancer cell lines (MCF-7, MCF-7/ADR, MDA-MB-231) with potency comparable to Tamoxifen.[83] Illustrated in *Figure 1.9* is a range of semi-synthetic bile acids that show therapeutic action against cancerous cell lines and labelled with respective IC_{50} and GI_{50} values.[i] [84]



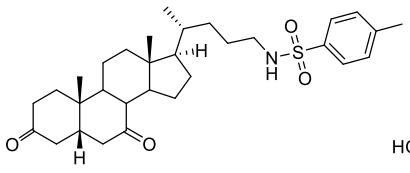
1.8 Obeticholic acid
IC_{50} (μM): HepG2: 1.07;
Huh7: 1.04; SNU-449: 0.71



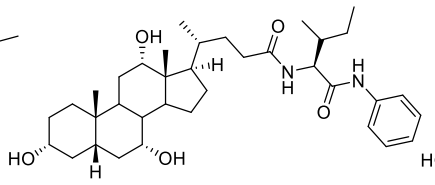
1.10
IC_{50} (μM): HepG2: 16.9;
MH-22a: 38.2; HuTu-80: 10.5



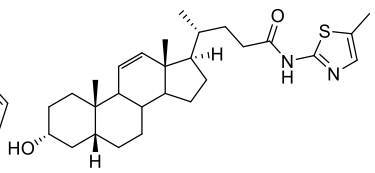
1.11
IC_{50} (μM): HepG2: 8.1;
MH-22a: 9.8; HuTu-80: 6.5



1.12
IC_{50} (μM): MCF-7: 8.62;
HCT-116: 3.71; K562: 4.94



1.13
GI_{50} (μM): MDA-MB-231: 1.35



1.14
IC_{50} (μM): MCF-7: 7.39;
MCF/ADR: 6.95; MDA-MB-231: 7.36

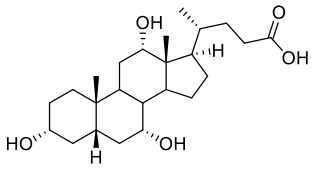
Figure 1.9. Semi-synthetic bile acids that are potent at cancerous cell lines.[79-83]

[i] IC_{50}: the drug concentration at which half-maximal inhibition of a given activity is elicited; GI_{50}: the drug concentration at which half-maximal inhibition of cell proliferation is elicited.[84]

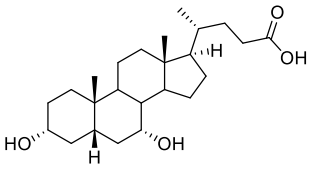
1.2.6 FXR and TGR5 selectivity of endogenous and semi-synthetic bile acids

The benefits of targeting the FXR and TGR5 have been discussed in detail (see: *Sections 1.2.3 and 1.2.4).* Given the differing roles of FXR and TGR5, selectivity is highly important; exploration of BA analogues to yield a selective agonist is prevalent across recent literature.[40, 50, 85]

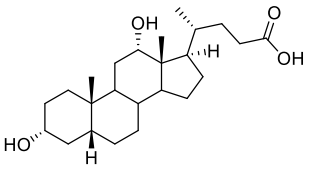
The endogenous human BAs display stark differences in the selectivity of FXR and TGR5 agonism.[33, 49, 85-88] Comparing the ratio of EC_{50} values illustrated in *Figure 1.10*,[ii] cholic acid (**1.1**) and deoxycholic acid (**1.3**) show high selectivity towards the TGR5, suggesting a poor compatibility of the 12α-OH group with the FXR, while CDCA (**1.2**) shows almost no selectivity but remained modestly potent at both receptor types. Lithocholic acid is notably very potent at the TGR5.



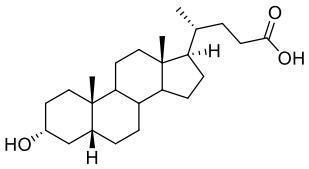
1.1. Cholic acid
EC_{50} (μM): FXR: >1000;
TGR5: 13.6
Ratio: >75:1



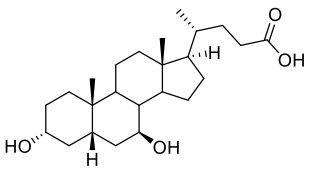
1.2. Chenodeoxycholic acid
EC_{50} (μM): FXR: 4.5;
TGR5: 6.7
Ratio: 2:3



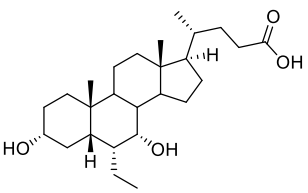
1.3 Deoxycholic acid
EC_{50} (μM): FXR: 100;
TGR5: 1.3
Ratio: 80:1



1.4 Lithocholic acid
EC_{50} (μM): FXR: 3.8;
TGR5: 0.6
Ratio: 6:1



1.5 Ursodeoxycholic acid
EC_{50} (μM): FXR: >1000;
TGR5: >1000
Ratio: N/A



1.8 Obeticholic acid
EC_{50} (μM): FXR: 0.18;
TGR5: 0.84
Ratio: 1:5

Figure 1.10. EC_{50} values of a selection of bile acids at the FXR and TGR5.[33, 49, 85-88]

Obeticholic acid (**1.8**) is one of many semi-synthetic bile acids developed by Pellicciari to further improve the potency of CDCA (**1.2**). At both receptor types we see a benefit to the introduction of the 6α-ethyl group but this is more pronounced at the FXR, thereby giving 5-fold selectivity.[40] The importance of such selectivity is demonstrated in the treatment of NASH with OCA (approved in 2016);[89] intentional and therapeutic agonism of the FXR is matched by the unintentional coactivation of the TGR5, giving pruritus (severe skin itching) as a prominent side effect.[90, 91]

[ii] EC_{50}: the drug concentration at which a half-maximal response is observed.

Therefore, while OCA can be regarded as the benchmark for activity at both receptors, there is clear room for improvement in regard to receptor selectivity.

Extensive further work from Pellicciari centred around 6-substituted BAs (*Figure 1.11*).[50] Introduction of a (*S*)-configured methyl group at the 23-position gave the highly-selective TGR5 agonist *S*-EMCA (**1.15**).[50] OCA organosulfate derivative **1.16** was found to be a dual FXR/TGR5 agonist and with unprecedented nanomolar activity.[92]

Interestingly, 3-deoxy OCA (**1.17**) maintained agonistic activity at the FXR; the same result is witnessed in comparison of 11β-hydroxy OCA (**1.18**) and 3-deoxy-11β-hydroxy OCA (**1.19**). 3,3-Difluoro OCA (**1.21**) also exhibited similar levels of FXR agonism. It is postulated that a hydrophobic cavity provides a strong binding interaction to the 6-ethyl group, negating a loss of binding affinity upon deoxygenation of the 3-position.[53, 91] Additional binding interactions of OCA to the FXR have also been discovered involving the 7α-hydroxyl group to amino acid residues tyrosine-366 and serine-329, advancing the original work of Mi from 2003 (see: *Section 1.2.3.1*) [91]

The introduction of the hydroxyl group at the 11-position to yield **1.18** from OCA (and **1.19** from **1.17**) eliminates activity at TGR5 and giving excellent selectivity towards the FXR. Similarly to organosulfate **1.16**, tetrazole derivative **1.22** shows modest selectivity but high potency at both receptors.

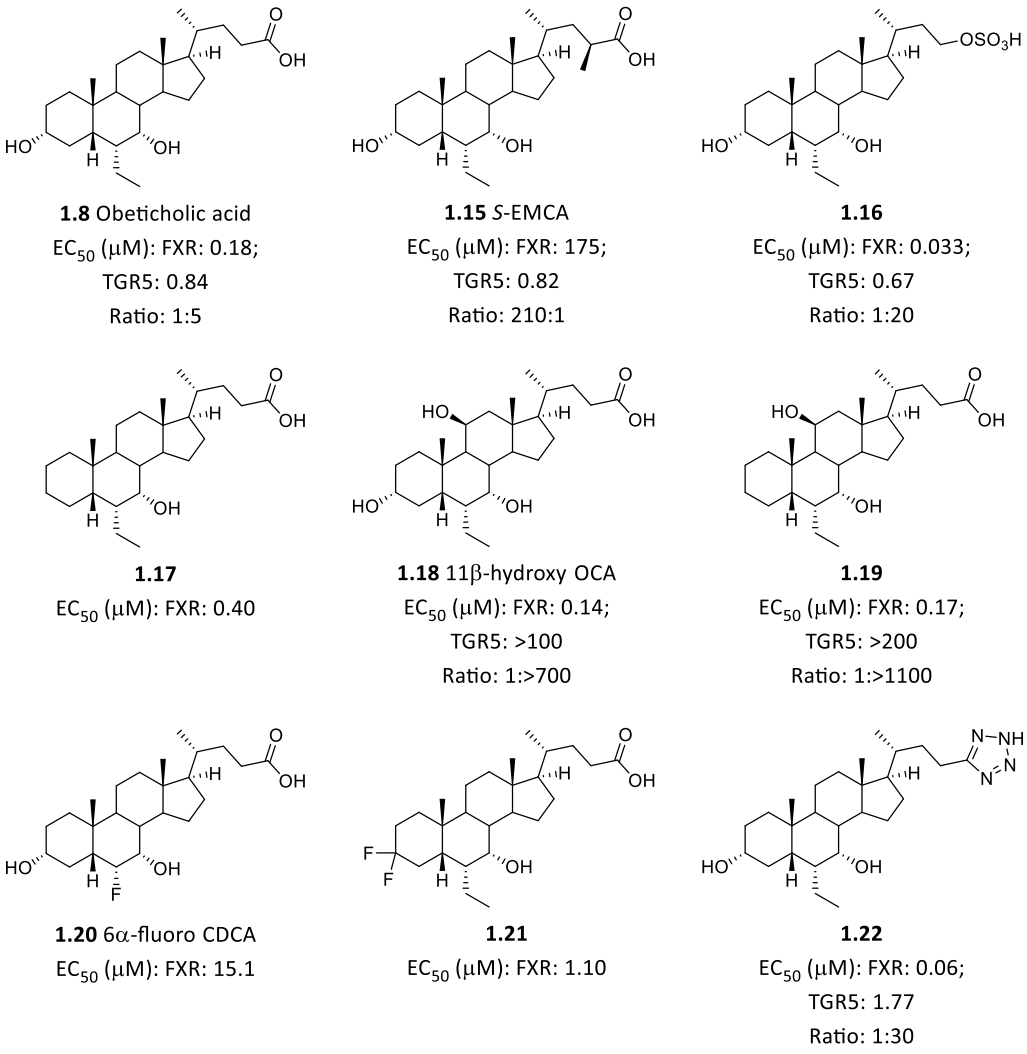


Figure 1.11. 6-position functionalised bile acids described by Pellicciari.[40, 50, 53, 91, 92]

On the topic of potent agonists for the FXR, it is important to introduce the most effective non-bile acid examples. The first key synthetic agonist with nanomolar potency at the FXR was GW4064 (**1.23**) from Glaxo-Wellcome in 2000, closely followed by Fexaramine (**1.24**) from the Scripps Research Institute in 2003 and compound **1.25** from Novartis in 2004 (*Figure 1.12*).[93-95]

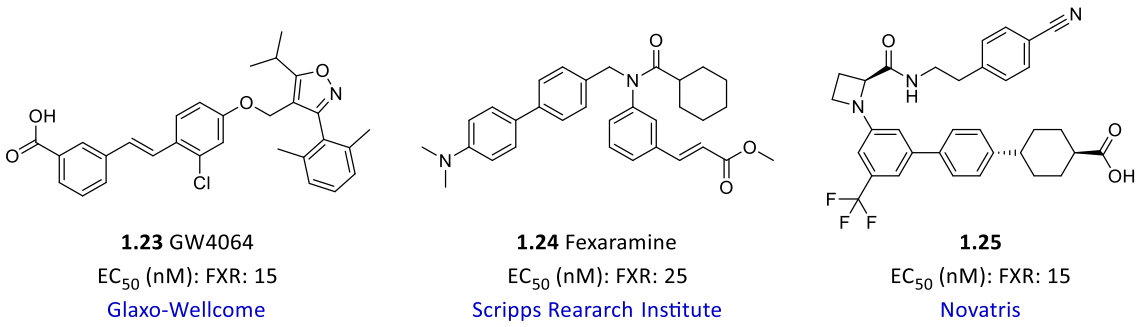


Figure 1.12. Non-bile acid FXR agonists from Glaxo-Wellcome, Scripps RI and Novartis.[93-95]

1.3 Organofluorine chemistry

Organofluorine molecules are ubiquitous within the chemical industry and feature in around 25% of available pharmaceuticals as well as in polymers, refrigerants and agrochemicals. This is a somewhat surprising statistic considering that fluorinated natural products are extremely rare (6 reported examples) and, of which, most are highly toxic.[96] In this section, fluorine and the C-F bond will be introduced, and the effects of C-F implementation discussed.[97-99]

1.3.1 General organofluorine chemistry

Fluorine is the most electronegative element in the periodic table.[100] It forms very short and very strong bonds to carbon,[98] and due to the large difference in electronegativity, the C-F bond is highly polarised and has considerable ionic character. Increasing fluorine substitution increases this ionic character at both carbon and fluorine, resulting in even stronger C-F bonds.[101] The strong electrostatic interaction between carbon and fluorine attenuates fluorine's participation in electron lone pair donation, and C-F groups can only weakly coordinate to hydrogen-bond donors. Given the strength of the C-F bond, fluoride is a poor leaving group.

C-X bond	Bond dissociation energy / eV	Bond length / Å	Electronegativity of X (Pauling)	VdW radii of X/Å
C-F	4.57	1.35	3.98	1.47
C-H	4.28	1.09	2.20	1.20
C-O	3.64	1.43	3.44	1.52
C-C	3.60	1.54	2.55	1.70
C-Cl	3.40	1.77	3.16	1.74
C-N	3.00	1.47	3.04	1.55
C-Br	2.90	1.94	2.96	1.85

Table 1.1. Properties of X-F bonds and atoms X.[98, 100, 101]

With a van der Waals radius of 1.47 Å, covalently bound fluorine occupies a smaller volume than a methyl group, hydroxyl group or amino group, but is larger than hydrogen (van der Waals radius 1.20 Å, *Table 1.1*).[102] The comparable size makes the C-F bond an excellent isostere for C-H replacement and has been adopted ubiquitously in medicinal chemistry to slow, or even prevent altogether, the oxidation of drugs by cytochromic enzymes, increasing drug half-life.[103] The C-F bond is also highly adopted in materials science to provide organic compounds of higher thermal and chemical stability, for example fluorinated polymer PTFE.[97]

Though most comparable in size to a C-H bond, organic fluorine is more similar electrostatically to a hydroxyl group. Replacement of a C-H bond with a C-F bond can therefore have profound effects

on proximal functional groups (see: *Section 1.3.3*). Replacement of a C-OH bond with a C-F bond retains similar dipole-dipole interactions (Δ = 0.16 D) but indeed precludes the ability to act as a hydrogen bond donor. The CF_2 motif is an also a possible isostere for the CH-OH group given the greater similarity in size; it also exhibits a comparable dipole moment, but this is less similar (Δ = 0.28 D) (*Figure 1.13*).[104-106]

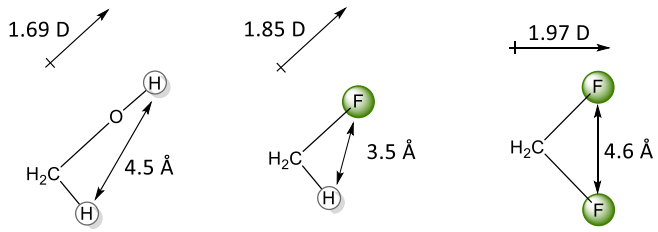


Figure 1.13. The CFH and CF_2 motifs; suitable isosteres for the hydroxyl group.[104-106]

1.3.2 Common organofluorination methodologies

There is a wealth of fluorination methodologies available.[107-111] Nevertheless, it is important to introduce a handful of the most-used and key reagents.

Diethylaminosulfur trifluoride (DAST) was developed in the mid 1970's as a replacement for sulfur tetrafluoride.[112] Sulfur tetrafluoride has long proven to be an effective reagent for the synthesis of alkyl fluorides from alcohols and geminal difluorides from ketones, however extreme toxicity and gaseous nature have always been of concern. As liquids, DAST and other dialkylaminosuflur trifluorides such as *bis*(2-methoxyethyl)aminosulfur trifluoride (Deoxo-Fluor) are safer alternatives and easier to handle.[113] Nevertheless, DAST suffers from thermal instability at higher temperatures (75 °C or higher), above which, the use of Deoxo-Fluoro is reccomended.[114] 'Convenient, crystalline deoxofluorination reagents' XtalFluor-E and XtalFluor-M have also recently emerged as direct competitors, yet our experiences have found their reactivity to be poorer than neat DAST.

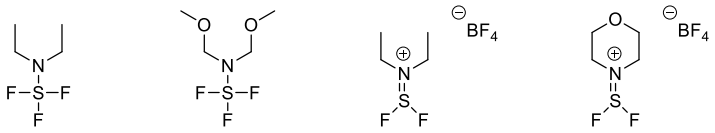
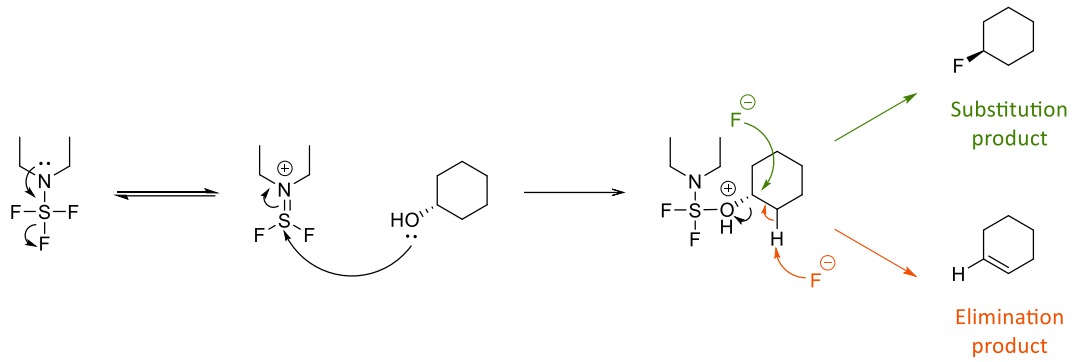


Figure 1.14. Contemporary alternative deoxyfluorination reagents to sulfur tetrafluoride.[108]

These deoxyfluorination reagents provide a source of nucleophilic fluoride for the establishment of C-F bonds while simultaneously activating the hydroxyl or carbonyl group. However, the low nucleophilicity and high basicity of the fluoride anion often results in a complex mixture of elimination side products (*Scheme 1.1*).



Scheme 1.1. Deoxyfluorination reactions are often plagued by competing elimination pathways.[112]

An acidic source of fluoride is pyridinium poly(hydrogen fluoride), otherwise known as HF.pyridine, or Olah's Reagent.[115] As an alternative to anhydrous hydrogen fluoride (with a low boiling point of 19.5 °C), HF.pyridine is vastly easier to handle but is still very toxic and corrosive so appropriate safety precautions are imperative; it is known to etch borosilicate glass even at cryogenic temperatures. It contains approximately 70% HF and 30% pyridine by weight, or a ~9:1 molar ratio. Other HF-organic base reagents include $Et_3N.3HF$ (HF.triethylamine) and HF.DMPU;[116, 117] they are known not to etch borosilicate glass because they have an inherently lower acidity (fewer equivalents of acid, 3 and 1 respectively). All of these examples of acidic fluoride sources show excellent reactivity in the opening of epoxides.[118, 119]

A library of electrophilic fluorine sources also are prevalent in modern fluorination chemistries for the introduction of a C-F bond at nucleophilic carbon. Common amongst their structures is a weak N-F bond with a bond enthalpy of 3 eV or less (2.84 eV for *N*-fluorosultam, *Figure 1.15*),[120] comparable to the O-H bond in water (2.78 eV).[121] Nucleophilic attack at these reagents pushes electron density into the σ* orbital of this weak N-F bond at fluorine, as the equivalent orbital is sterically inaccessible on the nitrogen atom, and is described to proceed by an S_N2 mechanism. Though disputed to be a single-electron transfer process, aryllithium and Grignard reagents provide similar yields of fluorobenzene when used in combination with electrophilic fluorine source such as NFOBS, supporting the accepted hypothesis of an S_N2 pathway.[122]

N-fluorosultam
N-F BDE: 2.84 eV

NFOBS
N-F BDE: 2.57 eV

NFSI
N-F BDE: 2.75 eV

SelectFluor
N-F BDE: 2.78 eV

Figure 1.15. Electrophilic fluorination reagents with labelled N-F BDE.[123]

1.3.3 The effects of fluorination

1.3.3.1 … on acidity (pK_a) and hydrogen bond donating capacity (pK_{aHY})

The substantial electronegativity of fluorine directly affects the acidity and basicity of neighbouring functional groups.[103] Through stabilisation of the conjugate anion by its inductive effect, acidity of the parent compound can significantly increase (*Figure 1.16*).[103]

pK_a:	4.76	2.59	1.24	0.23

Figure 1.16. Increasing acidity (pK_a) upon poly-fluorination of acetic acid.[103]

Likewise, for aliphatic amine groups, the increasing substitution of fluorine yields a greater acidity of the conjugate acid (pK_{aH}) (*Figure 1.17*).[103] This technique has been used to attenuate the basicity of amine-containing pharmaceutical molecules and has been rewarded with significant improvements in bioavailability.[124]

pK_{aH}:	10.7	9.0	7.5	5.7

Figure 1.17. Decreasing basicity upon poly-fluorination of ethylamine.[103]

Regarding hydrogen bond donating capacity, fluorine introduction for a long time was assumed to increase the hydrogen bond acidity of proximal functional groups,[125] as typically illustrated by polyfluorinated alcohols such as trifluoroethanol (TFE) and hexafluoroisopropyl alcohol (HFIP) (*Figure 1.18*).

[E^T_N]:	0.90	1.07

Figure 1.18. Polyfluorinated alcohols TFE and HFIP.[iii] [126]

However, our group has described that fluorine introduction can impart both significant increases and decreases on hydrogen bond acidity.[127] The pK_{AHY} (a measure of hydrogen bond acidity) of a

[iii] [E^T_N] is the normalised Reichardt scale with Me_4Si = 0 and H_2O = 1; for reference, EtOH = 0.65.[126]

series of rigid cyclohexanol fluorohydrins was determined using FTIR spectroscopy to examine complexation with *N*-methylpyrrolidinone (NMP) in carbon tetrachloride (*Figure 1.19*).[127]

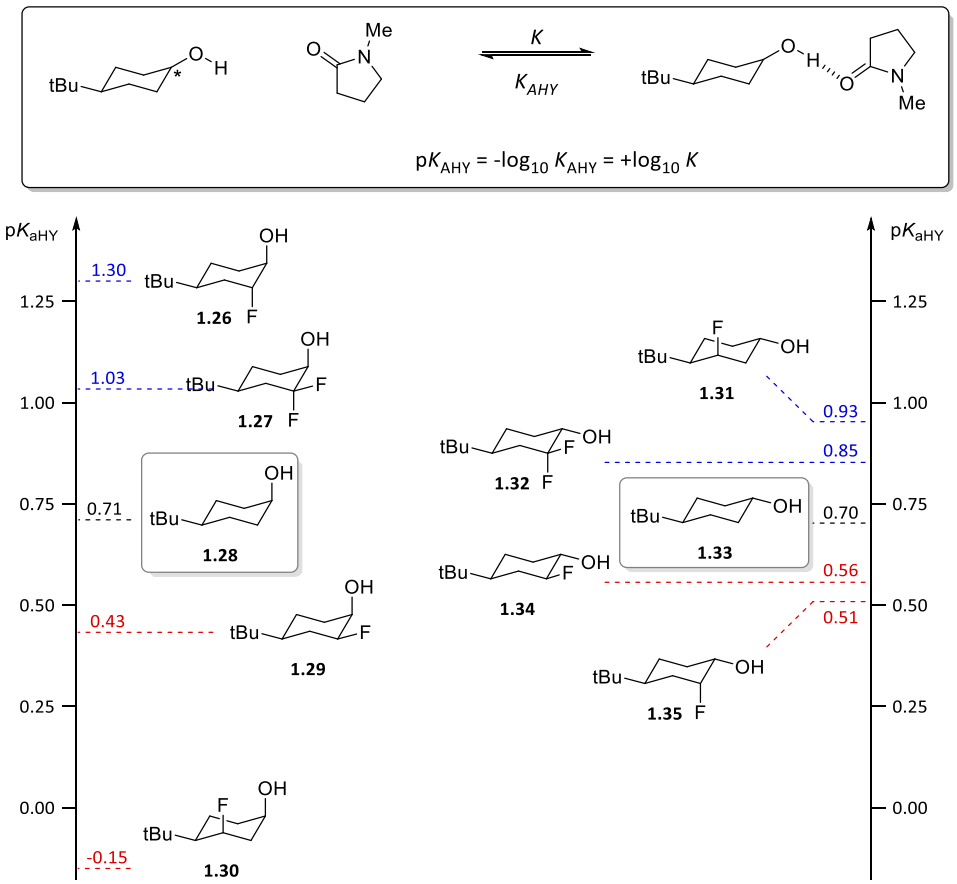


Figure 1.19. Hydrogen bond formation to NMP, examined by FTIR, to determine the pK_{AHY} values of a series of rigid cyclohexanol fluorohydrins.[127]

Fluorohydrins **1.26**, **1.27**, **1.31** and **1.32** (reported in blue) exhibit a greater hydrogen bond acidity than their non-fluorinated analogous **1.28** and **1.33**. The electron withdrawing effect of fluorine is responsible for this general observation. By contrast, compounds **1.29**, **1.34**, **1.35** and especially 1,3-diaxial fluorohydrin **1.30** (reported in red) have a lower hydrogen bond acidity than their parent non-fluorinated compounds, explained by an electrostatic interaction or hydrogen bond between the fluorine lone pairs and the exchangeable proton; the proton is then less readily available to interact as a hydrogen bond donor (*Figure 1.20*).

For 1,3-diaxial fluorohydrin **1.30** a F-H coupling constant of 12.1 Hz is observed in the ^{1}H NMR spectrum, and the calculated distance of 2.033 Å between atoms is significantly shorter than found in 1,2-fluorohydrins (2.3-2.4Å).[127] This observation also explains why difluoro compounds **1.27** and **1.32** exhibit a lower pK_{aHY} than mono-fluorinated derivatives **1.26** and **1.31**, despite the larger summative electron withdrawing effect of the difluoro motif.

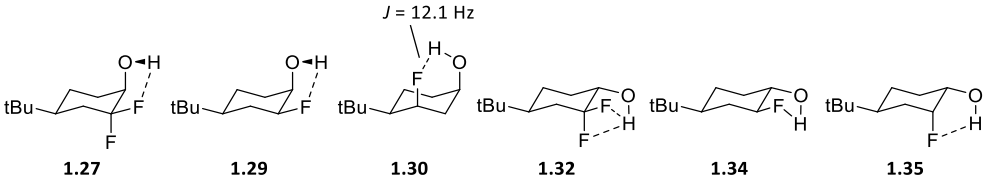


Figure 1.20. Interactions of fluorine and hydrogen attenuate alcohol HBD capacity.

Examples of this effect of fluorine can be found for steroidal substrates, the most classical of which is 9α-fluorohydrocortisone (*Figure 1.21*). Substitution of a C-H bond for a C-F bond at the 9α-position was observed to give a 10-fold increase in potency (anti-inflammatory properties) and is likely from the increase in hydrogen bond acidity of the 11-OH group;[128] the antiperiplanar arrangement of the C-F and C-OH bonds is analogous to rigid cyclohexanol fluorohydrin **1.26** (*Figure 1.19*).

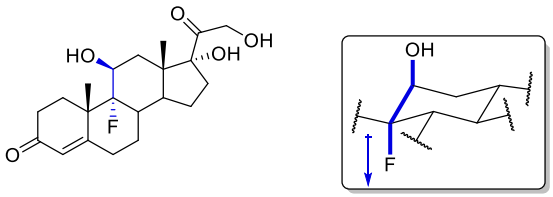
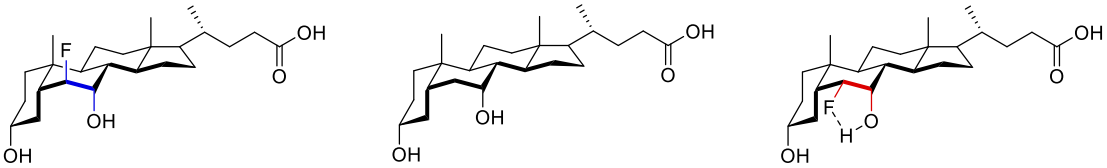


Figure 1.21. 9α-fluorohydrocortisone, with vicinal and antiperiplanar C-F and C-OH bonds.[128]

Regarding bile acids, specifically CDCA, the importance of the 7-OH group in binding at the FXR is illustrated by fluorination at the 6-position (*Figure 1.22*).



1.36 6β-fluoro CDCA
EC_{50}: FXR: 1.2 μM

1.2. CDCA
EC_{50}: FXR: 4.5 μM

1.19 6α-fluoro CDCA
EC_{50}: FXR: 15.1 μM

Figure 1.22. Fluorination at the 6-position of CDCA can systematically vary FXR potency.[50]

1.3.3.2 … on log*P* (lipophilicity)

Lipophilicity (log*P*) is a measure for membrane permeability, and is measured as the partition coefficient between octanol and water; lipophilicity can also be described as log*D*, the distribution coefficient of an ionisable compound for a specified pH.[129] With influences on solubility, potency, selectivity and metabolism, lipophilicity is the most important physiochemical property to consider for a drug molecule.[103, 130]

According to Lipinski in 1997, the log*P* value of orally available drugs is ideally less than five.[131] Any higher, a drug molecule will be plagued with poor aqueous solubility, high toxicity and rapid metabolic turnover. Recent reviews surrounding log*P* have however suggested an optimum range of between one and three.[132] A powerful tool to influence log*P* without the introduction of heteroatoms such as oxygen and nitrogen is site-selective fluorination.

Historically, log*P* studies have mostly concerned only aromatic substrates; measurement of log*P* was achieved by quantitative HPLC whereby UV-vis activity is essential. For these aromatic compounds, the introduction of fluorine almost always led to an increase in lipophilicity and so a generalised statement of 'substitution of a C-H bond for a C-F bond increases log*P*' has emerged but is wrongly applied to more than just these aryl fluorides. Rather, the effect of fluorination on lipophilicity is far more nuanced with a myriad of substrates, though mostly alkyl fluorides, showing a decrease in log*D* (*Figure 1.23*).

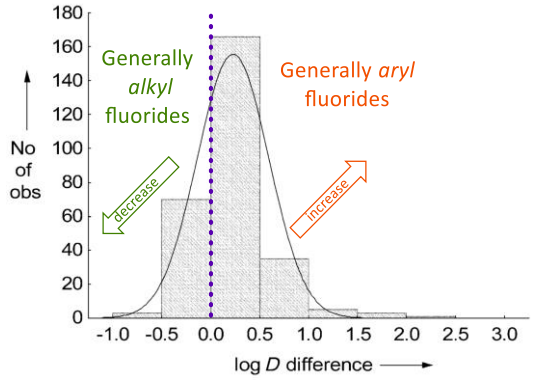


Figure 1.23. The effect of fluorination on logP. Adapted from Böhm.[103]

The exact nature of fluorine's influence on log*P* depends largely on two opposing effects. They are the introduction of the strong C-F dipole (that decreases lipophilicity), or the increase in hydrophobic surface area owing to the larger size of fluorine, than hydrogen, and poor polarizability of the fluorine lone pairs (that increases lipophilicity). [133] Compounds that are already very polar see little polarity change upon fluorination, so the increase in hydrophobic surface area dominates and their log*P* increases. By contrast, very apolar molecules will experience a greater influence from the introduction of the strong dipole upon fluorination and their log*P* will decrease;[134] this has been witnessed for short chain alkanes, *n*-propyl benzene derivatives and linear fluorohydrins.[125, 133, 135]

The study of fluorinated *n*-propyl benzene derivatives by Müller also gave valuable insight into the relative polarities of polyfluorinated methyl groups;[133] key to the understanding of these results is analysis of C-F bond dipole moment vectors (*Figure 1.24*). We observe that the CH_2F and CF_3 group have approximately the same overall dipole magnitude whereas the CF_2H group has a dipole of around 15% greater than that of a single C-F bond.

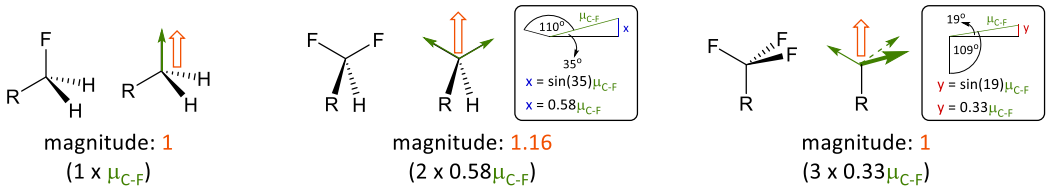


Figure 1.24. Bond vector analysis of polyfluorinated methyl groups.[133]

Extending these quantitative analyses further, we can compare the dipole magnitudes of the geminal and vicinal CF_2 motifs.[136] Because of the fluorine gauche effect, we can expect vicinal C-F bonds to be of gauche relationship to one another and the smaller dihedral angle (relative to geminal C-F bonds) thus yields a larger dipole moment of 64% greater than a single C-F bond. Examples of these fluorination patterns are found during Müller's investigation of fluorinated propyl indole substrates (*Figure 1.25*).

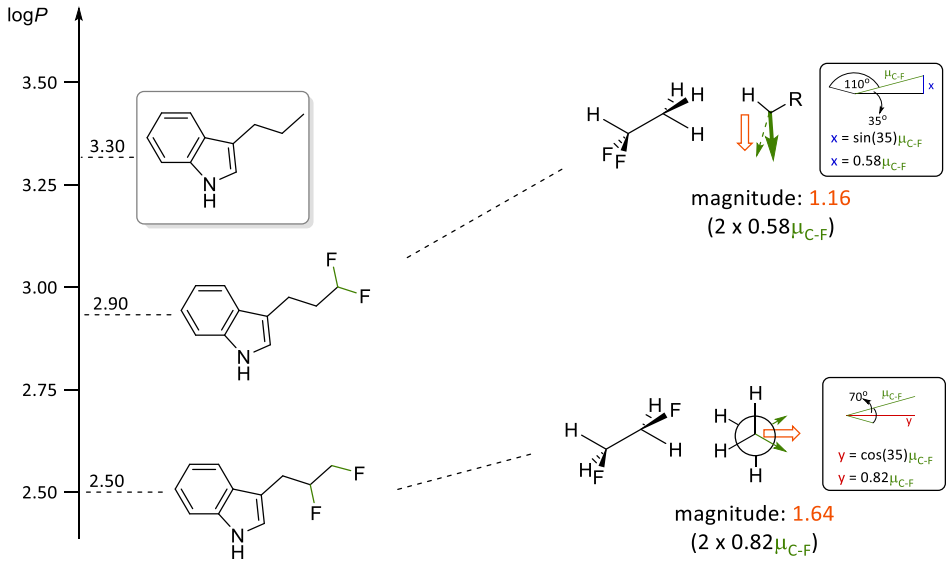


Figure 1.25. Bond vector analysis of geminal and vicinal CF_2 motifs.[136]

A recent publication by our group described a novel log*P* measurement method using ^{19}F NMR, drastically expanding the potential library of compounds for which reliable lipophilicity measurements can be taken.[135] From this new method, the lipophilicity of a range of fluorinated alkanols and fluorinated cyclohexanols was measured and selected results are presented in *Figure 1.26*. For the ethanol series, opposing dipole effects make 2,2,2-trifluoroethanol **1.37** more lipophilic than non-fluorinated ethanol **1.39**, whereas the aligning dipoles of 2-fluoroethanol **1.40** increase the overall molecular dipole and reduce lipophilicity; the alignment of dipoles in the most prevalent conformer is likely because of an prevelant intramolecular C-F•••H-O interaction. A combination of dipolar and hydrophobic surface area effects are in-play for 2,2-difluoroethanol **1.38** which is marginally more lipophilic than parent **1.39**. For the pentan-1-ol series, fluorination increased polarity in all cases (**1.42**-**1.44**) and therefore decreased lipophilicity, relative to parent

pentan-1-ol **1.41**. Examining the effect of fluorination of conformationally restricted alcohol derivatives **1.28** and **1.33**, opposing dipoles of *trans*-diaxial fluorohydrin **1.26** lead to an increase in lipophilicity and this is a rare example of aliphatic monofluorination yielding an increase in log*P*. Further, this result is despite the enhanced acidity of this fluorohydrin (*Figure 1.19*). By contrast, for examples **1.34**, **1.35** and especially **1.30**, the alignment of dipoles yields an overall enhanced molecular dipole, decreasing lipophilicity.

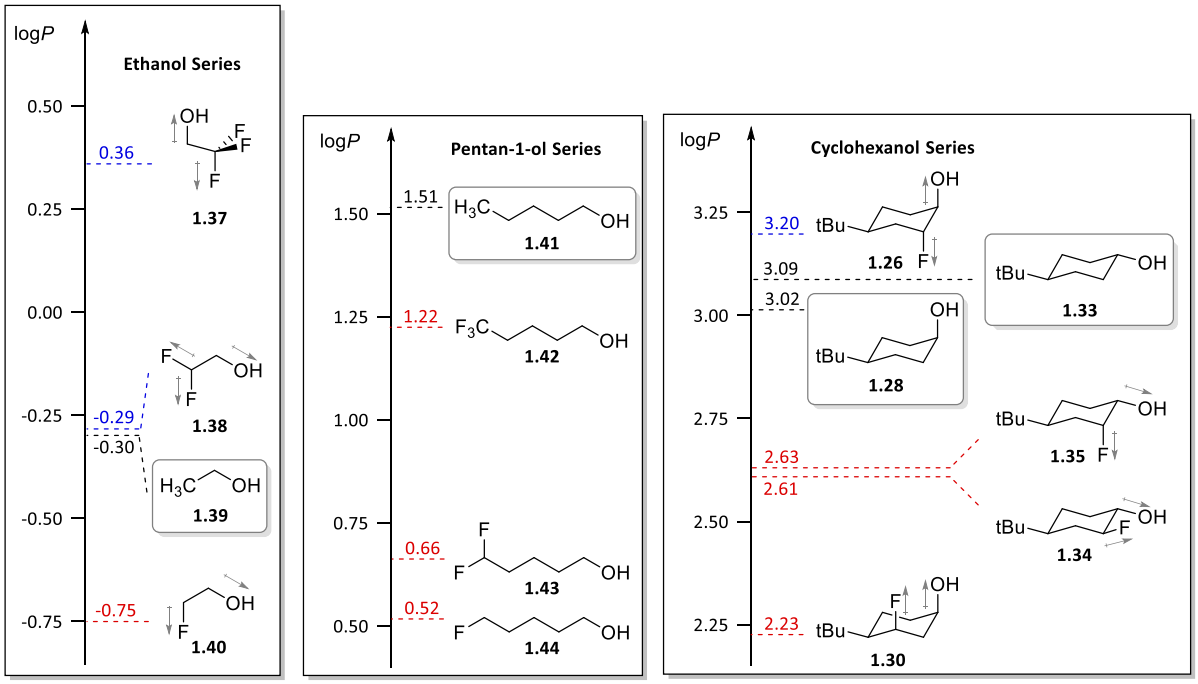


Figure 1.26. The effect of fluorination on logP of fluorinated alcohol derivatives.[135]

1.3.3.3 … on molecular conformation

The conformations of organofluorine molecules are heavily influenced by the large dipole moment of the C-F bond mainly through (solvent dependant) dipole alignment, attractive and repulsive electrostatic (dipolar) interactions and interactions of the C-F bond with formal charges.[98, 137, 138]

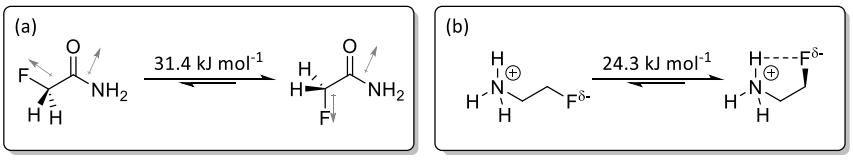


Figure 1.27. Effects of fluorination on conformational stability through a) dipolar alignment and b) electrostatic interactions involving the polar C-F bond and heteroatoms.[98]

Additionally, the fluorine gauche effect (hyperconjugation of electron density from a C-H σ-bond to the C-F σ* orbital) promotes an anti-configuration between vicinal fluorine and hydrogen atoms, namely yielding a gauche relationship between the two vicinal C-F bonds (*Figure 1.28*); this is

despite the typical preference for highly electronegative atoms (such as F) to minimise electronic repulsion in an anti-relationship.[137, 138]

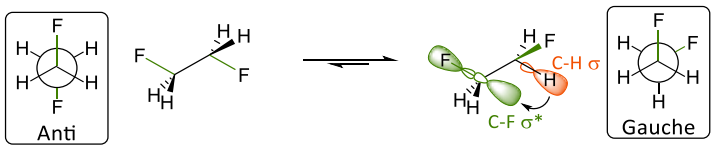


Figure 1.28. The fluorine gauche effect, demonstrated with 1,2-difluoroethane.

1.3.4 Intramolecular hydrogen bonding involving fluorine

The IUPAC definition of a hydrogen bond is: "*an attractive interaction between a hydrogen atom from a molecule or molecular fragment X-H in which X is more electronegative than H, and an atom or group of atoms in the same or different molecule, in which there is evidence of bond formation*".[139] A linear geometry of atoms C-X•••H-Y generally yields the strongest hydrogen bonds, but they have been reported with dihedral angles ranging 110° to 180°.[139] The electronegativity of fluorine is significantly greater than that of hydrogen, therefore it would seem logical that a C-F•••H-O bond is plausible.

The C-F bond is often used as an isostere for the C-OH motif given their comparable dipoles and volume (see: *Section 1.3.1*) although their hydrogen bonding properties are not so similar, and a sound understanding of how organic fluoride acts as a hydrogen bond acceptor is essential.

1.3.4.1 Towards the discovery of fluorine as a hydrogen bond acceptor

Though anionic fluoride has been reported extensively as an excellent hydrogen bond acceptor,[140] the ability of organic fluoride (C-F) to act as a HBA has been subject to extensive debate.[98, 101, 125, 141-143] Recent studies provide evidence for a weak C-F•••H-O interaction in structures lacking competing HBAs.[103, 125] A defining feature of a fluorine-hydrogen bond is that the proton experiences deshielding and exhibits a coupling constant to fluorine.[142] Bond lengths are typically around 2.5Å, compared to 1.97Å for hydrogen bonds in water with only ~25% of the strength of comparable hydrogen bonding to oxygen.[98]

1.3.4.1.1 Efforts toward discovery, in the solid phase

Historically, C-F•••H-O interactions were identified through crystallographic database scannings (such as the CSDS – Cambridge Structural Database System).[141-144] In a study by Howard in 1996 on the 146272 entries in the CSDS,[141] a mere 166 cases of a short C-F•••H-X motif (≤2.35 Å) were reported, though most unfortunately had the identity of X as an acidic carbon atom and so did not classify under the IUPAC definition of a hydrogen bond (see: *Section 1.3.4*). Surprisingly, of all data

within the database only 12 examples were found with X as oxygen and only 28 with X as nitrogen. Furthermore, only one example was found with a C-F•••H-X interaction of less than two angstroms (i.e. comparable to a true hydrogen bond in water) but was later described to be a result of conformational restraint elsewhere in the structure (*Figure 1.29*).

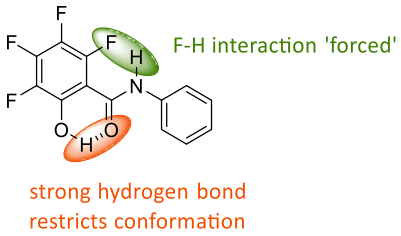


Figure 1.29. A single example from the CSDS of a fluorine hydrogen interaction of ≤2 Å.[141]

This work was later revisited and independently updated by D'Oria in 2008.[144] Alas, the population of C-F•••H-X interactions within the chemical space had not increased.

Crystal packing results in the minimum energy packing of molecules and is influenced by a number of factors such as molecular shape, hydrogen bonding and van der Waals interactions.[145] Competing interactions of stronger HBA atoms such as oxygen and nitrogen will dominate over those interactions involving fluorine, and illustrated by *Figure 1.29* is that C-F•••H-X interactions are possible when forced in proximity. The infrequence of fluorine hydrogen interactions within the database could be simply due to a lack of the relevant atoms being in proximity upon crystallisation.

1.3.4.1.2 Efforts toward discovery, in the solution phase

Historical investigation of solution phase hydrogen bond acceptor capacity was pioneered in the 1960's by Arnett and Taft and their IR and NMR methods were later updated in 1999 by Ouvrard and in 2014 by Dalvit (*Figure 1.30*).[146-149]

The method involved integration of the O-H band of the free HBD compound (4-fluorophenol) and the HB complex (found at a different wavenumber). The relative ratio of the HB complex and free HBD molecule can then be used to calculate the hydrogen bond acceptor capacity (pK_{BHX}) according to *Equation 1.1*.

$$\mathrm{pK_{BHX}} = \log_{10} K_f \quad where \quad K_f(\mathrm{dm^3 mol^{-1}}) = \frac{[\mathrm{HB\ complex}]}{[\mathrm{HBA}][\mathrm{HBD}]}$$

Equation 1.1. Calculation of hydrogen bond acceptor capacity (pK_{BHX}).[147]

Notably, the hydrogen bond acceptor capacities were poor and comparable to diethylthioether (0.22) and far less than typically regarded HBA compounds (ethylamine, 2.17). A second measure of hydrogen bond strength is the change in stretching frequency of the H-X bond ($\Delta\nu_{X\text{-}H}$) upon

interaction; bond strength is imperative to IR stretching frequency, and a decrease in this frequency ('red shift') is witnessed when H-X is involved in hydrogen bonding.[150, 151] The fluorinated HBA compounds were observed to elicit a small change in stretching frequency even compared to diethylthioether (Δ146 cm^{-1})(for reference: ethylamine Δ351 cm^{-1}) (*Figure 1.30*). The evidence suggests fluorine can act as a hydrogen bond acceptor, albeit weakly.

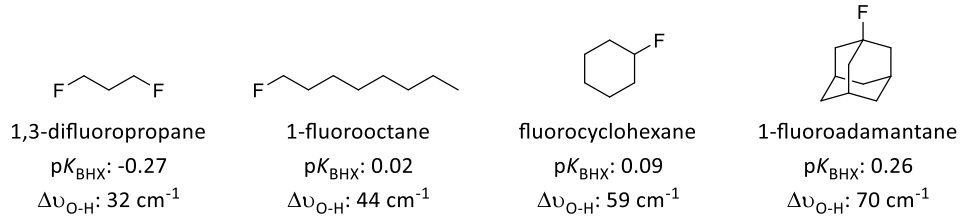


Figure 1.30. HBA capacity (pK_{BHX}) and the 'red-shift' power of fluorinated substrates.[148, 149]

Further evidence to suggest the existence of fluorine hydrogen bonds (though only weak interactions) came from ^{19}F NMR by Dalvit.[149] The method involved 4-fluorophenol as the hydrogen bond donor and the chemical shift of the fluorine atom was tracked by ^{19}F NMR; a value for K_f (*Equation 1.1*) can be recorded by titration of competing HBA acetophenone into a 1:1 mixture of the subject HBA and HBD and measuring the change in fluorine chemical shift of HBD (4-fluorophenol). Reporting the pK_{BHX} values of fluorinated compounds relative to acetophenone (1.56), 1-fluoroheptane gave the greatest HBA capacity (0.16) followed by (fluoromethyl)benzene (0.13) and (difluoromethyl)benzene (-0.19); (trifluoromethyl)benzene gave a value too low to accurately measure.

Hence, both IR and NMR methods evidence the ability of fluorine to participate in C-F•••H-X interactions.

1.3.4.2 Examples within cyclic systems of the C-F•••H-O hydrogen bond

The weak C-F•••H-X hydrogen bond is usually outcompeted by stronger hydrogen bond acceptors. In the solution phase, fluorine hydrogen bonds can be found in systems where the fluorine and hydrogen atoms are conformationally forced within a close proximity. A few key examples are presented in the following subsections.

8-Fluoro-napthol can exist as either the *cis*-or *trans*-conformer, and calculations by Rozas predicted the presence of a C-F•••H-O interaction to stabilise the *cis*-conformer by 16.4 $kJmol^{-1}$ (*Figure 1.31a*).[152] Synthesis and study of 8-fluoro-napthol by Takemura revealed the OH proton at 6.90 ppm as a doublet with $^1J_{OH-F}$ of 28.4 Hz.[153] Introduction of a competing HBA solvent reduced the coupling to 4.4 Hz in [D_8]-THF and to only 2.6 Hz in [D_6]-DMSO and clearly indicated the presence of the intramolecular fluorine-hydrogen bond. For reference, the OH proton of 4-fluoro-napthol was

observed at 5.07 ppm as a singlet. Surprisingly, in the solid phase the crystal packing did not feature this interaction, supporting the notion that it is weak and not controlling the packing. Rather, 8-fluoro-napthol tetramers were observed with intermolecular primary O-H•••H-O interactions and secondary intermolecular interactions involving fluorine and hydrogen (*Figure 1.31b*).

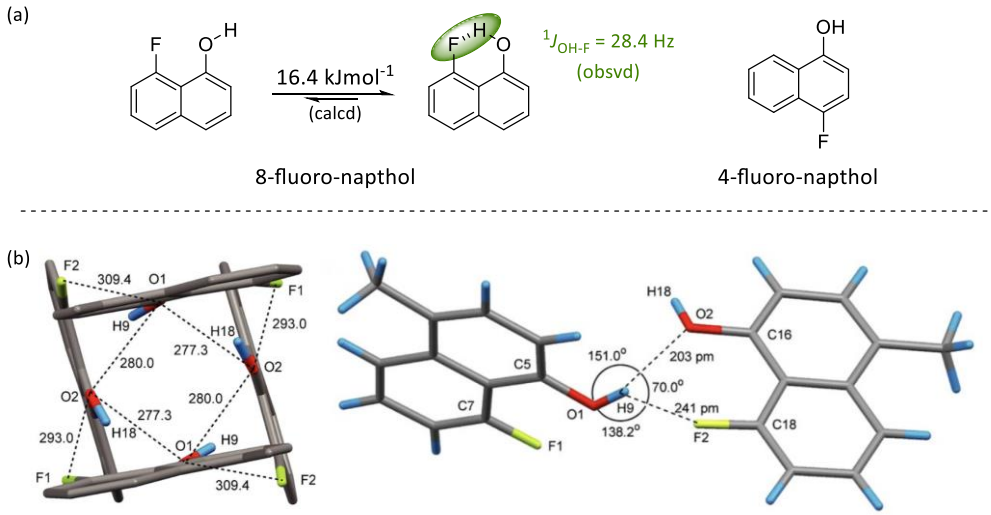


Figure 1.31. 8-fluoro-napthol exhibits a clear intramolecular fluorine hydrogen bond in solution phase (a), but not in the solid phase (b).[153]

A weak C-F•••H-O interaction was observed in an inositol derivative by Bernet and Vasella with $^{1}J_{OH\text{-}F}$ of 8.8 Hz (*Figure 1.32*).[154] Additionally, this hydroxyl group proton also exhibited a coupling constant of 8.3 Hz to the geminal proton, indicative of a relatively fixed ~150° angle between the C-H and O-H bonds (i.e. minimal rotation of the OH group). For the non-fluorinated analogue, a geminal coupling of 4.3 Hz was observed, typically for a freely rotating hydroxyl group. The intramolecular fluorine hydrogen coupling was disrupted in the presence of polar solvent ([D_6]-DMSO) and the $^{1}J_{OH\text{-}F}$ coupling was altogether lost.

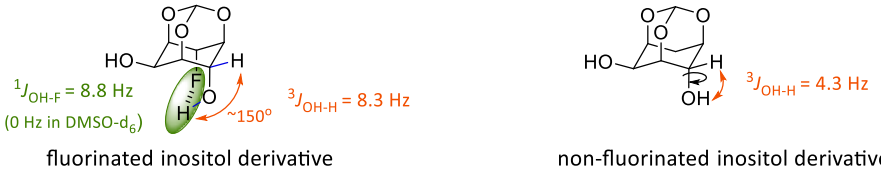


Figure 1.32. Fluorinated and non-fluorinated inositol derivatives.

A selection of other key examples of this rigid structure type is presented in *Figure 1.33*. A common observation is a coupling constant of 9 Hz or greater for a fixed ~antiperiplanar relationship of the C-H and O-H bonds within the H-C-OH motif, a result of the C-F•••H-O interaction preventing free rotation.[154-156] For those compounds where an NMR was reacquired in a strong HBA solvent, all examples were found to have lost (or thereabouts) the fluorine hydrogen coupling constant, owing

to the competing HBA diminishing this interaction. Furthermore, in polar solvent the aforementioned geminal coupling of ≥9 Hz for H-C-OH is significantly reduced to around 5 Hz, suggesting free rotation of the C-OH bond in the absence of the C-F•••H-O interaction.

A study by our group concerning the hydrogen bond acidity (pK_{AHY}) of fluorinated rigid cyclohexanols (see: *Section 1.3.3.1*) observed a 12.1 Hz fluorine hydrogen coupling for 1,3-diaxial fluorohydrin **1.30**. Smaller couplings of 1-2 Hz were witnessed for equatorially hydroxyl-substituted derivatives **1.34** and **1.35** (*Figure 1.33*). However, the abundance of protons on the cyclohexanol backbone gave a complex multiplet for the signal from geminal proton H_1 in all cases; therefore no anti/gauche relationship could be implied for the H-C-O-H motif.

Figure 1.33 also illustrates a comparison study of the CHF and CF_2 motifs by Bernet and Gouverneur.[157] Unsurprisingly, the electron withdrawing effect of the equatorial geminal fluorine atom impaired the HBA ability of the axial fluorine atom: a lower $^1J_{OH\text{-}F}$ of 1.5 Hz was observed, compared to 9.1 Hz for the equivalent monofluorinated carbohydrate. Described in the figure is also a tricyclic cage structure from Lectka *et al* where the fluorine and hydrogen atoms are forced to a distance of only 1.58 Å apart.[151] For this compound, a H-F coupling constant of 68 Hz was measured and is notably the largest H-F IMHB coupling constant to be found in the literature.

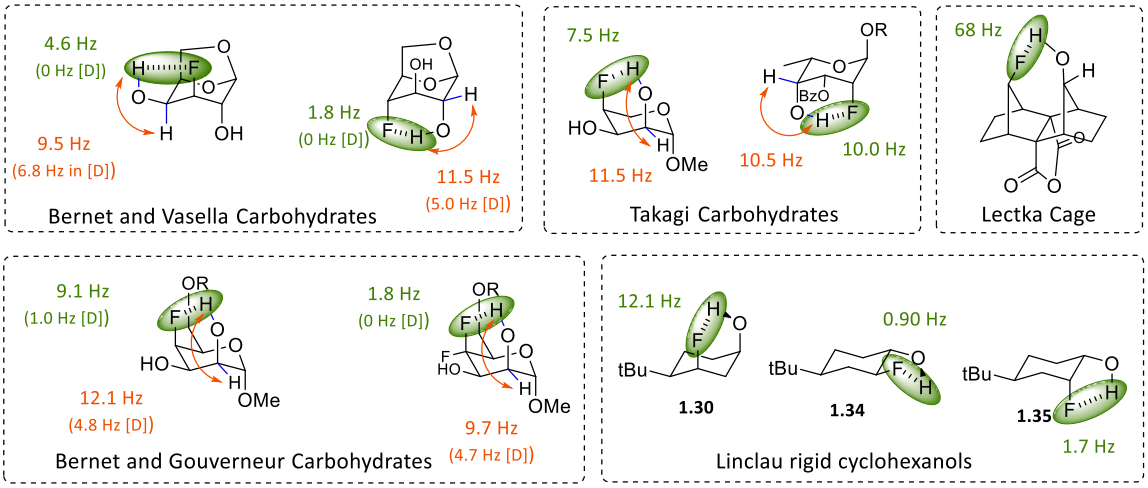


Figure 1.33. Examples of the C-F•••H-O interaction in carbohydrates and rigid cyclohexanols. All coupling constants in $CDCl_3$ unless otherwise denoted [D] for acquisition in ([D_6]-DMSO).

1.3.4.3 Examples within open-chain systems of the C-F•••H-O hydrogen bond

Bühl reported a lack of proton-fluorine coupling by NMR in a study of acyclic fluoroalkanols such as 3-fluoropropan-1-ol, 2-fluoroethanol and 4-fluorobutan-1-ol, where calculations all suggested that $^1J_{OH\text{-}F}$ should be observed.[158] The authors attributed the absence of an observable $^1J_{OH\text{-}F}$ to solvation effects and entropy penalties. Furthermore, they concluded that "*Such IMHB's may become detectable… upon introduction of suitable structural motifs… (for instance involving cyclic*

backbone)', suggesting that akin to cyclic systems (see: *Section 1.3.4.2*) the fluorine hydrogen bonds can only be observed if conformationally forced.

An investigation by our group has recently disproved this conclusion, by observing $^{1}J_{OH\text{-}F}$ couplings in a series of rigorously dried acyclic γ-fluorohydrins in $CDCl_3$.[159] Specifically, 3-fluoropropanol was observed to exhibit a coupling constant of 1.4 Hz at ambient temperature and of 1.7 Hz at -50 °C. Computational analysis revealed the strength of this bonding interaction to be of 20.4 kJmol^{-1} which is typical of 'moderate' hydrogen bonds generally (17-58 kJmol^{-1}).[144] The length of this bonding interaction was also calculated as 2.08 Å. Despite this, only 8% of conformer population (at ambient, and 10% at -50 °C) were reported to be for this IMHB conformer.

Of particular interest from the study by Linclau are γ-fluorohydrins **1.45** and **1.46** where the calculated major conformer (at ambient and also -50 °C) exhibits a clearly stabilising C-F•••H-O interaction (Figure 1.34). The length of the fluorine hydrogen bond of *syn*-4-fluoropropan-2-ol **1.45** was notably calculated less than 2 Å, a feature exclusive to 'forced' C-F•••H-O interactions in the solid phase (see: *Section 1.3.4.1.1*).

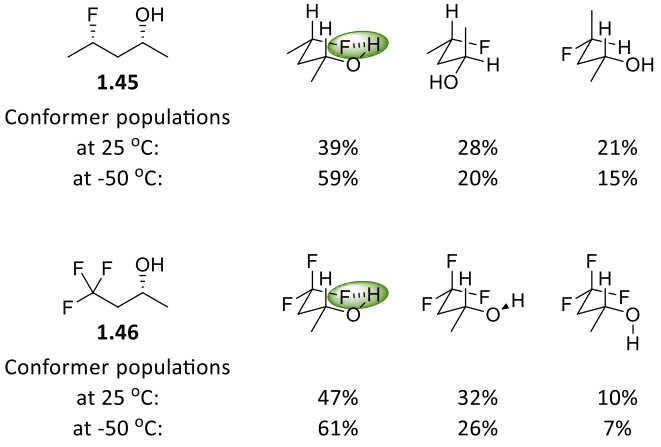
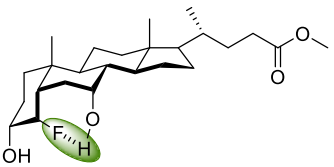


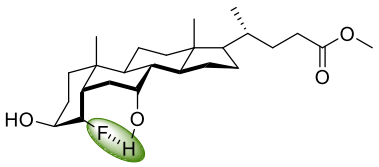
Figure 1.34. γ-fluorohydrins with intramolecular-hydrogen-bound major conformer.[159]

1.3.4.4 Recent unpublished results from within our group of bile acid examples

Synthesised previously within the group were a pair of 3-hydroxy-4α-fluoro CDCA methyl esters **1.47** and **1.48** as intermediates towards the synthesis of BA receptor agonists (*Figure 1.35*). The close proximity of the fluorine and hydrogen atoms suggested that a C-F•••H-O interaction was likely and indeed this hydrogen fluorine bond was confirmed without ambiguity. This section will report these findings.



3α-hydroxyl-4α-fluoro CDCA methyl ester **1.48**



3β-hydroxyl-4α-fluoro CDCA methyl ester **1.49**

Figure 1.35. 4-fluoro and 7α-OH group interactions on CDCA substrates.

1.3.4.4.1 NMR data

Presented in *Table 1.2* are the coupling constants from the $C_7O\underline{H}$ proton signal of 3-alcohol diastereomers **1.48** and **1.49**, as observed by ^1H NMR. In all cases and for both compounds, the signal was observed around 3 ppm as a doublet of doublets.

	3α-hydroxy **1.48**		3β-hydroxy **1.49**	
Solvent	$^1J_{OH\text{-}F}$	$^3J_{OH\text{-}H}$	$^1J_{OH\text{-}F}$	$^3J_{OH\text{-}H}$
$CDCl_3$	39.4	11.8	35.0	11.2
[D_6]-Acetone	38.9*	11.6*	31.8	10.6
[D_3]-Acetonitrile	38.2*	11.4*	30.4	10.4
[D_6]-DMSO	35.3*	10.3*	(17.9)	(6.7)

*Table 1.2. A solvent study of $^1J_{OH\text{-}F}$ and $^1J_{OH\text{-}H}$ coupling constants for $C_7O\underline{H}$ for **1.48** and **1.49**.*[iv]

In all solvents and for both the direct (^1J) and indirect (^3J) measurements of the C-F•••H-O interaction, the coupling constants recorded for compound **1.48** were greater than for compound **1.49**. Notably, the $^1J_{OH\text{-}F}$ value of 39.4 Hz is vast. To the best of our knowledge, it is almost the largest intramolecular C-F•••H-O interaction ever recorded, second only to the tricyclic cage structure from Lectka (see: *Section 1.3.4.2*).

This systematic variation between diastereomers **1.48** and **1.49** can be rationalised by considering the electron withdrawing effects (orbital interactions) of the 3β-substituent in either molecule and how they affect the HBA capacity of the 4α-fluoro atom. Firstly for **1.48** with hydrogen in the 3β-position, hyperconjugation of electron density from $\sigma_{C\text{-}H}$ to $\sigma^*_{C\text{-}F}$ is possible and strengthens the HBA capacity of the 4α-fluoro atom. By contrast for **1.49** with hydroxyl in the 3β-position, the electron withdrawing power of oxygen attenuates the HBA capacity of the antiperiplanar 4α-fluoro atom and we therefore observe a C-F•••H-O interaction ($^1J_{OH\text{-}F}$) of lesser magnitude.

[iv] All values reported in Hz. Those in parenthesis were recorded at 80 °C to avoid signal overlap of C-O$\underline{H}$. Those marked with an asterisk were recorded on the 24-COOH analogue owing to a lack of 24-COOMe compound in-hand; the change of functional group was found (by computational simulations) to bear no effect on the results. A D_2O experiment confirmed the assignment of the signal to $C_7O\underline{H}$ for both compounds and a fluorine decoupled ^1H NMR experiment confirmed the larger coupling (of the dd) was to fluorine at the 4-position.

Unlike the majority of fluorine hydrogen bonds reported in *Sections 1.3.4.2 and 1.3.4.3*, acquiring the NMR in a polar solvent (with strong HBA capacity) did not entirely eliminate this interaction (*Table 1.2*). Rather, for both **1.48** and **1.49**, a IMHB still existed even in deuterated DMSO though to a lesser extent (indicated by a smaller $^{1}J_{OH\text{-}F}$). Furthermore, the greater IMHB strength of compound **1.48** proved more resistive to competing HBA solvent and a $\Delta^{1}J_{OH\text{-}F}$ of only 1.2 Hz was observed when changing the solvent from deuterated chloroform to acetonitrile; by comparison for **1.49**, $\Delta^{1}J_{OH\text{-}F}$ was 4.6 Hz.

For all cases except for compound **1.49** in deuterated DMSO, the magnitude of the $^{3}J_{OH\text{-}H}$ coupling to $H_{7\beta}$ suggests an antiperiplanar relationship and a distinct lack of free rotation of the C_{7}-O bond, tethered by the interaction to $F_{4\alpha}$; non-bile acid examples of this rigid motif can be found in *Section 1.3.4.2*.

Considering the ^{19}F NMR of both compounds (not shown), **1.48** exhibits a well-defined triplet of triplets with coupling constants of 52.5 Hz ($^{2}J_{F\text{-}H}$ to geminal $H_{4\beta}$ and $^{3}J_{F\text{-}H}$ to axial H_{5}) and 36.0 Hz ($^{3}J_{F\text{-}H}$ to axial $H_{3\beta}$, attenuated by $HO_{3\alpha}$, and $^{1}J_{F\text{-}OH}$ to $C_{7}O\underline{H}$). Unusually, the ^{19}F NMR of **1.49** exhibited only a broad singlet that is yet to be fully explained and with no comparable example in the literature. Variation of temperature did not resolve this broad singlet any further.

1.3.4.4.2 Crystallographic data

In contrast to the solution phase data (see: *Section 1.3.4.1.1*), a clear IMHB was observed within the crystal structures of both CDCA derivatives. A short C-F•••H-O distance of only 1.93 Å was measured for 3α-hydroxyl derivative **1.48**. Surprisingly, an even shorter distance of 1.90 Å was measured for 3β-diastereomer **1.49** despite the reported lesser hydrogen bond strength, according to NMR data. Nevertheless, these lengths are shorter than the combined van der Waals radii of fluorine and hydrogen (2.67 Å) and the figure of 2.35 Å described by Howard as required for efficient hydrogen bonding. These data represent some of the shortest fluorine hydrogen bonds reported in the solid state.

1.3.4.4.3 Computational prediction of hydrogen bond acidity

When in a *trans*-diaxial arrangement, the electron withdrawing effect of a fluorine atom reduces the electron density at the oxygen of a vicinal hydroxyl group and hence leads to greater hydrogen bond acidity (pK_{AHY}) as illustrated by examples **1.28** and **1.26** (*Figure 1.36*) (see: *Section 1.3.3.1* for further examples). For the C-F•••H-O interaction (IMHB) within compound **1.49**, electron density from fluorine will be donated into the $\sigma^{*}_{C7O\text{-}H}$ orbital and this will give the fluorine atom a greater electron withdrawing effect in context of the existing 3β-hydroxy-4α-fluoro motif. The computationally predicted pK_{AHY} of **1.49** of 1.67 is therefore higher than for the isolated *trans*-

diaxial fluorohydrin motif of **1.26** and far surpasses the hydrogen bond acidity of any rigid fluorinated cyclohexanols previously reported.

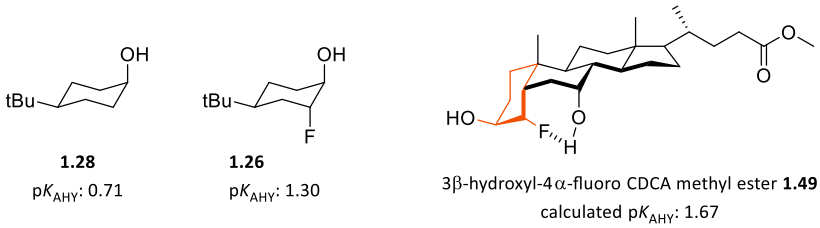


*Figure 1.36. The IMHB of **1.49** is predicted to increase the pK_{AHY} of the 3β-OH group.*

1.3.5 Literature precedence of fluorinated bile acid syntheses

While fluorinated examples of cholestane derivatives are commonplace in steroidal literature,[160-162] examples of fluorinated bile acids are far rarer. Notably, most fluorinated bile acid publications focus on the installation of a C-F bond or CF_2 motif at the 3-position and/or 7-position by relatively simple deoxyfluorination reactions (see: *Section 1.3.5.1*). Fluorination at other positions is less common or unprecedented. A brief summary of the existing literature surrounding fluorinated A-ring and B-ring steroids is presented in the following section.

1.3.5.1 3-fluoro and 7-fluoro derivatives

A search of chemical literature databases reports over 200 examples of 3-fluorinated steroids and over 90 examples of 7-fluorinated steroids as of July 2019, but constraining those results to compounds with a *cis*-A,B ring juncture returns only 35 and 15 hits, respectively. Within these results are a handful of examples that describe compounds with fluorine at both aforementioned positions and both CHF and CF_2 motif transformations have been published (*Figure 1.37*).

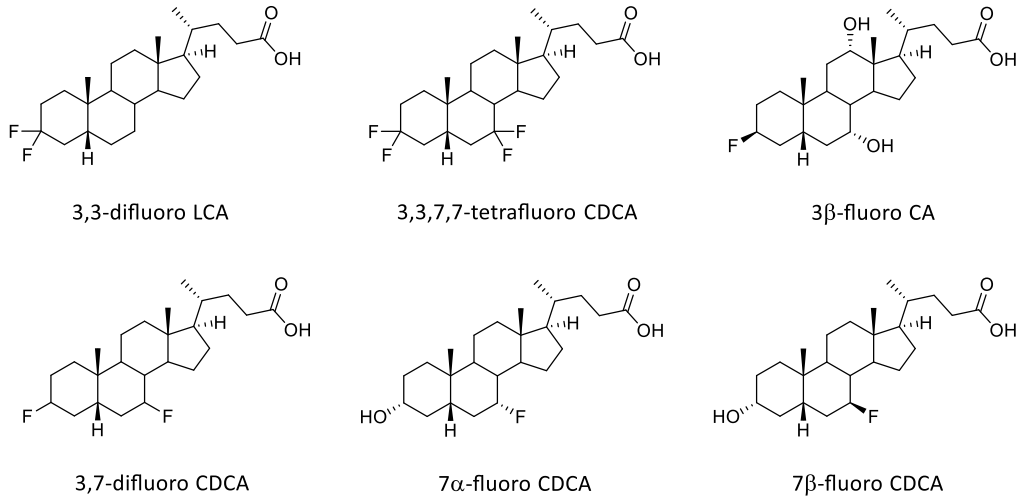


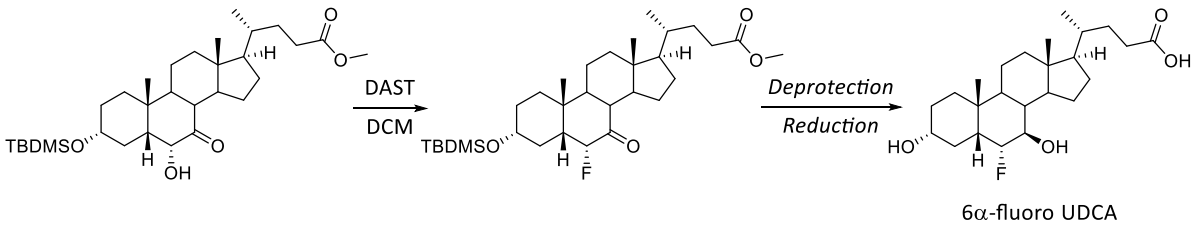
Figure 1.37. 3-fluoro and 7-fluoro BAs in the literature.[85, 163, 164]

The 3,3-difluoro group has been reported exclusively at LCA,[163] and the 3,3,7,7-tetrafluoro motif also reported.[164] Reported also at CDCA is the 3,7-difluorination pattern (though without stereochemical assignment),[164] and both diastereoisomers of monofluorination at the 7-position.[85, 164] 3β-Fluoro cholic acid is also reported, but the corresponding 3α-diastereomer is not.[165] Absent from the literature are 7,7-difluorinated bile acids (bearing only the 7,7-difluoro motif) and a reliable publication of a 3α-fluorinated bile acid. All other 'hits' reported by literature search engines for 3-position and 7-position fluorinated BA's are intermediates towards those compounds presented in *Figure 1.37*.

1.3.5.2 6-fluoro derivatives

Introduction of fluorine to the 6-position could involve the neighbouring 7-position; a 7-keto derivative would provide a handle for reaction of the nucleophilic 6-position with a source of electrophilic fluoride. For HDCA, akin to 3-and 7-position fluorination reactions on primary BAs, deoxyfluorination of the existing 6α-OH group (or keto derivative) could yield 6-fluorinated compounds.[166]

A literature search for 6-fluorinated steroids returned around 90 results and restriction to those only on BA structures narrowed this figure to 35. Notably, the first introduction of fluorine to the 6-position (6α-fluoro UDCA) was reported by Pellicciari in 1995 and involved the treatment of a 6α-hydroxy-7-keto BA with DAST (*Scheme 1.2*). Interestingly, the reaction (S_N2, with nucleophilic fluoride, see: *Section 1.3.2*) proceeded without apparent inversion of the 6-positon and we believe that *in-situ* epimerisation to the more stable and equatorial C-F bond is the likely explanation.

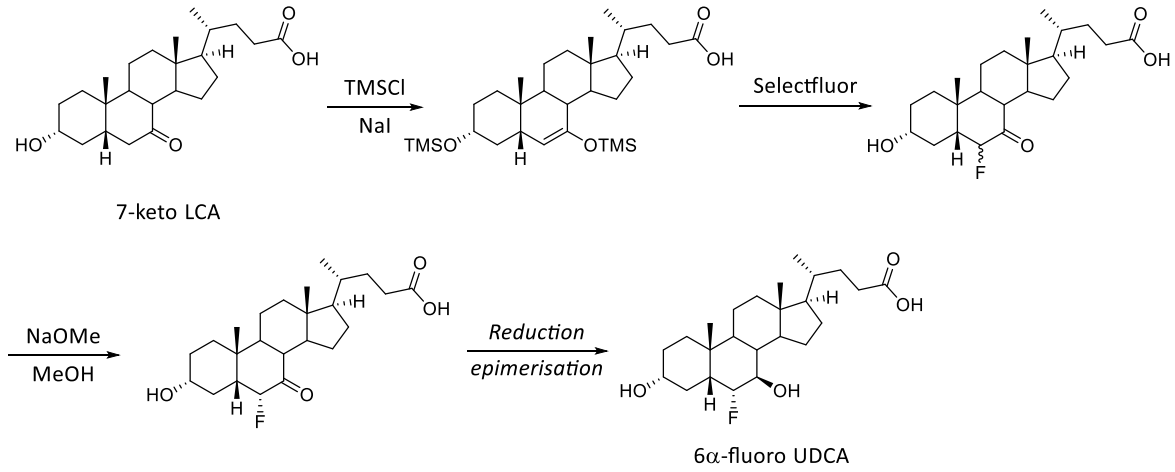


Scheme 1.2. Synthesis of 6α-fluoro UDCA by Pellicciari.[166]

The only search result of corresponding diastereomer 6β-fluoro UDCA is a subsequent paper by Pellicciari in 2002.[167] Reported is the stereochemical interconversion of the 7-OH group for 6-fluoro UDCA/CDCA compounds with the oxio-reductive bacteria *Xanthomonas maltophalia*.[167]

Synthesis of 6α-fluoro UDCA was also reported in 2002 by Prasad and significant improvement to the yield was achieved by electrophilic fluorination via the corresponding silyl enol ether.[168] A mixture of 6-fluoro diastereomers was yielded but epimerisation with sodium methoxide gave only the 6α-fluoro compound (*Scheme 1.3*). Then, stereoselective reduction of the 7-position with

platinum (IV) oxide gave the α-alcohol product (CDCA derivative, not shown) but epimerisation was lastly performed involving KO_2 and TDA-1 to yield 6α-fluoro UDCA.



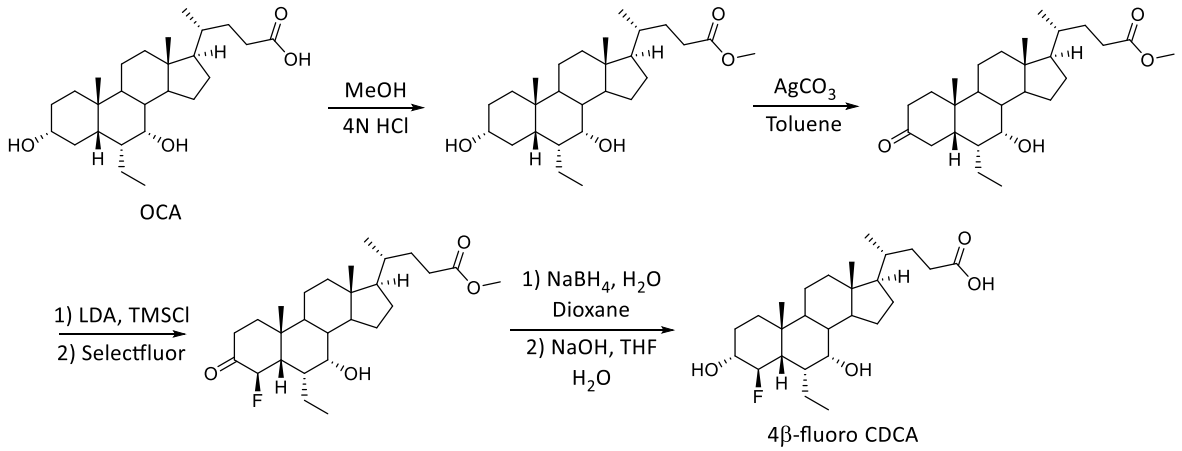
Scheme 1.3. 'Improved' synthesis of 6α-fluoro UDCA by Prasad.[168]

No results for 6,6-difluoro compounds were found across the literature. Otherwise, only advanced intermediates towards those compounds already described, were found.

1.3.5.3 4-fluoro derivatives

Installation of a C-F bond at the 4-position, like at the 6-position, could involve reaction with electrophilic fluoride after the deprotonation of an adjacent α-ketone group.

A search of chemical literature databases returned only 17 examples of steroidal 4-fluorinated compounds. After specifying the BA *cis*-A,B ring juncture, the search returned only three results, all from a patent by the Jiangsu Hansoh Pharmaceutical Group in 2018 (*Scheme 1.4*).[169] The narrative of the work was to synthesise bioactive analogues to target the FXR receptor and disclosed was the synthesis of 4β-fluoro OCA that mirrored our own-group strategy on CDCA (unpublished work). This route features a deprotonation of the 3-ketone with LDA and isolation of the resultant silyl enol ether(s), however the regioselectivity of this step is not described. The crude silyl enol ether(s) are then treated with Selectfluor® and only the desired 4β-fluoro regio-/stereoisomer is isolated.



Scheme 1.4. Synthesis of 4β-fluoro OCA by Jiangsu Hansoh Pharmaceutical Group.[169]

The 4β-fluoro motif has not been published on any other BA substrate, and 4α-fluoro BAs and 4,4-difluoro BAs remain entirely novel.

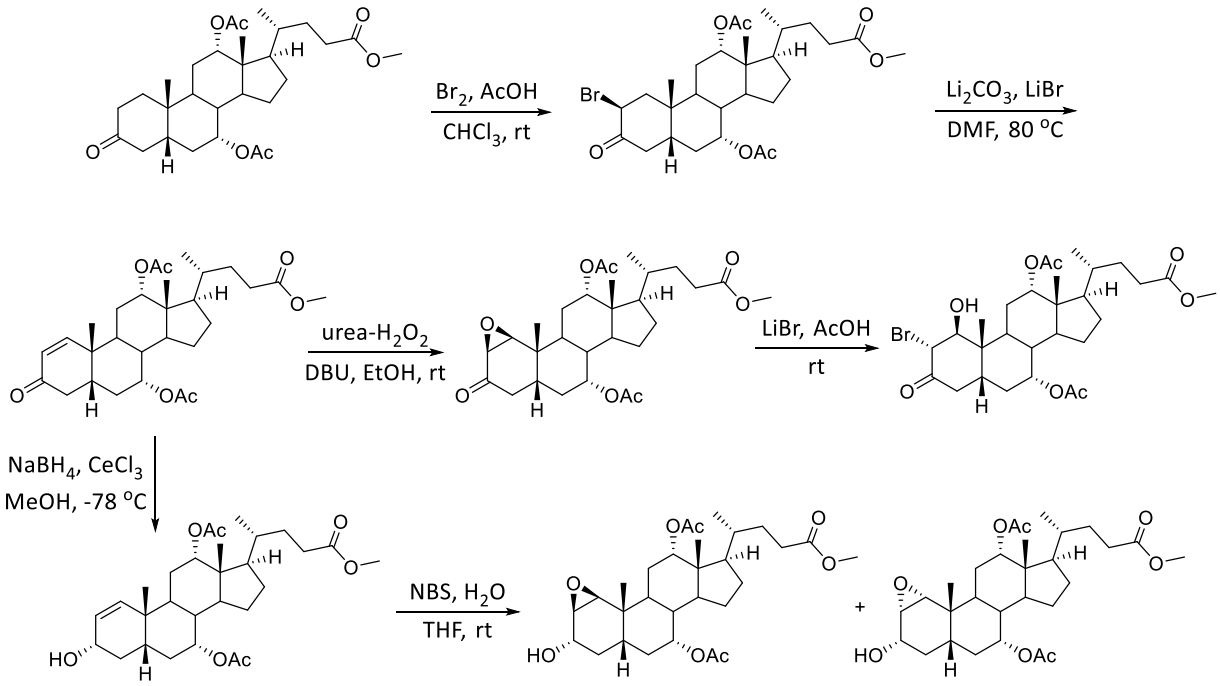
1.3.5.4 1-fluoro and 2-fluoro derivatives

Searching of chemical literature databases for 1-fluorinated steroids returned 5 results but only cholestane substrates were described. 1-Fluorinated bile acids are therefore novel, as of July 2019.

Equally, a search for 2-fluorinated steroids returned over 100 examples but all were found to be of cholestane skeleton. 2-Fluorinated bile acids are therefore also novel.

1.3.5.5 Carreira advanced intermediates at the A-ring.

While Carreira and colleagues are yet to introduce fluorine to bile acids, much of their chemistry involves the introduction of bromine, especially at the 1-position and 2-position.[170] Their published methods could be readily transferrable in our own work towards 1-fluorinated and 2-fluorinated OCA derivatives, specifically the synthesis of advanced epoxide intermediates. For example, an *O*-protected cholic acid derivative was brominated at the 2-position and subsequent treatment with lithium carbonate under heating furnished a C=C bond between carbons one and two (*Scheme 1.5***Error! Reference source not found.**). Epoxidation of the enone gave the corresponding 1β,2β-epoxide and opening with nucleophilic bromide yielded a vicinal bromohydrin. Additionally, with the enone in-hand, reduction of the 3-position and epoxidation of the resultant allylic 3α-alcohol substrate gave a mixture of epoxide diastereomers; opening of these epoxide diastereomers was however not reported.



Scheme 1.5. Synthesis of advanced A-ring BA intermediates by Carreira.[170]

1.4 Project Aims

1.4.1 Synthesis of OCA (NZP084) A-ring analogues to modify adjacent R-OH group interactions within the receptor

Considering the broad range of therapeutic actions possible from targeting the FXR and TGR5, the synthesis of selective agonists is of interest. Often, bile acid modifications have focussed on reactions at the side chain or simple deoxyfluorination reactions of carbon-oxygen bonds (see: *Section 1.3.5.1*), but there is clear scope for the reactions at other sites, specifically those on the A-ring.

The evidence presented in *Section 1.3.3* highlights fluorination as a handle in modifying a range of physiochemical properties that are relevant to drug molecules. Specifically, influences on pK_{AHY} at the 3α-OH group could strengthen the binding of BAs to both the FXR and TGR5. Obeticholic acid (**1.8**) is already potent at both receptor types and further derivatisation can be planned from this advanced bile acid skeleton. As such, the systematic installation of the C-F bond, according to experimentally determined hydrogen bond acidities of rigid cyclohexanols (see: *Section 1.3.3.1*), can be directly applied to the A-ring of OCA (*Figure 1.38*). Notably for 4α-fluoro OCA (**1.55**) we expect a secondary interaction of fluorine with the 7-positon hydroxyl group (see: *Section 1.3.4.4*).

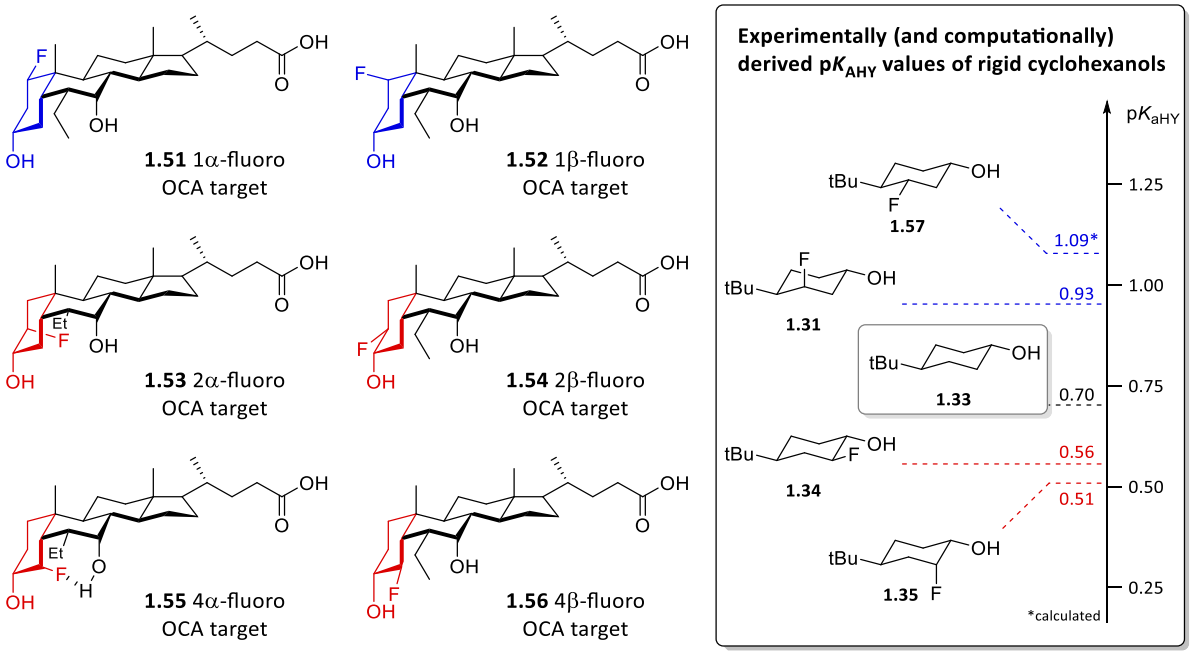


Figure 1.38. Systematic fluorination of the OCA A-ring could selectively modify pK_{AHY}. Structures shown in blue are expected to give an increase to pK_{AHY}, and red a decrease.[127]

Additionally, *in-silico* studies by our industrial collaborators had indicated that a modified side chain could explore an apolar pocket within the FXR binding domain, thereby giving potentially greater biological activity (unpublished). Hence, any targets (such as **1.51**-**1.56**, or others) that exhibit promising biological activity will undergo conversion to their corresponding aromatic-substituted sulfonyl urea analogue (*Figure 1.39*).

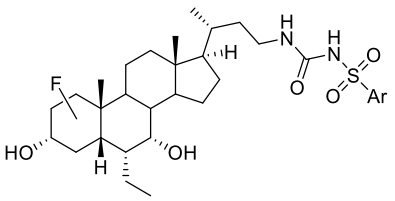
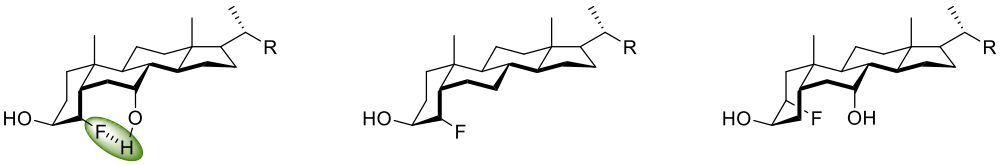


Figure 1.39. General structure of an A-ring fluorinated OCA aromatic-substituted sulfonyl urea analogue to be targeted.

Final compounds that require biological testing will be presented in a blue box and labelled with their corresponding NZP/JED catalogue numbers.

1.4.2 Synthesis of 3β,7α-hydroxyl-4α-fluoro CDCA (NZP318) analogues to investigate the effect of an intramolecular C-F•••H-O hydrogen bond on pK_{aHY}

Exploration of A-ring fluorination on CDCA by a previous group member yielded 4α-fluoro CDCA methyl ester diastereomers **1.48** and **1.49**. An interesting and strong C-F•••H-O interaction was observed (see: *Section 1.3.4.4*) and, as described above, is predicted to enhance the hydrogen bond donating capacity of the 3β-hydroxyl group of **1.49**. To study this in more detail (in collaboration with Dr Jérome Graton of Université de Nantes) and to record an experimental value of pK_{AHY}, approximately 1 gram of material is required. Crucially, other competing hydrogen bond acceptor groups must be removed and so conversion of methyl ester **1.49** to a corresponding C_{17}-alky/alkenyl compound **1.58** (or similar) is required (*Figure 1.40*). A lithocholic acid derivative **1.59** bearing the same A-ring motif, and a CDCA fluorohydrin analogue **1.60** not capable of IMHB, will also be targeted as reference compounds and also with an appropriate side-chain.



1.58 IMHB target compound

1.59 non-IMHB LCA reference compound

1.60 non-IMHB CDCA reference compound

*Figure 1.40. Target **1.58** and reference compounds **1.59**/**1.60** for pK_{AHY} study (R = alky/alkenyl).*

Chapter 2 Synthesis of OCA (NZP084) 1-fluoro analogues

2.1 Introduction

2.1.1 Retrosynthetic analysis

A retrosynthetic analysis of 1α-fluoro OCA (**1.51**) leads to 1β-hydroxyl intermediate **2.1** and then 1β,2β-epoxide **2.2** (*Figure 2.1*). Forward synthesis could be accomplished by epoxide opening with halide (such as bromide) and regioselectivity governs opening of epoxide **2.2** only at the 2-position (*Figure 2.2a*). A weak bond such as the C-Br bond could then be readily reduced to provide 1β-hydroxyl intermediate **2.1**. Nucleophilic deoxyfluorination at the 1-position with fluoride proceeds with inversion of configuration and could provide target **1.51**.

Retrosynthesis of 1β-fluoro OCA (**1.52**) leads to alternate 1α,2α-epoxide diastereomer **2.3**. Forward synthesis by epoxide opening with nucleophilic fluoride is regioselective only at the 1-position (*Figure 2.2b*) and could provide target **1.52** after deoxygenation of the resultant 2α-hydroxyl group.

Both epoxides lead to enone **2.4** as a common precursor and retrosynthesis of this enone leads to 3-keto OCA methyl ester (**2.6**) (not shown). The forward synthesis will follow the bromination/elimination strategy of Carreira who synthesised an analogous DCA enone (see: *Section 1.3.5.5*).[170]

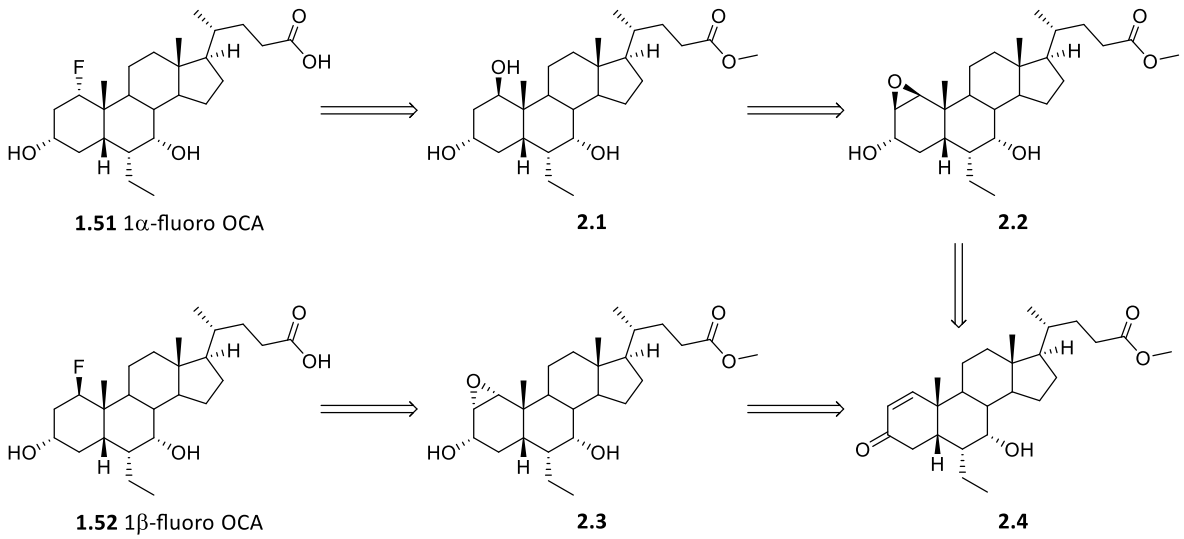


*Figure 2.1. Retrosynthesis of 1-fluoro target diastereomers **1.51** and **1.52**.*

2.1.2 Regioselectivity of epoxide opening

As cyclohexane epoxides open regioselectively to form the *trans*-diaxial product via a chair-like transition state, **2.2** and **2.3** will lead to different regioisomers (*Figure 2.2*). Hence, opening of 1β,2β-

epoxide **2.2** with halide will install the C-X bond only at the 2α-position. Equally, opening of 1α,2α-epoxide **2.3** will proceed only with installation of the C-X bond at the 1β-position.

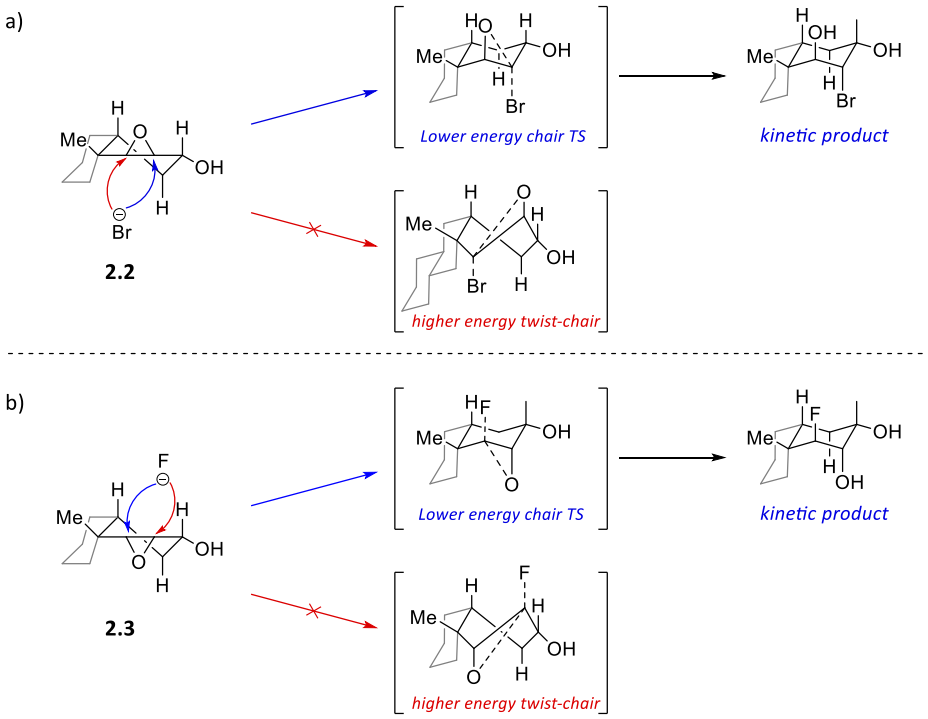


Figure 2.2. Regioselectivity of 1,2-epoxide opening via a chair transition state (blue) but not via a higher energy twist-chair transition state (red).

2.2 Synthesis and epoxidation of advanced enone intermediate 2.4

2.2.1 Selective 3α-OH oxidation

Starting the synthesis from commercially supplied OCA methyl ester (**2.5**), we required a selective oxidation procedure for the 3α-OH group**.** Historical computational analyses revealed that the 7α-OH group is more reactive than the 3α-OH group,[171] but a bulky oxidant can favour reactivity towards the lesser hindered 3-position; reactions are slow at the 7-position because of the adjacent tertiary 8-position. A procedure by Burns involves *N*-oxy 2,2,6,6,-tetramethylpiperidine (TEMPO) and is used in conjunction with sodium hypochlorite to give relatively good yields (60-90%) of the 3-keto product selectively.[172] The mechanism proceeds by a catalytic cycle under basic conditions where the alcohol attacks the sterically hindered oxidant (*Figure 2.3*).

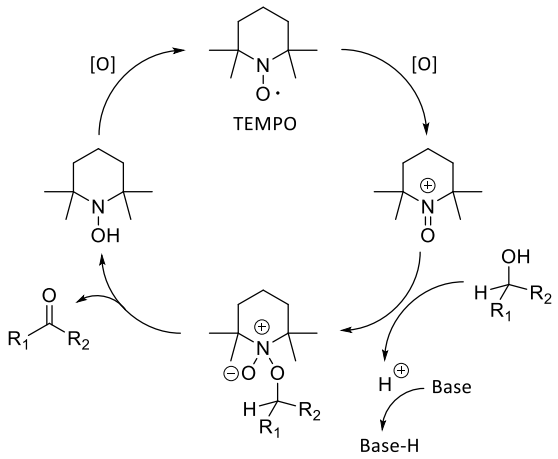
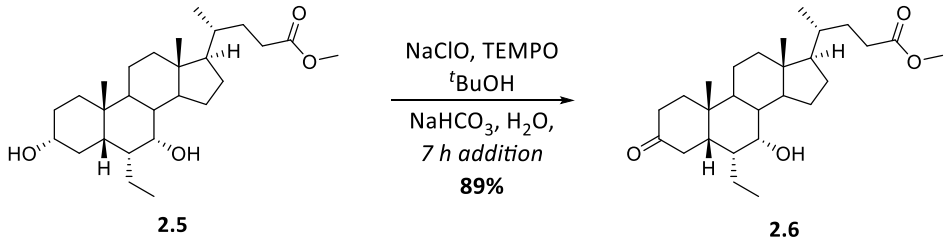


Figure 2.3. Proposed catalytic mechanism for TEMPO oxidation under basic aqueous conditions. [O] denotes secondary oxidant NaClO.[173]

The oxidation of OCA methyl ester (**2.5**) proceeded well and gave a very good yield of the 3-keto product **2.6** as a single regioisomer (*Scheme 2.1*). Bleach solution was added dropwise over 7 hours.



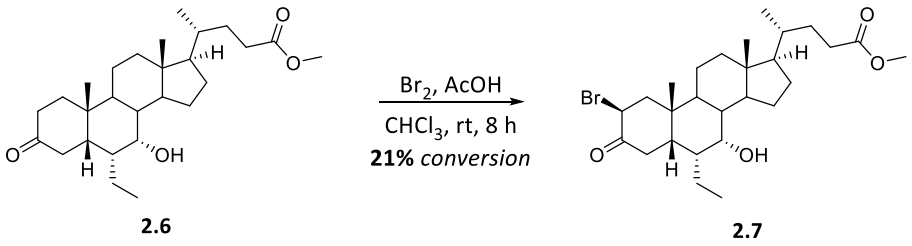
*Scheme 2.1. Regioselective 3α-OH oxidation of OCA methyl ester (**2.5**).*

2.2.2 Synthesis towards enone 2.4 via an α-bromoketone

2.2.2.1 Direct α-bromination

Within the group, reactions at the α-positions of the 3-keto group have been found to proceed with complete stereoselectivity towards the convex β-face due to steric hindrance at the concave α-face.[174] This observation is also reported by Carreira for the direct α-bromination of a 3-keto DCA derivative (see: *Section 1.3.5.5*), where the 2β-bromo group served as a handle for *syn*-elimination of the α-bromoketone.

In our own hands on 3-keto OCA methyl ester (**2.6**), the Carreira conditions of direct α-bromination gave poor conversion, determined by the emergence of product **2.7** by ^{1}H NMR (*Scheme 2.2*). No trace of the corresponding 4-bromo regioisomer (not shown) was found from ^{1}H NMR analysis of the crude material.

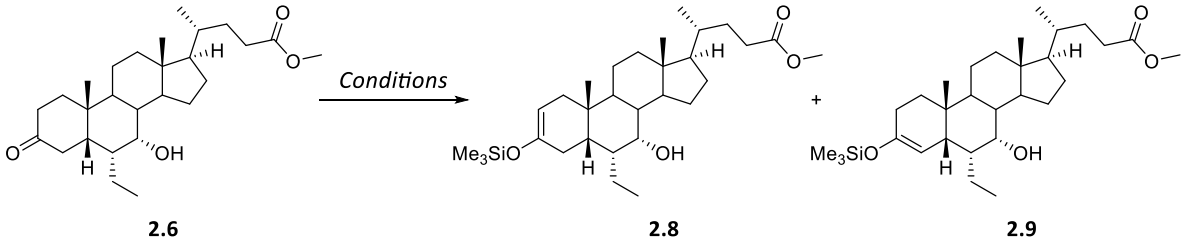


*Scheme 2.2. Direct α-bromination of 3-keto OCA methyl ester (**2.6**).*

2.2.2.2 Bromination via silyl enol ether

2.2.2.2.1 Silyl enol ether formation

A two-step silyl enol ether method could install the required 2-bromo moiety. Literature precedence exists for regioselective Δ2,3-silyl enol ether formation with trimethylsilyl chloride (TMSCl) and triethylamine.[175] Furtherment of this work involved the addition of sodium iodide to the reaction.[176] However, work by a previous member of the group with a CDCA substrate[174] showed that silyl enol ether formation was only successful with the more reactive trimethylsilyl trifluoromethanesulfonate (TMSOTf).[177] The application to my own 3-keto **2.6** is shown in *Table 2.1*.



Entry	Reagent	Base	Temp. / °C	Scale / mg	Conversion
1	TMSOTf (~1.1 equiv)	Et_3N (~2.0 equiv)	0 (7 h)	100	39% **2.8** 45% **2.9** 5% 7-OTMS **2.6**
2	TMSOTf (~1.1 equiv)	Et_3N (~2.0 equiv)	0 (1 h) rt (18 h)	100	0% **2.8** 0% **2.9** 40% 7-OTMS **2.6**
3	TMSCl (~5.0 equiv)	*n*-BuLi (~5.0 equiv)	-78 (1 h)	200	11% **2.8** 75% **2.9** 17% 7-OTMS **2.6**

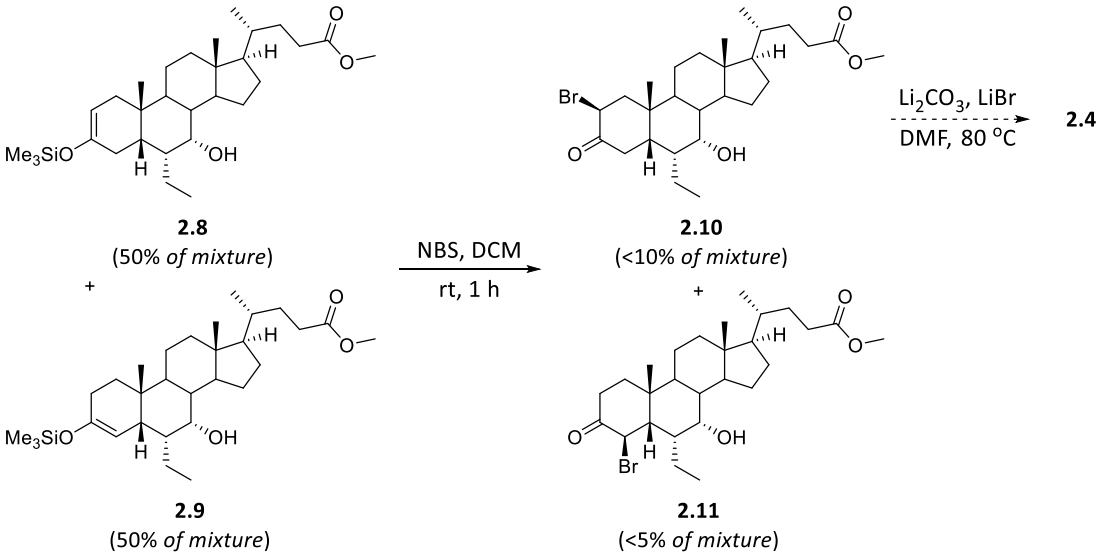
*Table 2.1. Screening of conditions for silyl enol ether formation from 3-keto derivative **2.6**.*

The conversion of the reaction was monitored by ^{1}H NMR by the emergence of characteristic product signals. For **2.9**, only coupling of H_4 (δ 4.87 ppm) to H_5 is possible and there is a 90° angle between these C-H bonds; no coupling constant is observed, hence this signal is a singlet. For compound **2.8**, a 90° interaction is also present between H_2 (δ 4.68 ppm) and $H_{1\beta}$, but a gauche interaction with $H_{1\alpha}$ gives this signal a characteristic doublet, and of 5.3 Hz.

Performing the reaction at 0 °C, an approximate 1:1 ratio of silyl enol ether regioisomers was observed after 7 hours alongside a small percentage of 7α-OTMS byproduct (*Entry 1*). Allowing the reaction to warm to rt overnight led to neither silyl enol ether regioisomer being found in the crude reaction mixture, but instead a greater proportion of byproduct from protection of the 7-position (*Entry 2*). The use of more powerful base *n*-BuLi led to the formation of the undesired Δ3,4-silyl enol ether regioisomer in excellent yield, with a small percentage of the 7α-OTMS protected starting material (**2.6**) as a byproduct observed once again (*Entry 3*).

2.2.2.2.2 Bromination

A ~1:1 mixture of silyl enol ether regioisomers **2.8** and **2.9** was prepared according to conditions outlined in *Table 2.1* and taken forward without purification (to avoid the inherent instability issues of silyl enol ethers when on silica). Reaction with *N*-bromosuccinimide (NBS) led to the expected β-stereoselectivity at both positions (*Scheme 2.3*), however in poor yield. The planned subsequent elimination of the 2β-bromo product **2.10** towards enone **2.4** according to Carreira conditions (see: *Section 1.3.5.5*) was therefore abandoned.



*Scheme 2.3. Reaction of silyl enol ethers **2.8** and **2.9** with electrophilic bromide (NBS).*

The conversion of the reaction was monitored by ^{1}H NMR by the emergence of characteristic product signals. For bromohydrin **2.10** the signal for $H_{2\alpha}$ was identified as a dd at δ 4.80 ppm with coupling constants of 14.2 Hz to $H_{1\beta}$ and of 5.1 Hz to $H_{1\alpha}$. For bromohydrin **2.11** the signal for $H_{4\alpha}$ was identified as a doublet at δ 4.67 ppm with a coupling constant of 5.4 Hz to H_5.

2.2.3 Synthesis of enone 2.4 via selenoxide elimination

The *syn*-elimination reaction of selenoxides was first described by Sharpless in 1973 and has since become a valuable one-pot pathway for the generation of olefins and α,β-unsaturated ketones.[178]

The mechanism of enone formation proceeds via a 5-membered coplanar transition state involving the α- and β-carbons, the selenoxide motif and *syn*-hydrogen atom (*Figure 2.4*).[81] The steric bulk of arylselenium reagents was expected to provide selectivity towards the lesser-hindered 2-position, as illustrated in the figure.

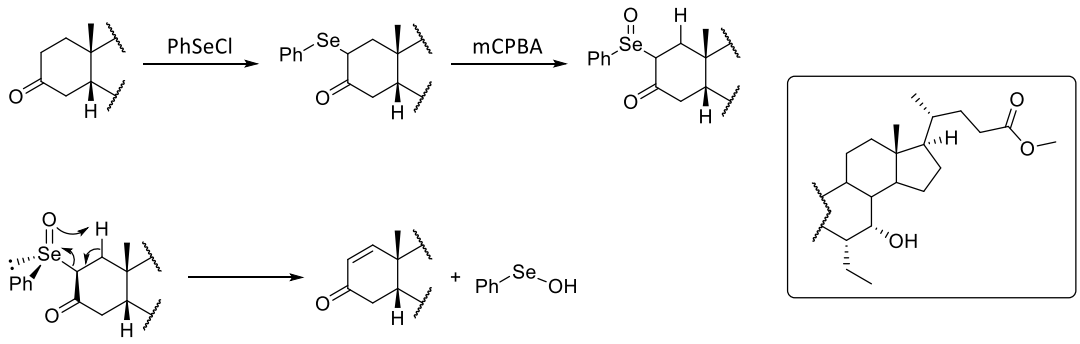
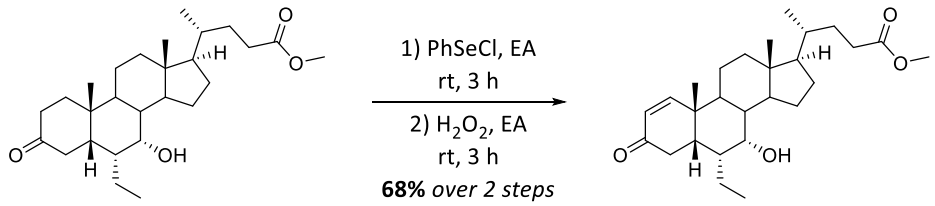


Figure 2.4. Mechanism of selenoxide elimination demonstrated at the bile acid 3-keto A-ring.

Hence, enone **2.4** was prepared from 3-keto derivative **2.6** in a good yield that was reproducible on scales of 100 mg to 2 g (*Scheme 2.4*). No base was needed for this reaction. The starting ketone and desired enone were however inseparable, even by HPLC purification, so were taken forward to the next reaction as a mixture.



2.6 **2.4**

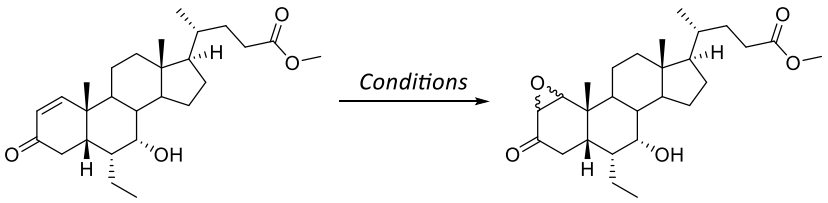
*Scheme 2.4. One pot α-selenation/elimination of 3-keto derivative **2.6** to provide target enone **2.4**.*

It was noted in all cases that starting ketone **2.6** was never entirely consumed, even at higher temperature, and the use of >1 equivalent of PhSeCl led to side reactions at the 4-position. Strictly 1 equivalent of *m*CPBA was used, as ketone **2.6** is susceptible to Baeyer-Villiger oxidation, and no evidence of lactone formation was observed. No epoxidation of target enone **2.4** by unreacted hydrogen peroxide was observed, which was attributed to fast selenoxide oxidation before elimination.[179]

2.2.4 Epoxidation studies of enone 2.4

2.2.4.1 Nucleophilic epoxidation

With enone **2.4** in-hand, epoxidation attempts were made with nucleophilic hydrogen peroxide according to the original work of Carreira.[170] Despite his reported success on CDCA substrates, our own work on OCA was unsuccessful and only starting material was observed (*Table 2.2*).



