UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

Synthesis of Bioactive Fluorinated Bile Acid Analogues

and

Investigating the Influence of (Deoxo)fluorination on Alkanol and Carbohydrate Lipophilicity by a New log*P* Determination Method

by

Zhong Wang

Thesis for the degree of Doctor of Philosophy

January 2016

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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Since the identification of bile acids as natural ligands for FXR and TGR5 receptors, huge interest has arisen in this field, including the synthesis of semi-synthetic bile acid derivatives to increase binding potency and selectivity. Fluorine introduction is an attractive strategy in drug design and property optimization. This thesis describes the synthesis of fluorinated analogues of bile acids and the lead compound 6-ECDCA. Interesting observations of effects of fluorination on crystal packing modes will also be discussed.

Another important application of fluorination is lipophilicity (log*P*) modulation. However, log*P* measurement of non-UV active compounds is rather cumbersome and hampers research toward the understanding how aliphatic fluorination influences lipophilicity. Over the course of this thesis, a novel and straightforward method was developed for accurate measurement of lipophilicity of fluorinated compounds by using ^{19}F NMR. Unlike many other methods, there is no requirement of UV activity for quantification, or the need for calibration curves for log*P* estimation. Using this method, log*P* values for a large number of fluorinated compounds were determined. Interesting effects and trends from different fluorination patterns were observed, along with the impact of stereochemistry on lipophilicity.

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DECLARATION OF AUTHORSHIP

I, Zhong Wang, 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.

Synthesis of Bioactive Fluorinated Bile Acid Analogues and Investigating the Influence of (Deoxo)fluorination on Alkanol and Carbohydrate Lipophilicity by a New log*P* Determination Method

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   B. Linclau, Z. Wang, G. Compain, V. Paumelle, C. Q. Fontenelle, N. Wells, A. Weymouth-Wilson, *Angew. Chem. Int. Ed.* **2016**, *55*, 674-678.

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Acknowledgements

I would like to sincerely thank Prof. Bruno Linclau for giving me the opportunity to carry out my PhD work in his research group and getting funding for my project. His guidance, patience and help were invaluable throughout my studies in Southampton. I would also like to thank Prof. Philip Gale and Dr. Alex Weymouth-Wilson for their advice and feedback.

I must thank all Linclau group members, past and present for their help and suggestions in and outside of the lab. Many thanks to Lewis, Krunal and Florent for sharing so many Saturdays with me. Joe, Gemma, Ben and Rachel for helping me to improve my English. Gemma (pride of Leeds) and Gert-Jan (nice Belgian guy) for exploring botanic gardens and Norman architectures together with me in North England. Clement, Carole, Julien, Thomas, Florent, Lucas, Guillaume, Stephanie, and Vincent to increase my knowledge in french culture (particularly wine and smelly cheeses). Julien, Gemma, Thomas, Victor, Karen and many other people who were involved in AI-Chem project for so many good memories. Also, I want to thank Guillaume, Florent and Thomas for playing badminton friendly, Rob for taking me to unhygienic takeaway, Alex (sportsman of the Year) for his positive energy, and Clement, Guillaume, Julien and Gert-Jan for many useful discussions in chemistry.

I would like to thank Joe for many helpful discussions in our bile acid project, for the proofreading of my thesis, for showing me how slow I am at a 5 k parkrun, and above all for his memorable spicy chicken wings. I must also thank Ramakrishna for checking my numbering of my thesis, and Jean-Baptiste for playing Chinese chess with me.

I want to thank Neil for running so many NMR experiments for me and his advice in NMR techniques, Julie for Mass Spec service (especially for analysing over 30 samples for HRMS in a single day, twice!), Mark and Mateuz for X-ray structure analysis of a large number of compounds. Also, I would like to thank Prof. James Emsley and Dr. Giuseppe Pileio for their advice and for many helpful discussions in NMR techniques and analysis, particularly regarding qNMR. Thanks also go to Karl, Keith and Mark in our chemical stores, and the staff in our glassblowing workshop. I have to thank everyone on the third floor. I also want to thank all staff in Dextra Laboratories for their help and suggestions in the lab during my placement, in particular Dr. Zofia Komsta.

I thank Dextra Laboratories and AI-Chem project (Interreg IVa Channel Programme) for funding.

Many thanks to my parents, uncles, aunts and my sister for their support over the years. And finally, I must thank my wife Haoyue for her support and understanding in the last three years.

Abbreviations

AC	Adenylate cyclase
ADMET	Absorption, distribution, metabolism, excretion, toxicity
AIM	Atoms in molecules
BA	Bile acid
BCP	Bond critical point
CA	Cholic acid
cAMP	Cyclic adenosine monophosphate
CDCA	Chenodeoxycholic acid
D1	Relaxation delay
DAST	(Diethylamino)sulphur trifluoride
DCA	Deoxycholic acid
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DFT	Density functional theory
DIPA	*N*,*N*-Diisopropylamine
DIPEA	*N*,*N*-Diisopropylethylamine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMPU	1,3-Dimethyl-3,4,5,6-tetrahydro-2(*1H*)-pyrimidinone
6-ECDCA	6α-Ethyl chenodeoxycholic aicd
FE	2-Fluoroethanol
FXR	Farnesoid X receptor
HB	Hydrogen bonding
HDCA	Hyodeoxycholic acid
HMPA	Hexamethylphosphoramide
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
IBX	2-Iodobenzoic acid
IMHB	Intramolecular hydrogen bonding
IPA	Isopropanol
IR	Infrared spectroscopy
LBD	Ligand binding domain
LCA	Lithocholic acid
LDA	Lithium diisopropylamide
mCPBA	3-Chloroperbenzoic acid
m.p.	Melting point
MS	Mass spectrometry

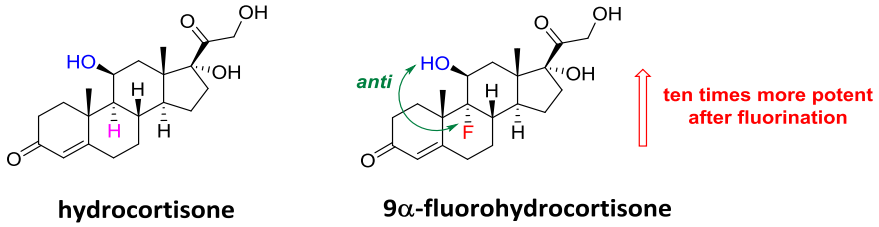
NASH	Nonalcoholic steatoheptitis
NBO	Natural bond orbital
NBS	*N*-Bromosuccinimide
NCI	Non-covalent interactions
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
NS	Number of scans
O/N	Overnight
O1P	Frequency offset point
PBC	Primary biliary cirrhosis
PCC	Pyridinium chlorochromate
PDC	Pyrdinium dichromate
PET	Positron emission tomography
QTAIM	Quantum theory of atoms in molecules
R_f	Retention factor
RP-HPLC	Reversed phase high-performance liquid chromatography
RT	Room temperature
SAR	Structure-activity relationship
SF	Shake-flask
SNR	Signal-to-noise ratio
SW	Spectral width
T1	Spin-lattice relaxation time
TBAB	Tetrabutylammonium bromide
TBAI	Tetrabuylammonium iodide
TBAF	Tetrabuylammonium fluoride
TEA	Triethylamine
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
TFE	2,2,2-Trifluoroethanol
TGR5	Takeda G-protein-coupled receptor 5
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSCl	Trimethylchlorosilane
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
UDCA	Ursodeoxycholic acid
UV	Ultraviolet

SECTION I INTRODUCTION

Chapter 1: Introduction

1.1 Overview of fluorine chemistry

One of the very early applications of fluorine in medicinal chemistry (1950s) is the synthesis of 9α-fluorohydrocortisone (**Figure 1.1**), which possessed increased potency by more than 10-fold compared with hydrocortisone,[1],[2] demonstrating the impact of fluorine in drug design. Accompanied by the significant development of synthetic methodologies for the introduction of fluorine in organic molecules in the last few decades,[3] the application of fluorine in the pharmaceutical, agricultural and organic materials industry is thriving. As of today, it is estimated that over 20% of drugs in the global pharma pipeline contain fluorine atom(s).[4] This is due to the fact that fluorination has influences on physicochemical and pharmacokinetic properties and can enhance the drug pharmacological profile.[5] In addition, [^{18}F]-fluorine is also a very attractive radioactive isotope for PET imaging to evaluate drug distribution and targeting.[5]



hydrocortisone **9α-fluorohydrocortisone**

Figure 1.1 Improved potency after fluorine insertion.

Due to the high electronegativity of fluorine, the C-F bond is highly polarised leading to a relatively large dipole.[6] This can significantly influence molecular conformations.[6] Fluorination can modulate the acidity (pK_a) or basicity (pK_{aH}) of nearby functional groups. For example, the pK_{aH} of 2,2,2-trifluoroethylamine is only 5.7,[7] and thus is not significantly protonated at physiological pH level. Replacement of C-H bonds by C-F bonds can improve drug bioavailability by blocking metabolically labile sites, and can sometimes even increase the binding affinity through additional polar interactions with protein residues.[7] In the following sections, the impact of fluorine on lipophilicty and hydrogen bond donating capacity of adjacent alcohols, as well as intramolecular OH···F hydrogen bonding, will be described in detail due to their relevance to the subject of this thesis.

1.2 Influence on lipophilicity

Lipophilicity, which is measured experimentally as partition or distribution coefficient (log*P*/log*D*), is a crucial physicochemical parameter and has impact on ADMET properties of the drug molecules. Lipophilicity contributes to drug solubility, membrane permeability, bioavailability, CNS penetration, toxicity and so forth.[8] From the classic Lipinski ''rule of 5'' to statistically obtained optimal log*P*/*D* ranges as well as the recent concept of lipophilic efficiency indices,[9] medicinal chemists are still trying hard to control lipophilicity in order to increase the prospect of identifying drug candidates. One of the key problems for drug design in the last few decades is that high lipophilicity (together with larger MW) was unsuitably used to increase binding affinities.[10] Therefore, it was suggested that lowering or at least maintaining lipophilicity level in the process of affinity optimisation can improve the likelihood of success for drug discovery.[10],[11]

Fluorination can modulate lipophilicity.[12],[13] Based on statistical analysis, it was concluded in several reviews that replacement of hydrogen by fluorine generally increases the lipophilicity of the molecule.[12],[14],[15] However, these results are generalized with a bias arising from the predominance of fluoroaryl compounds in the dataset,[16] and this has led to a misconception regarding the influence of fluorination on lipophilicity. As shown in **Figure 1.2**, aromatic fluorination can increase the lipophilicity due to the fact that C-F bond becomes less polarised caused by the orbital overlapping. However, hydrogen-to-fluorine exchange in aliphatic compounds can lead to decreased lipophilicity (**Figure 1.2**),.[14],[17],[18],[19]

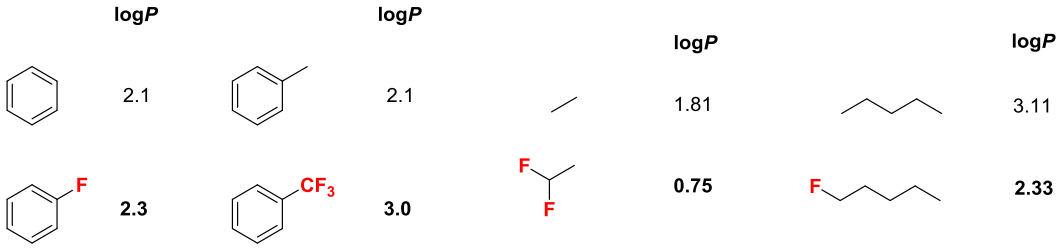


Figure 1.2 log*P* values of selected fluorinated compounds.[19]

The influence of fluorination on log*P* is further complicated by the presence of functional groups. Interesting results (**Figure 1.3**) have been reported on alkanols.[20] The change of lipophilicity upon fluorination depends on the fluorination positions relative to the hydroxyl group. β-Trifluorination on ethanol increases lipophilicity by 0.68 log*P* units. On the contrary, terminal trifluorination on *n*-pentanol and *n*-hexanol leads to more hydrophilic compounds.

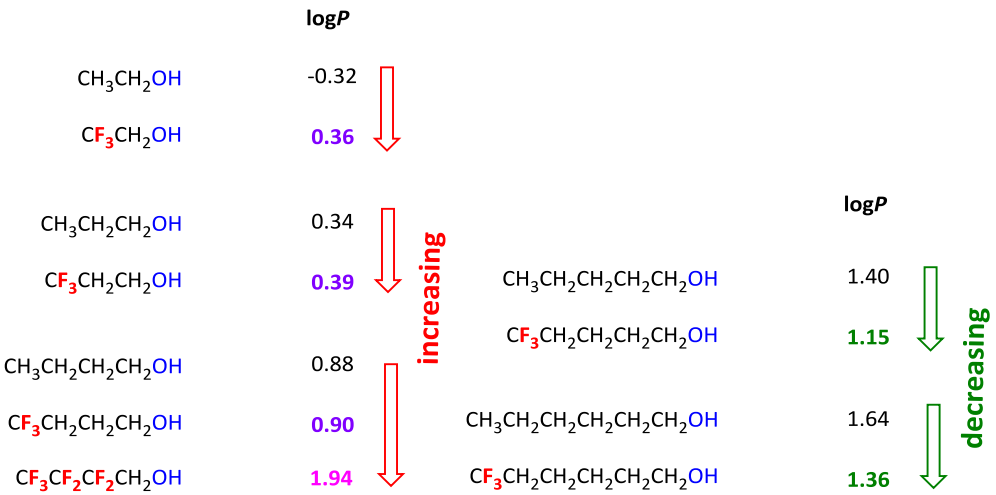


Figure 1.3 Log*P* values of fluorinated alkanols[20]

Encoding many intermolecular forces, lipophilicity was statistically summarized as **Equation 1**.[21]

Lipophilicity = Hydrophobicity – Polarity **(Eq. 1)**

Fluorination can have an impact on both molecular polarity and hydrophobicity, which have opposing effects on lipophilicity. This is in line with an experimental analysis published by Carreira, Mueller, *et al*. by investigating log*P* value differences of fluorinated propyl indole derivatives *via* comparing changes of local hydrophobic surface (volume) and polarity.[22] They also demonstrated that the influence of trifluorination on lipophilicity is also dependent on the absolute lipophilicity of the parent compounds. Impact on hydrophobicity from trifluorination for polar compounds overcomes the related impact on molecular polarity and therefore, log*P* increases. Mueller further extended this study by using simplified bond vector analysis to identify fluorinated log*P*-lowering motifs,[23] and recently they have found that *vic*-difluorination is more efficient in lowering lipophilicity than *gem*-difluorination through comparison of polarity analysis and experimentally obtained log*P* values, as illustrated in **Figure 1.4**.[24]

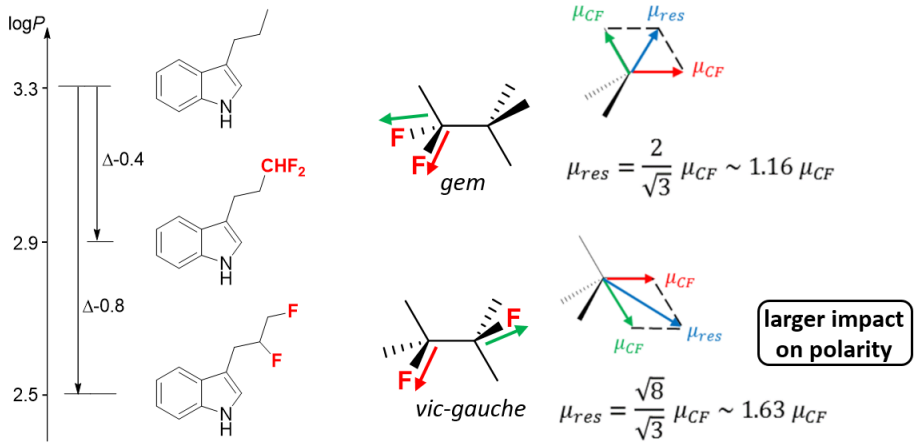


Figure 1.4 Comparison of polarity analysis and log*P* values. *Adapted from Ref. [24]*

1.3 Intramolecular OH···F hydrogen bonding

Hydrogen bonding (HB) involving covalently bound fluorine atom is still a controversial topic. An article by Dunitz and Taylor in 1997, entitled ''organic fluorine hardly ever accepts hydrogen bonds'',[25] arguably led to an unfavourable impression of fluorine's capacity of forming HB to many organic chemists. Strong reliance on the statistical analysis based on crystallographic data may have led to this.[25],[26],[27] However, recent experimental IR and NMR studies, as well as computational evidences, strongly indicated the existence of such weak intermolecular and intramolecular HB involving organic fluorine.[27],[28],[29],[30] In particular, intramolecular hydrogen bonding (IMHB) of fluorinated alcohols aroused our interest. As shown in **Figure 1.5**, Takemura *et al.* observed through-space ^{1h}J coupling constants (a necessary indicator for IMHB) between fluorine and hydroxyl hydrogen for cyclophane and naphthol derivatives, **1.1** and **1.2**.[31],[32]

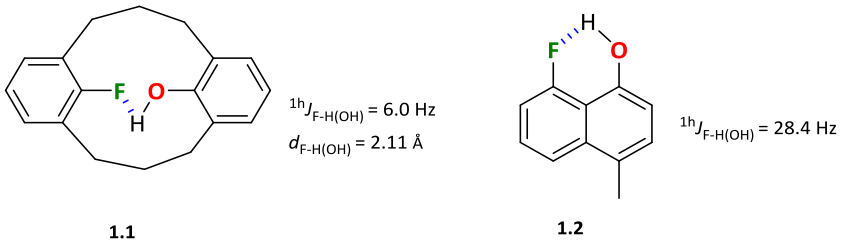


Figure 1.5 ^{1h}J coupling constants for cyclophane and naphthol derivatives.

This through-space coupling constant was also observed on the conformationally restricted cyclohexanol derivative **1.3** (**Figure 1.6**). Computational analysis indicated a single dominant IMHB conformer (99.7%, CCl_4) and significant lone pair charge transfer (17.1 kJ·mol^{-1}).[33]

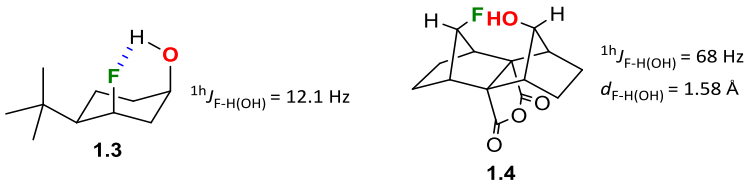


Figure 1.6 IMHB for cyclohexanol derivative and rigid caged compound.

In addition, Gouverneur, Bernet, *et al.* studied fluorinated carbohydrate derivatives and found that fluorine can even compete with endocyclic oxygen as HB acceptor based on ^{1}H NMR ^{1h}J coupling constant analysis.[34] They also illustrated that CF_2 was a worse acceptor than CHF to form hydrogen bonding.[34] As an extreme case, Lectka and co-workers have recently synthesized rigid caged compound **1.4** (**Figure 1.6**) for comprehensive IR, NMR, X-Ray and computational studies. Although the OH group of this caged substrate had no IR blue/red shift, a strong HB interaction was proven by a surprisingly large ^{1h}J coupling constant (68 Hz), a short contact distance between the fluorine

and hydroxyl hydrogen atoms (1.58 Å), and the profound presence of a bond critical point (BCP) based on AIM calculation.[35]

One common feature of these examples containing IMHB is the conformational rigidity of the molecular structures. It was also described in the literature that no experimental evidence was found for IMHB on linear fluoroalcohols (e.g., 3-fluoropropanol and 4-fluorobutanol) either by NMR (^{1h}J coupling constant) or IR (red shift), conflicting the computational calculation results from QTAIM and NCI analysis.[36] The assumed reason for the missing proof was the entropy penalty associated with hydrogen-bonded conformer formation.[36]

However, research in our group unexpectedly revealed IMHB for flexible acyclic fluorinated alkanols, including 3-fluoropropan-1-ol **1.8** (**Figure 1.7**).[37] We have obtained $^{1h}J_{F\text{-}HO}$ coupling constants even with large magnitude up to 9.9 Hz for compound **1.5** at lower temperature, which are all fully supported by theoretical calculations. Diastereoisomer **1.6** displayed different ^{1h}J coupling (1.9 Hz against 6.6 Hz) compared to compound **1.5**, due to a much lower population for the hydrogen-bonded conformers. Interestingly, a distinctive difference for the diastereotopic fluorine atoms of compound **1.7** was also measurable by ^{1}H NMR.[37]

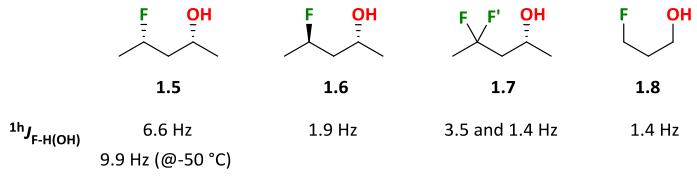


Figure 1.7 $^{1h}J_{F\text{-}HO}$ coupling constants in $CDCl_3$ for γ-fluoropropanol derivatives.

1.4 Influence on HB donating capacity of adjacent alcohols groups

Hydrogen bonding is one of the most important intermolecular interactions in many processes, such as in biological recognition, catalysis, crystal engineering, etc.[38],[39] It is also of particular interest in drug development due to its impact on drug properties.[40] Due to the high fluorine electronegativity, fluorine introduction close to functional groups (e.g., hydroxyl) is expected to influence their HB properties. Regarding the ability to act as HB donor (i.e., HB donating capacity or HB acidity), an early review concluded that ‘‘fluorination always increases hydrogen bond acidity’’.[12] However, only limited examples of polyfluorinated alcohols (e.g., hexafluoroisopropyl alcohol[41],[42]) had been examined.

Our group has recently investigated the influence of relative stereochemistry on the HB acidity (HB donating capacity) of fluorohydrins, using conformationally restricted substrates.[33] The HB acidity was determined by FTIR spectroscopy.[40] We have found that the general rule as formulated in the

literature was not correct, and that fluorination can significantly decrease the HB donating capacity. The influence depends on the relative configuration and the position of the inserted fluorine atom(s). As demonstrated in **Figure 1.8**, HB acidity can be modulated in both directions by single fluorine introduction. The result also showed that the inductive effect of fluorine is not the only factor to impact on HB acidity.

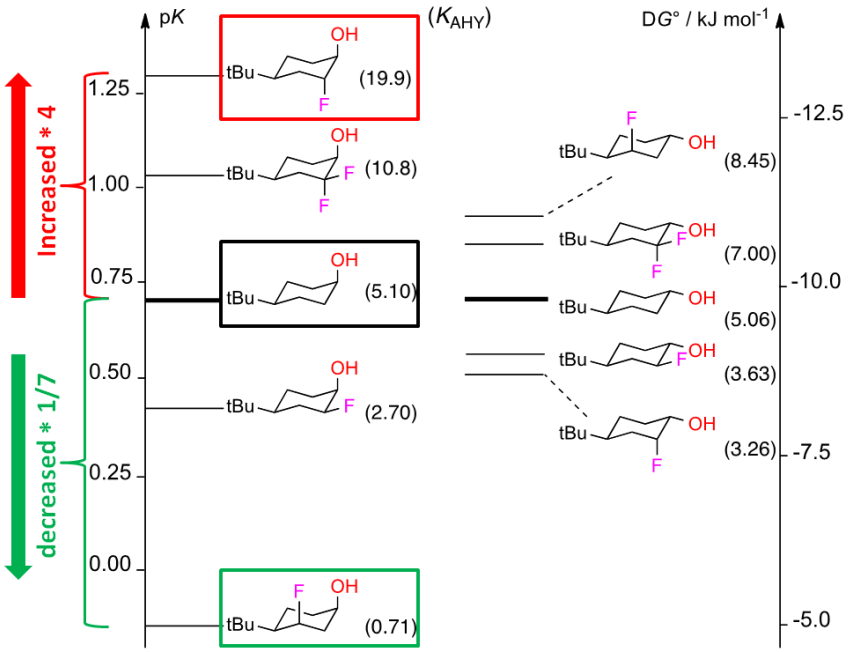


Figure 1.8 Impact of fluorination on HB acidity (pK_{AHY}). ***Adapted from Ref. [33]***

According to DFT calculations, the impact of the weak intramolecular OH···F interaction can surpass the strong inductive effect originated from a fluorine atom, leading to the unexpected decrease of HB acidity. In addition, we also concluded that the fluorine's electron-withdrawing effect is related to the dihedral angle, having the maximum effect at 180°.[33]

Our group has also extended this investigation into benzyl alcohol derivatives.[43] As shown in **Figure 1.9**, the HB acidity for the parent compound **1.10** can be mildly increased by *o*-monofluorination (compound **1.9**). However, additional *o*'-fluorine introduction (compound **1.11**) led to a decrease in pK_{AHY} value. This similar trend was also observed on its *meta*-substituted methoxy and nitro derivatives. Full computational (AIM, NCI and NBO) calculations indicated that there are several intramolecular interactions (F···OH, CH···F and CH···O) complicating the conformer population, as the hydroxyl arm of benzyl alcohol is flexible.[43]

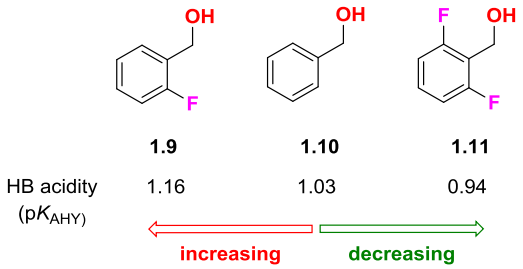


Figure 1.9 HB acidity of fluorinated benzyl alcohols.

1.5 Aim of the projects

This thesis is based on two different yet related projects:

- **Bile acid (BA) project** (synthesizing fluorinated bile acid analogues)
- **Lipophilicity project** (methodology development for log*P* measurement and investigation of influence of fluorination using this new method).

The insights into trends of the impact of fluorination on lipophilicity can provide useful guidance for the rational fluorine incorporation into the steroidal structure of bile acids project.

1.5.1 Objectives for bile acid project (SECTION II)

The aim of this project is to synthesize B-ring and C-ring fluorinated analogues of bile acids (*see section 2.1 for BA structures*), which will be used for biological screening. Obeticholic acid (6-ECDCA, **Figure 1.10**) was identified as a potent agonist of a ligand binding protein (FXR, *see section 2.2.1*), and 6α-ethyl group on CDCA led to significantly increased potency due to additional binding with hydrophobic pocket (*see section 2.2.3*). Therefore, it is of interest to synthesize analogues with polyfluorinated side chain on C-6. The larger hydrophobic surface can contribute to the binding with receptors.

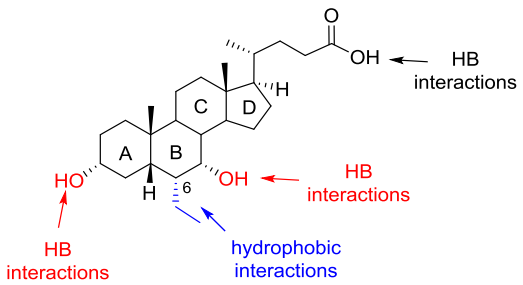


Figure 1.10 Structure of 6-ECDCA and simplified illustration of binding.

As the hydrogen bonding interactions are important for the binding (*see section 2.2*), we will apply our knowledge of modulating HB properties by fluorination. Fluorine atom(s) will be introduced in the vicinal positions of the hydroxyl groups.

Fluorine is also a classical bioisostere for hydrogen and hydroxyl as well.[44] In addition, the difluoromethylene moiety is regarded as isopolar and isosteric compared with a carbonyl group.[45] Therefore, selective deoxyfluorination will be conducted on OH groups and also their keto intermediates.

1.5.2 Objectives for lipophilicity project (SECTION III)

We are interested in systematic investigation of the impact of fluorination on lipophilicity of biological relevant groups or moieties (such as non-UV-active fluorohydrins and fluorinated carbohydrates). However, it is difficult and challenging (*see section 6.2*) for organic chemists to conduct efficient log*P* measurements merely by using general open-access facilities available to us. Commercial measurements are expensive and also difficult for non-UV-active compounds.

The objective of this project is to develop a novel and straightforward method for the accurate measurement of log*P* for fluorinated compounds by simply using ^{19}F NMR, and to demonstrate the potential of the method by investigating the influence of fluorination on the lipophilicity of alkanols.

SECTION II BILE ACIDS

Chapter 2: Introduction

2.1 Bile acids

Originating from cholesterol catabolism, bile acids have a crucial role in digestion and promotion of the absorption of dietary fat, lipids and lipid-soluble vitamins.[46],[47] In the human BA pool, there are mainly five types of bile acids, as shown in **Figure 2.1**.[46] Primary BAs (CA and CDCA) are formed in the liver by enzymatic pathways and further 7-dehydroxylation by bacterial flora in the ileum leads to the secondary BAs (DCA and LCA).[48] As tertiary BA, UDCA is synthesized *via* 7-keto LCA, which is metabolic product form CDCA by intestinal bacteria.[46] UDCA is currently used for the treatment of primary biliary cirrhosis (PBC).

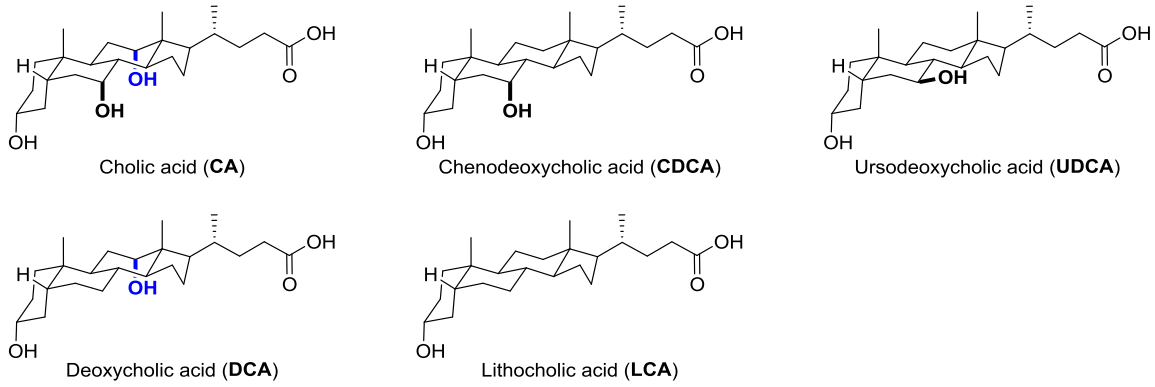


Figure 2.1 Structures of bile acids.

2.2 Bile acid receptors and ligand binding

2.2.1 Farnesoid X receptor

Bile acids were identified as natural ligands for the farnesoid X receptor (FXR) in 1999, sparking a huge interest.[49] CDCA displayed the highest potency, followed by LCA.[50] FXR is a nuclear receptor, expressed mainly in the liver, kidney and intestine as well as other cholesterol-rich tissues.[51] As one member of human nuclear receptor family (in total 48 known by far), FXR (class and gene ID: NR1H4) is a ligand-activated transcription factor, which can regulate target gene expression.[52],[53]

Activation of FXR by BAs led to inhibition of transcriptional processes involving the CYP7A1 gene for cholesterol metabolism, and therefore it was concluded that FXR is responsible for regulating bile acid synthesis and maintaining bile acid and cholesterol homeostasis.[54] Serum triglyceride level was lowered when rats were treated with nonsteroidal FXR agonists.[55] Besides, FXR agonists were found to be capable of reducing the levels of plasma glucose and lipid in wild-type/diabetic mice,

indicating the potential role of FXR in glucose homeostasis and lipid metabolism.[56] A recent review also highlighted the mechanisms behind glucose-lowering outcome *via* FXR signalling.[57] In addition, further studies showed that FXR agonism can reduce hepatic inflammation and steatosis, prevent cisplatin-induced kidney injury by SHP expression, and prevent experimental diarrhoea *via* inhibition of Cl^- secretion, and can also facilitate liver regeneration.[58],[59],[60],[61],[62] It is also worth mentioning that ^{18}F-labelled BA analogue was also reported as PET tracer for potential application for diagnosis of FXR-related diseases.[63]

2.2.2 TGR5 receptor

In 2002, BAs were also recognized as endogenous ligands for TGR5, with LCA and DCA more potent than CDCA and CA.[64],[65] TGR5 is a G protein-coupled membrane-type receptor (gene ID: GPBAR1/GPCR19/M-BAR) expressed in various cells and organs. Compared with nuclear receptor family, G protein-coupled receptors consist of over 800 genes, and they are among the most common targets for drug discovery.[66],[67] The simplified description of commonly accepted TGR5 signalling is illustrated in **Figure 2.2**. Upon BA (e.g., LCA) binding, TGR5 liberates a G-protein (α_s, β, γ) complex, followed by nucleoside exchange between guanosine diphosphate (GDP) and guanosine triphosphate (GTP). As a result, GTP bound G-protein-α_s is cleaved and activates adenylate cyclase (AC). This leads to cyclic adenosine monophosphate (cAMP) induction and enzyme activation, which can cause further cell response.[67]

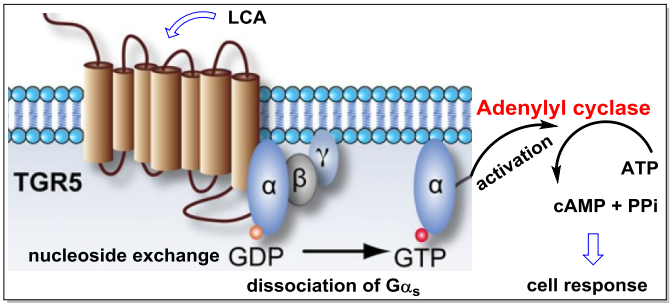


Figure 2.2 Brief illustration of signalling pathway of TGR5. ***Adapted from Ref. [67]***

Treatment of mice with BAs led to elevated energy consumption in brown adipose tissues, which is facilitated by thyroid hormone induction via cAMP signalling.[68] It was also found that glucagon-like peptide-1 (GLP-1) secretion can be enhanced by BAs *via* TGR5.[69] Production of pro-inflammatory cytokines can be reduced by TGR5 activation as well.[70] Numerous biological evidences were summarized in recent literature reviews regarding many key roles of TGR5 in BA metabolism, glucose metabolism, body weight, hepatic inflammation, energy metabolism, insulin sensitivity, immune response, etc.[67],[71],[72],[73]

Both FXR and TGR5 are becoming increasingly attractive for drug discovery projects as potential therapeutic targets for many diseases, such as metabolic syndrome and disorders, liver diseases, obesity, and type 2 diabetes. Besides FXR and TGR5, activities of some other G-protein coupled receptors and nuclear receptors can also be regulated by bile acids ligand binding.[67] Therefore, selectivity of BA analogues towards these receptors should be investigated fully to prevent any undesired side effects.

Increasing numbers of semisynthetic bile acid derivatives as well as nonsteroidal compounds have been reported as ligands for FXR and TGR5.[72],[74] Among them, the previously mentioned 6-ECDCA has been identified as a potent FXR agonist,[75] which currently has completed its Phase III clinical trials for the treatment of PBC and is under clinical trials for non-alcoholic steatohepatitis (NASH) as well.

2.2.3 Ligand binding with FXR

Pellicciari *et al.* reported that the introduction of ethyl group on natural ligand CDCA has led to an increased binding affinity towards FXR by nearly 100-fold (**Figure 2.3**), revealing the extra hydrophobic pocket in the ligand binding domain (LBD).[76],[77]

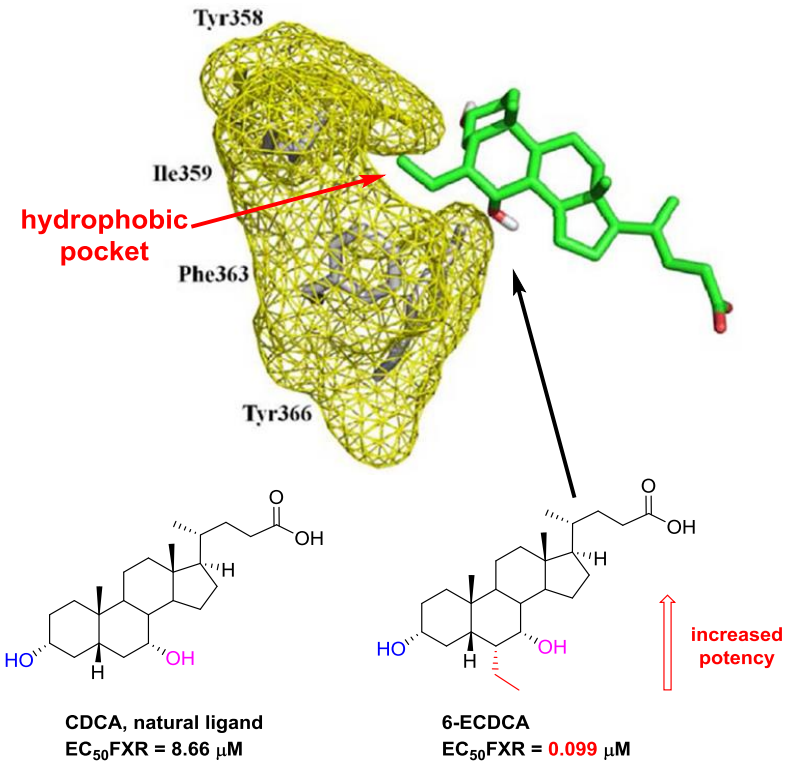


Figure 2.3 Increased potency due to 6-ethyl introduction. ***Adapted from Ref. [77]***

As illustrated in **Figure 2.4**, further docking analysis of binding mode of 6-ECDCA with FXR showed that 6α-ethyl group can accommodate itself in the hydrophobic pocket surrounded by the

hydrophobic residues (Phe284, Leu451 and Phe461). The 3α-OH, 7α-OH and carboxyl groups can form HB interactions with Trp469, His447 and Arg351 respectively.[78]

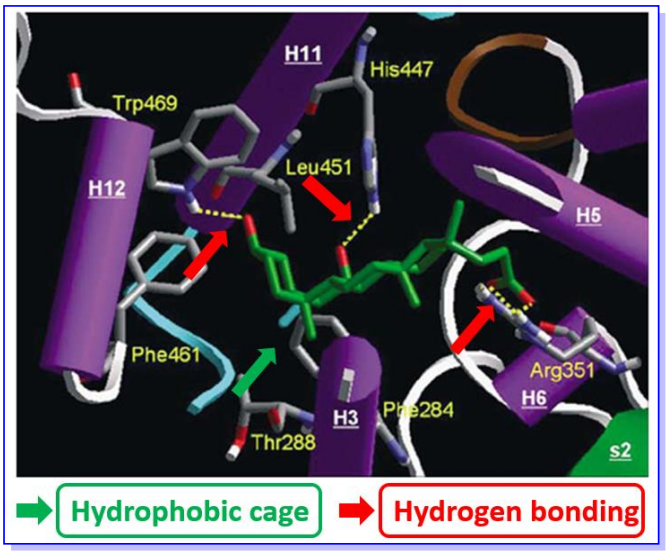


Figure 2.4 Docking analysis of 6-ECDCA/FXR binding mode. ***Adapted from Ref. [78]***

Mi *et al.* has also reported a crystal structure of 6-ECDCA bound *r*FXR (rat FXR), indicating the ligand binding interactions.[79] As shown in **Figure 2.5**, hydrogen bonding interactions as well as van der Waals interactions of 6α-ethyl group with FXR are of importance.

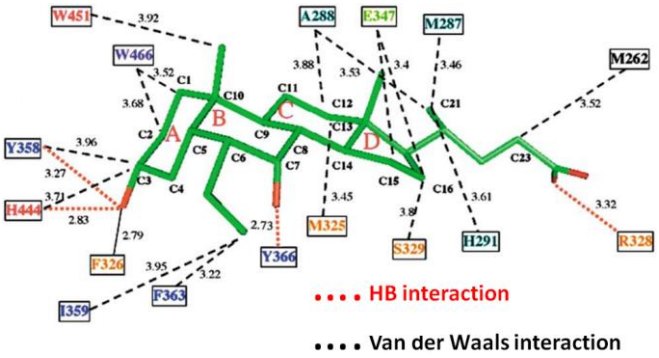


Figure 2.5 6-ECDCA and FXR binding interactions. ***Adapted from Ref. [79]***

2.2.4 Ligand binding with TGR5

Based on the structre-activity relationships (SAR) studies, Sato *et al*. predicted the presence of the hydrophobic pocket in proximity to C-6 and C-7 region (**Figure 2.6**) on TGR5 binding site, along with HB interactions close to C-3 and C-24 as well as an extra selectivity pocket at C-23.[80] Additional introduction of a methyl group at C-23 (6α-ethyl-23(*S*)-methylcholic acid, *S*-EMCA, **Figure 2.6**) led to a very selective and potent agonist towards TGR5.[81]

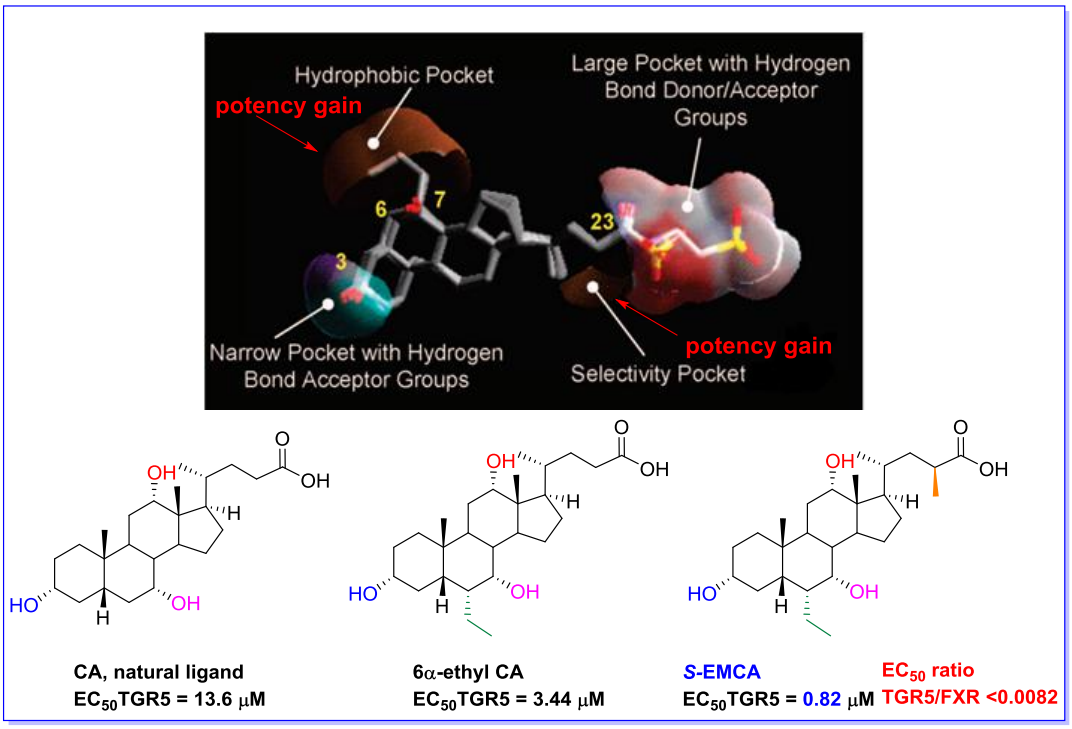


Figure 2.6 Binding sites and agonists of TGR5. ***Adapted from Ref. [80]***

Very recently, Macchiarulo *et al*. have also reported their investigation on the ligand binding site to TGR5.[82] Through combinational studies involving docking experiments, they were able to recognize crucial protein residues (as shown in **Figure 2.7**) which facilitated the binding. Hydrophobic interactions and HB interactions played key roles in the binding process.

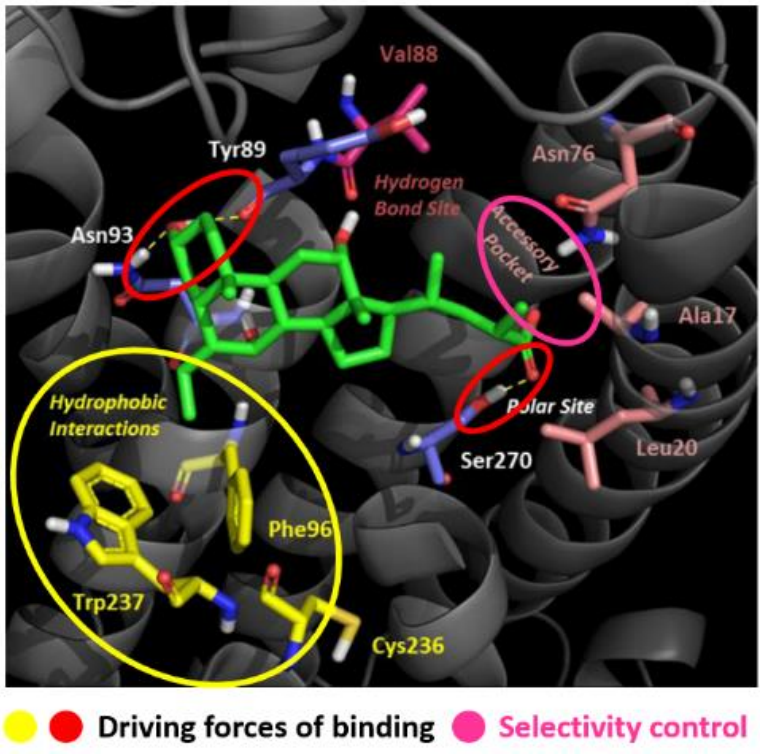


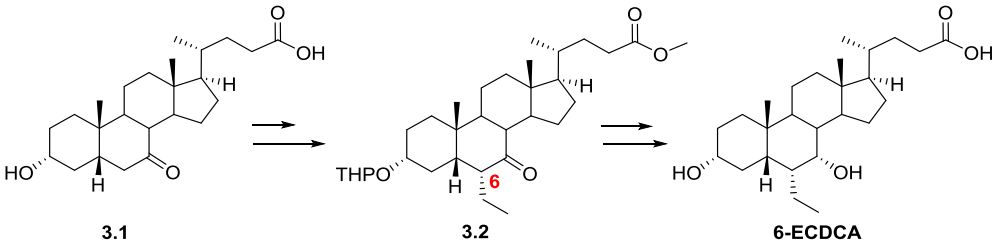
Figure 2.7 Binding mode of *S*-EMCA with TGR5. ***Adapted from Ref. [82]***

Chapter 3: Synthesis of 6α-Ethyl Chenodeoxycholic Acid and its Analogues

3.1 Synthesis of 6-ECDCA

3.1.1 Introduction: the Pellicciari and Sepe Synthesis of 6-ECDCA

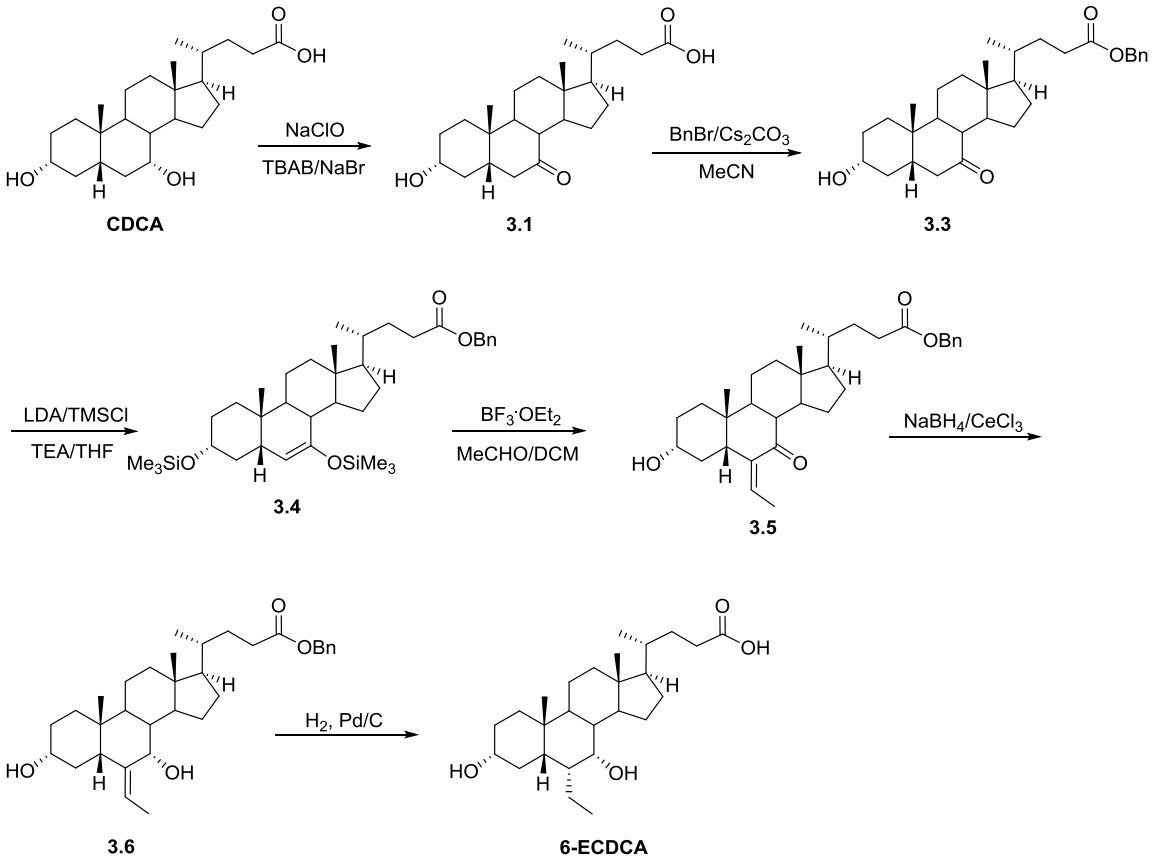
The synthesis of 6-ECDCA was initially reported by Pellicciari *et al.* starting from compound **3.1** (**Scheme 3.1**).[76] The 3α-OH group was first protected as THP ether and ethylation on C-6 was achieved using LDA and EtBr to give a low yield of 12%. The carboxyl group was then protected as a methyl ester, followed by reduction of the 7-keto using $NaBH_4$. Further hydrolysis under alkaline condition led to the desired 6-ECDCA product.



Scheme 3.1 Synthetic route by Pellicciari *et al.* [76]

More recently, they also reported an alternative method for ethylation on C-6, *via* a Mukaiyama aldol reaction of the corresponding silyl enol ether with acetaldehyde.[81] Starting from methyl ester of 7-keto LCA, the overall yield for the synthesis of 6-ECDCA was 58%. However, Sepe *et al*. reported difficulties in reproducing the high yield in the hydrolysis and reduction steps.[83] In addition, epimerisation at C-6 during the former, and a poor diastereoselectivity in the latter steps were observed.

Instead, Sepe and co-workers developed a new synthetic route for 6-ECDCA,[83] as shown in **Scheme 3.2**. The key step is to protect carboxylic acid as a benzyl ester (compound **3.3**), which can be conveniently deprotected *via* hydrogenolysis, together with reduction of the alkene in the final stage of the synthesis. The overall yield over six steps is 32%.



Scheme 3.2 Synthetic route developed by Sepe *et al.*[83]

3.1.2 Aim

A quantity of 6-ECDCA was needed as control in the biological evaluations of the fluorinated BA analogues. Hence, the route developed by Sepe and co-workers was selected for this purpose. Given reproducibility issues were also encountered with the Sepe route, a number of optimisation studies were required. Two carboxylic acid protecting groups were investigated (benzyl and methyl). The optimisation studies are grouped in three sections.

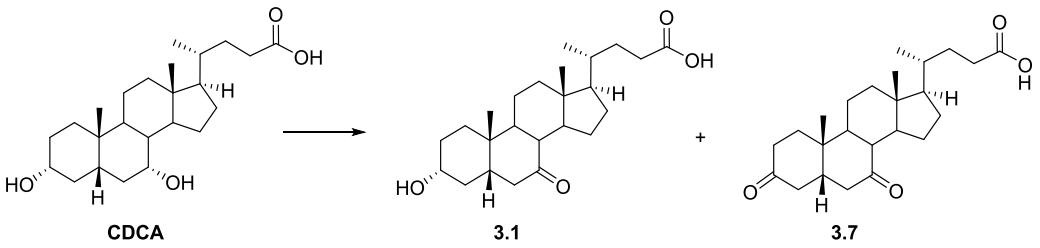
3.1.3 Synthesis of the 7-keto intermediate

3.1.3.1 Benzyl protecting group

3.1.3.1.1 Regioselective oxidation of 7α-OH group

DFT calculations suggested that the hydroxyl group on C-7 is more reactive than the group on C-3.[84] Hence, the regioselective oxidation of the 7α-OH group was described in the literature by several groups.[83],[84],[85] Oxidation of CDCA with PCC (reported yield: 78%)[85] was attempted first (**Scheme 3.3**). However in our hands a very low yield of 7-keto LCA **3.1** was obtained (**Entry 1**, **Table**

3.1). It was found that compound **3.1** has very low solubility in DCM, which was recommended as solvent for rinsing the solid residue during filtration.



Scheme 3.3 Oxidation of CDCA.

Table 3.1 Oxidation of CDCA.

Entry	Reagents	Solvent	Time	Temp.	Yield
1	PCC/Silica gel	DCM/$CHCl_3$	15 min	rt	trace
2	PCC/Silica gel	DCM/$CHCl_3$	15 min	rt	**3.1** (<6%[a]), **3.7** (<23%[a])
3	IBX	^{t}BuOH	O/N	reflux	**3.1** (23%), **3.7**
4	NaClO[b]/TBAB/NaBr	Solution[c]	6 h	rt	**SM** (22%), **3.7** (41%)
5	NaClO[d]/TBAB/NaBr	Solution[c]	6 h	rt	**3.1** (57%), **3.7** (33%)
6	NaClO[d]/TBAB/NaBr	Solution[c]	6 h	rt	**SM** (22%), **3.1** (66%), **3.7**

[a]Impure, calculated yield; [b]Reagent was potentially decomposed after long period of storage in the lab; [c]MeOH/MeCOOH/water/ethyl acetate v/v 3:1:0.25/6.5; [d]The regeant was fresh but the concentration was not accurate value.

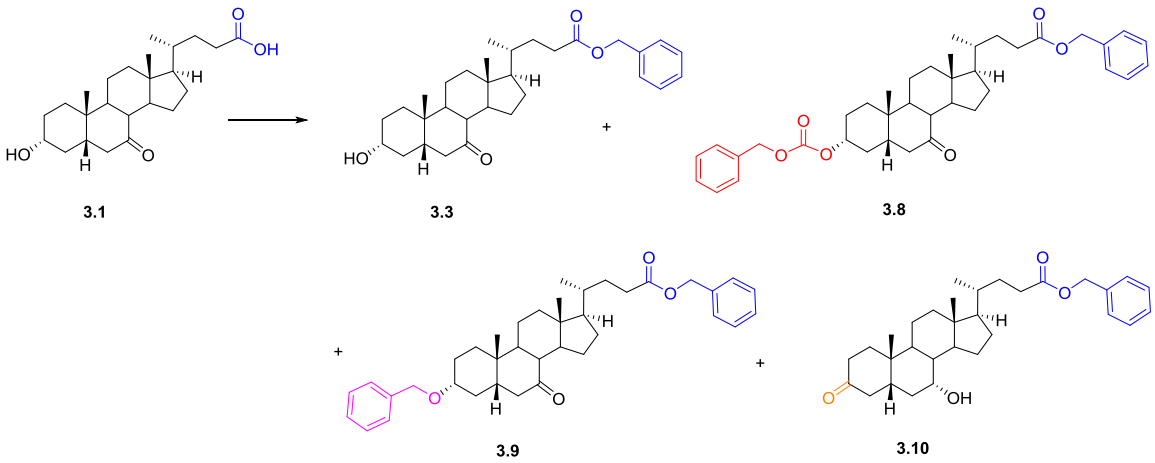
Thus, more polar solvents (ethyl acetate and acetone) were tried (**Entry 2**, **Table 3.1**), but as PCC and its dark-coloured derivatives were also soluble in these solvents, this was unsatisfactory. The isolated yield for the desired product was merely 6%. The over-oxidised by-product **3.7** could be recovered, which was not described in the related literatures.[83],[85]

Dangate *et al.* also reported regioselective oxidation of 7α-OH group of CA by IBX.[84] However, with extended reaction time and sufficient oxidant, the 3α-OH group can also be converted to a carbonyl group. In our hands, the reaction using IBX (**Entry 3**, **Table 3.1**) was not completed after 1 h, and when left to react overnight, di-keto by-product **3.7** was formed.

Finally, it was found that the use of sodium hypochlorite (**Entries 4-6**, **Table 3.1**) for the selective oxidation led to satisfactory results, with the best yield of 66% (**Entry 6**). The yield for product and by-product varies, depending on the reaction scale and the number of equivalents of oxidant used. The yield could be further improved with better time control of reaction monitored by TLC analysis, as well as by using oxidants with accurate concentration (NaClO concentration range 11%-14%).

3.1.3.1.2 Protection of the carboxyl group

The esterification of compound **3.1** (**Scheme 3.4**) was first attempted with benzyl alcohol under acidic conditions (**Entry 1**, **Table 3.2**). The reaction proceeded with side reactions, causing a low isolated yield of only 22%. The procedure from Sepe *et al.* was then repeated (**Entry 2**, **Table 3.2**). However, in our hands the reaction didn't reach completion after 24 h under reflux, and the starting material **3.1** was found to be not very soluble in acetonitrile. Thus, DMF was used as reaction solvent, increasing the yield up to 79% even at room temperature (**Entry 3**, **Table 3.2**).



Scheme 3.4 Protection of carboxyl group as benzyl ester

Table 3.2 Protection of the carboxylic acid as benzyl ester

Entry	Reagents	Solvent	Time	Temp.	Yield
1	pTSA/BnOH/Na_2SO_4	1,4-Dioxane	O/N	reflux	**SM** (20%), **3.3** (22%[a])
2[b]	BnBr/Cs_2CO_3	MeCN	24 h	reflux	**SM** (50%), **3.3** (14%)
3[c]	BnBr/Cs_2CO_3	DMF	O/N	rt	**3.3** (79%), **3.8**
4	BnBr/K_2CO_3	DMF	O/N	rt	**3.3** (63%), **3.8** (12%[d])
5[e]	BnBr/Cs_2CO_3	DMF	O/N	rt	**3.3** (71%[d]), **3.8** (13%[d]), **3.9** (0.7%[d]), **3.10** (2%[d])

[a]Calculated yield; [b]Procedure reported by Sepe *et al.*[83] and the yield in the paper was not reproducible; [c]Reactoin scale: 100 mg; [d]Isolated yield after HPLC; [e]Reaction scale: 6 g;

A novel by-product **3.8** was not isolated and identified until the reaction was performed on a large scale (**Entry 5**, **Table 3.2**). This was due to the fact that compound **3.8** had a similar R_f value compared with benzyl bromide. As both were UV-active, **3.8** had been mistaken for benzyl bromide. Its formation can be explained by reaction of the 3α-OH group with CO_2 (generated *in situ* from cesium carbonate with carboxylic acid) to result in a carbonate which was further alkylated by excess benzyl bromide. In fact, the synthesis of mixed carbonates from alcohols, CO_2 and alkyl bromide in DMF in the presence of TBAI/Cs_2CO_3 (or K_2CO_3) has been reported in the literature.[86],[87] This side reaction could potentially be reduced by using less caesium carbonate as well as less

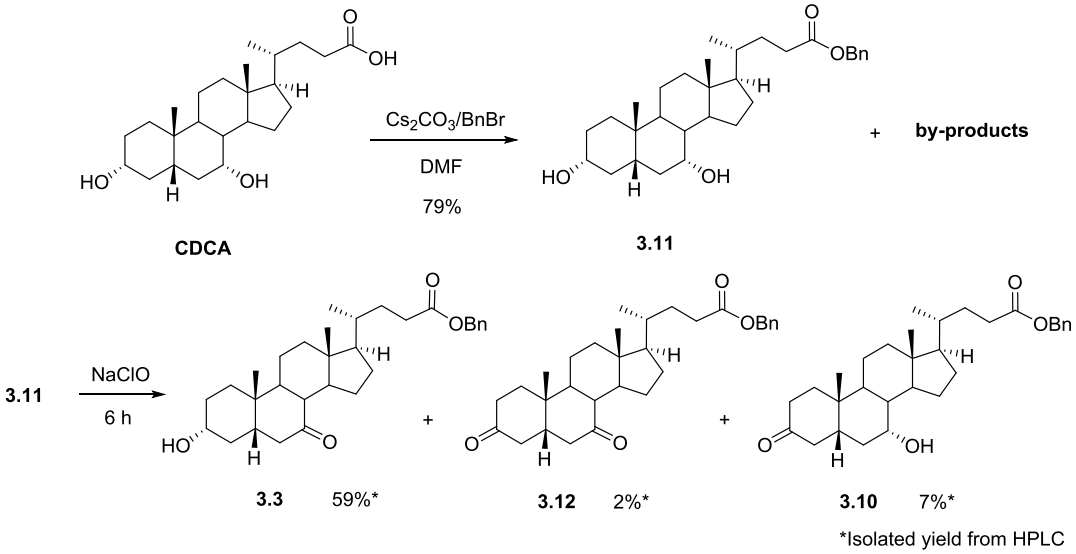
benzyl bromide. Interestingly, small amounts of compound **3.9** (**Entry 5**) were also obtained, due to the nucleophilic substitution of BnBr by the 3α-OH group.

As an alternative option for caesium carbonate, the cost-effective potassium carbonate was also used (**Entry 4**, **Table 3.2**), but this leads to a lower yield. The by-products made the purification by flash chromatography difficult and further purification by HPLC was needed.

In addition, for the large-scale experiment, compound **3.10** was also isolated by HPLC from the impure fractions in about 2% yield. Its formation must have originated by benzylation of the corresponding 3-keto-7α-OH derivative, which was possibly an impurity contaminating the starting material, even though NMR analysis of the starting material did not indicate that. Moreover, other by-products with steroid structure were also observed but not further analysed due to time constraints.

3.1.3.1.3 Alternative synthetic route for benzyl 7-keto-cholanoate derivative

Given the difficulties in the isolation of compounds **3.1** and **3.3**, it was investigated whether CDCA could first be esterified as benzyl ester, and then subjected to oxidation. As shown in **Scheme 3.5**, benzylation of CDCA was obtained with 79% yield, and isolated from other by-products by flash chromatography.

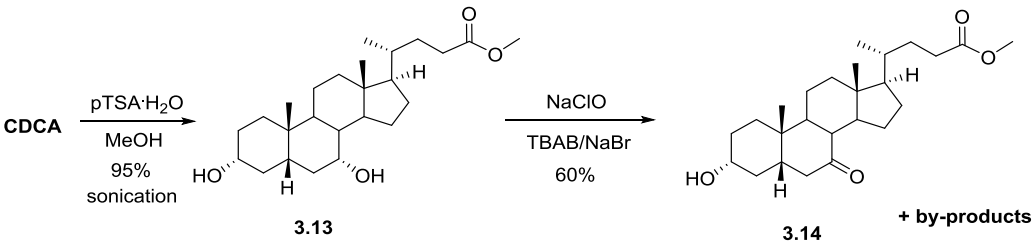


Scheme 3.5 Alternative route for 7-keto intermediate 3.3.

The oxidation was carried out by using NaOCl (**Scheme 3.5**). The isolated yield for **3.3** is less than 60%, and purification by HPLC was required to separate by-products **3.10** and **3.12**. Therefore, this synthetic route was similar in yield to the previous one, without significant improvement regarding purification.

3.1.3.2 Methyl protecting group

Following a reported procedure,[88] 24-carboxylic acid group of CDCA was protected as methyl ester **3.13** by sonication in 2 h (**Scheme 3.6**). Compared to the protection as benzyl ester, no side reactions occurred for this reaction, and the crude was directly used for further regioselective oxidation of the 7α-OH group using sodium hypochlorite, leading to the desired 7-keto intermediate **3.14.** with satisfactory yield. There was also formation of other by-products, but the isolation was relatively easier and achieved by flash chromatography.

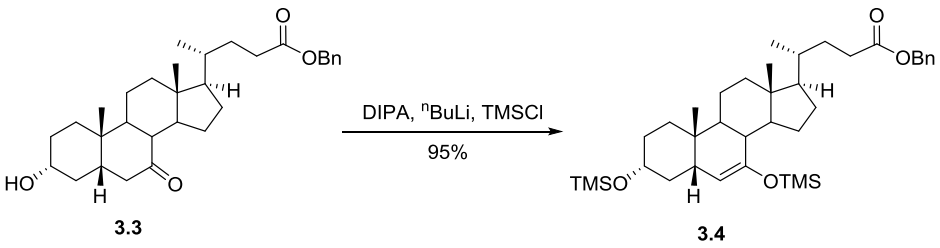


Scheme 3.6 Synthesis of 24-methyl ester derivative 3.14.

3.1.4 The Mukaiyama aldol process

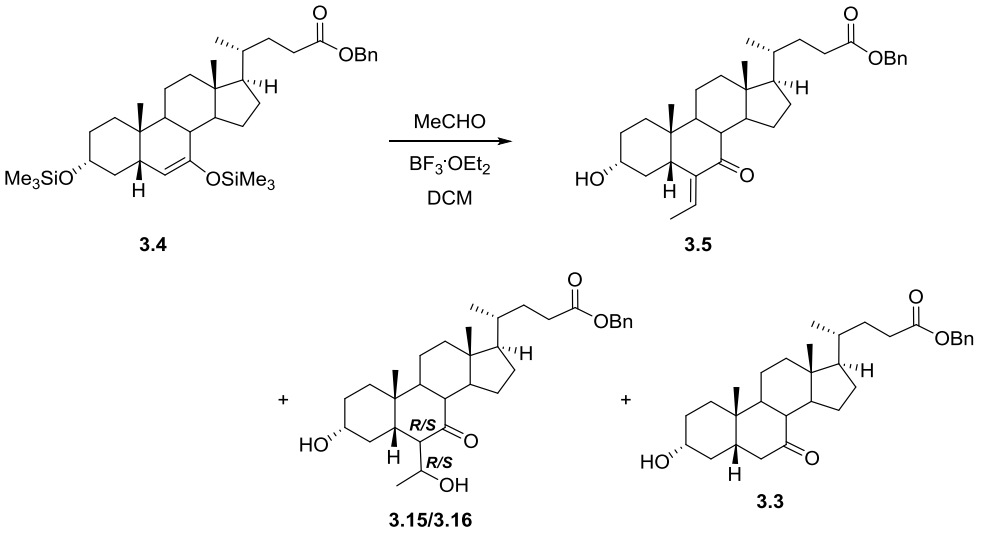
3.1.4.1 Benzyl ester

The silyl enol ether **3.4** was prepared in high yield (95%) on different scales (100 mg to 2.0 g) using Sepe's procedure,[83] as shown in **Scheme 3.7**. As compound **3.4** was unstable (confirmed by observation of decomposition over column), it was prepared and used directly for further Mukaiyama aldol reaction without any purification.



Scheme 3.7 Preparation of silyl enol ether 3.4.

The aldol condensation with acetaldehyde (**Scheme 3.8**), still following the reported procedure,[83] did not lead to the isolation of the expected enone **3.5**. Instead, diastereoisomers **3.15** and **3.16** (**Entry 1**, **Table 3.3**) were obtained. Compounds **3.15** and **3.16** were separable by flash chromatography, but assignment of the relative stereochemistry was not possible. In addition, the hydrolysis product **3.3** was also obtained.



Scheme 3.8 Aldol and elimination.

Table 3.3 Aldol condensation and elimination

Entry	Time	Temp.	Yield
1	2h	-60 °C	**3.15** (20%); **3.16** (36%); **3.3** (25%);
2	2h, O/N	-78 °C, rt	**3.5** (78%, isolated yield by HPLC); **3.3**;

Full conversion from compound **3.4** to **3.5** (**Entry 2**, **Table 3.3**) was achieved *via* extended reaction overnight at room temperature. Interestingly, the elimination led selectively to one enone isomer **3.5** (*tentative assignment* for enone stereochemistry, see beow), as indicated by crude ^{1}H NMR (**Figure 3.1**), with the alkene chemical shift value and coupling constant corresponding to both Sepe's and Gioiello's data (see below). No characteristic peak for the other isomer was observed on the crude spectrum. The ^{1}H NMR of very concentrated isolated compound indicated ca. 0.4% of an alkene isomer as deduced by the presence of a quartet at 5.40 ppm. Interestingly, Gioiello *et al*. reported an isomeric ratio of 85:15 in favour of *Z*-isomer for the enone (**Figure 3.2**) formation from methyl ester derivative of the silyl enol ether intermediate.[89]

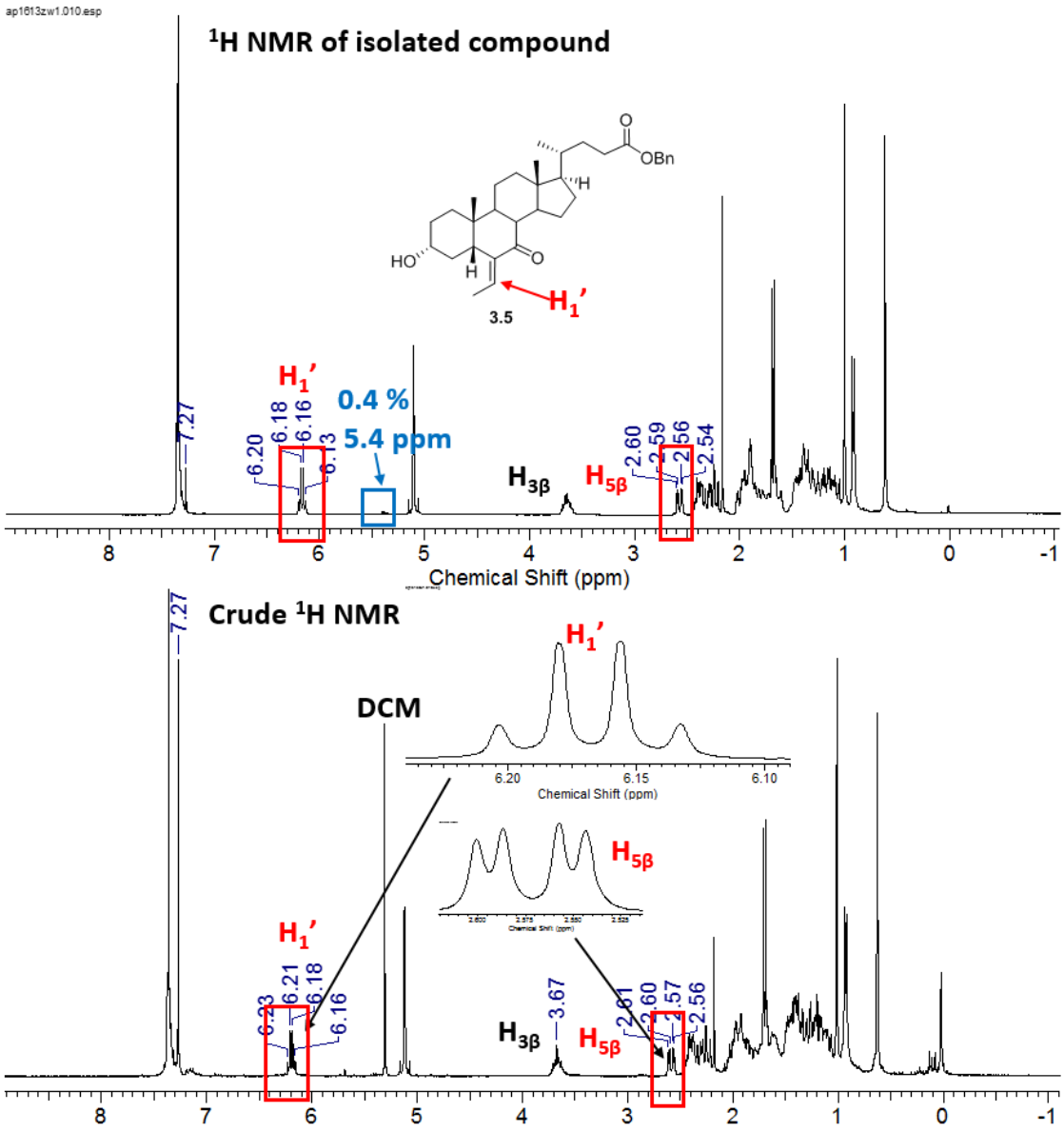
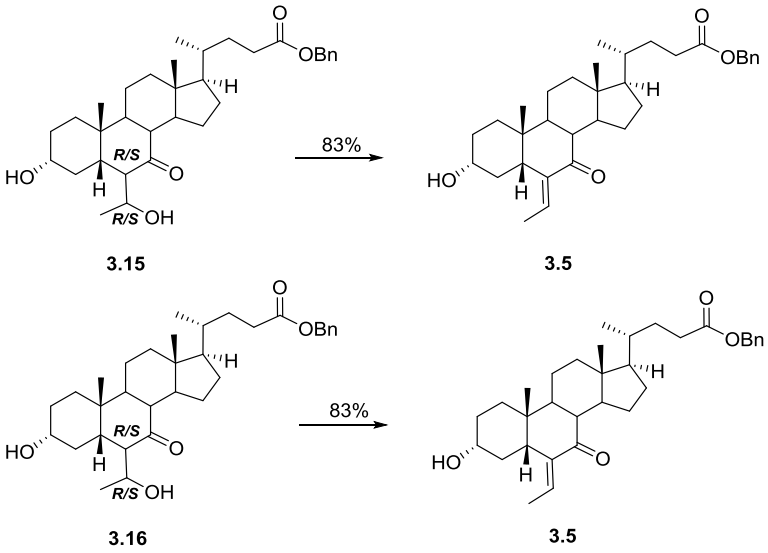


Figure 3.1 ^{1}H NMR spectra of crude mixture and isolated compound 3.5.

The elimination was also carried out starting from both isolated β-hydroxy ketones **3.15** and **3.16** (**Scheme 3.9**). Interestingly, when treated with boron trifluoride etherate overnight at room temperature, a single and the same enone isomer **3.5** was obtained.



Scheme 3.9 Elimination of compound 3.15 and 3.16.

The enone configuration was shown by Sepe, Gioiello and their co-workers to be the *Z*-isomer, though no details of the assignment were provided. (**Figure 3.2**).[83],[89]

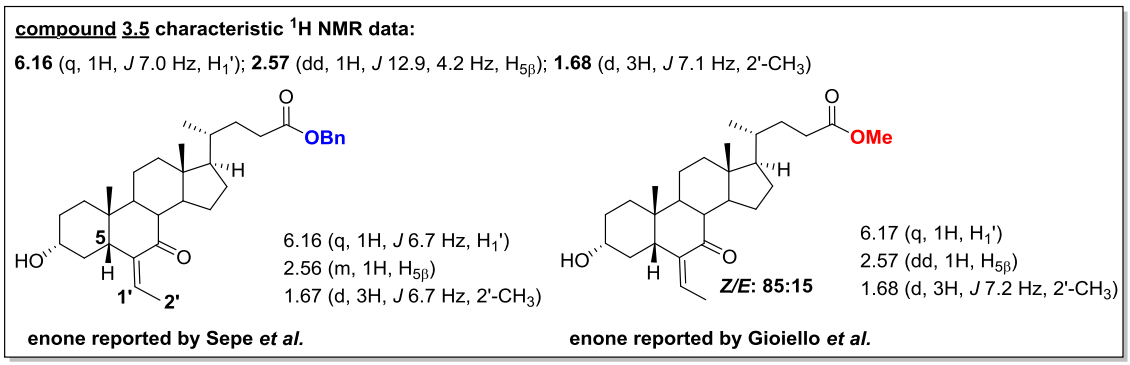


Figure 3.2 NMR data comparison with literature values.

However, this assignment did not correspond with our 1D NOE experiment results from compound **3.5** (**Figure 3.3**). Irradiation at H_1' caused no NOE at $H_{5\beta}$. Further irradiation at $H_{5\beta}$ induced NOE enhancement of 2'-methyl group, implying the close proximity between $H_{5\beta}$ and 2'-CH_3. In addition, the NOE results of **3.5** were also consistent with these of compound **3.66** (see section *3.2.2.2.2*), which configuration was confirmed by comparing NOE and COSY experiment with the corresponding *Z*-isomer **3.65**. Therefore, compound **3.5** was tentatively assigned with an *E*-configuration. Given both diastereomeric alcohols **3.15** and **3.16** led to the same alkene, it must be the thermodynamically more stable compound.

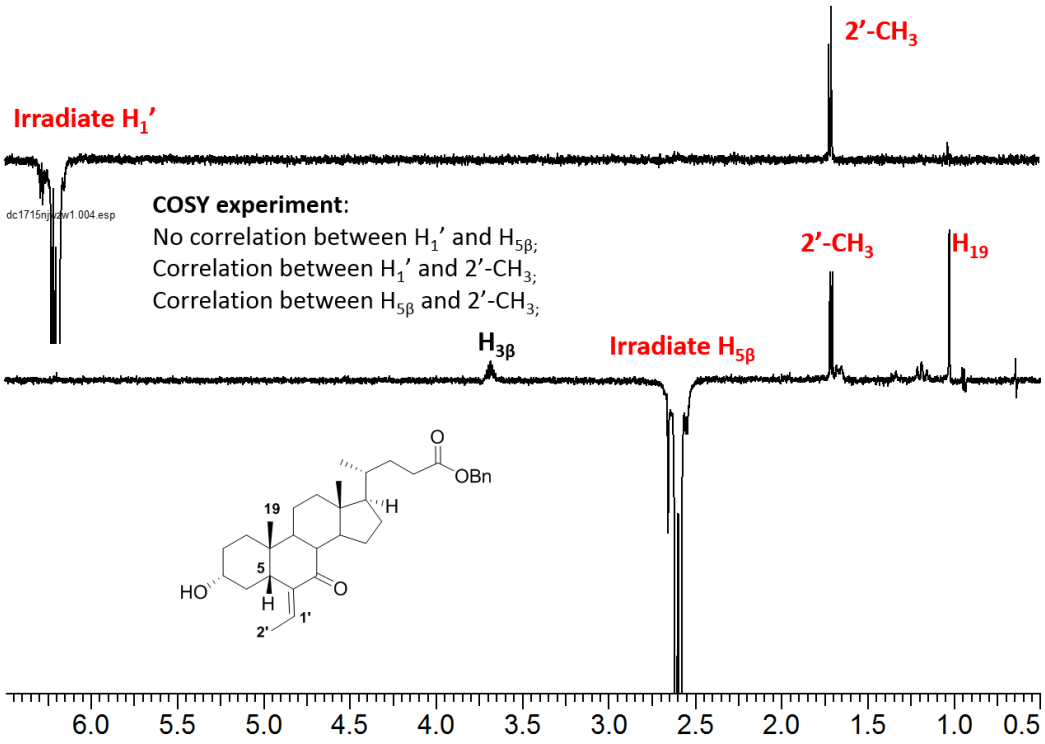


Figure 3.3 1D NOE experiments and COSY information for compound 3.5.

Surprisingly, small amount (3%) of compound **3.17** (**Figure 3.4**) was also isolated after HPLC purification. This suggests that compound **3.18** was also synthesized, but it could not be observed in the crude ^{1}H NMR of compound **3.4**, and it could not be isolated by flash chromtography. Clearly, the excess LDA effected deprotonation at the benzylic position. Such reaction has been reported in the literature.[90] The formation of the silylated benzyl moiety was confirmed by comparison of characteristic NMR data (^{1}H NMR: PhC*H* 5.68 ppm, singlet; Si(CH_3)$_3$ 0.01 ppm, singlet; ^{13}C NMR: Ph*C*H 71.3 ppm;) of the known compound **3.19**[91] to these of **3.17**, with almost identical chemical shifts (difference for ^{1}H NMR: 0.01 ppm; for ^{13}C NMR: <0.1 ppm).

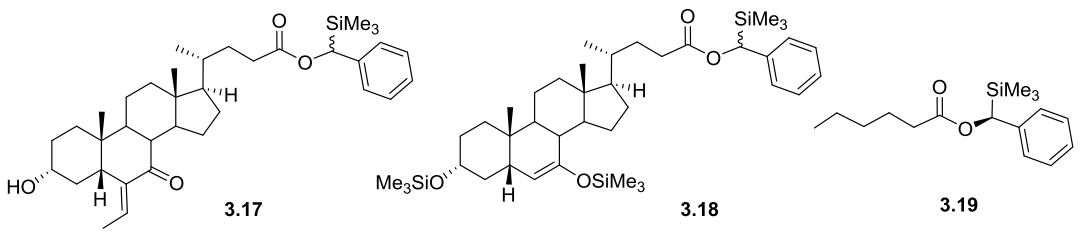
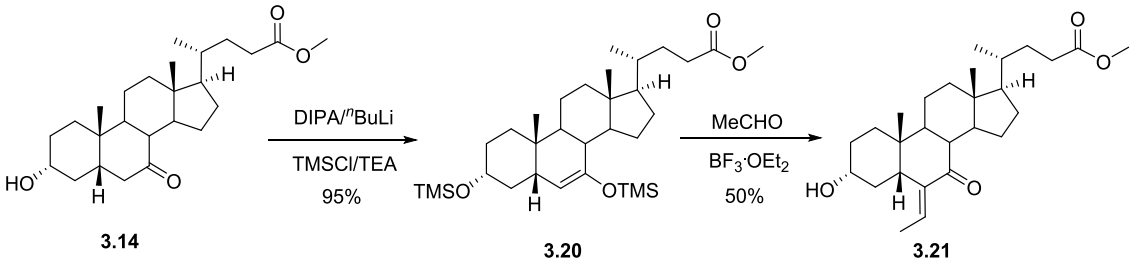


Figure 3.4 Silylated by-products 3.17 and 3.18, and compound 3.19.

3.1.4.2 Methyl ester

Similar to enone preparation from benzyl ester, silyl enol ether **3.20** was prepared from **3.14** using LDA/TMSCl with high yield (**Scheme 3.10**). The subsequent Mukaiyama aldol addition and dehydration led to the desired compound **3.21**, which was separated from other by-products by flash chromatography. The NMR data for **3.21** was consistent with the data of *Z*-configured enone

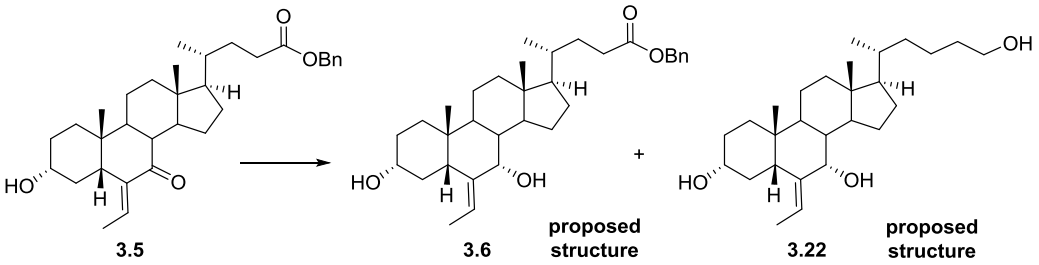
reported by Gioiello *et al*.[89] However, based on the previous analysis by 1D NOE experiments on benzyl ester derivative **3.5**, compound **3.21** was also assigned with an *E*-configuration.