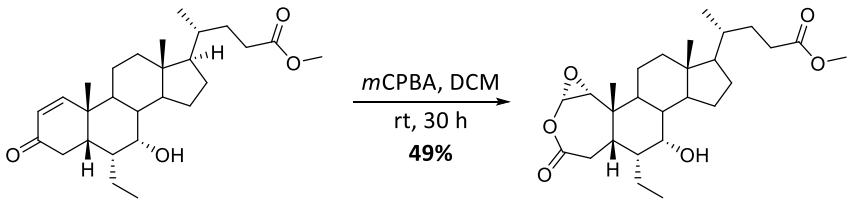
2.4 **2.12** α-epoxide **2.13** β-epoxide

Entry	Oxidant	Base	Solvent	Conversion / %
1	H_2O_2/urea complex	DBU	EtOH	0
2	H_2O_2/urea complex	DBU	THF	0
3	H_2O_2 solution	NaOH	MeOH	0

*Table 2.2. Epoxidation attempts of enone **2.4**.*[v]

2.2.4.2 Electrophilic epoxidation

Failure to yield either desired epoxide diastereoisomer **2.12** or **2.13** by published nucleophilic methods prompted us to consider electrophilic epoxidation. Reaction with enone **2.4** with 1-4 equivalents of *m*CPBA on 100 mg scale however gave lactone **2.14** as the only product as a result of successive epoxidation and Baeyer-Villiger reactions (*Scheme 2.5*).



2.4 **2.14**

*Scheme 2.5. Reaction of enone **2.4** and mCPBA gave lactone **2.14** as the only product.*

Confirmation of the product structure was attained from a single crystal XRD structure and the epoxide motif was found to exhibit α-stereochemistry (*Figure 2.5*, thermal ellipsoids drawn at the 50% probability level).

[v] Reactions were performed on 100 mg scale and conversion monitored by ^{1}H NMR for the disappearance of alkenyl proton signals: : δ 6.81 (d, *J* = 10.3 Hz, **2.4**-H_1); 5.89 (d, *J* = 10.2 Hz, **2.4**-H_2) ppm.

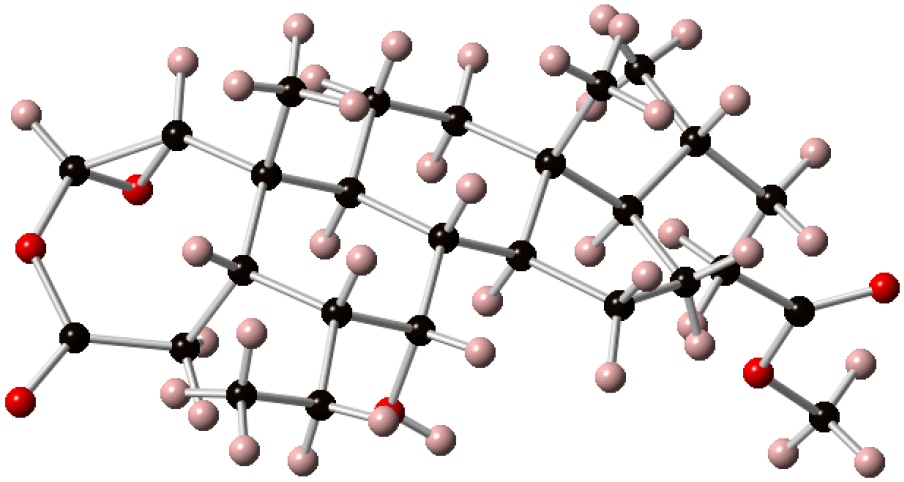


*Figure 2.5. Single crystal X-ray structure of lactone **2.14**.*

2.3 Synthesis of allylic alcohol derivatives

2.3.1 Screening of 3-keto reduction conditions

2.3.1.1 Introduction

The outcome of stereoselective reduction reactions of rigid cyclohexanones can be predicted. Typically, the axial alcohol product can be accessed using a bulky hydride source to deliver hydride at the lesser hindered α-face (along the equatorial trajectory, blue - *Figure 2.6*). Our group has described this reduction selectivity towards axial alcohols on rigid fluorinated cyclohexanone substrates.[127]

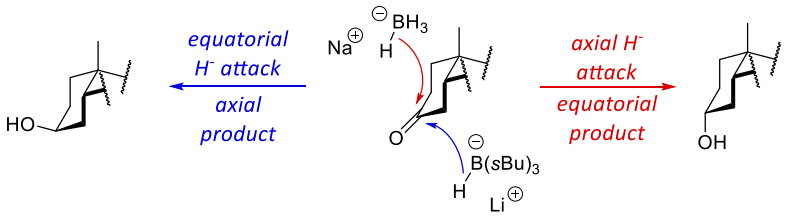


Figure 2.6. Stereoselectivity of hydride attack at rigid cyclohexanone systems is governed by the steric bulk of the hydride source.

The stereochemical outcome of cyclohexanone reduction with non-bulky hydride sources can be predicted by two arguments. The first argument is one based on electronics, and considers the relative stability of the reaction intermediates; the Cieplak effect. Considering the hyperconjugation that is possible involving the newly formed C-H σ* orbital with a parallel σ orbital (or orbitals),[180] because a C-H σ bond is a better electron donor than a C-C σ-bond, the reaction will favour the product where hyperconjugation is to C-H bonds rather than C-C bonds (*Figure 2.7a*). This occurs when hydride attack is at the axial position (to provide the equatorial alcohol, red - *Figure 2.6*). The

second argument to explain the predicted outcome of the reaction when using non-bulky hydride sources considers sterics, specifically the C=O bond torsion angle and the energy of the transition state during hydride attack. The sp^2 hybridised C=O bond on a rigid cyclohexanone has a trajectory of only 4° rotated away from the equatorial α-substituent and towards the direction of the corresponding equatorial α-substituent (*Figure 2.7b*). During equatorial hydride attack, the sp^2 carbon centre will transition to sp^3 hybridisation but must proceed *via* an eclipsing interaction with the proximal equatorial substituent (blue curved arrow in the figure). By contrast, hydride attack from the axial trajectory will not involve a transition state with an eclipsing C=O interaction (red curved arrow in the figure), so this pathway will require a lower activation energy.

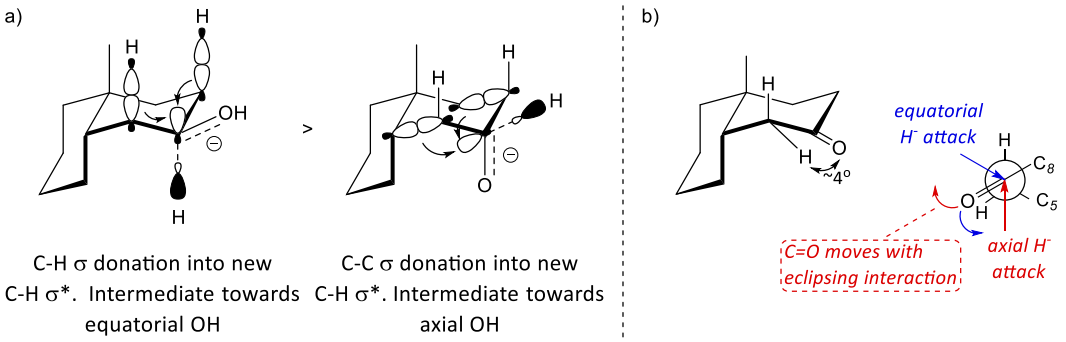
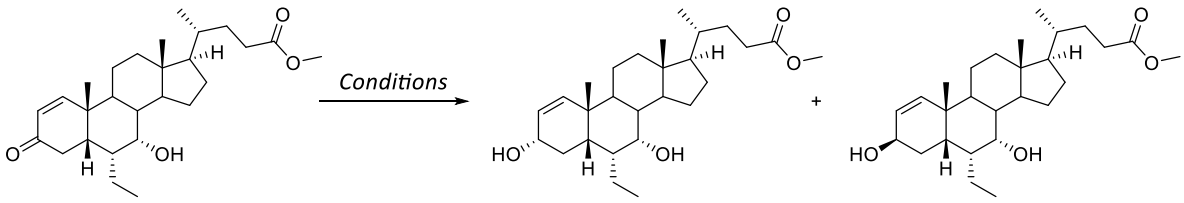


Figure 2.7. a) the Cieplak effect, predicting stereochemical outcome of reduction reactions with non-bulky hydride sources by comparison of hyperconjugation-stabilisation of the intermediates;[180] b) attack along the equatorial trajectory would require the C=O bond and a C-H bond to eclipse during formation of the product (blue arrow).

2.3.1.2 Chemistry

In addition to the epoxidation of a CDCA enone substrate, epoxidation of the corresponding allylic alcohol is also reported by Carreira (see: *Section 1.3.5.5*), and was investigated here. The synthesis of the required allylic alcohol substrates was achieved by Luche reduction of OCA enone **2.4** in excellent yield on milligram and gram scale, to afford allylic alcohol **2.15** cleanly as a single diastereomer in all attempts (*Entry 1, Table 2.3*). The rapid reaction was significantly slowed in the absence of $CeCl_3$ and when in THF, with only 50% conversion observed after 3 days at rt but maintaining selectivity towards the single α-diastereomer (*Entry 2*). For reduction of **2.4** with bulky *L*-selectride, **2.16** is the expected product. To our surprise, the reaction gave α-diastereomer **2.15** as the single product (*Entry 3*). No trace of conjugate addition products were observed.



2.4 **2.15** **2.16**

Entry	Reagents	Solvent	Temp. / °C	Time / h	Yield
1	$NaBH_4$, $CeCl_3$	MeOH	-78	0.5	86% **2.15** 0% **2.16**
2	$NaBH_4$	THF	rt	72	50% **2.15** 0% **2.16**
3	*L*-selectride	THF	-78	0.5	46% **2.15** 0% **2.16**

Table 2.3. Screening of 3-keto reduction conditions.

Reactions performed on 100 mg scale and their isolated yields reported.

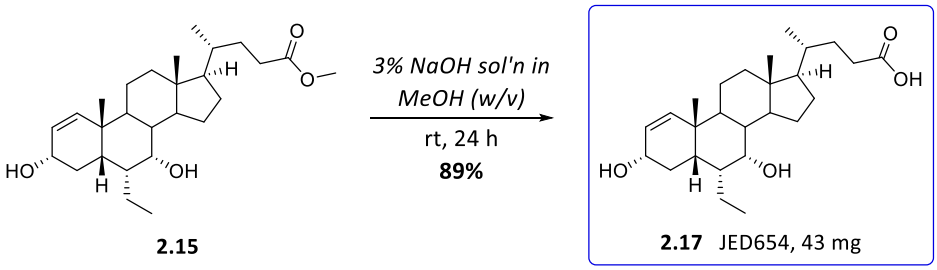
The identity of the product was confirmed by ^{1}H NMR. A distinct *pseudo*-antiperiplanar coupling constant of 7.8 Hz within the $H_{3\beta}$ signal (from coupling to $H_{4\alpha}$) was observed, indicating the formation of compound **2.15**. This coupling constant is smaller than an expected ~12-16 Hz because the structure of the product is a half-chair. A much smaller *pseudo*-gauche coupling constant of 2-5 Hz would be witnessed for corresponding diastereomer **2.16**.

The conditions to reduce enone **2.4** to 3β-hydroxyl diastereomer **2.16** are yet to be discovered. While those conditions presented in *Table 2.3* are not exhaustive, other reducing agents such as lithium aluminium hydride, sodium metal (Bouveault-Blanc reaction) or aluminium isopropoxide are known to reduce esters to primary alcohols and so were avoided.[181-183]

2.3.2 Synthesis of 1,2-enyl OCA (JED654)

The cyclohexene motif at the A-ring will cause a significant change in the shape of the molecule and could be influential to the potency of OCA at the FXR and TGR5. Despite not being included in the original synthetic plan, the activity of this compound was of interest, and a small amount was subjected to ester hydrolysis (*Scheme 2.6*).

Literature precedence for BA methyl ester hydrolysis typically uses very strong basic conditions such as potassium hydroxide in refluxing methanol,[184, 185] but we found saponification of methyl ester **2.15** to be successful overnight at rt with only three percent sodium hydroxide solution (w/v). Target **2.17** was isolated in excellent yield.

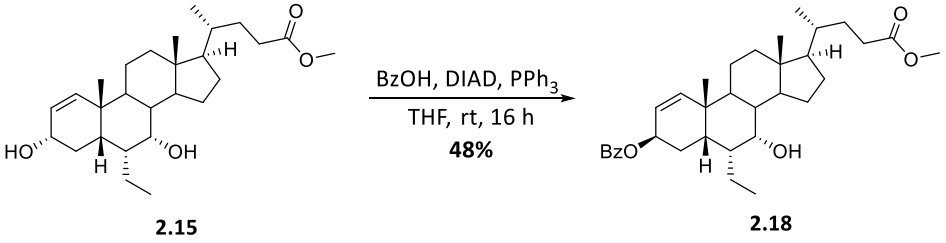


*Scheme 2.6. Saponification of allylic alcohol methyl ester **2.15** to yield target **2.17**.*

2.3.3 Mitsunobu inversion of 3α-hydroxyl diastereomer 2.15

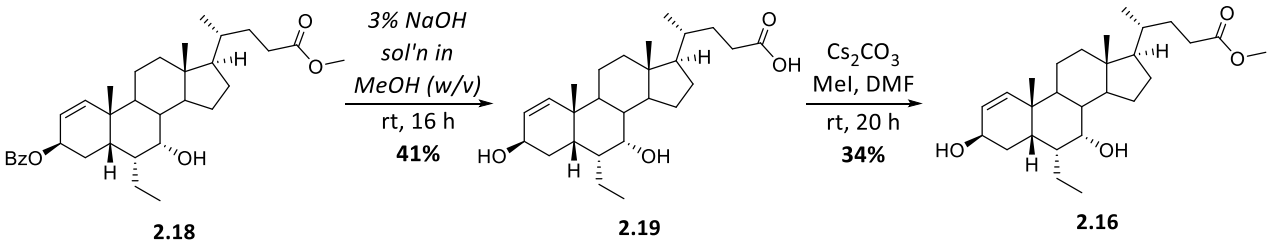
Given the inability to access the allylic 3β-alcohol diastereomer from reduction of parent enone **2.4**, we planned to invert 3α-diastereomer **2.15**. Literature precedence exists for such hydroxyl group epimerisation on bile acid substrates and includes the use of microbes such as *Xanthomonas maltophilia* to invert the 7-OH group,[186] and purely chemical routes have included the use of potassium superoxide on a 7α-mesylate;[187] these however suffer from being costly to set-up and an explosive reagent nature, respectively. Rather, a Mitsunobu reaction at the 7-OH group of a CDCA derivative has been reported in 92% yield and seemed the most favourable conditions to attempt at our own OCA 3-position.[188]

Reaction of allylic 3α-alcohol **2.15** under Mitsunobu conditions yielded 3β-benzoate derivative **2.18** in mediocre yield (*Scheme 2.7*). Additionally, removal of the byproducts (triphenylphosphine oxide, DIAD derivatives) was difficult by column chromatography.



*Scheme 2.7 Mitsunobu inversion of allylic 3α-alcohol **2.15**.*

Hydrolysis of benzoate alcohol **2.18** was successful but also gave hydrolysis of the methyl ester. The carboxylic ester was returned by reaction with caesium carbonate and iodomethane (*Scheme 2.8*).

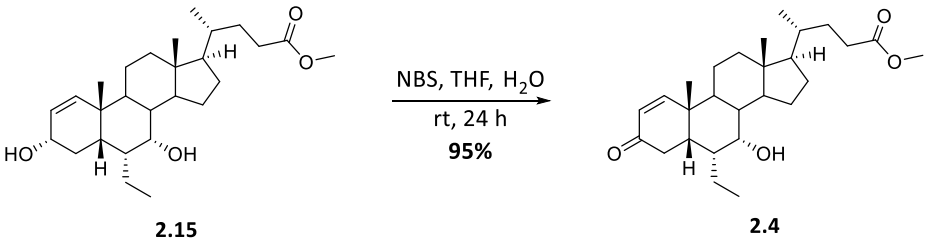


*Scheme 2.8. Global ester saponification and re-installation of the carboxylic ester to yield **2.16**.*

2.4 Epoxidation study of allylic alcohol derivatives

2.4.1 Epoxidation attempts with NBS

Following the work of Carreira (see: *Section 1.3.5.5*), reaction of **2.15** with NBS in a solution of water and THF yielded only preceding enone **2.4**, but cleanly and in high yield (*Scheme 2.9*).

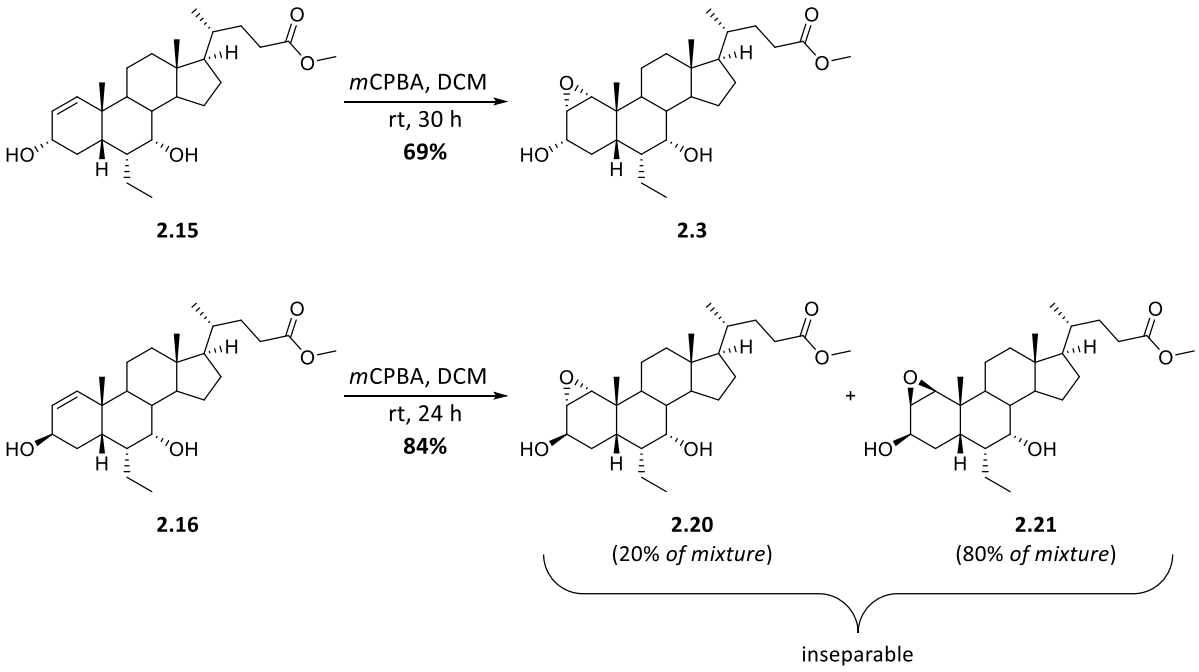


*Scheme 2.9. Treatment of allylic 3α-alcohol with NBS to yield only oxidation product **2.4**.*

2.4.2 Epoxidation with *m*CPBA

2.4.2.1 Epoxidation of free-alcohol diastereomers

Epoxidation was then attempted with *m*CPBA on both allylic 3-OH diastereomers (*Scheme 2.10*). Of interest was the effect of alcohol stereochemistry on the diastereoselectivity of the epoxidation. Epoxidation of α-diastereomer **2.15** gave only one epoxide product (alpha, **2.3**) while epoxidation of β-diastereomer **2.16** gave an inseparable mixture of both epoxide diastereomers **2.20** and **2.21**.



*Scheme 2.10. Epoxidation of allylic alcohol diastereomers **2.15** and **2.16** with mCPBA.*

2.4.2.2 *cis*-Directing effect

First introduced by Henbest and Wilson in 1957,[189] the *cis*-directing effect relies on the peracid acting as a hydrogen bond acceptor of a hydroxyl group, thereby defining the facial attack on the alkene. Its application to **2.15** is shown in *Figure 2.8*.

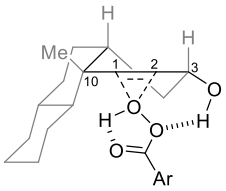


*Figure 2.8. cis-Directing effect of allylic 3α-alcohol **2.15** with mCPBA though hydrogen bond donation/acceptance, leading to only one epoxide diastereomer.*

However, the α-face is however significantly more sterically congested than the β-face (see: *Section 2.2.2.2.2*), which is not consistent with the diastereoselectivities described in *Scheme 2.10*. A possible explanation is the presence of a secondary *cis*-directing effect from the 7-OH group that directs epoxidation of **2.16** to the α-face (*Figure 2.9*). Hence, for **2.15** both directing effects work in tandem to provide only the α-epoxide, but for **2.16** the two competing directing effects lead to the formation of both epoxide diastereomers.

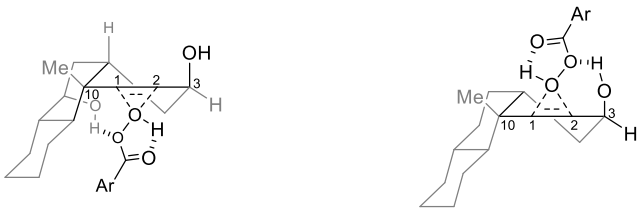


*Figure 2.9. Competing cis-directing effects from both the 3-OH and 7-OH of allylic 3β-alcohol **2.16** yield a mixture of epoxide diastereoisomers **2.20** and **2.21**.*

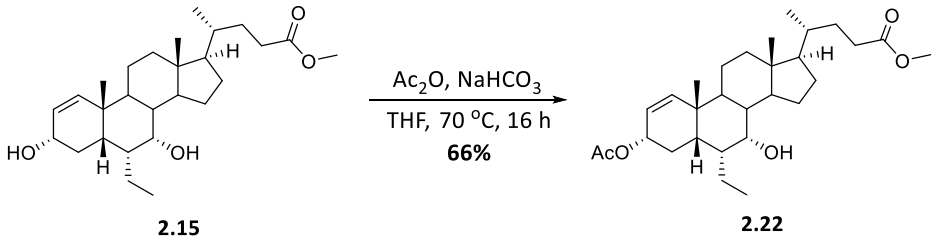
Because the epoxides distort the A-ring away from the perfect chair conformation, the typical axial and equatorial environments of cyclohexanol derivative substituents, and most importantly their distinctive coupling constants, are lost. Illustrated in *Figure 2.8* and *Figure 2.9* are the half-chair structures of the A-rings of compounds **2.15** and **2.16**, where carbons 10, 1, 2, and 3 are found within the same plane. The dihedral angle between the protons at the 2-position and 3-position would be between 50° and 80° and coupling by ^{1}H NMR would be in the region of 2-4 Hz for both diastereomers. NMR was therefore not conclusive in discerning the identity of the epoxide diastereoisomers and rather their assignment was made following epoxide opening with chloride (see: *Section 2.5.2*).

2.4.2.3 Epoxidation of *O*-protected alcohol diastereomers

To further investigate the possible *cis*-directing effect of the 7α-OH group, 3-*O*-protected analogues of both allylic alcohol diastereomers (without the ability to act as HBD to a peracid) were subjected to epoxidation.

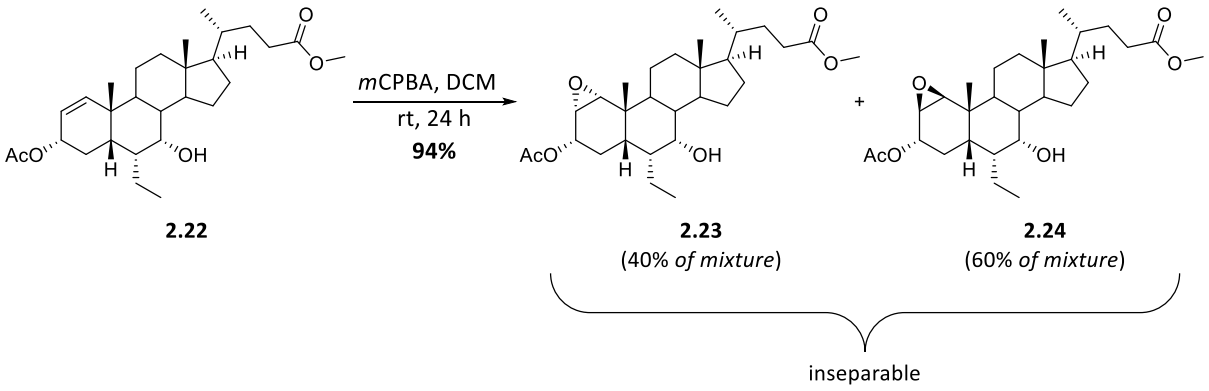
2.4.2.3.1 *O*-protected allylic 3α-alcohol

Regioselective acetate protection of allylic 3α-alcohol **2.15** was performed by reaction with acetic anhydride and potassium bicarbonate under reflux (*Scheme 2.11*). This selective method of *O*-protection was reported by Pellicciari on a CDCA substrate,[190] but the hindrance of the 6-ethyl group in **2.15** was expected to only further support the published regioselectivity. Indeed, no trace of 7α-acetylated product was observed in the crude reaction mixture. The yield of this reaction was below our expectations and without explanation.



*Scheme 2.11. Acetate protection of allylic 3α-alcohol **2.15**.*

Epoxidation of **2.22** with *m*CPBA provided an inseparable mixture of epoxide diastereomers that was now biased towards the β-product (*Scheme 2.12*). Without the *cis*-directing capacity of the 3α-OH group, we see the steric hindrance of the α-face dominate the stereoselectivity of the reaction to give the β-product **2.24** as the expected major diastereoisomer. However, the presence of a *cis*-directing effect from the 7-OH group would be consistent with the formation of the α-product **2.23** (see: *Section 2.4.2.2*).

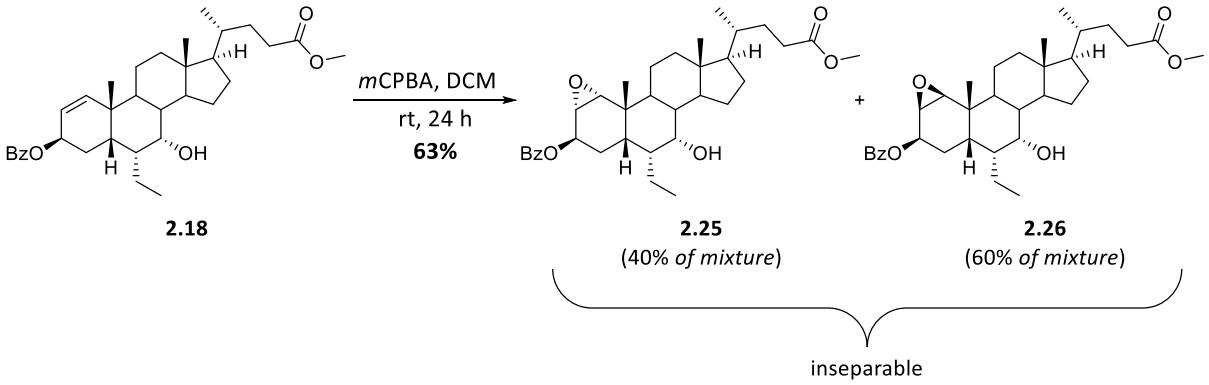


*Scheme 2.12. Epoxidation of O-protected allylic 3α-alcohol **2.22**.*

The assignment of the epoxide diastereoisomers **2.23** and **2.24** was achieved following reaction of one diastereomer with chloride (see: *Section 2.5.3*).

2.4.2.3.2 *O*-protected 3β-alcohol

Treatment of the benzoate **2.18**, the convenient product of the Mitsunobu reaction described above, with *m*CPBA gave an inseparable mixture of epoxide diastereomers (*Scheme 2.13*) in the same 40:60 ratio witnessed for the epoxidation of *O*-protected allylic 3α-alcohol diastereomer **2.22**. It is unexpected that the ratio is the same as for the 3α-acetate, but the β-face would be additionally hindered by the benzoate. The assignment of epoxide diastereomers was based on chemical shift comparison to the epoxide diastereomers **2.23** and **2.24** already in-hand.



*Scheme 2.13. Epoxidation of O-protected allylic 3*β*-alcohol **2.18**.*

2.4.3 Conclusions

Contrary to the seminal work of Carreira, reaction with NBS only resulted in oxidation of the 3-OH group. Epoxidation reactions of free 3-OH substrates with *m*CPBA were strongly influenced by the *cis*-directing effect and gave major epoxide products with the same stereochemistry as the 3-OH group. More so, in the case of allylic 3α-alcohol **2.15** this effect is strong enough to overcome steric hindrance at the α-face and even yield α-epoxide **2.3** as a single diastereomer, although this may be assisted by a directing effect of the 7α-OH group. We therefore have a clean route to the formation of the α-epoxide.

For *O*-protected 3-hydroxyl diastereomers where *cis*-directing effects are diminished, epoxidation gave a 40:60 ratio of α-epoxide to β-epoxide owing to competing steric hindrance at the α-face and a weak *cis*-directing effect from the 7α-OH group. We therefore do not have a route to cleanly access the β-epoxide selectively. Protection of the 7α-OH group was not investigated in this context, and we expect that installation of a protecting group here would prove slow and cumbersome because of steric hindrance.

2.5 Epoxide opening studies

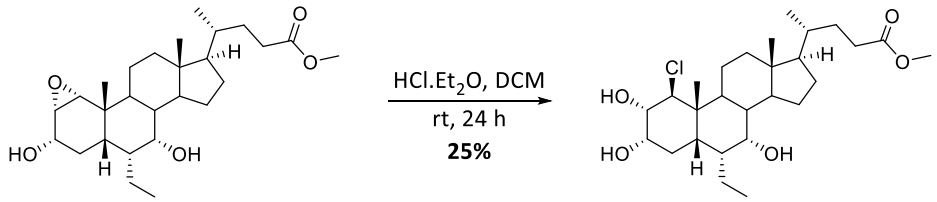
2.5.1 Rationale

Historically within the group, reactions of BAs with fluoride have given complex mixtures of products including fluoroalkene byproducts, owing to the competing nucleophilicity and basicity of fluoride. Chloride is an alternative nucleophile that can give fast and clean reactions in epoxide opening and is therefore suitable for reactions where the elucidation of product identity is imperative. Through such reactions we sought to unequivocally confirm the identity of some diastereomeric epoxides reported in *Section 2.4*. A 'reliable and robust' source of nucleophilic chloride is HCl in dry diethyl ether solution, available commercially at two molar concentration.

2.5.2 Opening of 3α-hydroxy-1α,2α-epoxide single diastereomer 2.3 with chloride

2.5.2.1 Chemistry

Reaction of 3α,7α-dihydroxy-1α,2α-epoxy OCA methyl ester (**2.3**) with anhydrous HCl gave chlorohydrin **2.27** as the single product in low yield (*Scheme 2.14*). No other products were observed in the crude ^{1}H NMR mixture and the mass balance of this reaction remains unexplained.



2.3 **2.27**

*Scheme 2.14. Epoxide opening of free 3α-hydroxy-1,2-epoxide **2.3** with chloride.*

2.5.2.2 NMR analysis

In the ^{1}H NMR spectrum of chlorohydrin **2.27**, we observe three signals above 4 ppm from the protons at the 1-, 2- and 3-positions (*Figure 2.11*). Assuming that cyclohexane epoxides open regioselectively to form the *trans*-diaxial product (see: *Section 2.1.2*), we would expect the protons at the 1-position and 2-position to be equatorial no matter the stereochemistry of the starting epoxide (*Figure 2.10*, *also showing* β-*epoxide **2.** to illustrate this*). The ^{1}H NMR signals for the protons at these two positions would therefore only exhibit small gauche coupling constants (2-5 Hz) to nearby ^{1}H nuclei.

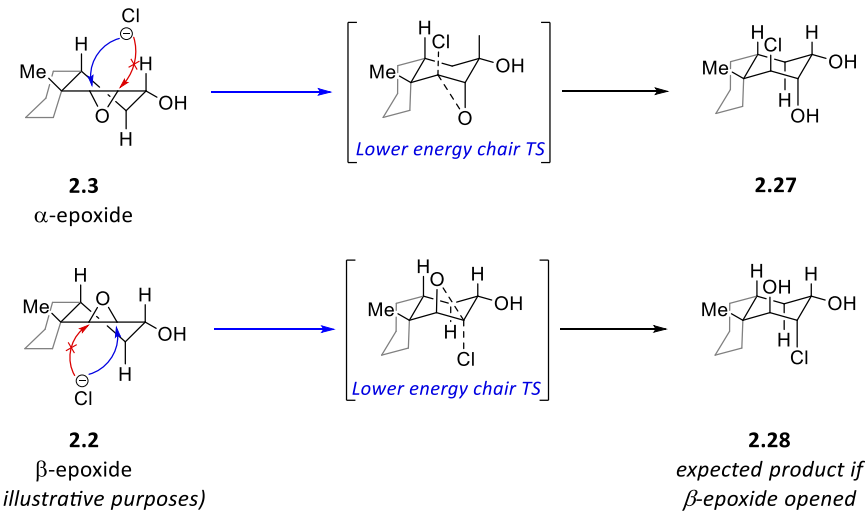


Figure 2.10. Opening of either epoxide diastereomers lead to a product with equatorial protons at the 1- and 2-positions.

Only one of the three key signals in the ^{1}H NMR spectrum (c, *Figure 2.11*) exhibits a distinctive antiperiplanar coupling constant of 11.5 Hz, whereas all three proton signals would exhibit antiperiplanar couplings to one another if a *trans*-equatorial product were instead formed (not shown). The signal at 4.11 ppm can therefore be assigned to $H_{3\beta}$.

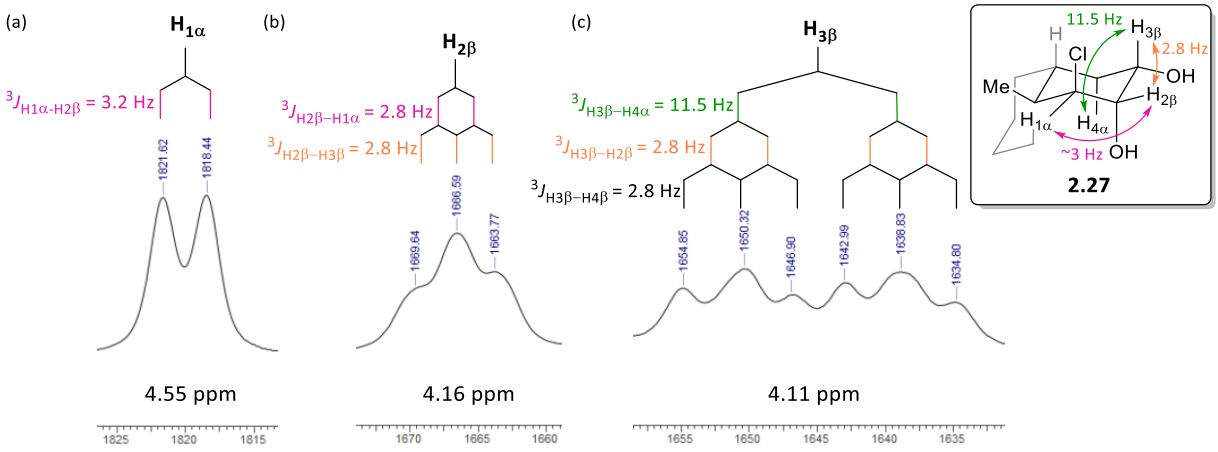


*Figure 2.11. ^{1}H NMR analysis of key signals above 4 ppm of chlorohydrin **2.27**.*[vi]

The relative assignment of protons $H_{1\beta}$ and $H_{2\alpha}$ to signals at 4.55 ppm and 4.16 ppm respectively was based on HMBC analyses, through a coupling of C_3 and H_2. The identity of the functional group at the 2-position was elucidated also by HMBC analyses through a coupling of C_2 and the $C_2O\underline{H}$ proton (no other cross peaks to carbon nuclei were observed).

[vi] COSY spectroscopy was used to confirm the cross-coupling of the nuclei described in *Figure 2.11*. The proton $H_{4\beta}$ was omitted from the 3D-structural drawing for concision.

2.5.3 Opening of 3α-acetate-1,2-epoxide single diastereomer 2.23 or 2.24 with chloride

Following prolonged and repeat HPLC purification of the diastereomeric mixture of epoxides **2.23** and **2.24**, a small amount of one diastereomer (~50 mg) was isolated pure; this was the minor isomer of the 60:40 mixture.

Considering the Fürst-Plattner rule of regioselective epoxide opening, treatment of this single compound with chloride was expected to provide either chlorohydrin **2.29** or **2.31** (*Figure 2.12*). However, "*anti*- Fürst-Plattner" products **2.30** or **2.32** were still possible (albeit extremely unlikely) from a reaction that would proceed by attack at C_2 and through a higher-energy transition state.

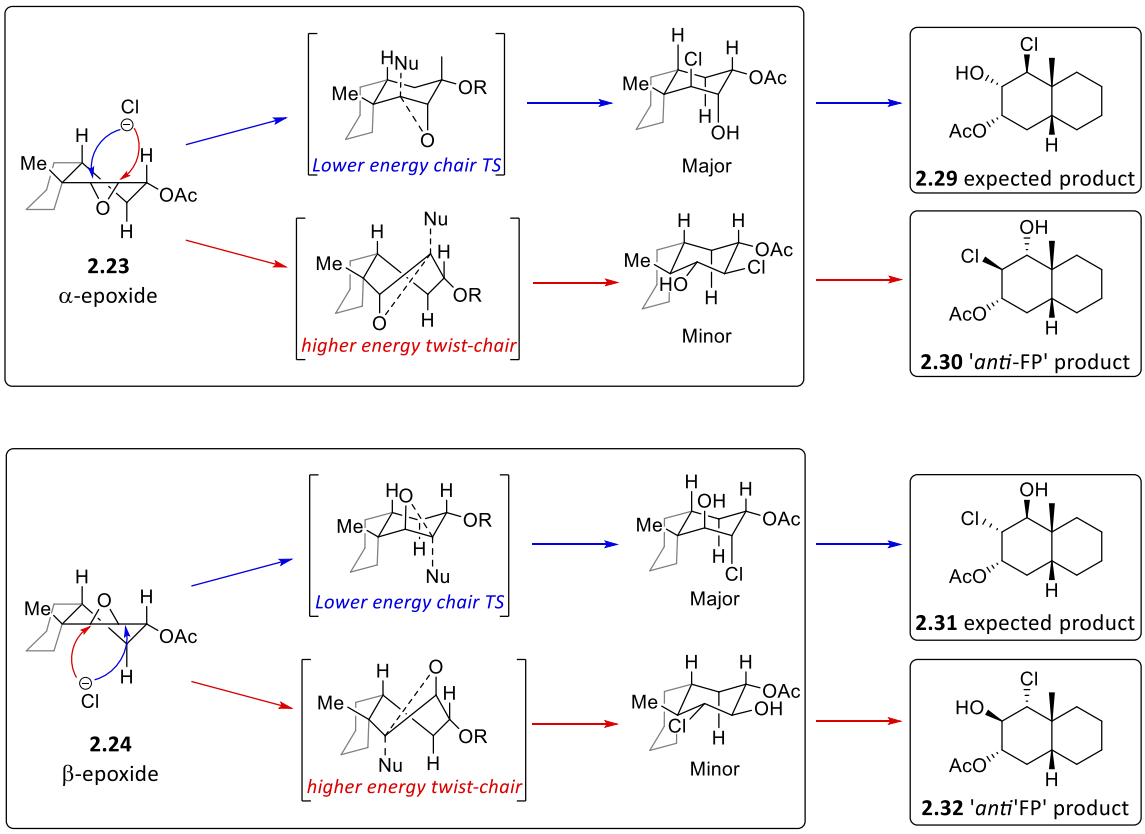
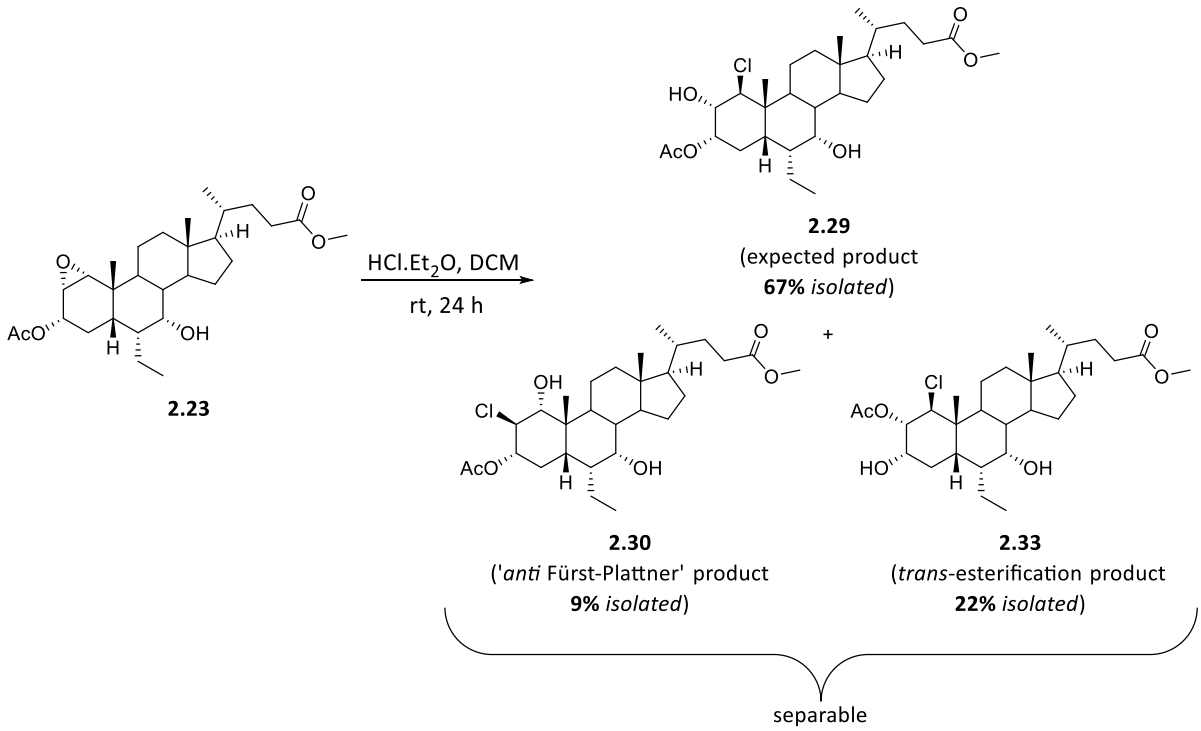


*Figure 2.12. Epoxide opening of α-epoxide **2.23** and β-epoxide **2.24** according to the Fürst-Plattner rule of regioselectivity. Only the BA A-ring and B-ring are drawn, for concision.*

2.5.3.1 Chemistry

Reaction of the pure single epoxide diastereomer with anhydrous HCl gave three products that were isolated pure after extensive HPLC purification (*Scheme 2.15*). Analyses by MS revealed that they were of identical mass and all contained chlorine. Combined, their isolated masses correspond to an excellent 98% overall yield, a figure significantly higher than for analogous non-protected 3α-alcohol **2.3**. Considering the structures of the chlorohydrin products (*Section 2.5.3.2*), the identity of the starting epoxide was defined as α-diastereoisomer **2.23**.



*Scheme 2.15. Epoxide opening of 3α-acetate-1α,2α-epoxide **2.23** with chloride.*

2.5.3.2 NMR analysis

For expected chlorohydrin product **2.29** the ^{1}H NMR spectrum was almost identical to free 3-OH derivative **2.27** (see: *Section 2.5.2.2*). The only notable difference was the deshielding of the $H_{3\beta}$ signal in the presence of the 3α-acetate group (5.26 ppm compared to 4.11 ppm) so will not be discussed further.

2.5.3.2.1 "*anti* Fürst-Plattner" product 2.30

For compound **2.30** the ^{1}H NMR spectrum exhibited a peak at 4.77 ppm that is consistent of being geminal to an acetate group. This ddd signal had coupling constants of 11.7 Hz (reasonably assigned to coupling with $H_{4\alpha}$) and 5.6 Hz (reasonably assigned to coupling with $H_{4\beta}$), and therefore this signal was assigned to $H_{3\beta}$ (*a*, *Figure 2.13*). Yet, this signal also exhibited a *further* coupling of 10.0 Hz, *indicative of an axial-axial relationship* to a proton at 4.14 ppm (confirmed by COSY spectroscopy). If this signal was from a re-arranged acetate group (for example, this C-H bond was geminal to a C-OAc motif at the 1-position), then we would expect this C_1-proton to exhibit (at most) one antiperiplanar coupling constant to the axial position at C_2; this is not the case, as we have indeed identified two axial-axial coupling constants here for this signal at 4.77 ppm.

Analysis of the dd signal at 4.14 ppm (*b, Figure 2.13*, and assigned to the proton at the 2-positon) further supports the formation of a *trans*-diequatorial product. In addition to the 10.0 Hz coupling to $H_{3\beta}$ already discussed, an additional 10.9 Hz coupling is witnessed to a peak at 3.26 ppm. This signal at 3.26 ppm was assigned as the proton at the 1-position and is observed as a dd (*c, Figure*

2.13). The signal has the aforementioned ~11 Hz reciprocal coupling to the 2-position and also a 2.3 Hz coupling to a geminal OH group proton (confirmed by a D_2O experiment).

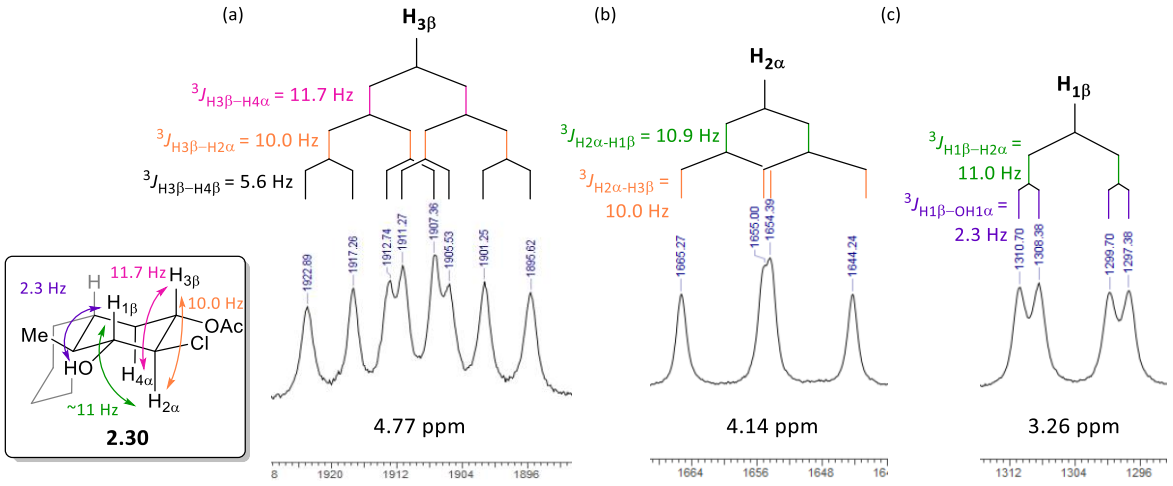


*Figure 2.13. ^{1}H NMR analysis of key signals above 3 ppm of "anti Fürst-Plattner" product **2.30**.*[vii]

The formation of **2.30** as the *trans*-diequatorial product contradicts the expected regiochemical outcome of any such epoxide opening reaction as is governed by the Fürst-Plattner rule.[191] Hence, compound **2.30** could have arisen from the "*anti*"-Fürst-Plattner epoxide opening of the α-epoxide and would have proceeded via the much higher-energy twist-chair transition state as shown in *Figure 2.12*. Alternatively, the product could be formed from a pathway involving: acid-catalysed S_N1 opening of the epoxide (anhydrous HCl is extremely acidic media) followed by attack from the nearby acetate group at the carbocation, then a displacement reaction with chloride to provide compound **2.30** (*Figure 2.14*).

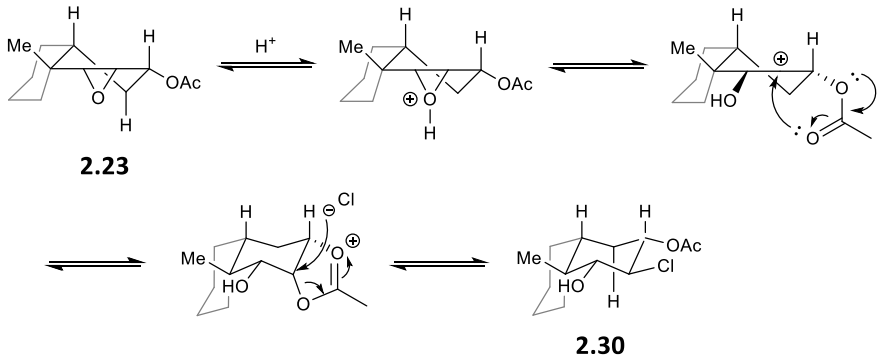


*Figure 2.14. A possible alternative reaction pathway to **2.30** via an S_N1 step.*

[vii] COSY spectroscopy was used to confirm the cross-coupling of the nuclei described in *Figure 2.13*. The proton $H_{4\beta}$ was omitted from the 3D-structural drawing for concision.

2.5.3.2.2 *Trans*-esterification product 2.33

Similarly to the expected product **2.29**, compound **2.33** also exhibited a signal at around 5.2 ppm. While this chemical shift remains indicative of a proton geminal to an acetate group, the multiplicity of this signal was a triplet of a mere 2.8 Hz and crucially did not contain an antiperiplanar coupling constant of around 8-15 Hz, as observed for **2.29**. Apart from an unlikely epimerisation of the 3-OH group, a *trans*-esterification of the ester group from the 3-position to the 2-position, possible under acidic conditions, is proposed to have occurred (*Figure 2.15*).

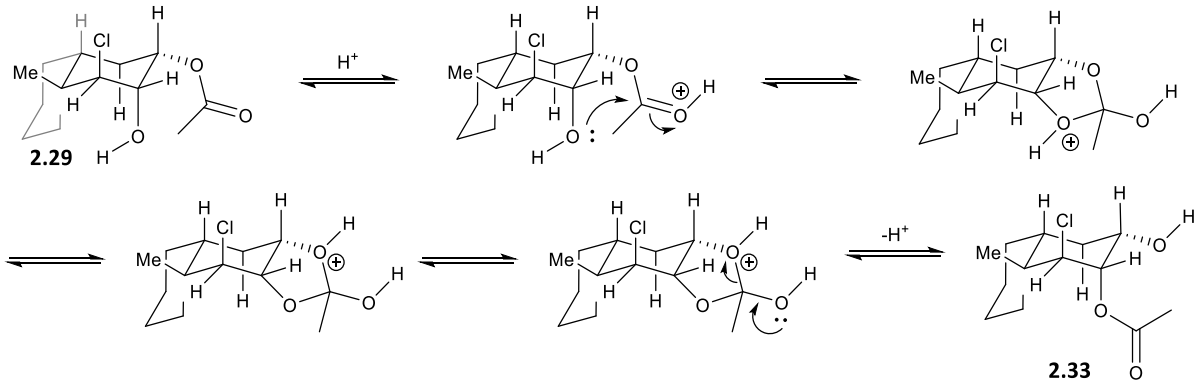


*Figure 2.15. Possible mechanism of trans-esterification of **2.29** to **2.33**.*

Under this pretence, assignment of the triplet at 5.24 ppm is to the proton at the 2-positon and the two gauche couplings would be to $H_{1\alpha}$ and $H_{3\beta}$. In the ^{1}H NMR spectrum of *trans*-esterification product **2.33**, we see two other key signals above 4 ppm (*Figure 2.16*). Note that the signal for $H_{3\beta}$ at 4.21 ppm is taken from a spectrum from a D_2O exchange experiment to increase resolution; the other signals were unchanged by D_2O. The coupling constants and multiplicities illustrated in the figure support the formation of *trans*-esterification product **2.33**.

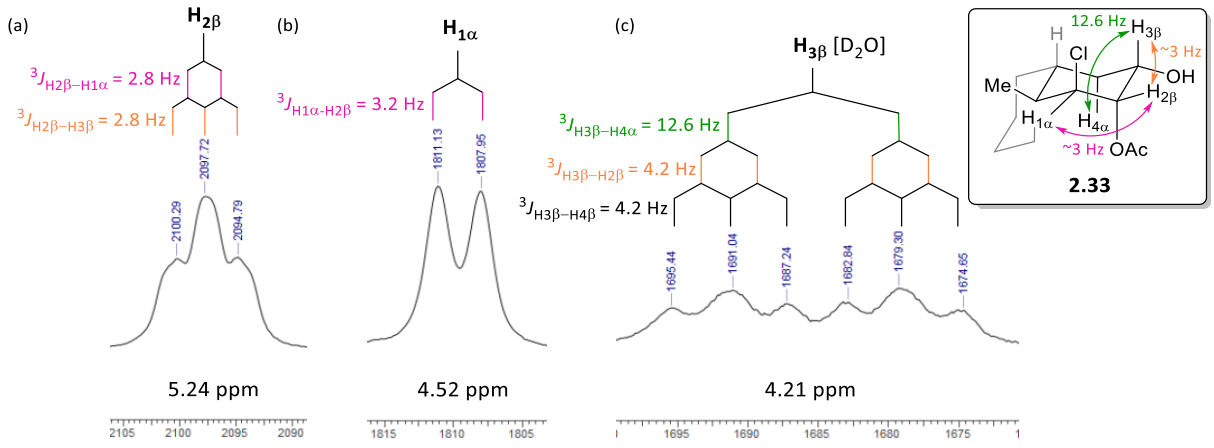
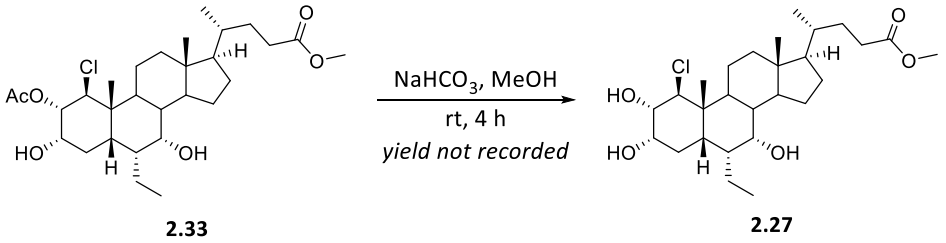


*Figure 2.16. 1H NMR analysis of key signals above 4 ppm of trans-esterification product **2.33**.*[viii]

[viii] COSY spectroscopy was used to confirm the cross-coupling of the nuclei described in *Figure 2.16*. The proton $H_{4\beta}$ was omitted from the 3D-structural drawing for concision.

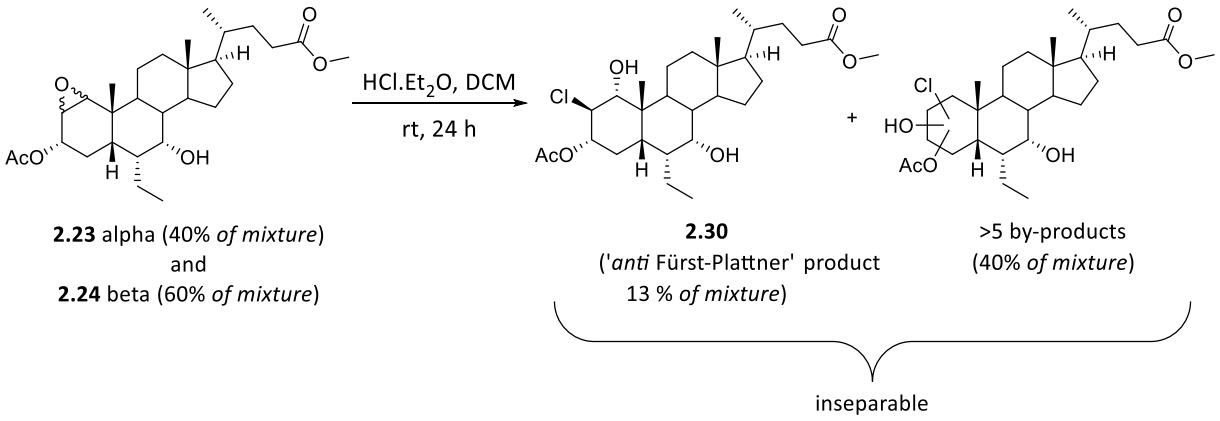
Cleavage of the 2α-acetate group of **2.33** led to chlorohydrin **2.27**, which is the reaction product of opening the single α-epoxide diastereomer **2.3** (see: *Section 2.5.2.1*). This means that chlorohydrin **2.33** originated from an α-epoxide, defining the stereochemistry of **2.23**. If β-epoxide **2.24** was instead opened with chloride, acetate-saponification would yield a chlorohydrin with a 1,3-hydroxyl motif that would *not* spectroscopically match **2.27**.



*Scheme 2.16. 2α-acetate **2.33** saponification to yield chlorohydrin **2.27**.*

2.5.4 Opening of 3α-acetate-1,2-epoxide diastereomer 2.23 and 2.24 mixture with chloride

Following the successful reaction of α-epoxide diastereomer **2.23** with chloride, the mixture of epoxide diastereomers **2.23** and **2.24** was next attempted. Alas, only "*anti* Fürst-Plattner" product **2.30** was identified in addition to at least 5 other chlorinated products that proved inseparable by HPLC (*Scheme 2.17*).



*Scheme 2.17. Chloride opening of 3α-acetate-1,2-epoxide diastereomers **2.23** and **2.24**.*

2.5.5 Conclusions

The selective synthesis of α-epoxide diastereomer **2.3** (see: *Section 2.4.2*) is complemented by a clean reaction to the expected chlorohydrin product **2.27**, albeit in low yield. We expect the analogous epoxide opening reaction with fluoride to proceed well and to thus install the 1β-fluoro motif; it will likely involve an analogous *O*-protected derivative akin to **2.23**.

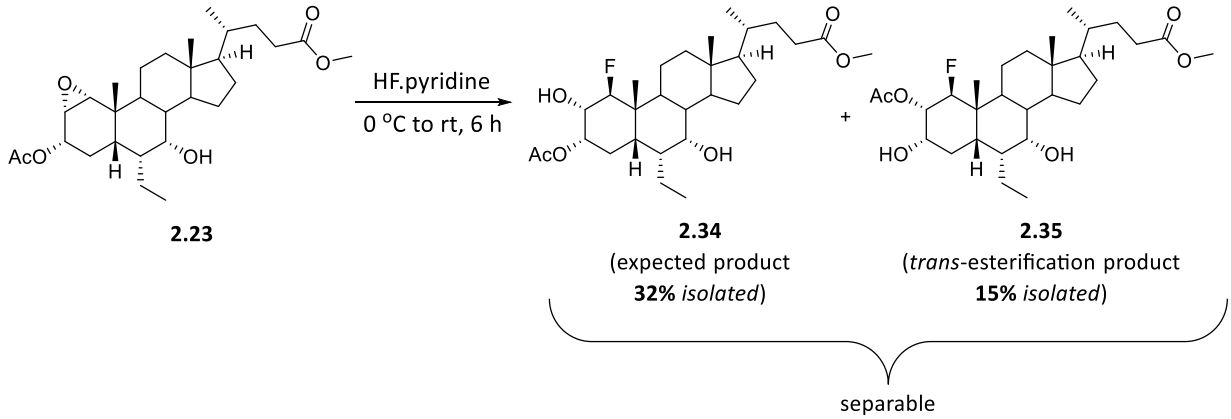
The corresponding β-epoxide could not be selectively synthesised or was not isolable from a mixture of epoxide diastereomers. Treatment of the epoxide mixture of **2.23** and **2.24** with chloride

unfortunately gave a complex mixture of products; no compounds from reaction of the β-epoxide were identified. Therefore, the synthesis towards 1α-fluorinated substrates remains precluded until a selective route to β-epoxides is first realised.

2.6 Synthesis of 1β-fluoro OCA analogues

2.6.1 Synthesis and opening of 1α,2α-epoxide 2.23 with fluoride

With *O*-protected α-epoxide **2.23** in-hand, reaction with an excess of fluoride was successful and led to the formation of two products that were readily separable by column chromatography (*Scheme 2.18*). In addition to expected product **2.35**, we observed the *trans*-esterification product **2.36** and is likely the result of the inherent acidity of HF.pyridine.[ix]



*Scheme 2.18. Epoxide opening of 3α-acetate-1α,2α-epoxide **2.23** with fluoride.*

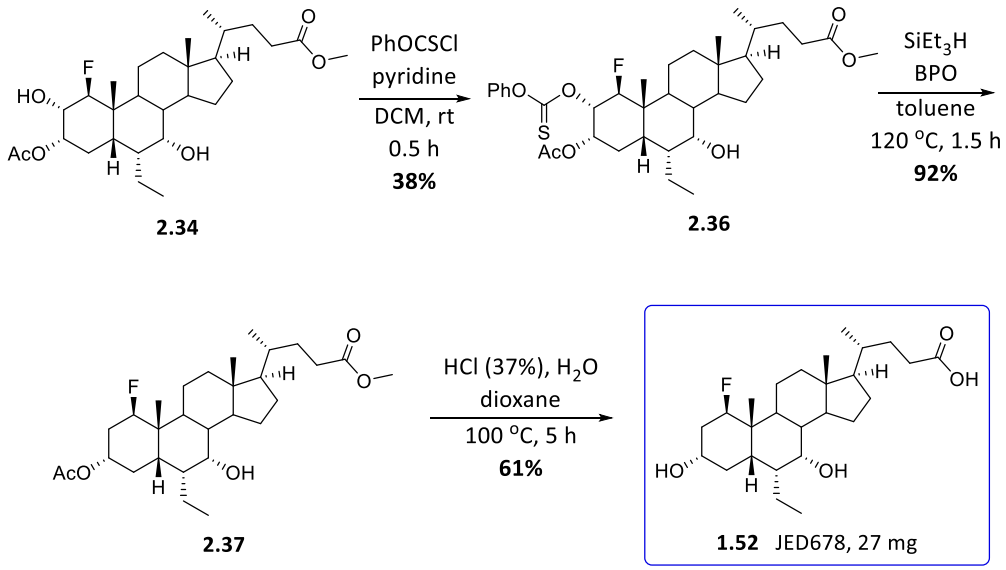
The ^{1}H NMR spectrum of expected product **2.34** was almost identical to that of chlorinated analogue **2.29**; the only difference was the addition of a H-F geminal coupling constant around 43 Hz in the signal of $H_{1\alpha}$ in both fluorinated products.

2.6.2 Synthesis of 1β-fluoro OCA (JED678)

2.6.2.1 Chemistry

With fluorohydrin **2.34** in hand, deoxygenation of the 2α-OH group was required (*Scheme 2.19*). Conversion firstly to the *O*-phenyl thiocarbonate ester **2.36** was of notably poor yield owing to the steric hindrance at the α-face and the congestion at the A-ring. Deoxygenation of **2.36** by heating at reflux and with triethylsilane as the source of hydride provided *O*-protected 1β-fluoro OCA methyl ester (**2.37**) in excellent yield (35% over two steps). Lastly, the two ester groups were saponified according to the acidic hydrolysis procedure described by Hopkins,[192] providing target 1β-fluoro OCA (**1.52**) in good yield.

[ix] A mechanism to describe acid-catalysed *trans*-esterification can be found in *Section 2.5.3.2.2*.



*Scheme 2.19. Deoxygenation at the 2-positon of 1β-fluoro intermediate **2.34** and synthesis of target 1β-fluoro OCA (**1.52**).*

2.6.2.2 NMR analysis

The ^{1}H NMR spectrum of target **1.52** (*Figure 2.17*) shows a dd signal for $H_{1\alpha}$ at 4.72 ppm with coupling constants of 46.6 Hz (geminal to fluorine) and 3.2 Hz (vicinal, gauche to $H_{2\alpha}$). We might expect a gauche equatorial-equatorial coupling to proton $H_{2\beta}$, but this is not observed.

No other H-F couplings are observed in other ^{1}H signals; specifically, the protons at the 2-position are situated within the 'messy' steroidal region of 1-2.2 ppm and $H_{3\beta}$ is too far away to be involved in coupling to the fluorine nucleus.

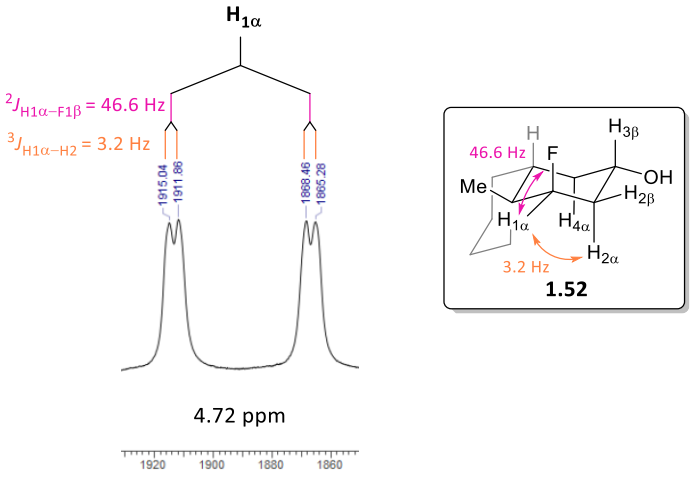


*Figure 2.17. ^{1}H NMR analysis of the signal exhibiting H-F coupling of target 1β-fluoro OCA (**1.52**).*

The ^{19}F NMR of target **1.52** (*Figure 2.18*) shows a td signal at -192.2 ppm with couplings of 46.8 Hz to geminal proton $H_{1\alpha}$ and to vicinal *trans*-diaxial proton $H_{2\alpha}$. A smaller coupling of 3.5 Hz is to vicinal gauche proton $H_{2\beta}$.

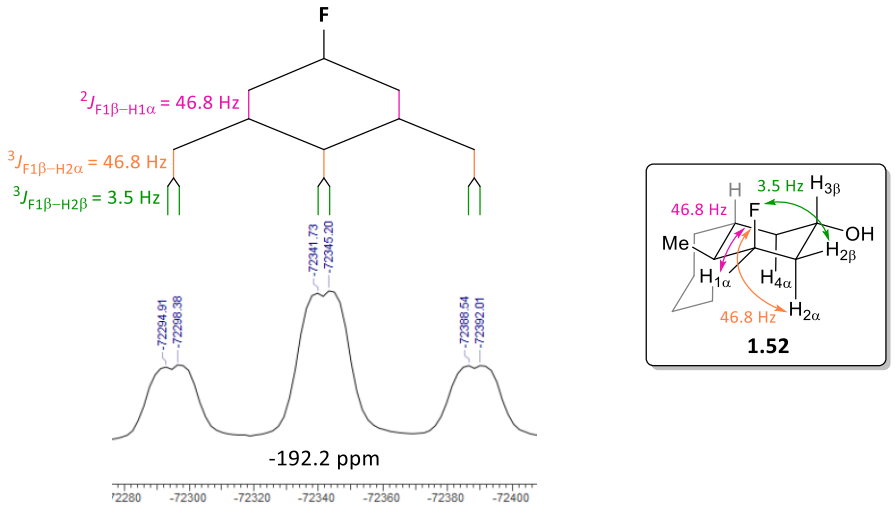
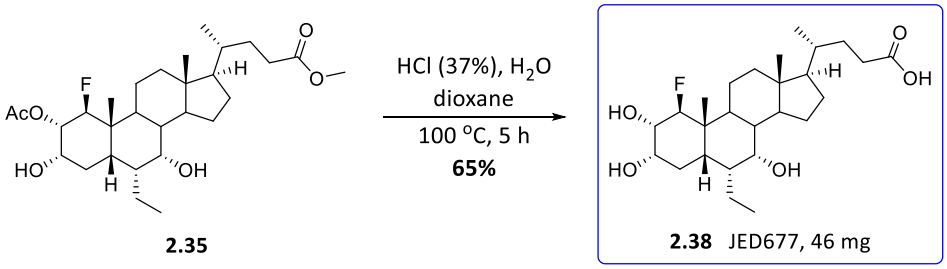


*Figure 2.18. ^{19}F NMR analysis of the $F_{1\beta}$ signal target 1β-fluoro OCA (**1.52**).*

2.6.3 Synthesis of 1β-fluoro-2α-hydroxy OCA (JED677)

Acidic hydrolytic conditions were applied to *trans*-esterification product **2.35** to simultaneously deprotect the 2-OH and C_{24}-acid groups in good yield (*Scheme 2.20*). Despite not being included in the original synthesis plan, the novel motif at the A-ring is of interest to test at the FXR and TGR5.



*Scheme 2.20. Reaction of fluorohydrin **2.35** under acidic conditions to yield compound **2.38**.*

2.6.4 Attempts towards 1β-fluoro OCA (1.52) by iodofluorination

It was previously published by the group that iodofluorination of allylic alcohol (1*S*, 4*S*)-4-(*tert*-butyl)cyclohex-2-en-1-ol with HF.pyridine complex and *N*-iodosuccinimide proceeded well with only 'much smaller amounts' of minor isomers and impurities otherwise observed.[127] Reaction of this product then with tributyltin hydride and AIBN was found to homolyse the weak C-I bond for hydride reduction to provide the desired axial fluorohydrin (*Figure 2.19*).

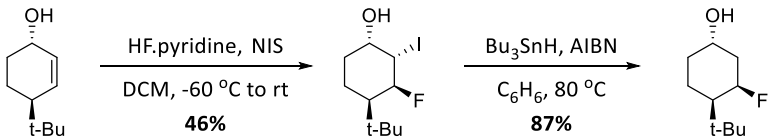
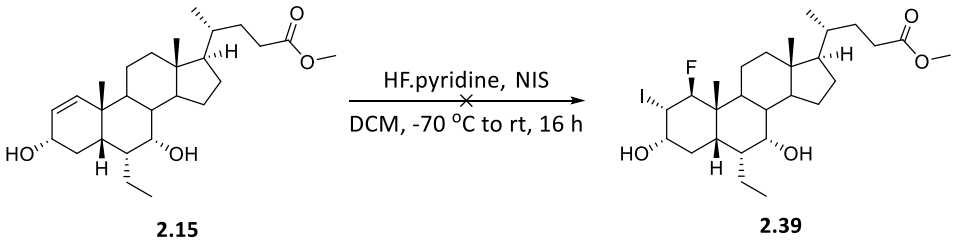


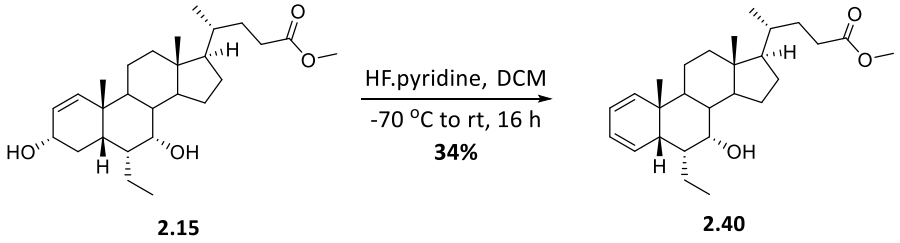
Figure 2.19. Iodofluorination and reduction of a rigid cyclohexanol.[127]

Alas, application of this fluorination chemistry to our own allylic alcohol **2.15** proved unsuccessful and a complex crude reaction mixture was instead recovered with over ten products visible by TLC (*Scheme 2.21*). The crude ^{19}F NMR spectrum further indicated a complex mixture of products.



*Scheme 2.21. Attempted iodofluorination of allylic alcohol **2.15** with HF.pyridine and NIS.*

Additionally, it was discovered that a reaction did occur in the absence of *N*-iodosuccinimide but to 1,3-diene derivative **2.40** (*Scheme 2.22*). Because of the equatorial stereochemistry of the C-OH bond, there are no antiperiplanar C-H bonds and so an E1 elimination involving alcohol protonation is likely the pathway from **2.15** to **2.40**.



*Scheme 2.22. Reaction of allylic alcohol **2.15** with HF.pyridine to yield 1,3-diene **2.40**.*

2.7 Conclusions

Two novel 1β-fluorinated OCA targets were synthesised (*Figure 2.20*). As a result of this work towards 1-fluoro targets, 1,2-enyl OCA (**2.17**) was also prepared. We were however unable to realise 1α-fluorinated targets. A key β-epoxide intermediate towards the synthesis of 1α-fluoro compounds could not be obtained in pure form. Reactions of this mixture were extremely cumbersome and precluded further investigation.

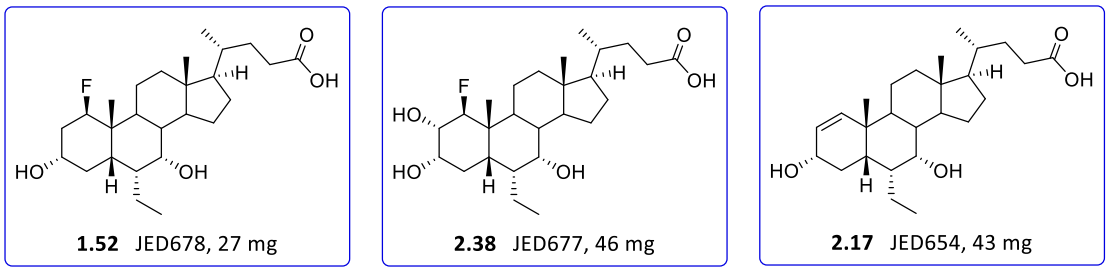


Figure 2.20. Synthesised 1-fluoro and 1-functionalised OCA derivatives.

Chapter 3 Synthesis of OCA (NZP084) 2α-fluoro and 4α-fluoro analogues

3.1 Introduction

3.1.1 Retrosynthetic analysis

The β-selectivity of reactions at the nucleophilic 2- and 4-positions meant that A-ring enol and enolate compounds would be suitable intermediates for the synthesis of β-fluorinated targets (see: *Section 4.1*). Precedence exists for the synthesis of 2α-fluoro and 4α-fluoro CDCA by a previous group member (see: *Section 6.1.2*),[174] and this strategy would be used to access α-fluorinated compounds **1.53** and **1.55**; a β-positioned leaving group is installed first a 2-step process, followed by a nucleophillic substitution reaction. A retrosynthetic analysis is presented in *Figure 3.1*.

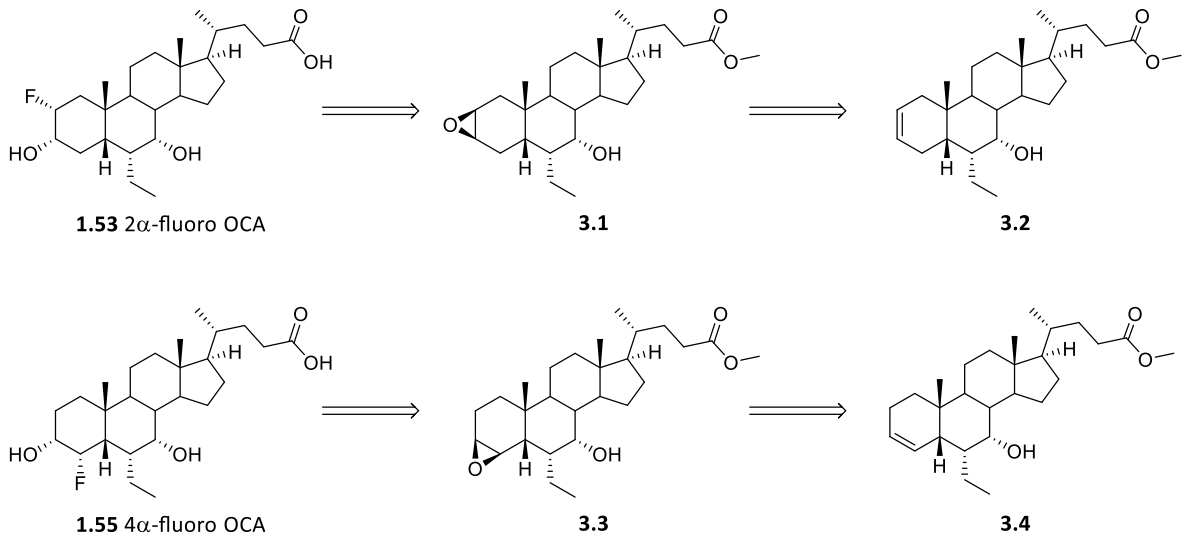


*Figure 3.1. Retrosynthesis of α-fluoro targets at the 2- and 4-positions **1.53** and **1.55**.*

Retrosynthetic analysis of 2α-fluoro OCA (**1.53**) requires 2β,3β-epoxide **3.1**. Forward synthesis by epoxide opening with nucleophilic fluoride is regioselective only at the 2-position (see below, *Figure 3.3a*) and could provide target **1.53** after epimerisation of the resultant 3β-hydroxyl group. Likewise, 4α-fluoro OCA (**1.55**) could be synthesised from the regioselective opening of 3β,4β-epoxide **3.3** at the 4-position (*Figure 3.3b*). Both β-epoxide regioisomers can be formed from the alkene regioisomers **3.2** and **3.4**, with the epoxidation reaction expected to be β-selective. Retrosynthesis of both alkene regioisomers leads back to OCA methyl ester (**2.5**) and then commercially available OCA (**1.8**) (*Figure 3.2*).

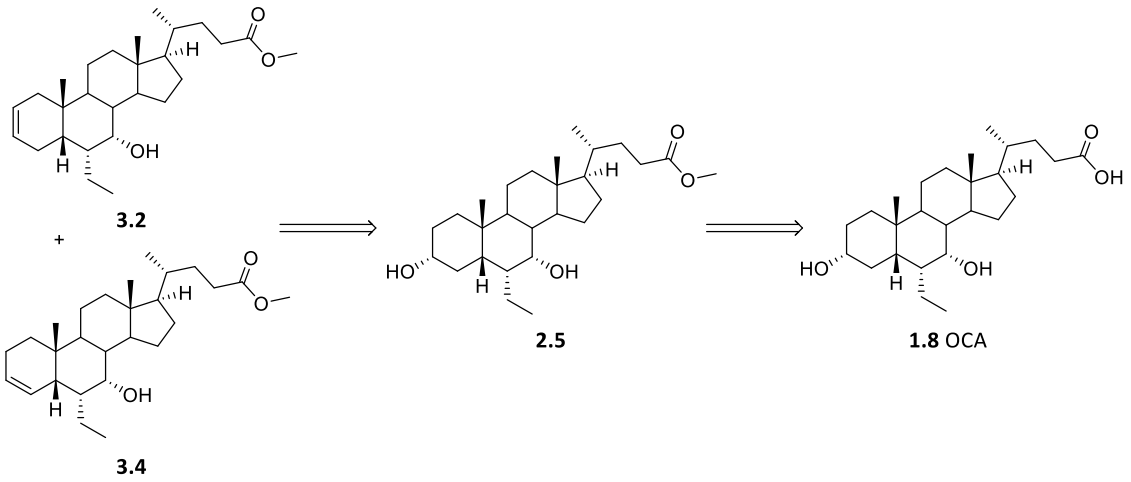


*Figure 3.2. Retrosynthesis of alkene regioisomers **3.2** and **3.4***

3.1.2 Regioselectivity of epoxide opening

As mentioned above, cyclohexane epoxides open regioselectively to form the *trans*-diaxial product from the lower energy chair transition state. Hence, opening of Δ2β,3β-epoxide **3.2** with fluoride is expected to form only fluorohydrin **3.5** with the desired axial C-F bond. Likewise, opening of Δ3β,4β-epoxide **3.4** with fluoride should only lead to fluorohydrin **3.6** with the desired axial C-F bond. Regioselectivity in opening at the 4-position of a Δ3β,4β-epoxide has been described in the literature with formic acid,[193] and also on CDCA substrates (unpublished work by the group).[174]

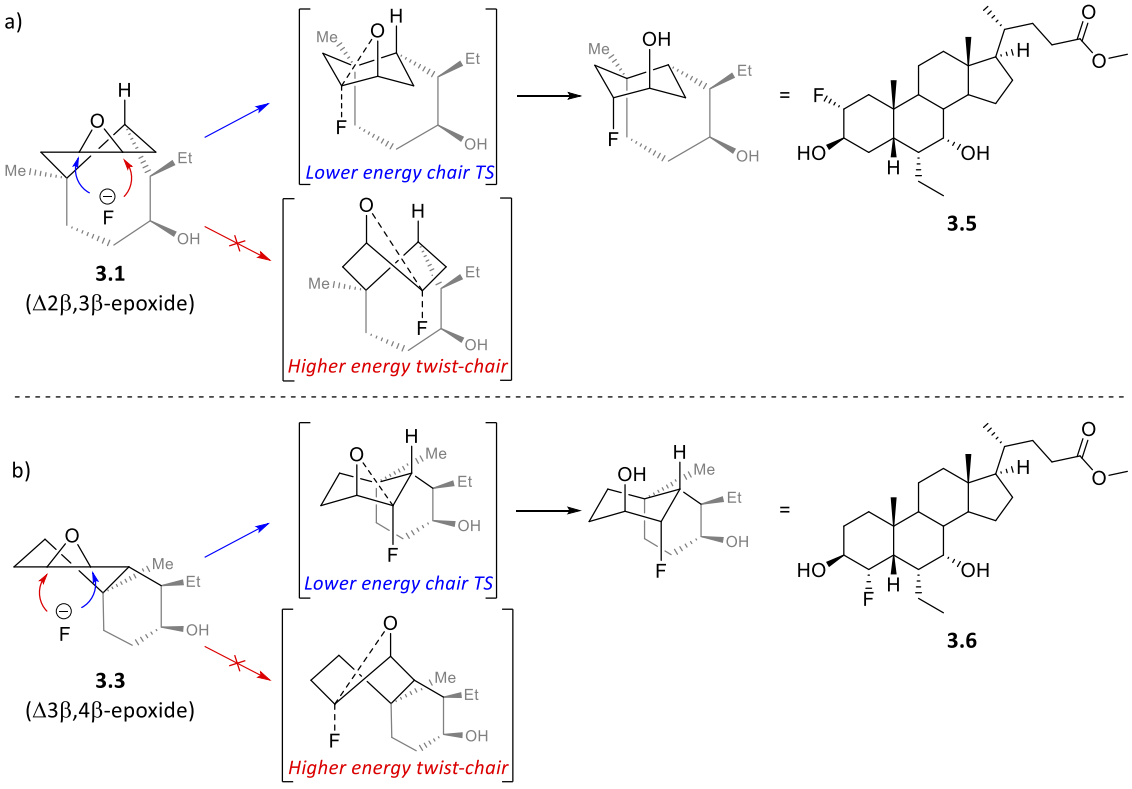


Figure 3.3. Regioselectivity of 2,3- and 3,4-epoxide opening via a chair transition state (blue) over a twist-chair transition state (red).

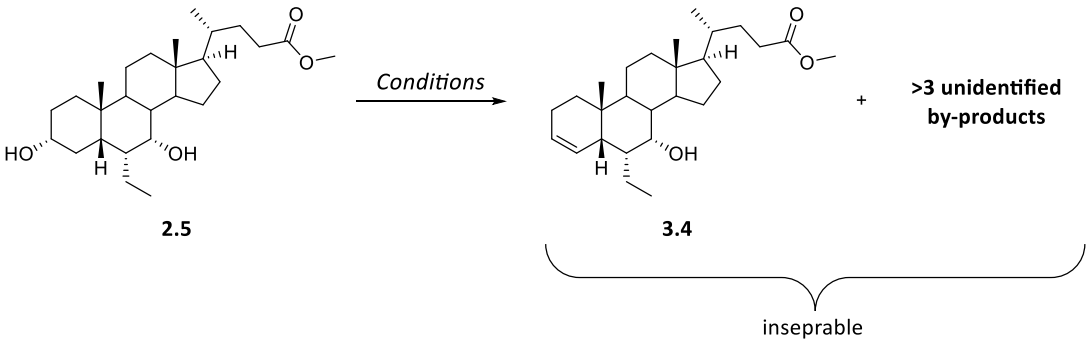
3.2 Synthesis towards fluorohydrin intermediates involving 7α-hydroxy substrates

The steric hindrance of the 6-ethyl group precludes almost all reactivity at the 7α-hydroxyl group of OCA. The installation of 7α-OH protecting groups has been described as extremely cumbersome by former members of the group; this difficulty is further compounded for the selective protection at the 7α-OH group in the presence of the lesser-hindered 3α-OH group. Considering these two observations, it was therefore of interest to work towards advanced alkene intermediates **3.2** and **3.4** in the absence of a protecting group at the 7-positon.

3.2.1 Dehydration

There is literature precedence for a one-pot elimination of secondary alcohol groups on steroidal skeletons; by reaction of the hydroxyl motif with triflic anhydride to generate an excellent leaving group, elimination is possible with a suitable base. The group of Kumar has reported this reaction at the 3 position of an LCA derivative to give a 3:7 mixture of Δ2,3- and Δ3,4-alkene regioisomers.[194] Applying this chemistry to the 3-position of a 7-keto CDCA derivative (**6.2**) gave a mixture of the corresponding alkene regioisomers **6.3** and **6.4** (see: *Section 6.1.2*), but the ratio was not recorded (unpublished work by the group).

These conditions were applied to OCA methyl ester (**2.5**) and the reaction temperature was varied (*Table 3.1*). In all cases, only one of the two expected alkene regioisomers was formed in addition to a complex mixture of at least 3 byproducts. The single alkene product was later determined to be Δ3,4-isomer **3.4** after a subsequent reaction with *m*CPBA (*Section 3.2.2*). Purification of **3.4** gave a sample of approximately 80% purity that was taken forward to the next reaction.



Entry	Time at 0 °C / mins	Total reaction time / mins	Temp. at reaction end / °C	Yield
1	240	240	0	25% **3.4** ~4% BP's
2	15	240	10	22% **3.4** ~6% BP's
3	15	240	20	21% **3.4** ~7% BP's
4	5	120	12	17% **3.4** ~11% BP's

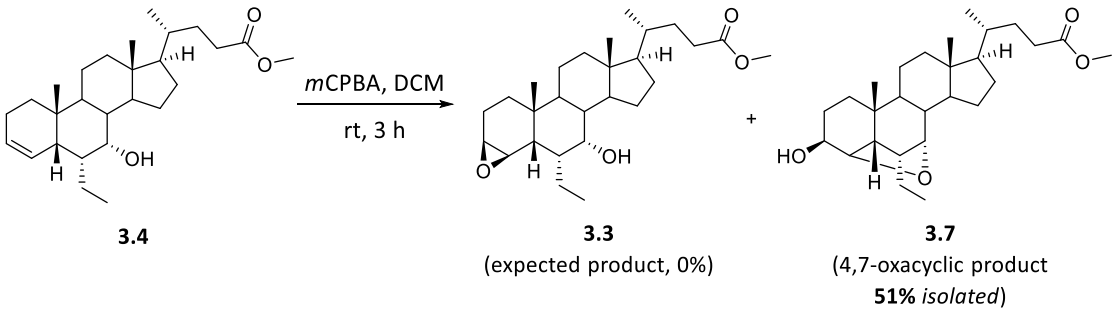
*Table 3.1. Screening of dehydration conditions of OCA methyl ester **2.5**.*

*Reactions were performed on 100 mg scale with 1.05 equivalents of Tf_2O and 2.0 equivalents of DMAP in dichloromethane and began at 0 °C. The isolated yield of product **3.4** is reported after HPLC purification and is based on the mass and composition of the sample, judged by ^1H NMR. The yields of the combined byproducts are estimated from these calculations of sample mass and composition.*

Maintaining 0 °C throughout the reaction gave the best yield of alkene **3.4** and led to the smallest quantity of byproduct mixture being formed (*Entry 1*). Warming the reaction mixture after an initial 15 min at 0 °C was detriment to the yield of alkene product (*Entries 2 and 3*). Holding the reaction at 0 °C for only 5 minutes before warming gave the worst yield of alkene product **3.4** and the highest quantity of the byproduct mixture (*Entry 4*). We conclude that an exotherm is present upon reagent addition and in the initial reaction towards the C-OTf species, and this localised heating, if not cooled properly within the first few minutes, is significant towards the occurrence of side-reactions.

3.2.2 Epoxidation and opening thereof

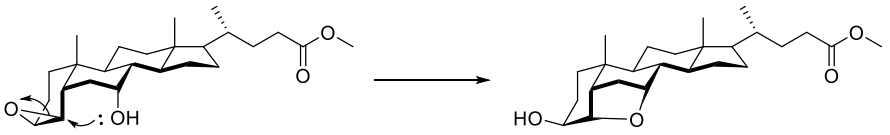
Epoxidation is expected to proceed at the least hindered convex β-face. Treatment of alkene **3.4** (the identity of which, at this point, remained unsolved) with *m*CPBA interestingly only yielded 4,7-oxacyclic product **3.7** (*Scheme 3.1*).



3.4 | **3.3** (expected product, 0%) | **3.7** (4,7-oxacyclic product **51%** *isolated*)

*Scheme 3.1. Epoxidation of alkene **3.4** to yield only 4,7-oxacyclic product **3.7**.*

Oxacyclic OCA derivative **3.7** is a product of an intramolecular reaction of expected product 3β,4β-epoxy OCA methyl ester (*Figure 3.4*). Compound **3.3** was therefore formed from the electrophilic epoxidation of alkene **3.4** but was rapidly consumed *in-situ* and so was merely an intermediate in the transformation of **3.4** to **3.7**. This result illustrates the close proximity of the 7α-position to the 4-position.



3.3 **3.7**

Figure 3.4. Intramolecular epoxide opening at the 4-position by the 7-OH group

The ^{1}H NMR signals for the expected epoxide product would have been in the range of 3.0 ppm to 3.5 ppm. For the alternative oxacyclic product we observe two key signals above 4.0 ppm corresponding to $H_{3\alpha}$ and $H_{4\beta}$ (*Figure 3.5*). Formation of this compound is evidence that the single alkene regioisomer (from dehydration of OCA methyl ester (**2.5**)) was indeed Δ3,4-alkene **3.4**.

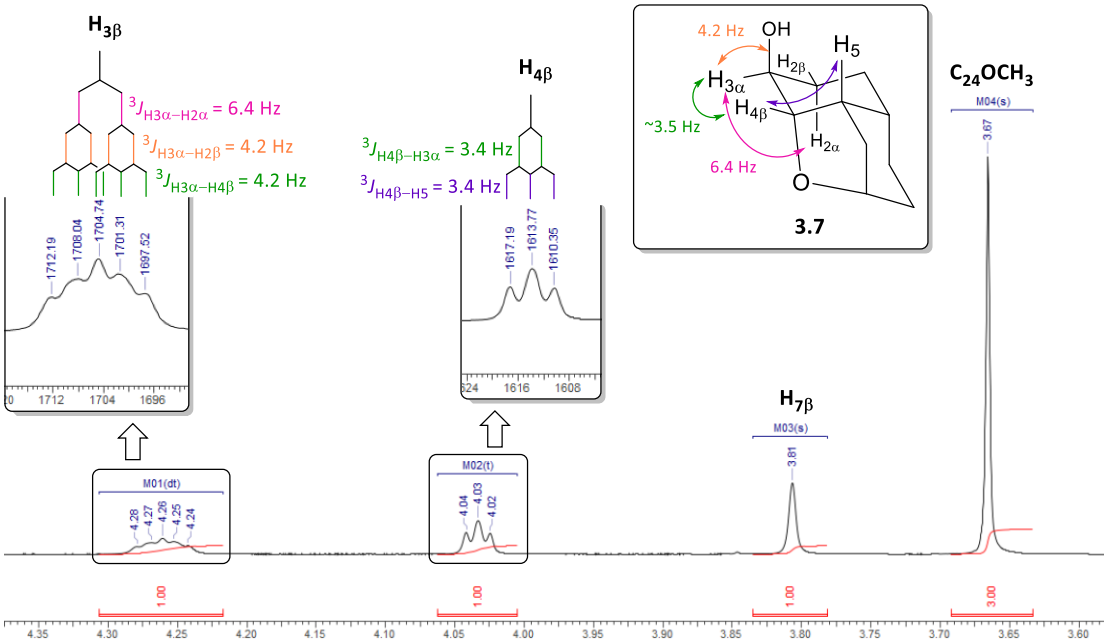


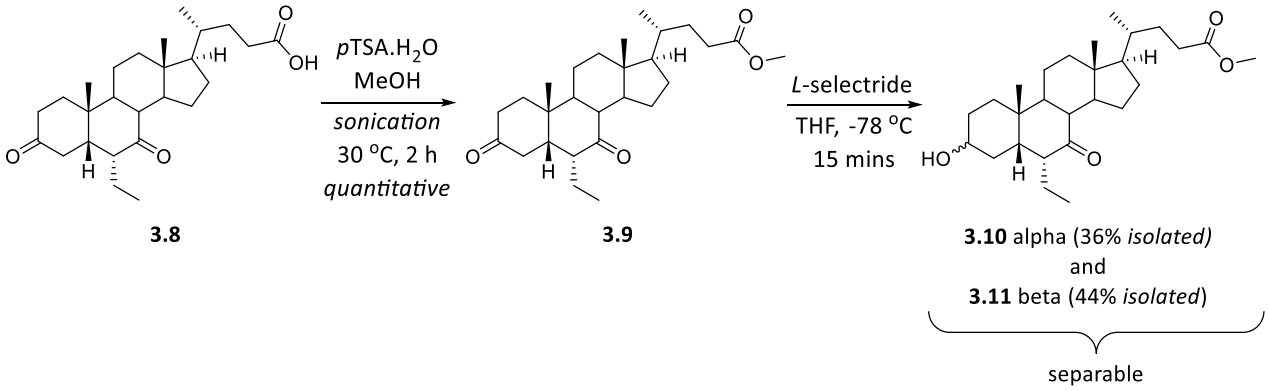
*Figure 3.5. ^{1}H NMR analysis of the key signals between 3.60 and 4.35 ppm of compound **3.7**.*

Hence, synthesis towards fluorohydrin intermediates in the presence of the 7α-hydroxyl group encountered two problems and this line of work was abandoned. We sought to overcome both in the presence of the 7-keto group.

3.3 Synthesis of fluorohydrin intermediates involving 7-keto substrates

3.3.1 Synthesis of 3-hydroxy-7-keto intermediates

From commercially sourced 3,7-diketo OCA (**3.8**), esterification by sonication on 36 g scale gave the corresponding methyl ester quantitatively (Scheme 3.2). Reduction with bulky *L*-selectride was not selective at the 3-OH group and provided an approximate 1:1 ratio of α- and β-alcohol diastereomers **3.10** and **3.11**. The optimal conditions of the reduction reaction involved 1.5 equivalents of the hydride source; test reactions with greater than 2 equivalents caused reduction at the 24-ester (not shown).

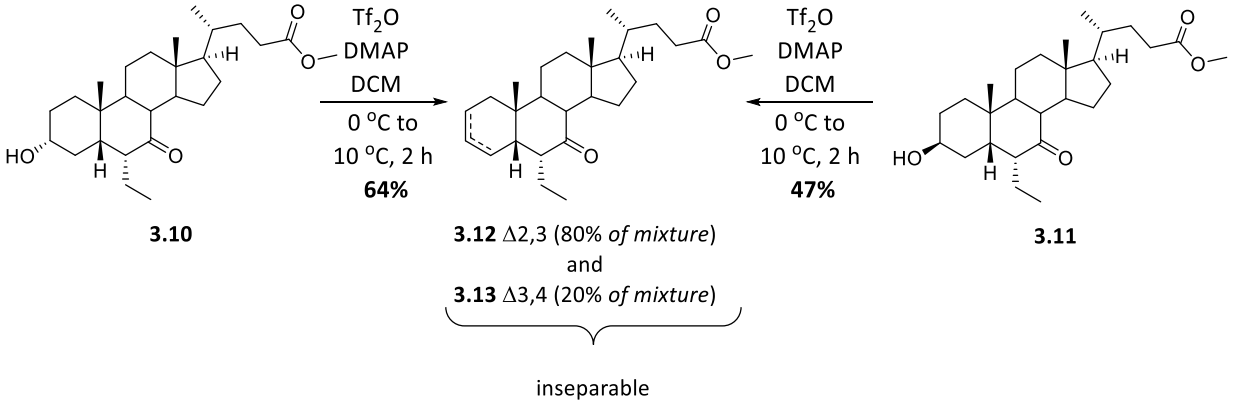


Scheme 3.2. Esterification of 3,7-diketo OCA and reduction thereof with *L*-selectride.

3.3.2 Dehydration and epoxidation

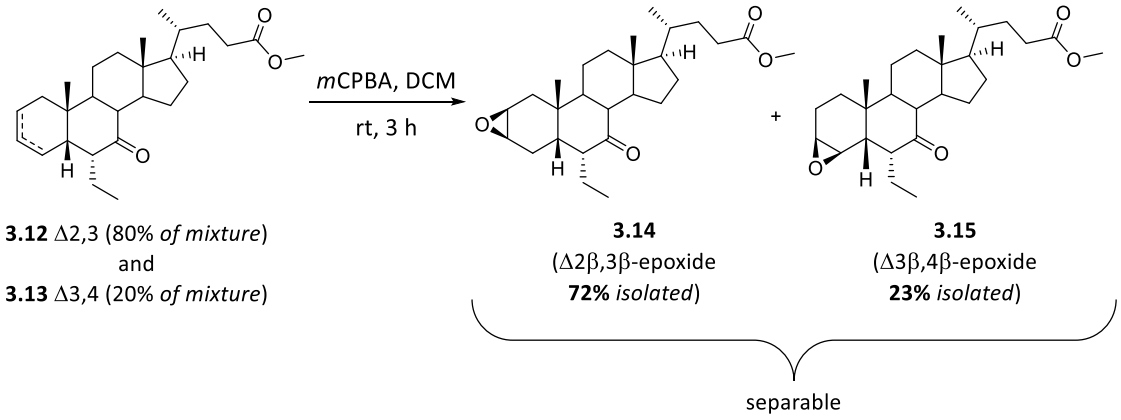
The dehydration reaction of the axial 3β-alcohol diastereomer **3.11** could proceed by an E2 elimination reaction involving either the antiperiplanar 2α- or 4α-proton. This could be more rapid (and perhaps higher yielding) than the dehydration reaction of the corresponding equatorial 3α-diastereomer **3.10** where an E2 dehydration mechanism requires conformational half-ring inversion to a boat A-ring structure. Against our expectations, the dehydration of the equatorial alcohol gave an approximate 35% better yield (*Scheme 3.3*). The elimination reaction of either alcohol diastereomer gave the same 80:20 ratio of alkene regioisomers **3.12** and **3.13**.[x]

[x] By ^{13}C NMR, the major alkene peak was identified at 125.3 ppm and 123.8 ppm. The minor isomer was identified at 129.7 ppm and 124.5 ppm. The identity of the major and minor alkene products was assigned retrospectively after epoxidation with *m*CPBA and opening with fluoride.



*Scheme 3.3. Dehydration of 3-alcohol diastereomers **3.10** and **3.11**.*

With a combined mass in-hand of ~5.0 g, epoxidation of the alkene regioisomers with *m*CPBA gave a 72% yield (3.7 g) of 2β,3β-epoxide **3.14** and a 23% yield (1.2 g) of 3β,4β-epoxide **3.15** (*Scheme 3.4*). Pleasingly, this represented a total of 95% isolated mass and, to our expectation, the epoxidation reaction fully proceeded with β-facial selectivity. Additionally, no traces of Baeyer-Villiger products were observed. The separation and isolation of these pure compounds was however difficult. In total, approximately 20 L of apolar solvent was used across four iterative purification runs.



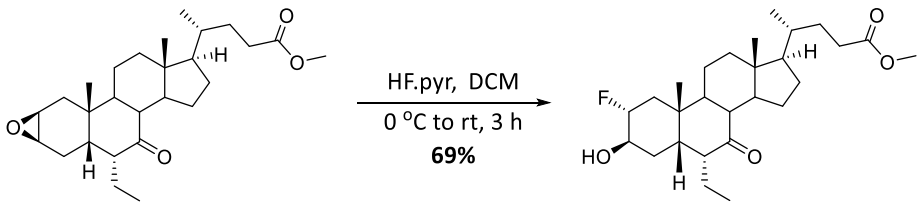
*Scheme 3.4. Selective β-epoxidation of alkene regioisomers **3.12** and **3.13**.*

3.3.3 Synthesis of 2α-fluoro fluorohydrin intermediate 3.16

3.3.3.1 Chemistry

With Δ2β,3β-epoxide **3.14** in-hand, epoxide opening with fluoride was performed on 3.3 g scale (*Scheme 3.5*). The handling of corrosive HF.pyridine on this scale was mitigated by the consumption of one new 100 mL bottle that could be poured (eliminating risks associated with syringe addition or cannula). Nevertheless, extreme care was taken when handling of the reagent given its inherent

toxicity.[xi] Given the high acidity (and thus reactivity with epoxides) of Olah's reagent, the reaction was began at 0 °C and warmed slowly to ambient temperature which proceeded to give a good yield of the expected fluorohydrin. Notably, no other compounds (such as "*anti* Fürst-Plattner" products, from reaction against expected regioselectivity (i.e. at the 3-position) were observed.



3.14 **3.16**

*Scheme 3.5. Opening of Δ2β,3β-epoxide **3.14** with nucleophilic fluoride.*

3.3.3.2 NMR analysis

The stereochemistry of the 2α-fluoro product was confirmed by ^{1}H NMR analysis of the $H_{2\beta}$ signal (*Figure 3.6*). The three gauche ^{3}J couplings of 2.3 Hz to both protons at the 1-position and to proton $H_{3\alpha}$ confirmed that H_2 is equatorial and the fluorine atom is therefore axial (alpha).

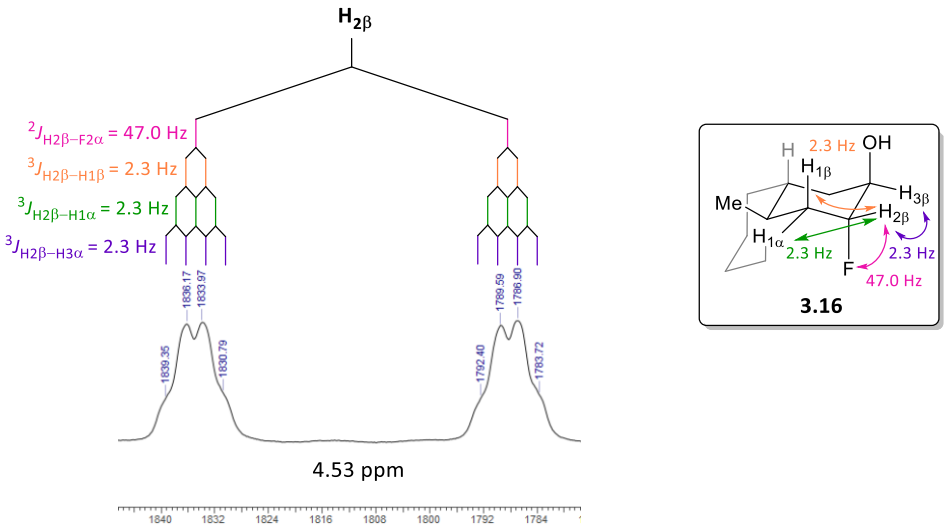


*Figure 3.6. ^{1}H NMR analysis of $H_{2\beta}$ signal of 2α-fluorinated intermediate **3.16.***

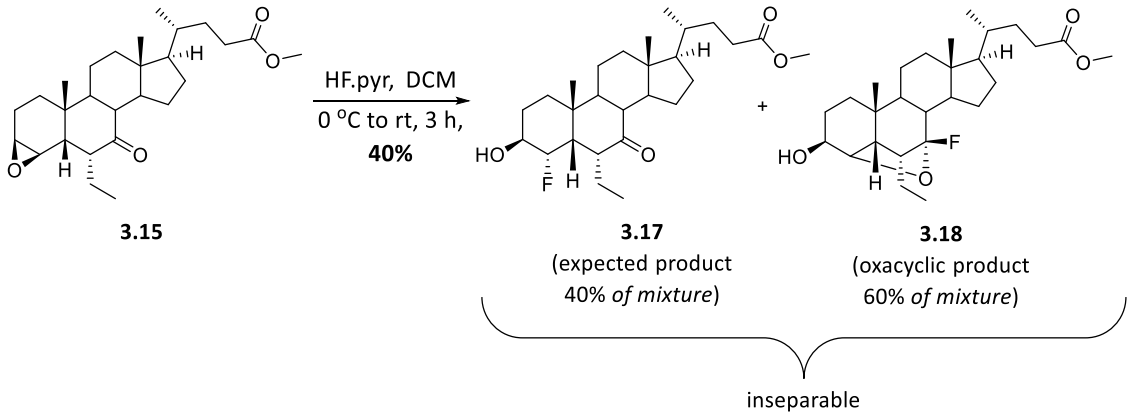
Further confirmation is realised from the ^{19}F NMR spectra of **3.16** (not shown). We observe one fluorine signal at -184.3 ppm that is a triplet of triplets. Couplings of 50.3 Hz to geminal proton $H_{2\beta}$ and to *trans*-diaxial proton $H_{1\beta}$ confirm the axial stereochemistry of the C-F bond while smaller gauche couplings of 8.7 Hz to $H_{1\alpha}$ and $H_{3\alpha}$ confirm the axial stereochemistry of the C-OH bond.

[xi] Heightened safety controls are imperative when handling such a toxic reagent on this scale – details of appropriate experimental controls can be found in the experimental procedure in *Section 8.3*.

3.3.4 Synthesis of 4α-fluoro fluorohydrin intermediate 3.17

3.3.4.1 Chemistry

Reaction of 3β,4β-epoxide **3.15** with Olah's reagent on 1.0 g scale in an identical fashion as before gave expected fluorohydrin **3.17** as the minor product. This compound was isolated as an inseparable mixture with a structural isomer, identified as fluorinated 4,7-oxacyclic byproduct **3.18** (*Scheme 3.6*). No regioisomers ("*anti* Fürst-Plattner" products) were observed from this reaction.



*Scheme 3.6. Opening of Δ3β,4β-epoxide **3.15** with nucleophilic fluoride.*[xii]

The formation of the fluorinated oxacyclic compound is proposed to occur by nucleophilic fluoride attack at the 7-keto group, followed by intramolecular reaction of the resultant 7α-alkoxide with the epoxide group (akin to intramolecular opening of **3.3**, *Figure 3.4*). The ^{1}H NMR of this product is similar to the 4,7-oxa derivative **3.7**, and with identical distinguishing features, therefore will not be discussed further. From the ^{13}C NMR spectrum of **3.18** we observe a signal at 122.8 ppm with a characteristic $^{1}J_{C\text{-}F}$ coupling constant of 237.7 Hz and only one carbon signal belonging to a carbonyl group (from the C_{24} ester, at 174.4 ppm).

3.3.4.2 NMR analysis of the expected fluorohydrin

The stereochemistry of 4α-fluoro product **3.17** was confirmed by ^{1}H NMR analysis of the $H_{4\beta}$ signal (*Figure 3.7*). This proton exhibits a characteristic geminal coupling to fluorine of 46.6 Hz in addition to two gauche ^{3}J couplings of 2.7 Hz to $H_{3\alpha}$ and H_5. Crucially the absence of a coupling constant in the region of 8-15 Hz confirms that this proton is equatorial and therefore the geminal C-F bond is axial (alpha).

[xii] Product ratio determined by ^{19}F NMR of the isolated fraction after column chromatography.

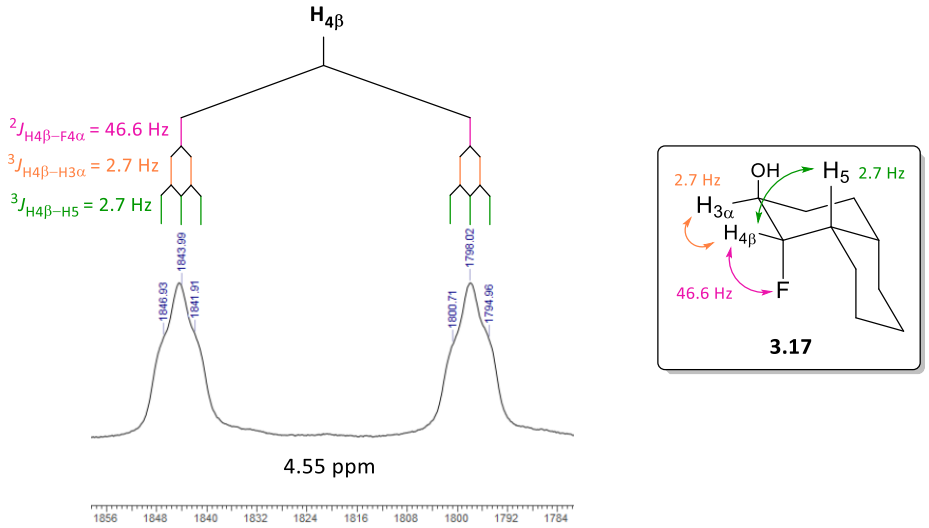


*Figure 3.7. ^{1}H NMR analysis of the $H_{4\beta}$ signal of 4α-fluorinated intermediate **3.17**.*

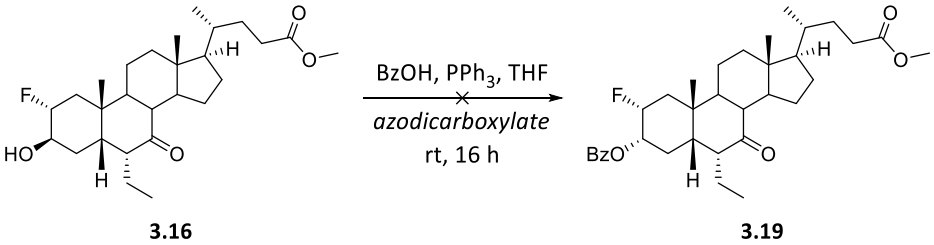
Further support as to structural identity of compound **3.17** is gained from analysis of the ^{19}F NMR spectrum of the compound (not shown). We observe one fluorine signal at -186.8 ppm that is a triplet of doublets. Couplings of 45.1 Hz to geminal proton $H_{4\beta}$ and to *trans*-diaxial proton H_5 confirm the axial stereochemistry of the C-F bond while a smaller gauche coupling of 6.9 Hz to equatorial $H_{3\alpha}$ confirms the axial stereochemistry of the C_3-OH bond.

3.4 Synthesis of 2α-fluoro OCA (JED665)

3.4.1 3β-OH group isomerisation of 2α-fluoro fluorohydrin intermediate 3.16

3.4.1.1 Mitsunobu inversion

The Mitsunobu reaction was first introduced in *Section 2.3.3*. Based on previous successes, it became our primary route of investigation. Both typically used azodicarboxylate species DIAD and DEAD were tested, but with equally lacking success (*Scheme 3.7*).



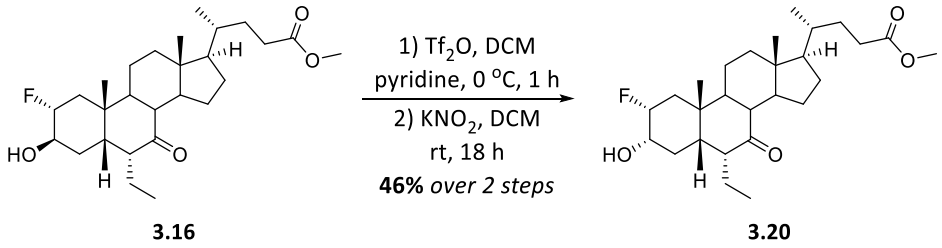
*Scheme 3.7. Attempts at Mitsunobu inversion of the 3β-OH group of **3.16**.*

The failure of this Mitsunobu reaction is likely down to simple sterics – the severe congestion at the α-face prohibits attack by benzoic acid at the 3-position from this trajectory. Notably, the previously successful Mitsunobu reaction was in the isomerisation of the 3-OH group from alpha to beta, so benzoic acid attacked here from the lesser hindered β-face.

Extensive test reactions by a previous group member have found that this poor reactivity at the α-face can be overcome by use of greater excesses of azodicarboxylate reagent or by using a smaller carboxylic acid (acetic acid compared to benzoic acid). These more forcing attempts also gave a significant proportion of elimination product so were not applied to our own fluorinated compound **3.16**.

3.4.1.2 Lattrell-Dax inversion

An established method for the inversion of alcohol groups in carbohydrates is the Lattrell-Dax method and involves potassium nitrite-mediated nucleophilic attack on the pre-formed triflate.[195] We were interested to see how this chemistry would be applied to our bile acid substrate **3.16** and in the first attempt we observed a yield of 46% (*Scheme 3.8*). However, the reaction led to a number of byproducts and purification was cumbersome; we therefore looked towards alternative routes for the isomerisation of the 3β-OH group.

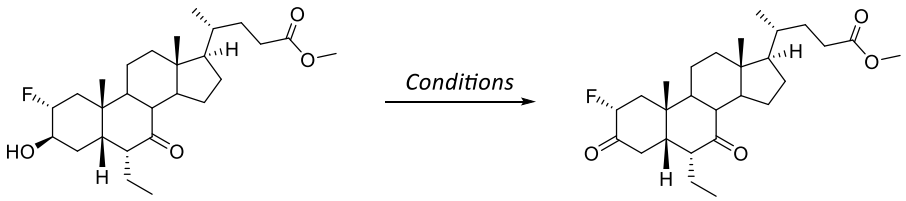


*Scheme 3.8. Lattrell-Dax nucleophilic inversion of the 3β-hydroxyl group of **3.16**.*

3.4.2 3β-OH group oxidation of 2α-fluoro fluorohydrin intermediate 3.16

The low yields associated with direct isomerisation reactions described above are inherent to the displacement of the OH-group occurring by attack from the α-face. We therefore considered an oxidation/reduction strategy where the reaction of hydride on a 3-keto intermediate would occur from the β-face; Luche conditions have already been observed to give high diastereoselectivity towards the desired α-diastereomer (see: *Section 2.3.1*).

The oxidation of 3β-alcohol **3.16** was not required to be selective and so a number of new conditions were explored (*Table 3.2*).



3.16 **3.21**

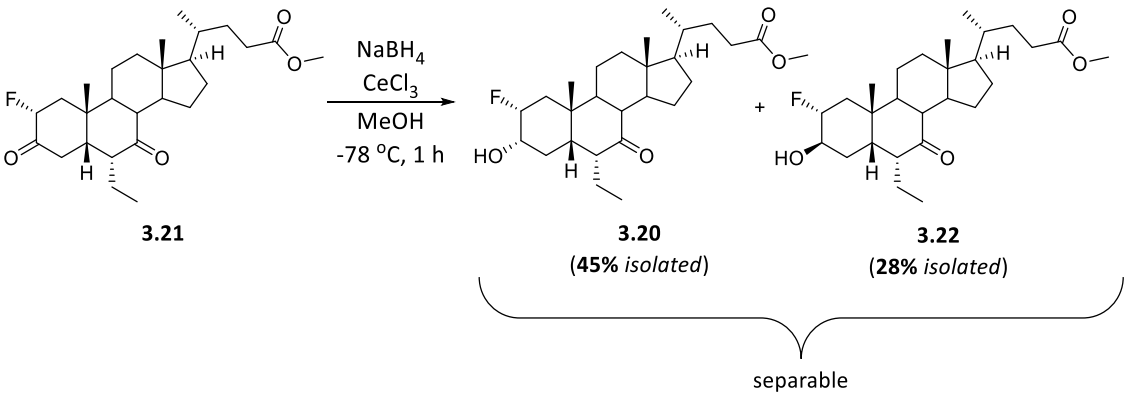
Entry	Reagent(s)	Solvent	Temp. / °C	Time / h	Yield / %
1	TEMPO/NaClO	*t*-BuOH	0	8	58
2	TEMPO/BAIB	DCM	rt	20	40
3	DMP/H_2O	DCM	rt	4	72
4	PCC	DCM	rt	5	73

*Table 3.2. Screening of oxidation conditions of 2α-fluorinated 3-keto intermediate **3.16**. All reactions performed on 200 mg scale and isolated yields are reported following HPLC purification.*

The conditions of TEMPO and bleach solution (used typically for selective oxidation at the 3-OH group) gave a respectable yield of the target diketone after a few hours *(Entry 1)*. Changing the co-oxidant to BAIB (*bis*-acetoxyiodobenzene) gave a significantly worse yield and the reaction was significantly slower (*Entry 2*). The reactions involving Dess-Martin periodinane (DMP) and pyridinium chlorochromate (PCC) were comparable in their yield and rate (*Entries 3* and *4*),[196, 197] however the noxious nature of chromium reagents disfavoured this reaction on larger scale. The oxidation reaction was thus repeated using Dess-Martin periodinane on 500 mg and 1 g scale with yields of 64% and 56% respectively.

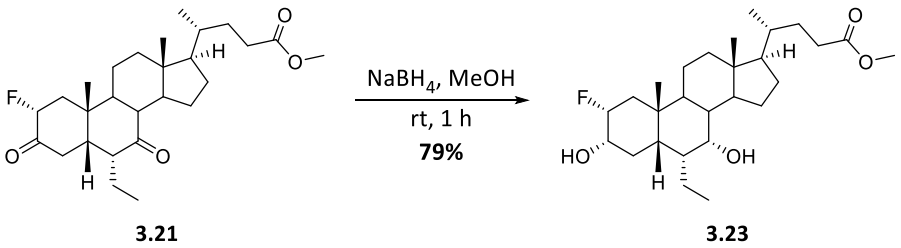
3.4.3 Synthesis of 2α-fluoro OCA

Reduction of the 3-keto group would complete the desired isomerisation of the 3β-alcohol moiety. Reduction of the 7-ketone is also required in the end game to 2α-fluoro OCA and we therefore planned to reduce both positions simultaneously in one reaction. Luche conditions have been previously successful in stereoselective 3-keto reduction to give the single desired 3α-alcohol diastereomer. Applying these conditions to **3.21** however gave an approximate 2:1 ratio of alcohol diastereomers **3.20** and **3.22** and no reaction at the 7-keto group (*Scheme 3.9*). The $H_{3\beta}$ ^{1}H NMR signal of 3α-alcohol **3.20** was observed at 3.50 ppm as a dddd (J = 29.0, 11.7, 4.7, 2.6 Hz) while for 3β-alcohol **3.22** the ^{1}H NMR signal for $H_{3\alpha}$ was observed at 4.01 ppm as a dsxt (J = 6.4, 2.8 Hz); the antiperiplanar H-F coupling constant of ~30 Hz is only possible for the 3α-alcohol product.



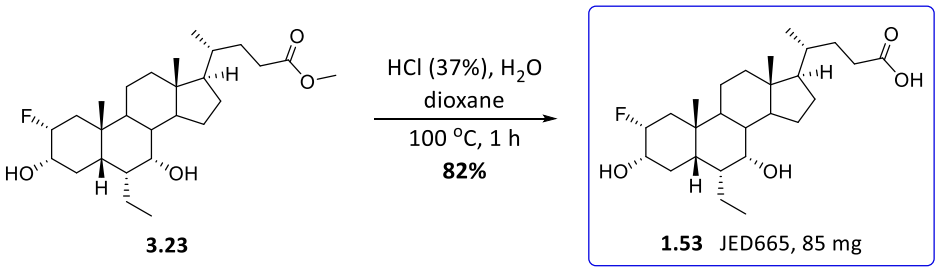
*Scheme 3.9. Luche reduction of 3,7-diketo intermediate **3.21**, proving regioselective to the 3-OH group yet poorly diastereoselective.*

Omitting the Lewis acid and performing the reaction at ambient temperature led to reaction at both ketone groups and, surprisingly, the reaction was highly diastereoselective at both positions to give the '3α,7α-product' in a high yield (*Scheme 3.10*).[xiii]



*Scheme 3.10. Stereoselective reduction of the 3-keto and 7-keto groups of **3.21** simultaneously.*

Finally, hydrolysis of the C_{24}-methyl ester under familiar acidic conditions gave a good yield of target 2α-fluoro OCA (*Scheme 3.11*).



*Scheme 3.11. Synthesis of target 2α-fluoro OCA (**1.53**).*

[xiii] The stereochemistry of the 7α-OH group was confirmed by the absence of an antiperiplanar coupling constant from the $H_{7\beta}$ signal (observed as a broad singlet from small merged gauche couplings (1-2 Hz) to $H_{6\beta}$ and H_8).

3.4.4 NMR analysis

The ^{1}H NMR of target **1.53** was used to confirm the identity of the product (not shown). The signal for $H_{2\beta}$ was observed as a broad doublet at 4.75 ppm with a characteristic geminal coupling to fluorine of 52.5 Hz, indicating three small vicinal gauche couplings to $H_{1\alpha}$, $H_{1\beta}$ and $H_{3\beta}$. Importantly, the absence of a distinct 8-15 Hz antiperiplanar coupling constant confirms that proton $H_{2\beta}$ is indeed equatorial. This was confirmed by ^{19}F NMR analysis (*Figure 3.8*) which featured a sharp tdt signal with a geminal coupling to $H_{2\beta}$ of 51.6 Hz and a ^{3}J antiperiplanar coupling to $H_{1\beta}$ also of 51.6 Hz. A large antiperiplanar ^{3}J coupling of 28.6 Hz was also observed to $H_{3\beta}$ and is attenuated to the lower end of the expected 30-50 Hz range by the adjacent electron withdrawing C-O bond. The reduction of *trans*-diaxial proton-proton coupling constants by proximal electron withdrawing motifs is well reported.[198]

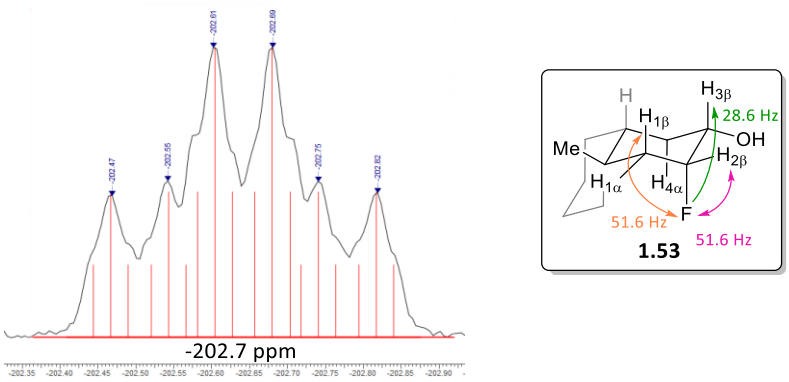


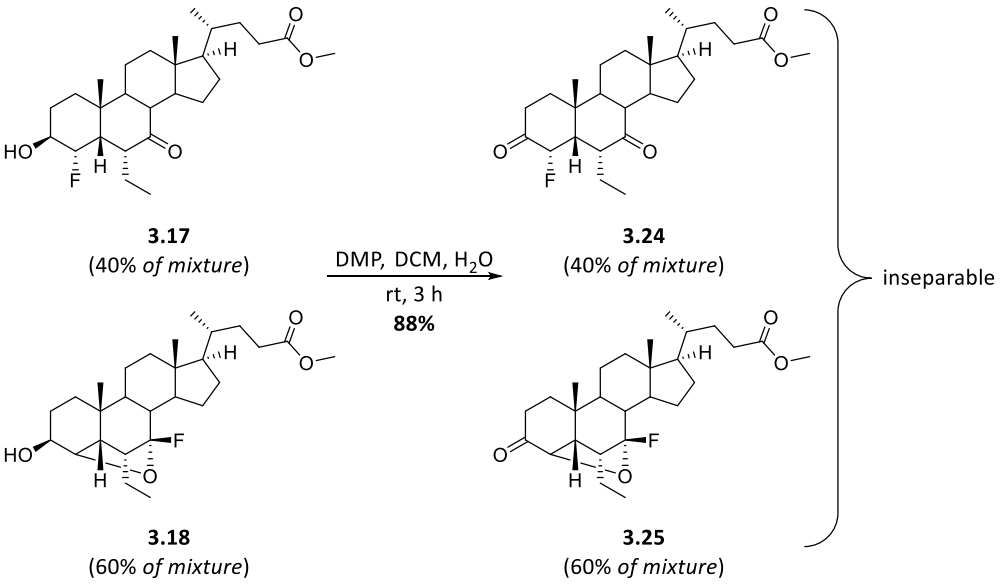
*Figure 3.8. ^{19}F NMR analysis of the $F_{2\alpha}$ signal of target 2α-fluoro OCA (**1.53**).*[xiv]

3.5 Synthesis of 4α-fluoro OCA (JED664)

3.5.1 3β-OH group isomerisation of 4α-fluoro fluorohydrin intermediate 3.17

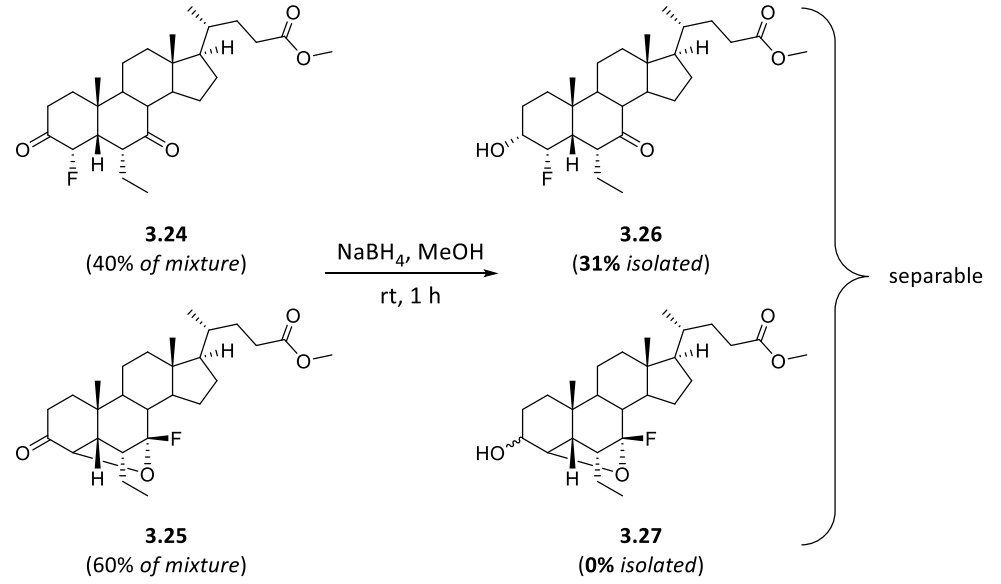
Direct nucleophilic inversion of the 3-OH group has already proven cumbersome (see: *Section 3.4.1*). A two-step oxidation/reduction strategy was therefore the primary route of investigation for 4α-fluoro intermediates. The inseparable mixture of fluorohydrin **3.17** and 4,7-oxacyclic byproduct **3.18** was oxidised using Dess-Martin periodinane. An excellent yield of the corresponding 3-keto products was achieved (*Scheme 3.12*), however they were still inseparable at this stage.

[xiv] Gauche couplings are omitted from the 3D-structural drawing for concision. The complexity of the signal precluded the illustration of a splitting tree.



*Scheme 3.12. Oxidation of fluorohydrin **3.17** and 4,7-oxacycle **3.18** mixture with DMP.*

Diastereoselective reduction of the 3-keto group was performed in an identical fashion as described for the analogous 2α-fluoro compound **3.21**. According to ^{19}F NMR, both starting 3-keto compounds **3.24** and **3.25** were consumed rapidly. Only one compound was however isolated after purification by column chromatography, namely 3α-hydroxy-4α-fluoro fluorohydrin **3.26** and this compound is the product of reduction of **3.24** at the 3-keto group only.[xv] No trace of a C_7-reduction products was observed. Additionally, the expected product **3.27** (from reduction of **3.25**) could not be isolated. (*Scheme 3.13*).



*Scheme 3.13. 3-keto reduction of 4α-fluoro intermediate **3.24**.*

[xv] Although reported in the experimental details section as an overall multiplet, the signal for $H_{3\beta}$ exhibited a clear doublet coupling of approximately 30 Hz that is only possible from a *trans*-diaxial coupling to fluorine.

We expect that the 7-keto group of diketo **3.24** is unreactive because hydride attack at the β-face will result in a 7α-alkoxide motif that would experience strong electronic repulsion with the 4α-fluoro atom, disfavouring the reaction under these mild conditions (*Figure 3.9*). Additionally, the alternate trajectory of hydride attack from the α-face is likely blocked altogether by the C-F bond. More forcing conditions (temperature, activating agents) will therefore be required for the reduction of this ketone group.

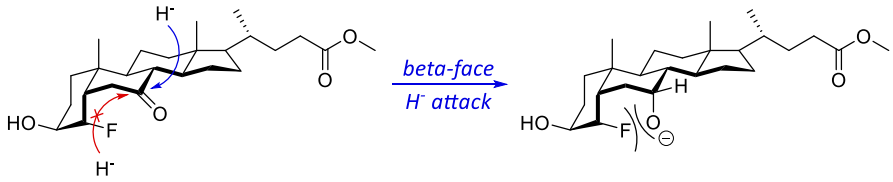
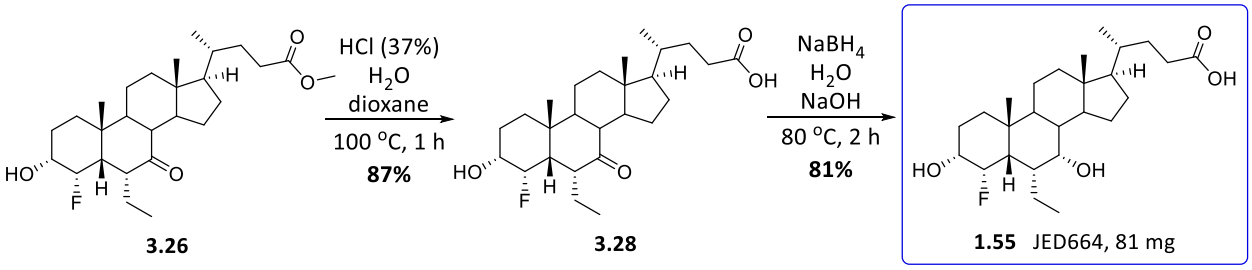


Figure 3.9. Mild reduction of the 7-keto group is prevented by electronic effects (for β-hydride attack, blue) and sterics (for α-hydride attack, red). 6-Ethyl group omitted for concision.

3.5.2 Synthesis of 4α-fluoro OCA

Forcing conditions could unintendedly reduce the C_{24}-methyl ester group to a primary alcohol group, so saponification to the carboxylic acid was performed first (*Scheme 3.14*).Using acidic conditions in an identical fashion to those described already, 4α-fluoro-7-keto OCA (**3.28**) was prepared in good yield. Lastly, in the presence of the chemically orthogonal 24-carboxylic acid group, forcing reduction of the 7-keto group was achieved with a mildly basic aqueous solution of sodium borohydride heated to 80 °C to give target 4α-fluoro OCA (**1.55**) in a good yield.



*Scheme 3.14. Synthesis of target 4α-fluoro OCA (**1.55**).*

3.5.3 NMR analysis

The $H_{4\beta}$ signal in the ^{1}H NMR of target **1.55** was a similar broad doublet (J = 51.4 Hz) to that observed for 2-fluoro regioisomer **1.53** (see: *Section 3.4.4*). From the ^{19}F NMR spectrum of target **1.55** we can however unequivocally prove the identity of the product (*Figure 3.10*). At -192.8 ppm we observe a triplet of doublets with a geminal coupling to $H_{4\beta}$ of 48.6 Hz and a ^{3}J antiperiplanar coupling to H_5 also of 48.6 Hz. A large antiperiplanar ^{3}J coupling of 33.0 Hz was also observed to $H_{3\beta}$ and is attenuated to the lower end of the expected 30-50 Hz range by the adjacent electron withdrawing

C-O bond. These key antiperiplanar coupling constants prove the axial stereochemistry of the fluorine atom and equatorial stereochemistry of the hydroxyl group.

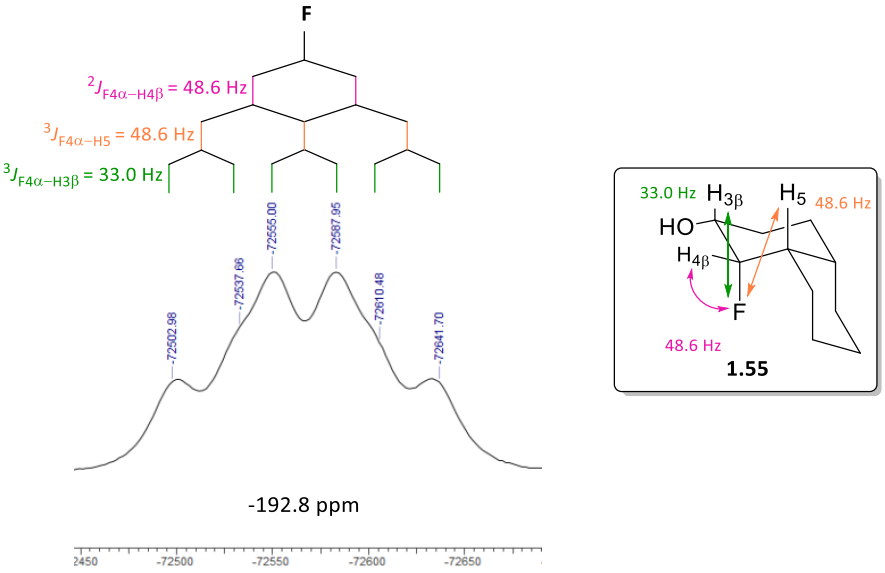


*Figure 3.10. ^{19}F NMR analysis of the $F_{4\alpha}$ signal of target 4α-fluoro OCA (**1.55**).*

3.6 Conclusions

To conclude, the two novel target 2- and 4-position α-fluorinated OCA analogues were synthesised (*Figure 3.11*) following a nucleophillic epoxide opening strategy with fluoride. The preparation of the preceding epoxide intermediates was successful in the presence of the 7-keto group but not the 7α-hydroxyl group.

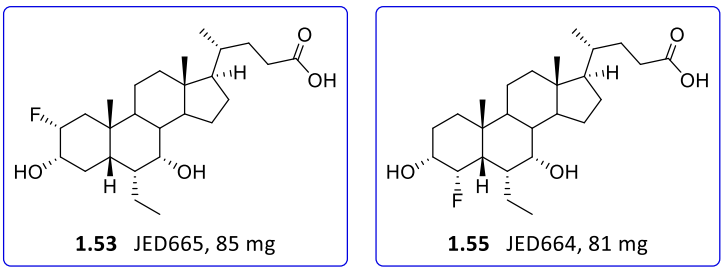


Figure 3.11. Synthesised 2- and 4-position α-fluorinated OCA derivatives.

The reduction of the 7-ketone proceeded under mild conditions for the 2-fluorinated intermediate. For the analogous 4-fluorinated intermediate, forcing conditions were required to overcome the electronic repulsion between the nearby 4α-fluoro group and resultant proximal alkoxide specie.

Chapter 4 Synthesis of OCA (NZP084) 2β-fluoro and 4β-fluoro analogues

4.1 Retrosynthetic analysis

Precedence for electrophilic fluorination reactions commonly involves nucleophilic enol and enolate derivatives,[199] such as silyl enol ethers.[200] The β-selectivity of reactions at the nucleophilic 2- and 4-positions has already been described and could give direct access to the desired β-fluorinated targets. Fluorination of 3-keto derivatives by this strategy became our primary route of investigation.

Retrosynthesis of 2β-fluoro OCA (**1.54**) leads to Δ2,3-silyl enol ether **2.8** (*Figure 4.1*) as the forward reaction with electrophilic fluoride is expected to be β-selective. An equivalent retrosynthesis of 4β-fluoro OCA (**1.56**) can be made via silyl enol ether **2.9**. Their synthesis was described in *Section 2.2.2.2.1* from OCA methyl ester (**2.5**) by selective oxidation of the 3α-OH group and a silyl enol ether formation reaction.

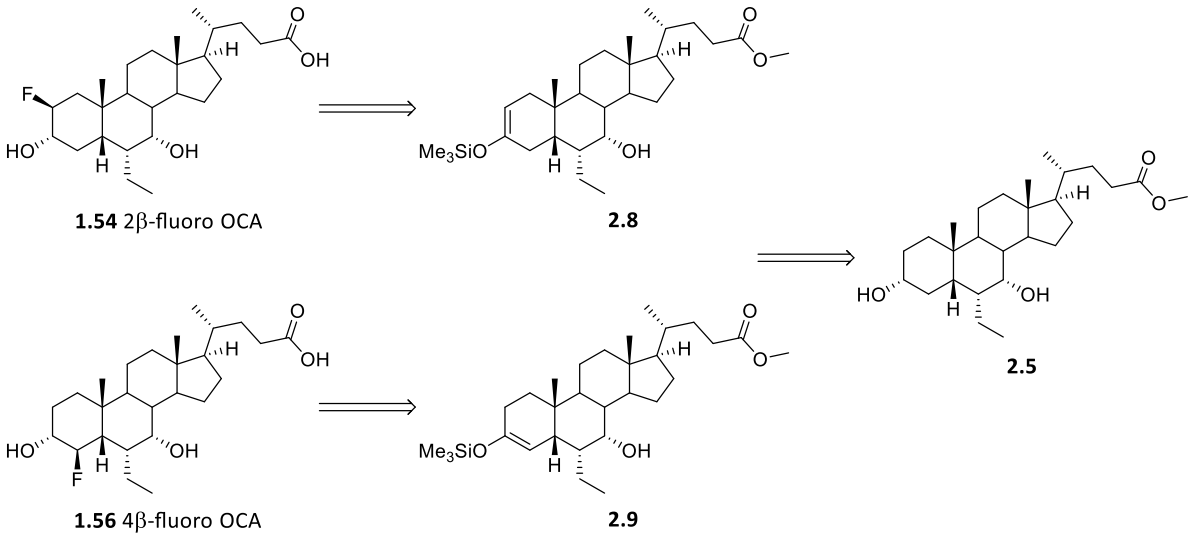
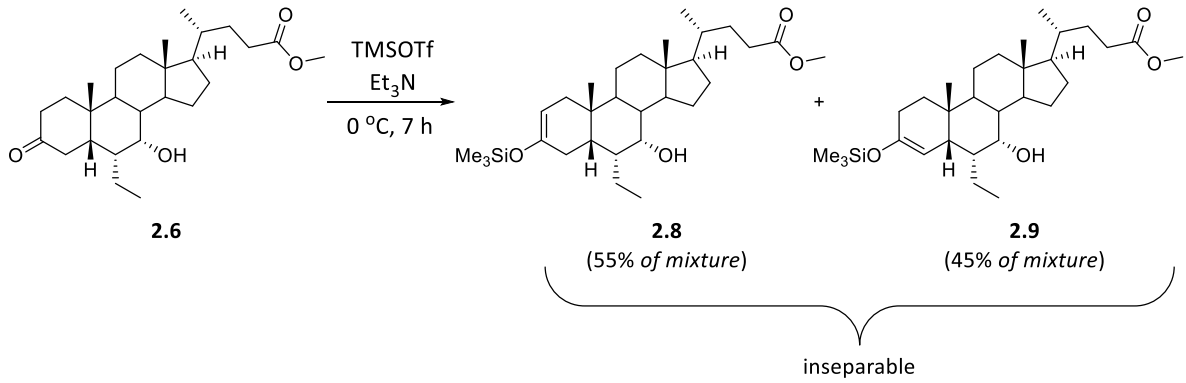


*Figure 4.1. Retrosynthesis of the 2-position and 4-position β-fluoro targets **1.54** and **1.56**.*

4.2 Synthesis of fluoroketone intermediates

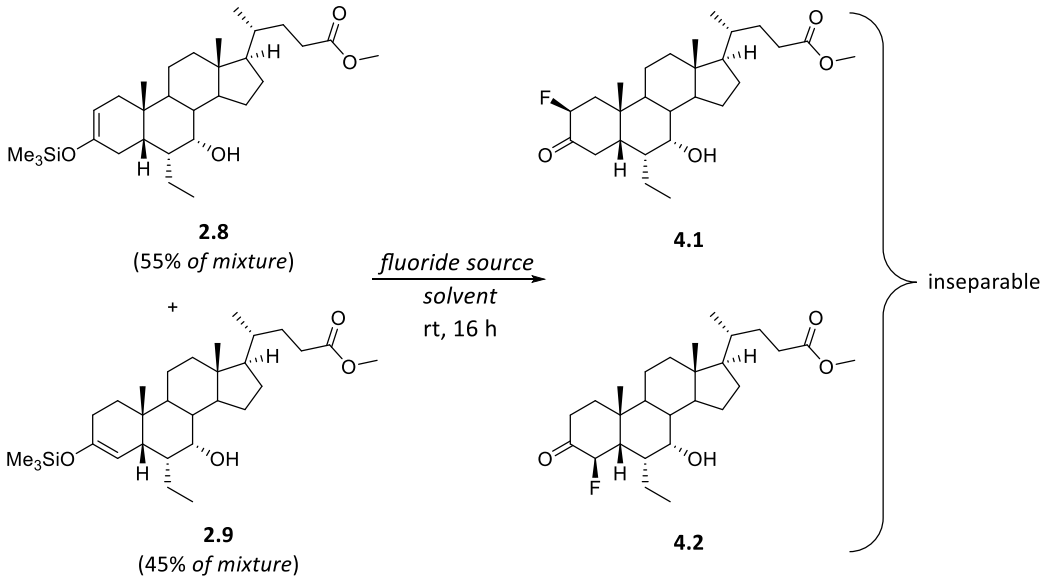
4.2.1 Chemistry

Starting from 3-keto OCA methyl ester (**2.6**), applying the previously described conditions towards a ~1:1 mixture of silyl enol ether regioisomers gave excellent conversion (>95% according to ^{1}H NMR) towards intermediates **2.8** and **2.9** (*Scheme 4.1*).



*Scheme 4.1. Synthesis of ~1:1 mixture of silyl enol ether regioisomers, from **2.6**.*

Treatment of the crude mixture of silyl enol ethers with electrophillic fluorination reagents was β-selective for both regioisomers but gave only appreciable quantities of 4β-fluorinated intermediate **4.2** (*Table 4.1*). Poor yields of 2β-fluoroketone **4.1** were encountered in all attempts.



Entry	Fluoride source	Solvent	Scale / mg	Conversion
1	NFSI	THF	60	2% **4.1** 44% **4.2**
2	SelectFluor®	THF	60	0% **4.1** 0% **4.2**
3	SelectFluor®	MeCN	270	1% **4.1** 52% **4.2**

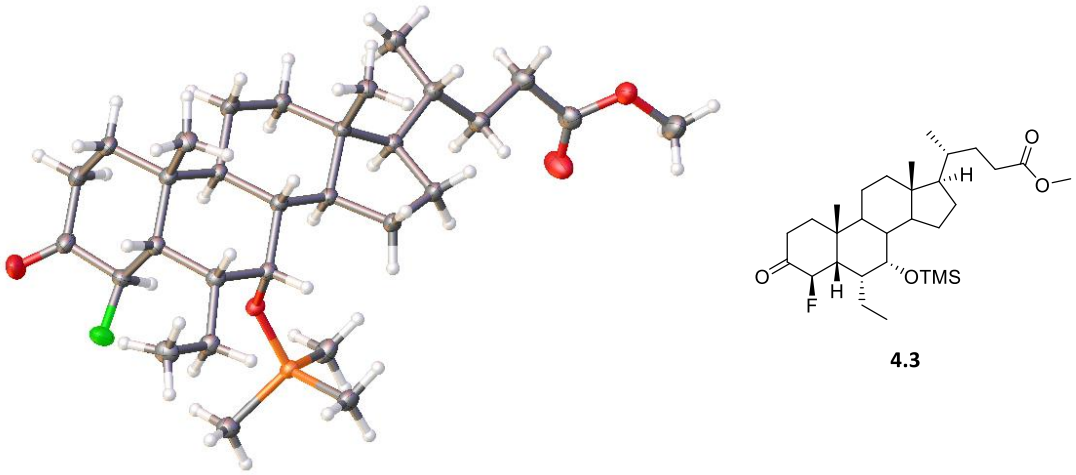
Table 4.1. Screening of electrophilic fluorination conditions.

Conversion judged by ^{1}H and ^{19}F NMR analysis of the crude material.

The use of NFSI in THF gave a mediocre yield of the 4-fluorinated regioisomer and a very poor yield of the corresponding 2-fluoro product (*Entry 1*). Using Selectfluor® in the same solvent gave no

reaction (*Entry 2*) likely owing to the poor solubility of this reagent in all but highly polar solvents. When used in combination with acetonitrile (*Entry 3)*, Selectfluor® gave almost no 2β-fluoro product **4.1** but an acceptable yield of 4β-fluoro product **4.2**.

A byproduct from the reactions was 7-OTMS protected variant **4.3** of the 4β-fluoroketone (not shown in scheme). A crystal of this compound was isolated from a crude reaction mixture and a single crystal XRD structure was attained (*Figure 4.2*, thermal ellipsoids drawn at the 50% probability level).



4.3

*Figure 4.2. Single crystal X-ray structure of 4β-fluorinated-7-OTMS byproduct **4.3**.*

Alternatives to the low-yielding electrophillic fluorination strategy towards 2β-fluoro intermediate **4.1** were explored and are described in *Sections 4.6* and *4.7*.

4.2.2 NMR analysis of 2β-fluoro fluoroketone intermediate 4.1

The $H_{2\alpha}$ signal in the ^{1}H NMR of fluoroketone **4.1** was identified as a ddd at 5.04 ppm (*Figure 4.3*), with the largest coupling constant being a characteristic geminal proton-fluorine interaction, measured at 49.6 Hz. There is also a *trans*-diaxial proton-proton coupling of 13.8 Hz to $H_{1\beta}$, and this confirms the stereochemistry of the C-H bond, and hence also the C-F bond, at C_2. There is also a proton-proton gauche coupling of 6.5 Hz to $H_{1\alpha}$.

Analysis of the ^{19}F NMR spectrum was precluded by the low signal to noise ratio of the sample.

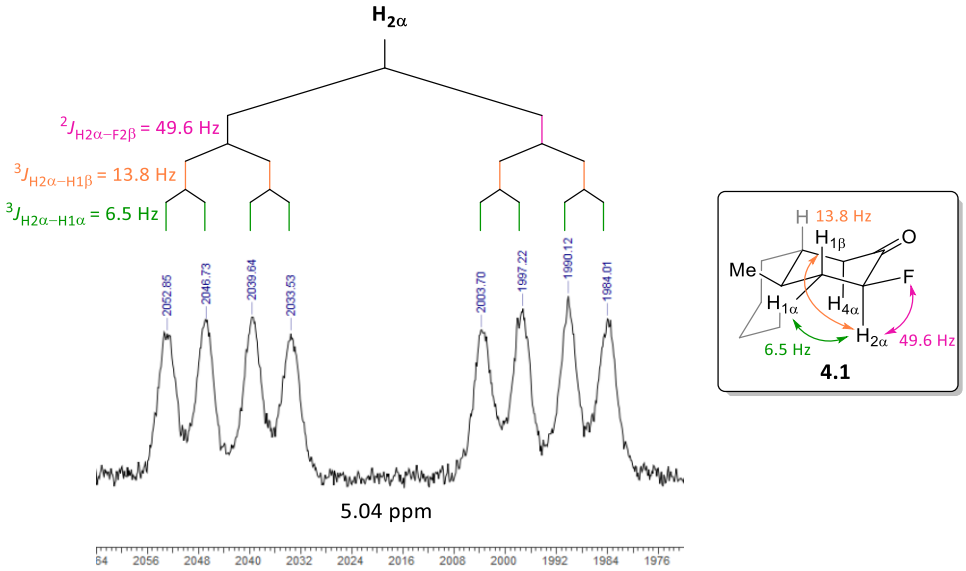


*Figure 4.3. ^{1}H NMR analysis of the $H_{2\alpha}$ signal of 2β-fluorinated intermediate **4.1**.*

4.2.3 NMR analysis 4β-fluoro fluoroketone intermediate 4.2

The $H_{4\alpha}$ signal in the ^{1}H NMR of fluoroketone **4.2** was identified as a dd at 5.94 ppm (*Figure 4.4*), with a 46.5 Hz geminal coupling to fluorine and a 10.9 Hz antiperiplanar coupling to H_5.

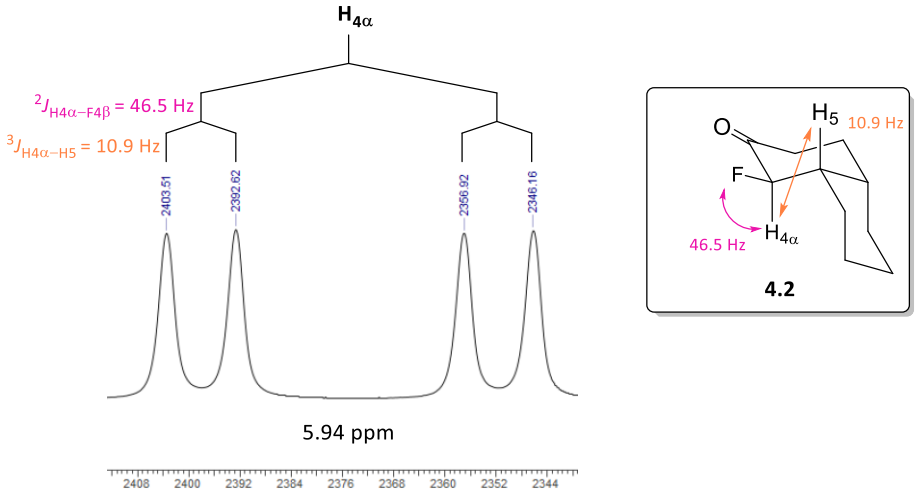


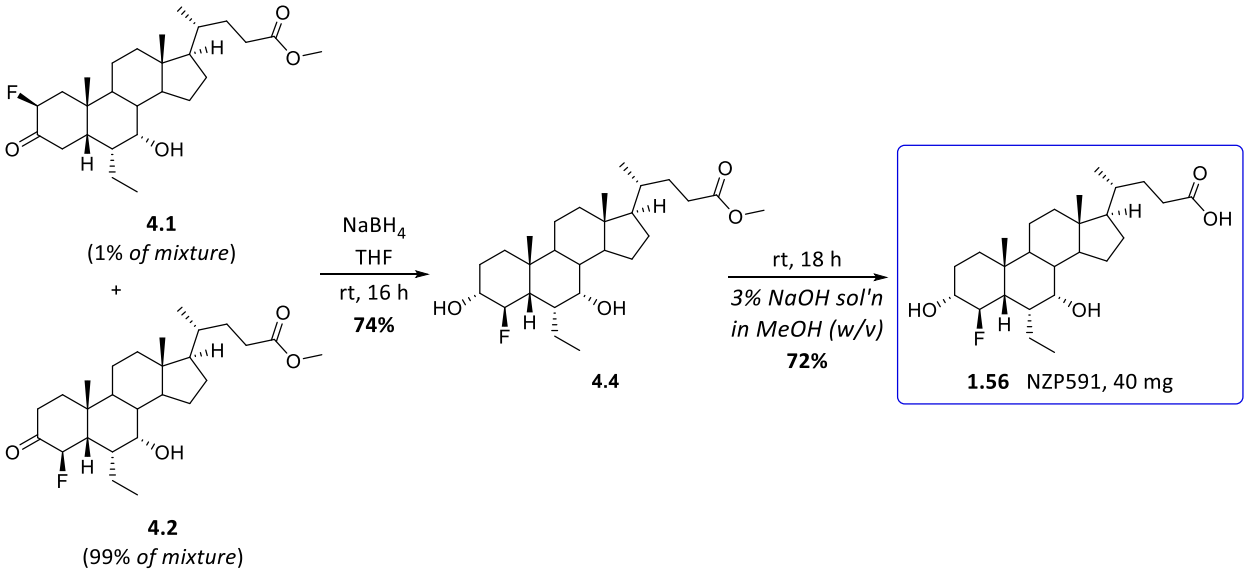
*Figure 4.4. ^{1}H NMR analysis of the $H_{4\alpha}$ signal of 4β-fluorinated intermediate **4.2**.*

The ^{19}F NMR of 4β-fluoro fluoroketone intermediate **4.2** exhibited one signal as a dd at -194.3 ppm (not shown). In addition to the geminal coupling to $H_{4\alpha}$ of 46.8 Hz is a gauche coupling to H_5 of 10.9 Hz , which further supports that the C-F bond is in the 4β-position.

4.3 Synthesis of 4β-fluoro OCA (NZP591)

4.3.1 Chemistry

Diastereoselective reduction of 4β-fluorinated fluoroketone **4.2** gave 4β-fluoro OCA methyl ester (**4.4**) in good yield (*Scheme 4.2*). No trace of the 2β-fluorinated contaminant (or its reduction product) was found after purification by column chromatography. Finally, saponification of the methyl ester intermediate also proceeded in good yield to give target 4β-fluoro OCA (**1.56**).



*Scheme 4.2. Synthesis of target 4β-fluoro OCA (**1.56**).*

4.3.2 NMR analysis

The ^{1}H NMR signal for H_4 (*Figure 4.5*) was observed at 5.31 ppm as a ddd with a 49.9 Hz geminal coupling to fluorine. Two antiperiplanar couplings, 10.3 Hz to H_5 and 9.1 Hz to $H_{3\beta}$, confirm the equatorial stereochemistry of the C_4-F and C_3-OH bonds.

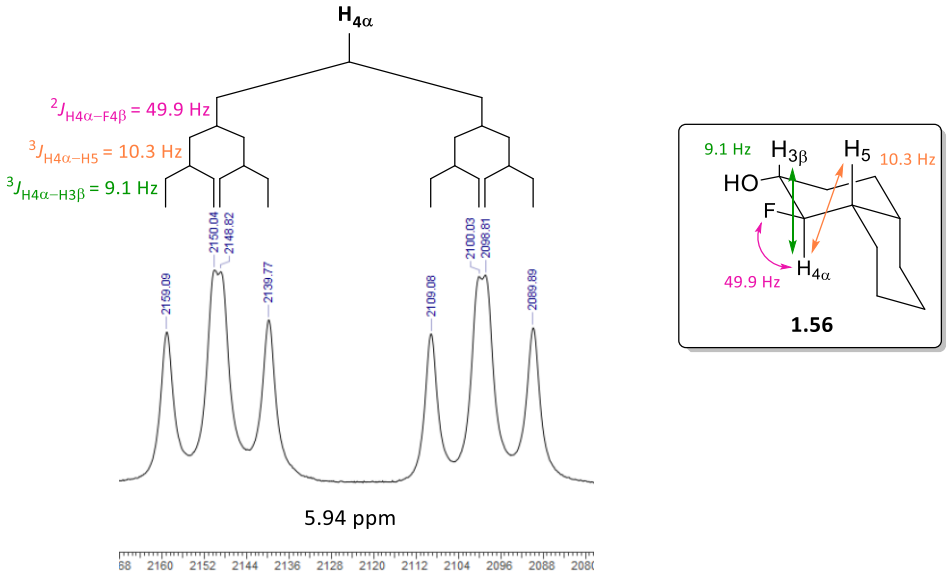


*Figure 4.5. ^{1}H NMR analysis of the $H_{4\alpha}$ signal of target 4β-fluoro OCA (**1.56**).*

The ^{19}F NMR of 4β-fluoro OCA (**1.56**) exhibited only one signal at -188.7 ppm as a broad doublet of 48.6 Hz from geminal coupling to $H_{4\alpha}$ (not shown). Gauche couplings to other adjacent protons are merged to give the doublet its broad character.

4.4 Synthesis of 4β-fluoro OCA sulfonyl ureas (NZP781, JED563, JED659, JED672 and JED673)

4.4.1 Rationale

The biological activity of 4β-fluoro OCA (**1.56**) was deemed 'of interest' (see: *Chapter 7*) and merited subsequent derivatisation to the corresponding aromatic-substituted sulfonyl urea analogues with shortened C_{17}-side chain.

The well-established Curtius rearrangement of a C_{24}-acyl azide intermediate could be highly synthetically useful.[201] Firstly, it could achieve the installation of the desired C-N bond at C_{23} and, secondly, the reactive isocyanate product could directly provide the urea motif upon reaction with an amine (*Figure 4.6*). Therefore, use of a pre-functionalised amine such as a sulfonamide could give rapid access to the desired class of target derivatives.

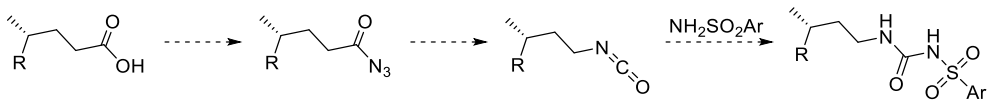
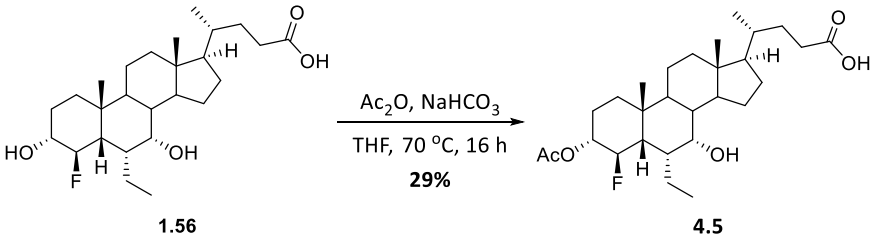


Figure 4.6. Proposed forward-synthesis of sulfonyl urea targets involving the Curtius rearrangement. Only the C_{17}-side chain is shown, for concision.

4.4.2 *O*-protection

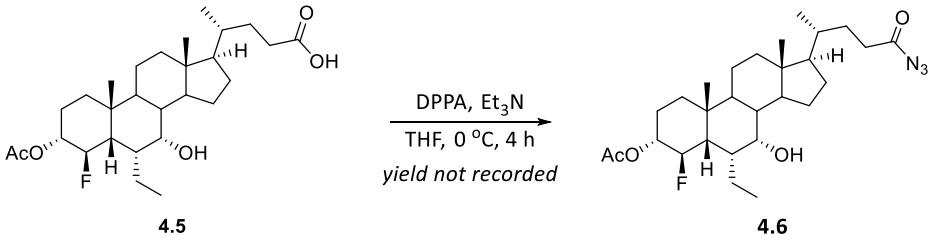
Protection of the labile 3-OH group was required. As seen already, reactivity at the 7α-OH group is mostly precluded by sterics and so simple acetate protection of the 3α-alcohol was our primary route of investigation. Reaction of 4β-fluoro OCA (**1.56**) with acetic anhydride under reflux in THF gave a poor 29% isolated yield of the corresponding 3-*O*-protected derivative **4.5** (*Scheme 4.3*), a yield significantly worse than for the analogous methyl ester compound. A byproduct of the reaction would be the formation of a mixed anhydride involving the C_{24}-carboxylic acid but would be hydrolysed upon aqueous workup. No trace of a *bis*-acetate protected compound was observed by NMR analysis. Time constraints precluded the re-synthesis of the starting material **1.56** that was entirely consumed in this one reaction, but further attempts at this reaction (for example, using acetyl chlorine with pyridine) would have been sought.



*Scheme 4.3. Selective acetate protection of the 3α-OH group of 4β-fluoro OCA (**1.56**).*

4.4.3 Curtius rearrangement

Deprotonation of the C_{24}-carboxylic acid and reaction with diphenylphosphoryl azide (DPPA) yielded acyl azide **4.6** (*Scheme 4.4*). Notably, the reaction was performed at 0 °C and on a maximum of 500 mg scale to reduce the explosive risks of azides.[xvi]



*Scheme 4.4. Conversion of carboxylic acid **4.5** to acyl azide **4.6** with diphenylphosphorylazide.*

IR analysis showed the carbonyl peak of the acetate group at 1735 cm^{-1} in both the isolated starting material and crude reaction mixture; the carbonyl peak of the starting carboxylic acid was identified

[xvi] The adoption of 0 °C also extended to the workup procedure (ice-cold solvents) and during rotary evaporation (ice-water bath). Yields were not recorded for this reaction to reduce excess-handling.

at 1708 cm^{-1} and was absent in the crude mixture, but a sharp peak at 2133 cm^{-1} confirmed the presence of the acyl azide motif (expected region 2160-2120).[202] In the ^{13}C NMR spectrum the starting carboxylic acid carbon signal at 179.6 ppm was completely absent while a sharp signal at 181.1 ppm had emerged. The sample totally degraded within one hour at ambient temperature.

The mechanism of the Curtius rearrangement involves concerted thermal decomposition of an acyl azide, whereby migration of the α-carbon (wrt the carbonyl) yields a C-N bond at the loss of dinitrogen gas (*Figure 4.7*),[203, 204] to yield an isocyanate.

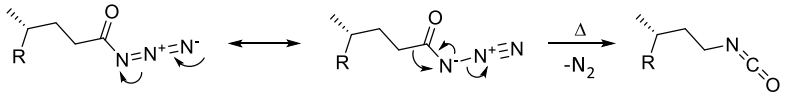
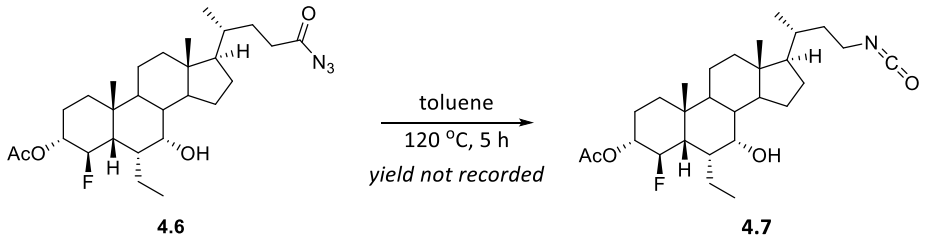


Figure 4.7. Mechanism of the Curtius rearrangement.[203, 204]

The thus formed reactive isocyanate derivative **4.7** (*Scheme 4.5*) was taken forward directly to the next reaction as a solution in toluene, after cooling to rt.

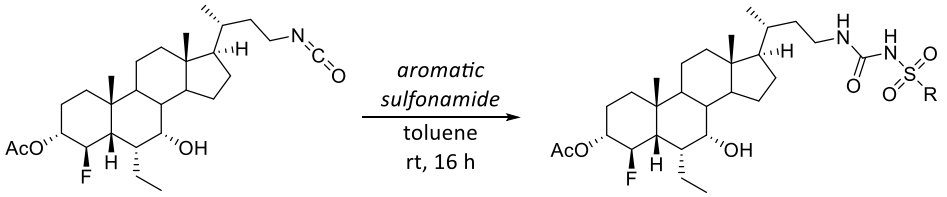


*Scheme 4.5. Curtius rearrangement of O-protected 4β-fluoro OCA acyl azide **4.6**.*

The identity of the product was again confirmed by IR spectroscopic analysis. The carbonyl peak of the acyl azide at 2133 cm^{-1} was replaced by a new peak at 2270 cm^{-1}, characteristic of an isocyanate group (expected 2270-2250 cm^{-1}).[202] The carbonyl peak of the acetate protecting group was still present (1737 cm^{-1}). Through ^{13}C NMR analysis we observed the isocyanate carbon atom at 121.8 ppm with no trace of the starting material.

4.4.4 Synthesis of *O*-protected sulfonyl ureas

Reaction of the crude solution of isocyanate **4.7** with aromatic sulfonamides gave the corresponding *O*-protected sulfonyl urea derivatives in yields ranging mediocre to excellent (*Table 4.2*).



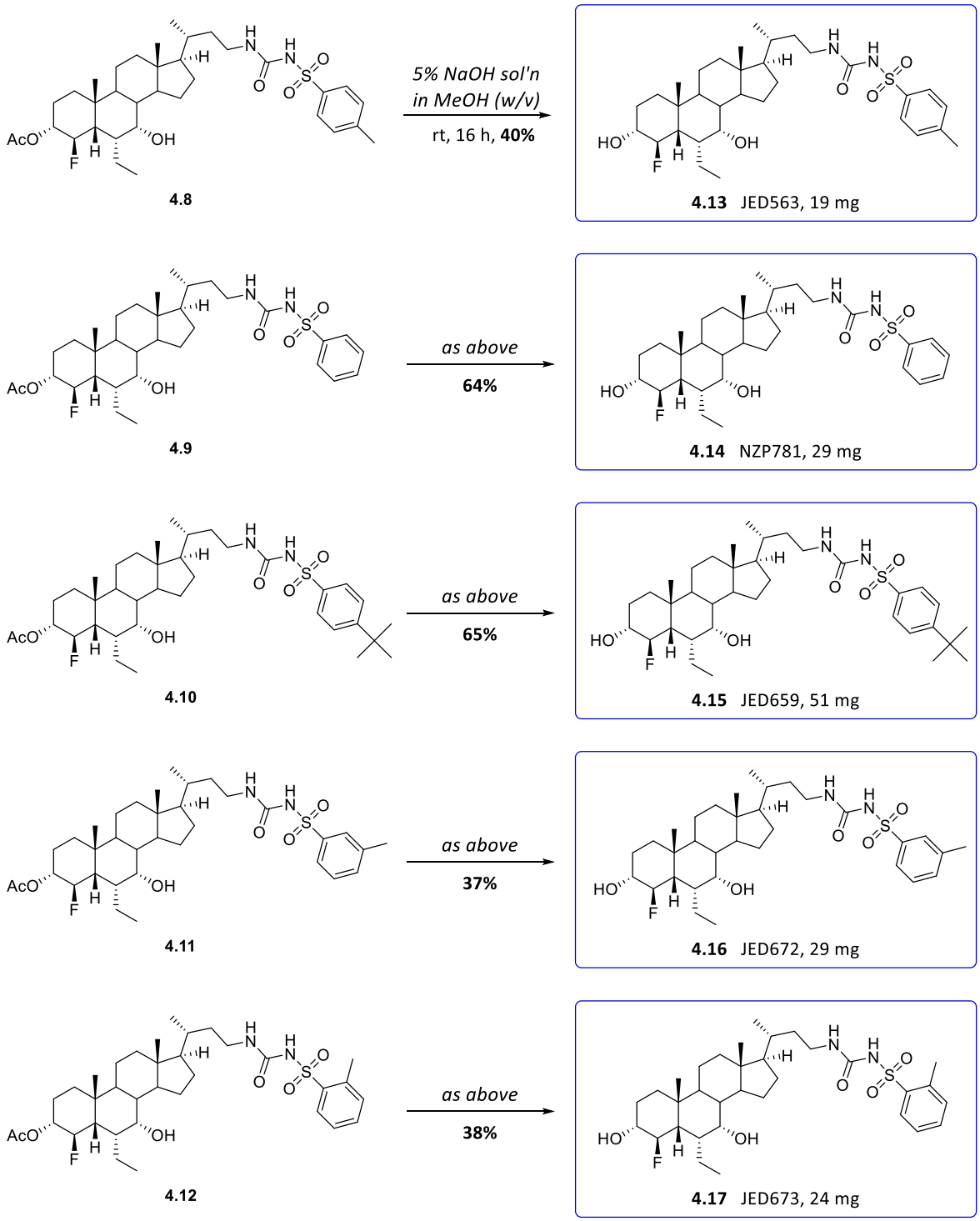
4.7 **4.8-4.12**

Compound	R group	Isolated yield / %
4.8	*p*-toluyl	38
4.9	phenyl	38
4.10	4-*tert*-butyl phenyl	71
4.11	*m*-toluyl	80
4.12	*o*-toluyl	51

*Table 4.2. Synthesis of O-protected sulfonyl ureas **4.8**-**4.12**.*

4.4.5 Synthesis of 4β-fluoro OCA sulfonyl ureas

Lastly, deprotection of the 3α-acetate sulfonyl ureas **4.8**-**4.12** was performed under mildly basic conditions. Synthesis of the target sulfonyl ureas **4.13**-**4.17** was accomplished in mediocre to good yields (*Scheme 4.6*).

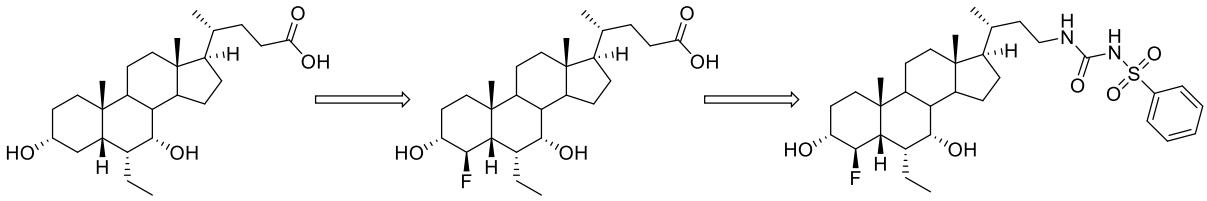


*Scheme 4.6. Synthesis of 4β-fluoro OCA sulfonyl urea targets **4.13**-**4.17**.*

Compound **4.14** is proven to be extremely potent at the FXR with a good safety profile (see: *Chapter 7*). As such, it is an attractive candidate for the treatment of NASH.

4.5 Contribution towards 'process-scale synthesis' of 4β-fluoro OCA phenyl-sulfonyl urea (NZP781)

4.5.1 Introduction



1.8 OCA | **1.44** 4β-fluoro OCA (NZP591) | **4.14** (NZP781)

Figure 4.8. Development of a highly active FXR agonist.

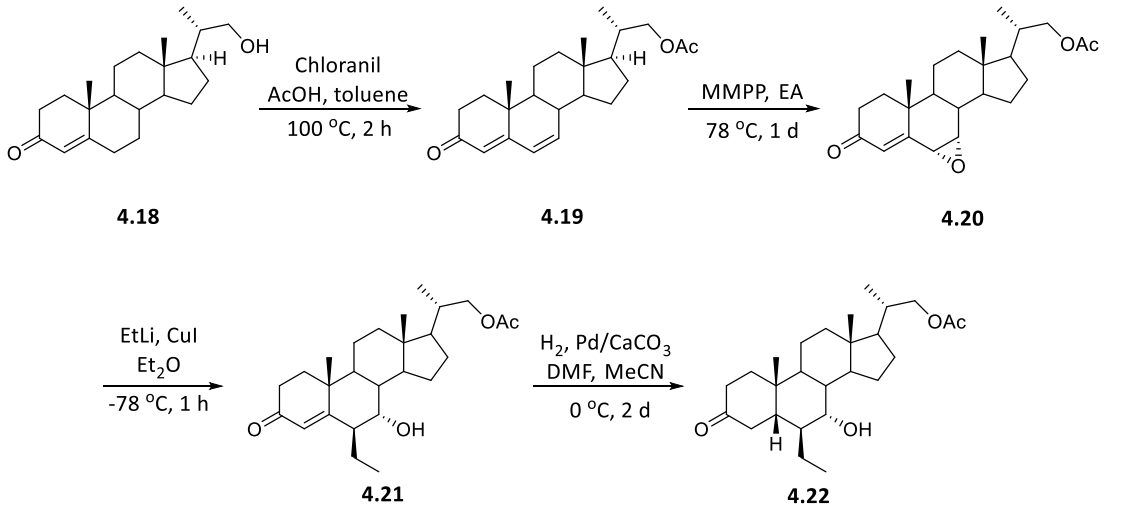
Unfortunately, the current synthesis of **4.14** involved chemistry that would be difficult on a multi-gram or kilogram scale. For example, installation of the isocyanate group on the side-chain previously involved the Curtius rearrangement of an unstable and potentially explosive acyl azide intermediate. It is therefore of importance to revaluate the synthesis of target **4.14** for process-scale.

The synthesis described herein was performed on-site at our industrial sponsor NZP UK Ltd. during a thirteen-week placement. Specifically, the focus was on optimising a key fluorination step that is part of the wider synthetic plan towards **4.14** (see: *Section 4.5.2.2*).

4.5.2 Synthesis plan

4.5.2.1 Established process chemistry

A well-established multi-kilogram synthesis of advanced intermediate **4.22** from **4.18** is reported by NZP (*Scheme 4.7*).[15] Importantly, the starting material is a plant extract; the avoidance of animal-derived steroids for large-scale campaigns is essential for moral reasons. Prior to the commencement of my placement work towards target **4.14**, sufficiently large quantities of advanced enone intermediate **4.21** were prepared by industrial colleagues with the view that I would complete the last step to **4.22** (see: *Section 4.5.3.1*).



*Scheme 4.7. Existing and optimised multi-kilogram synthesis of advanced intermediate **4.22**.*

4.5.2.2 Retrosynthesis

A retrosynthesis of target phenyl-sulfonyl urea **4.14** was envisioned that led to established-intermediates **4.22** and **4.21** (*Figure 4.9*). The previous work in Southampton on milligram-scale involved substrates with a C_{24}-carboxylic acid group whereas the advanced intermediates in the figure below (**4.21** and **4.22**) have a side-chain ending at C_{22}. Hence, functional group interconversion from target **4.14** involving the sulfonyl urea group led to primary amine **4.23**, and then to nitrile **4.24**. The forward synthesis could involve the reduction of the C-N triple bond and coupling with an *N*-sulfonyl carboxamide. Disconnection of the cyanide group leads to a good leaving group at C_{22} of **4.25**, which could be accessed from **4.22**; the forward synthesis would require 4β-fluorination of the C_3 ketone (see: *Section 4.2.1*) and the epimerisation of the 6-ethyl group proceeding via a 7-keto intermediate (not shown), further investigation would discover the optimal order of events (see: *Section 4.5.3*).

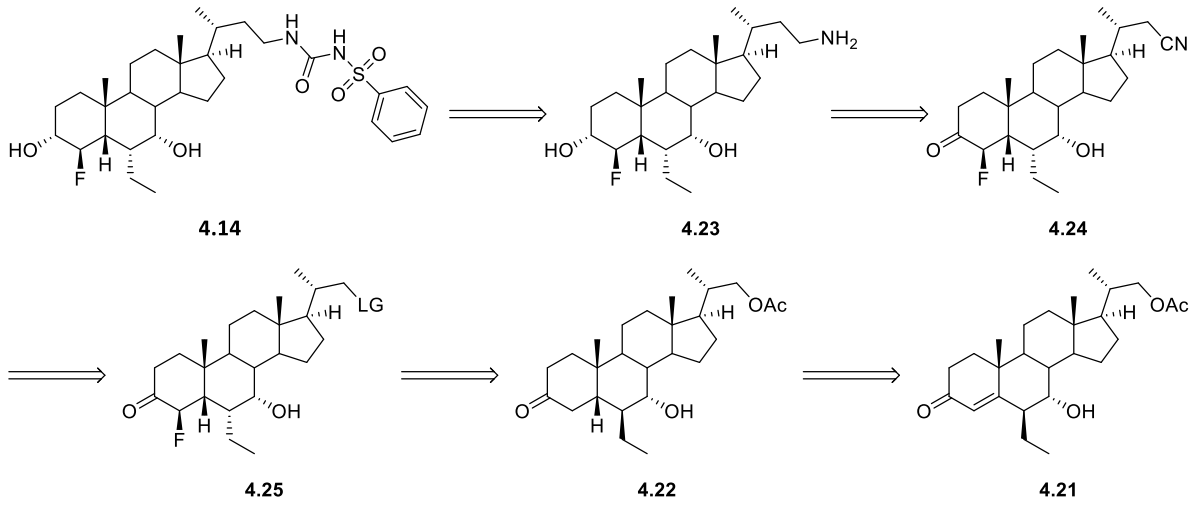


*Figure 4.9. Retrosynthesis of target **4.14** to advanced intermediate **4.22**.*

Where possible, chromatography was to be avoided. Instead, 'purge-points' would be incorporated throughout the synthesis where techniques appropriate to this scale (such as crystallisation) could be used to purify the intermediates. Between these purge-points, the identity and chemistries of impurities would need to be followed.

4.5.3 Fluorination studies

A range of intermediates, with varying *O*-protection, were considered for fluorination studies (*Figure 4.10*) and their synthesis (where required) is given in the following section. They were selected to probe the tolerance of the acetate-protecting group at the 22-OH group and also to discover the effect, if any, between the presence of an α-alcohol group and a ketone at the 7-positon.

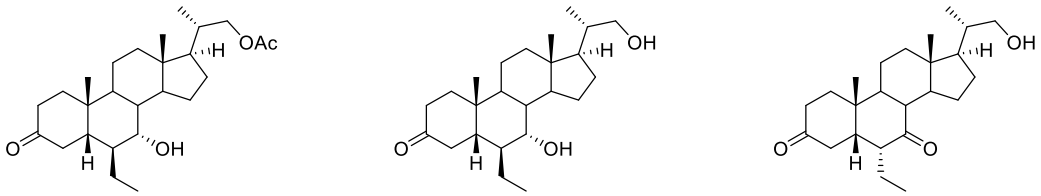
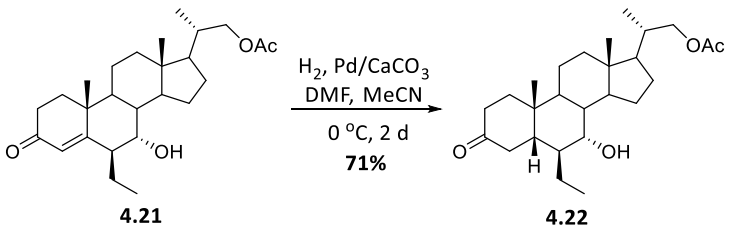


Figure 4.10. Substrates designed for fluorination studies.

4.5.3.1 3-keto-7-hydroxy-22-*O*-protected substrate 4.22

Hydrogenation of advanced enone intermediate **4.21** on 50 g scale yielded the target *cis*-decalin product **4.22** in good yield (*Scheme 4.8*). The reaction followed a well-established method developed in-house. A low reaction temperature (0-2 °C) was important to achieve full selectivity towards the target *cis*-diastereomer. A small quantity of starting material was however present in the crude reaction mixture after workup (<5 %).

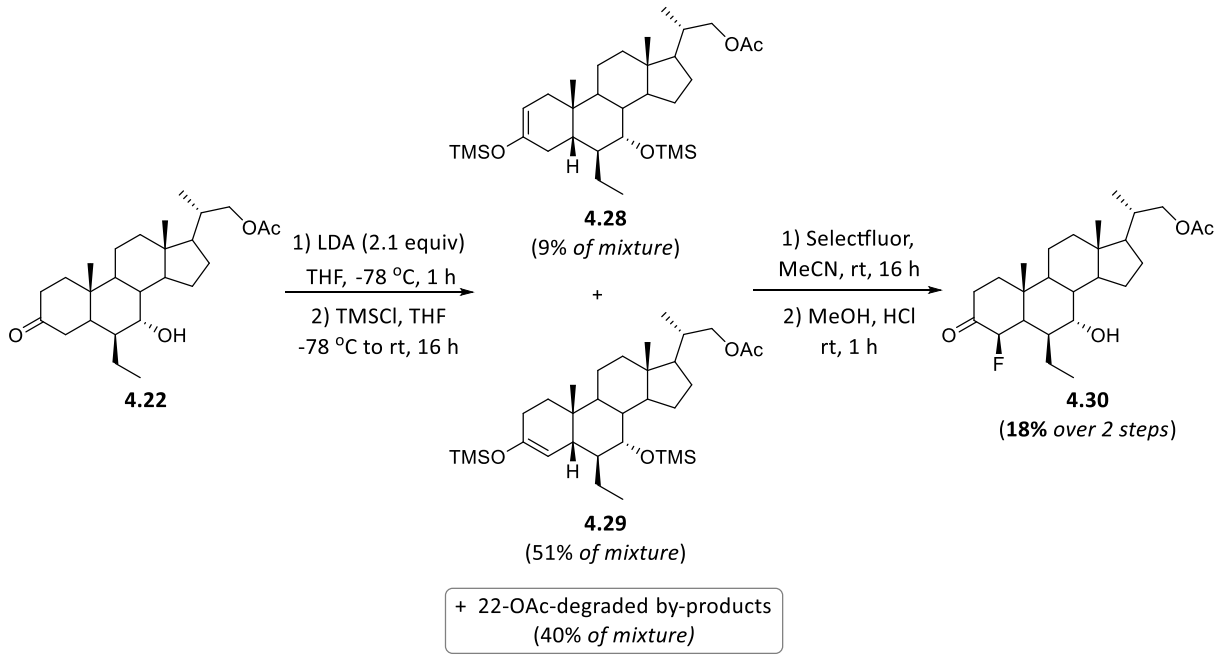


*Scheme 4.8. Hydrogenation of enone intermediate **4.21** on 50 g scale.*

Despite our aim to avoid column chromatography where possible, a small sample of **4.22** was purified to remove the trace of starting material **4.21**, before the fluorination study.

On 250 mg scale the established route of silyl enol ether formation (biased to the Δ3,4-regioisomer) was applied to test substrate **4.22** and gave a 15:85 ratio of the products (*Scheme 4.9*). Interestingly,

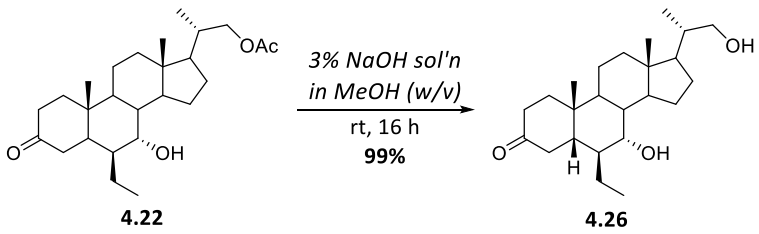
in the presence of the 6β-ethyl group here we observed quantitative conversion at the 7α-OH group to the *O*-TMS motif, whereas significantly lesser conversion was observed for the analogous 6α-ethyl diastereomer (see: *Section 2.2.2.2.1*). For this reaction where two equivalents of strong base were used, 40% of the crude material was observed as unidentified byproducts owing to the degradation of the 22-acetate group. Overall, all starting material was consumed. However, when using five equivalents of strong base these byproducts dominated the crude material at around 90% composition (not shown). At best, treatment of the crude silyl enol ethers with Selectfluor® gave an 18% isolated yield of the target 4β-fluorinated product alongside 5% of the starting material **4.22**. No trace of the 2β-fluorinated regioisomer was observed.



*Scheme 4.9. Fluorination of test substrate **4.22** via silyl enol ether intermediates.*

4.5.3.2 3-keto-7,22-dihydroxy substrate 4.26

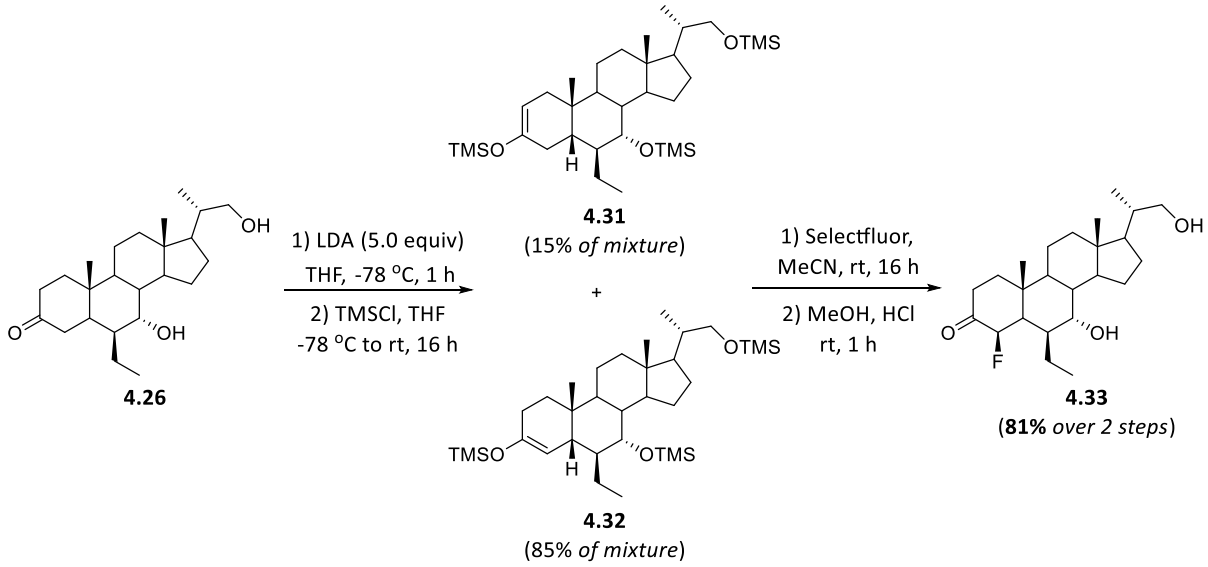
Diol substrate **4.26** was prepared from **4.22** by saponification of the C_{22}*O* acetate protecting group and was achieved on 22 g scale in a quantitative yield (*Scheme 4.10*).



*Scheme 4.10. C_{22}-O saponification to yield fluorination substrate **4.26**.*

For the subsequent reaction of **4.26** with LDA, more than two equivalents would be required to observe a reaction at the 3-ketone because the deprotonation of the 7-OH and 22-OH groups would

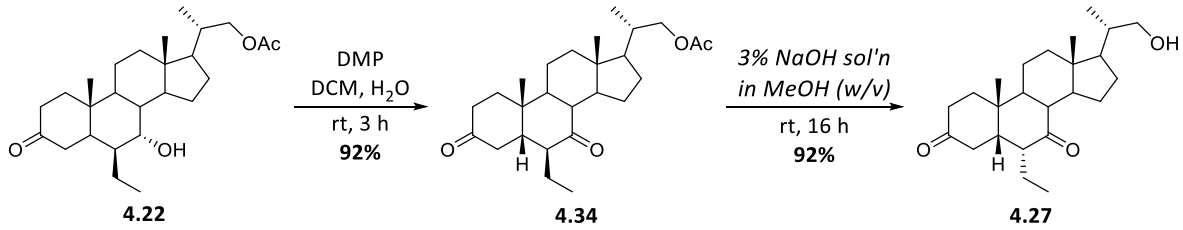
proceed first. Hence, using five equivalents of LDA on 250 mg scale, we observed the reliable and quantitative TMS protection of both alcohol groups alongside the desired (and mostly regioselective) reaction at the 3-keto group to yield a 15:85 ratio of silyl enol ether regioisomers **4.31** and **4.32** (*Scheme 4.11*). No other byproducts were observed. Reaction of the silyl enol ether intermediates with Selectfluor® gave 4β-fluoro derivative **4.33** in an excellent 81% isolated yield over two steps, in addition to around 10% of starting ketone **4.26**. No trace of the 2β-fluorinated regioisomer was observed, despite the clear formation of the Δ2,3-silyl enol ether regioisomer as an intermediate. This reaction was reproducible on scales between 250 mg and 31 g; the yields reported in the scheme below are from the reaction at 31 g scale.



*Scheme 4.11. Fluorination of test substrate **4.26** via silyl enol ether intermediates.*

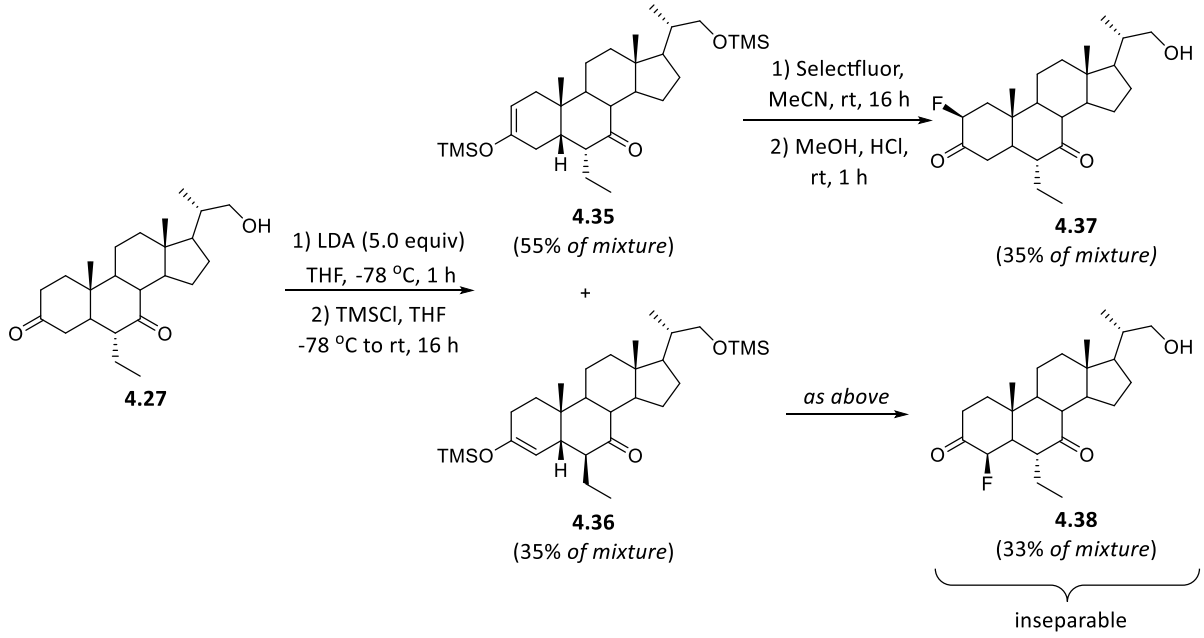
4.5.3.3 3,7-diketo-22-hydroxy substrate 4.27

The 3,7-diketone substrate **4.27** was prepared in two steps from **4.22**. Firstly, oxidation of the 7α-alcohol with Dess-Martin periodinane on 1.0 g scale gave an excellent yield of **4.34** (*Scheme 4.12*). Two drops of water were added to the reaction mixture as catalyst, as per the literature procedure of Meyer and Schreiber.[205] As a control experiment, we discovered that the absence of water did not reduce the overall yield of the reaction but that the reaction was significantly slower (2 days at rt before completion). Saponification of the 22-acetate group of **4.34** on 500 mg scale proceeded in excellent yield and also gave the expected epimerisation of the 6-ethyl group (from axial stereochemistry to the more stable equatorial stereochemistry).



*Scheme 4.12. Synthesis of fluorination test substrate **4.27** from **4.22** in two steps.*

The reaction of 3,7-diketo-22-hydroxy substrate **4.27** with 2 equivalents of LDA and on ~200 mg scale gave 45% conversion to regioisomers **4.35** and **4.36** but in a 60:40 ratio in favour of the Δ2,3-silyl enol ether (not shown). Forcing the reaction progress with 5 equivalents of LDA led to a greater 90% conversion to the silyl enol ether regioisomers and in a 60:40 ratio (*Scheme 4.13*). Treatment of this mixture with Selectfluor® provided the corresponding β-fluoroketones in good yields alongside a small quantity (<10 %) of recovered starting material **4.27**. Interestingly, when the 22-OH group was protected with an acetate group (compound **4.34**), only trace amounts of silyl enol ether products were observed (not shown) alongside a complex mixture of byproducts.



*Scheme 4.13. Fluorination of test substrate **4.27** via silyl enol ether intermediates.*

4.5.3.4 Conclusions

We first conclude that substrates bearing an acetate group on the side-chain are best avoided owing to the instability of this motif in the presence of strong base. Reactions of **4.22** and **4.34** demonstrate this, especially when base is used in greater excess (5 equivalents).

In the presence of the 7-OH group, silyl enol ether formation is biased heavily towards the Δ3,4-regioisomer and subsequent treatment of the regioisomer mixture with Selectfluor® leads only to

the 4β-fluoroketone product; the starting material is also isolated in small quantities, despite complete conversion to silyl enol ether intermediates in the reaction with LDA. No trace of the 2β-fluoroketone was found, suggesting that the Δ2,3-silyl enol ether does not react with fluoride and instead degrades back to the non-fluorinated parent 3-keto compound during workup. This would explain the returned starting material in reactions of substrates **4.22** and **4.26**. Contrastingly, in the presence of the 7-keto group, the undesired Δ2,3-silyl enol ether was identified as the major regioisomer and treatment with fluoride gave a mixture of fluoroketone products.

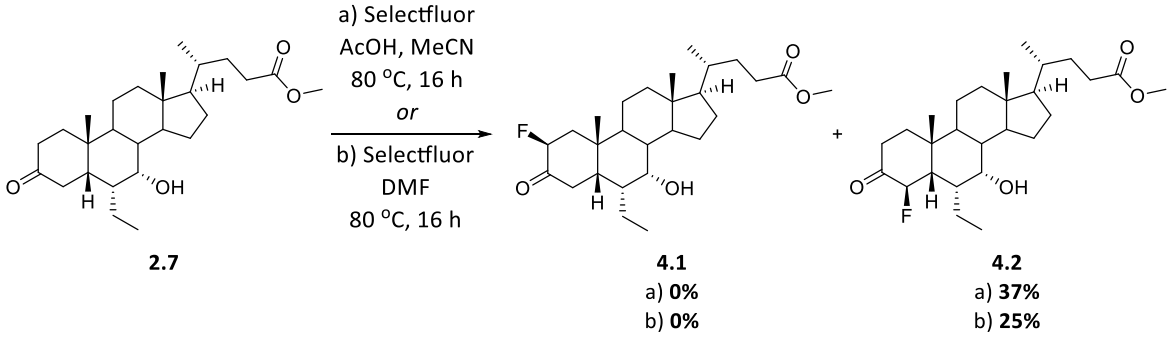
To avoid the inherent stability issues associated with the *O*-acetate group and to take advantage of the desired regioselectivity, 3-keto-7,22-dihydroxy substrate **4.26** was identified as the most suitable for proceeding syntheses.

4.6 Synthesis towards 2β-fluoro OCA (JED397) by direct fluorination

An alternative method for the synthesis of fluorinated 3-keto derivatives is by direct fluorination with acid/Selectfluor®.[206] After the failure to synthesise appreciable quantities of 2β-fluorinated fluoroketones by a silyl enol ether strategy, this became our primary route of investigation.

Two variations of this direct fluorination methodology were attempted, inspired by those having shown previous success within the group.[174] The first involved heating Selectfluor® in acetonitrile with a catalytic amount (10%) of acetic acid while the second omitted acid and instead used dimethylformamide as the solvent. Yet, both only provided the formerly synthesised 4β-fluorinated fluoroketone regioisomer **4.2** (

Scheme 4.14, conversion determined by ^{1}H NMR). Complete β-selectivity of the nucleophilic 4-position was observed (^{19}F NMR analysis).



*Scheme 4.14. Attempts at direct fluorination of 3-keto OCA methyl ester (**2.7**).*

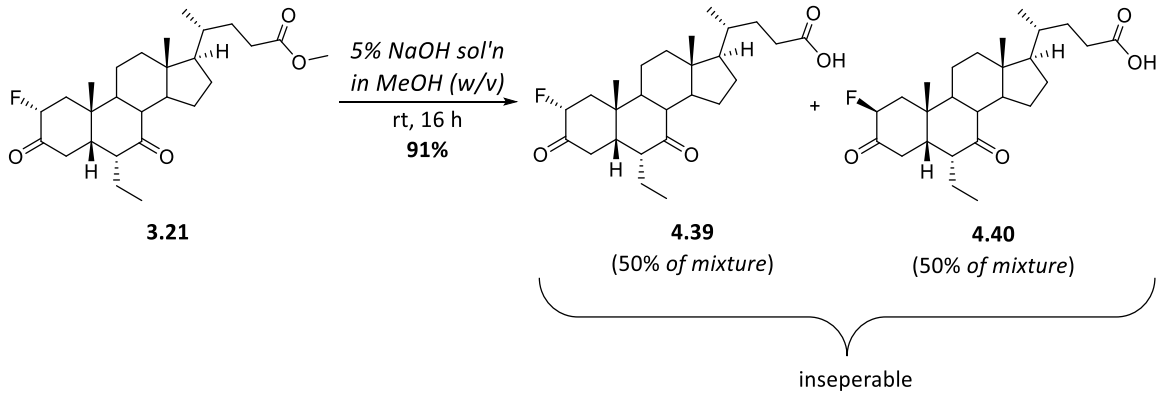
4.7 Synthesis of 2β-fluoro OCA (JED397) by epimerisation of an advanced 2α-fluoro OCA intermediate

4.7.1 Rationale

The shortcomings in accessing 2β-fluoro OCA (**1.54**) by a silyl enol ether strategy and by direct fluorination prompted us to consider other approaches. The successful synthesis of 2α-fluoro OCA (**1.53**), described in *Chapter 3*, presented a handle from which the desired β-stereochemistry could be accessed by epimerisation. Indeed, the 2α-fluoro substrates in-hand have an axial C-F bond and could epimerise to the provide the desired equatorial C-F bond.

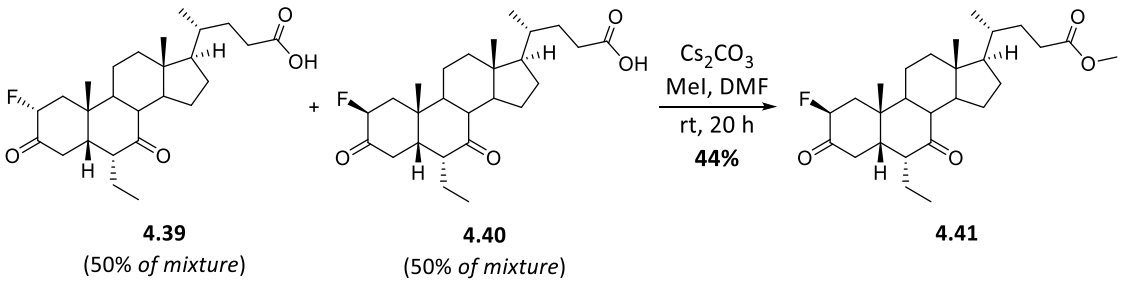
4.7.2 Epimerisation of the 2α-fluoro motif

2α-Fluoroketone **3.21** was a suitable substrate for testing epimerisation conditions. Stirring the compound in mildly basic methanol solution overnight at ambient temperature gave a ~1:1 inseparable mixture of 2-fluorinated diastereoisomers in the first attempt (*Scheme 4.15*). The 6-ethyl group remained of α-stereochemistry (equatorial) in both products, however the methyl ester was saponified cleanly to yield these products as their carboxylic acid derivatives.



*Scheme 4.15. Epimerisation of an advanced 2α-fluoro intermediate **3.21**.*

Compounds with a 24-carboxylic acid group are known to co-elute during column chromatography, and the mixture of 2-fluoro diastereomers was thus converted back to their corresponding methyl ester derivatives. Using a weak base and iodomethane, protection of the carboxylic acids proceeded with excellent conversion and both fluoroketone methyl esters were observed in the crude product mixture by NMR. The undesired diastereomer 2α-fluoro-3,7-diketo OCA methyl ester (**3.21**) had already been isolated during previous work towards 2α-fluoro OCA (see: *Section 3.4.2*), and so only 2β-fluoroketone **4.41** was isolated from the crude mixture following careful chromatographic purification (*Scheme 4.16*).



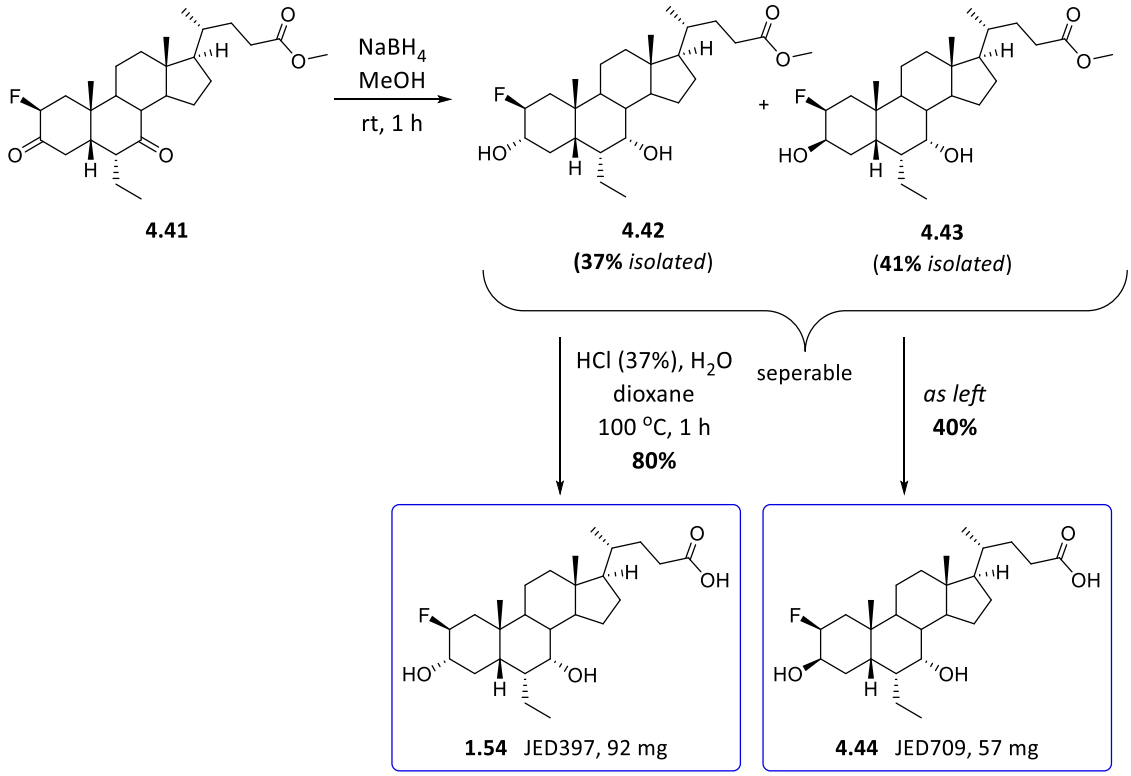
*Scheme 4.16. Methyl ester protection of 2-fluoro carboxylic acid intermediates **4.39** and **4.40**.*

4.7.3 Synthesis of 2β-fluoro analogues (JED397 and JED709)

4.7.3.1 Chemistry

The diketone reduction reaction of 2β-fluorinated intermediate **4.41** proceeded with excellent diastereoselectivity at the 7-keto group, but with poor diastereoselectivity at the 3-keto group, to yield a 1:1 mixture of separable C_3 diastereomers **4.42** and **4.43** (*Scheme 4.17*). The $H_{3\beta}$ ^{1}H NMR signal of 3α-alcohol **4.42** was observed at 3.52 ppm as a ddt (*J* = 13.0, 12.5, 6.0 Hz) while for 3β-alcohol **4.43** the ^{1}H NMR signal for $H_{3\alpha}$ was observed at 4.15 ppm as a quartet (*J* = 3.6 Hz); the antiperiplanar H-H coupling constants of ~13 Hz are only possible for the 3α-alcohol product.

Acidic hydrolysis of either diastereoisomer, in an identical fashion as before, led to target 2β-fluoro OCA (**1.54**) in excellent yield and 2β-fluoro-3β-hydroxyl OCA (**4.44**) in mediocre yield.



*Scheme 4.17. Synthesis of 2β-fluoro OCA (**1.54**) and 2β-fluoro-3β-hydroxy OCA (**4.44**).*

4.7.3.2 NMR analysis of 2β-fluoro OCA

The stereochemistry of target 2β-fluoro OCA (**1.54**) was confirmed by ^{1}H NMR (*Figure 4.11*). The $H_{2\alpha}$ signal was identified at 4.42 ppm as a dddd due to the geminal coupling of 52.6 Hz. Two antiperiplanar couplings of 12.5 Hz to $H_{1\beta}$ and 8.7 Hz to $H_{3\beta}$ confirmed this hydrogen is axial; the magnitude of the coupling to the axial C_3-proton was attenuated by both electron withdrawing C-F and C-OH bonds.

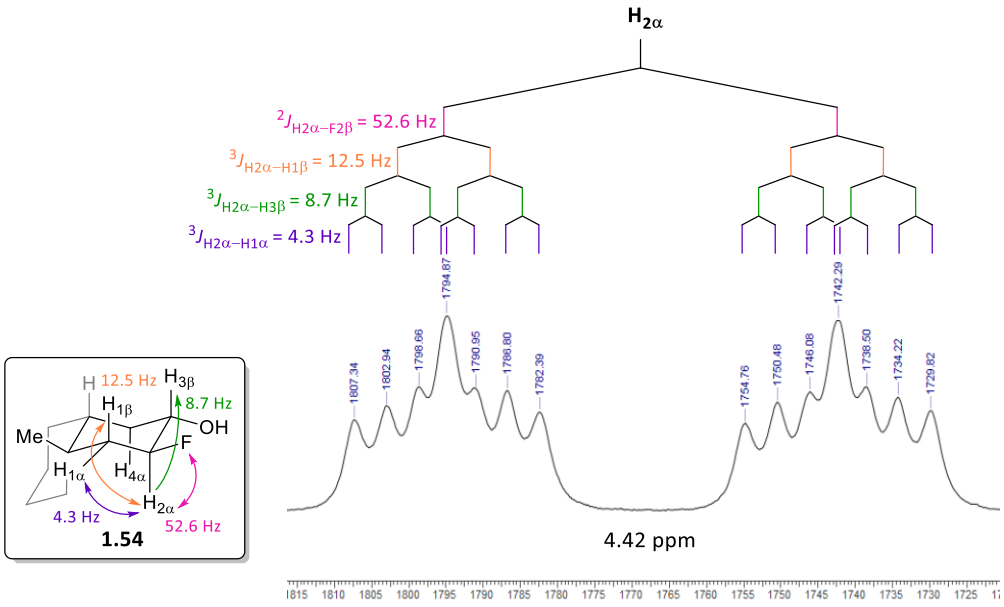


*Figure 4.11. ^{1}H NMR analysis of the $H_{2\alpha}$ signal of target 2β-fluoro OCA (**1.54**).*

The ^{19}F NMR signal of the fluorine atom was identified at -186.8 ppm as a ddq (not shown). Apart from the geminal coupling to proton $H_{2\alpha}$ (52.9 Hz) there is a large gauche coupling of 13.0 Hz to $H_{1\beta}$. Additionally, three smaller gauche couplings of 7.5 Hz are to protons $H_{3\beta}$, $H_{4\beta}$ and $H_{1\alpha}$. The absence of any antiperiplanar F-H couplings supports the equatorial stereochemistry of the C_2-F bond.

4.8 Conclusions

The two target 2- and 4-position β-fluorinated OCA targets were successfully synthesised (*Figure 4.12*). 2β-Fluoro OCA (**1.54**) is novel, while 4β-fluoro OCA (**1.56**) was described in the literature in 2018 (see: *Section 1.3.5.3*).[169] For the 4-fluoro compounds, an established route of electrophilic fluorination with silyl enol ether intermediates proved successful. This strategy however gave poor yields of 2β-fluorinated intermediates and direct fluorination attempts towards **1.56** also proved unsuccessful. Epimerisation of a 2α-fluorinated fluoroketone however gave practical access to 2β-fluorinated compounds. A 3β-OH diastereomer of 2β-fluoro OCA was also synthesised (**4.44**) as an interesting byproduct from non-diastereoselective 3-keto reduction.

A series of aromatic sulfonyl urea derivatives of 4β-fluoro OCA were also prepared (**4.13**-**4.17**) by the Curtius rearrangement. The foundation of a large-scale route to 4β-fluorinated compounds was also established following a screening of suitable substrates for an electrophilic fluorination reaction. The synthesis of target **4.14** is being continued by colleagues in industry according to the described rationale.

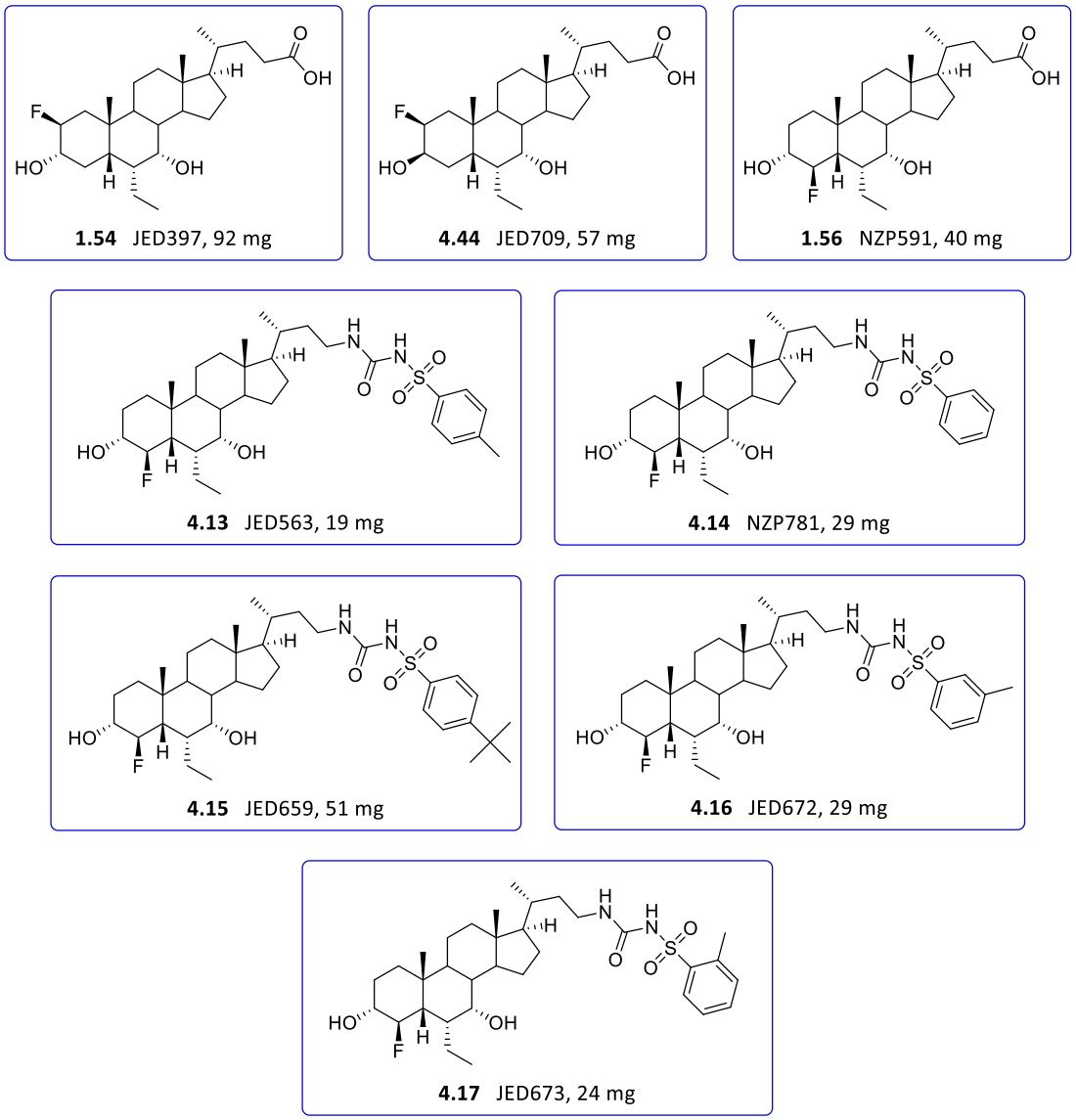


Figure 4.12. Synthesised 2- and 4-position β-fluorinated OCA derivatives.

Chapter 5 Synthesis of OCA (NZP084) 4,4-difluoro analogues

5.1 Rationale

The synthesis of 3-keto-4β-fluoro OCA (**4.2**) was outlined in *Section 4.2*. A second deprotonation at C_4 could provide a handle for the introduction of a second fluorine substituent in identical fashion (*Figure 5.1*).

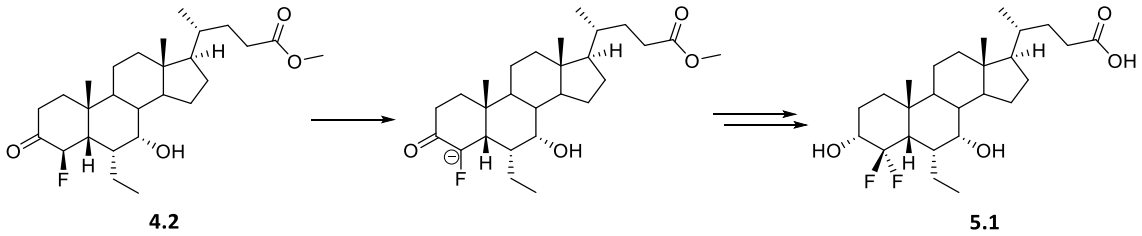


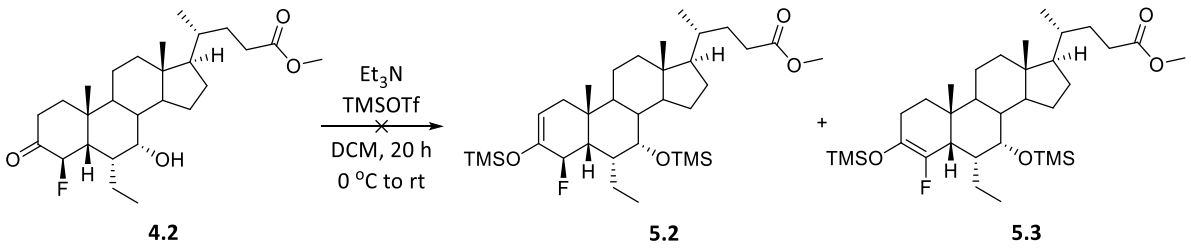
*Figure 5.1. Synthesis of 4,4-difluoro OCA (**5.1**) from fluorination of a 4β-fluoro derivative **4.2**.*

During the earlier and first fluorination reactions towards 1-fluoro substrates (see: *Section 2.2.2.2.1*) we reported that silyl enol ether formation using triethylamine/TMF triflate gave an approximate 1:1 mixture of Δ2,3- and Δ3,4-silyl enol ether regioisomers. A combination of LDA/TMS chloride improved the regioselectivity in favour of the 4-position to a 7:1 ratio. We were yet to discover how the presence of a 4β-fluorine atom would influence this regioselectivity.

A brief literature search discovered the only published steroidal example of such a fluorination reaction whereby CF_2 group is formed; the authors described the use of KHMDS to deprotonate the 2-position of a 2-fluoro-3-keto cholesterol derivative (with a *trans*-AB hydrindane ring system) and subsequent treatment with NFSI yielded their desired 2,2-difluoro compound.[207] All other literature examples of CF_2 formation reactions not involving steroidal substrates utilised Selectfluor® as the electrophilic fluoride source.[208-210] We have enjoyed our own successes with this reagent, so it became our primary choice for electrophilic fluorination reactions on **4.2** towards **5.1**.

5.2 Regioselective deprotonation

Thermodynamic conditions that were previously successful on the non-fluorinated 3-keto analogue were applied to 4β-fluorinated intermediate **4.2** by our industrial collaborators (unpublished). Alas, they proved altogether unsuccessful and only starting material was recovered from the reaction (*Scheme 5.1*).

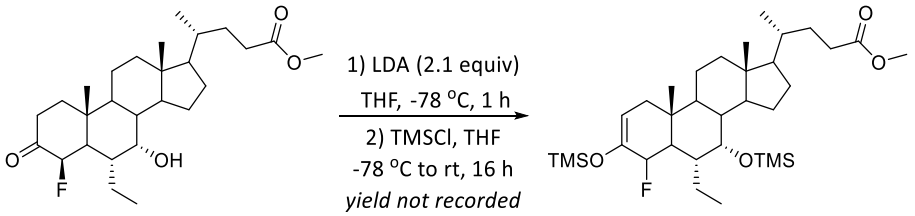


4.2 **5.2** **5.3**

*Scheme 5.1. Unsuccessful reaction of **4.2** with Et_3N/TMS triflate (by our industrial collaborators).*

5.2.1 At the 2-position

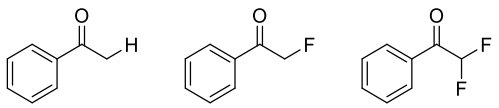
Treatment of **4.2** with an excess of kinetic base was proposed to first deprotonate the 7-OH group (pKa ~16), followed by a regioselective deprotonation at either the 2-position or 4-position (both pKa ~20). Using two to five equivalents of LDA solution and trapping the resulting lithium enolate with TMS chloride, our industrial collaborators observed only the 7-*O*-protected Δ2,3-fluorosilyl enol ether **5.2** (unpublished).



4.2 **5.2**

*Scheme 5.2. Reaction of **4.2** (by our industrial collaborators) using an excess of kinetic base yielded exclusively undesired Δ2,3silyl enol ether regioisomer **5.2**.*

The complete regioselectivity towards kinetic deprotonation at the 2-position was an interesting result. Typically, the introduction of fluorine at the ketone α-position leads to a greater acidity of any protons also at that carbon (*Figure 5.2*),[211] so the Δ3,4-silyl enol ether regioisomer would be expected from **4.2**. The observed regioselectivity can be explained by the deprotonated alcohol group disfavouring the approach of LDA towards $C_{4\alpha}$-H.



pKa	24.7	21.7	20.2

Figure 5.2. Fluorination shown to reduce the pK_a of geminal protons.[211]

5.2.2 At the 4-position

The close proximity of the 7α-OH group and 4-position has already been observed in our own chemistries (see: *Sections 3.2.2* and *3.3.4*) and is well reported by a previous group member (see:

Section 1.3.4.4). We propose to exploit this close spatial relationship to achieve deprotonation of the 4α-proton. For this reaction, one equivalent of strong base would be used for the kinetic deprotonation of the 7α-OH group of **4.2**, which would allow an intramolecular deprotonation of the 3-keto group at the 4-position, which is proposed to establish an equilibrium between those species. Trapping with TMS chloride would occur at the least sterically hindered position, and at the most reactive functional group, to then give the desired Δ3,4-fluorosilyl enol ether regioisomer **5.4** (*Figure 5.3*).

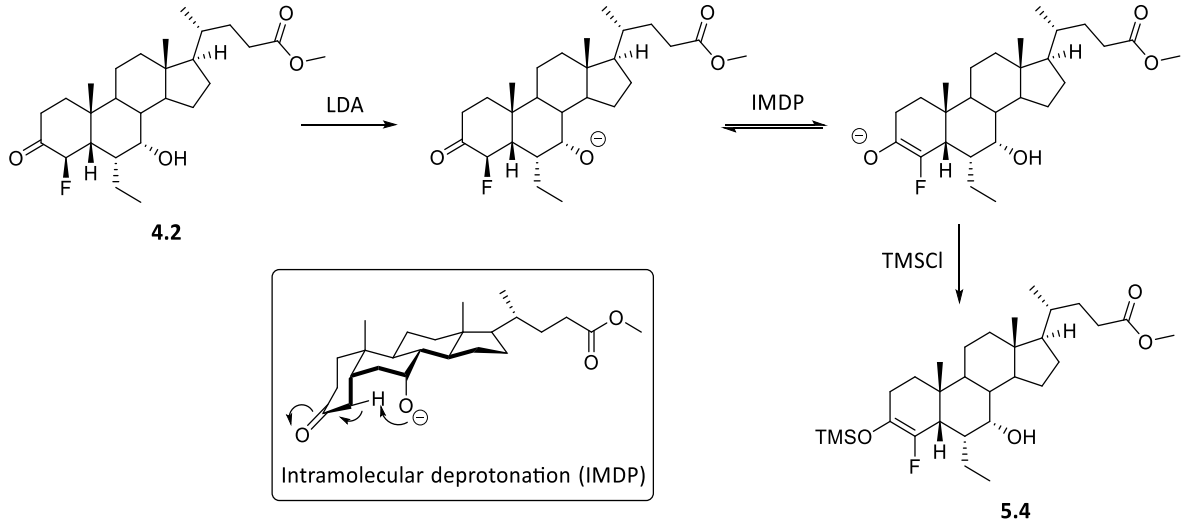
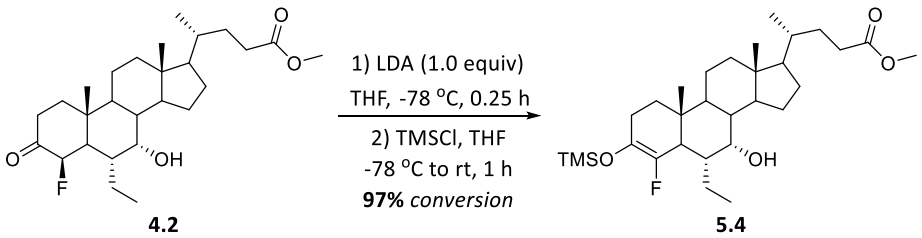


Figure 5.3. Proposed kinetic deprotonation of the 7α-hydroxyl group and subsequent intramolecular deprotonation of the 4α-proton.

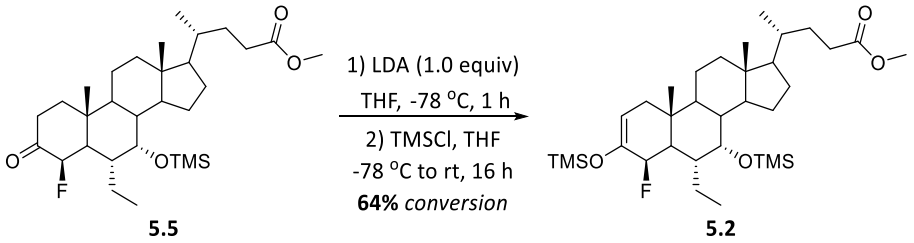
Pleasingly, our first attempt with one equivalent of LDA was successful, with no warming required to initiate the intramolecular deprotonation. The reaction was also very rapid (15 mins) and treatment of the *O*-lithiated species with one equivalent of TMS chloride yielded the desired Δ3,4-fluorosilyl enol ether (*Scheme 5.3*). After optimisation, the conversion of the reaction was measured at 97% by ^{19}F NMR analysis; a sharp singlet appeared at -135.2 ppm while the starting fluoroketone **4.2** was identified as a broad doublet at -188.7 ppm (J = 46.8 Hz).