Scheme 3.10 Preparation of methyl ester enone.

3.1.5 Reduction of the 7-keto group

The ketone at C-7 was reduced with sodium borohyride in the presence of cerium trichloride (**Scheme 3.11**), leading to the formation of compound **3.6**[1].



Scheme 3.11 Reduction of enone.

Table 3.4 Reduction of enone.

Entry	Reagents	Solvent	Time	Yield
1	$CeCl_3 \cdot 7H_2O/NaBH_4$[a] (>1.2 equiv)	THF/MeOH	72 h[b]	**3.6** (<65%[c])
2	$CeCl_3 \cdot 7H_2O/NaBH_4$[a] (>2.4 equiv)	THF/MeOH	O/N	**3.6** (<40%[c]), **3.22** (13%)
3	$CeCl_3/NaBH_4$ (1.2 equiv)	THF/MeOH	3 h	**3.6** (~90%[d])

[a,b]The product **3.6** had similar R_f compared to SM **3.5** which was found later during purification, and more sodium borohydride was added with extended reaction time; [c]Impure, and calculated yield by NMR analysis; [d]With solvent residues, and calculated yield by NMR analysis;

The reduction process was slow when cerium chloride heptahydrate was used (**Entry 1, Table 3.4**). Additional equivalents of sodium borohydride were then added (**Entry 2**). This did not lead to an

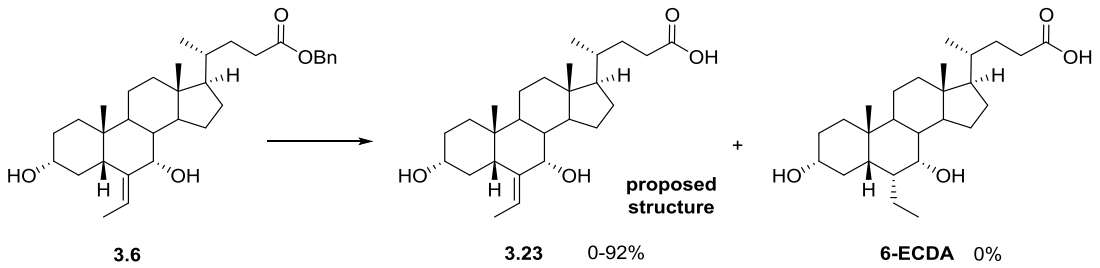
[1]Additional small coupling constant (ca. 2 Hz) was observed for H_1' and 2'-CH_3 for compound **3.6**. This was not reported by Sepe and co-workers presumably due to the peak broadening with unresolved small coupling constants. The chemical shifts for diagnostic protons (H_1', 2'-CH_3 and H_7) and carbons (alkene carbons, C3 and C7) were consistent with the reported data by Sepe *et al*. Therefore, the stereochemistry for compound **3.6** was tentatively assigned based on the comparison of NMR data with the reported values from Sepe *et al.* The alkene configuration was based on the previous conclusion from NOE experiments of enone **3.5**. Interestingly, Zampella and co-workers (*J. Med. Chem*. **2014**, *57*, 8477−8495) have recently reported the reduction on 7-keto-6-ethylidene derivative using $NaBH_4$ without $CeCl_3$, leading to a 7β-OH derivative.

increased yield, but caused further reduction of the benzyl ester group to give compound **3.22** (proposed structure based NMR and HRMS analysis). When anhydrous $CeCl_3$ (as utilized in the literature[83]) was used (**Entry 3**), reduction of the 7-keto group was achieved within 3 h.

3.1.6 Hydrogenolysis and reduction

3.1.6.1 Attempts on the 7α-OH allylic alcohol intermediate

According to the literature,[83] cleavage of the benzyl group and reduction of the carbon-carbon double bond (**Scheme 3.12**) was designed to be achieved in a single step. However, this was not reproducible in our hands. We found that only the benzyl ester was cleaved, at a very slow conversion rate (**Entry 1**, **Table 3.5**). No 6-ECDCA was obtained at all.



Scheme 3.12 Hydrogenolysis and reduction of benzyl ester 3.6.

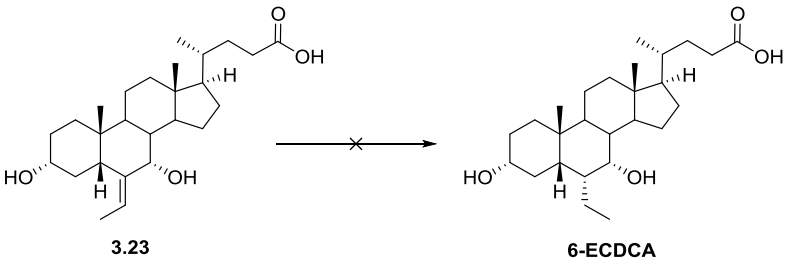
Table 3.5 Hydrogenolysis and reduction of benzyl ester 3.6.

Entry	Reagents	Solvent	Temp.	Time/Pressure	Yield
1	H_2, Pd/C	THF, MeOH	rt	72 h/1 atm	**SM** (17%), **3.23**[2] (50%)
2	H_2, PtO_2	AcOH, HCl	rt	24 h/1 atm	complex mixture
3	$TsNHNH_2$, NaOAc	THF	reflux	24 h	side reactions; **SM**
4	H_2, PtO_2, TEA	EtOH	rt	24 h/1 atm	**SM** (34%), **3.23** (55%)
5	H_2, Pd/C	MeOH	rt	24 h/10 bar	**3.23** (92%)

The use of Adams' catalyst[92] (PtO_2) in both acidic and basic media (**Entries 2** and **4**, **Table 3.5**), also only lead to the partial cleavage of benzyl protecting group, or to a complex mixture. *p*-Toluenesulfonylhydrazide ($TsNHNH_2$), in the presence of base (NaOAc), liberates diimide (N_2H_2) *in situ*, which has been described as a potent reducing agent towards alkenes.[93] However, no reduction was observed for compound **3.6** after 24 h under reflux (**Entry 3, Table 3.5**). When using high pressure (10 bar) (**Entry 5, Table 3.5**), a full and rapid hydrogenolysis of the benzyl group was achieved in high yield (92%). However, no alkene reduction was observed.

[2]Characterisation of compound **3.23** was based on the assignment of stereochemistry for compound **3.6**.

An experiment in which **3.23** (**Scheme 3.13**) was subjected to hydrogenation conditions using Adams' catalyst or palladium catalyst under atmospheric pressure (**Entries 1-2**, **Table 3.6**) were equally unsuccessful.



Scheme 3.13 Hydrogenation of carboxylic acid 3.23.

Table 3.6 Hydrogenation of carboxylic acid 3.23.

Entry	Reagents	Solvent	Temp.	Time/Pressure	Yield
1	H_2, Pd/C	THF, MeOH	rt	72 h/1 atm	0%
2	H_2, PtO_2, TEA	EtOH	rt	48 h/1 atm	0%
3	H_2, Pd/C (1.5 equiv.)	MeOH, EtOAc	rt	48 h/10 bar	0%*

*Crude NMR indicated the C=C double bond was reduced but no desired compound was obtained after purification by flash chromatography.

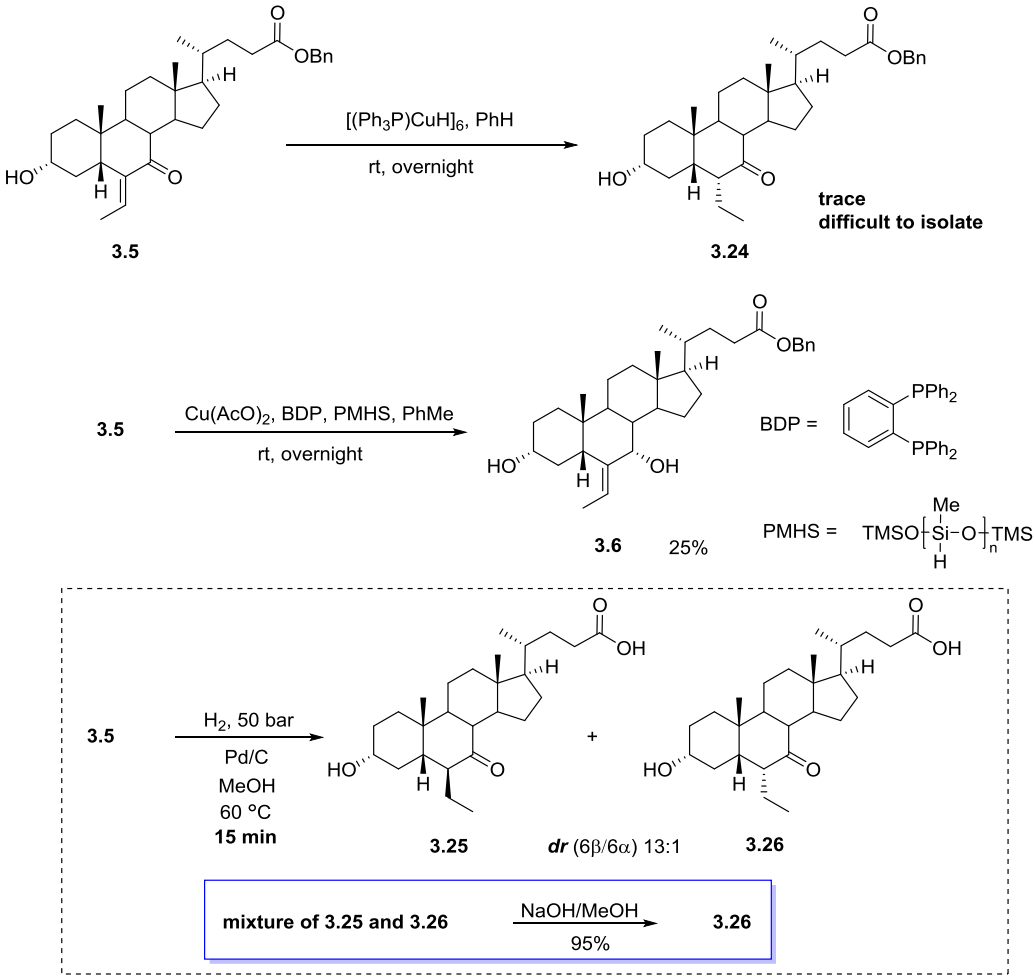
Under high pressure (10 bar, **Entry 3**) and catalyst loading, reduction of the carbon-carbon double bond was observed, though the desired compound was not obtained after purification and only a series of by-products were obtained which we were unable to identify. We were unable to solve this problem, which led to a reconsideration of the synthesis.

3.1.6.2 Attempts on the enone intermediate

Stryker *et al.* had also described a successful alkene reduction on the enone substrates using $[(Ph_3P)CuH]_6$,[94] which was thus also investigated. However, using the reported procedure, only little conversion was identified on compound **3.5** (**Scheme 3.14**). The compound **3.24** was also very difficult to be isolated from copper ligand, even after repeated acidic and basic work-up. The extremely low conversion was possibly due to the steric hindrance between the steroid structure and the bulky ligand surrounding the hydride source.

To minimize the steric effect, less hindered hydride source (BDP)CuH was also used (**Scheme 3.14**),[95] which was identified as an alternative option for Stryker's reagent. However, only reduction of the carbonyl group was identified on compound **3.5** after reaction overnight.

Finally, the alkene reduction and hydrogenolysis were achieved by using an H-Cube instrument (50 bar, 60 °C, see below). This led to two diasteroisomers **3.25** and **3.26** (6β/6α 13:1). Epimerisation of the mixture using NaOH/MeOH led to the desired isomer **3.26**.



Scheme 3.14 Reduction of the alkene bond of enone.

3.1.7 Final steps for the 6-ECDCA synthesis

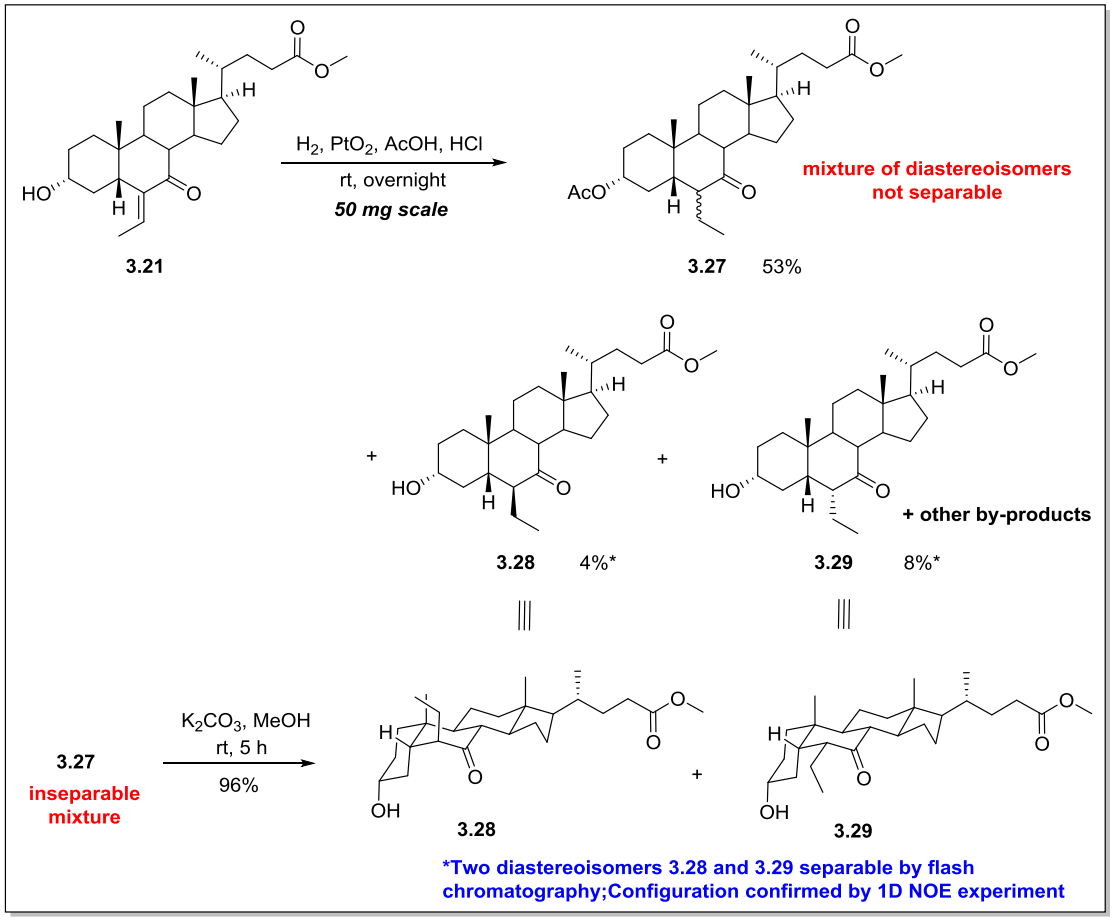
3.1.7.1 Hydrogenation of methyl ester enone 3.21 in AcOH/HCl

Starting from the 24-methyl ester enone **3.21**, Pellicciari and co-workers had synthesized 6-ECDCA by a multistep (hydrogenation at elevated pressure, hydrolysis and reduction) procedure without isolation of intermediates.[89] We tried to repeat this procedure at *ca.* 0.5 gram scale, but with hydrogenation under atmospheric pressure. Purification by flash chromatography was also conducted after the hydrolysis step to remove by-products. Nevertheless, after further reduction, this led to a mixture of a major by-product with unreduced alkene double bond and other by-products, and separation was not possible. Therefore, we decided to isolate the intermediates at each step.

Compound **3.21** was firstly hydrogenated using Adams' catalyst in acetic acid/hydrochloric acid solution under atmospheric pressure, adapted from procedeures described by Pellicciari *et al*.[89]

Double bond was successfully reduced, but leading to several interesting products (**Scheme 3.15**). Compound **3.28** and desired compound **3.29** was obtained relatively in a very low yield.

By-products **3.27** (inseparable mixture of diastereoisomers), due to acetylation of the 3α-OH group, were isolated as the major products. Under basic condition using K_2CO_3 in MeOH, acetyl group was removed and diastereoisomers **3.28** and **3.29** were obtained after purification by column chromatography.



Scheme 3.15 Hydrogenation of enone 3.21 in AcOH/HCl (50 mg scale).

The configuration on C-6 was determined by 1D NOE NMR experiment. As shown in **Figure 3.5 [a]**, irradiation on $H_{8\beta}$ of **3.28** led to identification of H_{19} and H_{18} through nuclear Overhauser effect. Instead, $H_{6\alpha}$ was not observed as it was not in close proximity with $H_{8\beta}$. Likewise, the identification of $H_{8\beta}$ of compound **3.29** was concluded *via* irradiation at H_{18} (**Figure 3.5 [c]**) and $H_{6\beta}$ (**Figure 3.5 [b]**) and as a result, this suggested the correct assignment of $H_{6\beta}$ for compound **3.29**.

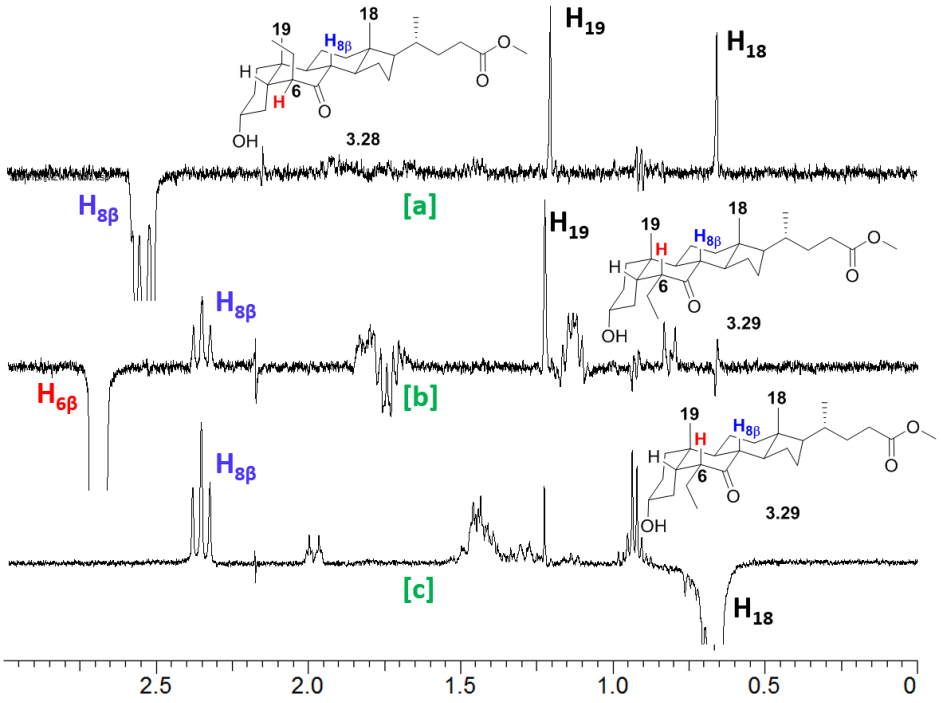
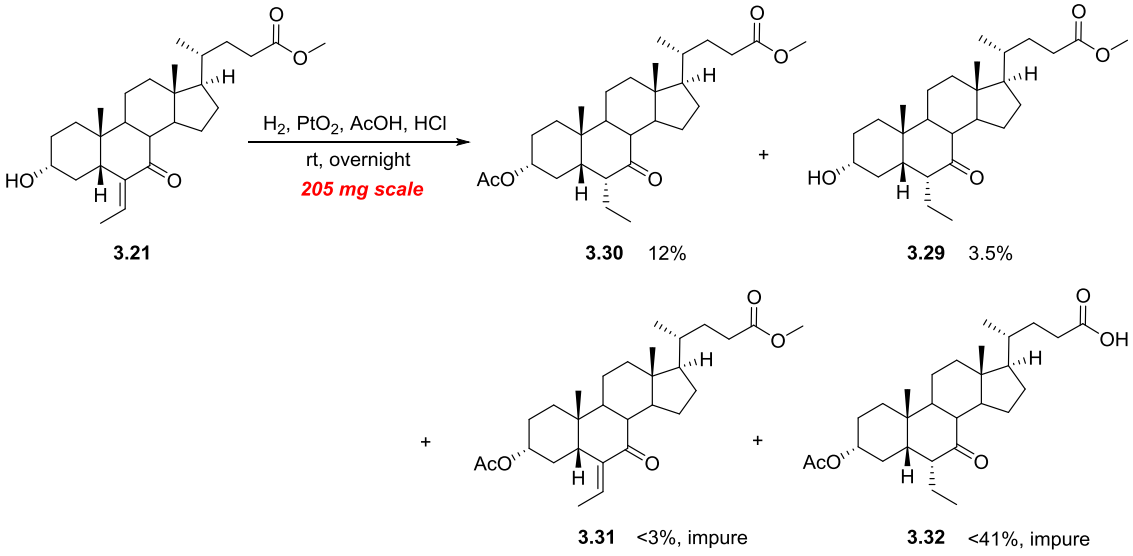


Figure 3.5 1D selective gradient NOESY experiment of compound 3.28 and 3.29.

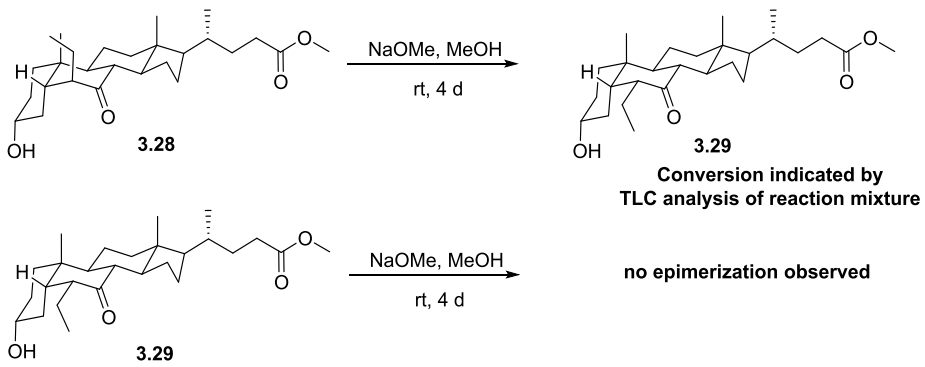
Unexpectedly, when this reaction was repeated on a slightly larger scale (205 mg rather than 50 mg), different compounds were isolated as shown in **Scheme 3.16**. Interestingly, only 6α-isomers (**3.30**, **3.29**, and **3.32**) were obtained, without the existence of 6β-isomers. It was assumed that the reduction of double bond was slow, which explained the existence of intermediate **3.31** (precursor for **3.30**).



Scheme 3.16 Hydrogenation of enone 3.21 in AcOH/HCl (205 mg scale).

Conversion of **3.28** to **3.29** was attempted under basic condition (**Scheme 3.17**). However, the epimerization process was rather slow, according to the TLC analysis. As to **3.29**, no epimerization

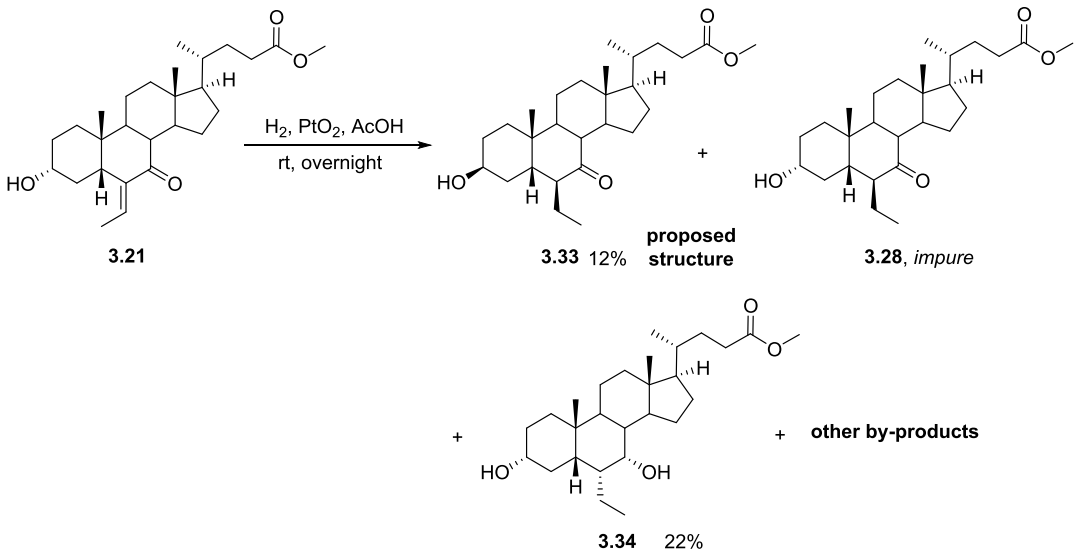
was observed, presumably due to the fact that equatorial ethyl group on C-6 was more thermodynamically favoured compared with the axial configuration.



Scheme 3.17 Epimerisation under basic condition.

3.1.7.2 Hydrogenation of enone 3.21 in AcOH

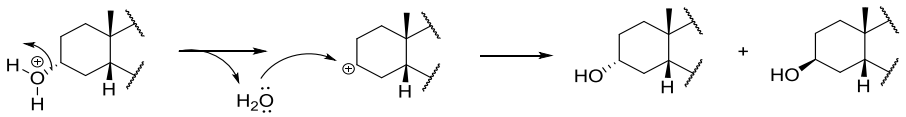
Hydrogenation of enone **3.21** was also carried out in acetic acid without hydrochloric acid (**Scheme 3.18**) according to the patent by Pellicciari *et al*.[96] Surprisingly, conversion of the configuration of the 3α-OH group (compound **3.33**) was observed according to the NMR analysis. Except for the anticipated product **3.28**, compound **3.34** (precursor for 6-ECDCA) was also isolated, with the reduction of carbonyl group. Similar reduction of ketone *via* hydrogenation under pressure using Adam's catalyst has been previously reported by Königsberger *et al*.[97] Acetylation of 3α-OH group was not observed without the addition of HCl. The desired product **3.29** was not found, and the rest of by-products were not reported by Pellicciari *et al*.[96]



Scheme 3.18 Hydrogenation of enone 3.21 in AcOH.

The possible mechanism for formation of **3.33** is shown in **Scheme 3.19**. Under acidic condition, 3α-OH group was protonated and acted as a leaving group *via* S_N1 mechanism to form the carbocation

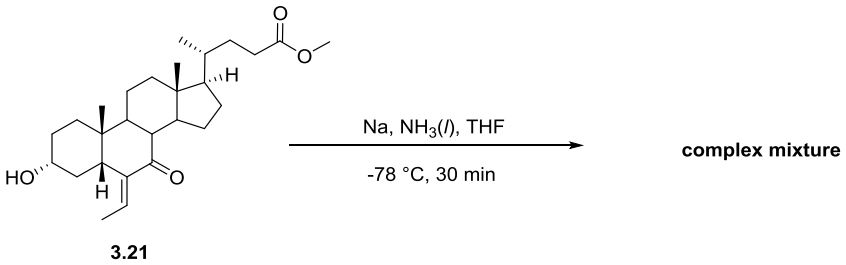
on C-3, which followed by nucleophilic attack by H_2O to afford both α- and β-isomer. Alternatively, 3α-OH group could also be oxidized first to 3-keto by PtO_2, and then reduced back to 3-OH group by hydrogenation using Adam's catalyst.



Scheme 3.19 Proposed mechanism for conversion of configuration of 3α-OH group.

3.1.7.3 Reduction of enone 3.21

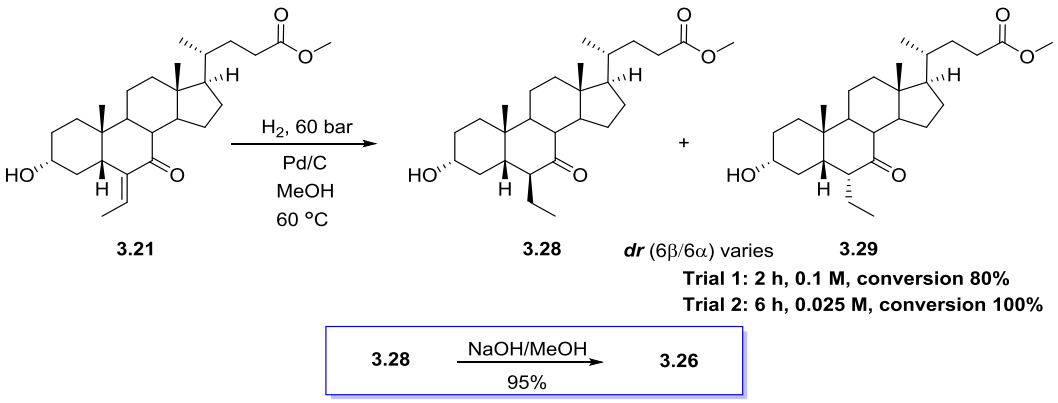
Birch reduction condition using sodium and liquid ammonia was reported as a possible route for selective conjugate reduction, demonstrated *via* similar reduction on the steroid scaffold by Surendra *et al*.[98] When this condition was applied on enone **3.21** (**Scheme 3.20**), a complex mixture was obtained. Crude NMR analysis indicated the methyl ester moiety had also reacted.



Scheme 3.20 Reduction of enone 3.21.

Literature evidences have shown that a carbonyl/ester group could be converted to secondary/primary alcohol using Na/NH_3, which was used as reducing agent for ketone and as deprotecting condition on esters.[97],[99] This can explain the complexity of the crude mixture to some extent.

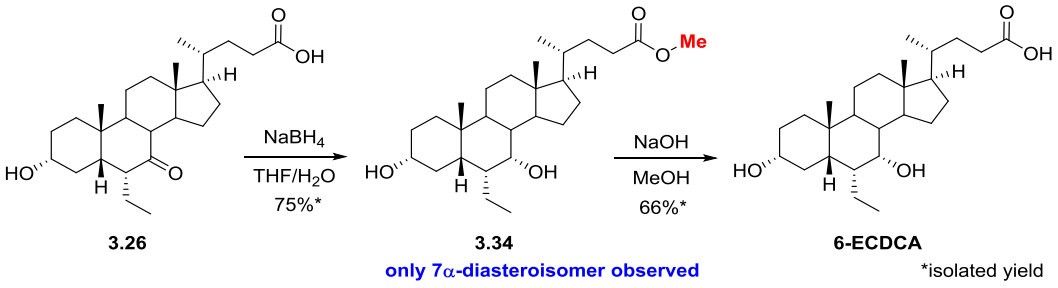
Finally, alkene reduction of enone **3.21** was satisfactorily achieved by using an H-Cube instrument. Under higher temperature and presussure (60 °C and 60 bar), hydrogenation with sample concentration of 0.1 M at flow rate 1 mL/min only led to a partial conversion (**Scheme 3.21**). Complete conversion was achieved by using less concentrated sample solution (0.025 M) after 6 h for gram-scale reaction. Subsequent alkaline epimerisation and hydrolysis of compound **3.28** led to the desired intermediate **3.26**.



Scheme 3.21 Hydrogenation of enone using H-Cube.

3.1.7.4 Preparation of 6-ECDCA

The synthesis of target compound 6-ECDCA should have been achieved *via* reduction of compound **3.26** using $NaBH_4$ (**Scheme 3.22**). Interestingly, 6-ECDCA was methylated to compoung **3.34** during the purification using flash chromatography (eluent: MeOH and DCM). Finally, additional step of alkaline hydrolysis led to the target compound 6-ECDCA**.**



Scheme 3.22 Preparation of 6-ECDCA.

3.2 Attempted synthesis of 6-ECDCA analogues

3.2.1 Attempted synthesis towards 6-polyfluoroalkyl analogues

3.2.1.1 Synthetic plan

The target compounds **3.35** and **3.36**, with polyfluorinated alkyl chain at C-6, are shown in **Figure 3.6**.

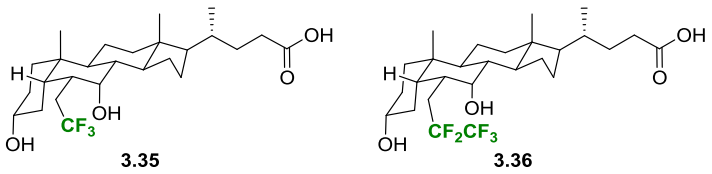
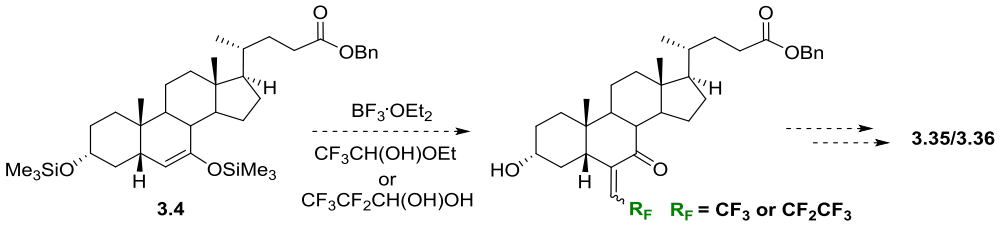


Figure 3.6 Target compounds with 6-polyfluoroalkyl group.

In order to synthesize **3.35** and **3.36**, aldol addition of compound **3.4** with the corresponding aldehyde hemiacetal or hydrate was envisioned, as shown in **Scheme 3.23**. Besides, aldolic condensation using trifluoroacetaldehyde aminal,[100] and other hemiaminals of fluoral,[101] as well as trifluoromethyl- or pentafluoroethyl iminium salts,[102] could also be applied for the synthesis.

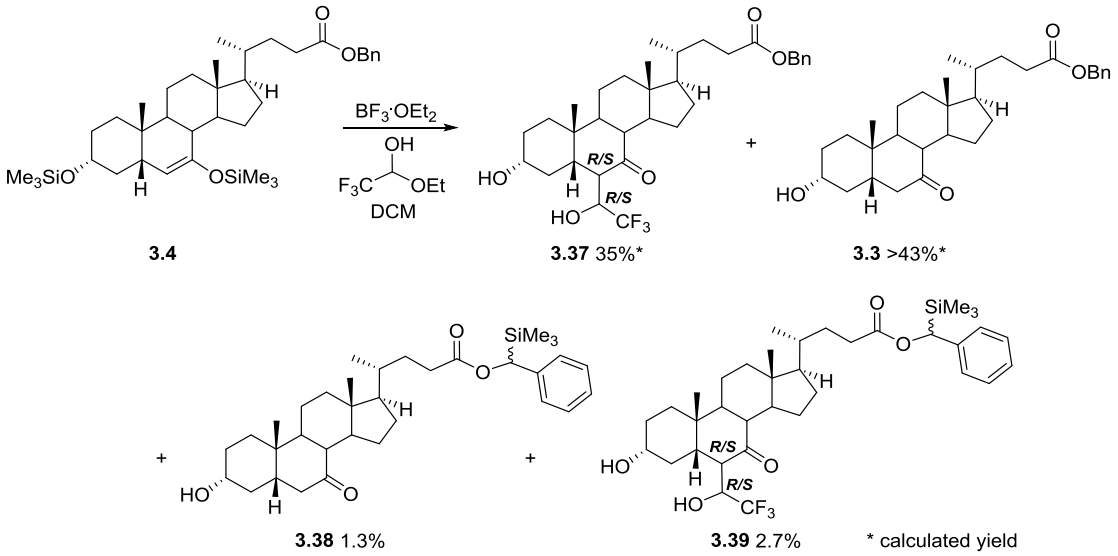


Scheme 3.23 Synthetic plan for compound 3.35 and 3.36.

3.2.1.2 Mukaiyama aldol with fluorinated aldehyde hemiacetal and hydrate

To introduce a trifluoroethyl moiety on C-6, compound **3.4** (**Scheme 3.24**) was reacted with trifluoracetaldehyde ethyl hemiacetal to afford compound **3.37**[3], which was a mixture of diastereoisomers (confirmed by ^{19}F $\{^{1}H\}$ NMR analysis). Unlike their unfluorinated derivatives, they were not separable *via* flash chromatography or HPLC. Surprisingly, compound **3.37** was extremely difficult to separate from by-product **3.3**, even with repeated purification *via* HPLC, although the R_f values of two compounds were significantly different. It was assumed that compound **3.37** had decomposed to **3.3** on silica gel, which was further confirmed by the decomposition of a HRMS sample. Therefore, the mixture was used without further purification for the elimination step.

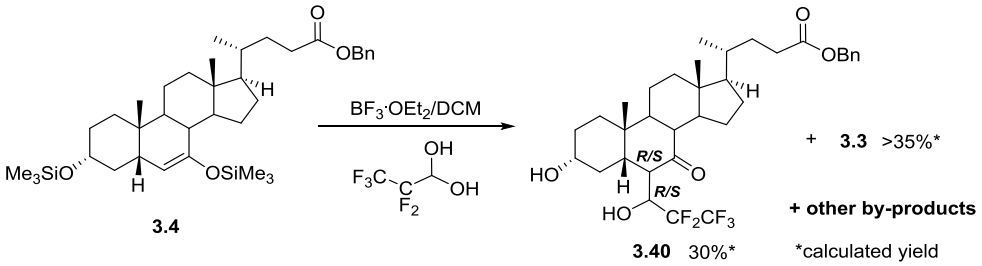
[3]Proposed structure based on NMR analysis.



Scheme 3.24 Mukaiyama aldol with fluorinated aldehyde hemiacetal.

Small amounts of compound **3.38** and **3.39**[4] were also obtained after HPLC purification, similarly caused by the minor by-product **3.18** (*see section 3.1.4.1*) during the preparation of compound **3.4**. For compound **3.4**, the aldol addition didn't reach completion, even with the extended reaction time and the addition of further trifluoracetaldehyde ethyl hemiacetal. Compared with the aldol addition with acetaldehyde, this reaction proceeded rather slowly.

Aldol addition using pentafluoropropionaldehyde hydrate was also carried out, as shown in **Scheme 3.25**. The reaction was incomplete after extended reaction time. Isolation of compound **3.40**[5] from **3.3** proved extremely difficult by flash chromatography, presumably due to the retro-aldol decomposition. This reaction also proceeded with numerous side reactions and the TLC analysis of crude reaction mixture indicated more than 7 spots. These by-products were not able to be further analyzed due to time limit.



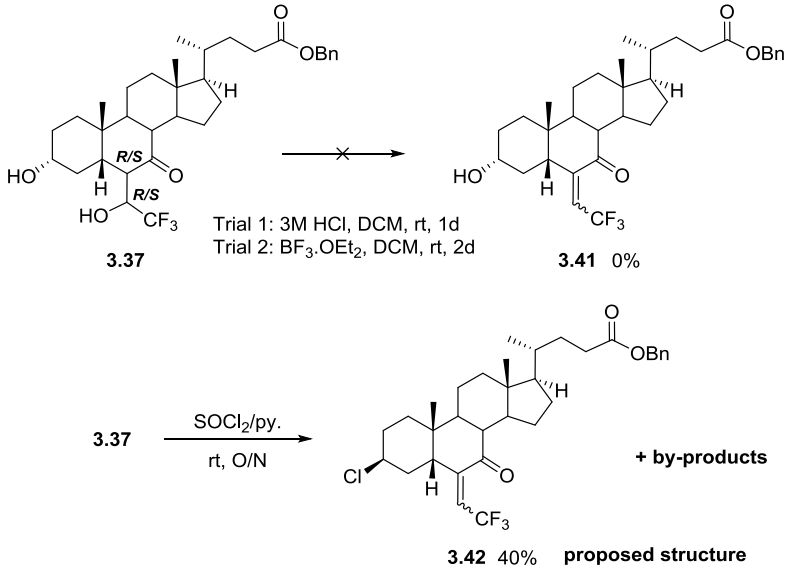
Scheme 3.25 Mukaiyama aldol with fluorinated aldehyde hydrate.

[4]Proposed structure based on NMR and MS analysis.
[5]Proposed structure based on NMR analysis.

3.2.1.3 Elimination

3.2.1.3.1 Lewis/Brønsted acid and $SOCl_2$-mediated elimination

In order to obtain compound **3.41**, the impure compound **3.37** was subjected to acidic conditions using 3 M HCl and similary using Lewis acid (BF_3) in DCM (**Scheme 3.26**). However, no reaction was observed after stirring at room temperature for 1 – 2 d. As reported in the literature,[103] (α-polyfluoroalkyl)hydroxylmethyl carbonyl compounds were resistant to the dehydration. Presumably, trifluoromethyl group withdraws the electron density from –OH group, making it a worse leaving group during the elimination (E1cb) step under this specific chemical environment.



Scheme 3.26 Attempted preparation of enone 3.41.

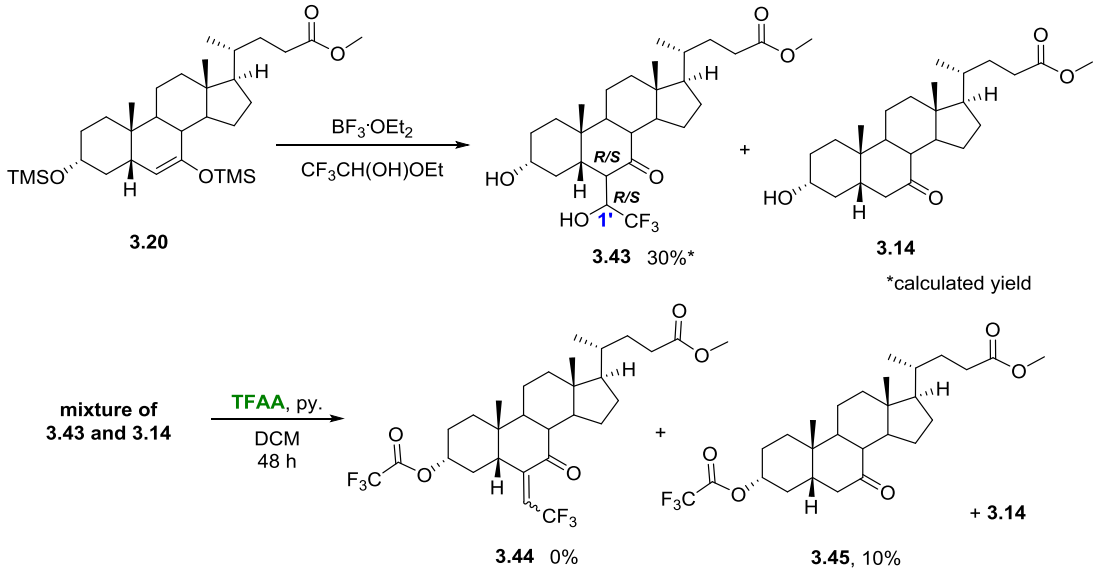
The elimination was also attempted by using thionyl chloride in pyridine (**Scheme 3.26**). This reaction proceeded with side reaction on C-3, leading to compound **3.42** (proposed structure based on NMR, IR and HRMS analysis). Thus, the 3α-OH group should be protected prior aldol and elimination steps if thionyl chloride is used (*see section 3.2.1.4*).

3.2.1.3.2 TFAA-mediated elimination

The elimination was trialed on compound **3.43**[6], which was prepared from compound **3.20** (**Scheme 3.27**). Treatment of mixture of compound **3.43** and **3.14** with trifluoroacetic anhydride led to the trifluoroacetylation on C-3. However, no desired intermediate **3.44** was formed. It was assumed that the esterification of C_1' was rather slow, due to the reduced nucleophilicity of hydroxyl group on C_1' by electron withdrawing effect from CF_3-group. Meanwhile, acetylation on C-3 could have

[6]Proposed structure based on NMR and HRMS analysis.

also slowed this process due to the increased steric hindrance. As expected, by-product **3.45** was also formed due to the trifluoroacetylation of the 3α-OH group, and it spontaneously recrystallised from acetone/petroleum ether. The X-Ray studies of compound **3.45**, in comparison with its unfluorinated 3α-acetyloxy derivative, are described in *Chapter 5.*

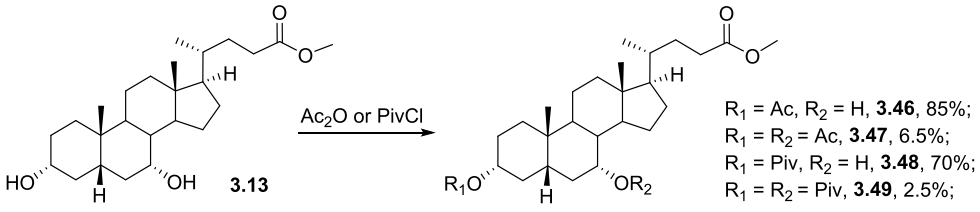


Scheme 3.27 TFAA-mediated elimination.

3.2.1.4 Mukaiyama aldol with the 3α-protected derivative

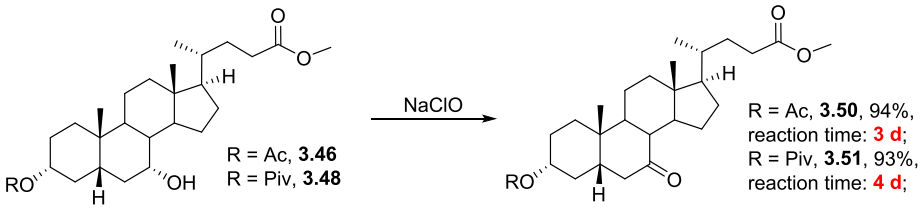
Due to the proposed side reaction on 3α-OH group for $SOCl_2$-mediated elimination, a 3α-protected 7-keto derivative became an interesting substrate for Mukaiyama aldol process. As over-oxidation occurred for preparation of both 7-keto benzyl and methyl ester intermediates, it is thus worth trying to protect 3α-OH group selectively before oxidation of the 7α-OH group.

As shown in **Scheme 3.28**, methyl ester of CDCA **3.13** was used for acetylation and pivaloylation with satisfactory yield (up to 85%). The high regioselectivity in the literature for compound **3.46** was not reproducible,[88] and its isolation from diacetylated compound **3.47** was achieved by HPLC purification. Higher regioselectivity was obtained with pivaloyl group due to its steric effect. The isolation of compound **3.49** can be done simply by flash chromatography.



Scheme 3.28 Synthesis of protected intermediate 3.46 and 3.48.

The 7α-OH groups of compounds **3.46** and **3.48** were then oxidized by sodium hypochlorite in high yield (**Scheme 3.29**). Unexpectedly, the oxidation process for both substrates (3 d and 4 d respectively) was much slower than the regioselective oxidation of CDCA, which was nearly completed within 6 hr.



Scheme 3.29 Oxidation of the 7α-OH group.

This can be rationalized by the increased steric hindrance from the steroid sculpture against the approaching of the oxidant due to either assumed intramolecular hydrophobic interactions or possible intramolecular hydrogen bonding interactions, as illustrated in **Figure 3.7**.

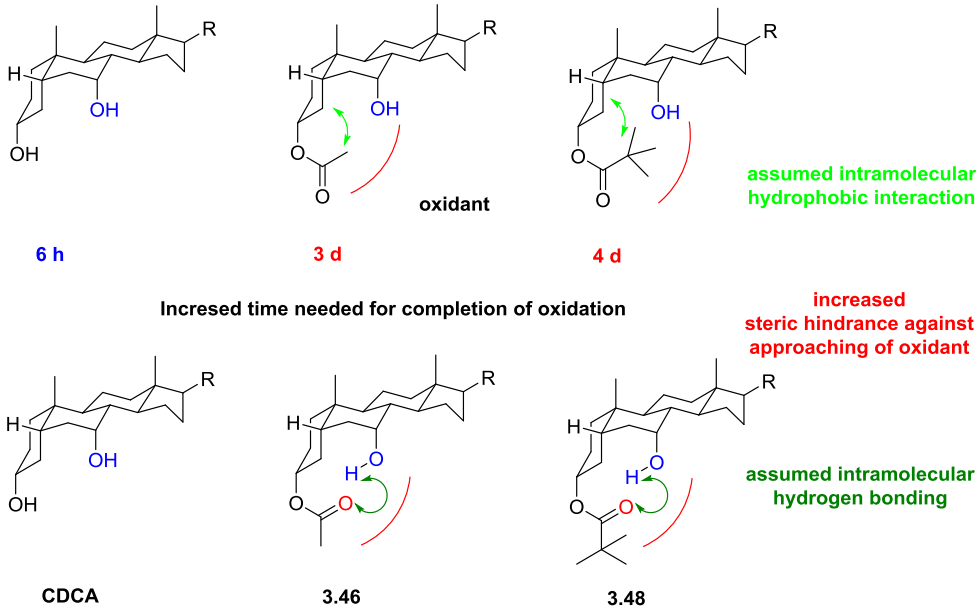
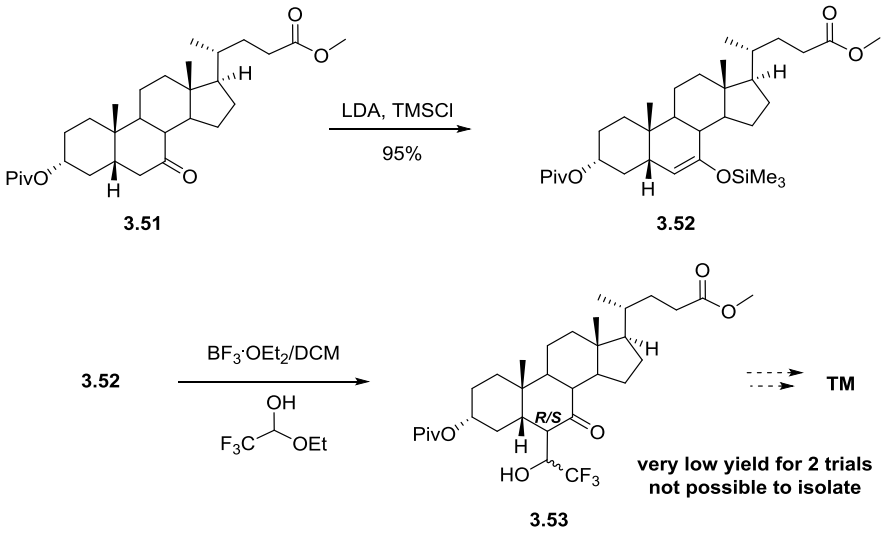


Figure 3.7 Possible reason for slowed oxidation process.

Because the acetyl group is labile in the presence of strong bases (e.g., LDA), silyl enol ether **3.52** was prepared from 3-pivaloyloxy methyl ester derivative **3.51** (**Scheme 3.30**). However, further Mukaiyama aldol addition with fluorinated aldehyde hemiacetal was very difficult (with two trials) that ^{19}F NMR indicated only a very low yield of fluorinated compound **3.53**.



Scheme 3.30 Mukaiyama aldol with 3-pivaloyloxy derivative 3.52.

It is assumed that the steric hindrance from steroid scaffold was increased, presumably due to the intramolecular hydrophobic interactions, which could prevent the approach of the aldehyde hemiacetal as illustrated in **Figure 3.8**.

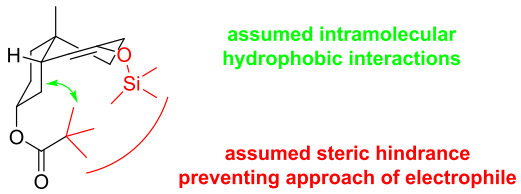
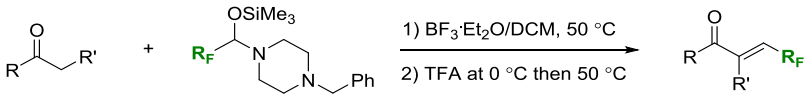


Figure 3.8 Proposed intramolecular interactions.

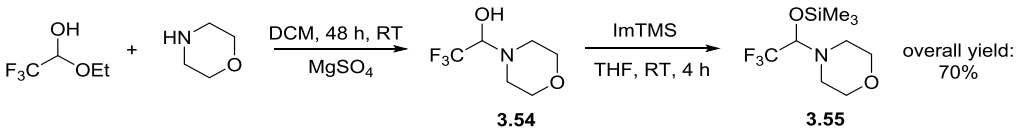
3.2.1.5 One-pot synthesis using morpholino hemiaminal

It was reported by Blond *et al.* that using hemiaminals of fluoral, the enolizable carbonyl compounds could be converted to β-polyfluoroalkyl β-dialkylamino ketones,[101] which could be further transformed to β-polyfluoroalkylenones under acidic conditions (**Scheme 3.31**).



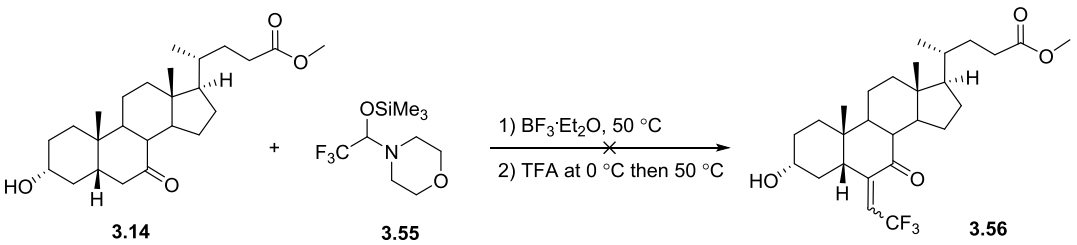
Scheme 3.31 Preparation of enones[101]

Due to the unavailability of benzyl piperazine, morpholine was used for the preparation according to the reported procedures.[104] As shown in **Scheme 3.32**, compound **3.55** was prepared in two steps with overall yield of 70%, which was used directly for next step.



Scheme 3.32 Preparation of hemiaminal of fluoral.

The reaction between compound **3.14** and **3.55** was catalyzed by Lewis acid, followed by the treatment with trifluoroacetic acid (**Scheme 3.33**). However, the desired compound **3.56** was not obtained. Instead, trifluoroacetylation was observed on the 3α-OH group.



Scheme 3.33 One-pot synthesis towards enone.

3.2.2 Attempted synthesis towards 6β-fluoro-6α-ethyl CDCA

3.2.2.1 Synthetic plan

The key precursor for compound **3.57** is the corresponding 7-keto derivative **3.58** (**Figure 3.9**), which can be synthesized *via* two different pathways (electrophilic fluorination on α-ethylated ketone or direct ethylation on α-fluoroketone).

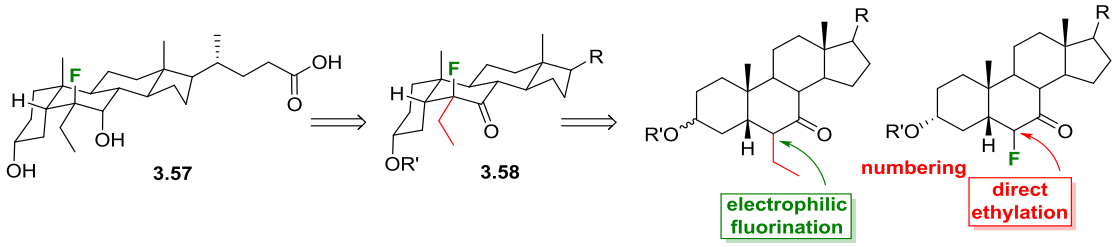
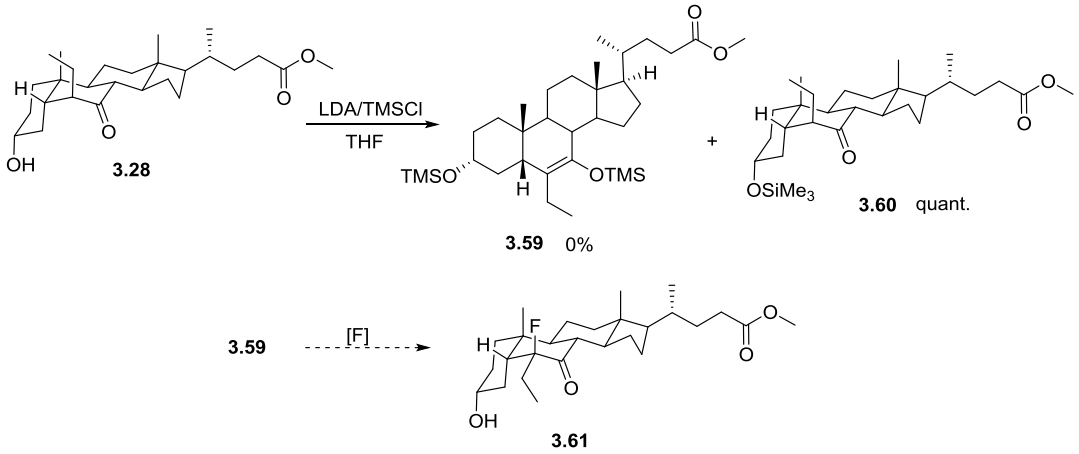


Figure 3.9 Retrosynthetic analysis for compound 3.57.

3.2.2.2 Electrophilic fluorination on 6-alkylated intermediate

3.2.2.2.1 Attempted synthesis on the derivatives with 3α-configuration

The silyl enol ether **3.59** (**Scheme 3.34**) was envisioned as substrate for a subsequent eletrophilic fluorination towards compound **3.61**. Hence, deprotonation/silylation of **3.28** was investigated. Unfortunately, the formation of the silyl enol ether **3.59** could never be observed, even in the crude reaction mixture (NMR analysis). Instead, by-product **3.60** was obtained quantitatively after work-up. A likely reason, apart from steric hindrance, was the relatively high pKa of $H_{6\alpha}$ in **3.28**, given the non-overlap of the C_6-$H_{6\alpha}$ bond with the $\pi^*_{C7=O}$ orbital, leading to a difficult deprotonation of $H_{6\alpha}$. Compound **3.60** was recrystallized from acetone/petroleum ether, with the X-Ray structure shown in **Figure 3.10**, which was the supplementary evidence for the configuration of 6-ethyl group (previously determined by 1D NOE experiments).



Scheme 3.34 Electrophilic fluorination pathway towards compound 3.61.

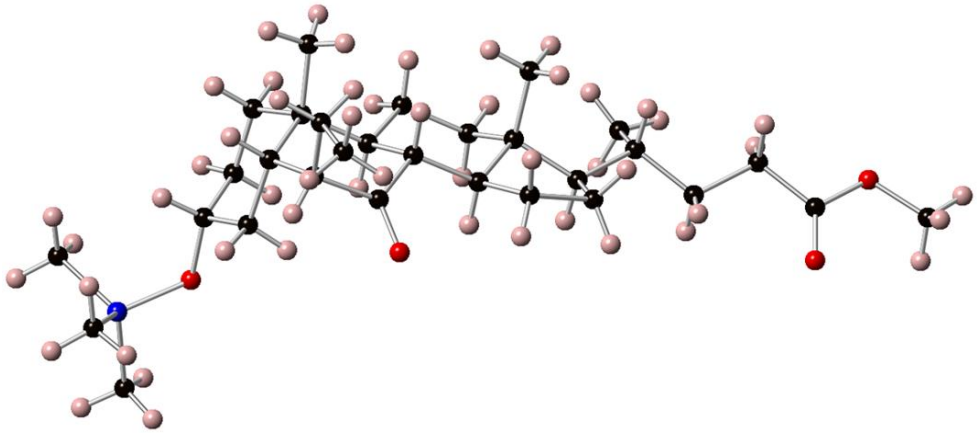
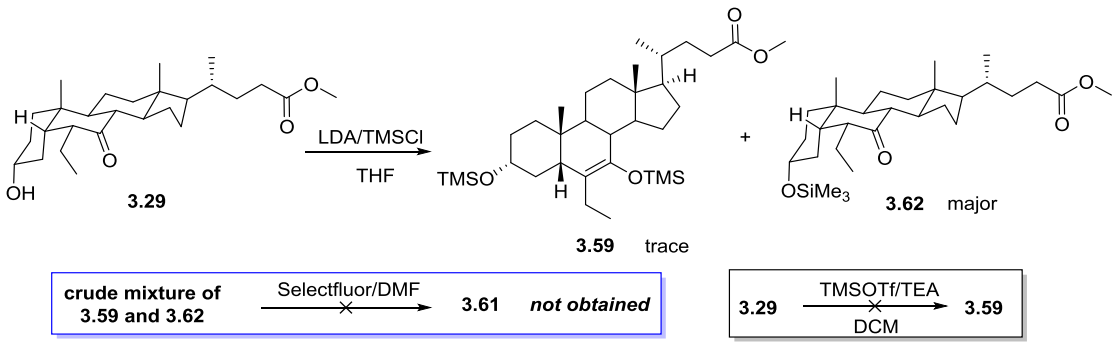


Figure 3.10 X-Ray structure of compound 3.60.

A similar approach was attempted on compound **3.29** with an equatorial 6-ethyl group (**Scheme 3.35**), but the formation of intermediate **3.59** again proved difficult. ^{1}H NMR analysis of the crude showed that compound **3.62** was the major product of the reaction. An attempt to effect fluorination by treating the crude mixture with Selectfluor, lead to the formation of only trace amount of fluorinated compound as indicated by ^{19}F NMR. In this case, deprotonation of $H_{6\beta}$ was difficult presumably due to steric hindrance. As an alternative method for silyl enol ether **3.59** formation by treating compound **3.29** with TMSOTf/TEA in DCM, was also not successful (**Scheme 3.35**).



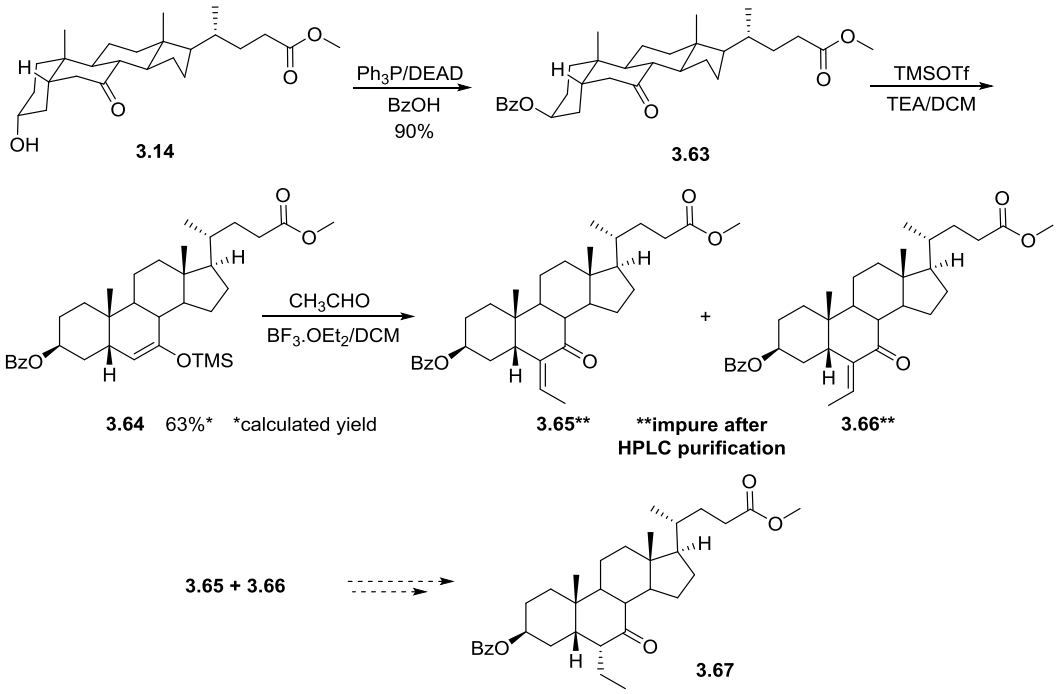
Scheme 3.35 Fluorination towards compound 3.61.

3.2.2.2.2 Attempted synthesis on derivative with 3β-configuration

As mentioned in *section 3.2.1.4*, the 3α-acetyl and pivaloyl group reduced the rate of reactions on the B-ring. A 3α-TMS group could have a similar effect, made worse by the additional 6-ethyl group. Therefore, it was deemed worth investigating to conduct silyl enol ether formation using substrates with a 3β-configuration.

The synthesis of the required substrate started from the keto-alcohol **3.14** (**Scheme 3.36**). The conversion of stereochemistry at C-3 was achieved *via* Mitsunobu reaction in good yield (90%), leading to compound **3.63**. Formation of silyl enol ether **3.64** didn't reach completion and the crude reaction mixture was use directly for the Mukaiyama aldol reaction, leading to enone **3.65** (minor)

and **3.66** (major). Both enones were impure even after HPLC purification. Therefore, the alternative route was required towards the synthesis of compound **3.67**.



Scheme 3.36 Attempted synthesis towards compound 3.67.

The assignment of stereochemistery for enone isomers **3.65** and **3.66** was confirmed by 1D NOE and COSY experiments. Although COSY experiment for **3.65** displayed a correlation between $H_{5\beta}$ and 2'-CH_3, no NOE effect was observed when $H_{5\beta}$ was irradiated (**Figure 3.11**). However, irradiation at $H_{5\beta}$ of **3.65** caused NOE on H_1', and no correlation between them was observed from COSY experiment. This indicated the close proximity between $H_{5\beta}$ and H_1' of compound **3.65**, which was further confirmed by NOE experiment with irradiation at H_1' (**Figure 3.11**). Therefore, enone **3.65** was assigned with a *Z*-configuration. In contrast, irradiation at $H_{5\beta}$ of *E*-configured enone **3.66** led to an NOE effect on 2'-CH_3 (**Figure 3.12**).

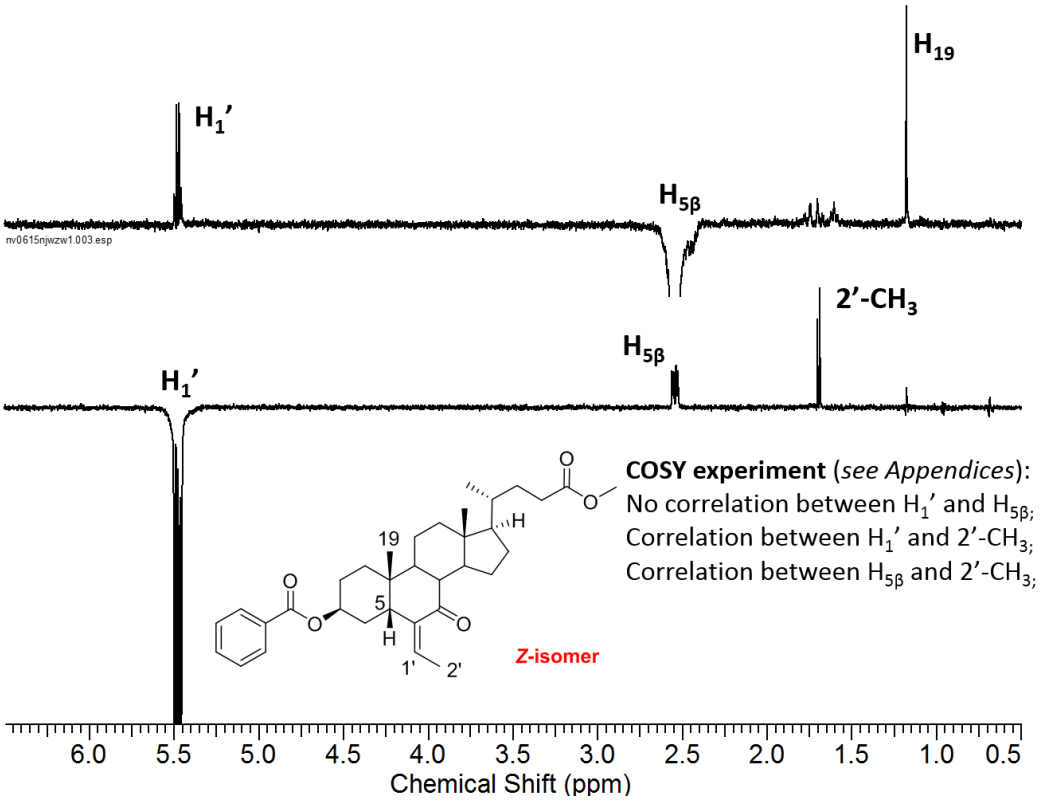


Figure 3.11 1D NOE experiments and COSY information for enone 3.65.

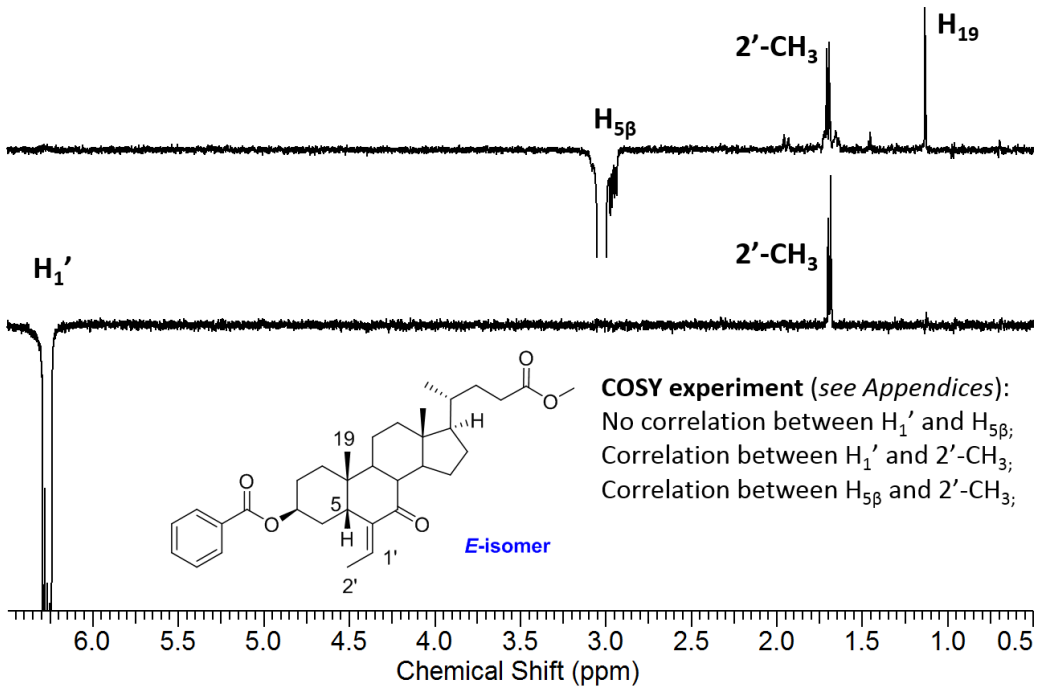
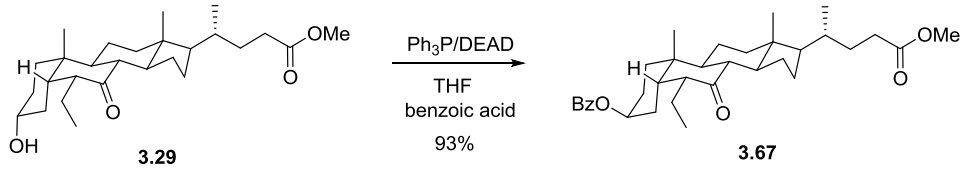


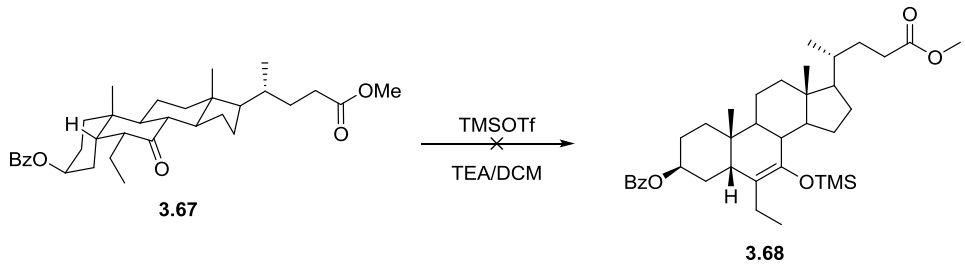
Figure 3.12 1D NOE experiments and COSY information for enone 3.66.

As the ethylated ketone derivative **3.29**[7] was available, Mitsunobu reaction (**Scheme 3.37**) was also performed, leading to a high yield (93%) for the synthesis of compound **3.67**.



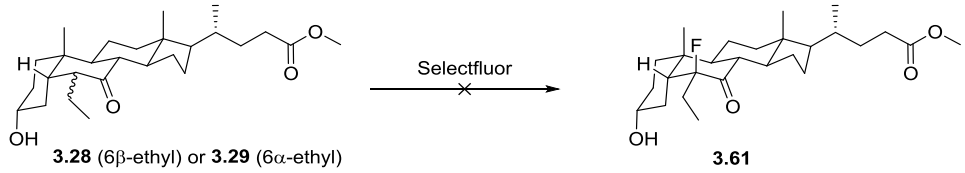
Scheme 3.37 Mitsunobu reaction on compound 3.1.33.

As shown in **Scheme 3.38**, compound **3.67** was treated with TMSOTf under basic conditions. No desired compound **3.68** was obtained, despite the reduction in steric hindrance from the α-face of the steroid skeleton.



Scheme 3.38 Preparation of compound 3.68.

It was reported in the literature[105] that α-fluorination next to carbonyl group could be achieved *via* acid-catalyzed (H_2SO_4) electrophilic fluorination using Selectfluor. In our group, we have also found that acetic acid was a more effective alternative. Ketone intermediate **3.28** (**Scheme 3.39**) was treated with fluorinating agent catalyzed by acetic acid under reflux (**Entry 1**, **Table 3.7**).



Scheme 3.39 Direct electrophilic fluorination.

Table 3.7 Direct fluorination using Selectfluor on ketone intermediates.

Entry	SM	Solvent	Additive	Condition	Result
1	**3.28**	MeCN	MeCOOH	reflux	complex mixture
2	**3.29**	DMF	n/a	40 °C	no conversion
3	**3.29**	DMF	n/a	95 °C	trace (^{19}F NMR)

[7]Synthesis of compound **3.29** was also attempted *via* fluoride-mediated alkylation using tetrabutylammonium difluorotriphenylstannate (anhydrous fluoride) with ethyl iodide on compound **3.20**. However, it was not successful and only 3-silyloxy-7-keto derivative (**exp.01**) was obtained.

However, fluorination proceeded together with numerous side reactions, as illustrated by ^{19}F {^{1}H] NMR spectrum of the crude mixture in **Figure 3.13**. Mild reaction condition (**Entry 2**, **Table 3.7**) was then used for compound **3.29** in DMF, without addition of acid. But no conversion was observed. Thus, the reaction temperature was elevated to 95 °C (**Entry 3**), leading to only trace amount of fluorinated compounds as indicated by ^{19}F NMR.

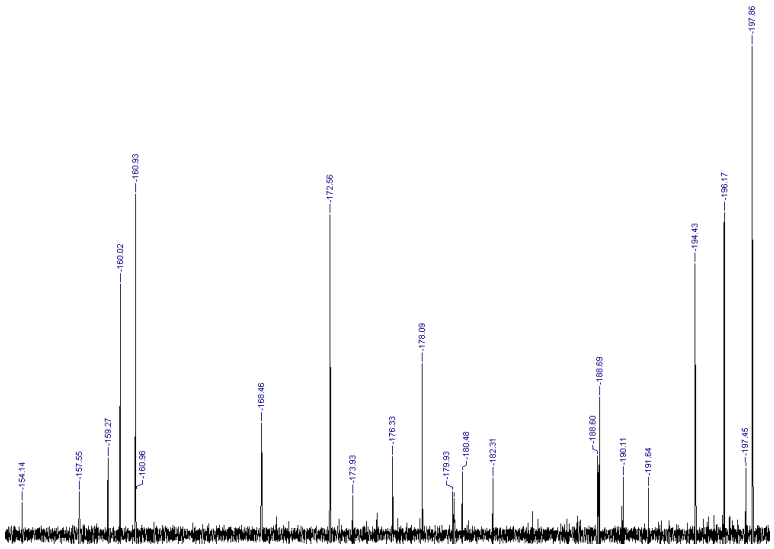
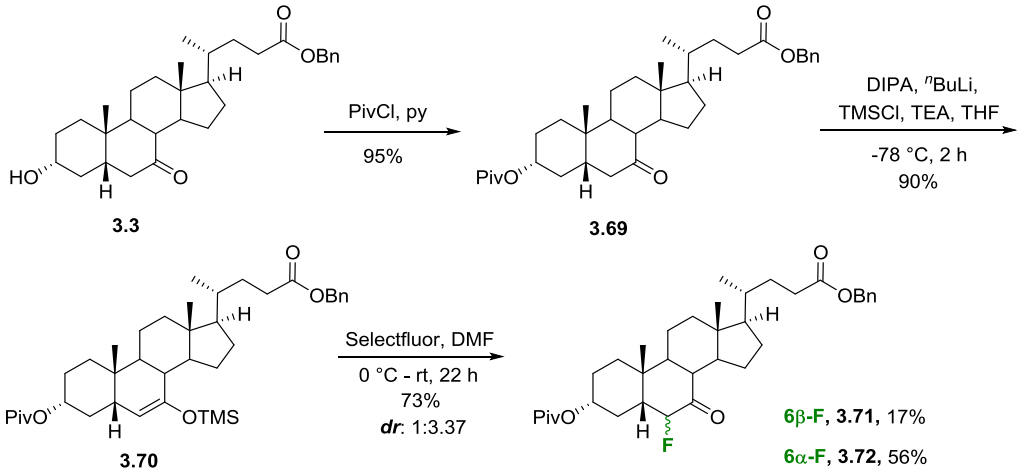


Figure 3.13 ^{19}F {^{1}H} NMR spectrum of crude mixture from acid-catalyzed fluorination.

3.2.2.3 Direct ethylation on 6-fluorinated intermediate

3.2.2.3.1 Preparation of 6-fluoro-7-keto intermediate 3.72

The 3α-OH group of compound **3.3** (**Scheme 3.40**) was protected as pivaloate **3.69** with excellent yield, which was used for the preparation of silyl enol ether **3.70** for further eletrophilic fluorination using Selectfluor. The fluoroketones **3.71** and **3.72** were then obtained, with the diastereoselectivity towards 6α-fluoro ketone **3.72**.

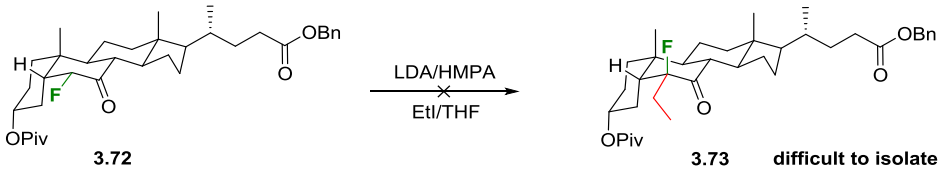


Scheme 3.40 Synthesis of fluoroketone intermediate 3.72.

Interestingly, the retention time of diastereoisomer **3.71** and **3.72** was remarkably different, which enabled an easy separation *via* flash chromatography.

3.2.2.3.2 Synthesis towards compound 3.73

As shown in **Scheme 3.41**, compound **3.72** was deprotonated with LDA and the nucleophilic enolate was treated with ethyl iodide. However, the alkylation proceeded with numerous side reactions. The desired intermediate **3.72** was not able to be isolated from the complex mixture *via* flash chromatography and purification using HPLC.

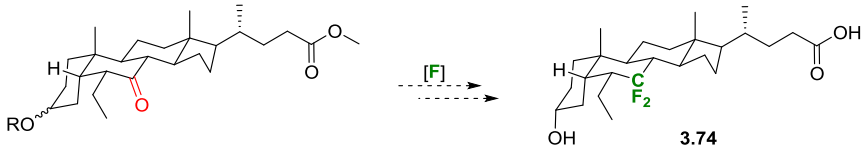


Scheme 3.41 Ethylation on compound 3.72.

3.2.3 Attempted synthesis towards 7,7-difluoro LCA

3.2.3.1 Synthetic plan

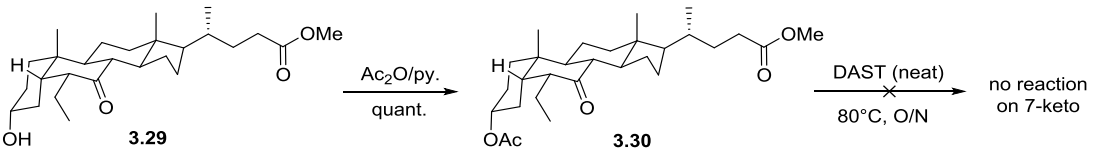
The synthesis of target compound **3.74** (**Scheme 3.42**) was planned by direct deoxyfluorination of a 7-keto precursor. As described in *section 4.1.2.2*, it was only possible to successfully deoxofluorinate a 7-keto group under harsh reaction conditions (neat DAST with heating) .



Scheme 3.42 Synthetic plan for compound 3.74.

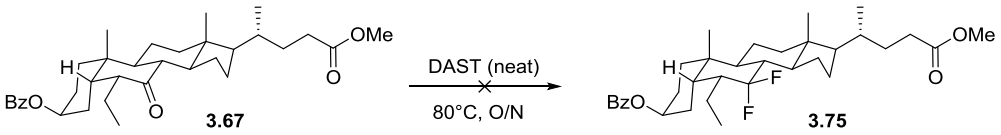
3.2.3.2 Fluorination

In order to avoid deoxofluorination at the 3-position, the 3α-OAc derivative **3.30** was prepared from compound **3.29** by using acetic anhydride in pyridine (**Scheme 3.43**). However, no conversion was observed after further treatment of **3.30** using neat DAST at 80 °C, and the starting material was fully recovered. The difficulty for the nucleophilic fluorination is presumably due to the increased steric hindrance caused by the 6α-ethyl group.



Scheme 3.43 Fluorination on compound 3.30.

In order to decrease the steric hindrance around the 7-keto group, a 3β-derivative (**3.67**) was also subjected to the fluorination condition (**Scheme 3.44**). However, this was not successful. No further conditions were investigated, as the ethyl group was assumed to obstruct the approach of fluorinating reagent.



Scheme 3.44 Fluorination on compound 3.67.

3.2.4 Alternative synthesis towards 6-ECDCA analogues

3.2.4.1 Synthetic plan

As described in *section 3.2.1* and *3.2.2*, the syntheses towards target compound **3.35** and **3.57** were attempted but not successful. Therefore, new synthetic plans were proposed, as shown in **Figure 3.14**, for the synthesis of compounds **3.35** and **3.57**, as well as 6-ECDCA. The key precursor for these targets would be 6-keto derivative **3.76**, which could be prepared from hyodeoxycholic acid (HDCA).

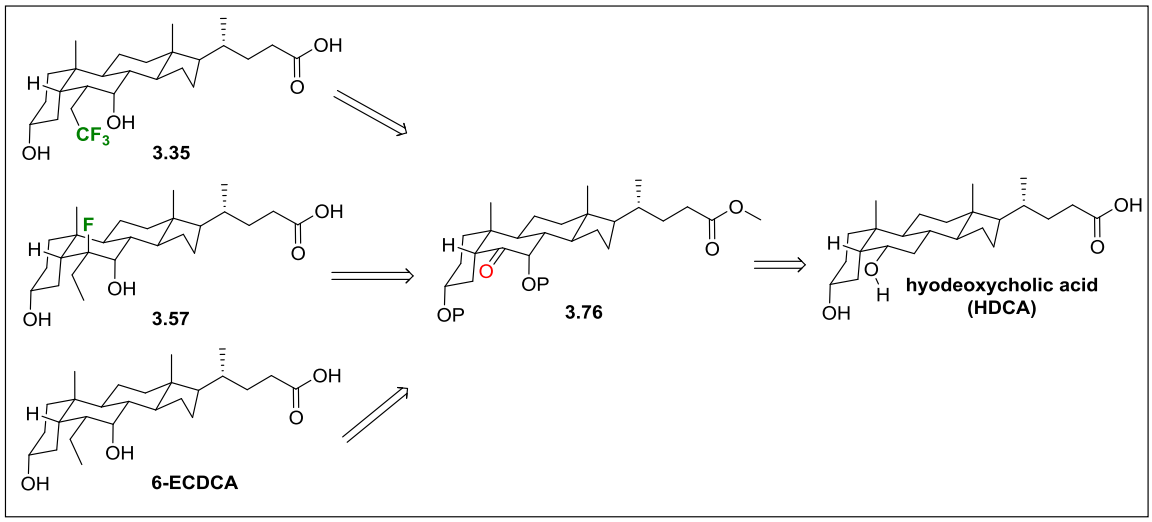


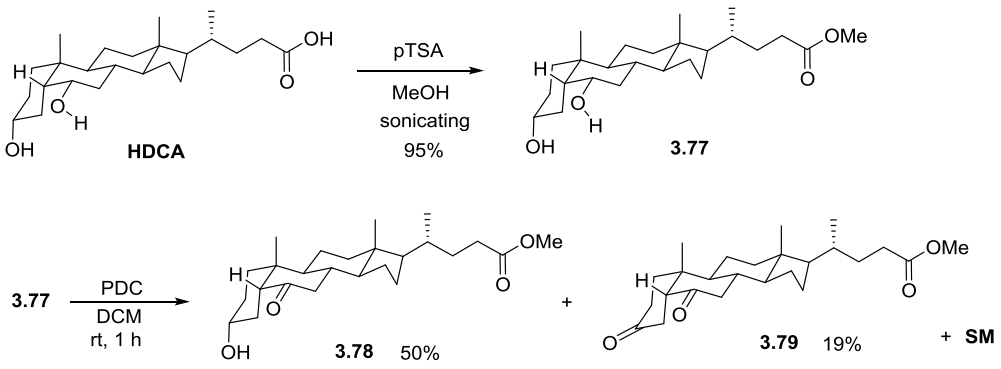
Figure 3.14 Retrosynthetic analysis for 6-ECDCA and its analogues.

Wittig olefination on compound **3.76** can potentially incorporate methylidene groups at C-6. Further hydrotrifluoromethylation on the 6-methylidene derivative is designed for the introduction

of trifluoroethyl group towards target compound **3.35**. Gouverneur and co-worker have recently reported such hydrotrifluoromethylation method on unactivated alkenes.[106] Also, Grignard reaction on the precursor **3.76** and subsequent deoxyfluorination could lead to the synthesis of compound **3.57**. In addition, 6-ethylidene group could also be introduced at C-6 *via* Wittig olefination, and further hydrogenation could provide a novel synthetic approach for the synthesis of 6-ECDCA.

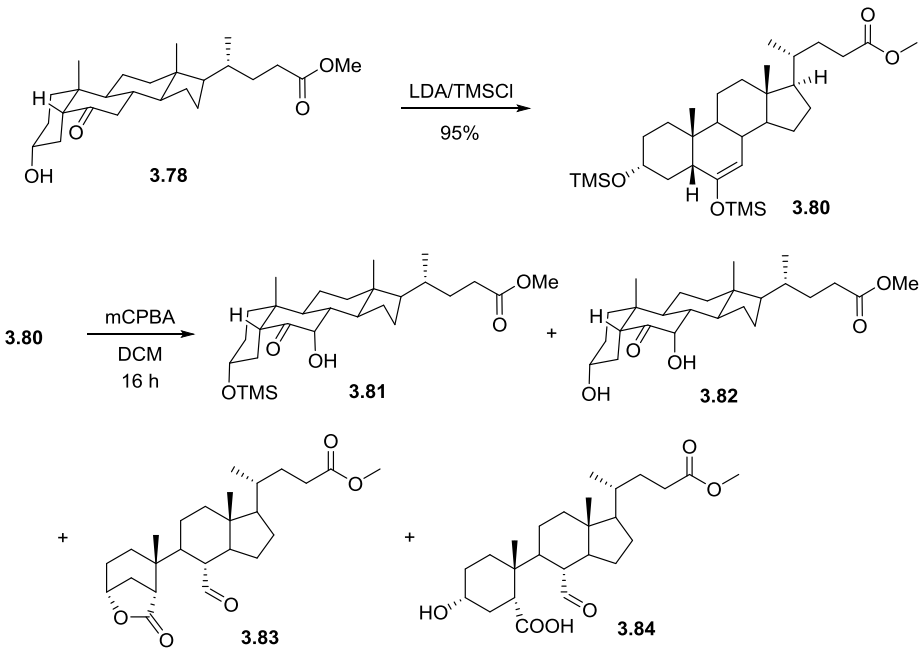
3.2.4.2 Synthesis of the key 6-keto intermediates

As shown in **Scheme 3.45**, HDCA was methylated to compound **3.77** by sonicating with high yield (95%). Through further oxidation using pyridinium dichromate (PDC), 6-keto derivative **3.78** was obtained with satisfactory selectivity, along with the diketo by-product **3.79**.



Scheme 3.45 Preparation of compound 3.78.

Compound **3.80** was selectively prepared by using LDA/TMSCl in THF with a yield of 95% (**Scheme 3.46**), and was used directly for further α-hydroxylation reaction. Following the reported procedure,[107] compound **3.80** was treated with 3-chloroperbenzoic acid (mCPBA) for the synthesis of compound **3.80** (**Scheme 3.46**). Compound **3.81** was obtained due to the partial cleavage of the 3-trimethylsilyl group. However, this α-hydroxylation proceeded also with other side reactions. Compound **3.83** and **3.84** (isolated yield: 65%, **Entry 1**, **Table 3.8**) with a opened B-ring were isolated after purification, which were not reported in the literature.[107]



Scheme 3.46 Silyl enol ether preparation and α-hydroxylation.

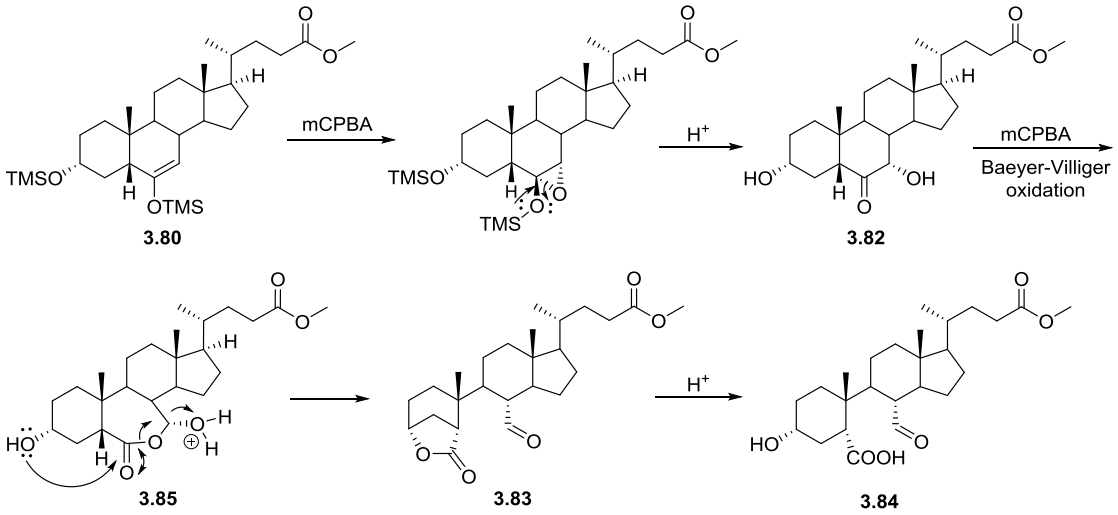
The side reactions were assumed to be caused by slight excess of mCPBA, leading to the over-oxidation. This was further proven by using less equivalents of mCPBA (**Entry 2**, **Table 3.8**), with significantly reduced formation of undesired by-products. The reaction yield for compound **3.82** was increased up to 50% with only 1.05 equivalents of mCPBA (**Entry 3**). However, change of the reaction scale caused fluctuations in yield.

Table 3.8 α-Hydroxylation using mCPBA.

Entry	mCPBA	Yield
1	2.8 eq.	**3.81**, 3%; **3.82**, 23%; **3.83**, 19%; **3.84**, major;
2	1.1 eq.	**3.81**, 57%; **3.82**, 20%; **3.83**; **3.84**, 3%;
3	1.05 eq.	**3.81**, ~18%; **3.82**, 37%-50%*; **3.83**; **3.84**; **SM**;

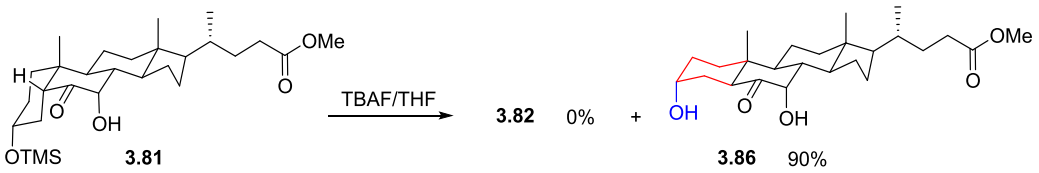
*reaction scale: 170 mg – 1 g;

The proposed mechanism pathway is shown in **Scheme 3.47**. Compound **3.82** can be further oxidized by mCPBA (Baeyer-Villiger oxidation), leading to a strained lactone **3.85** with a seven-membered ring. B-ring opening by nucleophilic attack from the 3α-OH group afforded more stable lactone **3.83**. Compound **3.84** can form due to the hydrolysis of the lactone. Synthesis of similar lactones on steroidal structures using periodic acid was also reported by Zhou and co-workers.[108]



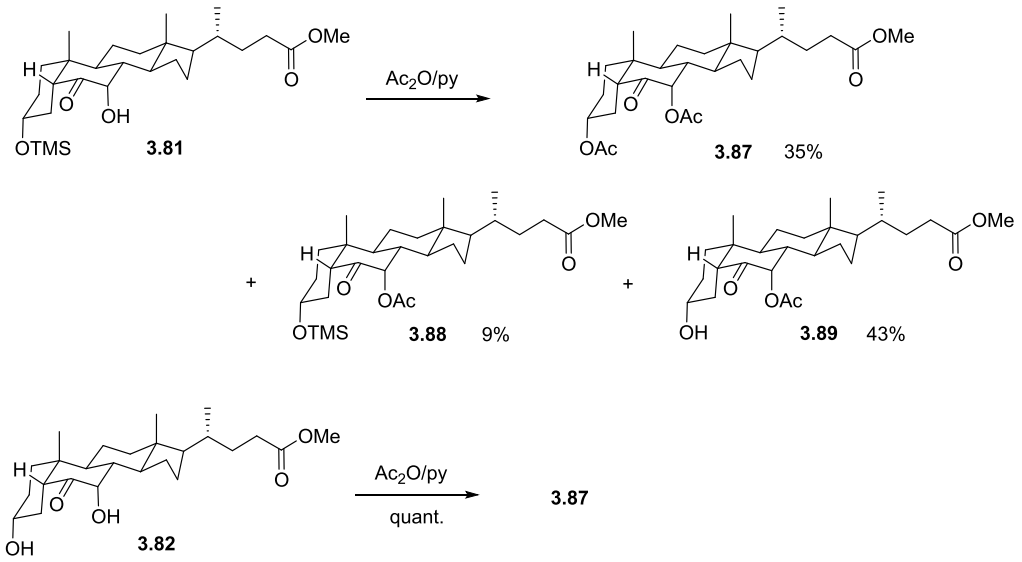
Scheme 3.47 Proposed mechanism for the by-products formation.

Silyl ether **3.81** was treated with tetrabutylammonium fluoride (TBAF), as shown in **Scheme 3.48**. Interestingly, no desired compound **3.82** was formed. Instead, by-product **3.86** was isolated, with the epimerisation on C-5 due to the basicity of TBAF.



Scheme 3.48 Deprotection silyl ether using TBAF.

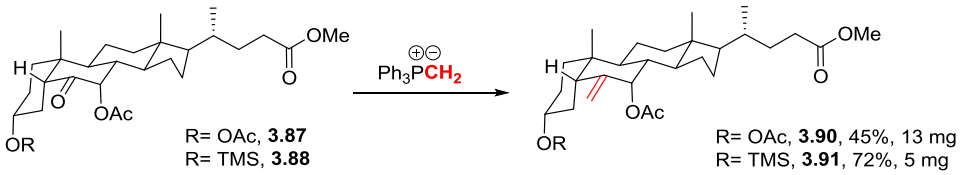
As the silyl deprotection proceeded with C5-epimerisation, compound **3.81**, as well as **3.82**, were directly acetylated for the preparation of the key percursors (**Scheme 3.49**). Acetylation on compound **3.82** afforded the desired intermediate **3.87** quantitatively. Identical conditions on compound **3.81** also led to compound **3.87**, as well as **3.88** and **3.89**, which were used for further reactions.



Scheme 3.49 Acetylation of compounds 3.81 and 3.82.

3.2.4.3 Wittig Olefination

The ylide (**Scheme 3.50**), prepared from Ph_3PMeBr/KO^tBu, was used for the Wittig reaction, and this led to a successful introduction of the methylidene group at C-6. A higher yield (72%) was obtained from 3-TMS derivative **3.88**.



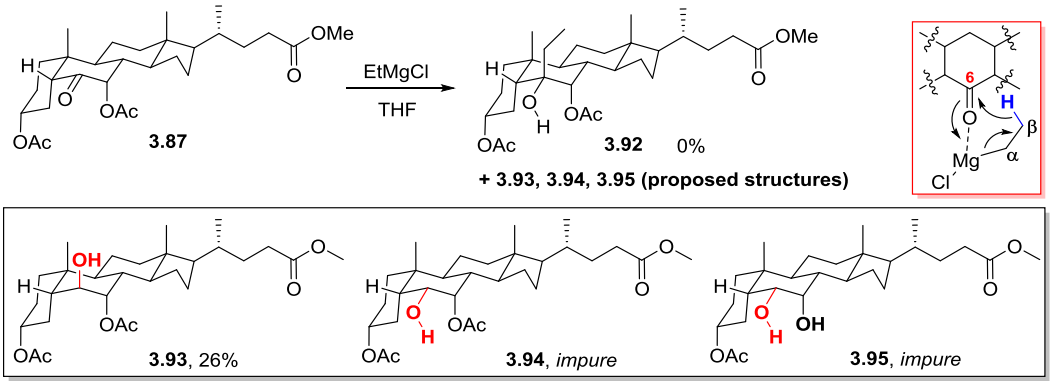
Scheme 3.50 Wittig olefination using Ph_3PMeBr/KO^tBu.

Because only small amounts of compound **3.90** and **3.91** (~10 mg) have been synthesized so far, further repeatition of these reactions are necessary to obtain sufficient substrates for the intended hydrotrifluorination reaction.

3.2.4.4 Grignard reaction

For the synthesis of 6β-fluoro-6α-ethyl CDCA, compound **3.87** is treated with Grignard reagent (**Scheme 3.51**). No desired intermediate **3.92** was obtained after purification. Surprisingly, by-product **3.93**, **3.94** and **3.95** were isolated, as a result of the reduction of 6-keto and/or 7-OAc group. It is interesting to find out that several side reactions (such as enolization, condensation and reduction) can occur when using Grignard reagents, as reported in the literature.[109] In fact, the reduction of carbonyl groups by Grignard reagents was initially reported by Grignard himself in 1901, when he observed that benzyl alcohol was formed as minor product from Grignard reaction on benzaldehyde.[110] This is due to the reduction by β-hydride. Because of the steric hindrance, the

reaction rate of addition process can be significantly reduced, promoting the occurrence of reduction.[109] Therefore, in our case, the intrinsic steric effect of steroid skeleton inhibited the addition to 6-carbonyl group, leading to the formation of these reduced by-products (assumed mechanism shown in **Scheme 3.51**). Further Grignard reactions were not attempted, as the steric hindrance originates from the steroid itself and is difficult to resolve.



Scheme 3.51 Grignard reaction on compound 3.87.

The identification of C-6 stereochemistry for compounds **3.93**-**3.95** is based on NMR analysis and comparison with the literature values.[111] As shown in **Figure 3.15**, chemical shift of H_{19} is distinctive for both 6β- and 6α-OH derivatives. H_{19} is more deshielded (compound **3.93**, δ_H 1.11 ppm) when the 6-OH group is in the axial position.

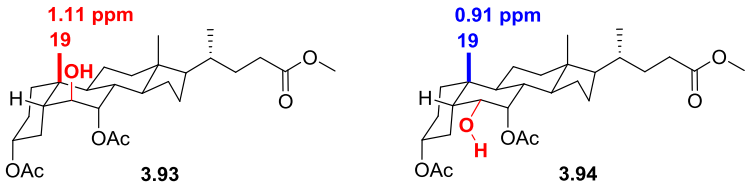
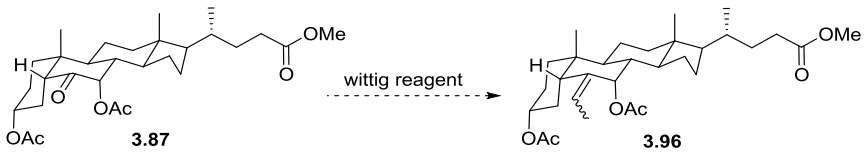


Figure 3.15 Assignment of 6-OH configuration by NMR analysis.

3.2.4.5 Possible alternative synthesis of 6-E-CDCA?

The Wittig reagent was prepared from the treatment of Ph_3EtBr with the base (**Table 3.9**). The formation of the ylide was indicated by the orange colour of the reaction mixture. Reaction between compound **3.87** and the ylide didn't yield the desired product **3.96** (**Scheme 3.52, Entries 1-2, Table 3.9**). Additon of excessive ylide (**Entry 3**) resulted in side reactions. Further elevation of the reaction temperature (**Entry 4**) was trialed but only led to undesired by-products.



Scheme 3.52 Wittig reaction towards compound 3.87.

Table 3.9 Wittig reaction towards compound 3.96.

Entry	Ph_3PEtBr	Base	Temp./Time	Results
1	1.2 eq.	^{n}BuLi (1.0 eq.)	rt/4 h	no conversion
2	1.2 eq.	KO^{t}Bu (1.0 eq.)	rt/4 h	no conversion
3	12 eq.	KO^{t}Bu (10 eq.)	rt/4 h	side reactions
4	6 eq.	KO^{t}Bu (5 eq.)	reflux/24 h	side reactions

It is assumed that the steric hindrance surrounding the 6-carbonyl group might block the approach of the ylide. In addition, the formation of oxaphophetane intermediate within the cavity between A- and B-ring can also be sterically unfavoured. Sterically less hindered substrate **3.89** was also used for the olefination but was not successful.

As mentioned above (**Scheme 3.50**), methylidene group was introduced with a slightly less bulky ylide. Therefore, steric impact must have played a negative role for the introduction of the ethylidene group *via* Wittig reaction. For future work, less bulky Peterson reagents can be used for this preparation.[112] It is also worth trying Tebbe reagent,[113] which was reported for the olefination on relatively hindered carbonyl groups.[114]

3.3 Conclusion

In order to synthesise 6-ECDCA, the reported procedures from Sepe, Pellicciari and their co-workers were first repeated, which proceeded with side reactions and poor reproducibility. The alkene reduction was then satisfactorily achieved by using an H-Cube instrument, leading to the synthesis of 6-ECDCA after tremendous efforts.

For the introduction of polyfluoroalkyl at C-6, formation of the desired enone was not successful due to the difficulty of dehydrating the aldol adducts, as well as the possible steric hindrance from steroid skeleton, blocking the approach of reagents. Also, the synthesis towards 6β-fluoro-6α-ethyl CDCA was unsuccessful *via* the attempted synthetic routes, due to the side reactions, and also the difficulties to obtained key 6-alkylated silyl enol ether intermediates because of steric challenges. Besdies, deoxofluorination of the 7-keto group was obstructed in the presence of the 6α-ethyl group, precluding the synthesis towards 7,7-difluoro-6α-ethyl LCA.

In addition, Wittig and Grignard reactions were also attempted on 6-keto derivatives in the approach to target compounds. 6-Methylidene intermediates were successfully obtained, and can be used for further hydrotrifluoromethylation reaction. However, introduction of 6-ethylidene group and Grignard reaction proved difficult, due to the inherent steric hindrance from the steroid sculpture.

Chapter 4: Synthesis of B-ring and C-ring fluorinated BA Analogues

4.1 Synthesis of C-7 and C-12 deoxyfluorinated BA analogues

4.1.1 Introduction of synthetic plan

In this chapter, deoxyfluorination at the C-7 and C-12 positions to give analogues **4.1**-**4.6** is discussed (**Figure 4.1**).

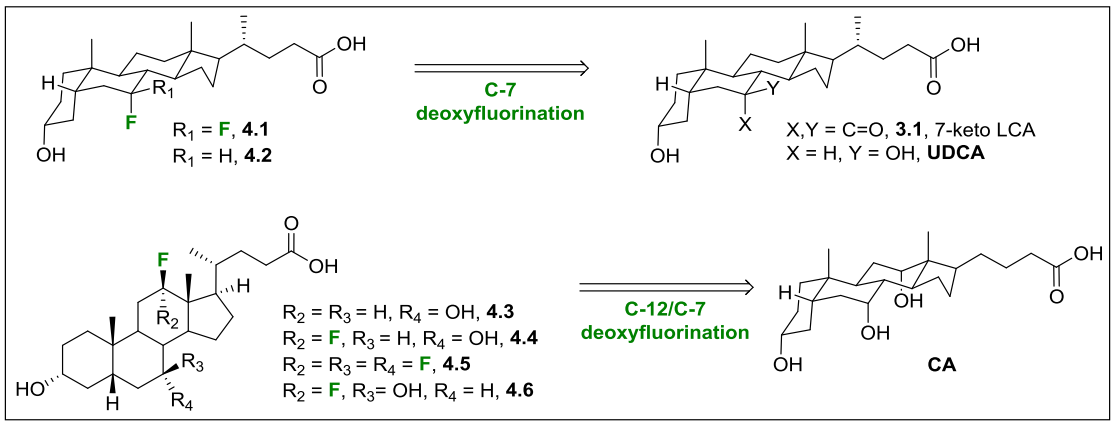


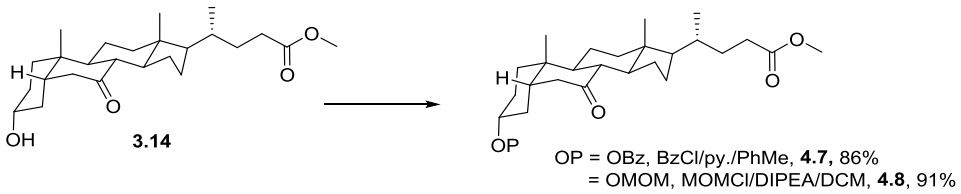
Figure 4.1 Synthetic plan for deoxyfluorinated BA analogues.

The synthetic strategy is directed at selective protection to allow for deoxyfluorination at the required oxidation level. Introduction of CF_2 at C-7 could be achieved *via* deoxyfluorination of 7-keto LCA derivative **3.1**. The desire to obtain fluorination at the 7-position with CDCA configuration leads to the selection of the available UDCA as a starting material. For compounds with fluorination at the 12-position, CA is the most reasonable starting material.

4.1.2 Synthesis of 7,7-difluoro LCA

4.1.2.1 Protection of 3α-OH group

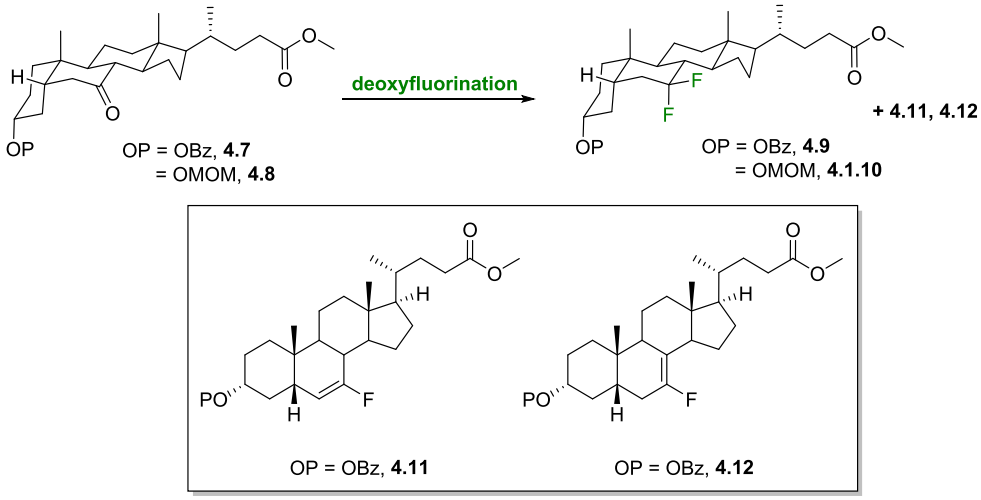
The 3α-OH group of 7-keto lithocholate **3.14** (**Scheme 4.1**) was protected as benzoate **4.7** as well as the MOM ether **4.8**.



Scheme 4.1 Protection of 3α-OH group.

4.1.2.2 Nucleophilic fluorination

Nucleophilic fluorination on 7-keto intermediates (**Scheme 4.2**) required extensive screening of reaction conditions and fluorinating reagents. Fluorinations on **4.7** using DAST in toluene, with increasing reaction temperature and time (**Table 4.1**, **Entries 1-3**), were not successful and only trace amounts of fluorinated compounds were obtained according to ^{19}F NMR analysis of the crude reaction mixture.



Scheme 4.2 Deoxyfluorination on 7-keto intermediates.

Table 4.1 Deoxyfluorination of the 7-keto group.

Entry	OP	Reagents	Solvent/Conditions	Product and yield
1	OBz	DAST	toluene, 24 h, rt	**4.9**, trace
2	OBz	DAST	toluene, 3 h, 80 °C	**4.9**, trace
3	OBz	DAST	toluene, 16 h, 80 °C	**4.9**, trace
4	OMOM	DAST	toluene, 16 h, rt	**4.10**, trace (slightly improved)
5	OMOM	DAST	toluene, 6 h, 80 °C	**4.10**, trace (slightly improved)
6	OMOM	DAST/HF·DMPU	DCM, 16 h, rt	**4.10**, trace
7	OMOM	DAST/HF·DMPU	DCM, 6 h, 80 °C	**4.10**, trace
8	OBz	DAST/HF·py	DCM, 5 d, rt	**4.9**, trace
9	OBz	Deoxofluor	4 d, reflux	**4.9**, 15%; **4.11**/**4.12**, 8%;
10	OBz	DAST (neat)	16 h, 80 °C	**4.9**, 52%; **4.11**/**4.12**, 10%;

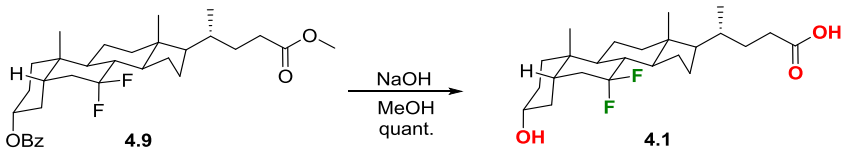
It was assumed that 3α-benzoate could potentially increase the steric hindrance for fluorination on the α-side of the steroid skeleton. Thus, the less bulky MOM-protected intermediate **4.8** was subjected to similar reaction conditions (**Entries 4-5, Table 4.1**) leading to a slightly higher but still far from satisfactory conversion. HF-mediated fluorination (**Entries 6-8**) was also performed. However, the fluorination yield was not improved, while, cleavage of the MOM protecting group was observed.

Fluorination using Deoxofluor (50% in THF, without addition of solvent) under reflux temperature (**Entry 9**) finally led to an improved yield (15%) after 4 d, together with fluoroalkene by-products **4.11** and **4.12** and remaining starting material. In order to increase conversion, DAST was then used in the absence of solvent (**Entry 10**). Interestingly, the overall yield was increased to more than 50%, with relatively reduced formation of fluoroalkene by-products. HPLC purification to separate the desired product **4.9** from the mixture of fluoroalkenes was possible.

Presumably the difficulty in fluorinating the 7-keto group was mainly due to the inherent steric hindrance from the bile acid skeleton. The identification of compounds **4.7** and **4.9** was further achieved by X-ray crystallographic analysis, and an interesting comparison of their crystal packing structures and crystalline geometries is described in *Chapter 5*.

4.1.2.3 Hydrolysis towards 7,7-difluoro LCA

Compound **4.9** was finally converted to the target compound **4.1** in quantitative conversion through alkaline hydrolysis of both the methyl ester and the 3α-benzoate groups (**Scheme 4.3**). Unfortunately, the final compound **4.1** was rather difficult to separate from the benzoic acid hydrolysis by-product, requiring repeated chromatography for purification.



Scheme 4.3 Hydrolysis towards the target compound 4.1.

The identity of compound **4.1** was also further proven by X-Ray crystallographic analysis, as shown in **Figure 4.2**. Co-crystallisation of a water molecule was observed.

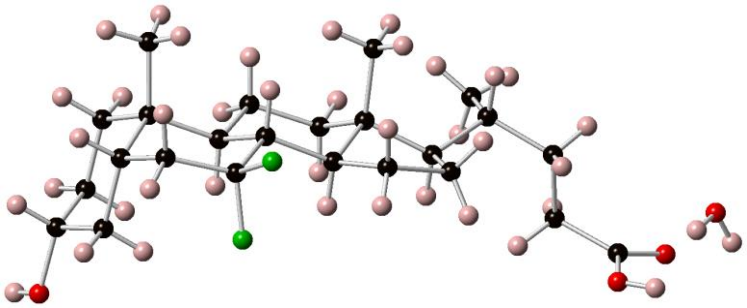
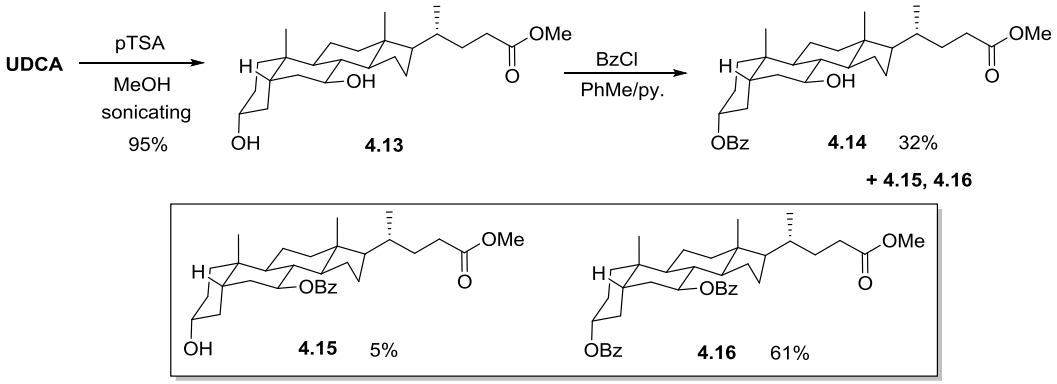


Figure 4.2 X-Ray structure of compound 4.1.

4.1.3 Towards the synthesis of 7α-fluoro LCA

4.1.3.1 Preparation of 3-protected UDCA methyl ester

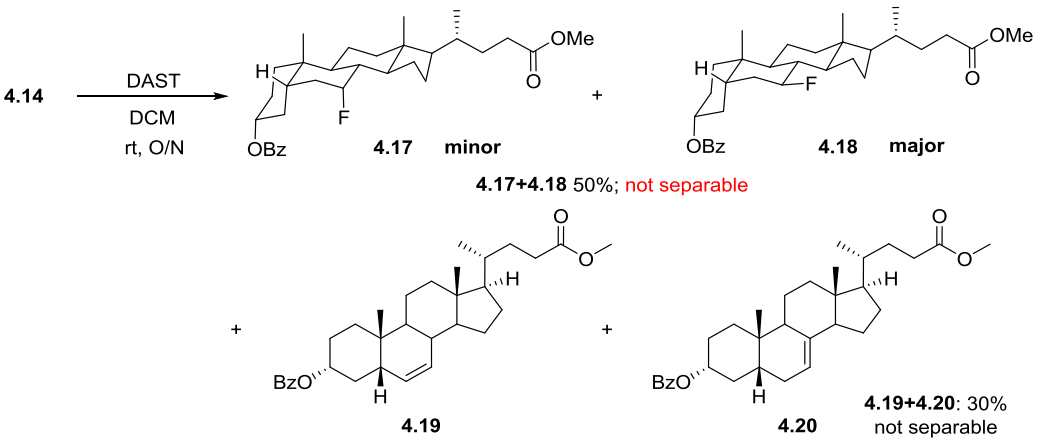
Following the procedure for the methyl ester formation of CDCA, UDCA was methylated through sonicating in acidic methanol (**Scheme 4.4**). The subsequent protection of the 3α-OH group occurred with poor regioselectivity (compound **4.14**, 32%), due to the fact that both hydroxyl groups are equatorial and not sterically hindered. Therefore, by-products **4.15** and **4.16** were also obtained by flash chromatography.



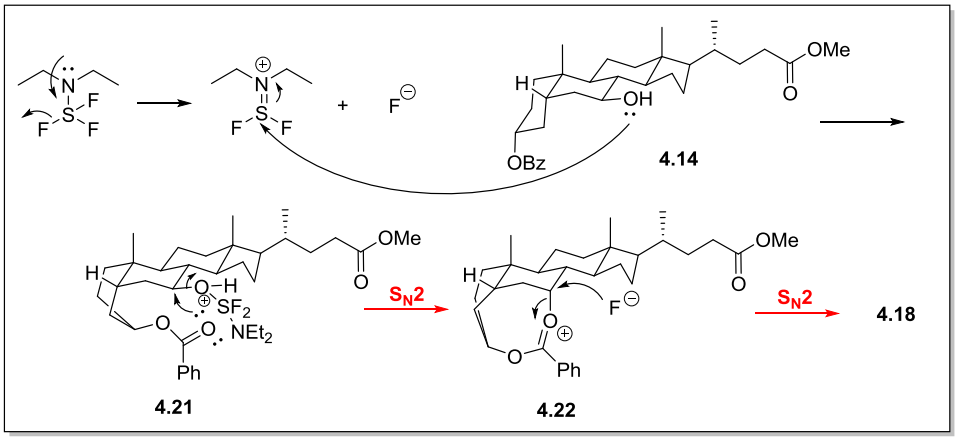
Scheme 4.4 Preparation of 3α-protected UDCA ester.

4.1.3.2 Fluorination on compound 4.14 using DAST

Fluorination of 7β-OH group using DAST in DCM (**Scheme 4.5**) only led to the desired **4.17** as minor product. Surprisingly, the major product turned out to be the 7β-fluoro derivative **4.18**, suggesting deoxyfluorination with retention of configuration. Two elimination products **4.19** and **4.20** were also isolated. The equatorial disposition of the starting C7 alcohol on the rigid steroid framework would preclude an E2-type mechanism for the formation of the alkenes. Instead, an E1 mechanism would need to be invoked, which would also explain the retentive deoxofluorination. A mechanism involving neighbouring group participation of the C3-ester group (**Scheme 4.6**) was also considered, but with an 8-membered ring involving a distorted A-ring. However, an E2-type elimination on intermediate **4.22** would then be possible.

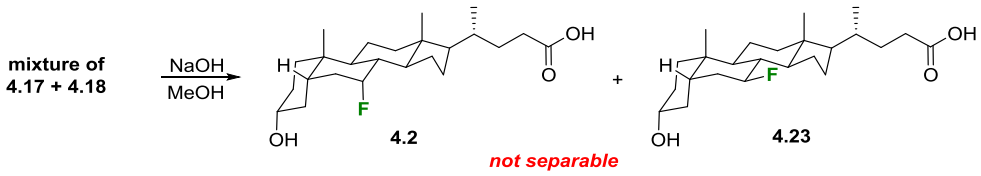


Scheme 4.5 Fluorination on compound 4.14 using DAST.



Scheme 4.6 Proposed mechanisms for retention of configuration of 4.18.

It was not possible to separate **4.17** from **4.18** by flash chromatography. Therefore, the mixture of both compounds was then directly hydrolysed under alkaline condition (**Scheme 4.7**), but separation between compound **4.2** and **4.23** was still not possible, both using normal-phase TLC analysis or RP-HPLC.

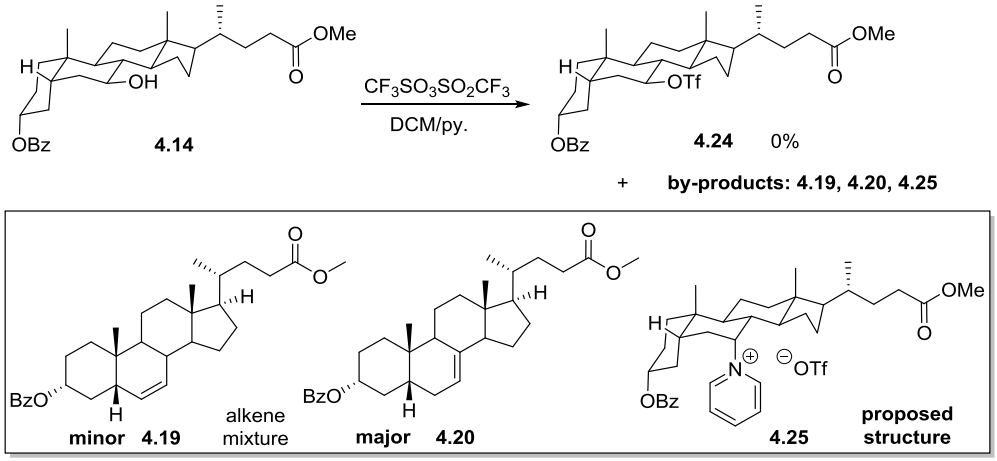


Scheme 4.7 Hydrolysis of mixture of compound 4.17 and 4.18.

Therefore, another synthetic route (*see section 4.1.3.3*) was envisioned *via* preparation of a leaving group (e.g., tosylate or triflate) on 7β-OH group, followed by S_N2 fluorination using anionic fluoride (e.g., TBAF).

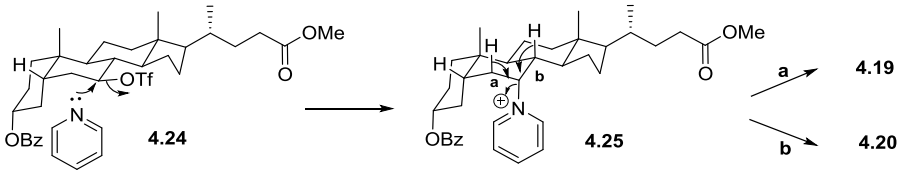
4.1.3.3 Attempted fluorination route using fluoride

The synthesis of triflate **4.24** was considered first for subsequent fluorination using TBAF (**Scheme 4.8**).



Scheme 4.8 Triflation on compound 4.14.

However, the desired intermediate **4.24** was not obtained *via* triflation catalyzed by pyridine. Instead, alkene by-product **4.19** and **4.20** were formed, presumably due to E1 elimination from triflate or E2 elimination (proposed mechanism shown in **Scheme 4.9**).



Scheme 4.9 Proposed mechanism of E2 elimination.

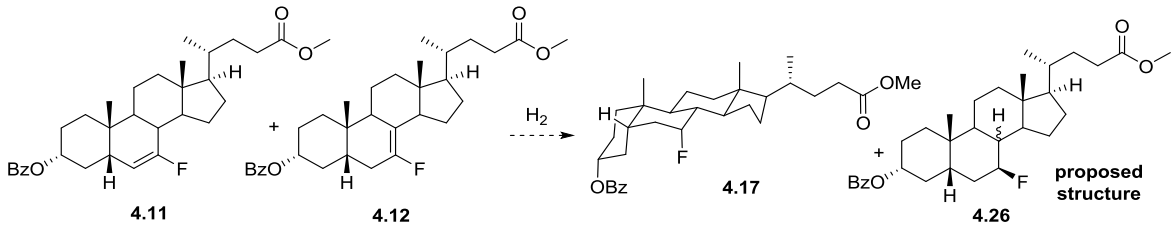
Surprisingly, pyridinium salt **4.25** was also isolated. The proposed structure was based on full NMR, IR and HRMS data analysis. The crystallization of compound **4.25** was attempted using various solvent systems, but was not successful. Interestingly, ^{1}H NMR of **4.25** indicated a quartet for $H_{7\beta}$ with a coupling constant of 6.2 Hz, which is much larger than the *J* values (q, 2.4 Hz to 3.1 Hz) of $H_{7\beta}$ for other 7α-configured derivatives. This strongly suggested that the axial bulky pyridinium ring might lead to a slightly distorted B-ring.

The bulkier 2,6-lutidine was then used to avoid E2 elimination mentioned above, but similarly only alkene products were isolated from a complex mixture of fluorinated compounds.

4.1.3.4 Hydrogenation on fluoroalkene intermediates

The fluoroalkene by-products **4.11** and **4.12**, obtained from the fluorination attempts on the 7-keto derivative towards target compound **4.1** (*see section 4.1.2.2*), were also evaluated as possible

starting materials towards the synthesis of 7α-fluoro LCA *via* hydrogenation (**Scheme 4.10**). The hydrogenation reaction was trialed under different reaction conditions (**Table 4.2**).



Scheme 4.10 Hydrogenation on fluoroalkenes.

Table 4.2 Hydrogenation of fluoroalkene mixture.

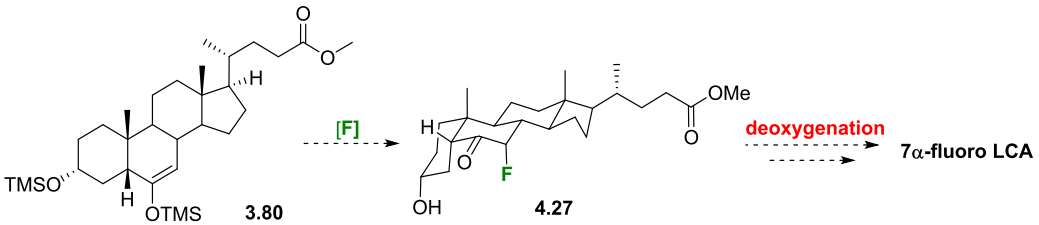
Entry	Catalyst	Solvent	Pressure	Temperature	Results
1	Pd/C	EtOH	1 atm	rt	**4.17**, 0%;
2	PtO_2	AcOH/HCl	1 atm	rt	**4.17**, 0%;
3	Pd/C	MeOH	60 bar	60 °C	**4.17**, 0 %; **4.26***;

*Based on NMR analysis (chemical shift and multiplicity).

Hydrogenation using Pd/C (**Entry 1**, **Table 4.2**) or Adam's catalyst (**Entry 2**) under atmospheric pressure were not successful. With increased pressure (60 bar, **Entry 3**) using an H-cube instrument, fluoroalkenes (**4.114.12**) were partially reduced. However, the desired product **4.17** was not obtained. According to ^{1}H and ^{19}F NMR analysis, 7β-fluorinated by-product **4.26** was formed.

4.1.3.5 Alternative route towards 7α-fluoro LCA

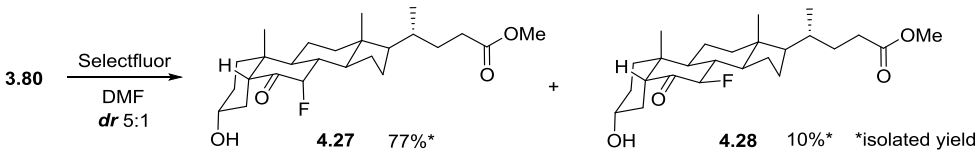
In order to obtain the target compound, an alternative synthetic route was proposed (**Scheme 4.11**). It was envisioned that the key intermediate **4.27**, with C7-fluorination, could be synthesized by electrophilic fluorination of silyl enol ether **3.80**. Further deoxygenation on C-6 would then be required to give 7α-fluoro LCA.



Scheme 4.11 Alternative synthetic plan towards 7α-fluoro LCA.

4.1.3.5.1 Synthesis of compound 4.27

Silyl enol ether **3.80** was treated with Selectfluor in DMF (**Scheme 4.12**), leading to the formation of fluoroketones **4.27** (major product, *dr* 5:1) and **4.28**.



Scheme 4.12 Preparation of fluoroketone 4.27.

To confirm the configuration at both C-5 (as epimerisation was possible) and C-7 of compound **4.27**, 1D NOE experiments were conducted with irradiation of H_{19} and H_{18}. The spectra are shown in **Figures 4.3** and **4.4** respectively. As expected, irradiation of H_{19} led to the identification of $H_{1\beta}$, $H_{5\beta}$, $H_{8\beta}$ and $H_{11\beta}$ (**Figure 4.3**). The assignments of the individual protons were based on coupling constants and multipilicity. Identification of $H_{8\beta}$ and $H_{11\beta}$ was further confirmed by NOE *via* irradiation on H_{18} (**Figure 4.4**).

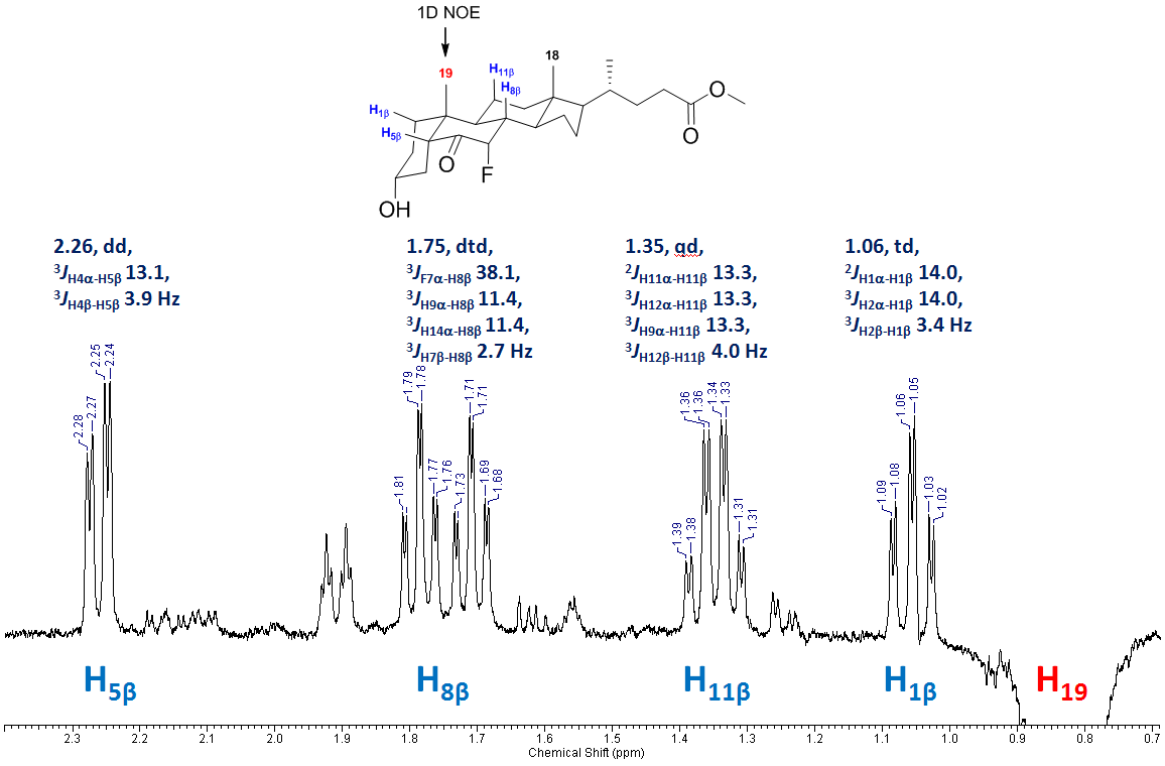


Figure 4.3 1D selective gradient NOESY at H_{19}.

Even though the multipilicity of H_7 (^{1}H NMR) implied axial fluorine substitution at C-7, the F_7 configuration was not verifyable by ^{19}F NMR analysis (only broad peak observed). However, with a coupling constant up to 38.1 Hz for $^3J_{F7\text{-}H8\beta}$, F_7 was deemed to be axial.

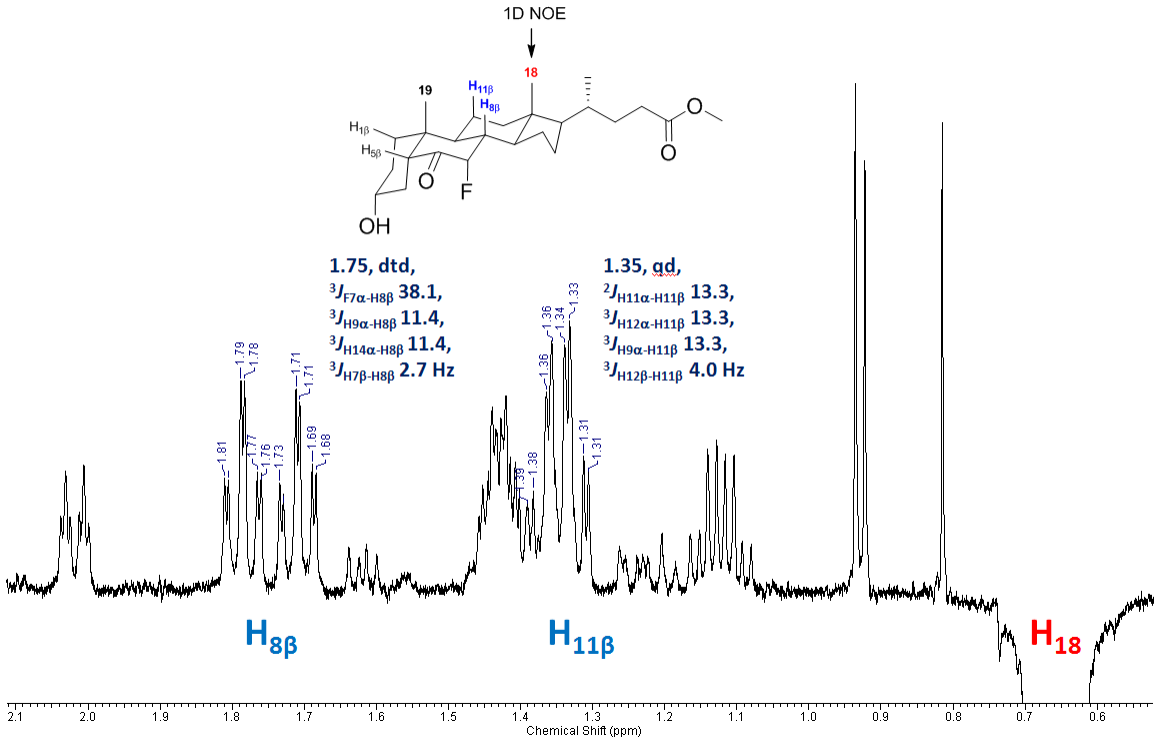


Figure 4.4 1D selective gradient NOESY at H_{18}.

The configurations of **4.27** and **4.28** were ultimately confirmed by X-Ray crystallographic analysis as shown in **Figure 4.5**. Further discussion regarding the crystal packing of these two diastereoisomers is included in *Chapter 5.*

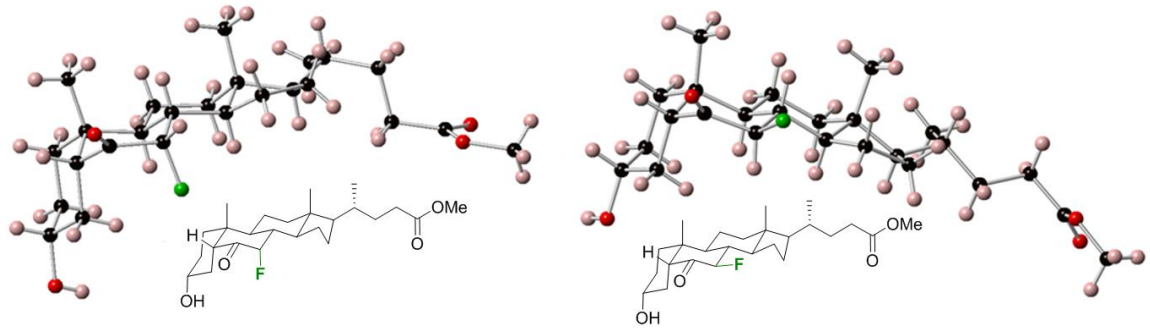


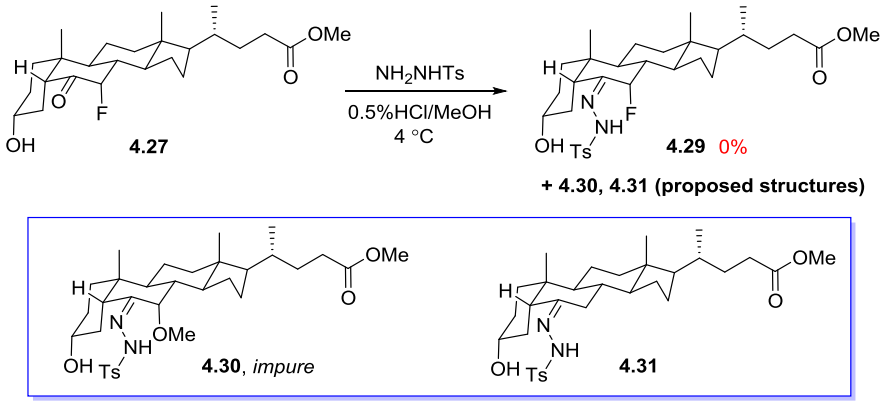
Figure 4.5 X-Ray structure of fluoroketone 4.27 and 4.28.

4.1.3.5.2 Deoxygenation via modified Wolff-Kishner reduction

In order to convert the carbonyl group to methylene, a synthesis of the corresponding tosyl hydrazone and subsequent reduction by hydride donor (e.g., $NaBH_3CN$) according to Caglioti, was envisioned.[115],[116] The main advantage of the Caglioti reaction over the Wolff-Kishner reduction[117] is the mild reaction conditions, which can tolerate other functional groups such as esters.

Hence, fluoroketone **4.27** was treated with tosyl hydrazide at 4 °C overnight in order to obtain intermediate **4.29** (**Scheme 4.13**). Unexpectedly, ^{19}F NMR analysis of the crude reaction mixture

indicated the disappearance of 7α-F. After purification *via* flash chromatography, compound **4.30** was obtained and identified based on NMR and HRMS analysis, together with by-product **4.31** (proposed structure based on HRMS analysis).



Scheme 4.13 Preparation of tosylhydrozone 4.29.

The presence of the 7α-methoxy and 6-(2-tosylhydrazono) groups in compound **4.30** was further confirmed by the comparison of NMR data with the known compound **4.32** (**Figure 4.6**),[118] even though a difference in proton chemical shift for $H_{7\beta}$ and C_7OCH_3 ($\Delta\delta$ ca. 0.3 ppm) was observed. This could be due to the difference between their electronic and/or steric environments.

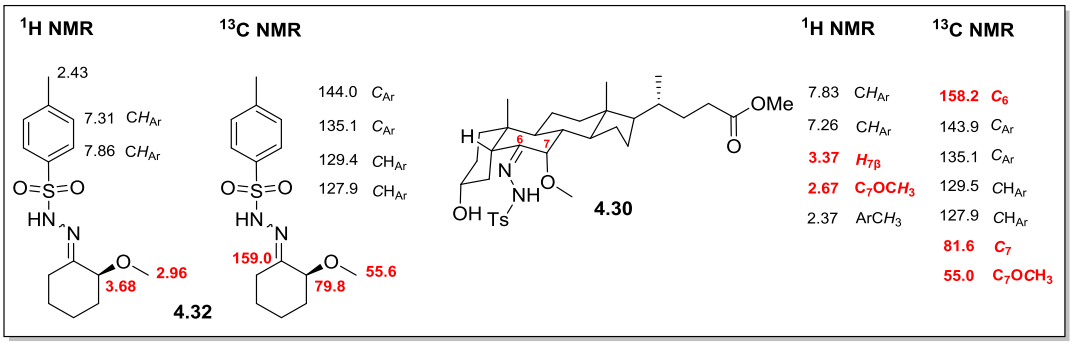
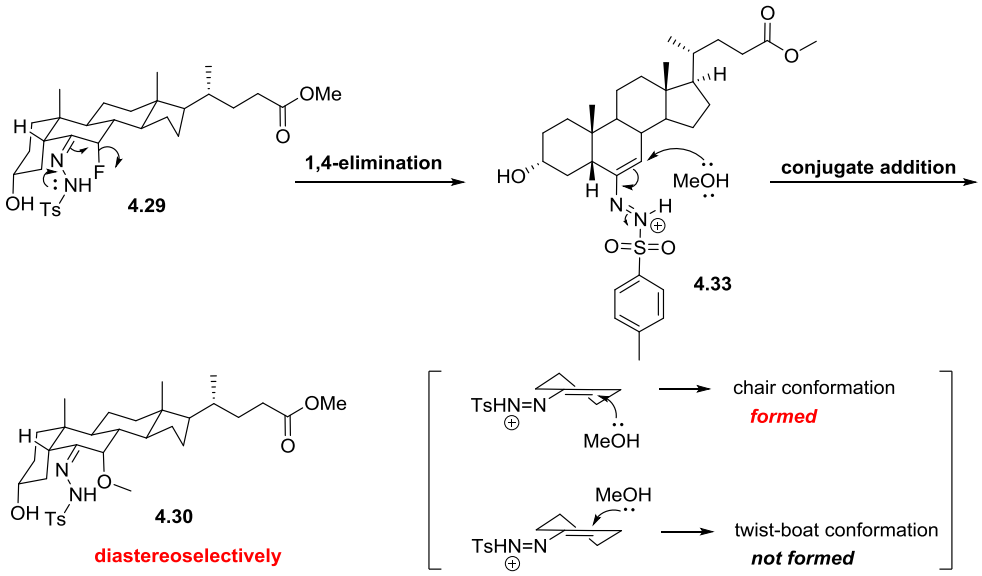


Figure 4.6 NMR data comparison between compound 4.30 and 4.32.[118]

The possible mechanism for the formation of compound **4.30** is shown in **Scheme 4.14**. Fluoride elimination on hydrazone **4.29** to give the azoalkene **4.33** is followed by diastereoselective nucleophilic conjugate addition of methanol to afford compound **4.30**. This step would preferentially proceed *via* the more favourable chair conformation, rather than a twist-boat conformation. Recrystallisation of **4.30** was not successful, leading to product decomposition.

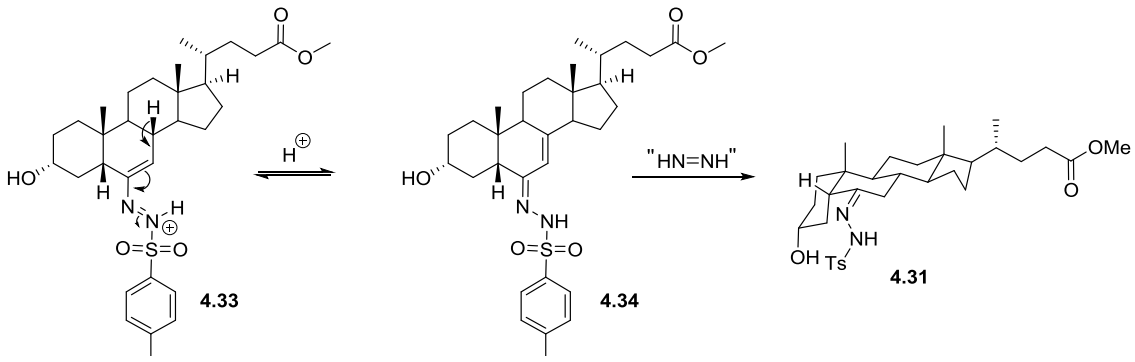


Scheme 4.14 Proposed mechanism for by-product 4.30.

A similar elimination process had been described in the literature for the substrates with α-substituents (α-hydroxyketone, α-aminoketones, etc).[119],[120] Collins *et al.* also explored the functionalization on glycosides *via* base-mediated elimination of benzoic acid and 1,4-addition starting from phenylhydrazone derivatives of pyranosides.[121] Applications of α-polyfluoro hydrazone to assist cyclisation through key intermediates from 1,4-elimination were also reported.[122],[123],[124]

However, the proposed mechanism for the diastereoseletive formation of **4.30** *via* 1,4-elimination of fluoride and conjugate addition of a nucleophile without base-catalysis has not been reported yet, which could be an interesting pathway for a one-pot synthesis to functionalize of α-position of carbonyl groups *via* α-fluoroketo intermediates.

The proposed by-product **4.31** is thought to be formed through reduction of compound **4.34** (**Scheme 4.15**) by diimide, which could be formed from tosyl hydrazide.

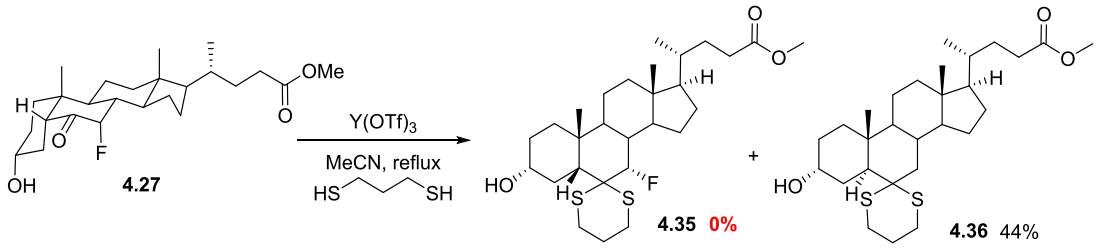


Scheme 4.15 Proposed mechanism for by-product 4.31.

Reduction of alkenes using tosyl hydrazide is often performed with addition of base.[93] But it was also reported in the literature that 2-nitrobenzenesulfonylhydrazide could play the same role without the additive.[125] Possibly, a slight excess of tosyl hydrazide could have acted in the same manner for this side reaction.

4.1.3.5.3 Deoxygenation *via* reductive desulfurisation of dithiane

Intermediate **4.35** (**Scheme 4.16**) was envisaged to achieve deoxygenation *via* reductive desulfurisation. Reaction towards the 1,3-dithiane was attempted using yttrium triflate as catalyst,[126] but no conversion was observed at room temperature. Thus, the reaction was heated under reflux, leading to the appearance of new compound(s) based on TLC analysis. Surprisingly, 7α-F disappeared according to ^{19}F NMR analysis of the crude reaction mixture.



Scheme 4.16 Preparation of dithiane intermediate 4.35.

A by-product **4.36** was obtained, with a proposed structure as shown, after NMR (Full 1D/2D NMR experiments) and HRMS analysis. A 1D NOE experiment with irradiation on H_{19} of **4.36** indicated no nuclear Overhauser effect for $H_{1\beta}$ and $H_{5\beta}$ (compared with 1D NOE experiments of compound **4.27**). In addition, epimerisation on C-5 was further confirmed by ^{1}H NMR analysis, as the multiplicity of H_3 was changed from a triplet of triplets to a broad singlet. Recrystallisation was attempted but unsuccessful.

The disappearance of 7α-F could be due to substitution by thiol or E2/E1 elimination but the mechanism towards the formation of **4.36** is still unclear.

4.1.4 Towards the synthesis of 12β-fluoro CDCA

4.1.4.1 Deoxyfluorination of 12α-OH group

Compound **4.37** was donated by Dextra Laboratories and its synthesis from CA was reported with very high yield in the literature.[127] The first attempt for fluorine introduction was by using DAST, as shown in **Scheme 4.17**.

Scheme 4.17 Deoxyfluorination of 12α-OH group.

The reaction led to a mixture of unidentifiable unfluorinated and fluorinated by-products, as illustrated by ^{19}F NMR spectrum in **Figure 4.7**. The separation of the major fluorinated product was not successful by using a Biotage (chromatographic purification) apparatus as the by-products had nearly the same R_f values. Further trial with Fluolead[128] (4-*tert*-butyl-2,6-dimethylphenylsulfur trifluoride) failed but with less side reactions.

As shown in **Figure 4.7**, the main fluorinated peak indicated a quartet with a coupling constant of ca. 30 Hz, which is not consistent with the multiplicity of desired product **4.38** (only one large $^2J_{HF}$ coupling constant was expected), if this compound had formed.

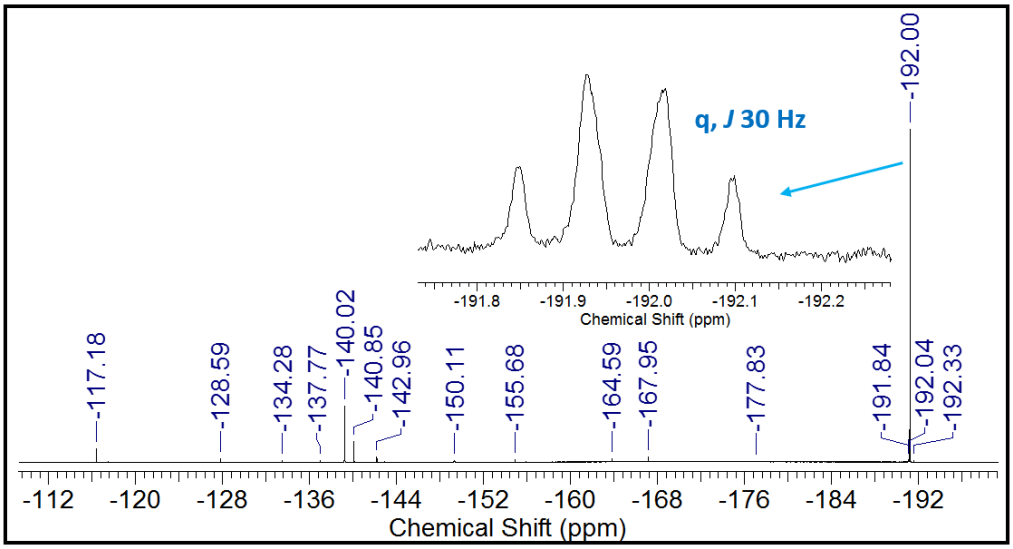
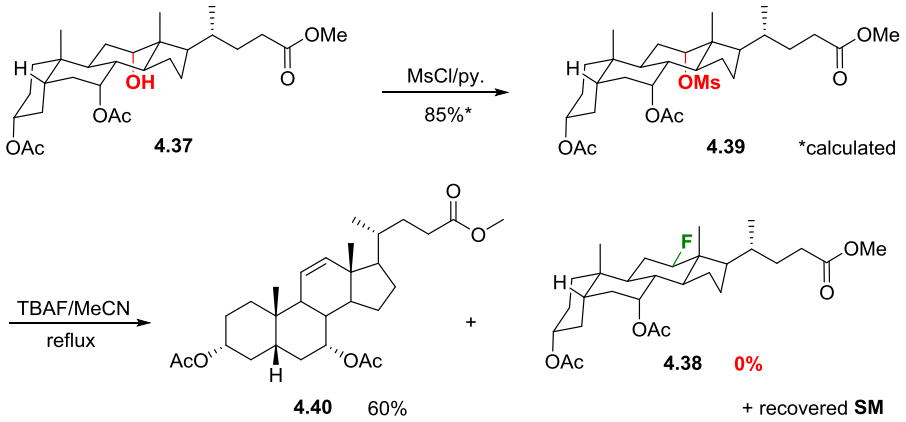


Figure 4.7 ^{19}F NMR of fraction containing fluorinated compounds after purification.

The formation of the by-products may be due to C-14 or C-18 migration, which will be discussed in detail in *section 4.3.2.1.3*.

4.1.4.2 S_N2 pathway *via* mesylate intermediate

As direct deoxyfluorination led to the undesired by-products, an approach *via* mesylate displacement was also attempted (**Scheme 4.18**). Mesylate **4.39** was prepared from **4.37** with a satisfactory yield. It was then subjected to fluorination using TBAF under reflux. However, only the corresponding 11-ene elimination product **4.40** was obtained, and no desired product **4.38** was formed. It is likely that the nucleophilic attack next to a quarternary carbon (C-13) is sterically unfavoured. The elimination then occurred due to the basicity of TBAF.

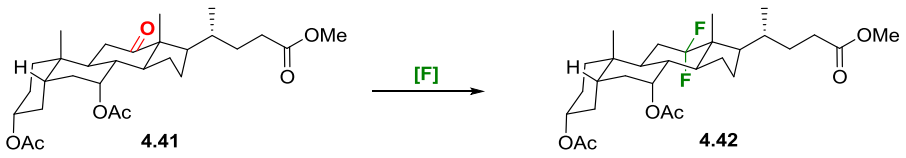


Scheme 4.18 S_N2 pathway *via* mesylation intermediate.

4.1.5 Synthesis of 12,12-difluoro CDCA

4.1.5.1 Deoxyfluorination on 12-keto intermediate 4.41

The synthesis of compound **4.41** was reported in the literature,[127] starting from CA with an overall yield of 88% over 3 steps. It was donated to us by Dextra Laboratories. Deoxofluorination of the 12-keto group (**Scheme 4.19**) was investigated, as listed in **Table 4.3**.



Scheme 4.19 Deoxyfluorination on 12-keto intermediate 4.41.

Table 4.3 Fluorination on 12-keto intermediate.

Entry	Solvent	Reagent	Condition	Yield
1	DCM	DAST	RT, O/N	0%
2	DCM	Fluorolead/HF	RT, 20 hr	0%
3	DCM	Fluorolead/HF	RT, 5 d	0%
4	n/a	Deoxofluor (50% in THF)	70 °C, O/N	trace
5	n/a	DAST (neat)	50 °C, 2 hr, then 4 d, RT	17%[1]
6	n/a	DAST (neat)	50 °C, 4 d	30%[2,3]
7	**n/a**	**DAST (neat, portionwise)**	**50 °C, 6 d**	**60%[2,3]**

[1]Based on ^{1}H NMR analysis; [2]Isolated yield; [3]Separated from starting material.

There was no conversion observed (**Entries 1-4, Table 4.3**) when DAST, Fluolead and Deoxofluor were used as solution. With neat DAST under mild heating, the yield of fluorination was up to 30% after 4 days (**Entries 5-6**). The yield was then improved to 60% by adding DAST portionwise over 6 days (**Entry 7**). The conversion can be potentially further increased, if the reaction time is extended. The quick isolation of the difluoro compound was achieved by using a Biotage apparatus, although compounds **4.42** and **4.41** had very similar R_f values.

The harsh reaction conditions were necessary for deoxyfluorination step, likely due to the 12-keto group being sterically hindered. This was clearly shown by the X-Ray structure of compound **4.41**. As illustrated in **Figure 4.8**, the hydrogen atoms on C-11, along with the 18- and 21-methyl groups can obstruct the nucleophilic attack on the C-12 carbonyl group.

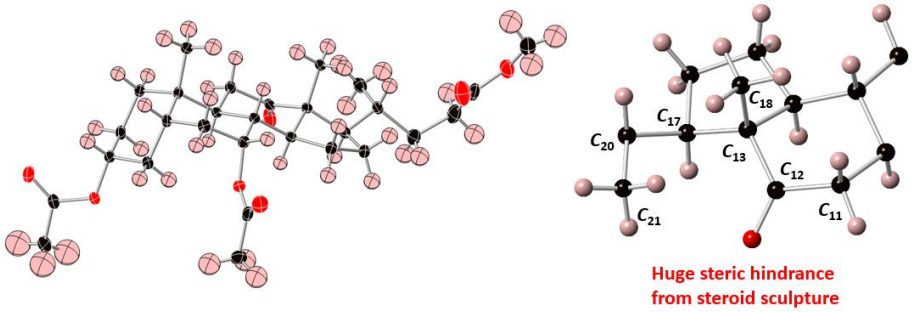


Figure 4.8 X-Ray structure of compound 4.41.

A coupling constant of $^{6}J_{F12\text{-}H21}$ 1.9 Hz was observed for compound **4.42**, which can be a through-space coupling. Similar six-bond coupling constants in rigid systems have been reported in the literature.[129] Surprisingly, a very large coupling constant of $^{5}J_{F12\text{-}C21}$ 10.6 Hz was also found, presumably due to a close proximity between the nuclei involved. Indeed, this is in good accordance with their spatial distance ($d_{F12\beta\text{-}C21}$: 2.89 Å; $d_{F12\beta\text{-}H21}$: 2.32 Å) as measured by X-ray crystallographic analysis of compound **4.42** (**Figure 4.9**). Interestingly, after fluorine introduction on C-12, as many as 12 out of 24 steroidal carbon atoms display multiplicity, due to the coupling with fluorine.

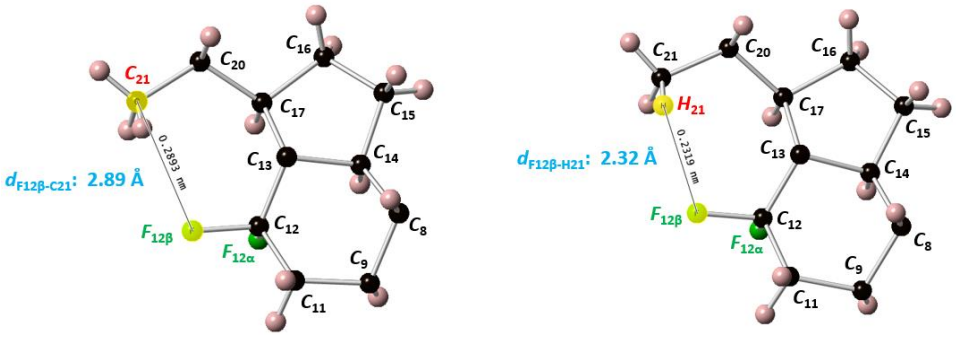
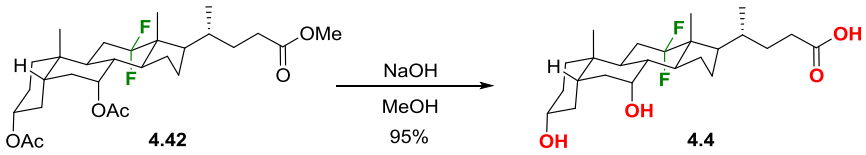


Figure 4.9 Close proximity between $F_{12\beta}$ and 21-methyl group from X-Ray structure of 4.42.

Interesting similarities regarding crystal packing and molecular geometries were observed between compound **4.41** and **4.42**, which will be discussed in *Chapter 5*.

4.1.5.2 Preparation of 12,12-difluoro CDCA

The target compound **4.4** was obtained after hydrolysis under basic condition (**Scheme 4.20**), and the structure was further confirmed by X-Ray analysis (**Figure 4.10**).



Scheme 4.20 Preparation of target compound 4.4.

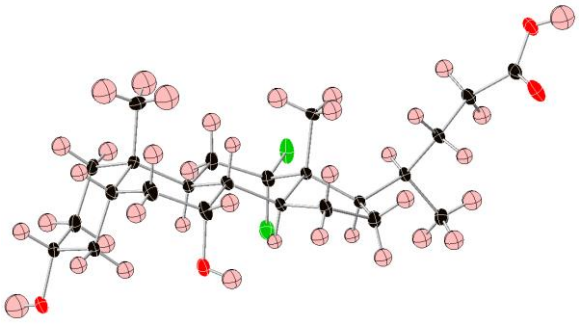
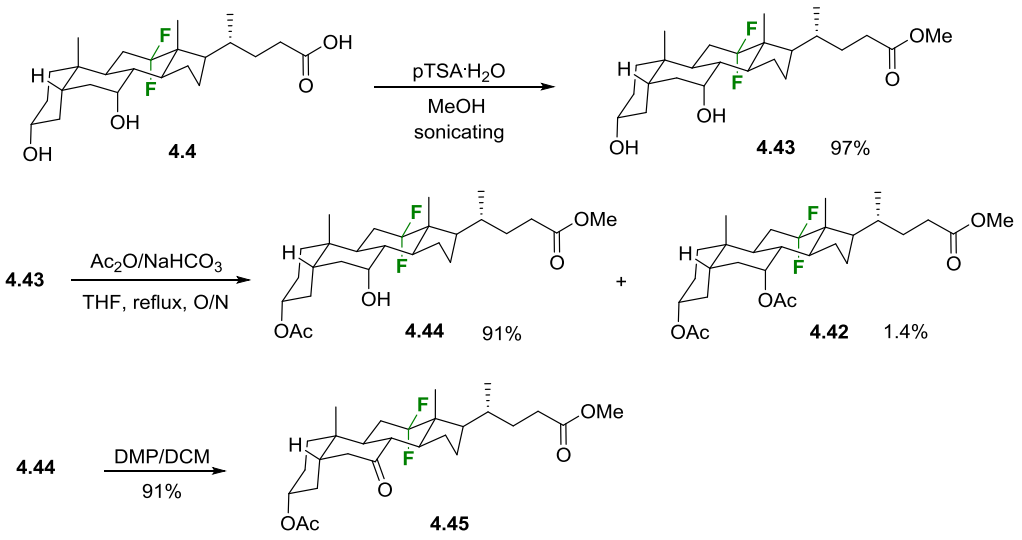


Figure 4.10 X-ray structure of target compound 4.4.

4.1.6 Synthesis of 7,7,12,12-tetrafluorinated LCA

4.1.6.1 Preparation of 7-keto intermediate

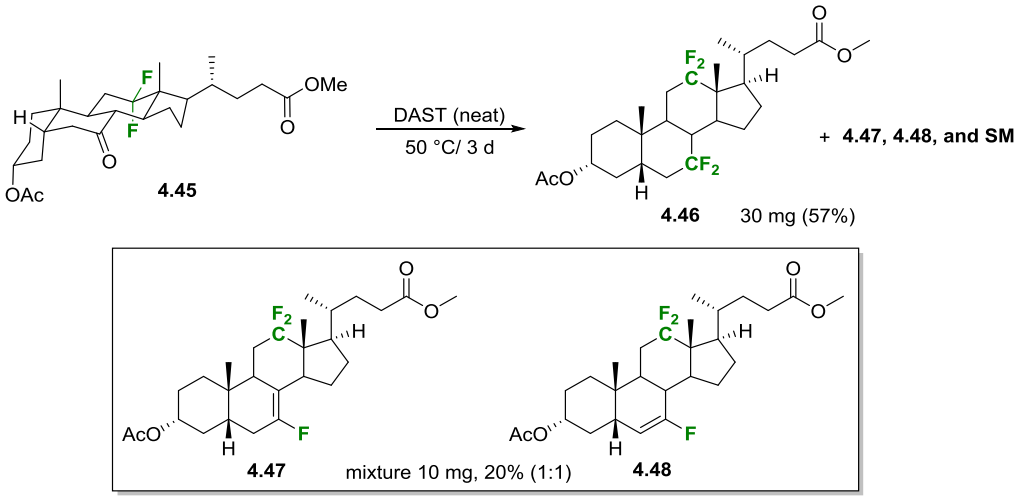
Compound **4.4** was esterified *via* sonication, leading to the methyl ester **4.43** (**Scheme 4.21**). Regioselective protection of 3α-OH group was achieved in high yield (91%) using acetic anhydride. The 7-keto intermediate **4.45** was then obtained from **4.44** by Dess-Martin periodinane (DMP) oxidation.



Scheme 4.21 Preparation of 7-keto intermediate 4.45.

4.1.6.2 Fluorination on 7-keto intermediate using neat DAST

Following the conditions established earlier, the ketone **4.45** was treated with neat DAST under heating (**Scheme 4.22**).

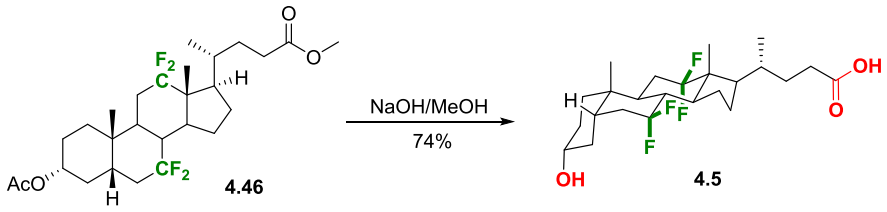


Scheme 4.22 Fluorination on compound 4.45 using DAST.

The desired tetrafluorinated compound **4.46** was obtained as major product. ^{13}C NMR analysis of **4.46** indicated that 16 carbon atoms had multiplicity due to the coupling with fluorine. Besides, fluoroalkenes **4.47** and **4.48** were also formed as elimination by-products.

4.1.6.3 Preparation of tetrafluorinated LCA

The target compound **4.5** was obtained after alkaline hydrolysis (**Scheme 4.23**).

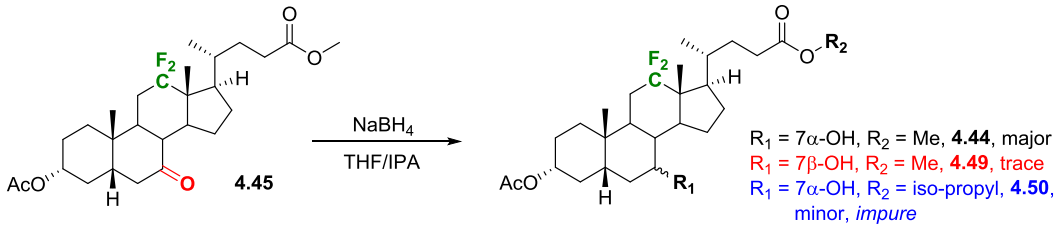


Scheme 4.23 Preparation of the target compound 4.5.

4.1.7 Synthesis of 12,12-difluoro UDCA

4.1.7.1 Reduction of 7-keto using $NaBH_4$

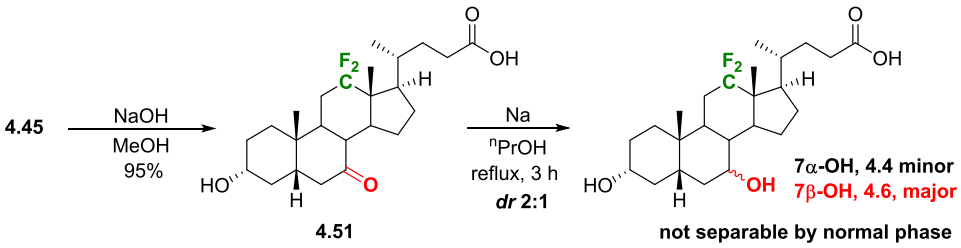
To access the corresponding 12,12-UDCA derivative, reduction of the 7-keto intermediate **4.45** with sodium borohydride (**Scheme 4.24**) was attempted. Unfortunately, this proceeded to give the 7α-configuration as the major product. Only a trace amount of the desired UDCA derivative **4.49** was formed, based on ^{19}F NMR analysis. Interestingly, isopropyl ester **4.50** was also isolated as by-product due to a transesterification process.



Scheme 4.24 Reduction of compound 4.45.

4.1.7.2 Hydrolysis and reduction using sodium

It was also reported in the literature that reduction of 7-keto LCA using alkali metals (such as Na and K) led to high diastereoselectivity for UDCA.[130] Thus, compound **4.51** was first prepared from **4.45** *via* hydrolysis (**Scheme 4.25**). Further reduction using sodium metal in *n*-propanol under reflux indeed resulted in higher selectivity for the formation of the target compound **4.6**.