*Scheme 5.3. Intramolecular deprotonation selectively yielding target Δ3,4-regioisomer **5.4**.*

To confirm the intramolecular deprotonation pathway, a control experiment was performed involving 7-*O*-protected analogue **5.5** (*Scheme 5.4*). Here, with one equivalent of base and

subsequent trapping with TMS chloride we observed only the Δ2,3-fluorosilyl enol ether **5.2**; the ^{19}F NMR signal of **5.2** was identified as a multiplet at -170.0 ppm while the signal of starting fluoroketone **5.5** was identified as ddd at -195.2 ppm (*J* = 46.8, 13.9, 3.5 Hz); no trace of the fluorosilyl enol ether motif (at approximately -135 ppm) was observed. The lower 64% conversion of this reaction was attributed to the poor quality of this batch of LDA. Other control experiments are ongoing to see this interesting chemistry through to publication.

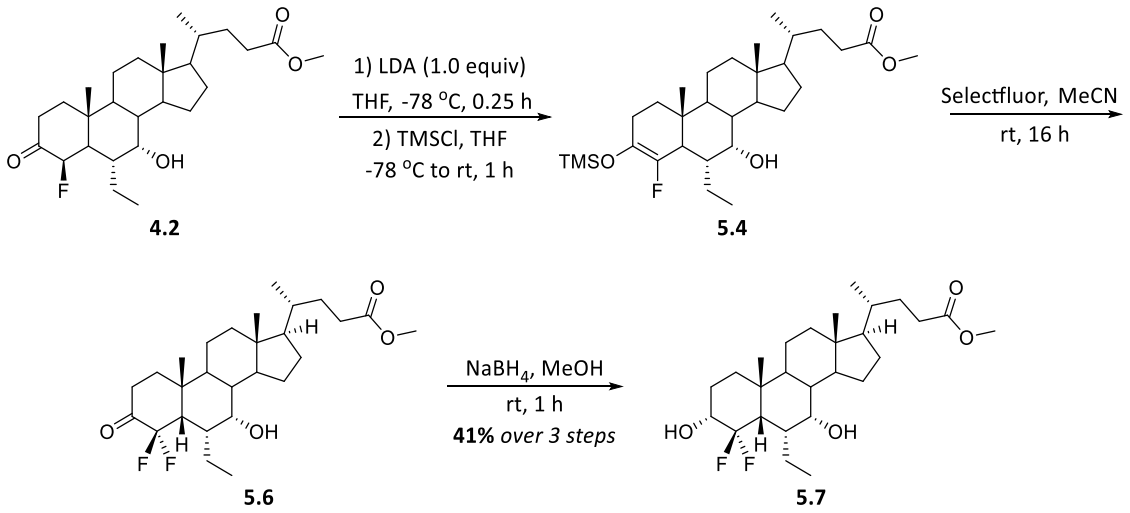


*Scheme 5.4. O-protected derivative **5.5** led to only the undesired Δ2,3-regioisomer **5.2**.*

5.3 Synthesis of 4,4-difluoro OCA (JED556)

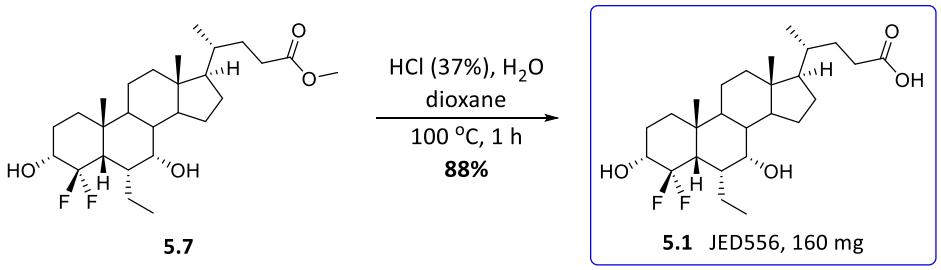
5.3.1 Chemistry

Following the procedure for regioselective Δ3,4-fluorosilyl enol ether formation via intramolecular deprotonation of the 7α-OH group (*Scheme 5.3*), reaction of 3-keto-4β-fluoro OCA (**4.2**) on 7.3 g scale gave a 97% conversion to the expected fluorosilyl enol ether intermediate **5.4** with 3% observed starting material by ^{19}F NMR analysis (*Scheme 5.5*). Reaction of the crude fluorosilyl enol ether with Selectfluor® in acetonitrile gave a good conversion (~80%) to the expected 3-keto-4,4-difluouro derivative **5.6** alongside small percentages of unidentified fluorinated byproducts.



*Scheme 5.5. Synthesis of target 4,4-difluoro OCA methyl ester (**5.7**).*

Partial formation of a hydrate at the 3-keto group of compound **5.6** was observed (not shown). Hydration of difluoro ketones is favoured due to the strongly electron withdrawing effect of the CF_2 motif to yield a greater electrophilicity on the carbonyl carbon.[212] The hydrate compound 'streaked' severely on silica (TLC or otherwise) and this led to cumbersome purification. Even on smaller scale (1 g), there was no separation of **5.6**/**5.6**-hydrate from the starting 4β-fluoroketone **4.2**. Note that no trace of the hydrate was observed by NMR likely due to the interconversion rates of the two species on the NMR timescale. Subsequent crude mixtures of **5.6** were immediately taken forward to the next step; reduction of the 3-keto group to **5,7**. Treatment with sodium borohydride in an identical fashion to before was successful, rapid and selective, and provided the desired 3α-OH diastereomer **5.7** in a good yield of 41% over three steps from **4.2**. Lastly, acidic deprotection of methyl ester derivative **5.7** was high-yielding to give target of 4,4-difluoro OCA (**5.1**) (*Scheme 5.6*).



*Scheme 5.6. Synthesis of target 4,4-difluoro OCA (**5.1**).*

5.3.2 NMR analysis

Analysis of the geminal difluoro motif of target **5.1** and preceding intermediates was undertaken using ^{19}F NMR.

5.3.2.1 3-keto-4,4-difluoro OCA methyl ester (5.6)

The signal from $F_{4\beta}$ of 3-keto-4-4-difluoro OCA (**5.6**) is observed at -99.2 ppm as a dd (*Figure 5.4*). A characteristic geminal fluorine-fluorine coupling of 263.6 Hz is observed.

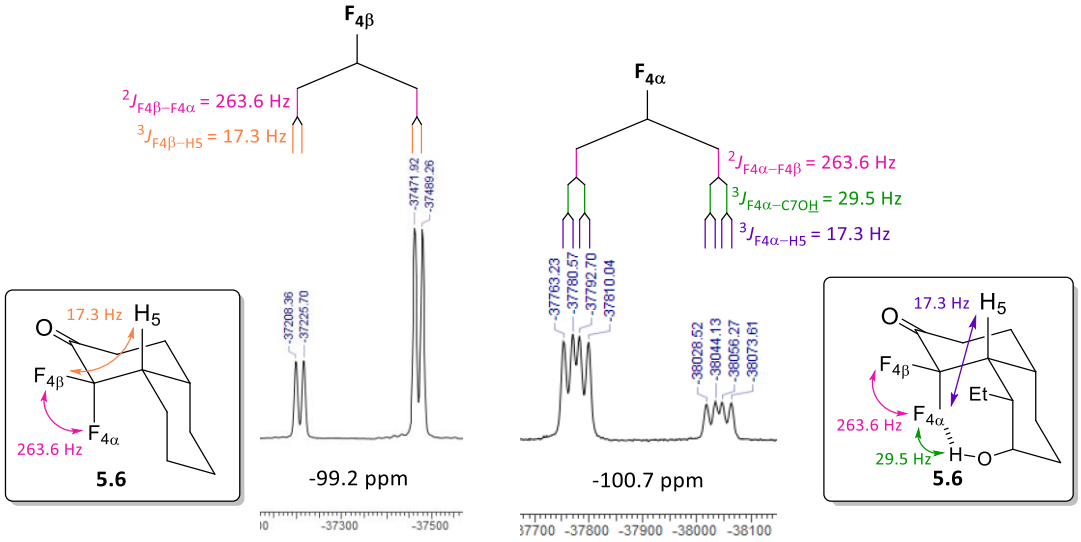


*Figure 5.4. ^{19}F NMR analysis of the F_4 signals of 3-keto-4,4-difluoro OCA methyl ester (**5.6**).*

The signal from $F_{4\alpha}$ of compound **5.6** is observed at -100.7 ppm as a ddd and we observe the reciprocating geminal fluorine-fluorine coupling of 263.6 Hz. For the antiperiplanar coupling to H_5 we expect a magnitude of 30-50 Hz but a combined presence of the electron withdrawing $F_{4\beta}$ atom and the 3-keto group attenuates this coupling to a mere 17.3 Hz. We also observe a coupling constant of 29.5 Hz that is the expected magnitude for coupling to the 7-OH proton through an intramolecular hydrogen bond (see: *Section 1.3.4.4*). This coupling constant was not observed in the ^{19}F NMR of compound **5.6** after a D_2O experiment, thereby confirming its origin as from a C-F•••H-O interaction.

5.3.2.2 4,4-difluoro OCA methyl ester (5.7)

The signal from $F_{4\beta}$ of 4-4-difluoro OCA (**5.7**) is observed at -99.3 ppm as a doublet after the apparent loss of coupling to H_5 (*Figure 5.5*). The magnitude of the geminal fluorine-fluorine coupling was observed to decrease marginally to 239.3 Hz.

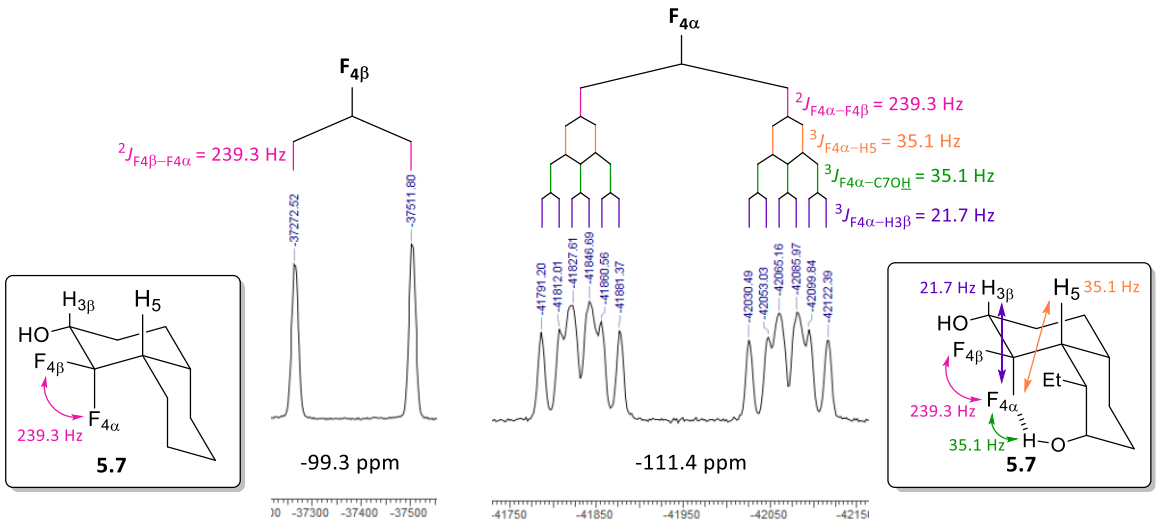


*Figure 5.5. ^{19}F NMR analysis of the F_4 signals of 4,4-difluoro OCA methyl ester (**5.7**).*

The signal from $F_{4\alpha}$ of compound **5.7** is identified further upfield at -111.4 ppm relative to the 3-keto derivative **5.6** and is now observed as a dtd. The reciprocation of the fluorine-fluorine coupling is again witnessed with a magnitude of 239.3 Hz. The magnitude of coupling to H_5 has increased significantly to 35.1 Hz (from 17.4 Hz in **5.6**) following the loss of the sp^2 stereocentre at the 3-position but the presence of $F_{4\beta}$ still attenuates this coupling constant towards the lower end of the expected 30-50 Hz range. The EWG-mediated reduction in fluorine-proton axial-axial coupling magnitudes is further witnessed for the coupling of $F_{4\alpha}$ to $H_{3\beta}$ at only 21.7 Hz because of the adjacent C-F and C_3-OH bonds. The intramolecular hydrogen bonding coupling is witnessed again and at a marginally increased magnitude of 35.1 Hz following the loss of the sp^2 stereocentre, thereby likely reducing the proximity between these atoms. The IMHB coupling was also again confirmed by a D_2O experiment.

5.3.2.3 4,4-difluoro OCA (5.1)

As one would expect, we observe the same coupling multiplicities and magnitudes (to within a few Hz) for 4,4-difluoro OCA (**5.1**) as was described for preceding methyl ester analogue **5.7** (*Figure 5.6*). The only notable absence is the absence of the ~35 Hz intramolecular hydrogen bonding coupling to the 7α-OH group. The exchangeable readily-available proton from the C_{24}-carboxylic acid group (pKa ~5) is likely involved in an exchange process in solution with the 7α-OH group, resulting in complete loss of coupling with fluorine.[213]

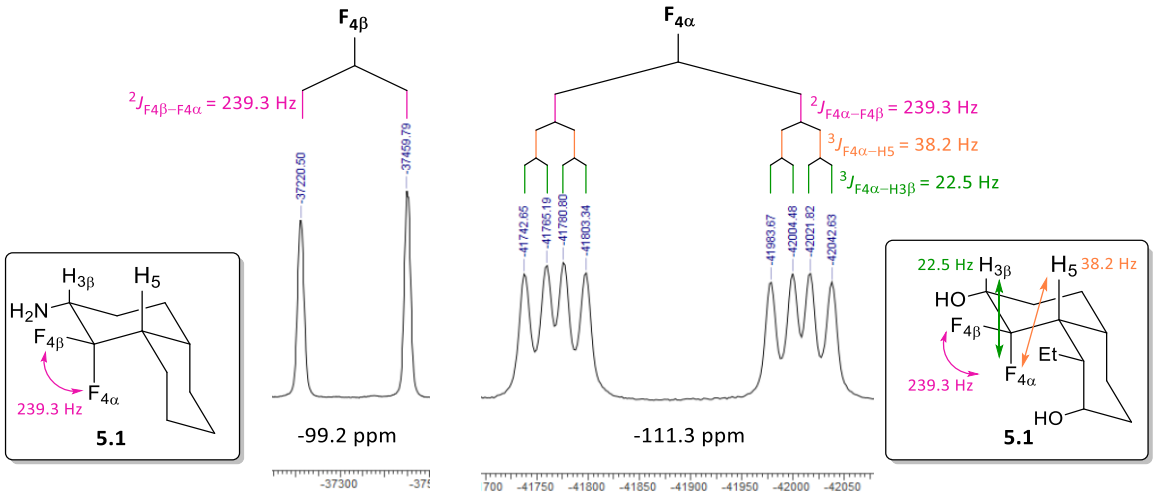
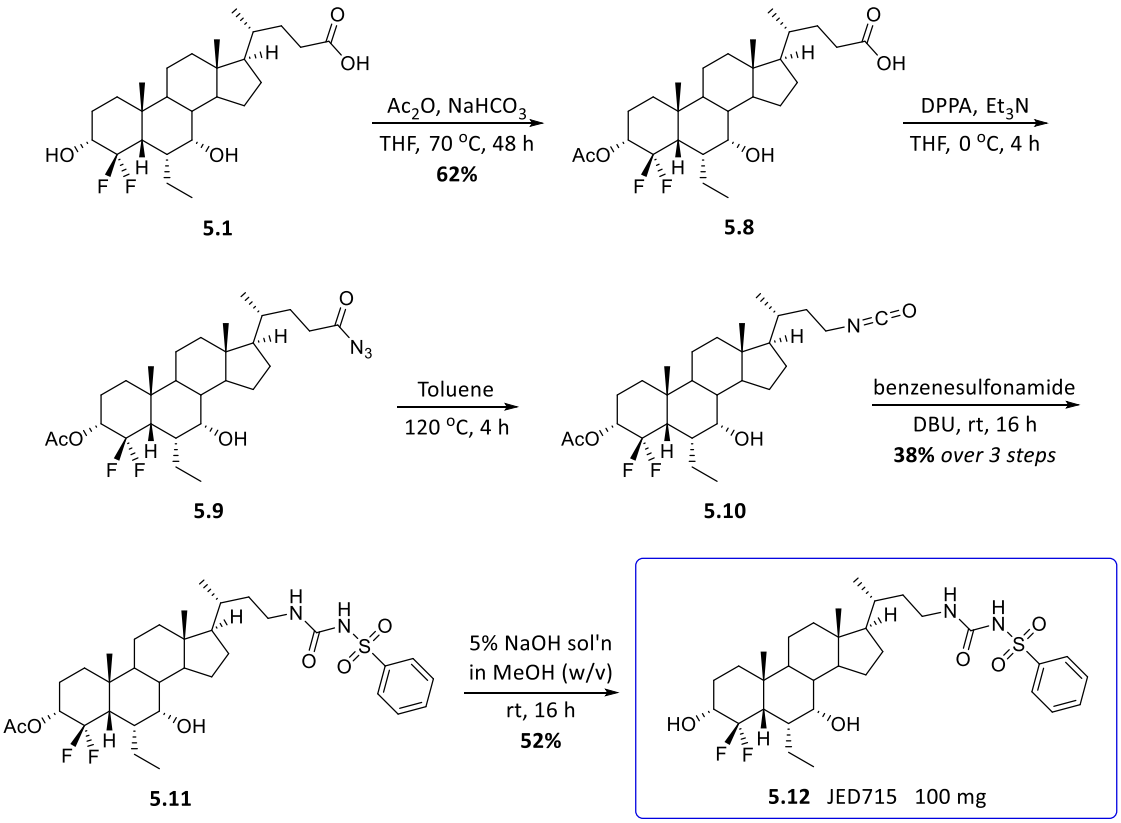


*Figure 5.6. ^{19}F NMR analysis of the F_4 signals of target 4,4-difluoro OCA (**5.1**).*

5.4 Synthesis of 4,4-difluoro OCA phenyl-sulfonyl urea (JED715)

In analogy with the 4β-fluoro OCA (**1.56**) derivative, the phenyl sulfonyl urea side chain motif of the 4,4-difluorinated analogue was targeted.

Acetate protection of the labile 3α-OH group was successful using the established conditions presented so-far (*Scheme 5.7*). In identical fashion to 4β-fluoro OCA (**1.56**), the poor reactivity of the free 7α-OH group was expected to precluded side-reactions and so its protection was not necessary (see: *Section 4.4.2*). 3-*O*-Protected intermediate **5.8** was then converted to the corresponding acyl azide **5.9**. As described in previous acyl azide syntheses (see: *Section 4.4.3*), care was taken to maintain 0 °C during the reaction, during workup and when concentrating *in vacuo*, owing to the explosive nature of azides. The acyl azide group was observed by IR analysis at 2169 cm^{-1} and by ^{13}C NMR analysis at 181.0 ppm, and heating at reflux in toluene towards isocyanate **5.10** was monitored by the emergence of a peak at 2270 cm^{-1} and a singlet at 121.8 ppm. After cooling, the crude solution of isocyanate derivative **5.10** received benzenesulfonamide and DBU. Stirring overnight at ambient temperature yielded 75% of sulfonyl urea **5.11** over three steps from **5.8**. Lastly, deprotection of the 3-*O*-acetate **5.11** using standard basic conditions gave target 4,4-difluoro OCA phenyl-sulfonyl urea derivative **5.12.**

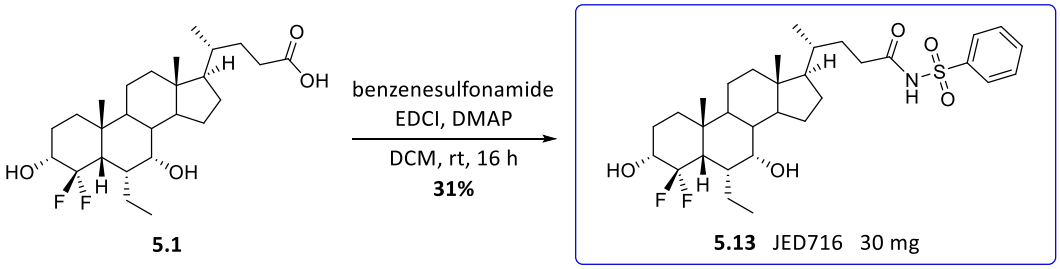


*Scheme 5.7. Synthesis of 4,4-difluoro OCA phenyl-sulfonyl urea target **5.12**.*

5.5 Synthesis of 4,4-difluoro OCA phenyl-acylsulfonamide (JED716)

In-parallel to the synthesis of 4β-fluoro aromatic sulfonyl urea analogues **4.13**-**4.17**, a series of aromatic acylsulfonamide analogues were also synthesised by colleagues within the group and at our industrial partner, given the promising biological activities of related aromatic acylsulfonamides. As such, the corresponding 4,4-difluorinated phenyl acylsulfonamide of OCA was targeted.

Hence, an EDCI-mediated amide-bond formation reaction involving 4,4-difluoro OCA **5.1** and benzenesulfonamide yielded the target acylsulfonamide derivative **5.13** in a good yield for this type of reaction (typically 20-50% as reported by members of the group) (*Scheme 5.8*).



*Scheme 5.8. Synthesis of 4,4-difluoro OCA phenyl-acylsulfonamide target **5.13**.*

The mechanism of the reaction proceeds via a carbamimidic anhydride intermediate. Regioselective substitution by the amine reagent leads to the expected product and 1-(3-(dimethylamine)propyl)-3-ethylurea as an expected byproduct (*Figure 5.7*).

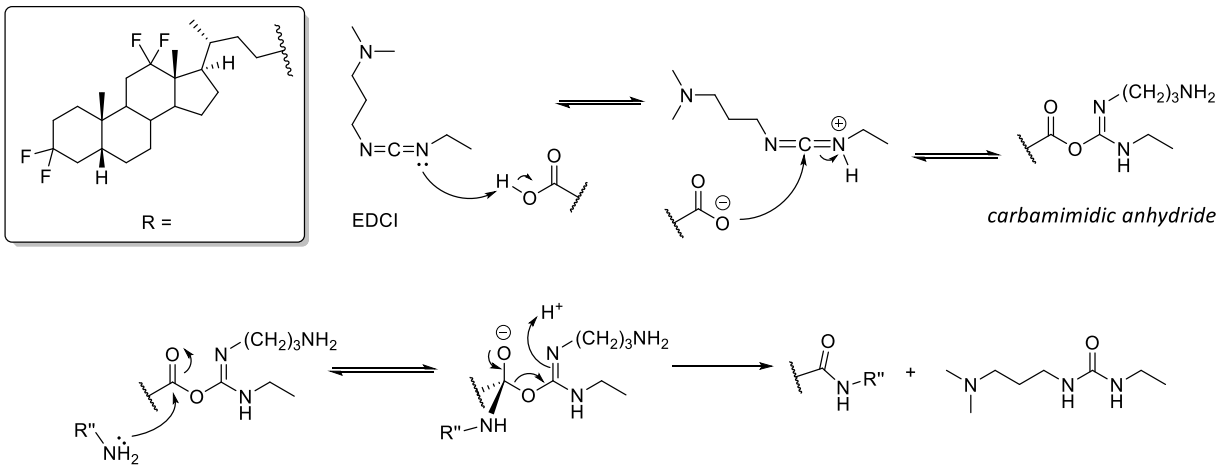


*Figure 5.7. EDCI-mediated amide bond formation mechanism to give **7.17**-**7.19**.*

5.6 Conclusions

An interesting (proposed) intramolecular deprotonation pathway has given convenient access to novel 4,4-difluorinated analogues (*Figure 5.8*). The close proximity of the 7α-OH group and the 4-position is evidenced by an intramolecular hydrogen bond between the resultant 4α-fluoro atom and this OH motif, an interaction first witnessed on BA substrates by a previous group member on 4α-fluoro CDCA derivatives (see: *Section 1.3.4.4*). Following direction from biological data, the corresponding phenyl sulfonyl urea and phenyl acylsulfonamide analogues of 4,4-difluoro OCA (**5.1**) were synthesised in addition to the parent carboxylic acid.

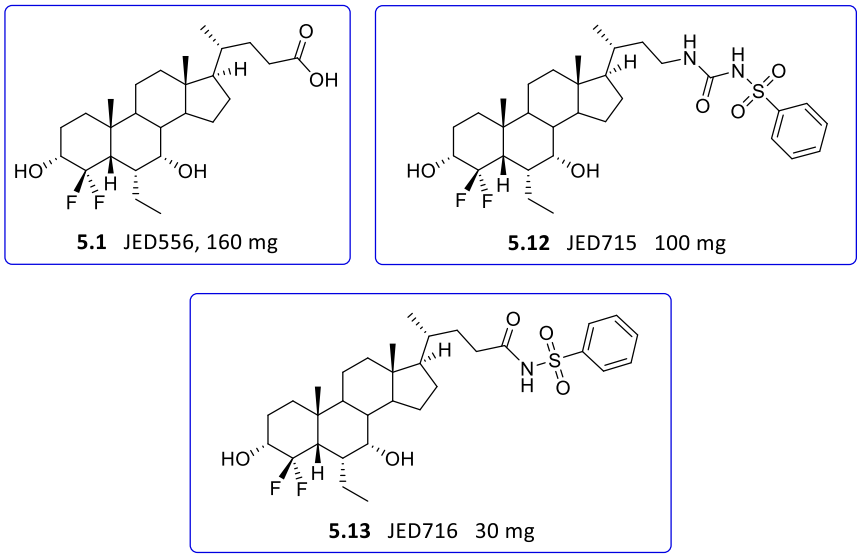


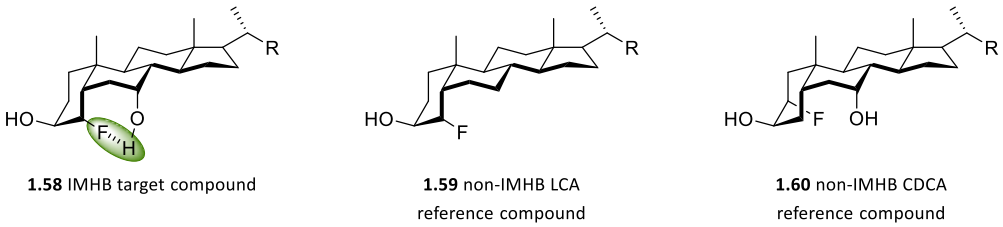
Figure 5.8. Synthesised 4,4-difluorinated OCA derivatives.

Chapter 6 Synthesis of 3β-hydroxyl-4α-fluoro CDCA (NZP318) analogues on large scale

6.1 Introduction

6.1.1 Rationale

We require approximately one gram of a 4α-fluoro CDCA derivative **1.58** for a pK_{AHY} study to quantify the influence of the strong C-F•••H-O interaction (see: *Section 1.3.4.4*) on the hydrogen bond donating capacity of the 3β-hydroxyl group. The analogous lithocholic acid analogue **1.59**, or a non-IMHB CDCA fluorohydrin analogue **1.60**, will also be targeted on one-gram scale as reference compounds (*Figure 6.1*). A key requirement was the removal of the C_{24} ester group to avoid interference with the measurements.



1.58 IMHB target compound

1.59 non-IMHB LCA reference compound

1.60 non-IMHB CDCA reference compound

*Figure 6.1. Target **1.58** and reference compounds **1.59/1.60** for pK_{AHY} study (R = alky/alkenyl).*

6.1.2 Recap and critical evaluation of existing route

3β-Hydroxyl-4α-fluoro CDCA methyl ester (**1.49**) and 2α-fluoro-3β-hydroxyl CDCA methyl ester (**1.50**) were previously synthesised by a member of the Linclau group in yields of 0.8% and 1.5%, respectively, over 6 steps from commercially available CDCA (**1.2**). Given their close structural relationship with the target compounds **1.58**, **1.59** and **1.60**, this synthetic route (*Figure 6.2*) was considered first.

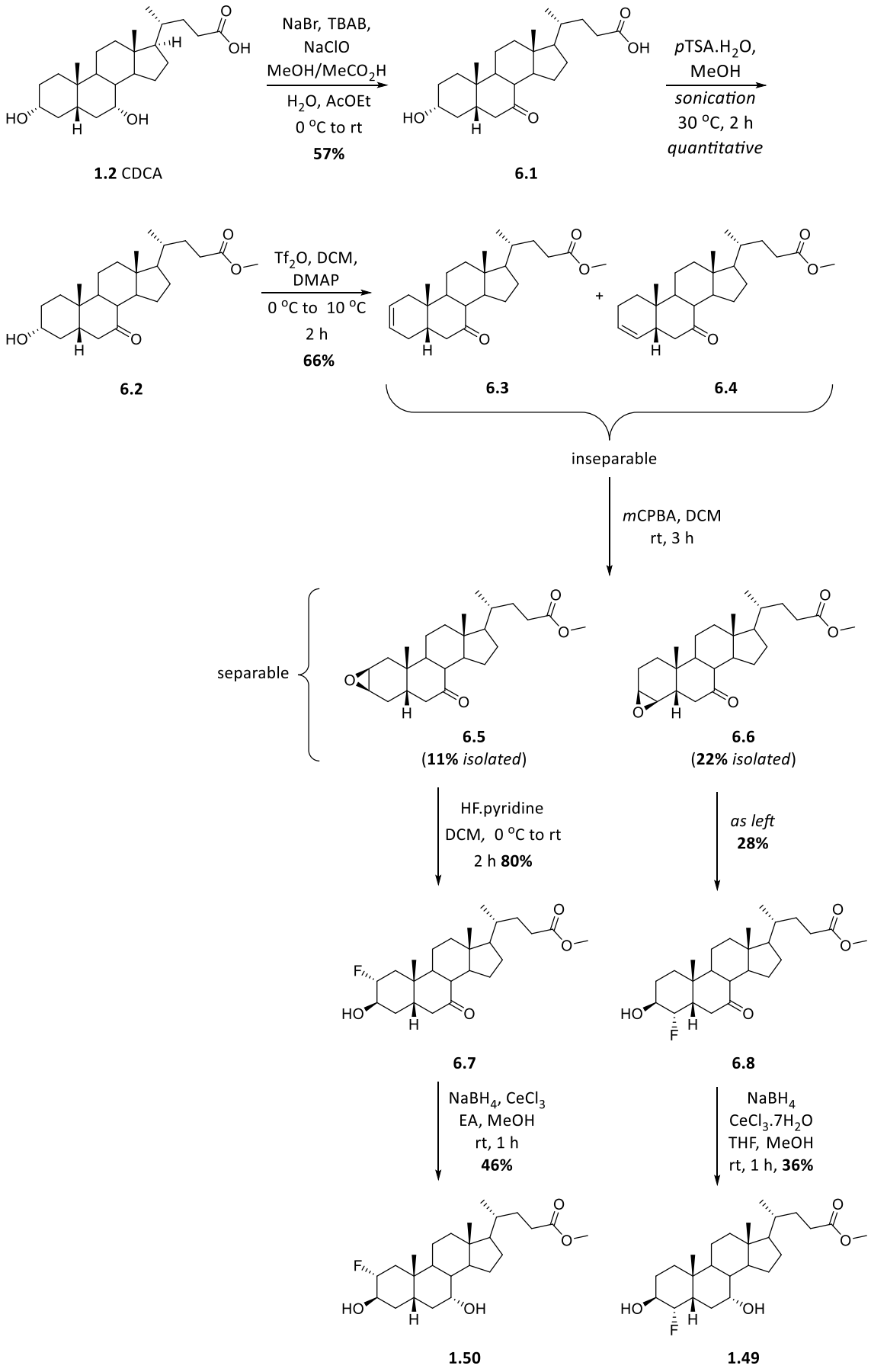


Figure 6.2. Historical route to 2α- and 4α-fluoro CDCA methyl ester analogues by a previous group member (unpublished).

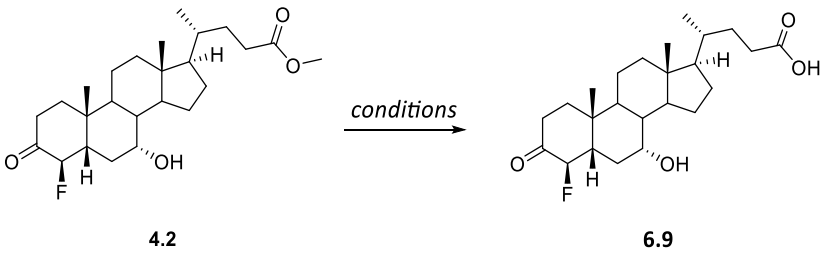
The selective 7-OH oxidation of CDCA (**1.2**) proceeded in mediocre yield owing to the formation of diketone byproducts. Significant side-reactions were also observed during the dehydration of the 3-OH group. Furthermore, two regioisomers **6.3** and **6.4** were formed upon elimination, contributing to a marked reduction in efficiency. The ratio of these alkene regioisomers was not reported. Epoxidation of the alkene mixture with *m*CPBA gave a poor isolated yield of epoxides **6.5** and **6.6**; this result was reproduced in all attempts and is postulated to be from, at least, Baeyer-Villiger byproducts. Epoxide opening of **6.6** with nucleophilic fluoride gave poor conversion to fluorohydrin **6.8** and, for both fluorohydrins, final reduction of the 7-ketone group was not diastereoselective so gave a poor yield of the corresponding targets **1.49** and **1.50**.

Overall, there is clear room for improvement in the synthesis of 3β-hydroxyl-4α-fluoro CDCA methyl ester (**1.49**) and 2α-fluoro-3β-hydroxyl CDCA methyl ester (**1.50**), despite significant optimisation already of the above route. Some steps are not regioselective or diastereoselective, and therefore other routes were explored first.

6.2 Epimerisation attempts of a more readily-accessible 4β-fluoro intermediate

6.2.1 Direct epimerisation

The first and simplest approach to the synthesis of 4α-fluorinated CDCA derivatives would be the epimerisation of readily accessible 3-keto-4β-fluorinated derivatives. It has been shown already that 2α-fluorinated OCA analogue **3.21** readily epimerises to give a mixture with 2β-fluorinated diastereomer **4.39** (see: *Section 4.7.2*). Yet, at the 4-position, the additional stabilisation of a C-F•••H-O interaction could bias the equilibrium towards the desired α-diastereomer. However, thermodynamic epimerisation of **4.2** was unsuccessful in all attempts. Only methyl ester saponification occurred (but was quantitative) to yield the carboxylic acid derivative **6.9** (*Scheme 6.1*).



4.2 **6.9**

*Scheme 6.1. Attempts at thermodynamic epimerisation of **4.2** were unsuccessful.*

Solvents (methanol, chloroform and THF), temperatures (rt and 0 °C) and concentration of sodium hydroxide (2% and 5% solutions w/v) were screened. We believe that the resultant carboxylic acid group could interfere with IMHB formation (as described in the NMR of 4,4-difluorinated substrates, see: *Section 5.3.2*), thereby reducing the likelihood of the reverse-epimerisation process (axial to equatorial). It would be of interest to repeat this reaction with the C_{24}-carboxylic acid suitably protected, which has not been attempted.

6.2.2 Epimerisation via intramolecular deprotonation

6.2.2.1 Rationale

The intramolecular deprotonation of the 4α-proton involving the 7α-OH group has been described on OCA substrates (see: *Section 5.2.2*); trapping of the resultant intermediate with TMS chloride led to an isolable OCA Δ3,4-fluorosilyl enol ether **5.4** and reaction with an F^+ source gave access to 4,4-difluorinated OCA substrates. The reaction of the non-fluorinated nucleophilic 4-position proceeds with complete β-selectivity for CDCA and OCA substrates, as described by previous members of the group and as confirmed in our own work. If the same β-selectivity is possible at the fluorinated 4-position, a proton could be introduced in the β-position (*Figure 6.3*), thus achieving the epimerisation.

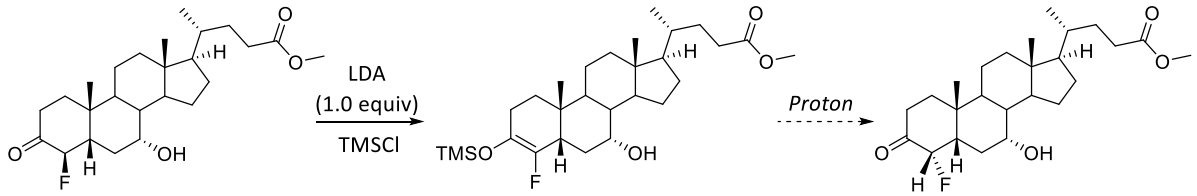
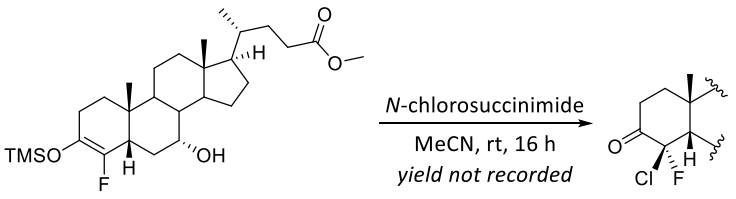


*Figure 6.3. 'Epimerisation' of the 4-fluoro atom by stereoselective reaction of silyl enol ether **6.10**.*

6.2.2.2 Confirmation of reaction diastereoselectivity

Th reaction with F^+ at the analogous OCA substrate **5.4** proceeded well. However, it is not possible to differentiate the two fluorine atoms in the product. Hence, as a control experiment to investigate the β-selectivity, Δ3,4-fluorosilyl enol ether of CDCA **6.10** was treated with an electrophillic chlorine source (*Scheme 6.2*).



6.10 **6.12**

*Scheme 6.2. β-selective reaction of CDCA Δ3,4-fluorosilyl enol ether **6.10** with chloride.*

The exact structure of 4β-chlorinated product **6.12** could not be determined, although the absence of the $H_{7\beta}$ signal by ^{1}H NMR (not shown) suggests that side reaction(s) at the B-ring had occurred; NCS is a potent oxidant. The connectivity and stereochemical relationship of the atoms on the A-ring of **6.12** was however confirmed by ^{19}F NMR (*Figure 6.4*).

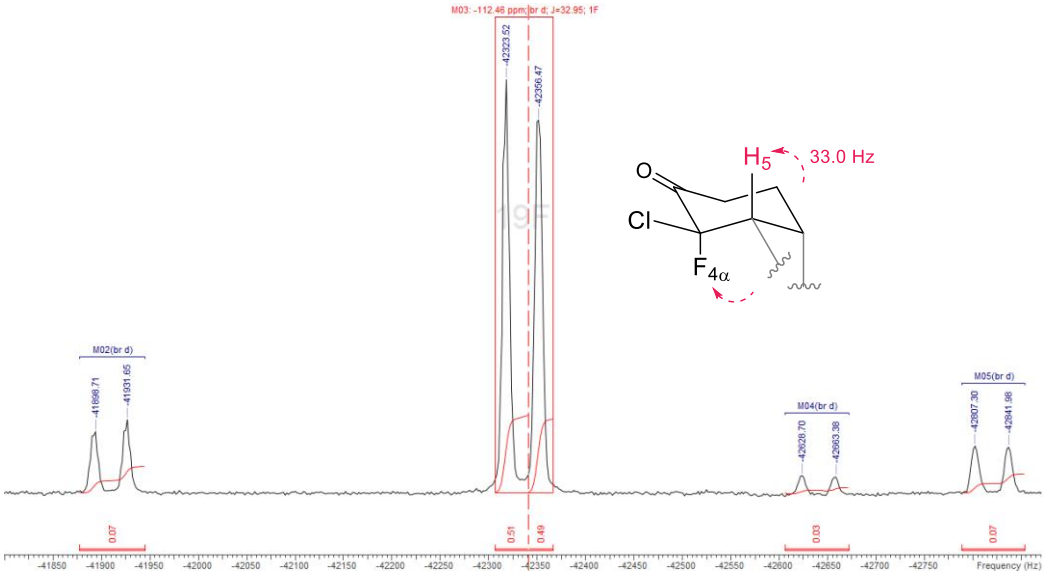
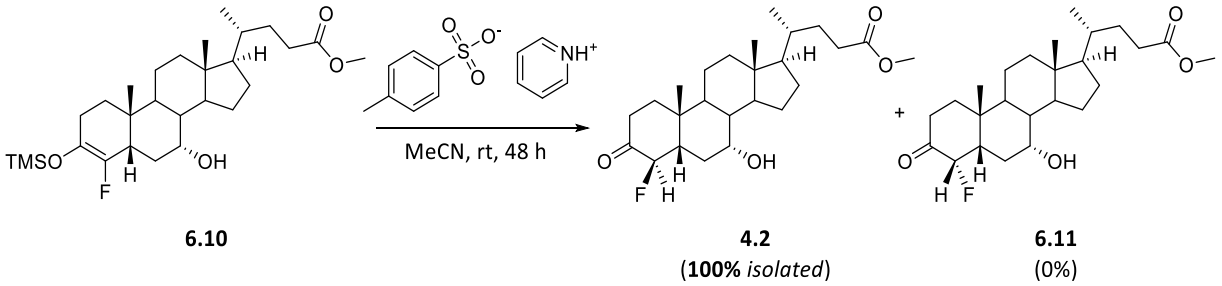


*Figure 6.4. ^{19}F NMR viewed -113.9 to -111.0 ppm with 4α-fluoro-4β-chloro product **6.12** visible.*

For the major peak (85%) at -112.5 ppm a doublet was observed with a coupling constant of 33.0 Hz. The ^{19}F chemical shift of this geminal fluoro-chloro motif, α-to a ketone group, is similar to that reported in the literature.[214] Only three other peaks (3-7% each) are present in the spectrum and deviate by less than 2.5 ppm, yet they share the same multiplicity and coupling constant. From this information we conclude that all mass within this crude sample bears the same A-ring motif, yet the molecules indeed differ at other positions on the BA owing to the aforementioned likelihood of side-reactions. Crucially, the coupling constant of 33.0 Hz would suggest an axial-axial interaction that is attenuated by an electron withdrawing substituent nearby. This indeed matches the proposed structure at the A-ring of **6.12** with chlorine in the 4β-position and fluorine in the 4α-position; the fluorine-proton coupling of 33.0 Hz is to H_5.

6.2.2.3 Epimerisation attempts using fluorosilyl enol ether substrates

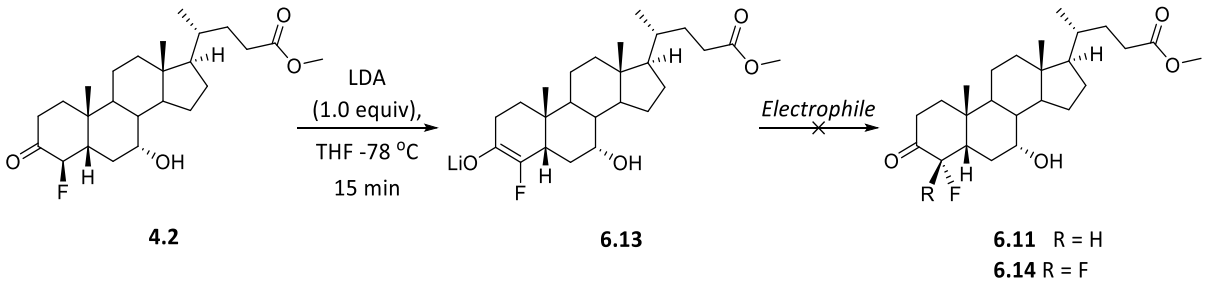
The successful reaction of OCA fluorosilyl enol ether **5.4** with Selectfluor® inspired an attempt with CDCA fluorosilyl enol ether derivative **6.10** and pyridinium *para*-toluenesulfonate (PTTS), a quarternary-ammonium proton source However, the product was identified as the 4β-fluoro diastereomer **4.2** (*Scheme 6.3*). We suspect that an intramolecular protonation reaction of the fluorosilyl enol ether motif and the proximal 7α-OH group could outcompete the desired intermolecular reaction with PTTS.



*Scheme 6.3. Reaction of fluorosilyl enol ether **6.10** with PTTS yielded only the preceding 4β-fluoro compound **4.2**.*

6.2.2.4 Epimerisation attempts using fluoroenolate substrates

The treatment of TMS fluorosilyl enol ether **6.10** with TBAF leads to the formation of the corresponding reactive fluoroenolate intermediate, driven by the formation of a strong silicon-fluorine bond as a result of this *in-situ* deprotection reaction. The fluoroenolate is then expected to rapidly react via C_4. The next attempt therefore involved treatment of **6.10** with 1M TBAF solution (containing trace amounts of water as the electrophile). Unfortunately, the reaction proceeded to yield only the preceding and undesired 4β-fluoro diastereomer **4.2** (not shown). The analogous lithium enolate **6.13** was prepared directly from **4.2** and then investigated (*Scheme 6.4*), but also only returned **4.2** in all attempts.



*Scheme 6.4. Reaction of lithium fluoroenolate **6.13** with electrophiles.*

The first attempt involved quenching the intermediate lithium fluoroenolate **6.13** with 1M HCl solution and led to the recovery of **4.2** quantitatively within a matter of minutes. The same result

was observed upon reaction of **6.13** with *t*-butyl bromide in THF solution. It is postulated that the enolate oxygen is kinetically protonated, with tautomerisation of the resultant enol species leading to the more stable 3-ketone. During this tautomerisation, the C-F bond would be re-established in the equatorial position. Interestingly, a reaction with Selectfluor® as the electrophile also failed and, instead of **6.14**, also gave **4.2** as the only product.

6.3 Study of contemporary decarboxylation methods on commercially available BAs

6.3.1 Introduction

6.3.1.1 Rationale

After the failure to epimerise advanced 3-keto-4β-fluorinated CDCA intermediates, our attention returned to the original synthesis of 3β-hydroxyl-4α-fluoro CDCA methyl ester (**1.49**) and 2α-fluoro-3β-hydroxyl CDCA methyl ester (**1.50**) (see: *Section 6.1.2*). Given the close structural relationship to C_{17}-alkyl/alkenyl targets **1.58**, **1.59** and **1.60**, this underpinning and well-established chemistry represented foundations on which to base a strategy. Considering the overall transformation required, we opted to begin with contemporary side-chain modification reactions of inexpensive and readily available primary/secondary BAs and *then* transform the A-ring to the desired fluorohydrin motif.

6.3.1.2 Retrosynthetic analysis

A retrosynthesis of side-chain modified targets **1.58**-**1.60** was considered. CDCA-derived targets **1.58** and **1.60** led to commercially available CDCA (**1.2**) while LCA-derived target **1.59** led to commercially available LCA (**1.4**). In the forward synthesis, the decarboxylation of the starting BAs could provide intermediates **6.15** and **6.16** and then the employment of the epoxide opening strategy, discussed in *Section 6.1.2*, could then lead to targets **1.58**-**1.60**.

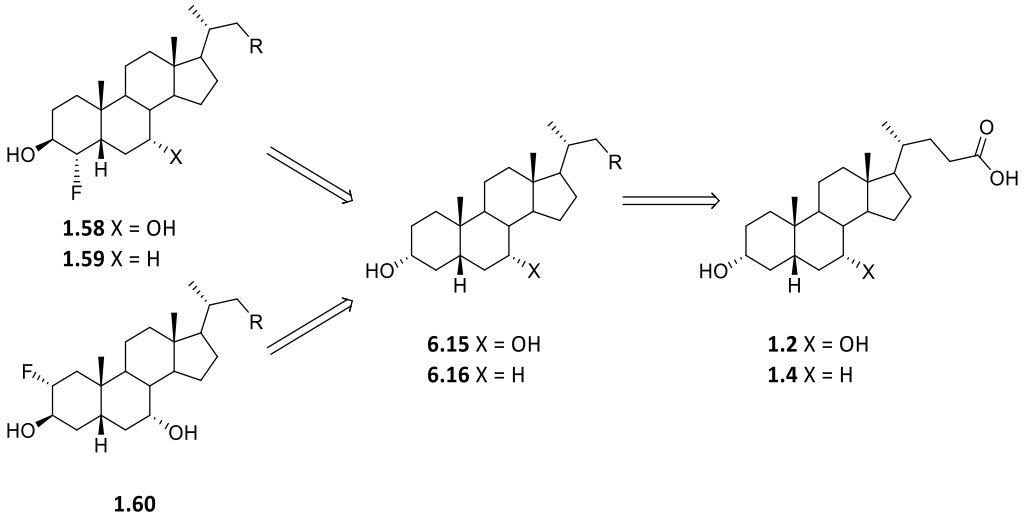


*Figure 6.5. Retrosynthesis of targets **1.58**-**1.60** to commercially available BAs **1.2** and **1.4**.*

6.3.2 Irradiative decarboxylation

6.3.2.1 Background

The first attempt at decarboxylation of CDCA (**1.2**) or LCA (**1.4**) involved a photochemical method from the group of Yoshimi using a 400 W high-pressure mercury lamp.[215] Crucially, the decarboxylation of deoxycholic acid (**1.3**) was described (*Figure 6.6*).

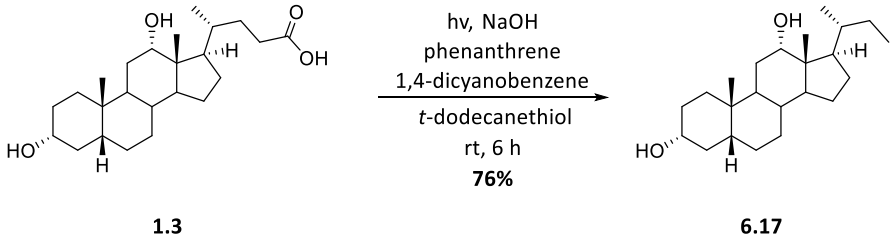


*Figure 6.6. Published irradiative decarboxylation of DCA (**1.3**).[215]*

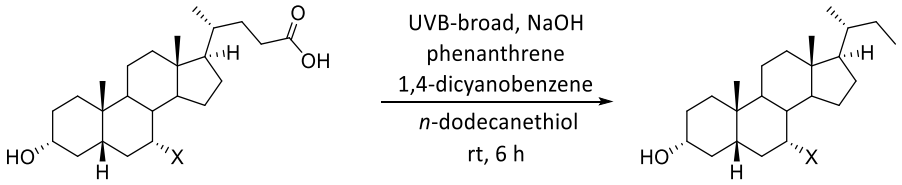
Alas, the required *t*-dodecanethiol was not commercially available and could not be sourced internally. Furthermore, we did not have access to a high-pressure mercury lamp as described in the original paper.

The decarboxylation process begins with the photo-excitation of phenanthrene, followed by a single-electron transfer (SET) with 1,4-dicyanobenzene (DCB) to provide a phenanthrene cation radical and the anionic radical of DCB. An electron transfer process can then occur between the cationic phenanthrene radical and the carboxylate anion, formed by deprotonation of the carboxylic acid under basic conditions, to afford the carboxylate radical and return phenanthrene. The carboxylate radical decomposes to form a terminal alkyl radical and, finally, hydrogen abstraction from thiol yields the desired terminal alkane.

From the mechanism described, photoexcitation of phenanthrene is the pivotal initiation point of this decarboxylative electron transfer cascade. Therefore, despite our lack of high-pressure mercury lamp, UVA (360-395 nm), UVB (310-310 nm) and UVB broad (280-370 nm) lamps were deemed suitable. Considering an absorption spectrum of phenanthrene, the UVB broad emission range gave the greatest overlap.

6.3.2.2 Chemistry

However, attempts to decarboxylate both CDCA and LCA were unsuccessful using UVB-broad (*Scheme 6.5*). The bulbs were part of an existing flow-chemistry setup and this apparatus was used for the test reactions. The conversion of the reactions was monitored by ^{1}H NMR.



1.2 X =OH
1.4 X = H

6.15 X = OH (0%)
6.16 X = H (0%)

*Scheme 6.5. Irradiative decarboxylation attempts of CDCA (**1.2**) and LCA (**1.4**).*

For these reactions, *n*-dodecanethiol was the most suitable thiol alternative. A handful of shorter chained, more-hindered thiols were found in our inventory but were all significantly more volatile. Their pungent odour and low boiling points therefore left us uninspired to use these reagents in the communal flow apparatus. Nevertheless, having observed no conversion of the starting materials by ^{1}H NMR analysis, we believe the failure of the reactions to be from unsuccessful radical initiation processes and not the choice of thiol; initiation of the reaction involving phenanthrene and DCB would have led to *some* consumption of **1.2** and **1.4**, even if not to the expected products after the final step of the reaction with a thiol. Several other attempts at this reaction were made using UVA and UVB irradiation but no conversion was observed. Additionally, sodium hydroxide had to be omitted from a number of attempts because deprotonation of the BA starting materials caused precipitation within the flow apparatus.

The significant issues encountered from this methodology led us to abandon any further attempts.

6.3.3 Transition metal-mediated decarboxylation

6.3.3.1 Background

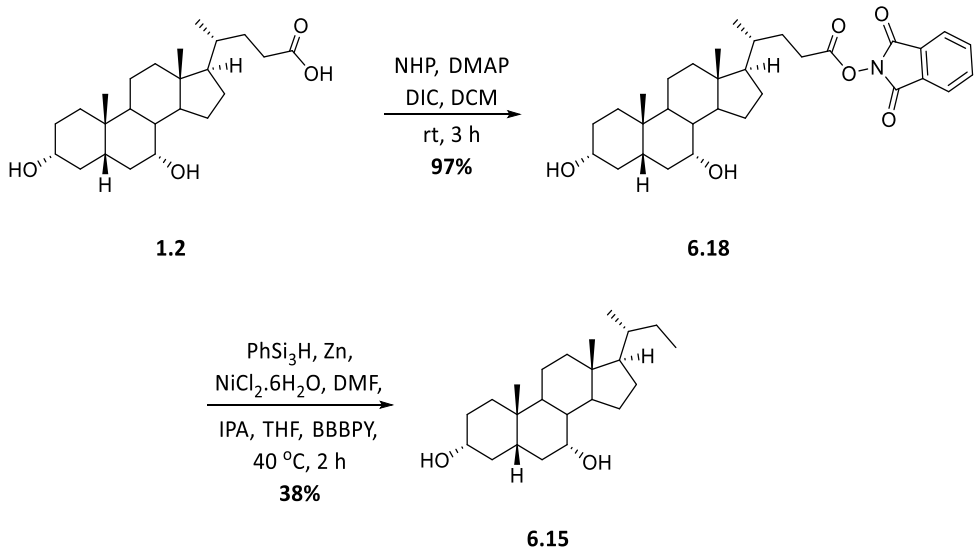
Attempts at decarboxylation of CDCA (**1.2**) and LCA (**1.4**) were made using a transition metal strategy. Barton esters were first introduced in 1983 as a convenient and simple way to synthesise

alkyl radical intermediates from carboxylic acids.[216] Hydride abstraction thereafter provides alkanes from an overall reductive decarboxylation pathway.

Despite the success of this methodology in the wider synthetic field, Barton esters are characteristically photo-sensitive and thermally unstable making these intermediates often capricious to work with. A more contemporary method that is described to overcome these inherent difficulties involves coupling of the carboxylic acid motif to *N*-hydroxyphthalamide (NHP).[217] The addition of inexpensive nickel catalyst (nickel (II) chloride hexahydrate) then initiates a rapid decarboxylative radical fragmentation to yield the same alkyl radical that would be expected from the Barton reaction. With this pathway involving NHP esters, the successful decarboxylation of a limited number of steroidal substrates has been described.

6.3.3.2 Chemistry

Coupling of CDCA (**1.2**) with *N*-hydroxyphthalamide yielded the corresponding NHP ester **6.18** in excellent yield on 100 mg scale (*Scheme 6.6*). On 7 g scale this yield reduced to 81%. In both cases, the target compound could not be isolated pure and was contaminated with 1,3-diisopropylurea byproduct from coupling agent DIC. Decarboxylation of the NHP ester according to the published procedure was successful, though poorly yielding, and gave CDCA C_{17}-alkane **6.15**.



1.2 **6.18** **6.15**

*Scheme 6.6. Formation of CDCA NHP ester **6.18** and decarboxylation thereafter to **6.15**.*

Purification of compound **6.15** was very cumbersome. Despite the hydroxyl groups at the 3- and 7-positions, the presence of the C_{17}-alkyl motif caused this compound to be very apolar. It was therefore inseparable from triphenyl silane and phthalimide-derived byproducts. The yield reported for the decarboxylation reaction was calculated by ^{1}H NMR of the isolated impure fraction. Bearing only one hydroxyl group, we expected the corresponding C_{17}-alkyl derivative **6.16** of LCA to be even more apolar and so further exploration of this route was abandoned.

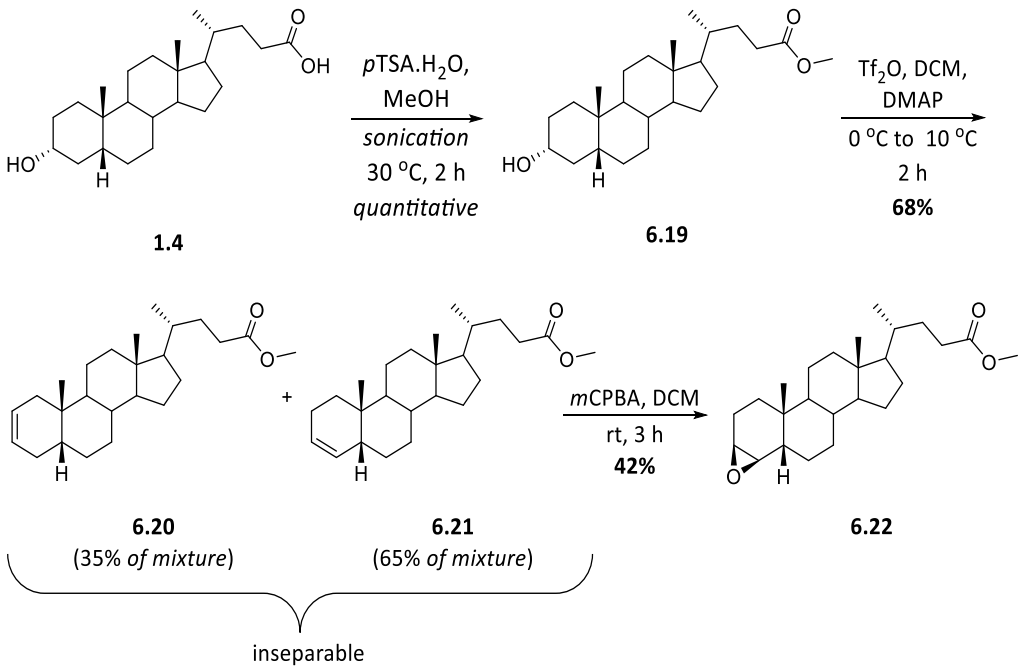
6.4 Synthesis towards 3β-OH-4α-fluoro LCA reference compound 1.59

6.4.1 Introduction

To avoid the high apolarity of compounds with a C_{17}-alkyl motif, decarboxylation was planned for after the introduction of the fluorohydrin motif to the A-ring. Hence, the epoxide opening strategy was employed as described in *Section 6.1.2*, but now on significantly larger scale; decarboxylation could then be explored at a later stage of the synthesis.

6.4.2 Synthesis of advanced LCA 3β,4β-epoxide intermediate

The quantitative esterification of LCA (**1.4**) was followed by the dehydration of the 3α-OH group of **6.19** using established conditions (*Scheme 6.7*). An approximate 1:2 ratio of alkene regioisomers was formed; the yield of this reaction on the analogous CDCA substrate was reported at 66%. Epoxidation of the inseparable alkene regioisomers gave a 42% isolated yield of Δ3,4-epoxide regioisomer **6.22**, considerably higher than the yield reported for the analogous CDCA substrate (22%).

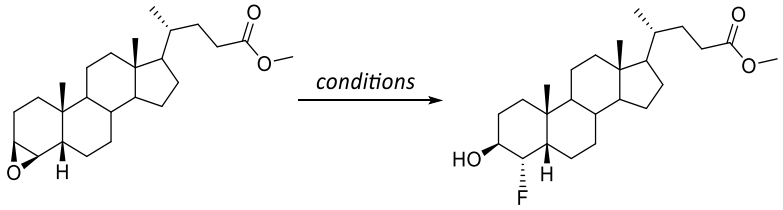


*Scheme 6.7. Synthesis of Δ3β,4β-epoxy LCA methyl ester (**6.22**).*

6.4.3 Epoxide opening attempts

With Δ3β,4β-epoxy LCA methyl ester (**6.22**) in-hand, fluorination conditions were screened (*Table 6.1*). Sources of nucleophilic fluoride included Olah's reagent, inorganic fluoride (KHF_2), and TBAF and the reaction was forced in some cases using high temperatures or vast excesses of reagent. However, in contrast to the reaction involving the corresponding 7-keto substrate (see: *Section*

6.1.2), most attempts gave no reaction whatsoever and only returned starting material. Of those where conversion was indeed witnessed, the expected fluorohydrin product was observed in very low quantities in the crude reaction mixture (by ^{19}F and ^{1}H NMR).



6.22 **6.23**

Entry	Fluoride Source	Equivalents	Temp. / °C	Duration / h	Solvent	Conversion
1	TBAF 1M solution (old)	4	0	8	THF	0
2	TBAF 1M solution (old)	2.5	70	22	THF	0
3	TBAF (solid from old solution)	5	70	22	THF	0
4	TBAF 1M solution (new)	2.5	70	20	THF	0
5	$TBAF.H_2O$	2.5	70	8	*t*-BuOH	0
6	$TBAF.H_2O/KHF_2$	1.5/2	115	60	*neat*	0
7	$Bu_4NH_2F_3/KHF_2$	½	115	60	*neat*	0
8	HF.pyridine	5	0 to rt	6	DCM	0
9	HF.pyridine	30	0 to rt	6	DCM	(<4)
10	HF.pyridine	30	0 to rt	16	DCM	(<13)
11	HF.pyridine	400	0 to rt	6	DCM	(<8)
12	HF.pyridine	400	0 to rt	4	DCM	(<5)

Table 6.1. Screening of epoxide opening conditions with nucleophilic fluoride.[xvii]

[xvii] All reactions performed on 250 mg scale. Those entries marked with an asterisk gave complete conversion to the corresponding carboxylic acid analogue. The conversions of those entries in parentheses were determined by ^{1}H NMR of the crude reaction mixture after aqueous workup.

A screening of nucleophilic fluoride sources was conducted to try and optimise this reaction. Alas, no conversion was observed in any of the preliminary reactions (*Entries 1* to *8*). Forcing attempts with HF.pyridine gave consistently poor yields of expected fluorohydrin **6.23** and a multitude of fluorinated byproducts (*Entries 8* to *12*). This result was of some surprise given the reported success of this reaction between 7-keto CDCA epoxide **6.6** and fluoride by a previous group member. Additionally, it was discovered that trace amounts of water in some reactions with TBAF (from an old solution) led only to hydrolysis of the methyl ester group (*Entries 2* and *3*).

6.4.4 Insight into failed epoxide opening

Analysis of a crystal structure of epoxide **6.22** provided insight into the failed epoxide opening.

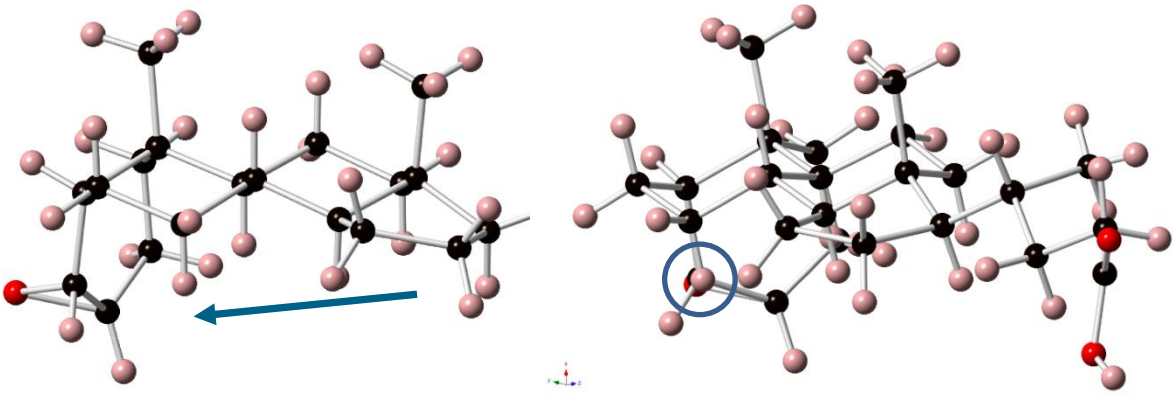


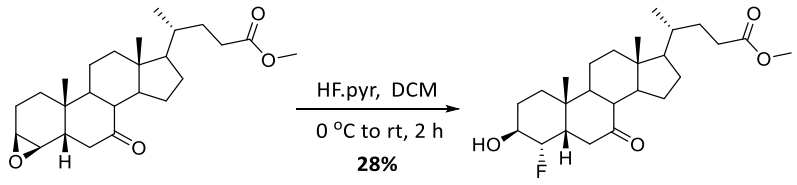
*Figure 6.7. Left: epoxide **6.22** viewed in traditional 3D orientation; Right: epoxide viewed along the trajectory of the C_4-O bond. Methyl ester not shown for concision.*

As expected for cyclohexane epoxides, the A-ring appears as a half-chair. A view along the C-O bond at the 4-position (right-hand image) reveals that C_4 (black, in the navy circle) is almost blocked from view by the hydrogen in the 7α-position (pink). This proton lies directly along the linear trajectory of the C_4-O bond and in close proximity.

Opening of the epoxide at C_4 with fluoride requires insertion of a nucleophilic lone pair of electrons into the σ* orbital of the C_4-O bond. For this to occur, attack of the nucleophile must be at 180 ° to that of the leaving group (oxygen, for this reaction) to maximise orbital interactions. The proton $H_{7\alpha}$ entirely blocks this trajectory, so only in very forcing cases (*Table 6.1, Entries 9* to *12*) do we see the reaction proceed at all.

6.4.5 Exploiting epoxide stability: Synthesis of JED656

In the original work on 7-keto CDCA, the rationale for the oxidation of the 7α-OH group was to protect the alcohol during the dehydration reaction of the 3α-OH group. Hence, this decision proved fortuitous, as it allowed 4α-fluoro introduction, albeit in low yield (*Scheme 6.8*).



*Scheme 6.8. Opening of 7-keto CDCA epoxide **6.6** with fluoride (taken from Figure 6.2).*

The 3-position of this LCA Δ3β,4β-epoxide will not react because of Fürst-Plattner rules and the four position will not react due to steric blocking from the 7α-substituent. It could therefore be quite stable towards hydrolysis (*Figure 6.8*). The epoxide motif is a suitable bioisostere to the 3-position hydroxyl group because it can act as a hydrogen bond acceptor. It could therefore exhibit similar binding affinity but with a significantly longer biological half-life and was thus of interest for biological screening.

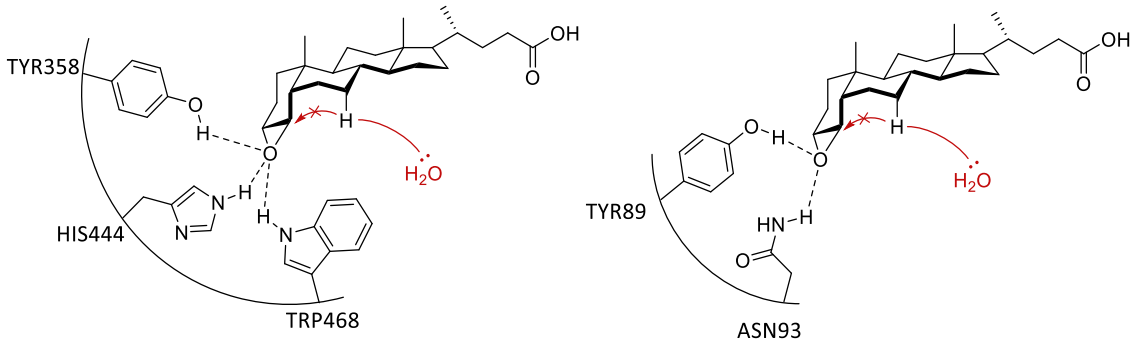
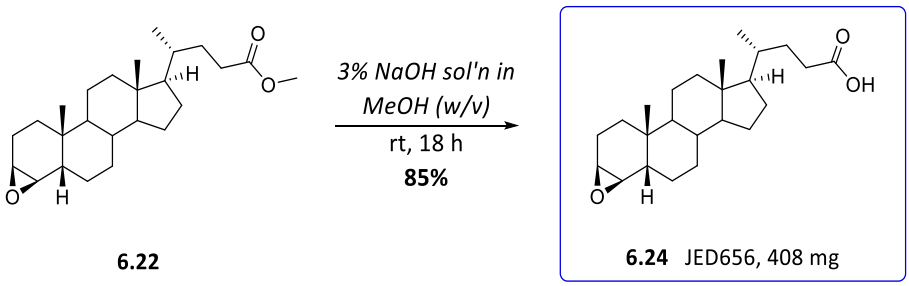


Figure 6.8. The Δ3β,4β-epoxide motif could be a more stable hydroxyl-group bioisostere at the FXR (left) and TGR5 (right).

Saponification of Δ3β,4β-epoxy LCA methyl ester **6.22** was performed under mildly basic conditions and proceeded in excellent yield to give target **6.24** for biological testing (*Scheme 6.9*).



*Scheme 6.9. Synthesis of target Δ3β,4β-epoxy LCA (**6.24**).*

6.5 Synthesis of 3β-OH-4α-fluoro CDCA target compound 1.58 and of 2α-fluoro-3β-OH CDCA reference compound 1.60

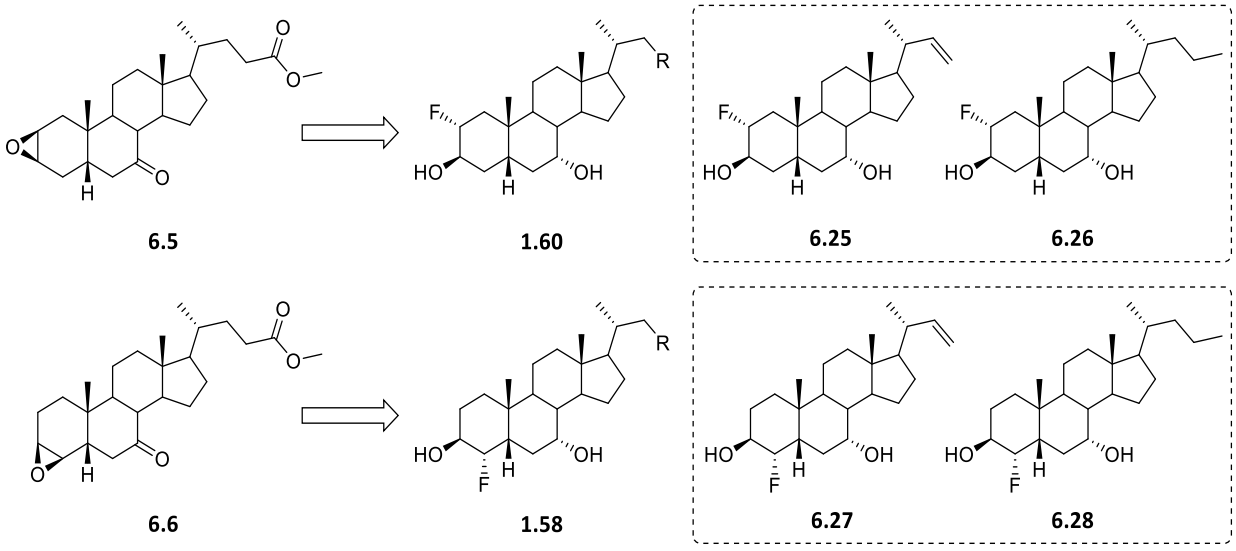
6.5.1 Introduction

6.5.1.1 Rationale

The high apolarity of compounds with a C_{17}-alkyl motif described in *Section 6.3* was to be avoided. Hence, in identical fashion as for the synthesis towards LCA reference compound **1.59** (see: *Section 6.4*), decarboxylation of CDCA compounds was envisioned *after* fluorohydrin motif incorporation, and a 7-keto group is required for the epoxide opening step.

6.5.1.2 Synthetic plan

Separable epoxide regioisomers **6.5** and **6.6** can be synthesised from CDCA (**1.2**) (see: *Section 6.1.2*) and epoxide opening with fluoride followed by 7-keto reduction and a decarboxylation reaction could then provide targets **1.60** and **1.58**. Specifically, oxidative decarboxylation would lead to alkenyl derivatives **6.25** and **6.27** while a reduction/deoxygenation strategy would lead to alkyl derivatives **6.26** and **6.28**.



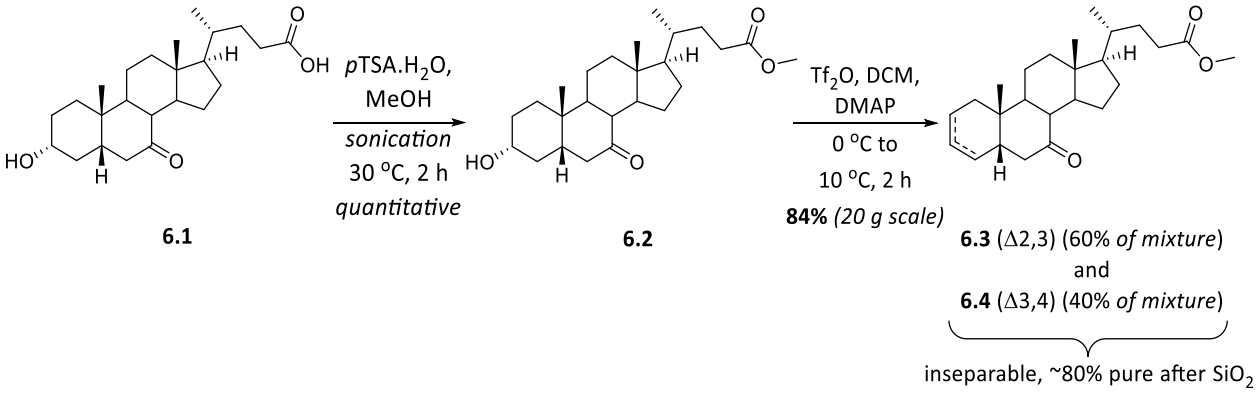
*Scheme 6.10. Synthesis of targets **1.58** and **1.60** and illustration of possible side-chain derivatives.*

6.5.2 Synthesis of compounds via oxidative decarboxylation

6.5.2.1 Synthesis of 7-keto fluorohydrin methyl ester intermediates

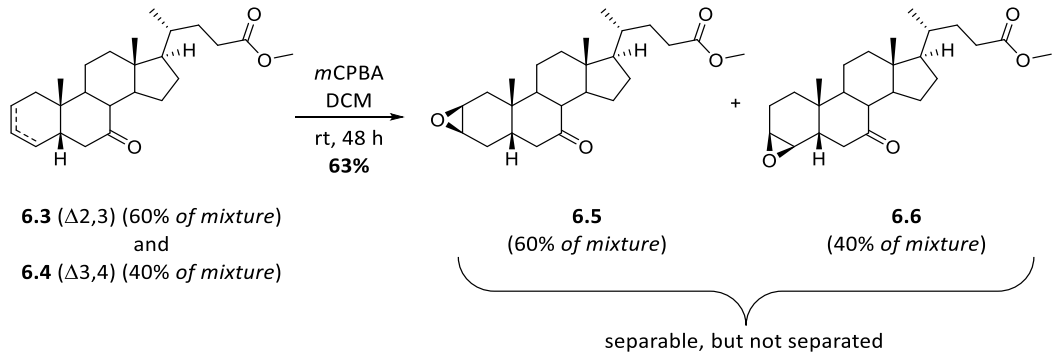
Esterification of 7-keto CDCA (**6.1**), provided by our industrial sponsor, on 80 g scale was quantitative and led to **6.2** (*Scheme 6.11*). Quantitative yields were also attained on 60 g and 20 g scales. This aligns with the quantitative yields observed when performed by a previous group

member. Dehydration of the 3α-OH group gave the best yield on 20 g scale of 82% but larger scales proved detrimental (53% on 40 g scale and 32% on 60 g scale). On all scales the ratio of regioisomers was consistently 60:40 in favour of the Δ2,3-product **6.3**. This reaction was performed on 30 g scale by a previous group member with a yield reported of 66% which fits the observed trend of yield/scale. The addition of triflic anhydride is highly exothermic and is therefore added dropwise, but we suspect that inefficient cooling on these larger scales provided local 'hot-spots' where byproduct formation became more prevelant. Indeed, later attempts involved internal monitoring of the reaction and recorded sudden fluctuations in temperature as high as 28 °C, far higher than the recommended upper limit of ~10 °C that was set during optimisation. The large quantity of methyl ester **6.2** was therefore taken forward to alkene regioisomers **6.3** and **6.4** in many 20 g batches.



*Scheme 6.11. Esterification and dehydration reactions on 7-keto CDCA ester **6.2** on large scale.*

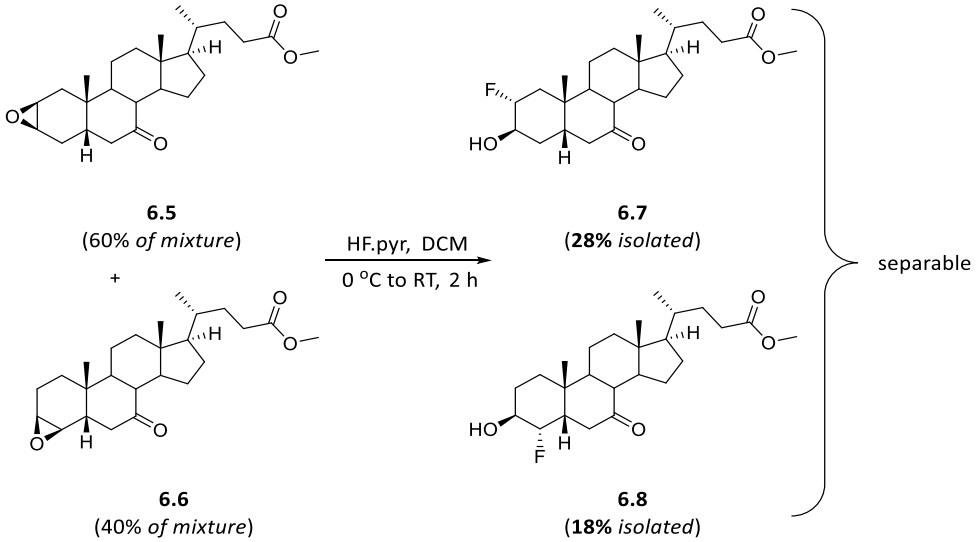
Each batch of inseparable alkenes was epoxidised separately and gave a consistent 60:40 ratio in favour of Δ2β,3β-epoxide **6.5**. An average yield of around 60% was attained from all 8 batches, with the highest yield recorded at 63% (*Scheme 6.12*), double the yield previously obtained in the group. A small-scale scouting reaction found the subsequent fluorohydrin intermediates to be more separable; the epoxides were therefore isolated as a mixture and taken forward together.



*Scheme 6.12. Epoxidation of alkene regioisomers **6.3** and **6.4**.*

The batches of mixed epoxide regioisomers were now combined and treatment of **6.5** and **6.6** with HF.pyridine gave mediocre yields of the two expected fluorohydrin products following their

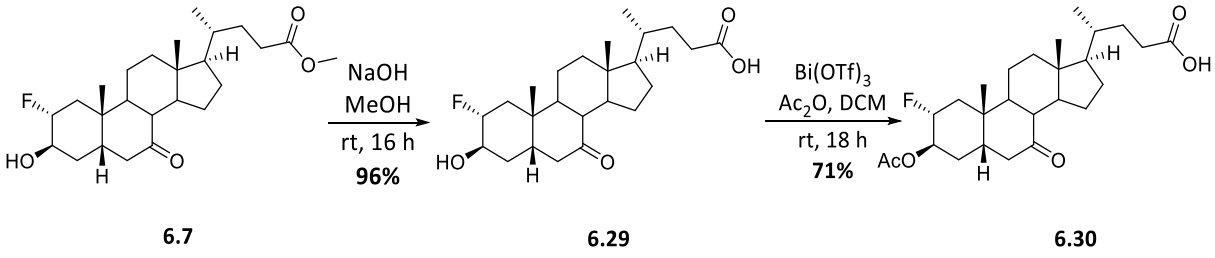
separation by column chromatography (*Scheme 6.13*). These yields are however comparable with those reported by the group.



*Scheme 6.13. Treatment of epoxide regioisomers **6.5** and **6.6** with fluoride.*

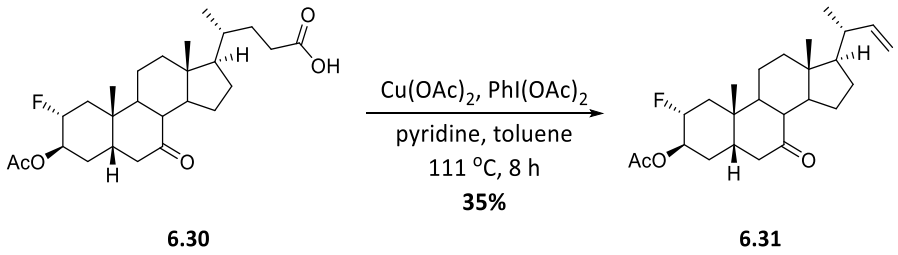
6.5.2.2 Synthesis of 2α-fluoro-3β-hydroxyl-C_{17}-alkenyl CDCA (JED722)

With 2α-fluoro-3β-hydroxy-7-keto CDCA methyl ester (**6.7**) in-hand, saponification under basic conditions gave the corresponding carboxylic acid **6.29** in excellent yield (*Scheme 6.14*). Acetylation of the 3β-OH group was first attempted using our classical method involving acetic anhydride and DMAP but we observed a poor yield of 41% (not shown). We expect that the electron withdrawing effect of the antiperiplanar 2-fluoro atom significantly reduces the nucleophilicity of the hydroxyl group. An alternative method involving catalytic bismuth (III) triflate gave a better yield of 71% of compound **6.30**.[218]



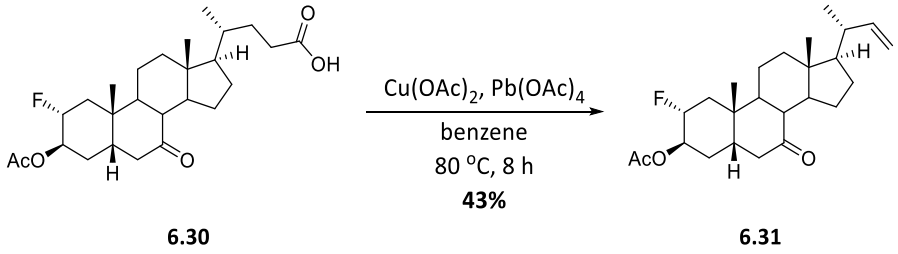
*Scheme 6.14. Methyl ester saponification and acetylation **6.29** with bismuth (III) triflate.[218]*

Oxidative decarboxylation was first attempted on 1 g scale with a catalytic amount of copper (II) acetate (0.2 equiv). After 8 hours at reflux in toluene, the target alkene **6.31** was isolated in only 15% alongside 15% of recovered starting material **6.30** (not shown). Using 0.4 equivalents of copper (II) acetate gave a 27% isolated yield of **6.30** and using 0.6 equivalents gave the best isolated yield of 35% (*Scheme 6.15*); even greater equivalents were of no benefit but made the crude organic material increasingly more cumbersome to separate against aqueous media during workup.



*Scheme 6.15. Oxidative decarboxylation of advanced 2α-fluoro intermediate **6.30**.*

A number of modifications were made to this procedure, including the substitution of *bis*-acetoxyiodobenzene for lead (IV) acetate. After 8 hours at reflux in benzene and adding six equivalents of lead (IV) acetate portionwise over the allotted time, a 43% isolated yield of alkene derivative **6.31** was accomplished (*Scheme 6.16*).



*Scheme 6.16. Improved oxidative carboxylation of **6.30**, involving lead (IV) acetate.*

Clear multiplet signals for the alkenyl protons were observed in the ^{1}H NMR spectrum of advanced alkene intermediate **6.31** (*Figure 6.9*) with the expected coupling pattern.

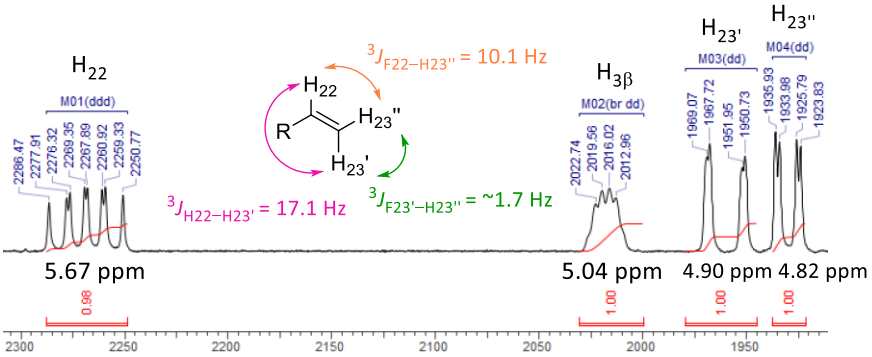
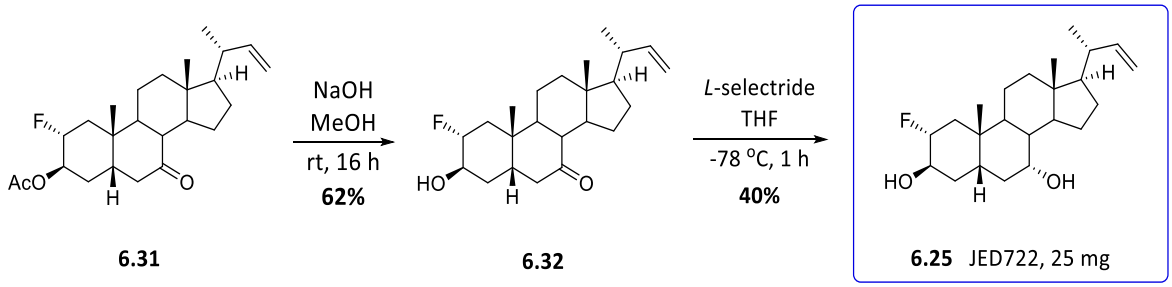


*Figure 6.9. ^{1}H NMR analysis of key alkenyl proton signals of compound **6.31**.*

Splitting trees not included for concision.

Removal of the 3β-acetate group under standard basic conditions proceeded in a mediocre 62% yield to give fluorohydrin **6.32** (*Scheme 6.17*). Deprotonation of the resultant hydroxyl group *in-situ* and reaction at the 2-positon is a potential side-reaction and could contribute to the lower-than-expected yield, but no epoxide byproducts were observed. Finally, reduction of the 7-ketone group was first attempted using sodium borohydride and cerium (III) trichloride (not shown). These were the conditions used within the group for reduction of ester derivative **6.8** and this reaction was

reported to proceed with high diastereoselectivity (*Figure 6.2*). In our own hands however, we obtained a 50:50 mixture of alcohol diastereoisomers. Using *L*-selectride to promote hydride attack along the more hindered equatorial trajectory, the diastereoselectivity improved to a 60:40 ratio in favour of the desired 7α-hydroxylated product **6.25**, which was isolated in a 40% yield after column chromatography. The corresponding 7β-diastereomer was not isolated.

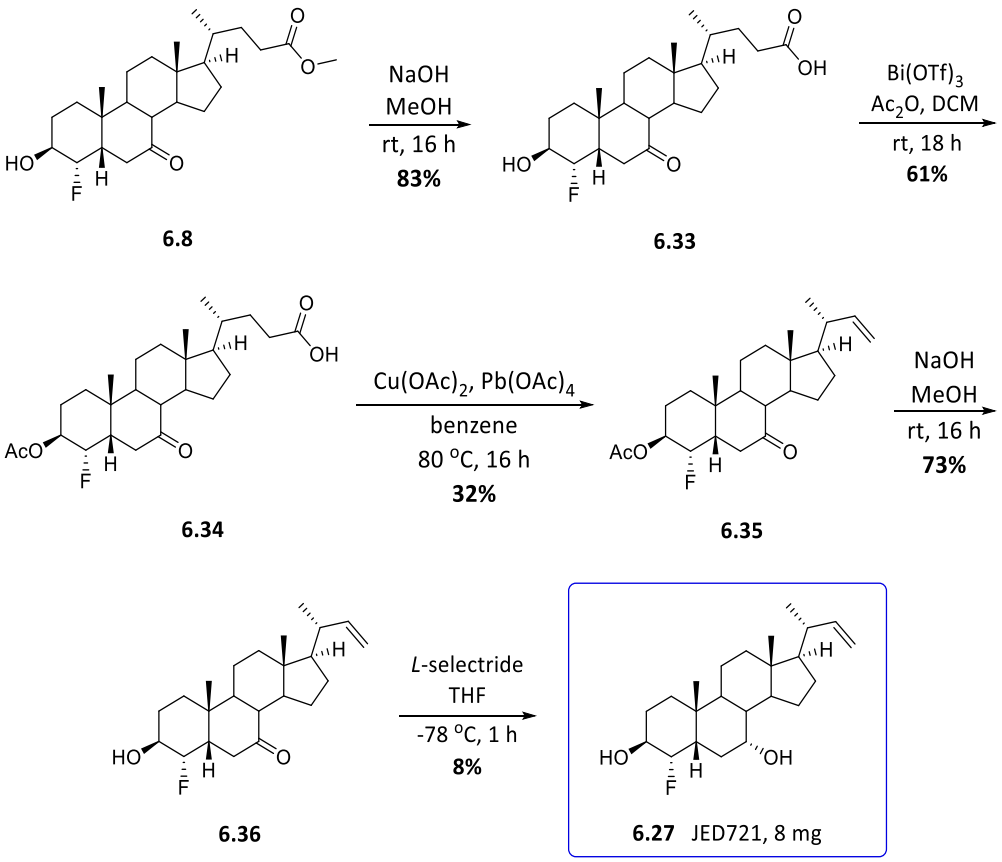


6.31 **6.32** **6.25** JED722, 25 mg

*Scheme 6.17. Synthesis of target 2α-fluoro-3β-hydroxyl-C_{17}-alkenyl CDCA (**6.25**).*

6.5.2.3 Synthesis of 3β-hydroxyl-4α-fluoro-C_{17}-alkenyl CDCA (JED721)

Equally, with the 3β-hydroxy-4α-fluoro-7-keto methyl ester (**6.8**) in-hand, synthesis towards the target 4α-fluoro alkenyl derivative **6.27** (*Scheme 6.18*) was completed in identical fashion to its 2α-fluoro regioisomer **6.25**. Firstly, saponification of the ester group gave carboxylic acid **6.33** and acetylation of the 3β-OH group using catalytic bismuth (III) triflate then gave a good 61% yield of compound **6.34**. The oxidative decarboxylation step was then performed using the improved method involving lead (IV) acetate and copper (II) acetate (see: *Section 6.5.2.2*) but gave only 27% of expected 4-fluoro alkene **6.35** with 29% starting material (not shown). Repeating this reaction and stirring at reflux overnight (following the last addition of lead (IV) acetate), we isolated an improved 32% of alkene **6.35** and only 3% starting material. Saponification of the acetate group proceeded in mediocre yield and, finally, reduction of the 7-keto group using *L*-selectride gave a complex mixture of products in the crude material. Purification by column chromatography gave an impure fraction of ~20 mg containing target 4-fluorinated alkene **6.27** at only 43% purity (determined by ^{1}H NMR, thus a calculated yield of only 8%). Unfortunately, the poor quality of sample precluded observation of an IMHB.



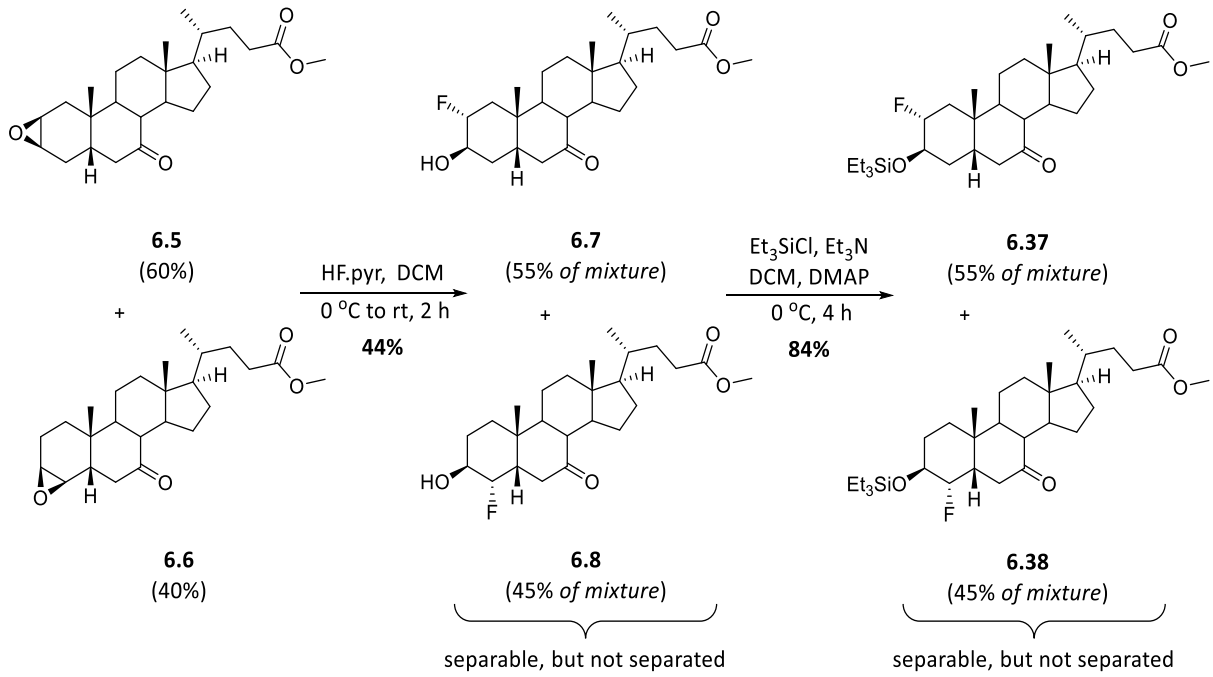
*Scheme 6.18. Synthesis of target 3β-hydroxyl-4α-fluoro C_{17}-alkenyl CDCA (**6.27**).*

6.5.3 Synthesis of compounds via a reduction/deoxygenation strategy

6.5.3.1 Synthesis of 2α-fluorinated and 4α-fluorinated intermediates

During the synthesis of C_{17}-alkenyl compounds **6.25** and **6.27**, the preceding 2-fluoro and 4-fluoro fluorohydrin intermediates were separated and taken forward in parallel separate reactions, which slowed the overall progress of the synthesis. Going forward, we therefore proposed to separate the 2-fluorinated and 4-fluorinated material at a much later stage when working towards C_{17}-alkyl compounds **6.26** and **6.28**. Additionally, the free 3β-alcohol will be protected with a silyl protecting group that we expect to remain in-place until the very last step.

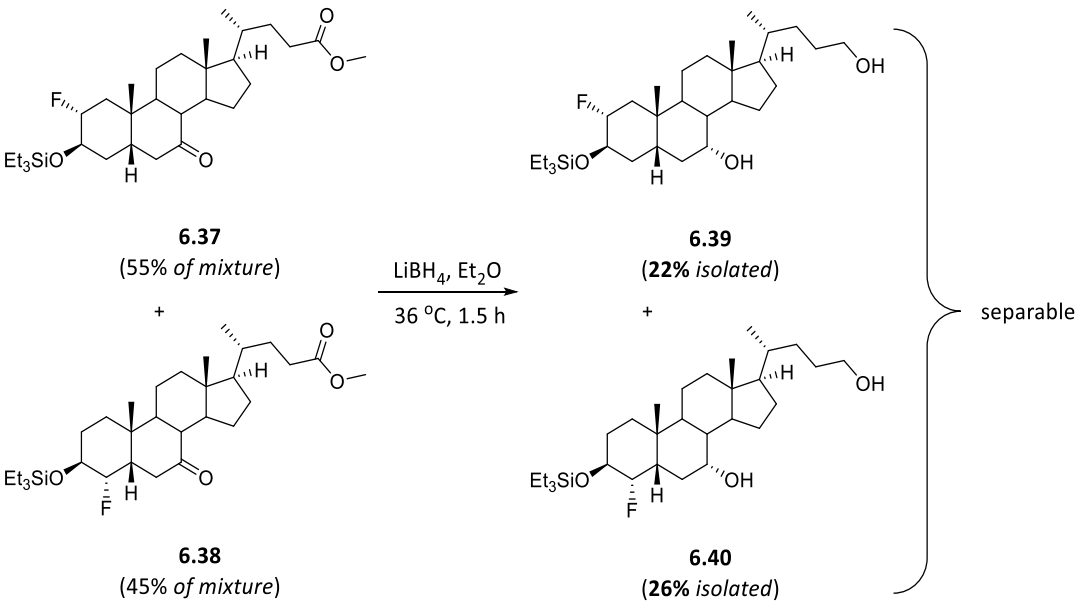
The fluoride opening of the 7-keto CDCA epoxide regioisomers **6.5** and **6.6** was performed on 22 g scale and gave a 44% yield of the expected fluorohydrin intermediates (*Scheme 6.19*). Protection of the 3β-hydroxyl groups of **6.7** and **6.8** as *tert*-butyldimethysilyl ethers gave no conversion (not shown) but installation of the less bulky triethylsilyl group proceeded in excellent yield. This now allowed reduction of the 7-keto group and C_{24}- ester group in one-pot, using forcing conditions.



*Scheme 6.19. Epoxide opening of **6.5** and **6.6** with fluoride and subsequent O-TES protection.*

A first attempt at a one-pot reduction reaction involved sodium borohydride in mildly basic aqueous solution heated to 80 °C (not shown). Yet, only reduction at the 7-keto group was successful and no conversion was observed for the C_{24}-ester group. Changing to lithium borohydride and when in refluxing diethyl ether then gave the desired reaction outcome (*Scheme 6.20*). Notably, the same reaction at ambient temperature gave no conversion after 12 hours. In all cases using lithium borohydride, the diastereoselectivity at the 7-keto group was towards the desired axial alcohol. The 4α-fluorinated diol product **6.40** exhibited a significantly lesser polarity than 2α-fluorinated diol product **6.39** and the two compounds were therefore readily separable by column chromatography. The polarity difference is explained by the existence of an IMHB in the 4-fluoro compound; the exchangeable 7α-O<u>H</u> is involved in a C-F•••H-O interaction, so binds with less affinity to silica.[xviii]

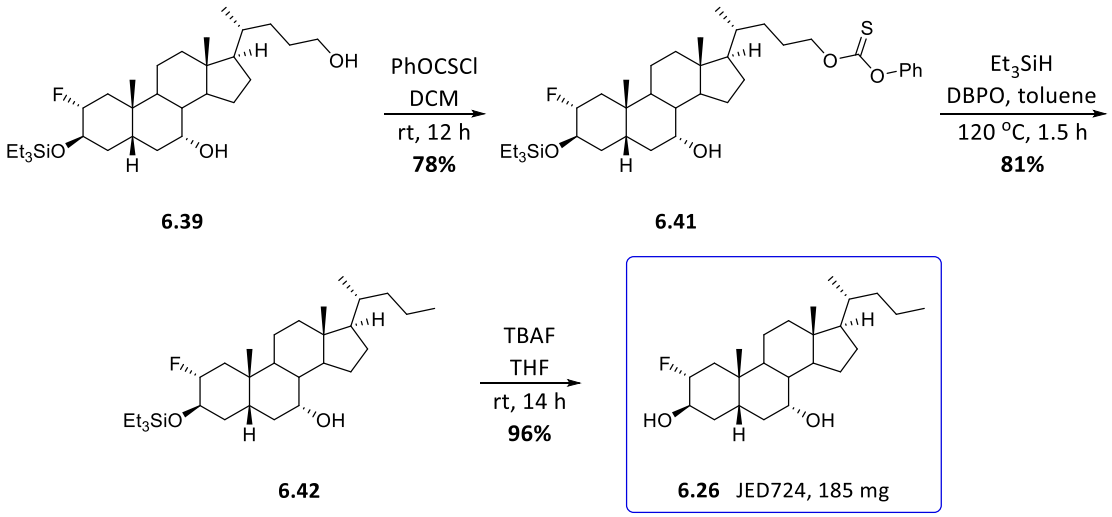
[xviii] Evidence to support this IMHB was also observed in the ^{1}H NMR: 3.16 ppm (dd, *J* = 38.0, 11.5 Hz, C_7-O<u>H</u>). 38.0 Hz coupling to $F_{4\alpha}$. 11.5 Hz coupling to $H_{7\beta}$.



*Scheme 6.20. One-pot reduction of the C_7-keto and C_{24}-ester groups of **6.37** and **6.38**.*

6.5.3.2 Synthesis of 2α-fluoro-3β-hydroxyl-C_{17}-alkyl CDCA (JED724)

We aimed to selectively deoxygenate the primary C_{24}-OH group. Considering the hindrance at the secondary 7α-OH group, we expected a bulky reagent such as *O*-phenyl thiocarbonate to give the desired regioselectivity. Conversion of advanced 2-fluorinated intermediate **6.39** to the corresponding *O*-phenyl thiocarbonate ester **6.41** proceeded in excellent yield (*Scheme 6.21*). No trace of the corresponding 7-*O*-thiocarbonate ester was observed by NMR analysis. Deoxygenation was then completed by refluxing in toluene with triethylsilane, in excellent yield. Chromatographic purification of this apolar advanced intermediate from triethylsilane byproducts was cumbersome, but nevertheless successful. Finally, deprotection of the 3-*O*-silyl protecting group of **6.42** was accomplished by stirring in TBAF solution and provided an almost quantitative yield of target **6.26**.



Scheme 6.21. Synthesis of target 2α-fluoro-3β-hydroxyl-C_{17}-alkyl CDCA (**6.26**).

After purification by column chromatography, target 4-fluorinated CDCA alkyl compound **6.26** was observed to be approximately only 90% pure by NMR analysis.[xix] The only contaminant to this sample was identified by ^{19}F NMR at 10% (by mole) and exhibited identical multiplicity and coupling constants to the major and expected signal (*Figure 6.10*). Unfortunately, attempts to purify this compound further by HPLC only enriched the mixture with greater proportions of the byproduct (to 12% by mole). Its identity remains unknown. We expect *O*-protection of compound **6.26** to give greater separability on silica, but this was not achieved due to time constraints.

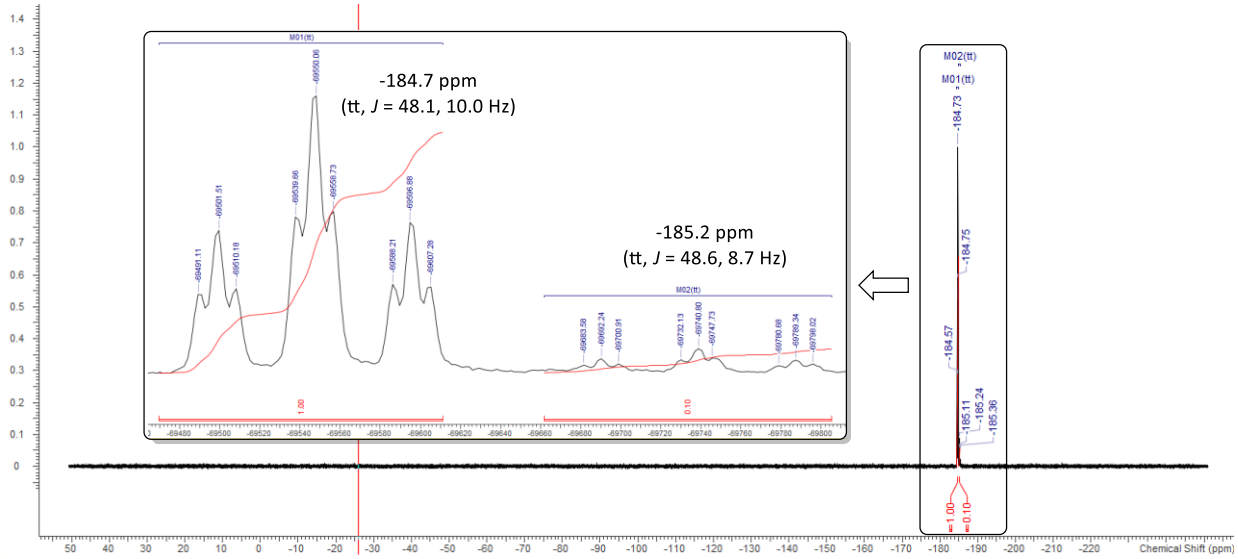
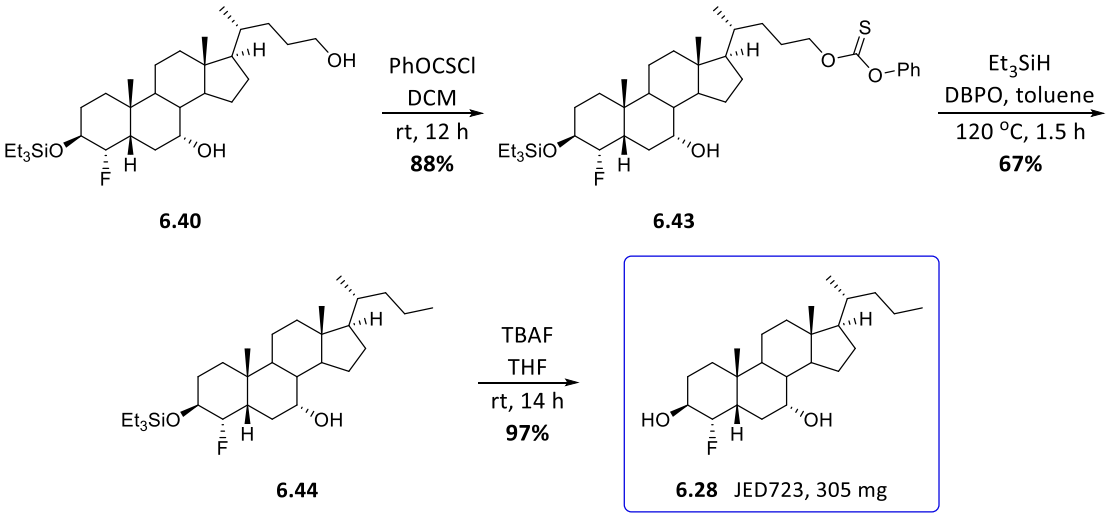


*Figure 6.10. ^{19}F NMR of alkyl target **6.26** containing 10% of an unknown byproduct.*

6.5.3.3 Synthesis of 3β-hydroxyl-4α-fluoro-C$_{17}$-alkyl CDCA (JED723)

Synthesis of analogous 4α-fluorinated regioisomer **6.28** was performed in an identical fashion as described for 2-fluorinated regioisomer **6.26** (*Scheme 6.22*). The conversion towards the corresponding thiocarbonate ester was once again regioselective. Comparable yields were attained over the three reactions to provide target JED723.

[xix] The yield reported in scheme 6.21 of 96% is therefore from attaining greater than 100% of the expected theoretical mass and, after analysis and calculation of the mass composition, recorded as 96%.



*Scheme 6.22. Synthesis of target 3β-hydroxyl-4α-fluoro C_{17}-alkyl CDCA (**6.28**).*

The earlier reported evidence of an IMHB for compound **6.40** was still present in target **6.28**, evidenced by a ^{1}H NMR signal from proton $C_{7\alpha}$O<u>H</u>. This peak was observed at 2.99 ppm for JED723 with coupling constants of 34.9 Hz to $F_{4\alpha}$ and 10.9 Hz to $H_{7\beta}$. This signal was first reported for 3β-hydroxyl-4α-fluoro CDCA methyl ester **1.49** (see: *section 1.3.4.4.1*). Likewise, the ^{19}F NMR of **6.28** and **1.49** both exhibit an unexpected broad singlet that is yet to be fully explained. Alas, by ^{19}F NMR (*Figure 6.11*) we can also observe a minor ~5% impurity that precludes the ability to take accurate pK_{AHY} measurements. Unfortunately, attempts to purify the compound by HPLC only enriched the mixture with greater portions of this byproduct (to 11% by mole). Its identity remains unknown, but it exhibits a discernible doublet structure with a coupling constant of 54 Hz and this does not fit the expected tt for a 4α-fluorine group. Re-purification of this compound was not performed owing to time constraints.

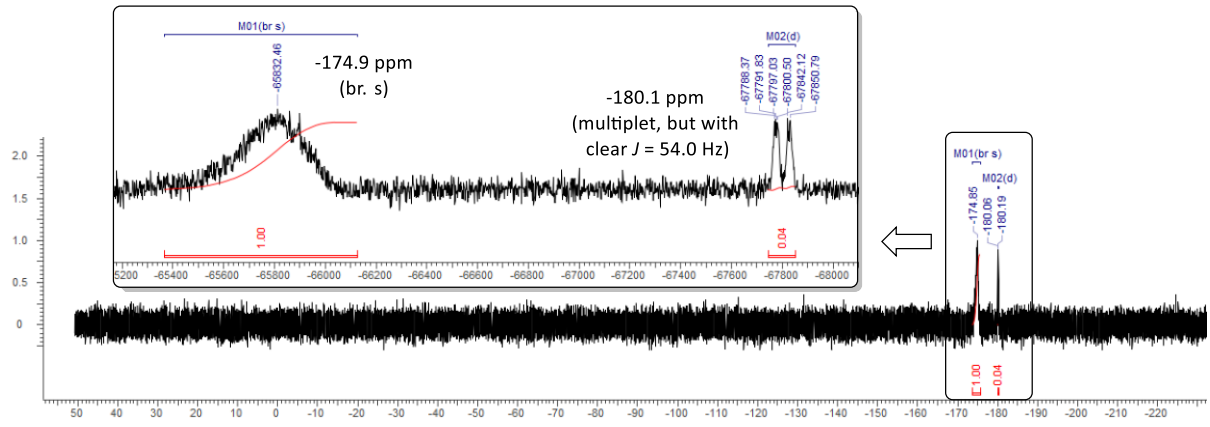


*Figure 6.11. ^{19}F NMR of alkyl target **6.28** containing ~5% of an unknown byproduct.*

6.6 Conclusions

Our aim for this theme of work was to synthesise approximately 1 gram of a 3β-hydroxy-4α-fluorinated CDCA compound with a modified C_{17}-side chain bearing no HBA/HBD groups. This compound would be used to investigate the pK_{AHY} of the 3β-hydroxyl group in the presence of the IMHB involving the 4-fluoro and 7-hydroxyl groups. Additionally, we originally targeted the analogous LCA derivative where the absence of the 7-OH group precludes IMHB formation; this compound would provide a reference pK_{AHY} value of the 3β-hydroxyl group in the absence of the aforementioned IMHB.