Scheme 4.25 Hydrolysis and reduction using sodium.

However, the two isomers **4.4** and **4.6** with unprotected carboxylic aicd group were not separable by normal-phase chromatography, as shown in **Figure 4.11**. Interestingly, retention time for both

compounds were rather different in RP-HPLC, which implied that purification *via* reversed-phase chromatography could be possible. Alternatively, both of isomers can also be further esterified to examine their separation.

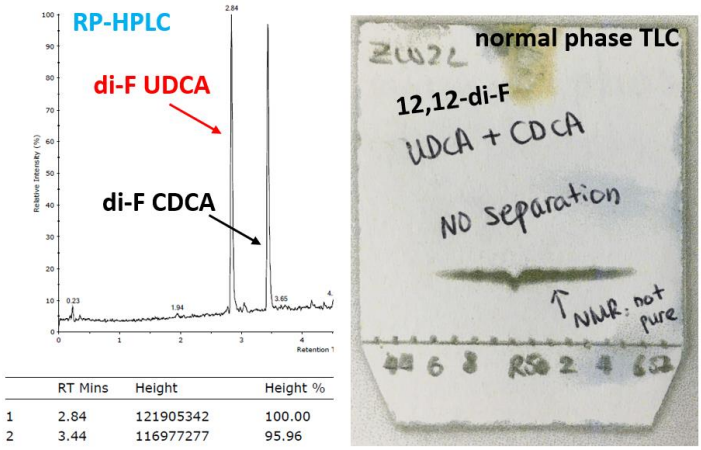
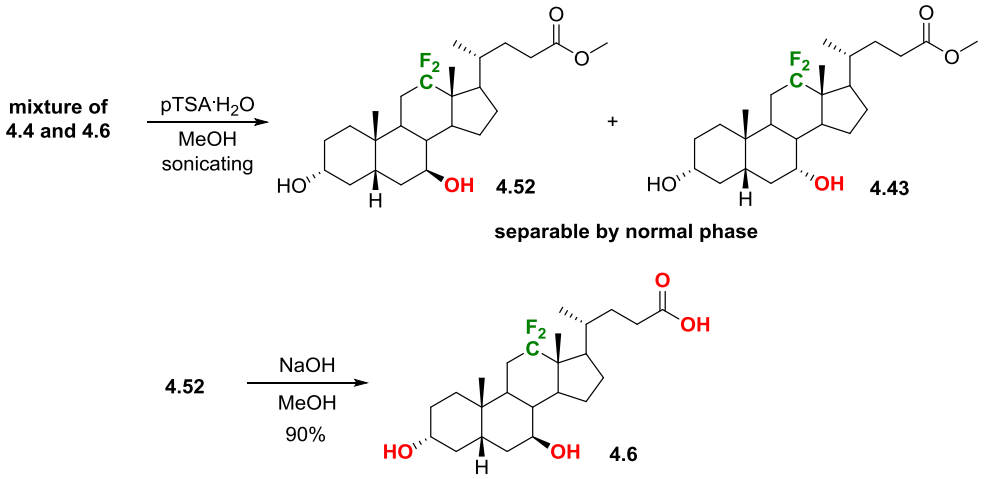


Figure 4.11 Comparison between separation in reversed-phase and normal-phase.

4.1.7.3 Isolation of 12,12-difluoro UDCA *via* methyl ester intermediate

As shown in **Scheme 4.26**, methyl esters of compound **4.4** and **4.6** were prepared and separated by flash chromatography. Further hydrolysis of compound **4.52** led to the target compound **4.6**.



Scheme 4.26 Isolation of compound 4.6 via methyl ester intermediate.

4.2 Synthesis of C-6 fluorinated B-Ring BA analogues

4.2.1 Synthetic plan

The insertion of fluorine on C-6 is envisioned *via* electrophilic fluorination, as shown in **Figure 4.12**. Reduction of the fluoroketones obtained from compound **4.57** can lead to target compound **4.53** and **4.54**. The synthesis of both compounds was also previously reported *via* enzymatic reduction of fluoroketones using *Xanthomonas maltophilia*, which contains 7α- and 7β-hydroxysteroid dehydrogenases (HSDH).[131],[132] We planned to use commercially available $NaBH_4$ as the reductant. Similarly, target compounds **4.55** and **4.56** could be synthesized starting from fluoroketone intermediate **4.58**.

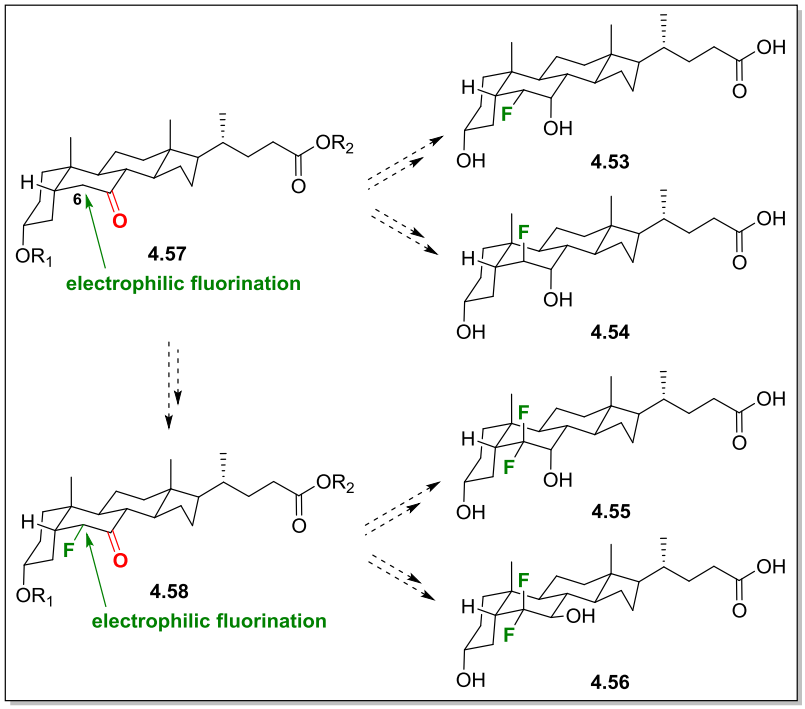


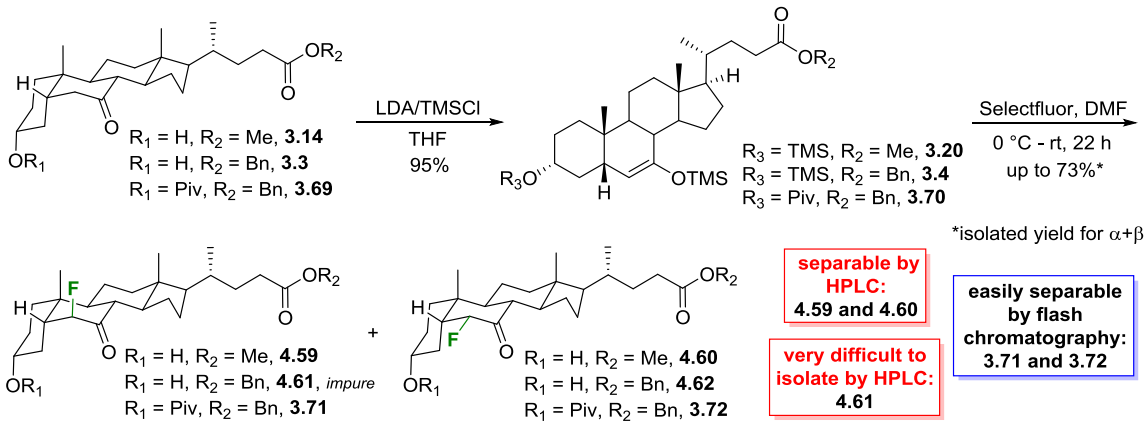
Figure 4.12 Synthetic plan for C-6 fluorinated analogues.

4.2.2 Synthesis of 6α-fluoro CDCA

4.2.2.1 Preparation of fluoroketone intermediates

As shown in **Scheme 4.27**, fluorination at the 6-position was achieved *via* silyl enol ether formation. Given the difficulty to separate fluorine diastereomers, a number of different protecting groups were tried. Treatment of the silyl enol ethers with Selectfluor in DMF led to the 6α-fluoro derivatives as the major products. The separation between compound **4.59** and **4.60** was achieved by using HPLC. In contrast, benzyl ester derivative **4.61** was not separable from **4.62**, even by HPLC.

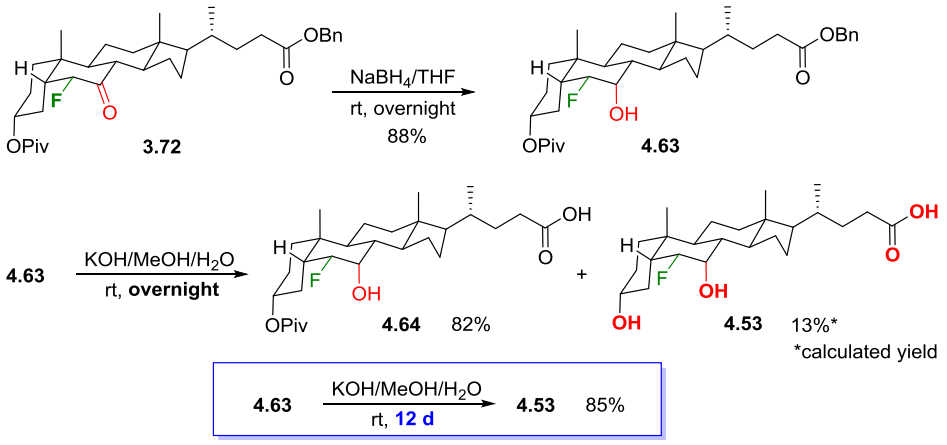
Interestingly, pivaloylation of 3α-OH group significantly simplified the purification, and diastereoisomers **3.71** and **3.72** were easily separated by column.



Scheme 4.27 Electrophilic fluorination.

4.2.2.2 Preparation of 6α-fluoro CDCA

Reduction of fluoroketone **3.72** led selectively to the desired diastereoisomer **4.63** (**Scheme 4.28**). Deprotection of benzyl ester and pivaloyl group were designed to be achieved by alkaline hydrolysis. However, cleavage of pivaloyl group proceeded rather slowly, due to its intrinsic steric hindrance hampering hydroxide attack. Only 13% of conversion towards the target compound **4.53** was observed after hydrolysis overnight. Due to the possibility of side reactions upon heating, the hydrolysis was left at room temperature up to 12 days to reach full conversion, leading to the target compound **4.53**.

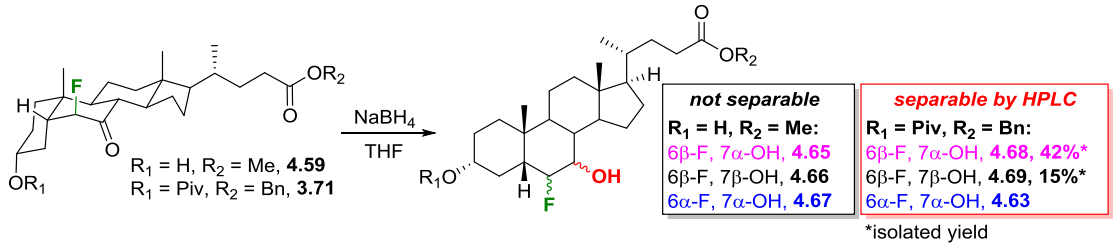


Scheme 4.28 Preparation of the target compound 4.53.

4.2.3 Synthesis of 6β-fluoro CDCA

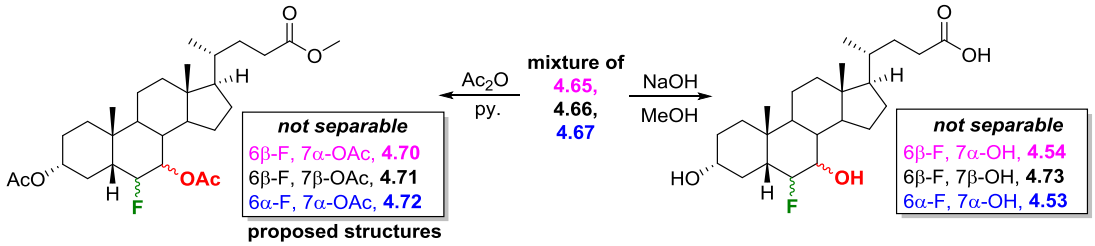
4.2.3.1 *Via* fluoroketone reduction

Fluoroketone derivatives **4.59** and **3.71** were reduced by $NaBH_4$ in THF (**Scheme 4.29**). Surprisingly, 3 diastereoisomers were formed. The formation of compound **4.67** and **4.63** was due to epimerisation on C-6. The isomer separations again proved cumbersome, with the methyl esters **4.65**-**4.67** being inseparable, in contrast to the 3α-protected benzyl ester derivatives **4.69**, **4.69**, and **4.63**, which were separable by HPLC.



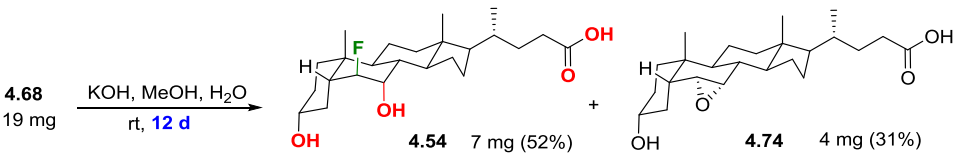
Scheme 4.29 Reduction of 6β-fluoro ketone derivatives.

The mixture of methyl esters (**4.65**-**4.67**) was also acetylated or hydrolysed, to examine the separation (**Scheme 4.30**). However, no improvement was observed, based on TLC analysis.



Scheme 4.30 Acetylation and hydrolysis to examine separation.

The hydrolysis of compound **4.68** again required about 12 days at room temperature to give target compound **4.54** (**Scheme 4.31**). An epoxide by-product **4.74** was also isolated, which structure was unambiguously confirmed by X-ray crystallographic analysis (**Figure 4.13**).



Scheme 4.31 Preparation of the target compound 4.54.

However, when this reaction was repeated on a larger scale (250 mg), no desired product **4.54** was obtained. Instead, only small amount of epoxide **4.74** was isolated from a complex mixture containing fluorine products.

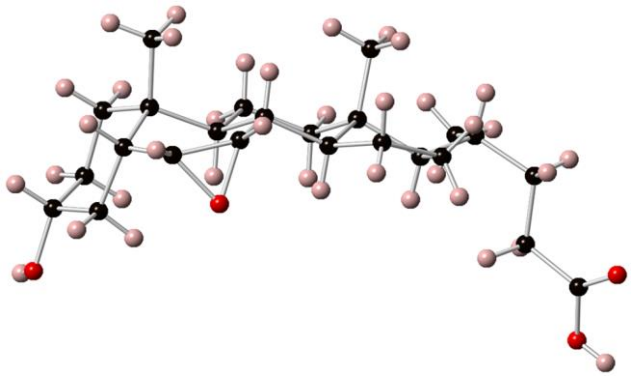
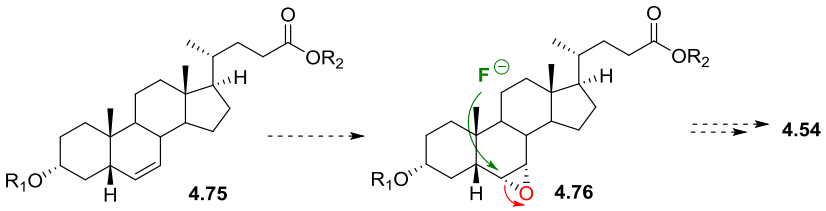


Figure 4.13 X-ray structure of compound 4.74.

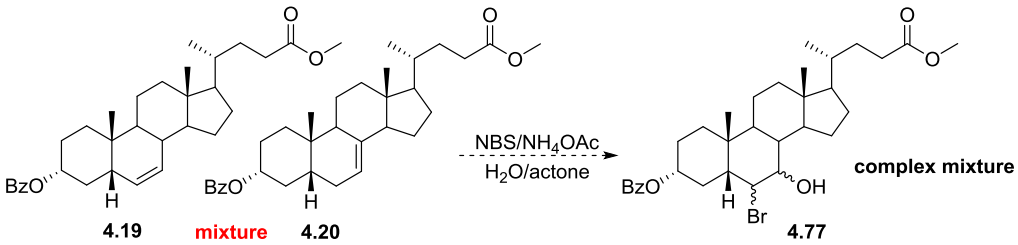
4.2.3.2 Alternative route towards 6β-fluoro CDCA

As side reactions occurred with compound **4.68** (containing diaxial fluorohydrin moiety) under strong alkaline condition, the synthetic route *via* 6α,7α-epoxide **4.76** was also envisioned, as shown in **Scheme 4.32**. Epoxide ring opening with fluoride source can lead to the key precursor for 6β-fluoro CDCA.



Scheme 4.32 Alternative plan towards compound 4.54.

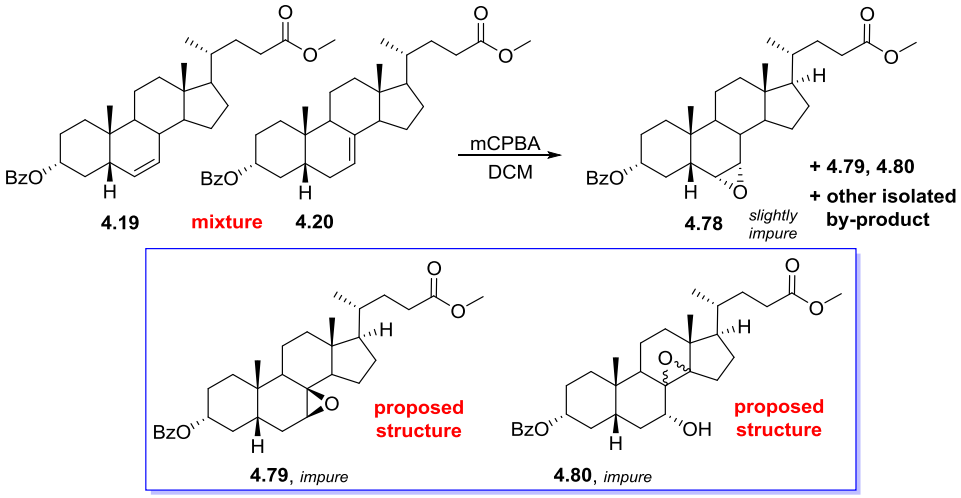
The first attempt was to synthesize bromohydrin intermediates, which can be used to prepare the 6α,7α-epoxide. As the mixture of alkene **4.19** and **4.20** was available, it was used directly for this purpose (**Scheme 4.33**). However, a complex mixture was obtained and the desired product **4.77** was difficult to identify and to isolate.



Scheme 4.33 Preparation of bromohydrins.

The mixture of alkenes was also treated directly with mCPBA (**Scheme 34**). Apart from the desired compound **4.78**, several other by-products were also formed. The separation by flash chromatography was very challenging and time-consuming. The stereochemistry for compound

4.78 was comfirmed by comparision of NMR data with **4.74** and also with the known 6α,7α-epoxy-5β-cholanoate derivative.[133]



Scheme 4.34 Epoxidation on alkene mixture.

The proposed structure for compound **4.79** was supported by full NMR, IR and HRMS analysis. In addition, the chemical shift and multiplicity of $H_{7\alpha}$[8] (**Figure 4.14**) was consistent with literature data for 7β,8β-epoxide derivative **4.81**.[134] The $H_{7\beta}$ of the corresponding 7α,8α-epoxide **4.82** is more deshielded, with a chemical shift close to 3.30 ppm.[134],[135]

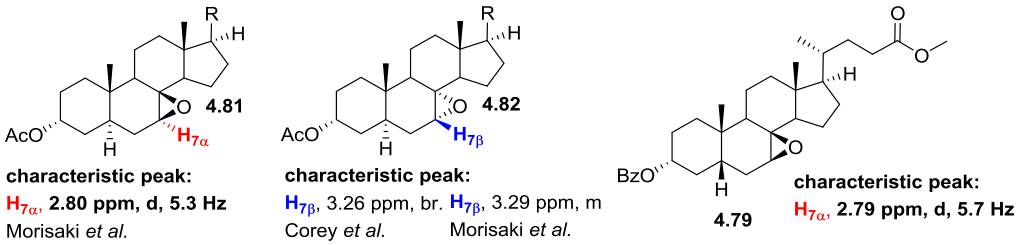
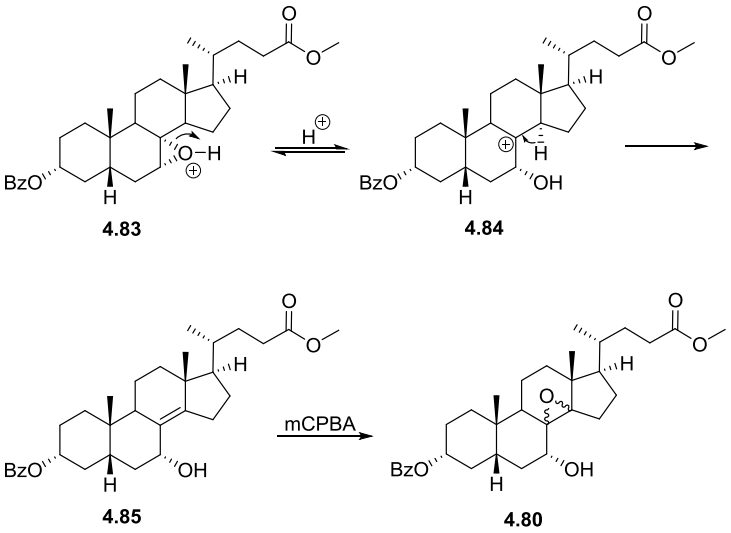


Figure 4.14 Identification of epoxide configuration by NMR data.

Interestingly, the 7α,8α-epoxide **4.83** (**Scheme 4.35**) was not found after purification. Instead, compound **4.80** was isolated (**Scheme 4.34**), proposed structure based on full NMR, IR and HRMS analysis). The possible mechanism is shown in **Scheme 4.35**. The acid catalysis on **4.83** led to allylic alcohol derivative **4.85**. Under further oxidation by mCPBA, 8,14-epoxy by-product **4.80** was then formed. In fact, treatment of compound **4.82** with acid and mCPBA similarly resulted in 7α-hydroxy-8,14-epoxide as well as 7α-hydroxy-8,9-epoxide derivative.[134] The assignment of the obtained regioisomer **4.80** was based on $H_{7\beta}$ chemical shift (found: δ 3.54 ppm; reported: 3.56 ppm for 7α-hydroxy-8,14-epoxide derivative, and 3.94 ppm for 7α-hydroxy-8,9-epoxide derivative).[134]

[8]From molecular models, it was assumed that the nature of the AB ring junction would not have a major influence on the H_7 chemical shift value.



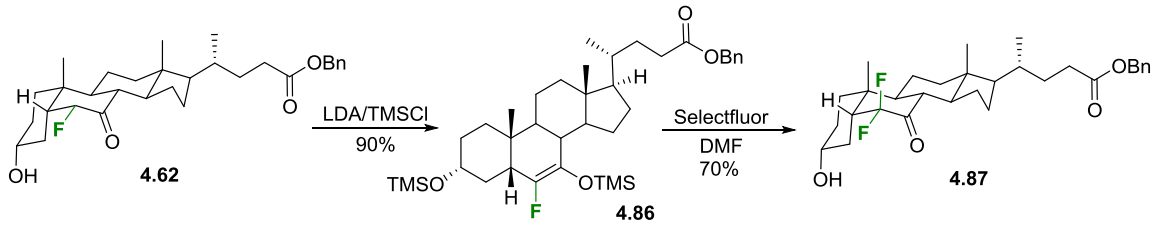
Scheme 4.35 Proposed mechanism for formation of compound 4.80.

Due to the low yield and difficulty in isolation of desired epoxide **4.78**, it is planned to use the isolated 6-ene intermediate for preparation of epoxide for future work. The systhesis of 6-ene derivative was reported in the literature (overall yield 38% in five steps from methyl ester of CDCA).[111]

4.2.4 Synthesis of 6,6-difluoro CDCA/UDCA

4.2.4.1 Synthesis of difluoro ketone intermediate

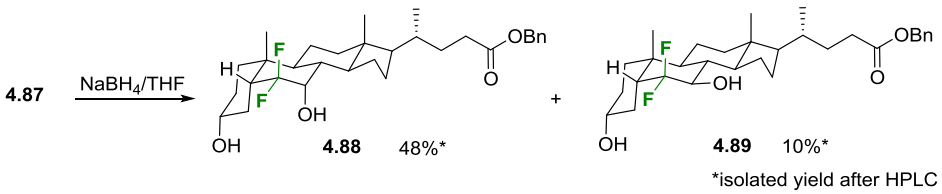
Starting from compound **4.62**, difluoro ketone **4.87** was obtained *via* treatment of fluorinated silyl enol ether with Selectfluor (**Scheme 4.36**). Interestingly, ^{1}H and ^{13}C NMR of **4.86** indicated a long-range 6-bond fluorine-hydrogen coupling ($^{6}J_{F\text{-}H(OTMS)}$ 1.7 Hz), and a 5-bond fluorine-carbon coupling ($^{5}J_{F\text{-}C(OTMS)}$ 3.5 Hz).



Scheme 4.36 Preparation of difluoro ketone 4.87.

4.2.4.2 Reduction of ketone intermediate

The reduction of **4.87** led to the desired CDCA derivative **4.88** as major product (**Scheme 4.37**) along with UDCA isomer **4.89**, with separation achieved by HPLC.



Scheme 4.37 Reduction of difluoro ketone 4.87.

Due to the overlapping of key protons in the ^{1}H NMR spectrum, and the poor resolution of the fluorine multiplets, the ^{13}C NMR spectrum was analysed for determination of the configuration on C-7. It was reported by Wray that the magnitude of $^{2}J_{FC}$ coupling constant was dependent on the orientation of the electronegative substituents on the coupled carbon.[136] He concluded that $^{2}J_{FC}$ coupling constant of a vicinal *trans*-orientation was larger than that of a vicinal *gauche*-orientation.[136]

As illustrated in **Figure 4.15**, C_7 of compound **4.88** was observed as doublet of doublets, with the larger coupling constant being about 36 Hz. In comparison with **4.89**, where the C_7 appeared as triplet (*J* 21 Hz), it was concluded that **4.88** has an axial hydroxyl group. The configuration of **4.88** and **4.89** was further confirmed upon comparison with the C_1 coupling constants of 4-*t*-butyl-2,2-difluorocyclohexan-1-ol isomers (**Figure 4.15**).[33]

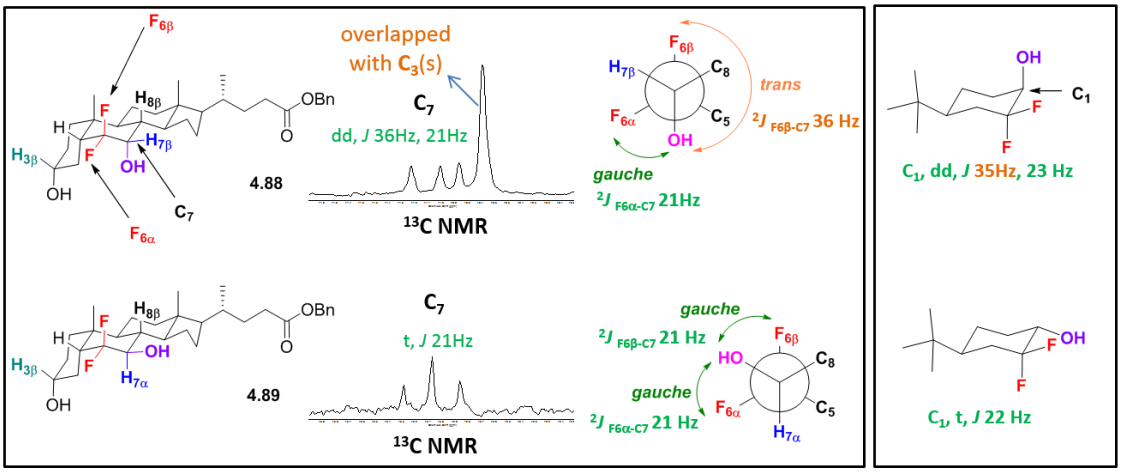
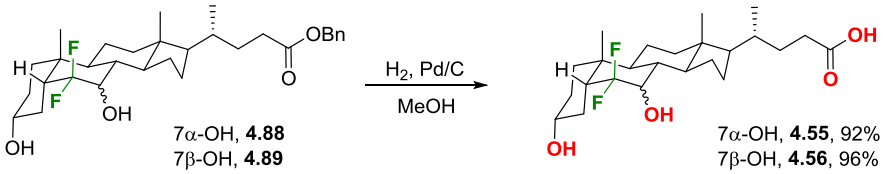


Figure 4.15 Identification of configuration on C-7 by ^{13}C NMR.

4.2.4.3 Preparation of 6,6-difluoro CDCA and UDCA

The target compounds **4.55** and **4.56** was finally obtained after cleavage of the benzyl group by hydrogenolysis (**Scheme 4.38**).



Scheme 4.38 Hydrogenolysis on benzyl ester.

4.3 Towards the synthesis of C-11 fluorinated C-Ring BA analogues

4.3.1 Introduction of synthetic plan

As shown in **Figure 4.16**, target compounds **4.90** and **4.91** were designed to be synthesised from alkene precursor **4.40**, which can be prepared from compound **4.39**. Fluorine introduction on C-11 was envisaged, either *via* epoxide ring opening using nucleophilic fluoride (for compound **4.90**) or *via* bromofluorination (for compound **4.91**).

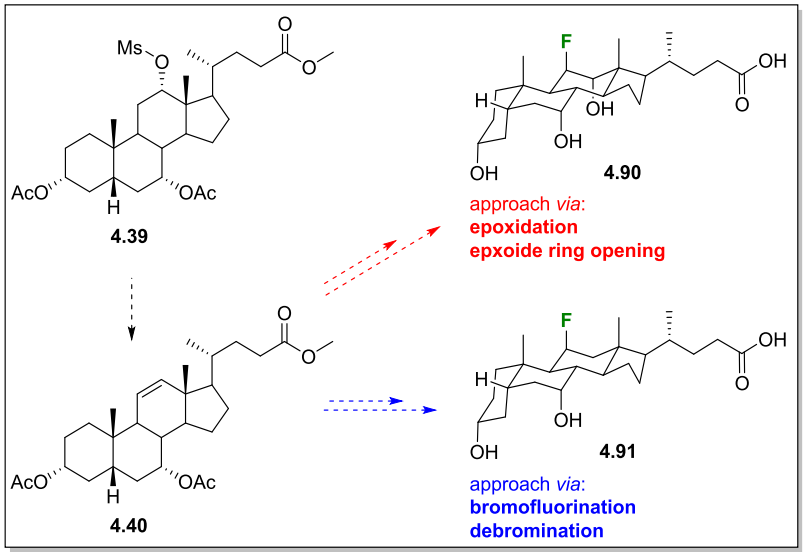


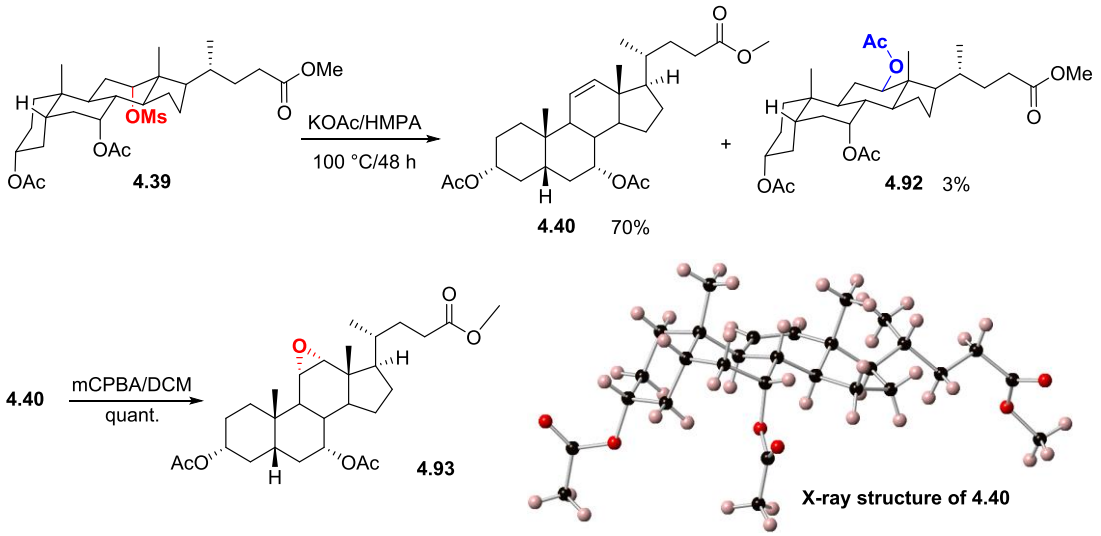
Figure 4.16 Synthetic plans for target compounds 4.90 and 4.91.

4.3.2 Attempted synthesis of 11β-fluoro CA 4.90

4.3.2.1 Proposed route *via* the epoxide intermediate

4.3.2.1.1 Preparation of the epoxide

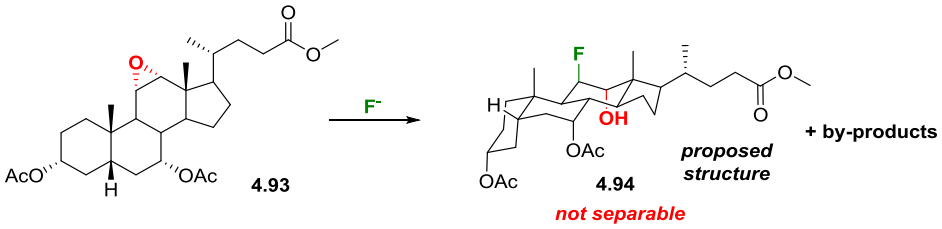
According to the literature procedures,[137] mesylate **4.39** was treated with KOAc in HMPA for 2 days to achieve complete elimination (**Scheme 4.39**), and the isolated yield for alkene **4.40** after crystallization was 70%. Compound **4.92** was also obtained as by-product after purification, because carboxylate can act as a nucleophile. Further epoxidation using mCPBA led diastereoselectively to 11α,12α-epoxide **4.93**. As described in the literature, the 18-methyl group directs facial attack to the β-face.[137]



Scheme 4.39 Preparation of epxide 4.93.

4.3.2.1.2 Epoxide ring opening

The nucleophilic attack at C-11 of the 11α,12α-epoxide **4.93** is stereoelectronically favoured as it leads to a stable C-ring chair conformation. Regioselective ring opening at C-11 by amines and thiols has been reported in the literature.[137],[138] As shown in **Table 4.4**, the epoxide was treated with several nucleophilic fluoride sources and reaction conditions, for the formation of the desired fluorohydrin **4.94** (**Scheme 4.40**).



Scheme 4.40 Epoxide ring opening.

Table 4.4 Epoxide ring opening.

Entry	Solvent	Reagent	Condition	Reaction outcome
1	DCM (1 mL/mmol)	HF·py (20 eq)	RT, O/N	side reactions; **4.94**, minor;
2	DCM (40 mL/mmol)	HF·py (10 eq)	RT, 2 hr then O/N	Side reactions; **4.94**, minor;
3	DCM (40 mL/mmol)	HF·py (3 eq)	RT, 2 hr then O/N	side reactions; very low conversion;
4	DCM (2 mL/mmol)	HF·py (77 eq)	-78 °C, 2 hr	side reactions; low conversion;
5	DCM (20 mL/mmol)	HF.TEA (10 eq)	RT, 2 hr then O/N	**4.94**, trace;
6	MeCN	TBAF	reflux, O/N	side reactions;

Treatment of concentrated **4.93** with HF·py led to the full consumption of epoxide (**Entry 1**, **Table 4.4**). Apart from the unidentifiable unfluorinated by-products, there were two fluorinated compounds formed. As shown by TLC analysis (**Figure 4.17**), there was no separation between those two products. ^{1}H and ^{19}F NMR analysis (**Figure 4.17**) suggested the formation of the desired compound **4.94** as a minor product. The other fluorinated compound was assumed to have a tertiary fluoride (δ_F ca. -152 ppm), based on the comparison with reported fluorine chemical shifts.[139] No significant improvement was observed for trials at lower concentration and temperature (**Entries 2-4**). When HF·TEA was used (**Entry 5**), only trace amounts of fluorinated compounds were formed based on ^{19}F NMR analysis. The epoxide was also treated with TBAF under reflux (**Entry 6**). No desired compound was obtained, and cleavage of ester groups was also observed.

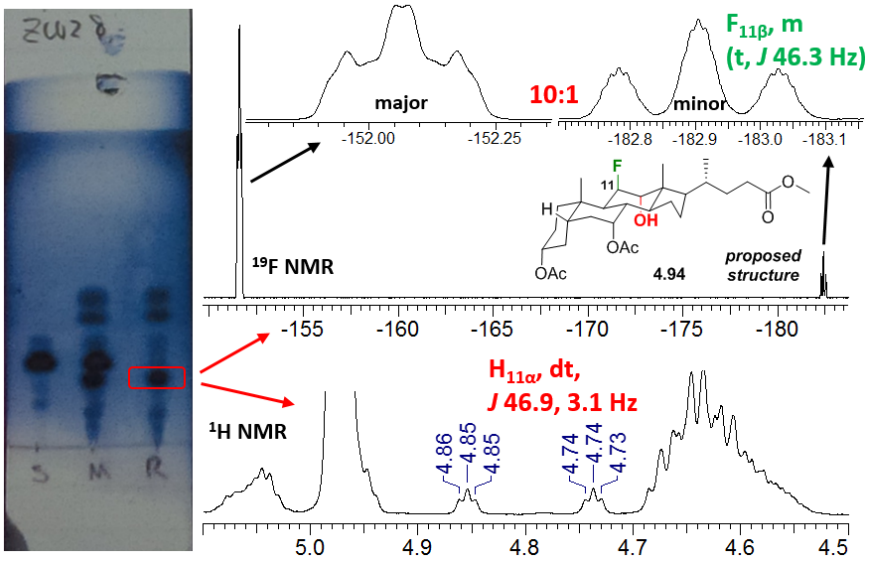
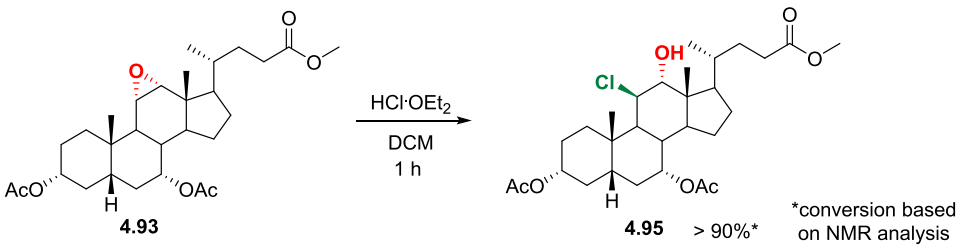


Figure 4.17 TLC and NMR analysis of mixture fraction containing fluorine.

For comparison purposes, compound **4.93** was also treated with HCl (**Scheme 4.41**). Interestingly, chlorohydrin derivative **4.95** was obtained with high conversion after 1 h, based on NMR analysis of crude reaction mixture. As chloride is a better nucleophile than fluoride in acidic media, epoxide ring opening by chloride proceeded much faster than the competitive side reactions.



Scheme 4.41 Formation of chlorohydrin.

4.3.2.1.3 Possible side reactions

Both the deoxyfluorination of the 12α-OH group (*see section 4.1.4.1*), and the epoxide ring opening by fluoride were constrained by side reactions. It was interesting to find that the presence of a good leaving group on C-12 can induce C-18 migration,[140],[141] or C-14 migration onto C-12,[142],[143],[144] as illustrated in **Figure 4.18**.

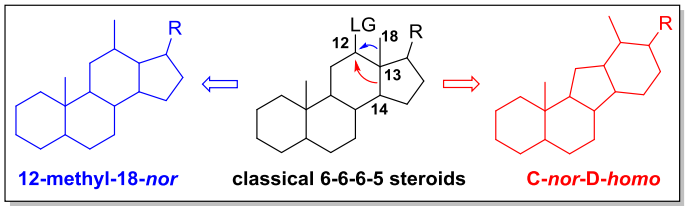


Figure 4.18 Possible side reactions on C-12.

Therefore, it is assumed that similar rearrangements could also occur on epoxide ring opening process (**Figure 4.19**). Indeed, the formation of a tertiary fluoride was deduced from ^{19}F NMR analysis as major fluorinated compound (**Figure 4.17**).

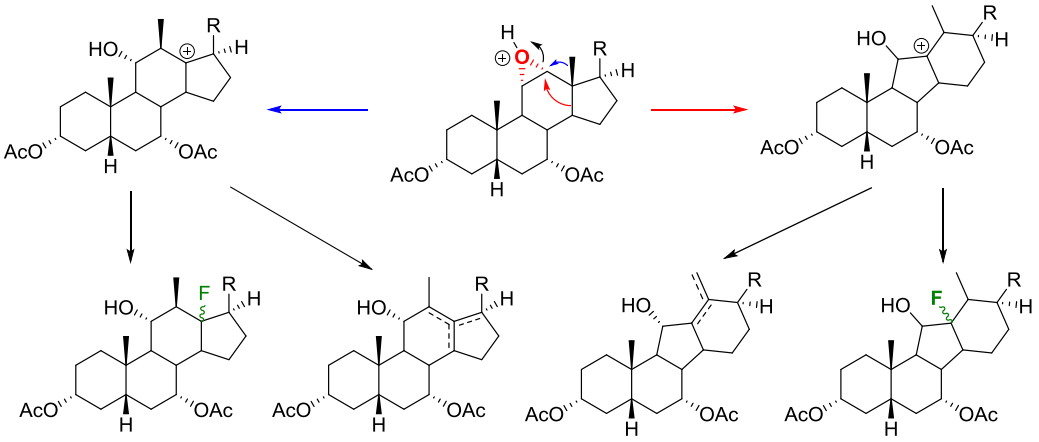
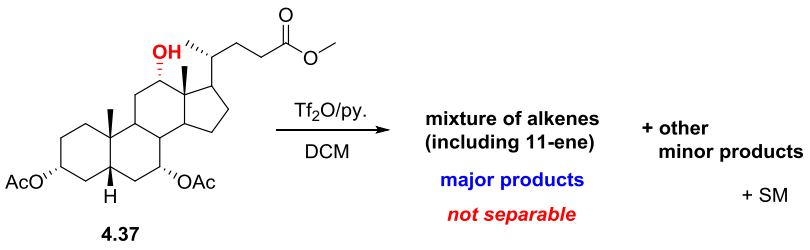


Figure 4.19 Proposed side reactions and by-products for epoxide ring opening.

The assumed side reactions on the bile acid structure were consistent with results from triflation of the 12α-OH group (**Scheme 4.42**), where ^{19}F NMR analysis indicated the possible formation of only a small amount of the triflate product.

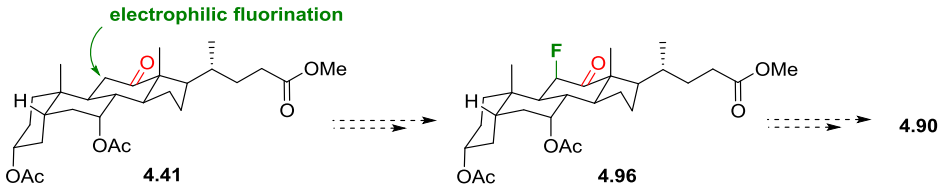


Scheme 4.42 Triflation of the 12α-OH group.

Instead, mixture of alkene products (based on HRMS and NMR analysis) was obtained after purification. Interestingly, 11-ene **4.40** was only a minor product. This strongly indicated the formation of other alkenes as a result of rearrangement mechanisms. Recrystallisation was further attempted but not successful.

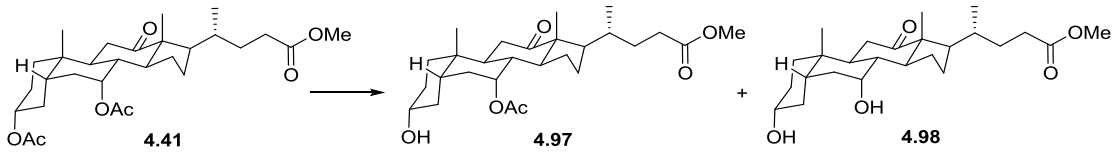
4.3.2.2 Alternative route *via* electrophilic fluorination

As it was difficult to introduce 11β-fluoro on C-11 through epoxide ring opening, an electrophilic fluorination pathway was also attempted, as shown in **Scheme 4.43**.



Scheme 4.43 Alternative route towards target compound 4.90.

Compound **4.41** was subjected to alcohol deprotection conditions for the formation of compound **4.98** (**Scheme 4.44**, **Table 4.5**). The cleavage of 7α-acetyl group was rather slow (**Entry 1**), presumably due to the steric hindrance. No significant improvement was observed when NaOMe solution was used (**Entry 2**).



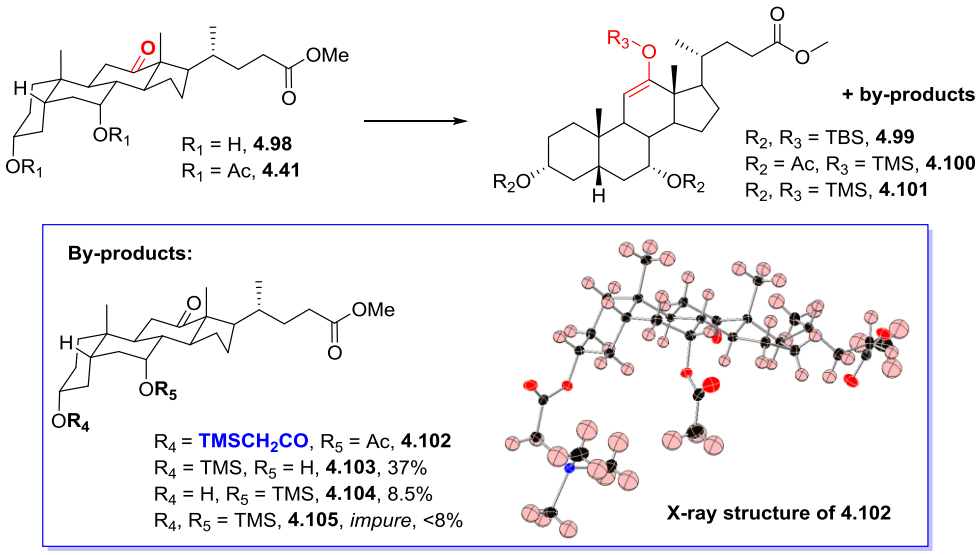
Scheme 4.44 Deprotection of acetate groups.

Table 4.5 Deprotection of acetate groups.

Entry	Solvent	Reagent	Condition	Yield
1	MeOH	K_2CO_3	RT, O/N	**4.97**: 80%; **4.98**: 20%; (^{1}H NMR)
2	MeOH	NaOMe	RT, 4 d	**4.97**: 15%; **4.98**: 68%; (isolated yield)

The preparation of silyl enol ether intermediates (**Scheme 4.45**) was attempted by using different reagents and conditions (**Table 4.6**). Treatment of compound **4.98** with TBSOTf mainly caused side reactions (**Entry 1**). TMSOTf was also attempted with **4.41** (**Entry 2**) but interestingly, the C-silylated side-product **4.102** was obtained (confirmed by X-ray structure, as shown in **Scheme 4.45**). When LDA was used for direct deprotonation (**Entry 3**), no desired **4.101** was formed. Instead, silyated by-products **4.103**-**4.105** were obtained after purification. Sterically less hindered NaH was then used, but no conversion was observed (**Entries 4-5**). With increased equivalents of base and extended reaction time (**Entry 6**), only side reactions occurred, such as cleavage of ester groups.

It is assumed that the failure of the formation of silyl enol ethers was mainly due to the high ring strain for enolate formation, or the steric hindrance which prevented the nucleophilic attack of enolate on TBS/TMS derivatives.



Scheme 4.45 Preparation of silyl enol ether intermediates.

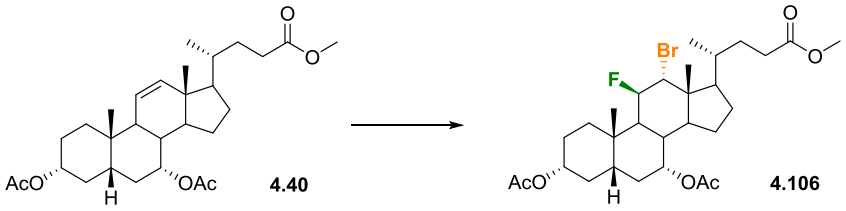
Table 4.6 Preparation of silyl enol ethers.

Entry	Reagent	R_1	R_2	R_3	Reaction outcome
1	TBSOTf/TEA	H	TBS	TBS	**4.99**, 0%; side reactions;
2	TMSOTf/TEA	Ac	Ac	TMS	**4.100**, 0%; by-product: **4.102**, 90%;
3	LDA/TMSCl	H	TMS	TMS	**4.101**, 0%; by-products: **4.103**, **4.104**, **4.105**; SM recovered;
4	a) NaH (1.05 eq) b)TMSCl	Ac	Ac	TMS	**4.100**, 0%; SM recovered;
5	a)NaH (10 eq) b)TMSCl	Ac	Ac	TMS	**4.100**, 0%; SM recovered;
6	a)NaH (15 eq) b)TMSCl	Ac	Ac	TMS	**4.100**, 0%; side reactions after O/N;

In addition, direct electrophilic fluorination on compound **4.41** (using Selectfluor under acid catalysis) was also attempted. But the reaction led to a complex mixture containing many fluorinated compounds.

4.3.3 Attempted synthesis of 11β-fluoro CDCA 4.91

Based on a literature procedure,[145] 11-ene **4.40** was treated with NBS and nucleophilic fluoride for the preparation of compound **4.106**, as shown in **Scheme 4.46**.



Scheme 4.46 Bromofluorination on 11-ene 4.40.

Table 4.7 Bromofluorination on 11-ene derivative.

Entry	Solvent	Reagents	Condition	Reaction outcome
1	MeCN	NBS/TBAF	RT, 1 hr	**4.106**, trace; SM recovered;
2	Et_2O	NBS/HF·py	RT, 2 hr	**4.106**, formed but not separable;

When TBAF was used as fluoride source (**Entry 1**, **Table 4.7**), only a trace amount of fluorinated compound was formed, as indicated by ^{19}F NMR analysis. Replacement of TBAF by HF·py led to the formation of the desired product **4.106** (**Entry 2**). However, it was not possible to separate this bromofluorinated intermediate from by-products by flash chromatography.

For future work, radical debromination on compound **4.106** is planned, and the separation between debrominated compound and the impurities will be examined.

4.4 Conclusion

The following target compounds (**Figure 4.20**) have been successfully synthesised *via* different synthetic approaches.

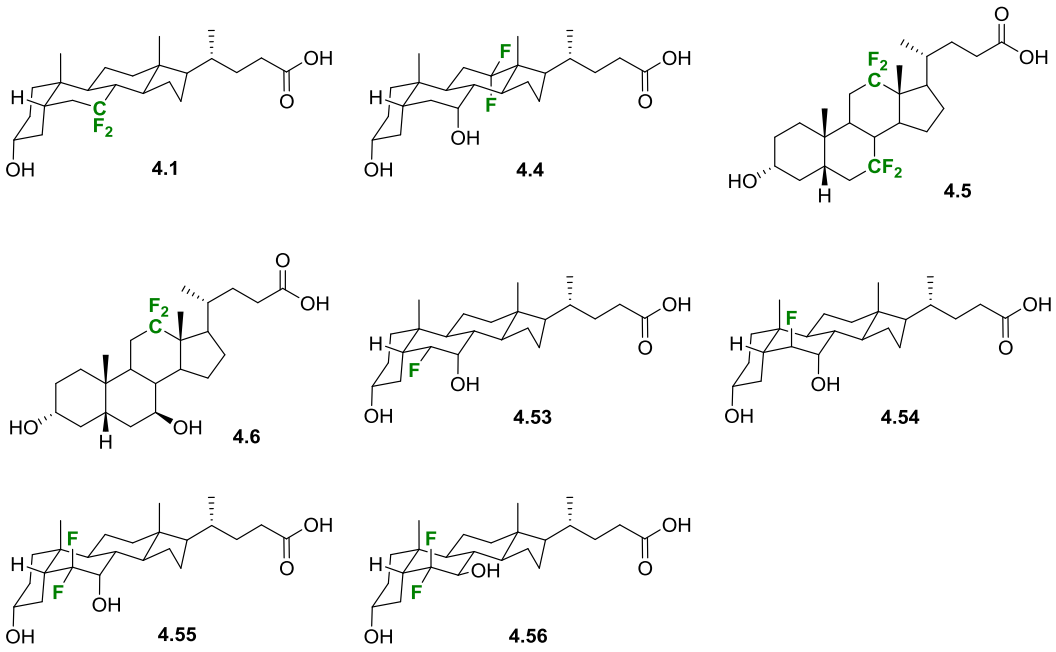


Figure 4.20 Successfully synthesised fluorinated BA analogues.

The 7,7- difluoro analogue of LCA was obtained *via* deoxyfluorination under harsh conditions. Same strategy also led to the syntheses of 12,12-difluoro CDCA/UDCA, and also tetrafluorinated LCA analogue. The syntheses of 6-monofluoro CDCA analgues and 6,6-difluoro CDCA/UDCA were achieved *via* electrophilic fluorination.

For 7α-fluoro LCA, nucleophilic fluorination on alcohol intermediate and the preparation of triflate for intended fluorination were not successful. It was either due to challenges of separation of diastereoisomers, or becasue of the failure of obtaining desired intermediate due to side reactions. Hydrogenation of fluoroalkenes also ended up with failure due to the undesired diastereoselectivity. Elimination of 7α-F also occurred for deoxygenation pathways.

With the C-12 deoxyfluorination step and the mesylate displacement unsuccessful, no further reactions were tried towards 12β-fluoro CDCA, given the steric hindrance and potential for rearrangements appeared unsurmountable.

Besides, the synthesis of 11β-fluoro CA was not successful, due to the side reactions for epoxide ring opening and the difficulties in preparation of 11-fluoro ketone intermediates. Regarding 11β-fluoro CDCA, fluorine was successfully inserted to the C-11 position *via* bromofluorination, and further debromination is needed to examine the separation towards the target compound.

Chapter 5: X-ray Studies of BA Derivatives

5.1 Introduction

Crystal engineering is defined as ''the understanding of intermolecular interactions in the context of crystal packing and in the utilisation of such understanding in the design of new solids with desired physical and chemical properties''.[146],[147] It has found significant applications in pharmaceutical industry, regarding modulating drug solid-state properties, processing and formulation.[148],[149],[150],[151] For crystal packing, hydrogen bond interactions have been identified as one of the key directional forces for supramolecular assembly.[152] In addition, the weak interactions (e.g., halogen···halogen interactions, π-stacking, C-H···π and C-H···O interaction, etc.) also contribute to stable packing.[153],[154],[155],[156]

Recently, increasing amounts of crystallographic evidence suggested that interactions involving fluorine are also of importance in this regard.[157],[158],[159],[160] Main fluorine-induced intermolecular interactions (C-F···H, C-F···π_F, F···F and π···π_F) have been identified, which can contribute to the overall packing motif.[161] Therefore, fluorine can be applied as a tool for crystal engineering.

Interestingly, it has been reported that crystal packing modes can be altered upon replacement of C-H by C-F bonds in drug precursors, and in model compounds.[158],[162],[163] In addition, trifluorination (CH_3->CF_3) on diketoarylhydrazones resulted in significant changes in crystal packing (as illustrated in **Figure 5.1**), although the molecular geometries were not noticeably changed.[164] However, studies also indicated that crystal packing can remain identical after trifluorination, when dominant hydrogen bonding or van der Waals interactions are present.[165]

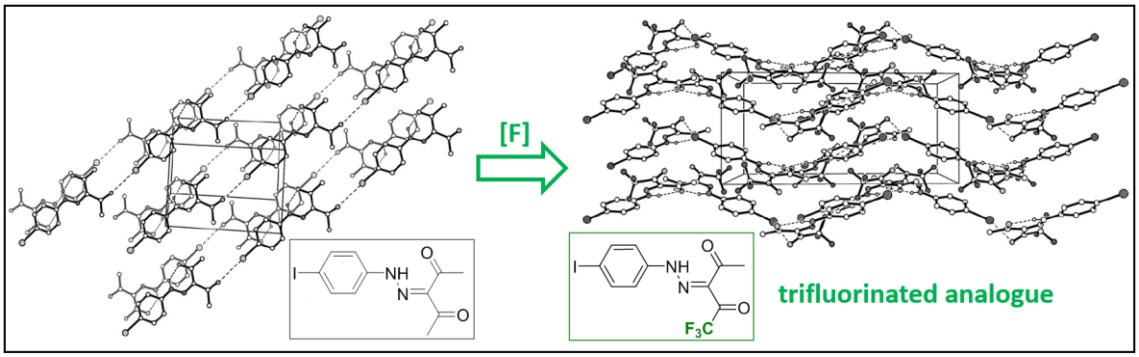


Figure 5.1 Example for change of crystal packing upon trifluorination. ***Adapted from Ref [164].***

In the course of this thesis, many crystal structures of BA derivatives have been determined. In this chapter, interesting observations regarding the effect of fluorination on molecular geometry and crystal packing modes will be described and briefly discussed.

5.2 Results and Discussion

5.2.1 Isostructurality after fluorination

5.2.1.1 Trifluorination

As shown in **Figure 5.2**, replacement of a methyl group in compound **3.50** by a trifluoromethyl group in **3.45** led to a similar molecular geometry and crystal packing mode. As concluded in the literature, isostructurality between a trifluorinated analogue and its parent compound can be facilitated by large molecular size and structural rigidity.[165] Indeed, bile acid derivatives having a polycyclic structure fit into this category. The small volumetric increase due to trifluorination can be tolerated, as crystal packing coefficients for crystals of organic compounds is generally between 0.6 and 0.7 (i.e., ca. 30% unoccupied space).[166] Therefore, it is assumed that the inserted fluorine atoms for compound **3.50** only filled the existing cavity, without inducing additional intermolecular interactions which can alter the original crystal packing. The crystal packing for both compounds **3.50** and **3.45** is most likely controlled by van der Waals interactions between the steroid hydrocarbon skeletons.

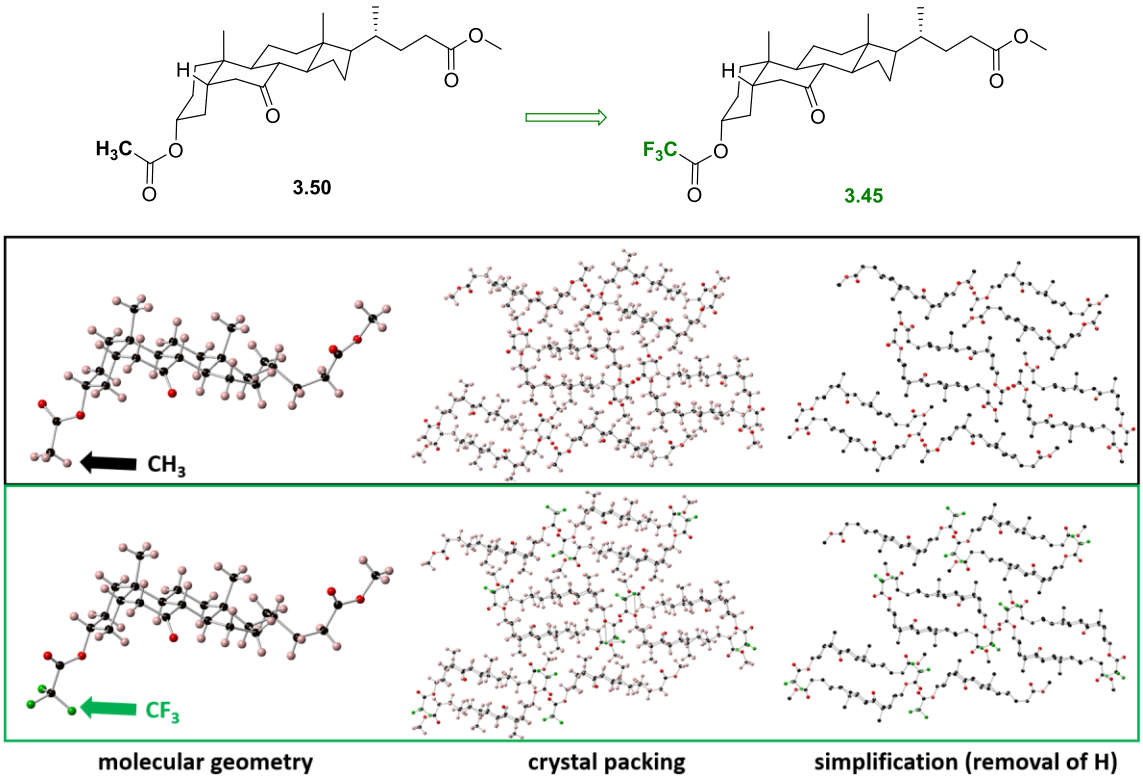


Figure 5.2 Comparison of molecular conformation and crystal packing after trifluorination.

5.2.1.2 Carbonyl – CF_2

Very surprisingly, C=O/CF_2 isostructurality was also observed on BA intermediates **4.41** and **4.42**, as shown in **Figure 5.3**. After deoxofluorination, the 12,12-difluorinated analogue exhibited a nearly

identical molecular conformation and crystal packing mode. Although the carbonyl and difluoromethylene groups have similar steric and polarity features, the change of hybridization on C-12 (from sp^2 to sp^3) could have caused a knock-on effect on the structural conformation. In fact, a slightly larger C-ring distortion was observed after fluorination (**Figure 5.4**). Nevertheless, no noticeable changes were observed for crystal self-assembly and spatial orientations.

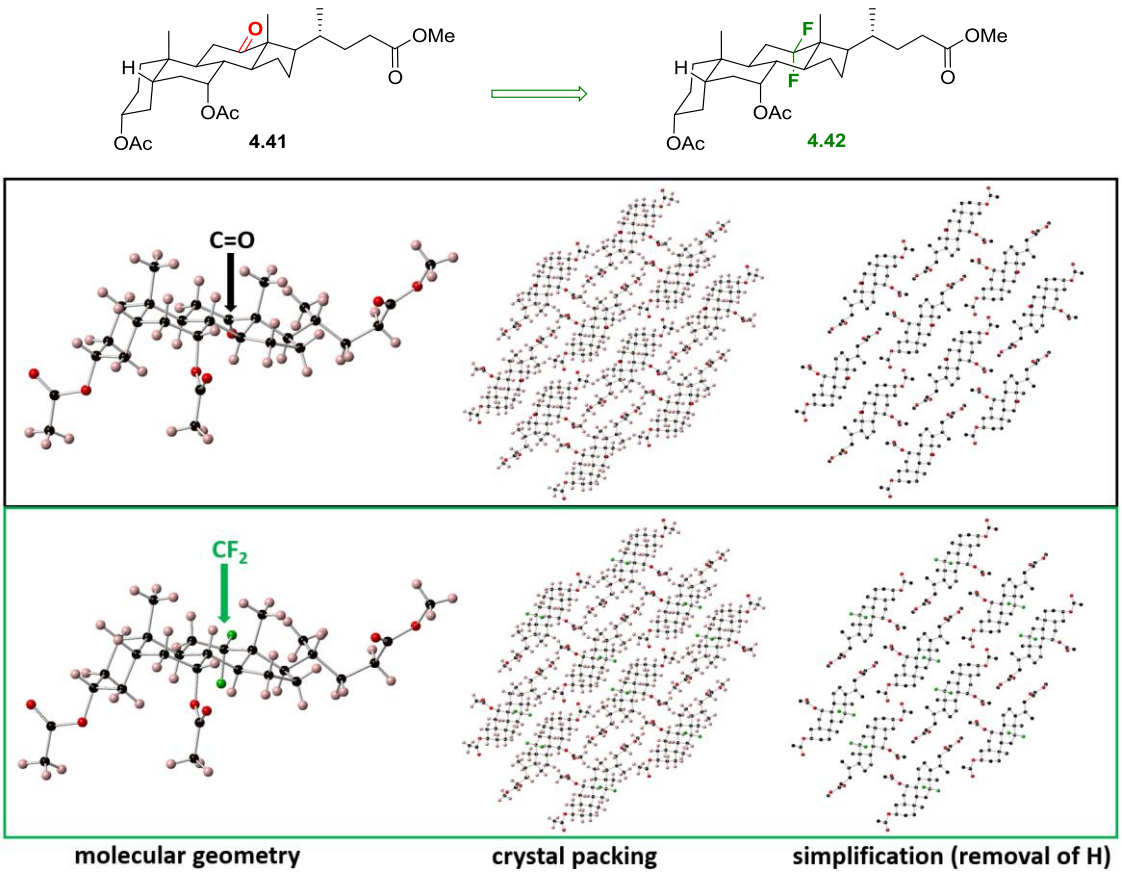


Figure 5.3 Comparison of molecular conformation and crystal packing after deoxofluorination.

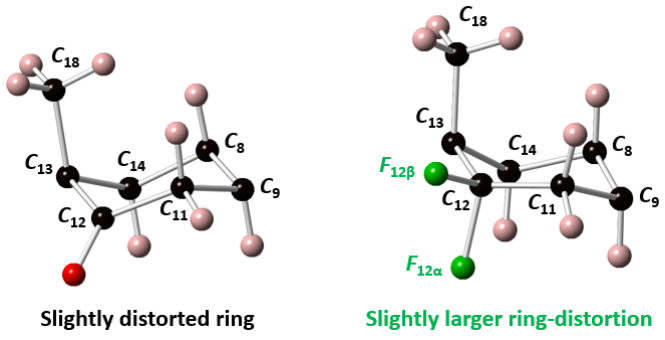


Figure 5.4 C-ring distortion before and after deoxofluorination.

The crystal packing for both compounds is assumed to be dominated by the accumulated van der Waals interactions between the steroid skeletons. For compound **4.42**, the weak C-H···F (closest contact: *d* 2.3 Å) interactions along *b* axis could also favour the stacking between crystals. Besides,

the formation of the aggregated layer (**Figure 5.5**) for ester groups was most probably the outcome of the aforementioned driving forces.

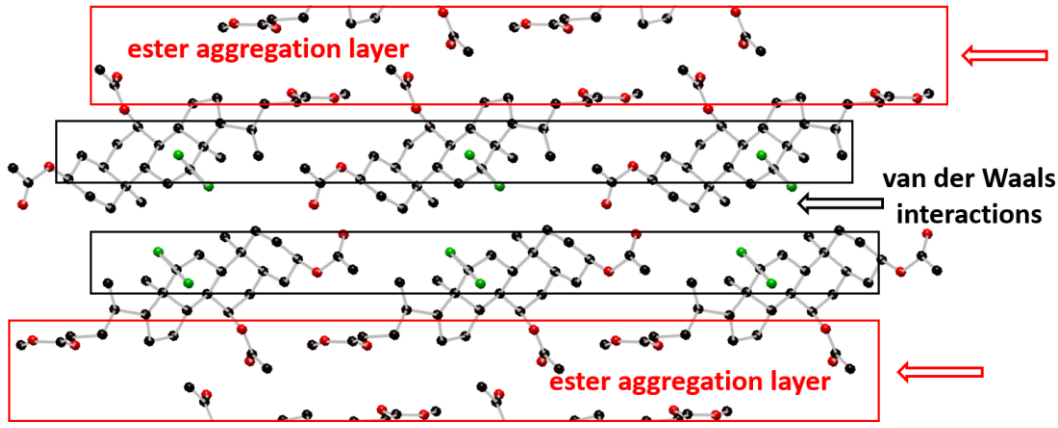


Figure 5.5 Intermelocular interactions for crystal packing.

This C=O/CF_2 isostructurality was also observed on BA derivatives **5.1** and **4.4** (**Figure 5.6**). The X-ray structure for compound **5.1** was reported in the literature,[167] and structural data were retrieved from Cambridge Structural Database[9].

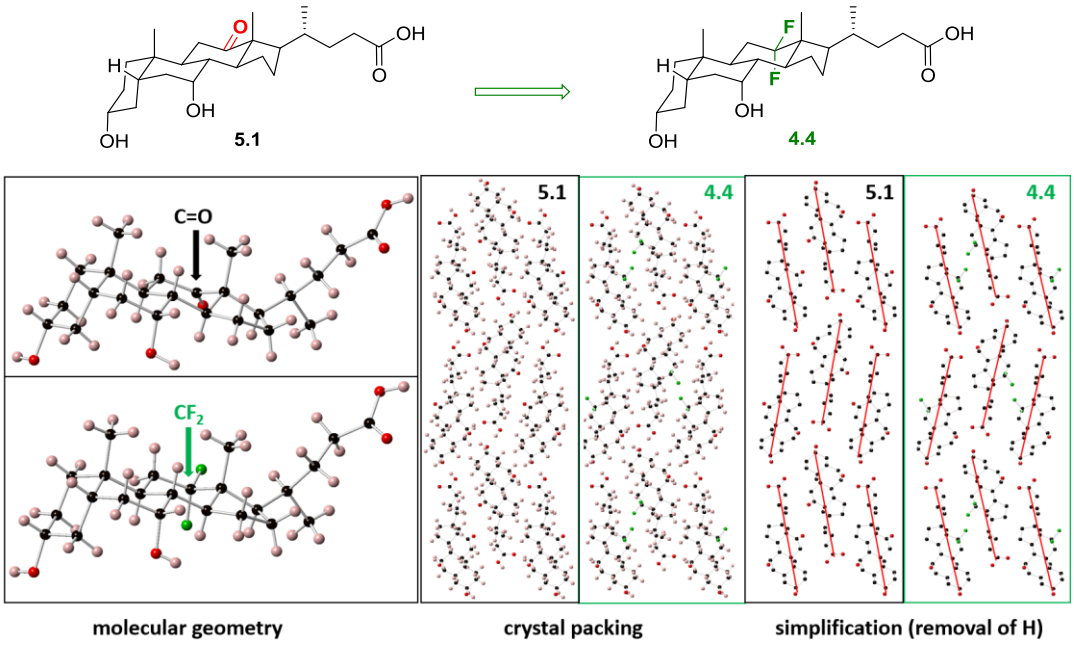


Figure 5.6 Comparison of molecular conformation and crystal packing

In addition to the close-packing induced by steroidal rigidity, hydrogen bonding interactions also played a dominant role for both compound **5.1** and **4.4** regarding crystal aggregation. As shown in **Figure 5.7**, a cyclic network of hydrogen bonding interactions among the 3α-OH, 7α-OH and carboxyl groups was formed on crystals of compound **4.4**. The OH (donor) of 24-carboxyl group

[9] http://webcsd.ccdc.cam.ac.uk/index.php;

formed the strongest HB with oxygen on C-3, which potentially contributed to the head-to-tail linkage pattern in crystal packing.

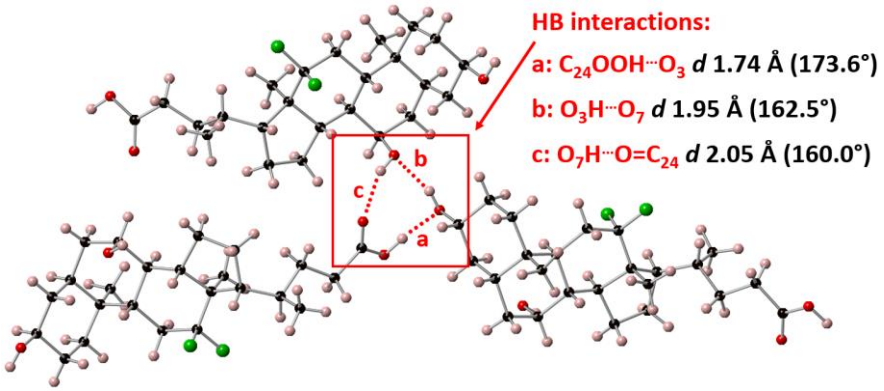


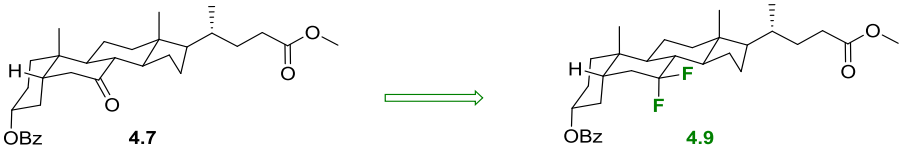
Figure 5.7 Hydrogen bonding interactions for compound 4.4.

With the presence of dominant hydrogen bonding and van der Waals interactions between BA skeletons, C-F···H-C interactions are too weak to affect the crystal self-assembly. Therefore, crystal packing remained nearly identical after replacement of carbonyl by CF_2.

5.2.2 Alteration of crystal packing mode

5.2.2.1 C=O to CF_2

In contrast to previous observations, deoxofluorination can also lead to an altered packing mode, as observed by replacement of the 7-keto group in **4.7** by CF_2 (**Figure 5.8**).



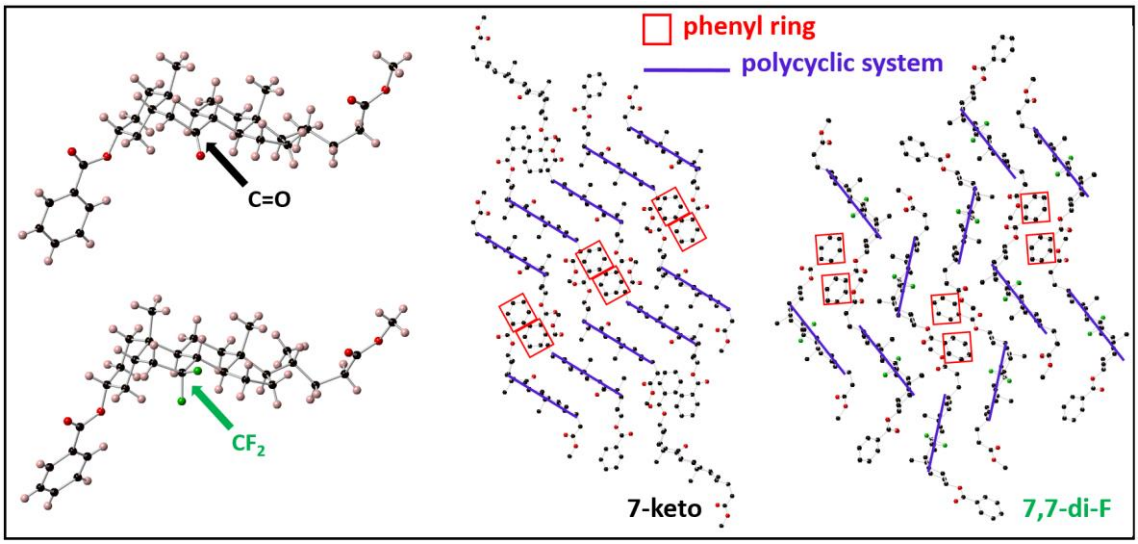


Figure 5.8 Crystal packing of compound 4.7 and 4.9 (removal of H for simplification).

As shown in **Figure 5.9**, molecular conformation was also changed after fluorination, which can be possibly due to the chemical change on C-7, resulting in the shape modification of steroid skeleton. However, based on DFT calculations,[10] it was found that the overall geometries of the isolated and gas phase molecules were barely affected by deoxofluorination. Therefore, it can be concluded that the alteration in molecular geometry and crystal packing mode should be due to the changes of intermolecular interactions, rather than the fluorination-associated shape change.

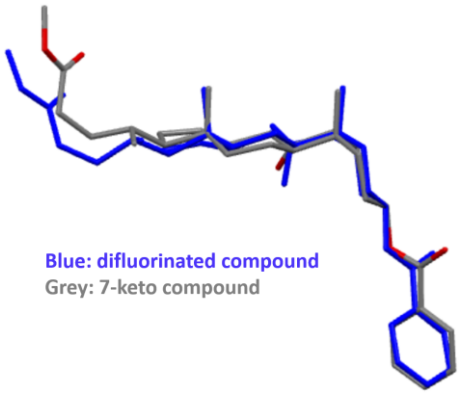


Figure 5.9 Comparison of molecular conformation of compound 4.7 and 4.9.

The tutorial review from Hulliger and co-workers suggested that contact distances less than 2.9 Å could be considered as F···H interactions.[161] Interestingly, both fluorine atoms ($F_{7\alpha}$ and $F_{7\beta}$) were observed in close proximity (2.75 Å and 2.71 Å respectively) with hydrogen atoms, as shown in **Figure 5.10**. Although such C-H···F interactions are rather weak, the accumulation of them can potentially impact on crystal aggregation.

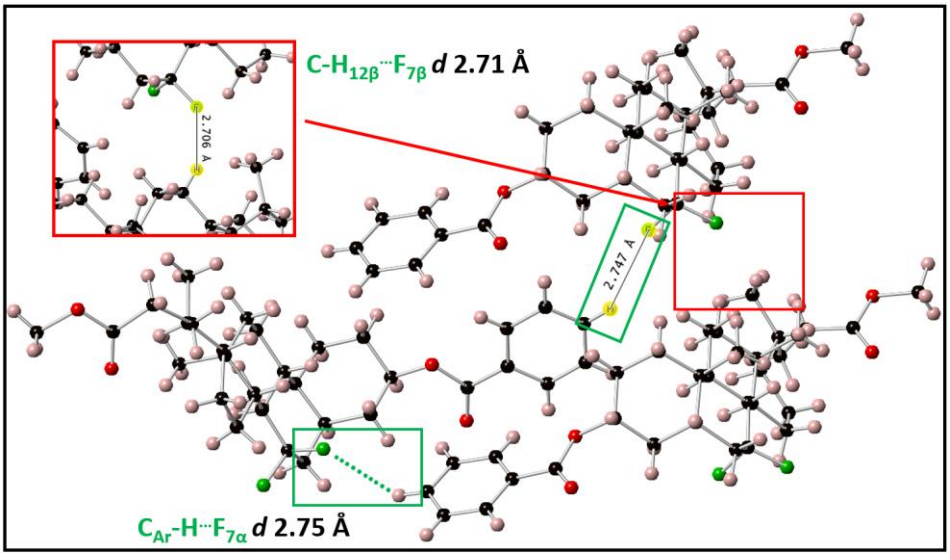


Figure 5.10 Possible weak C-H···F intermolecular interactions for compound 4.9.

[10]DFT calculations were conducted and analysed by our collaborator in University of Southampton (Prof Graeme Day and his PhD student Thomas Gee).

Therefore, deoxofluorination on compound **4.7** has affected the established intermolecular interactions. It is assumed that the alteration in crystal packing can be due to the accumulated C-H···F interactions. In comparison with previous examples of C=O/CF_2 isostructurality, the effect on crystal packing by deoxofluorination can be substantial, when supramolecular synthons (such as hydrogen bonding interactions) are absent and the sum of overall C-H···F interactions is competitive against the original directional forces for crystal packing.

5.2.2.2 C–F diastereoisomers

Alteration of crystal packing mode was also observed on monofluorinated diastereoisomers. As shown in **Figure 5.11**, conversion of fluorine stereochemistry on C-7 led to a drastic rearrangement of crystal self-assembly.

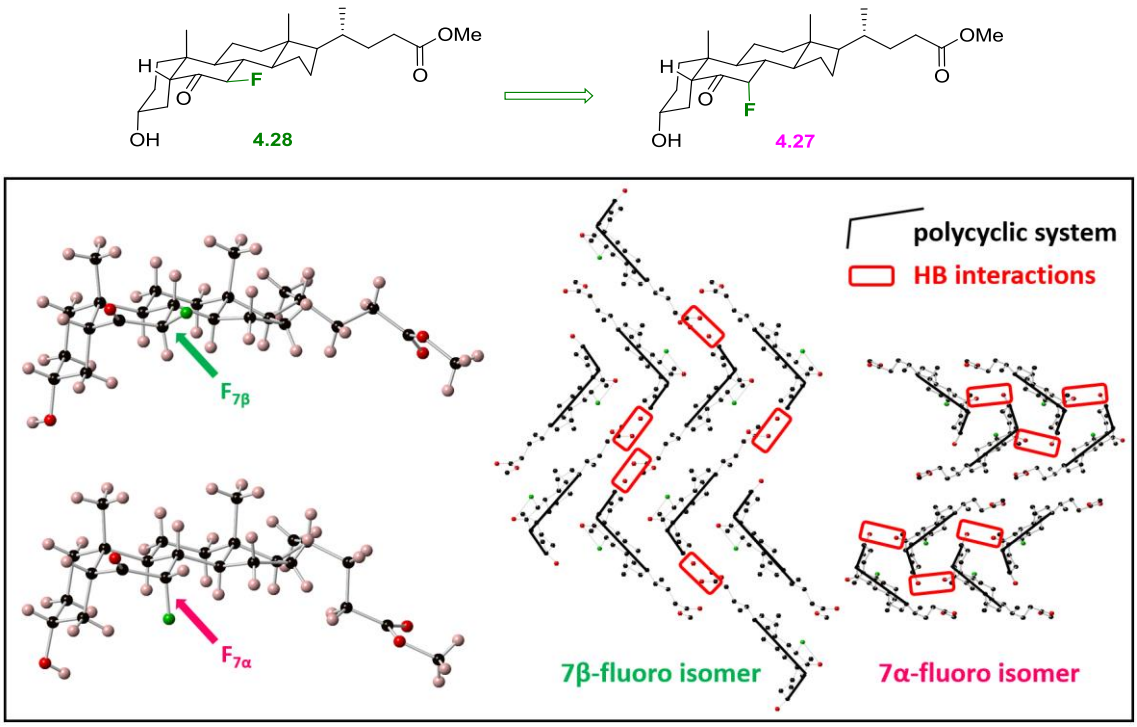


Figure 5.11 Crystal packing modes for diastereoisomers (removal of H for simplification).

Along the *a* axis, weak C-H···F interactions were observed for both compound **4.28** ($d_{H...F}$ 2.45 Å, 2.81 Å) and **4.27** ($d_{H...F}$ 2.49 Å, 2.58 Å), which can facilitate the crystal stacking. However, the directional forces along *a* axis are van der Waals interactions between rigid steroid skeletons. The crystal packing for both diastereoisomers is also dominated by hydrogen bonding interactions. However, hydrogen of 3α-hydroxyl group (donor) interacts with carbonyl oxygen in a different manner. For the 7β-fluoro isomer **4.28**, the 3-OH hydrogen formed hydrogen bond with the C-24-carbonyl oxygen (**Figure 5.12**), leading to a head-to-tail linkage pattern in crystal aggregation. In contrast, 3-hydroxyl hydrogen of 7α-fluoro isomer had hydrogen bonding interactions with the 6-carbonyl

oxygen (**Figure 5.13**). This is rather interesting because the only difference in such large and rigid molecules is the configuration of fluorine.

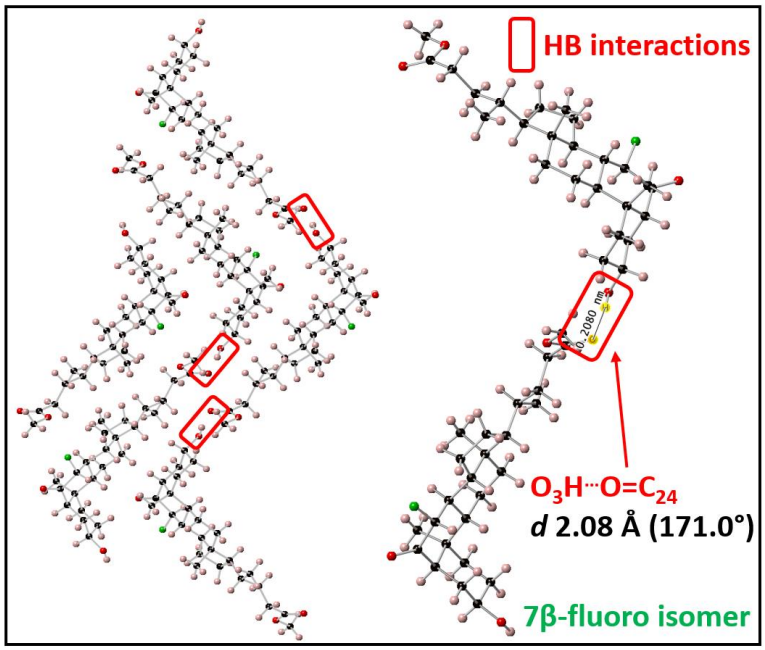


Figure 5.12 Packing mode and HB interactions for compound 4.28.

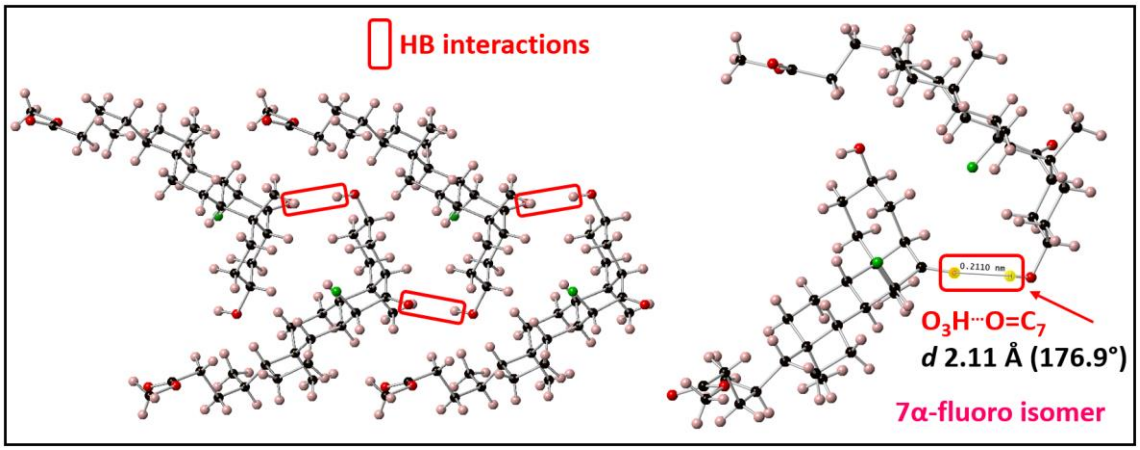


Figure 5.13 Packing mode and HB interactions for compound 4.27.

There are two hydrogen bond acceptors for both isomers: carboxyl oxygen of ketone and carbonyl oxygen of ester. It can be possible that hydroxyl hydrogen tends to interact with a better hydrogen bond acceptor to form a more stable packing structure with lower energy level. Lommerse and co-workers didn't observed any large difference regarding hydrogen bonding accepting capacity for carbonyl oxygen of esters and ketones, based on combinational studies of crystallographic data and computational calculations.[168] Therefore, it is possible that fluorination next to 6-keto group could have influenced its ability to act as HB acceptor. This impact can also be dependent on the relative configuration of fluorine. Further studies and computational calculations might provide further explanation to this phenomenon.

5.3 Conclusion

Interesting effects of fluorination on crystal packing have been observed, with either isostructurality or alteration of packing modes. With the inherent rigidity of polycyclic system of BA derivatives and/or the presence of intermolecular hydrogen bond interactions, the established packing structure can tolerate the introduction of fluorine. However, the accumulation of the weak C-H···F interactions can lead to changes in crystal aggregation. In addition, fluorination adjacent to functional groups can also influence their behaviour in supramolecuar assembly.

SECTION III LIPOPHILICITY

Chapter 6: A New Method for Lipophilicity Determination

6.1 Introduction

Lipophilicity is one of the most informative physicochemical parameters in drug design and development. It is defined by the partition coefficient (log*P*, for un-ionized form of substrate) and distribution coefficient (log*D*, for both ionized and un-ionized form of substrate) of a compound between *n*-octanol and water, which is taken as a measure for a lipid membrane.[169] The precise measurement of lipophilicity is thus crucial during the drug development process. Currently, there are many established methods available for lipophilicity determination.[8] The 'shake-flask' method and its variations are used for direct and accurate measurement of lipophilicity, requiring quantitative concentration determination (commonly using UV spectroscopy). Indirect approaches include potentiometric titration,[170] chromatographic methods (such as RP-HPLC), electrophoretic methods,[171],[172] NMR-based methods, and mass-spectrometry-based methods.[173] The values obtained from those indirect methods are estimations, and calibrated against compounds with known lipophilicity. In the following part, several relevant methods will be discussed in detail. In addition, it is necessary to mention that there are several calculation methods available to give the so-called clog*P* values, which are typically used in industry when dealing with large numbers of compounds, or as a preliminary filter when a series of compounds are proposed for synthesis. However, this has a low accuracy which is strongly dependent on the training set used, and errors of multiple log*P* units are not uncommon.[174],[175]

6.2 Log*P*/log*D* measurements

6.2.1 Shake-flask methods

The classic shake-flask (SF) method is a standard and simple procedure (OECD Guidelines,[176] 1992) for lipophilicity measurement. The substrate is added into *n*-octanol and water (or buffer solution) to reach equilibrium through shaking. After layer separation, the concentration can be quantified, typically by using UV/Vis absorption spectroscopy, or also *via* GC-LRMS, HPLC, NMR, etc.[20],[177],[178],[179] The inherent problems of this labour-intensive SF method are time consumption and also the potential for emulsion formation (particularly for highly lipophilic compounds).[177],[180] Several variations of SF methods were also designed to circumvent these disadvantages, such as methods using dialysis tubing and ultrasonic agitation, flow injection analysis, etc.[180],[181]

6.2.2 Chromatographic methods

Indirect measurement of lipophilicity based on retention time from chromatographic methods was also developed from the 1980s onwards, including reversed phase thin layer chromatography (RP-TLC),[182] and reversed phase high performance liquid chromatography (RP-HPLC),[183] etc. Since then, RP-HPLC method has become increasingly popular due to its time-effective and user-friendly features. Lipophilicty can be easily obtained by using a calibration curve, which should be established from the structurally related reference compounds with known lipophilicity data. However, the accuracy of the prediction using calibration curves is limited to individual compound families and is also dependent on the partition system.[184],[172] Besides, it is crucial to consider the polarity character of the column (stationary phase, e.g. C18), as intermolecular hydrogen bonding and other electrostatic interactions also contributed to the overall lipophilicity profile of the solute.[185] Incorporation of other parameters into the equation, application of modified columns, and other measures can certainly increase the correlation quality, but at the expense of the simplicity of the method.[186],[187]

6.2.3 NMR-based methods

Kitamura and co-workers developed a method for partition coefficient measurement based on the correlation between ^{19}F spin-lattice relaxation time (T_1) of the fluorinated compound and the concentration of lecithin small unilamellar liposome (mimicking lipid environment), without layer separation.[188] T1 values in liposomes differ from these in the water phase. Single broadened peaks in ^{19}F NMR spectrum of this partition system indicated that the exchange rate of the studied substrate (trifluprom azine) between both layers was fast on the ^{19}F NMR time scale,[189] which was a prerequisite for the proposed correlation equation. However, this study regrettably didn't consider the possible influence of concentration on individual T1 values, leaving the validity of the method under question. Nevertheless, this method provided alternative implications for measuring the lipophilicity of fluorinated compounds (e.g., without UV activity) by ^{19}F NMR experiments.

Similarly, based on the fast exchange rate of investigated substrates on NMR time scale, Kitamura *et al.* were also able to precisely determine lipophilicity from the established relationship of phosphatidylcholine (PC) bilayer concentration and the observed chemical shift difference of ^{19}F NMR after addition of PC bilayer.[190]

Recently, Mo, Colby and co-workers have reported a deuterium-free ^{1}H NMR method for lipophilicity measurement.[191] They used partition solvents (proton concentration in neat water and *n*-octanol: 110.7 M and 114 M respectively) directly as native references to examine the concentration of the solute in both layers based on NMR integration.[191] However, the method is

restricted by the chemical shift ranges of the compounds. For example, it may not be suitable for measurement of log*P* of alkanols due to the possible overlapping of their characteristic peaks with *n*-octanol signals. Apart from one compound, all the other substrates used in their studies contain aromatic protons. Besides, NMR experiments involving the application of a small excitation angle or solvent suppression are difficult to perform with multiuser open-access NMR facilities.

The dependence on UV spectroscopy for quantification, the need for calibration curves for concentration/lipophilicity calculation and also the limitation of reported NMR methods, hamper progress in this field. This is especially the case for aliphatic compounds that don't contain chromophores, and these types of substrates are of interest to our group. Therefore, the need arose to develop a novel and straightforward method for lipophilicity measurement of fluorinated compounds using ^{19}F NMR, which is also compatible with non-UV-active compounds.

6.3 Methodology development

6.3.1 Principle of the method based on ^{19}F NMR

The key principle for our variation of the shake-flask method (**Figure 6.1**) involves the use of fluorinated reference compound with known log*P* value (log P^R). The reference compound (R) and the log*P*-unknown compound (A) are partitioned, as a mixture, between the *n*-octanol and water layers. After equilibrating, an aliquot is taken from each layer for ^{19}F NMR experiments. The signal intensities depend on the compound concentration (C) and the number of fluorine atoms (n) in the molecule. The integration ratios between two compounds in both layers are defined as r_o (for octanol layer) and r_w (for water layer), which are described as in **Eq. 1** and **Eq. 2**. The ratio of the *r* values (r_o/r_w) is equal to the ratio of partition coefficient (P) of compound A and R (**Eq. 3**). This leads to the final calculation equation for log*P* for the compound A (**Eq. 4**). Therefore, the log*P* value of an unknown compound (log P^A) can be obtained simply by measuring the concentration ratios (r_o and r_w).

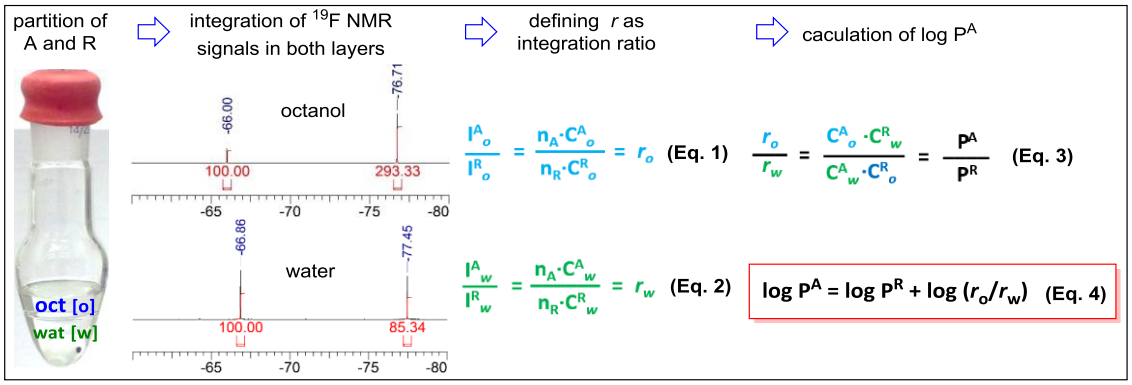


Figure 6.1 Principle of the log*P* determination method.

This method has several practical advantages compared to the current shake-flask method and its variations. There is no requirement of measuring compound mass, volume of the octanol and water layers and also of the NMR sample volume. High purity of compound is not compulsory as long as the impurity signal is not overlapping with diagnostic peaks of the compounds in the NMR spectra. Besides, systematic errors are cancelled out due to the compensation effect inherent to working with the ratio of a ratio. In addition, this NMR-based method is suitable for log*P* measurement of non-UV-active compounds.

As the determination of concentration ratios are essential to this method, it is therefore crucial to carry out accurate quantitative integration. In addition to the standard data processing (wave function, zero-filling, phase and baseline correction), NMR experiment settings for data acquisition are also of importance, which are investigated by parameter screening in the following sections.

6.3.2 Optimisation of the NMR method

The NMR setting and parameters that could impact on log*P* measurement investigated include signal-to-noise ratio (SNR), spectral width (SW), frequency offset point (O1P), and pulse delay time (D1).

6.3.2.1 Influence of proton decoupling (nuclear Overhauser effect)

2,2,2-Trifluoroethanol (TFE, logP^{LIT} +0.36) was used to determine the log*P* value of 2-fluoroethanol (FE, logP^{EXP} -0.75) for the following NMR parameter screening experiments (**Table 6.1**). The log*P* values from odd-number entries (**Entries 1**, **3**, **5**, …, **23**) represent the ^{19}F {^{1}H} acquisitions, while results from even-number entries (**Entries 2**, **4**, **6**, …, **24**) were obtained without proton decoupling. With the same SW, O1P and D1 parameter settings, no large difference (Δlog*P* up to 0.04 log*P* units) was observed for 12 pairs of experiments, either with or without proton decoupling. The individual integration ratios in NMR samples for both layers are also barely different (e.g., comparing r_o or r_w values between **Entries 1** and **2**, or **Entries 23** and **24**) by using inverse-gated decoupling technique.

As described in the literature, inverse-gated decoupling can remove nuclear Overhauser effects (nOe) and lead to a decoupled spectrum without nOe enhancements, and ''This is because any NOE that builds up during the acquisition time affects only longitudinal magnetization so does not influence the detected (transverse) signals''.[192] The benefit from using ^{19}F {^{1}H} NMR is that the decoupled spectrum has a higher signal-to-noise ratio, which provides higher accuracy for quantitative integration. Therefore, proton-decoupled ^{19}F NMR is recommended for log*P* measurement.

6.3.2.2 Influence of spectra width

With narrowing SW ranges from 300 ppm to 160 ppm (**Entries 1**-**8**, **Table 6.1**), the integration ratios from NMR experiments in both layers and the final log*P* results remained nearly the same. Therefore, there is no influence from changing spectral width. However, for compounds which are highly hydrophilic or liphophilic (causing a very low concentration in one of the phases), a narrower SW can result in a higher digital resolution and higher SNR, which is beneficial for quantitative integration.

6.3.2.3 Influence of frequency offset point

As indicated in **Table 6.1**, the O1P was set in the middle of two fluorine signals (**Entries 13**-**14**), which was the optimal setting for quantitative integration (equal excitation of both nuclei). The integration ratios from individual layers changed dramatically when changing the O1P setting, e.g., O1P at -70 ppm (r_o 0.0401, **Entry 9**) to O1P at -350 ppm (r_o 0.1686, **Entry 17**).

Table 6.1 NMR setting and parameter screening experiments.

Entry	SW (ppm)	O1P (ppm)	D1 (sec)	decoupled	r_o	r_w	log*P*	Δlog*P*
1	**300**	-150	O 18.36/ W 34.97	*yes*	0.0832	1.0625	**-0.75**	**0.00**
2	**300**	-150	O 18.36/ W 34.97	**no**	0.0834	1.0749	**-0.75**	
3	**250**	-150	O 18.36/ W 34.97	*yes*	0.0821	1.0766	**-0.76**	**-0.02**
4	**250**	-150	O 18.36/ W 34.97	**no**	0.0860	1.0727	**-0.74**	
5	**200**	-150	O 18.36/ W 34.97	*yes*	0.0826	1.0704	**-0.75**	**0.00**
6	**200**	-150	O 18.36/ W 34.97	**no**	0.0832	1.0651	**-0.75**	
7	**160**	-150	O 18.36/ W 34.97	*yes*	0.0828	1.0675	**-0.75**	**0.01**
8	**160**	-150	O 18.36/ W 34.97	**no**	0.0806	1.0662	**-0.76**	
9	350	**-70**	O 18.36/ W 34.97	*yes*	0.0401	0.5438	**-0.77**	**-0.01**
10	350	**-70**	O 18.36/ W 34.97	**no**	0.0408	0.5379	**-0.76**	
11	350	**-113**	O 18.36/ W 34.97	*yes*	0.0660	0.8721	**-0.76**	**0.00**
12	350	**-113**	O 18.36/ W 34.97	**no**	0.0661	0.8672	**-0.76**	
13	350	**-150**	O 18.36/ W 34.97	*yes*	0.0847	1.0727	**-0.74**	**0.01**
14	350	**-150**	O 18.36/ W 34.97	**no**	0.0840	1.0723	**-0.75**	
15	350	**-187**	O 18.36/ W 34.97	*yes*	0.1069	1.3203	**-0.73**	**0.00**
16	350	**-187**	O 18.36/ W 34.97	**no**	0.1067	1.3217	**-0.73**	
17	350	**-230**	O 18.36/ W 34.97	*yes*	0.1643	2.0622	**-0.74**	**-0.01**
18	350	**-230**	O 18.36/ W 34.97	**no**	0.1686	2.0921	**-0.73**	
19	300	-150	**O 1 / W 1**	*yes*	0.0660	0.8082	**-0.73**	**-0.04**
20	300	-150	**O 1 / W 1**	**no**	0.0640	0.7209	**-0.69**	
21	300	-150	**O 5 / W 5**	*yes*	0.0746	0.8908	**-0.72**	**-0.02**
22	300	-150	**O 5 / W 5**	**no**	0.0748	0.8496	**-0.70**	
23	300	-150	**O 18.36 / W 34.97**	*yes*	0.0823	1.0732	**-0.76**	**-0.01**
24	300	-150	**O 18.36/ W 34.97**	**no**	0.0837	1.0704	**-0.75**	

However, the integration ratio changes seen in the two layers of a given partition experiment were increased/decreased proportionally and as a result, the obtained log*P* results remained nearly the same. Because from the equation $\log P^{A} = \log P^{ref} + \log(r_o/r_w)$, the final log*P* result depends on r_o/r_w, which is a ratio of a ratio. Therefore, all the systematic errors are cancelled out. Due to this compensation effect, the log*P* results obtained have only very small error up to 0.02 units. For practical log*P* determination, the optimal O1P value (centered between the diagnostic fluorine peaks) will be used. These chemical shifts, if unknown, can be obtained by initial acquisition of the respective ^{19}F NMR spectra in each solvent.

6.3.2.4 Influence of pulse delay time

As shown in **Entries 19-24** (**Table 6.1**), NMR parameter screening experiments to evaluate the pulse delay time (D1) were carried out, from the default D1 setting of 1 sec to 5*T1 (T1, spin-lattice relaxation time). Five times of T1 is the standard D1 setting for quantitative integration, to allow over 99% of nuclei to fully relax before the next pulse. Errors exist for the integration ratios from

spectra acquired with short D1, as insufficient pulse delay leads to the perturbation of the signal intensities.[192]

However, as the spin-lattice relaxation time (T1, *see section 6.3.3*) for both diagnostic F nuclei is different in the octanol and water phase, systematic errors are not cancelled out. This is a case where the compensation effect doesn't apply. Comparing **Entries 20** and **24** (with non-proton decoupling), the error was up to 0.06 logP unit. Therefore, it is recommended to have a sufficient pulse delay (≥ least 5*T1) to obtain a more accurate integration ratio for the log*P* measurement.

6.3.2.5 Importance of Signal-to-Noise Ratio (SNR)

For highly lipophilic compounds, most of the compound will partition to the octanol layer. Thus, the compound concentration in the water layer will be very low. Similarly, the concentration for highly hydrophilic compounds in octanol will be very low. The lower the concentration, the lower the signal-to-noise ratio for the observed peaks, which, from a certain point, renders accurate quantitative integration impossible. The appropriate S/N ratio for quantitative analysis is recommended to be above 300:1.[193] A simple way to increase the S/N ratio is to increase the number of scans (NS) for the NMR experiment.

As illustrated by one example below (comparing data shown in **Figure 6.2** with **Figure 6.3**), the S/N ratios obtained were increased by ca. 10 times when the number of scans was increased from NS 64 to NS 4096. Interestingly, the integration ratios (0.6523 against 0.6582) remained nearly identical, leading to nearly same log*P* results (Δlog*P* 0.004). Similar results were observed with two other examples when measuring lipophilic compounds. Based on these examples, it is found that reliable integration ratios can still be obtained with S/N ratio lower than 100:1.

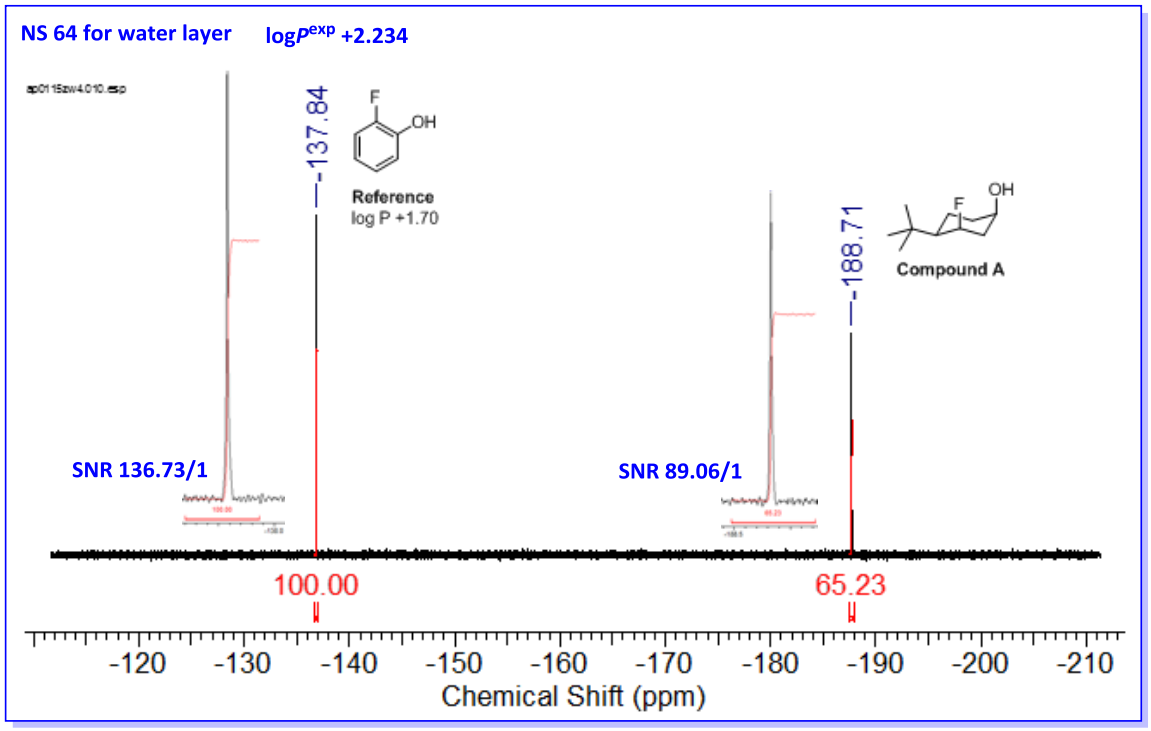


Figure 6.2 SNR and log*P* result from NMR experiment with NS 64.

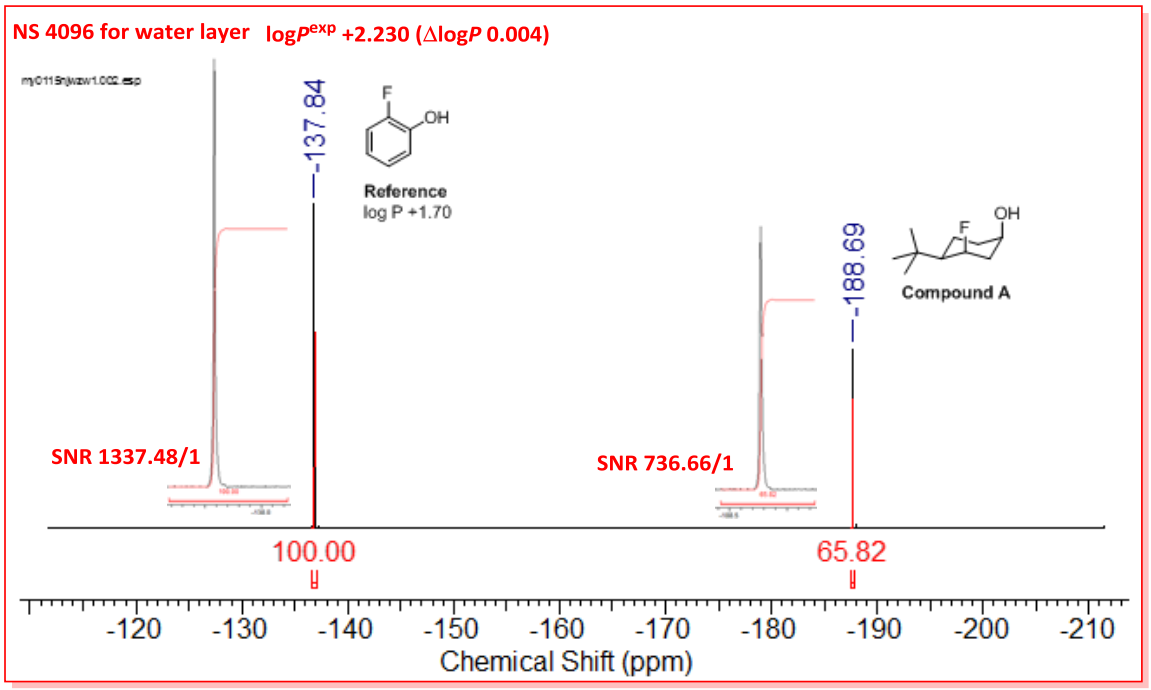


Figure 6.3 SNR and log*P* result from NMR experiment with NS 4096.

6.3.3 Spin-lattice relaxation time

The spin-lattice relaxation time (T1) is a molecular property, depending on many external factors, such as impurities, oxygen content, temperature, solvent/solvent systems, compound concentration, etc.[194] Because of this, each NMR sample prepared for a log*P* experiment is

individually different (e.g., compound concentration, and compound mixture). In addition, a certain amount of acetone-d_6 is added for signal lock, the volume of which is not precisely measured. Besides, there are no reported T1 values in the literature for such compounds in this solvent mixture. Therefore, it was decided to measure T1 values for the diagnostic F nuclei in individual NMR tubes, in order to gauge the level of appropriate D1 settings for accurate quantitative integration. The T1 values were measured by using an inversion-recovery sequence.[195]

Table 6.2 Selected examples for T1 measurements.

Entry	Exp. Code	CF_3CH_2OH		$CF_3CF_2CH_2OH$		$CF_3CF_2CH_2OH$	
		Oct	Wat	Oct	Wat	Oct	Wat
1	ZW7157-26-3	2.394	4.223	2.393	3.951	2.101	3.492
2	ZW7157-26-1	2.363	4.062	2.406	3.892	2.131	3.414
		$CF_3CF_2CH_2OH$		$CF_3CF_2CH_2OH$		FCH_2CH_2OH	
3	ZW7157-28-1	2.424	3.948	2.195	3.743	4.589	7.663
4	ZW7157-28-2	2.466	3.779	2.241	3.666	3.953	7.615
5	ZW7157-28-3	2.185	3.917	1.947	3.378	3.347	7.229

As shown in **Table 6.2**, T1 values were determined for fluorinated alcohols in different NMR tubes, which were prepared independently for log*P* measurement. **Entries 1-2** represent two experiments in which a mixture of trifluoroethanol and pentafluoropropanol is used. It is easily seen that the determined T1 values vary between two experiments. The same is observed in **Entries 3-5**. Overall, it can be observed that the T1's for monofluorinated compounds are longer than these of di- and trifluorinated compounds. Besides, T1 values in the octanol samples were shorter than those from the water samples. The longest T1 value in octanol was observed for 2-fluoroethanol with T1 value of 4.589 sec (**Entry 3**). Similarly, the longest T1 value in water NMR sample was observed for 2-fluoroethanol with T1 value of 7.663 sec (**Entry 3**).

Because T1 determinations are very time-consuming, for practical purposes it is recommended (based on the dataset collected) to use a D1 of 30 sec for the octanol sample and 60 sec for the water sample.

6.3.4 Influence of intermolecular interactions

It is assumed that intermolecular interactions between fluorohydrins in a dilute solvent system are negligible, and hence do not have an impact on log*P* values. In the literature, it is also common to use compound solutions in DMSO for log*P* measurement.[196] As shown in **Table 6.3**, the difference of log*P* value due to significantly increased concentration (from 2 mg to 120 mg, regarding substrate load of compound A in 4 mL mixture of octanol and water) is only -0.025. Therefore, it is concluded that intermolecular interactions have negligible influence on log*P* measurement for the substrates used. The quantities that were generally used were between 1-10 mg.

Table 6.3 log*P* measurement with different concentrations.

Entry	m_A^* (mg)	m_R^* (mg)	r_o	r_w	log*P*	average (error)
1	2.0	0.8	2.1983	0.3163	1.202	+1.200 (±0.001)
2	2.0	0.8	1.9761	0.2854	1.200	
3	2.0	0.8	2.3148	0.3352	1.199	
4	10.0	4.0	2.1598	0.3117	1.201	+1.199 (±0.003)
5	10.0	4.0	2.1723	0.3140	1.200	
6	10.0	4.0	2.1231	0.3105	1.195	
7	70.0	30.0	2.2576	0.3404	1.182	+1.181 (±0.001)
8	70.0	70.0	2.2214	0.3346	1.182	
9	70.0	70.0	2.2991	0.3488	1.179	
10	120.0	60.0	1.8726	0.2886	1.172	+1.175 (±0.002)
11	120.0	60.0	1.8875	0.2872	1.178	
12	120.0	60.0	1.9581	0.3002	1.174	

*The amount of compound (A: pentafluoropropanol; R: trifluoroethanol) used is the approximate mass.

6.3.5 Reproducibility of the method

6.3.5.1 Multiple acquisitions

In this control experiment, 2-fluoro-pentan-1-ol and 2,2,2-trifluoroethanol were partitioned between *n*-octanol and water, giving two NMR samples. For each tube, seven acquisitions were taken (**Entries 1-7**, **Table 6.4**), leading to seven sets of *r* values and the resulting log*P* values. As can be seen in the Table, the error (standard deviation ca. 1%) was negligible.

Table 6.4 Multiple acquisitions of NMR samples.

Entry	r_o	r_w	log*P*
1	0.5138	0.3580	0.5169
2	0.5138	0.3550	0.5206
3	0.5119	0.3574	0.5160
4	0.5116	0.3520	0.5224
5	0.5159	0.3493	0.5294
6	0.5085	0.3510	0.5210
7	0.5038	0.3475	0.5213
Average	0.5113	0.3529	0.5211
Standard deviation	**±0.0037 (0.73%)**	**±0.0037 (1.06%)**	**±0.0040 (0.77%)**

6.3.5.2 Multiple reprocessing

The obtained FID file of each sample was reprocessed using the following conditions: WFunction (LB 2 Exponential), Zero Filling (from 65536 to 262144) and then Fourier transform, followed by manual phasing and auto baseline correction. The integration ratio was obtained by manual integration. Bias correction can be applied *via* adjusting tilt and slope if integral curve is not parallel

to the baseline. This was repeated 7 times independently (**Entries 1-7**, **Table 6.5**). As can be seen in the Table, the error (standard derivation ca. 0.5%) was negligible.

Table 6.5 Multiple reprocessing of FID files.

Entry	r_o	r_w	log*P*
1	0.5138	0.3580	0.5169
2	0.5108	0.3594	0.5127
3	0.5143	0.3554	0.5205
4	0.5128	0.3575	0.5167
5	0.5146	0.3588	0.5166
6	0.5140	0.3544	0.5215
7	0.5146	0.3572	0.5186
Average	0.5136	0.3572	0.5176
Standard deviation	**±0.0013 (0.25%)**	**±0.0017 (0.46%)**	**±0.0027 (0.52%)**

6.3.6 Validation of the method

6.3.6.1 Internal validation

As shown in **Figure 6.4 (A)**, the log*P* values of fluoroethanol and pentafluoropropanol were measured using trifluoroethanol as a reference. Then, the log*P* value of fluoroethanol was measured using the just determined log*P* value (+1.20) of pentafluoropropanol as the reference. Comparing the two log*P* values for fluoroethanol (-0.75 against -0.76), the difference was only 0.01 log*P* units. Another example was also given in **Figure 6.4 (B)**, the log*P* values differ also by only 0.01 units. This also indicates that the nature of the reference does not influence the log*P* determination. In addition to the excellent internal consistency, the reproducibility of the method was demonstrated by standard deviation values (<0.01 log*P* units).

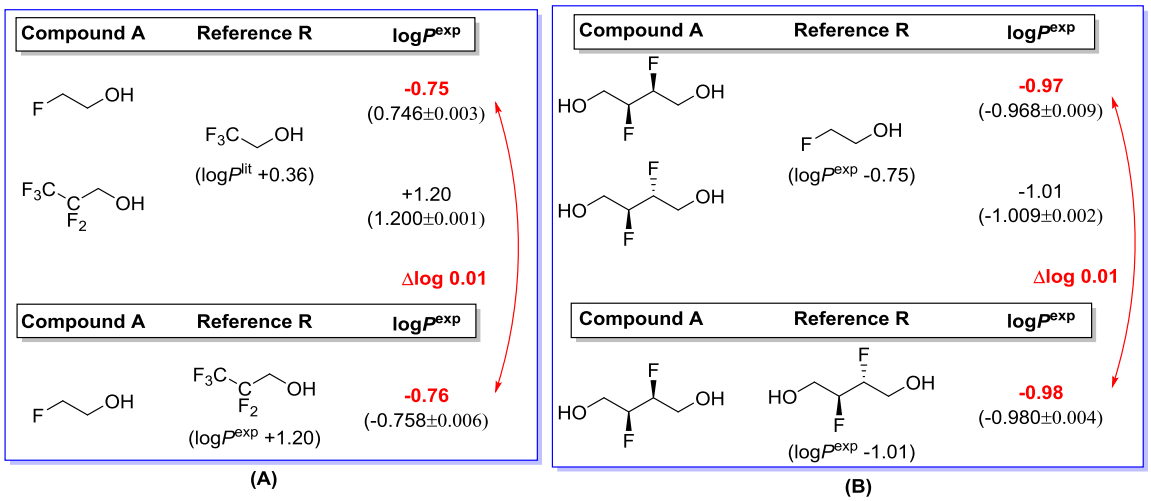
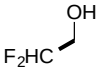
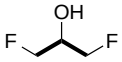
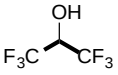
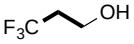
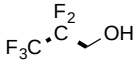
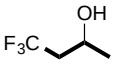
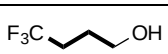
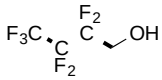
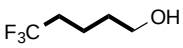
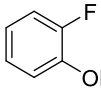


Figure 6.4 Examples for internal validation.

6.3.6.2 Comparison between literature log*P* and the measured log*P* values

Overall, the experimentally obtained log*P* values have good to excellent accordance with the literature values, as shown in **Table 6.6**.

Table 6.6 Comparison between literature and experimental log*P* values.

Compound	log*P* (lit)	Reference[1]	exp. log*P*[2]	Δlog*P*
	-0.67[3]	TFE[4]	-0.75	-0.08
	-0.92[5,6]	TFE		+0.17
	-0.33[3]	TFE	-0.29	+0.04
	-0.36[3]	TFE	-0.42	-0.06
	+0.70[4]	TFE	+0.71	+0.01
	+0.71[3,5,6]			+0.00
	+1.66[3]	TFE	+1.69	+0.03
	-0.28[3]	FE[7]	-0.26	+0.02
	+0.39[3,4]	TFE	+0.41	+0.02
	+1.23[3,6,9]	TFE	+1.20	-0.03
	+1.04[3,4]	TFE	+1.03	-0.01
	0.71[4]	TFE	+0.72	+0.01
	+0.90[3,4]	TFE	+0.91	+0.01
	+1.94[3]	TFE	+1.98	+0.04
	+1.81[9]			+0.18
	+1.15[4]	TFE	+1.22	+0.07
	+1.71[3,6,8]	TFE	+1.70	-0.01
	+1.64[6]			+0.06

[1]2,2,2-Trifluoroethanol (TFE); log*P* +0.36[11] (method used for partition: shake-flask; method used for analysis: ^{1}H or ^{19}F NMR; temperature for log*P* measurement: not reported). **This value used as reference value**. Other values (+0.41 and +0.32) reported in a table in ref[12] and in ref[13] (original source not published);

[11]N. Mueller, *J. Pharm. Sci.* **1986**, *75*, 987-991.

[12]C. Hansch, A. Leo, in *Substituent constants for correlation analysis in chemistry and biology*. Wiley, **1979**.; partition and analysis method as well as temperature for log*P* values are not provided.

[13]A. Leo, C. Hansch, D. Elkins, *Chem. Rev.* **1971**, *71*, 525-616.

[2]Average log*P* value (at 25 °C, temperature controlled by recirculating chiller) from at least three experiments as listed in experimental part;
[3]log*P* data retrieved from U.S. National Library of Medicine online database: http://chem.sis.nlm.nih.gov/chemidplus/; partition and analysis method as well as temperature for log*P* values are not provided;
[4]Value in ref[11]; method used for partition: shake-flask; method used for analysis: ^{1}H or ^{19}F NMR; temperature for log*P* measurement: not reported;
[5]Value in ref[14]; method used for partition: shake-flask (method of Hansch and Muir[15]); method used for analysis: gas chromatography (method developed by Bluestein and Posmanter[16]); temperature for log*P* measurement: not reported;
[6]Value in ref[12]. Partition and analysis method as well as temperature for log*P* values are not provided;
[7]FE = 2-fluoroethanol. Experimentally determined log*P* value (-0.75) for 2-fluoroethanol was used here as reference;
[8]Value in ref[17]. Method used for partition: shake-flask; analysis with Cary Model 14 spectrophotometer; temperature for log*P* measurement: 25 ± 5 °C.
[9]Value in ref[13], p534. Partition method: not described; analysis method: using vapor-phase chromatography; temperature for log*P* measurement: room temperature.

[14]E. O. Dillingham, R. W. Mast, G. E. Bass, J. Autian, *J. Pharm. Sci.* **1973**, *62*, 22-30.
[15]C. Hansch, R. M. Muir, T. Fujita, P. P. Maloney, F. Geiger, M. Streich, *J. Am. Chem. Soc.* **1963**, *85*, 2817-2824.
[16]C. Bluestein, H. N. Posmanter, *Anal. Chem.* **1966**, *38*, 1865-1869.
[17]T. Fujita, J. Iwasa, C. Hansch, *J. Am. Chem. Soc.* **1964**, *86*, 5175-5180.

6.4 Application of the methodology: lipophilicity determination of a range of fluorinated alcohols, including carbohydrates

6.4.1 Lipophilicity of fluorinated alkanols

The developed methodology was then applied in the measurement of alkanol lipophilicity. Next to the measurement of known compounds, the log*P* of many novel compounds was determined. The amount of data obtained allowed making generalisations about the effect of fluorination on the lipophilicity of alkanols.

6.4.1.1 Monofluorination

As shown in **Figure 6.5**, monofluorination of acyclic fluorohydrins led to decreased log*P* values, regardless of the fluorination positions. β-Monofluorination on ethanol **6.1** and 2-propanol **6.3** lowered lipophilicity by up to 0.45 log*P* units. Additional β'-fluorination on compound **6.4** had only a minor effect. Besides, no large difference was observed between β- and γ-fluorination on 1-propanol **6.6**. The same was also seen between γ- and δ-fluorination on 1-butanol **6.11**.

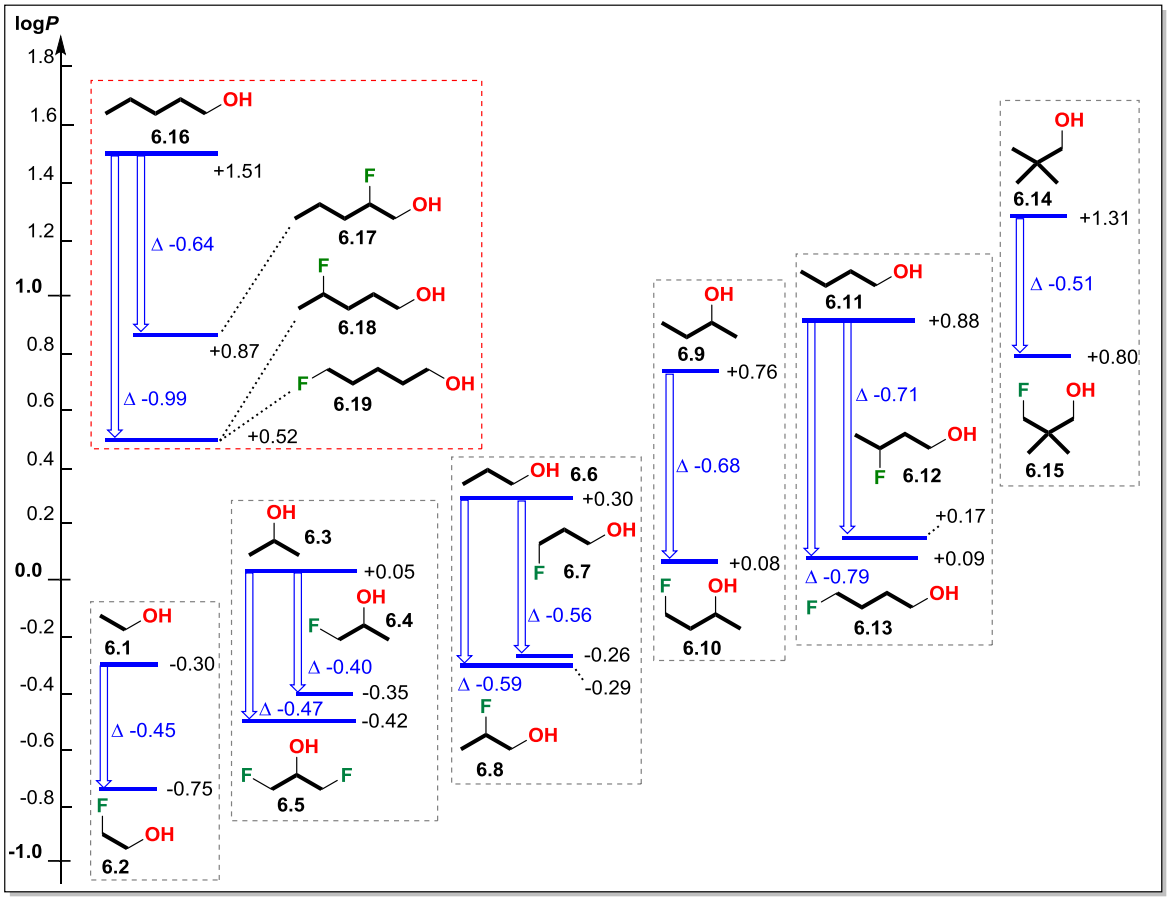


Figure 6.5 Monofluorination on acyclic fluorohydrins.

A larger log*P* decrease for neopentyl alcohol **6.14** could have been expected, based on the trend of impact of fluorination (Δlog*P* values) from ethanol to 1-butanol. However, only a relatively small influence was observed, in comparison with γ-fluorination on 2- and 1-butanol (**6.9** and **6.11**). It is possible that the absence of a polarisable C-H bond in β-position of compound **6.15** could weaken fluorine's effect on molecular polarity.

For 1-pentanol **6.16**, fluorination on 5- and 4-position (compound **6.19** and **6.18**) resulted in a rather large log*P* decrease (0.99 units). Interestingly, fluorine insertion on 2-position (compound **6.17**[18]) had a lesser impact.

Overall, monofluorination on aliphatic fluorohydrins displayed the opposite effect on lipophilicity, compared to the generally observed increase seen upon aromatic monofluorination.

[18]Synthesis of fluorohydrins **6.19** and **6.22** is included in the experimental part.

6.4.1.2 Difluorination

Except for ethanol **6.1** (Δlog*P* +0.01), *gem*-difluorination reduced the lipophilicity of all studied substrates (**Figure 6.6**). Compared with the impact of β- or γ-monofluorination on 1-propanol **6.6** and 2-butanol **6.9** (**Figure 6.5**, Δlog*P* 0.56-0.68), the influence of difluorination on lipophilicity was much reduced (Δlog*P* <0.34). Nevertheless, difluorination can also lead to a substantial decrease in log*P* values (compound **6.26**, Δlog*P* -0.80).

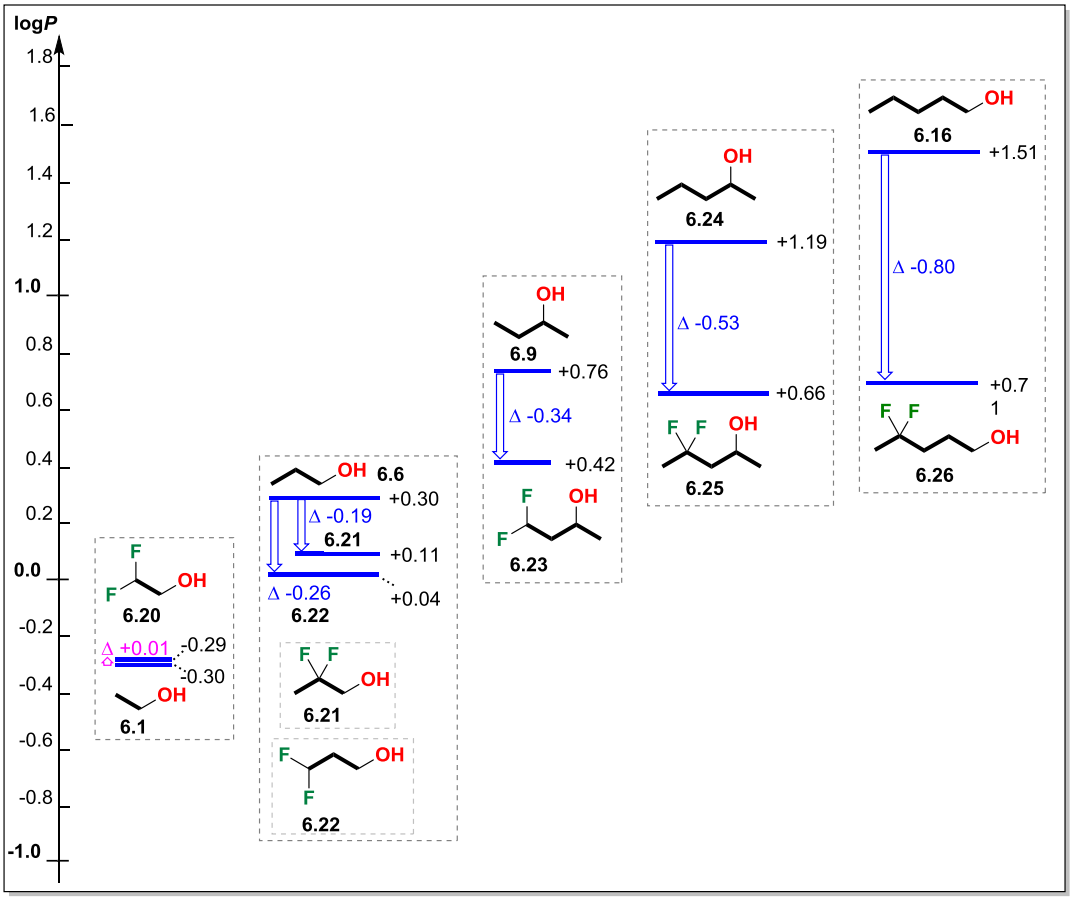


Figure 6.6 Difluorination on acyclic fluorohydrins.

6.4.1.3 Trifluorination

Trifluorination can modulate lipophilicity in both directions (**Figure 6.7**), depending on the relative fluorination position to hydroxyl group and also the lipophilic nature of the parent compounds. This is consistent with the observation,[20] and the explanation regarding polarity effect of CF_3 group in the literature.[22]

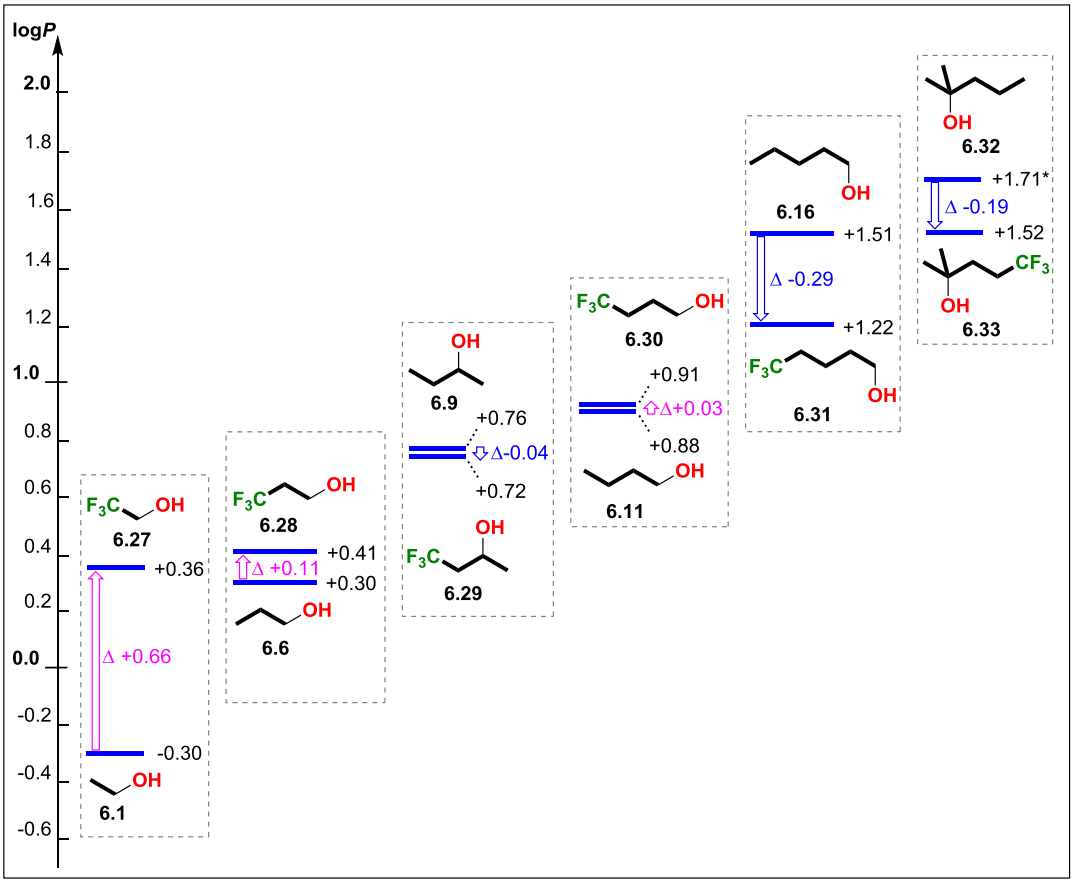


Figure 6.7 Trifluorination of acyclic fluorohydrins.

However, it was observed that the impact of β-trifluorination was independent of the absolute lipophilicity of the parent compounds. As shown in **Figure 6.8**, log*P* values were increased by nearly the same magnitude (ca. 0.65 log*P* units) after β-trifluorination, even though the log*P* values for ethanol **6.1** and 2-pentanol **6.24** are rather different. Lipophilicity can be further increased approximately by one log*P* units *via* second β-trifluorination (compound **6.35** and **6.38**).

It is assumed that the antiperiplanar orientation of C-O and C-F bond (**Figure 6.8**) causes the counteraction of bond dipoles, leading to a reduced polarity effect from the CF_3 group. Thus, hydrophobicity impact from CF_3 group dominates, resulting in increased lipophilicity.

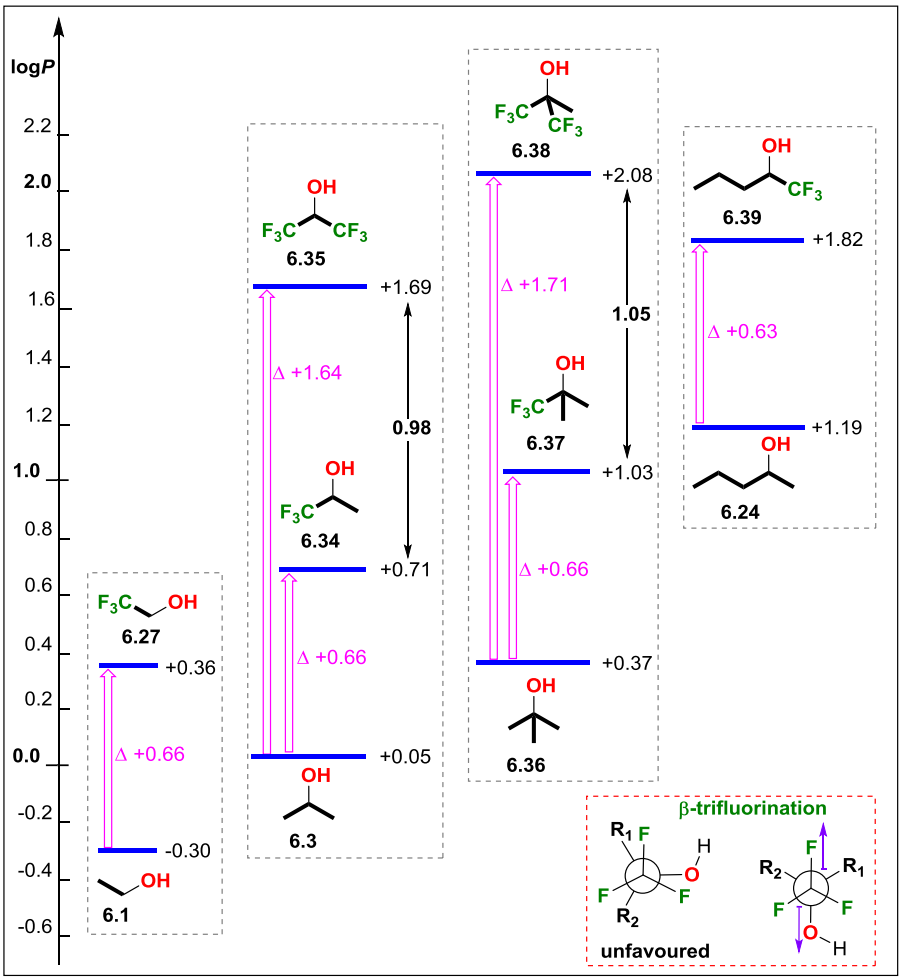


Figure 6.8 β-trifluorination on fluoroalkanols.

6.4.1.4 Polyfluorination

As shown in **Figure 6.9**, perfluorination of alkanols from the β-position onwards can substantially increase lipophilicity, with larger impact from heptafluorination (Δlog*P* up to 1.22 units, compound **6.45**). Presumably, this is also due to the counteraction of dipole moment, as shown in **Figure 6.8** (with one fluorine replaced by perfluoroalkyl groups). The effect of terminal pentafluorination (CF_3CF_2) was undermined, when it was further away from the hydroxyl group. Certainly, the impact from polarity of CF_3CF_2 group can potentially outcompete its contribution in hydrophobicity, similar to a CF_3 group. As can be seen on 1-pentanol **6.16**, terminal pentafluorination increased its log*P* value only by 0.21 units. With further elongated chains, this effect could be reversed.

Interestingly, terminal tetrafluorination (HCF_2CF_2) from β-position of 2-butanol **6.9** led to a much smaller log*P* increase (Δlog*P* +0.29), compared with pentafluorination. When the HCF_2CF_2 group was further away from alcohol group (compound **6.47**), it resulted in a large decrease in lipophilicity by 0.54 log*P* units. This tetrafluorinated compound was even less lipophilic than the trifluorinated

compound **6.31**, although it has a slightly larger hydrophobic surface based on calculation[19] (**Figure 6.9**). This implied that HCF_2CF_2 is a more polar group than CF_3CH_2.

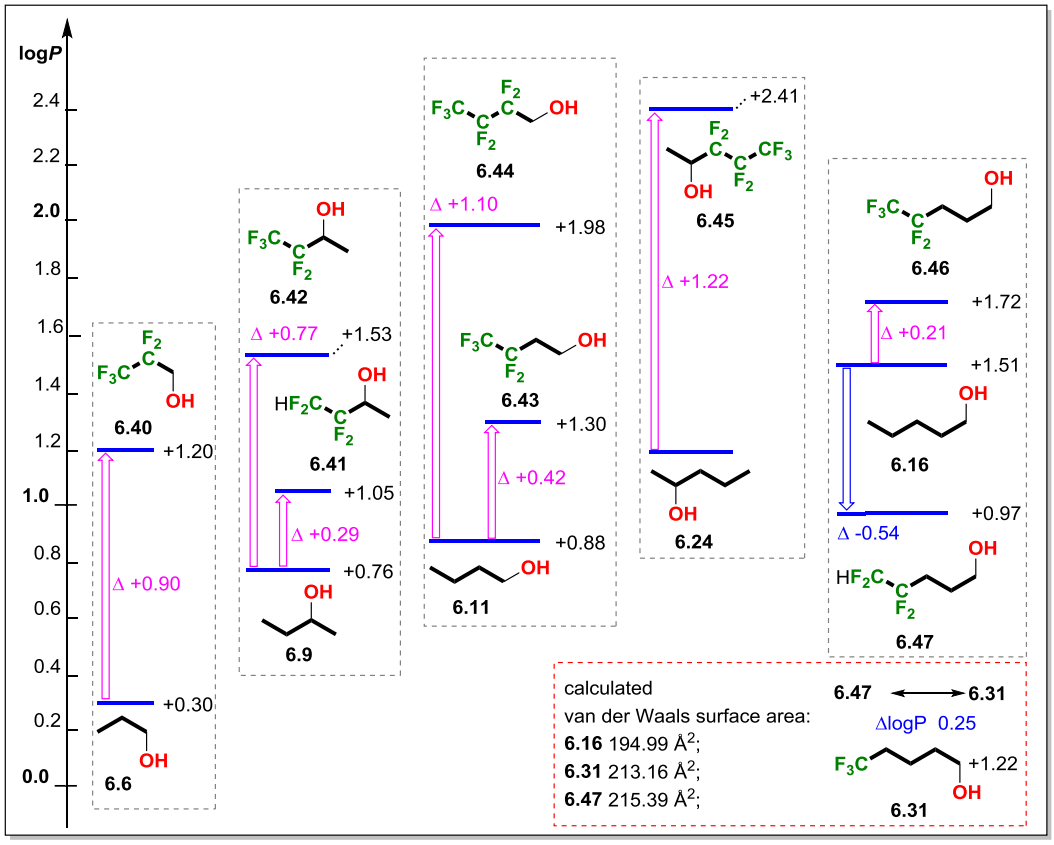


Figure 6.9 Polyfluorination on acyclic fluorohydrins.

It is assumed that terminal tetrafluorinated compounds mainly adopted a *gauche* conformation (**Figure 6.10**), with a large impact on the molecular polarity. Instead, dipole contribution of HCF_2CF_2 from the *anti*-conformer is relatively small. Indeed, this *gauche* conformation was also observed in crystal structures.[197],[198],[199],[200],[201]

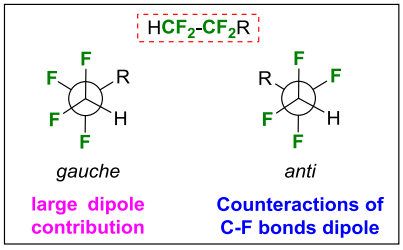


Figure 6.10 Conformation for terminal tetrafluorinated compounds.

[19]Van der Waals surface area was calculated by using MarvinSketch 14.8.4.0.

6.4.1.5 Comparision of different fluorinated motifs

As shown in **Figure 6.11**, fluorine introduction on monofluorinated alkanols (CHF to CF_2) always led to an increased log*P* (trend highlighted *in magenta*) of the corresponding *gem*-difluorinated compounds. Further fluorination (CHF_2 to CF_3) resulted in another log*P* rise (*in orange*), with larger effect on β-*gem*-difluoro compound **6.20**. As can be seen for the studied substrates, monofluorinated alkanols were always less liphophilic compared to the corresponding *gem*-difluorinated and trifluorinated compounds.

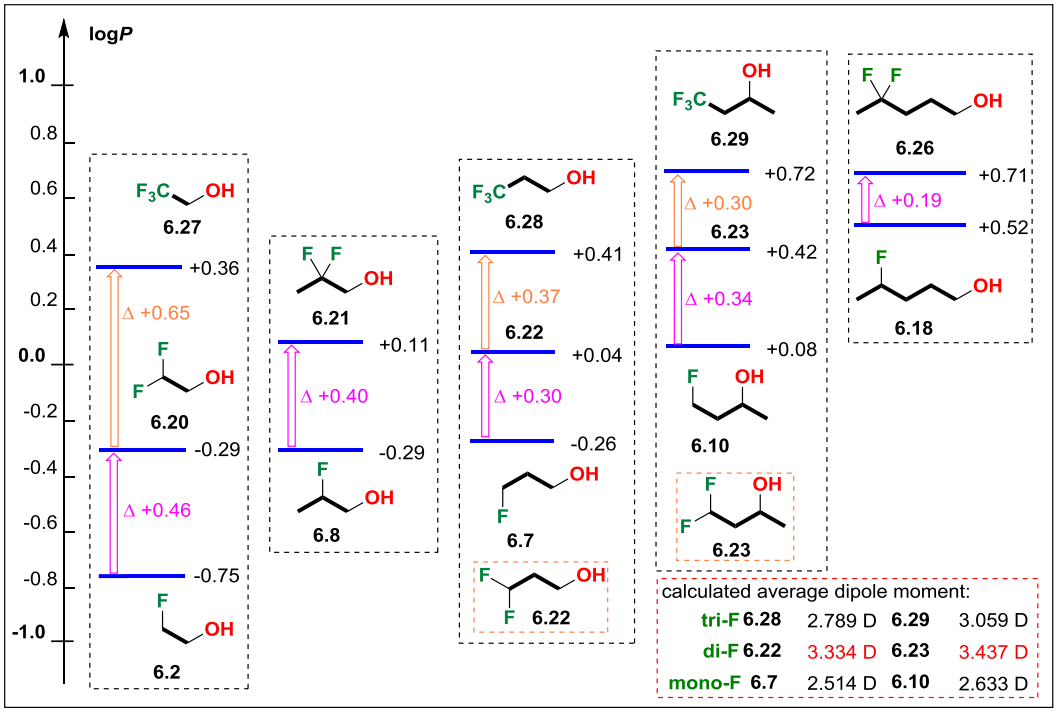


Figure 6.11 Comparison between mono-, di- and trifluorination.

Based on the calculated dipole moment (**Figure 6.11**),[20] it was observed that the CF_2 moiety had the strongest effect on molecular polarity. The increase in log*P* values from CHF_2 to CF_3 can be easily rationalized by a decline in polarity and an increased hydropobic surface. Regarding the log*P* rise from CHF to CF_2, it is assumed that the effect from enlarged hydrophobicity surpassed the polarity impact.

Interesting trends were also observed among polyfluorinated moieties. As illustrated in **Figure 6.12**, *gem*-difluorination on trifluorinated alkanols (CF_3CH_2 to CF_3CF_2) led to an increased lipophilicity (trend highlighted *in magenta*), with substantial effect for difluorination on β-position of the alcohol

[20]Calculated by our collaborator in University of Nantes, calculated at the IEFPCM-MP2/6-311++G(2d,p)//MPWB1K/6-31+G(d,p) level of theory; μ (D) in $CHCl_3$ at 298K;

group (Δlog*P* ca. 0.80 units for compound **6.40** and **6.42**). Fluorine substitution on terminal tetrafluorinated compounds (HCF_2CF_2 to CF_3CF_2) resulted in similar lipophilicity enhancement (*in orange*). Interestingly, this effect was more profound when the polyfluorinated moiety was further away from hydroxyl group (Δlog*P* 0.75 unit for compound **6.46**).

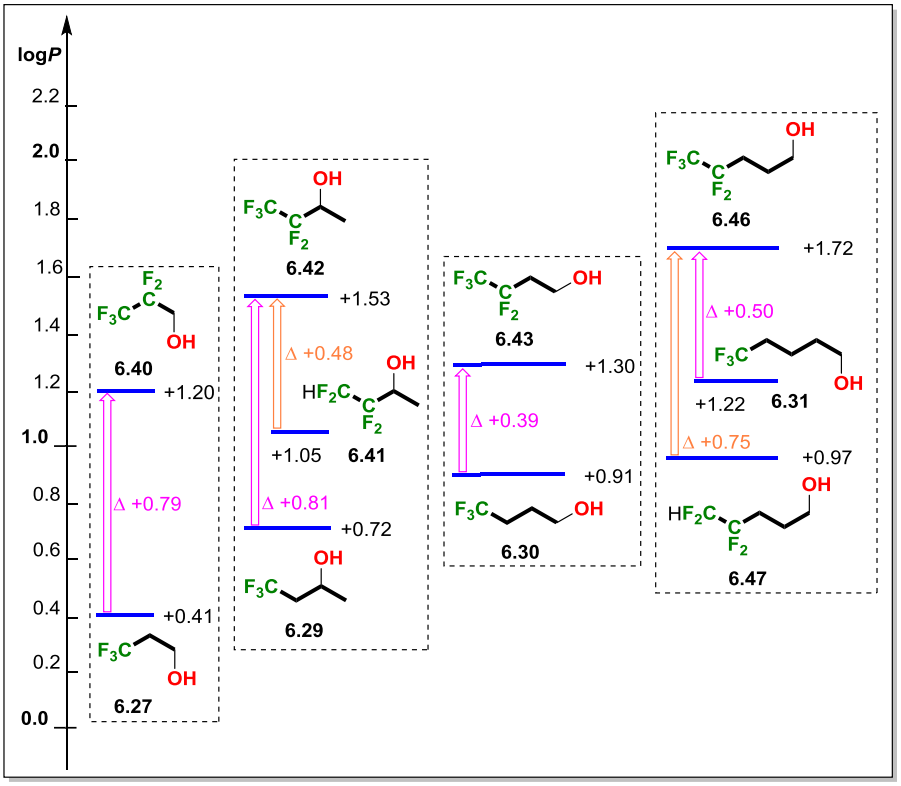


Figure 6.12 Comparison between polyfluorination.

6.4.1.6 Lipophilicity of fluorinated diastereoisomers

The lipophilicities of fluorinated diastereoisomers were also measured, as shown in **Figure 6.13** (**A-C**). In all three cases, inversion of stereochemistry resulted in differences in log*P* values. The ΔΔlog*P* is up to 0.13 units between monofluorinated isomers **6.55** and **6.56** (**Figure 6.13-C**). Although *vic*-difluoro moiety has been recently identified as an effective motif in lowering molecular polarity and lipophilicity in model compounds,[24] only a small decrease (Δlog*P* < 0.2 units) was observed for *vic*-difluorination on compound **6.48** (**Figure 6.13-A**). Interestingly, further *vic*-difluorination (CHFCHF to CF_2CF_2) led to a significant log*P* increase. This is possibly due to the enlarged hydrophobic surface and also the counteractions between bond dipole, as C-H bonds at both β-positions in the diol were all replaced by C-F bonds (compound **6.51**).

For γ-fluorinated diastereoisomers (**Figure 6.13 B-C**), the *anti*-isomers were more lipophilic than the corresponding *syn*-isomers. It is assumed that the most polar conformation of *syn*-isomers is

more favourable by water stabilisation (**Figure 6.13-D**). Indeed, the calculations[21] suggested that *syn*-isomer **6.53** has much higher molecular polarity compared to its *anti*-isomer. Therefore, for *syn*-isomers, larger effect on polarity by fluorination resulted in relatively lower lipophilicity.

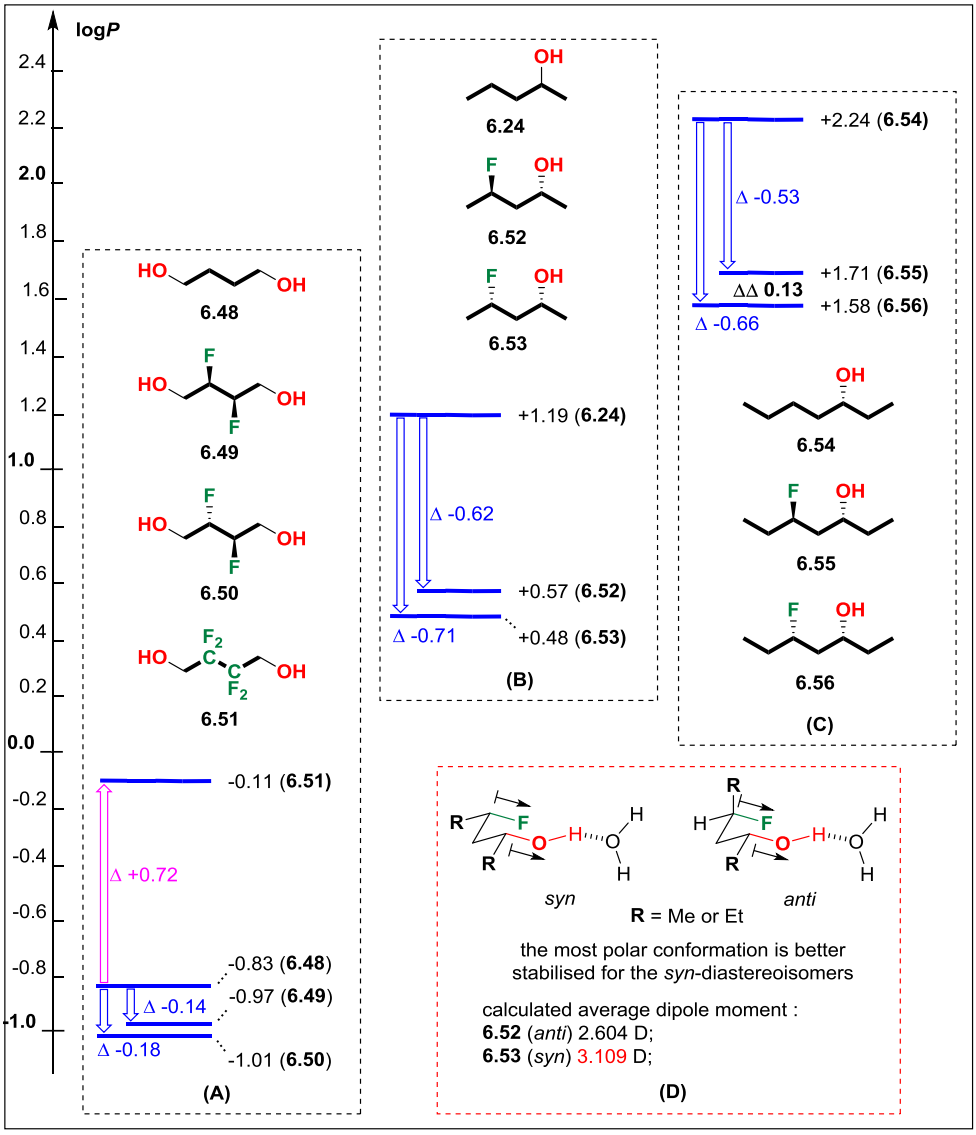


Figure 6.13 Lipophilicity of fluorinated diastereoisomers.

6.4.1.7 Monofluorination on rigid cyclohexanols

The impact on lipophilcity by fluorination was also investigated on the conformationally restricted cyclohexanols, as shown in **Figure 6.14**. For parent compound **6.57** (with equatorial OH group), β-monofluorination decreased its lipophilicity by similar magnitude (log*P* ca. 0.5 units) regardless of the configuration of fluorine.

[21]See footnote 20;

However, β-monofluorination on compound **6.60** surprisingly led to an increased log*P*. Instead, insertion of an axial fluorine on γ-position caused a substantial decrease by 0.79 log*P* units. This is rather interesting, as single fluorination can modulate lipophilicity in both directions. It is assumed that the log*P* rise for **6.61** was due to the counteraction between C-F/C-O bond dipoles (**Figure 6.14**). For 1,3-diaxial alkanol **6.62**, alignment of C-F/C-O bond dipoles maximized the polarity impact by fluorination, and thus resulted in a reduction of log*P*.

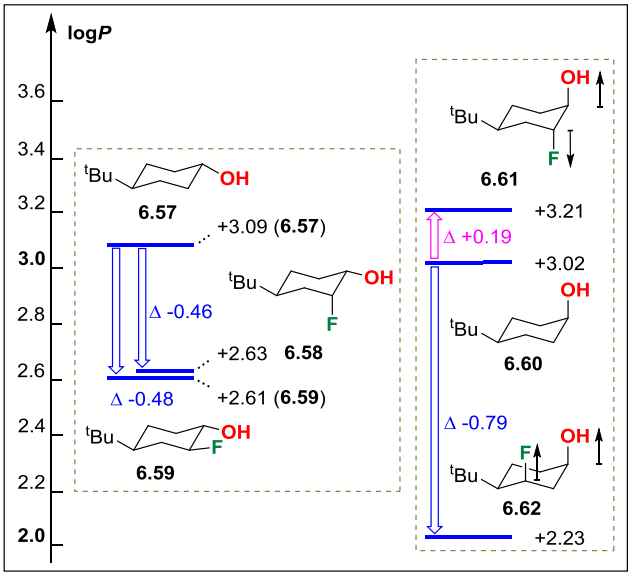


Figure 6.14 Monofluorination on rigid cyclohexanols.

6.4.2 Lipophilicity of fluorinated carbohydrates

Fluorinated carbohydrates have been increasingly exploited in chemical biology for the understanding of biological processes.[202],[203],[204] For example, deoxyfluorination can be used to investigate the role of hydroxyl groups in carbohydrate-protein binding.[205] As lipophilicity also contributes to potency gain,[10] the interpretation of the biological results can be biased without thorough evaluation of influence of deoxyfluorination on lipophilicity. However, the log*P* quantification of non-UV-active carbohydrates is rather challenging, which makes these compounds suitable substrates for our method.

As shown in **Figure 6.15**, monodeoxyfluorination led to a substantial increase in log*P* values (Δlog*P* ca. 1 units), compared to log*P* value of glucose. The impact of stereochemistry can also be observed, e.g., between C-4 epimers **6.64** and **6.66** (Δlog*P* 0.16 units). Dideoxyfluorination resulted in further reduction of its hydrophilicity. The log*P* value for difluoro analogue **6.69** was increased by over 2.1 log*P* units. Interestingly, monofluorination of compound **6.68** to **6.69** caused a log*P* rise by over 0.3 units. This is presumably due to the counteraction between dipoles of the two *vic-trans* C-F bonds (**Figure 6.15**).

Further *vic*-difluorination (CHFCHF to CF_2CF_2) on **6.69** led to another log*P* rise by ca. 0.8 units, which is similar to the previous observation on tetrafluorinated 1,4-butanediol derivative. A larger difference (0.26 units) was also seen between the C-4 epimers **6.70** and **6.71**. Replacement of three hydroxyl groups by fluorine substitution (compound **6.72**) resulted in a log*P* leap by 3 log*P* units.

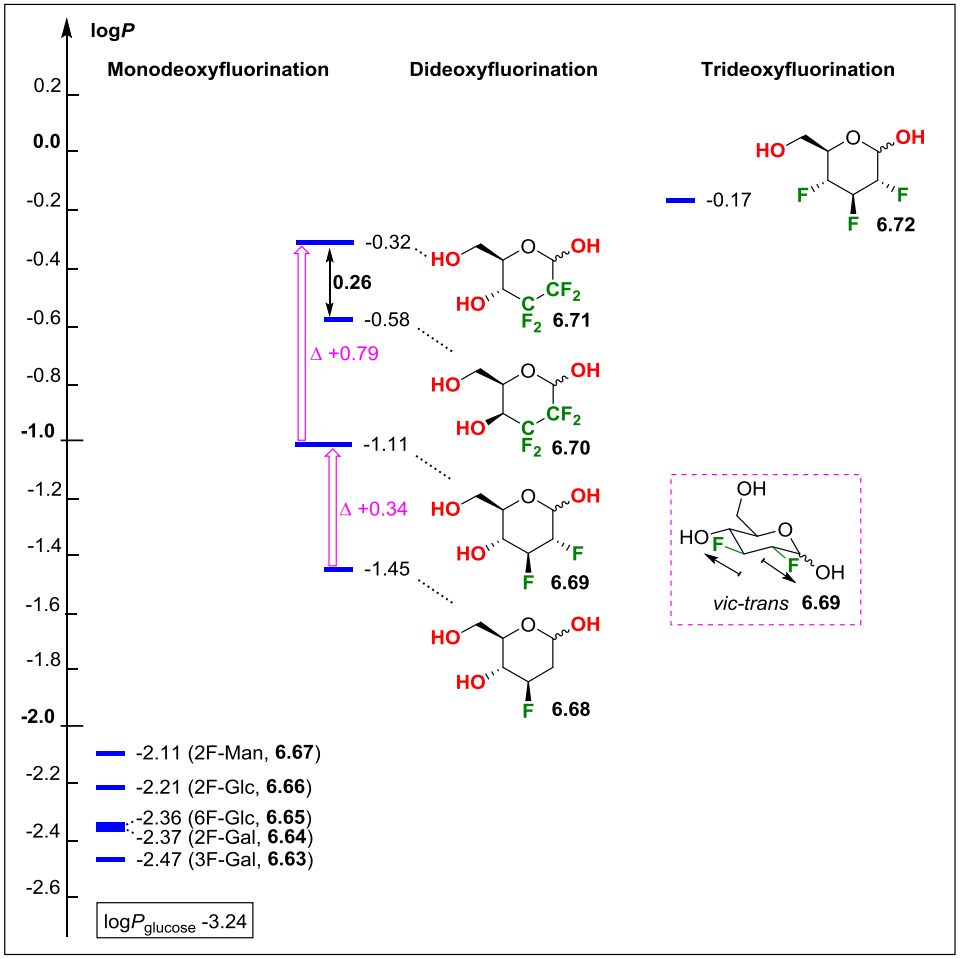


Figure 6.15 Lipophilicity of fluorinated carbohydrates.

6.5 Conclusion

A novel and straightforward method was developed for the accurate measurement of lipophilicity of fluorinated compounds by using ^{19}F NMR, which is compatible with non-UV-active fluorinated compounds. Optimal NMR parameters and settings were identified as the following for log*P* measurement: proton-decoupling (for higher SNR), centered O1P, sufficient D1 (recommended settings: 30 sec for octanol sample and 60 sec for water sample), narrower SW, and increased number of transients. Extended NS and narrower SW lead to better SNR but require longer NMR experiment time. The reproducibility of the method was confirmed by control experiments. The feasibility and validity of the method was further proven by the internal consistency and also the overall consistency of measured log*P* values with literature data.

Using this method, large numbers of fluorohydrins were measured to investigate the influence of fluorination. It was found that monofluorination can cause significant reduction in lipophilicity (with exceptions). The log*P*-lowering effect from *gem*-difluorination is slightly less. Trifluorination can either increase or decrease lipophilicity. However, β-trifluorination, as well as terminal penta-/heptafluorination from β-position, always leads to a large log*P* increase. Besides, the impact of fluorination is dependent on the relative stereochemistry. The first log*P* values for fluorinated carbohydrates were also determined, and the impact of deoxyfluorination was shown to depend on the stereochemistry, fluorination position(s), and also the fluorinated moieties.

SECTION IV EXPERIMENTAL PART

Chapter 7: Experimental for Bile Acids

7.1 General Methods

Chemical reagents were obtained from commercial sources and used without further purification, unless stated otherwise. Anhydrous solvents were distilled immediately prior to use, with the exception of anhydrous DMF which was purchased in sealed containers from commercial sources. THF was distilled from Na/benzophenone immediately prior to use. DCM and Et_3N were dried over CaH_2. All glassware was flame-dried under vacuum and cooled under N_2 prior to use. Water or air sensitive reactions were performed under inert atmosphere, using dry solvents.

Reactions were monitored by TLC (Merck Keiselgel 60 F_{254}, aluminium sheet). Detection was carried out using one of the following dying reagents. Anisaldehyde-reagent: A solution of 5.1 mL p-anisaldehyde, 2.1 mL AcOH and 6.9 mL H_2SO_4 in 186 mL EtOH gives a reagent that will show varied coloured spots after development with a heat gun. $KMnO_4$-reagent: A solution of 3 g $KMnO_4$, 20 g K_2CO_3 and 5 mL NaOH (aq., 5%) in 300 mL H_2O gives a reagent that will show yellow spots after development with a heat gun.

Column chromatography was performed on silica gel (60 Å, particle size 35-70 µm). All reported solvent mixtures are volume measures. Preparative HPLC was carried out using a Biorad Bio-Sil D 90-10 column (250×22 mm at 15mL min^{-1}).

^{1}H, ^{19}F, ^{13}C NMR spectra were recorded at room temperature on a BRUKER AV300/400/500 spectrometer. ^{1}H and ^{13}C chemical shifts (δ) are quoted in ppm relative to residual solvent peaks as appropriate. ^{19}F spectra were externally referenced to $CFCl_3$. The coupling constants (J) are given in Hertz (Hz). The proton NMR signals were designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sxt (sextet), spt (septet), m (multiplet), or a combination of the above.

IR spectra were recorded as neat films on a Nicolet 380 FT-IR. Absorption peaks are given in cm^{-1} and the intensities were designated as follows: w (weak), m (medium), s (strong), br (broad). Optical rotations were recorded on an OPTICAL ACTIVITY POLAAR 2001 polarimeter at 589 nm. Melting points were recorded on a Reichert melting point apparatus, equipped with a Reichert microscope. Low resolution ES mass spectra were recorded on a WATERS ZMD single quadrupole system. High resolution mass spectra were recorded on the Bruker Apex III FT-ICR-MS.

7.2 General procedures

7.2.1 Procedure A for oxidation of 7α-hydroxy group using NaClO

To a round-bottom flask were added chenodeoxycholic acid or its derivatives (1 eq), sodium bromide (0.057 eq), TBAB (3.30 eq) and a solution of MeOH/MeCOOH/H_2O/AcOEt (v/v: 3/1/0.25/6.5, 8.43 mL/mmol) and the reaction mixture was stirred till the solution was homogeneous. Sodium hypochlorite solution (11-14%, 1.1 eq) was added at 0 °C till the hypochlorite test (peroxide test paper) was positive. The reaction mixture was stirred at room temperature for 6 h. After TLC indicated the completion of the reaction, the reaction mixture was quenched with sodium bisulfite solution (3.3%) till the hypochlorite test was negative. Water was added and stirred for 15 min at room temperature. The mixture was extracted with ethyl acetate (three times) and the combined organic phase was washed with aqueous sodium bisulfite solution (3.3%), water and dried over anhydrous sodium sulphate, filtered and concentrated. The crude was purified by flash chromatography to afford the desired compound.