Two approaches were considered. The first attempted contemporary decarboxylation reactions on commercially available CDCA, and, would have included LCA, but the apolarity of the products led to cumbersome purification. We then looked to the second route, where A-ring fluorination was first performed, and side-chain modification performed at a later stage. While studying LCA substrates we discovered that any 7α-position substituent blocked epoxide opening at the regiochemically desired 4-position; this was confirmed by a single crystal X-ray structure, and the unreactive LCA epoxide **6.26** was of interest for biological studies. Following from this discovery, an LCA-derived reference compound was no longer feasible. Instead, we looked to synthesise a 2α-fluorinated regioisomer where the distance between fluorine and C_7-OH precluded IMHB formation. Following this second approach, both alkenyl and alkyl derivatives of 3β-hydroxylated 2α-and 4α-fluoro CDCA were prepared. The synthesis of alkenyl compounds **6.25** and **6.27** was more cumbersome and lower yielding than the synthesis of corresponding alkyl compounds **6.26** and **6.28**, however inseparable and unidentified byproducts were found in the final alkyl targets.

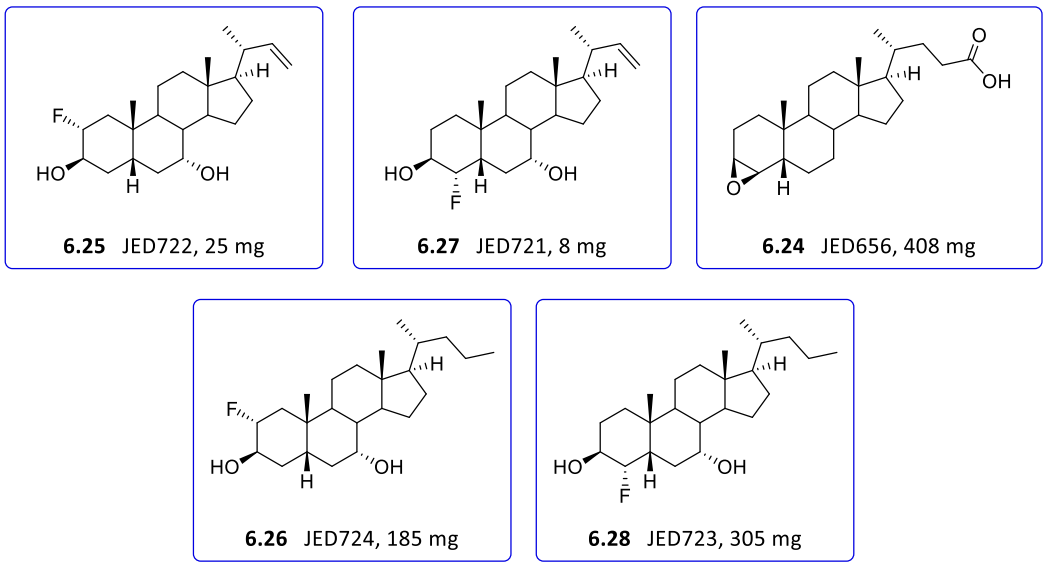
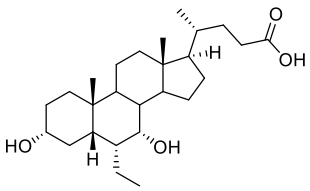


Figure 6.12. Synthesised modified-C_{17}-side chain derivatives of 2α/4α-fluoro-3β-hydroxy CDCA ***6.25***-***6.28*** *and biologically interesting LCA epoxide intermediate* ***6.24****.*

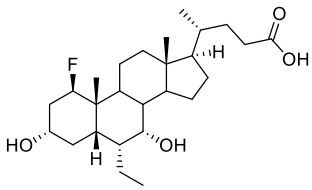
Chapter 7 Biological Testing Results

The fluorinated bile acids described in chapters 2-5 were synthesised as targets to treat NASH, through activation of the FXR, and were sent to our collaborators for testing. A selection of these compounds was screened against an FXR Gal4 DBD hybrid receptor with a Gal4 UAS-Luciferase reporter gene, and the agonist GW4064 (**1.23**) was used as a reference to standardise the performance of the assay.[xx]

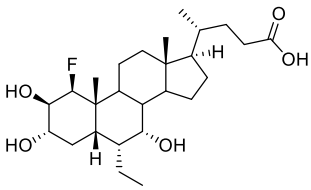
Both 1β-fluoro derivatives **1.52** and **2.39** (*Figure 7.1*) exhibit less potency at the FXR than non-fluorinated OCA (**1.8**). Hence, the increased 3α-OH hydrogen bond donating capacity resulting from 1β-fluorination (see: *Section 1.4.1*) does not increase affinity. This would support that the 3α-OH group functions as a hydrogen bond acceptor (see: *Section 1.2.3.1*) and could also suggest unfavourable interactions of the 1β-fluoro substituent with a residue in the FXR binding site. Additionally, we observe that the presence of the 2β-hydroxyl group gives a significant decrease to potency.



1.8 OCA
EC_{50} (nM): 270



1.52 1β-fluoro OCA
EC_{50} (nM): 1600



2.39 1β-fluoro-2β-hydroxy OCA
EC_{50} (nM): >10,000

*Figure 7.1. Measured EC_{50} values of OCA and 1-fluorinated OCA derivatives **1.52** and **2.39**.*

Both 2β-fluoro OCA (**1.54**) and 4β-fluoro OCA (**1.56**) (*Figure 7.2*) exhibit 20% and 100% improvements in FXR affinity, respectively. This would suggest that deactivation of the 3α-OH group as a hydrogen bond donor (thereby improving HBA capacity) is beneficial for binding to the FXR.

[xx] Conditions of a typical study quoted from a study report: "In brief, *Step* 1: A suspension of reporter cells prepared in Indigo's cell recover medium (CRM: containing 10% charcoal-stripped FBS). *Step 2*: Immediately prior to assay setup, test compound master stocks were diluted in DMSO to generate solutions at '1,000 x-concentration' relative to each final treatment concentration. These immediate stocks were subsequently diluted directly into Indigo's compound screening medium (CSM: containing 10% charcoal-stripped FBS) to generate '2x-concentration' treatment media. 100 µL of each prepared treatment medium was dispensed into triplicate assay wells pre-dispensed with a 100 µL suspension of reporter cells, thereby achieving the desired final treatment concentrations. The concentration of residual DMSO in all assay wells was 0.1%. Assay plates were incubated for 22-24 hr in a cell culture incubator (37 °C, 5% CO_2, 85% humidity). *Step 3*: Following the incubation period, treatment media were discarded and 100 µL/well of each Luciferase detection reagent was added. RLUs were quantified from each assay well to determine agonist activity." Dr Kayla Smith, 9th July 2018.

The EC_{50} value for 4α-fluoro OCA (**1.55**) shows a massive increase relative to parent non-fluorinated OCA (**1.8**). We propose that the fluorine-IMHB (involving the 7-OH group) could reduce the HBA capacity of the 3-OH group. This postulation is modestly supported by the EC_{50} value for 2α-fluoro OCA (**1.53**) which exhibits very poor activity, but activity that is not entirely 'knocked out' by the axial C-F bond. The activity of 4,4-difluoro OCA (**5.1**) is somewhere between the 'active' 4β-derivative and 'inactive' 4α-derivative, and higher than non-fluorinated OCA (**1.8**).

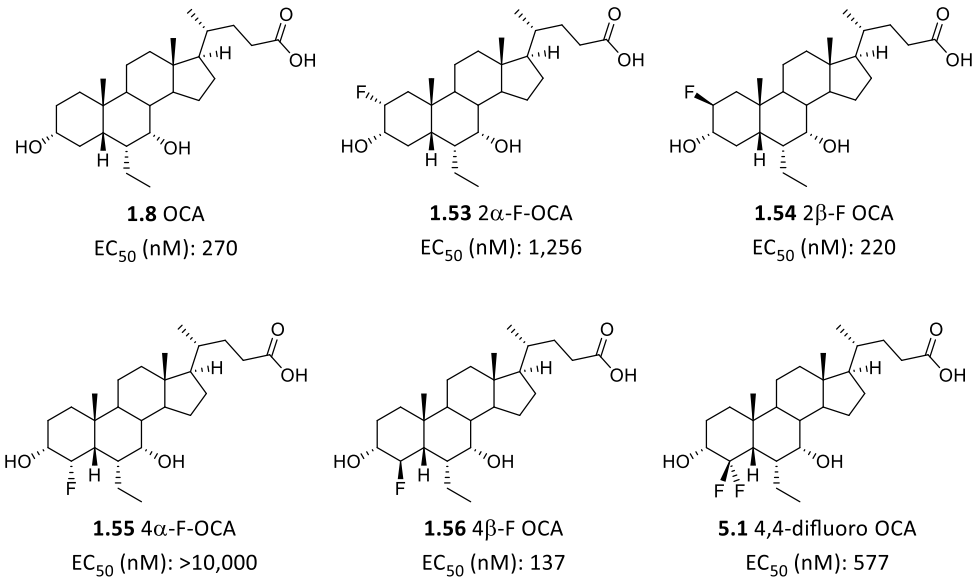


*Figure 7.2. Measured EC_{50} values of OCA and 2- and 4-fluorinated OCA derivatives **1.53**-**1.56**, **5.1**.*

A phenyl-sulfonyl urea derivative **8.1** was prepared by industrial collaborators and gave approximately 100% improvement in potency at the FXR, akin to 4β-fluoro OCA (**1.56**). However, none of the bile acids presented so far have proved as potent as classical non-BA example GW4064 (**1.23**) at 25 nanomoles.

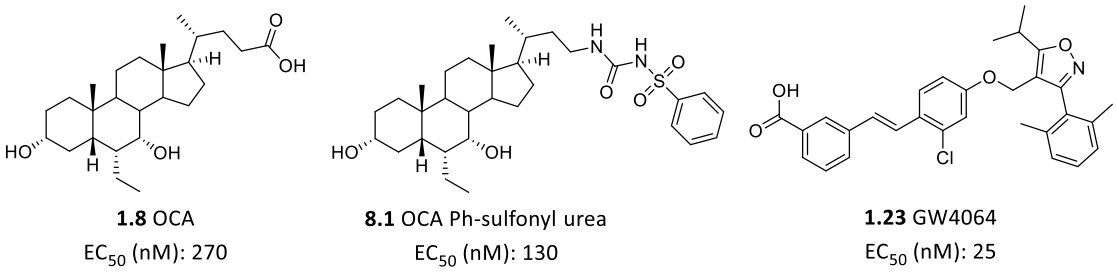
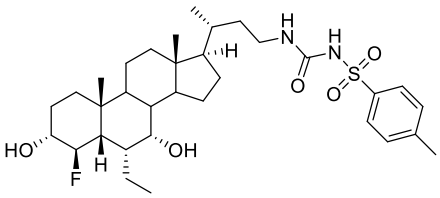


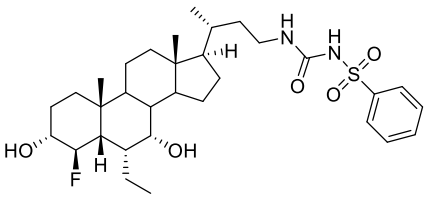
*Figure 7.3. Measured EC_{50} values of OCA, phenyl sulfonyl urea derivative **8.1** and GW6046 **1.23**.*

Hence, aromatic sulfonyl urea derivatisation was carried out on 4β-fluoro **1.56** and 4,4-difluoro **5.1**. A sulfonamide derivative of **5.1** was also made. Pleasingly, improved potency was observed in all cases relative to the parent carboxylic acid compounds, with the 4β-fluoro sulfonyl urea derivatives arriving at the same order of magnitude as that of GW4064 (*Figure 7.4*).



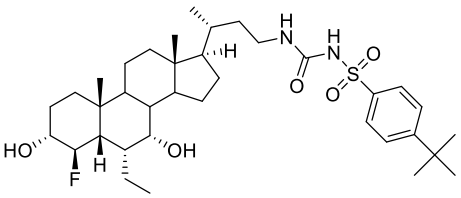
4.13

EC_{50} (nM): 17



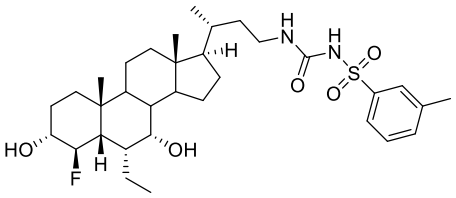
4.13

EC_{50} (nM): 31



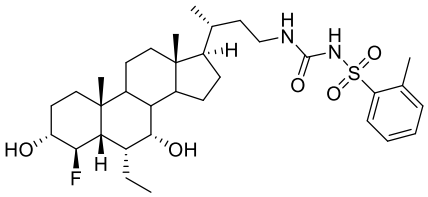
4.15

EC_{50} (nM): 18



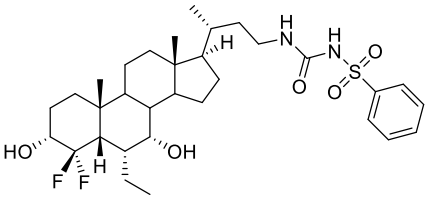
4.16

EC_{50} (nM): 22



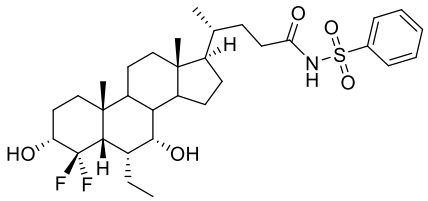
4.17

EC_{50} (nM): 28



5.12

EC_{50} (nM): 308



5.13

EC_{50} (nM): 128

*Figure 7.4. Measured EC_{50} values of 4β-fluorinated OCA phenyl sulfonyl urea derivatives **4.13**-**4.17** and 4,4-difluoro side-chain derivatives **5.12** and **5.13**.*

These EC_{50} values indicate that a 4β-fluorinated OCA aromatic-sulfonyl urea derivative could be a suitable lead-compound for NASH treatment. Further studies of other ADME parameters were important and included microsome stability, Caco2 permeability, efflux ratio, Cyp inhibition, blood plasma ratios as well as oral PK mouse data and off-target safety profiling, but the details of which shall not be discussed.

Chapter 8 Experimental Details

8.1 Preface

All reagents were obtained from commercial suppliers and used without further purification as received, unless otherwise stated. Anhydrous solvents were from dry and sealed bottles under nitrogen/argon, purchased from commercial sources. Water and air sensitive reactions were performed under inert atmosphere (argon) in glassware that had been dried at greater than 400 °C under vacuum.

Reactions were monitored by thin layer chromatography (TLC) using aluminium foil supported silica plates (MERCK 60 F254) and eluted by the described eluent system. Subsequently, they were visualised under UV light and developed with potassium permanganate solution, *p*-anisaldehyde solution, or CAM solution

Chromatography columns were prepared using Merck Geduran Si 60 40-63 µm silica gel. Column chromatography was also performed using a Biotage® Isolera One instrument with the solvents and prepacked Biotage® column stated.

Nuclear magnetic resonance spectra were recorded using a Bruker Ultrashield 400 MHz spectrometer with spectra being assigned using COSY, HSQC, HMBC, Dept Q and Dept 135. All chemical shifts are quoted on the δ scale in ppm using trimethylsilane (TMS) as an internal reference standard.

Mass spectra were recorded using a Waters TDQ mass spectrometer equipped with a triple quadrupole analyser. Samples were introduced to the mass spectrometer via an Acquity H-Class quaternary solvent manager (with TUV detector at 254 nm, sample and column manager). Ultraperformance liquid chromatography was undertaken via a Waters BEH C18 column (50 mm x 2.1mm 1.7µm). Gradient 20% acetonitrile (0.2% formic acid) to 100% acetonitrile (0.2% formic acid) in five minutes at a flow rate of 0.6 mL/min. Low resolution mass spectra were recorded using positive ion electrospray ionisation.

Optical rotations were recorded on an OPTICAL ACTIVITY POLAAR 2001 polarimiter at 589 nm on pure and novel final compounds that were isolated.

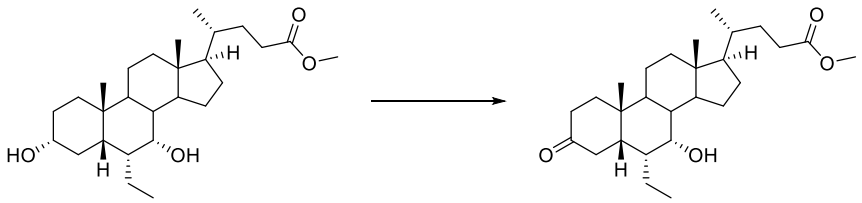
IR spectra were recorded on a Nicolet 380 FT-IR with absorption peaks given in cm^{-1}.

Melting points were recorded on a Gallenkamp melting point apparatus fitted with a microscope and are uncorrected.

8.2 Synthesis of OCA (NZP084) 1-fluoro analogues

The synthesis and characterisation of the following compounds is presented in the order of reactions discussed in Chapter 2.

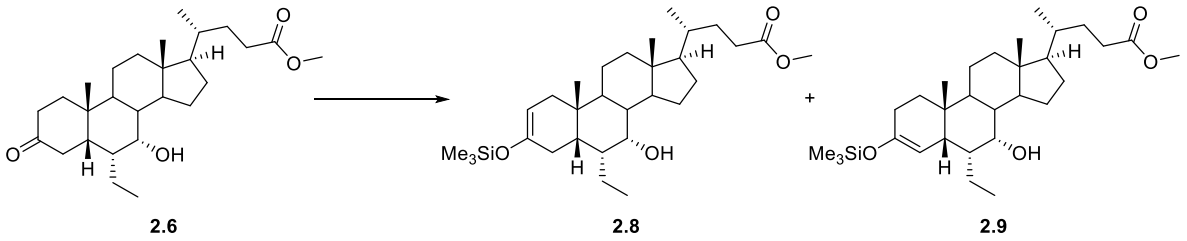
Methyl-3-oxo-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (2.6)



To a solution of **2.5** (9.53 g, 21.9 mmol, 1.0 equiv) in water (22 mL) and *tert*-butanol (88 mL) at rt was added potassium bromide (5.22 g, 43.9 mmol, ~2.0 equiv), potassium bicarbonate (22.0 g, 219 mmol, ~10 equiv) and TEMPO (4.45 g, 28.5 mmol, ~1.3 equiv). The reaction mixture was cooled to 0 °C and received sodium hypochlorite (28 mL, 32.9 mmol, ~1.5 equiv) dropwise at a rate of 4 mL per hour over 7 h. After a further 4 h at 0 °C, the reaction was deemed complete by TLC and the reaction mixture was quenched by the slow addition of 1:1 sat. $Na_2S_2O_3$ solution in water (250 mL). The aqueous phase was separated and extracted with ethyl acetate (3 x 180 mL) and the combined organic layers were washed with brine (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 14.2 g of crude material as an orange oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 120 g cartridge) using PE 40-60/acetone (100/0 to 80/20) as the eluent yielded compound **2.6** as a white solid (8.48 g, 19.6 mmol, 89%).

m.p. 66-67 °C (evaporated from PE 40-60/acetone). **R_f** (PE 40-60/acetone), 80/20) 0.28. **$[\alpha]_D$:** +12.5 (c = 0.53, methanol, 21 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.78 (1H, d, *J* = 2.2 Hz, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.07 (1H, dd, *J* = 15.2, 13.5 Hz), 2.46-2.33 (2H, m), 2.29-1.91 (7H, m), 1.84-1.77 (1H, m), 1.71 (1H, dt, *J* = 13.2, 4.4 Hz), 1.67-1.15 (16H, m), 1.00 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.5 Hz, H_{21}), 0.91(3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.70 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.4 (C_3), 174.7 (C_{24}), 70.9 (C_7), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.4 (C_{14}), 46.9 (C_5), 42.8 (C_{13}), 41.3 (CH), 40.6 (CH_2), 40.1 (CH), 39.5 (CH_2), 36.92 (CH_2), 36.86 (CH_2), 35.8 (C_{10}), 35.3 (CH), 33.6 (CH), 30.98 (CH_2), 30.96 (CH_2), 28.1 (CH_2), 23.7 (CH_2), 22.3 (C_{19}), 22.0 (CH_2), 21.1, (CH_2), 18.3 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.5 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 450.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{44}O_4Na$ $[M+Na]^+$, m/z calculated: 455.3132; found: 455.3137. **IR** (neat) 3525 (m), 2935 (s), 2869 (s), 1737 (s), 1707 (s), 1436 (m), 1377 (m), 1165 (m) cm^{-1}.

Methyl-3-trimethylsilyl-6α-ethyl-7α-hydroxyl-5β-chol-2-en-24-oate (2.8) and methyl-3-trimethylsilyl-6α-ethyl-7α-hydroxyl-5β-chol-3-en-24-oate (2.9)



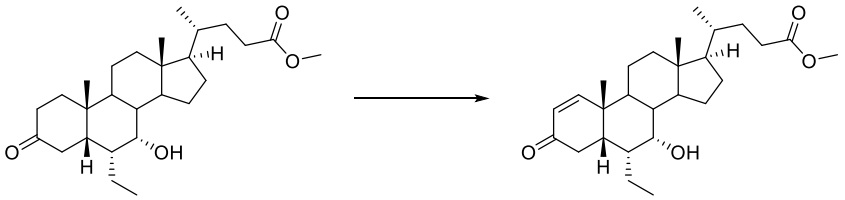
To a solution of **2.6** (2.00 g, 4.62 mmol, 1.0 equiv) in dry dichloromethane (40 mL) under argon at 0 °C was added triehthylamine (1.29 mL, 9.24 mmol, ~2.0 equiv) and trimethylsilyl triflate (0.92 mL, 5.08 mmol, ~1.1 equiv). After 7 h at 0 °C, the reaction was deemed complete by TLC and the reaction mixture was diluted with dichloromethane (200 mL) and quenched by the addition of sat. $NaHCO_3$ solution (200 mL). The aqueous phase was separated and extracted with dichloromethane (3 x 100 mL) and the combined organic layers were washed with brine (300 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 2.74 g of crude material as a pale-yellow oil that was used without further purification. From this procedure, a ratio of 1 : ~1.15 of **2.8** : **2.9** was observed by ^{1}H NMR.

*Preparation of compounds **2.8/2.9** was also successful by the following method:* To a solution of diisopropylamine (0.78 mL, 5.54 mmol, ~12 equiv) in dry THF (6.9 mL) under argon at -78 °C was added *n*-BuLi (1.44 mL, 2.31 mmol, ~5.0 equiv) dropwise. After stirring for 15 min, to the reaction mixture was then added trimethylsily chloride (0.29 mL, 2.31 mmol, ~5.0 equiv) and was stirred for a further 20 min. To the reaction mixture was then added a solution of **2.6** (200 mg, 0.46 mmol, 1.0 equiv) in dry THF (3 mL) dropwise and triethylamine (1.16 mL, 8.32 mmol, ~18 equiv). After one hour at -78 °C, the reaction mixture was warmed to -20 °C and quenched with sat. $NaHCO_3$ solution (5 mL) and warmed to rt over 2 h. The aqueous phase was separated and extracted with ethyl acetate (3 x 10 mL) and the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 271 mg of crude material as a pale-yellow gummy paste that was used without further purification. From this procedure, a ratio of 1 : ~6.8 of **2.8** : **2.9** was observed by ^{1}H NMR.

The data presented for compounds **2.8** and **2.9** was attained from an impure crude mixture. Characteristic signals are reported to help identify these intermediates in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.87 (0.55H, s, H_4, **2.9**), 4.68 (0.45H, d, *J* = 5.3 Hz, H_2, **2.8**), 3.75-3.74 (1H, m), 3.68 (1.35H, s, $C_{24}OC\mathbf{H}_3$, **2.8**), 3.67 (1.65H, s, $C_{24}OC\mathbf{H}_3$, **2.9**), 3.63 (1H, t, *J* = 1.6 Hz), 3.15-3.06 (0.55H, m), 2.95-2.87 (0.45H, m), 2.37 (1H, ddd, *J* = 15.3, 10.2, 4.9 Hz, $H_{23'}$), 0.69 (1.35H, s, H_{18}, **2.8**), 0.65 (1.65H, s, H_{18}, **2.9**), 0.18 (9H, s, $OSi(C\mathbf{H}_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.72 (C_{24}, **2.9**), 172.66 (C_{24}, **2.8**), 150.6 (C_3, **2.8**), 145.9 (C_3, **2.9**), 108.1 (C_4, **2.9**), 100.4 (C_2, **2.8**), 74.1 (C_7, **2.8**), 73.0, C_7, **2.9**),

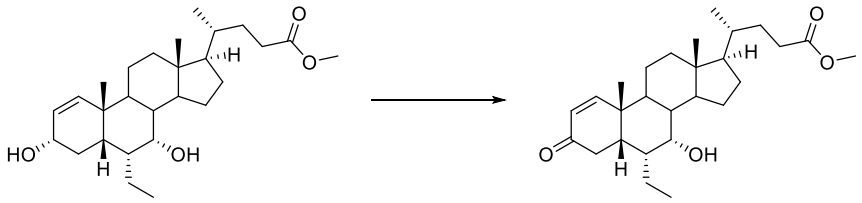
55.9 (C_{17}, **2.9**), 55.7 (C_{17}, **2.8**), 51.48 ($C_{24}O\mathbf{C}H_3$, **2.8**), 51.45 ($C_{24}O\mathbf{C}H_3$, **2.9**), 18.32 (C_{21}, **2.8**), 18.30 (C_{21}, **2.9**) ppm. **LRMS** (ESI^+) m/z: 522.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{30}H_{52}O_4SiNa$ $[M+Na]^+$, m/z calculated: 527.3527; found: 527.3531.

Methyl-3-oxo-6α-ethyl-7α-hydroxyl-5β-chol-1-ene-24-oate (2.4)



2.6 **2.4**

Prepared according to the conditions given by Salakhutdinov *et al*.[219] To a solution of **2.6** (500 mg, 1.16 mmol, 1.0 equiv) in ethyl acetate (20 mL) at rt was added phenylselenium chloride (232 mg, 1.21 mmol, ~1.05 equiv), notably turning the solution a dark red colour. After 3 h at rt, the solution had become a pale yellow indicating the consumption of the harmful selenium reagent and the reaction mixture received ethyl acetate (20 mL) and sat. $NaHCO_3$ solution (30 mL). The organic phase was separated and received 30% hydrogen peroxide solution (1.3 mL, 12.1 mmol, ~10.5 equiv). After 19 h stirring at rt, the reaction mixture was separated against brine (500 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 580 mg of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 10 g cartridge) using PE 40-60/acetone (99/1 to 90/10) as the eluent yielded compound **2.4** as a pale-yellow residue (340 mg, 0.79 mmol, 68%).

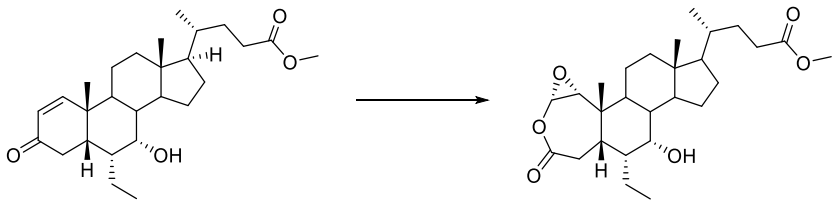


2.15 **2.4**

*Preparation of compound **2.4** was also successful by the following method:* To a solution of **2.15** (72 mg, 0.17 mmol, 1.0 equiv) in THF (1.5 mL) at rt was added water (0.5 mL) and *N*-bromosuccinimide (30 mg, 0.17 mmol, ~1.0 equiv). After 24 h at rt the reaction was deemed complete by TLC and the reaction mixture was quenched with sat. $NaHCO_3$ solution (4 mL) and diluted with ethyl acetate (10 mL). The aqueous phase was separated and extracted with ethyl acetate (2 x 10 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 74 mg of crude material as white residue. Purification by HPLC using hexane/acetone (90/10) as the eluent yielded compound **2.4** as a colourless oil (70.8 mg, 0.16 mmol, 95%).

R_f (PE 40-60/acetone), 80/20) 0.52. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.82 (1H, d, *J* = 10.2 Hz, H_1), 5.90 (1H, d, *J* = 10.4 Hz, H_2), 3.77 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.05 (1H, dd, *J* = 17.4, 14.6 Hz), 2.41-2.32 (2H, m), 2.27-2.19 (1H, m), 2.05-1.76 (5H, m), 1.67-1.21 (14H, m), 1.18 (3H, s, H_{19}), 1.16-1.14 (1H, m), 0.931 (3H, d, *J* = 6.2 Hz, H_{21}), 0.926 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.71 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 201.1 (C_3), 174.7 (C_{24}), 161.1 (C_1), 126.9 (C_2), 70.5 (C_7), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.8 (C_{14}), 43.8 (CH), 42.6 (C_{13}), 41.0 (CH), 40.2 (CH), 39.5 (C_{10}), 39.3 (CH_2), 38.2 (CH), 37.2 (CH_2), 35.3 (CH), 31.0 (CH_2), 30.9, CH_2), 27.9 (CH_2), 23.8 (CH_2), 22.1 (CH_2), 21.9 (CH_2), 20.7 (C_{19}), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 431.2 $[M+H]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{42}O_4Na$ $[M+Na]^+$, m/z calculated: 453.2975; found: 453.2947. **IR** (neat) 2934 (s), 2870 (s), 1736 (s), 1670 (s), 1458 (m), 1436 (m), 1376 (m), 1167 (m) cm^{-1}.

Methyl-1α,2α-epoxy-3-oxo-6α-ethyl-7α-hydroxyl-*A*-homo-2-oxa-5β-cholan-24-oate (2.14)



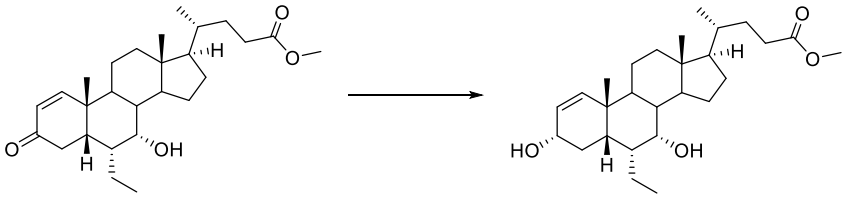
2.4 **2.14**

Prepared according to the conditions given by Carreria *et* al.[170] To a solution of **2.4** (100 mg, 0.23 mmol, 1.0 equiv) in dichloromethane (3 mL) at rt was added *meta*-perchlorobenzoic acid (160 mg, 0.92 mmol, ~4.0 equiv). After 30 h at rt, the reaction was deemed complete by TLC and the reaction mixture was quenched with H_2O (10 mL). The aqueous phase was separated and extracted with dichloromethane (3 x 20 mL) and the combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 192 mg of crude material as a white solid. Purification by HPLC using hexane/acetone (80/20) as the eluent yielded compound **2.14** as a white solid (51 mg, 0.11 mmol, 49%).

R_f (PE 40-60/acetone, 80/20) 0.16. **$[\alpha]_D$:** +14.1 (c = 0.18, chloroform, 25 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.12 (1H, d, *J* = 2.7 Hz, H_2), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.63 (1H, s, $H_{7\beta}$), 2.98 (1H, dd, *J* = 16.0, 10.5 Hz, $H_{4\alpha}$), 2.91 (1H, d, *J* = 2.6 Hz, H_1), 2.63 (1H, d, *J* = 16.0 Hz, $H_{4\beta}$), 2.36 (1H, ddd, *J* = 15.3, 10.0, 5.3 Hz, $H_{23'}$), 2.23 (1H, ddd, *J* = 15.9, 9.9, 6.7 Hz, $H_{23''}$), 2.01 (2H, dt, *J* = 12.25, 3.2 Hz), 1.92 (1H, ddd, *J* = 13.0, 9.4, 3.2 Hz, $H_{23''}$), 1.84-1.78 (1H, m, $H_{22'}$), 1.75 (1H, dd, *J* = 10.5, 4.0 Hz, H_5), 1.74-1.70 (1H, m), 1.64-1.25 (12H, m), 1.21 (3H, s, H_{19}), 1.20-1.08 (2H, m), 0.93 (3H, d, *J* = 6.5 Hz, H_{21}), 0.88 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.70 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 171.3 (C_3), 81.9 (C_2), 69.7 (C_7), 64.1 (C_1), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.0 (CH), 42.5 (C_{13}), 41.7 (C_6), 39.9 (CH), 39.7 (CH), 39.2 (CH_2), 38.2 (C_{10}), 35.4 (CH), 35.3 (C_5), 33.1 (C_4), 31.0 (C_{23}), 30.9 (C_{22}), 28.0 (CH_2), 23.5 (CH_2), 22.4 ($C_6\mathbf{C}H_2CH_3$), 21.4 (CH_2), 19.8 (C_{19}), 18.3 (C_{21}), 11.8 (C_{18}), 11.5 ($C_6CH_2\mathbf{C}H_3$) ppm. **LRMS** (ESI^+)

m/z: 480.3 $[M+NH_4]^+$ 100%; 463.3 $[M+H]^+$ 25% . **HRMS** (ESI^+) $C_{27}H_{43}O_6$ $[M+H]^+$, m/z calculated: 463.3054; found: 463.3053. **IR** (neat) 3527 (m), 2942 (m), 2875 (m), 1736 (s), 1728 (s), 1468 (m), 1437 (m), 1304 (m), 1161 (m), 1151 (m) cm^{-1}.

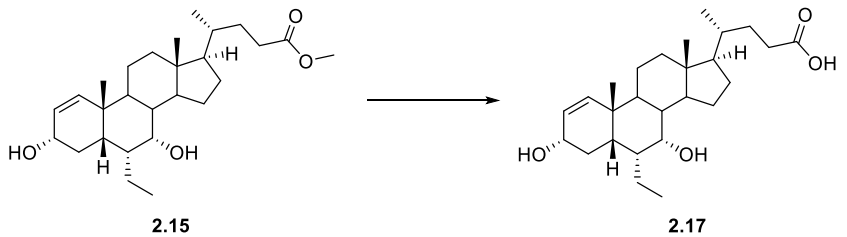
Methyl-3α,7α-dihydoxyl-6α-ethyl-5β-chol-1-ene-24-oate (2.15)



To a solution of **2.4** (1.90 g, 4.41 mmol, 1.0 equiv) in dry methanol (130 mL) under argon at -78 °C was added sodium borohydride (500 mg, 13.2 mmol, ~3.0 equiv) and cerium trichloride (3.29 g, 8.82 mmol, ~2.0 equiv). After 1 hour at -78 °C, the reaction mixture was warmed to rt and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate (300 mL), washed with brine (2 x 300 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 2.6 g of crude material as an off-white oil. Purification by flash column chromatography (Biotage SNAP KP-Sil 30 g cartridge) using PE 40-60/acetone (95/5 to 80/20) as the eluent yielded compound **2.15** as a colourless gummy oil (1.65 g, 3.81 mmol, 86%).

R_f (PE 40-60/acetone, 70/30) 0.33. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.66 (1H, dd, *J* = 10.3, 1.6 Hz, H_1), 5.58 (1H, dt, *J* = 10.0, 1.6 Hz, H_2), 4.11 (1H, ddd, *J* = 7.8, 5.8, 2.0 Hz, $H_{3\beta}$), 3.68 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.36 (1H, ddd, *J* = 15.4, 10.2, 5.3 Hz), 2.26-2.18 (1H, m), 2.03 (1H, dd, *J* = 12.6, 5.8 Hz), 1.95 (1H, dt, *J* = 13.0, 3.4 Hz), 1.92-1.86 (1H, m), 1.82-1.76 (1H, m), 1.73-1.26 (16H, m), 1.19-1.11 (3H, m), 1.01 (3H, s, H_{19}), 0.93 (3H, d, *J* = 6.1 Hz, H_{21}), 0.92 (3H, t, *J* = 7.0 Hz, $C_6CH_2C\mathbf{H}_3$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 139.6 (C_1), 129.3 (C_2), 70.7 (CH), 69.6 (CH), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.2 (C_{14}), 43.0 (CH), 42.6 (C_{13}), 41.1 (CH), 40.5 (CH), 39.53 (CH_2), 39.46 (CH), 38.3 (C_{10}), 35.3 (CH), 32.0 (CH_2), 30.99 (CH_2), 30.96 (CH_2), 28.0 (CH_2), 23.6 (CH_2), 22.4 (CH_2), 21.6 (CH_2), 21.3 (C_{19}), 18.3 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 450.2 $[M+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{27}H_{44}O_4Na$ $[M+Na]^+$, m/z calculated: 455.3132; found: 455.3135. **IR** (neat) 2934 (m) 2869 (s), 1738 (s), 1456 (m), 1374 (s), 1329 (m), 1167 (m) cm^{-1}.

3α,7α-dihydoxyl-6α-ethyl-5β-chol-1-enoic acid (2.17)

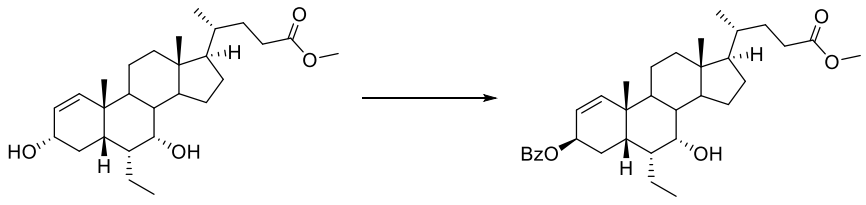


To a solution of **2.15** (50 mg, 0.12 mmol, 1.0 equiv) in methanol (5 mL) at rt was added sodium hydroxide (150 mg, 3% solution). After 24 h at rt, the reaction was deemed complete by TLC and the reaction mixture was acidified to pH ~4 with 2M hydrochloric acid and concentrated *in vacuo*. The residue was dissolved in ethyl acetate (30 mL) and H_2O (50 mL) and the aqueous phase was separated and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with 1M hydrochloric acid (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 47 mg of crude material as a colourless oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 5 g cartridge) using dichloromethane/methanol (95/5 to 85/15) as the eluent yielded compound **2.17** as a colourless oil (43 mg, 0.10 mmol, 89%).

The basic saponification of ester groups using sodium hydroxide will herein be referred to as *General Procedure A.*

R_f (dichloromethane/methanol, 80/20) 0.33. **$[\alpha]_D$:** +15.0 (c = 0.39, methanol, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.65 (1H, dd, *J* = 10.2, 1.1 Hz, H_1), 5.57 (1H, dt, *J* = 10.2, 1.6 Hz, H_2), 4.10 (1H, ddd, *J* = 7.8, 5.8, 2.0 Hz, $H_{3\beta}$), 3.68 (1H, s, $H_{7\beta}$), 2.38 (1H, ddd, *J* = 15.7, 10.4, 5.4 Hz), 2.27-2.21 (1H, m), 2.01 (1H, dd, *J* = 12.2, 5.6 Hz), 1.96-1.30 (20H, m), 1.18-1.08 (3H, m), 1.00 (3H, s, H_{19}), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.91 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.1 (C_{24}), 139.6 (C_1), 129.3 (C_2), 70.7 (CH), 69.6 (CH), 55.8 (C_{17}), 50.1 (C_{14}), 43.0 (CH), 42.6 (C_{13}), 41.1 (CH), 40.5 (CH), 39.5 (CH_2), 39.4 (CH), 38.3 (C_{10}), 35.4 (CH), 31.7 (CH_2), 31.1 (CH_2), 30.8 (CH_2), 28.0 (CH_2), 23.6 (CH_2), 22.4 (CH_2), 21.6 (CH_2), 21.3 (C_{19}), 18.3 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 436.3 $[M+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{26}H_{42}O_4Na$ $[M+Na]^+$, m/z calculated: 441.2975; found: 441.2976. **IR** (neat) 2932 (s), 2868 (s), 2159 (m), 1707 (s), 1456 (m), 1374 (s), 1143 (m), 1053 (m) cm^{-1}.

Methyl-3β-benzoyl-6α-ethyl-7α-hydroxyl-5β-chol-1-en-24-oate (2.18)



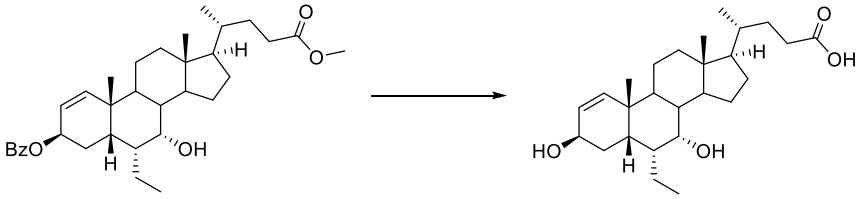
2.15 **2.18**

To a solution of **2.15** (550 mg, 1.27 mmol, 1.0 equiv) in dry THF (7 mL) under argon at rt was added triphenylphosphine (500 mg, 1.91 mmol, ~1.5 equiv), benzoic acid (233 mg, 1.91 mmol, ~1.5 equiv) and diisopropylazodicarboxylate (374 µL, 1.91 mmol, ~1.5 equiv) dropwise over 5 min. After 16 h at rt, the reaction was deemed complete by TLC (note. Vanillin dip was required in order to distinguish the product) and the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography (Biotage SNAP KP-Sil 30 g cartridge) using PE 40-60/acetone (95/5 to

90/10) as the eluent yielded compound **2.18** as a white solid (497 mg, ~0.61 mmol, ~48%; considering 66 mol% purity by ^{1}H NMR, owing to benzoic acid residue).

R_f (PE 40-60/acetone, 70/30) 0.37. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.03 (2H, dd, *J* = 8.4, 1.3 Hz, C_oArH), 7.54 (1H, tt, *J* = 7.5, 1.3 Hz, C_pArH), 7.43 (2H, t, *J* = 7.8 Hz, C_mArH), 5.97 (1H, d, *J* = 10.0 Hz, H_1), 5.86 (1H, ddd, *J* = 9.9, 4.7, 0.7 Hz, H_2), 5.40 (1H, t, *J* = 3.4 Hz, $H_{3\alpha}$), 3.72 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.36 (1H, ddd, *J* = 15.4, 9.8, 5.1 Hz), 2.27-2.21 (1H, m), 2.18 (1H, d, *J* = 2.6 Hz), 2.06-2.01 (1H, m), 1.99-1.77 (5H, m), 1.69-1.13 (15H, m), 1.10 (3H, s, H_{19}), 0.941 (3H, t, *J* = 7.2 Hz, $C_6CH_2C\mathbf{H}_3$), 0.937 (3H, d, *J* = 6.4 Hz, H_{21}), 0.70 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 166.2 ($C_3O\mathbf{C}$Bz), 144.6 (C_1), 132.6 (C_pH_{Ar}), 131.0 ($C_{i\text{-}Ar}$), 129.5 (CH_{Ar}), 128.3 (CH_{Ar}), 122.0 (C_2), 71.0 (C_7), 68.0 (C_3), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.1 (C_{14}), 42.7 (C_{13}), 40.8 (CH), 40.2 (CH), 39.5 (CH_2), 38.8 (CH), 38.3 (C_{10}), 38.1 (CH), 35.4 (CH), 31.01 (CH_2), 30.97 (CH_2), 28.0 (CH_2), 27.2 (CH_2), 23.7 (CH_2), 22.3 (CH_2), 21.9 (CH_2), 21.2 (C_{19}), 18.3 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.5 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 432.3 $[M\text{-}BzOH+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{34}H_{48}O_5Na$ $[M+Na]^+$, m/z calculated: 559.3394; found: 539.3389. **IR** (neat) 3546 (m), 2956 (s), 2870 (s), 1730 (s), 1712 (s), 1450 (m), 1375 (s), 1270 (m), 1173 (m) cm^{-1}.

3β,7α-dihydroxyl-6α-ethyl-5β-chol-1-enoic acid (2.19)



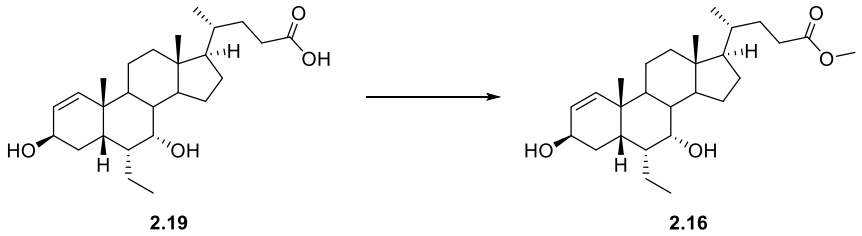
2.18 **2.19**

Prepared according to *General Procedure A* (see: *page 147*) using 530 mg of compound **2.18** (0.98 mmol, 1.0 equiv) and 25 mL of a 10% sodium hydroxide in methanol solution. Purification of the resultant crude material by flash column chromatography (Biotage ZIP KP-Sil 10 g cartridge) using PE 40-60/acetone (80/20 to 70/30) as the eluent yielded compound **2.19** as a colourless residue (173 mg, 0.41 mmol, 41%).

R_f (dichloromethane/methanol, 90/10) 0.48. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.81 (1H, d, *J* = 10.0 Hz, H_1), 5.75 (1H, dd, *J* = 10.0, 4.0 Hz, H_2), 4.12 (1H, dd, *J* = 4.0, 1.3 Hz, $H_{3\alpha}$), 3.72 (1H, s, $H_{7\beta}$), 2.40 (1H, ddd, *J* = 15.5, 10.2, 5.3 Hz), 2.30-2.24 (1H, m), 2.04-1.78 (5H, m), 1.69-1.10 (17H, m), 1.05 (3H, s, H_{19}), 0.95 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.94 (3H, d, *J* = 6.4 Hz, H_{21}), 0.69 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 178.8 (C_{24}), 142.5 (C_1), 125.7 (C_2), 70.9 (C_7), 64.2 (C_3), 55.8 (C_{17}), 50.1 (C_{14}), 42.7 (C_{13}), 40.9 (CH), 40.2 (CH), 39.5 (CH_2), 38.4 (C_{10}), 38.2 (CH), 38.1 (CH), 35.3 (CH), 30.74 (CH_2), 30.73 (CH_2), 30.1 (CH_2), 28.0 (CH_2), 23.7 (CH_2), 22.2 (CH_2), 21.9 (CH_2), 21.2 (C_{19}), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 432.3 $[M\text{-}H_2O\text{-}OH]^+$ 100%. **HRMS** (ESI^+) $C_{26}H_{42}O_4Na$ $[M+Na]^+$, m/z

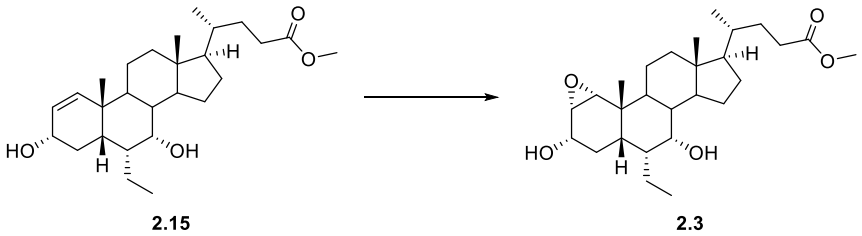
calculated: 441.2975; found: 441.2978. **IR** (neat) 3445 (m), 2934 (s), 2870 (s), 1707 (s), 1457 (m), 1377 (s), 1270 (m) cm^{-1}.

Methyl-3β,7α-dihydroxyl-6α-ethyl-5β-chol-1-en-24-oate (2.16)



To a solution of **2.19** (110 mg, 0.26 mmol, 1.0 equiv) in dimethylformamide (2 mL) at rt was added caesium carbonate (116 mg,0.34 mmol, ~1.3 equiv). After 5 min at rt, the reaction mixture received methyl iodide (102 µL, 1.58 mmol, ~6.0 equiv). After 10 h at rt, the reaction mixture was quenched by the addition of H_2O (10 mL) and the aqueous phase was separated and extracted with ethyl acetate (3 x 75 mL). The combined organic layers were washed with brine (300 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 69 mg of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage KP-Sil 10 g cartridge) using PE 40-60/acetone (90/10 to 80/20) as the eluent yielded compound **2.16** as a colourless oil (39 mg, 0.09 mmol, 34%). $\mathbf{R_f}$ (PE 40-60/acetone, 70/30) 0.25. ^{1}**H NMR** (400 MHz, $CDCl_3$): δ 5.79 (1H, d, *J* = 10.0 Hz, H_{1}), 5.73 (1H, ddd, *J* = 10.0, 4.7, 1.1 Hz, H_{2}), 4.10 (1H, dd, *J* = 4.0, 1.3 Hz, $H_{3\alpha}$), 3.69 (1H, s, $H_{7\beta}$), 3.65 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.34 (1H, ddd, *J* = 15.5, 10.3, 5.4 Hz), 2.25-2.18 (1H, m), 2.04-1.73 (5H, m), 1.68-1.60 (3H, m), 1.55-1.28 (11H, m), 1.17-1.08 (3H, m), 1.03 (3H, s, H_{19}), 0.93 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.91 (3H, d, *J* = 6.4 Hz, H_{21}) , 0.67 (3H, s, H_{18}) ppm. ^{13}**C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 142.4 (C_{1}), 125.7 (C_{2}), 70.8 (C_{7}), 64.1 (C_{3}), 55.8 (C_{17}), 51.4 ($C_{24}O\mathbf{C}H_3$), 50.1 (C_{14}), 42.6 (C_{13}), 40.9 (CH), 40.1 (CH), 39.5 (CH_2), 38.3 (C_{10}), 38.10 (CH), 38.06 (CH), 35.3 (CH), 30.92 (CH_2), 30.86 (CH_2), 30.1 (CH_2), 28.0 (CH_2), 23.7 (CH_2), 22.2 (CH_2), 21.9 (CH_2), 21.2 (C_{19}), 18.2 (C_{21}), 1.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 397.2 $[M\text{-}H_2O\text{-}OH]^+$ 100%. **HRMS** (ESI^+) $C_{27}H_{44}O_4Na$ $[M+Na]^+$, m/z calculated: 455.3142; found: 455.3132. **IR** (neat) 3431 (m), 2934 (s), 2870 (s), 1734 (s), 1457 (m), 1436 (s), 1374 (m), 1265 (m) cm^{-1}.

Methyl-1α,2α-epoxy-3α,7α-dihydroxyl-6α-ethyl-5β-cholan-24-oate (2.3)

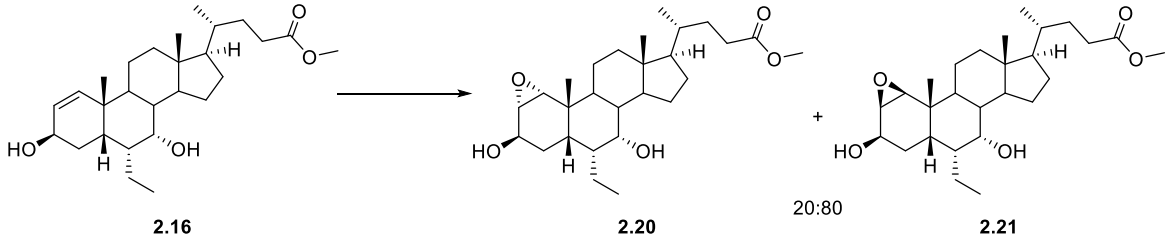


To a solution of **2.15** (200 mg, 0.46 mmol, 1.0 equiv) in DCM (5 mL) at rt was added meta-perchlorobenzoic acid (230 mg, 0.93 mmol, ~2.0 equiv). After 18 h at rt, the reaction was deemed complete by TLC and the reaction mixture was quenched with sat. $Na_2S_2O_3$ solution (20 mL) and stirred at rt for 30 min. The aqueous phase was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated in vacuo to afford 234 mg of crude material as a colourless oil. Purification by HPLC using hexane/acetone (75/25) as the eluent yielded compound **2.3** as a white solid (144 mg, 0.32 mmol, 69%).

The epoxidation of alkenes with *m*CPBA in dichloromethane will herein be referred to as *General Procedure B.*

R_f (PE 40-60/acetone, 65/35) 0.38. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.77 (1H, dd, *J* = 10.4, 5.6 Hz, $H_{3\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.62 (1H, s, $H_{7\beta}$), 3.37 (1H, d, *J* = 3.6 Hz, H_2), 3.03 (1H, d, *J* = 3.9 Hz, H_1), 2.36 (1H, ddd, *J* = 15.5, 10.3, 5.4 Hz), 2.27-2.19 (1H, m), 2.00-1.76 (5H, m), 1.71-1.12 (18H, m), 1.08 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.5 Hz, H_{21}), 0.87 (3H, t, *J* = 7.1 Hz, $C_6CH_2C\mathbf{H}_3$), 0.69 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 71.0 (C_3), 70.1 (C_7), 64.0 (C_1), 58.2 (C_2), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.1 (CH), 43.7 (CH), 42.8 (C_{13}), 41.5 (CH), 39.9 (CH), 39.4 (CH_2), 35.3 (CH), 35.0 (C_{10}), 34.5 (CH), 30.96 (CH_2), 30.94 (CH_2), 28.0 (CH_2), 27.3 (CH_2), 23.5 (CH_2), 22.51 (CH_2), 22.48 (CH_2), 20.2 (C_{19}), 18.3 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 466.3 $[M+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{27}H_{48}O_5N$ $[M+NH_4]^+$, m/z calculated: 466.3527; found: 466.3534. **IR** (neat) 3384 (m), 2849 (s), 1730 (s), 1456 (m), 1377 (s), 1264 (m), 1178 (m) cm^{-1}.

Methyl-1α,2α-epoxy-3β,7α-dihydroxyl-6α-ethyl-5β-cholan-24-oate (2.20) and methyl 1β,2β-epoxy-3β,7α-dihydroxyl-6α-ethyl-5β-cholan-24-oate (2.21)

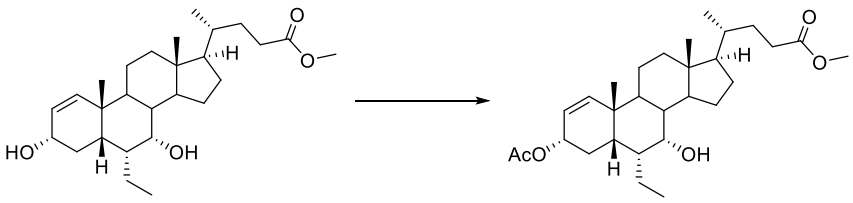


Prepared according to *General Procedure B* (see: *page 150*) using 35 mg of compound **2.16** (0.08 mmol, 1.0 equiv). Purification of the resultant crude material by HPLC using hexane/acetone (75/25) as the eluent yielded an inseparable mixture of compounds **2.20** and **2.21** in a 20:80 ratio as a colourless residue (31 mg, 0.07 mmol, 84%).

R_f (PE 40-60/acetone, 70/30) 0.18. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.30 (0.2H, d, *J* = 2.1 Hz, $H_{3\alpha}$, **2.20**), 4.09 (0.8H, t, *J* = 4.7 Hz, $H_{3\alpha}$, **2.21**), 3.71 (0.8H, d, *J* = 7.6 Hz, $H_{7\beta}$, **2.21**), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.62

(0.2H, s, $H_{7\beta}$, **2.20**), 3.43 (0.8, t, *J* = 4.2 Hz, $H_{2\alpha}$, **2.21**), 3.30 (0.8, d, *J* = 3.7 Hz, $H_{1\alpha}$, **2.21**), 3.19 (0.2, s, $H_{2\beta}$, **2.20**), 2.93 (0.2, d, *J* = 3.4 Hz, $H_{1\beta}$, **2.20**), 2.35 (1H, ddd, *J* = 15.5, 10.2, 5.3 Hz), 2.26-2.18 (1H, m), 2.02-1.74 (4H, m), 1.65-1.58 (3H, m), 1.52-1.19 (16H, m), 1.17 (3H, s, H_{19}), 0.93 (3H, d, *J* = 6.4 Hz, H_{21}), 0.89 (3H, t, *J* = 7.2 Hz, $C_6CH_2C\mathbf{H}_3$), 0.69 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.72 (C_{24}, **2.20**), 174.67 (C_{24}, **2.21**), 70.5 (C_7, **2.21**), 70.3 (C_7, **2.20**), 66.0 (C_3, **2.20**), 63.5 (C_3, **2.21**), 63.1 (C_1, **2.21**), 61.3 (C_1, **2.20**), 56.5 (C_2, **2.21**), 56.0 (C_2, **2.20**), 55.80 (C_{17}, **2.21**), 55.78 (C_{17}, **2.20**), 51.46 ($C_{24}O\mathbf{C}H_3$, **2.21**), 51.44 ($C_{24}O\mathbf{C}H_3$, **2.20**), 50.2 (C_{14}, **2.21**), 50.1 (C_{14}, **2.20**), 42.7 (C_{13}, **2.20**), 42.4 (C_{13}, **2.21**), 41.1 (CH, **2.20**), 40.8 (CH, **2.20**), 40.03 (CH, **2.21**), 39.97 (CH, **2.21**), 39.6 (CH_2, **2.20**), 39.4 (CH_2, **2.21**), 36.6 (C_{10}, **2.21**), 36.0 (C_{10}, **2.20**), 35.33 (CH, **2.21**), 35.28 (CH, **2.20**), 34.9 (CH, **2.21**), 34.4 (CH, **2.20**), 33.4 (CH, **2.21**), 32.5 (CH, **2.20**), 30.97 (CH_2), 30.91 (CH_2), 30.7 (CH_2, **2.20**), 30.1 (CH_2, **2.20**), 29.7 (CH_2, **2.21**), 29.6 (CH_2, **2.21**), 23.7 (CH_2, **2.21**), 23.6 (CH_2, **2.20**), 22.5 (CH_2, **2.21**), 22.4 (CH_2, **2.20**), 21.9 (CH_2, **2.20**), 21.8 (CH_2, **2.21**), 20.4 (C_{19}, **2.20**), 18.9 (C_{19}, **2.21**), 18.23 (C_{21}, **2.20**), 18.21 (C_{21}, **2.21**), 11.79 ($C_6CH_2\mathbf{C}H_3$, **2.21**), 11.76 ($C_6CH_2\mathbf{C}H_3$, **2.20**), 11.54 (C_{18}, **2.20**), 11.48 (C_{18}, **2.21**) ppm. **LRMS** (ESI^+) m/z: 466.3 $[M+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{27}H_{48}O_5N$ $[M+NH_4]^+$, m/z calculated: 466.3527; found: 466.3529. **IR** (neat) 3381 (m), 2921 (s), 2851 (s), 1734 (s), 1721 (s), 1457 (m), 1377 (s), 1260 (m), 1176 (m) cm^{-1}.

Methyl-3α-acetoxy-6α-ethyl-7α-hydroxyl-5β-chol-1-en-24-oate (2.22)



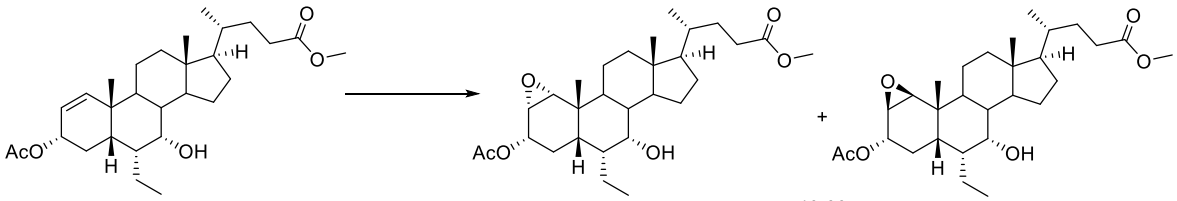
To a solution of **2.15** (825 mg, 1.91 mmol, 1.0 equiv) in dry THF (55 mL) under argon at rt was added sodium hydrogen carbonate (800 mg, 19.53 mmol, ~5.0 equiv) and acetic anhydride (0.90 mL, 9.53 mmol, ~5.0 equiv). After 20 h at 70 °C, the reaction was deemed complete by TLC and the reaction mixture quenched by the addition of H_2O (50 mL) and the aqueous phase was extracted with ethyl acetate (3 x 150 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 955 mg of crude material as a colourless gummy oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 10 g cartridge) using PE 40-60/acetone (90/10) as the eluent yielded compound **2.22** as a colourless oil (599 mg, 1.26 mmol, 66%).

The acetate protection of alcohols using acetic anhydride and sodium hydrogen carbonate will herein be referred to as *General Procedure C.*

R_f (PE 40-60/acetone, 70/30) 0.40. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.74 (1H, dd, *J* = 10.2, 1.8 Hz, H_1), 5.58 (1H, dt, *J* = 10.2, 1.5 Hz, H_2), 5.22 (1H, tt, *J* = 8.2, 1.7 Hz, $H_{3\beta}$), 3.68 (1H, s, $H_{7\beta}$), 3.66 (3H, s,

$C_{24}OC\mathbf{H}_3$), 2.35 (1H, ddd, *J* = 15.4, 10.3, 5.3 Hz), 2.26-2.17 (1H, m), 2.04 (3H, s, $C_3OC\mathbf{H}_3$), 2.01-1.25 (17H, m), 1.18-1.11 (4H, m), 1.01 (3H, s, H_{19}), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.91 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 170.9 ($C_3O\mathbf{C}$), 141.5 (C_1), 125.0 (C_2), 72.1 (C_7), 70.4 (C_3), 55.8 (C_{17}), 51.4 ($C_{24}O\mathbf{C}H_3$), 50.1 (C_{14}), 42.8 (CH), 42.6 (C_{13}), 41.1 (CH), 40.5 (CH), 39.5 (CH_2), 39.3 (CH), 38.2 (C_{10}), 35.3 (CH), 31.0 (CH_2), 30.9 (CH_2), 28.0 (CH_2), 27.3 (CH_2), 23.6 (CH_2), 22.4 (CH_2), 21.5 (CH_2), 21.4 (C_{19}), 21.1 ($C_3OC\mathbf{C}H_3$), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 432.3 $[M\text{-}AcOH+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{29}H_{46}O_5Na$ $[M+Na]^+$, m/z calculated: 497.3237; found: 497.3244. **IR** (neat) 3549 (m), 2962 (m), 2926 (s), 2864 (s), 1720 (s), 1455 (m), 1437 (s), 1366 (m), 1251 (s), 1015 (s) cm^{-1}.

Methyl-1α,2α-epoxy-3α-acetoxy-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (2.23) and methyl-1β,2β-epoxy-3α-acetoxy-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (2.24)



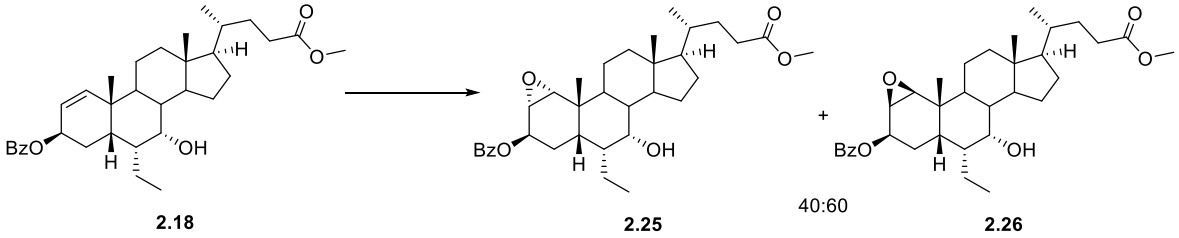
2.22 **2.23** **2.24**

Prepared according to *General Procedure B* (see: *page 150*) using 80 mg of compound **2.22** (0.17 mmol, 1.0 equiv). Purification of the resultant crude material by HPLC using hexane/acetone (75/25) as the eluent yielded an inseparable mixture of compounds **2.23** and **2.24** in a 40:60 ratio as a colourless residue (78 mg, 0.16 mmol, 94%).

R_f (PE 40-60/acetone, 65/35) 0.48. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.99 (0.4H, ddd, *J* = 11.0, 5.5, 1.5 Hz, $H_{3\beta}$, **2.23**), 4.82 (0.6H, ddd, *J* = 10.8, 6.8 Hz, $H_{3\beta}$, **2.24**), 3.68 (3.6H, s, $C_{24}OCH_3$ + $H_{7\beta}$, **2.24**), 3.62 (0.4H, s, $H_{7\beta}$, **2.23**), 3.37 (0.4H, d, *J* = 4.0 Hz, $H_{2\beta}$, **2.23**), 3.18 (0.6H, d, *J* = 3.4 Hz, $H_{2\alpha}$, **2.24**), 3.13 (0.6H, d, *J* = 3.2, 0.9 Hz, $H_{1\alpha}$, **2.24**), 3.00 (0.4H, d, *J* = 4.0 Hz, $H_{1\beta}$, **2.23**), 2.35 (1.0H, ddd, *J* = 15.7, 10.3, 5.4 Hz, $H_{23'}$), 2.28-2.19 (1.0H, m, $H_{23''}$), 2.10 (1.2H, s, $C_3OC\mathbf{H}_3$, **2.23**), 2.09 (1.8H, s, $C_3OC\mathbf{H}_3$, **2.24**), 2.05-1.99 (1.0H, m), 1.97-1.87 (2.0H, m), 1.85-1.78 (2.0H, m), 1.75-1.19 (16H, m), 1.16 (1.8H, s, H_{19}, **2.24**), 1.11 (1.2H, s, H_{19}, **2.23**), 0.94 (3.0H, d, *J* = 6.4 Hz, H_{21}), 0.89 (1.8H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$, **2.24**), 0.88 (1.2H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$, **2.23**), 0.71 (1.8H, s, H_{18}, **2.24**), 0.70 (1.2H, s, H_{18}, **2.23**) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.72 (C_{24}, **2.23**), 174.68 (C_{24}, **2.24**), 171.0 ($C_3O\mathbf{C}$, **2.23**), 168.5 ($C_3O\mathbf{C}$, **2.24**), 73.4 (C_3, **2.23**), 70.3 (C_7, **2.24**), 69.9 (C_7, **2.23**), 69.2 (C_3, **2.24**), 62.9 (C_1, **2.23**), 61.2 (C_2, **2.24**), 57.4 (C_1, **2.24**), 55.9 (C_{17}, **2.24**), 55.8 (C_{17}, **2.23**), 55.1 (C_2, **2.23**), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.3 (C_{14}, **2.24**), 50.1 (C_{14}, **2.23**), 43.7 (CH, **2.23**), 43.6 (CH, **2.23**), 42.8 (C_{13}, **2.23**), 42.5 (C_{13}, **2.24**), 41.6 (CH, **2.23**), 40.12 (CH, **2.24**), 40.06 (CH, **2.24**), 39.5 (CH_2, **2.24**), 39.4 (CH_2, **2.23**), 36.6 (C_{10}, **2.24**), 35.5 (CH, **2.24**), 35.37 (CH, **2.24**), 35.36 (CH, **2.23**), 35.13 (C_{10}, **2.23**), 35.05 (CH, **2.24**), 34.4 (CH, **2.23**), 31.02 (CH_2), 30.96 (CH_2),

28.02 (CH_2, **2.23**), 27.97 (CH_2, **2.24**), 26.2 (CH_2, **2.24**), 23.7 (CH_2, **2.24**), 23.6 (CH_2, **2.23**), 23.3 (CH_2, **2.23**), 22.7 (CH_2, **2.24**), 22.5 (CH_2, **2.23**), 22.4 (CH_2, **2.23**), 22.1 (CH_2, **2.24**), 21.24 ($C_3OC\mathbf{C}H_3$, **2.23**), 21.19 ($C_3OC\mathbf{C}H_3$, **2.24**), 20.16 (C_{19}, **2.23**), 18.4 (C_{19}, **2.24**), 18.3 (C_{21}), 11.9 (C_{18}, **2.24**), 11.8 (C_{18}, **2.23**), 11.62 ($C_6CH_2\mathbf{C}H_3$, **2.24**), 11.60 ($C_6CH_2\mathbf{C}H_3$, **2.23**) ppm. **LRMS** (ESI^+) m/z: 508.3 $[M+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{29}H_{46}O_6Na$ $[M+Na]^+$, m/z calculated: 513.3187; found: 513.3195. **IR** (neat) 3441 (m), 2949 (m), 2871 (m), 2360 (m), 1734 (s), 1714 (s) 1457 (m), 1436 (m), 1374 (m), 1258 (s), 1240 (s) cm^{-1}.

Methyl-1α,2α-epoxy-3β-benzoyl-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (2.25) and methyl-1β,2β-epoxy-3β-benzoyl-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (2.26)



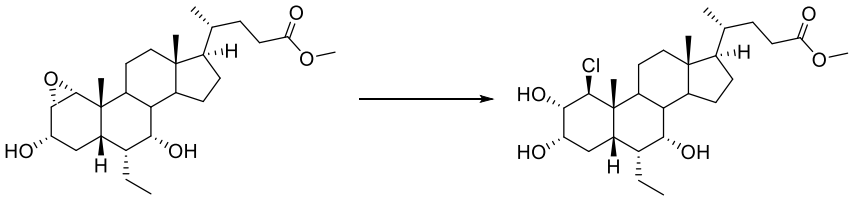
2.18 **2.25** **2.26**

Prepared according to *General Procedure B* (see: *page 150*) using 92 mg of compound **2.18** (0.19 mmol, 1.0 equiv). Purification of the resultant crude material by HPLC using hexane/acetone (80/20) as the eluent yielded an inseparable mixture of compounds **2.25** and **2.26** in a 40:60 ratio as a colourless residue (65 mg, 0.12 mmol, 63%).

$\mathbf{R_f}$ (PE 40-60/acetone, 75/25) 0.28. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.10-8.04 (3H, m, ArH), 8.00 (1H, dt, *J* = 7.8, 1.5 Hz, ArH), 7.61-7.53 (2H, m, ArH), 7.48-7.41 (3H, m, ArH), 5.56 (0.6H, q, *J* = 2.3 Hz, $H_{3\alpha}$, **2.26**), 5.34 (0.4H, ddd, *J* = 5.9, 4.4, 1.3 Hz, $H_{3\alpha}$, **2.25**), 3.69 (0.4H, s, $H_{7\beta}$, **2.25**), 3.68 (1.2H, s, $C_{24}OC\mathbf{H}_3$, **2.25**), 3.67 (1.8H, s, $C_{24}OC\mathbf{H}_3$, **2.26**), 3.63 (0.6H, s, $H_{7\beta}$, **2.26**), 3.61 (0.4, d, *J* = 4.0 Hz, $H_{2\beta}$, **2.25**), 3.38 (0.6, d, *J* = 2.2 Hz, $H_{2\alpha}$, **2.26**), 3.23 (0.4, d, *J* = 3.6 ,Hz, $H_{1\beta}$, **2.25**), 3.00 (0.6, d, *J* = 3.4 Hz, $H_{1\alpha}$, **2.26**), 2.37 (1H, ddd, *J* = 15.3, 10.0, 5.3 Hz), 2.26 (1H, dd, *J* = 9.5, 6.5 Hz), 2.20-1.26 (18H, m), 1.22 (1.2H, s, H_{19}, **2.25**), 1.19 (1.8H, s, H_{19}, **2.26**), 0.94 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.85 (3H, d, *J* = 6.9 Hz, H_{21}), 0.71 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.8 (C_{24}, **2.26**), 169.8 (C_{24}, **2.25**), 166.3 ($C_3O\mathbf{C}Bz$, **2.25**), 165.9 ($C_3O\mathbf{C}Bz$, **2.26**), 70.9 (C_7, **2.25**), 70.5 (C_7, **2.26**), 69.5 (C_3, **2.26**), 68.1 (C_3, **2.25**), 61.3 (C_1, **2.26**), 61.1 (C_1, **2.25**), 55.82 (C_{17}, **2.25**), 55.78 (C_{17}, **2.26**), 53.7 (C_2, **2.25**), 53.6 (C_2, **2.26**), 51.50 ($C_{24}O\mathbf{C}H_3$, **2.25**), 51.49 ($C_{24}O\mathbf{C}H_3$, **2.26**), 50.2 (C_{14}, **2.25**), 50.1 (C_{14}, **2.26**), 42.7 (C_{13}, **2.26**), 42.5 (C_{13}, **2.25**), 41.1 (CH, **2.26**), 40.1 (CH, **2.25**), 39.9 (CH, **2.25**), 39.8 (CH, **2.26**), 39.42 (CH_2, **2.25**), 39.37 (CH_2, **2.26**), 37.4 (CH, **2.26**), 36.7 (C_{10}, **2.25**), 35.35 (CH, **2.25**), 35.33 (CH, **2.26**), 35.2 (C_{10}, **2.26**), 35.0 (CH, **2.25**), 34.4 (CH, **2.26**), 34.2 (CH, **2.25**), 30.99 (CH_2), 30.95 (CH_2), 28.00 (CH_2, **2.26**), 27.94 (CH_2, **2.25**), 26.4 (CH_2, **2.25**), 23.7 (CH_2, **2.25**), 23.62 (CH_2, **2.26**), 23.57 (CH_2, **2.26**), 22.6 (CH_2, **2.26**), 22.4 (CH_2, **2.26**), 22.1 (CH_2, **2.26**), 21.8 (CH_2, **2.25**), 20.5 (C_{19}, **2.26**), 18.9 (C_{19}, **2.25**), 18.3 (C_{21}, **2.26**), 18.2 (C_{21}, **2.25**), 11.84 ($C_6CH_2\mathbf{C}H_3$, **2.25**), 11.79 ($C_6CH_2\mathbf{C}H_3$, **2.26**), 11.44 (C_{18}, **2.25**), 11.35 (C_{18}, **2.26**) ppm. **LRMS**

(ESI$^+$) m/z: 570.4 [M+NH$_4$]$^+$ 100%. **HRMS** (ESI$^+$) $C_{34}H_{52}O_6N$ [M+NH$_4$]$^+$, m/z calculated: 570.3789; found: 570.3793. **IR** (neat) 3451 (m), 2950 (m), 2254 (m), 1727 (s), 1713 (s) 1452 (m), 1378 (m), 1274 (s) cm^{-1}.

Methyl-1β-chloro-2α,3α,7α-trihydroxyl-6α-ethyl-5β-cholan-24-oate (2.27)

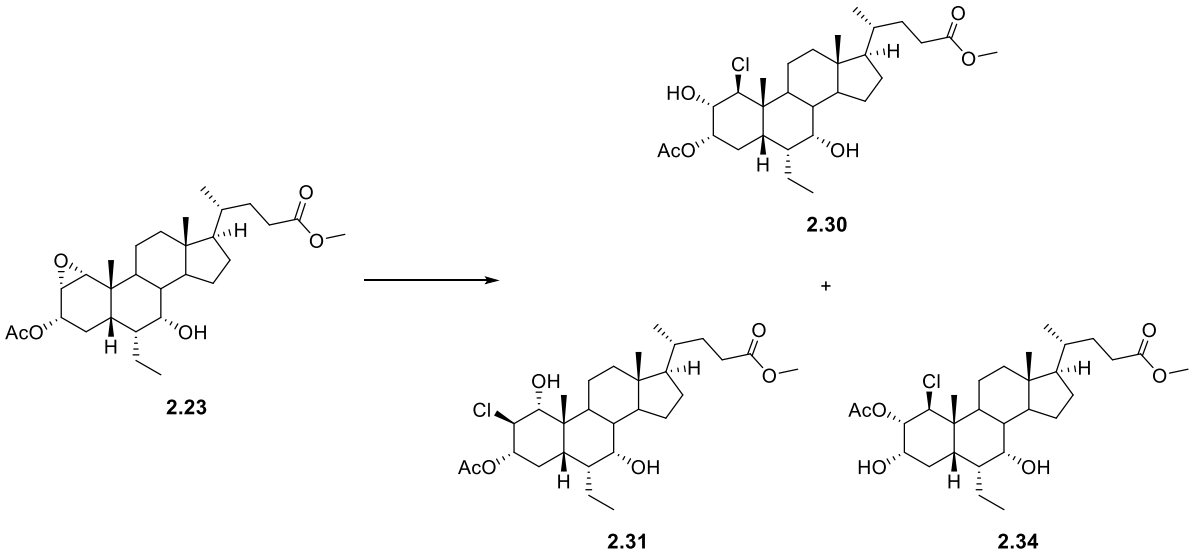


2.3 **2.27**

To a solution of **2.3** (50 mg, 0.12 mmol, 1.0 equiv) in dichloromethane (2.5 mL) at rt was added 2M HCl in diethyl ether solution (170 µL, 0.35 mmol, ~3.0 equiv). After 21 h at rt, the reaction was deemed complete by TLC and the reaction mixture was quenched with sat. $NaHCO_3$ solution (5 mL) and the aqueous phase was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were washed with brine (60 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 56 mg of crude material as a colourless oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 5 g cartridge) using PE 40-60/acetone (90/10 to 80/20) as the eluent yielded compound **2.27** as a colourless oil (14 mg, 0.03 mmol, 25%).

R$_f$ (PE 40-60/acetone, 80/20) 0.09. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.55 (1H, d, *J* = 3.2 Hz, H$_{1}$), 4.16 (1H, t, *J* = 2.8 Hz, H$_{2}$), 4.11 (1H, dt, *J* = 11.5, 3.4 Hz, H$_{3\beta}$) , 3.71 (1H, s, H$_{7\beta}$), 3.67 (3H, s, C$_{24}$OC**H**$_3$), 2.79 (1H, s, C$_2$OH), 2.39-2.32 (2H, m), 2.26-2.18 (3H, m),1.93 (1H, dt, *J* = 12.5, 2.8 Hz), 1.92-1.14 (19H, m) 1.10 (3H, s, H$_{19}$), 0.93 (3H, t, *J* = 7.5 Hz, C$_6$CH$_2$C**H**$_3$), 0.92 (3H, d, *J* = 6.5 Hz, H$_{21}$), 0.68 (3H, s, H$_{18}$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.8 (C$_{24}$), 74.3 (C$_2$), 70.3 (C$_7$), 69.2 (C$_1$), 67.6 (C$_3$), 55.7 (C$_{17}$), 51.5 (C$_{24}$O**C**H$_3$), 50.5 (C$_{14}$), 42.6 (C$_{13}$), 40.8 (C$_6$), 40.7 (CH), 40.4 (CH), 39.44 (CH), 39.36 (CH), 36.3 (CH$_2$), 35.3 (C$_{10}$), 30.99 (CH$_2$), 30.96 (CH$_2$), 28.0 (CH$_2$), 27.5 (C$_4$), 23.7 (CH$_2$), 22.4 (C$_6$**C**H$_2$CH$_3$), 21.8 (C$_{19}$), 21.7 (CH$_2$), 18.2 (C$_{21}$), 11.9 (C$_{18}$), 11.7 (C$_6$CH$_2$**C**H$_3$) ppm. **LRMS** (ESI$^+$) m/z: 502.3 [MCl^{35}+NH$_4$]$^+$ 100%, 504.3 [MCl^{37}+NH$_4$]$^+$ 35%. **HRMS** (ESI$^+$) $C_{27}H_{45}ClO_5Na$ [MCl^{35}+Na]$^+$, m/z calculated: 507.2848; found: 507.2852. **IR** (neat) 3402 (m), 2946 (s), 2871 (m), 2360 (m), 2342 (m), 1734 (s) 1457 (m), 1437 (m), 1377 (m), 1265 (s), 1168 (m) cm^{-1}.

Methyl-1β-chloro-2α,7α-dihydroxyl-3α-acetoxy-6α-ethyl-5β-cholan-24-oate (2.30), methyl-1α,7α-dihydroxyl-2β-chloro-3α-acetoxy-6α-ethyl-5β-cholan-24-oate (2.31) and methyl-1β-chloro-2α-acetoxy-3α,7α-dihydroxyl-6α-ethyl-5β-cholan-24-oate (2.34)



To a solution of **2.23** (50 mg, 0.10 mmol, 1.0 equiv) in dichloromethane (2.5 mL) at rt was added 2M HCl in diethyl ether solution (170 µL, 0.31 mmol, ~3.0 equiv). After 48 h at rt, the reaction was deemed complete by TLC and the reaction mixture concentrated *in vacuo* to afford 61 mg of crude material as a colourless oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 5 g cartridge) using PE 40-60/acetone (94/6) as the eluent yielded compound **2.30** as a white residue (35.9, 0.07 mmol, 67%), compound **2.31** as a colourless oil (5 mg, 0.01 mmol, 9%) and compound **2.34** as a colourless oil (12 mg, 0.02 mmol, 22%).

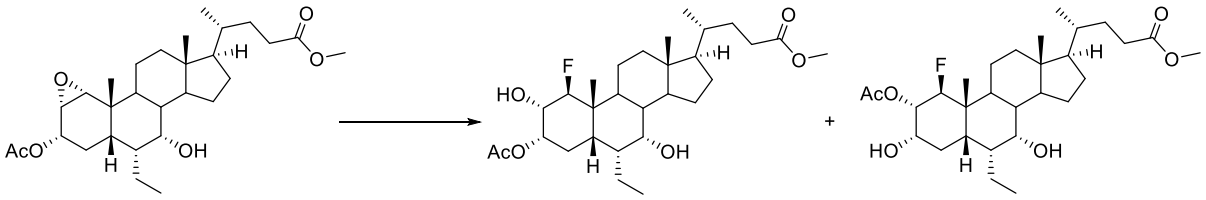
Compound **2.30**: $\mathbf{R_f}$ (PE 40-60/acetone, 70/30) 0.29. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.26 (1H, ddd, *J* = 12.2, 4.4, 3.3 Hz, $H_{3\beta}$), 4.53 (1H, d, *J* = 3.2 Hz, $H_{2\beta}$), 4.25 (1H, s, $H_{1\alpha}$), 3.72 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.44 (1H, ddd, *J* = 11.7, 4.0 Hz), 2.39-2.13 (4H, m), 2.07 (3H, s, $C_3OC\mathbf{H}_3$), 1.99-1.14 (20H, m), 1.11 (3H, s, H_{19}), 0.93 (3H, t, *J* = 6.7 Hz, $C_6CH_2C\mathbf{H}_3$), 0.92 (3H, d, *J* = 6.9 Hz, H_{21}) , 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 169.8 ($C_3O\mathbf{C}$), 73.2 (C_2), 71.5 (C_3), 70.2 (C_7), 68.1 (C_1), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.5 (C_{14}), 42.7 (C_{13}), 40.9 (CH), 40.7 (CH), 40.5 (C_{10}), 39.5 (CH), 39.4 (CH_2), 36.3 (CH), 35.3 (CH), 31.01 (CH_2), 30.96 (CH_2), 28.1 (CH_2), 23.8 (CH_2), 23.7 (CH_2), 22.3 CH_2), 21.8 (C_{19}), 21.7 (CH_2), 21.3 ($C_3OC\mathbf{C}H_3$), 18.2 (C_{21}), 11.9 (C_{18}), 11.7 ($C_6CH_2\mathbf{C}H_3$) ppm. **LRMS** (ESI^+) m/z: 544.4 $[MCl^{35}+NH_4]^+$ 100%, 546.4 $[MCl^{37}+NH_4]^+$ 35%. **HRMS** (ESI^+) $C_{29}H_{47}ClO_6Na$ $[MCl^{35}+Na]^+$, m/z calculated: 549.2953; found: 549.2957. **IR** (neat) 3452 (m), 2950 (s), 2871 (m), 1734 (s), 1716 (s), 1458 (m), 1436 (m), 1369 (m), 1263 (s), 1167 (m) cm^{-1}.

Compound **2.31**: $\mathbf{R_f}$ (PE 40-60/acetone, 70/30) 0.36. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.77 (1H, ddd, *J* = 11.7, 10.0, 5.6 Hz, $H_{3\beta}$), 4.14 (1H, dd, *J* = 10.9. 10.0 Hz, $H_{2\alpha}$), 3.71 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.26 (1H, dd, *J* = 11.0, 2.3 Hz, $H_{1\beta}$), 2.43 (1H, d, *J* = 2.57 Hz, $OH_{1\alpha}$), 2.27-2.21 (1H, m), 2.10 (3H, s,

$C_3OC\mathbf{H}_3$), 1.98-1.77 (4H, m), 1.70-1.27 (17H, m), 1.22 (3H, s, H_{19}), 1.19-1.13 (2H, m), 0.93 (3H, d, *J* = 6.5 Hz, H_{21}) , 0.91 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 170.2 ($C_3O\mathbf{C}$), 81.8 (C_1), 75.5 (C_3), 69.9 (C_7), 66.6 (C_2), 55.9 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.2 (C_{14}), 43.2 (C_{13}), 42.9 (CH), 42.3 (C_{10}), 40.7 (CH), 40.3 CH), 39.7 (CH_2), 35.4 (CH), 34.9 (CH), 31.02 (CH_2), 30.97 (CH_2), 29.6 (CH_2), 27.8 (CH_2) , 24.0 (CH_2), 23.2 (CH_2), 22.4 (CH_2), 21.2 ($C_3OC\mathbf{C}H_3$), 19.3 (C_{19}), 18.2 (C_{21}), 11.8 (C_{18}), 11.6 ($C_6CH_2\mathbf{C}H_3$) ppm. **LRMS** (ESI^+) m/z: 544.4 $[MCl^{35}+NH_4]^+$ 100%, 546.4 $[MCl^{37}+NH_4]^+$ 35%. **HRMS** (ESI^+) $C_{29}H_{47}ClO_6Na$ $[M+Na]^+$, m/z calculated: 549.2953; found: 549.2938. **IR** (neat) 3529 (m), 2957 (s), 2872 (m), 1736 (s), 1458 (m), 1376 (m), 1242 (s), 1167 (m) cm^{-1}.

Compound **2.34**: $\mathbf{R_f}$ (PE 40-60/acetone, 70/30) 0.19. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.24 (1H, t, *J* = 2.8 Hz, $H_{2\beta}$), 4.52 (1H, d, *J* = 3.2 Hz, $H_{1\alpha}$), 4.24-4.21 (1H, m, $H_{3\beta}$), 3.72 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.36 (1H, ddd, *J* = 15.5, 10.2, 5.4 Hz), 2.25-2.16 (3H, m), 2.13 (3H, s, $C_3OC\mathbf{H}_3$), 2.02 (1H, dt, *J* = 12.7, 3.2 Hz), 1.98-1.15 (20H, m), 1.11 (3H, s, H_{19}), 0.94 (3H, t, *J* = 7.0 Hz, $C_6CH_2C\mathbf{H}_3$), 0.92 (3H, d, *J* = 6.5 Hz, $\mathbf{H}_{21}$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.6 (C_{24}), 170.6 ($C_3O\mathbf{C}$), 75.5 (C_2), 70.1 (C_7), 66.2 (C_3), 65.8 (C_1), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.7 (C_{14}), 42.6 (C_{13}), 40.9 (CH), 40.7 (CH), 40.6 (C_{10}), 39.8 (CH_2), 39.4 (CH), 36.2 (CH), 35.4 (CH), 31.0 (CH_2), 30.9 (CH_2), 28.4 (CH_2), 28.1 (CH_2), 23.7 (CH_2), 22.3 (CH_2), 21.7 (C_{19}), 21.6 (CH_2), 21.2 ($C_3OC\mathbf{C}H_3$), 18.2 (C_{21}), 11.9 (C_{18}), 11.6 ($C_6CH_2\mathbf{C}H_3$) ppm. **LRMS** (ESI^+) m/z: 544.4 $[MCl^{35}+NH_4]^+$ 100%, 546.4 $[MCl^{37}+NH_4]^+$ 35%. **HRMS** (ESI^+) $C_{29}H_{47}ClO_6Na$ $[M+Na]^+$, m/z calculated: 549.2953; found: 549.2938. **IR** (neat) 3511 (m), 2948 (s), 2871 (m), 1727 (s), 1458 (m), 1437 (m), 1374 (m), 1264 (s), 1234 (s), 1167 (m) cm^{-1}.

Methyl 1β-fluoro-2α,7α-dihydroxyl-3α-acetoxy-6α-ethyl-5β-cholan-24-oate (2.35) and methyl 1β-fluoro-2α-acetoxy-3α,7α-dihydroxyl-6α-ethyl-5β-cholan-24-oate (2.36)



2.23 2.35 2.36

To a solution of **2.23** (350 mg, 0.71 mmol, 1.0 equiv) in dry dichloromethane (15 mL) under argon at 0 °C was added HF.pyridine complex (70%, 9.3 mL, 357 mmol, ~500 equiv) using a plastic syringe. After 8 h at 0 °C, the reaction mixture was diluted with dichloromethane (5 mL) and quenched by the slow addition of sat. $NaHCO_3$ solution (50 mL) and stirred at rt for 18 h. The aqueous phase was then separated and extracted with dichloromethane (3 x 50 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 310 mg of crude material as a yellow oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 25 g cartridge) using PE

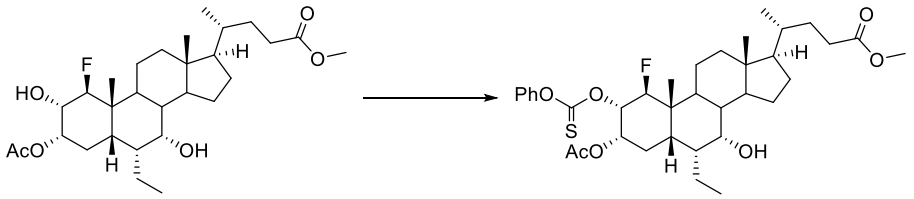
40-60/acetone (90/10 to 85/15) as the eluent yielded compound **2.35** as a colourless oil (115 mg, 0.23 mmol, 32%) and compound **2.36** as a colourless oil (55 mg, 0.11 mmol, 15%).

The opening of epoxides with HF.pyridine complex in dichloromethane will herein be referred to as *General Procedure D.*

Compound **2.35**: $\mathbf{R_f}$ (PE 40-60/acetone, 50/50) 0.54. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.93 (1H, ddd, *J* = 12.0, 7.0, 4.8 Hz, $H_{3\beta}$), 4.76 (1H, dd, *J* = 43.2, 3.3 Hz, $H_{1\alpha}$), 4.20 (1H, t, *J* = 2.9 Hz, $H_{2\beta}$), 3.70 (1H, s, $H_{7\beta}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.39-2.18 (4H, m), 2.07 (3H, s, $C_3OC\mathbf{H}_3$), 2.02-1.11 (20H, m), 1.08 (3H, d, *J* = 4.0 Hz, H_{19}), 0.921 (3H, t, *J* = 6.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.920 (3H, d, *J* = 6.1 Hz, H_{21}), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 169.9 ($C_3O\mathbf{C}$), 94.9 (d, *J* = 166.5 Hz, C_1), 72.7 (C_3), 70.3 (C_7), 69.9 (d, *J* = 33.8 Hz, C_2), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.3 (C_{14}), 42.5 (C_{13}), 40.6 (CH), 40.0 (d, *J* = 16.1 Hz, C_{10}), 39.6 (2 x CH), 39.4 (CH_2), 35.3 (CH), 33.4 (d, *J* = 5.9 Hz, CH), 30.99 (CH_2), 30.96 (CH_2), 28.0 (CH_2), 23.7 (CH_2), 23.5 (CH_2), 22.3 (CH_2), 21.8 (CH_2), 21.2 ($C_3OC\mathbf{C}H_3$), 18.2 (C_{21}), 17.8 (d, *J* = 10.3 Hz, C_{19}), 11.8 (C_{18}), 11.6 ($C_6CH_2\mathbf{C}H_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -199.0 (1F, d, *J* = 43.4 Hz, $F_{1\beta}$) ppm. **LRMS** (ESI^+) m/z: 528.3 $[M+NH_4]$+ 100%. **HRMS** (ESI^+) $C_{29}H_{47}FO_6$ $[M+Na]^+$, m/z calculated: 533.3249; found: 533.3259. **IR** (neat) 3493 (w), 2948 (m), 2871 (m), 1719 (s), 1457 (m), 1437 (m), 1369 (m), 1238 (s), 1167 cm^{-1}.

Compound **2.36**: $\mathbf{R_f}$ (PE 40-60/acetone, 50/50) 0.48. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.24 (1H, dt, *J* = 5.4, 3.6 Hz, $H_{2\beta}$), 4.74 (1H, dd, *J* = 42.8, 3.8 Hz, $H_{1\alpha}$), 3.89 (1H, dq, *J* = 12.1, 4.0 Hz, $H_{3\beta}$), 3.70 (1H, s, $H_{7\beta}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.35 (1H, ddd, *J* = 15.5, 10.2, 5.3 Hz), 2.26-2.20 (1H, m), 2.11 (3H, s, $C_3OC\mathbf{H}_3$), 2.02-1.09 (23H, m), 1.07 (3H, d, *J* = 4.4 Hz, H_{19}), 0.92 (3H, t, *J* = 7.2 Hz, $C_6CH_2C\mathbf{H}_3$), 0.91 (3H, d, *J* = 6.5 Hz, H_{21}), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 170.6 ($C_3O\mathbf{C}$), 93.0 (d, *J* = 169.5 Hz, C_1), 72.3 (d, *J* = 33.8 Hz, C_2), 70.2 (C_7), 67.7(C_3), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.4 (C_{14}), 42.5 (C_{13}), 40.6 (CH), 40.0 (d, *J* = 16.8 Hz, C_{10}), 39.7 (2 x CH), 39.5 (CH_2), 35.4 (CH), 33.4 (d, *J* = 5.9 Hz, CH), 31.0 (CH_2), 30.9 (CH_2), 28.0 (CH_2), 23.7 (CH_2), 22.3 (CH_2), 21.8 (CH_2), 21.1 ($C_3OC\mathbf{C}H_3$), 18.2 (C_{21} + CH_2), 17.7 (d, *J* = 10.3 Hz, C_{19}), 11.8 (C_{18}), 11.6 ($C_6CH_2\mathbf{C}H_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -197.6 (1F, dd, *J* = 43.4, 3.5 Hz, $F_{1\beta}$) ppm. **LRMS** (ESI^+) m/z: 528.3 $[M+NH_4]$+ 100%. **HRMS** (ESI^+) $C_{29}H_{47}FO_6$ $[M+Na]^+$, m/z calculated: 533.3249; found: 533.3257. **IR** (neat) 3492 (w), 2946 (m), 2871 (m), 1720 (s), 1458 (m), 1437 (m), 1375 (m), 1233 (s), 1166 (m) cm^{-1}.

Methyl 1β-fluoro-2α-(phenoxycarbonothioyl)oxy-3α-acetoxy-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (2.37)

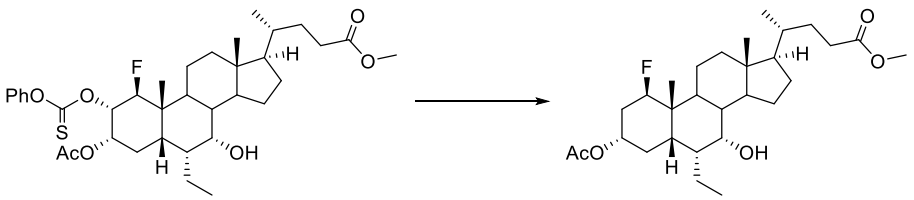


2.35 **2.37**

To a solution of **2.35** (170 mg, 0.33 mmol, 1.0 equiv) in dry dichloromethane (3 mL) under argon at rt was added pyridine (0.08 mL, 1.00 mmol, ~3.0 equiv) and *O*-phenylchlorothionoformate (61 µL, 0.37 mmol, ~1.1 equiv). After 48 h at rt the reaction was deemed complete by TLC and the reaction mixture was diluted with ethyl acetate (10 mL) and washed with water (20 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 242 mg of crude material as a green oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 10 g cartridge) using PE 40-60/acetone (100/0 to 90/10) as the eluent yielded compound **2.37** as a pale-yellow residue (130 mg, ~0.13 mmol, ~38%; considering 65 mol% purity by ^{19}F NMR).

R_f (PE 40-60/acetone, 70/30) 0.52. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.45-7.38 (2H, m, ArH), 7.33-7.28 (1H, m, ArH), 7.18-7.09 (2H, m, ArH), 5.81 (1H, dd, *J* = 7.6, 3.8 Hz, $H_{2\beta}$), 5.15 (1H, dq, *J* = 12.7, 4.0 Hz, $H_{3\beta}$), 4.96 (1H, dd, *J* = 42.6, 3.7 Hz, $H_{1\alpha}$), 3.72 (1H, s, $H_{7\beta}$), 3.65 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.44-2.30 (2H, m), 2.26-2.11 (2H, m), 2.09 (3H, s, $C_3OC\mathbf{H}_3$), 2.05-1.77 (5H, m), 1.67-1.17 (14H, m), 1.13 (3H, d, *J* = 4.3 Hz, H_{19}), 0.96-0.90 (6H, m, $C_6CH_2C\mathbf{H}_3$ and H_{21}), 0.69 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 194.4 (C=S), 174.6 (C_{24}), 170.1 ($C_3O\mathbf{C}$), 153.3 (C_i Ar), 129.6 (C_mHAr), 126.7 (C_pHAr), 121.7 (C_oHAr), 91.5 (d, *J* = 170.9 Hz, C_1) , 78.9 (d, *J* =37.4 Hz, C_2), 69.9 (C_7), 69.5 (C_3), 55.7 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.1 (C_{14}), 42.5 (C_{13}), 40.5 (CH), 40.2 (d, *J* = 16.1 Hz, C_{10}), 39.7 (CH), 39.6 (CH), 39.5 (CH_2), 35.3 (CH), 33.3 (d, *J* = 6.6 Hz, CH), 31.0 (CH_2), 30.9 (CH_2), 27.9 CH_2), 24.8 (CH_2), 23.6 (CH_2), 22.3 (CH_2), 21.9 (CH_2), 21.2 ($C_3OC\mathbf{C}H_3$), 18.2 (C_{21}), 17.6 (d, *J* = 10.3 Hz, C_{19}), 11.8 (C_{18}), 11.6 ($C_6CH_2\mathbf{C}H_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -198.5 (1F, dd, *J* = 41.6, 3.5 Hz, $F_{1\beta}$) ppm. **LRMS** (ESI^+) m/z: 664.3 $[M+NH_4]$+ 100%. **HRMS** (ESI^+) $C_{36}H_{51}FO_6SNa$ $[M+Na]^+$, m/z calculated: 669.3232; found: 669.3243.

Methyl 1β-fluoro-3α-acetoxy-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (2.38)

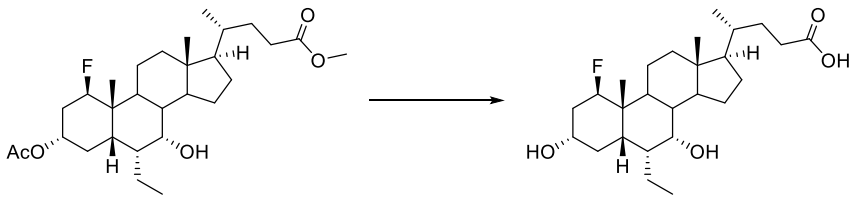


2.37 **2.38**

To a solution of **2.37** (80 mg, 0.12 mmol, 1.0 equiv) in dry toluene (2 mL) at rt was added triethylsilane (0.76 mL, 4.8 mmol, ~40 equiv) and the reaction mixture degassed under argon. The reaction mixture was heated to 120 °C and received dibenzoyldiperoxide (~10 mg, 0.02 mmol, ~0.2 equiv) portionwise every 30 min until the final addition of dibenzoyldiperoxide to total ~1.0 equiv. After 30 min at 120 °C of the final addition, the reaction mixture was cooled to rt and concentrated *in vacuo*. Purification by flash column chromatography (Biotage ZIP KP-Sil 10 g cartridge) using PE 40-60/acetone (95/5 to 85/15) as the eluent yielded compound **2.38** as a pale-yellow residue (95 mg, ~0.11 mmol, ~92%; considering 60 mol% purity by ^{1}H NMR, owing to aromatic impurities).

R_f (PE 40-60/acetone, 70/30) 0.41. **$[\alpha]_D$:** +14.1 (c = 0.82, methanol, 22 °C) **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.96 (1H, tt, *J* = 11.6, 4.8 Hz, $H_{3\beta}$), 4.73 (1H, dd, *J* = 46.3, 3.3 Hz, $H_{1\alpha}$), 3.73 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.36 (2H, ddd, *J* = 15.5, 10.2, 5.3 Hz), 2.27-2.18 (2H, m), 2.02 (3H, s, $C_3OC\mathbf{H}_3$), 2.02-1.12 (22H, m), 1.08 (3H, d, *J* = 3.1 Hz, H_{19}), 0.924 (3H, d, *J* = 6.5 Hz, H_{21}), 0.916 (3H, t, *J* = 7.0 Hz, $C_6CH_2C\mathbf{H}_3$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 170.5 ($C_3O\mathbf{C}$), 96.4 (d, *J* = 168.7 Hz, C_1), 70.4 (C_7), 69.9 (d, *J* =2.9 Hz, C_3), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.1 (C_{14}), 42.4 (C_{13}), 40.7 (CH), 39.78 (CH), 39.77 (d, *J* = 16.1 Hz, C_{10}), 39.3 (CH_2), 38.9 (CH), 35.3 (CH), 34.4 (d, *J* = 6.6 Hz, CH), 31.8 (d, *J* = 20.5 Hz, C_2), 31.0 (CH_2), 30.9 (CH_2), 28.9 CH_2), 27.9 CH_2), 23.7 (CH_2), 22.2 (CH_2), 21.4 (C_3OCCH_3), 20.8 (CH_2), 18.2 (C_{21}), 17.4 (d, J_n= 7.3 Hz, C_{19}), 11.7 (C_{18}), 11.6 ($C_6CH_2\mathbf{C}H_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -193.0 (1F, td, *J* = 46.4, 6.1 Hz, $F_{1\beta}$) ppm. **LRMS** (ESI^+) m/z: 512.3 $[M+NH_4]$+ 100%. **HRMS** (ESI^+) $C_{27}H_{47}FO_5Na$ $[M+Na]^+$, m/z calculated: 517.3300; found: 517.3312. **IR** (neat) 2949 (m), 2871 (m), 1721 (s), 1451 (m), 1366 (m), 1318 (m), 1240 (s), 1161 cm^{-1}.

1β-fluoro-3α,7α-dihydroxyl-6α-ethyl-5β-cholanic acid (1.52)



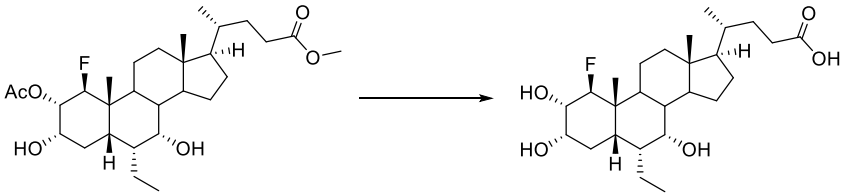
To a solution of **2.38** (50 mg, 0.10 mmol, 1.0 equiv) in a solution of 1,4-dioxane (9.8 mL) and water (3.6 mL) at rt was added concentrated (37%) hydrochloric acid (1.2 mL, 9:3:1 ratio). After 2 h at reflux, the reaction was deemed complete by TLC and the reaction mixture was neutralised with sat. $NaHCO_3$ solution (10 mL). The aqueous phase was extracted with ethyl acetate (3 x 20 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 46 mg of crude material as a colourless oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 10 g cartridge) using dichloromethane/methanol

(100/00 to 90/10) as the eluent yielded compound **1.52** as a colourless residue (27 mg, 0.06 mmol, 61%).

The acidic hydrolysis of ester groups using sodium hydroxide will herein be referred to as *General Procedure E.*

R_f (dichloromethane/methanol, 80/20) 0.24. **$[\alpha]_D$:** +12.4 (c = 0.43, methanol, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.72 (1H, dd, *J* = 46.6, 3.2 Hz, $H_{1\alpha}$), 3.86 (1H, tt, *J* = 11.4, 4.4 Hz, $H_{3\beta}$), 3.72 (1H, s, $H_{7\beta}$), 2.40 (1H, ddd, *J* = 15.5, 10.3, 5.3 Hz), 2.28-2.18 (2H, m), 1.99-1.26 (20H, m), 1.21-1.12 (3H, m), 1.08 (3H, d, *J* = 3.2 Hz, H_{19}), 0.94 (3H, d, *J* = 6.4 Hz, H_{21}), 0.92 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.3 (C_{24}), 97.1 (d, *J* = 168.0 Hz, C_1), 70.6 (C_7), 69.8 (d, *J* =2.9 Hz, C_3), 55.8 (C_{17}), 50.1 (C_{14}), 42.4 (C_{13}), 40.7 (CH), 39.8 (CH), 39.7 (d, *J* = 18.3 Hz, C_{10}), 39.3 (CH_2), 39.1 (CH), 35.37 (d, *J* =20.5 Hz, C_2), 35.35 (CH), 34.4 (d, *J* = 5.9 Hz, CH), 33.1(CH_2), 30.9 (C_{23}), 30.8 (C_{22}), 28.0 (CH_2), 23.7 (CH_2), 22.2 (CH_2), 20.8 (CH_2), 18.2 (C_{21}), 17.5 (d, *J* = 7.3 Hz, C_{19}), 11.8 (C_{18}), 11.6 ($C_6CH_2\mathbf{C}H_3$) ppm.**^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -192.2 (1F, td, *J* = 46.8, 3.4 Hz, $F_{1\beta}$) ppm. **LRMS** (ESI^+) m/z: 456.3 $[M+NH_4]$+ 100%. **HRMS** (ESI^+) $C_{26}H_{43}FO_4Na$ $[M+Na]^+$, m/z calculated: 461.3038 found: 461.3043. **IR** (neat) 3442 (w), 2943 (m), 1708 (s), 1463 (m), 1311 (m), 1201 (m), 1057 (m) cm^{-1}.

1β-fluoro-2α,3α,7α-trihydroxyl-6α-ethyl-5β-cholanic acid (2.39)



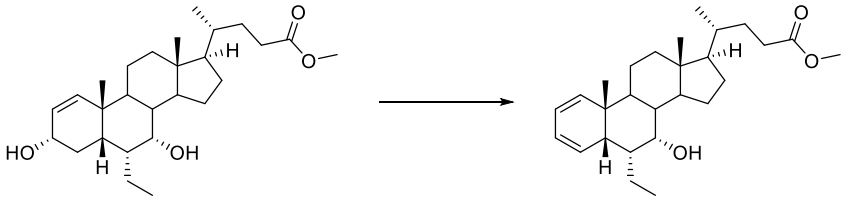
2.36 **2.39**

Prepared according to *General Procedure E* (see: *page 160*) using 80 mg of compound **2.36** (0.16 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage ZIP KP-Sil 10 g cartridge) using dichloromethane/methanol (100/0 to 70/30) as the eluent yielded compound **2.39** as a pale-yellow residue (46 mg, 0.10 mmol, 65%).

R_f (dichloromethane/methanol, 70/30) 0.48. **$[\alpha]_D$:** -4.68 (c = 0.79, methanol, 23 °C). **^{1}H NMR** (400 MHz, MeOD-d_4): δ 4.67 (1H, dd, *J* = 44.0, 3.7 Hz, $H_{1\alpha}$), 3.97 (1H, dt, *J* = 6.2, 3.6 Hz, $H_{2\beta}$), 3.64 (1H, s, $H_{7\beta}$), 3.59 (1H, ddd, *J* = 12.1, 9.1, 4.7 Hz, $H_{3\beta}$), 2.33 (1H, ddd, *J* = 15.3, 9.5, 5.3 Hz), 2.25-2.16 (1H, m), 1.96 (1H, dt, *J* = 12.6, 2.8 Hz), 1.90-1.10 (20H, m), 1.05 (3H, d, *J* = 4.3 Hz, H_{19}), 0.95 (3H, t, *J* = 6.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.92 (3H, d, *J* = 7.5 Hz, H_{21}), 0.71 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, MeOD-d_4): δ 178.3 (C_{24}), 97.6 (d, *J* = 168.0 Hz, C_1), 72.5 (d, *J* = 31.5 Hz, C_2), 70.9 (C_7), 70.5 (C_3), 57.6 (C_{17}), 51.7 (C_{14}), 43.7 (C_{13}), 42.8 (CH), 42.0 (CH), 41.4 (CH), 41.2 (d, *J* = 16.8 Hz, C_{10}), 41.0 (CH_2), 36.9 (CH), 34.7 (d, *J* = 6.6 Hz, CH), 32.5 (CH_2), 32.1 (CH_2), 29.3 (CH_2), 28.2 (CH_2), 24.7 (CH_2), 23.8 (CH_2), 23.1 (CH_2), 18.9 (C_{21}),

18.4 (d, *J* = 10.3 Hz, C_{19}), 12.5 (C_{18}), 12.2 ($C_6CH_2\mathbf{C}H_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, MeOD-d_4): δ -194.6 (1F, d, *J* = 45.1 Hz, $F_{1\beta}$) ppm. **LRMS** (ESI$^+$) m/z: 472.2 [M+NH_4]+ 100%. **HRMS** (ESI$^+$) $C_{26}H_{43}FO_5Na$ [M+Na]$^+$, m/z calculated: 477.2987; found: 477.2989. **IR** (neat) 3372 (w), 2943 (s), 2872 (m), 1706 (s), 1457 (m), 1377 (m), 1067 (s), 1023 (m) cm^{-1}.

Methyl-6α-ethyl-7α-hydroxyl-5β-chol-1,3-dien-24-oate (2.41)



2.15 **2.41**

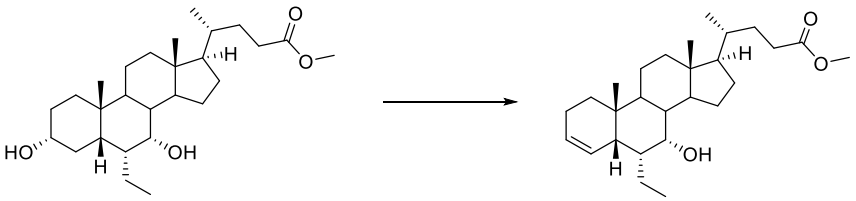
To a solution of **2.15** (100 mg, 0.23 mmol, 1.0 equiv) in dry dichloromethane (1 mL) under argon at -78 °C was added HF.pyridine complex (70%, 9 µL, 0.32 mmol, ~1.4 equiv) using a plastic syringe and stirred for 2 h. After warming to rt over 18 h, the reaction mixture was diluted with dichloromethane (5 mL) and quenched by the slow addition of sat. $NaHCO_3$ solution (10 mL). The aqueous phase was separated and extracted with dichloromethane (3 x 5 mL) and the combined organic layers were washed with sat. $Na_2S_2O_3$ solution (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 105 mg of crude material as a pale-yellow residue. Purification by HPLC using hexane/acetone (90/10) as the eluent yielded compound **2.41** as a colourless oil (32 mg, 0.08 mmol, 34%).