7.2.2 Procedure B for 24-carboxylic acid protection as methyl ester

To a round-bottom flask were added CDCA (1 g, 2.55 mmol, 1 eq), MeOH (20 mL, 8 mL/mmol) and *p*-toluenesulfonic acid monohydrate (50 mg, 0.26 mmol, 0.1 eq). The reaction mixture was stirred until the solution was homogeneous, and then sonicated at 30 °C for 2 h. The reaction mixture was concentrated and the residue was dissolved in chloroform (30 mL), washed with saturated $NaHCO_3$ (2 × 10 mL), water (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and concentrated to afford the methyl ester quantitatively as gummy solid, and used for next step without any purification.

7.2.3 Procedure C for preparation of silyl enol ether from ketone derivatives

To a round-bottom flask were added DIPA (2.75 mL, 19.66 mmol, 12.6 eq) and THF (25 mL, 1.25 mL/mmol). The solution was cooled to -78 °C, then n-butyllithium (2.5 M in hexanes, 7.45 mL, 18.72 mmol, 12 eq) was added dropwise and stirred at -78 °C for 30 min. TMSCl (1.98 mL, 15.6 mmol, 10 eq) was added and stirred for 20 min. A solution of benzyl 3α-hydroxy-7-keto-5β-cholan-24-oate **3.3** (750 mg, 1.56 mmol, 1 eq) in THF (10.2 mL, 6.5 mL/mmol) was then added dropwise in 10 min and stirred at this temperature for 45 min, followed by the addition of triethylamine (3.92 mL, 28.1 mmol, 18 eq) and stirred for 1 h. The reaction mixture was warmed to -20 °C, quenched with saturated $NaHCO_3$ solution (5 mL) and warmed to room temperature in 2 h. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 25 mL). The combined organic layer was washed with saturated $NaHCO_3$ solution (40 mL), water (40 mL), brine (40 mL),

dried over Na_2SO_4, filtered and concentrated to afford the desired sily enol ether intermediate (95%) and was used for further reaction without any purification.

7.2.4 Procedure D for Mukaiyama aldol addition (preparation of 6-ethylidene derivatives)

To a round-bottom flask were added benzyl 3α,7-bis(trimethylsilyloxy)-5β-cholan-6-en-24-oate **3.4** (975 mg, 1.56 mmol, 1 eq), DCM (16 mL) and MeCHO (0.175 mL, 2 eq). Boron trifluoride diethyl etherate (1.93 mL) was added dropwise at -78 °C. The reaction mixture was stirred at -78 °C for 2 h and then stirred at room temperature overnight. The reaction mixture was quenched with saturated $NaHCO_3$ solution (10 mL), and extracted with DCM (3 × 25 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography, and by HPLC if needed.

7.2.5 Procedure E for hydrogenation/hydrogenolysis using catalyst

To a round-bottom flask was added benzyl 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oate **3.6** [or its derivatives] (1.0 eq), catalyst [Pd/C or PtO_2] (10 mol%) and solvent [MeOH, EtOH, etc]. The reaction mixture was degassed with hydrogen gas and then stirred under H_2 at atmospheric pressure or high pressure for 16-72 hours. The catalyst was filtered through Celite and the filtrate was concentrated and purified by flash chromatography.

7.2.6 Procedure F for eletrophilic fluorination of silyl enol ether using Selectfluor in DMF

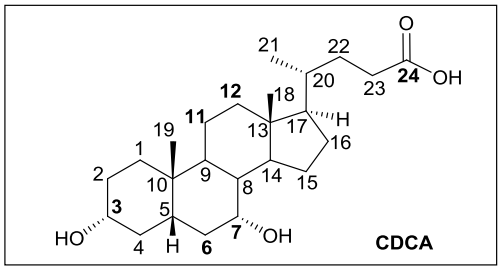
To a round bottom flask were added benzyl 3α-pivaloyloxy-7-trimethylsilyloxy-5β-cholan-6-en-24-oate **3.70** (574 mg, 0.90 mmol, 1.0 eq), DMF (4 mL, 2 mL/mmol) and a solution of Selectfluor (480 mg, 1.35 mmol, 1.5 eq) in DMF (5 mL, 3 mL/mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight, quenched with H_2O (10 mL) and extracted with ethyl acetate (50 mL × 4). The combined organic layer was washed with brine (70 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography, and by HPLC if needed.

7.2.7 Procedure G for reduction of ketone derivatives using $NaBH_4$

To a round bottom flask were added benzyl 3α-pivaloyloxy-6β-fluoro-7α-keto-5β-cholan-24-oate **3.71** (78 mg, 0.134 mmol, 1.0 eq), sodium borohydride (12 mg, 0.32 mmol, 2.4 eq) and anhydrous THF (3 mL). The reaction mixture was stirred at room temperature overnight, quenched with H_2O (5 mL) and extracted with ethyl acetate (15 mL × 3). The combined organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography, and by HPLC if needed.

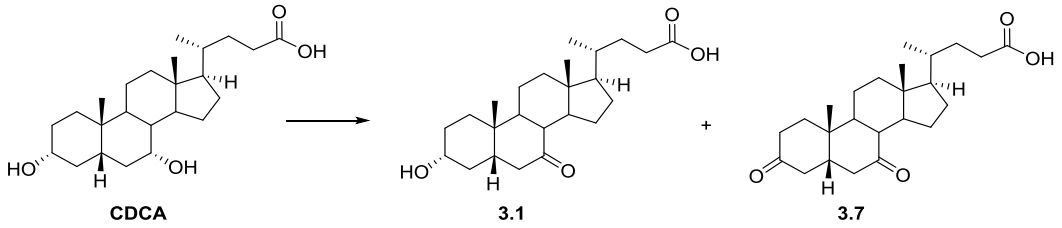
7.3 Synthesis of 6-ECDCA and its analogues

The order of the following compound synthesis and characterisation is based on the order of reactions discussed from *section 3.1.3* in *Chapter 3*. The numbering of steroid structure (*see example below*) is used for the assignment of all compounds.



7.3.1 Synthesis of 6-ECDCA

Synthesis of 3α-hydroxy-7-keto-5β-cholanic acid (3.1) and 3,7-diketo-5β-cholanic acid (3.7)

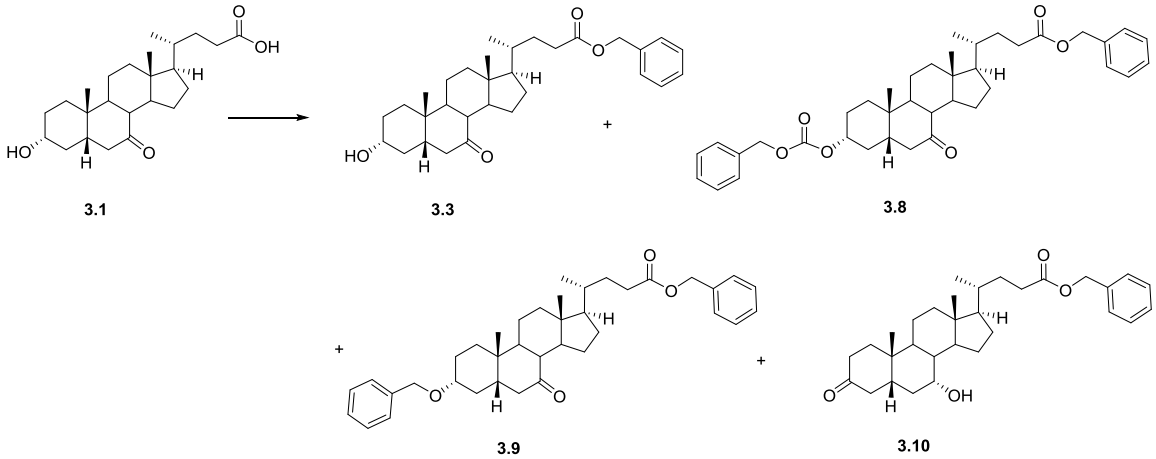


Following general procedure A, chenodeoxycholic acid (1.0 g, 2.55 mmol) was oxidized and purified by flash chromatography (MeOH/DCM 2:98–30:70) to afford the desired compound **3.1** (white solid, 568mg, 1.46 mmol, 57%) and the by-product **3.7** (white solid, 328mg, 0.844 mmol, 33%). Compound **3.1** was recrystallized from methanol to afford transparent crystals.

3.1, **Formula** $C_{24}H_{38}O_4$; **MW** 390.56; **R_f** 0.46 (MeOH/DCM 8:92); **^{1}H NMR** (300 MHz, CD_3OD) δ 3.45 - 3.61 (1H, m, $H_{3\beta}$), 2.99 (1H, dd, *J* 12.4, 6.0 Hz, $H_{6\beta}$), 2.54 (1H, t, *J* 11.3 Hz, $H_{8\beta}$), 1.23 (3H, s, H_{19}), 0.97 (3H, d, *J* 6.5 Hz, H_{21}), 0.71 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, CD_3OD) δ 215.2 (C_7), 178.2 (C_{24}), 71.6 (C_3), 56.4, 50.8, 50.6, 47.6, 46.5, 44.5, 44.0, 40.5, 38.4, 36.7, 36.4, 35.3, 32.5, 32.1, 30.8, 29.4, 25.9, 23.6, 23.0, 19.0, 12.6 ppm; NMR data were consistent with the data in the literature.[85]

3.7, **Formula** $C_{24}H_{36}O_4$; **MW** 388.54; **R_f** 0.55 (MeOH/DCM 8:92); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 2.88 (1H, dd, *J* 12.9, 4.9 Hz), 2.47 (1H, d, *J* 11.4 Hz), 1.30 (3H, s, H_{19}), 0.94 (3H, d, *J* 6.3 Hz, H_{21}), 0.69 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 211.3 (C_3 or C_7), 210.5 (C_3 or C_7), 180.2 (C_{24}), 54.7, 49.5, 48.8, 47.7, 44.9, 42.9, 42.8, 42.6, 38.8, 36.7, 35.4, 35.4, 35.1, 31.0, 30.7, 28.2, 24.7, 22.4, 22.1, 18.3, 12.1 ppm; ^{1}H NMR data were consistent with the data in the literature.[206]

Synthesis of benzyl 3α-hydroxy-7-keto-5β-cholan-24-oate (3.3), benzyl 3α-(((benzyloxy)carbonyl)oxy)-7-keto-5β-cholan-24-oate (3.8), benzyl 3α-benzyloxy-7-keto-5β-cholan-24-oate (3.9) and Benzyl 3-keto-7α-hydroxy-5β-cholan-24-oate (3.10)



To a round-bottom flask were added 3α-hydroxy-7-keto-5β-cholanic acid **3.1** (5.98 g, 15.3 mmol, 1 eq), DMF (60 mL, 4 mL/mmol) and cesium carbonate (7.59 g, 23.0 mmol, 1.5 eq). The reaction mixture was stirred at room temperature for 40 min and benzyl bromide (9.1 mL, 75.6 mmol, 5 eq) was added and stirred overnight. After removal of DMF under reduced pressure, the residue was dissolved in ethyl acetate (100 mL) and water (70 mL), and then extracted with ethyl acetate (3 × 150 mL). The combined organic layer was washed with brine (200 mL), dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (MeOH/DCM 0:100–3:97) and repeated HPLC (acetone/hexane 13:87–30:70) to afford **3.3** (5.5 g, 71%, gummy solid [after under high vac for 3 d]), **3.8** (1.6 g, 13%, gummy solid), **3.9** (0.7%, gummy solid) and **3.10** (2%, gummy solid).

3.3, **Formula** $C_{31}H_{44}O_4$; **MW** 480.68; **$[\alpha]_D$** -27.0 (c 1.0, $CHCl_3$, 21 °C), lit.[83] **$[\alpha]_D$** -10.9 (c 0.99, $CHCl_3$, 25 °C); **R_f** 0.34 (acetone/hexane 30:70); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.28 - 7.43 (5 H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, CH_2Ph), 5.10 (1H, d, *J* 12.4 Hz, CH_2Ph), 3.49 - 3.71 (1H, m, $H_{3\beta}$), 2.86 (1H, dd, *J* 12.3, 6.0 Hz, $H_{6\beta}$), 1.20 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.3 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 211.9 (C_7), 174.0 (C_{24}), 136.1 (C_{Ar}), 128.5 (C_{Ar}), 128.2 (C_{Ar}), 128.1 (C_{Ar}), 70.9 (C_3), 66.1(*C*H_2Ph), 54.8, 49.5, 48.9, 46.1, 45.4 , 42.7, 42.6, 38.9, 37.4, 35.16, 35.14, 34.1, 31.3, 31.0, 29.9, 28.2, 24.8, 23.0, 21.7, 18.3, 12.0 ppm; ^{1}H NMR data were consistent with the data in the literature.[83]

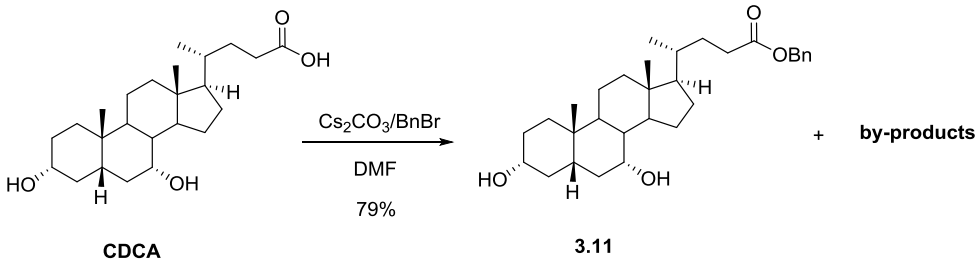
3.8, **Formula** $C_{39}H_{50}O_6$; **MW** 614.81; **$[\alpha]_D$** +1.7 (c 0.5, $CHCl_3$, 21 °C); **R_f** 0.32 (ethyl acetate/hexane 20:80); **IR** (neat) 2949 (m), 2873 (w), 1735 (s), 1709 (s), 1455 (w), 1387 (w), 1247 (s), 1157 (m), 971 (w), 952 (w), 733 (m), 699 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.30 - 7.41 (10H, m, H_{Ar}), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2OCOSt), 5.13 (2H, s, PhCH_2OCO$_2$St), 5.08 (1H, d, *J* 12.3 Hz, PhCH_2OCOSt), 4.49 - 4.65 (1H, m, $H_{3\beta}$), 2.85 (1H, dd, *J* 12.8, 6.3 Hz), 1.21 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.6 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 211.3 (C_7), 173.9 (C_{24}), 154.5 (BnO*C*O$_2$), 136.1 (C_{Ar}), 135.3 (C_{Ar}), 128.6 (*C*H$_{Ar}$), 128.5 (*C*H$_{Ar}$), 128.4 (*C*H$_{Ar}$), 128.21 (*C*H$_{Ar}$), 128.21 (*C*H$_{Ar}$), 128.1 (*C*H$_{Ar}$), 77.0 (C_3),

69.4 (Ph*C*H$_2$OCO$_2$St), 66.1 (Ph*C*H$_2$OCOSt), 54.7 (*C*H), 49.4 (*C*H), 48.9 (*C*H), 45.7 (*C*H), 45.1 (*C*H$_2$), 42.59 (*C*H), 42.59(*C*), 38.9 (*C*H$_2$), 35.15 (*C*H), 35.10 (*C*), 33.7 (*C*H$_2$), 33.0 (*C*H$_2$), 31.3 (*C*H$_2$), 30.9 (*C*H$_2$), 28.2 (*C*H$_2$), 26.0 (*C*H$_2$), 24.8 (*C*H$_2$), 22.9 (C_{19}), 21.7 (*C*H$_2$), 18.3(C_{21}), 12.0 (C_{18}) ppm; **MS** (ESI+) *m/z* 632.3 ([M+NH$_4$]$^+$, 100%); **HRMS** (ESI+) for $C_{39}H_{54}NO_6$ [M+NH$_4$]$^+$ calcd 632.3946, found 632.3946 (-0.1 ppm error).

3.9, **Formula** $C_{38}H_{50}O_4$; **MW** 570.80; **R$_f$** 0.29 (acetone/hexane 13:87); **IR** (neat) 2933 (s), 2869 (m), 1739 (s), 1705 (s), 1455 (m), 1383 (m), 1365 (m), 1164 (s), 1096 (s), 737 (m), 699 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 - 7.40 (10H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, PhC*H*$_2$OCOSt), 5.10 (1H, d, *J* 12.4 Hz, PhC*H*$_2$OCOSt), 4.56 (1H, d, *J* 11.9 Hz, PhC*H*$_2$OSt), 4.50 (1H, d, *J* 11.7 Hz, PhC*H*$_2$OSt), 3.36 (1H, tt, *J* 11.2, 4.3 Hz, $H_{3\beta}$), 2.85 (1H, dd, *J* 12.6, 5.7 Hz), 1.19 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.2 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 211.7 (C_7), 174.0 (C_{24}), 138.8 (C_{Ar}), 136.1 (C_{Ar}), 128.5 (*C*H$_{Ar}$), 128.3 (*C*H$_{Ar}$), 128.2 (*C*H$_{Ar}$), 128.1 (*C*H$_{Ar}$), 127.5 (*C*H$_{Ar}$), 127.4 (*C*H$_{Ar}$), 77.3 (C_3), 69.8 (Ph*C*H$_2$OSt), 66.1 (Ph*C*H$_2$OCOSt), 54.7, 49.5, 48.9, 46.0, 45.5, 42.6, 42.5, 38.9, 35.4, 35.2, 34.2, 33.9, 31.3, 31.0, 28.3, 26.9, 24.8, 23.0 (C_{19}), 21.6, 18.3 (C_{21}), 12.0 (C_{18}) ppm; **MS** (ESI+) *m/z* 593.3 ([M+Na]$^+$, 100%), 1163.6 ([2M+Na]$^+$, 72%); **HRMS** (ESI+) for $C_{38}H_{54}NO_4$ [M+NH$_4$]$^+$ calcd 588.4047, found 588.4056 (-1.5 ppm error).

3.10, **Formula** $C_{31}H_{44}O_4$; **MW** 480.68; **IR** (neat) 3450 (br. w), 2926 (m), 2858 (m), 1727 (m), 1712 (s), 1455 (w), 1376 (w), 1160 (s), 726 (s), 703 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.30 - 7.42 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, PhC*H*$_2$), 5.10 (1H, d, *J* 12.4 Hz, PhC*H*$_2$), 3.86 - 3.99 (1H, m, $H_{7\beta}$), 3.40 (1H, dd, *J* 15.3, 13.8 Hz), 1.01 (3H, s, H_{19}), 0.94 (3H, d, *J* 6.3 Hz, H_{21}), 0.69 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 213.1 (C_3), 174.0 (C_{24}), 136.1 (C_{Ar}), 128.5 (*C*H$_{Ar}$), 128.2 (*C*H$_{Ar}$), 128.2 (*C*H$_{Ar}$), 68.5 (C_7), 66.1 (Ph*C*H$_2$), 55.8 (*C*H), 50.3 (*C*H), 45.6 (*C*H$_2$), 43.2 (*C*H), 42.7 (*C*), 39.5 (*C*H$_2$), 39.4 (*C*H), 36.9 (*C*H$_2$), 36.8 (*C*H$_2$), 35.28 (*C*H), 35.28 (*C*), 33.9 (*C*H$_2$), 33.3 (*C*H), 31.2 (*C*H$_2$), 30.9 (*C*H$_2$), 28.1 (*C*H$_2$), 23.7 (*C*H$_2$), 21.9 (C_{19}), 21.0 (*C*H$_2$), 18.2 (C_{21}), 11.8 (C_{18}) ppm; **MS** (ESI+) *m/z* 961.3 ([2M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{31}H_{48}NO_4$ [M+NH$_4$]$^+$ calcd 498.3578, found 498.3585 (-1.3 ppm error).

Synthesis of benzyl 3α,7α-dihydroxy-5β-cholan-24-oate (3.11)



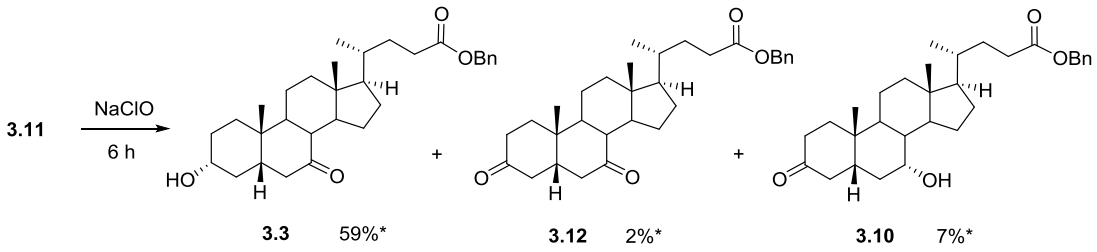
CDCA **3.11**

To a round-bottom flask were added CDCA (400 mg, 1.02 mmol, 1.0 eq), DMF (6 mL, 6 mL/mmol). The resulting mixture was cooled to 0 °C, followed by the addition of Cs_2CO_3 (500 mg, 1.53 mmol, 1.50 eq). The suspension was stirred for 40 min, and BnBr (0.61 mL, 5.1 mmol, 5 eq) was added. The reaction mixture was stirred at 0 °C for 30 min and then left at room temperature overnight.

Following TLC analysis, DMF was removed under reduced pressure. The residue was dissolved in EtOAc and water, followed by extraction with EtOAc (× 3). The combined organic layer was washed with brine, dried over $MgSO_4$/Na_2SO_4, and concentrated. The crude mixture was purified by flash chromatography (MeOH/DCM 1:99 – 5:95) to afford compound **3.11** (389 mg, 0.81 mmol, 79%) as white solid.

3.11, **Formula** $C_{31}H_{46}O_4$; **MW** 482.69; **R_f** 0.24 (MeOH/DCM 3:97); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.30 - 7.40 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, CH_2Ph), 5.10 (1H, d, *J* 12.4 Hz, CH_2Ph), 3.80 - 3.89 (1H, m, $H_{7\beta}$), 3.38 - 3.55 (1H, m, $H_{3\beta}$), 0.92 (3H, d, *J* 7.7 Hz, H_{21}), 0.91 (3H, s, H_{19}), 0.64 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.0 (C_{24}), 136.1 (C_{Ar}), 128.5 (C_{Ar}), 128.2 (C_{Ar}), 128.1 (C_{Ar}), 72.0(C_3), 68.5(C_7), 66.1(CH$_2$Ph), 55.8, 50.4, 42.7, 41.5, 39.9, 39.6, 39.4, 35.3, 35.2, 35.0, 34.6, 32.8, 31.2, 30.9, 30.6, 28.1, 23.7, 22.7, 20.5, 18.2, 11.7 ppm; NMR data were consistent with the data in the literature.[207]

Synthesis of benzyl 3,7-diketo-5β-cholan-24-oate (3.12)

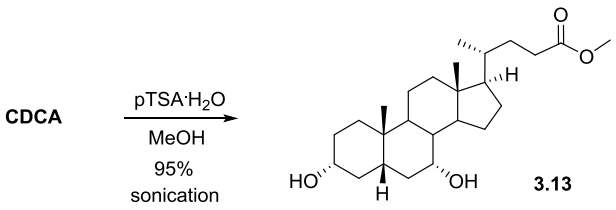


*Isolated yield from HPLC

Following general procedure A, benzyl 3α,7α-dihydroxy-5β-cholan-24-oate **3.11** (290 mg, 0.60 mmol) was oxidized and purified by flash chromatography (MeOH/DCM 0.5:99.5-1:99) and HPLC purification (MeOH/DCM 2.5:97.5) to afford compound **3.3** (170 mg, 0.354 mmol, 59%), compound **3.10** (19 mg, 0.04 mmol, 7%) and compound **3.12** (5 mg, 0.01 mmol, 2%) as gummy solids. Please see above for the characterisation of compounds **3.3** and **3.10**.

3.12, **Formula** $C_{31}H_{42}O_4$; **MW** 478.66; **IR** (neat) 2934 (m), 2872 (m), 1735 (s), 1710 (s), 1457 (w), 1382 (w), 1257 (w), 1166 (m), 741 (w), 694 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 - 7.41 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, PhCH_2), 5.10 (1H, d, *J* 12.4 Hz, PhCH_2), 2.88 (1H, dd, *J* 12.7, 5.1 Hz), 1.31 (3H, s, H_{19}), 0.93 (3H, d, *J* 6.3 Hz, H_{21}), 0.68 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 211.1 (C_3 or C_7), 210.2 (C_3 or C_7), 173.9 (C_{24}), 136.1 (C_{Ar}), 128.6 (CH$_{Ar}$), 128.3 (CH$_{Ar}$), 128.2 (CH$_{Ar}$), 66.1 (PhCH_2), 54.8 (CH), 49.6 (CH), 48.9 (CH), 47.8 (CH), 45.0 (CH$_2$), 42.94 (CH$_2$), 42.86 (CH), 42.7 (C), 38.9 (CH$_2$), 36.8 (CH$_2$), 35.5 (CH$_2$), 35.4 (C), 35.2 (CH), 31.3 (CH$_2$), 30.9 (CH$_2$), 28.2 (CH$_2$), 24.8 (CH$_2$), 22.4 (C_{19}), 22.1 (CH$_2$), 18.4 (C_{21}), 12.1 (C_{18}) ppm; **MS** (ESI+) *m/z* 957.6 ([2M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{31}H_{46}NO_4$ [M+NH_4]$^+$ calcd 496.3421, found 496.3428 (-1.4 ppm error).

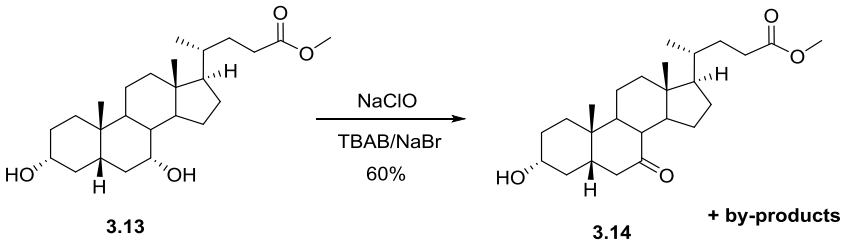
Synthesis of methyl 3α,7α-dihydroxy-5β-cholan-24-oate (3.13)



Following general procedure B, **3.13** was obtained from CDCA as gummy solid in high yield (95%), and used for further reaction without purification.

3.13, **Formula** $C_{25}H_{42}O_4$; **MW** 406.60; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.79 - 3.90 (1H, m, $H_{7\beta}$), 3.66 (3H, s, OCH_3), 3.37 - 3.55 (1H, m, $H_{3\beta}$), 0.92 (3H, d, *J* 6.4 Hz, H_{21}), 0.90 (3H, s, H_{19}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.7 (C_{24}), 71.9 (C_3 or C_7), 68.5 (C_3 or C_7), 55.8, 51.4, 50.4, 42.6, 41.5, 39.9, 39.6, 39.4, 35.33, 35.31, 35.0, 34.6, 32.8, 30.97, 30.95, 30.6, 28.1, 23.7, 22.7, 20.5, 18.2, 11.7 ppm; NMR data were consistent with the data in the literature.[208]

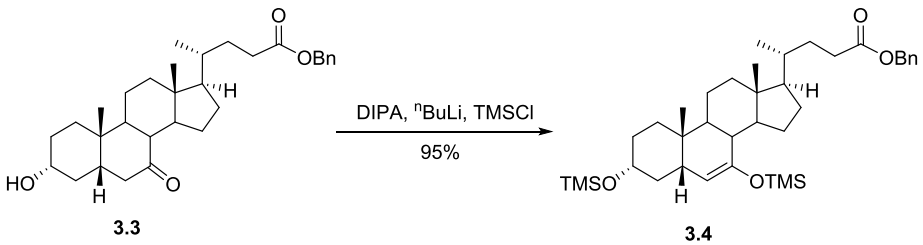
Synthesis of methyl 3α-hydroxy-7-keto-5β-cholan-24-oate (3.14)



Following general procedure A, compound **3.13** (3.59 g, 8.83 mmol) was oxidized and purified by flash chromatography (acetone/petroleum ether 12:88-80:20) to afford **3.14** (2.15 g, 60%) as gummy solid.

3.14, **Formula** $C_{25}H_{40}O_4$; **MW** 404.58; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.65 (3H, s, OCH_3), 3.52 - 3.63 (1H, m, $H_{3\beta}$), 2.85 (1H, dd, *J* 12.6, 6.3 Hz, $H_{6\beta}$), 2.28 - 2.44 (2H, m), 2.12 - 2.27 (2H, m), 1.19 (3H, s, H_{19}), 0.91 (3H, d, *J* 6.2 Hz, H_{21}), 0.64 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 212.0 (C_7), 174.6 (C_{24}), 70.8 (C_3), 54.7, 51.4, 49.5, 48.9, 46.1, 45.4, 42.7, 42.6, 38.9, 37.4, 35.2, 35.1, 34.1, 31.0, 31.0, 29.8, 28.2, 24.8, 23.0, 21.6, 18.3, 12.0 ppm; NMR data were consistent with the data in the literature.[209]

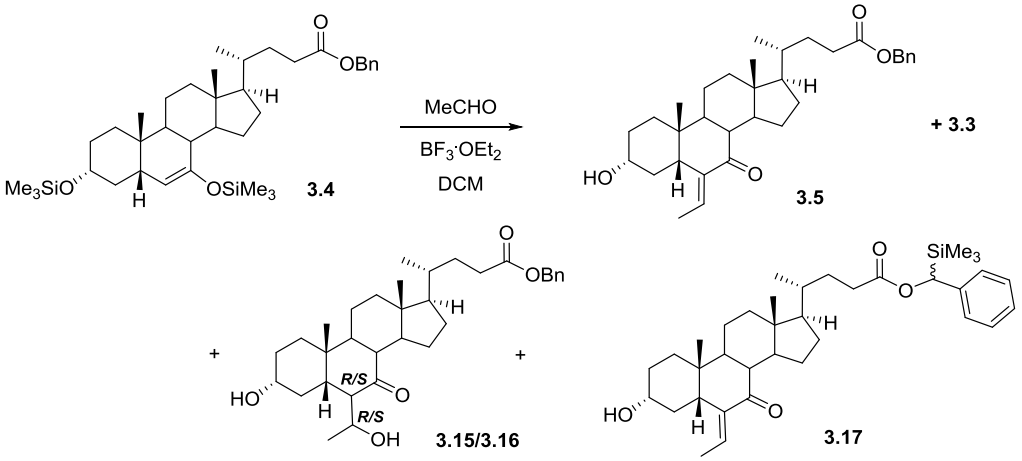
Synthesis of benzyl 3α,7-bis(trimethylsilyloxy)-5β-cholan-6-en-24-oate (3.4)



Following general procedure C, silyl enol ether **3.4** was prepared from benzyl 3α-hydroxy-7-keto-5β-cholan-24-oate **3.3** with high yield (95%), and the crude mixture was used directly for next step.

3.4, **Formula** $C_{37}H_{60}O_4Si_2$; **MW** 625.04; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.31 - 7.41 (5H, m, H_{Ar}), 5.15 (1H, d, *J* 12.7 Hz, CH_2Ph), 5.10 (1H, d, *J* 12.7 Hz, CH_2Ph), 4.73 (1H, dd, *J* 5.8, 1.7 Hz, C*H*=C), 3.44 - 3.59 (1H, m, $H_{3\beta}$), 0.92 (3H, d, *J* 6.1 Hz, H_{21}), 0.83 (3H, s, H_{19}), 0.66 (3H, s, H_{18}), 0.17 (9H, s, Si(CH_3)$_3$), 0.12 (9H, s, Si(CH_3)$_3$) ppm; NMR data were consistent with the data in the literature.[83]

Synthesis of (*E*)-benzyl 3α-hydroxy-6-ethyliden-7-keto-5β-cholan-24-oate (3.5, tentative assignment of *E*-configuration based on NOE experiments**), benzyl 3α-hydroxy-6-(1-hydroxyethyl)-7-keto-5β-cholan-24-oate (3.15/3.16**, stereochemistry for both isomers not determined**), (*E*)-phenyl(trimethylsilyl)methyl 3α-hydroxy-6-ethyliden-7-keto-5β-cholan-24-oate (3.17,** two isomers**)**



Following general procedure D, benzyl 3α,7-trimethylsilyloxy-5β-cholan-6-en-24-oate **3.4** (975 mg, 1.56 mmol) reacted with acetaldehyde and the crude was purified by flash chromatography (MeOH/DCM 0:100-10:90) and HPLC (acetone/hexane 23:77) afford **3.5** (619 mg, 78%) and by-product **3.17** (27 mg, 3%) as gummy solids.

3.5, **2.1.9**, **Formula** $C_{33}H_{46}O_4$; **MW** 506.72; **R_f** 0.27 (acetone/hexane 23:77); **IR** (neat) 3400 (br. w), 2937 (w), 2865 (w), 1735 (m), 1705 (w), 1640 (w), 1455 (w), 1380 (w), 1157 (m), 1062 (w), 911 (s), 729 (s) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.28 - 7.42 (5H, m, H_{Ar}), 6.16 (1H, q, *J* 7.0 Hz, C=C*H*), 5.13 (1H, d, *J* 12.3 Hz, PhCH_2), 5.08 (1H, d, *J* 12.3 Hz, PhCH_2), 3.55 - 3.72 (1H, m, $H_{3\beta}$), 2.57 (1H, dd, *J* 12.9, 4.2 Hz), 1.68 (3H, d, *J* 7.1 Hz, C=CHCH_3), 1.00 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.1 Hz, H_{21}), 0.61 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 204.9, 173.9, 143.3, 136.0, 129.7, 128.4, 128.2, 128.1, 70.3, 66.0, 54.4, 50.6, 48.6, 45.5, 43.5, 39.0, 38.9, 37.5, 35.1, 34.5, 34.4, 31.2, 30.9, 29.6, 28.3, 25.9, 22.8, 21.3, 18.3, 12.6, 12.0 ppm; **MS** (ESI+) *m/z* 529.3 ([M+Na]$^+$, 28%), 1035.6 ([2M+Na]$^+$, 100%). ^{1}H NMR data were consistent with the data in the literature.[83]

3.17, **Formula** $C_{36}H_{54}O_4Si$; **MW** 578.90; **R_f** 0.22 (acetone/hexane 23:77); **IR** (neat) 3435 (br. w), 2931 (s), 2860 (m), 1732 (s), 1689 (m), 1462 (w), 1381 (w), 1242 (s), 1168 (m), 870 (m), 852 (s), 696 (m) cm^{-1}; **^{1}H NMR** (400 MHz, CDCl3) δ 7.25 - 7.32 (2H, m, H_{Ar}), 7.10 - 7.20 (3H, m, H_{Ar}), 6.18 (1H, q, *J* 6.9 Hz, C=C*H*), 5.68 (1H, s, C*H*SiMe$_3$), 3.61 - 3.72 (1H, m, $H_{3\beta}$), 2.58 (1H, dd, *J* 12.9, 4.3 Hz), 1.69 (3H, d, *J*

7.2 Hz, C=CHCH_3), 1.01 (3H, s, H_{19}), 0.95 (3H, d, *J* 6.3 Hz, H_{21} of ***R***- or ***S***-isomer), 0.95 (3H, d, *J* 6.3 Hz, H_{21} of ***R***- or ***S***-isomer), 0.63 (3H, s, H_{18}), 0.02 (9H, s, Si(CH_3)$_3$) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 204.8 (C_7), 173.84 (C_{24} of ***R***- or ***S***-isomer), 173.77 (C_{24} of ***R***- or ***S***-isomer), 143.3 (CH=*C*), 140.2 (C_{Ar} of ***R***- or ***S***-isomer), 140.1 (C_{Ar} of ***R***- or ***S***-isomer), 129.8 (*C*H=C), 128.1 (*C*H$_{Ar}$), 126.00 (*C*H$_{Ar}$ of ***R***- or ***S***-isomer), 125.97 (*C*H$_{Ar}$ of ***R***- or ***S***-isomer), 125.23 (*C*H$_{Ar}$ of ***R***- or ***S***-isomer), 125.17 (*C*H$_{Ar}$ of ***R***- or ***S***-isomer), 71.32 (*C*HSiMe$_3$ of ***R***- or ***S***-isomer), 71.30 (*C*HSiMe$_3$ of ***R***- or ***S***-isomer), 70.5 (C_3), 54.6 (*C*H of ***R***- or ***S***-isomer), 54.5 (*C*H of ***R***- or ***S***-isomer), 50.7 (*C*H), 48.7 (*C*H), 45.5 (*C*H), 43.6 (*C*), 39.1 (*C*H), 39.0 (*C*H$_2$), 37.6 (*C*H$_2$), 35.14 (*C*H of ***R***- or ***S***-isomer), 35.10 (*C*H of ***R***- or ***S***-isomer), 34.6 (*C*), 34.5 (*C*H$_2$), 31.5 (*C*H$_2$), 31.25 (*C*H$_2$ of ***R***- or ***S***-isomer), 31.20 (*C*H$_2$ of ***R***- or ***S***-isomer), 29.7 (*C*H$_2$), 28.4 (*C*H$_2$), 25.9 (*C*H$_2$), 22.8 (C_{19}), 21.3 (*C*H$_2$), 18.4 (C_{21} of ***R***- or ***S***-isomer), 18.3 (C_{21} of ***R***- or ***S***-isomer), 12.7 (C=CH*C*H$_3$), 12.1 (C_{18}), -3.9 (Si(*C*H$_3$)$_3$) ppm; **MS** (ESI+) *m/z* 1179.8 ([2M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{36}H_{55}O_4Si$ [M+H]$^+$ calcd 579.3864, found 579.3868 (-0.7 ppm error).

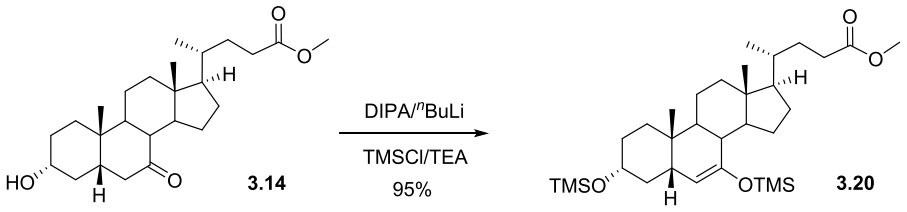
When the literature procedures[83] were repeated without extended stirring at room temperature overnight, the aldol addition products **3.15** (20%) and **3.16** (36%) were isolated *via* flash chromatography (ethyl acetate/hexane 10:90; ethyl acetate 100%; acetone 100%, with addition of 0.5% triethylamine) as gummy solids. **3.15** and **3.16** were converted (yield: 83%) to the elimination product **3.5** after stirring overnight in the presence of boron trifluoride.

3.15, **Formula** $C_{33}H_{48}O_5$; **MW** 524.73; **R$_f$** 0.32 (MeOH/DCM 3:97); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.29 - 7.41 (5H, m, H$_{Ar}$), 5.14 (1H, d, *J* 12.3 Hz, C*H*$_2$Ph),5.09 (1H, d, *J* 12.3 Hz, C*H*$_2$Ph), 4.23 - 4.42 (1H, m, C*H*(OH)CH$_3$), 3.50 - 3.70 (1H, m, $H_{3\beta}$), 2.53 (1H, t, *J* 11.3 Hz), 1.30 (3H, d, *J* 6.0 Hz, CH(OH)C*H*$_3$), 1.13 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.0 Hz, H_{21}), 0.65 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 214.7 (C_7), 174.0 (C_{24}), 136.1 (C_{Ar}), 128.5 (*C*H$_{Ar}$), 128.2 (*C*H$_{Ar}$), 128.1 (*C*H$_{Ar}$), 70.2, 68.3, 67.7, 66.1, 54.9, 49.3, 47.2, 46.6, 42.7, 42.7, 39.7, 38.9, 35.5, 35.2, 34.9, 31.2, 30.9, 29.5, 28.1, 25.4, 24.7, 22.5, 21.4, 18.3, 12.1 ppm.

3.16, **Formula** $C_{33}H_{48}O_5$; **MW** 524.73; **R$_f$** 0.24 (MeOH/DCM 3:97); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.29 - 7.40 (5H, m, H_{Ar}), 5.13 (1H, d, *J* 12.4 Hz, C*H*$_2$Ph), 5.09 (1H, d, *J* 12.4 Hz, C*H*$_2$Ph), 4.19 - 4.37 (1H, m, C*H*(OH)CH$_3$), 3.51 - 3.66 (1H, m, $H_{3\beta}$), 1.24 (3H, s, H_{19}), 1.08 (3H, d, *J* 6.2 Hz, CH(OH)C*H*$_3$), 0.91 (3H, d, *J* 6.2 Hz, H_{21}), 0.64 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 213.9 (C_7), 174.0 (C_{24}), 136.1 (C_{Ar}), 128.5 (*C*H$_{Ar}$), 128.2 (*C*H$_{Ar}$), 128.1 (*C*H$_{Ar}$), 70.5, 68.5, 66.8, 66.1, 54.8, 48.9, 48.1, 45.1, 43.2, 42.5, 39.7, 38.8, 35.4, 35.2, 35.2, 31.2, 30.9, 29.6, 28.1, 25.4, 24.7, 22.9, 21.4, 18.3, 12.1 ppm.

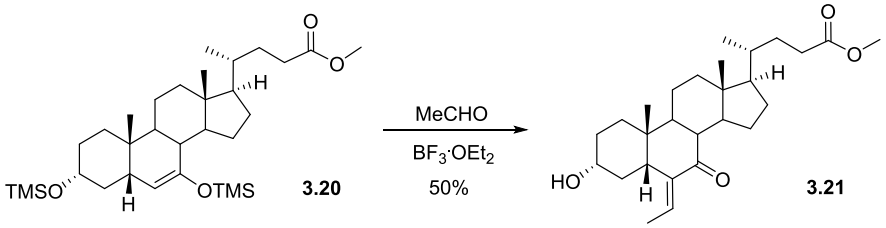
Synthesis of methyl 3α,7-bis(trimethylsilyloxy)-5β-cholan-6-en-24-oate (3.20)

Following general procedure C, **3.14** was converted to **3.20** with high yield (95%), and used directly for the next step without purification.



3.20, **Formula** $C_{31}H_{56}O_4Si_2$; **MW** 548.94; **^{1}H NMR** (300 MHz, *CDCl$_3$*) δ 4.73 (1H, dd, *J* 5.9, 1.7 Hz, *H*$_6$), 3.66 (3H, s, OC*H*$_3$), 3.45 - 3.58 (1H, m, *H*$_{3β}$), 0.92 (3H, d, *J* 6.4 Hz, *H*$_{21}$), 0.83 (3H, s, *H*$_{19}$), 0.68 (3H, s, *H*$_{18}$), 0.16 (9H, s, Si(C*H*$_3$)$_3$), 0.11 (9H, s, Si(C*H*$_3$)$_3$) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.7 (*C*$_{24}$), 151.7 (*C*$_7$), 108.8 (*C*$_6$), 71.5 (*C*$_3$), 54.8, 54.1, 51.4, 44.4, 42.7, 41.0, 40.9, 40.3, 40.1, 35.3, 34.6, 33.0, 31.1, 31.0, 30.6, 28.7, 27.1, 22.5, 20.9, 18.4, 12.2, 0.3 (Si(*C*H$_3$)$_3$), 0.2 (Si(*C*H$_3$)$_3$) ppm; NMR data were consistent with the data in the literature.[89]

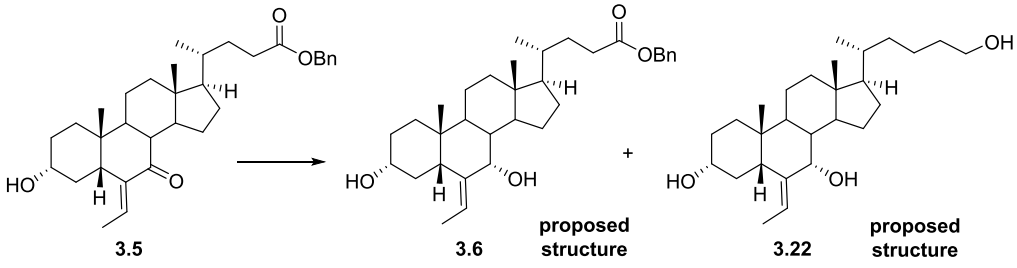
Syntheis of (*E*)-methyl 3α-hydroxy-6-ethyliden-7-keto-5β-cholan-24-oate (3.21, *E*-configuration assigned based on compound **3.5)**



Following general procedure D, compound **3.20** (5.10 mmol) reacted with acetaldehyde and the crude was purified by flash chromatography (acetone/petroleum ether 12:88-30:70) to afford **3.21** (1.1 g, 2.55 mmol, 50%) as gummy solid.

3.21, **Formula** $C_{27}H_{42}O_4$; **MW** 430.62; **R$_f$** 0.25 (aceonte/petroleum ether 20:80); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 6.19 (1H, q, *J* 7.1 Hz, C*H*=C), 3.58 - 3.75 (4H, m, OC*H*$_3$ + *H*$_{3β}$), 2.59 (1H, dd, *J* 13.1, 4.2 Hz), 1.70 (3H, d, *J* 7.0 Hz, C*H*$_3$CH=C), 1.01 (3H, s, *H*$_{19}$), 0.94 (3H, d, *J* 6.3 Hz, *H*$_{21}$), 0.65 (3H, s, *H*$_{18}$) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 204.7, 174.7, 143.3, 129.9, 70.5, 54.5, 51.5, 50.7, 48.7, 45.5, 43.6, 39.1, 39.0, 37.6, 35.2, 34.6, 34.5, 31.1, 31.0, 29.8, 28.4, 26.0, 22.8, 21.3, 18.4, 12.7, 12.1 ppm; NMR data were consistent with the data in the literature.[89]

Synthesis of (*E*)-benzyl 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oate (3.6, *E*-configuration assigned based on compound **3.5**; *discussion of comparison with literature values included in *section 3.1.5*), **and (*E*)-3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-ol (3.22)**

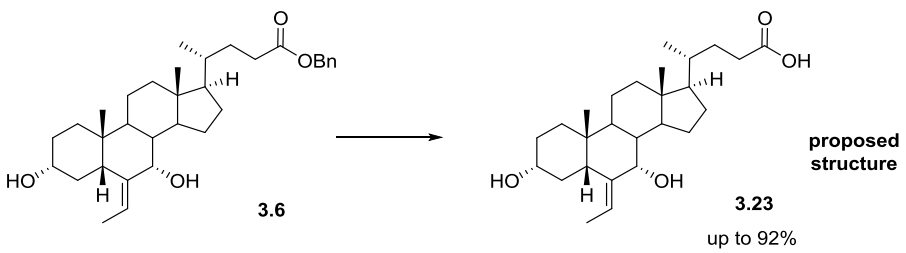


To a round-bottom flask were added (*E*)-benzyl 3α-hydroxy-6-ethyliden-7-keto-5β-cholan-24-oate **3.5** (40 mg, 0.079 mmol, 1 eq), THF (anhydrous)/MeOH (anhydrous) (v/v: 4/1, 2 mL, 25 mL/mmol), cerium chloride heptahydrate (90 mg, 0.24 mmol, 3 eq) and $NaBH_4$ (4 mg, 0.095 mmol, 1.2 eq). The reaction mixture was stirred at room temperature overnight. After completion of the reaction indicated by TLC analysis, water (2 mL) and methanol (2 mL) were added. The solvent was evaporated and the residue was dissolved in ethyl acetate (5 mL) and water (5 mL), extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine (15 mL), dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (DCM/MeOH 99:1-98:2) to afford **3.6** (<65%, impure, calculated yield) as gummy solid. When more than 2.4 eq of $NaBH_4$ was used, **3.22** was also obtained as a by-product (13%) together with **3.6** (<40%, impure, calculated yield). Following the literature procedures by using anhydrous $CeCl_3$,[83] **3.6** was obtained with much higher yield (90%).

3.6, **Formula** $C_{33}H_{48}O_4$; **MW** 508.73; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.29 - 7.43 (5H, m, H_{Ar}), 5.65 (1H, qd, *J* 6.8, 2.1 Hz, C=C*H*), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2), 5.09 (1H, d, *J* 12.3 Hz, PhCH_2), 3.91 - 4.11 (1H, m, $H_{7\beta}$), 3.66 (1H, tt, *J* 10.3, 5.7 Hz, $H_{3\beta}$), 1.62 (3H, dd, *J* 6.8, 2.0 Hz, C=CHCH_3), 0.93 (3H, d, *J* 6.1 Hz, H_{21}), 0.79 (3H, s, H_{19}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.0, 141.6, 136.1, 128.5, 128.2, 128.2, 114.4, 73.3, 71.2, 66.1, 56.0, 54.9, 45.5, 44.7, 44.0, 40.0, 39.5, 36.2, 35.3, 35.2, 34.7, 31.3, 31.0, 30.2, 28.5, 27.1, 22.8, 21.2, 18.4, 12.4, 12.1 ppm; **MS** (ESI+) *m/z* 531.3 ([M+Na]$^+$, 17%); 1039.7 ([2M+Na]$^+$, 29%); ^{1}H NMR data were consistent* with the data in the literature.[83]

3.22, **Formula** $C_{26}H_{44}O_3$; **MW** 404.63; **R_f** 0.14 (acetone/hexane: 20:80); **IR** (neat) 3327 (br. w), 2932 (m), 2863 (m), 1721 (w), 1452 (w), 1380 (w), 1333 (w), 1053 (m), 908 (s), 727 (s), 640 (w) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 5.65 (1H, qd, *J* 6.6, 2.1 Hz, C=C*H*), 3.89 - 4.11 (1 H, m, $H_{7\beta}$), 3.52 - 3.74 (3H, m, $H_{3\beta}$ + H_{24}), 2.49 (1H, dd, *J* 11.9, 5.3 Hz), 1.62 (3H, dd, *J* 6.8, 2.0 Hz, C=CHCH_3), 0.95 (3H, d, *J* 6.5 Hz, H_{21}), 0.79 (3H, s, H_{19}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 141.6, 114.4, 73.3, 71.2, 63.5, 56.1, 55.1, 45.6, 44.7, 44.0, 40.0, 39.6, 36.3, 35.5, 35.4, 34.7, 31.8, 30.2, 29.4, 28.7, 27.2, 22.8, 21.2, 18.8, 12.4, 12.2 ppm; **MS** (ESI+) *m/z* 387.0 ([M-OH]$^+$, 100%); **HRMS** (ESI+) for $C_{26}H_{44}NaO_3$ [M+Na]$^+$ calcd 427.3183, found 427.3175 (1.8 ppm error); **Other analysis** 1D NOE/Homonuclear decoupling/TOSCY.

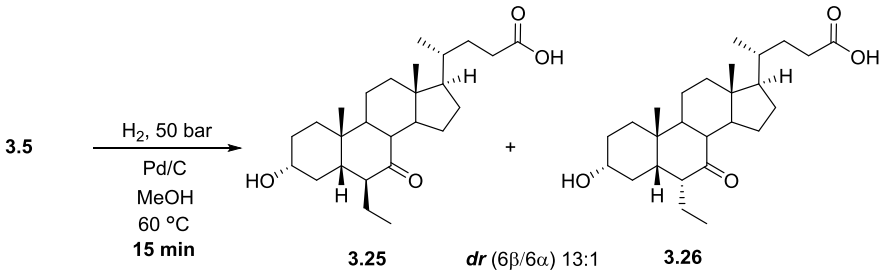
Synthesis of (*E*)-3α,7α-dihydroxy-6-ethyliden-5β-cholanic acid (3.23)



Following procedure E starting from (*E*)-benzyl 3α,7α-dihydroxy-6-ethyliden-5β-cholan-24-oate **3.6** using Pd/C or PtO_2 as catalyst, **3.23** was obtained (up to 92%) as gummy solid.

3.23, **Formula** $C_{26}H_{42}O_4$; **MW** 418.61; **^{1}H NMR** (300 MHz, CD_3OD) δ 5.66 (1H, qd, *J* 6.9, 2.2 Hz, C=C*H*), 3.85 - 3.96 (1H, m, $H_{7\beta}$), 3.57 (1H, tt, *J* 10.9, 4.5 Hz, $H_{3\beta}$), 2.53 (1H, dd, *J* 13.0, 4.2 Hz), 1.62 (3H, dd, *J* 6.9, 1.9 Hz, C=CHCH_3), 0.97 (3H, d, *J* 6.4 Hz, H_{21}), 0.80 (3H, s, H_{19}), 0.69 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 179.6, 143.9, 115.5, 74.3, 72.0, 58.1, 56.9, 46.3, 46.1, 45.2, 41.7, 41.3, 37.2, 37.1, 36.5, 36.0, 33.3, 31.1, 30.8, 29.8, 28.3, 23.6, 22.6, 19.2, 12.9, 12.7 ppm; **MS** (ESI-) *m/z* 417.2 ([M-H]$^-$, 100%); **HRMS** (ESI+) for $C_{26}H_{42}NaO_4$ [M+Na]$^+$ calcd 441.2975, found 441.2972 (0.7 ppm error).

Synthesis of 3α-hydroxy-6α-ethyl-7-keto-5β-cholanic acid (3.26)

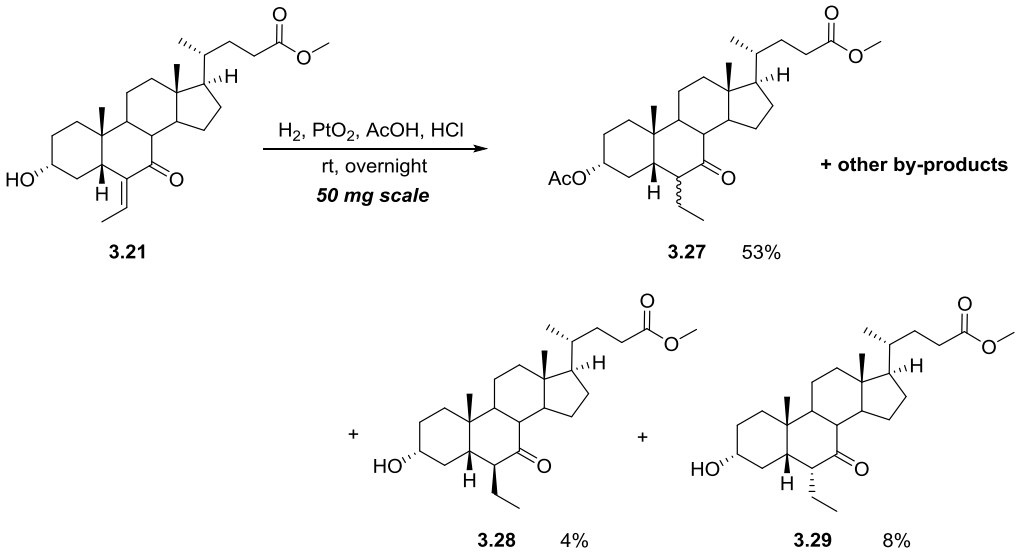


Using an H-Cube instrument, benzyl ester enone **3.5** in MeOH was reduced and deprotected under 50 bar at 60 °C within 15 min. The diastereosiomer **3.25** was epimerised using NaOH, leading to the desired compound **3.26** with high yield (95%).

3.26, **Formula** $C_{26}H_{42}O_4$; **MW** 418.61; **^{1}H NMR** (300 MHz, CD_3OD) δ 3.38 - 3.55 (1H, m, $H_{3\beta}$), 2.74 - 2.89 (1H, m, $H_{6\beta}$), 2.50 (1H, t, *J* 11.0 Hz, $H_{8\beta}$), 1.26 (3H, s, H_{19}), 0.96 (3H, d, *J* 6.6 Hz, H_{21}), 0.82 (3H, t, *J* 7.4 Hz, CH_2CH_3), 0.71 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, CD_3OD) δ 215.6 (C_7), 178.2 (C_{24}), 71.8 (C_3), 56.5, 53.4, 52.3, 51.3, 50.6, 45.5, 44.0, 40.5, 37.0, 36.7, 35.4, 32.7, 32.5, 32.1, 30.7, 29.4, 25.7, 24.1, 23.1, 20.2, 19.0, 12.7, 12.4 ppm; **MS** (ESI-) *m/z* 417.3 ([M-H]$^-$, 100%); **HRMS** (ESI+) for $C_{26}H_{42}NaO_4$ [M+Na]$^+$ calcd 441.2975, found 441.2976 (-0.1 ppm error); NMR data were consistent with the data in the literature.[210]

Synthesis of methyl 3α-acetyloxy-6-ethyl-7-keto-5β-cholan-24-oate (3.27, mixture of isomers**), methyl 3α-hydroxy-6β-ethyl-7-keto-5β-cholan-24-oate (3.28), and methyl 3α-hydroxy-6α-ethyl-7α-keto-5β-cholan-24-oate (3.29)**

Following general procedure E starting from **3.21** (50 mg, 0.116 mmol) using Adam's catalyst in AcOH/HCl (3 mL), compounds **3.27** (29 mg, 53%), **3.28** (2 mg, 4%) and **3.29** (4 mg, 8%) were obtained as gummy solids after purification. Further treatment of **3.27** with K_2CO_3 in anhydrous MeOH also led to **3.28** and **3.29**.



3.27, **Formula** $C_{29}H_{46}O_5$; **MW** 474.67; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.55 - 4.73 (1H, m, $H_{3\beta}$), 3.66 (3H, s, COOCH_3), 2.64 - 2.75 (1H, m, $H_{6\beta}$ for 6α-ethyl isomer), 2.56 (1H, t, *J* 11.3 Hz, $H_{8\beta}$ for 6β-ethyl isomer), 2.02 (3H, s, CH_3COOSt), 1.23 (3H, s, H_{19}), 0.93 (3H, m, H_{21} of two isomers overlapped), 0.67 (3H, s, H_{18} of 6α- or 6β-ethyl isomer), 0.65 (3H, s, H_{18} of 6α- or 6β-ethyl isomer) ppm; **Selected ^{13}C NMR** (75 MHz, $CDCl_3$) δ 215.3 (C_7 of 6α- or 6β-ethyl isomer), 212.6 (C_7 of 6α- or 6β-ethyl isomer), 174.7 (C_{24} or CH_3COOSt of two isomers, overlapped), 170.6 (C_{24} or CH_3COOSt of two isomers, overlapped), 73.19 (C_3 of 6α- or 6β-ethyl isomer), 72.77 (C_3 of 6α- or 6β-ethyl isomer) ppm. [only key peaks were described and the rest of peaks were difficult to differentiate]

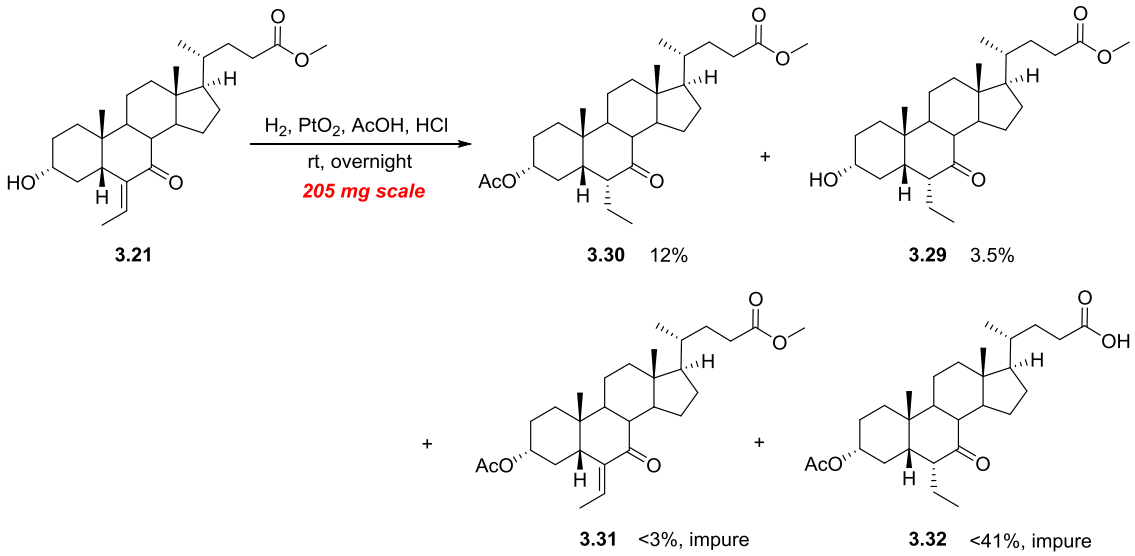
3.28, **Formula** $C_{27}H_{44}O_4$; **MW** 432.64; **$[\alpha]_D$** -2.0 (c 1.7, $CHCl_3$, 20 °C); **R_f** 0.24 (acetone/hexane 25:75); **IR** (neat) 3403 (br. w), 2932 (s), 2870 (m), 1737 (s), 1703 (s), 1450 (m), 1381 (m), 1260 (w), 1173 (m), 1059 (m), 910 (w), 726 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.66 (3H, s, OCH_3), 3.48 - 3.61 (1H, m, $H_{3\beta}$), 2.55 (1H, t, *J* 11.3 Hz, $H_{8\beta}$), 2.27 - 2.42 (1H, m), 1.21 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.4 Hz, H_{21}), 0.84 (3H, t, *J* 7.2 Hz, CH_2CH_3), 0.67 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 215.5 (C_7), 174.7 (C_{24}), 70.6 (C_3), 62.1 (*C*H), 54.8 (*C*H), 51.5 (OCH_3), 49.6 (*C*H), 48.7 (*C*H), 45.6 (*C*H), 42.9 (*C*H), 42.5 (*C*), 39.9 (CH_2), 38.8 (CH_2), 35.6 (*C*), 35.4 (CH_2), 35.2 (*C*H), 31.1 (CH_2), 31.0 (CH_2), 29.6 (CH_2), 28.2 (CH_2), 26.6 (CH_2), 26.0 (C_{19}), 24.9 (CH_2), 21.4 (CH_2), 18.4 (C_{21}), 13.1 (CH_2CH_3), 12.1 (C_{18}) ppm; **MS** (ESI+) *m/z* 433.3 ([M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{27}H_{44}NaO_4$ [M+Na]$^+$ calcd 455.3132, found 455.3128 (0.9 ppm error); **Other analysis** 1D NOE analysis.

3.29, **Formula** $C_{27}H_{44}O_4$; **MW** 432.64; **IR** (neat) 3416 (br. w), 2933 (s), 2865 (s), 1734 (s), 1702 (s), 1614 (w), 1455 (m), 1378 (m), 1254 (w), 1167 (m), 1057 (w), 1047 (w) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.67 (3H, s, OCH_3), 3.44 - 3.59 (1H, m, $H_{3\beta}$), 2.69 (1 H, q, *J* 7.3 Hz, $H_{6\beta}$), 2.29 - 2.43 (2H, m), 1.22 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.2 Hz, H_{21}), 0.81 (3H, t, *J* 7.4 Hz, CH_2CH_3), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 212.6 (C_7), 174.7 (C_{24}), 71.2 (C_3), 54.8, 52.0, 51.5, 50.6, 49.9, 49.0, 43.7, 42.6, 39.0, 35.7, 35.2, 34.2, 31.8, 31.02, 30.97, 29.9, 28.3, 24.6, 23.5, 21.8, 18.8, 18.3, 12.1, 12.0 ppm; **MS**

(ESI+) *m/z* 433.2 ([M+H]$^+$, 100%); 865.3 ([2M+H]$^+$, 68%); **HRMS** (ESI+) for $C_{27}H_{45}O_4$ [M+H]$^+$ calcd 433.3312, found 433.3310 (0.6 ppm error); **Other analysis** 1D NOE analysis.

Synthesis of methyl 3α-acetyloxy-6α-ethyl-7-keto-5β-cholan-24-oate (3.30), (*E*)-methyl 3α-acetyloxy-6-ethyliden-7-keto-5β-cholan-24-oate (3.31), and 3α-acetoxy-6α-ethyl-7-keto-5β-cholanic acid (3.32)

Interestingly, when this reaction (following general procedure E) was repeated on a larger scale with **3.21** (205 mg) in AcOH/HCl (10 mL) using Adam's catalyst, the desired compound **3.29** (3.5%) was obtained, together with by-product **3.30** (12%), **3.31** (<3%, impure) and **3.32** (<41%, impure) as gummy solids.



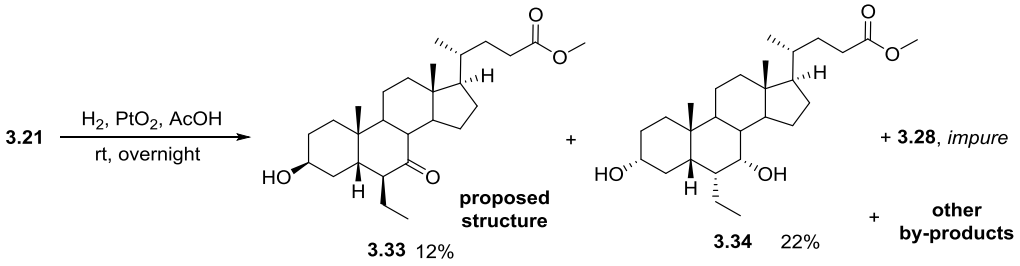
3.30, **Formula** $C_{29}H_{46}O_5$; **MW** 474.67; **IR** (neat) 2948 (m), 2869 (m), 1737 (s), 1708 (s), 1450 (w), 1387 (w), 1361 (w), 1242 (s), 1175 (w), 1036 (m) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.64 (1H, tt, *J* 11.5, 4.4 Hz, $H_{3\beta}$), 3.66 (3H, s, OCH_3), 2.65 - 2.75 (1H, m, $H_{6\beta}$), 2.29 - 2.42 (2H, m), 2.12 - 2.27 (2H, m), 1.97 (3H, s, CH_3COOSt), 1.23 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.2 Hz, H_{21}), 0.80 (3H, t, *J* 7.4 Hz, CH_2CH_3), 0.65 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 212.6 (C_7), 174.6, 170.5, 73.2(C_3), 54.8, 52.0, 51.5, 50.5, 49.9, 48.9, 43.7, 42.6, 38.9, 35.7, 35.2, 33.9, 31.01, 30.98, 28.3, 27.7, 26.0, 24.6, 23.5, 21.9, 21.3, 18.8, 18.3, 12.04, 12.01 ppm; **MS** (ESI+) *m/z* 415.2 ([M-OAc]$^+$, 100%); 497.1 ([M+Na]$^+$, 48%); 949.4 ([2M+H]$^+$, 33%); **HRMS** (ESI+) for $C_{29}H_{50}NO_5$ [M+NH_4]$^+$ calcd 492.3684, found 492.3683 (0.0 ppm error).

3.31, **Formula** $C_{29}H_{44}O_5$; **MW** 472.66; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 6.19 (1H, q, *J* 7.1 Hz, C*H*=C), 4.71 - 4.83 (1H, m, $H_{3\beta}$), 3.67 (3H, s, OCH_3), 2.63 (1H, dd, *J* 13.3, 4.4 Hz), 2.00 (3H, s, C$\underline{H}_3$COOSt), 1.69 (2H, d, *J* 7.4 Hz, CH_3CH=C), 1.22 (3H, s, H_{19}), 0.93 (3H, d, *J* 5.9 Hz, H_{21}), 0.65 (3H, s, H_{18}) ppm;

3.32, **Formula** $C_{28}H_{44}O_5$; **MW** 460.65; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.65 (1H, tt, *J* 11.0, 4.3 Hz, $H_{3\beta}$), 2.63 - 2.78 (1H, m, $H_{6\beta}$), 2.00 (3H, s, CH_3CO), 1.24 (3H, s, H_{19}), 0.94 (3H, d, *J* 6.3 Hz, H_{21}), 0.81 (3H, t, *J* 7.3 Hz, CH_2CH_3), 0.67 (3H, s, H_{18}) ppm; **MS** (ESI+) *m/z* 401.0 ([M-OAc]$^+$, 100%); 461.1 ([M+H]$^+$, 28%);

921.3 ([2M+H]$^+$, 68%); **HRMS** (ESI+) for $C_{28}H_{48}NO_5$ [M+NH_4]$^+$ calcd 478.3527, found 478.3521 (1.2 ppm error).

Synthesis of methyl 3β-hydroxy-6β-ethyl-7α-keto-5β-cholan-24-oate (3.33), and methyl 3α,7α-dihydroxy-6α-ethyl-5β-cholan-24-oate (3.34)



Following general procedure E starting from **3.21** (50 mg) using Adam's catalyst in neat AcOH, the unexpected by-product **3.33** (12%) and **3.34** (22%) were also obtained, in addition to **3.28**.

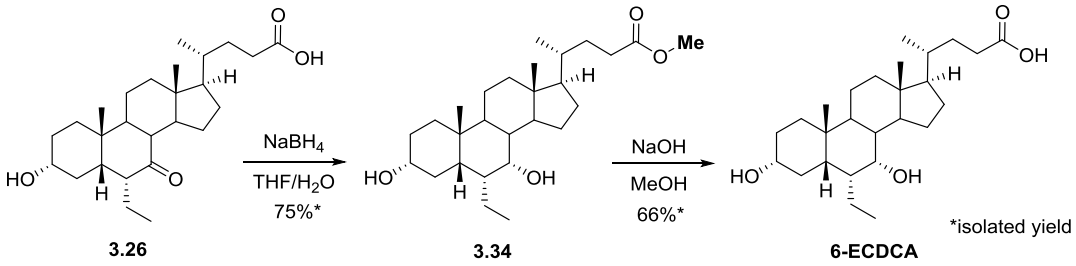
3.33, **Formula** $C_{27}H_{44}O_4$; **MW** 432.64; **IR** (neat) 3524 (br. w), 2938 (s), 2869 (s), 1734 (s), 1703 (s), 1454 (m), 1377 (m), 1252 (m), 1166 (s), 1103 (s), 1065 (m), 892 (w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.78 (1H, br. s, $H_{3\alpha}$), 3.67 (3H, s, OCH_3), 3.07 (1H, dd, *J* 15.2, 13.6 Hz, $H_{8\beta}$), 1.00 (3H, s, H_{19}),0.96 (3H, d, *J* 6.6 Hz, H_{21}), 0.91 (3H, t, *J* 7.3 Hz, CH_2CH_3), 0.71 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 213.1 (C_7), 174.7 (C_{24}), 70.9 (C_3), 55.8, 51.5, 50.4, 46.9, 42.8, 41.3, 40.6, 40.0, 39.5, 36.94, 36.88, 35.8, 35.4, 33.6, 31.00, 30.96, 28.1, 23.7, 22.3, 22.0, 21.1, 18.3, 11.8, 11.5 ppm; **MS** (ESI+) *m/z* 865.6 ([2M+H]$^+$, 100%); 887.6 ([2M+Na]$^+$, 93%); **HRMS** (ESI+) for $C_{27}H_{48}NO_4$ [M+NH_4]$^+$ calcd 450.3578, found 450.3579 (-0.4 ppm error).

3.34, **Formula** $C_{27}H_{46}O_4$; **MW** 434.65; **[α]$_D$** +2.3 (c 0.8, $CHCl_3$, 22 °C); **IR** (neat) 3406 (br. w), 2927 (s), 2869 (s), 1728 (s), 1447 (m), 1375 (m), 1166 (m), 1062 (m), 907 (m), 738 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.68 - 3.73 (1H, m, $H_{7\beta}$), 3.66 (3H, s, OCH_3), 3.34 - 3.45 (1H, m, $H_{3\beta}$), 2.29 - 2.42 (1H, m), 2.17 - 2.28 (1H, m), 1.00 (1H, td, *J* 14.5, 3.5 Hz), 0.86 - 0.95 (9H, m, H_{21} + CH_2CH_3 + H_{19}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (C_{24}), 72.3 (C_3), 70.9 (C_7), 55.7, 51.4, 50.5, 45.2, 42.7, 41.2, 40.0, 39.6, 35.5, 35.5, 35.3, 34.0, 33.2, 31.0, 31.0, 30.6, 28.1, 23.7, 23.1, 22.2, 20.7, 18.2, 11.8, 11.6 ppm; **MS** (ESI+) *m/z* 891.6 [2M+Na]$^+$; **HRMS** (ESI+) for $C_{27}H_{46}NaO_4$ [M+Na]$^+$ calcd 457.3288, found 457.3292 (-0.7 ppm error).

Synthesis of 6α-ethyl chenodeoxycholic acid (6-ECDCA)

To a round-bottom flask were added **3.26** (47 mg, 0.11 mmol, 1.0 eq), THF (8 mL), water (2 mL) and $NaBH_4$ (17 mg, 0.44 mmol, 4 eq). The resulting mixture was stirred at room temperature overnight. Upon completion, the solvent was removed under reduced pressure. The residue was directly purified by flash chromatography (MeOH/DCM 5:95 – 15/85), leading to compound **3.34** (36 mg, 0.083 mmol, 75%). The esterification on 24-carboxylic acid group occurred during the purification

step. Compound **3.34** (35 mg) was then subjected to alkaline hydrolysis with NaOH (500 mg) in MeOH (5 mL) to afford the desired target compound 6-ECDCA (22 mg, 0.053 mmol, 66%).

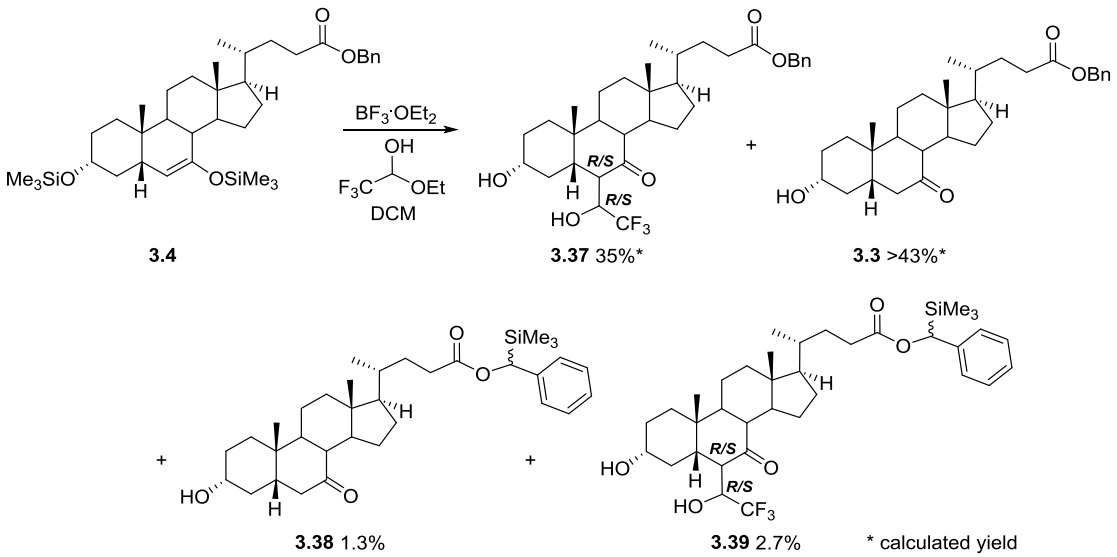


3.26 3.34 6-ECDCA

6-ECDCA, **Formula** $C_{26}H_{44}O_4$; **MW** 420.63; **IR** (neat) 3406 (br. w), 2954 (m), 2926 (m). 2863 (m), 1712 (s), 1454 (m), 1381 (m), 1159 (w), 1061 (m), 901 (s), 734 (s) cm^{-1}; **^{1}H NMR** (400 MHz, CD_3OD) δ 3.60 - 3.71 (1H, m, $H_{7\beta}$), 3.27 - 3.34 (1H, m, $H_{3\alpha}$), 2.27 - 2.44 (1H, m), 0.96 (3H, d, *J* 6.8 Hz, H_{21}), 0.92 (3H, s, H_{19}), 0.90 (3H, t, *J* 7.3 Hz, CH_2CH_3), 0.70 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 178.3 (C_{24}), 73.3 (C_3), 71.3 (C_7), 57.5 (*C*H), 51.8 (*C*H), 47.1 (*C*H), 43.9 (*C*), 43.3 (*C*H), 41.7 (*C*H), 41.2 (CH_2), 36.92 (CH_2), 36.90 (*C*H), 36.8 (*C*), 34.6 (*C*H), 34.5 (CH_2), 32.5 (CH_2), 32.1 (CH_2), 31.4 (CH_2), 29.4 (CH_2), 24.7 (CH_2), 23.9 (C_{19}), 23.6 (CH_2), 22.1 (CH_2), 19.0 (C_{21}), 12.4 (C_{18}), 12.2 (CH_2CH_3) ppm; **MS** (ESI+) *m/z* 841.7 ($[2M+H]^+$, 100%); **HRMS** (ESI+) for $C_{26}H_{44}NaO_4$ $[M+Na]^+$ calcd 443.3132, found 443.3129 (0.6 ppm error); ^{1}H NMR data were consistent with the data in the literature.[89]

7.3.2 Attempted synthesis of 6-ECDCA analogues

Synthesis of benzyl 3α-hydroxy-6-(2,2,2-trifluoro-1-hydroxyethyl)-7-keto-5β-cholan-24-oate (3.37, proposed structure based on NMR analysis**), phenyl(trimethylsilyl)methyl 3α-hydroxy-7-keto-5β-cholan-24-oate (3.38), and phenyl(trimethylsilyl)methyl 3α-hydroxy-6-(2,2,2-trifluoro-1-hydroxyethyl)-7-keto-5β-cholan-24-oate (3.39**, proposed structure based on NMR and MS analysis**)**



3.4 3.37 35%* 3.3 >43%*

3.38 1.3% 3.39 2.7% * calculated yield

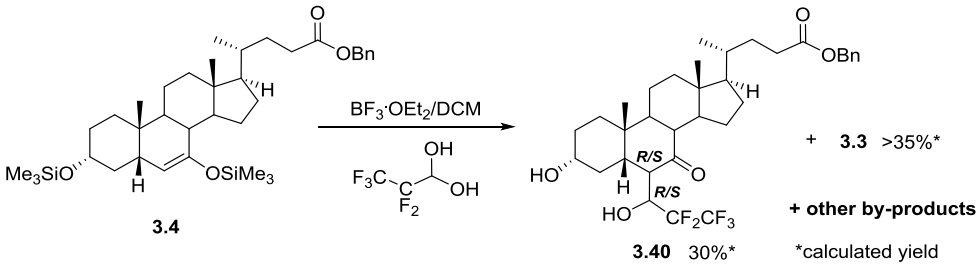
Following general procedure D, **3.4** (175mg) was reacted with trifluoroacetaldehyde ethyl hemiacetal to afford **3.37** (35%*, *calculated yield), **3.3** (>43%*), **3.38** (2 mg, 1.3%) and **3.39** (5 mg, 2.7%) after purification *via* flash chromatography (acetone/hexane 20:80) and HPLC (acetone/hexane 20:80, 1st trial, 37 mg/injection; 2nd trial, 10 mg/injection).

3.37, **Formula** $C_{33}H_{45}F_3O_5$; **MW** 578.70; **R_f** 0.28 (acetone/hexane 20:80); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.31 - 7.40 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2), 5.09 (1H, d, *J* 12.4 Hz, PhCH_2), 4.80 - 4.98 (1H, m, C*H*CF_3), 3.58 - 3.83 (1H, m, $H_{3\beta}$), 2.78 - 2.93 (1H, m), 1.20 (3H, s, H_{19}), 0.91 (3H, d, *J* 5.9 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -84.2 - -84.1 (1F, m, CF_3), -84.3 - -84.2 (1F, m, CF_3) ppm. [**^{13}C NMR** N/A Peak signals were difficult to be identified due to the presence of the diastereoisomers as well the decomposition by-product; Configuration was not able to be determined with 1D NOE due to the overlapping of key protons as well as the presence of decomposition by-product.]

3.38, **Formula** $C_{34}H_{52}O_4Si$; **MW** 552.86; **R_f** 0.22 (acetone/hexane 23:77); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.24 - 7.32 (2H, m, H_{Ar}), 7.10 - 7.21 (3H, m, H_{Ar}), 5.69 (1H, s, C*H*$SiMe_3$), 3.50 - 3.70 (1H, m, $H_{3\beta}$), 2.86 (1H, dd, *J* 12.9, 5.7 Hz, $H_{6\beta}$), 1.21 (3H, s, H_{19}), 0.94 (3H, d, *J* 5.8 Hz, H_{21}), 0.64 (3H, s, H_{18}), 0.02 (9H, s, Si(CH_3)$_3$) ppm; **MS** (ESI+) *m/z* 575.2 ([M+Na]$^+$, 100%), 1128.1 ([2M+Na]$^+$, 82%); **HRMS** (ESI+) for $C_{34}H_{56}NO_4Si$ [M+NH_4]$^+$ calcd 570.3973, found 570.3981 (-1.5 ppm error).

3.39, **Formula** $C_{36}H_{53}F_3O_5Si$; **MW** 650.88; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.24 - 7.33 (2H, m, H_{Ar}), 7.05 - 7.22 (3H, m, H_{Ar}), 5.69 (1H, s, C*H*$SiMe_3$), 4.75 - 4.99 (1H, m, C*H*CF_3), 3.57 - 3.83 (1H, m, $H_{3\beta}$), 2.80 - 2.93 (1H, m), 2.53 - 2.67 (1H, m), 1.21 (3H, s, H_{19}), 0.94 (3H, d, *J* 5.9 Hz, H_{21}), 0.64 (3H, s, H_{18}), 0.02 (9H, s, Si(CH_3)$_3$) ppm; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -84.5 - -84.4 (3F, m, CF_3), -84.6 - -84.5 (3F, m, CF_3) ppm; **MS** (ESI+) *m/z* 668.1 ([M+NH_4]$^+$, 100%), 1323.0 ([2M+Na]$^+$, 97%); **HRMS** (ESI+) for $C_{34}H_{56}NO_4Si$ [M-CF_3CHO+NH_4]$^+$ calcd 570.3973, found 570.3985 (-2.1 ppm error).

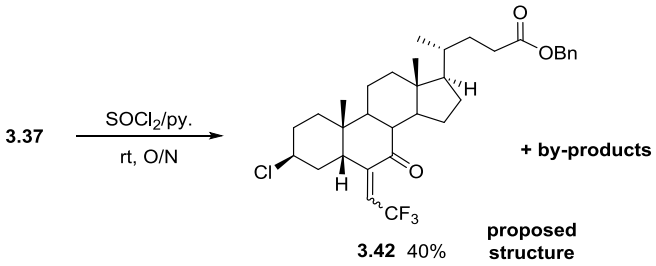
Synthesis of benzyl 3α-hydroxy-6-(3,3,3,2,2-pentafluoro-1-hydroxypropyl)-7-keto-5β-cholan-24-oate (3.40, proposed structure based on NMR analysis**)**



Following general procedure D, **3.4** was treated with pentafluoropropionaldehyde hydrate and purified by flash chromatography (acetone/hexane 20:80-30:70-40:60) to afford **3.40** (30%, calculated yield) and **3.3** (>35%, calculated yield) as gummy solids.

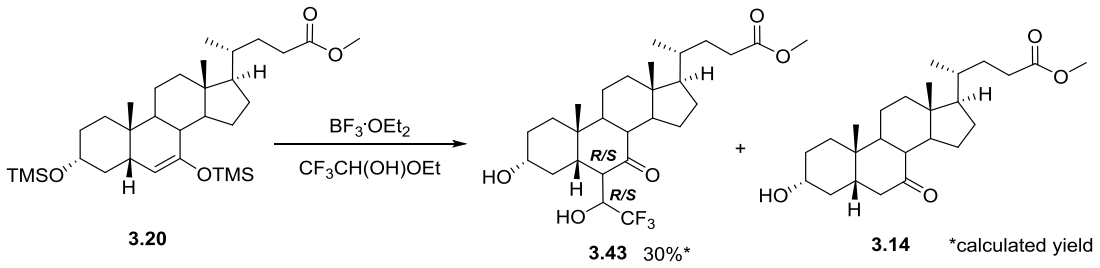
3.40, **Formula** $C_{34}H_{45}F_5O_5$; **MW**: 628.71; $\mathbf{R_f}$ 0.29 (acetone/hexane 25:75); **IR** (neat) 3384 (br. w), 2935 (m), 2870 (w), 1722 (m), 1708 (s), 1451 (m), 1385 (m), 1216 (s), 1191 (s), 1143 (s), 1094 (s), 1008 (s), 730 (s), 635 (m) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.28 - 7.42 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2), 5.09 (1H, d, *J* 12.4 Hz, PhCH_2), 4.92 - 5.04 (1H, m, CHCF$_2$CF$_3$), 3.44 - 3.80 (1H, m, $H_{3\beta}$), 2.86 (1H, dd, *J* 13.1, 6.1 Hz), 1.20 (3H, s, H_{19}), 0.92 (3H, d, *J* 5.9 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** N/A Peak signals were difficult to be identified due to the presence of the diastereoisomers; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -81.69 (3F, s, CF_3), -81.71 (3F, s, CF_3), -127.7 (1F, dd, *J* 275.1, 6.4 Hz, CF_2), -128.1 (1F, dd, *J* 276.2, 6.4 Hz, CF_2), -133.8 (1F, dd, *J* 276.2, 8.6 Hz, CF_2), -134.2 (1F, dd, *J* 275.1, 8.6 Hz, CF_2) ppm; **^{19}F {^{1}H} NMR** (282 MHz, $CDCl_3$) δ -81.69 (3F, s, CF_3), -81.71 (3F, s, CF_3), -127.7 (1F, d, *J* 277.2 Hz, CF_2), -128.1 (1F, d, *J* 276.2 Hz, CF_2), -133.8 (1F, d, *J* 275.1 Hz, CF_2), -134.2 (1F, d, *J* 276.2 Hz, CF_2) ppm.

Synthesis of benzyl 3β-chloro-6-trifluoroethyliden-7-keto-5β-cholan-24-oate (3.42, proposed structure based on NMR and HRMS analysis**)**



To a round-bottom flask were added benzyl 3α-hydroxy-6-(2,2,2-trifluoro-1-hydroxyethyl)-7-keto-5β-cholan-24-oate **3.37** (76 mg, impure, 1 eq), pyridine (4 mL) and thionyl chloride (0.2 mL). The reaction mixture was stirred overnight at room temperature. After removal of the solvent, the residue was dissolved in water (2 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine (15 mL), dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (acetone/hexane 10:90-30:70) to afford compound **3.42** (18 mg, 40%, adjusted yield based on percentage of **3.37** in starting material) as gummy solid. **3.42**, **Formula** $C_{33}H_{42}ClF_3O_3$; **MW** 579.13; $\mathbf{R_f}$ 0.27 (acetone/hexane 15:85); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.28 - 7.42 (5H, m, H_{Ar}), 5.67 - 5.75 (1H, m, C=CH), 5.12 (1H, d, *J* 12.3 Hz, PhCH_2), 5.11 (1H, d, *J* 12.4 Hz, PhCH_2), 3.65 - 3.90 (1H, m, $H_{3\alpha}$), 2.76 - 2.97 (1H, m), 1.23 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.3 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** N/A mixture of two isomers; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -80.0 (3F, d, *J* 4.3 Hz, CF_3), -80.1 (3F, d, *J* 4.3 Hz, CF_3) ppm; **^{19}F {^{1}H} NMR** (282 MHz, $CDCl_3$) δ -80.0 (1F, s, CF_3), -80.1 (1F, s, CF_3) ppm; **IR** (neat) (IR indicated no presence of hydroxyl group); **MS** (ESI+) *m/z* 619.0 ([M+H_2O+Na]$^+$, 73%); 1195.2 ([2M+K]$^+$, 67%); 1215.3 ([2M+MeCN+NH_4]$^+$, 100%); [No isotopic (^{35}Cl and ^{37}Cl) pattern observed]

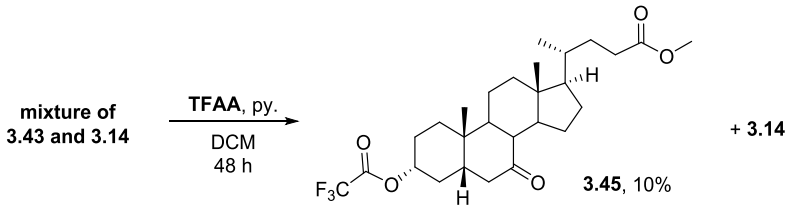
Synthesis of methyl 3α-hydroxy-6-(2,2,2-trifluoro-1-hydroxyethyl)-7-keto-5β-cholan-24-oate (3.43, proposed structure based on NMR and HRMS analysis**)**



Following general procedure D, **3.20** was treated with acetaldehyde to afford **3.43** (30%, calculated yield) and **3.14**. The mixture was used directly for next step.

3.43, **Formula** $C_{27}H_{41}F_3O_5$; **MW** 502.61; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.77 - 4.97 (1H, m, C*H*CF_3), 3.68 - 3.80 (1H, m, $H_{3\beta}$), 3.67 (3H, s, OCH_3), 2.77 - 2.96 (1H, m), 2.51 - 2.75 (1H, m), 1.21 (3H, s, H_{19}), 0.93 (3H, d, *J* 6.3 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** N/A Peak signals were difficult to be identified due to the presence of the diastereoisomers; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -84.5 - -84.4 (1F, m, CF_3), -84.6 - -84.5 (1F, m, CF_3) ppm; **^{19}F {^{1}H} NMR** (282 MHz, $CDCl_3$) δ -84.47 (1F, s, CF_3), -84.55 (1F, s, CF_3) ppm; **MS** (ESI+) *m/z* 525.4 $[M+Na]^+$, 1027.4 $[2M+Na]^+$; **HRMS** (ESI+) for $C_{27}H_{41}F_3NaO_5$ $[M+Na]^+$ calcd 525.2798, found 525.2804 (-1.0 ppm error).

Synthesis of methyl 3α-trifluoroacetoxy-7-keto-5β-cholan-24-oate (3.45)

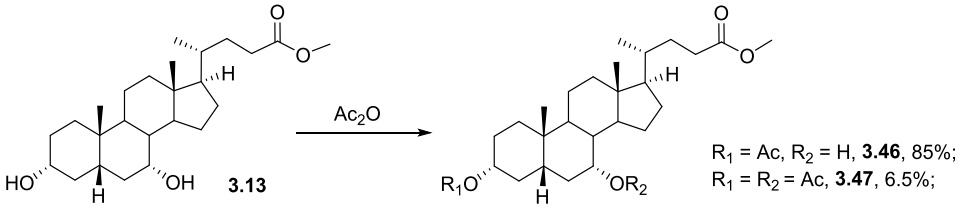


To a round-bottom flask were added a mixture of **3.43** and **3.14** (ca. 2 mmol) and DCM (30 mL) under argon. The mixture was cooled to 0 °C, followed by addition pyridine (0.94 mL) and TFAA (1.23 mL). The resulting mixture was stirred for 1 h. Upon completion indicated by TLC analysis, the mixture was washed with $NaHCO_3$ solution, 0.1 M HCl solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 10:90-35:65) to by-product **3.45** (10%, recrystallised from acetone/petroleum ether).

3.45, **Formula** $C_{27}H_{39}F_3O_5$; **MW** 500.59; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.87 (1H, tt, *J* 11.6, 4.9 Hz, $H_{3\beta}$), 3.64 (3H, s, OCH_3), 2.87 (1H, dd, *J* 12.7, 6.1 Hz), 2.40 (1H, t, *J* 11.1 Hz), 2.28 - 2.36 (1H, m), 1.22 (3H, s, H_{19}), 0.90 (3H, d, *J* 6.4 Hz, H_{21}), 0.64 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 211.2 (C_7), 174.5 (C_{24}), 156.8 (q, *J* 42.0 Hz, CF_3CO), 114.4 (q, *J* 286.1 Hz, CF_3), 77.7 (C_3), 54.7, 51.4, 49.4, 48.8, 45.7, 45.0, 42.7, 42.5, 38.8, 35.1, 35.0, 33.4, 32.4, 31.0, 30.9, 28.2, 25.5, 24.7, 22.8, 21.7, 18.3, 12.0 ppm;

^{19}F NMR (376 MHz, $CDCl_3$) δ -75.6 (3F, s, CF_3) ppm; **Other analysis** X-ray analysis included in the Appendices; NMR data were consistent with the data in the literature.[211]

Synthesis of methyl 3α-acetoxy-7α-hydroxy-5β-cholan-24-oate (3.46), and methyl 3α,7α-diacetoxy-5β-cholan-24-oate (3.47)

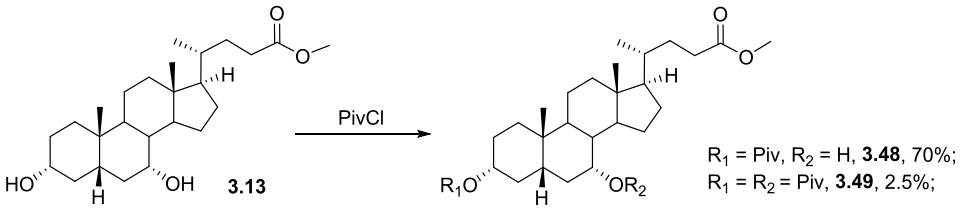


To a round bottom flask were added methyl 3α,7α-dihydroxy-5β-cholan-24-oate **3.13** (1 g, 2.46 mmol, 1 eq), THF (anhydrous, 25 mL, 10 mL/mmol), acetic anhydride (4.30 mL, 44.3 mmol, 18 eq) and $NaHCO_3$ (4.26 g, 49.2 mmol, 20 eq). The reaction mixture was heated under reflux overnight, then cooled to room temperature and diluted with water (25 mL). The mixture was extracted with ethyl acetate (3 × 20 mL) and the combined organic layer was washed with water (2 × 20 mL), brine (20 mL), dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography, followed by HPLC to afford compound **3.46** (943 mg, 2.10 mmol, 85%) and compound **3.47** (78 mg, 0.16 mmol, 6.5%) as white solids.

3.46, **Formula** $C_{27}H_{44}O_5$; **MW** 448.64; **R_f** 0.30 (acetone/hexane 20:80); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.49 - 4.66 (1H, m, $H_{3\beta}$), 3.80 - 3.90 (1H, m, $H_{7\beta}$), 3.66 (3H, s, OCH_3), 2.01 (3H, s, CH_3COOSt), 0.92 (3H, d, *J* 7.0 Hz, H_{21}), 0.91 (3H, s, H_{19}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.7, 170.7, 74.3, 68.4, 55.8, 51.5, 50.4, 42.7, 41.2, 39.6, 39.4, 35.35, 35.27, 35.1, 35.0, 34.4, 32.8, 30.99, 30.97, 28.1, 26.7, 23.7, 22.7, 21.5, 20.6, 18.2, 11.7 ppm; NMR data were consistent with the data in the literature.[88],[212]

3.47, **Formula** $C_{29}H_{46}O_6$; **MW** 490.67; **R_f** 0.36 (acetone/hexane 20:80); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.80 - 4.92 (1 H, m, $H_{7\beta}$), 4.57 (1H, tt, *J* 11.1, 4.9 Hz, $H_{3\beta}$), 3.64 (3H, s, OCH_3), 2.03 (3H, s, CH_3COOSt), 2.01 (3H, s, CH_3COOSt), 0.91 (3H, s, H_{19}), 0.90 (3H, d, *J* 6.0 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.6, 170.5, 170.3, 74.1, 71.1, 55.7, 51.4, 50.3, 42.6, 40.9, 39.4, 37.8, 35.2, 34.8, 34.7, 34.5, 34.0, 31.2, 30.9, 30.9, 27.9, 26.7, 23.5, 22.6, 21.5, 21.4, 20.6, 18.2, 11.6 ppm; NMR data were consistent with the data in the literature.[213]

Synthesis of methyl 3α-pivaloyloxy-7α-hydroxy-5β-cholan-24-oate (3.48), and methyl 3α,7α-dipivaloyloxy-5β-cholan-24-oate (3.49)

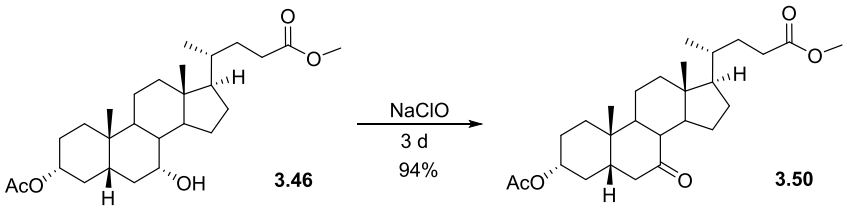


To a round-bottom flask were added methyl 3α,7α-dihydroxy-5β-cholan-24-oate **3.13** (500 mg, 1.23 mmol, 1 eq), PivCl (0.228 mL, 1.85 mmol, 1.5 eq) and pyridine (3.1 mL, 2.5 mL/mmol). The reaction mixture was stirred at room temperature overnight and quenched with brine (20 mL) and then extracted with ethyl acetate (40 mL × 3). The combined organic layer was washed with HCl (1 M, 40 mL), sat. $NaHCO_3$ (40 mL), brine (40 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography (acetone/petroleum ether 7:93-30:70) to afford **3.48** (425 mg, 70%) and **3.49** (17 mg, 2.5%) as gummy solids.

3.48, **Formula** $C_{30}H_{50}O_5$; **MW** 490.72; **R_f** 0.30 (acetone/petroleum ether 10:90); **IR** (neat) 3512 (br. w), 2937 (s), 2869 (m), 1746 (s), 1720 (s), 1455 (m), 1281 (m), 1168 (s), 1077 (w), 726 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.46 - 4.63 (1H, m, $H_{3\beta}$), 3.87 (1H, br. s, $H_{7\beta}$), 3.67 (3H, s, OCH_3), 1.17 (9H, s, (CH_3)$_3$C), 0.93 (3H, d, *J* 7.1 Hz, H_{21}), 0.92 (3H, s, H_{19}), 0.67 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.2 (*C*OO), 174.7 (*C*OO), 74.0 (C_3), 68.5 (C_7), 55.8 (*C*H), 51.5 (O*C*H$_3$), 50.5 (*C*H), 42.7 (*C*), 41.2 (*C*H), 39.5 (*C*H$_2$), 39.3 (*C*H), 38.6 (*C*), 35.4 (*C*H), 35.2 (*C*H$_2$), 35.1 (*C*), 35.0 (*C*H$_2$), 34.6 (*C*H$_2$), 32.9 (*C*H), 31.00 (*C*H$_2$), 30.98 (*C*H$_2$), 28.1 (*C*H$_2$), 27.2 ((*C*H$_3$)$_3$C), 26.7 (*C*H$_2$), 23.7 (*C*H$_2$), 22.7 (*C*H$_3$), 20.6 (*C*H$_2$), 18.3 (*C*H$_3$), 11.7 (*C*H$_3$) ppm; **MS** (ESI+) *m/z* 513.1 ([M+Na]$^+$, 100%); 1003.5 ([2M+Na]$^+$, 11%); **HRMS** (ESI+) for $C_{30}H_{54}NO_5$ [M+NH_4]$^+$ calcd 508.3997, found 508.3994 (0.6 ppm error).

3.49, **Formula** $C_{35}H_{58}O_6$; **MW** 574.83; **IR** (neat) 2932 (m), 2862 (m), 1726 (s), 1458 (w), 1288 (m), 1160 (s), 1074 (w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.84 - 4.90 (1H, m, $H_{7\beta}$), 4.57 (1H, tt, *J* 11.4, 4.7 Hz, $H_{3\beta}$), 3.66 (3H, s, OCH_3), 1.21 (9H, s, (CH_3)$_3$C), 1.16 (9H, s, (CH_3)$_3$C), 0.94 (3H, s, H_{19}), 0.93 (3H, d, *J* 6.6 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.0 (*C*OO), 177.4 (*C*OO), 174.6 (*C*OO), 73.5 (C_3), 71.1 (C_7), 56.0 (*C*H), 51.5 (O*C*H$_3$), 50.7 (*C*H), 42.7 (*C*), 40.8 (*C*H), 39.7 (*C*H$_2$), 39.0 (*C*), 38.6 (*C*), 38.1 (*C*H), 35.4 (*C*H), 35.1 (*C*H$_2$), 34.8 (*C*H$_2$), 34.7 (*C*), 34.4 (*C*H), 31.3 (*C*H$_2$), 31.1 (*C*H$_2$), 31.0 (*C*H$_2$), 27.9 (*C*H$_2$), 27.17 ((*C*H$_3$)$_3$C), 27.14 ((*C*H$_3$)$_3$C), 26.6 (*C*H$_2$), 23.6 (*C*H$_2$), 22.7 (*C*H$_3$), 20.7 (*C*H$_2$), 18.3 (*C*H$_3$), 11.7 (*C*H$_3$) ppm; **MS** (ESI+) *m/z* 597.0 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{35}H_{62}NO_6$ [M+NH4]$^+$ calcd 592.4572, found 592.4563 (1.5 ppm error).

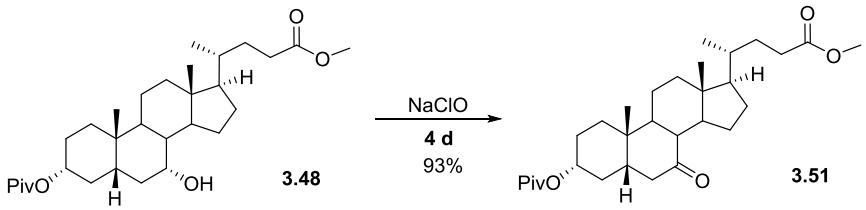
Synthesis of methyl 3α-acetyloxy-7-keto-5β-cholan-24-oate (3.50)



Following general procedure A, methyl 3α-acetyloxy-7α-hydroxy-5β-cholan-24-oate **3.46** (556 mg, 1.24 mmol) was oxidized over 3 days and purified by flash chromatography (acetone/petroleum ether 10:90-15:85) to afford **3.50** (476 mg, 94%) as white solid and the crystals was obtained *via* recrystallisation from acetone/petroleum ether.

3.50, **Formula** $C_{27}H_{42}O_5$; **MW** 446.62; $\mathbf{R_f}$ 0.21 (acetone/petroleum ether 10:90); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.56 - 4.73 (1H, m, $H_{3\beta}$), 3.63 (3H, s, OCH_3), 2.82 (1H, dd, *J* 12.5, 6.0 Hz, $H_{6\beta}$), 2.25 - 2.41 (2H, m), 2.09 - 2.25 (2H, m), 1.96 (3H, s, CH_3COOSt), 1.17 (3H, s, H_{19}), 0.89 (3H, d, *J* 6.3 Hz, H_{21}), 0.62 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 211.6, 174.5, 170.4, 72.8, 54.7, 51.4, 49.3, 48.8, 45.7, 45.1, 42.6, 42.5, 38.8, 35.1, 35.0, 33.7, 33.0, 30.9, 30.9, 28.2, 25.9, 24.7, 22.9, 21.6, 21.2, 18.2, 11.9 ppm; **Other analysis** X-Ray analysis included in the Appendices. NMR data were consistent with the data in the literature.[214]

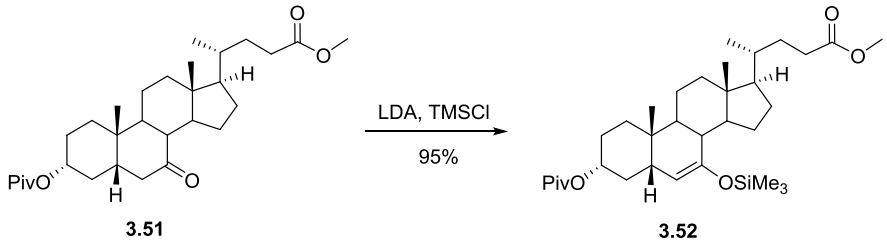
Synthesis of methyl 3α-pivaloyloxy-7-keto-5β-cholan-24-oate (3.51)



Following general procedure A, methyl 3α-pivaloyloxy-7α-hydroxy-5β-cholan-24-oate **3.48** (400 mg, 0.81 mmol) was oxidized over 4 days and purified by flash chromatography (acetone/petroleum ether 8:92) to afford **3.51** (368 mg, 93%) as gummy solid.

3.51, **Formula** $C_{30}H_{48}O_5$; **MW** 488.70; $\mathbf{R_f}$ 0.32 (acetone/petroleum ether 10:90); **IR** (neat) 2939 (m), 2868 (m), 1722 (s), 1464 (w), 1435 (w), 1381 (w), 1288 (w), 1163 (s) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.59 - 4.72 (1H, m, $H_{3\beta}$), 3.67 (3H, s, OCH_3), 2.87 (1H, dd, *J* 12.5, 6.0 Hz, $H_{6\beta}$), 2.29 - 2.47 (2H, m), 2.13 - 2.28 (2H, m), 1.21 (3H, s, H_{19}), 1.15 (9H, s, (CH_3)$_3$C), 0.92 (3H, d, *J* 6.2 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 212.1 (C_7), 178.0 (*C*OO), 174.6 (*C*OO), 72.5 (C_3), 54.8 (*C*H), 51.5 (*C*H_3), 49.5 (*C*H), 48.9 (*C*H), 45.9 (*C*H), 45.3 (*C*H_2), 42.8 (*C*H), 42.6 (*C*), 38.9 (*C*H_2), 38.6 (*C*), 35.19 (*C*H), 35.18 (*C*), 33.8 (*C*H_2), 33.0 (*C*H_2), 31.03 (*C*H_2), 30.99 (*C*H_2), 28.3 (*C*H_2), 27.1 (*C*H_3), 26.0 (*C*H_2), 24.8 (*C*H_2), 23.0 (*C*H_3), 21.7 (*C*H_2), 18.4 (*C*H_3), 12.0 (*C*H_3) ppm; **MS** (ESI+) *m/z* 511.2 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{30}H_{52}NO_5$ [M+NH_4]$^+$ calcd 506.3840, found 506.3837 (0.7 ppm error);

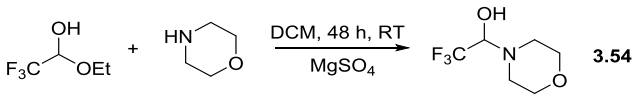
Synthesis of methyl 3α-pivaloyloxy-7-trimethylsilyloxy-5β-cholan-6-en-24-oate (**3.52**)



Following general procedure C, silyl enol ether **3.52** was prepared from 7-keto derivative **3.51** with high yield (95%).

3.52, **Formula** $C_{33}H_{56}O_5Si$; **MW** 560.88; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.72 (1H, dd, *J* 5.9, 1.6 Hz, H_6), 4.62 (1H, tt, *J* 11.3, 4.5 Hz, $H_{3\beta}$), 3.67 (3H, s, COOCH_3), 1.17 (9H, s, (CH_3)$_3$C), 0.94 (3H, d, *J* 6.3 Hz, H_{21}), 0.86 (3H, s, H_{19}), 0.70 (3H, s, H_{18}), 0.16 (9H, s, Si(CH_3)$_3$) ppm.

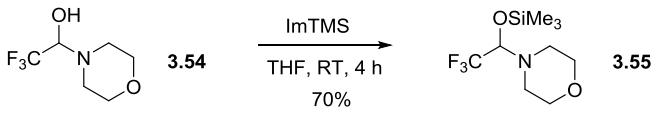
Synthesis of 2,2,2-trifluoro-1-morpholinoethanol (3.54)



To a round-bottom flask were added $MgSO_4$ (15 g), DCM (80 mL), morpholine (7 mL, 80 mmol, 1.0 eq) and acetaldehyde methyl hemiacetal (8.42 mL, 88 mmol, 1.1 eq). The resulting mixture was stirred for 48 h. Upon completion indicated by TLC analysis, the mixture was filtered and concentrated to afford **3.54** nearly quantitatively as yellow oil.

3.54, **Formula** $C_6H_{10}F_3NO_2$; **MW** 185.14; **IR** (neat) 3334 (br.) 2971 (w), 2922 (w), 2892 (w), 2858 (w), 1463 (w), 1270 (m), 1157 (s), 1108 (s), 1005 (s), 934 (w), 843 (w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.42 (1H, q, *J* 5.9 Hz, C*H*), 4.34 (1H, br. s., O*H*), 3.65 - 3.75 (4H, m, OCH_2), 2.70 - 2.88 (4H, m, NCH_2) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 123.3 (q, *J* 286.1 Hz, *C*F_3), 84.4 (q, *J* 32.3 Hz, *C*H), 66.9 (O*C*H_2), 47.4 (N*C*H_2) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -76.4 (3F, d, *J* 5.6 Hz, CF_3) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -76.4 (3 F, s, CF_3) ppm; **MS** (ESI+) *m/z* 186.0 ([M+H]$^+$, 93%); **HRMS** (ESI+) for $C_6H_9F_3NO$ [M-OH]$^+$ calcd 168.0631, found 168.0631 (-0.2 ppm error).

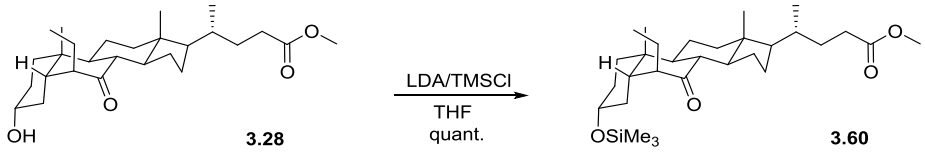
Synthesis of 4-(2,2,2-trifluoro-1-((trimethylsilyl)oxy)ethyl)morpholine (3.55)



To a round-bottom flask were added **3.54** (4 g, 21.6 mmol, 1.0 eq), THF (35 mL) and ImTMS (9.51 mL, 64.8 mmol, 3 eq) at 0 °C. The resulting mixture was stirred at 0 °C for 20 min and then stirred at rt for 5 h. The reaction mixture was treated with pentane and washed with 6% aq. $NaHCO_3$ solution. The organic layer was then dried over Na2SO4, filtered and concentrated to afford **3.55** (70%) as yellow oil.

3.55, **Formula** $C_9H_{18}F_3NO_2Si$; **MW** 257.32; **IR** (neat) 2958 (w), 2919 (w), 2895 (w), 2852 (w), 1458 (w), 1279 (w), 1259 (m), 1166 (s), 1149 (s), 1119 (s), 1063 (s), 877 (s), 834 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.35 (1H, q, *J* 5.7 Hz, C*H*), 3.58 - 3.71 (4H, m, OCH_2), 2.61 - 2.79 (4H, m, NCH_2), 0.15 (9H, s, CH_3) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 123.3 (q, *J* 286.8 Hz, *C*F_3), 85.4 (d, *J* 33.7 Hz, *C*H), 66.9 (O*C*H_2), 47.8 (N*C*H_2), -0.3 (*C*H_3) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -76.9 (3F, d, *J* 5.5 Hz, CF_3) ppm; **MS** (ESI+) *m/z* 257.99 ([M+H]$^+$, 100%); **HRMS** (ESI+) for $C_9H_{19}F_3NO_2Si$ $[M+H]^+$ calcd 258.1132, found 258.1133 (-0.7 ppm error).

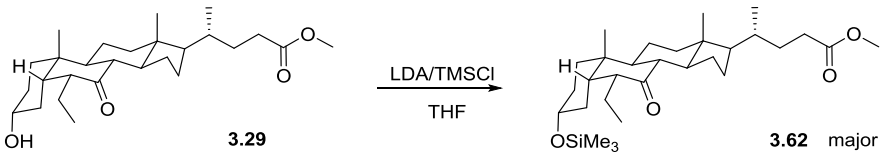
Synthesis of methyl 3α-trimethylsilyloxy-6β-ethyl-7-keto-5β-cholan-24-oate (3.60)



Following general procedure C, the corresponding silyl enol ether from **3.28** was not formed. Instead, by-product **3.60** was isolated after column chromatography (acetone/petroleum ether 5:95) quantitatively.

3.60, **Formula** $C_{30}H_{52}O_4Si$; **MW** 504.82; **$[\alpha]_D$** +10.0 (c 0.6, $CHCl_3$, 20 °C); **m.p.** 130-134 °C (acetone/petroleum ether); **IR** (neat) 2929 (s), 2865 (s), 1744 (s), 1702 (s), 1449 (m), 1372 (m), 1251 (s), 1171 (m), 1084 (s), 886 (s), 834 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.67 (3H, s, OCH_3), 3.52 (1H, tt, *J* 10.5, 4.5 Hz, $H_{3\beta}$), 2.52 (1H, t, *J* 11.4 Hz, $H_{8\beta}$), 2.29 - 2.42 (1H, m), 1.20 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.4 Hz, H_{21}), 0.85 (3H, t, *J* 7.3 Hz, CH_2CH_3), 0.66 (3H, s, H_{18}), 0.09 (9H, s, Si(CH_3)$_3$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 215.0 (C_7), 174.7 (C_{24}), 71.0 (C_3), 62.0 (*C*H), 54.8 (*C*H), 51.5 (O*C*H_3), 49.6 (*C*H), 48.7 (*C*H), 45.6 (*C*H), 42.5 (*C*H), 42.5 (*C*) [overlapped with CH at 42.5 ppm leading to peak enhancement when C NMR and DEPT135 were compared], 40.0 (*C*H_2), 38.8 (*C*H_2), 35.54 (*C*), 35.52 (*C*H_2), 35.2 (*C*H), 31.03 (*C*H_2), 30.98 (*C*H_2), 29.9 (*C*H_2), 28.2 (*C*H_2), 26.6 (*C*H_2), 25.9 (C_{19}), 25.0 (*C*H_2), 21.4 (*C*H_2), 18.3 (C_{21}), 13.1 (CH_2CH_3), 12.1 (C_{18}), 0.2 (Si(*C*H_3)$_3$) ppm; **MS** (ESI+) *m/z* compound decomposed to **3.28** over C18 acid column for RP-HPLC-MS; **HRMS** (ESI+) for $C_{30}H_{52}NaO_4Si$ $[M+Na]^+$ calcd 527.3527, found 527.3527 (0.1 ppm error). **Other analysis** X-Ray analysis included in the Appendices.

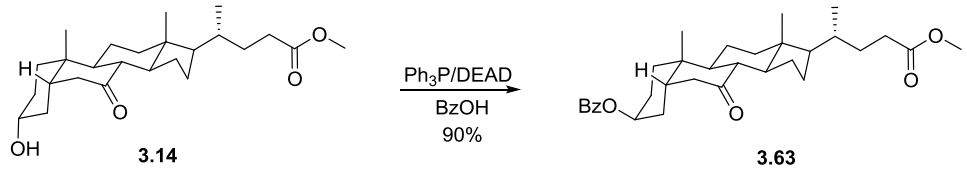
Synthesis of methyl 3α-trimethylsilyloxy-6α-ethyl-7-keto-5β-cholan-24-oate (3.62)



Following general procedure C, the corresponding silyl enol ether from **3.29** was not formed. Instead, by-product **3.62** was identified as major product by NMR analysis.

3.62, **Formula** $C_{30}H_{52}O_4Si$; **MW** 504.82; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.67 (3H, s, OCH_3), 3.50 (1H, tt, *J* 11.0, 4.4 Hz, $H_{3\beta}$), 2.58 - 2.73 (1H, m, $H_{6\beta}$), 1.21 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.4 Hz, H_{21}), 0.81 (3H, t, *J* 7.5 Hz, CH_2CH_3), 0.65 (3H, s, H_{18}), 0.09 (9H, s, Si(CH_3)$_3$) ppm;

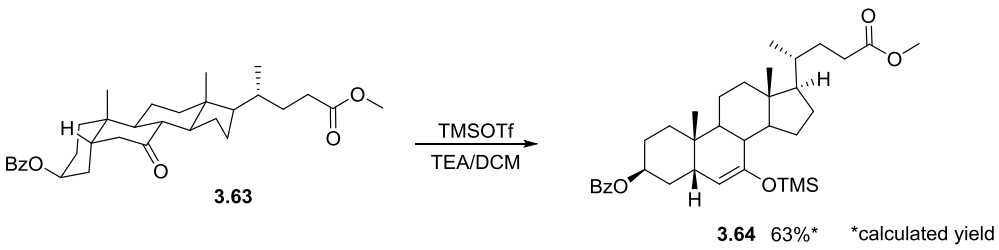
Synthesis of methyl 3β-benzoyloxy-7-keto-5β-cholan-24-oate (3.63)



To a round-bottom flask were added compound **3.14** (6.56 g, 16.2 mmol, 1.0 eq), Ph_3P (6.37 g, 24.3 mmol, 1.5 eq), benzoic acid (2.97 g, 24.3 mmol, 1.5 eq) and THF (80 mL). DEAD (3.82 mL, 24.3 mmol, 1.5 eq) was then added dropwise over 5 min. The resulting reaction mixture was stirred at room temperature overnight. Upon completion indicated by TLC analysis, the solvent was removed under reduced pressure. The residue was directly purified by flash chromatography (acetone/petroleum ether 5:95 – 7:93 – 10:90) to afford the desired product **3.63** (7.41 g, 14.57 mmol, 90%).

3.63, **Formula** $C_{32}H_{44}O_5$; **MW** 508.70; **IR** (neat) 3089 (w), 3064 (w), 2950 (s), 2860 (m), 1736 (s), 1704 (s), 1601 (w), 1577 (w), 1454 (m), 1373 (m), 1274 (s), 1254 (s), 1205 (m), 1160 (s), 1099 (s), 1021 (m), 735 (s), 702 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.01 - 8.06 (2H, m, H_{Ar}), 7.56 (1H, tt, *J* 7.4, 1.3 Hz, H_{Ar}), 7.41 - 7.48 (2H, m, H_{Ar}), 5.27 - 5.30 (1H, m, $H_{3\alpha}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.92 (1H, dd, *J* 12.5, 6.2 Hz, $H_{6\beta}$), 2.45 (1H, t, *J* 11.4 Hz, $H_{8\beta}$), 1.30 (3H, s, H_{19}), 0.93 (3H, d, *J* 6.4 Hz, H_{21}), 0.67 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 212.2 (C_7), 174.6 (C_{24}), 165.6 (Ph*C*OO), 132.8 (CH_{Ar}), 130.8 (C_{Ar}), 129.5 (CH_{Ar}), 128.3 (CH_{Ar}), 70.2 (C_3), 54.7 (*C*H), 51.5 ($C_{24}OCH_3$), 49.6 (*C*H), 48.9 (*C*H), 45.0 (CH_2), 42.6 (*C*), 42.4 (*C*H), 42.3 (*C*H), 38.9 (CH_2), 35.5 (*C*), 35.2 (*C*H), 31.8 (CH_2), 31.01 (CH_2), 30.96 (CH_2), 30.1 (CH_2), 28.2 (CH_2), 24.8 (CH_2), 24.7 (CH_2), 23.7 (C_{19}), 22.0 (CH_2), 18.3 (C_{21}), 12.0 (C_{18}) ppm; **MS** (ESI+) 531.4 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{32}H_{44}NaO_5$ [M+Na]$^+$, calcd 531.3081, found 531.3087 (-1.1 ppm error).

Synthesis of methyl 3β-benzoyloxy-7-trimethylsilyloxy-5β-cholan-6-en-24-oate (**3.64**)

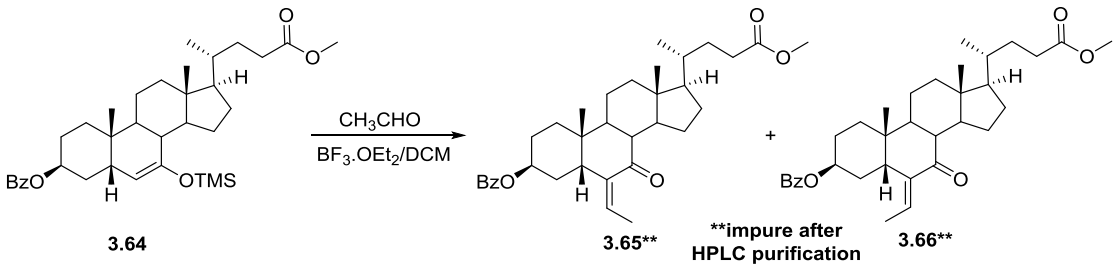


To a round-bottom flask were added compound **3.63** (298 mg, 0.586 mmol, 1 eq) and DCM (10 mL). The solution was cooled to 0 °C, followed by addition of TEA (0.163 mL, 1.172 mmol, 2 eq) and TMSOTf (0.12 mL, 0.645 mmol, 1.1 eq). The resulting reaction mixture was stirred at 0 °C for 1 h,

and further monitored by TLC analysis. Upon completion, the reaction was quenched by sat. aq. $NaHCO_3$ solution, and then extracted with DCM (× 4). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude mixture was used directly for further Mukaiyama aldo reaction. The calculated yield for the desired product **3.64** was 63%, based on ^{1}H NMR analysis.

3.64, **Formula** $C_{35}H_{52}O_5Si$; **MW** 580.88; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.02 - 8.09 (2H, m, H_{Ar}), 7.54 - 7.60 (1H, m, H_{Ar}), 7.42 - 7.49 (2H, m, H_{Ar}), 5.24 - 5.28 (1H, m, $H_{3\alpha}$), 4.76 (1H, dd, *J* 5.9, 1.7 Hz, H_6), 3.68 (3H, s, $C_{24}OCH_3$), 0.951 (3H, d, *J* 6.4 Hz, H_{21}), 0.946 (3H, s, H_{19}), 0.72 (3H, s, H_{18}), 0.17 (9H, s, $Si(CH_3)_3$) ppm.

Synthesis of (*Z*)-methyl 3β-benzoyloxy-6-ethyliden-7-keto-5β-cholan-24-oate (3.65), and (*E*)-methyl 3β-benzoyloxy-6-ethyliden-7-keto-5β-cholan-24-oate (3.66)



Following general procedure C, **3.64** (0.58 mmol) was reacted with acetaldehyde, leading to the formation of compounds **3.65** and **3.66**. The crude mixture was purified by flash chromatography (acetone/petroleum ether 8:92) and also by HPLC (acetone/hexane 10:90). However, it was not possible to isolate both compounds from impurities. The assignment of the enone configuration was based on 1D NOE NMR experiments.

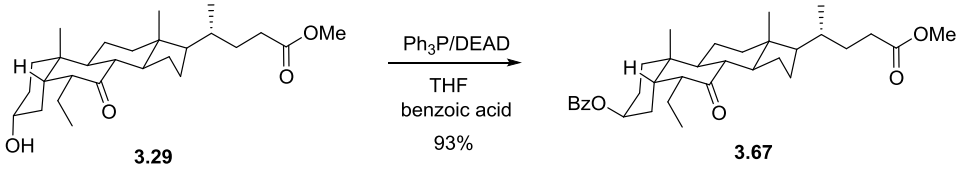
3.65, **Formula** $C_{34}H_{46}O_5$; **MW** 534.74; **IR** (neat) 2954 (s), 2864 (m), 1732 (s), 1708 (s), 1687 (s), 1603 (w), 1583 (w), 1454 (m), 1376 (m), 1266 (s), 1111 (s), 1025 (m), 923 (m), 735 (s), 711 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03 - 8.08 (2H, m, H_{Ar}), 7.55 - 7.61 (1H, m, H_{Ar}), 7.44 - 7.50 (2H, m, H_{Ar}), 5.46 (1H, q, *J* 7.0 Hz, C_6=C*H*CH_3), 5.26 - 5.31 (1H, m, $H_{3\alpha}$), 3.68 (3H, s, $C_{24}OCH_3$), 2.53 (1H, dd, *J* 12.6, 5.0 Hz, $H_{5\beta}$), 1.68 (4H, d, *J* 7.0 Hz, C_6=CHCH_3), 1.16 (3H, s, H_{19}), 0.95 (3H, d, *J* 6.4 Hz, H_{21}), 0.67 (3H, s, H_{18}) ppm; **MS** (ESI+) 557.4 ($[M+Na]^+$, 51%); **HRMS** (ESI+) for $C_{34}H_{46}NaO_5$ $[M+Na]^+$, calcd 557.3237, found 557.3233 (0.9 ppm error); **Other analysis** 1D NOE NMR experiments.

3.66, **Formula** $C_{34}H_{46}O_5$; **MW**: 534.74; **IR** (neat) 2946 (s), 2872 (m), 1744 (s), 1716 (s), 1603 (w), 1583 (w), 1454 (m), 1377 (w), 1274 (s), 1107 (s), 1017 (m), 907 (s), 727 (s), 706 (s), 645 (w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03 - 8.09 (2H, m, H_{Ar}), 7.55 - 7.61 (1H, m, H_{Ar}), 7.44 - 7.50 (2H, m, H_{Ar}), 6.24 (1H, q, *J* 7.1 Hz, C_6=C*H*CH_3), 5.25 - 5.35 (1H, m, $H_{3\alpha}$), 3.66 (3H, s, $C_{24}OCH_3$), 3.00 (1H, dd, *J* 12.1, 5.3 Hz, $H_{5\beta}$), 1.67 (3H, d, *J* 7.2 Hz, C_6=CHCH_3), 1.10 (3H, s, H_{19}), 0.94 (3H, d, *J* 6.3 Hz, H_{21}), 0.67 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 204.6 (C_7), 174.6 (C_{24}), 165.6 (Ph*C*O), 143.0 (C_6), 132.8 (CH_{Ar}),

130.8 (*C*$_{Ar}$), 130.6 (*C*H=C$_6$), 129.4 (*C*H$_{Ar}$), 128.4 (*C*H$_{Ar}$), 69.7 (*C*$_3$), 54.5 (*C*H), 51.4 (C$_{24}$O*C*H$_3$), 50.7 (*C*H), 48.6 (*C*H), 43.6 (*C*), 41.4 (*C*H), 39.0 (*C*H$_2$), 38.6 (*C*H), 35.2 (*C*H), 34.8 (*C*), 32.4 (*C*H$_2$), 30.99 (*C*H$_2$), 30.97 (*C*H$_2$), 30.2 (*C*H$_2$), 28.4 (*C*H$_2$), 25.9 (*C*H$_2$), 24.6 (*C*H$_2$), 23.6 (*C*$_{19}$), 21.8 (*C*H$_2$), 18.4 (*C*$_{21}$), 12.6 (*C*H$_3$CH=C$_6$), 12.0 (*C*$_{18}$) ppm; **MS** (ESI+) 535.4 ([M+H]$^+$, 100%), 557.4 ([M+Na]$^+$, 48%); **HRMS** (ESI+) for $C_{34}H_{46}NaO_5$ [M+Na]$^+$, calcd 557.3237, found 557.3247 (-1.6 ppm error); **Other analysis** 1D NOE NMR experiments (see Appendices).

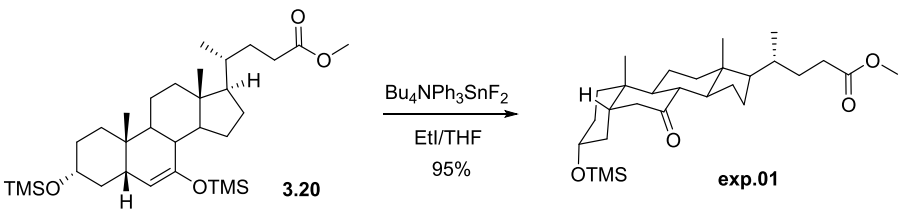
Synthesis of methyl 3β-benzoyloxy-6α-ethyl-7-keto-5β-cholan-24-oate (3.67)

To a round-bottom flask were added **3.29** (347 mg, 0.802 mmol, 1 eq), Ph_3P (316 mg, 1.203 mmol, 1.5 eq), benzoic acid (147 mg, 1.203 mmol, 1.5 eq) and THF (8 mL). DEAD was then added dropwise over 5 min. The resulting mixture was stirred at room temperature overnight. Upon completion indicated by TLC analysis, the solvent was removed under reduced pressure. The residue was directly purified by flash chromatography (acetone/petroleum ether 5:95 – 8:92) to afford the desired product **3.67** (400 mg, 0.745 mmol, 93%) as white solid.



3.67, **Formula** $C_{34}H_{48}O_5$; **MW** 536.75; **IR** (neat) 3061 (w), 3033 (w), 2939 (m), 2868 (m), 1736 (s), 1704 (s), 1603 (w), 1583 (w), 1262 (s), 1171 (m), 1108 (s), 1026 (m), 911 (s), 734 (s), 710 (s), 651 (w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03 (2H, dd, *J* 8.4, 1.3 Hz, *H*$_{Ar}$), 7.57 (1H, tt, *J* 7.5, 1.3 Hz, *H*$_{Ar}$), 7.42 - 7.49 (2H, m, *H*$_{Ar}$), 5.25 - 5.31 (1H, m, *H*$_{3α}$), 3.67 (3H, s, C$_{24}$OC*H*$_3$), 2.79 (1H, q, *J* 6.5 Hz, *H*$_{6β}$), 2.42 (1H, t, *J* 11.2 Hz, *H*$_{8β}$), 1.33 (3H, s, *H*$_{19}$), 0.94 (3H, d, *J* 6.4 Hz, *H*$_{21}$), 0.79 (3H, t, *J* 7.4 Hz, CH$_2$C*H*$_3$), 0.68 (3H, s, *H*$_{18}$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 213.0 (*C*$_7$), 174.6 (*C*$_{24}$), 165.6 (Ph*C*O), 132.8 (*C*H$_{Ar}$), 130.8 (*C*$_{Ar}$), 129.4 (*C*H$_{Ar}$), 128.3 (*C*H$_{Ar}$), 70.0 (*C*$_3$), 54.8 (*C*H), 51.6 (*C*H), 51.4 (C$_{24}$O*C*H$_3$), 50.0 (*C*H), 48.9 (*C*H), 46.8 (*C*H), 43.3 (*C*H), 42.6 (*C*), 38.9 (*C*H$_2$), 36.0 (*C*), 35.2 (*C*H), 30.99 (*C*H$_2$), 30.95 (*C*H$_2$), 30.3 (*C*H$_2$), 28.2 (*C*H$_2$), 26.4 (*C*H$_2$), 24.58 (*C*H$_2$), 24.56 (*C*H$_2$), 24.2 (*C*$_{19}$), 22.1 (*C*H$_2$), 18.6 (*C*H$_2$), 18.3 (*C*$_{21}$), 12.0 (*C*$_{18}$), 11.9 (CH$_2$*C*H$_3$) ppm; **MS** (ESI+) 559.4 ([M+Na]$^+$, 78%); **HRMS** (ESI+) for $C_{34}H_{48}NaO_5$ [M+Na]$^+$, calcd 559.3394, found 559.3389 (0.9 ppm error).

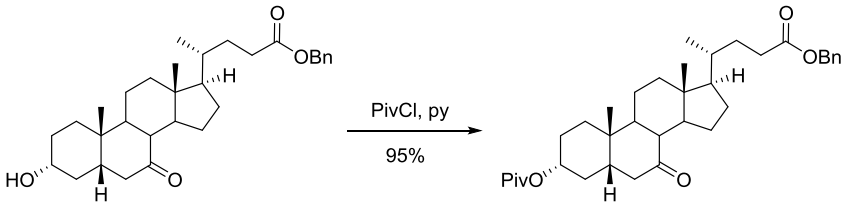
Synthesis of methyl 3α-trimethylsilyloxy-7-keto-5β-cholan-24-oate (exp.01)



To a round-bottom flask were added **3.20** (0.60 mmol, 1.0 eq), THF (8 mL, 5 mL/mmol) and EtI (0.48 mL, 10 eq). The solution was stirred at -78 °C for 15 min. Tetrabutylammonium difluorotriphenylstannate (416 mg, 0.66 mmol, 1.1 eq) was then added at -78 °C in one portion. The reaction mixture was warmed to room temperature and stirred overnight, followed by filtration over silica gel. The remaining solvent was concentrated. The desired C6-ethylated product **3.29** was not formed. Instead, by-product **exp.01** was obtained with high yield (95%).

exp.01, **Formula** $C_{28}H_{48}O_4Si$; **MW** 476.76; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.67 (3H, s, OCH_3), 3.56 (1H, tt, *J* 10.5, 4.9 Hz, $H_{3\beta}$), 2.83 (1H, dd, *J* 12.3, 5.9 Hz), 2.30 - 2.43 (2H, m), 1.18 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.2 Hz, H_{21}), 0.65 (3H, s, H_{18}), 0.10 (9H, s, Si(CH_3)$_3$) ppm.

Synthesis of benzyl 3α-pivaloyloxy-7-keto-5β-cholan-24-oate (3.69)

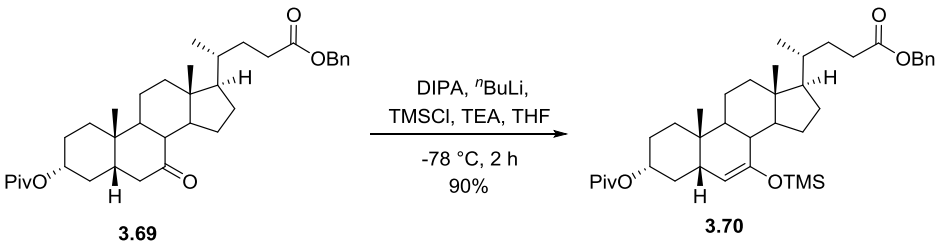


3.3 **3.69**

To a round-bottom flask were added benzyl 3α-hydroxy-7-keto-5β-cholan-24-oate **3.3** (500 mg, 1.04 mmol, 1 eq), pyridine (2.6 mL, 2.5 mL/mmol) and PivCl (0.192 mL, 1.56 mmol, 1.5 eq). The reaction mixture was stirred at room temperature overnight and quenched with brine (20 mL), and further extracted with ethyl acetate (40 mL × 3). The combined organic layer was washed with HCl (1 M, 40 mL), sat. aq. $NaHCO_3$ solution (40 mL), brine (40 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography (acetone/petroleum ether 8:92-10:90) to afford **3.69** (558 mg, 95%) as gummy solid.

3.69, **Formula** $C_{36}H_{52}O_5$; **MW** 564.80; **R_f** 0.33 (acetone/petroleum ether 10:90); **IR** (neat) 3086 (w), 3066 (w), 3034 (w), 2937 (m), 2863 (m), 1722 (s), 1482 (w), 1453 (m), 1376 (w), 1279 (m), 1216 (w), 1153 (s), 976 (w), 741 (w), 696 (m) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.29 - 7.50 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, PhCH_2O), 5.10 (1H, d, *J* 12.4 Hz, PhCH_2O), 4.58 - 4.73 (1H, m, $H_{3\beta}$), 2.87 (1H, dd, *J* 13.0, 6.3 Hz, $H_{6\beta}$), 1.22 (3H, s, H_{19}), 1.16 (9H, s, (CH_3)$_3$C), 0.92 (3H, d, *J* 6.3 Hz, H_{21}), 0.64 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 212.0 (C_7), 178.0 (*C*OO), 173.9 (*C*OO), 136.1 (C_{Ar}), 128.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}), 72.5 (C_3), 66.0 (PhCH_2), 54.8 (*C*H), 49.4 (*C*H), 48.8 (*C*H), 45.9 (*C*H), 45.2 (CH_2), 42.8 (*C*H), 42.6 (*C*), 38.8 (CH_2), 38.5 (*C*), 35.13 (*C*), 35.11 (*C*H), 33.8 (CH_2), 33.0 (CH_2), 31.2 (CH_2), 30.9 (CH_2), 28.2 (CH_2), 27.1 (CH_3), 26.0 (CH_2), 24.7 (CH_2), 23.0 (CH_3), 21.7 (CH_2), 18.3 (CH_3), 12.0 (CH_3) ppm; **MS** (ESI+) *m/z* 587.1 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{36}H_{56}NO_5$ [M+NH_4]$^+$ calcd 582.4153, found 582.4166 (-2.2 ppm error).

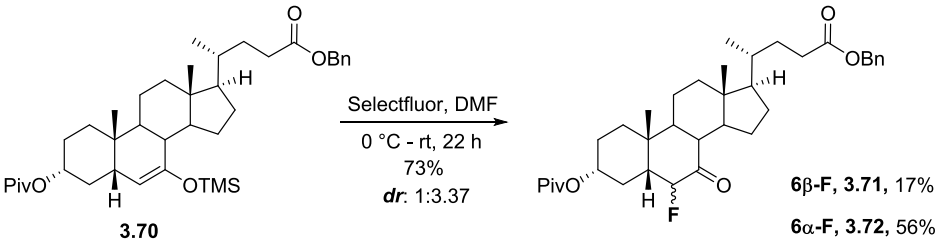
Synthesis of benzyl 3α-pivaloyloxy-7-trimethylsilyloxy-5β-cholan-6-en-24-oate (3.70)



Following general procedure C, **3.70** was obtained from **3.69** with satisfactory yield (90%).

3.70, **Formula** $C_{39}H_{60}O_5Si$; **MW** 636.98; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.31 - 7.40 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, PhCH_2), 5.10 (1H, d, *J* 12.4 Hz, PhCH_2), 4.72 (1H, dd, *J* 6.0, 1.7 Hz, H_6), 4.62 (1H, tt, *J* 10.8, 4.2 Hz, $H_{3\beta}$), 2.35 - 2.48 (1H, m), 2.22 - 2.35 (1H, m), 1.17 (9H, s, (CH_3)$_3$C), 0.93 (3H, d, *J* 6.2 Hz, H_{21}), 0.86 (3H, s, H_{19}), 0.68 (3H, s, H_{18}), 0.17 (9H, s, Si(CH_3)$_3$) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 178.1 (^{t}Bu*C*OO), 174.1 (C_{24}), 151.9 (C_7), 136.1 (C_{Ar}), 128.5 (C_{Ar}), 128.2 (C_{Ar}), 128.1 (C_{Ar}), 108.3 (C_6), 72.9 (C_3), 66.1 (Ph*C*H$_2$), 54.8, 54.1, 44.4, 42.2, 41.0, 40.5, 40.1, 38.6, 36.4, 35.2, 34.1, 33.0, 31.3, 31.0, 28.7, 27.1, 27.0, 26.4, 22.5, 21.0, 18.5, 12.2, 0.3 (Si(*C*H$_3$)$_3$) ppm.

Synthesis of benzyl 3α-pivaloyloxy-6β-fluoro-7-keto-5β-cholan-24-oate (3.71), and benzyl 3α-pivaloyloxy-6α-fluoro-7-keto-5β-cholan-24-oate (3.72)



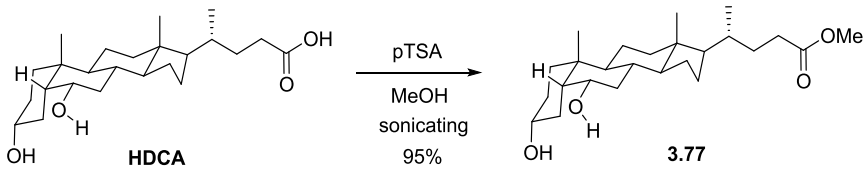
Following general procedure F, **3.70** (574 mg) was treated with Selectfluor in DMF. The crude mixture was purified by column (acetone/petroleum ether 5:95-10:90) to afford **3.71** (90 mg, 0.154 mmol, 17%) and **3.72** (291 mg, 0.50 mmol, 56%) as gummy solids.

3.71, **Formula** $C_{36}H_{51}FO_5$; **MW** 582.79; **[α]$_D$** -18.2 (c 0.6, $CHCl_3$, 22 °C); **R$_f$** 0.29 (acetone/petroleum ether 5:95); **IR** (neat) 2944(m), 2870(w), 1720(s), 1457(m), 1279(m), 1153(s), 1031(m), 908(m), 730(s), 699(s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 - 7.40 (5H, m, H_{Ar}), 5.13 (1H, d, *J* 12.4 Hz, PhCH_2), 5.09 (1H, d, *J* 12.4 Hz, PhCH_2), 4.60 - 4.73 (1H, m, $H_{3\beta}$), 4.28 (1H, dd, *J* 51.8, 3.2 Hz, $H_{6\alpha}$), 3.00 (1H, td, *J* 11.5, 5.2 Hz, $H_{8\beta}$), 2.35 - 2.45 (H, m), 2.21 - 2.34 (2H, m), 1.27 (3H, d, *J* 3.4 Hz, H_{19}), 1.15 (9H, s, (CH_3)$_3$C), 0.92 (3H, d, *J* 6.3 Hz, H_{21}), 0.68 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 208.4 (d, *J* 22.0 Hz, C_7), 177.9 (^{t}Bu*C*OO), 173.9 (C_{24}), 136.0 (C_{Ar}), 128.5 (C_{Ar}), 128.2 (C_{Ar}), 128.1 (C_{Ar}), 97.6 (d, *J* 184.4 Hz, C_6), 71.8 (d, *J* 2.9 Hz, C_3), 66.0 (Ph*C*H$_2$), 54.9, 49.5 (d, *J* 19.0 Hz), 48.1, 45.6, 44.3, 42.4, 38.8, 38.6, 35.1, 34.9, 34.1, 31.2, 30.9, 28.12 (d, *J* 8.8 Hz), 28.08, 27.0, 25.8, 24.8 (d, *J* 5.9 Hz), 24.2, 21.7, 18.3, 11.9 ppm; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -178.5 (1F, dd, *J* 51.6, 11.8 Hz, $F_{6\beta}$) ppm; **MS** (ESI+) *m/z*

605.4 [M+Na]$^+$; **HRMS** (ESI+) for $C_{36}H_{51}FNaO_5$ [M+Na]$^+$ calcd 605.3613, found 605.3619 (-1.0 ppm error);

3.72, **Formula** $C_{36}H_{51}FO_5$; **MW** 582.79; **[α]$_D$** -7.6 (c 1.1, $CHCl_3$, 22 °C); **R$_f$** 0.21 (acetone/petroleum ether 10:90); **IR** (neat) 2947(m), 2866(w), 1725(s), 1454(m), 1279(m), 1160(s), 1014(m), 917(m), 727(s), 699(s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.32 (5H, s, H_{Ar}), 5.21 (1H, dd, *J* 49.5, 6.4 Hz, $H_{6\beta}$), 5.11 (1H, d, *J* 12.4 Hz, PhCH_2O), 5.07 (1H, d, *J* 12.4 Hz, PhCH_2O), 4.54 - 4.71 (1H, m, $H_{3\beta}$), 1.22 (3H, s, H_{19}), 1.13 (9H, s, (CH_3)$_3$C), 0.90 (3H, d, *J* 6.1 Hz, H_{21}), 0.61 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 205.6 (d, *J* 13.5 Hz, C_7), 177.7 (^{t}Bu*C*OO), 173.7 (C_{24}), 136.0 (C_{Ar}), 128.4 (*C*H$_{Ar}$), 128.1 (*C*H$_{Ar}$), 128.0 (*C*H$_{Ar}$), 91.6 (d, *J* 195.0 Hz, C_6), 71.6 (C_3), 66.0 (Ph*C*H$_2$), 54.6 (*C*H), 50.6 (d, *J* 15.4 Hz, *C*H), 48.3 (*C*H), 47.5 (*C*H), 42.5 (*C*H), 42.5 (*C*, overlapping confirmed by peak enhancement and integration), 38.48 (*C*H$_2$), 38.46 (*C*), 35.8 (d, *J* 7.0 Hz, *C*), 35.0 (*C*H), 33.8 (*C*H$_2$), 31.1 (*C*H$_2$), 30.8 (*C*H$_2$), 28.1 (*C*H$_2$), 27.0 ((*C*H$_3$)$_3$*C*), 26.0 (d, *J* 5.9 Hz, *C*H$_2$), 25.8 (*C*H$_2$), 24.2 (*C*H$_2$), 23.2 (C_{19}), 21.5 (*C*H$_2$), 18.2 (C_{21}), 11.9 (C_{18}) ppm; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -202.0 (1F, br. d, *J* 49.4 Hz, $F_{6\alpha}$) ppm; **MS** (ESI+) *m/z* 605.3 [M+Na]$^+$; **HRMS** (ESI+) for $C_{36}H_{51}FNaO_5$ [M+Na]$^+$ calcd 605.3613, found 605.3616 (-0.6 ppm error).

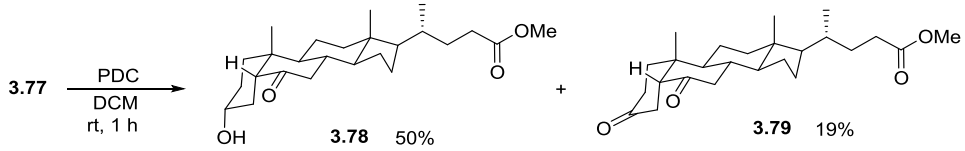
Synthesis of methyl 3α,6α-dihydroxy-5β-cholan-24-oate (3.77)



Following general procedure B, **3.77** was prepared from HDCA in high yield (95%), and used directly for next step without any purification.

3.77, **Formula** $C_{25}H_{42}O_4$; **MW** 406.60; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.03 (1H, dt, *J* 12.0, 4.7 Hz, $H_{6\beta}$), 3.66 (3H, s, OCH_3), 3.59 (1H, tt, *J* 11.0, 4.5 Hz, $H_{3\beta}$), 0.91 (3H, d, *J* 7.6 Hz, H_{21}), 0.90 (3H, s, H_{19}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7, 71.5, 68.0, 56.2, 55.9, 51.4, 48.4, 42.8, 39.9, 39.8, 35.9, 35.6, 35.3, 34.9, 34.8, 31.0, 30.9, 30.1, 29.2, 28.1, 24.2, 23.5, 20.7, 18.2, 12.0 ppm; NMR data were consistent with data reported in the literature.[215]

Synthesis of methyl 3α-hydroxy-6-keto-5β-cholan-24-oate (3.78), and methyl 3,6-diketo-5β-cholan-24-oate (3.79)



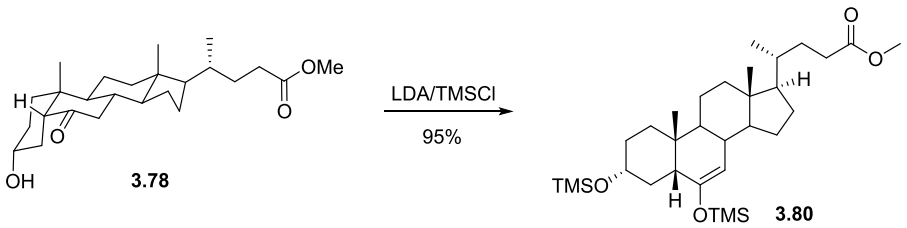
To a round-bottom flask were added **3.77** (2 g, 4.92 mmol, 1 eq), PDC (2.78 g, 7.38 mmol, 1.5 eq), $MgSO_4$ (10 g) and DCM (50 mL). The reaction mixture was stirred for 5.5 h. Upon completion, the reaction mixture was filtered over short layer of silica gel. The solvent was concentrated and

purified by flash chromatography (acetone/petroleum ether 15:85-20:80-30:70), followed by HPLC purification (ethyl acetate/hexane 50:50) to afford the desired compound **3.78** (50%) and by-product **3.79** (19%).

3.78, **Formula** $C_{25}H_{40}O_4$; **MW** 404.58; $\mathbf{R_f}$ 0.23 (acetone/petroleum ether 20:80); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.66 (3H, s, OCH_3), 3.63 (1H, tt, *J* 10.4, 5.4 Hz, $H_{3\beta}$), 2.28 - 2.43 (1H, m), 0.92 (3H, d, *J* 6.5 Hz, H_{21}), 0.84 (3H, s, H_{19}), 0.65 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 213.8 (C_6), 174.6 (C_{24}), 70.1 (C_3), 59.4, 56.8, 55.8, 51.5, 43.1, 42.9, 40.0, 39.6, 37.9, 37.0, 35.2, 34.8, 34.3, 31.0, 30.9, 29.8, 27.9, 23.9, 23.1, 20.8, 18.2, 11.9 ppm; NMR data were consistent with data reported in the literature.[107],[216]

3.79, **Formula** $C_{25}H_{38}O_4$; **MW** 402.57; $\mathbf{R_f}$ 0.34 (acetone/petroleum ether 20:80); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.64 (3H, s, OCH_3), 2.63 (1H, t, *J* 13.7 Hz), 0.93 (3H, s, H_{19}), 0.91 (3H, d, *J* 6.5 Hz, H_{21}), 0.67 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 210.7, 208.5, 174.4, 59.6, 56.7, 55.7, 51.4, 43.0, 42.1, 40.8, 39.8, 39.4, 38.2, 36.6, 36.4, 35.7, 35.2, 30.9, 30.8, 27.9, 23.8, 22.4, 21.2, 18.2, 11.9 ppm; ^{1}H NMR data were not consistent with data in the literature.[217] Epimerisation could occur on C-5, leading to 5α-cholan-24-oate. However, ^{13}C NMR was different from 5α-cholan-24-oate derivative in the literature.[133] Thus, methyl 3,6-diketo-5β-cholan-24-oate should been obtained, as both 3α- and 6α-OH groups were oxidized.

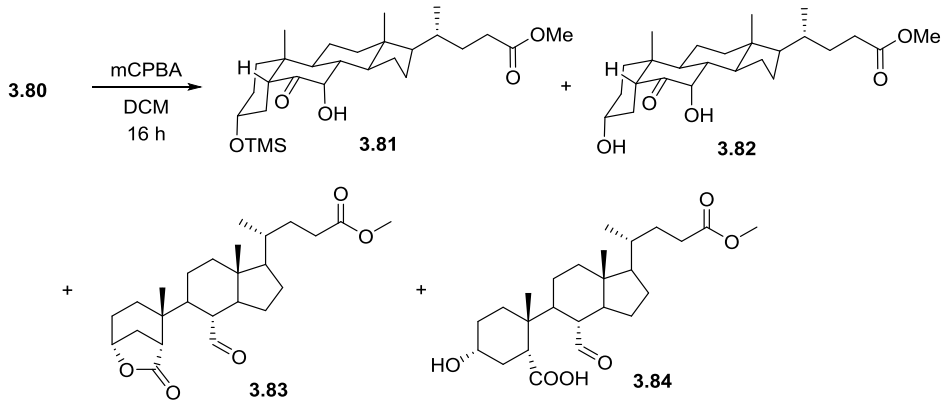
Synthesis of methyl 3α,6-bis(trimethylsilyloxy)-5β-cholan-6-en-24-oate (3.80)



Following general procedure C, compound **3.80** was prepared from **3.78** with high yield (95%).

3.80, **Formula** $C_{31}H_{56}O_4Si_2$; **MW** 548.94; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.59 (1H, d, *J* 1.8 Hz, H_7), 3.67 (3H, s, OCH_3), 3.54 (1H, tt, *J* 11.1, 4.4 Hz, $H_{3\beta}$), 2.31 - 2.43 (1H, m), 2.19 - 2.29 (1H, m), 0.92 (3H, d, *J* 6.5 Hz, H_{21}), 0.85 (3H, s, H_{19}), 0.68 (3H, s, H_{18}), 0.18 (9H, s, Si(CH_3)$_3$), 0.12 (9H, s, Si(CH_3)$_3$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (C_{24}), 153.0 (C_6), 104.4 (C_7), 71.5 (C_3), 55.9, 55.4, 51.5, 48.3, 43.3, 40.0, 39.8, 38.3, 35.8, 35.3, 34.4, 34.1, 31.0, 30.9, 30.7, 28.2, 24.0, 22.7, 20.5, 18.2, 12.1, 0.5 (Si(*C*H$_3$)$_3$), 0.2 (Si(*C*H$_3$)$_3$) ppm.

Synthesis of methyl 3α-trimethylsilyloxy-6-keto-7α-hydroxy-5β-cholan-24-oate (3.81), methyl 3α,7α-dihydroxy-6-keto-5β-cholan-24-oate (3.82), B-ring by-product 01 (3.83), and B-ring by-product 02 (3.84)



To a round-bottom flask were added **3.80** (0.43 mmol), DCM (10 mL), and a solution of mCPBA (208 mg, 1.2 mmol, 2.8 eq) in DCM (10 mL) at 5 °C. The reaction mixture was then warmed to room temperature and stirred for 26 h. Upon completion, the reaction solution was washed with sat. aq. $NaHCO_3$ solution, brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by flash chromatography (acetone/petroleum ether 15:85 – 18:82 – 25:38, then neat MeOH) to afford compounds **3.81** (7 mg, 0.014 mmol, 3%), **3.82** (42 mg, 0.1 mmol, 23%), **3.83** (34 mg, 0.08 mmol, 19%) and **3.84** (major product, *slightly impure*) as gummy solids. The yield for the desired product **3.82** was increased up to 50% by using less equivalents of mCPBA.

3.81, **Formula** $C_{28}H_{48}O_5Si$; **MW** 492.77; **IR** (neat) 3399 (br. m), 2942 (s), 2856 (s), 1740 (s), 1704 (s), 1450 (m), 1385 (m), 1250 (m), 1156 (m), 1050 (s), 849 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.77 - 3.85 (1H, m, $H_{7\beta}$), 3.68 (3H, s, $C_{24}OCH_3$), 3.47 (1H, tt, *J* 10.6, 4.8 Hz, $H_{3\beta}$), 0.94 (3H, d, *J* 6.4 Hz, H_{21}), 0.81 (3H, s, H_{19} or H_{18}), 0.66 (3H, s, H_{18} or H_{19}), 0.11 (9H, s, $Si(CH_3)_3$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 212.6 (C_6), 174.6 (C_{24}), 75.7 (C_7), 71.2 (C_3), 59.3 (*C*H), 55.5 (*C*H), 51.4 ($C_{24}OCH_3$), 49.1 (*C*H), 42.7 (*C*), 40.1 (*C*H), 39.2 (CH_2), 37.9 (*C*), 35.3 (*C*H), 34.9 (CH_2), 34.7 (CH_2), 32.6 (*C*H), 30.88 (CH_2), 30.85 (CH_2), 30.2 (CH_2), 28.0 (CH_2), 23.2 (C_{19} or C_{18}), 23.1 (CH_2), 20.7 (CH_2), 18.2 (C_{21}), 11.6 (C_{18} or C_{19}), 0.0 ($Si(CH_3)_3$) ppm; **MS** (ESI+) *m/z* 493.2 ($[M+H]^+$, 100%); **HRMS** (ESI+) for $C_{28}H_{48}NaO_5Si$ $[M+Na]^+$ calcd 515.3163, found 515.3156 (1.4 ppm error).

3.82,**Formula** $C_{25}H_{40}O_5$; **MW** 420.59; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.81 - 3.85 (1H, m, $H_{7\beta}$), 3.64 (3H, s, $C_{24}OCH_3$), 3.52 - 3.59 (1H, m, $H_{3\beta}$), 0.91 (3H, d, *J* 6.4 Hz, H_{21}), 0.85 (3H, s, H_{19}), 0.65 (3H, s, H_{18}) ppm; **MS** (ESI+) *m/z* 421.0 ($[M+H]^+$, 100%), 863.5 ($[2M+Na]^+$, 50%); **HRMS** (ESI+) for $C_{25}H_{41}O_5$ $[M+H]^+$ calcd 421.2949, found 421.2953 (-1.0 ppm error); NMR data were consistent with the reported data in the literature.[107]

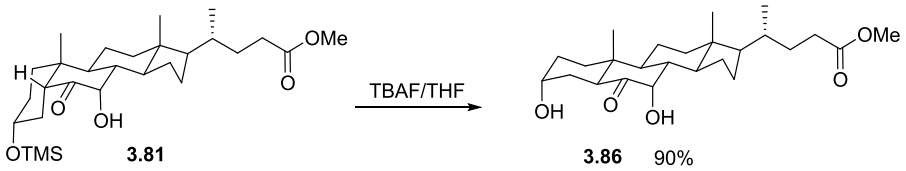
3.83,**Formula** $C_{25}H_{38}O_5$; **MW** 418.57; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.67 (1H, d, *J* 5.8 Hz, H_7), 4.70 (1H, t, *J* 5.0 Hz, H_3), 3.66 (3H, s, $C_{24}OCH_3$), 1.03 (3H, s, H_{19} or H_{18}), 0.92 (3H, d, *J* 6.4 Hz, H_{21}), 0.69 (3H, s, H_{18} or H_{19}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 207.2 (C_7), 177.4 (C_6), 174.5 (C_{24}), 77.2 (C_3), 55.3 (*C*H),

53.2 (*C*H), 51.5 ($C_{24}OCH_3$), 47.7 (*C*H), 46.0 (*C*H), 42.6 (*C*H), 41.7 (*C*), 38.9 (CH_2), 37.3 (*C*), 35.3 (*C*H), 32.7 (CH_2), 31.3 (CH_2), 30.9 (CH_2), 30.8 (CH_2), 27.7 (CH_2), 26.0 (CH_2), 24.5 (CH_2), 20.7 (CH_2), 18.1 (C_{21}), 16.0 (C_{19} or C_{18}), 11.8 (C_{18} or C_{19}) ppm; **MS** (ESI+) *m/z* 419.0 ($[M+H]^+$, 100%), 441.0 ($[M+Na]^+$, 36%), 859.4 ($[2M+Na]^+$, 87%); **HRMS** (ESI+) for $C_{25}H_{39}O_5$ $[M+H]^+$ calcd 419.2792, found 419.2791 (0.2 ppm error).

3.84, **Formula** $C_{25}H_{40}O_6$; **MW** 436.59; **^{1}H NMR** (400 MHz, CD_3OD) δ 9.70 (1H, d, *J* 6.2 Hz, H_7), 3.73 - 3.79 (1H, m, H_3), 3.64 (3H, s, $C_{24}OCH_3$), 0.98 (3H, s, H_{19} or H_{18}), 0.94 (3H, d, *J* 6.5 Hz, H_{21}), 0.73 (3H, s, H_{18} or H_{19}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 211.0 (C_7), 188.1 (C_6), 176.4 (C_{24}), 66.2 (C_3), 56.9 (*C*H), 55.5 (*C*H), 52.2 ($C_{24}OCH_3$), 52.1 (*C*H), 50.2 (*C*H), 47.7 (*C*H), 43.3 (*C*), 41.1 (CH_2), 38.7 (*C*), 36.7 (*C*H), 32.2 (CH_2), 31.9 (CH_2), 31.3 (CH_2), 30.7 (CH_2), 29.0 (CH_2), 27.5 (CH_2), 25.7 (CH_2), 23.1 (CH_2), 19.3 (CH_3), 18.8 (CH_3), 12.5 (CH_3) ppm; **MS** (ESI+) *m/z* 437.1 ($[M+H]^+$, 100%); **HRMS** (ESI+) for $C_{25}H_{41}O_6$ $[M+H]^+$ calcd 437.2898, found 437.2906 (-1.8 ppm error);

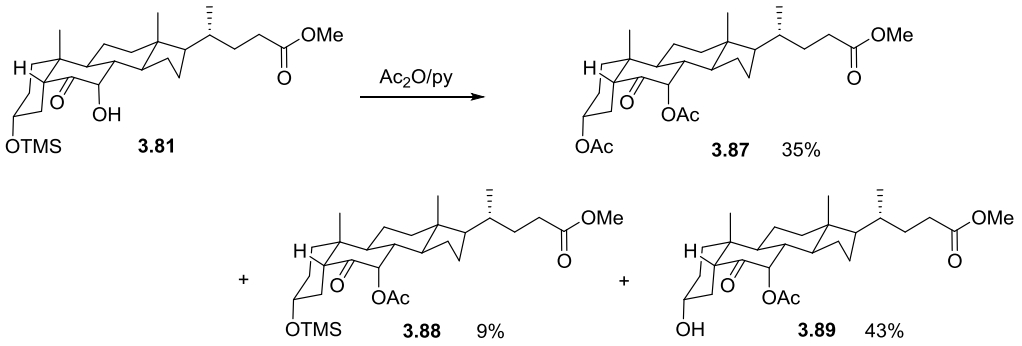
Synthesis of methyl 3α,7α-dihydroxy-6-keto-5α-cholan-24-oate (3.86)

To a round-bottom flask were added **3.81** (138 mg, 0.28 mmol, 1.0 eq), THF (5 mL) and TBAF (0.56 mL (1M in THF), 0.56 mmol, 2 eq). The resulting reaction mixture was stirred at room temperature overnight. Upon completion indicated by TLC analysis, water (15 mL) was added, followed by the extraction with EtOAc (25 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 20:80 – 30:70) to afford by-product **3.86** (106 mg, 0.252 mmol, 90%). The desired product **3.82** was not obtained due to the epimerisation at C-5.



3.86, **Formula** $C_{25}H_{40}O_5$; **MW** 420.59; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.17 - 4.24 (1H, m, -O*H* [confirmed by HSQC and H/D Exchange NMR experiment]), 4.06 - 4.13 (1H, m, $H_{3\beta}$ or $H_{7\beta}$), 3.70 - 3.75 (1H, m, $H_{7\beta}$ or $H_{3\beta}$), 3.64 (3H, s, $C_{24}OCH_3$), 3.38 (1H, dd, *J* 12.6, 2.8 Hz, $H_{5\alpha}$), 3.12 (1H, br. s, -O*H*, [confirmed by HSQC and H/D Exchange NMR experiment]), 0.91 (3H, d, *J* 6.4 Hz, H_{21}), 0.65 (3H, s, H_{19} or H_{18}), 0.63 (3H, s, H_{18} or H_{19}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 215.0 (C_6), 174.7 (C_{24}), 75.8 (C_7 or C_3), 65.2 (C_3 or C_7), 55.6 (*C*H), 51.4 ($C_{24}OCH_3$), 49.4 (*C*H), 45.9 (*C*H), 45.5 (*C*H), 42.8 (*C*), 42.6 (*C*), 41.9 (*C*H), 39.0 (CH_2), 35.3 (*C*H), 31.8 (CH_2), 31.0 (CH_2), 30.9 (CH_2), 27.9 (CH_2), 27.8 (CH_2), 26.9 (CH_2), 23.1 (CH_2), 20.9 (CH_2), 18.2 (C_{21}), 11.7 (C_{19} or C_{18}), 11.5 (C_{18} or C_{19}) ppm; **MS** (ESI+) *m/z* 420.9 ($[M+H]^+$, 100%), 859.0 ($[2M+NH_4]^+$, 44%); **Other analysis** H/D Exchange NMR experiment; NMR data were consistent with the reported data in the literature.[108],[218]

Synthesis of methyl 3α,7α-diacetoxy-6-keto-5β-cholan-24-oate (3.87), methyl 3α-trimethylsilyloxy-6-keto-7α-acetoxy-5β-cholan-24-oate (3.88), and methyl 3α-hydroxy-6-keto-7α-acetoxy-5β-cholan-24-oate (3.89)



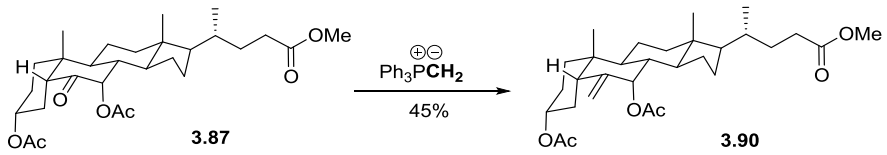
To a round-bottom flask were added **3.81** (80 mg, 0.162 mmol), pyridine (4 mL) and acetic anhydride (1 mL). The resulting reaction mixture was stirred at room temperature overnight. Upon completion indicated by TLC analysis, the reaction mixture was quenched with icy water and extracted with EtOAc (× 3). The combined organic layer was washed with 10% aq HCl solution, sat. aq. $NaHCO_3$ solution, brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by flash chromatography (acetone/petroleum ether 10:90 – 20:80 – 30:70) to afford compounds **3.87** (29 mg, 0.057 mmol, 35%), **3.88** (8 mg, 0.015 mmol, 9%) and **3.89** (32 mg, 0.069 mmo, 43%) as gummy solids.

3.87, **Formula** $C_{29}H_{44}O_7$; **MW** 504.66; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.89 (1H, d, *J* 3.3 Hz, $H_{7\beta}$), 4.59 (1H, tt, *J* 10.8, 4.3 Hz, $H_{3\beta}$), 3.66 (3H, s, $C_{24}OCH_3$), 2.11 (3H, s, CH_3COOC_3 or CH_3COOC_7), 2.03 (3H, s, CH_3COOC_3 or CH_3COOC_7), 0.94 (3H, d, *J* 6.9 Hz, H_{21}), 0.85 (3H, s, H_{19}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 205.8 (C_6), 174.5 (C_{24}), 170.3 (CH_3COOC_3 or CH_3COOC_7), 169.3 (CH_3COOC_3 or CH_3COOC_7), 77.3 (C_7), 72.3 (C_3), 59.1 (CH), 55.6 (CH), 51.5 ($C_{24}OCH_3$), 49.6 (CH), 43.0 (C), 39.7 (CH), 39.0 (CH_2), 38.7 (C), 35.2 (CH), 34.24 (CH_2), 34.21 (CH), 30.9 (CH_2), 30.8 (CH_2), 29.5 (CH_2), 27.9 (CH_2), 26.4 (CH_2), 23.3 (CH_2), 22.9 (C_{19}), 21.3 (CH_3COOC_3 or CH_3COOC_7), 20.9 (CH_3COOC_3 or CH_3COOC_7), 20.8 (CH_2), 18.2 (C_{21}), 11.6 (C_{18}) ppm; **MS** (ESI+) *m/z* 505.2 ([M+H]$^+$, 62%), 1031.6 ([2M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{29}H_{44}NaO_7$ [M+Na]$^+$ calcd 527.2979, found 527.2984 (-0.8 ppm error); ^{1}H NMR data were consistent with the reported data in the literature.[107]

3.88, **Formula** $C_{30}H_{50}O_6Si$; **MW** 534.81; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.87 (1H, d, *J* 3.4 Hz, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 3.46 (1H, tt, *J* 10.2, 4.8 Hz, $H_{3\beta}$), 2.11 (3H, s, CH_3COOC_7), 0.94 (3H, d, *J* 6.4 Hz, H_{21}), 0.84 (3H, s, H_{19}), 0.66 (3H, s, H_{18}), 0.11 (9H, s, $Si(CH_3)_3$) ppm.

3.89, **Formula** $C_{27}H_{42}O_6$; **MW** 462.63; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.96 (1H, d, *J* 2.7 Hz, $H_{7\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 3.60 (1H, tt, *J* 9.4, 4.1 Hz, $H_{3\beta}$), 2.12 (3H, s, CH_3COOC_7), 0.94 (3H, d, *J* 6.5 Hz, H_{21}), 0.89 (3H, s, H_{19}), 0.67 (3H, s, H_{18}) ppm.