R_f (PE 40-60/acetone), 75/25) 0.56. **$[\alpha]_D$:** +12.9 (c = 0.79, methanol, 21 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.92-5.87 (2H, m), 5.84-5.80 (1H, m, H_4), 5.62 (1H, dd, *J* = 9.5, 0.7 Hz, H_1), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.64 (1H, s, $H_{7\beta}$), 2.38-2.30 (1H, m), 2.25-2.17 (1H, m), 1.93-1.88 (1H, m), 1.86-1.61 (7H, m), 1.53-1.19 (11H, m), 1.13 (3H, s, H_{19}), 0.93 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.90 (3H, d, *J* = 6.4 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 138.6 (C_1), 127.8 (**C**=C), 123.2 (C=**C**), 121.7 (C_4), 70.9 (C_7), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.2 (C_{14}), 44.0 (C_5), 42.8 (C_{13}), 41.2 (CH), 40.3 (CH), 39.5 (CH_2), 36.7 (C_{10}), 36.2 (CH), 35.3 (CH), 31.0 (CH_2), 30.9 (CH_2), 28.0 (CH_2), 23.7 (CH_2), 22.3 (CH_2), 22.1 (CH_2), 21.9 (C_{19}), 18.2 (C_{21}), 11.9 ($C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **LRMS** (ESI$^+$) m/z: 434.2 [M+NH_4]$^+$, 100%. **HRMS** (ESI$^+$) $C_{27}H_{46}O_3$ [M+NH_4]$^+$, m/z calculated: 437.3026; found: 437.3033. **IR** (neat) 2934 (s), 2871 (s), 1735 (s), 1458 (m), 1377 (m), 1244 (m), 1195 (m), 1165 (s) cm^{-1}.

8.3 Synthesis of OCA (NZP084) 2α-fluoro and 4α-fluoro analogues

The synthesis and characterisation of the following compounds is presented in the order of reactions discussed in Chapter 3Chapter 2.

Methyl-6α-ethyl-7α-hydroxyl-5β-chol-3-en-24-oate (3.4)



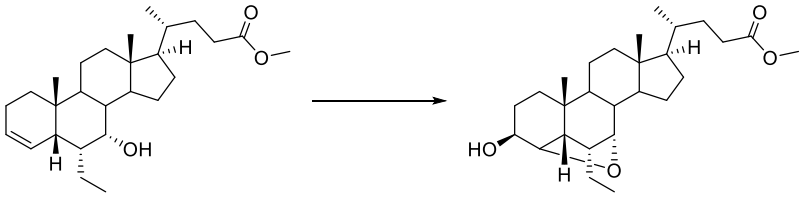
2.5 **3.4**

Prepared according to the conditions given by Kagan *et al*.[220] To a solution of **2.5** (100 mg, 0.23 mmol, 1.0 equiv) in dichloromethane (2 mL) at rt was added DMAP (56 mg, 0.46 mmol, ~2.0 equiv). The reaction mixture was cooled to 0 °C and received triflic anhydride (41 µL, 0.24 mmol, ~1.05 equiv) dropwise over 5 min. After 2 h at 0 °C the reaction was deemed complete by TLC and the reaction mixture was quenched with 2M hydrochloric acid (1 mL). The aqueous phase was separated and extracted with dichloromethane (3 x 5 mL) and the combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 95 mg of crude material as a colourless oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 5 g cartridge) using PE 40-60/acetone (98/2 to 80/20) as the eluent yielded compound **3.4** as a pale-yellow oil (32 mg, ~0.06 mmol, ~25%; considering 80 mol% purity by ^{1}H NMR).

The dehydration of secondary alcohol groups with triflic anhydride and DMAP will herein be referred to as *General Procedure F.*

The data presented for compound **3.4** was attained from an impure mixture. Characteristic signals are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.85-5.82 (1H, m), 5.75-5.71 (1H, m), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.57 (1H, d, *J* = 10.5, $H_{7\beta}$), 2.32 (1H, dd, *J* = 10.2, 5.2 Hz), 2.25-2.19 (1H, m), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 127.71 (CH), 127.67 (CH), 80.8 (C_7), 75.5 (CH), 71.6 (CH), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 42.6 (C_{13}), 34.3 (C_{10}), 22.7 (C_{19}), 18.3 (C_{21}), 11.9 ($C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 434.4 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{44}O_3Na$ $[M+Na]^+$, m/z calculated: 439.3183; found: 439.3181.

Methyl-3β-hydroxyl-4α,7α-epoxy-6α-ethyl-5β-cholan-24-oate (3.7)



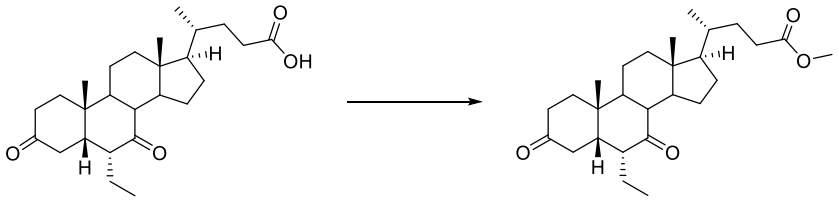
3.4 **3.7**

Prepared according to *General Procedure B* (see: *page 150*) using 35 mg of compound **3.4** (0.08 mmol, 1.0 equiv). Purification of the resultant crude material by HPLC using hexane/acetone

(90/10) as the eluent yielded an inseparable mixture of compound **3.7** as a colourless oil (19 mg, 0.04 mmol, 51%).

R_f (PE 40-60/acetone, 80/20) 0.72. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 4.26 (1H, dt, *J* = 7.7, 3.4 Hz), 4.04 (1H, t, *J* = 3.5 Hz), 3.81 (1H, s, $H_{7\beta}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.35 (1H, ddd, *J* = 15.4, 10.3, 5.1 Hz), 2.21 (1H, ddd, *J* = 16.1, 9.9, 6.7 Hz), 1.94 (1H, dt, *J* = 12.5, 2.8 Hz), 1.90-1.70 (6H, m), 1.59 (1H, dt, *J* = 13.7, 6.7 Hz), 1.51 (1H, dd, *J* = 14.1, 7.0 Hz), 1.46-1.06 (15H, m), 0.92 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.90 (3H, d, *J* = 6.4 Hz, H_{21}), 0.88 (3H, s, H_{19}), 0.64 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 83.8 (C_4), 81.0 (C_7), 69.0 (C_3), 55.8 (C_{17}), 52.5 (CH), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.4 (CH), 47.7 (CH), 44.9 (CH), 44.6 (CH), 43.6 (C_{13}), 40.4 (CH_2), 35.3 (CH), 34.3 (C_{10}), 31.3 (CH_2), 31.01 (C_{23}), 30.95 (C_{22}), 28.2 (CH_2), 26.8 (CH_2), 23.8 (CH_2), 23.5 (CH_2), 23.1 (C_{19}), 21.9 (CH_2), 18.2 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 12.0 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 433.6 $[M+H]^+$, 63%; 866.0 $[2M+H]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{45}O_4$ $[M+H]^+$, m/z calculated: 433.3312; found: 433.3323. **IR** (neat) 2929 (s), 2870 (m), 2360 (s), 2342 (m), 1739 (s), 1457 (m), 1436 (m), 1377 (m), 1251 (m) cm^{-1}.

Methyl-3,7-dioxo-6α-ethyl-5β-cholan-24-oate (3.9)



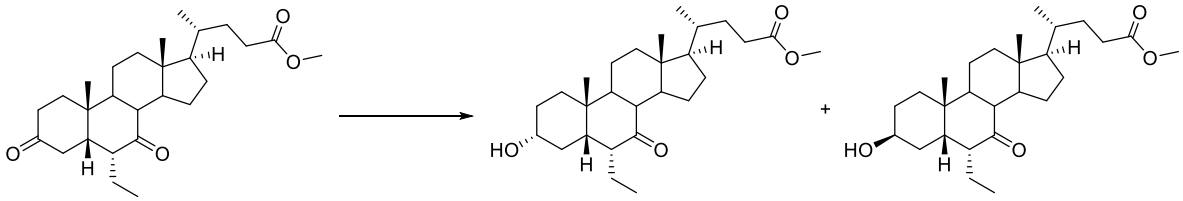
To a solution of **3.8** (36.0 g, 87.7 mmol, 1.0 equiv) in methanol (800 mL) at rt was added *para*-toluenesulfonic acid (1.67 g, 8.78 mmol, ~0.1 equiv) and sonicated at 30 °C for 4 h. The reaction was deemed complete by TLC and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in chloroform (400 mL) and washed with sat. $NaHCO_3$ solution (400 mL) and brine (400 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 37.6 g of crude material as a white solid that was used without further purification (87.3 mmol, 99%).

The esterification of carboxylic acids with methanol and *para*-toluenesulfonic acid will herein be referred to as *General Procedure G.*

m.p. 139-141 °C (evaporated from methanol). **R_f** (PE 40-60/acetone, 80/20) 0.59. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.74 (1H, dd, *J* = 12.2, 5.5 Hz), 2.47 (1H, t, *J* = 11.3 Hz), 2.35 (1H, ddd, *J* = 15.4, 10.0, 5.3 Hz), 2.26-2.14 (6H, m), 2.10-1.77 (6H, m), 1.74-1.35 (7H, m), 1.33 (3H, s, H_{19}), 1.31-1.26 (1H, m), 1.21-0.96 (4H, m), 0.93 (3H,d, *J* = 6.5 Hz, H_{21}), 0.80 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.69 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.1 (C_7), 210.5 (C_3), 174.6 (C_{24}), 54.8 (C_{17}), 52.4 (CH), 52.2 (CH), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.3 (CH), 48.7 (CH), 43.7 (CH), 42.6 (C_{13}), 38.9 (CH_2),

38.3 (CH_2), 36.6 (CH_2), 35.9 (C_{10}), 35.5 (CH_2), 35.2 (CH), 31.0 (CH_2), 30.9 (CH_2), 28.2 (CH_2), 24.5 (CH_2), 22.9 (C_{19}), 22.2 (CH_2), 18.6 (C_6**C**H_2CH_3), 18.3 (C_{21}), 12.1 (C_6CH_2**C**H_3), 11.8 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 448.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{42}O_4Na$ $[M+Na]^+$, m/z calculated: 453.2975; found: 453.2977. **IR** (neat) 2965 (s), 2938 (m), 2870 (m), 1733 (s), 1711 (s), 1698 (s), 1466 (m), 1436 (m), 1167 (m) cm^{-1}.

Methyl-3α-hydroxyl-6α-ethyl-7-oxo-5β-cholan-24-oate (3.10) and methyl-3β-hydroxyl-6α-ethyl-7-oxo-5β-cholan-24-oate (3.11)

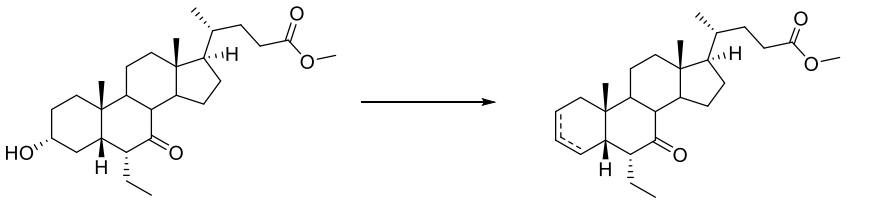


To a solution of **3.9** (10.0 g, 23.2 mmol, 1.0 equiv) in dry THF (340 mL) under argon at -78 °C was added *L*-selectride (35.0 mL, 34.8 mmol, ~2.5 equiv) dropwise over 15 min. After 10 min, the reaction mixture received a solution of hydrogen peroxide (40 mL, 30% v/v) and 2M sodium hydroxide (40 mL) in water (400 mL) at 0 °C. After a further 10 min, the reaction mixture received 2M hydrochloric acid (130 mL) at rt. The aqueous phase was separated and extracted with ethyl acetate (2 x 250 mL) and the combined organic layers were washed with water (500 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 11.0 g of crude material as a colourless oil. Purification by flash column chromatography (Biotage SNAP KP-Sil 100 g cartridge) using PE 40-60/acetone (90/10 to 80/20) as the eluent yielded a 2:1 mixture of compounds **3.10** and **3.11** as a white residue (5.62 g, 13.0 mmol, 56%) and compound **3.11** pure as a white residue (2.41 g, 5.57 mmol, 24%). Overall, the yield of **3.10** was 36% and the yield of **3.11** was 44%.

Compound **3.10**: R_f (hexane/acetone, 65/35) 0.39. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.66 (3H, s, $C_{24}OC$**H**$_3$), 3.54 (1H, ddd, *J* = 15.4, 10.6, 4.5 Hz, $H_{3\beta}$), 2.69 (1H, dd, *J* = 13.1, 5.9 Hz), 2.41-2.32 (2H, m), 2.26-2.14 (3H, m), 2.00-1.88 (2H, m), 1.84-1.59 (7H, m), 1.55-1.29 (9H, m), 1.22 (3H, s, H_{19}), 1.17-1.07 (4H, m), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.80 (3H, t, *J* = 7.4 Hz, C_6CH_2C**H**$_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.8 (C_7), 174.7 (C_{24}), 71.2 (C_3), 54.7 (C_{17}), 52.0 (CH), 51.5 ($C_{24}O$**C**H_3), 50.6 (CH), 49.9 (CH), 48.9 (CH), 43.7 (CH), 42.6 (C_{13}), 39.0 (CH_2), 35.7 (C_{10}), 35.2 (CH), 34.2 (CH_2), 31.8 (CH_2), 31.02 (CH_2), 30.97 (CH_2), 29.8 (CH_2), 28.3 (CH_2), 24.6 (CH_2), 23.5 (C_{19}), 21.8 (CH_2), 18.8 (C_6**C**H_2CH_3), 18.3 (C_{21}), 12.04 (C_6CH_2**C**H_3), 11.96 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 450.4 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{45}O_4$ $[M+H]^+$, m/z calculated: 433.3312; found: 433.3308. IR (neat) 2935 (m), 2873 (m), 1735 (s), 1706 (s), 1448 (m), 1436 (m), 1379 (m), 1265 (s), 1166 (m) cm^{-1}.

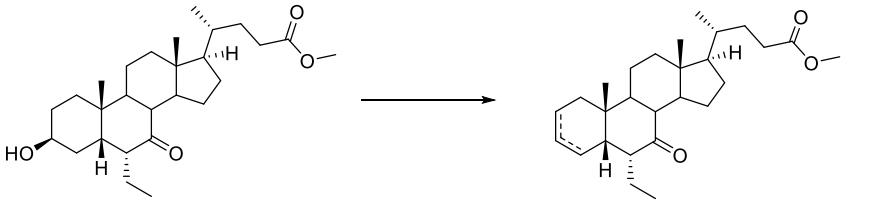
Compound **3.11**: R_f (hexane/acetone, 65/35) 0.42. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.05 (1H, t, *J* = 2.5 Hz, $H_{3\alpha}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.75 (1H, dd, *J* = 12.8, 5.6 Hz) 2.41-2.31 (2H, m), 2.26-2.14 (3H, m), 2.00-1.88 (2H, m), 1.84-1.58 (6H, m), 1.55-1.29 (10H, m), 1.25 (3H, s, H_{19}), 1.15-1.07 (4H, m), 0.92 (3H, d, *J* = 6.5 Hz, H_{21}), 0.81 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.66 (3H, s, H_{18}). **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.6 (C_7), 174.7 (C_{24}), 65.9 (C_3), 54.8 (C_{17}), 51.8 (CH), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.0 (CH), 49.0 (CH), 45.8 (CH), 43.2 (CH), 42.7 (C_{13}), 39.0 (CH_2), 36.2 (C_{10}), 35.2 (CH), 31.02 (CH_2), 30.98 (CH_2) , 29.2 (CH_2), 29.0 (CH_2), 28.3 (CH_2), 27.3 (CH_2), 24.6 (CH_2), 24.0 (C_{19}), 22.1 (CH_2), 18.7 ($C_6\mathbf{C}H_2CH_3$), 18.3 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 12.0 (C_{18}). **LRMS** (ESI^+) m/z: 450.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{45}O_4$ $[M+H]^+$, m/z calculated: 433.3312; found: 433.3309. **IR** (neat) 2936 (m), 1729 (s), 1704 (s), 1437 (m), 1380 (m), 1250 (s) 1166 (m), 1051 (s) cm^{-1}.

Methyl-6α-ethyl-7oxo-5β-chol-2-en-24-oate (3.12) and methyl-6α-ethyl-7-oxo-5β-chol-3-en-24-oate (3.13)



3.10 2-ene **3.12** and 3-ene **3.13** in 80:20 ratio

Prepared according to *General Procedure F* (see: *page 162)* using 360 mg of compound **3.10** (0.83 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil 5 g cartridge) using PE 40-60/acetone (90/10) as the eluent yielded an inseparable mixture of compounds **3.12** and **3.13** in an 80:20 ratio as a colourless oil (216 mg, 0.52 mmol, 64%).



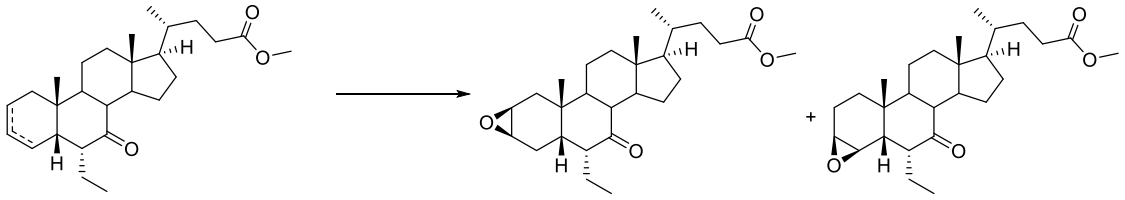
3.11 2-ene **3.12** and 3-ene **3.13** in 80:20 ratio

*Preparation of compounds **3.12/3.13** was also successful by the following method:* Prepared according to *General Procedure F* (see: *page 162)* using 980 mg of compound **3.11** (2.27 mmol, 1.0 equiv). Purification by flash column chromatography (Biotage SNAP KP-Sil 25 g cartridge) using PE 40-60/acetone (90/10) as the eluent yielded an inseparable mixture of compounds **3.12** and **3.13** in an 80:20 ratio as a colourless oil (438 mg, 1.06 mmol, 47%).

R_f (PE 40-60/acetone, 80/20) 0.45. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.63-5.40 (2H, m), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.74 (1H, dd, *J* = 12.0, 6.6 Hz), 2.34 (2H, tt, *J* = 10.3, 5.1 Hz), 2.27-1.29 (19H, m), 1.27 (2.4H,

s, H_{19}, **3.13**), 1.26 (0.6H, s, H_{19}, **3.12**), 1.18-0.94 (3H, m), 0.91 (3H, d, *J* = 6.5 Hz, H_{21}), 0.83 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.664 (0.6H, s, H_{18}, **3.13**), 0.657 (2.4H, s, H_{18}, **3.12**) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.4 (C_7, **3.12**), 212.9 (C_7, **3.13**), 174.7 (C_{24}), 127.9 (**C**=C, **3.13**), 125.3 (**C**=C, **3.12**), 124.5 (C=**C**, **3.13**), 123.8 (C=**C**, **3.12**), 54.8 (C_{17}, **3.13**), 54.7 (C_{17}, **3.12**), 52.4 (CH, **3.13**), 52.2 (CH, **3.12**), 51.6 ($C_{24}O\mathbf{C}H_3$, **3.13**), 51.5 ($C_{24}O\mathbf{C}H_3$, **3.12**), 50.3 (CH, **3.12**), 49.9 (CH, **3.13**), 49.2 (CH, **3.12**), 48.8 (CH, **3.13**), 47.0 (CH, **3.12**), 43.99 (CH, **3.13**), 43.97 (CH, **3.12**), 42.9 (C_{13}, **3.12**), 42.6 (C_{13}, **3.13**), 39.1 (CH_2, **3.13**), 39.0 (CH_2, **3.12**), 35.3 (CH_2), 35.2 (CH), 35.1 (C_{10}, **3.12**), 34.8 (C_{10}, **3.13**), 32.9 (CH, **3.13**), 31.04 (CH_2), 31.01 (CH_2), 28.33 (CH_2, **3.12**), 28.28 (CH_2, **3.13**), 24.8 (CH_2, **3.12**), 24.5 (CH_2, **3.13**), 23.9 (CH_2, **3.12**), 23.6 (C_{19}, **3.12**), 22.9 (C_{19}, **3.13**), 22.6 (CH_2, **3.12**), 22.3 (CH_2, **3.13**), 22.0 (CH_2, **3.13**), 18.8 (CH_2, **3.12**), 18.6 (CH_2, **3.13**), 18.4 (C_{21}), 12.1 (C_{18}), 12.0 ($C_6CH_2\mathbf{C}H_3$, **3.13**), 11.9 ($C_6CH_2\mathbf{C}H_3$, **3.12**) ppm. **LRMS** (ESI^+) m/z: 432.2 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{42}O_3Na$ $[M+Na]^+$, m/z calculated: 437.3026; found: 437.3030. **IR** (neat) 2936 (m), 2360 (w), 1736 (s), 1706 (s), 1437 (m), 1379 (m). 1250 (m), 1166 (m) cm^{-1}.

Methyl-2β,3β-epoxy-6α-ethyl-7-oxo-5β-cholan-24-oate (3.14) and methyl-3β,4β-epoxy-6α-ethyl-7-oxo-5β-cholan-24-oate (3.15)



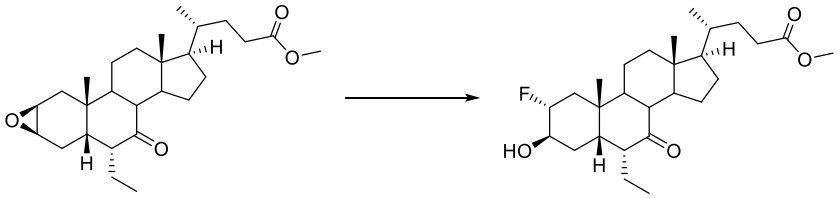
Prepared according to *General Procedure B* (see: *page 150*) using 5.0 g of a mixture of **3.12** and **3.13** (12.1 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil 100 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded an inseparable mixture of compound **3.14** and **3.15** as a colourless oil (4.94 g, 11.5 mmol, 95%). Further purification by flash column chromatography (Biotage SNAP KP-Sil 340 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **3.14** as a colourless oil (3.71 g, 8.62 mmol, 72%) and compound **3.15** as a colourless oil (1.18 g, 2.74 mmol, 23%).

Compound **3.14**: R_f (PE 40-60/acetone, 70/30) 0.47. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.13 (1H, t, *J* = 2.6 Hz), 3.01 (1H, dd, *J* = 5.5, 4.2 Hz), 2.67 (1H, dd, *J* = 11.5, 6.6 Hz, $H_{6\beta}$), 2.35 (1H, ddd, *J* = 15.4, 10.2, 5.1 Hz, ,$H_{23'}$), 2.30-2.18 (3H, m), 2.01-1.65 (7H, m), 1.55-1.20 (10H, m), 1.17 (3H, s, H_{19}), 1.15-0.95 (3H, m), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.81 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.0 (C_7), 174.6 (C_{24}), 54.7 (C_{17}), 51.8 (CHO), 51.6 (CHO), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.5 (CH), 49.3 (CH), 49.2 (CH), 45.1 (CH), 43.5 (CH), 42.9 (C_{13}), 38.9 (CH_2), 35.2 (CH), 34.3 (CH_2), 34.1 (C_{10}), 31.01 (C_{23}), 30.95 (C_{22}), 28.3 (CH_2), 24.7 (CH_2), 23.7 (C_{19}), 22.6 (CH_2), 22.2 (CH_2), 18.5 (CH_2), 18.3 (C_{21}), 12.2 ($C_6CH_2\mathbf{C}H_3$), 11.9 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 448.3

$[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{43}O_4$ $[M+H]^+$, m/z calculated: 431.3156; found: 431.3168. **IR** (neat) 2937 (m), 2870 (m), 1733 (s), 1704 (s), 1435 (m), 1381 (m), 1191 (m), 1166 (m) cm^{-1}.

Compound **3.15**: R_f (PE 40-60/acetone), 70/30) 0.48. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.10-3.09 (1H, m, CHO), 2.79-2.74 (2H, m, CHO + $H_{6\beta}$), 2.41-2.31 (2H, m), 2.26-2.04 (3H, m), 2.00-1.88 (4H, m), 1.84-1.58 (2H, m), 1.53-1.20 (11H, m), 1.17 (3H, s, H_{19}), 1.14-0.94 (2H, m), 0.91 (3H, d, *J* = 6.3 Hz, H_{21}), 0.90 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.4 (C_7), 174.6 (C_{24}), 54.8 (C_{17}), 52.9 (CHO), 52.1 (CHO), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.8 (CH), 50.4 (CH), 49.8 (CH), 48.7 (CH), 46.1 (CH), 42.5 (C_{13}), 38.2 (CH_2), 35.2 (CH), 33.8 (C_{10}), 31.0 (C_{23}), 30.9 (C_{22}), 28.4 (CH_2), 28.2 (CH_2), 24.5 (CH_2), 22.4 (C_{19}), 22.2 (CH_2), 20.0 (CH_2), 18.4 (CH_2), 18.3 (C_{21}), 12.03 ($C_6CH_2\mathbf{C}H_3$), 11.96 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 448.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{43}O_4$ $[M+H]^+$, m/z calculated: 431.3156; found: 431.3150. **IR** (neat) 2937 (m), 2870 (m), 1733 (s), 1704 (s), 1435 (m), 1381 (m), 1327 (m), 1191 (m), 1166 (m) cm^{-1}.

Methyl-2α-fluoro-3α-hydroxyl-6α-ethyl-7-oxo-5β-cholan-24-oate (3.16)

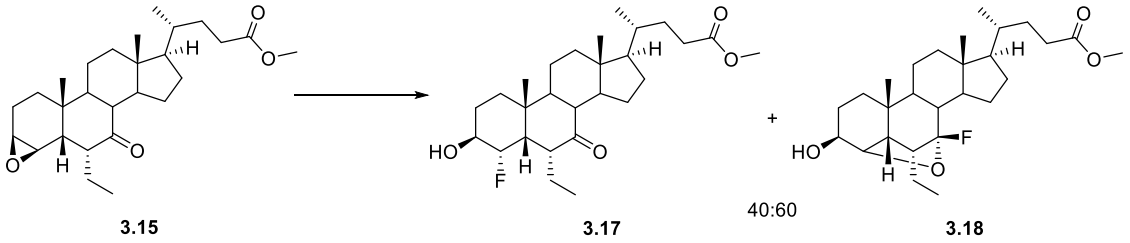


Prepared according to *General Procedure D* (see: *page 157*) using 3.33 g of compound **3.14** (7.73 mmol, 1.0 equiv) and using a fresh 100 mL bottle of HF.pyridine (70%) complex poured directly into the reaction mixture via a funnel whilst under a steady flow of argon. CAUTION: exercise extensive and appropriate controls for the large scale of this hazardous reagent. Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil 100 g cartridge) using PE 40-60/acetone (95/5 to 90/150 as the eluent yielded compound **3.16** as a white residue (2.39 g, 5.30 mmol, 69%).

R_f (PE 40-60/acetone, 50/50) 0.54. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.53 (1H, dq, *J* = 47.0, 2.3 Hz, $H_{2\beta}$), 4.01 (1H, dq, *J* = 6.6, 2.9 Hz, $H_{3\alpha}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.75 (1H, dd, *J* = 13.3, 5.8 Hz, $H_{6\beta}$), 2.38-2.30 (2H, m), 2.25-2.11 (4H, m), 2.01 (1H, dd, *J* = 12.0, 3.8 Hz), 1.94-1.28 (14H, m), 1.25 (3H s, H_{19}), 1.22-1.10 (3H, m), 0.91 (3H, d, *J* = 6.5 Hz, H_{21}) , 0.82 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.4 (C_7), 174.7 (C_{24}), 91.0 (d, *J* = 171.7 Hz, C_2), 66.7 (d, *J* = 29.3 Hz, C_3), 54.7 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 51.3 (CH), 50.4 (CH), 48.8 (CH), 45.9 (d, *J* = 5.9 Hz, CH), 45.2 (CH), 42.6 (C_{13}), 38.8 (CH_2), 36.1 (d, J = 1.5 Hz, C_{10}), 35.2 (CH), 34.4 (d, *J* = 19.1 Hz, C_1), 31.00 (C_{23}), 30.98 (C_{22}), 28.3 (CH_2), 25.0 (CH_2), 24.6 (CH_2), 24.0 (C_{19}), 22.4 (CH_2), 18.7 (CH_2), 18.3 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 12.0 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.3 (1F, tt, *J* = 50.3, 8.7 Hz, $F_{2\alpha}$) ppm.

LRMS (ESI$^+$) m/z: 468.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI$^+$) $C_{27}H_{44}FO_4$ $[M+H]^+$, m/z calculated: 451.3128; found: 451.3226. **IR** (neat) 3493 (w), 2948 (m), 2871 (m), 1719 (s), 1703 (s), 1457 (m), 1437 (m), 1369 (m), 1238 (s), 1167 cm^{-1}.

Methyl-3β-hydroxyl-4α-fluoro-6α-ethyl-7-oxo-5β-cholan-24-oate (3.17) and methyl-3β-hydroxy-4α,7α-epoxy-6α-ethyl-7β-fluoro-5β-cholan-24-oate (3.18)



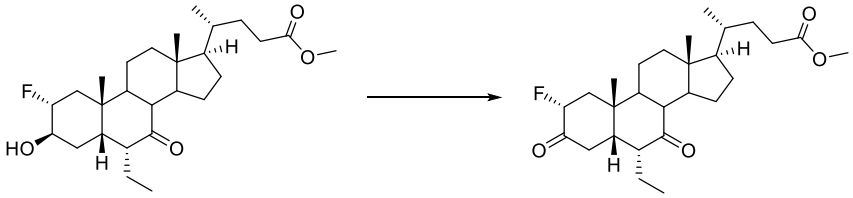
Prepared according to *General Procedure D* (see: *page 157*) using 990 mg of compound **3.15** (2.29 mmol, 1.0 equiv) and using a fresh 20 mL bottle of HF.pyridine (70%) complex poured directly into the reaction mixture via a funnel whilst under a steady flow of argon. CAUTION: exercise extensive and appropriate controls for the large scale of this hazardous reagent. Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil 100 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded a mixture of compound **3.17** and **3.18** in a 40:60 ratio as a white residue (412 mg, 0.91 mmol, 40%). Further purification by HPLC using hexane/acetone (95/5) as the eluent yielded a pure analytical sample of compound **3.18** (~25 mg) as a white residue.

Compound **3.17**: R_f (PE 40-60/acetone, 70/30) 0.38. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.55 (1H, dt, *J* = 46.6, 2.7 Hz, $H_{4\beta}$), 4.04 (1H, dq, *J* = 7.8, 2.7 Hz, $H_{3\alpha}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.59 (1H, dd, *J* = 13.3, 6.6 Hz, $H_{6\beta}$), 2.28-1.03 (26H, m), 0.94-0.88 (9H, m, H_{19}, H_{21} and $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.8 (C_7), 174.7 (C_{24}), 90.1 (d, *J* = 175.3 Hz, C_4), 66.7 (d, *J* = 30.1 Hz, C_3), 54.6 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.5 (d, *J* = 12.5 Hz, CH), 48.9 (d, *J* = 17.6 Hz, C_5), 48.7 (CH), 44.6 (d, *J* = 5.1 Hz, C_6), 43.0 (C_{13}), 38.9 (CH_2), 36.0 (d, *J* = 2.2 Hz, C_{10}), 35.1 (CH), 31.1 (C_{23}), 30.99 (C_{22}), 28.44 (CH_2), 28.35 (CH_2), 25.1 (CH_2), 24.1 (CH), 23.8 (C_{19}), 23.0 (CH_2), 22.4 (CH_2), 18.5 (CH_2), 18.4 (C_{21}), 12.11 ($C_6CH_2\mathbf{C}H_3$), 12.09 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.8 (1F, td, *J* = 45.1 Hz, 6.9 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI$^+$) m/z: 468.2 $[M+NH_4]^+$, 100%.

Compound **3.18**: R_f (PE 40-60/acetone, 70/30) 0.38. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.38 (1H, t, *J* = 2.9 Hz, $H_{3\alpha}$), 4.21 (1H, ddd, *J* = 8.2, 5.4, 3.6 Hz, $H_{4\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.35 (1H, ddd, *J* = 15.3, 10.3, 5.4 Hz), 2.25-2.18 (1H, m), 2.02-1.74 (7H, m), 1.60-1.22 (14H, m), 1.19-1.05 (3H, m), 0.93 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.92 (3H, d, *J* = 6.5 Hz, H_{21}), 0.90 (3H, s, H_{19}), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 122.8 (d, *J* = 237.7 Hz, C_7), 82.9 (C_4), 67.3 (d, *J* = 2.2 Hz, C_3), 54.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.6 (CH), 49.8 (CH), 49.4 (d, *J* = 17.6 Hz, C_5), 47.3 (d, *J* = 26.7 Hz, C_8), 46.9 (d, *J* = 6.6

Hz, CH), 44.4 (C_{13}), 40.2 (CH_2), 35.2 (CH), 34.4 (C_{10}), 31.1 (C_{23}), 31.0 (C_{22}), 30.4 (CH_2), 28.6 (d, *J* = 2.2 Hz, CH_2), 26.4 (CH_2), 25.5 (d, *J* = 5.9 Hz, CH_2), 22.7 (C_{19}), 22.4 (CH_2), 18.4 (C_{21}), 18.3 (d, *J* = 7.3 Hz, CH_2), 12.3 ($C_6CH_2\mathbf{C}H_3$), 12.2 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -131.6 (1F, s, $F_{7\beta}$) ppm. **LRMS** (ESI^+) m/z: 468.5 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{44}FO_4$ $[M+H]^+$, m/z calculated: 451.3218; found: 451.3220. **IR** (neat) 3458 (w), 2950 (m), 2874 (m), 1736 (s), 1456 (m), 1379 (m), 1165 (m), 1056 (s), 1018 cm^{-1}.

Methyl-2α-fluoro-3,7-dioxo-6α-ethyl-5β-cholan-24-oate (3.21)

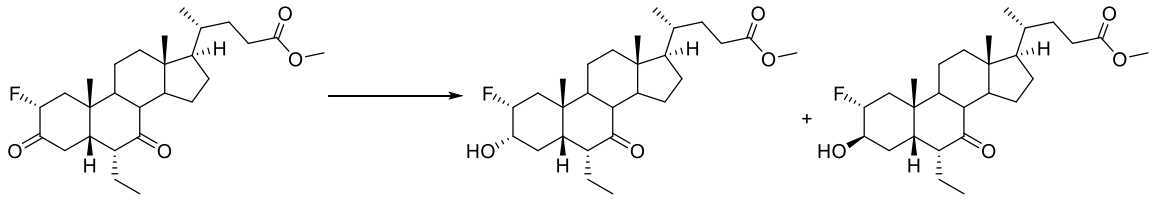


3.16 **3.21**

To a solution of **3.16** (1.00 g, 2.26 mmol, 1.0 equiv) in dichloromethane (20 mL) at rt was added Dess-Martin periodinane (1.92 g, 4.52 mmol, ~2.0 equiv) and H_2O (0.25 mL). After 3 h at rt, the reaction was deemed complete by TLC and the reaction mixture was quenched with sat. $NaHCO_3$ solution (25 mL) and filtered over Celite and washed with dichloromethane (90 mL). The aqueous phase was then separated and extracted with dichloromethane (2 x 50 mL) and the combined organic layers were washed with sat. $Na_2S_2O_3$ solution (150 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 1.21 g of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP KP-Sil 25 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **3.21** as a white residue (730 mg, 1.63 mmol, 72%).

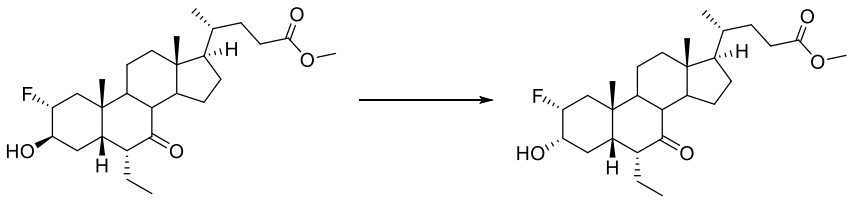
R_f (PE 40-60/acetone, 60/40) 0.54. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.67 (1H, ddd, *J* = 50.6, 5.1, 3.3 Hz, $H_{2\beta}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.75 (1H, dt, *J* = 7.8, 4.8 Hz, $H_{6\beta}$), 2.48-1.90 (11H, m), 1.84-1.38 (8H, m), 1.35 (3H, s, H_{19}), 1.33-1.04 (5H, m), 0.92 (3H, d, *J* = 6.5 Hz, H_{21}), 0.81 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.6 (C_7), 205.8 (d, *J* = 18.3 Hz, C_3), 174.6 (C_{24}), 91.9 (d, *J* = 181.2 Hz, C_2), 54.7 (C_{17}), 52.6 (CH), 51.7 (CH), 51.4 ($C_{24}O\mathbf{C}H_3$), 50.4 (CH), 48.6 (CH), 47.3 (d, *J* = 4.4 Hz, CH), 42.5 (C_{13}), 40.8 (d, *J* = 19.8 Hz, C_1), 38.7 (CH_2), 36.7 (C_{10}), 35.1 (2 x CH), 31.0 (C_{23}), 30.9 (C_{22}), 28.2 (CH_2), 24.4 (CH_2), 23.1 (C_{19}), 22.5 (CH_2), 18.8 (CH_2), 18.3 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 11.9 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -188.2 (1F, ddd, *J* = 51.2, 40.8, 12.1 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 466.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{42}FO_4$ $[M+H]^+$, m/z calculated: 449.3062; found: 449.3056. **IR** (neat) 3668 (w), 2944 (m), 2875 (m), 1734 (s), 1708 (s), 1457 (m), 1436 (m), 1379 (m), 1253 (s), 1197 cm^{-1}.

Methyl-2α-fluoro-3α-hydroxyl-6α-ethyl-7-oxo-5β-cholan-24-oate (3.20) and methyl-2α-fluoro-3β-hydroxyl-6α-ethyl-7-oxo-5β-cholan-24-oate (3.22)



3.21 3.20 3.22

To a solution of **3.21** (140 mg, 0.31 mmol, 1.0 equiv) in dry methanol (7 mL) under argon at -78 °C was added sodium borohydride (59 mg, 1.56 mmol, ~5.0 equiv) and cerium trichloride (233 mg, 0.62 mmol, ~2.0 equiv). After 1 hour at -78 °C, the reaction mixture was warmed to rt and then concentrated *in vacuo*. The residue was dissolved in dichloromethane (20 mL) and water (20 mL) and the aqueous phase was then separated and extracted with dichloromethane (3 x 20 mL) and the combined organic layers were washed with sat. Na_2SO_4, filtered and concentrated *in vacuo* to afford 141 mg of crude material as a colourless oil. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **3.20** as a colourless oil (64 mg, 0.14 mmol, 45%) and compound **3.22** as a white residue (39 mg, 0.09 mmol, 28%).



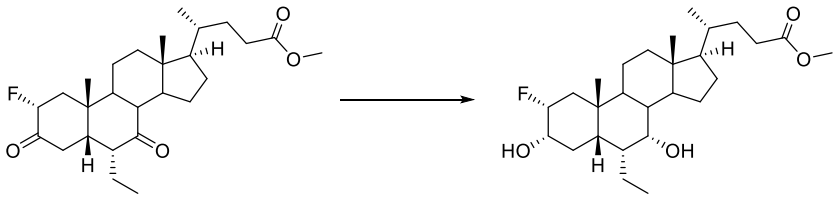
3.16 3.20

*Compound **3.20** was also prepared by the following method:* To a solution of **3.16** (200 mg, 0.44 mmol, 1.0 equiv) in dry dichloromethane (1.5 mL) under argon at 0 °C was added dry pyridine (0.4 mL) and triflic anhydride (0.15 mL, 0.88 mmol, ~2.0 equiv). After 1 h at 0 °C, the reaction was quenched water (10 mL) and the reaction mixture extracted with dichloromethane (3 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a pale-yellow oil. The crude oil was dissolved in dry DMF (2 mL) under argon at rt and received KNO_2 (113 mg, 1.33 mmol, ~3.0 equiv). After 18 h at rt, the reaction mixture was diluted with dichloromethane (5 mL) and quenched with water (5 mL). The aqueous layer was separated and extracted with dichloromethane (3 x 10 mL) and the combined organic layers dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a yellow oil (130 mg). Purification by flash column chromatography (Biotage SNAP KP-Sil 10 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **3.20** as a colourless oil (93 mg, 0.21 mmol, 46%).

Compound **3.20**: R_f (PE 40-60/acetone, 60/40) 0.48. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.73 (1H, dtd, *J* = 51.7, 3.7, 1.1 Hz, $H_{2\beta}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.50 (1H, dddd, 29.0, 11.7, 4.7, 2.6 Hz, $H_{3\beta}$), 2.70 (1H, dd, *J* = 13.1, 5.5 Hz, $H_{6\beta}$), 2.42-2.32 (2H, m), 2.25-2.13 (2H, m), 2.01-1.76 (7H, m), 1.72-1.60 (2H, m), 1.50-1.29 (7H, m), 1.23 (3H, s, H_{19}), 1.20-1.10 (5H, m), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.82 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.5 (C_7), 174.7 (C_{24}), 92.0 (d, *J* = 171.7 Hz, C_2), 71.0 (d, *J* = 19.1 Hz, C_3), 54.7 (C_{17}), 51.6 (CH), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.9 (CH), 50.3 (CH), 48.8 (CH), 45.9 (d, *J* = 5.9Hz, CH), 42.6 (C_{13}), 38.8 (CH_2), 38.2 (d, *J* = 18.3 Hz, C_1), 35.9 (d, *J* = 1.5 Hz, C_{10}), 35.2 (CH), 31.00 (C_{23}), 30.97 (C_{22}), 28.3 (CH_2), 26.6 (CH_2), 24.5 (CH_2), 23.5 (C_{19}), 22.6 (CH_2), 18.9 (CH_2), 18.3 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 12.0 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -202.3 (1F, tdd, *J* = 51.2, 29.5, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 468.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{44}FO_4$ $[M+H]^+$, m/z calculated: 451.3218; found: 451.3214. **IR** (neat) 3479 (w), 2948 (m), 2875 (m), 2254 (m), 1730 (s), 1707 (s), 1436 (m), 1379 (m), 1256 (s), 1196 cm^{-1}.

Compound **3.22**: R_f (PE 40-60/acetone, 60/40) 0.56. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.53 (1H, dq, *J* = 46.8, 2.3 Hz, $H_{2\beta}$), 4.01 (1H, dsxt, 6.4, 2.8 Hz, $H_{3\alpha}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.75 (1H, dd, *J* = 13.0, 5.9 Hz, $H_{6\beta}$), 2.38-2.30 (2H, m), 2.25-2.08 (4H, m), 2.03-1.86 (3H, m), 1.82-1.60 (5H, m), 1.56-1.28 (8H, m), 1.26 (3H, s, H_{19}), 1.17-1.10 (3H, m), 0.91 (3H, d, *J* = 6.4 Hz, H_{21}), 0.82 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.4 (C_7), 174.8 (C_{24}), 91.0 (d, *J* = 171.7 Hz, C_2), 66.7 (d, *J* = 30.1Hz, C_3), 54.7 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 51.3 (CH), 50.4 (CH), 48.8 (CH), 45.9 (d, *J* = 35.1Hz, CH), 45.2 (CH), 42.6 (C_{13}), 38.8 (CH_2), 36.1 (d, *J* = 1.5 Hz, C_{10}), 35.2 (CH), 34.4 (d, *J* = 19.1 Hz, C_1), 31.00 (C_{23}), 30.98 (C_{22}), 28.3 (CH_2), 25.0 (CH_2), 24.6 (CH_2), 24.0 (C_{19}), 22.4 (CH_2), 18.7 (CH_2), 18.3 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 12.0 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.3 (1F, tt, *J* = 46.8, 8.7 Hz $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 468.4 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{44}FO_4$ $[M+H]^+$, m/z calculated: 451.3218; found: 451.3228. **IR** (neat) 3479 (w), 2946 (m), 2254 (m), 1730 (s), 1704 (s), 1437 (m), 1381 (m), 1256 (s), 1198 cm^{-1}.

Methyl-2α-fluoro-3α,7α-dihydroxyl-6α-ethyl-5β-cholan-24-oate (3.23)



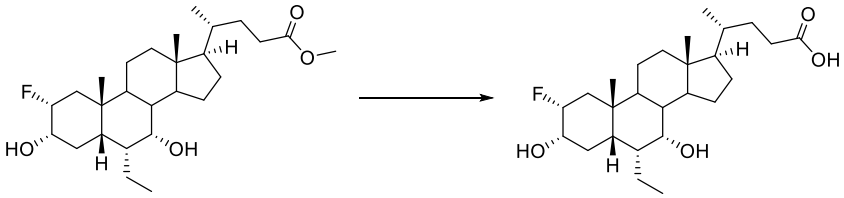
To a solution of **3.21** (140 mg, 0.31 mmol, 1.0 equiv) in dry methanol (7 mL) under argon at rt was added sodium borohydride (59 mg, 1.6 mmol, ~5.0 equiv). After 1 hour at rt, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in dichloromethane (10 mL) and H_2O (10 mL) and the aqueous phase was separated and extracted with dichloromethane (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 184

mg of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **3.23** as a white residue (111 mg, 0.25 mmol, 79%).

The reduction of the ketone group with sodium borohydride in methanol at rt will herein be referred to as *General Procedure H*.

R_f (PE 40-60/acetone, 60/40) 0.32. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.74 (1H, dtd, *J* = 52.2, 3.7, 1.5 Hz, $H_{2\beta}$), 3.71 (1H, s $H_{7\beta}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.41-3.34 (1H, m, $H_{3\beta}$), 2.42-2.31 (2H, m), 2.26-2.09 (3H, m), 1.99-1.86 (3H, m), 1.82-1.73 (2H, m), 1.70-1.51 (4H, m), 1.46-1.26 (10H, m), 1.21-1.13 (3H, m), 0.93 (3H, d, *J* = 6.5 Hz, H_{21}), 0.916 (3H, s, H_{19}), 0.916 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 93.0 (d, *J* = 171.7 Hz, C_2), 72.1 (d, *J* = 19.1 Hz, C_3), 70.7 (C_7), 55.7 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.5 (CH), 45.4 (CH), 42.8 (C_{13}), 41.0 (CH), 40.3 (CH), 39.5 (CH_2), 39.3 (d, *J* = 19.1 Hz, C_1), 35.8 (d, *J* = 1.5 Hz, C_{10}), 35.6 (d, *J* = 4.4 Hz, CH), 35.4 (CH), 31.0 (C_{23}, C_{22}), 28.21 (d, *J* = 2.2 Hz, C_4), 28.16 (CH_2), 23.6 (CH_2), 23.3 (C_{19}), 22.3 (CH_2), 21.5 (CH_2), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -202.5 (1F, tdd, *J* = 52.0, 29.5, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 470.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{49}NFO_4$ $[M+NH_4]^+$, m/z calculated: 470.3640; found: 470.3635. **IR** (neat) 3452 (w), 2937 (m), 2871 (m), 1728 (s), 1435 (m), 1376 (m), 1196 (m), 1159 (m) cm^{-1}.

2α-fluoro-3α,7α-dihydroxyl-6α-ethyl-5β-cholanic acid (1.53)

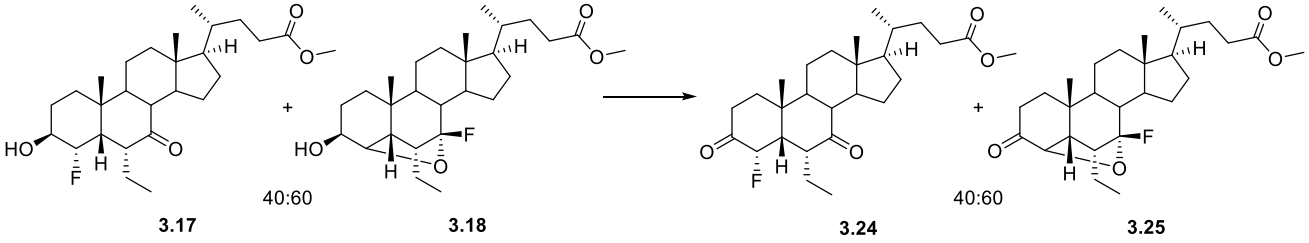


Prepared according to *General Procedure E* (see: *page 160*) using 110 mg of compound **3.23** (0.24 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using dichloromethane/methanol (95/5 to 90/10) as the eluent yielded compound **1.53** as a white residue (88 mg, 0.21 mmol, 82%).

R_f (dichloromethane/methanol), 90/10) 0.34. **$[\alpha]_D$:** -2.90 (c = 1.63, methanol, 25 °C). **$[\alpha]_D$:** +19.7 (c = 0.48, chloroform, 28 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.75 (1H, dt, *J* = 52.5, 3.7 Hz, $H_{2\beta}$), 3.72 (1H, s $H_{7\beta}$), 3.39 (1H, dd, *J* = 28.2, 10.2 Hz, $H_{3\beta}$), 2.43-2.36 (2H, m), 2.30-2.22 (1H, m), 2.19-2.09 (2H, m), 1.98-1.89 (3H, m), 1.86-1.75 (2H, m), 1.71-1.52 (4H, m), 1.47-1.14 (13H, m), 0.94 (3H, d, *J* = 6.1 Hz, H_{21}), 0.93 (3H, t, *J* = 7.1 Hz, $C_6CH_2C\mathbf{H}_3$), 0.92 (3H, s, H_{19}), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.2 (C_{24}), 92.9 (d, *J* = 171.7 Hz, C_2), 72.1 (d, *J* = 19.8 Hz, C_3), 70.7 (C_7), 55.7 (C_{17}), 50.4 (CH),

45.4 (CH), 42.8 (C_{13}), 41.0 (CH), 40.3 (CH), 39.5 (CH_2), 39.3 (d, *J* = 18.3 Hz, C_1), 35.9 (d, *J* = 1.5 Hz, C_{10}), 35.6 (d, *J* = 4.4 Hz, CH), 35.3 (CH), 30.80 (C_{23}), 30.75 (C_{22}), 28.3 (d, *J* = 1.5 Hz, CH_2), 28.2 (CH_2), 23.6 (CH_2), 23.3 (C_{19}), 22.3 (CH_2), 21.5 (CH_2), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -202.7 (1F, tdt, *J* = 51.6, 28.6, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 456.4 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{26}H_{43}FO_4Na$ $[M+Na]^+$, m/z calculated: 461.3038; found: 461.3041. **IR** (neat) 3427 (w), 2936 (m), 2870 (m), 1705 (s), 1457 (m), 1377 (m), 1189 (m), 1158 (m) cm^{-1}.

Methyl-3,7-dioxo-4α-fluoro-6α-ethyl-5β-cholan-24-oate (3.24) and methyl-3-oxo-4α,7α-epoxy-6α-ethyl-7β-fluoro-5β-cholan-24-oate (3.25)

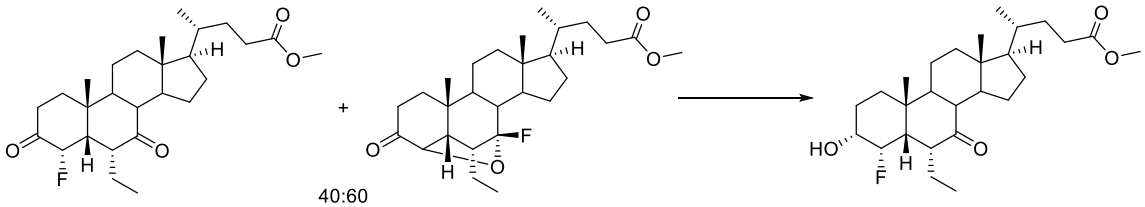


To a solution of a 40:60 ratio of **3.17** and **3.18** (430 mg, 0.96 mmol, 1.0 equiv) in dichloromethane (12 mL) at rt was added Dess-Martin periodinane (815 mg, 1.92 mmol, ~2.0 equiv) and H_2O (0.15 mL). After 3 h at rt, the reaction was deemed complete by TLC and the reaction mixture was quenched with sat. $NaHCO_3$ solution (10 mL) and filtered over Celite and washed with dichloromethane (50 mL). The aqueous phase was then separated and extracted with dichloromethane (3 x 25 mL) and the combined organic layers were washed with sat. $Na_2S_2O_3$ solution (50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 529 mg of crude material as a colourless oil. Purification by flash column chromatography (Biotage SNAP KP-Sil 25 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded an inseparable mixture of compound **3.24** and **3.25** in a 40:60 ratio as a white residue (379 mg, 0.84 mmol, 88%).

Compound **3.24**: R_f (PE 40-60/acetone, 70/30) 0.49. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.55 (1H, ddd, *J* = 51.0, 2.5, 1.2 Hz, $H_{4\beta}$), 3.67 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.89 (1H, tt, *J* = 13.9, 4.5 Hz, $H_{2\alpha}$), 2.59 (1H, dd, *J* = 13.3, 6.5 Hz), 2.08-1.34 (16H, m), 1.30 (3H, s, H_{19}), 1.18-1.02 (7H, m), 0.99-0.88 (6H, m, H_{21} and $C_6CH_2C\mathbf{H}_3$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 210.7 (C_7), 205.5 (d, *J* = 21.3 Hz, C_3), 174.6 (C_{24}), 92.1 (d, *J* = 175.3 Hz, C_4), 56.4 (d, *J* = 20.5 Hz, C_5), 54.6 (C_{17}), 51.4 ($C_{24}O\mathbf{C}H_3$), 49.5 (CH), 44.7 (d, *J* = 5.1 Hz, CH), 43.0 (C_{13}), 40.8 (CH_2), 38.8 (CH_2), 36.6 (C_{10}), 36.3 (CH_2), 35.2 (CH), 33.7 (d, *J* = 2.2 Hz, C_1), 31.00 (C_{23} + C_{22}), 25.0 (CH_2), 23.3 (C_{19}), 22.7 (CH_2), 18.4 (C_{21}), 17.5 (CH), 17.3 (CH_2), 12.1 ($C_6CH_2\mathbf{C}H_3$), 12.0 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -188.0 (1F, dd *J* = 48.6, 43.4 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 466.5 $[M+NH_4]^+$, 100%.

Compound **3.25**: R_f (PE 40-60/acetone, 70/30) 0.49. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.41 (1H, d, *J* = 4.9 Hz, $H_{4\beta}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.28-1.00 (25H, m), 0.99-0.88 (9H, m, H_{19}, H_{21} and $C_6CH_2C\mathbf{H}_3$), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 209.8 (d, *J* = 1.5 Hz, C_3), 174.7 (C_{24}), 122.0 (d, *J* = 239.2 Hz, C_7), 78.3 (d, *J* = 2.9 Hz, CH), 54.5 (C_{17}), 51.4 ($C_{24}O\mathbf{C}H_3$), 49.6 (CH), 48.7 (d, *J* = 16.9 Hz, CH), 46.6 (d, *J* = 24.9 Hz, CH), 46.3 (d, *J* = 7.3 Hz, CH), 44.2 (C_{13}), 39.6 (CH_2), 35.1 (CH), 34.9 (C_{10}), 34.4 (CH_2), 32.5 (CH_2), 30.97 (C_{23}), 30.9 (C_{22}), 28.4 (d, *J* = 2.2 Hz, CH_2), 25.4 (d, *J* = 7.3 Hz, CH_2), 22.2 (CH_2), 21.9 (C_{19}), 20.7 (CH), 18.33 (C_{21}), 12.3 ($C_6CH_2\mathbf{C}H_3$), 11.9 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -134.0 (1F, s, $F_{7\beta}$) ppm. **LRMS** (ESI^+) m/z: 466.5 $[M+NH_4]^+$, 100%.

Methyl-3α-hydroxyl-4α-fluoro-6α-ethyl-7-oxo-5β-cholan-24-oate (3.26)

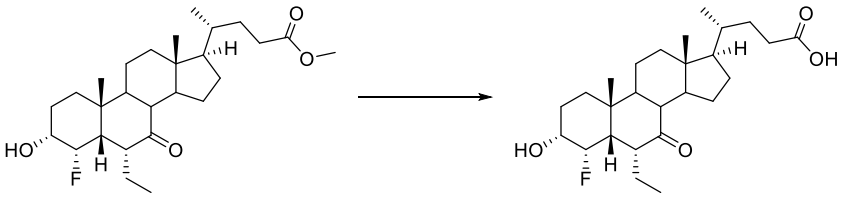


3.24 **3.25** **3.26**

Prepared according to *General Procedure H* (see: *page 172*) using 440 mg of a 40:60 ratio of compounds **3.24** and **3.25** (0.99 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP Ultra KP-Sil 25 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **3.26** as a white residue (140 mg, 0.31 mmol, 31%). The corresponding 4-O-7 oxacyclic-derivative, from **3.25**, was not isolated.

R_f (PE 40-60/acetone, 60/40) 0.32. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.74 (1H, dt, *J* = 51.4, 2.7 Hz, $H_{4\beta}$), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.51-3.41 (1H, m, $H_{3\beta}$), 2.55 (1H, dd, *J* = 13.3, 6.5 Hz), 2.40-2.33 (2H, m), 2.28-2.18 (2H, m), 2.01-1.98 (6H, m), 1.87-1.26 (11H, m), 1.23 (3H, s, H_{19}), 1.20-0.95 (4H, m), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.88 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}), ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 210.8 (C_7), 174.7 (C_{24}), 91.5 (d, *J* = 176.1 Hz, C_4), 71.5 (d, *J* = 19.1 Hz, C_3), 54.6 (C_{17}), 53.3 (d, *J* = 18.3 Hz, C_5), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.6 (CH), 49.4 (CH), 49.1 (CH), 44.9 (d, *J* = 5.9 Hz, C_6), 43.0 (C_{13}), 38.9 (CH_2), 35.7 (d, *J* = 1.5 Hz, C_{10}), 35.2 (CH), 33.9 (CH_2), 31.04 (C_{23}), 30.99 (C_{22}), 28.4 (CH_2), 25.1 (CH_2), 24.8 (d, *J* = 2.2 Hz, C_2), 23.8 (C_{19}), 22.3 (CH_2), 18.6 (CH_2), 18.4 (C_{21}), 12.13 ($C_6CH_2\mathbf{C}H_3$), 12.09 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -205.0 (1F, ddd, *J* = 51.6, 42.9, 28.6 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 468.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{44}FO_4$ $[M+H]^+$, m/z calculated: 451.3218; found: 451.3226. **IR** (neat) 3455 (w), 2941 (m), 2874 (m), 1735 (s), 1713 (s), 1453 (m), 1379 (m), 1259 (m), 1196 (m), 1163 (m) cm^{-1}.

Methyl-3α-hydroxyl-4α-fluoro-6α-ethyl-7-oxo-5β-cholan-24-oate (3.28)

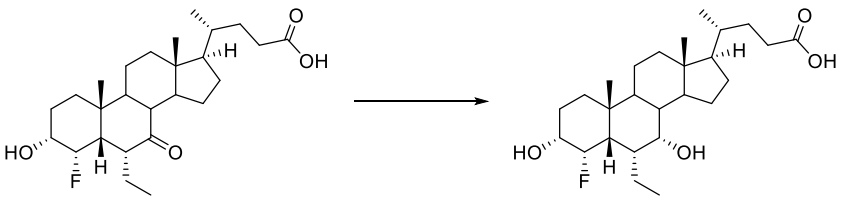


3.26 3.28

Prepared according to *General Procedure E* (see: *page 160*) using 145 mg of compound **3.26** (0.33 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using dichloromethane/methanol (98/2 to 90/10) as the eluent yielded compound **3.28** as a white residue (124 mg, 0.28 mmol, 87%).

R_f (dichloromethane/methanol, 90/10) 0.42. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.74 (1H, dt, *J* = 51.5, 2.3 Hz, $H_{4\beta}$), 3.66 (1H, dddd, *J* = 28.9, 11.6, 5.1, 2.5 Hz, $H_{3\beta}$), 2.56 (1H, q, *J* = 6.6 Hz, $H_{6\beta}$), 2.43-2.35 (2H, m), 2.33-2.18 (2H, m), 2.01-1.90 (5H, m), 1.87-1.68 (4H, m), 1.58 (1H, d, *J* = 12.6 Hz), 1.50-1.26 (8H, m), 1.23 (3H, s, H_{19}), 1.19-0.99 (4H, m), 0.93 (3H, d, *J* = 6.5 Hz, H_{21}), 0.88 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 210.9 (C_7), 179.7 (C_{24}), 91.5 (d, *J* = 175.3 Hz, C_4), 71.5 (d, *J* = 19.8 Hz, C_3), 54.6 (C_{17}), 53.3 (d, *J* = 17.6 Hz, C_5), 49.6 (CH), 49.4 (CH), 49.1 (CH), 44.9 (d, *J* = 5.9 Hz, C_6), 43.0 (C_{13}), 38.9 (CH_2), 35.7 (C_{10}), 35.1 (CH), 33.9 (CH_2), 30.9 (C_{23}), 30.7 (C_{22}), 28.3 (CH_2), 25.1 (CH_2), 24.8 (C_2), 23.8 (C_{19}), 22.3 (CH_2), 18.6 (CH_2), 18.3 (C_{21}), 12.14 ($C_6CH_2\mathbf{C}H_3$), 12.08 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -204.9 (1F, ddd, *J* = 51.6, 42.9, 28.6 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 454.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{26}H_{42}FO_4$ $[M+H]^+$, m/z calculated: 437.3062; found: 437.3063. **IR** (neat) 3398 (w), 2942 (m), 2874 (m), 1710 (s), 1457 (m), 1411 (m), 1381 (m), 1266 (m), 1163 (m) cm^{-1}.

3α,7α-dihydroxyl-4α-fluoro-6α-ethyl-5β-cholanic acid (1.55)



3.28 1.55

To a solution of **3.28** (100 mg, 0.23 mmol, 1.0 equiv) in methanol (10 mL) at rt was added H_2O (2 mL) and heated to 80 °C without a reflux condenser for 2 h to remove methanol. The reaction mixture received 0.05M NaOH solution (0.10 mL) and was stirred at 80 °C for 1 hour. The reaction mixture received sodium borohydride (40 mg, 1.15 mmol, ~5.0 equiv) and was stirred at 80 °C for 2 h. The reaction mixture then received another portion of sodium borohydride (40 mg, 1.15 mmol, ~5.0 equiv). After a further 2 h at 80 °C the reaction was deemed complete and the reaction mixture

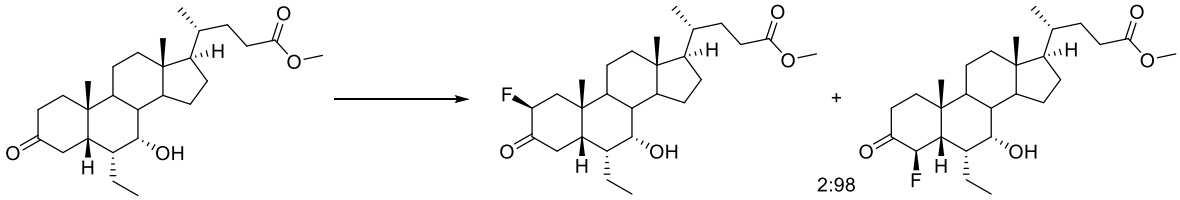
was cooled to rt and quenched by the addition of 1M hydrochloric acid (10 mL). The reaction mixture was extracted with ethyl acetate (3 x 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 101 mg of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using dichloromethane/methanol (98/2 to 90/10) as the eluent yielded compound **1.55** as a white residue (81 mg, 0.18 mmol, 81%).

R_f (PE 40-60/acetone, 70/30) 0.18. **$[\alpha]_D$:** +19.7 (c = 0.48, methanol, 28 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.83 (1H, dt, *J* = 51.4, 2.3 Hz, $H_{4\beta}$), 3.61 (1H, s, $H_{7\beta}$), 3.55 (1H, dddd, *J* = 28.7, 11.0, 6.5 Hz, $H_{3\beta}$), 2.37 (1H, ddd, *J* = 15.3, 10.5, 5.0 Hz), 2.26-2.17 (2H, m), 2.01-1.02 (25H, m), 0.95 (3H, s, H_{19}), 0.94-0.91 (6H, m, $\mathbf{H}_{21}$ and $C_6CH_2C\mathbf{H}_3$), 0.64 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.3 (C_{24}), 93.7 (d, *J* = 168.7 Hz, C_4), 72.1 (d, *J* = 21.3 Hz, C_3), 67.9 (C_7), 55.7 (C_{17}), 53.6 (d, *J* = 33.8 Hz, C_5), 50.9 (CH), 48.3 (d, *J* = 18.3 Hz, C_6), 42.4 (C_{13}), 40.5 (CH), 40.0 (CH), 39.5 (CH_2), 35.53 (C_{10}), 35.47 (d, *J* = 5.1 Hz, CH), 34.8 (CH_2), 31.0 (C_{23}), 30.8 (C_{22}), 28.1 (CH_2), 24.5 (CH_2), 23.6 (C_2), 23.5 (C_{19}), 21.7 (CH_2), 21.4 (CH_2), 18.2 (C_{21}), 11.9 ($C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -204.9 (1F, td, *J* = 48.6, 33.0 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 456.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{26}H_{43}FO_4Na$ $[M+Na]^+$, m/z calculated: 461.3038; found: 461.3041. **IR** (neat) 2934 (m), 2872 (m), 17105 (s), 1451 (m), 1413 (m), 1377 (m), 1230 (m), 1166 (m), 1069 (m) cm^{-1}.

8.4 Synthesis of OCA (NZP084) 2β-fluoro and 4β-fluoro analogues

The synthesis and characterisation of the following compounds is presented in the order of reactions discussed in Chapter 4.

Methyl-2β-fluoro-3-oxo-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (4.1) and methyl-3-oxo-4β-fluoro-6α-ethyl-7α-hydroxyl-5β-cholan-24-oate (4.2)



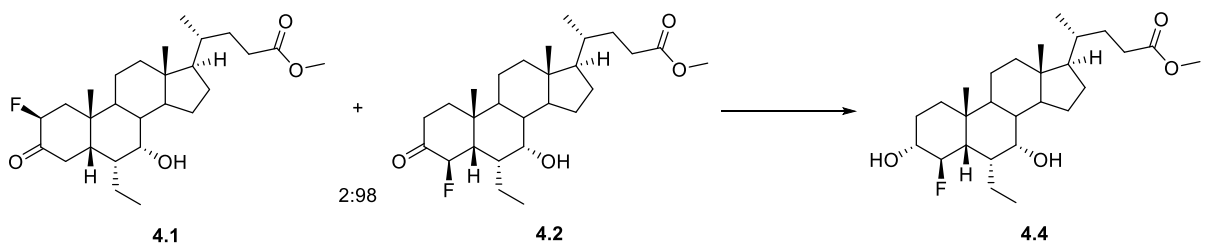
2.6 **4.1** **4.2**

To a solution of diisopropylamine (0.78 mL, 5.54 mmol, ~12 equiv) in dry THF (6.9 mL) under argon at -78 °C was added *n*-BuLi (1.44 mL, 2.31 mmol, ~5.0 equiv) dropwise. After stirring for 15 min, to the reaction mixture was then added trimethylsily chloride (0.29 mL, 2.31 mmol, ~5.0 equiv) and was stirred for a further 20 min. To the reaction mixture was then added a solution of **2.6** (200 mg, 0.46 mmol, 1.0 equiv) in dry THF (3 mL) dropwise and triethylamine (1.16 mL, 8.32 mmol, ~18 equiv). After 1 hour at -78 °C, the reaction mixture was warmed to -20 °C and quenched by the

addition of sat. $NaHCO_3$ solution (5 mL) and warmed to rt over 2 h. The aqueous phase was separated and extracted with ethyl acetate (3 x 10 mL) and the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 271 mg of crude material as a pale-yellow gummy paste. To a solution of the crude material in acetonitrile (13 mL) was added Selectfluor® (285 mg, 0.81 mmol, ~1.5 equiv). After 16 h at rt, the solvent was removed *in vacuo* and the residue was diluted with ethyl acetate (20 mL) and received 2M hydrochloric acid (30 mL). The aqueous phase was separated and extracted with ethyl acetate (3 x 15 mL) and the combined organic layers were washed with brine (200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 196 mg of crude material as pale-green solid. Purification by HPLC using hexane/acetone (90/10) as the eluent yielded an inseparable mixture of compounds **4.1** and **4.2** in a 2:98 ratio as a colourless oil (79 mg, 0.18 mmol, 38% over two steps). Characteristic signals for compound **4.1**, found as a trace impurity in the sample described isolated above, are reported to help identify this molecule in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.04 (1H, ddd, *J* = 49.6, 13.8, 6.5 Hz, H_2). **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -194.9 (1F, dq, *J* = 48.6, 7.5 Hz, $F_{2\beta}$) ppm.

Compound **4.2**: **R_f** (PE 40-60/acetone), 80/20) 0.22. **$[\alpha]_D$:** +27.2 (c = 0.49, methanol, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.94 (1H, dd, *J* = 46.5, 10.9 Hz, $H_{4\alpha}$), 3.88 (1H, s, $H_{7\beta}$), 3.65 (3H, s, $C_{24}OCH_3$), 2.49 (1H, td, *J* = 14.6, 5.0 Hz), 2.35 (1H, ddd, *J* = 15.4, 10.2, 5.1 Hz, $H_{23'}$), 2.30-2.26 (1H, m), 2.21 (1H, ddd, *J* = 15.8, 9.2, 6.2 Hz, $H_{23''}$), 2.11 (1H, ddd, *J* = 14.2, 3.9, 3.2 Hz), 1.99 (1H, dt, *J* = 12.5, 3.2 Hz), 1.95-1.30 (17H, m), 1.25-1.14 (3H, m), 1.04 (3H, s, H_{19}), 0.92 (3H, d, *J* = 6.6 Hz, H_{21}), 0.91 (3H, t, *J* = 7.1 Hz, $C_6CH_2C\mathbf{H}_3$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 206.4 (d, *J* = 14.7 Hz, C_3), 174.7 (C_{24}), 95.2 (d, *J* = 187.1 Hz, C_4), 71.0 (C_7), 55.7 (C_{17}), 53.5 (d, *J* = 14.7 Hz, C_5), 51.4 ($C_{24}O\mathbf{C}H_3$), 50.3 (C_{14}), 42.6 (C_{13}), 42.3 (d, *J* = 1.5 Hz, CH), 40.1 (CH), 39.3 (CH_2), 38.4 (d, *J* = 8.8 Hz, C_{10}), 36.7 (CH_2), 35.4 (CH), 35.27 (d, *J* = 2.9 Hz, CH_2), 35.2 (CH), 30.91 (CH_2), 30.87 (CH_2), 28.1 (CH_2), 23.9 (d, *J* = 8.1 Hz, CH_2), 23.5 (CH_2), 22.6 (C_{19}), 21.2 (CH_2), 18.2 (C_{21}), 12.3 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -194.3 (1F, dd, *J* = 46.8, 13.9 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 468.4 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{43}FO_4Na$ $[M+Na]^+$, m/z calculated: 473.3038; found: 473.3040. **IR** (neat) 2931 (s), 2870 (s), 2360 (w), 1732 (s), 1707 (s), 1438 (m), 1375 (m), 1168 (m) cm^{-1}.

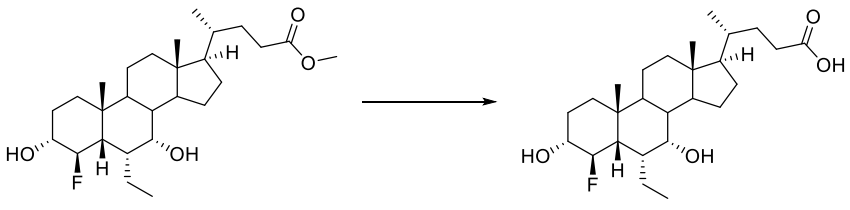
Methyl-3α,7α-dihydroxyl-4β-fluoro-6α-ethyl-5β-cholan-24-oate (4.4)



To a solution of a 2:98 ratio of **4.1** and **4.2** (75 mg, 0.17 mmol, 1.0 equiv) in dry THF (6.7 mL) under argon at rt was added sodium borohydride (19 mg, 0.50 mmol, ~3.0 equiv). After 16 h at rt, the reaction was deemed complete by TLC and the reaction mixture was diluted with ethyl acetate (10 mL) and quenched by the slow addition of water (8 mL). The aqueous phase was separated and extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were washed with water (100 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 77 mg of crude material as a white residue. Purification by HPLC using hexane/acetone (90/10) as the eluent yielded compound **4.4** as a colourless oil (55 mg, 0.12 mmol, 74%).

R_f (PE 40-60/acetone), 80/20) 0.17. **$[\alpha]_D$:** +26.3 (c = 0.14, methanol, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.31 (1H, ddd, *J* = 50.0, 10.4, 8.9 Hz, $H_{4\alpha}$), 3.82 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 3.54 (1H, dddd, *J* = 19.8, 14.1, 8.7, 5.1 Hz, $H_{3\beta}$), 2.36 (1H, ddd, *J* = 15.4, 10.1, 5.7 Hz, $H_{23'}$), 2.23 (1H, ddd, *J* = 16.0, 9.3, 6.2 Hz, $H_{23''}$), 1.96-1.92 (2H, m), 1.83-1.05 (23H, m), 0.97 (3H, s, H_{19}), 0.94-0.92 (6H, m, H_{21} and $C_6CH_2C\mathbf{H}_3$), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 99.7 (d, *J* = 168.0 Hz, C_4), 74.8 (d, *J* = 19.8 Hz, C_3), 70.9 (C_7), 55.7 (C_{17}), 51.5 ($C_{24}OCH_3$), 50.4 (C_{14}), 49.3 (d, *J* = 13.1 Hz, C_5), 42.7 (C_{13}), 42.1 (d, *J* = 1.5 Hz, CH), 40.3 (CH), 39.4 (CH_2), 39.0 (d, *J* = 9.5 Hz, C_{10}), 35.4 (CH), 35.3 (CH), 34.4 (d, *J* = 1.5 Hz, CH_2), 31.0 (CH_2), 30.9 (CH_2), 28.1 (CH_2), 26.0 (d, *J* = 8.1 Hz, CH_2), 24.3 (d, *J* = 8.1 Hz, CH_2), 23.6 (CH_2), 23.3 (C_{19}), 20.7 (CH_2), 18.2 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -189.0 (1F, d, *J* = 50.3 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 470.4 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{49}FO_4N$ $[M+NH_4]^+$, m/z calculated: 470.3640; found: 470.3647. **IR** (neat) 3480 (m), 2931 (s), 2870 (s), 1727 (s), 1456 (m), 1377 (m), 1195 (m), 1163 (m), 1139 (m) cm^{-1}.

3α,7α-dihydroxyl-4β-fluoro-6α-ethyl-5β-cholanic acid (1.56)

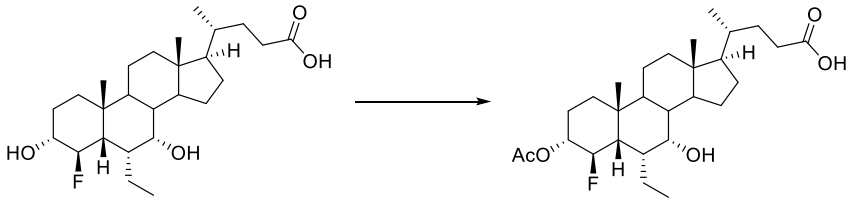


Prepared according to *General Procedure A* (see: *page 147*) using 58 mg of compound **4.4** (0.13 mmol, 1.0 equiv) and 5 mL of a 5% sodium hydroxide in methanol solution. Purification by HPLC using hexane/acetone (70/30) as the eluent yielded compound **1.56** as a colourless oil (40 mg, 0.09 mmol, 72%).

R_f (PE 40-60/acetone), 70/30) 0.18. **$[\alpha]_D$:** +20.0 (c = 0.11, chloroform, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.31 (1H, dt, *J* = 49.9 9.5 Hz, $H_{4\alpha}$), 3.83 (1H, s, $H_{7\beta}$), 3.55 (1H, ddq, *J* = 13.9, 8.9, 5.1 Hz, $H_{3\beta}$), 2.40 (1H, ddd, *J* = 15.5, 10.4, 5.3 Hz, $H_{23'}$), 2.26 (1H, ddd, *J* = 15.8, 9.5, 6.6 Hz, $H_{23''}$), 1.97-1.91 (2H,

m), 1.85-1.08 (21H, m), 0.97 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.5 Hz, H_{21}), 0.93 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.7 (C_{24}), 99.6 (d, *J* = 168.7 Hz, C_4), 74.9 (d, *J* = 19.8 Hz, C_3), 70.9 (C_7), 55.7 (C_{17}), 50.4 (C_{14}), 49.3 (d, *J* = 13.2 Hz, C_5), 42.7 (C_{13}), 42.1 (d, *J* = 1.5 Hz, C_6), 40.3 (C_8), 39.4 (C_{12}), 39.0 (d, *J* = 9.5 Hz, C_{10}), 35.32 (C_9), 35.31 (C_{20}), 34.4 (d, *J* = 1.5 Hz, C_1), 30.9 (C_{23}), 30.7 (C_{23}), 28.1 (C_{16}), 26.0 (d, *J* = 8.1 Hz, C_2), 24.3 (d, *J* = 8.8 Hz, $C_6\mathbf{C}H_2CH_3$), 23.6 (CH_2), 23.3 (C_{19}), 20.7 (CH_2), 18.2 (C_{21}), 12.5 (d, *J* = 2.2 Hz, $C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -188.7 (1F, d, *J* = 48.6 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 456.2, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{26}H_{43}FO_4Na$ $[M+Na]^+$, m/z calculated: 461.3038; found: 461.3037. **IR** (neat) 2926 (m), 2871 (m), 1717 (s), 1462 (m), 1378 (m), 758 (m), 731 (s) cm^{-1}.

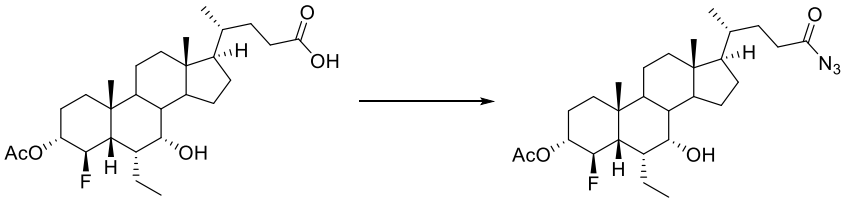
3α-acetoxy-4β-fluoro-6α-ethyl-7α-hydroxyl-5β-cholanic acid (4.5)



Prepared according to *General Procedure C* (see: *page 151*) using 2.08 g of compound **1.56** (4.74 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil 100 g cartridge) using dichloromethane/methanol (100/0 to 97/3) as the eluent yielded compound **4.5** as a colourless oil (660 mg, 1.37 mmol, 29%).

R_f (dichloromethane/methanol, 95/5) 0.33. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.47 (1H, dt, *J* = 49.4, 9.4 Hz, $H_{4\alpha}$), 4.78 (1H, dddd, *J* = 14.1, 11.9, 9.3, 5.0 Hz, $H_{3\beta}$), 3.84 (1H, s, $H_{7\beta}$), 2.41 (1H, ddd, *J* = 15.5, 10.2, 5.3 Hz, $H_{23'}$), 2.27 (1H, ddd, *J* = 15.8, 9.7, 6.6 Hz, $H_{23''}$), 2.06 (3H, s, $C_3OC\mathbf{H}_3$), 1.97-1.89 (2H, m), 1.86 - 1.80 (3H, m), 1.70-1.14 (19H, m), 0.99 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.2 Hz, H_{21}), 0.92 (3H, t, *J* = 7.1 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.6 (C_{24}), 170.8 ($C_3\mathbf{C}OCH_3$), 95.1 (d, *J* = 174.6 Hz, C_4), 76.1 (d, *J* = 19.8 Hz, C_3), 71.0 (C_7), 55.8 (C_{17}), 50.4 (C_{14}), 49.8 (d, *J* = 13.2 Hz, C_5), 42.7 (C_{13}), 42.1 (d, *J* = 1.5 Hz, C_6), 40.2 (C_8), 39.4 (C_{12}), 38.5 (d, *J* = 9.5 Hz, C_{10}), 35.3 (2 x CH), 34.1 (CH_2), 30.9 (CH_2), 30.7 (CH_2), 28.1 (CH_2), 24.2 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 24.1 (d, *J* = 6.6 Hz, C_2), 23.6 (CH_2), 23.2 (C_{19}), 21.2 ($C_3O\mathbf{C}H_3$), 20.7 (CH_2), 18.2 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -188.6 (1F, dt, *J* = 50.3 Hz, 12.1 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 498.2, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{28}H_{45}FO_5Na$ $[M+Na]^+$, m/z calculated: 503.3143; found: 503.3157. **IR** (neat) 2936 (s), 2905 (s), 2871 (m), 1736 (s, $C_3O\mathbf{CO}CH_3$), 1708 (s, **CO**OH), 1378 (m), 1377 (m), 1242 (s), 1162 (m), 1043 (m) cm^{-1}.

3α-acetoxy-4β-fluoro-6α-ethyl-7α-hydroxyl-5β-cholan-24-azide (4.6)

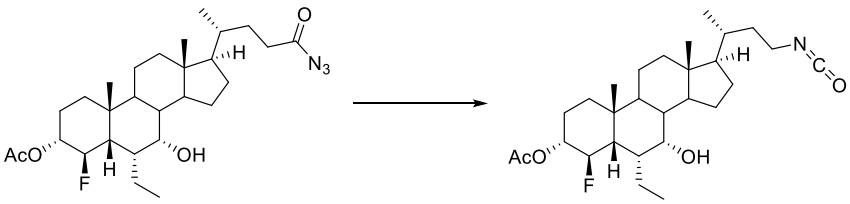


4.5 4.6

To a solution of **4.5** (200 mg, 0.42 mmol, 1.0 equiv) in dry THF (4 mL) under argon at rt was added triethylamine (0.12 mL, 0.83 mmol, ~2.0 equiv) dropwise. The reaction mixture was then cooled to 0 °C and recieved diphenylphosphoryl azide (0.13 mL, 0.62 mmol, ~1.5 equiv) dropwise. The reaction mixture was then stirred for 3 h behind a blast shield and then quenched with ice-cold brine (5 mL) and diluted with dichloromethane (5 mL). The aqueous phase was separated and extracted with dichloromethane (3 x 5 mL) and the combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo* at 0 °C to yield a yellow oil that was used without further purification.

The data presented for compound **4.6** was attained from an impure crude mixture. Characteristic signals are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.48 (1H, ddd, *J* = 49.4, 10.4, 9.2 Hz, $H_{4\alpha}$), 4.78 (1H, dddd, 14.2, 11.9, 9.2, 5.4 Hz, $H_{3\beta}$), 3.83 (1H, s, $H_{7\beta}$), 2.38 (1H, ddd, *J* = 15.8, 10.0, 5.3 Hz, $H_{23'}$), 2.29-2.23 (1H, m, $H_{23''}$), 2.06 (3H, s, $C_3OC\mathbf{H}_3$), 0.98 (3H, s, H_{19}), 0.920 (3H, d, *J* = 6.5 Hz, H_{21}) , 0.919 (3H, t, *J* = 7.2 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 181.1 (C_{24}), 170.7 ($C_3\mathbf{C}OCH_3$), 95.1 (d, *J* = 174.5 Hz, C_4), 76.1 (d, *J* = 19.3 Hz, C_3), 71.0 (C_7), 56.0 (C_{17}), 50.4 (C_{14}), 49.8 (d, *J* = 13.6 Hz, C_5), 42.8 (C_{13}), 42.1 (d, *J* = 1.0 Hz, CH), 38.5 (d, *J* = 9.3 Hz, C_{10}), 24.21 (d, *J* = 8.6 Hz, CH_2), 24.15 (d, *J* = 8.1 Hz, CH_2), 23.1 (C_{19}), 21.2 ($C_3O\mathbf{C}H_3$), 18.2 (C_{21}), 12.5 (d, *J* = 3.1 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.8 (1F, d, *J* = 50.3 Hz, $F_{4\beta}$) ppm. **IR** (neat) 2169 (s, CON_3), 2133 (s, CON_3), 1736 (s, $C_3O\mathbf{CO}CH_3$) cm^{-1}.

3α-acetoxy-4β-fluoro-6α-ethyl-7α-hydroxyl-24-*nor*-5β-cholan-23-isocyanate (4.7)

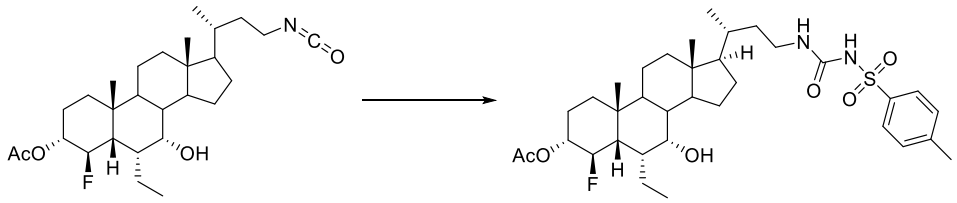


4.6 4.7

A solution of crude **4.6** (105 mg assumed, 0.42 mmol) in dry toluene (3.1 mL) under argon was heated to 125 °C. After 5 h the reaction was cooled to rt and the resulting colourless solution was used without further purification.

The data presented for compound **4.7** was attained from an impure crude mixture. Characteristic signals are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.48 (1H, ddd, *J* = 49.4, 10.4, 9.2 Hz, $H_{4\alpha}$), 4.81-4.73 (1H, m, $H_{3\beta}$), 3.83 (1H, s, $H_{7\beta}$), 3.35 (1H, ddd, *J* = 12.9, 7.8, 4.5 Hz, $H_{23'}$), 3.30-3.24 (1H, m, $H_{23''}$), 2.05 (3H, s, $C_3OC\mathbf{H}_3$), 0.98 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.6 Hz, H_{21}), 0.92 (3H, t, *J* = 7.2 Hz, $C_6CH_2C\mathbf{H}_3$), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.6 ($C_3\mathbf{C}OCH_3$), 121.8 (NCO), 95.1 (d, *J* = 174.8 Hz, C_4), 76.1 (d, *J* = 19.1 Hz, C_3), 70.9 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.8 (d, *J* = 13.8 Hz, C_5), 42.7 (C_{13}), 42.0 (d, *J* = 1.2 Hz, CH), 38.4 (d, *J* = 9.3 Hz, C_{10}), 24.2 (d, *J* = 8.6 Hz, CH_2), 24.1 (d, *J* = 8.8 Hz, CH_2), 23.1 (C_{19}), 21.2 ($C_3O\mathbf{C}H_3$), 18.2 (C_{21}), 12.4 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.5 (1F, dt, *J* = 49.3, 12.7 Hz, $F_{4\beta}$) ppm. **IR** (neat) (2270 (s, NCO), 1737 (s, $C_3O\mathbf{CO}CH_3$) cm^{-1}.

_N_-(3α-acetoxy-4β-fluoro-6α-ethyl-7α-hydroxyl-24-_nor_-5β-cholan-23-yl)-_p_-toluenesulfonyl urea (4.8)



4.7 **4.8**

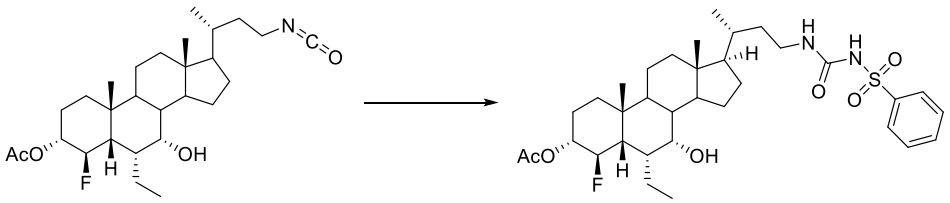
To a crude solution of **4.7** (99 mg assumed, 0.21 mmol) in toluene (3.1 mL) was added *p*-toluenesulfonamide (53 mg, 0.31 mmol, ~1.5 equiv). After 16 h at rt, the reaction was deemed complete by TLC and the reaction mixture was quenched via dropwise addition of 1M hydrochloric acid (2 mL) and diluted with ethyl acetate (5 mL). The aqueous phase was separated and extracted with ethyl acetate (3×5 mL) and the combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column chromatography (Biotage SNAP KP-Sil 10 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **4.8** as a yellow oil (52 mg, 0.08 mmol, 38%).

The reaction of a crude isocyanate with an aromatic sulfonamide will herein be referred to as *General Procedure I.*

R_f (PE 40-60/acetone, 60/40) 0.30. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.78 (2H, d, *J* = 8.2 Hz, C_oH_{Ar}), 7.32 (2H, d, *J* = 8.3 Hz, C_mH_{Ar}), 6.50 (1H, t, *J* = 4.8 Hz, $C_{23}NH$), 5.47 (1H, dt, *J* = 49.4, 9.8 Hz, $H_{4\alpha}$), 4.78 (1H, ddt, *J* = 20.3, 9.1, 4.9 Hz, $H_{3\beta}$), 3.83 (1H, s, $H_{7\beta}$), 3.29 (1H, ddd, *J* = 18.5, 9.8, 5.0 Hz, $H_{23'}$), 3.15 (1H, td, 13.5, 7.7 Hz, $H_{23''}$), 2.44 (3H, s, $C_{Ar}C\mathbf{H}_3$), 2.06 (3H, s, $C_3OC\mathbf{H}_3$), 1.96-1.80 (5H, m), 1.72 - 1.38 (15H, m), 1.26-1.14 (5H, m), 0.99 (3H, s, H_{19}), 0.93 (3H, d, *J* = 6.6 Hz, H_{21}) , 0.92 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.7 ($C_3\mathbf{C}OCH_3$), 151.6 ($C_{23}NH\mathbf{C}O$), 144.7 (C_pH_{Ar}), 136.8 (C_oH_{Ar}), 129.9 (C_mH_{Ar}), 126.9 ($C_{i\,Ar}$), 95.1 (d, *J* = 174.6 Hz, C_4), 76.1 (d, *J* = 19.1 Hz, C_3), 71.0 (C_7),

55.9 (C_{17}), 50.4 (C_{14}), 49.8 (d, *J* = 13.9 Hz, C_5), 42.7 (C_{13}), 42.1 (C_6), 40.2 (C_8), 39.4 (C_{12}), 38.5 (d, *J* = 9.5 Hz, C_{10}), 37.9 (C_{23}), 35.6 (C_{22}), 35.3 (C_9), 34.1 (C_1), 33.8 (C_{20}), 28.3 (C_{16}), 24.2 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 24.1 (d, *J* = 6.6 Hz, C_2), 23.6 (CH_2), 23.1 (C_{19}), 21.6 ($CH_{Ar}C(\mathbf{C}H_3)$), 21.2 ($C_3O\mathbf{C}H_3$), 20.7 (CH_2), 18.5 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.6 (1F, dt, *J* = 49.0, 12.8 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 666.4, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{35}H_{53}FO_6SN_2Na$ $[M+Na]^+$, m/z calculated: 671.3501; found: 671.3511. **IR** (neat) 2934 (m), 2872 (m), 1721 (s), 1668 (s), 1541 (m), 1457 (m), 1247 (m) cm^{-1}.

_N_-(3α-acetoxy-4β-fluoro-6α-ethyl-7α-hydroxyl-24-_nor_-5β-cholan-23-yl)-benzenesulfonyl urea (4.9)

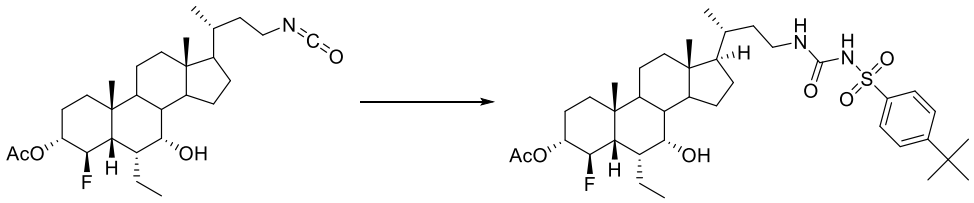


4.7 **4.9**

Prepared according to *General Procedure I* (see: *page 181*) using 99 mg (assumed) of compound **4.7** (0.21 mmol, 1.0 equiv) and 49 mg of benzenesulfonamide to afford, after purification, compound **4.9** as a yellow oil (50 mg, 38%).

R_f (PE 40-60/acetone, 60/40) 0.28. **^{1}H NM**R (400 MHz, $CDCl_3$): δ 7.90 (2H, d, *J* = 7.6 Hz, C_oH_{Ar}), 7.63 (1H, t, *J* = 7.0 Hz, C_pH_{Ar}), 7.50 (2H, t, *J* = 7.7 Hz, C_mH_{Ar}), 6.60 (1H, s, $C_{23}NH$), 5.48 (1H, dt, *J* = 49.2, 9.8 Hz, $H_{4\alpha}$), 4.84-4.74 (1H, m, $H_{3\beta}$), 3.83 (1H, s, $H_{7\beta}$), 3.31-3.25 (1H, m $H_{23'}$), 3.18-3.10 (1H, m, $H_{23''}$), 2.06 (3H, s, $C_3OC\mathbf{H}_3$), 1.95-1.80 (5H, m), 1.69-1.38 (13H, m), 1.29-1.12 (7H, m), 0.99 (3H, s, H_{19}), 0.93 (3H, d, *J* = 6.7 Hz, H_{21}) , 0.92 (3H, t, *J* = 7.0 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.7 ($C_3\mathbf{C}OCH_3$), 151.6 ($C_{23}NH\mathbf{C}O$), 139.7 ($C_{i\,Ar}$), 133.7 (C_pH_{Ar}), 129.3 (C_mH_{Ar}), 126.9 (C_oH_{Ar}), 95.1 (d, *J* = 174.6 Hz, C_4), 76.1 (d, *J* = 19.1 Hz, C_3), 71.0 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.8 (d, *J* = 13.9 Hz, C_5), 42.7 (C_{13}), 42.1 (C_6), 40.2 (C_8), 39.4 (C_{12}), 38.5 (d, *J* = 9.5 Hz, C_{10}), 37.9 (C_{23}), 35.5 (C_{22}), 35.3 (C_9), 34.1 (C_1), 33.8 (C_{20}), 28.3 (C_{16}), 24.2 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 24.1 (d, *J* = 6.6 Hz, C_2), 23.6 (CH_2), 23.2 (C_{19}), 21.2 ($C_3O\mathbf{C}H_3$), 20.7 (CH_2), 18.5 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.5 (1F, dt, *J* = 48.6, 12.1 Hz, $F_{4\beta}$) ppm **LRMS** (ESI^+) m/z: 652.3, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{34}H_{51}FO_6SN_2Na$ $[M+Na]^+$, m/z calculated: 657.3344; found: 657.3355. **IR** (neat) 2933 (m), 2872 (m), 2359 (w), 1716 (s), 1671 (s), 1590 (m), 1488 (m), 1456 (m), 1245 (m) cm^{-1}.

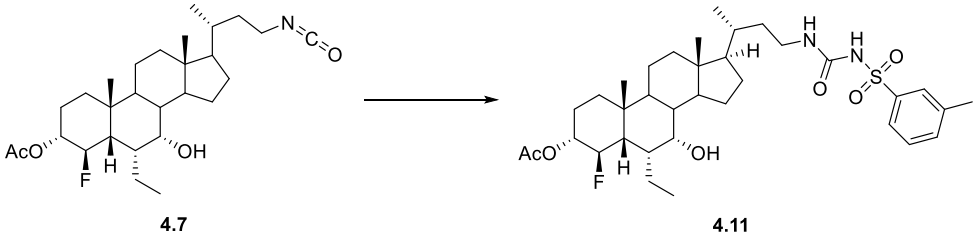
***N*-(3α-acetoxy-4β-fluoro-6α-ethyl-7α-hydroxyl-24-*nor*-5β-cholan-23-yl)-4-(*tert*-butyl)benzenesulfonyl urea (4.10)**



Prepared according to *General Procedure I* (see: *page 181*) using 99 mg (assumed) of compound **4.7** (0.21 mmol, 1.0 equiv) and 53 mg of 4-(*tert*-butyl)benzenesulfonamide to afford, after purification, compound **4.10** as a colourless oil (82 mg, 71%).

R_f (PE 40-60/acetone, 60/40) 0.22. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.81 (2H, d, *J* = 8.7 Hz, C_oH_{Ar}), 7.54 (2H, d, *J* = 8.7 Hz, C_mH_{Ar}), 6.56 (1H, s, $C_{23}NH$), 5.48 (1H, ddd, *J* = 49.4, 10.4, 9.3 Hz, $H_{4\alpha}$) , 4.78 (1H, dddd, 13.9, 11.4, 8.9, 4.7 Hz, $H_{3\beta}$), 3.84 (1H, s, $H_{7\beta}$), 3.34-3.28 (1H, m $H_{23'}$), 3.21-3.14 (1H, m, $H_{23''}$), 2.07 (3H, s, $C_3OC\mathbf{H}_3$), 1.97-1.80 (4H, m), 1.74 - 1.44 (13H, m), 1.36 (9H, s, $C_{Ar}C(C\mathbf{H}_3)_3$), 1.29-1.16 (7H, m), 0.99 (3H, s, H_{19}), 0.95 (3H, d, *J* = 6.6 Hz, H_{21}) , 0.93 (3H, t, *J* = 7.0 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.7 ($C_3\mathbf{C}OCH_3$), 157.8 ($\mathbf{C}_{Ar}C(CH_3)_3$), 151.1 ($C_{23}NH\mathbf{C}O$), 136.6 (C_{iAr}), 126.7 (C_oH_{Ar}), 126.4 (C_mH_{Ar}), 95.1 (d, *J* = 174.6 Hz, C_4), 76.1 (d, *J* = 18.3 Hz, C_3), 71.0 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.8 (d, *J* = 13.9 Hz, C_5), 42.8 (C_{13}), 42.1 (C_6), 40.2 (C_8), 39.4 (C_{12}), 38.5 (d, *J* = 8.8 Hz, C_{10}), 38.1 (C_{23}), 35.6 (C_{22}), 35.3 (C_9 + $C_{Ar}\mathbf{C}(CH_3)_3$), 34.1 (d, *J* = 1.5 Hz, C_1), 33.8 (C_{20}), 31.0 ($C_{Ar}C(\mathbf{C}H_3)_3$), 28.3 (C_{16}), 24.2 (d, *J* = 8.8 Hz, $C_6\mathbf{C}H_2CH_3$), 24.1 (d, *J* = 7.3 Hz, C_2), 23.6 (CH_2), 23.2 (C_{19}), 21.2 ($C_3O\mathbf{C}H_3$), 20.7 (CH_2), 18.6 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.6 (1F, dt, *J* = 49.9, 13.2 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 708.4, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{38}H_{59}FO_6SN_2Na$ $[M+Na]^+$, m/z calculated: 713.3970; found: 713.3977. **IR** (neat) 2961 (m), 2872 (m), 2360 (w), 1726 (s), 1670 (s), 1541 (m), 1458 (m), 1250 (m) cm^{-1}.

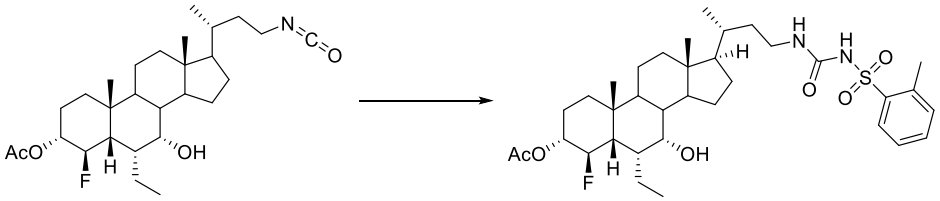
***N*-(3α-acetoxy-4β-fluoro-6α-ethyl-7α-hydroxyl-24-*nor*-5β-cholan-23-yl)-*m*-toluenesulfonyl urea (4.11)**



Prepared according to *General Procedure I* (see: *page 181*) using 99 mg (assumed) of compound **4.7** (0.21 mmol, 1.0 equiv) and 43 mg of *m*-toluenesulfonamide to afford, after purification, compound **4.11** as a colourless oil (86 mg, 80%).

R_f (PE 40-60/acetone, 60/40) 0.17. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.71-7.68 (2H, m, CH_{Ar}), 7.45-7.38 (2H, m, CH_{Ar}), 6.55 (1H, s, $C_{23}NH$), 5.47 (1H, ddd, *J* = 49.4, 10.3, 9.4 Hz, $H_{4\alpha}$), 4.83-4.73 (1H, m, $H_{3\beta}$), 3.83 (1H, s, $H_{7\beta}$), 3.30 (1H, ddd, *J* = 13.6, 8.9, 4.7 Hz, $H_{23'}$), 3.16 (1H, dt, *J* = 13.3, 7.9 Hz, $H_{23''}$), 2.42 (3H, s, $C_{Ar}C\mathbf{H}_3$), 2.06 (3H, s, $C_3OC\mathbf{H}_3$), 1.96-1.80 (4H, m), 1.72-1.11 (21H, m), 0.99 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.6 Hz, H_{21}) , 0.93 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.7 ($C_3\mathbf{C}OCH_3$), 151.6 ($C_{23}NH\mathbf{C}O$), 139.6 (C_{Ar}), 139.5 (C_{Ar}), 138.5 (CH_{Ar}), 129.1 (CH_{Ar}), 127.1 (CH_{Ar}), 123.9 (CH_{Ar}), 95.1 (d, *J* = 174.6 Hz, C_4), 76.1 (d, *J* = 19.1 Hz, C_3), 71.0 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.8 (d, *J* = 13.9 Hz, C_5), 42.7 (C_{13}), 42.1 (C_6), 40.2 (C_8), 39.4 (C_{12}), 38.5 (d, *J* = 9.5 Hz, C_{10}), 38.0 (C_{23}), 33.6 (C_{22}), 35.3 (C_9), 34.1 (C_1), 33.8 (C_{20}), 28.3 (C_{16}), 24.2 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 24.1 (d, *J* = 6.6 Hz, C_2), 23.6 (CH_2), 23.1 (C_{19}), 21.4 ($C_{Ar}\mathbf{C}H_3$), 21.2 ($C_3O\mathbf{C}H_3$), 20.7 (CH_2), 18.5 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.6 (1F, dt, *J* = 49.4, 13.4 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 666.3, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{35}H_{53}FO_6SN_2Na$ $[M+Na]^+$, m/z calculated: 671.3501; found: 671.3492. **IR** (neat) 2934 (m), 2872 (m), 2361 (w), 1717 (s), 1670 (s), 1541 (m), 1457 (m), 1247 (m) cm^{-1}.

_N_-(3α-acetoxy-4β-fluoro-6α-ethyl-7α-hydroxyl-24-_nor_-5β-cholan-23-yl)-_o_-toluenesulfonyl urea (4.12)



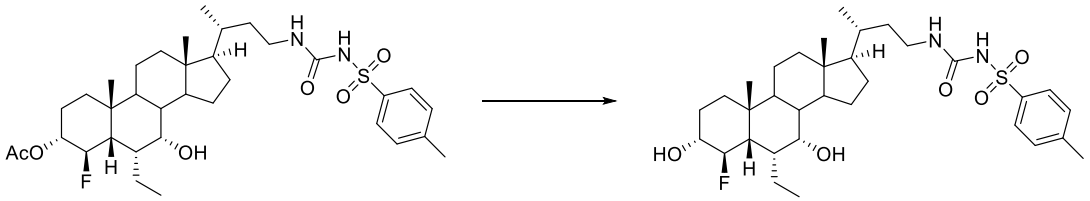
4.7 **4.12**

Prepared according to *General Procedure I* (see: *page 181*) using 99 mg (assumed) of compound **4.7** (0.21 mmol, 1.0 equiv) and 43 mg of *o*-toluenesulfonamide to afford, after purification, compound **4.12** as a colourless oil (55 mg, 51%).

R_f (PE 40-60/acetone, 60/40) 0.16. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.94 (1H, dd, *J* = 8.3, 1.0 Hz, CH_{Ar}), 7.51 (1H, td, *J* = 7.6, 1.2 Hz, CH_{Ar}), 7.34 (2H, d, *J* = 7.3 Hz, CH_{Ar}), 6.47 (1H, s, $C_{23}NH$), 5.47 (1H, dt, *J* = 49.3, 9.5 Hz, $H_{4\alpha}$) , 4.83-4.73 (1H, m, $H_{3\beta}$), 3.83 (1H, s, $H_{7\beta}$), 3.25 (1H, ddd, *J* = 13.3, 8.8, 4.7 Hz, $H_{23'}$), 3.11 (1H, dt, *J* = 13.3, 8.0 Hz, $H_{23''}$), 2.65 (3H, s, $C_{Ar}C\mathbf{H}_3$), 2.21-2.17 (1H, m), 2.06 (3H, s, $C_3OC\mathbf{H}_3$), 1.94-1.80 (4H, m), 1.68-1.10 (20H, m), 0.98 (3H, s, H_{19}), 0.92 (3H, d, *J* = 6.7 Hz, H_{21}), 0.90 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.63 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.7 ($C_3\mathbf{C}OCH_3$), 151.8 ($C_{23}NH\mathbf{C}O$), 137.8 (C_{Ar}), 137.5 (C_{Ar}), 133.6 (CH_{Ar}), 133.0 (CH_{Ar}), 128.7 (CH_{Ar}), 126.3 (CH_{Ar}), 95.1 (d, *J* = 174.6 Hz, C_4), 76.1 (d, *J* = 19.1 Hz, C_3), 71.0 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.8 (d, *J* = 13.9 Hz, C_5), 42.7 (C_{13}), 42.1 (C_6), 40.2 (C_8), 39.4 (C_{12}), 38.5 (d, *J* = 9.5 Hz, C_{10}), 38.0 (C_{23}), 35.6 (C_{22}), 35.3 (C_9), 34.1 (C_1), 33.8 (C_{20}), 28.3 (C_{16}), 24.2 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 24.1 (d, *J* = 6.6 Hz, C_2), 23.6 (CH_2), 23.1 (C_{19}), 21.2 ($C_3O\mathbf{C}H_3$), 20.7 (CH_2), 20.1 ($C_{Ar}\mathbf{C}H_3$), 18.5 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-

decoupled, 376 MHz, $CDCl_3$): δ -186.6 (1F, dt, *J* = 49.9, 12.4 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 666.3, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{35}H_{53}FO_6SN_2Na$ $[M+Na]^+$, m/z calculated: 671.3511; found: 671.3497. **IR** (neat) 2934 (m), 2872 (m), 2361 (w), 1717 (s), 1670 (s), 1541 (m), 1457 (m), 1247 (m) cm^{-1}.

***N*-(3α,7α -dihydroxyl-4β-fluoro-6α-ethyl-24-*nor*-5β-cholan-23-yl)-*p*-toluenesulfonyl urea (4.13)**

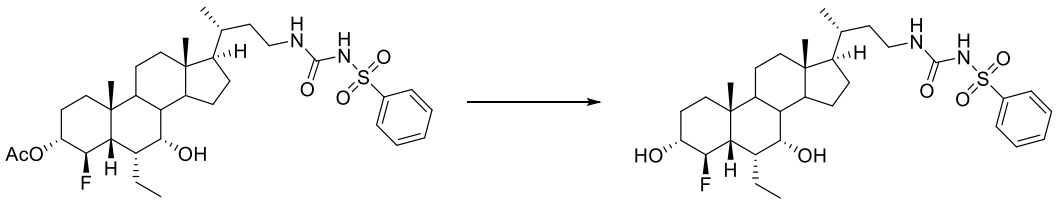


4.8 4.13

Prepared according to *General Procedure A* (see: *page 147*) using 50 mg of compound **4.8** (0.08 mmol, 1.0 equiv) and 10 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage SNAP KP-Sil 10 g cartridge) using dichloromethane/methanol (100/0 to 95/5) as the eluent yielded compound **4.13** as a colourless residue (19 mg, 0.03 mmol, 40%).

R_f (dichloromethane/methanol, 95/5) 0.33. **$[\alpha]_D$:** +14.3 (c = 0.30, chloroform, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.78 (2H, d, *J* = 8.3 Hz, C_oH_{Ar}), 7.34 (2H, d, *J* = 8.1 Hz, C_mH_{Ar}), 6.50 (1H, t, *J* = 4.9 Hz, $C_{23}NH$), 5.32 (1H, ddd, *J* = 50.0, 10.0, 9.2 Hz, $H_{4\alpha}$), 3.83 (1H, s, $H_{7\beta}$), 3.60-3.50 (1H, m, $H_{3\beta}$), 3.34-3.26 (1H, m $H_{23'}$), 3.21-3.12 (1H, m, $H_{23''}$), 2.46 (3H, s, $C_{Ar}C\mathbf{H}_3$), 1.96-1.06 (26H, m), 0.98 (3H, s, H_{19}), 0.94 (3H, t, *J* = 6.2 Hz, H_{21}), 0.93 (3H, d, *J* = 6.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 151.6 ($C_{23}NH\mathbf{C}O$), 144.9 (C_pH_{Ar}), 136.7 (C_oH_{Ar}), 130.0 (C_mH_{Ar}), 126.9 ($C_{i\ Ar}$), 99.7 (d, *J* = 168.7 Hz, C_4), 74.8 (d, *J* = 19.8 Hz, C_3), 70.9 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.3 (d, *J* = 13.9 Hz, C_5), 42.8 (C_{13}), 42.1 (C_6), 40.3 (C_8), 39.4 (C_{12}), 39.0 (d, *J* = 9.5 Hz, C_{10}), 38.0 (C_{23}), 35.6 (C_{22}), 35.3 (C_9), 34.4 (C_1), 33.8 (C_{20}), 28.4 (C_{16}), 26.0 (d, *J* = 8.1 Hz, C_2), 24.3 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 23.6 (CH_2), 23.3 (C_{19}), 21.6 ($C_{Ar}\mathbf{C}H_3$), 20.7 (CH_2), 18.5 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -188.8 (1F, dt, *J* = 50.3, 12.1 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 624.4, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{33}H_{51}FO_5SN_2Na$ $[M+Na]^+$, m/z calculated: 629.3395; found: 629.3389. **IR** (neat) 3359 (w), 2930 (m), 2871 (m), 1671 (s), 1541 (m), 1457 (m), 1341 (m), 1162 (m) cm^{-1}.

***N*-(3α,7α -dihydroxyl-4β-fluoro-6α-ethyl-24-*nor*-5β-cholan-23-yl)-benzenesulfonyl urea (4.14)**

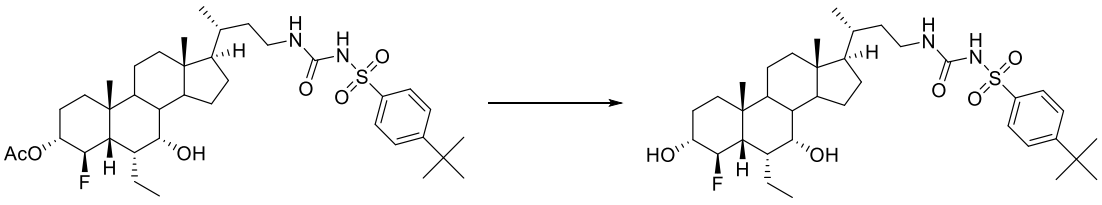


4.9 4.14

Prepared according to *General Procedure A* (see: *page 147*) using 48 mg of compound **4.9** (0.08 mmol, 1.0 equiv) and 10 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage SNAP KP-Sil 10 g cartridge) using dichloromethane/methanol (100/0 to 95/5) as the eluent yielded compound **4.14** as a colourless residue (29 mg, 0.05 mmol, 64%).

R_f (dichloromethane/methanol, 95/5) 0.28. **$[\alpha]_D$:** +12.3 (c = 0.37, chloroform, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.91 (2H, d, *J* = 7.5 Hz, C_oH_{Ar}), 7.65 (1H, t, *J* = 7.3 Hz, C_pH_{Ar}), 7.54 (2H, t, *J* = 7.8 Hz, C_mH_{Ar}), 6.51 (1H, s, $C_{23}NH$), 5.31 (1H, ddd, *J* = 50.1, 10.3, 9.1 Hz, $H_{4\alpha}$), 3.82 (1H, s, $H_{7\beta}$), 3.60-3.50 (1H, m, $H_{3\beta}$), 3.35-3.26 (1H, m $H_{23'}$), 3.20-3.12 (1H, m, $H_{23''}$), 1.95-1.36 (17H, m), 1.27-1.11 (9H, m), 0.97 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.2 Hz, H_{21}), 0.93 (3H, t, *J* = 6.7 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 151.4 ($C_{23}NH\mathbf{C}O$), 139.7 ($C_{i\,Ar}$), 133.7 (C_pH_{Ar}), 129.3 (C_mH_{Ar}), 126.8 (C_oH_{Ar}), 99.7 (d, *J* = 168.7 Hz, C_4), 74.8 (d, *J* = 19.8 Hz, C_3), 70.9 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.3 (d, *J* = 13.3 Hz, C_5), 42.7 (C_{13}), 42.1 (C_6), 40.3 (C_8), 39.4 (C_{12}), 39.0 (d, *J* = 9.5 Hz, C_{10}), 38.0 (C_{23}), 35.6 (C_{22}), 35.4 (C_9), 34.4 (C_1), 33.8 (C_{20}), 28.3 (C_{16}), 26.0 (d, *J* = 8.1 Hz, C_2), 24.3 (d, *J* = 8.8 Hz, $C_6\mathbf{C}H_2CH_3$), 23.6 (CH_2), 23.3 (C_{19}), 20.7 (CH_2), 18.5 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -189.0 (1F, dt, *J* = 50.3, 12.1 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 610.2, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{32}H_{49}FO_5SN_2Na$ $[M+Na]^+$, m/z calculated: 615.3238; found: 615.3234. **IR** (neat) 2932 (m), 2871 (m), 2359 (w), 1698 (s), 1540 (m), 1449 (m), 1336 (m), 1162 (m) cm^{-1}.

_N_-(3α,7α-dihydroxyl-4β-fluoro-6α-ethyl-24-_nor_-5β-cholan-23-yl)-4-(_tert_-butyl)benzenesulfonyl urea (4.15)



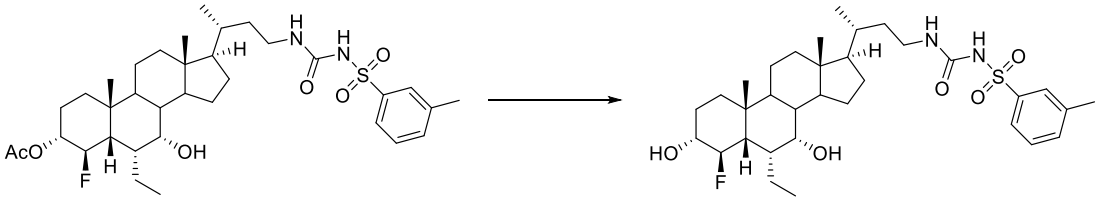
4.10 **4.15**

Prepared according to *General Procedure A* (see: *page 147*) using 80 mg of compound **4.10** (0.12 mmol, 1.0 equiv) and 10 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage SNAP KP-Sil 10 g cartridge) using dichloromethane/methanol (100/0 to 95/5) as the eluent yielded compound **4.15** as a colourless residue (51 mg, 0.08 mmol, 65%).

R_f (dichloromethane/methanol, 95/5) 0.31. **$[\alpha]_D$:** +11.6 (c = 1.34, chloroform, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.82 (2H, d, *J* = 8.6 Hz, C_oH_{Ar}), 7.53 (2H, t, *J* = 8.4 Hz, C_mH_{Ar}), 6.54 (1H, s, $C_{23}NH$), 5.32 (1H, ddd, *J* = 49.9, 10.3, 9.1 Hz, $H_{4\alpha}$), 3.82 (1H, s, $H_{7\beta}$), 3.60-3.50 (1H, m, $H_{3\beta}$), 3.35-3.25 (1H, m $H_{23'}$), 3.19-3.11 (1H, m, $H_{23''}$), 1.95-1.41 (16H, m), 1.34 (9H, s, $C_{Ar}C(C\mathbf{H}_3)_3$), 1.28-1.08 (10H, m), 0.97 (3H, s,

H_{19}), 0.930 (3H, t, *J* = 6.9 Hz, $C_6CH_2C\mathbf{H}_3$), 0.927 (3H, d, *J* = 6.0 Hz, H_{21}) , 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 157.6 ($\mathbf{C}_{Ar}C(CH_3)_3$), 151.7 ($C_{23}NH\mathbf{C}O$), 136.7 ($C_{i\,Ar}$), 126.7 (C_oH_{Ar}), 126.3 (C_mH_{Ar}), 99.7 (d, *J* = 168.0 Hz, C_4), 74.8 (d, *J* = 19.8 Hz, C_3), 70.9 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.3 (d, *J* = 13.2 Hz, C_5), 42.7 (C_{13}), 42.1 (C_6), 40.3 (C_8), 39.4 (C_{12}), 39.0 (d, *J* = 9.5 Hz, C_{10}), 38.0 (C_{23}), 35.6 (C_{22}), 35.4 (C_9), 34.4 (C_1), 33.8 (C_{20}), 31.0 ($C_{Ar}C(\mathbf{C}H_3)_3$), 28.3 (C_{16}), 26.0 (d, *J* = 8.1 Hz, C_2), 24.3 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 23.6 (CH_2), 23.3 (C_{19}), 20.7 (CH_2), 18.5 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -188.8 (1F, dt, *J* = 50.3, 12.1 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 666.4, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{36}H_{57}FO_5SN_2Na$ $[M+Na]^+$, m/z calculated: 671.3864; found: 671.3872. **IR** (neat) 2932 (m), 2870 (m), 2361 (w), 1670 (s), 1541 (m), 1458 (m), 1198 (m) cm^{-1}.

***N*-(3α,7α -dihydroxyl-4β-fluoro-6α-ethyl-24-*nor*-5β-cholan-23-yl)-*m*-toluenesulfonyl urea (4.16)**

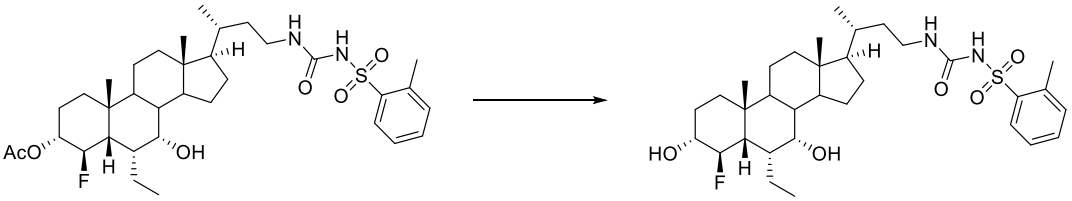


4.11 **4.16**

Prepared according to *General Procedure A* (see: *page 147*) using 84 mg of compound **4.11** (0.13 mmol, 1.0 equiv) and 10 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage SNAP KP-Sil 10 g cartridge) using dichloromethane/methanol (100/0 to 95/5) as the eluent yielded compound **4.16** as a colourless residue (29 mg, 0.05 mmol, 37%).

R_f (dichloromethane/methanol, 95/5) 0.30. **$[\alpha]_D$:** +12.1 (c = 0.89, chloroform, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.71-7.69 (2H, m, CH_{Ar}), 7.45-7.38 (2H, m, CH_{Ar}), 6.52 (1H, s, $C_{23}NH$), 5.32 (1H, ddd, *J* = 49.9, 10.4, 8.9 Hz, $H_{4\alpha}$), 3.82 (1H, s, $H_{7\beta}$), 3.60-3.50 (1H, m, $H_{3\beta}$), 3.35-3.26 (1H, m $H_{23'}$), 3.20-3.10 (1H, m, $H_{23''}$), 2.43 (3H, s, $C_{Ar}CH_3$), 1.95-1.39 (15H, m), 1.28-1.11 (10H, m), 0.97 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.2 Hz, H_{21}) , 0.93 (3H, t, *J* = 6.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 151.5 ($C_{23}NH\mathbf{C}O$), 139.7 (C_{Ar}), 139.6 (C_{Ar}), 134.5 (CH_{Ar}), 129.1 (CH_{Ar}), 127.1 (CH_{Ar}), 123.9 (CH_{Ar}), 99.7 (d, *J* = 168.0 Hz, C_4), 74.8 (d, *J* = 19.8 Hz, C_3), 70.9 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.3 (d, *J* = 13.9 Hz, C_5), 42.7 (C_{13}), 42.1 (C_6), 40.3 (C_8), 39.4 (C_{12}), 39.0 (d, *J* = 9.5 Hz, C_{10}), 38.0 (C_{23}), 35.6 (C_{22}), 35.3 (C_9), 34.4 (C_1), 33.8 (C_{20}), 28.3 (C_{16}), 26.0 (d, *J* = 8.8 Hz, C_2), 24.3 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 23.6 (CH_2), 23.3 ($\mathbf{C}_{19}$), 21.4 ($C_{Ar}\mathbf{C}H_3$), 20.7 (CH_2), 18.5 (C_{21}), 12.5 (d, *J* = 2.9 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -188.9 (1F, dt, *J* = 48.6, 10.4 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 624.3, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{33}H_{51}FO_5SN_2Na$ $[M+Na]^+$, m/z calculated: 629.3395; found: 629.3381. **IR** (neat) 3359 (m), 2932 (s), 2870 (m), 2360 (w), 1670 (s), 1540 (m), 1456 (m), 1340 (m), 1157 (m) cm^{-1}.

_N_-(3α,7α -dihydroxyl-4β-fluoro-6α-ethyl-24-_nor_-5β-cholan-23-yl)-_o_-toluenesulfonyl urea (4.17)

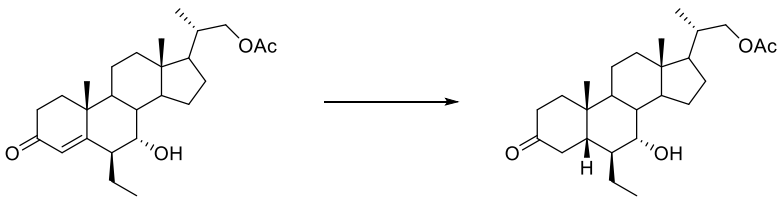


4.12 4.17

Prepared according to *General Procedure A* (see: *page 147*) using 53 mg of compound **4.12** (0.08 mmol, 1.0 equiv) and 10 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage SNAP KP-Sil 10 g cartridge) using dichloromethane/methanol (100/0 to 95/5) as the eluent yielded compound **4.17** as a colourless residue (24 mg, 0.04 mmol, 48%).

R_f (dichloromethane/methanol, 95/5) 0.29. **$[\alpha]_D$:** +13.2 (c = 0.53, chloroform, 22 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.94 (1H, d, *J* = 8.1 Hz, CH_{Ar}), 7.52 (1H, td, *J* = 7.6, 1.1 Hz, CH_{Ar}), 7.35 (2H, d, *J* = 7.7 Hz, CH_{Ar}), 6.47 (1H, t, *J* = 4.6 Hz, $C_{23}NH$), 5.31 (1H, ddd, *J* = 49.9, 10.4, 8.9 Hz, $H_{4\alpha}$), 3.82 (1H, s, $H_{7\beta}$), 3.60-3.50 (1H, m, $H_{3\beta}$), 3.30-3.22 (1H, m $H_{23'}$), 3.17-3.08 (1H, m, $H_{23''}$), 2.67 (3H, s, $C_{Ar}CH_3$), 1.94-1.06 (25H, m), 0.97 (3H, s, H_{19}), 0.91 (3H, t, *J* = 7.5 Hz, $C_6CH_2C\mathbf{H}_3$), 0.90 (3H, d, *J* = 6.6 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 151.3 ($C_{23}NH\mathbf{C}O$), 137.8 (C_{Ar}), 137.4 (C_{Ar}), 133.7 (CH_{Ar}), 133.0 (CH_{Ar}), 128.7 (CH_{Ar}), 126.3 (CH_{Ar}), 99.7 (d, *J* = 168.7 Hz, C_4), 74.8 (d, *J* = 19.8 Hz, C_3), 70.9 (C_7), 55.9 (C_{17}), 50.4 (C_{14}), 49.3 (d, *J* = 13.2 Hz, C_5), 42.7 (C_{13}), 42.1 (C_6), 40.3 (C_8), 39.4 (C_{12}), 39.0 (d, *J* = 9.5 Hz, C_{10}), 37.9 (C_{23}), 35.5 (C_{22}), 35.3 (C_9), 34.4 (C_1), 33.7 (C_{20}), 28.3 (C_{16}), 26.0 (d, *J* = 8.8 Hz, C_2), 24.3 (d, *J* = 8.1 Hz, $C_6\mathbf{C}H_2CH_3$), 23.6 (CH_2), 23.3 (C_{19}), 20.7 (CH_2), 20.2 ($C_{Ar}\mathbf{C}H_3$), 18.4 (C_{21}), 12.5 (d, *J* = 2.2 Hz, $C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -189.0 (1F, dt, *J* = 50.3, 12.1 Hz, $F_{4\beta}$) ppm. **LRMS** (ESI^+) m/z: 624.3, $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{33}H_{51}FO_5SN_2Na$ $[M+Na]^+$, m/z calculated: 629.3395; found: 629.3401. **IR** (neat) 3356 (w), 2930 (m), 2871 (m), 1669 (s), 1542 (m), 1457 (m), 1337 (m), 1162 (m) cm^{-1}.

3-keto-6β-ethyl-7α-hydroxyl-23,24-_dinor_-cholane-22-ol acetate (4.22)



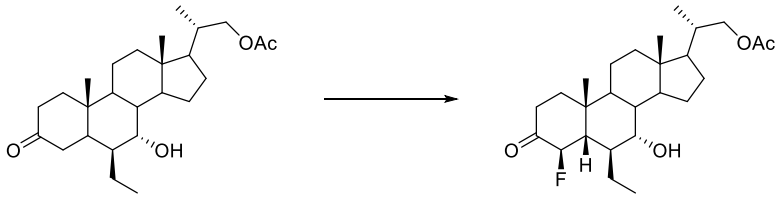
4.21 4.22

To a solution of **4.21** (50.0 g, 120 mmol, 1.0 equiv) in dry dimethylformamide (150 mL) and acetonitrile (300 mL) under argon at 0 °C was added $Pd/CaCO_3$ (~5% by mass, 6.40 g, 60 mmol, ~0.5 equiv). The reaction vessel was evacuated *in vacuo* and filled with hydrogen gas to a pressure of

approximately 1.05 atmospheres. After 2 days under a hydrogen atmosphere, the reaction was deemed complete by TLC and the reaction mixture was filtered over Whatman® GF/B grade filter pad (glass fibre pore size 1 µm). The solids were washed with ethyl acetate (150 mL) and then quenched with water (100 mL). The filtrate was washed with sat. $NaHCO_3$ solution (2 x 250 mL), dried and $MgSO_4$, filtered over a thin pad of silica and concentrated *in vacuo* to afford 59.5 g of crude material as a brown residue. Purification by flash column chromatography (Biotage KP-Sil SNAP 50 g cartridge) using heptane/ethyl acetate (80/20 to 70/30) as the eluent yielded the title compound **4.22** as a pale-yellow solid (35.7 g, 85.2 mmol, 71%).

The data presented for (industrial placement) compound **4.22** is not a full characterisation. Characteristic features are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.09 (1H, dd, *J* = 10.7, 3.4 Hz, C_{22}**H**HO), 3.81 (1H, dd, *J* = 10.7, 7.3 Hz, C_{22}H**H**O), 3.72 (1H, s, $H_{7\beta}$), 3.35 (1H, dd, *J* = 15.6, 13.6 Hz, $H_{4\alpha}$), 2.37 (1H, ddd, *J* = 14.3, 13.2, 4.9 Hz, $H_{2\alpha}$), 2.06 (3H, s, C_{22}OCOC**H**$_3$), 1.06 (3H, s, H_{19}), 1.05 (3H, d, *J* = 6.8 Hz, H_{21}), 0.95 (3H, t, *J* = 7.0 Hz, C_6CH_2C**H**$_3$), 0.74 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 213.4 (C_3), 171.3 (C_{22}O**C**O), 71.2 (C_7), 69.4 (C_{22}), 52.8 (C_{17}), 42.8 (C_{13}), 35.7 (C_{10}), 21.0 (C_{19}), 17.2 (C_{21}), 13.9 (C_6CH_2**C**H$_3$), 11.8 (C_{18}) ppm.

3-keto-4β-fluoro-6β-ethyl-7α-hydroxyl-23,24-*dinor*-cholane-22-ol acetate (4.30)



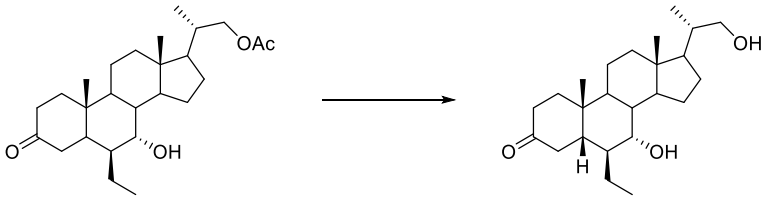
4.22 **4.30**

To a solution of **4.22** (250 mg, 0.60 mmol, 1.0 equiv) in dry THF (10 mL) under argon at -78 °C was added a 1M solution of LDA in hexanes (1.25 mL, 1.25 mmol, ~2.1 equiv). After 15 min at -78 °C the colourless solution had become a yellow gelatinous consistency that was agitated to resume stirring. To the reaction mixture was added a solution of trimethylsilyl chloride (0.16 mL, 1.25 mmol, ~2.1 equiv) in dry THF (5 mL) under argon. After 16 h warming to rt, the reaction was quenched by the addition of sat. $NaHCO_3$ solution (5 mL) and stirred at rt for 1 hour. The aqueous phase was separated and extracted with ethyl acetate (2 x 15 mL) and the combined organic layers were washed with sat. $NaHCO_3$ solution (15 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 321 mg of crude material as a pale-yellow oil. To a solution of the crude material in acetonitrile (15 mL) was added Selectfluor® (233 mg, 0.66 mmol, ~1.1 equiv). After 17 h at rt, the solvent was removed *in vacuo* and the residue was diluted with ethyl acetate (10 mL) and sat.$NaHCO_3$ solution (10 mL). The aqueous phase was separated and extracted with ethyl acetate (2 x 15 mL) and the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue received methanol (25 mL) and was acidified

to pH 2 with 2M HCl and stirred at rt to remove TMS residue at C_7. After 2 h, the reaction mixture was concentrated *in vacuo* and the residue was diluted with sat. $NaHCO_3$ solution (25 mL) and dichloromethane (25 mL). The aqueous phase was separated and extracted with dichloromethane (2 x 25 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 299 mg of crude material as pale-yellow oil. Analyses of the crude material by ^{1}H NMR determined an 18% yield of title compound **4.30** alongside approximately 5% of the starting compound **4.22**.

The data presented for (industrial placement) compound **4.30** was taken from an impure mixture and is not a full characterisation. Characteristic features are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.72 (1H, dd, *J* = 46.6, 11.2 Hz, $H_{4\alpha}$), 4.03 (1H, dd, *J* = 10.7, 3.1 Hz, C_{22}**H**HO), 3.79 (1H, dd, *J* = 10.7, 6.9 Hz, C_{22}H**H**O), 2.54 (1H, td, *J* = 14.3, 4.6 Hz, $H_{2\alpha}$), 2.06 (3H, s, C_{22}OCOC**H**$_3$), 1.07 (3H, s, H_{19}), 0.88 (3H, t, *J* = 6.9 Hz, C_6CH_2C**H**$_3$), 0.72 (3H, s, H_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -198.3 (1F, ddd, *J* = 46.4, 13.9, 6.1 Hz, $F_{4\beta}$) ppm.

3-keto-6β-ethyl-7α-hydroxyl-23,24-*dinor*-cholane-22-ol (4.26)

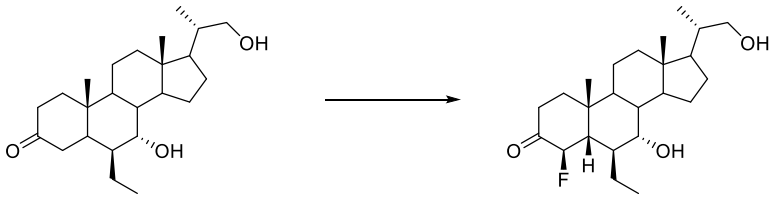


4.22 **4.26**

Prepared according to *General Procedure A* (see: *page 147*) using 22.0 g of compound **4.22** (52.6 mmol 1.0 equiv) and 700 mL of a 5% sodium hydroxide in methanol solution. After workup, a crude mass of 21.9 g of **4.26** was isolated as a yellow residue that was used without further purification.

The data presented for (industrial placement) compound **4.26** was taken from an impure mixture and is not a full characterisation. Characteristic features are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.72 (1H, s, $H_{7\beta}$), 3.66 (1H, dd, *J* = 10.4, 3.1 Hz, C_{22}**H**HO), 3.39 (1H, dd, *J* = 10.3, 7.2 Hz, C_{22}H**H**O), 3.35 (1H, dd, *J* = 15.4, 13.6 Hz, $H_{4\alpha}$), 2.37 (1H, td, *J* = 14.2, 4.9 Hz, $H_{2\alpha}$), 1.08 (3H, d, *J* = 6.7 Hz, H_{21}), 1.06 (3H, s, H_{19}), 0.95 (3H, t, *J* = 7.0 Hz, C_6CH_2C**H**$_3$), 0.75 (3H, s, H_{18}) ppm.

3-keto-4β-fluoro-6β-ethyl-7α-hydroxyl-23,24-*dinor*-cholane-22-ol (4.33)

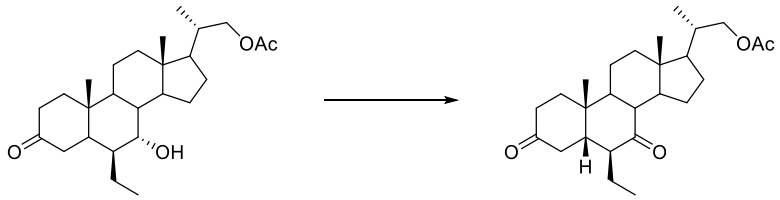


4.26 **4.33**

To a solution of **4.26** (31.1 g, 83.1 mmol, 1.0 equiv) in dry THF (1250 mL) under argon at -78 °C was added a 1M solution of LDA in hexanes (420 mL, 416 mmol, ~5.0 equiv). After 15 min at -78 °C the colourless solution had become a yellow gelatinous consistency that was agitated to resume stirring. To the reaction mixture was added a solution of trimethylsilyl chloride (53.0 mL, 416 mmol, ~5.0 equiv) in dry THF (650 mL) under argon. After 16 h warming to rt, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ solution (1000 mL) and stirred at rt for 1 hour. The aqueous phase was then separated and extracted with ethyl acetate (3 x 500 mL) and the combined organic layers were washed with sat. $NaHCO_3$ solution (2 x 500 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 57.4 g of crude material as an orange oil. To a solution of the crude material in acetonitrile (1500 mL) was added Selectfluor® (58.9 g, 166 mmol, ~ 2.0 equiv). After 17 h at rt, the solvent was removed *in vacuo* and the residue was diluted with ethyl acetate (500 mL) and sat.$NaHCO_3$ solution (500 mL). The aqueous phase was separated and extracted with ethyl acetate (2 x 500 mL) and the combined organic layers were washed with brine (500 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude residue received methanol (1000 mL) and was acidified to pH 2 with 2M HCl and stirred at rt to remove TMS residue at C_7. After 2 h, the reaction mixture was concentrated *in vacuo* and the residue was diluted with sat. $NaHCO_3$ solution (1000 mL) and ethyl acetate (1000 mL). The aqueous phase was separated and extracted with ethyl acetate (2 x 500 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 35.1 g of crude material as yellow oil. Analyses of the crude material by ^{1}H NMR determined an 81% yield of title compound **4.33** alongside approximately 10% of the starting compound **4.26**.

The data presented for (industrial placement) compound **4.33** was taken from an impure mixture and is not a full characterisation. Characteristic features are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.78 (1H, dd, *J* = 46.4, 11.3 Hz, $H_{4\alpha}$), 3.78 (1H, s, $H_{7\beta}$), 3.66 (1H, dd, *J* = 10.5, 3.2 Hz, C_{22}**H**HO), 3.39 (1H, dd, *J* = 10.9, 6.5 Hz, C_{22}H**H**O), 2.54 (1H, td, *J* = 14.4, 4.8 Hz, $H_{2\alpha}$), 1.09 (3H, s, H_{19}), 1.07 (3H, d, *J* = 6.7 Hz, H_{21}), 0.97 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.75 (3H, s, H_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -199.1 (1F, ddd, *J* = 46.4, 13.7, 6.3 Hz, $F_{4\beta}$) ppm

3,7-diketo-6β-ethyl-23,24-*dinor*-cholane-22-ol acetate (4.34)

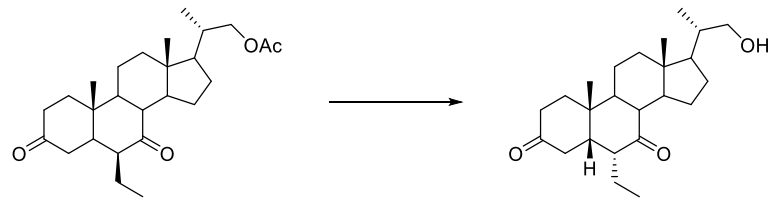


4.22 **4.34**

To a solution of **4.22** (1.00 g, 2.39 mmol, 1.0 equiv) in dichloromethane (20 mL) at rt was added Dess-Martin periodinane (2.03 g, 4.78 mmol, ~2.0 equiv) and two drops of water. After 3 h at rt, the reaction was quenched by the addition of 5% aqueous $NaHCO_3$ solution (10 mL). The reaction mixture was filtered over Whatman® GF/A grade filter pad (glass fibre pore size 1 µm) and the filtrate was separated and extracted with dichloromethane (2 x 40 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 1.52 g of crude material as an orange oil. Purification by flash column chromatography (Biotage KP-Sil Ultra SNAP 25 g cartridge) using heptane/ethyl acetate (90/10 to 78/22) as the eluent yielded the title compound **4.34** as a pale-yellow residue (920 mg, 2.21 mmol, 92%).

The data presented for (industrial placement) compound **4.34** is not a full characterisation. Characteristic features are reported to help identify this intermediate in future syntheses: $\mathbf{R_f}$ (heptane/ethyl acetate, 60/40) 0.61. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.08 (1H, dd, *J* = 10.7, 3.4 Hz, C_{22}**H**HO), 3.80 (1H, dd, *J* = 10.7, 7.3 Hz, C_{22}H**H**O), 2.44 (1H, t, *J* = 11.4 Hz), 2.05 (3H, s, $C_{22}OCOC\mathbf{H}_3$), 2.00 (1H, dt, *J* = 9.6, 4.6 Hz), 1.36 (1H, td, *J* = 9.5, 1.1 Hz), 1.16 (3H, s, H_{19}), 1.03 (3H, d, *J* = 6.7 Hz, H_{21}), 0.85 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.75 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 214.4 (C_3), 211.4 (C_7), 171.3 ($C_{22}O\mathbf{C}O$), 69.3 (C_{22}), 52.0 (C_{17}), 43.2 (C_{13}), 35.3 (C_{10}), 21.7 (C_{19}), 17.2 (C_{21}), 12.6 ($C_6CH_2\mathbf{C}H_3$), 12.2 (C_{18}) ppm.

3,7-diketo-6β-ethyl-23,24-*dinor*-cholane-22-ol (4.27)



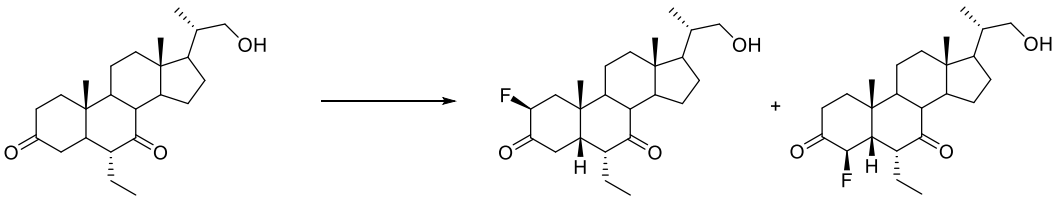
4.34 **4.27**

Prepared according to *General Procedure A* (see: *page 147*) using 500 mg of compound **4.34** (1.20 mmol, 1.0 equiv) and 6 mL of a 25% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage KP-Sil SNAP 10 g cartridge) using heptane/ethyl acetate (60/40

to 40/60) as the eluent yielded the title compound **4.27** as a pale-yellow residue (410 mg, 1.09 mmol, 92%).

The data presented for (industrial placement) compound **4.27** is not a full characterisation. Characteristic features are reported to help identify this intermediate in future syntheses: $\mathbf{R_f}$ (heptane/ethyl acetate, 60/40) 0.32. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.65 (1H, dd, *J* = 10.5, 3.2 Hz, C_{22}**H**HO), 3.37 (1H, dd, *J* = 10.4 7.0 Hz, C_{22}H**H**O), 2.74 (1H, td, *J* = 6.5, 5.4 Hz), 2.47 (1H, t, *J* = 11.3 Hz), 1.33 (3H, s, H_{19}), 1.05 (3H, d, *J* = 6.7 Hz, H_{21}), 0.80 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.71 (3H, s, H_{18}) ppm.

3,7-diketo-2β-fluoro-6β-ethyl-23,24-*dinor*-cholane-22-ol (4.37) and 3,7-diketo-4β-fluoro-6β-ethyl-23,24-*dinor*-cholane-22-ol (4.38)



4.27 **4.37** **4.38**

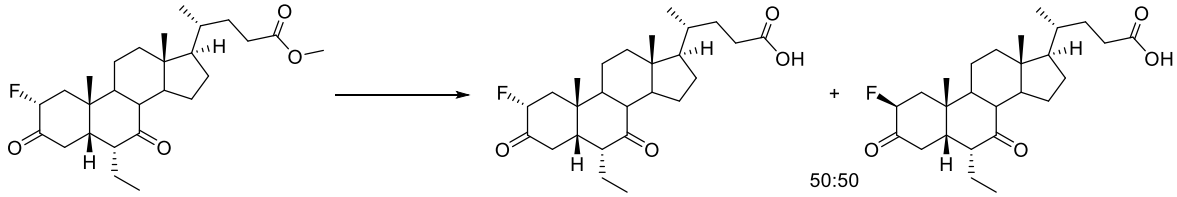
To a solution of **4.27** (190 mg, 0.51 mmol, 1.0 equiv) in dry THF (4 mL) under argon at -78 °C was added a 1M solution of LDA in hexanes (2.54 mL, 2.54 mmol, ~5.0 equiv). After 15 min at -78 °C the colourless solution had become a yellow gelatinous consistency that was agitated to resume stirring. To the reaction mixture was added a solution of trimethylsilyl chloride (0.33 mL, 2.54 mmol, ~5.0 equiv) in dry THF (4 mL) under argon. After 16 h warming to rt, the reaction mixture was quenched by the addition of sat. $NaHCO_3$ solution (10 mL) and stirred at rt for 1 hour. The aqueous phase was then separated and extracted with ethyl acetate (2 x 15 mL) and the combined organic layers were washed with sat. $NaHCO_3$ solution (30 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 265 mg of crude material as a pale-yellow oil. To a solution of the crude material in acetonitrile (10 mL) was added Selectfluor® (198 mg, 0.56 mmol, ~ 1.1 equiv). After 16 h at rt, the solvent was removed *in vacuo* and the residue was diluted with dichloromethane (10 mL) and sat.$NaHCO_3$ solution (10 mL). The aqueous phase was separated and extracted with dichloromethane (10 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 66.0 mg of crude material as a pale-yellow oil. Analyses of the crude material by ^{1}H NMR determined a 35% yield of title compound **4.37** and a 33% yield of title compound **4.38** alongside approximately 10% of the starting compound **4.27**.

The data presented for (industrial placement) compounds **4.37** and **4.38** was taken from an impure mixture and is not a full characterisation. Characteristic features are reported to help identify these intermediates in future syntheses:

Compound **4.37** $\mathbf{R_f}$ (heptane/acetone, 70/30) 0.28. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.90 (1H, ddd, *J* = 48.2, 13.3, 6.3 Hz, $H_{2\alpha}$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -195.0 (1F, ddt, *J* = 53.1, 9.0, 5.1 Hz, $F_{2\beta}$) ppm.

Compound **4.38**: $\mathbf{R_f}$ (heptane/acetone, 70/30) 0.28. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.62 (1H, dd, *J* = 47.4, 11.2 Hz, $H_{4\alpha}$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -193.0 (1F, dd, *J* = 47.7, 8.9 Hz, $F_{4\beta}$) ppm.

2α-fluoro-3,7-dioxo-6α-ethyl-5β-cholanic acid (4.39) and 2β-fluoro-3,7-dioxo-6α-ethyl-5β-cholanic acid (4.40)



3.21 **4.39** **4.40**

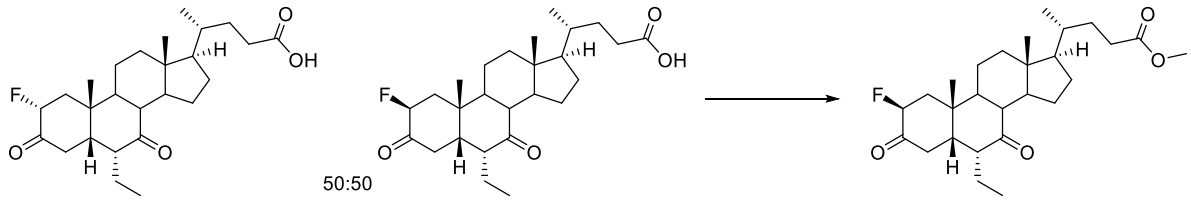
Prepared according to *General Procedure A* (see: *page 147*) using 878 mg of compound **3.21** (1.95 mmol, 1.0 equiv) and 20 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage SNAP KP-Sil 25 g cartridge) using dichloromethane/methanol (98/2 to 90/10) as the eluent yielded an inseparable mixture of compound **4.39** and **4.40** in a 50:50 ratio as a white residue (772 mg, 1.77 mmol, 91%). Using a longer reaction time of 48 h, the ratio of products was observed at 35:65 but in a lower yield of 78%.

Compound **4.39**: R_f (dichloromethane/methanol, 95/5) 0.50. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.68 (1H, ddd, *J* = 50.7, 5.1, 3.6 Hz, $\mathbf{H}_{2\beta}$), 2.75 (1H, ddd, *J* = 8.4, 5.3, 4.3 Hz, $\mathbf{H}_{6\beta}$), 2.18-1.44 (19H, m), 1.36 (3H, s, $\mathbf{H}_{19}$), 1.30-1.01 (6H, m), 0.94 (3H, d, *J* = 6.4 Hz, $\mathbf{H}_{21}$), 0.82 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.69 (3H, s, $\mathbf{H}_{18}$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.7 (C_7), 205.9 (d, *J* = 18.3 Hz, C_3), 178.0 (C_{24}), 91.9 (d, *J* = 180.5 Hz, C_2), 54.7 (C_{17}), 52.6 (CH), 51.7 (CH), 50.4 (CH), 48.6 (CH), 47.3 (d, *J* = 4.4 Hz, CH), 42.5 (C_{13}), 40.8 (d, *J* = 19.8 Hz, C_1), 38.69 (CH_2), 36.7 (C_{10}), 35.2 (CH_2), 35.1 (CH), 30.9 (C_{23}), 30.7 (C_{22}), 28.2 (CH_2), 24.4 (CH_2), 23.1 (C_{19}), 22.5 (CH_2), 18.8 (CH_2), 18.3 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 11.9 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -188.2 (1F, ddd, *J* = 51.6, 42.1, 12.1 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 452.5 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{26}H_{40}FO_4$ $[M+H]^+$, m/z calculated: 435.2905; found: 435.2902. **IR** (neat) 2942 (m), 2875 (m), 1732 (s), 1704 (s), 1457 (m), 1382 (m), 1284 (m), 1209 (m), 1093 cm^{-1}.

Compound **4.40**: R_f (dichloromethane/methanol, 95/5) 0.39. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.90 (1H, ddt, *J* = 48.6, 10.4, 5.2 Hz, $H_{2\alpha}$), 2.75 (1H, ddd, *J* = 8.4, 5.3, 4.3 Hz, $H_{6\beta}$), 2.31-1.54 (18H, m), 1.39 (3H, s, H_{19}), 1.37-1.25 (2H,m), 1.26-0.98 (5H, m), 0.95 (3H, d, *J* = 6.4 Hz, H_{21}), 0.82 (3H, t, *J* = 7.3 Hz,

$C_6CH_2C\mathbf{H}_3$), 0.70 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.3 (C_7), 204.1 (d, *J* = 13.9 Hz, C_3), 179.9 (C_{24}), 88.8 (d, *J* = 190.7 Hz, C_2), 54.8 (C_{17}), 52.9 (CH), 51.9 (CH), 49.9 (CH), 48.7 (CH), 45.0 (CH), 42.5 (C_{13}), 42.0 (d, *J* = 16.9 Hz, C_1), 38.7 (CH_2), 38.6 (C_{10}), 37.5 (CH_2), 35.1 (CH), 30.9 (C_{23}), 30.7 (C_{22}), 28.2 (CH_2), 24.4 (CH_2), 22.7 (C_{19}), 22.4 (CH_2), 18.9 (CH_2), 18.3 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -195.1 (1F, ddd, *J* = 48.6, 10.4, 5.2 Hz, $F_{2\beta}$) ppm. **LRMS** (ESI^+) m/z: 452.5 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{26}H_{40}FO_4$ $[M+H]^+$, m/z calculated: 435.2905; found: 435.2902. **IR** (neat) 2942 (m), 2875 (m), 1732 (s), 1704 (s), 1457 (m), 1382 (m), 1284 (m), 1209 (m), 1093 cm^{-1}.

Methyl-2β-fluoro-3,7-dioxo-6α-ethyl-5β-cholan-24-oate (4.41)



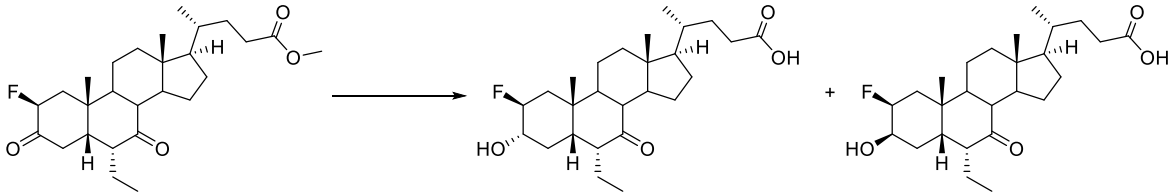
4.39 **4.40** **4.41**

To a solution of a 50:50 ratio of **4.39** and **4.40** (750 mg, 1.72 mmol, 1.0 equiv) in dimethylformamide (17 mL) at rt was added caesium carbonate (840 mg, 2.58 mmol, ~1.5 equiv). After 20 min at rt, iodomethane (0.54 mL, 8.59 mmol, ~5.0 equiv) was added dropwise. After 19 h at rt, the reaction was deemed complete by TLC and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (25 mL) and H_2O (20 mL). The aqueous layer was separated and extracted with ethyl acetate (3 x 25 mL) and the combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford a 917 mg of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 25 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **4.41** as a white residue (339 mg, 0.76 mmol, 54%). The corresponding 2α-fluoro derivative, from **4.39**, was not isolated.

R_f (PE 40-60/acetone, 70/30) 0.49. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.90 (1H, ddd, *J* = 48.8, 13.3, 6.4 Hz, $H_{2\alpha}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.74 (1H, dd, *J* = 13.1, 5.0 Hz, $H_{6\beta}$), 2.54-2.44 (2H, m), 2.40-2.32 (2H, m), 2.29-2.13 (3H, m), 2.09 (1H, dt, *J* = 13.0, 3.3 Hz), 2.01-1.91 (2H, m), 1.85-1.65 (5H, m), 1.54-1.42 (2H, m), 1.39 (3H, s, H_{19}), 1.38-1.05 (7H, m), 0.94 (3H, d, *J* = 6.5 Hz, H_{21}), 0.82 (3H, t, *J* = 7.4 Hz, $C_6CH_2C\mathbf{H}_3$), 0.70 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.3 (C_7), 204.1 (d, *J* = 13.9 Hz, C_3), 174.6 (C_{24}), 88.8 (d, *J* = 190.7 Hz, C_2), 54.8 (C_{17}), 52.9 (CH), 51.9 (CH), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.9 (CH), 48.7 (CH), 45.0 (CH), 42.5 (C_{13}), 42.0 (d, *J* = 16.9 Hz, C_1), 38.72 (CH_2), 38.67 (d, *J* = 11.0 Hz, C_{10}), 37.5 (CH_2), 35.2 (CH), 31.0 (C_{23}), 30.9 (C_{22}), 28.2 (CH_2), 24.4 (CH_2), 22.7 (C_{19}), 22.4 (CH_2), 18.9 (CH_2), 18.3 (C_{21}), 12.1 ($C_6CH_2\mathbf{C}H_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -195.1 (1F, ddt, *J*

= 48.6, 10.4, 5.2 Hz, $F_{2\beta}$) ppm. **LRMS** (ESI^+) m/z: 466.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{42}FO_4$ $[M+H]^+$, m/z calculated: 449.3062; found: 449.3071. **IR** (neat) 2946 (m), 2874 (m), 1734 (s), 1708 (s), 1436 (m), 1380 (m), 1305 (m), 1265 (s), 1168 cm^{-1}.

Methyl-2β-fluoro-3α,7α-dihydroxyl-6α-ethyl-5β-cholan-24-oate (4.42) and methyl-2β-fluoro-3β,7α-dihydroxyl-6α-ethyl-5β-cholan-24-oate (4.43)



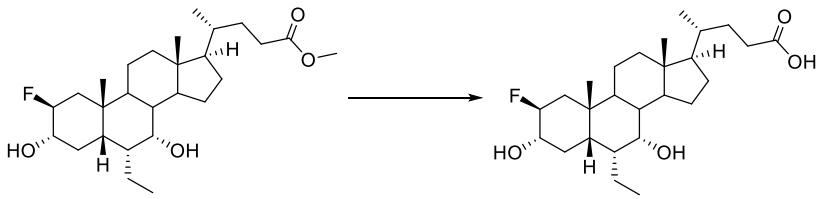
Prepared according to *General Procedure H* (see: *page 172*) using 390 mg of compound **4.41** (0.87 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP Ultra KP-Sil 25 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **4.42** as a colourless oil (146 mg, 0.32 mmol, 37%) and **4.43** as a colourless oil (161 mg, 0.36 mmol, 41%).

Compound 4.42: R_f (PE 40-60/acetone, 70/30) 0.29. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.42 (1H, dddd, *J* = 52.7, 12.5, 8.7, 4.5 Hz, $H_{2\alpha}$), 3.70 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 3.52 (1H, ddt, 13.8, 12.5, 6.0 Hz, $H_{3\beta}$), 2.36 (1H, ddd, *J* = 15.4, 10.2, 5.3 Hz, $H_{23'}$), 2.27-2.17 (3H, m), 2.09-1.97 (2H, m), 1.95-1.86 (2H, m), 1.84-1.76 (1H, m), 1.66-1.29 (14H, m), 1.22-1.13 (4H, m), 0.98 (3H, s, H_{19}), 0.93 (3H, d, *J* = 6.5 Hz, H_{21}), 0.92 (3H, t, *J* = 7.2 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 93.7 (d, *J* = 171.7 Hz, C_2), 74.8 (d, *J* = 17.6 Hz, C_3), 70.4 (C_7), 55.7 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.3 (CH), 44.8 (d, *J* = 1.5 Hz, CH), 42.7 (C_{13}), 40.8 (CH), 40.5 (d, *J* = 15.4 Hz, C_1), 40.0 (CH), 39.4 (CH_2), 38.9 (d, *J* = 11.0 Hz, C_{10}), 35.3 (CH), 34.5 (CH), 31.0 (C_{23}), 30.9 (C_{22}), 30.5 (d, *J* = 8.1 Hz, C_4), 28.1 (CH_2), 23.6 (CH_2), 22.9 (C_{19}), 22.6 (CH_2), 21.0 (CH_2), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -187.1 (1F, ddq, *J* = 52.8, 13.1, 7.5 Hz, $F_{2\beta}$) ppm. **LRMS** (ESI^+) m/z: 470.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{45}FO_4Na$ $[M+Na]^+$, m/z calculated: 475.3194; found: 475.3204. **IR** (neat) 3469 (w), 2936 (m), 2870 (m), 1731 (s), 1457 (m), 1377 (m), 1266 (s), 1196 (m), 1167 cm^{-1}.

Compound 4.43: R_f (PE 40-60/acetone, 70/30) 0.36. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.63 (1H, dddd, *J* = 47.4, 12.5, 4.4, 3.1 Hz, $H_{2\alpha}$), 4.15 (1H, q, *J* = 3.6 Hz, $H_{3\alpha}$), 3.71 (1H, s, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.36 (1H, ddd, *J* = 15.5, 10.2, 5.3 Hz, $H_{23'}$), 2.33 (1H, ddd, *J* = 16.0, 9.7, 6.6 Hz, $H_{23''}$), 2.12 (1H, td, *J* = 13.2, 2.2 Hz), 2.00 (1H, dt, *J* = 12.5, 3.1 Hz), 1.94-1.78 (5H, m), 1.77-1.54 (6H, m), 1.52-1.25 (8H, m), 1.20-1.12 (4H, m), 1.00 (3H, s, H_{19}), 0.93 (3H, d, *J* = 6.8 Hz, H_{21}), 0.92 (3H, t, *J* = 7.1 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 90.6 (d, *J* = 170.9 Hz, C_2), 70.7 (C_7), 67.6 (d, *J* = 17.6 Hz, C_3), 55.8 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.4 (CH), 42.7 (C_{13}), 40.3 (CH),40.0 (CH), 39.5

(CH_2), 38.52 (CH), 38.45 (d, *J* = 9.5 Hz, C_{10}), 35.5 (CH), 34.9 (d, *J* = 16.1 Hz, C_1), 33.7 (CH), 31.0 (C_{23}), 30.9 (C_{22}), 28.6 (d, *J* = 6.6 Hz, C_4), 28.1 (CH_2), 23.7 (CH_2), 23.3 (C_{19}), 22.4 (CH_2), 21.2 (CH_2), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -187.1 (1F, dquin, *J* = 46.9, 7.8 Hz, $F_{2\beta}$) ppm. **LRMS** (ESI^+) m/z: 470.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{45}FO_4Na$ $[M+Na]^+$, m/z calculated: 475.3194; found: 475.3194. **IR** (neat) 2942 (m), 2875 (m), 1732 (s), 1704 (s), 1457 (m), 1382 (m), 1284 (m), 1209 (m), 1093 cm^{-1}.

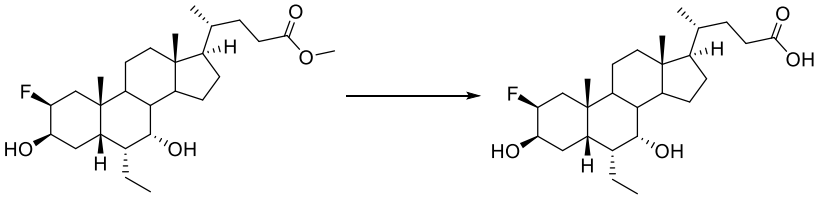
2β-fluoro-3α,7α-dihydroxyl-6α-ethyl-5β-cholanic acid (1.54)



Prepared according to *General Procedure E* (see: *page 160*) using 199 mg of compound **4.42** (0.26 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using dichloromethane/methanol (95/5 to 90/10) as the eluent yielded compound **1.54** as a white residue (92 mg, 0.21 mmol, 80%).

R_f (dichloromethane/methanol), 40/10) 0.29. **$[\alpha]_D$:** -1.1 (c = 0.43, chloroform, 28 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.42 (1H, dddd, *J* = 52.6, 12.5, 8.7, 4.3 Hz, $H_{2\alpha}$), 3.69 (1H, s, $H_{7\beta}$), 3.52 (1H, tdd, 12.0, 8.8, 5.4 Hz, $H_{3\beta}$), 2.39 (1H, ddd, *J* = 15.5, 10.3, 5.1 Hz, $H_{23'}$), 2.29-2.19 (3H, m), 2.01 (2H, t, *J* = 13.0 Hz), 1.93-1.77 (3H, m), 1.65-1.54 (3H, m), 1.53-1.26 (12H, m), 1.23-1.10 (4H, m), 0.97 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.5 Hz, H_{21}), 0.91 (3H, t, *J* = 7.1 Hz, $C_6CH_2C\mathbf{H}_3$), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.6 (C_{24}), 93.7 (d, *J* = 171.7 Hz, C_2), 74.8 (d, *J* = 16.9 Hz, C_3), 70.5 (C_7), 55.8 (C_{17}), 50.3 (CH), 44.8 (CH), 42.7 (C_{13}), 40.8 (CH), 40.6 (d, *J* = 14.7 Hz, C_1), 40.0 (CH), 39.4 (CH_2), 38.9 (d, *J* = 11.0 Hz, C_{10}), 35.4 (CH), 34.5 (CH), 31.0 ($C_{23,}$), 30.8 (C_{22}), 30.5 (d, *J* = 8.1 Hz, C_4), 28.1 (CH_2), 23.6 (CH_2), 22.9 (C_{19}), 22.6 (CH_2), 21.1 (CH_2), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.6 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.8 (1F, ddq, *J* = 52.9, 13.0, 7.5 Hz, $F_{2\beta}$) ppm. **LRMS** (ESI^+) m/z: 456.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{26}H_{43}FO_4Na$ $[M+Na]^+$, m/z calculated: 461.3038; found: 461.3039. **IR** (neat) 3413 (w), 2935 (m), 2870 (m), 1706 (s), 1457 (m), 1377 (m), 1245 (s), 1152 (m), 1066 cm^{-1}.

2β-fluoro-3β,7α-dihydroxyl-6α-ethyl-5β-cholanic acid (4.44)



4.43 4.44

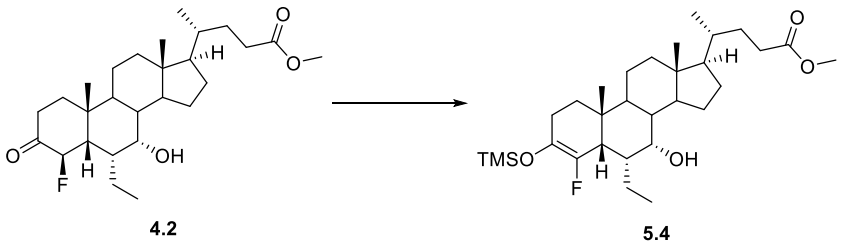
Prepared according to *General Procedure E* (see: *page 160*) using 145 mg of compound **4.43** (0.32 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using dichloromethane/methanol (98/2 to 90/10) as the eluent yielded compound **4.44** as a white residue (57 mg, 0.13 mmol, 40%).

R_f (dichloromethane/methanol, 90/10) 0.43. **$[\alpha]_D$:** -5.68 (c = 0.19, chloroform, 28 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.63 (1H, ddt, *J* = 47.8, 12.2, 3.6 Hz, $H_{2\alpha}$), 4.15 (1H, q, *J* = 3.2 Hz, $H_{3\alpha}$), 3.71 (1H, s, $H_{7\beta}$), 2.40 (1H, ddd, *J* = 15.4, 10.2, 5.1 Hz, $H_{23'}$), 2.33 (1H, ddd, *J* = 15.8, 9.5, 6.4 Hz, $H_{23''}$), 2.15-2.08 (1H, m), 2.00 (1H, dt, *J* = 12.4, 3.3 Hz), 1.94-1.77 (4H, m), 1.75-1.54 (7H, m), 1.50-1.29 (9H, m), 1.21-1.12 (4H, m), 1.00 (3H, s, H_{19}), 0.94 (3H, d, *J* = 6.2 Hz, H_{21}), 0.92 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.7 (C_{24}), 90.6 (d, *J* = 171.7 Hz, C_2), 70.7 (C_7), 67.7 (d, *J* = 16.9 Hz, C_3), 55.8 (C_{17}), 50.4 (CH), 42.7 (C_{13}), 40.3 (CH), 40.0 (CH), 39.5 (CH_2), 38.52 (CH), 38.49 (d, *J* = 11.7 Hz, C_{10}), 35.3 (CH), 34.9 (d, *J* = 16.1 Hz, C_1), 33.8 (CH), 31.0 (C_{23}), 30.8 (C_{22}), 28.6 (d, *J* = 5.9 Hz, C_4), 28.1 (CH_2), 23.7 (CH_2), 23.3 (C_{19}), 22.4 (CH_2), 21.2 (CH_2), 18.2 (C_{21}), 11.8 ($C_6CH_2\mathbf{C}H_3$), 11.7 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -187.0 (1F, dquin, *J* = 47.8, 7.6 Hz, $F_{2\beta}$) ppm. **LRMS** (ESI^+) m/z: 456.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{26}H_{43}FO_4Na$ $[M+Na]^+$, m/z calculated: 461.3038; found: 461.3030. **IR** (neat) 3448 (w), 2935 (m), 2872 (m), 2360 (m), 1706 (s), 1458 (m), 1378 (s), 1333 (m), 1244 (s), 1148 (m), 1048 cm^{-1}.

8.5 Synthesis of OCA (NZP084) 4,4-difluoro analogues

The synthesis and characterisation of the following compounds is presented in the order of reactions discussed in Chapter 5.

Methyl-3-trimethylsilyloxy-4-fluoro-6α-ethyl-7α-hydroxyl-5β-cholan-3-ene-24-oate (5.4)

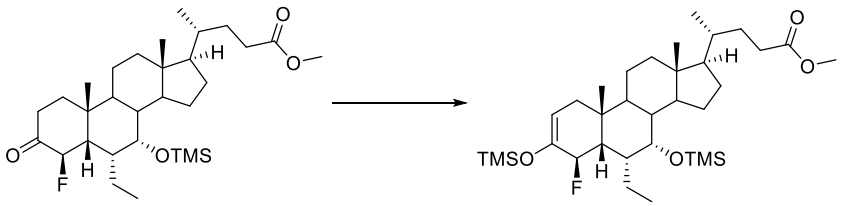


4.2 5.4

To a solution of **4.2** (7.30 g, 16.0 mmol, 1.0 equiv) in dry THF (300 mL) under argon at -78 °C was added a solution of LDA in hexanes (1M, 21.1 mL, 21.1 mmol, ~1.3 equiv) dropwise over 15 min. After stirring for a further 15 min, to the reaction mixture was then added a solution of trimethylsilylchloride (2.70 mL, 21.1 mmol, ~1.3 equiv) in dry THF (15 mL) and stirred at -78 °C under argon for 1 h. The reaction mixture was then quenched by the addition of sat. $NaHCO_3$ solution (300 mL) and warmed to rt over 15 min. The aqueous phase was separated and extracted with dichloromethane (2 x 150 mL) and the combined organic layers were washed with brine (300 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 8.06 g of crude material as a yellow residue that was used without further purification.

The data presented for compound **5.4** was attained from an impure crude mixture. Characteristic signals are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.66 (3H, s, $C_{24}OCH_3$), 0.99 (3H, s, H_{19}), 0.94 (3H, t, *J* = 7.0 Hz, $C_6CH_2CH_3$), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.66 (3H, s, H_{18}), 0.18 (9H, s, $OSi(CH_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 147.8 (d, *J* = 248.0 Hz, C_4), 130.6 (d, *J* = 11 Hz, C_3), 70.2 (C_7), 55.7 (C_{17}), 51.4 ($C_{24}O\mathbf{C}H_3$), 50.5 (CH), 45.1 (d, *J* = 15.4 Hz, C_5), 43.5 (d, *J* = 2.2 Hz, CH), 42.6 (C_{13}), 40.7 (CH), 39.7 (CH_2), 37.1 (d, *J* = 7.3 Hz, C_{10}), 35.3 (CH), 33.9 (CH), 31.0 (C_{23}), 30.9 (C_{22}), 28.1 (CH_2), 25.6 (CH_2), 25.5 (d, *J* = 1.5 Hz, CH_2), 24.7 (d, *J* = 9.5 Hz, CH_2), 23.5 (CH_2), 22.4 (d, *J* = 1.5 Hz, C_{19}), 21.6 (CH_2), 18.2 (C_{21}), 12.8 (d, *J* = 2.2 Hz, $C_6CH_2C\mathbf{H}_3$), 11.8 (C_{18}), 0.5 (d, *J* = 1.5 Hz, $OSi(CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -135.2 (1F, s, F_4) ppm.

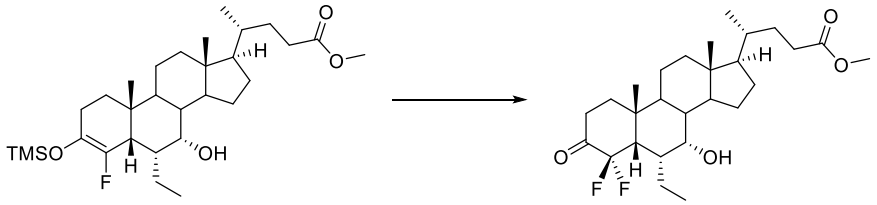
Methyl-3,7α-*bis*-trimethylsilyloxy-4β-fluoro-6α-ethyl-5β-cholan-2-ene-24-oate (5.2)



To a solution of **5.5** (291 mg, 0.56 mmol, 1.0 equiv) in dry THF (10 mL) under argon at -78 °C was added a solution of LDA in hexanes (1M, 0.58 mL, 0.58 mmol, ~1.2 equiv) dropwise over 15 min. After stirring for a further 15 min, to the reaction mixture was then added a solution of trimethylsilylchloride (0.08 mL, 0.67 mmol, ~1.3 equiv) in dry THF 5 mL) and stirred at -78 °C under argon for 1 h. The reaction mixture was then quenched by the addition of sat. $NaHCO_3$ solution (20 mL) and warmed to rt over 15 min. The aqueous phase was separated and extracted with dichloromethane (3 x 25 mL) and the combined organic layers were washed with brine (75 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 376 g of crude material as a yellow residue that was used without further purification.

The data presented for compound **5.2** was attained from an impure crude mixture. Characteristic signals are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.67 (1H, ddd, *J* = 51.5, 5.8, 1.5 Hz, $H_{4\alpha}$), 3.67 (3H, s, $C_{24}OCH_3$), 0.64 (3H, s, H_{18}), 0.17 (9H, s, $OSi(CH_3)_3$), 0.15 (9H, s, $OSi(CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -169.9 - -170.1 (1F, m, $F_{4\beta}$) ppm.

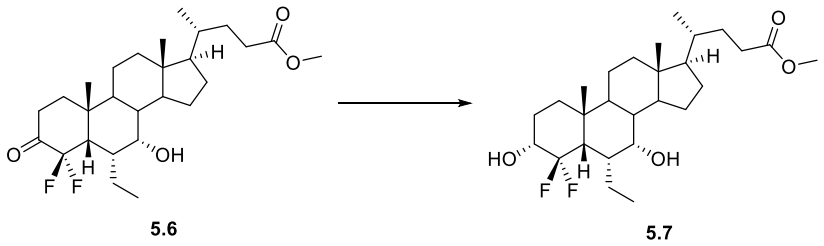
Methyl-3-keto-4,4-difluoro-6α-ethyl-7α-hydroxyl-5β-cholan-3-ene-24-oate (5.6)



To a solution of the crude **5.4** (8.06 g, assumed 16.0 mmol, 1.0 equiv) in acetonitrile (360 mL) at rt was added Selectfluor® (11.4 g, 32.0 mmol, ~2.0 equiv) and stirred for 16 h. The solvent was removed *in vacuo* and the residue was diluted with dichloromethane (500 mL) and received H_2O (500 mL). The aqueous phase was separated and extracted with dichloromethane (2 x 250 mL) and the combined organic layers were washed with brine (250 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 7.51 g of crude material as a yellow residue that was used without further purification.

R_f (PE 40-60/acetone), 60/40) 0.34. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.70 (1H, dq, *J* = 7.7, 3.2 Hz, $H_{7\beta}$), 3.66 (3H, s, $C_{24}OCH_3$), 2.70 (1H, tdd, *J* = 14.2, 5.1, 3.4 Hz, $H_{2\alpha}$), 2.44 (1H, dq, *J* = 15.2, 3.8 Hz, $H_{2\beta}$), 2.35 (1H, ddd, *J* = 15.5, 10.2, 5.4 Hz, $H_{23'}$), 2.24 (1H, dd, *J* = 9.5, 6.6 Hz, $H_{23''}$), 2.18 (1H, dd, *J* = 10.2, 2.9 Hz), 2.14 (1H, dt, *J* = 17.0, 5.6 Hz), 2.08 (1H, td, *J* = 14.6, 5.8 Hz), 2.00-1.86 (3H, m), 1.84-1.75 (3H, m), 1.73-1.63 (3H, m), 1.58 (1H, dd, *J* = 13.9, 4.8 Hz), 1.54-1.28 (6H, m), 1.25-1.11 (3H, m), 1.09 (3H, s, H_{19}), 0.98 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.93 (3H, d, *J* = 6.4 Hz, H_{21}), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 197.4 (dd, *J* = 29.3, 26.4 Hz, C_3), 174.7 (C_{24}), 117.1 (dd, *J* = 257.1, 254.2 Hz, C_4), 67.8 (C_7), 55.6 (C_{17}), 53.7 (dd, *J* = 20.2, 18.0 Hz, C_5), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.4 (CH), 42.7 (CH), 42.4 (C_{13}), 40.0 (CH), 39.1(CH_2), 37.3 (d, *J* = 5.9 Hz, C_{10}), 35.3 (CH), 34.6 (d, *J* = 2.9 Hz, CH), 34.4 (CH_2), 33.0 (CH_2), 31.0 (C_{23}), 30.9 (C_{22}), 28.1 (CH_2), 23.6 (CH_2), 23.5 (d, *J* = 3.7 Hz, CH_2), 23.2 (C_{19}), 21.4 (CH_2), 18.3 (C_{21}), 12.5 (d, *J* = 2.2 Hz, $C_6CH_2C\mathbf{H}_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -99.2 (1F, dd, *J* = 263.6, 17.3 Hz, $F_{4\beta}$), -100.7 (1F, ddd, *J* = 263.6, 29.5, 15.6 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 486.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{42}F_2O_4Na$ $[M+Na]^+$, m/z calculated: 491.2943; found: 491.2939. **IR** (neat) 3634 (m), 2937 (m), 2873 (m), 2361 (w), 2252 (w), 1756 (s), 1732 (s), 1437 (m), 1379 (m), 1197 (m), 1168 (m), 1137 (m) cm^{-1}.

Methyl-3α,7α-dihydroxy-4,4-difluoro-6α-ethyl-5β-cholan-24-oate (5.7)

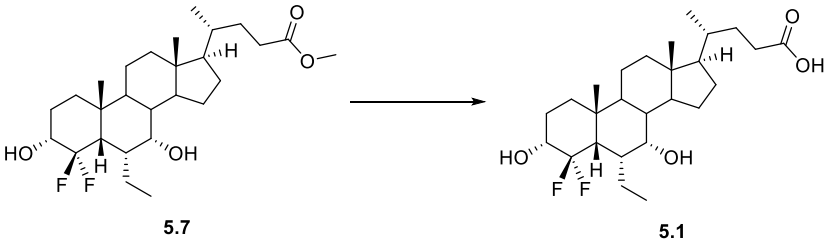


5.6 5.7

Prepared according to *General Procedure H* (see: *page 172*) using 7.51 g of compound **5.6** (assumed 16.0 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil 100 g cartridge) using hexane/acetone (100/0 to 80/20) as the eluent yielded compound **5.7** as a colourless residue (3.09 g, 6.57 mmol, 41% *over three steps*).

R_f (PE 40-60/acetone), 50/50) 0.47. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.76-3.65 (2H, m, $H_{7\beta}$ + $H_{3\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.36 (1H, ddd, *J* = 15.5, 10.3, 5.4 Hz, $H_{23'}$), 2.31-2.19 (2H, m), 2.11 (1H, d, *J* = 5.4 Hz), 2.00-1.92 (3H, m), 1.91-1.30 (16H, m), 1.22-1.10 (4H, m), 1.04 (3H, s, H_{19}), 0.97 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.92 (3H, d, *J* = 6.5 Hz, H_{21}), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 73.4 (dd, *J* = 24.2, 22.7 Hz, C_3), 68.0 (C_7), 55.6 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.8 (CH), 50.4 (dd, *J* = 22.4, 15.0 Hz, C_5), 42.4 (C_{13}), 41.7 (CH), 40.0 (CH), 39.3 (CH_2), 38.4 (dd, *J* = 8.8, 1.5 Hz, C_{10}), 35.4 (CH), 34.2 (d, *J* = 4.4 Hz, CH), 33.9 (CH_2), 31.01 (C_{23}), 30.96 (C_{22}), 28.2 (CH_2), 25.3 (d, *J* = 6.6 Hz, CH_2), 23.9 (C_{19}), 23.7 (CH_2), 23.6 (d, *J* = 7.3 Hz, CH_2), 21.1 (CH_2), 18.3 (C_{21}), 12.6 (d, *J* = 3.7 Hz, $C_6CH_2C\mathbf{H}_3$), 11.9 (C_{18}) ppm; difluorinated carbon C_4 not observed. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -99.3 (1F, d, *J* = 239.3 Hz, $F_{4\beta}$), -111.4 (1F, dtd, *J* = 239.3, 34.7, 22.5 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 488.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{27}H_{44}F_2O_4Na$ $[M+Na]^+$, m/z calculated: 493.3100; found: 493.3090. **IR** (neat) 3630 (m), 2935 (s), 2871 (s), 2360 (w), 2184 (w), 1734 (s), 1453 (m), 1412 (m), 1378 (m), 1200 (s), 1168 (s), 1090 (m) cm^{-1}.

3α,7α-Dihydroxy-4α,4β-difluoro-6α-ethyl-5β-cholanic acid (5.1)

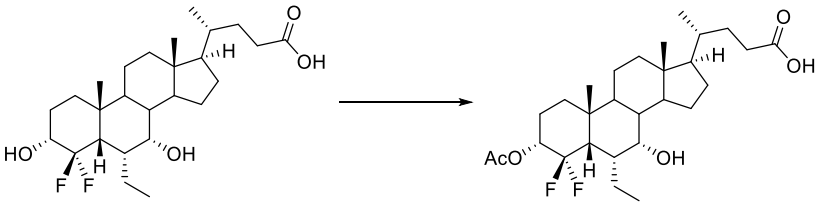


5.7 5.1

Prepared according to *General Procedure E* (see: *page 160*) using 1.77 g of compound **5.7** (3.75 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil 50 g cartridge) using hexane/acetone (100/0 to 90/10) as the eluent yielded compound **5.1** as a colourless oil (1.51 g, 3.30 mmol, 88%).

R_f (PE 40-60/acetone), 60/40) 0.25. **$[\alpha]_D$:** +4.67 (c = 0.15, chloroform, 21 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.78-3.64 (2H, m, $H_{7\beta}$ + $H_{3\beta}$), 2.39 (1H, ddd, *J* = 15.8, 10.3, 5.3 Hz, $H_{23'}$), 2.25 (1H, ddd, *J* = 15.7, 9.6, 6.4 Hz, $H_{23''}$), 1.98-1.93 (3H, m), 1.86-1.65 (7H, m), 1.60 (1H, d, *J* = 13.0 Hz), 1.49-1.41 (5H, m), 1.40-1.29 (3H, m), 1.25-1.06 (6H, m), 1.04 (3H, s, H_{19}), 0.96 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.93 (3H, d, *J* = 6.4 Hz, H_{21}), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 179.4 (C_{24}), 124.6 (dd, *J* = 252.0, 247.6 Hz, C_4), 73.4 (t, *J* = 23.1 Hz, C_3), 68.1 (C_7), 55.6 (C_{17}), 50.7 (CH), 50.4 (dd, *J* = 22.0, 15.4 Hz, C_5), 42.4 (C_{13}), 41.7 (CH), 40.0 (CH), 39.3 (CH_2), 38.3 (d, *J* = 8.1 Hz, C_{10}), 35.3 (CH), 34.1 (d, *J* = 5.1 Hz, CH), 33.9 (CH_2), 30.9 (C_{23}), 30.8 (C_{22}), 28.2 (CH_2), 25.3 (d, *J* = 6.6 Hz, CH_2), 23.9 (C_{19}), 23.7 (CH_2), 23.5 (d, *J* = 7.3 Hz, CH_2), 21.1 (CH_2), 18.2 (C_{21}), 12.6 (d, *J* = 2.9 Hz, $C_6CH_2C\mathbf{H}_3$), 11.9 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -99.1 (1F, d, *J* = 239.3 Hz, $F_{4\beta}$), -111.1 (1F, dtd, *J* = 241.0, 38.2, 19.1 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI$^+$) m/z: 474.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI$^+$) $C_{26}H_{42}F_2O_4Na$ $[M+Na]^+$, m/z calculated: 479.2943; found: 479.2949. **IR** (neat) 3618 (m), 2936 (s), 2873 (s), 2362 (w), 2251 (w), 2159 (w), 1707 (s), 1456 (m), 1379 (m), 1201 (s), 1119 (s), 1090 (m) cm^{-1}.

3α-Acetoxy-4α,4β-difluoro-6α-ethyl-7α-hydroxy 5β-cholanic acid (5.8)



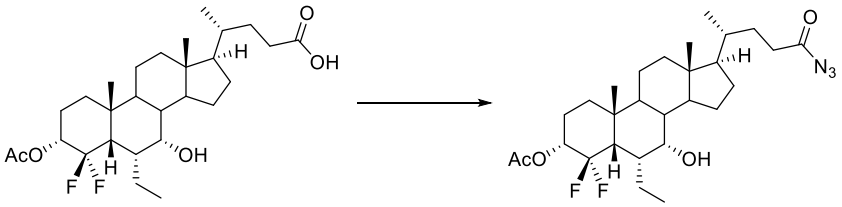
5.1 **5.8**

Prepared according to *General Procedure C* (see: *page 151*) using 400 mg of compound **5.1** (0.88 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil Ultra 25 g cartridge) using hexane/acetone (100/0 to 80/20) as the eluent yielded compound **5.8** as a colourless oil (270 mg, 0.54 mmol, 62%).

R_f (PE 40-60/acetone), 60/40) 0.41. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.97 (1H, ddd, *J* = 27.5, 10.2, 6.0 Hz, $H_{3\beta}$), 3.67 (1H, s, $H_{7\beta}$), 2.54-2.33 (2H, m), 2.30-2.26 (1H, m), 2.24-2.18 (1H, m), 2.13 (3H, s, C_3OCOCH_3), 2.02-1.79 (6H, m), 1.77-1.62 (3H, m), 1.56-1.42 (6H, m), 1.41-1.21 (5H, m), 1.20-1.13 (3H, m), 1.05 (3H, s, H_{19}), 0.95 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.94 (3H, d, *J* = 6.5 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 178.7 (C_{24}), 170.1 ($C_3O\mathbf{C}OCH_3$), 73.1 (t, *J* = 20.5 Hz, C_3), 68.0 (C_7), 55.6 (C_{17}), 50.7 (CH), 42.4 (C_{13}), 41.8 (CH), 40.0 (CH), 39.3 (CH_2), 38.2 (d, *J* = 9.5 Hz, C_{10}), 35.3 (CH), 34.1 (d, *J* = 4.4 Hz, CH), 33.7 (CH_2), 30.82 (C_{23}), 30.77 (C_{22}), 28.2 (CH_2), 23.8 (C_{19}), 23.7 (CH_2), 23.5 (d, *J* = 7.3 Hz, CH_2), 23.0 (d, *J* = 5.9 Hz, CH_2), 21.1 (CH_2), 20.9 ($C_3OCO\mathbf{C}H_3$), 18.2 (C_{21}), 12.6 (d, *J* = 3.7 Hz, $C_6CH_2C\mathbf{H}_3$), 11.9 (C_{18}) ppm; difluorinated carbon C_4 and adjacent carbon C_5 not observed. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -98.2 (1F, d, *J* = 244.5 Hz, $F_{4\beta}$), -107.1 (1F, ddd, *J* = 242.8, 36.4, 22.5 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI$^+$) m/z: 516.5 $[M+NH_4]^+$, 100%. **HRMS** (ESI$^+$) $C_{28}H_{44}F_2O_5Na$

$[M+Na]^+$, m/z calculated: 521.3049; found: 521.3059. **IR** (neat) 3638 (m), 2934 (s), 2871 (s), 1978 (w), 1742 (s), 1708 (s), 1456 (m), 1378 (m), 1231 (s), 1166 (s) cm^{-1}.

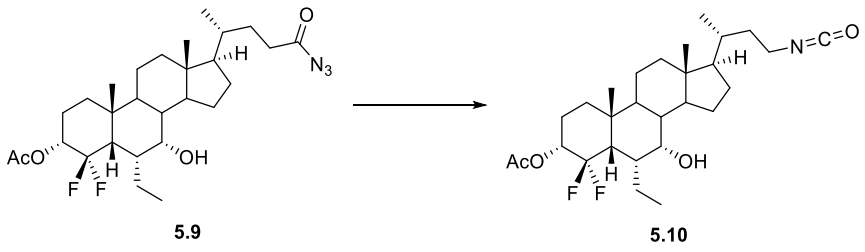
3α-Acetoxy-4,4-difluoro-6α-ethyl-7α-hydroxyl-5β-cholan-24-azide (5.9)



To a stirred solution of **5.8** (240 mg, 0.48 mmol, 1.0 equiv) in dry THF (5.3 mL) at rt was added triethylamine (0.14 mL, 0.48 mmol, ~1.0 equiv) dropwise under argon and then cooled to 0 °C. To the reaction mixture was then added diphenylphosphoryl azide (0.16 mL, 0.72 mmol, ~1.5 equiv) dropwise and stirred at 0 °C. for 3 h behind a blast shield. The reaction mixture was then quenched by the addition of ice-cold brine (10 mL) and diluted with dichloromethane (5 mL). The aqueous phase was separated and extracted with dichloromethane (3 x 20 mL) and the combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo* at 0 °C to yield a pale-yellow oil that was used without further purification.

The data presented for compound **5.9** was attained from an impure crude mixture. Characteristic signals are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.89 (1H, ddd, *J* =27.4, 10.8, 4.2 Hz, $H_{3\beta}$), 3.58 (1H, s, $H_{7\beta}$), 2.30 (1H, ddd, *J* = 15.7, 10.0, 5.4 Hz, $H_{23'}$), 2.04 (3H, s, C_3OCH_3), 0.97 (3H, s, H_{19}), 0.87 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.84 (3H, d, *J* = 6.4 Hz, H_{21}) , 0.57 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 181.0 (C_{24}), 170.0 ($\mathbf{C}_3OCH_3$), 73.0 (t, *J* = 21.6 Hz, C_3), 68.0 (C_7), 55.7 (C_{17}), 50.8 (dd, *J* = 21.6, 15.0 Hz, C_5), 42.4 (C_{13}), 41.7 (CH), 38.1 (d, *J* = 8.1 Hz, C_{10}), 34.0 (d, *J* = 5.1 Hz, CH), 23.7 (C_{19}), 20.8 (C_3OCOCH_3), 18.2 (C_{21}), 12.5 (d, *J* = 3.7 Hz, $C_6CH_2C\mathbf{H}_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -98.2 (1F, d, *J* = 242.8 Hz, $F_{4\beta}$), -107.2 (1F, dtd, *J* = 242.8, 33.0, 26.0 Hz, $F_{4\alpha}$) ppm. **IR** (neat) 2169 (s, CON_3), 2134 (s, CON_3), 1748 (s, $C_3O\mathbf{CO}$) cm^{-1}.

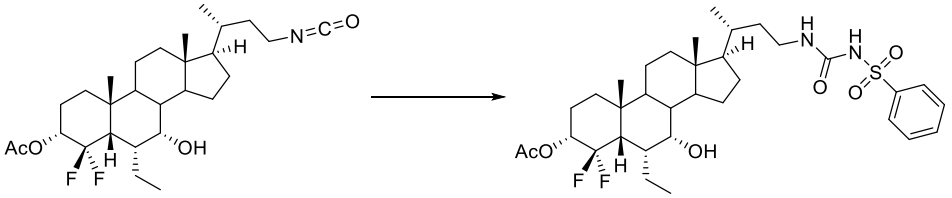
3α-Acetoxy-4,4-difluoro-6α-ethyl-7α-hydroxyl-24-nor-5β-cholan-23-isocyanate (5.10)



A stirred solution of crude **5.9** (252 mg assumed, 0.48 mmol) in dry toluene (7.5 mL) was heated to 125 °C under argon for 4 h. The reaction was then cooled to rt and the resulting colourless solution was used without further purification.

The data presented for compound **5.10** was attained from an impure crude mixture. Characteristic signals are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.89 (1H, ddd, *J* = 27.5 10.2, 6.5 Hz, $H_{3\beta}$), 3.59 (1H, q, *J* = 3.2 Hz, $H_{7\beta}$), 3.27 (1H, ddd, *J* = 13.1, 7.8, 4.5 Hz, $H_{23'}$), 3.22-3.15 (1H, m, $H_{23''}$), 2.04 (3H, s, C_3OCH_3), 0.97 (3H, s, H_{19}), 0.88 (3H, t, *J* = 7.1 Hz, $C_6CH_2C\mathbf{H}_3$), 0.86 (3H, d, *J* = 6.4 Hz, H_{21}), 0.59 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.0 ($\mathbf{C}_3OCH_3$), 121.8 (NCO), 73.1 (t, *J* = 21.3 Hz, C_3), 67.9 (C_7), 55.8 (C_{17}), 50.8 (dd, *J* = 21.6, 15.0 Hz, C_5), 42.5 (C_{13}), 41.7 (CH), 38.1 (d, *J* = 7.3 Hz, C_{10}), 34.0 (d, *J* = 5.1 Hz, CH), 23.8 (C_{19}), 20.8 ($C_3OCO\mathbf{C}H_3$), 18.2 (C_{21}), 12.6 (d, *J* = 3.7 Hz, $C_6CH_2C\mathbf{H}_3$), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -98.2 (1F, d, *J* = 242.8 Hz, $F_{4\beta}$), -107.2 (1F, dtd, *J* = 243.2, 37.7, 22.5 Hz, $F_{4\alpha}$) ppm. **IR** (neat) 2270 (s, NCO), 1747 (s, $C_3O\mathbf{CO}$) cm^{-1}.

***N,N'*-(3α-acetoxy-4,4-difluoro-6α-ethyl-7α-hydroxyl-24-*nor*-5β-cholan-23-yl)-benzenesulfonylurea (5.11)**



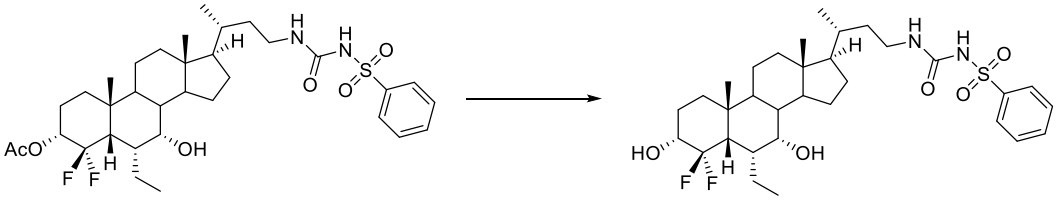
5.10 **5.11**

Prepared according to *General Procedure I* (see: *page 181*) using 238 mg (assumed) of compound **5.19** (0.48 mmol, 1.0 equiv) and 113 mg of benzenesulfonamide to afford, after purification, compound **5.11** as a white residue (235 mg, 38%).

R_f (PE 40-60/acetone), 60/40) 0.38. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.90 (2H, dd, *J* = 8.4, 1.1 Hz, C_oH_{Ar}), 7.63 (1H, tt, *J* = 7.6, 1.1 Hz, C_pH_{Ar}), 7.49 (2H, t, *J* = 8.1 Hz, C_mH_{Ar}), 6.53 (1H, s, $C_{23}N\mathbf{H}$), 4.98 (1H, ddd, *J* = 27.1, 11.3, 4.5 Hz, $H_{3\beta}$), 3.67 (1H, s, $H_{7\beta}$), 3.27 (1H, ddd, *J* = 13.5, 8.9, 4.5 Hz, $H_{23'}$), 3.18-3.11 (1H, m, $H_{23''}$), 2.13 (3H, s, C_3OCH_3), 2.02-1.79 (6H, m), 1.75-1.64 (4H, m), 1.62 - 1.43 (5H, m), 1.42-1.36 (1H, m), 1.28-1.10 (7H, m), 1.05 (3H, s, H_{19}), 0.95 (3H, t, *J* = 7.3 Hz, $C_6CH_2CH_3$), 0.92 (3H, d, *J* = 6.5 Hz, H_{21}) , 0.63 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.1 ($C_3\mathbf{C}OCH_3$), 151.6 ($C_{23}NH\mathbf{C}O$), 139.6 ($C_{i\,Ar}$), 133.7 (C_pH_{Ar}), 129.3 (C_mH_{Ar}), 126.8 (C_oH_{Ar}), 73.1 (t, *J* = 22.4 Hz, C_3), 68.0 (C_7), 55.8 (C_{17}), 50.7 (CH), 42.4 (C_{13}), 41.7 (CH), 40.0 (CH), 39.3 (CH_2), 38.2 (d, *J* = 10.3 Hz, C_{10}), 38.0 (CH_2), 35.5 (CH_2), 34.1 (d, *J* = 4.4 Hz, CH), 33.8 (CH), 33.7 (CH_2), 28.4 (CH_2), 23.8 (C_{19}), 23.7 (CH_2), 23.5 (d, *J* = 6.6 Hz, CH_2), 22.9 (d, *J* = 5.9 Hz, CH_2), 21.1 (CH_2), 20.9 ($C_3OCO\mathbf{C}H_3$), 18.5 (C_{21}), 12.6 (d, *J* = 2.9 Hz, $C_6CH_2C\mathbf{H}_3$), 11.9 (C_{18}) ppm; difluorinated carbon C_4 and adjacent carbon C_5 not observed. **^{19}F NMR** (^{1}H non-

decoupled, 376 MHz, $CDCl_3$): δ -98.2 (1F, d, *J* = 242.8 Hz, $F_{4\beta}$), -107.1 (1F, dtd, *J* = 243.2, 37.7, 22.5 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 635.8 $[M\text{-}OH]^+$, 100%. **HRMS** (ESI^+) $C_{34}H_{50}F_2N_2O_6SNa$ $[M+Na]^+$, m/z calculated: 675.3250; found: 675.3241. **IR** (neat) 3352 (w), 2937 (m), 2875 (m), 1745 (s), 1679 (s), 1589 (m), 1538 (m), 1489 (m), 1448 (m) 1337 (s), 1233 (s), 1160 (s) cm^{-1}.

***N,N'*-(3α,7α-dihydroxyl-4,4-difluoro-6α-ethyl-24-*nor*-5β-cholan-23-yl)-benzenesulfonylurea (5.12)**

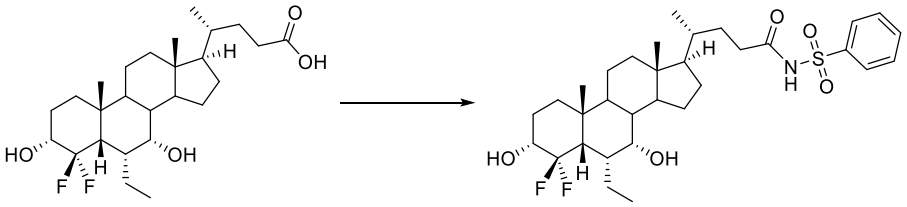


5.11 **5.12**

Prepared according to *General Procedure A* (see: *page 147*) using 210 mg of compound **5.11** (0.32 mmol, 1.0 equiv) and 10 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage SNAP KP-Sil 10 g cartridge) using dichloromethane/methanol (100/0 to 95/5) as the eluent yielded compound **5.12** as a colourless residue (102 mg, 0.17 mmol, 52%).

$\mathbf{R_f}$ (PE 40-60/acetone), 60/40) 0.31. **$[\alpha]_D$:** +5.65 (c = 0.12, chloroform, 25 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.19 (1H, s, C_{23}NHCON**H**), 7.90 (2H, dt, *J* = 7.3, 1.3 Hz, C_oH_{Ar}), 7.65 (1H, tt, *J* = 7.5, 1.1 Hz, C_pH_{Ar}), 7.54 (2H, tt, *J* = 7.3, 1.6 Hz, C_mH_{Ar}), 6.51 (1H, t, *J* = 5.2 Hz, C_{23}NH), 3.78-3.67 (2H, m, $H_{7\beta}$ + $H_{3\beta}$), 3.29 (1H, ddt, *J* = 13.9, 10.2, 5.1 Hz, $H_{23'}$), 3.18 (1H, ddd, *J* = 13.6, 8.0, 6.1 Hz, $H_{23''}$), 2.30 (1H, dd, *J* = 31.4, 11.0 Hz, C_7OH), 1.99-1.94 (3H, m), 1.88-1.57 (9H, m), 1.52-1.37 (5H, m), 1.23-1.11 (7H, m), 1.04 (3H, s, H_{19}), 0.97 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.93 (3H, d, *J* = 6.5 Hz, H_{21}), 0.64 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 151.8 (C_{23}NH**C**O), 139.7 ($C_{i\ Ar}$), 133.8 (C_pH_{Ar}), 129.4 (C_mH_{Ar}), 126.8 (C_oH_{Ar}), 73.4 (t, *J* = 23.5 Hz, C_3), 68.1 (C_7), 55.8 (C_{17}), 50.8 (CH), 50.4 (dd, *J* = 22.0, 14.7 Hz, C_5), 42.5 (C_{13}), 41.7 (CH), 40.0 (CH), 39.3 (CH_2), 38.3 (d, *J* = 8.1 Hz, C_{10}), 38.1 (CH_2), 35.6 (CH_2), 34.1 (d, *J* = 4.4 Hz, CH), 33.92 (CH_2), 33.86 (CH), 28.4 (CH_2), 25.3 (d, *J* = 5.9 Hz, CH_2), 23.9 (C_{19}), 23.7 (CH_2), 23.6 (d, *J* = 7.3 Hz, CH_2), 21.1 (CH_2), 18.5 (C_{21}), 12.6 (d, *J* = 3.7 Hz, $C_6CH_2C\mathbf{H}_3$), 11.9 (C_{18}) ppm; difluorinated carbon C_4 not observed. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -99.3 (1F, d, *J* = 239.3 Hz, $F_{4\beta}$), -111.3 (1F, dtd, *J* = 241.0, 33.0, 22.5 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 593.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{32}H_{48}F_2N_2O_5SNa$ $[M+Na]^+$, m/z calculated: 633.3114; found: 633.3138. **IR** (neat) 3381 (w), 2939 (m), 2596 (w), 2225 (w), 1702 (s), 1540 (m), 1449 (m) 1376 (s), 1347 (s), 1161 (s) cm^{-1}.

***N*-(3α,7α-dihydroxyl-4,4-difluoro-6α-ethyl-5β-cholan-24-yl)-benzene acylsulfonamide (5.13)**



5.1 5.13

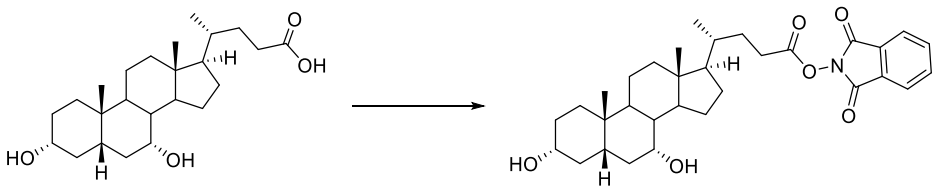
To a stirred solution of **5.1** (400 mg, 0.88 mmol, 1.0 equiv) in dry dichloromethane (16 mL) at rt under argon was added EDCI (345 mg, 1.75 mmol, ~2.0 equiv), DMAP (214 mg, 1.75 mmol, ~2.0 equiv) and benzenesulfonamide (275 mg, 1.75 mmol, ~2.0 equiv) and stirred at rt for 26 h. The reaction mixture was quenched by the addition of H_2O (20 mL). The aqueous phase was separated and extracted with dichloromethane (2 x 20 mL) and the combined organic phases were dried over MgSO4, filtered and concentrated in vacuo to yield a pale-pink oil of 284 mg. Purification by flash column chromatography (Biotage SNAP KP-Sil Ultra 25 g cartridge) using hexane/acetone (95/5 to 80/20) as the eluent yielded compound **5.13** as a colourless residue (161 mg, 0.27 mmol, 31%).

R_f (PE 40-60/acetone), 60/40) 0.26. **$[\alpha]_D$:** +7.38 (c = 0.17, chloroform, 25 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.06 (2H, dd, *J* = 7.6, 1.3 Hz, C_oH_{Ar}), 7.64 (1H, tt, *J* = 7.6, 0.8 Hz, C_pH_{Ar}), 7.54 (2H, t, *J* = 7.3 Hz, C_mH_{Ar}), 3.85-3.69 (2H, m, $H_{7\beta}$ + $H_{3\beta}$), 2.45 (1H, dd, *J* = 32.1, 11.0 Hz, C_7OH), 2.27 (1H, ddd, *J* = 15.3, 10.2, 5.0 Hz, $H_{23'}$), 2.16-2.10 (1H, m), 1.97-1.89 (3H, m), 1.86-1.64 (8H, m), 1.61-1.54 (1H, m), 1.47-1.43 (4H, m), 1.37-1.29 (2H, m), 1.22-1.06 (6H, m), 1.03 (3H, s, H_{19}), 0.97 (3H, t, *J* = 7.3 Hz, $C_6CH_2C\mathbf{H}_3$), 0.84 (3H, d, *J* = 6.2 Hz, H_{21}), 0.59 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 171.5 (C_{24}), 138.8 (C_{iAr}), 133.8 (C_pH_{Ar}), 128.9 (C_mH_{Ar}), 128.3 (C_oH_{Ar}), 73.4 (t, *J* = 23.1 Hz, C_3), 68.4 (C_7), 55.4 (C_{17}), 50.6 (CH), 50.2 (dd, *J* = 23.5, 13.2 Hz, C_5), 42.4 (C_{13}), 41.6 (CH), 39.9 (CH), 39.3 (CH_2), 38.3 (d, *J* = 8.8 Hz, C_{10}), 35.0 (CH), 34.1 (d, *J* = 4.4 Hz, CH), 33.9 (CH_2), 33.1 (CH_2), 30.4 (CH_2), 28.0 (CH_2), 25.2 (d, *J* = 6.6 Hz, CH_2), 23.8 (C_{19}), 23.63 (CH_2), 23.57 (d, *J* = 7.3 Hz, CH_2), 21.1 (CH_2), 18.2 (C_{21}), 12.6 (d, *J* = 2.9 Hz, $C_6CH_2C\mathbf{H}_3$), 11.9 (C_{18}) ppm; difluorinated carbon C_4 not observed. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -99.0 (1F, d, *J* = 239.3 Hz, $F_{4\beta}$) -111.0 (1F, dtd, *J* = 239.3, 33.0, 26.0 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 613.6 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{32}H_{47}F_2NO_5SNa$ $[M+Na]^+$, m/z calculated: 618.3035; found: 618.3033. **IR** (neat) 3631 (w), 2957 (s), 2930 (s), 2872 (s), 1712 (s), 1450 (m), 1378 (m) 1354 (s), 1173 (s), 1087 (s) cm^{-1}.

8.6 Synthesis of 3β-hydroxyl-4α-fluoro CDCA (NZP318) analogues on large scale

The synthesis and characterisation of the following compounds is presented in the order of reactions discussed in Chapter 6.

***O*-(3α,7α-dihydroxyl-5β-cholan)hydroxylpthalamide (6.18)**

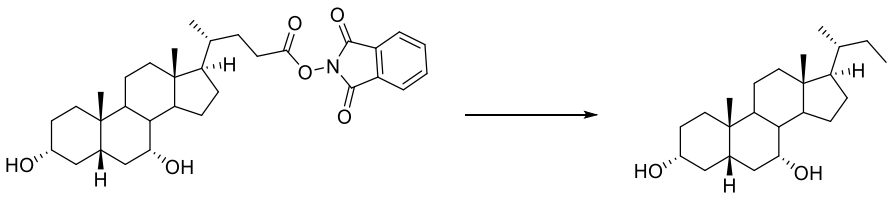


1.2 6.18

Prepared according to the conditions given by Baran *et* al.[221] To a solution of **1.2** (100 mg, 0.26 mmol, 1.0 equiv) in dichloromethane (3 mL) at rt was added *N*-hydroxyphthalamide (46 mg, 0.28 mmol, ~1.1 equiv), 4-dimethylaminopyridine (3 mg, 0.03 mmol, ~0.1 equiv) and *N*,*N*'-diisopropylcarbodiimide (44 µL, 0.28 mmol, ~1.1 equiv). After 3 h at rt, the reaction was deemed complete by TLC and the reaction mixture was filtered over a thin pad of silica, washing with dichloromethane (20 mL) and ethyl acetate (20 mL). The retentate was concentrated *in vacuo* to afford 190 mg of crude material as a pale-yellow oil. Purification by flash column chromatography using PE 40-60/acetone (60/40) as the eluent yielded compound **6.18** as a colourless oil (194 mg, ~0.25 mmol, ~97%; considering 60 mol% purity by ^{1}H NMR, owing to 1,3-diisopropylurea.)

R$_f$ (PE 40-60/acetone, 60/40) 0.34. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.88 (2H, dd, *J* = 5.4, 3.1 Hz, ArH), 7.78 (2H, dd, *J* = 5.5, 3.2 Hz, ArH), 4.39 (2H, d, *J* = 1.8 Hz, OH), 3.87-3.81 (1H, m, $H_{7\beta}$), 3.45 (1H, ddd, *J* = 15.3, 10.9, 4.3 Hz, $H_{3\beta}$), 2.70 (1H, ddd, *J* = 15.9, 9.7, 5.3 Hz, $H_{23'}$), 2.63-2.54 (1H, m, $H_{23''}$), 2.26-2.16 (1H, m), 1.99-1.80 (6H, m), 1.73-1.61 (4H, m), 1.54-1.19 (12H, m) , 0.99 (3H, d, *J* = 6.4 Hz, H_{21}), 0.90 (3H, s, H_{19}), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.1 (C_{24}), 162.0 (2 x **C**O), 134.7 ($C_{Ar}CH_{Ar}$**C**H_{Ar}), 128.9 (C_{Ar}), 124.0 (C_{Ar}**C**H_{Ar}), 72.0 (C_7), 68.4 (C_3), 55.7 (C_{17}), 50.4 (C_{14}), 42.7 (C_{13}), 41.5 (CH), 39.9 (CH_2), 39.6 (CH_2), 39.4 (CH), 35.3 (CH_2), 35.2 (CH), 35.0 (C_{10}), 34.6 (CH_2), 32.8 (CH), 30.71 (CH_2), 30.67 (CH_2), 28.13 (CH_2), 28.05 (CH_2), 23.7 (CH_2), 22.8 (C_{19}), 20.6 (C_{11}), 18.2 (C_{21}), 11.8 (C_{18}) ppm. **LRMS** (ESI$^+$) m/z: 538.3 $[M+H]^+$ 100%. **HRMS** (ESI$^+$) $C_{32}H_{43}O_6NNa$ $[M+MeOH+Na]^+$, m/z calculated: 592.3245; found: 592.3253.

3α,7α-dihydroxy-24-*nor*-5β-cholan-23-ane (6.15)



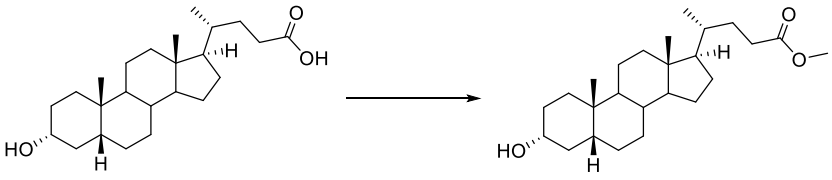
6.18 6.15

Prepared according to the conditions given by Baran *et* al.[221] To a solution of **6.18** (1.0 g, 1.86 mmol, 1.0 equiv) in dry THF under argon was added zinc powder (61 mg, 0.93 mmol, 0.5 ~equiv) and isopropyl alcohol (1.0 mL). After 5 min at rt, the reaction mixture received a solution of nickel (II)

chloride hexahydrate (44 mg, 0.19 mmol, ~0.1 equiv) and 4,4′-di-tert-butyl-2,2′-dipyridyl (100 mg, 0.37 mmol ~0.2 equiv) in dimethylformamide (2 mL) immediately followed by triphenylsilane (340 µL, 2.79 mmol, ~1.5 equiv). After 2 h at 40 °C, the reaction was deemed complete by TLC and the reaction mixture was quenched with a 1:1 mixture of sat. NH_4Cl solution and water (10 mL). The aqueous phase was extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were washed with brine (150 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 1.69 g of crude material as a grey solid. Purification by flash column chromatography using PE 40-60/acetone (70/30) as the eluent yielded compound **6.15** as a white foamy residue (504 mg, ~0.71 mmol, ~38%; considering 40 mol% purity by ^{1}H NMR, owing to a phthalimide-derived impurity).

The data presented for compound **5.9** was attained from an impure mixture. Characteristic signals are reported to help identify this intermediate in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.87-3.85 (1H, m, $H_{7\beta}$), 3.48-3.45 (1H, m, $H_{3\beta}$), 2.27-2.17 (1H, m), 2.02-1.96 (2H, m), 0.919 (3H, d, *J* = 6.5 Hz, H_{21}), 0.917 (3H, s, H_{19}), 0.83 (3H, t, *J* = 7.4 Hz, H_{23}), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 72.0 (C_3), 68.6 (C_7), 55.6 (C_{17}), 50.5 (C_{14}), 42.6 (C_8), 41.5 (C_{13}), 39.9 (CH_2), 39.6 (CH), 39.5 (CH_2), 37.0 (CH), 35.3 (CH_2), 35.1 (C_{10}), 34.5 (CH_2), 32.9 (CH), 30.7 (CH_2), 28.3 (CH_2), 28.1 (CH_2), 23.7 (CH_2), 22.8 (C_{19}), 20.6 (C_{11}), 18.0 (C_{21}), 11.8 (C_{18}), 10.3 (C_{23}) ppm. **LRMS** (ESI^+) m/z: 331.2 $[M-OH]^+$ 100%.

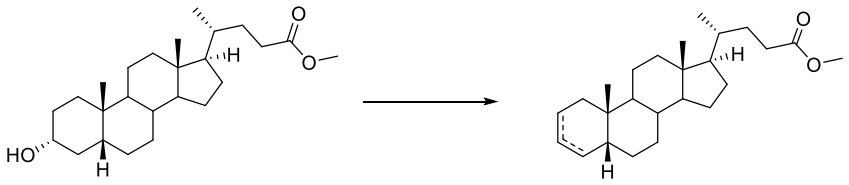
Methyl-3α-hydroxy-5β-cholan-24-oate (6.19)



Prepared according to *General Procedure G* (see: *page 163*) using 25.0 g of compound **1.4** (66.8 mmol, 1.0 equiv) and sonicated for 2 h. After workup, a crude mass of 25.1 g of **6.19** was isolated as a white solid that was used without further purification (66.8 mmol, 99%).

R_f (PE 40-60/acetone, 75/25) 0.44. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.66 (3H, s, $C_{24}OCH_3$), 3.62 (1H, ddd, *J* = 15.8, 11.0, 4.7 Hz, $H_{3\beta}$), 2.35 (1H, ddd, *J* = 15.4, 10.3, 5.3 Hz, $H_{23'}$), 2.25-2.17 (1H, m), 1.95 (1H, dt, *J* = 12.0, 3.3 Hz), 1.87-1.47 (10H, m), 1.42-0.96 (16H, m), 0.914 (3H, s, H_{19}), 0.907 (3H, d, *J* = 6.2 Hz, H_{21}), 0.64 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.8 (C_{24}), 71.8 (C_3), 56.5 (C_{14}), 55.9 (C_{17}), 51.4 ($C_{24}O\mathbf{C}H_3$), 42.7 (C_{13}), 42.1 (CH), 40.4 (CH), 40.1 (CH_2), 36.4 (CH_2), 35.8 (CH), 35.33 (CH), 35.32 (CH_2), 34.5 (C_{10}), 31.02 (C_{23}), 30.97 (C_{22}), 30.5 (CH_2), 28.2 (CH_2), 27.2 (CH_2), 26.4 (CH_2), 24.2 (CH_2), 23.3 (C_{19}), 20.8 (C_{11}), 18.2 (C_{21}), 12.0 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 408.3 $[M+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{25}H_{46}O_6Na$ $[M+Na]^+$, m/z calculated: 413.3026; found: 413.3030. **IR** (neat) 3348 (m), 2926 (s), 2863 (s), 2360 (m), 2342 (m), 1737 (s), 1447 (m), 1375 (m), 1263 (m), 1166 (m) cm^{-1}.

Methyl-5β-chol-2-en-24-oate (6.20) and methyl-5β-chol-3-en-24-oate (6.21)

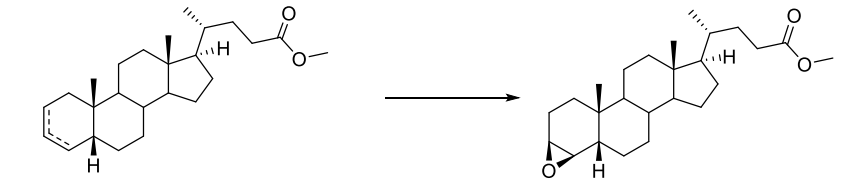


6.19 2-ene **6.20** and 3-ene **6.21** in 35:65 ratio

Prepared according to *General Procedure F* (see: *page 162)* using 10.0 g of compound **6.19** (25.6 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography using PE 40-60/acetone (95/5) as the eluent yielded an inseparable mixture of compounds **6.20** and **6.21** in a 35:65 ratio as a pale-yellow oil (6.49 g, 17.4 mmol, 68%).

R_f (PE 40-60/acetone, 70/30) 0.41. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.88-5.60 (1H, C**H**=CH), 5.57-5.51 (0.35H, CH=C**H**, **6.20**), 5.34 (1H, dd, *J* = 19.9, 1.8 Hz, CH=C**H**, **6.21**), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.35 (1H, ddd, *J* = 15.4, 10.3, 5.3 Hz, $H_{23'}$), 2.22 (1H, ddd, *J* = 15.6, 9.6, 6.5 Hz, $H_{23''}$), 2.03 (1H, d, *J* = 2.1 Hz), 1.98-1.75 (6H, m), 1.70-1.64 (1H, m), 1.60-1.54 (2H, m), 1.44-0.99 (14H, m), 0.97 (1.05H, s, H_{19}, **6.20**), 0.95 (1.95H, s, H_{19}, **6.21**), 0.913 (1.95H, d, *J* = 6.5 Hz, H_{21}, **6.21**), 0.906 (1.05H, d, *J* = 6.4 Hz, H_{21}, **6.20**), 0.664 (1.95H, s, H_{18}, **6.21**), 0.658 (1.05H, s, H_{18}, **6.20**) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.8 (C_{24}), 132.4 (C_4, **6.21**), 127.0 (C_3, **6.21**), 136.4 (C_4, **6.20**), 124.5 (C_3, **6.20**), 56.6 (C_{14}, **6.20**), 56.2 (C_{14}, **6.21**), 56.0 (C_{17}), 51.4 ($C_{24}O\mathbf{C}H_3$), 43.5 (CH), 42.8 (C_{13}, **6.20**), 42.7 (C_{13}, **6.21**), 40.84 (CH, **6.21**), 40.81 (CH, **6.20**), 40.3 (CH_2, **6.21**), 40.2 (CH_2, **6.20**), 35.6 (CH), 35.4 (CH), 34.0 (CH_2, **6.21**), 33.97 (CH_2, **6.20**), 33.5 (C_{10}), 31.1 (C_{23}), 31.0 (C_{22}), 28.21 (CH_2, **6.20**), 28.16 (CH_2, **6.21**), 27.6 (CH_2, **6.21**), 27.5 (CH_2, **6.21**), 27.1 (CH_2, **6.20**), 25.7 (CH_2, **6.20**), 25.6 (CH_2, **6.20**), 25.2 (CH_2, **6.21**), 24.1 (CH_2, **6.20**), 23.3 (C_{19}, **6.20**), 22.9 (C_{19}, **6.21**), 22.4 (CH_2, **6.21**), 21.7 (CH_2, **6.20**), 21.5 (CH_2, **6.21**), 18.28 (C_{21}, **6.21**), 18.25 (C_{21}, **6.20**), 12.1 (C_{18}, **6.20**), 12.0 (C_{18}, **6.21**) ppm. **LRMS** (ESI^+) m/z: 390.3 $[M+NH_4]^+$, 100%. **HRMS** (ESI^+) $C_{25}H_{40}O_2Na$ $[M+Na]^+$, m/z calculated: 395.2921; found: 395.2928. **IR** (neat) 3399 (m), 2933 (s), 2868 (s), 2360 (m), 2342 (m), 1736 (s), 1683 (m), 1437 (m), 1378 (m), 1169 (m) cm^{-1}.

Methyl-3β,4β-epoxy-5β-cholan-24-oate (6.22)



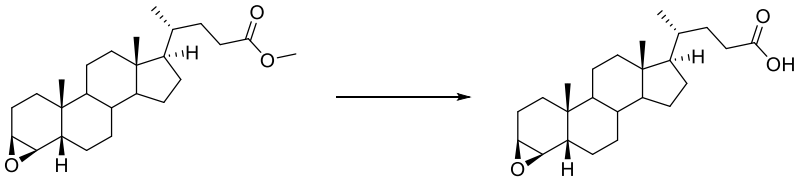
2-ene **6.20** and 3-ene **6.21** in 35:65 ratio **6.22**

Prepared according to *General Procedure B* (see: *page 150*) using 6.03 g of a 35:65 mixture of compounds **6.20** and **6.21** (16.2 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage ZIP KP-Sil 120 g cartridge) using PE 40-60/acetone (95/5) as

the eluent yielded an inseparable mixture of epoxide regioisomers (5.7 g). Purification by HPLC using PE 40-60/acetone (95/5) as the eluent yielded compound **6.22** as a colourless oil (2.64, 6.80 mmol, 42%). The corresponding 2β,3β-derivative, from **6.20**, was not isolated.

R_f (PE 40-60/acetone, 70/30) 0.44. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.66 (3H, s, $C_{24}OCH_3$), 3.21 (1H, s, CHO), 2.85 (1H, d, *J* = 3.8 Hz, CHO), 2.35 (1H, ddd, *J* = 15.4, 10.3, 5.1 Hz, $H_{23'}$), 2.22 (1H, ddd, *J* = 15.6, 9.5, 6.4 Hz, $H_{23''}$), 1.97 (1H, dt, *J* = 12.3, 3.1 Hz), 1.93-1.67 (4H, m), 1.64-1.56 (3H, m), 1.50-0.95 (16H, m), 0.91 (3H, d, *J* = 6.5 Hz, H_{21}), 0.87 (3H, s, H_{19}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 174.7 (C_{24}), 56.5 (CHO), 56.3 (C_{14}), 56.0 (C_{17}), 53.3 (CHO), 51.4 ($C_{24}O\mathbf{C}H_3$), 42.8 (CH), 42.64 (C_{13}), 42.59 (CH), 40.0 (CH_2), 35.3 (CH), 35.0 (CH), 33.3 (C_{10}), 31.02 (C_{23}), 30.98 (C_{22}), 29.0 (CH_2), 28.6 (CH_2), 28.1 (CH_2), 26.9 (CH_2), 24.2 (CH_2), 22.3 (C_{19}), 20.9 (CH_2), 20.6 (CH_2), 18.3 (C_{21}), 11.9 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 389.2 $[M+H]^+$, 100%, 406.2 $[M+NH_4]^+$, 47%. **HRMS** (ESI^+) $C_{25}H_{40}O_3Na$ $[M+Na]^+$, m/z calculated: 411.2870; found: 411.2879. **IR** (neat) 2928 (s), 2867 (m), 2360 (m), 2342 (m), 1735 (s), 1445 (m), 1264 (m), 1164 (m) cm^{-1}.

3β,4β-epoxy-5β-cholanic acid (6.24)

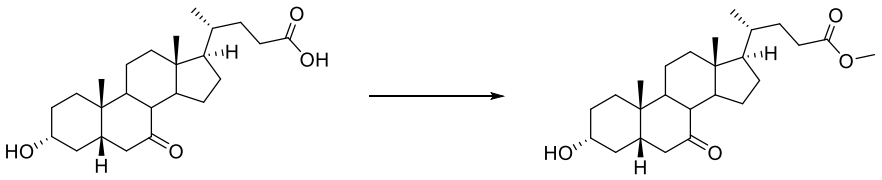


6.22 6.24

Prepared according to *General Procedure A* (see: *page 147*) using 500 mg of compound **6.22** (1.29 mmol, 1.0 equiv) and 25 mL of a 3% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage ZIP KP-Sil 10 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **6.24** as a colourless residue (408 mg, 1.09 mmol, 85%).

R_f (PE 40-60/acetone, 70/30) 0.44. **$[\alpha]_D$:** +24.4 (c = 0.68, methanol, 23 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.23 (1H, s, CHO), 2.86 (1H, d, *J* = 3.8 Hz, CHO), 2.39 (1H, ddd, *J* = 15.3, 10.2, 5.0 Hz, $H_{23'}$), 2.29-2.21 (1H, m, $H_{23''}$), 1.97 (1H, dt, *J* = 12.5, 2.7 Hz), 1.93-1.68 (4H, m), 1.64-1.22 (12H, m), 1.19-0.96 (7H, m), 0.93 (3H, d, *J* = 6.4 Hz, H_{21}), 0.87 (3H, s, H_{19}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 180.1 (C_{24}), 56.6 (CHO), 56.3 (C_{14}), 55.9 (C_{17}), 53.4 (CHO), 42.8 (CH), 42.7 (C_{13}), 42.6 (CH), 40.0 (CH_2), 35.3 (CH), 35.0 (CH), 32.3 (C_{10}), 31.0 (C_{23}), 30.7 (C_{22}), 29.0 (CH_2), 28.6 (CH_2), 28.1 (CH_2), 26.9 (CH_2), 24.2 (CH_2), 22.2 (C_{19}), 20.9 (CH_2), 20.6 (CH_2) 18.2 (C_{21}), 11.9 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 375.2 $[M+H]^+$, 100%, 392.3 $[M+NH_4]^+$, 65%. **HRMS** (ESI^+) $C_{24}H_{38}O_3Na$ $[M+Na]^+$, m/z calculated: 397.2713; found: 397.2709. **IR** (neat) 2929 (m), 1715 (s), 1653 (m), 1445 (m), 1383 (m), 1302 (s), 1260 (s) cm^{-1}.

Methyl-3α-hydroxy-7-keto-5β-cholan-24-oate (6.2)

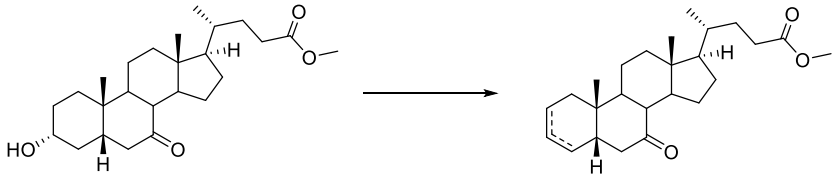


6.1 **6.2**

Prepared according to *General Procedure G* (see: *page 163*) using 24.2 g of compound **6.1** (61.8 mmol, 1.0 equiv) and sonicated for 3 h. After workup, a crude mass of 25.0 g of **6.2** was isolated as a white solid that was used without further purification (61.8 mmol, 99%).

R_f (PE 40-60/acetone, 70/30) 0.34. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 3.59, 1H, dddd, *J* = 15.4, 10.8, 9.4, 4.5 Hz, $H_{3\beta}$), 2.85 (1H, ddd, *J* = 12.5, 6.0, 0.6 Hz, $H_{6\beta}$), 2.40-2.31 (2H, m, H_8 +$H_{23'}$), 2.25-2.15 (2H, m), 2.00-1.65 (10H, m), 1.50-1.25 (10H, m), 1.19 (3H, s, H_{19}), 1.16-1.09 (2H, m), 0.91 (3H, d, *J* = 6.4 Hz, H_{21}), 0.65 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.0 (C_7), 174.6 (C_{24}), 70.8 (C_3), 54.7 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.5 (C_8), 48.9 (C_{14}), 46.1 (CH), 45.4 (C_6), 42.7 (CH), 42.6 (C_{13}), 38.9 (C_{12}), 37.4 (CH_2), 35.2 (C_{20}), 35.1 (C_{10}), 34.1 (CH_2), 31.02 (C_{23}), 30.95 (C_{22}), 29.8 (CH_2), 28.2 (CH_2), 24.8 (CH_2), 23.0 (C_{19}), 21.6 (C_{11}), 18.3 (C_{21}), 12.0 (C_{18}) ppm. **LRMS** (ESI^+) m/z: 422.3 $[M+NH_4]^+$ 100%. **HRMS** (ESI^+) $C_{25}H_{41}O_4$ $[M+H]^+$, m/z calculated: 405.2999; found: 405.3009. **IR** (neat) 3399 (m), 2932 (s), 2868 (s), 1735 (s), 1707 (s), 1435 (m), 1378 (m), 1261 (m), 1167 (m) cm^{-1}. *This data is consistent with that reported in the literature*.[222]

Methyl-7-keto-5β-chol-2-en-24-oate (6.3) and methyl-7-keto-5β-chol-3-en-24-oate (6.4)



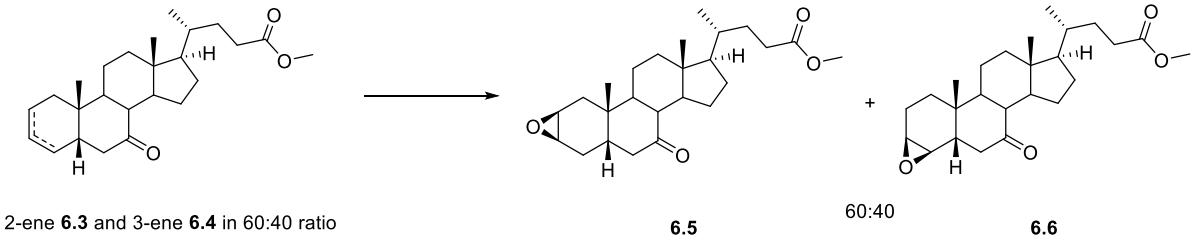
6.2 2-ene **6.3** and 3-ene **6.4** in 60:40 ratio

Prepared according to *General Procedure F* (see: *page 162)* using 20.0 g of compound **6.2** (49.3 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage ZIP KP-Sil 80 g cartridge) using PE 40-60/acetone (85/15) as the eluent yielded an inseparable mixture of compounds **6.3** and **6.4** in a 60:40 ratio, and in approximately 80% purity, as a pale-yellow oil (15.6 g, 40.4 mmol, 84%, considering 80 mol% purity by ^{1}H NMR).

The data presented for compounds **6.3** and **6.4** was attained from an impure mixture. Characteristic signals are reported to help identify these intermediates in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.65-5.61 (0.40H, m, C**H**=CH, **6.4**), 5.59-5.49 (1.2H, m, C**H**=CH + CH=C**H**, **6.3**), 5.36 (0.40H, dq, *J* = 10.0, 1.9 Hz, CH=C**H**, **6.4**), 3.66 (3H, s, $C_{24}OC\mathbf{H}_3$), 2.84 (1H, td, *J* = 12.7, 5.1 Hz, $\mathbf{H}_{6\beta}$), 2.56-2.52

(0.4H, m, **6.4**), 2.42-2.30 (2H, m, $\mathbf{H}_{8}$ + $\mathbf{H}_{23}$), 1.24 (1.8H, s, $\mathbf{H}_{19}$, **6.3**), 1.22 (1.2H, s, $\mathbf{H}_{19}$, **6.4**), 0.913 (1.2H, d, *J* = 6.4 Hz, $\mathbf{H}_{21}$, **6.4**), 0.910 (1.8H, d, *J* = 6.4 Hz, $\mathbf{H}_{21}$, **6.3**), 0.663 (1.2H, s, $\mathbf{H}_{18}$, **6.4**), 0.658 (1.8H, s, $\mathbf{H}_{18}$, **6.3**) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.7 ($\mathbf{C}_{7}$, **6.3**), 212.3 ($\mathbf{C}_{7}$, **6.4**), 174.6 ($\mathbf{C}_{24}$), 129.5 (C=**C**, **6.4**), 127.5 (C=**C**, **6.4**), 125.4 (C=**C**, **6.3**), 125.2 (C=**C**, **6.3**), 54.8 ($\mathbf{C}_{17}$, **6.4**), 54.7 ($\mathbf{C}_{17}$, **6.3**), 51.4 ($C_{24}O\mathbf{C}H_3$), 50.0 ($\mathbf{C}_{8}$, **6.3**), 49.2 ($\mathbf{C}_{14}$, **6.3**), 49.0 ($\mathbf{C}_{8}$, **6.4**), 48.7 ($\mathbf{C}_{14}$, **6.4**), 45.0 ($\mathbf{C}_{6}$, **6.4**), 44.9 ($\mathbf{C}_{6}$, **6.3**), 43.12 (CH, **6.3**), 43.06 (CH, **6.4**), 42.9 ($\mathbf{C}_{13}$, **6.3**), 42.5 ($\mathbf{C}_{13}$, **6.4**), 39.0 ($\mathbf{C}_{12}$, **6.4**), 38.9 ($\mathbf{C}_{12}$, **6.3**), 35.20 ($\mathbf{C}_{20}$, **6.4**), 35.19 ($\mathbf{C}_{20}$, **6.3**), 34.5 ($\mathbf{C}_{10}$, **6.3**), 34.2 ($\mathbf{C}_{10}$, **6.4**), 31.03 ($\mathbf{C}_{23}$), 30.98 ($\mathbf{C}_{22}$), 25.0 (CH_2, **6.3**), 24.8 (CH_2, **6.4**), 23.1 ($\mathbf{C}_{19}$, **6.3**), 22.5 ($\mathbf{C}_{19}$, **6.4**), 22.4 (CH_2, **6.3**), 22.1 (CH_2, **6.4**), 18.3 ($\mathbf{C}_{21}$), 12.1 ($\mathbf{C}_{18}$, **6.3**), 12.0 ($\mathbf{C}_{18}$, **6.4**) ppm. **LRMS** (ESI^+) m/z: 387.3 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{25}H_{39}O_3$ $[M+H]^+$, m/z calculated: 387.2894; found: 387.2888.

Methyl-2β,3β-epoxy-7-keto-5β-cholan-24-oate (6.5) and methyl-3β,4β-epoxy-7-keto-5β-cholan-24-oate (6.6)

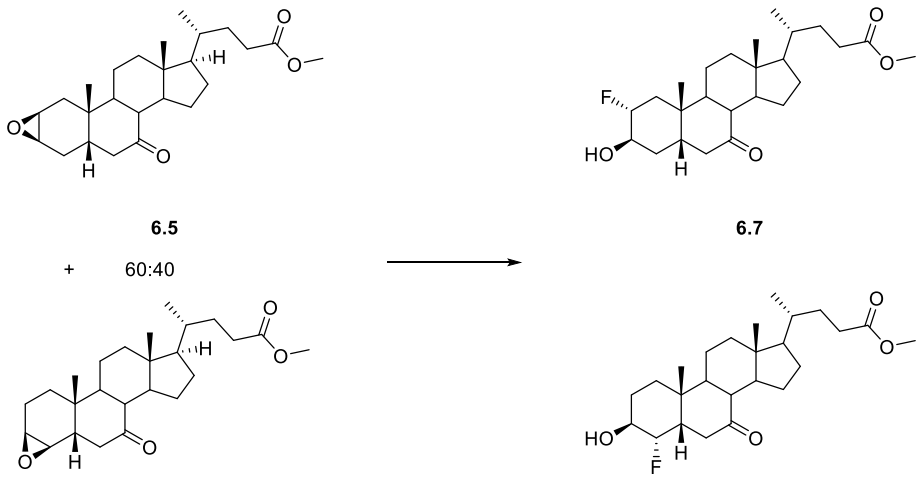


Prepared according to *General Procedure B* (see: *page 150*) using 12.0 g of a 60:40 mixture of compounds **6.3** and **6.4** (31.0 mmol, 1.0 equiv). Purification of the resultant crude material by flash column chromatography (Biotage ZIP KP-Sil 340 g cartridge) using PE 40-60/acetone (90/10) as the eluent yielded an inseparable mixture of compounds **6.5** and **6.6** in a 60:40 ratio as a pale-yellow oil (7.86 g, 19.5 mmol, 63%).

R_f (PE 40-60/acetone, 70/30) 0.44. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.660 (1.8H, s, $C_{24}OC\mathbf{H}_3$, **6.5**), 3.656 (1.2H, s, $C_{24}OC\mathbf{H}_3$, **6.6**), 3.17-3.16 (0.4H, m, $\mathbf{H}_{3\alpha}$, **6.6**), 3.15-3.11 (0.6H, m, $\mathbf{H}_{3\alpha}$, **6.5**), 3.02 (0.6H, dd, *J* = 5.4, 4.3 Hz, $\mathbf{H}_{2\alpha}$, **6.5**), 2.91 (0.4H, ddd, *J* = 12.8, 6.9, 0.7 Hz, $\mathbf{H}_{6\beta}$, **6.6**), 2.85 (0.4H, d, *J* = 3.8 Hz, $\mathbf{H}_{4\alpha}$, **6.6**), 2.80 (0.6H, dd, *J* = 12.3, 4.1 Hz, $\mathbf{H}_{6\beta}$, **6.5**), 2.43-2.09 (5H, m), 2.02-1.88 (4H, m), 1.85-1.60 (3H, m), 1.56-1.25 (9H, m), 1.15 (3H, s, $\mathbf{H}_{19}$), 1.13-0.95 (2H, m), 0.92 (1.8H, d, *J* = 6.4 Hz, $\mathbf{H}_{21}$, **6.5**), 0.91 (1.2H, d, *J* = 6.2 Hz, $\mathbf{H}_{21}$, **6.6**), 0.66 (1.2H, s, $\mathbf{H}_{18}$, **6.6**), 0.65 (1.8H, s, $\mathbf{H}_{18}$, **6.5**) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.3 ($\mathbf{C}_{7}$, **6.6**), 212.1 ($\mathbf{C}_{7}$, **6.5**), 174.6 ($\mathbf{C}_{24}$), 54.8 ($\mathbf{C}_{17}$, **6.6**), 54.7 ($\mathbf{C}_{17}$, **6.5**), 54.6 ($\mathbf{C}_{4}$, **6.6**), 53.1 ($\mathbf{C}_{3}$, **6.6**), 51.9 ($\mathbf{C}_{3}$, **6.5**), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.3 ($\mathbf{C}_{8}$, **6.5**), 49.4 ($\mathbf{C}_{2}$, **6.5**), 49.2 ($\mathbf{C}_{14}$, **6.6**), 49.0 ($\mathbf{C}_{8}$, **6.6**), 48.6 ($\mathbf{C}_{14}$, **6.6**), 47.3 ($\mathbf{C}_{5}$, **6.6**), 45.0 ($\mathbf{C}_{9}$, **6.6**), 44.33 ($\mathbf{C}_{9}$, **6.5**), 44.25 ($\mathbf{C}_{6}$, **6.5**), 44.1 ($\mathbf{C}_{6}$, **6.6**), 42.9 ($\mathbf{C}_{13}$, **6.5**), 42.4 ($\mathbf{C}_{13}$, **6.6**), 39.1 ($\mathbf{C}_{5}$, **6.5**), 38.9 ($\mathbf{C}_{12}$, **6.5**), 38.7 ($\mathbf{C}_{12}$, **6.6**), 35.16 ($\mathbf{C}_{20}$, **6.6**), 35.15 ($\mathbf{C}_{20}$, **6.5**), 34.1 (CH_2, **6.5**), 33.4 ($\mathbf{C}_{10}$, **6.5**), 33.1 ($\mathbf{C}_{10}$, **6.6**), 30.98 ($\mathbf{C}_{23}$, **6.5**), 30.96 ($\mathbf{C}_{23}$, **6.6**), 30.90 ($\mathbf{C}_{22}$), 28.3 (CH_2, **6.5**), 28.2 (CH_2, **6.6**), 28.0 (CH_2, **6.6**), 26.8 (CH_2, **6.5**), 24.8 (CH_2, **6.5**), 24.6 (CH_2, **6.6**), 23.3 ($\mathbf{C}_{19}$, **6.5**), 22.5

(CH_2, **6.5**), 22.1 (**C**$_{19}$, **6.6**), 21.9 (CH_2, **6.6**), 20.1 (CH_2, **6.6**), 18.3 (**C**$_{21}$), 12.1 (**C**$_{18}$, **6.5**, 11.9 (**C**$_{18}$, **6.6**) ppm. **LRMS** (ESI^+) m/z: 420.3 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{25}H_{39}O_4$ $[M+H]^+$, m/z calculated: 403.2843; found: 403.2848. **IR** (neat) 2941 (m), 2870 (m), 1734 (s), 1703 (s), 1436 (m), 1301 (m), 1342 (m), 1167 (m) cm^{-1}.

Methyl-2α-fluoro-3β-hydroxy-7-keto-5β-cholan-24-oate (6.7) and methyl-3β-hydroxy-4α-fluoro-7-keto-5β-cholan-24-oate (6.8)

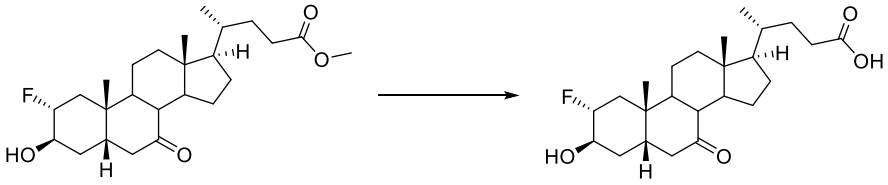


Prepared according to *General Procedure D* (see: *page 157*) using 10.0 g of a mixture of compounds **6.5** and **6.6** (24.8 mmol, 1.0 equiv) and 23 mL (~30 equiv) of HF.pyridine (70%) complex. CAUTION: exercise extensive and appropriate controls for the large scale of this hazardous reagent. Purification of the resultant crude material by flash column chromatography (Biotage SNAP KP-Sil 340 g cartridge) using PE 40-60/acetone (100/0 to 80/20) as the eluent yielded compound **6.7** as a white residue (2.90 g, 6.86 mmol, 28%) and compound **6.8** as a white residue (1.88 g, 4.44 mmol, 18%).

Compound **6.7**: $\mathbf{R_f}$ (PE 40-60/acetone, 70/30) 0.26. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.55 (1H, dq, *J* = 46.8, 2.8 Hz, $H_{2\beta}$), 4.03 (1H, dquin, *J* = 6.9, 3.4 Hz, $H_{3\alpha}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.91 (1H, dd, *J* = 12.7, 6.0 Hz, $H_{6\beta}$), 2.39-2.16 (5H, m), 2.13 (1H, dt, *J* = 14.6, 2.9 Hz), 2.01-1.89 (4H, m), 1.82-1.76 (2H, m), 1.71-1.26 (10H, m), 1.24 (3H, s, H_{19}), 1.16-1.10 (2H, m), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.6 (C_7), 174.7 (C_{24}), 91.3 (d, *J* = 171.7, C_2), 61.2 (d, *J* = 28.6, C_3), 54.7 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.8 (CH), 48.8 (CH), 45.0 (d, *J* = 5.1, C_5), 44.8 (CH_2), 42.6 (C_{13}), 40.5 (CH), 38.8 (CH_2), 35.7 (C_{10}), 35.2 (CH), 34.4 (d, *J* = 18.3 Hz, CH_2), 31.03 (C_{23}), 30.99 (C_{22}), 30.7 (CH_2), 28.3 (CH_2), 24.8 (CH_2), 23.5 (C_{19}), 22.3 (CH_2), 18.3 (C_{21}), 12.1 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.7 (1F, tt, *J* = 48.6, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 440.3 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{25}H_{40}FO_4$ $[M+H]^+$, m/z calculated: 423.2095; found: 423.2909. **IR** (neat) 3455 (w), 2941 (m), 1735 (s), 1707 (s), 1435 (m), 1379 (m), 1260 (m), 1200 (m) cm^{-1}.

Compound **6.8**: **R_f** (PE 40-60/acetone, 70/30) 0.25. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.52 (1H, dt, *J* = 47.0, 2.7 Hz, $H_{4\beta}$), 4.06 (1H, dd, *J* = 7.6, 3.2 Hz, $H_{3\alpha}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.80 (1H, dd, *J* = 14.6, 7.7 Hz, $H_{6\beta}$), 2.40-2.30 (2H, m), 2.27-2.18 (3H, m), 2.12 (1H, dt, *J* = 7.5, 2.6 Hz), 2.00-1.89 (3H, m), 1.88-1.76 (2H, m), 1.71-1.58 (3H, m), 1.53-1.24 (8H, m), 1.22 (3H, s, H_{19}), 1.17-1.01 (2H, m), 0.92 (3H, d, *J* = 6.4 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.4 (C_7), 174.7 (C_{24}), 89.3 (d, *J* = 169.3, C_4), 67.4 (d, *J* = 27.9, C_3), 54.6 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.8 (CH), 49.3 (CH), 49.0 (CH), 48.8 (CH), 44.7 (CH_2), 43.0 (CH_2), 42.6 (C_{13}), 40.5 (CH_2), 38.9 (CH_2), 35.6 (C_{10}), 35.2 (CH), 31.1 (C_{23}), 31.0 (C_{22}), 28.4 (CH_2), 24.8 (CH_2), 23.5 (C_{19}), 22.2 (CH_2), 18.4 (C_{21}), 12.1 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.2 (1F, t, *J* = 42.5 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 440.3 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{25}H_{40}FO_4$ $[M+H]^+$, m/z calculated: 423.2905 found: 423.2909. **IR** (neat) 3455 (w), 2941 (m), 1735 (s), 1707 (s), 1435 (m), 1379 (m), 1260 (m), 1201 (m) cm^{-1}.

2α-fluoro-3β-hydroxy-7-keto-5β-cholanic acid (6.29)

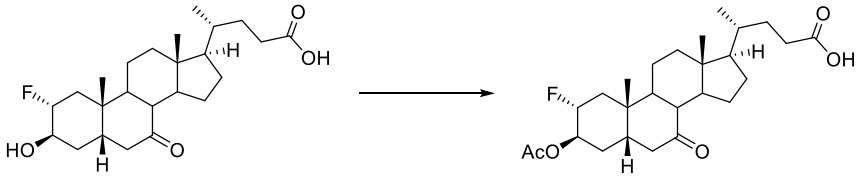


6.7 **6.29**

Prepared according to *General Procedure A* (see: *page 147*) using 4.56 g of compound **6.7** (10.8 mmol, 1.0 equiv) and 100 mL of a 5% sodium hydroxide in methanol solution. After workup, a crude mass of 4.27 g of **6.29** was isolated as a white residue that was used without further purification (10.4 mmol, 96%).

R_f (PE 40-60/acetone, 70/30) 0.16. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.55 (1H, dq, *J* = 46.7, 2.8 Hz, $H_{2\beta}$), 4.03 (1H, dquin, *J* = 6.9, 3.2 Hz, $H_{3\alpha}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.89 (1H, dd, *J* = 12.7, 6.0 Hz, $H_{6\beta}$), 2.43-2.14 (6H, m), 2.01-1.78 (7H, m), 1.70-1.30 (9 H, m), 1.24 (3H, s, H_{19}), 1.16-1.08 (2H, m), 0.93 (3H, d, *J* = 6.4 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.8 (C_7), 180.0 (C_{24}), 91.3 (d, *J* = 171.7, C_2), 61.2 (d, *J* = 28.6, C_3), 54.7 (C_{17}), 49.8 (CH), 48.8 (CH), 45.0 (d, *J* = 4.4, C_5), 44.8 (CH_2), 42.6 (C_{13}), 40.5 (CH), 38.8 (CH_2), 35.7 (C_{10}), 35.2 (CH), 34.4 (d, *J* = 18.3 Hz, CH_2), 30.9 (C_{23}), 30.7 (C_{22}), 30.6 (CH_2), 28.2 (CH_2), 24.8 (CH_2), 23.5 (C_{19}), 22.3 (CH_2), 18.3 (C_{21}), 12.1 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.7 (1F, tt, *J* = 48.6, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 426.4 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{24}H_{37}FO_4$ $[M+H]^+$, m/z calculated: 409.2749; found: 409.2758. **IR** (neat) 2939 (m), 1702 (s), 1380 (m), 1204 (m), 1048 (m), 1003 (m) cm^{-1}.

2α-fluoro-3β-acetoxy-7-keto-5β-cholanic acid (6.30)

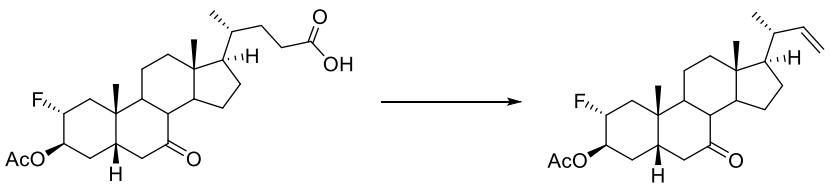


6.29 6.30

To a solution of **6.29** (1.00 g, 2.44 mmol, 1.0 equiv) in dichloromethane (60 mL) at rt was added acetic anhydride (920 µL, 9.76 mmol, ~4.0 equiv) and bismuth (III) triflate (80 mg, 0.12 mmol, ~0.05 equiv). After 18 h at rt, the reaction was deemed complete by TLC and the reaction mixture filtered over Celite. The filtrate received 1M NaOH solution (20 mL) and was extracted with dichloromethane (3 x 50 mL) and the combined organic layers dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 1.07 g of crude material as a white residue. Purification by flash column chromatography (Biotage ZIP KP-Sil 25 g cartridge) using PE 40-60/acetone (100/0 to 95/5) as the eluent yielded compound **6.30** as a white residue (785 mg, 1.74 mmol, 71%).

R_f (dichloromethane/methanol, 90/10) 0.55. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.04 (1H, dd, *J* = 6.7, 2.9 Hz, $H_{3\alpha}$), 4.58 (1H, dq, *J* = 46.0, 2.2 Hz, $H_{2\beta}$), 2.90 (1H, dd, *J* = 12.6, 6.1 Hz, $H_{6\beta}$), 2.43-2.34 (2H, m), 2.31-2.14 (5H), 2.07 (3H, s, C_3OCOCH_3), 2.05-1.76 (8H, m), 1.66-1.57 (2 H, m), 1.55-1.31 (8H, m), 1.26 (3H, s, H_{19}), 1.22-1.08 (2H, m), 0.93 (3H, d, *J* = 6.5 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.1 (C_7), 179.0 (C_{24}), 169.6 (C_3O**C**OCH_3), 88.2 (d, *J* = 172.4, C_2), 68.8 (d, *J* = 32.3, C_3), 54.7 (C_{17}), 49.9 (CH), 48.7 (CH), 44.8 (d, *J* = 5.1, C_5), 44.7 (CH_2), 42.6 (C_{13}), 41.4 (CH), 38.7 (CH_2), 35.4 (C_{10}), 35.2 (CH), 30.98 (C_{23}), 30.96 (C_{22}), 30.8 (CH_2), 28.2 (CH_2), 27.7 (CH_2), 24.8 (CH_2), 23.6 (C_{19}), 22.3 (CH_2), 21.1(C_3OCO**C**H_3), 18.3 (C_{21}), 12.1 (C_{18}) ppm.**^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.1 (1F, tt, *J* = 48.6, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 468.4 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{26}H_{40}FO_5$ $[M+H]^+$, m/z calculated: 451.2854; found: 451.2867. **IR** (neat) 2942 (m), 2875 (m), 1736 (s), 1707 (s), 1436 (m), 1376 (m), 1234 (s), 1207 (m), 1034 (m) cm^{-1}.

2α-fluoro-3β-acetoxy-7-keto-24-*nor*-5β-cholan-22-ene (6.31)



6.30 6.31

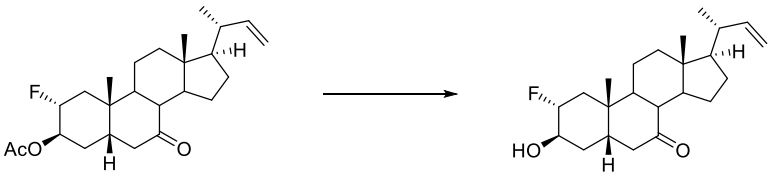
To a solution of **6.30** (750 mg, 1.66 mmol, 1.0 equiv) in dry toluene (70 mL) under argon at rt was added copper (II) acetate (60 mg, 0.33 mmol, ~0.2 equiv) and pyridine (1.35 mL, 16.6 mmol, ~10.0 equiv) and stirred for 1 hour. The reaction mixture was then heated to 111 °C and received

diacetoxyiodobenzene (540 mg, 1.66 mmol, ~1.0 equiv) at 90 min intervals until the fifth successive addition of BAIB was complete; copper (II) acetate (60 mg, 0.33 mmol, ~0.2 equiv) was also added alongside BAIB on the third and fifth additions. After a further 90 min at 111 °C, the reaction was deemed complete by TLC and cooled to rt over 15 h. The reaction mixture was then quenched by the addition of sat. $Na_2S_2O_3$ solution (50 mL). The aqueous phase was separated and extracted with dichloromethane (20 mL) and the combined organic phases dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 1.00 g of crude material as a yellow oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 30 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **6.31** as a pale-yellow residue (232 mg, 0.58 mmol, 35%).

*Preparation of compound **6.31** was also successful by the following method:* To a solution of **6.30** (500 mg, 1.10 mmol, 1.0 equiv) in dry benzene (35 mL) under argon at rt was added copper (II) acetate (202 mg, 1.10 mmol, ~1.0 equiv) and pyridine (180 µL, 2.20 mmol, ~2.0 equiv). The reaction mixture was then heated to 80 °C and received lead (IV) acetate (490 mg, 1.10 mmol, ~1.0 equiv) at 1 h intervals until the sixth successive addition of lead (IV) acetate was complete. After a further 1 hour at 80 °C, the reaction was deemed complete by TLC and cooled to rt over 15 h. The reaction mixture was then filtered over Celite and silica gel and the resultant solid was washed with dichloromethane (20 mL). The filtrate was quenched by the addition of sat. $Na_2S_2O_3$ solution (15 mL) and the aqueous phase was separated and extracted with dichloromethane (3 x 50 mL) and the combined organic phases dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 870 mg of crude material as a yellow residue. Purification by flash column chromatography (Biotage ZIP KP-Sil 30 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **6.31** as a white foamy residue (194 mg, 0.47 mmol, 43%).

R_f (PE 40-60/acetone, 60/40) 0.60. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.66 (1H, ddd, 17.1, 10.2, 8.4 Hz, H_{22}), 5.03 (1H, dd, *J* = 6.4, 3.1 Hz, $H_{3\alpha}$), 4.89 (1H, ddd, 17.1, 1.8, 0.9 Hz, $H_{23'}$), 4.81 (1H, dd, 10.2, 1.9 Hz, $H_{23''}$), 4.58 (1H, dq, *J* = 46.0, 2.1 Hz, $H_{2\beta}$), 2.90 (1H, dd, *J* = 12.6, 6.1 Hz, $H_{6\beta}$), 2.37 (1H, td, *J* = 10.9 1.6 Hz), 2.25-2.09 (4H, m), 2.06 (3H, s, C_3OCOCH_3), 2.04-1.92 (3H, m), 1.87-1.38 (8H, m), 1.26 (3H, s, H_{19}), 1.24-1.14 (2H, m), 1.03 (3H, d, *J* = 6.6 Hz, H_{21}), 0.99-0.79 (1H, m), 0.68 (3H, s, **H_{18}**) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.0 (C_7), 169.6 (C_3O**C**OCH_3), 145.1 (C_{22}), 111.6 (C_{23}), 88.2 (d, *J* = 172.4, C_2), 68.8 (d, *J* = 33.0, C_3), 54.3 (C_{17}), 49.9 (CH), 48.7 (CH), 44.9 (d, *J* = 5.1, C_5), 44.7 (CH_2), 42.5 (C_{13}), 41.4 (CH), 41.0 (CH), 38.6 (CH_2), 35.4 (d, *J* = 1.5 Hz, C_{10}), 35.0 (d, *J* = 18.3 Hz, CH_2), 28.5 (CH_2), 27.8 (CH_2), 24.8 (CH_2), 23.6 (C_{19}), 22.3 (CH_2), 21.1(C_3OCO**C**H_3), 20.2 (C_{21}), 12.3 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.1 (1F, tt, *J* = 48.6, 10.4 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 422.4 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{25}H_{37}FNaO_3$ $[M+Na]^+$, m/z calculated: 427.2619; found: 427.2621. **IR** (neat) 2943 (m), 2874 (m), 1745 (s), 1710 (s), 1458 (m), 1435 (m), 1371 (m), 1234 (s), 1207 (m), 1036 (m) cm^{-1}.

2α-fluoro-3β-hydroxyl-7-keto-24-*nor*-5β-cholan-22-ene (6.32)

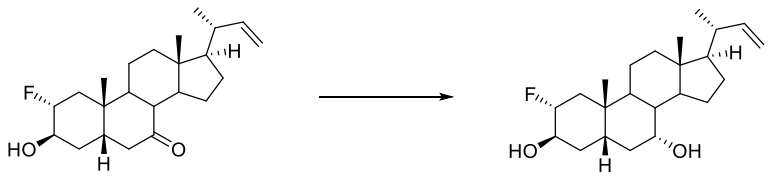


6.31 6.32

Prepared according to *General Procedure A* (see: *page 147*) using 199 mg of compound **6.31** (10.8 mmol, 1.0 equiv) and 10 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **6.32** as an orange oil (110 mg, 0.30 mmol, 62%).

R_f (PE 40-60/acetone, 70/30) 0.10. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.66 (1H, ddd, 17.1, 10.1, 8.5 Hz, H_{22}), 4.89 (1H, ddd, 17.1, 1.1 Hz, $H_{23'}$), 4.82 (1H, dd, 10.2, 2.0 Hz, $H_{23''}$), 4.55 (1H, dq, *J* = 47.0, 2.7 Hz, $H_{2\beta}$), 4.02 (1H, td, *J* = 7.0, 3.3 Hz, $H_{3\alpha}$), 2.88 (1H, dd, *J* = 12.8, 6.1 Hz, $H_{6\beta}$), 2.37 (1H, td, *J* = 10.8, 1.5 Hz), 2.31-2.25 (1H, m), 2.18-1.92 (6H, m), 1.84-1.40 (8H, m), 1.24 (3H, s, H_{19}), 1.21-1.13 (3H, m), 1.03 (3H, d, *J* = 6.7 Hz, H_{21}), 0.98-0.81 (2H, m), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.9 (C_7), 145.1 (C_{22}), 111.6 (C_{23}), 91.3 (d, *J* = 171.7, C_2), 67.1 (d, *J* = 28.6, C_3), 54.3 (C_{17}), 49.8 (CH), 48.8 (CH), 45.0 (d, *J* = 5.1, C_5), 44.8 (CH_2), 42.6 (C_{13}), 41.0 (CH), 40.5 (CH), 38.7 (CH_2), 35.7 (C_{10}), 34.4 (d, *J* = 18.3 Hz, CH_2), 30.7 (CH_2), 28.5 (CH_2), 24.9 (CH_2), 23.5 (C_{19}), 22.3 (CH_2), 20.2 (C_{21}), 12.3 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.6 (1F, tt, *J* = 48.6, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 363.4 $[M+H]^+$, 100%. HRMS (ESI^+) $C_{23}H_{36}FO_2$ $[M+H]^+$, m/z calculated: 363.2694; found: 3632697. **IR** (neat) 3409 (m), 2952 (m), 2940 (s), 1697 (s), 1636 (s), 1610 (s), 1508 (s), 1467 (m), 1457 (m), 1433 (s), 1331 (m), 1328 (m), 1240 (s) cm^{-1}.

2α-fluoro-3β,7α-dihydroxyl-24-*nor*-5β-cholan-22-ene (6.25)



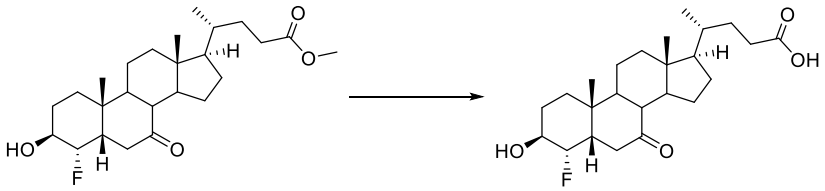
6.32 6.25

To a solution of **6.32** (61 mg, 0.17 mmol, 1.0 equiv) in dry in THF (10 mL) under argon at -78 °C was added *L*-selectride (0.25 mL, 0.25 mmol, ~1.5 equiv) dropwise over 2 min. After 10 min at -78 °C, the reaction mixture received hydrogen peroxide (1 mL), 2M sodium hydroxide solution (1 mL) and water (10 mL). After 10 min warming to rt, the reaction mixture received 2M hydrochloric acid solution (5 mL) and the aqueous phase was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 68

mg of crude material as a white residue. Purification by flash column chromatography (Biotage ZIP KP-Sil 30 g cartridge) using PE 40-60/acetone (95/5 to 75/25) as the eluent yielded compound **6.25** as an orange oil (25 mg, 0.07 mmol, 40%).

R_f (PE 40-60/acetone, 70/30) 0.24. **[**α]$_D$: +20.0 (c = 0.30, chloroform, 25 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.66 (1H, ddd, 17.1, 10.1, 8.5 Hz, H_{22}), 4.90 (1H, ddd, 17.1, 2.0, 0.7 Hz, $H_{23'}$), 4.82 (1H, dd, 10.2, 2.0 Hz, $H_{23''}$), 4.55 (1H, dq, *J* = 47.3, 3.6 Hz, $H_{2\beta}$), 4.01 (1H, dd, *J* = 7.8, 3.5 Hz, $H_{3\alpha}$), 3.86 (1H, q, *J* = 2.9 Hz, $H_{7\beta}$), 2.72 (1H, tt, *J* = 14.2, 2.9 Hz, $H_{6\beta}$), 2.13-1.92 (4H, m), 1.79-1.64 (4H), 1.62-1.56 (2H, m), 1.52 (1H, dt, *J* = 15.0, 2.4 Hz), 1.44 (1H, dt, *J* = 11.7, 2.5 Hz), 1.40-1.34 (2H, m), 1.31 (1H, d, *J* = 3.4 Hz), 1.28-1.20 (4H, m), 1.17-1.10 (1H, td, *J* = 11.8, 6.1 Hz), 1.04 (3H, d, *J* = 6.7 Hz, H_{21}), 0.98 (3H, s, H_{19}), 0.93-0.75 (1H, m), 0.70 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 145.2 (C_{22}), 111.6 (C_{23}), 92.1 (d, *J* = 171.7, C_2), 68.6 (C_7), 68.0 (d, *J* = 27.9, C_3), 55.3 (C_{17}), 50.3 (CH), 42.7 (C_{13}), 41.2 (CH), 39.6 (CH), 39.4 (CH_2), 35.7 (C_{10}), 35.4 (CH), 35.32 (d, *J* = 18.3 Hz, C_4), 35.31 (CH), 34.0 (CH_2), 32.9 (CH_2), 28.4 (CH_2), 23.7 (CH_2), 23.1 (C_{19}), 21.3 (CH_2), 20.1 (C_{21}), 12.0 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.7 (1F, tt, *J* = 48.6, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI$^+$) m/z: 382.9 $[M+NH_4]^+$, 100%. HRMS (ESI$^+$) $C_{23}H_{37}FNaO_2$ $[M+Na]^+$, m/z calculated: 387.2670; found: 387.2671. **IR** (neat) 3392 (w), 2934 (s), 2869 (m), 1437 (m), 1378 (m), 1045 (m), 1009 (m), 906 (m) cm^{-1}.

3β-hydroxy-4α-fluoro-7-keto-5β-cholanic acid (6.33)

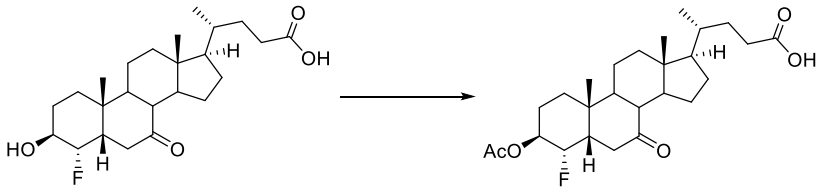


Prepared according to *General Procedure A* (see: *page 147*) using 3.45 g of compound **6.8** (8.16 mmol, 1.0 equiv) and 100 mL of a 5% sodium hydroxide in methanol solution. Purification by flash column chromatography (Biotage ZIP KP-Sil 50 g cartridge) using PE 40-60/acetone (100/0 to 95/5) as the eluent yielded compound **6.33** as a white residue (2.87 g, 6.79 mmol, 83%).

R_f (PE 40-60/acetone, 60/40) 0.19. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.52 (1H, dt, *J* = 47.1, 2.9 Hz, $H_{4\beta}$), 4.06 (1H, dt, *J* = 7.7, 4.3 Hz, $H_{3\alpha}$), 2.80 (1H, dd, *J* = 14.5, 7.6 Hz, $H_{6\beta}$), 2.44-2.20 (5H, m), 2.00-1.79 (5H, m), 1.71-1.57 (3H, m), 1.54-1.24 (8H, m), 1.22 (3H, s, H_{19}), 1.22-0.99 (4H, m), 0.93 (3H, d, *J* = 6.4 Hz, H_{21}), 0.66 (3H, s, H_{18}), ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.3 (C_7), 179.5 (C_{24}), 95.2 (d, *J* = 176.1, C_4), 67.2 (d, *J* = 29.3, C_3), 54.6 (C_{17}), 49.4 (CH), 49.0 (CH), 44.6 (d, *J* = 16.9, C_5), 43.8 (d, *J* = 5.1, CH), 43.0 (C_{13}), 41.7 (CH_2), 38.9 (CH_2), 35.5 (C_{10}), 35.2 (CH), 30.9 (C_{23}), 30.7 (C_{22}), 28.6 (CH_2), 28.3 (CH_2), 25.3 (CH_2), 23.5 (CH_2), 23.4 (C_{19}), 22.2 (CH_2), 18.4 (C_{21}), 12.1 (**C_{18}**) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.2 (1F, t, *J* = 42.5 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI$^+$) m/z: 426.5 $[M+NH_4]^+$,

100%. HRMS (ESI$^+$) $C_{24}H_{38}FO_4$ $[M+H]^+$, m/z calculated: 409.2749; found: 409.2758. **IR** (neat) 3411 (w), 2940 (m), 2873 (m), 1703 (s), 1429 (m), 1381 (m), 1303 (m), 1262 (m), 1172 (m) cm^{-1}.

3β-acetoxy-4α-fluoro-7-keto-5β-cholanic acid (6.34)

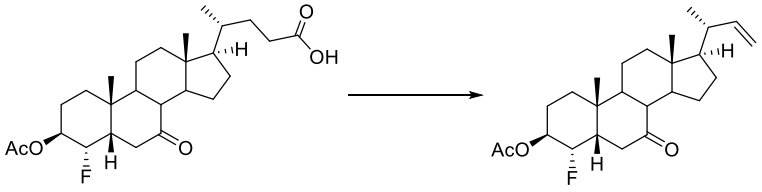


6.33 **6.34**

To a solution of **6.33** (2.85 g, 6.98 mmol, 1.0 equiv) in dichloromethane (170 mL) at rt was added acetic anhydride (2.63 mL, 27.9 mmol, ~4.0 equiv) and bismuth (III) triflate (230 mg, 0.35 mmol, ~0.05 equiv). After 16 h at rt, the reaction was deemed complete by TLC and the reaction mixture filtered over Celite. The filtrate received 1M NaOH solution (20 mL) and water (100 mL) and was extracted with dichloromethane (3 x 75 mL) and the combined organic layers dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 3.09 g of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP KP-Sil 100 g cartridge) using PE 40-60/acetone (100/0 to 95/5) as the eluent yielded compound **6.34** as a pale-yellow residue (1.91 g, 4.23 mmol, 61%).

R_f (PE 40-60/acetone, 60/40) 0.26. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.07 (1H, dd, *J* = 8.2, 2.9 Hz, $H_{3\alpha}$), 4.53 (1H, dt, *J* = 46.3, 2.5 Hz, $H_{4\beta}$), 2.82 (1H, dd, *J* = 14.3, 7.6 Hz, $H_{6\beta}$), 2.44-2.18 (5H), 2.08 (3H, s, C_3OCOCH_3), 2.03-1.72 (6H), 1.62 (1H, dd, *J* = 14.6, 2.6 Hz), 1.52-1.28 (8H, m), 1.24 (3H, s, H_{19}), 1.21-1.02 (4H, m), 0.93 (3H, d, *J* = 6.4 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 210.6 (C_7) ,179.2 (C_{24}), 169.6 (C_3O**C**OCH_3), 95.2 (d, *J* = 176.1, C_4), 69.0 (d, *J* = 33.0, C_3), 54.5 (C_{17}), 49.3 (CH), 49.1 (CH), 45.5 (d, *J* = 17.6, C_5), 43.6 (d, *J* = 5.1, CH), 43.0 (C_{13}), 41.8 (CH_2), 38.8 (CH_2), 35.24 (d, *J* = 1.5 Hz, C_{10}), 35.15 (CH), 30.8 (C_{23}), 30.7 (C_{22}), 29.2 (CH_2), 28.3 (CH_2), 25.3 (CH_2), 23.5 (C_{19}), 22.2 (CH_2), 21.1(C_3OCO**C**H_3), 20.5 (CH_2), 18.4 (C_{21}), 12.1 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -187.4 (1F, td, *J* = 45.1, 6.9 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI$^+$) m/z: 468.4 $[M+NH_4]^+$, 100%. HRMS (ESI$^+$) $C_{26}H_{40}FO_5$ $[M+H]^+$, m/z calculated: 451.2854; found: 451.2863. **IR** (neat) 2944 (m), 2874 (m), 1735 (s), 1707 (s), 1445 (m), 1377 (m), 1307 (m), 1234 (m), 1174 (m), 1034 (m) cm^{-1}.

3β-acetoxy-4α-fluoro-7-keto-24-*nor*-5β-cholan-22-ene (6.35)

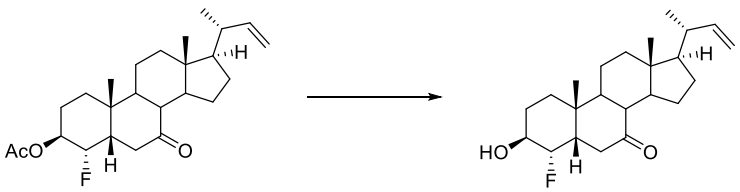


6.34 **6.35**

To a solution of **6.34** (750 mg, 1.66 mmol, 1.0 equiv) in dry benzene (62 mL) under argon at rt was added copper (II) acetate (302 mg, 1.66 mmol, ~1.0 equiv) and pyridine (270 µL, 3.33 mmol, ~2.0 equiv). The reaction mixture was then heated to 80 °C and received lead (IV) acetate (740 mg, 1.10 mmol, ~1.0 equiv) at 1 h intervals until the seventh successive addition of lead (IV) acetate was complete. After a further 1 hour at 80 °C, the reaction was deemed complete by TLC and cooled to rt over 14 h. The reaction mixture was then filtered over Celite and silica gel and the resultant solid was washed with ethyl acetate (50 mL). The filtrate was quenched by the addition of sat. $Na_2S_2O_3$ solution (15 mL) and 2M HCl (5 mL) and the aqueous phase was separated and extracted with ethyl acetarte (3 x 50 mL) and the combined organic phases dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 1.2 g of crude material as a yellow oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 30 g cartridge) using PE 40-60/acetone (95/5 to 75/25) as the eluent yielded compound **6.35** as a white foamy residue (214 mg, 0.53 mmol, 32%) and compound **6.34** as a white residue (23 mg, 0.06 mmol, 3%).

R_f (PE 40-60/acetone, 60/40) 0.62. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.68 (1H, ddd, 17.1, 10.1, 8.5 Hz, H_{22}), 5.07 (1H, dd, *J* = 8.0, 2.8 Hz, $H_{3\alpha}$), 4.90 (1H, ddd, 17.1, 2.0, 0.7 Hz, $H_{23'}$), 4.83 (1H, dd, 10.2, 1.7 Hz, $H_{23''}$), 4.53 (1H, dt, *J* = 46.3, 2.6 Hz, $H_{4\beta}$), 2.82 (1H, dd, *J* = 14.3, 7.7 Hz, $H_{6\beta}$), 2.38-2.29 (2H, m), 2.23 (1H, dd, *J* = 14.4, 1.8 Hz), 2.12-2.10 (1H, m), 2.08 (3H, s, C_3OCOCH_3), 1.97 (2H, dt, *J* = 2.7, 3.4 Hz), 1.96-1.72 (4H, m), 1.62 (1H, dq, *J* = 14.8, 2.7 Hz), 1.56-1.42 (5H, m), 1.24 (3H, s, H_{19}), 1.21-1.11 (3H, m), 1.03 (3H, d, *J* = 6.4 Hz, H_{21}), 1.00-0.84 (1H, m), 0.69 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 210.5 (C_7), 169.6 (C_3O**C**OCH_3), 145.1 (C_{22}), 111.7 (C_{23}), 92.5 (d, *J* = 176.8, C_4), 69.0 (d, *J* = 33.0, C_3), 54.2 (C_{17}), 49.4 (CH), 49.2 (CH), 45.5 (d, *J* = 17.6, C_5), 43.6 (d, *J* = 5.1, CH), 42.9 (C_{13}), 41.8 (d, *J* = 2.2 Hz, CH_2), 41.0 (CH), 38.7 (CH_2), 35.3 (d, *J* = 1.5 Hz, C_{10}), 29.2 (CH_2), 28.5 (CH_2), 25.4 (CH_2), 23.5 (C_{19}), 22.2 (CH_2), 21.1(C_3OCO**C**H_3), 20.6 (CH_2), 20.3 (C_{21}), 12.3 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -187.4 (1F, td, *J* = 44.2, 6.9 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 422.6 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{25}H_{37}FNaO_3$ $[M+Na]^+$, m/z calculated: 427.2619; found: 427.2628. **IR** (neat) 2942 (m), 2873 (m), 1740 (s), 1714 (s), 1431 (m), 1370 (s), 1306 (m), 1231 (s), 1175 (m), 1033 (m) cm^{-1}.

3β-hydroxyl-4α-fluoro-7-keto-24-*nor*-5β-cholan-22-ene (6.36)



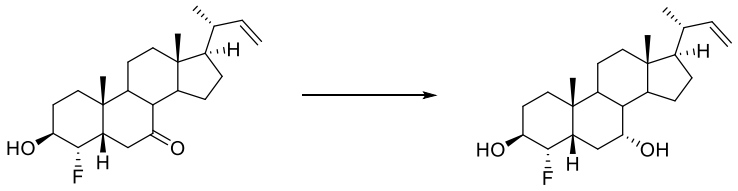
6.35 **6.36**

Prepared according to *General Procedure A* (see: *page 147*) using 77 mg of compound **6.35** (0.19 mmol, 1.0 equiv) and 10 mL of a 5% sodium hydroxide in methanol solution. Purification by flash

column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using PE 40-60/acetone (95/5 to 85/15) as the eluent yielded compound **6.36** as as colourless oil (51 mg, 0.14 mmol, 73%).

R_f (PE 40-60/acetone, 60/40) 0.30. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.67 (1H, ddd, 17.1, 10.2, 8.4 Hz, H_{22}), 4.90 (1H, dd, 17.1, 1.2 Hz, $H_{23'}$), 4.82 (1H, dd, 10.2, 1.7 Hz, $H_{23''}$), 4.51 (1H, dt, *J* = 47.0, 2.6 Hz, $H_{4\beta}$), 4.05 (1H, dd, *J* = 7.8, 3.4 Hz, $H_{3\alpha}$), 2.80 (1H, dd, *J* = 14.6, 7.6 Hz, $H_{6\beta}$), 2.36-2.28 (2H, m), 2.12 (1H, dt, *J* = 7.6, 2.8 Hz), 2.07 (1H, dt, *J* = 6.6, 2.0 Hz), 2.05-1.92 (2H, m), 1.90-1.80 (2H, m), 1.74 (2H, ddt, *J* = 19.1, 9,6, 3.6 Hz), 1.66 (1H, dd, *J* = 7.8, 3.5 Hz), 1.62-1.40 (4H), 1.31-1.24 (2H, m), 1.22 (3H, s, H_{19}), 1.19-1.12 (2H, m), 1.03 (3H, d, *J* = 6.7 Hz, H_{21}), 0.99-0.86 (1H, m), 0.68 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.4 (C_7), 145.1 (C_{22}), 111.6 (C_{23}), 92.5 (d, *J* = 176.1, C_4), 67.2 (d, *J* = 28.6, C_3), 54.2 (C_{17}), 49.4 (CH), 49.0 (CH), 44.6 (d, *J* = 17.6, C_5), 43.9 (d, *J* = 5.1, CH), 42.9 (C_{13}), 41.7 (d, *J* = 2.2 Hz, CH_2), 41.0 (CH), 38.8 (CH_2), 35.6 (C_{10}), 28.6 (CH_2), 28.5 (CH_2), 25.4 (CH_2), 23.43 (CH_2), 23.42 (C_{19}), 22.2 (CH_2), 20.3 (C_{21}), 12.3 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -186.1 (1F, t, *J* = 43.4 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 362.5 $[M+H]^+$, 100%. HRMS (ESI^+) $C_{23}H_{36}FO_2$ $[M+H]^+$, m/z calculated: 363.2694; found: 363.2703. **IR** (neat) 3430 (w), 2942 (m), 2872 (m), 1703 (s), 1459 (m), 1381 (s), 1297 (m), 1176 (m), 1043 (s) cm^{-1}.

3β,7α-dihydroxyl-4α-fluoro-24-*nor*-5β-cholan-22-ene (6.27)



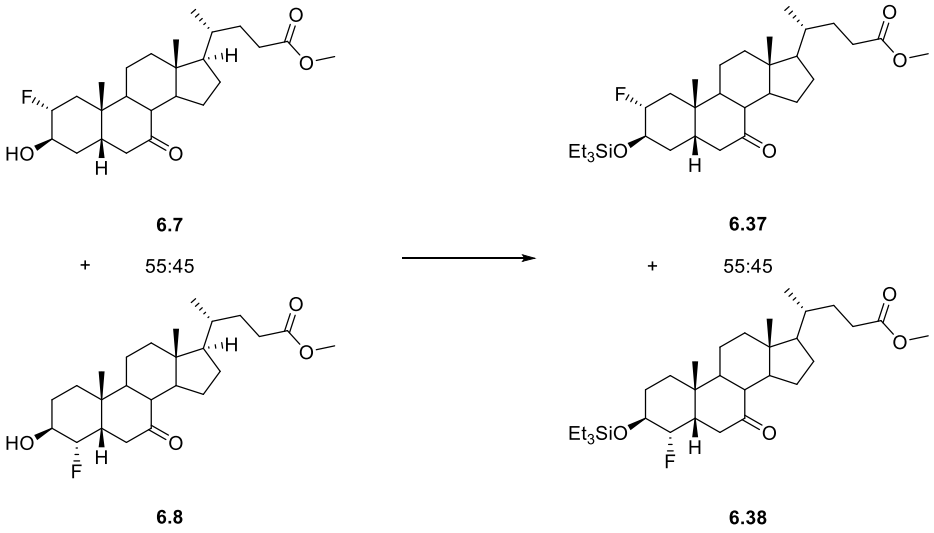
6.36 **6.27**

To a solution of **6.36** (95 mg, 0.26 mmol, 1.0 equiv) in dry in THF (10 mL) under argon at -78 °C was added *L*-selectride (0.65 mL, 0.66 mmol, ~2.5 equiv) dropwise over 5 min. After 10 min at -78 °C, the reaction mixture received hydrogen peroxide (2 mL), 2M sodium hydroxide solution (2 mL) and water (20 mL). After 10 min warming to rt, the reaction mixture received 2M hydrochloric acid solution (10 mL) and the aqueous phase was extracted with dichloromethane (3 x 30 mL) and the combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 165 mg of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage ZIP KP-Sil 30 g cartridge) using PE 40-60/acetone (95/5 to 75/25) yielded compound **6.27** as a pale-yellow residue (25 mg, ~0.02 mmol, 8%; considering 43 mol% purity by ^{1}H NMR).

The data presented for compound **6.27** was attained from an impure mixture. Characteristic signals are reported to help identify this product in future syntheses: **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.66 (1H, ddd, 17.1, 10.2, 8.3 Hz, H_{22}), 4.91 (1H, dd, 16.8, 1.8 Hz, $H_{23'}$), 4.82 (1H, dd, 10.3, 1.2 Hz, $H_{23''}$), 4.56 (1H, dt, *J* = 47.4, 3.7 Hz, $H_{4\beta}$), 4.01 (1H, dd, *J* = 8.1, 3.8 Hz, $H_{3\alpha}$), 3.87 (1H, q, *J* = 2.9 Hz, $H_{7\beta}$), 2.73 (1H,

tt, *J* = 14.6, 2.6 Hz, $H_{6\beta}$), 1.04 (3H, d, *J* = 6.7 Hz, H_{21}), 0.98 (3H, s, H_{19}), 0.70 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.4 (C_7), 145.2 (C_{22}), 111.6 (C_{23}), 92.1 (d, *J* = 171.7, C_4), 68.6 (C_7), 68.0 (d, *J* = 27.9, C_3), 55.3 (C_{17}), 50.4 (CH), 42.7 (C_{13}), 23.1 (C_{19}), 20.1 (C_{21}), 12.0 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.8 (1F, tt, *J* = 48.8, 9.3 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 382.4 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{23}H_{36}FO_2$ $[M-H]^+$, m/z calculated: 363.2694; found: 363.2695.

Methyl-2α-fluoro-3β-triethylsilyloxy-7-keto-5β-cholan-24-oate (6.37) and Methyl-3β-triethylsilyloxy-4α-fluoro-7-keto-5β-cholan-24-oate (6.38)



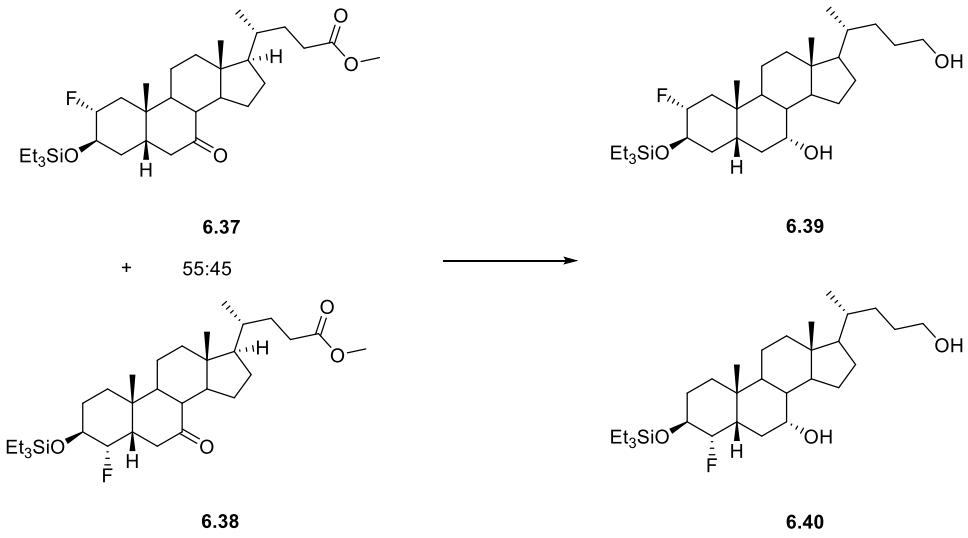
To a solution of inseparable mixture **6.7** and **6.8** (9.15 g, 21.7 mmol, 1.0 equiv) in dichloromethane (100 mL) at rt was added triethylsilylchloride (10.9 mL, 65.0 mmol, ~3.0 equiv), DMAP (1.32 g, 10.8 mmol, ~0.5 equiv) and triethylamine (9.1 mL, 65.0 mmol, ~3.0 equiv). After 17 h at rt, the reaction was deemed complete by TLC and the reaction mixture was concentrated *in vacuo*. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 100 g cartridge) using PE 40-60/acetone (100/0 to 95/5) as the eluent yielded an inseparable mixture of compounds **6.37** and **6.38** in a 55:45 ratio as a yellow oil (9.70 g, 18.0 mmol, 84%).

Compound **6.37**: **R_f** (PE 40-60/acetone, 70/30) 0.54. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.42 (1H, dq, *J* = 47.4, 2.6 Hz, $H_{2\beta}$), 3.93 (1H, dd, *J* = 6.1, 3.2 Hz, $H_{3\alpha}$), 3.66 (3H, s, $C_{24}OCH_3$), 2.90 (1H, dd, *J* = 12.4, 6.1 Hz, $H_{6\beta}$), 2.39-2.16 (5H, m), 2.10-1.87 (4H, m), 1.84-1.62 (4H, m), 1.55-1.26 (8H, m), 1.22 (3H, s, H_{19}), 1.17-1.03 (2H, m), 1.00-0.91 (12H, m, H_{21} + $OSi(CH_2C\mathbf{H}_3)_3$), 0.65 (3H, s, H_{18}), 0.63-0.55 (6H, m, $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 212.8 (C_7), 174.7 (C_{24}), 95.9 (d, *J* = 176.8, C_2), 67.4 (d, *J* = 27.9, C_3), 54.6 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 50.0 (CH), 49.3 (CH), 48.7 (CH), 45.1 (CH_2), 45.0 (d, *J* = 5.1 Hz, CH), 42.6 (C_{13}), 38.8 (CH_2), 35.6 (d, *J* = 1.5 Hz, $\mathbf{C}_{10}$), 35.2 (CH), 34.2 (CH_2), 31.1 (C_{23}), 31.0 (C_{22}), 28.4 (CH_2), 28.3 (CH_2), 24.8 (CH_2), 23.7 (C_{19}), 22.3 (CH_2), 18.3 (C_{21}), 12.1 (C_{18}), 6.76 $OSi(CH_2C\mathbf{H}_3)_3$), 4.70 $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -183.7 (1F, tt, *J* = 50.3, 8.7 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 537.6 $[M+H]^+$, 100%. HRMS (ESI^+) $C_{31}H_{53}FO_4Si$ $[M+H]^+$, m/z calculated:

537.3770; found: 537.3771. **IR** (neat) 2949 (w), 2875 (m), 1739 (s), 1712 (s), 1459 (m), 1435 (m), 1378 (m), 1239 (m), 1205 (m), 1170 (s cm^{-1}.

Compound **6.38**: $\mathbf{R_f}$ (PE 40-60/acetone, 70/30) 0.54. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.37 (1H, dt, *J* = 47.6, 3.3 Hz, $H_{4\beta}$), 3.96 (1H, dd, *J* = 6.8, 2.9 Hz, $H_{3\alpha}$), 3.66 (3H, s, $C_{24}OCH_3$), 2.81 (1H, dd, *J* = 14.1, 7.9 Hz, $H_{6\beta}$), 2.39-2.16 (5H, m), 2.10-1.87 (4H, m), 1.84-1.62 (4H, m), 1.55-1.26 (8H, m), 1.20 (3H, s, H_{19}), 1.17-1.03 (2H, m), 1.00-0.91 (12H, m, H_{21} + $OSi(CH_2C\mathbf{H}_3)_3$), 0.65 (3H, s, H_{18}), 0.63-0.55 (6H, m, $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 211.4 (C_7), 174.7 (C_{24}), 91.8 (d, *J* = 173.1, C_4), 67.3 (d, *J* = 27.9, C_3), 54.5 (C_{17}), 51.5 ($C_{24}O\mathbf{C}H_3$), 49.2 (CH), 44.8 (d, *J* = 16.9 Hz, CH), 43.8 (d, *J* = 5.1 Hz, CH), 43.0 (C_{13}), 42.4 (d, *J* = 2.2 Hz, CH), 40.8 (CH_2), 38.9 (CH_2), 35.4 (d, *J* = 1.5 Hz, C_{10}), 35.21 (CH), 34.0 (CH_2), 31.1 (C_{23}), 31.01 (C_{22}), 28.3 (CH_2), 25.3 (CH_2), 23.7 (CH_2), 23.6 (C_{19}), 22.2 (CH_2), 18.4 (C_{21}), 12.1 (C_{18}), 6.76 $OSi(CH_2C\mathbf{H}_3)_3$), 4.72 $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.5 (1F, td, *J* = 46.5, 5.2 Hz, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 537.6 $[M+H]^+$, 100%. HRMS (ESI^+) $C_{31}H_{53}FO_4Si$ $[M+H]^+$, m/z calculated: 537.3770; found: 537.3771. **IR** (neat) 2949 (w), 2875 (m), 1739 (s), 1712 (s), 1459 (m), 1435 (m), 1378 (m), 1239 (m), 1205 (m), 1170 (s) cm^{-1}.

2α-fluoro-3β-triethylsilyloxy-7-keto-5β-cholan-24-ol (6.39) and 3β-triethylsilyloxy-4α-fluoro-7-keto-5β-cholan-24-ol (6.40)



To a solution of an inseparable mixture of **6.37** and **6.38** (500 mg, 0.93 mmol, 1.0 equiv) in dry diethyl ether (25 mL) under argon at rt was added lithium borohydride (100 mg, 4.66 mmol, ~5.0 equiv). The reaction mixture was then heated to 36 °C and after 1 hour was deemed complete by TLC. The reaction mixture was cooled to rt and received water (25 mL). The aqueous phase was separated and extracted with ethyl acetate (25 mL) and the combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 476 mg of crude material as a white residue. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 25 g cartridge)

using PE 40-60/acetone (100/0 to 90/10) as the eluent yielded compound **6.39** as a colourless oil (106 mg, 0.21 mmol, 22%) and compound **6.40** as a colourless oil (123 mg, 0.24 mmol, 26%).

Compound **6.39**: $\mathbf{R_f}$ (PE 40-60/acetone, 70/30) 0.34. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.43 (1H, dq, *J* = 47.9, 2.8 Hz, $H_{2\beta}$), 3.93 (1H, dd, *J* = 6.1, 3.0 Hz, $H_{3\alpha}$), 3.85 (1H, q, *J* = 2.8 Hz, $H_{7\beta}$), 3.61 (2H, td, *J* = 6.4, 1.6 Hz, $H_{23'}$ + $H_{23''}$), 2.59 (1H, tt, *J* = 14.1, 2.8 Hz), 2.05-1.93 (4H, m), 1.91-1.82 (2H,m), 1.86-1.58 (3H, m), 1.54-1.06 (16H, m), 0.95 (3H, s, H_{19}), 0.943 (9H, t, *J* = 8.0 Hz, $OSi(CH_2C\mathbf{H}_3)_3$), 0.942 (3H, d, *J* = 6.4 Hz, H_{21}), 0.67 (3H, s, H_{18}), 0.58 (6H, q, *J* = 7.9 Hz, $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 92.6 (d, *J* = 172.4, C_2), 68.7 (C_7), 67.8 (d, *J* = 28.6, C_3), 63.6 (C_{24}), 55.9 (C_{17}), 50.4 (CH), 42.7 (C_{13}), 39.8 (CH), 39.6 (CH_2), 35.6 (CH), 35.5 (C_{10}), 35.2 (CH), 35.1 (d, *J* = 3.7 Hz, CH), 34.9 (d, *J* = 18.3, C_1), 34.2 (CH_2), 33.3 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 28.3 (CH_2), 23.7 (CH_2), 23.3 (C_{19}), 21.3 (CH_2), 18.6 (C_{21}), 11.8 (C_{18}), 6.8 $OSi(CH_2C\mathbf{H}_3)_3$), 4.8 $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -183.5 (1F, tt, *J* = 50.3, 8.8 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 528.6 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{30}H_{55}FNaO_3Si$ $[M+Na]^+$, m/z calculated: 533.3797; found: 533.3806. **IR** (neat) 3367 (w), 2934 (s), 2874 (s), 1457 (m), 1376 (m), 1235 (m), 1116 (s), 1074 (s), 1009 (s) cm^{-1}.

Compound **6.40**: $\mathbf{R_f}$ (PE 40-60/acetone, 70/30) 0.38. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.43 (1H, dt, *J* = 47.2, 2.9 Hz, $H_{4\beta}$), 4.02 (1H, dq, *J* = 9.4, 2.8 Hz, $H_{3\alpha}$), 3.67 (1H, m, $H_{7\beta}$), 3.60 (2H, t, *J* = 6.1 Hz, $H_{23'}$ + $H_{23''}$), 3.16 (1H, dd, *J* = 38.0, 11.5 Hz, C_7OH), 2.35 (1H, ddd, *J* = 15.1, 8.0, 4.8 Hz, $H_{2\alpha}$), 1.94 (1H, dt, *J* = 12.4, 2.9 Hz), 1.87-1.34 (16H, m), 1.27-1.06 (7H, m), 0.95 (3H, s, $\mathbf{H}_{19}$), 0.94 (9H, t, *J* = 7.1 Hz, $OSi(CH_2C\mathbf{H}_3)_3$), 0.93 (3H, d, *J* = 6.4 Hz, H_{21}), 0.65 (3H, s, H_{18}), 0.59 (6H, q, *J* = 8.0 Hz, $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 98.2 (d, *J* = 170.9, C_4), 67.8 (d, *J* = 35.2, C_3), 66.0 (C_7), 63.6 (C_{24}), 55.9 (C_{17}), 50.8 (CH), 42.4 (C_{13}), 39.53 (CH_2), 39.48 (CH), 39.2 (d, *J* = 17.6 Hz, CH), 35.63 (CH), 35.61 (d, *J* = 3.7 Hz, CH), 35.1 (C_{10}), 32.7 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 28.8 (CH_2), 28.3 (CH_2), 23.9 (CH_2), 23.8 (CH_2), 23.2 (C_{19}), 21.4 (CH_2), 18.6 (C_{21}), 11.9 (C_{18}), 6.8 $OSi(CH_2C\mathbf{H}_3)_3$), 4.7 $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -172.7 (1F, br. s, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 528.6 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{30}H_{55}FNaO_3Si$ $[M+Na]^+$, m/z calculated: 533.3797; found: 533.3805. **IR** (neat) 3606 (w), 3367 (w), 2935 (s), 2874 (s), 1457 (m), 1412 (m), 1378 (m), 1224 (m), 1156 (s), 1130 (s), 1093 (s) cm^{-1}.

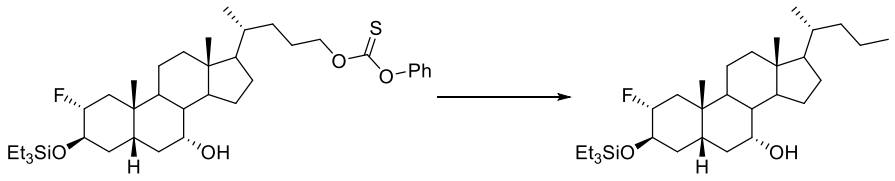
(2α-fluoro-3β-triethylsilyloxy-7α-hydroxy-5β-cholan-24)-*O*-phenyl carbonothioate (6.41)

6.39 **6.41**

To a solution of **6.39** (660 mg, 1.29 mmol, 1.0 equiv) in dry dichloromethane (18 mL) under argon at rt was added pyridine (313 µL, 3.88 mmol, ~3.0 equiv) and *o*-phenylchlorothionoformate (245 mg, 1.42 mmol, ~1.1 equiv). After 12 h at rt, the reaction was deemed complete by TLC and the reaction mixture concentrated *in vacuo*. The residue received water (25 mL) and ethyl acetate (10 mL) and the aqueous phase separated and extracted with ethyl acetate (50 mL) and the combined organic phases dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 820 mg of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 25 g cartridge) using PE 40-60/acetone (100/0 to 90/10) as the eluent yielded compound **6.41** as a colourless oil (650 mg, 1.00 mmol, 78%)

R_f (PE 40-60/acetone, 70/30) 0.48. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.49-7.41 (2H, ArH), 7.32-7.27 (1H, ArH), 7.13-7.10 (2H, ArH), 4.52-4.37 (1H, m, $H_{2\beta}$), 4.50 (2H, td, *J* = 6.7, 2.5 Hz, $H_{23'}$ + $H_{23''}$), 3.94 (1H, dq, *J* = 6.3, 3.1 Hz, $H_{3\alpha}$), 3.86 (1H, quin, *J* = 2.5 Hz, $H_{7\beta}$), 2.60 (1H, tt, *J* = 14.1, 2.6 Hz), 2.06-1.94 (4H, m), 1.92-1.83 (2H,m), 1.76-1.59 (3H, m), 1.57-1.09 (13H, m), 0.98-0.93 (15H, m, H_{19}, $OSi(CH_2C\mathbf{H}_3)_3$), H_{21}), 0.68 (3H, s, H_{18}), 0.59 (6H, q, *J* = 8.1 Hz, $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 195.2 (C=S), 153.4 ($C_{i\ Ar}$), 129.5 (C_mH_{Ar}), 126.4 (C_pH_{Ar}), 122.0 (C_oH_{Ar}), 92.6 (d, *J* = 172.4, C_2), 75.1 (C_{24}), 68.7 (C_7), 67.8 (d, *J* = 28.6, C_3), 55.8 (C_{17}), 50.4 (CH), 42.8 (C_{13}), 39.8 (CH), 39.6 (CH_2), 35.5 (C_{10}), 35.4 (CH), 35.2 (CH), 35.1 (d, *J* = 3.7 Hz, CH), 34.9 (d, *J* = 18.3, C_1), 34.2 (CH_2), 33.3 (CH_2), 31.8 (CH_2), 28.2 (CH_2), 24.8 (CH_2), 23.7 (CH_2), 23.3 (C_{19}), 21.3 (CH_2), 18.5 (C_{21}), 11.8 (C_{18}), 6.8 $OSi(CH_2C\mathbf{H}_3)_3$), 4.8 $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -183.5 (1F, tt, *J* = 50.3, 6.9 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 664.7 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{37}H_{59}FNaO_4SSi$ $[M+Na]^+$, m/z calculated: 669.3780; found: 669.3792. **IR** (neat) 2951 (w), 2875 (m), 2360 (w), 1490 (m), 1380 (m), 1279 (m), 1202 (m), 1077 (s) cm^{-1}.

2α-fluoro-3β-triethylsilyloxy-7α-hydroxy-5β-cholan-24-ane (6.42)



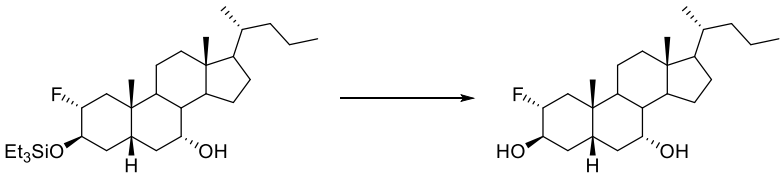
6.41 **6.42**

To a solution of **6.41** (648 mg, 1.00 mmol, 1.0 equiv) in dry toluene (25 mL) under argon at rt was added triethylsilane (6.4 mL, 40.1 mmol, ~40 equiv) and the solution degassed with argon, under sonication. The reaction mixture was then heated to 120 °C and received dibenzoyl peroxide (73 mg, 0.30 mmol, ~0.3 equiv) at 30 min intervals until the fifth successive addition of dibenzoyl peroxide was complete. After a further 1 hour at 120 °C, the reaction was deemed complete by TLC. The reaction mixture was cooled to rt and concentrated *in vacuo* to yield 707 mg of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 25 g

cartridge) using PE 40-60/acetone (100/0 to 90/10) as the eluent yielded compound **6.42** as a colourless oil (413 mg, 0.81 mmol, 81%)

R_f (PE 40-60/acetone, 70/30) 0.63. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.43 (1H, dq, *J* = 47.9, 3.4 Hz, $H_{2\beta}$), 3.93 (1H, dq, *J* = 6.5, 3.1 Hz, $H_{3\alpha}$), 3.86 (1H, quin, *J* = 2.6 Hz, $H_{7\beta}$), 2.60 (1H, tt, *J* = 14.1, 3.0 Hz), 2.05-1.93 (4H, m), 1.91-1.81 (2H, m), 1.68-1.10 (17H, m), 1.06-1.00 (1H, m), 0.952 (3H, s, H_{19}), 0.949 (9H, t, *J* = 8.2 Hz, $OSi(CH_2C\mathbf{H}_3)_3$), 0.92 (3H, d, *J* = 6.6 Hz, H_{21}), 0.87 (3H, t, *J* = 7.0 Hz, H_{24}), 0.67 (3H, s, H_{18}), 0.58 (6H, q, *J* = 7.8 Hz, $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 92.6 (d, *J* = 172.4, C_2), 68.8 (C_7), 67.8 (d, *J* = 28.6, C_3), 56.1 (C_{17}), 50.4 (CH), 42.7 (C_{13}), 39.8 (CH), 39.6 (CH_2), 38.3 (CH_2), 35.6 (C_{10}), 35.5 (CH), 35.2 (CH), 35.1 (d, *J* = 3.7 Hz, CH), 34.9 (d, *J* = 18.3, C_1), 34.1 (CH_2), 33.3 (CH_2), 28.3 (CH_2), 23.7 (CH_2), 23.3 (C_{19}), 21.3 (CH_2), 19.2 (CH_2), 18.6 (C_{21}), 14.6 (C_{24}), 11.8 (C_{18}), 6.8 $OSi(CH_2C\mathbf{H}_3)_3$), 4.8 $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -183.5 (1F, tt, *J* = 50.3, 6.9 Hz, $F_{2\alpha}$) ppm. **LRMS** inconclusive.

2α-fluoro-3β,7α-dihydroxy-5β-cholan-24-ane (6.26)



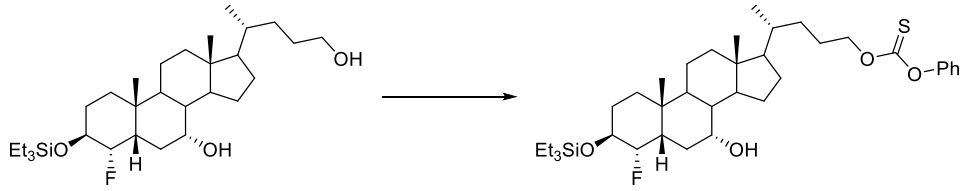
6.42 **6.26**

To a solution of **6.42** (33 mg, 0.07 mmol, 1.0 equiv) in THF (2 mL) at rt was added a solution of TBAF in THF (1M, 0.13 mL, 0.13 mmol, ~2.0 equiv). After 12 h at rt, the reaction was deemed complete by TLC and the reaction mixture concentrated *in vacuo*. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using PE 40-60/acetone (100/0 to 90/10) as the eluent yielded compound **6.26** as a white residue (24 mg, 0.06 mmol, 96%).

R_f (PE 40-60/acetone, 70/30) 0.34. **$[\alpha]_D$:** +7.69 (c = 0.02, chloroform, 25 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.56 (1H, dq, *J* = 47.3, 3.7 Hz, $H_{2\beta}$), 4.01 (1H, dquin, *J* = 7.7, 4.0 Hz, $H_{3\alpha}$), 3.87 (1H, quin, *J* = 2.7 Hz, $H_{7\beta}$), 2.74 (1H, tt, *J* = 14.1, 2.7 Hz), 2.11-2.03 (2H, m), 2.01-1.84 (3H, m), 1.76 (1H, ddt, *J* = 13.6, 5.5, 3.1 Hz, 1.66-1.48 (6H, m), 1.45-1.26 (7H, m), 1.25-1.10 (6H, m), 0.98 (3H, s, H_{19}), 0.91 (3H, d, *J* = 6.5 Hz, H_{21}), 0.87 (3H, d, *J* = 7.0 Hz, H_{24}), 0.67 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 92.1 (d, *J* = 171.7, C_2), 68.6 (C_7), 68.0 (d, *J* = 27.9, C_3), 56.1 (C_{17}), 50.3 (CH), 42.7 (C_{13}), 39.64 (CH), 39.57 (CH_2), 38.3 (CH_2), 35.7 (C_{10}), 35.6 (CH), 35.40 (CH), 35.35 (d, *J* = 17.6, C_1), 35.3 (CH), 33.9 (CH_2), 32.9 (CH_2), 28.3 (CH_2), 23.7 (CH_2), 23.2 (C_{19}), 21.3 (CH_2), 19.2 (CH_2), 18.6 (C_{21}), 14.6 (C_{24}), 11.8 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -184.7 (1F, tt, *J* = 48.1, 10.0 Hz, $F_{2\alpha}$) ppm. **LRMS** (ESI^+) m/z: 363.5 $[M\text{-}OH]^+$, 100%. HRMS (ESI^+) $C_{24}H_{41}FNaO_2$ $[M+Na]^+$, m/z calculated:

403.2983; found: 403.2986. **IR** (neat) 3375 (w), 2931 (s), 2869 (s), 2359 (w), 2167 (w), 1457 (m), 1437 (m), 1377 (m), 1076 (s) 1047 (s) cm^{-1}.

(3β-triethylsilyloxy-4α-fluoro-7α-hydroxy-5β-cholan-24)-*O*-phenyl carbonothioate (6.43)

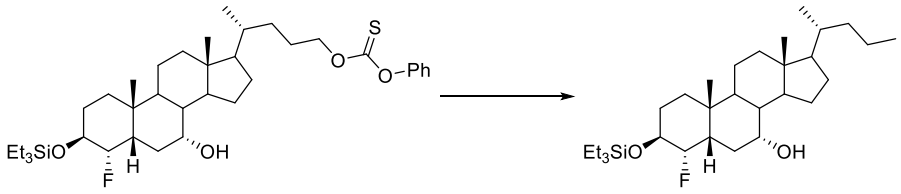


6.40 **6.43**

To a solution of **6.40** (790 mg, 1.55 mmol, 1.0 equiv) in dry dichloromethane (21 mL) under argon at rt was added pyridine (375 µL, 4.64 mmol, ~3.0 equiv) and *o*-phenylchlorothionoformate (293 mg, 1.70 mmol, ~1.1 equiv). After 12 h at rt, the reaction was deemed complete by TLC and the reaction mixture concentrated *in vacuo*. The residue received water (50 mL) and ethyl acetate (50 mL) and the aqueous phase separated and extracted with ethyl acetate (50 mL) and the combined organic phases dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford 1.05 g of crude material as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 25 g cartridge) using PE 40-60/acetone (100/0 to 90/10) as the eluent yielded compound **6.43** as a colourless oil (877 mg, 1.36 mmol, 88%)

R_f (PE 40-60/acetone, 70/30) 0.52. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.44-7.40 (2H, ArH), 7.31-7.27 (1H, ArH), 7.13-7.11 (2H, ArH), 4.50 (2H, td, *J* = 6.7, 2.7 Hz, $H_{23'}$ + $H_{23''}$), 4.52-4.38 (1H, m, $H_{4\beta}$), 4.03 (1H, dq, *J* = 9.5, 2.8 Hz, $H_{3\alpha}$), 3.70-3.67 (1H, m, $H_{7\beta}$), 3.16 (1H, dd, *J* = 38.0, 11.5 Hz, C_7OH), 2.35 (1H, ddd, *J* = 14.8, 7.5, 4.8 Hz, $H_{2\alpha}$), 1.95 (1H, dt, *J* = 12.0, 3.1 Hz), 1.91-1.35 (13H, m), 1.32-1.07 (7H, m), 0.98 (3H, s, H_{19}), 0.960 (9H, t, *J* = 7.8 Hz, $OSi(CH_2C\mathbf{H}_3)_3$), 0.957 (3H, d, *J* = 6.6 Hz, H_{21}), 0.67 (3H, s, H_{18}), 0.60 (6H, q, *J* = 8.0 Hz, $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 195.2 (C=S), 153.4 ($C_{i\ Ar}$), 129.5 (C_mH_{Ar}), 126.4 (C_pH_{Ar}), 122.0 (C_oH_{Ar}), 98.2 (d, *J* = 170.9, C_4), 75.1 (C_{24}), 67.8 (d, *J* = 36.0, C_3), 66.0 (C_7), 55.7 (C_{17}), 50.9 (CH), 42.4 (C_{13}), 39.53 (CH_2), 39.49 (CH), 39.2 (d, *J* = 17.6 Hz, CH), 35.6 (d, *J* = 3.7 Hz, CH), 35.4 (CH), 35.1 (C_{10}), 32.7 (CH_2), 31.8 (CH_2), 28.8 (CH_2), 28.3 (CH_2), 24.8 (CH_2), 23.9 (CH_2), 23.8 (CH_2), 23.2 (C_{19}), 21.4 (CH_2), 18.6 (C_{21}), 11.9 (C_{18}), 6.8 $OSi(CH_2C\mathbf{H}_3)_3$), 4.7 $OSi(C\mathbf{H}_2CH_3)_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -172.7 (1F, br. s, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 664.8 $[M+NH_4]^+$, 100%. HRMS (ESI^+) $C_{37}H_{59}FNaO_4SSi$ $[M+Na]^+$, m/z calculated: 669.3780; found: 669.3766. **IR** (neat) 3607 (w), 2939 (m), 2874 (m), 2359 (w), 1490 (m), 1279 (s), 1202 (s), 1072 (m) cm^{-1}.

3β-triethylsilyloxy-4α-fluoro-7α-hydroxy-5β-cholan-24-ane (6.44)

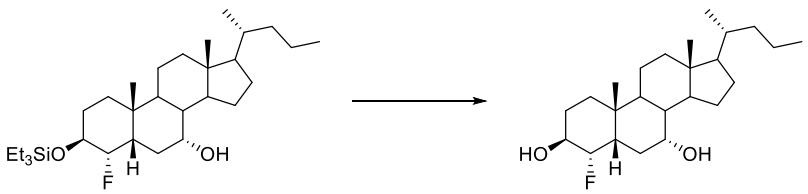


6.43 **6.44**

To a solution of **6.43** (870 mg, 1.35 mmol, 1.0 equiv) in dry toluene (30 mL) under argon at rt was added triethylsilane (8.6 mL, 53.8 mmol, ~40 equiv) and the solution degassed with argon, under sonication. The reaction mixture was then heated to 120 °C and received dibenzoyl peroxide (97 mg, 0.40 mmol, ~0.3 equiv) at 30 min intervals until the fifth successive addition of dibenzoyl peroxide was complete. After a further 1 hour at 120 °C, the reaction was deemed complete by TLC. The reaction mixture was cooled to rt and concentrated *in vacuo* to yield 904 mg of crude as a pale-yellow oil. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 25 g cartridge) using PE 40-60/acetone (95/5 to 90/10) as the eluent yielded compound **6.44** as a colourless oil (568 mg, 0.90 mmol, 67%; considering 50 mol% purity by ^{1}H NMR, owing to benzoic acid residue).

R_f (PE 40-60/acetone, 70/30) 0.68. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.44 (1H, dt, *J* = 47.3, 1.7 Hz, $H_{4\beta}$), 4.03 (1H, dq, *J* = 9.4, 2.9 Hz, $H_{3\alpha}$), 3.69 (1H, dq, *J* = 8.2, 2.2 Hz, $H_{7\beta}$), 3.15 (1H, dd, *J* = 38.0, 11.6 Hz, C_7OH), 2.36 (1H, ddd, *J* = 15.4, 7.5, 4.8 Hz), 1.96 (1H, dt, *J* = 12.2, 3.2 Hz), 1.91-1.08 (22H, m), 0.98 (3H, s, H_{19}), 0.97 (9H, t, *J* = 7.8 Hz, OSi(CH_2C**H**$_3$)$_3$), 0.91 (3H, d, *J* = 6.5 Hz, H_{21}), 0.87 (3H, t, *J* = 7.0 Hz, H_{24}), 0.66 (3H, s, H_{18}), 0.61 (6H, q, *J* = 8.0 Hz, OSi(C**H**$_2$$CH_3$)$_3$) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 98.2 (d, *J* = 170.9, C_4), 67.8 (d, *J* = 35.2, C_3), 66.1 (C_7), 56.0 (C_{17}), 50.9 (CH), 42.4 (C_{13}), 39.6 (CH_2), 39.5 (CH), 39.2 (d, *J* = 16.9 Hz, CH), 38.3 (CH_2), 35.7 (d, *J* = 4.4 Hz, CH), 35.6 (CH), 35.2 (C_{10}), 32.7 (CH_2), 28.8 (CH_2), 28.3 (CH_2), 23.90 (CH_2), 23.86 (CH_2), 23.2 (C_{19}), 21.4 (CH_2), 19.2 (CH_2), 18.7 (C_{21}), 14.6 (C_{24}), 11.9 (C_{18}), 6.8 OSi(CH_2C**H**$_3$)$_3$), 4.8 OSi(C**H**$_2$$CH_3$)$_3$) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -172.7 (1F, br. s, $F_{4\alpha}$) ppm. **LRMS** inconclusive.

3β,7α-dihydroxy-4α-fluoro-5β-cholan-24-ane (6.28)



6.44 **6.28**

To a solution of **6.44** (65 mg, 0.13 mmol, 1.0 equiv) in THF (25 mL) at rt was added a solution of TBAF in THF (1M, 0.26 mL, 0.26 mmol, ~2.0 equiv). After 12 h at rt, the reaction was deemed

complete by TLC and the reaction mixture concentrated *in vacuo*. Purification by flash column chromatography (Biotage SNAP Ultra KP-Sil 10 g cartridge) using PE 40-60/acetone (100/0 to 90/10) as the eluent yielded compound **6.28** as a white residue (47 mg, 0.12 mmol, 97%)

R_f (PE 40-60/acetone, 70/30) 0.45. **$[\alpha]_D$:** +8.33 (c = 0.02, chloroform, 25 °C). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.57 (1H, dt, *J* = 46.3, 2.9 Hz, $H_{4\beta}$), 4.11 (1H, dq, *J* = 6.8, 3.3 Hz, $H_{3\alpha}$), 3.71 (1H, dq, *J* = 7.7, 3.8 Hz, $H_{7\beta}$), 2.99 (1H, dd, *J* = 34.9, 10.9 Hz, C_7OH), 2.37 (1H, ddd, *J* = 15.2, 7.7, 5.5 Hz, $H_{2\alpha}$), 1.96 (1H, dt, *J* = 12.4, 3.3 Hz), 1.92-1.21 (19H, m), 1.20-1.07 (4H, m), 1.00 (3H, s, H_{19}), 0.91 (3H, d, *J* = 6.6 Hz, H_{21}), 0.87 (3H, d, *J* = 7.0 Hz, H_{24}), 0.66 (3H, s, H_{18}) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ 97.5 (d, *J* = 170.2, C_4), 67.8 (d, *J* = 34.5, C_3), 66.1 (C_7), 56.0 (C_{17}), 50.7 (CH), 42.3 (C_{13}), 39.5 (CH + CH_2), 39.3 (2 x CH), 38.3 (CH_2), 35.6 (CH), 35.4 (C_{10}), 32.3 (CH_2), 29.2 (CH_2), 28.3 (CH_2), 23.9 (CH_2), 23.7 (CH_2), 22.9 (C_{19}), 21.4 (CH_2), 19.2 (CH_2), 18.7 (C_{21}), 14.6 (C_{24}), 11.9 (C_{18}) ppm. **^{19}F NMR** (^{1}H non-decoupled, 376 MHz, $CDCl_3$): δ -174.9 (1F, br. s, $F_{4\alpha}$) ppm. **LRMS** (ESI^+) m/z: 363.5 $[M\text{-}OH]^+$, 100%. HRMS (ESI^+) $C_{24}H_{41}FNaO_2$ $[M+Na]^+$, m/z calculated: 403.2983; found: 403.2987. **IR** (neat) 3566 (m), 3384 (m), 2930 (s), 2869 (s), 2359 (w), 2166 (w), 1456 (m), 1436 (m), 1378 (m), 1224 (m), 1040 (s) 1021 (s) cm^{-1}.

Appendix A Crystal Structure Data

A.1 X-ray structure analysis for compound 2.4

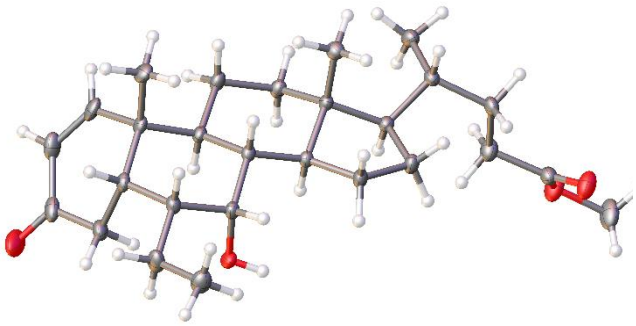


*Figure 8.1: Thermal ellipsoids drawn at the 50% probability level for compound **2.4**.*

Experimental. Single clear colourless prism-shaped crystals of (**DE-8265-30**) were recrystallised from a mixture of acetone and petrol by slow evaporation. A suitable crystal (0.19×0.07×0.05) mm^3 was selected and mounted on a MITIGEN holder silicon oil on a Rigaku AFC12 FRE-HF diffractometer. The crystal was kept at T = 120(2) K during data collection. Using **Olex2** (Dolomanov et al., 2009), the structure was solved with the **ShelXT** (Sheldrick, 2015) structure solution program, using the Intrinsic Phasing solution method. The model was refined with version 2016/6 of **ShelXL** (Sheldrick, 2015) using Least Squares minimisation.

Crystal Data. $C_{27}H_{42}O_4$, M_r = 430.60, orthorhombic, $P2_12_12$ (No. 18), a = 26.5692(9) Å, b = 14.8636(5) Å, c = 6.1040(2) Å, $\alpha = \beta = \gamma = 90^\circ$, V = 2410.56(14) Å^3, T = 120(2) K, Z = 4, Z' = 1, μ(MoK$_\alpha$) = 0.077, 19609 reflections measured, 6109 unique (R_{int} = 0.0387) which were used in all calculations. The final wR_2 was 0.1110 (all data) and R_1 was 0.0524 (I > 2(I)).

Compound	**DE-8265-30**
Formula	$C_{27}H_{42}O_4$
$D_{calc.}$/ g cm^{-3}	1.187
μ/mm^{-1}	0.077
Formula Weight	430.60
Colour	clear colourless
Shape	prism
Size/mm^3	0.19×0.07×0.05
T/K	120(2)
Crystal System	orthorhombic
Flack Parameter	-0.8(5)
Hooft Parameter	-1.0(5)
Space Group	$P2_12_12$
a/Å	26.5692(9)
b/Å	14.8636(5)
c/Å	6.1040(2)
α/°	90
β/°	90
γ/°	90
V/Å^3	2410.56(14)
Z	4
Z'	1
Wavelength/Å	0.71073
Radiation type	MoK$_\alpha$
Θ_{min}/°	3.067
Θ_{max}/°	28.500
Measured Refl.	19609
Independent Refl.	6109
Reflections with I > 2(I)	5537
R_{int}	0.0387
Parameters	289
Restraints	0
Largest Peak	0.327
Deepest Hole	-0.184
GooF	1.069
wR_2 (all data)	0.1110
wR_2	0.1076
R_1 (all data)	0.0600
R_1	0.0524

A.2 X-ray structure analysis for compound 2.14

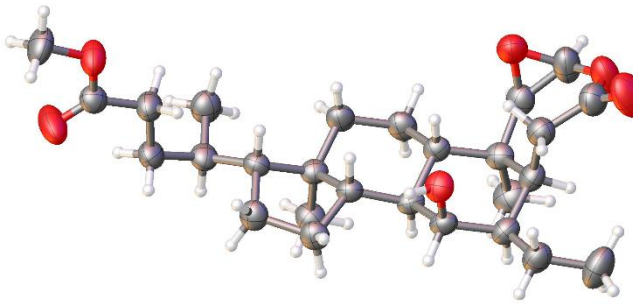


*Figure 8.2: Thermal ellipsoids drawn at the 50% probability level for compound **2.14**.*

Experimental. Single clear colourless block-shaped crystals of **2019sot0027_D1_100K** were obtained by recrystallisation from .... A suitable crystal 0.20×0.20×0.20 mm³ was selected and mounted on a MITIGEN holder with silicon oil on an Rigaku AFC11 007-HF diffractometer. The crystal was kept at a steady T = 100(2) K during data collection. The structure was solved with the **ShelXS** (Sheldrick, 2008) structure solution program using the Direct Methods solution method and by using **Olex2** (Dolomanov et al., 2009) as the graphical interface. The model was refined with version 2016/6 of **ShelXL** (Sheldrick, 2015) using Least Squares minimisation.

Crystal Data. $C_{27}H_{42}O_6$, M_r = 462.60, orthorhombic, $P2_12_12_1$ (No. 19), a = 9.6158(3) Å, b = 12.2715(6) Å, c = 21.1259(4) Å, $\alpha = \beta = \gamma = 90^\circ$, V = 2492.86(15) Å³, T = 100(2) K, Z = 4, Z' = 1, $\mu(CuK_\alpha)$ = 0.687, 2206 reflections measured, 1609 unique (R_{int} = 0.0208) which were used in all calculations. The final wR_2 was 0.0819 (all data) and R_1 was 0.0320 (I > 2(I)).

Compound	**DE-8265-37**
Formula	$C_{27}H_{42}O_6$
$D_{calc.}$/ g cm^{-3}	1.233
μ/mm^{-1}	0.687
Formula Weight	462.60
Colour	clear colourless
Shape	block
Size/mm³	0.20×0.20×0.20
T/K	100(2)
Crystal System	orthorhombic
Flack Parameter	0.5(3)
Hooft Parameter	0.4(2)
Space Group	$P2_12_12_1$
a/Å	9.6158(3)
b/Å	12.2715(6)
c/Å	21.1259(4)
α/°	90
β/°	90
γ/°	90
V/Å³	2492.86(15)
Z	4
Z'	1
Wavelength/Å	1.54184
Radiation type	CuK_α
Θ_{min}/°	14.513
Θ_{max}/°	51.529
Measured Refl.	2206
Independent Refl.	1609
Reflections with I > 2(I)	1500
R_{int}	0.0208
Parameters	304
Restraints	0
Largest Peak	0.086
Deepest Hole	-0.082
GooF	1.040
wR_2 (all data)	0.0819
wR_2	0.0799
R_1 (all data)	0.0347
R_1	0.0320

A.3 X-ray structure analysis for compound 3.15

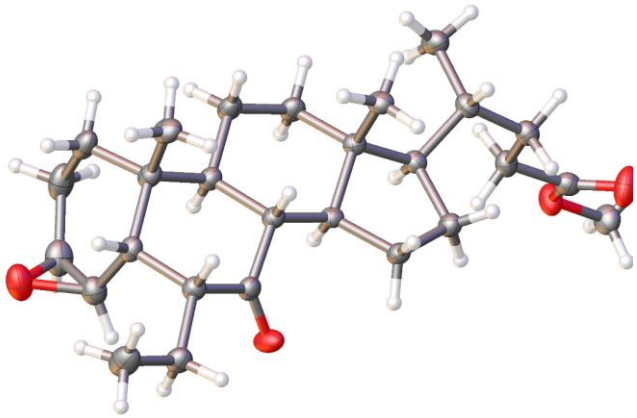


*Figure 8.3. Thermal ellipsoids drawn at the 50% probability level for compound **3.15**.*

Experimental. Single clear colourless plate-shaped crystals of **DE-8472-49-A** were recrystallised from a mixture of acetone and petrol by slow evaporation. A suitable crystal 0.40×0.30×0.03 mm3 was selected and mounted on a MITIGEN holder with silicon oil on a Rigaku R-AXIS Spider diffractometer. The crystal was kept at a steady T = 120(2) K during data collection. The structure was solved with the **ShelXT** (Sheldrick, 2015) structure solution program using the Intrinsic Phasing solution method and by using **Olex2** (Dolomanov et al., 2009) as the graphical interface. The model was refined with version 2018/3 of **ShelXL** (Sheldrick, 2015) using Least Squares minimisation.

Crystal Data. $C_{27}H_{42}O_4$, M_r = 430.60, orthorhombic, $P2_12_12_1$ (No. 19), a = 9.2111(2) Å, b = 12.1255(2) Å, c = 21.2003(4) Å, $\alpha = \beta = \gamma = 90°$, V = 2367.84(8) Å^3, T = 120(2) K, Z = 4, Z' = 1, μ(CuK_α) = 0.620, 26149 reflections measured, 4341 unique (R_{int} = 0.0319) which were used in all calculations. The final wR_2 was 0.0808 (all data) and R_1 was 0.0329 (I > 2(I)).

Compound	**DE-8472-49-A**
Formula	$C_{27}H_{42}O_4$
$D_{calc.}$/ g cm^{-3}	1.208
μ/mm^{-1}	0.620
Formula Weight	430.60
Colour	clear colourless
Shape	plate
Size/mm^3	0.40×0.30×0.03
T/K	120(2)
Crystal System	orthorhombic
Flack Parameter	0.2(2)
Hooft Parameter	0.21(5)
Space Group	$P2_12_12_1$
a/Å	9.2111(2)
b/Å	12.1255(2)
c/Å	21.2003(4)
α/°	90
β/°	90
γ/°	90
V/Å^3	2367.84(8)
Z	4
Z'	1
Wavelength/Å	1.54187
Radiation type	CuK
Θ_{min}/°	4.171
Θ_{max}/°	68.480
Measured Refl.	26149
Independent Refl.	4341
Reflections with I > 2(I)	4172
R_{int}	0.0319
Parameters	286
Restraints	0
Largest Peak	0.181
Deepest Hole	-0.147
GooF	1.044
wR_2 (all data)	0.0808
wR_2	0.0798
R_1 (all data)	0.0346
R_1	0.0329

A.4 X-ray structure analysis for compound 4.3

*Figure 8.4. Thermal ellipsoids drawn at the 50% probability level for compound **4.3**.*

Experimental. Single clear colourless block-shaped crystals of (DE**GPDX298-198C**) were recrystallised from a mixture of ethyl acetate and heptane by slow evaporation. A suitable crystal (0.22×0.21×0.18) mm^3 was selected and mounted on a MITIGEN holder silicon oil on a Rigaku AFC11 007-HF diffractometer. The crystal was kept at T = 100(2) K during data collection. Using **Olex2** (Dolomanov et al., 2009), the structure was solved with the **ShelXT** (Sheldrick, 2015) structure solution program, using the Intrinsic Phasing solution method. The model was refined with version 2016/6 of **ShelXL** (Sheldrick, 2015) using Least Squares minimisation.

Crystal Data. $C_{30}H_{51}FO_4Si$, M_r = 522.79, monoclinic, $P2_1$ (No. 4), a = 7.40470(10) Å, b = 18.5600(3) Å, c = 10.87060(10) Å, α = 98.5130(10)°, $\beta = \gamma = 90°$, V = 1477.50(3) Å^3, T = 100(2) K, Z = 2, Z' = 1, μ(CuK$_\alpha$) = 1.004, 27329 reflections measured, 5409 unique (R_{int} = 0.0407) which were used in all calculations. The final wR_2 was 0.0745 (all data) and R_1 was 0.0290 (I > 2(I)).

Compound	**DEGPDX298-198C**
Formula	$C_{24}H_{38}O_3$
$D_{calc.}$/ g cm^{-3}	$C_{30}H_{51}FO_4Si$
μ/mm^{-1}	1.175
Formula Weight	1.004
Colour	522.79
Shape	clear colourless
Size/mm^3	block
T/K	0.22×0.21×0.18
Crystal System	100(2)
Flack Parameter	monoclinic
Hooft Parameter	-0.017(12)
Space Group	-0.021(9)
a/Å	$P2_1$
b/Å	7.40470(10)
c/Å	18.5600(3)
α/°	10.87060(10)
β/°	90
γ/°	98.5130(10)
V/Å^3	90
Z	1477.50(3)
Z'	2
Wavelength/Å	1
Radiation type	1.54184
Θ_{min}/°	CuK$_\alpha$
Θ_{max}/°	4.112
Measured Refl.	68.482
Independent Refl.	27329
Reflections with I > 2(I)	5409
R_{int}	5266
Parameters	0.0407
Restraints	333
Largest Peak	1
Deepest Hole	0.176
GooF	-0.295
wR$_2$ (all data)	1.064
wR$_2$	0.0745
R$_1$ (all data)	0.0739
R$_1$	0.0298

A.5 X-ray structure analysis for compound 6.28

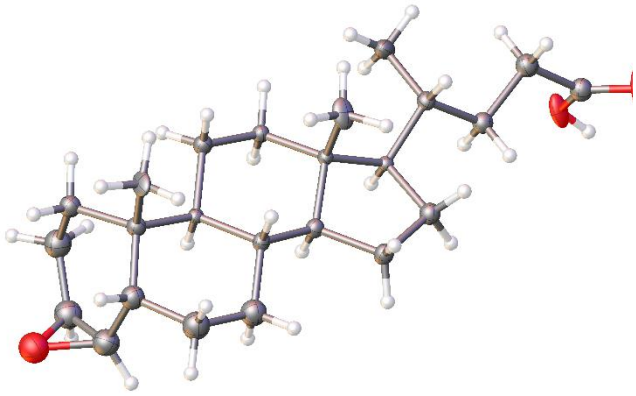


*Figure 8.5. Thermal ellipsoids drawn at the 50% probability level for compound **6.28**.*

Experimental. Single clear colourless plate-shaped crystals of (**DE-8265-41**) were recrystallised from a mixture of acetone and petrol by slow evaporation. A suitable crystal (0.21×0.10×0.01) mm^3 was selected and mounted on a MITIGEN holder with silicon oil on a Rigaku AFC12 FRE-HF diffractometer. The crystal was kept at T = 120(2) K during data collection. Using **Olex2** (Dolomanov et al., 2009), the structure was solved with the **ShelXT** (Sheldrick, 2015) structure solution program, using the Intrinsic Phasing solution method. The model was refined with version 2016/6 of **ShelXL** (Sheldrick, 2015) using Least Squares minimisation.

Crystal Data. $C_{24}H_{38}O_3$, M_r = 374.54, monoclinic, $P2_1$ (No. 4), a = 13.0333(3) Å, b = 12.3790(3) Å, c = 26.9198(8) Å, β = 103.848(3)°, $\alpha = \gamma$ = 90°, V = 4217.0(2) Å^3, T = 120(2) K, Z = 8, Z' = 4, μ(MoK$_\alpha$) = 0.075, 57934 reflections measured, 21342 unique (R_{int} = 0.0580) which were used in all calculations. The final wR_2 was 0.3280 (all data) and R_1 was 0.1394 (I > 2(I)).

Compound	**DE-8265-41**
Formula	$C_{24}H_{38}O_3$
$D_{calc.}$/ g cm^{-3}	1.180
μ/mm^{-1}	0.075
Formula Weight	374.54
Colour	clear colourless
Shape	plate
Size/mm^3	0.21×0.10×0.01
T/K	120(2)
Crystal System	monoclinic
Flack Parameter	-0.2(5)
Hooft Parameter	0.0(6)
Space Group	$P2_1$
a/Å	13.0333(3)
b/Å	12.3790(3)
c/Å	26.9198(8)
α/°	90
β/°	103.848(3)
γ/°	90
V/Å^3	4217.0(2)
Z	8
Z'	4
Wavelength/Å	0.71073
Radiation type	MoK$_\alpha$
Θ_{min}/°	2.988
Θ_{max}/°	28.500
Measured Refl.	57934
Independent Refl.	21342
Reflections with I > 2(I)	17754
R_{int}	0.0580
Parameters	954
Restraints	1586
Largest Peak	1.113
Deepest Hole	-0.722
GooF	1.129
wR_2 (all data)	0.3280
wR_2	0.3149
R_1 (all data)	0.1541
R_1	0.1394

A.6 X-ray structure analysis for compound 6.42

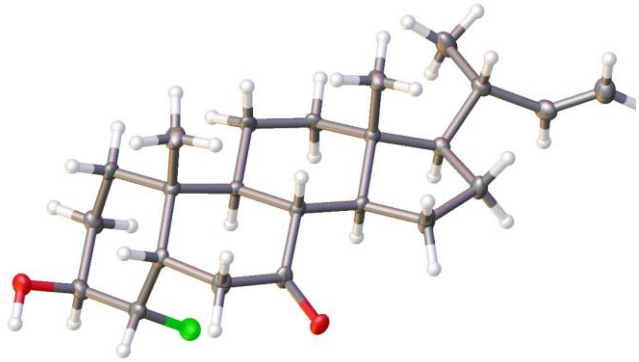


*Figure 8.6. Thermal ellipsoids drawn at the 50% probability level for compound **6.42**.*

Experimental. Single clear colourless prism-shaped crystals of **DE-8580-72** were recrystallised from hexane by slow evaporation. A suitable crystal 0.45×0.27×0.10 mm³ was selected and mounted on a MITIGEN holder in perfluoroether oil on a Rigaku AFC12 FRE-HF diffractometer. The crystal was kept at a steady T = 100(2) K during data collection. The structure was solved with the **ShelXT** (Sheldrick, 2015) structure solution program using the Intrinsic Phasing solution method and by using **Olex2** (Dolomanov et al., 2009) as the graphical interface. The model was refined with version 2016/6 of **ShelXL** (Sheldrick, 2015) using Least Squares minimisation.

Crystal Data. $C_{23}H_{35}FO_2$, M_r = 362.51, orthorhombic, $P2_12_12_1$ (No. 19), a = 11.6298(2) Å, b = 11.7190(2) Å, c = 14.5967(2) Å, $\alpha = \beta = \gamma = 90^\circ$, V = 1989.38(6) Å³, T = 100(2) K, Z = 4, Z' = 1, μ(MoK_α) = 0.081, 25338 reflections measured, 5037 unique (R_{int} = 0.0488) which were used in all calculations. The final wR_2 was 0.1144 (all data) and R_1 was 0.0446 (I > 2(I)).

Compound	**DE-8580-72**
Formula	$C_{23}H_{35}FO_2$
$D_{calc.}$/ g cm^{-3}	1.210
μ/mm^{-1}	0.081
Formula Weight	362.51
Colour	clear colourless
Shape	prism
Size/mm³	0.45×0.27×0.10
T/K	100(2)
Crystal System	orthorhombic
Flack Parameter	-0.2(3)
Hooft Parameter	-0.2(3)
Space Group	$P2_12_12_1$
a/Å	11.6298(2)
b/Å	11.7190(2)
c/Å	14.5967(2)
α/°	90
β/°	90
γ/°	90
V/Å³	1989.38(6)
Z	4
Z'	1
Wavelength/Å	0.71073
Radiation type	MoK
Θ_{min}/°	3.288
Θ_{max}/°	28.498
Measured Refl.	25338
Independent Refl.	5037
Reflections with I > 2(I)	4852
R_{int}	0.0488
Parameters	242
Restraints	0
Largest Peak	0.537
Deepest Hole	-0.219
GooF	1.121
wR_2 (all data)	0.1144
wR_2	0.1130
R_1 (all data)	0.0464
R_1	0.0446

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