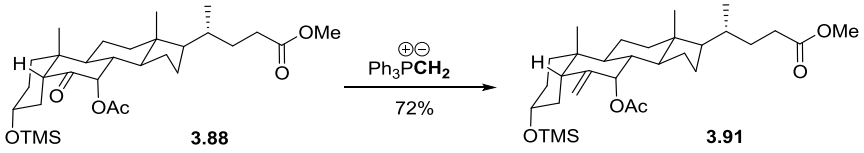
Synthesis of methyl 3α,7α-diacetoxy-6-methyliden-5β-cholan-24-oate (3.90)



To a round-bottom flask were added Ph_3PMeBr (250 mg, 0.696 mmol, 12 eq), THF (3 mL), and ^tBuOK (72 mg, 0.638 mmol, 11 eq). The mixture was stirred for 30 min, followed by the slow addition of a solution of compound **3.87** (29 mg, 0.058 mmol, 1.0 eq) in THF (3 mL). The resulting reaction mixture was stirred at room temperature overnight. Upon completion by TLC analysis, the reaction was quenched by sat. aq. NH_4Cl solution (15 mL) and then extracted with EtOAc (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by flash chromatography (acetone/petroleum ether 10:90) to afford compound **3.90** (13 mg, 0.26 mmol, 45%) as gummy solid.

3.90, **Formula** $C_{30}H_{46}O_6$; **MW** 502.69; **IR** (neat) 2942 (m), 2968 (m), 1744 (s), 1647 (w), 1458 (w), 1438 (w), 1364 (m), 1242 (s), 1160 (w), 1037 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.24 (1H, d, *J* 3.4 Hz, $H_{7\beta}$), 5.12 (1H, d, *J* 1.9 Hz, C_6=C*H*H'), 4.94 (1H, d, *J* 1.9 Hz, C_6=CH*H'*), 4.64 (1H, tt, *J* 11.2, 4.5 Hz, $H_{3\beta}$), 3.67 (3 H, s, $C_{24}OCH_3$), 2.36 (1H, ddd, *J* 15.6, 10.2, 5.1 Hz, H_{23}), 2.23 (1H, ddd, *J* 16.1, 9.7, 6.7 Hz, H_{23}'), 2.033 (3H, s, CH_3COOC_3 or CH_3COOC_7), 2.031 (3H, s, CH_3COOC_3 or CH_3COOC_7), 0.93 (3H, d, *J* 6.4 Hz, H_{21}), 0.81 (3H, s, H_{19}), 0.65 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.6 (C_{24}), 170.5 (CH_3COOC_3 or CH_3COOC_7), 169.9 (CH_3COOC_3 or CH_3COOC_7), 145.2 (C_6), 117.6 (C_6=CH_2), 75.0 (C_7), 73.6 (C_3), 55.7 (CH), 51.5 ($C_{24}OCH_3$), 51.3 (CH), 50.1 (CH), 42.8 (C), 39.3 (CH_2), 38.9 (CH), 35.7 (C), 35.3 (CH), 34.6 (CH_2), 34.4 (CH_2), 34.0 (CH), 31.0 (CH_2), 30.9 (CH_2), 28.0 (CH_2), 26.6 (CH_2), 23.5 (CH_2), 22.8 (C_{19}), 21.6 (CH_3COOC_3 or CH_3COOC_7), 21.4 (CH_3COOC_3 or CH_3COOC_7), 20.8 (CH_2), 18.3 (C_{21}), 11.7 (C_{18}) ppm; **MS** (ESI+) *m/z* 525.2 ([M+Na]$^+$, 75%); **HRMS** (ESI+) for $C_{30}H_{46}NaO_6$ [M+Na]$^+$, calcd 525.3187, found 525.3196 (-1.8 ppm error).

Synthesis of methyl 3α-trimethylsilyloxy-6-methyliden-7α-acetoxy-5β-cholan-24-oate (3.91)



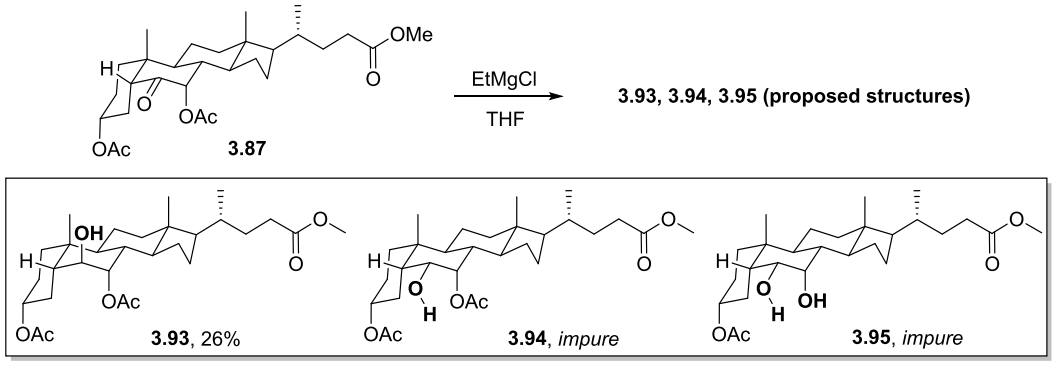
To a round-bottom flask were added Ph_3PMeBr (56 mg, 12 eq), THF (2 mL) and ^tBuOK (16 mg, 11 eq). The mixture was stirred for 30 min, followed by the slow addition of a solution of compound **3.88** (7 mg, 0.013 mmol, 1 eq) in THF (1mL). The resulting reaction mixture was stirred at room temperature overnight. Upon completion indicated by TLC analysis, the reaction was quenched by sat. aq. NH_4Cl solution (5 mL) and extracted with EtOAc (10 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by

flash chromatography (acetone/petroleum ether 5:95) to afford compound **3.91** (5 mg, 0.0094 mmol, 72%) as gummy solid.

3.91, **Formula** $C_{31}H_{52}O_5Si$; **MW** 532.84; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.22 (1H, d, *J* 3.5 Hz, $H_{7\beta}$), 5.10 (1H, d, *J* 2.2 Hz, C_6=C*H*H'), 4.93 (1H, d, *J* 2.2 Hz, C_6=CH*H*'), 3.67 (3H, s, $C_{24}OCH_3$), 3.51 (1H, tt, *J* 10.9, 4.4 Hz, $H_{3\beta}$), 2.37 (1H, ddd, *J* 15.3, 10.0, 5.3 Hz, H_{23}), 2.23 (1H, ddd, *J* 15.9, 9.6, 6.5 Hz, H_{23}'), 2.03 (3H, s, CH_3COOC_7), 0.93 (3H, d, *J* 6.4 Hz, H_{21}), 0.79 (3H, s, H_{19}), 0.64 (3H, s, H_{18}), 0.12 (9H, s, $Si(CH_3)_3$) ppm; **MS** (ESI+) *m/z* 555.2 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{28}H_{44}NaO_5$ [M-TMS*+H+Na]$^+$, calcd 483.3081, found 483.3083 (-0.5 ppm error, *cleavage of TMS group under acidic column condition).

Synthesis of methyl 3α,7α-diacetoxy-6β-hydroxy-5β-cholan-24-oate (3.93, proposed structure**), methyl 3α,7α-diacetoxy-6α-hydroxy-5β-cholan-24-oate (3.94**, proposed structure**), and methyl 3α-acetoxy-6α,7α-dihydroxy-5β-cholan-24-oate (3.95**, proposed structure**)**

To a round-bottom flask were added **3.87** (50 mg, 0.1 mmol, 1 eq), THF (1.5 mL), and then EtMgCl (2M, 0.06 mL, 0.12 mmol, 1.2 eq) dropwise at 0 °C. The resulting reaction mixture was stirred at 0 °C for 2 h and then left at room temperature overnight. Upon completion, the reaction was quenched with sat. aq. NH_4Cl solution, followed by the extraction with EtOAc (× 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by flash chromatography (acetone/petroleum ether 15:85 – 20:80) to afford only by-products **3.93** (13 mg, 0.026 mmol, 26%), **3.94** and **3.95** as gummy solids. No desired addition product was obtained, and compound **3.94** and **3.95** were not separable.



3.93, **Formula** $C_{29}H_{46}O_7$; **MW** 506.68; **IR** (neat) 3448 (br. w), 2943 (m), 2872 (m), 1724 (s), 1444 (w), 1369 (m), 1246 (s), 1172 (w), 1030 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.70 - 4.75 (1H, m, $H_{7\beta}$), 4.60 (1H, tt, *J* 11.2, 4.5 Hz, $H_{3\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 3.62 - 3.65 (1H, m, $H_{6\alpha}$), 2.36 (1H, ddd, *J* 15.7, 10.2, 5.1 Hz, H_{23}), 2.23 (1H, ddd, *J* 16.1, 9.8, 6.7 Hz, H_{23}'), 2.08 (3H, s, CH_3COOC_3 or CH_3COOC_7), 2.03 (3H, s, CH_3COOC_3 or CH_3COOC_7), 1.11 (3H, s, H_{19}), 0.93 (3H, d, *J* 6.5 Hz, H_{21}), 0.68 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (C_{24}), 170.6 (CH_3COOC_3 or CH_3COOC_7), 170.4 (CH_3COOC_3 or CH_3COOC_7), 74.5 (C_7), 73.7 (C_3), 73.1 (C_6), 55.7 (CH), 51.5 ($C_{24}OCH_3$), 49.9 (CH), 47.0 (CH), 42.7 (C), 39.4 (CH_2), 35.34 (CH_2), 35.29 (CH), 34.5 (C), 34.0 (CH), 33.6 (CH), 31.6 (CH_2), 31.0 (CH_2), 30.9 (CH_2), 28.0 (CH_2), 26.4 (CH_2), 25.0 (C_{19}), 23.6 (CH_2), 21.4 (CH_3COOC_3 or CH_3COOC_7), 21.3 (CH_3COOC_3 or

*C*H$_3$COOC$_7$), 20.4 (*C*H$_2$), 18.3 (*C*$_{21}$), 11.6 (*C*$_{18}$) ppm; **MS** (ESI+) *m/z* 529.2 ([M+Na]$^+$, 94%), 1035.7 ([2M+Na]$^+$, 6%); **HRMS** (ESI+) for $C_{29}H_{46}NaO_7$ [M+Na]$^+$, calcd 529.3136, found 529.3145 (-1.8 ppm error).

3.94, **Formula** $C_{29}H_{46}O_7$; **MW** 506.68; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.11 (1H, t, *J* 3.1 Hz, *H*$_{7β}$), 4.48 - 4.64 (1H, m, *H*$_{3β}$), 4.06 (1H, t, *J* 3.9 Hz, *H*$_{6β}$), 3.67 (3H, s, C$_{24}$OC*H*$_3$), 2.03 (3H, s, C*H*$_3$COOC$_7$ or C*H*$_3$COOC$_3$), 2.02 (3H, s, C*H*$_3$COOC$_3$ or C*H*$_3$COOC$_7$), 0.92 (3H, d, *J* 6.4 Hz, *H*$_{21}$), 0.91 (3H, s, *H*$_{19}$), 0.65 (3H, s, *H*$_{18}$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ (only characteristic peaks given due to overlapping) 174.6 (*C*$_{24}$), 172.7 (CH$_3$*C*OOC$_7$), 170.5 (CH$_3$*C*OOC$_3$), 74.4 (*C*$_7$), 74.1 (*C*$_3$), 69.2 (*C*$_6$) ppm; **MS** (ESI+) *m/z* 529.3 ([M+Na]$^+$, 100%).

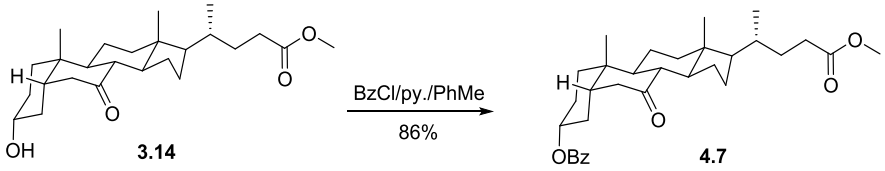
3.95, **Formula** $C_{27}H_{44}O_6$; **MW** 464.64; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.55 (1H, tt, *J* 10.1, 4.8 Hz, *H*$_{3β}$), 3.82 - 3.90 (2H, m, *H*$_{6β}$ + *H*$_{7β}$), 3.67 (3H, s, C$_{24}$OC*H*$_3$), 2.02 (3H, s, C*H*$_3$COOC$_3$), 0.93 (3H, d, *J* 6.5 Hz, *H*$_{21}$), 0.92 (3H, s, *H*$_{19}$), 0.66 (3H, s, *H*$_{18}$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (*C*$_{24}$), 170.7 (CH$_3$*C*OOC$_3$), 74.2 (*C*$_3$), 71.9 (*C*$_7$ or *C*$_6$), 69.2 (*C*$_6$ or *C*$_7$), 55.7 (*C*H), 51.5 (C$_{24}$O*C*H$_3$), 50.1 (*C*H), 47.6 (*C*H), 42.7 (*C*), 39.4 (*C*H$_2$), 38.5 (*C*H), 36.0 (*C*), 35.4 (*C*H), 35.2 (*C*H$_2$), 32.6 (*C*H), 31.0 (*C*H$_2$), 30.9 (*C*H$_2$), 28.4 (*C*H$_2$), 28.1 (*C*H$_2$), 26.7 (*C*H$_2$), 23.6 (*C*H$_2$), 22.9 (*C*$_{19}$), 21.4 (*C*H$_3$COOC$_3$), 20.5 (*C*H$_2$), 18.2 (*C*$_{21}$), 11.7 (*C*$_{18}$) ppm; **MS** (ESI+) *m/z* 487.3 ([M+Na]$^+$, 88%), 951.7 ([2M+Na]$^+$, 65%); **HRMS** (ESI+) for $C_{27}H_{44}NaO_6$ [M+Na]$^+$, calcd 487.3030, found 487.3033 (-0.5 ppm error).

7.4 Synthesis of B-ring and C-ring fluorinated BA analogues

The order of the following compound synthesis and characterisation is based on the order of reactions discussed from *section 4.1.2* in *Chapter 4*.

7.4.1 Synthesis of C-7 and C-12 deoxyfluorinated BA analogues

Synthesis of methyl 3α-benzoyloxy-7-keto-5β-cholan-24-oate (4.7)

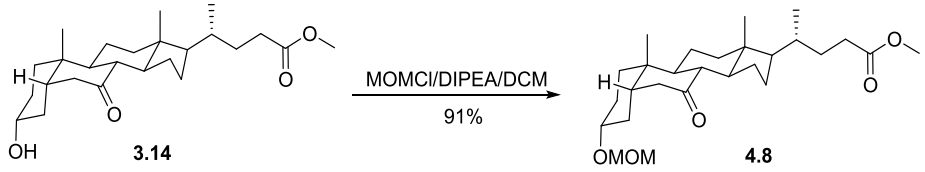


To a round-bottom flask were added **3.14** (5 g, 12.4 mmol, 1.0 eq), toluene (50 mL), pyridine (1.6 mL) and BzCl (2.3 mL, 1.5 eq). The reaction mixture was stirrerd at rt overnight. Upon completion indicated by TLC analysis, the reaction was quenched with H_2O and extracted with ethyl acetate. The organic layer was washed with aq. HCl (2.0 M), H_2O, brine, and then dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 8:92-20:80) to afford **4.7** (86%) as white solid. The crystals of **4.7** were obtained from recrystallisation in acetone/petroleum ether.

4.7, **Formula** $C_{32}H_{44}O_5$; **MW**: 508.69; **$[\alpha]_D$** -14.0 (c 0.7, $CHCl_3$, 20 °C); **m.p.** 136-138°C (acetone/petroleum ether); **R_f** 0.22 (acetone/petroleum ether 10:90); **IR** (neat) 2940 (m), 2869 (m), 1736 (m), 1710 (s), 1445 (w), 1380 (w), 1274 (s), 1173 (w), 1112 (m), 756 (w), 711 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.00 (2H, dd, *J* 8.4, 1.3 Hz, $H_{Ar(o)}$), 7.53 (1H, tt, *J* 7.3, 1.8 Hz, $H_{Ar(p)}$), 7.41 (2H, t, *J* 7.9 Hz, $H_{Ar(m)}$), 4.94 (1H, tt, *J* 11.4, 4.6 Hz, $H_{3\beta}$), 3.66 (3H, s, OCH_3),2.89 (1H, dd, *J* 12.4, 5.9 Hz, $H_{6\beta}$), 2.42 (1H, t, *J* 11.3 Hz, $H_{8\beta}$), 2.30 - 2.38 (1H, m), 2.15 - 2.28 (2H, m), 1.24 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.3 Hz, H_{21}), 0.67 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 211.8 (C_7), 174.6 (C_{24}), 165.9 (Ph*C*O), 132.8 ($C_{Ar(p)}$), 130.5 (C_{Ar}CO), 129.5 ($C_{Ar(o)}$), 128.2 ($C_{Ar(m)}$), 73.4 (C_3), 54.7 (*C*H), 51.4 (O*C*H_3), 49.5 (C_8), 48.8 (*C*H), 45.9 (*C*H), 45.2 (C_6), 42.8 (*C*H), 42.6 (*C*), 38.9 (*C*H_2), 35.17 (*C*), 35.16 (*C*H), 33.8 (*C*H_2), 33.2 (*C*H_2), 31.0 (*C*H_2), 30.9 (*C*H_2), 28.2 (*C*H_2), 26.1 (*C*H_2), 24.7 (*C*H_2), 23.0 (C_{19}), 21.7 (*C*H_2), 18.3 (C_{21}), 12.0 (C_{18}) ppm; **MS** (ESI+) *m/z* 526.2 ([M+NH_4]$^+$, 100%), 531.2 ([M+Na]$^+$, 17%); **HRMS** (ESI+) for $C_{32}H_{44}NaO_5$ [M+Na]$^+$ calcd 531.3081, found 531.3072 (1.7 ppm error); **Other analysis** X-Ray structure included in the Appendices.

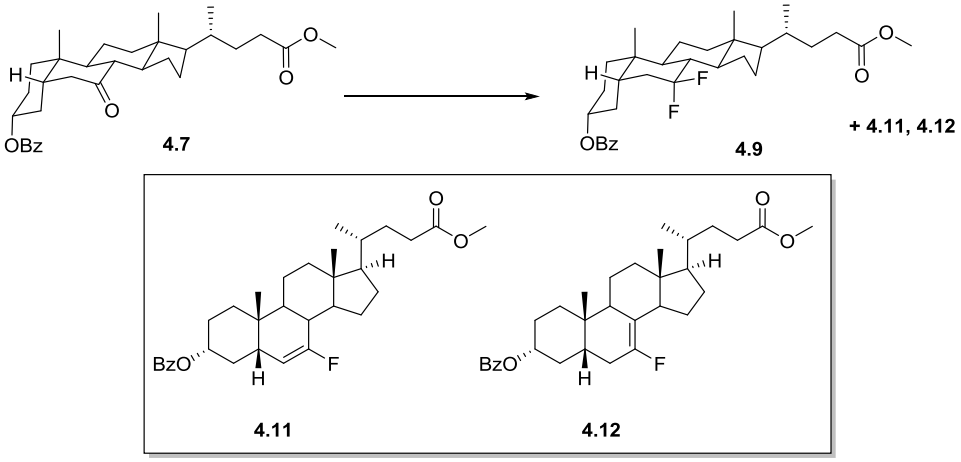
Synthesis of methyl 3α-(methoxymethoxy)-7-keto-5β-cholan-24-oate (4.8)

To a round-bottom flask were added **3.14** (500 mg, 1.24 mmol, 1.0 eq), DCM (4 mL), DIPEA (0.65 mL, 3 eq) and MOMCl (0.48 mL, 6.20 mmol, 5 eq) at 0 °C. The resulting mixture was stirred at rt overnight. Upon completion indicated by TLC analysis, the reaction was quenched by MeOH and H_2O. The aqueous layer was extracted with ethyl acetate, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 10:90) to afford **4.8** (512 mg, 1.41 mmol, 91%) as gummy solid.



4.8, **Formula** $C_{27}H_{44}O_5$; **MW** 448.64; **R_f** 0.20 (acetone/petroleum ether 10:90); **IR** (neat) 2933 (s), 2864 (m), 1740 (s), 1710 (s), 1468 (w), 1441 (w), 1372 (w), 1175 (w), 1150 (m), 1101 (m), 1046 (s), 960 (w) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 4.65 (1H, d, *J* 7.0 Hz, OCH_2O), 4.63 (1H, d, *J* 7.0 Hz, OCH_2O), 3.66 (3H, s, OCOCH_3), 3.49 (1H, tt, *J* 11.3, 4.7 Hz, $H_{3\beta}$), 3.34 (3H, s, OCH_2OCH_3), 2.84 (1H, dd, *J* 12.6, 5.9 Hz), 2.28 - 2.44 (2H, m), 1.19 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.4 Hz, H_{21}), 0.65 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 211.7, 174.6, 94.6, 75.8, 55.2, 54.7, 51.4, 49.5, 48.9, 46.0, 45.4, 42.6, 42.5, 38.9, 35.2, 35.2, 34.4, 34.1, 31.0, 31.0, 28.2, 27.3, 24.8, 23.1, 21.6, 18.3, 12.0 ppm; **MS** (ESI+) *m/z* 471.2 ([M+Na]$^+$, 47%); 919.3 ([2M+Na]$^+$, 76%); **HRMS** (ESI+) for $C_{27}H_{48}NO_5$ [M+NH_4]$^+$ calcd 466.3527, found 466.3531 (-0.9 ppm error);

Synthesis of methyl 3α-benzoyloxy-7,7-difluoro-5β-cholan-24-oate (4.9), methyl 3α-benzyloxy-5β-7-fluoro-cholan-6-en-24-oate (4.11), and methyl 3α-benzyloxy-5β-7-fluoro-cholan-7-en-24-oate (4.12)



To a round-bottom flask were added **4.7** (1.36 g) and DAST (15 mL). The reaction mixture was heated at 80 °C overnight. Upon completion monitored by TLC analysis, the reaction mixture was quenched with sat. aq. $NaHCO_3$ solution then extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 4:96-8:92-20:80) and HPLC (acetone/hexane 5:95) to afford the desired compound **4.9** (52%), and inseparable alkenes mixture **4.11**/**4.12** (10%).

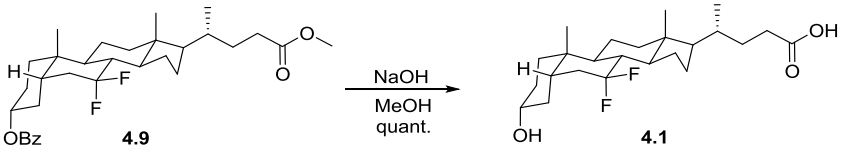
4.9, **Formula** $C_{32}H_{44}F_2O_4$; **MW** 530.69; **$[\alpha]_D$** +30.4 (c 1.1, $CHCl_3$, 20 °C); **m.p.** 140-144°C (acetone/hexane); **R_f** 0.29 (acetone/petroleum ether 10:90); **IR** (neat) 2940 (m), 2868 (w), 1740 (s), 1711 (s), 1455 (w), 1321 (w), 1275 (s), 1176 (m), 1141 (m), 1121 (s), 1065 (s), 1026 (m), 977 (m), 760 (s), 718 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.02 (2H, dd, *J* 8.5, 1.5 Hz, $H_{Ar(o)}$), 7.51 (1H, tt, *J* 7.5, 1.4 Hz, $H_{Ar(p)}$), 7.40 (2H, t, *J* 7.5 Hz, $H_{Ar(m)}$), 4.87 (1H, tt, *J* 11.0, 4.5 Hz, $H_{3\beta}$), 3.64 (3H, s, OCH_3), 2.27 - 2.40 (1H, m), 0.97 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.5 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.6 (C_{24}), 166.0 (Ph*C*O), 132.7 ($C_{Ar(p)}$), 130.7 (C_{Ar}CO), 129.5 ($C_{Ar(o)}$), 128.2 ($C_{Ar(m)}$), 124.6 (t, *J* 244.5 Hz, C_7), 74.0 (C_3), 55.0 (*C*H), 51.4 (O*C*H$_3$), 48.6 (d, *J* 3.5 Hz, *C*H), 43.1 (*C*), 41.8 (dd, *J* 22.9, 19.3 Hz, C_8), 41.1 (d, *J* 10.6 Hz, *C*H), 39.4 (*C*H$_2$), 37.1 (d, *J* 9.0 Hz, *C*H), 36.1 (t, *J* 23.7 Hz, C_6), 35.3 (*C*H), 34.40 (*C*H$_2$), 34.36 (*C*), 33.1 (d, *J* 4.4 Hz), 31.01 (*C*H$_2$), 30.98 (*C*H$_2$), 28.3 (*C*H$_2$), 26.5 (*C*H$_2$), 25.3 (d, *J* 3.9 Hz, *C*H$_2$), 22.6 (C_{19}), 20.9 (*C*H$_2$), 18.3 (C_{21}), 11.8 (C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -84.7 (1F, br. d, *J* 241.0 Hz, $F_{7\beta}$), -101.4 (1F, dddd, *J* 241.0, 38.1, 25.1, 14.7 Hz, $F_{7\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -84.7 (1F, d, *J* 241.0 Hz, $F_{7\beta}$), -101.4 (1 F, d, *J* 241.0 Hz, $F_{7\alpha}$) ppm; **MS** (ESI+) *m/z* 553.3 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{32}H_{44}F_2NaO_4$ [M+Na]$^+$ calcd 553.3100, found 553.3101 (-0.2 ppm error); **Other analysis** X-Ray structure included in the Appendices.

4.11, **Formula** $C_{32}H_{43}FO_4$; **MW** 510.7; **R_f** 0.32 (acetone/hexane 6:94); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03 (2H, m, *J* 7.2 Hz, H_{Ar}), 7.50 - 7.58 (1H, m, H_{Ar}), 7.42 (2H, t, *J* 7.6 Hz, H_{Ar}), 5.10 (1H, ddd, *J* 18.2, 6.2, 1.9 Hz, H_6), 4.87 - 5.03 (1H, m, $H_{3\beta}$), 3.67 (3H, s, OCH_3), 0.95 (3H, d, *J* 6.4 Hz, H_{21}), 0.91 (3H, s,

H_{19}), 0.73 (3H, s, H_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -107.1 (1F, d, *J* 17.8 Hz, F_7) ppm; **MS** (ESI+) *m/z* 533.2 ([M+Na]$^+$, 91%); 1021.8 ([2M+H]$^+$, 6%); **HRMS** (ESI+) for $C_{32}H_{47}FNO_4$ [M+NH_4]$^+$ calcd 528.3484, found 528.3484 (-0.1 ppm error).

4.12, **Formula** $C_{32}H_{43}FO_4$; **MW** 510.7; **R_f** 0.32 (acetone/hexane 6:94); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03 (2H, m, *J* 7.2 Hz, H_{Ar}), 7.50 - 7.58 (1H, m, H_{Ar}), 7.43 (2H, t, *J* 7.6 Hz, H_{Ar}), 4.87 - 5.03 (1H, m, $H_{3\beta}$), 3.67 (3H, s, OCH_3), 0.95 (3H, d, *J* 6.4 Hz, H_{21}), 0.93 (3H, s, H_{19}), 0.67 (3H, s, H_{18}) ppm; **MS** (ESI+) *m/z* 533.2 ([M+Na]$^+$, 91%); 1021.8 ([2M+H]$^+$, 6%); **HRMS** (ESI+) for $C_{32}H_{47}FNO_4$ [M+NH_4]$^+$ calcd 528.3484, found 528.3484 (-0.1 ppm error).

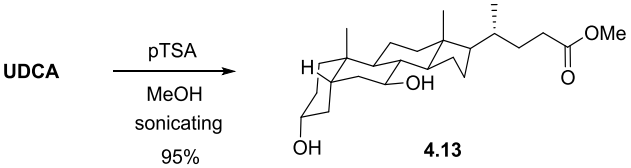
Synthesis of 7,7-difluoro lithocholic acid (4.1)



To a round-bottom flask were added **4.9** (40 mg), NaOH (200 mg) and MeOH (2 mL). The reaction mixture was stirred at rt overnight. Upon the completion indicated by TLC analysis, the solvent was firstly removed and then acidified with aq. 6 M HCl solution. The solution was further extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. NMR analysis of the crude mixture indicated a quantitative conversion to the desired product. The crude was purified by flash chromatography (DCM/MeOH 97:3, addition of 0.5% formic acid) to afford **4.1** as white solid. Compound **4.1** was recrystallised from neat MeOH.

4.1, **Formula** $C_{24}H_{38}F_2O_3$; **MW** 412.6; **IR** (neat) 3361 (br. w), 2952 (s), 2927 (s), 2869 (s), 1708 (s), 1470 (m), 1455 (m), 1379 (m), 1072 (s), 978 (m), 916 (m), 732 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.47 (1H, tt, *J* 11.0, 5.3 Hz, $H_{3\beta}$), 2.29 - 2.45 (1H, m), 0.98 (3H, s, H_{19}), 0.97 (3H, d, *J* 6.9 Hz, H_{21}), 0.71 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.2 (C_{24}), 126.0 (dd, *J* 244.5, 243.2 Hz, C_7), 72.1 (C_3), 56.6, 50.2 (d, *J* 3.9 Hz), 44.4, 43.3 (dd, *J* 23.3, 20.0 Hz), 42.7 (d, *J* 10.6 Hz), 41.0, 38.6 (d, *J* 9.0 Hz), 38.2 (d, *J* 4.0 Hz), 37.5 (t, *J* 23.5 Hz), 36.8, 35.9, 35.5, 32.4, 32.1, 31.0, 29.4, 26.7 (d, *J* 3.9 Hz), 23.2, 22.1, 19.0, 12.4 ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -84.7 (1F, br. d, *J* 241.0 Hz, $F_{7\beta}$), -102.0 (1F, dddd, *J* 241.0, 38.6, 25.1, 14.7 Hz, $F_{7\alpha}$) ppm; **MS** (ESI+) *m/z* 825.4 ([2M+H]$^+$, 46%); **HRMS** (ESI+) for $C_{24}H_{42}F_2NO_3$ [M+NH_4]$^+$ calcd 430.3127, found 430.3124 (0.7 ppm error); **Other analysis** X-Ray analysis included in the Appendices.

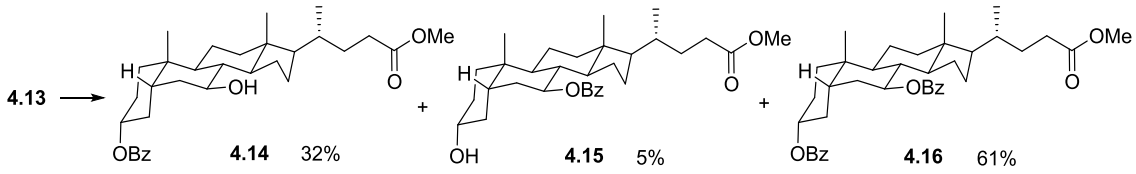
Synthesis of methyl 3α,7β-dihydroxy-5β-cholan-24-oate (4.13)



Following general procedure B, compound **4.13** was prepared from UDCA with high yield (95%), and used directly for next step.

4.13, **Formula** $C_{25}H_{42}O_4$; **MW** 406.60; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 3.66 (3H, s, OCH_3), 3.50 - 3.63 (2H, m, $H_{3\beta}$ + $H_{7\alpha}$), 2.28 - 2.43 (1H, m), 2.13 - 2.28 (1H, m), 1.94 - 2.05 (1H, m), 0.94 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.4 Hz, H_{21}), 0.67 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 174.7, 71.3, 71.3, 55.7, 54.9, 51.5, 43.72, 43.72, 42.4, 40.1, 39.2, 37.3, 36.8, 35.2, 34.9, 34.0, 31.03, 30.98, 30.3, 28.6, 26.8, 23.3, 21.1, 18.3, 12.1 ppm; NMR data were consistent with data reported in the literature.[219],[215]

Synthesis of methyl 3α-benzoyloxy-7β-hydroxy-5β-cholan-24-oate (4.14), methyl 3α-hydroxy-7β-benzoyloxy-5β-cholan-24-oate (4.15), and methyl 3α,7β-dibenzoyloxy-5β-cholan-24-oate (4.16)



To a round-bottom flask were added **4.13** (500 mg, 1.0 eq), toluene (5 mL), pyridine (0.16 mL) and BzCl (0.23 mL, 1.5 eq). The reaction mixture was stirrerd at rt overnight. Upon completion indicated by TLC analysis, the reaction was quenched with H_2O and extracted with ethyl acetate. The organic layer was washed with aq. HCl (2.0 M), H_2O, brine, and then dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 8:92-10:90-20:80) to afford **4.14** (203 mg, 32 **%**), **4.15** (31 mg, 5%) and **4.16** (460 mg, 61%) as gummy solids.

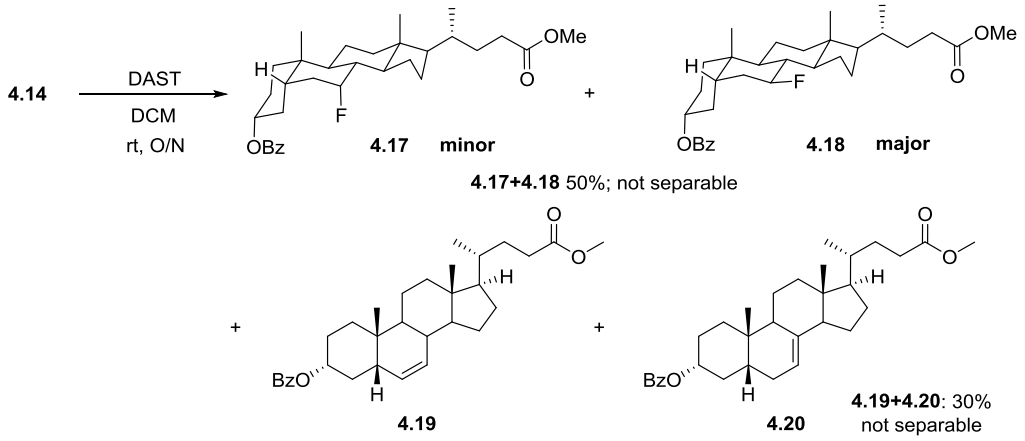
4.14, **Formula** $C_{32}H_{46}O_5$; **MW** 510.70; **IR** (neat) 3476 (br. w), 2937 (m), 2872 (m), 1742 (s), 1713 (s), 1448 (m), 1318 (m), 1278 (s), 1177 (m), 1111 (s), 1025 (m), 713 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.01 (2H, dd, *J* 8.6, 1.4 Hz, H_{Ar}), 7.50 (1H, tt, *J* 7.5, 1.2 Hz, H_{Ar}), 7.39 (2H, t, *J* 7.8 Hz, H_{Ar}), 4.89 (1H, tt, *J* 10.5, 5.1 Hz, $H_{3\beta}$), 3.62 (3H, s, OCH_3), 3.56 (1H, td, *J* 10.5, 5.6 Hz, $H_{7\alpha}$), 2.26 - 2.39 (1H, m), 2.11 - 2.25 (1H, m), 1.93 - 2.03 (1H, m), 0.95 (3H, s, H_{19}), 0.90 (3H, d, *J* 6.4 Hz, H_{21}), 0.65 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (*C*OO), 166.0 (*C*OO), 132.7 (*C*H_{Ar}), 130.7 (*C*$_{Ar}$), 129.5 (*C*H_{Ar}), 128.2 (*C*H_{Ar}), 74.4 (C_3 or C_7), 71.2 (C_3 or C_7), 55.7 (*C*H), 54.9 (*C*H), 51.5 (O*C*H_3), 43.74 (*C*), 43.74 (*C*H), 42.3 (*C*H), 40.1 (*C*H_2), 39.2 (*C*H), 36.7 (*C*H_2), 35.2 (*C*H), 34.7 (*C*H_2), 34.1 (*C*), 33.3 (*C*H_2), 31.03 (*C*H_2), 31.01 (*C*H_2), 28.6 (*C*H_2), 26.8 (*C*H_2), 26.6 (*C*H_2), 23.4 (*C*H_3), 21.2 (*C*H_2), 18.4 (*C*H_3), 12.1 (*C*H_3) ppm; **MS** (ESI+) *m/z* 533.1 ([M+Na]$^+$, 25%); 1021.7 ([2M+H]$^+$, 22%); **HRMS** (ESI+) for $C_{32}H_{50}NO_5$ [M+NH_4]$^+$ calcd 528.3684, found 528.3686 (-0.4 ppm error).

4.15, **Formula** $C_{32}H_{46}O_5$; **MW** 510.70; **IR** (neat) 3394 (br. w), 2927 (s), 2862 (m), 1741 (s), 1719 (s), 1455 (m), 1271 (s), 1177 (m), 1115 (s), 1028 (m), 743 (m), 710 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.03 (2H, dd, *J* 8.4, 1.4 Hz, H_{Ar}), 7.55 (1H, tt, *J* 7.5, 1.4 Hz, H_{Ar}), 7.44 (2H, t, *J* 7.9 Hz, H_{Ar}), 5.06 (1H, td,

J 10.9, 5.3 Hz, $H_{7\alpha}$), 3.64 (3H, s, OCH_3), 3.56 - 3.64 (1H, m, $H_{3\beta}$), 2.26 - 2.38 (1H, m), 2.14 - 2.25 (1H, m), 1.95 - 2.09 (1H, m), 1.02 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.5 Hz, H_{21}), 0.72 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.6 (*C*OO), 166.0 (*C*OO), 132.7 (CH_{Ar}), 130.9 (C_{Ar}), 129.5 (CH_{Ar}), 128.3 (CH_{Ar}), 74.6 (C_7), 71.4 (C_3), 55.2 (*C*H), 54.9 (*C*H), 51.4 (OCH_3), 43.6 (*C*), 42.3 (*C*H), 40.2 (*C*H), 39.9 (CH_2), 39.4 (*C*H), 37.2 (CH_2), 35.2 (*C*H), 34.8 (CH_2), 34.0 (*C*), 33.0 (CH_2), 31.0 (CH_2), 30.9 (CH_2), 30.1 (CH_2), 28.4 (CH_2), 26.1 (CH_2), 23.3 (CH_3), 21.2 (CH_2), 18.4 (CH_3), 12.1 (CH_3) ppm; **MS** (ESI+) *m/z* 1021.7 ($[2M+H]^+$, 100%); **HRMS** (ESI+) for $C_{32}H_{50}NO_5$ $[M+NH_4]^+$ calcd 528.3684, found 528.3696 (-2.4 ppm error).

4.16, **Formula** $C_{39}H_{50}O_6$; **MW** 614.81; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.97 - 8.12 (4H, m, H_{Ar}), 7.50 - 7.61 (2H, m, H_{Ar}), 7.39 - 7.49 (4H, m, H_{Ar}), 5.10 (1H, td, *J* 11.0, 5.3 Hz, $H_{7\alpha}$), 4.96 (1H, tt, *J* 10.2, 5.7 Hz, $H_{3\beta}$), 3.65 (3H, s, OCH_3), 2.27 - 2.39 (1H, m), 2.15 - 2.26 (1H, m), 1.07 (3H, s, H_{19}), 0.93 (3H, d, *J* 6.4 Hz, H_{21}), 0.75 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.6, 166.1, 166.0, 132.74, 132.70, 130.6, 129.6, 129.5, 128.9, 128.29, 128.26, 74.4, 74.2, 55.3, 54.9, 51.4, 43.6, 42.2, 40.3, 39.9, 39.6, 35.2, 34.6, 34.1, 33.03, 32.99, 31.0, 30.9, 28.4, 26.6, 26.1, 23.3, 21.3, 18.4, 12.2 ppm; NMR data were consistent with data reported in the literature.[220]

Synthesis of methyl 3α-benzoyloxy-7α/β-fluoro-5β-cholan-24-oate (4.17/4.18, not separable**), methyl 3α-benzyloxy-5β-cholan-6-en-24-oate (4.19,** not separable from 4.20**), and Methyl 3α-benzyloxy-5β-cholan-7-en-24-oate (4.20,** not separable from 4.19**)**



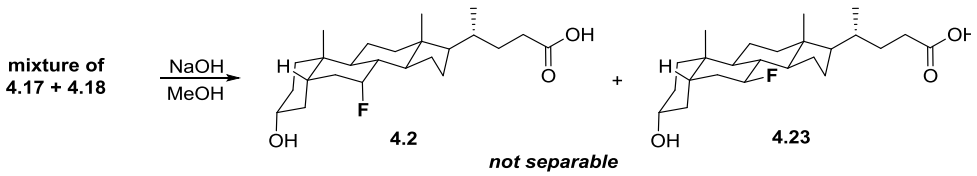
To a round-bottom flask were added **4.14** (10 mg), DAST (0.1 mL) and DCM (1 mL). The reaction mixture was stirred at rt overnight. Upon completion indicated by TLC analysis, the reaction was quenched with sat. aq. $NaHCO_3$ solution and then extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 4:96) to afford the desired compound **4.17** as minor product, together with other by-products **4.18**, **4.19** and **4.20** [**4.17**+**4.18** (inseparable) 50%; **4.19**+**4.20** (inseparable) 30%].

4.17/4.18, **Formula** $C_{32}H_{45}FO_4$; **MW** 512.70; **R_f** 0.23 (acetone/petroleum ether 5:95); **Selected ^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.57 - 4.73 (1H, m [d, *J* 47.7 Hz observed], $H_{7\beta}$ of 7α-fluoro isomer), 4.35 -

4.56 (1H, m [d, *J* 49.2 Hz observed], $H_{7\alpha}$ of 7β-fluoro isomer) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -173.0 (1F, br. d, *J* 50.3 Hz, $F_{7\beta}$ of 7β-fluoro isomer), -187.1 - -186.5 (1F, m, $F_{7\alpha}$ 7α-fluoro isomer) ppm; **MS** (ESI+) *m/z* 535.4 ([M+Na]$^+$, 88%); **HRMS** (ESI+) for $C_{32}H_{45}FNaO_4$ [M+Na]$^+$, calcd 535.3194, found 535.3207 (-2.4 ppm error).

4.19/4.20, **Formula** $C_{32}H_{44}O_4$; **MW** 492.7; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.94 - 8.10 (2H, m, H_{Ar}), 7.49 - 7.59 (1H, m, H_{Ar}), 7.36 - 7.47 (2H, m, H_{Ar}), 5.53 (1H, br. d, *J* 10.2 Hz, H_6 of **4.19**), 5.47 (1H, d, *J* 10.2 Hz, H_7 of **4.19**), 5.13 (1H, br. d, *J* 3.5 Hz, H_7 of **4.20**), 4.86 - 5.08 (1H, m, $H_{3\beta}$), 3.67 (3H, s, OCH_3), 0.79 - 1.00 (6H, m, H_{21} + H_{19}), 0.57 (3H, s, H_{18}) ppm; **MS** (ESI+) *m/z* 515.0 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{32}H_{48}NO_4$ [M+NH_4]$^+$ calcd 510.3578, found 510.3577 (0.2 ppm error).

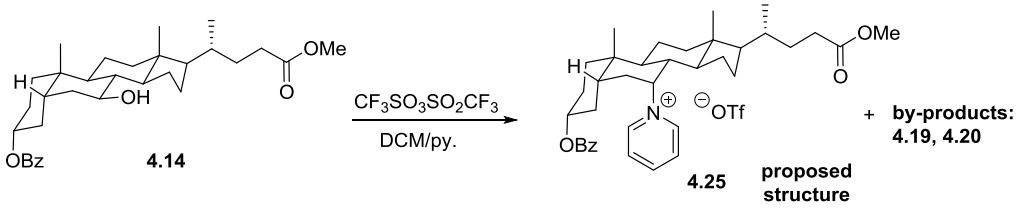
Synthesis of 7-fluoro lithocholic acid (4.2/4.23, inseparable mixture**)**



To a vial were added a mixture of **4.17/4.18** (3 mg), NaOH (100 mg) and MeOH (1 mL). The reaction mixture was stirred at rt overnight. Upon the completion indicated by TLC analysis, the solvent was firstly removed and then acidified with aq. 6 M HCl solution. The solution was further extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The desired compound **4.2** was not separable from its isomer **4.23**.

4.2/4.23, **Formula** $C_{24}H_{39}FO_3$; **MW** 394.57; **Selected ^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.54 - 4.71 (m, a doublet with 49.2 Hz can be observed, $H_{7\beta}$ of 7α-F LCA), 4.43 (dtd, *J* 49.5, 10.9, 5.7 Hz, $H_{7\alpha}$ of 7β-F LCA) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -172.5 (br. d, *J* 49.0 Hz, $F_{7\beta}$ of 7β-F LCA), -187.1 - -186.2 (m, $F_{7\alpha}$ of 7α-F LCA) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -172.5 (s, $F_{7\beta}$ of 7β-F LCA), -186.6 (s, $F_{7\alpha}$ of 7α-F LCA) ppm; **MS** (ESI+) 357.3 ([M-HF-OH]$^+$, 100%); **HRMS** (ESI+) for $C_{24}H_{39}FNaO_3$ [M+Na]$^+$, calcd 417.2775, found 417.2778 (-0.7 ppm error).

Synthesis of 7α-pyridinium triflate salt (4.25, proposed structure**)**

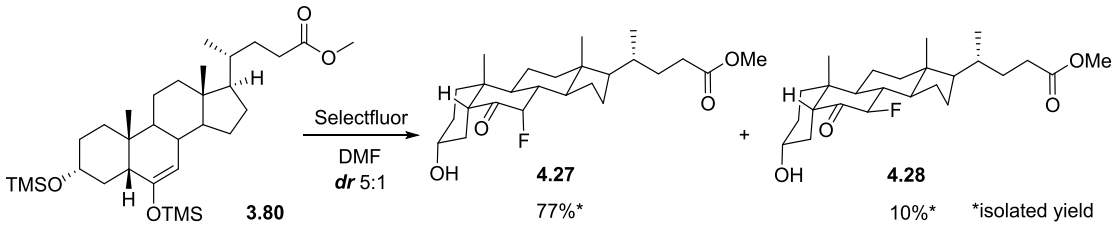


To a round-bottom flask were added **4.14** (1.32 g, 2.585 mmol, 1 eq), DCM (30 mL), pyridine (5.17 mL), and triflic anhydride (0.522 mL, 3.102 mmol, 1.2 eq) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. Upon completion indicated by TLC analysis, the reaction mixture was diluted with EtOAc, and washed with water, dilute aq. HCl solution, sat. aq. $NaHCO_3$ solution and brine. The

organic layer was then dried over Na_2SO_4 and concentrated.The crude mixture was purified by flash chromatography (acetone/petroleum ether 5:95 – 10:90, and then neat MeOH) to afford by-products **4.19/4.20** and triflate salt **4.25**. No 7β-triflate product was formed.

4.25, **Formula** $C_{38}H_{50}F_3NO_7S$; **MW** 721.87; **IR** (neat) 3134 (w), 3089 (w), 3060 (w), 2950 (m), 2872 (m), 1712 (s), 1622 (m), 1593 (w), 1573 (w), 1483 (m), 1446 (m), 1385 (w), 1250 (s), 1217 (s), 1156 (s), 1111 (m), 1025 (s), 919 (m), 719 (s), 637 (s) cm^{-1}; **^{1}H NMR** (400 MHz, CD_3OD) δ 9.26 (2H, d, *J* 5.9 Hz, H_{Py}), 8.59 (1H, t, *J* 7.7 Hz, H_{Py}), 8.14 (2H, t, *J* 7.1 Hz, H_{Py}), 7.92 (2H, dd, *J* 8.4, 1.3 Hz, H_{Ph}), 7.52 (1H, tt, *J* 7.5, 1.1 Hz, H_{Ph}), 7.37 - 7.42 (2H, m, H_{Ph}), 5.05 (1H, q, *J* 6.2 Hz, $H_{7\beta}$), 4.89 - 4.97 (1H, m, $H_{3\beta}$), 3.61 (3H, s, $C_{24}OCH_3$), 1.09 (3H, s, H_{19}), 0.91 (3H, d, *J* 6.5 Hz, H_{21}), 0.73 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 176.5 (C_{24}), 167.4 (Ph*C*OOC_3), 147.5 (CH_{Py}), 146.5 (CH_{Py}), 134.2 (CH_{Ph}), 131.7 (C_{Ph}), 130.5 (CH_{Ph}), 129.6 (CH_{Ph}), 129.5 (CH_{Py}), 121.8 (q, *J* 318.9 Hz, CF_3SO_3St), 74.2 (C_7 or C_3), 72.4 (C_3 or C_7), 56.1 (*C*H), 52.2 ($C_{24}OCH_3$), 51.5 (*C*H), 44.8 (*C*), 40.0 (*C*H), 39.7 (*C*H), 39.6 (CH_2), 37.4 (*C*H), 37.4 (CH_2, overlapped with another *C*H and confirmed by ^{13}C NMR integration and DEPT analysis), 36.4 (*C*H), 36.1 (*C*), 34.0 (CH_2), 33.7 (CH_2), 32.0 (CH_2), 31.8 (CH_2), 28.7 (CH_2), 27.3 (CH_2), 25.0 (CH_2), 22.5 (CH_2), 22.2 (CH_3), 18.8 (CH_3), 12.0 (CH_3) ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ -79.8 (3F, s, CF_3SO_3St) ppm; **MS** (ESI+) *m/z* 572.3 ($[M\text{-}OTf]^+$, 100%); **HRMS** (ESI+) for $C_{37}H_{50}NO_4$ $[M\text{-}OTf]^-$, calcd 572.3734, found 572.3744 (-1.7 ppm error).

Synthesis of methyl 3α-hydroxy-6-keto-7α-fluoro-5β-cholan-24-oate (4.27), and methyl 3α-hydroxy-6-keto-7β-fluoro-5β-cholan-24-oate (4.28)



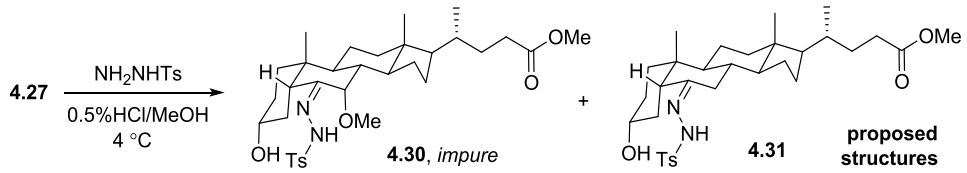
Following general procedure F, **3.80** was treated with Selectfluor and the crude mixture was purified by flash chromatography (ethyl acetate/petroleum ether 35:65; acetone/petroleum ether 25:75) to afford the desired compound **4.27** (77%*)and its diastereoisomer **4.28** (10%*, *isolated yield) as white solids. **4.27** and **4.28** were recrystallised from acetone/petroleum ether solution.

4.27, **Formula** $C_{25}H_{39}FO_4$; **MW** 422.57; **$[\alpha]_D$** -86.9 (c 0.5, $CHCl_3$, 19 °C); **m.p.** 168-172°C (acetone/petroleum ether); **R_f** 0.24 (acetone/petroleum ether 20:80); **IR** (neat) 3416 (br. w), 2935 (m), 2866 (m), 1736 (s), 1715 (s), 1457 (m), 1447 (m), 1375 (w), 1251 (w), 1169 (m), 1059 (m), 1004 (m), 736 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.43 (1H, br. d, *J* 51.0 Hz, $H_{7\beta}$), 3.66 (3H, s, OCH_3), 3.49 - 3.60 (1H, m, $H_{3\beta}$), 2.30 - 2.42 (1H, m), 0.93 (3H, d, *J* 6.4 Hz, H_{21}), 0.82 (3H, s, H_{19}), 0.66 (3H, s, H_{18}) ppm; **^{1}H NMR** (500 MHz, $CDCl_3$) [according to 1D Selective Gradient NOESY at H_{19} and H_{18}] δ 2.26 (1H, dd, $^3J_{H4\alpha\text{-}H5\beta}$ 13.1, $^3J_{H4\beta\text{-}H5\beta}$ 3.9 Hz, $H_{5\beta}$), 1.75 (1H, dtd, $^3J_{F7\alpha\text{-}H8\beta}$ 38.1, $^3J_{H9\alpha\text{-}H8\beta}$ 11.4, $^3J_{H14\alpha\text{-}H8\beta}$ 11.4,

$^{3}J_{H7\beta\text{-}H8\beta}$ 2.7 Hz, $H_{8\beta}$), 1.35 (1H, qd, $^{2}J_{H11\alpha\text{-}H11\beta}$ 13.3, $^{3}J_{H12\alpha\text{-}H11\beta}$ 13.3, $^{3}J_{H9\alpha\text{-}H11\beta}$ 13.3, $^{3}J_{H12\beta\text{-}H11\beta}$ 4.0 Hz, $H_{11\beta}$), 1.06 (1H, td, $^{2}J_{H1\alpha\text{-}H1\beta}$ 14.0, $^{3}J_{H2\alpha\text{-}H1\beta}$ 14.0, $^{3}J_{H2\beta\text{-}H1\beta}$ 3.4 Hz, $H_{1\beta}$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 207.0 (d, *J* 16.3 Hz, C_6), 174.6 (C_{24}), 94.6 (d, *J* 180.1 Hz, C_7), 69.9 (C_3), 58.9 (*C*H), 55.5 (*C*H), 51.5 (O*C*H_3), 49.2 (d, *J* 4.2 Hz, *C*H), 42.9 (*C*), 40.8 (d, *J* 21.1 Hz, C_8), 39.0 (*C*H_2), 38.5 (*C*), 35.3 (*C*H), 34.3 (*C*H_2), 34.0 (*C*H), 32.9 (d, *J* 7.2 Hz, *C*H_2), 30.9 (*C*H_2), 30.8 (*C*H_2), 29.8 (*C*H_2), 27.9 (*C*H_2), 23.1 (*C*H_2), 22.8 (C_{19}), 20.7 (*C*H_2), 18.2 (C_{21}), 11.5 (C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -195.9 - -194.0 (1F, m, $F_{7\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -194.9 (1F, s, $F_{7\alpha}$) ppm; **MS** (ESI+) *m/z* 423.2 ([M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{25}H_{39}FNaO_4$ [M+Na]$^+$ calcd 445.2725, found 445.2721 (0.8 ppm error); **Other analysis** X-Ray analysis included in the Appendices, and 1D NOESY.

4.28, **Formula** $C_{25}H_{39}FO_4$; **MW** 422.57; **R_f** 0.24 (acetone/petroleum ether 25:75); **IR** (neat) 3418 (br. w), 2945 (s), 2866 (m), 1726 (s), 1450 (m), 1382 (m), 1170 (m), 1084 (m), 1059 (m), 1009 (w), 679 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.84 (1H, dd, *J* 48.5, 10.0 Hz, $H_{7\alpha}$), 3.65 (3H, s, O*C*H_3), 3.58 (1H, tt, *J* 10.5, 5.4 Hz, $H_{3\beta}$), 0.92 (3H, d, *J* 6.4 Hz, H_{21}), 0.86 (3H, s, H_{19}), 0.65 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 206.8 (d, *J* 14.3 Hz, C_6), 174.6 (C_{24}), 94.0 (d, *J* 192.9 Hz, C_7), 69.6 (C_3), 58.8 (*C*H), 55.7 (*C*H), 55.0 (*C*H), 51.5 (O*C*H_3), 43.6 (d, *J* 16.1 Hz, *C*H), 43.5 (*C*), 39.3 (*C*H_2), 38.5 (d, *J* 8.3 Hz, *C*H), 37.8 (*C*), 35.1 (*C*H), 34.1 (*C*H_2), 34.0 (*C*H_2), 31.0 (*C*H_2), 30.8 (*C*H_2), 29.6 (*C*H_2), 28.1 (d, *J* 1.8 Hz, *C*H_2), 25.6 (d, *J* 4.8 Hz, *C*H_2), 22.9 (*C*H_3), 20.9 (*C*H_2), 18.2 (*C*H_3), 11.8 (*C*H_3) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -194.4 - -194.0 (1F, m [d, *J* 49.9 Hz observed], $F_{7\beta}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -194.2 (1F, s, $F_{7\beta}$) ppm; **MS** (ESI+) *m/z* 423.0 ([M+H]$^+$, 100%); 867.3 ([2M+Na]$^+$, 45%); **HRMS** (ESI+) for $C_{25}H_{40}FO_4$ [M+H]$^+$ calcd 423.2905, found 423.2904 (0.2 ppm error); **Other analysis** X-Ray analysis included in the Appendices.

Synthesis of methyl 3α-hydroxy-6-(2-tosylhyrazono)-7α-methoxy-5β-cholan-24-oate (4.30, proposed structure based on NMR and HRMS analysis**), and methyl 3α-hydroxy-6-(2-tosylhyrazono)-5β-cholan-24-oate (4.31**, proposed structure based on HRMS analysis**)**

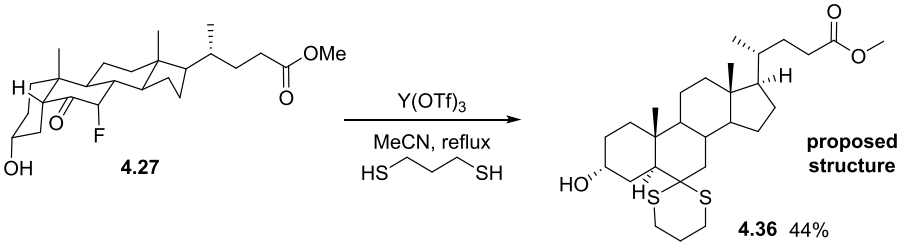


To a round-bottom flask were added **4.27** (150 mg, 0.36 mmol, 1 eq), 0.5% HCl-MeOH (30 mL) and $TsNNH_2$ (133 mg, 0.71 mmol, 2 eq). The resulting mixture was stirred at 5 °C for 24 h. The solution was washed with aq. sat. $NaHCO_3$ solution and then concentrated. The residue was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude mixture was purified by flash chromatography (acetone/petroleum ether 20:80, acetone/hexane: 30:70) to afford impure by-products **4.30** and **4.31**. The desired 7α-fluoro derivative was not obtained due to side reactions.

4.30, **Formula** $C_{33}H_{50}N_2O_6S$; **MW** 602.82; **Selected ^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.83 (2H, d, *J* 8.3 Hz, H_{Ar}), 7.26 (2H, d, *J* 8.1 Hz, H_{Ar}), 3.63 (3H, s, $C_{24}OCH_3$), 3.54 (1H, tt, *J* 11.0, 4.6 Hz, $H_{3\beta}$), 3.37 (1H, d, *J* 2.6 Hz, $H_{7\beta}$), 2.67 (3H, s, C_7OCH_3), 2.37 (3H, s, $ArCH_3$) ppm; **Selected ^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.6 (C_{24}), 158.2 (C_6), 143.9 (C_{Ar}), 135.1 (C_{Ar}), 129.5 (CH_{Ar}), 127.9 (CH_{Ar}), 81.6 (C_7), 69.9 (C_3), 55.5 (*C*H), 55.0 (C_7OCH_3), 51.3 ($C_{24}OCH_3$), 49.6 (*C*H) ppm; **MS** (ESI+) *m/z* 603.2 ([M+H]$^+$, 75%), 1205.9 ([2M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{33}H_{51}N_2O_6S$ [M+H]$^+$ calcd 603.3462, found 603.3454 (1.5 ppm error).

4.31, **Formula** $C_{32}H_{48}N_2O_5S$; **MW** 572.81; **MS** (ESI+) *m/z* 573.3 ([M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{32}H_{49}N_2O_5S$ [M+H]$^+$ calcd 573.3357, found 573.3350 (1.1 ppm error).

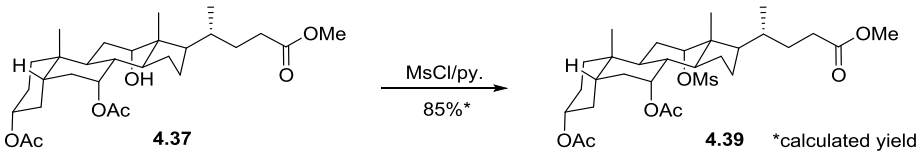
Synthesis of methyl 3α-hydroxy-6-(1,3-dithian-2-yl)-5α-cholan-24-oate (4.36, proposed structure**)**



To a round-bottom flask were added **4.27** (100 mg, 0.236 mmol, 1 eq), $Y(OTf)_3$ (6.4 mg, 0.012 mmol, 5mol%), MeCN (3 mL) and 1,3-propanedithiol (0.03 mL, 1.2 eq). The reaction mixture was stirred under reflux overnight. Upon completion indicated by TLC analysis, the reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (10 mL). The organic layer was then dried over Na_2SO_4, filtered and concentrated. The crude mixture was purified by flash chromatography (acetone/petroleum ether 10:90-15:85) to afford only by-product **4.36** (51 mg, 0.103 mmol, 44%). The desired fluorinated derivative was not obtained.

4.36, **Formula** $C_{28}H_{46}O_3S_2$; **MW** 494.79; **$[\alpha]_D$** +44.5 (c 0.9, $CHCl_3$, 20 °C); **m.p.** 82-86 °C (acetone/petroleum ether); **R_f** 0.55 (acetone/petroleum ether 25:75); **IR** (neat) 3429 (br.), 2937 (s), 2862 (m), 1731 (s), 1433 (m), 1172 (m), 1005 (m), 915 (m), 729 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.08 - 4.22 (1H, m, $H_{3\beta}$), 3.65 (3H, s, OCH_3), 3.05 - 3.19 (1H, m), 2.86 - 2.98 (1H, m), 2.83 (1H, dd, *J* 13.6, 2.7 Hz), 2.65 (1H, dt, *J* 15.0, 4.3 Hz), 2.51 (1H, dt, *J* 14.4, 3.9 Hz), 1.01 (3H, s, H_{19}), 0.90 (3H, d, *J* 6.4 Hz, H_{21}), 0.69 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (C_{24}), 66.3 (C_3), 55.7 (*C*H), 55.15 (*C*H), 55.13 (*C*), 54.0 (*C*H), 51.4 (OCH_3), 48.5 (*C*H), 44.3 (CH_2), 42.6 (*C*), 39.7 (CH_2), 38.4 (*C*), 35.3 (*C*H), 35.0 (CH_2), 32.6 (*C*H), 31.0 (CH_2), 30.9 (CH_2), 30.6 (CH_2), 28.2 (CH_2), 28.0 (CH_2), 26.4 (CH_2), 25.3 (CH_2), 25.2 (CH_2), 24.2 (CH_2), 20.5 (CH_2), 18.2 (C_{21}), 16.0 (C_{19}), 12.1 (C_{18}) ppm; **MS** (ESI+) *m/z* 495.2 ([M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{28}H_{47}O_3S_2$ [M+H]$^+$ calcd 495.2961, found 495.2961 (-0.1 ppm error); **Other analysis** 1D NOE experiments.

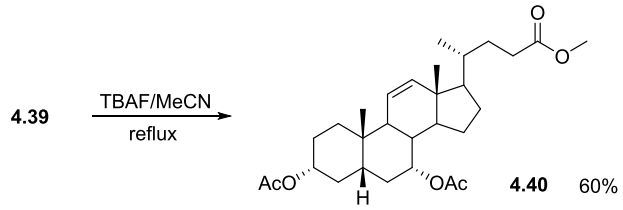
Synthesis of methyl 3α,7α-diacetoxy-12α-methylsulfonyloxy-5β-cholan-24-oate (4.39)



To a round-bottom flask (100 mL, three-neck) were added **4.37** (2 g, 3.95 mmol, 1 eq), pyridine (20 mL, 5 mL/mmol), and MsCl (1.22 mL, 15.79 mmol, 4 eq) dropwise at 0 °C. The resulting reaction mixture was warmed to room temperature and stirred overnight. Upon completion by TLC analysis, the reaction was quenched with MeOH (2 mL) and sat. aq. $NaHCO_3$ solution, followed by the extraction with DCM (× 3). The combined organic layer was washed with aq. 2M HCl solution, dried over $MgSO_4$ and concentrated. The crude mixture was purified by using a Biotage apparatus (Biotage SP1, 50 g cartridge, acetone/heptane 0% - 40%, with 20 CV) to afford the desired product **4.39** (85%*, *calculated yield), containing methyl methanesulfonate** (**not separable, to avoid using MeOH to quench MsCl as the by-product methyl methanesulfonate was difficult to remove and it is also a possible carcinogen for human).

4.39, **Formula** $C_{30}H_{48}O_9S$; **MW** 584.77; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.11 (1H, t, *J* 2.6 Hz, $H_{12\beta}$), 4.92 (1H, q, *J* 2.6 Hz, $H_{7\beta}$), 4.57 (1H, tt, *J* 11.4, 4.3 Hz, $H_{3\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 3.10 (3H, s, CH_3SO_3), 2.09 (3H, s, CH_3COOC_3 or CH_3COOC_7), 2.04 (3H, s, CH_3COOC_3 or CH_3COOC_7), 1.00 (3H, d, *J* 6.5 Hz, H_{21}), 0.94 (3H, s, H_{19} or H_{18}), 0.78 (3H, s, H_{18} or H_{19}) ppm; **MS** (ESI+) *m/z* 607.4 ($[M+Na]^+$, 100%); compound **4.39** was cited in the literature with similar preparation procedures.[137],[138]

Synthesis of methyl 3α,7α-diacetoxy-5β-chol-11-en-24-oate (4.40)

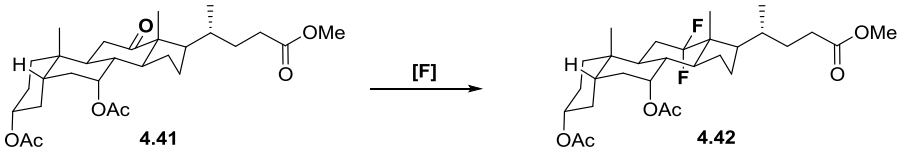


To a round-bottom flask (100 mL, 3-neck) were added **4.39** (0.91 g, 1.56 mmol, 1 eq), MeCN (16 mL, 10 mL/mmol) and TBAF (1M in THF, 4.67 mL, 4.67 mmol, 3 eq). The reaction mixture was heated at 80 °C overnight. The reaction solution was then diluted with water, and extracted with DCM (× 3). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/heptane) to afford the by-product **4.40** (455 mg, 0.931 mmol, 60%) as white solid. No desired 12β-fluoro derivative was obtained.

4.40, **Formula** $C_{29}H_{44}O_6$; **MW** 488.67; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.16 (1H, dd, *J* 10.3, 2.8 Hz, H_{12}), 5.46 (1H, dd, *J* 10.3, 1.7 Hz, H_{11}), 4.97 (1H, q, *J* 2.8 Hz, $H_{7\beta}$), 4.61 (1H, tt, *J* 11.2, 4.5 Hz, $H_{3\alpha}$), 3.66 (3H, s, $C_{24}OCH_3$), 2.73 (1H, dt, *J* 10.9, 2.2 Hz, $H_{9\alpha}$), 2.30 - 2.46 (1H, m, H_{23}), 2.16 - 2.30 (1H, m, H_{23}'), 2.06

(3H, s, CH_3COOC$_3$ or CH_3COOC$_7$), 2.02 (3H, s, CH_3COOC$_3$ or CH_3COOC$_7$), 1.01 (3H, d, *J* 6.4 Hz, H_{21}), 0.89 (3H, s, H_{19}), 0.74 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5 (C_{24}), 170.6 (CH$_3$*C*OOC$_3$ or CH$_3$*C*OOC$_7$), 170.4 (CH$_3$*C*OOC$_3$ or CH$_3$*C*OOC$_7$), 139.1 (C_{12}), 124.8 (C_{11}), 73.9 (C_3), 71.3 (C_7), 51.53 (*C*H or C$_{24}$O*C*H$_3$), 51.46 (*C*H or C$_{24}$O*C*H$_3$), 49.2 (*C*H), 45.1 (*C*), 40.2 (*C*H), 36.7 (*C*H), 36.3 (*C*H), 35.9 (*C*H), 35.18 (*C*), 35.15 (*C*H$_2$), 34.8 (*C*H$_2$), 31.8 (*C*H$_2$), 30.9 (*C*H$_2$), 30.8 (*C*H$_2$), 28.4 (*C*H$_2$), 26.7 (*C*H$_2$), 23.0 (C_{19}), 22.4 (*C*H$_2$), 21.5 (*C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$), 21.4 (*C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$), 18.3 (C_{21}), 16.7 (C_{18}) ppm; **Other analysis** X-Ray analysis included in the Appendices; NMR data were consistent with the reported values.[138]

Synthesis of methyl 3α,7α-diacetoxy-12,12-difluoro-5β-cholan-24-oate (4.42)

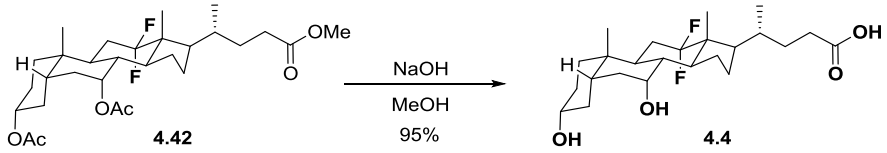


To a round-bottom flask (250 mL, 3-neck) were added **4.41** (4 g, 1 eq) and DAST (11.2 mL, 10.7 eq). The mixture was stirred at 50 °C for 6 d behind a blast shield, with further addition of DAST (2 mL) each day (except for weekend). The reaction was monitored by TLC analysis, ^{1}H and ^{19}F NMR analysis. The reaction mixture was then diluted with 150 mL of anhydrous DCM, and poured very slowly into an ice-cold sat. aq. $NaHCO_3$ solution under stirring, followed by further extraction with DCM (× 3). The combined organic layer was washed with sat. aq. $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/heptane) to afford the desired product **4.42** (2.52 g, 4.785 mmol, 60%) as white solid, with recovered starting material **4.41** (1.18 g, 30%). Compound **4.42** was further recrystallised from acetone/heptane solution.

4.42, **Formula** $C_{29}H_{44}F_2O_6$; **MW** 526.66; **IR** (neat) 2954 (m), 2876 (m), 1728 (s), 1462 (w), 1430 (m), 1373 (m), 1368 (m), 1246 (s), 1225 (s), 1180 (m), 1152 (m), 1025 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.92 (1H, q, *J* 3.1 Hz, $H_{7\beta}$), 4.59 (1H, tt, *J* 11.3, 4.3 Hz, $H_{3\beta}$), 3.67 (3H, s, C$_{24}$OCH_3), 2.22 - 2.44 (2H, m, H_{23} + H_{23}'), 2.06 (3H, s, CH_3COOC$_3$ or CH_3COOC$_7$), 2.03 (3H, CH_3COOC$_3$ or CH_3COOC$_7$), 0.99 (3H, dd, $^3J_{H20\text{-}H21}$ 6.6 Hz, $^6J_{F12\text{-}H21}$1.9 Hz [confirmed by ^{1}H {^{19}F} NMR], H_{21}), 0.94 (3H, s, H_{19}), 0.83 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5 (s, C_{24}), 170.5 (s, CH$_3$*C*OOC$_3$ or CH$_3$*C*OOC$_7$), 170.2 (s, CH$_3$*C*OOC$_3$ or CH$_3$*C*OOC$_7$), 126.0 (dd, ^1J 250.3, 247.0 Hz, C_{12}), 73.7 (s, C_3), 70.1 (d, $^5J_{F12\beta\text{-}C7}$ 1.5 Hz, C_7), 51.4 (s, C$_{24}$O*C*H$_3$), 49.0 (dd, ^2J 21.1, 20.0 Hz, C_{13}), 47.6 (dd, *J* 2.9, 0.9 Hz, *C*H), 46.7 (d, *J* 7.2 Hz, *C*H), 40.5 (d, *J* 0.6 Hz, *C*H), 36.8 (d, *J* 1.1 Hz, *C*H), 34.7 (s, *C*H$_2$), 34.5 (s, *C*H$_2$), 34.4 (s, C_{10}), 33.0 (s, *C*H), 31.6 (d, *J* 9.7 Hz, *C*H), 31.5 (s, *C*H$_2$), 31.13 (s, *C*H$_2$), 31.05 (t, ^2J 26.0 Hz, C_{11}), 30.3 (s, *C*H$_2$), 26.6 (s, *C*H$_2$), 26.0 (s, *C*H$_2$), 22.4 (d, *J* 0.7 Hz, *C*H$_2$), 22.3 (s, C_{19}), 21.5 (s, *C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$), 21.4 (s, *C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$), 19.0 (d, $^5J_{F12\text{-}C21}$ 10.6 Hz, C_{21} [$d_{F12\beta\text{-}C21}$ 2.89 Å, from X-Ray structure analysis]), 10.4 (dd, ^3J 5.3, 1.5 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -91.7 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 230.9 Hz, $F_{12\beta}$), -112.0 (1F,

ddd, $^{2}J_{F12\beta\text{-}F12\alpha}$ 231.6 Hz, $^{3}J_{H11\beta\text{-}F12\alpha}$ 35.1 Hz, $^{3}J_{H11\alpha\text{-}F12\alpha}$ 12.3 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -91.7 (1F, d, $^{2}J_{F12\alpha\text{-}F12\beta}$ 230.9 Hz, $F_{12\beta}$), -112.0 (1F, d, $^{2}J_{F12\beta\text{-}F12\alpha}$ 231.3 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 549.4 ([M+Na]$^{+}$, 100%); **HRMS** (ESI+) for $C_{29}H_{44}F_2NaO_6$ [M+Na]$^{+}$, calcd 549.2998, found 549.3013 (-2.8 ppm error); **Other analysis** X-Ray analysis included in the Appendices, and ^{1}H {^{19}F} NMR experiment.

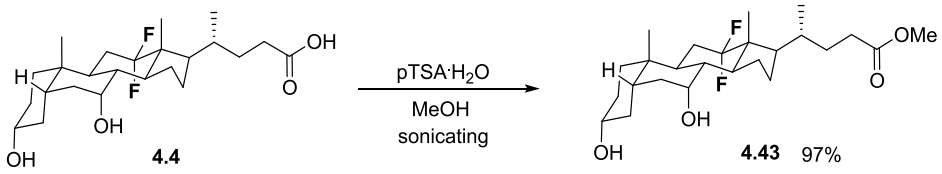
Synthesis of 12,12-difluoro chenodeoxycholic acid (4.4)



To a round-bottom flask were added **4.42 (**200 mg), NaOH (1.0 g) and MeOH (10 mL). The resulting solution was stirred at room temperature overnight. Upon completion indicated by TLC analysis, the solvent was first removed under reduced pressure, and then acidified with aq. 6 M HCl solution. This solution was further extracted with EtOAc (× 3). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated to afford the desired product **4.4** (95%). Compound **4.4** was recrstallised from MeOH.

4.4, **Formula** $C_{24}H_{38}F_2O_4$; **MW** 428.56; **IR** (neat) 3416 (br. m), 2966 (s), 2921 (s), 2844 (s), 2684 (br. w), 1704 (s), 1458 (m), 1377 (m), 1311 (w), 1201 (m), 1127 (w), 1066 (m), 1050 (m), 1025 (s), 972 (m), 919 (m), 727 (m) cm^{-1}; **^{1}H NMR** (400 MHz, CD_3OD) δ 3.83 (1H, q, *J* 2.6 Hz, $H_{7\beta}$), 3.38 (1H, tt, *J* 11.2, 4.3 Hz, $H_{3\beta}$), 1.01 (3H, dd, $^{3}J_{H20\text{-}H21}$ 6.6 Hz, $^{6}J_{F12\text{-}H21}$ 1.4 Hz [confirmed by ^{1}H {^{19}F} NMR], H_{21}), 0.94 (3H, s, H_{19}), 0.86 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 178.2 (s, C_{24}), 127.8 (dd, $^{1}J_{F12\text{-}C12}$ 250.5, 245.9 Hz, C_{12}), 72.7 (s, C_3), 68.3 (d, $^{5}J_{F12\text{-}C7}$ 1.5 Hz, C_7), 50.3 (dd, $^{2}J_{F12\text{-}C13}$ 21.1, 19.6 Hz, C_{13}), 49.3 (m, a coupling of 2.9 Hz can be observed, *C*H [overlapped with NMR solvent reference peak in ^{13}C NMR and confirmed by DEPT135 and HSQC), 48.4 (d, *J* 7.2 Hz, *C*H), 42.9 (d, *J* 0.7 Hz, *C*H), 40.5 (s, CH_2), 39.7 (d, *J* 1.1 Hz, *C*H), 36.5 (s, CH_2), 36.0 (s, C_{10}), 35.9 (s, CH_2), 34.7 (s, *C*H), 32.7 (s, CH_2), 32.4 (dd, $^{2}J_{F12\text{-}C11}$ 26.6, 25.7 Hz, C_{11}), 31.97 (d, *J* 0.7 Hz, CH_2), 31.96 (d, *J* 9.5 Hz, *C*H), 31.4 (s, CH_2), 27.5 (s, CH_2), 23.6 (d, *J* 1.1 Hz, CH_2), 23.1 (s, C_{19}), 19.7 (d, $^{5}J_{F12\text{-}C21}$ 11.0 Hz [$d_{F12\beta\text{-}C21}$ 4.3 Å, from X-Ray structural analysis], C_{21}), 11.0 (dd, *J* 5.0, 1.2 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ -92.3 (1F, d, $^{2}J_{F12\alpha\text{-}F12\beta}$ 230.6 Hz, $F_{12\beta}$), -113.2 (1F, ddd, $^{2}J_{F12\beta\text{-}F12\alpha}$ 230.6 Hz, $^{3}J_{H11\beta\text{-}F12\alpha}$ 35.4 Hz, $^{3}J_{H11\alpha\text{-}F12\alpha}$ 12.9 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, CD_3OD) δ -92.0 (1F, d, $^{2}J_{F12\alpha\text{-}F12\beta}$ 230.9 Hz, $F_{12\beta}$), -113.2 (1F, d, $^{2}J_{F12\beta\text{-}F12\alpha}$ 230.6 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 451.3 ([M+Na]$^{+}$, 17%); **HRMS** (ESI+) for $C_{24}H_{38}F_2NaO_4$ [M+Na]$^{+}$, calcd 451.2630, found 451.2625 (1.3 ppm error); **Other analysis** X-Ray analysis included in the Appendices, and ^{1}H {^{19}F} NMR experiment.

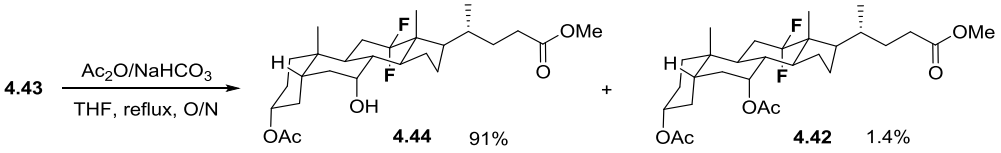
Synthesis of methyl 3α,7α-dihydroxy-12,12-difluoro-5β-cholan-24-oate (4.43)



Following general procedure B, compound **4.43** (2 g) was prepared from 12,12-difluoro CDCA **4.4** in high yield (97%) as gummy solid.

4.43, **Formula** $C_{25}H_{40}F_2O_4$; **MW** 442.59; **IR** (neat) 3400 (br. w), 2954 (m), 2919 (m), 2864 (m), 1728 (m), 1432 (m), 1377 (w), 1325 (w), 1258 (w), 1183 (w), 1081 (m), 1033 (m), 903 (s), 726 (s), 647 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.90 (1H, q, $^3J_{H8\beta\text{-}H7\beta}$ 2.9 Hz, $^3J_{H6\beta\text{-}H7\beta}$ 2.9 Hz, $^3J_{H6\alpha\text{-}H7\beta}$ 2.9 Hz, $H_{7\beta}$), 3.67 (3H, s, OCH_3), 3.47 (1H, tt, $^3J_{H2\alpha\text{-}H3\beta}$ 11.2 Hz, $^3J_{H4\alpha\text{-}H3\beta}$ 11.2 Hz, $^3J_{H2\beta\text{-}H3\beta}$ 4.5 Hz, $^3J_{H4\beta\text{-}H3\beta}$ 4.5 Hz, $H_{3\beta}$), 2.39 (1H, ddd, $^2J_{H23'\text{-}H23}$ 15.6 Hz, $^3J_{H22\text{ or }H22'\text{-}H23}$ 9.9 Hz, $^3J_{H22'\text{ or }H22\text{-}H23}$ 5.3 Hz, H_{23}), 2.27 (1H, ddd, $^2J_{H23\text{-}H23'}$ 16.3 Hz, $^3J_{H22\text{ or }H22'\text{-}H23'}$ 9.7 Hz, $^3J_{H22'\text{ or }H22\text{-}H23'}$ 7.0 Hz, H_{23}'), 1.00 (3H, dd, $^3J_{H20\text{-}H21}$ 6.7 Hz, $^6J_{F12\text{-}H21}$ 2.0 Hz [confirmed by ^{1}H {^{19}F} NMR], H_{21}), 0.92 (3H, s, H_{19}), 0.84 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (C_{24}), 126.2 (dd, *J* 250.0, 247.4 Hz, C_{12}), 71.6 (s, C_3), 67.5 (d, $^5J_{F12\text{-}C7}$ 1.7 Hz, C_7), 51.5 (s, $C_{24}OCH_3$), 48.9 (dd, *J* 20.9, 20.0 Hz, C_{13}), 47.6 (dd, *J* 2.9, 0.9 Hz, *C*H), 46.8 (d, *J* 7.2 Hz, *C*H), 41.0 (s, *C*H), 39.5 (s, CH_2), 38.2 (d, *J* 1.1 Hz, *C*H), 35.1 (s, CH_2), 34.63 (s, CH_2), 34.61 (s, C_{10}), 33.1 (s, *C*H), 31.5 (s, CH_2), 31.0 (dd, *J* 26.4, 25.3 Hz, C_{11}), 30.42 (d, *J* 10.5 Hz, *C*H), 30.40 (s, CH_2), 30.38 (s, CH_2), 26.2 (s, CH_2), 22.5 (br. s, CH_2), 22.4 (s, C_{19}), 18.9 (d, $^5J_{F12\text{-}C21}$ 10.8 Hz, C_{21}), 10.4 (dd, *J* 5.3, 1.5 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz,$CDCl_3$) δ -91.4 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 230.9 Hz, $F_{12\beta}$),-111.9 (1F, ddd, $^2J_{F12\beta\text{-}F12\alpha}$ 230.2 Hz, $^3J_{H11\beta\text{-}F12\alpha}$ 35.1 Hz, $^3J_{H11\alpha\text{-}F12\alpha}$ 13.3 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -91.4 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 230.6 Hz, $F_{12\beta}$), -111.9 (1F, d, $^2J_{F12\beta\text{-}F12\alpha}$ 230.6 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 465.4 ([M+Na]$^+$, 54%); **HRMS** (ESI+) for $C_{25}H_{40}F_2NaO_4$ [M+Na]$^+$, calcd 465.2787, found 465.2783 (0.8 ppm error); **Other analysis** ^{1}H {^{19}F} NMR experiment.

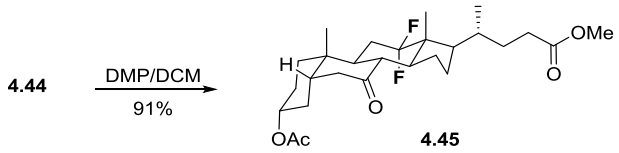
Synthesis of methyl 3α-acetoxy-7α-hydroxy-12,12-difluoro-5β-cholan-24-oate (4.44)



To a round-bottom flask were added **4.43** (430 mg, 0.972 mmol, 1.0 eq), $NaHCO_3$ (1.90 g), THF (12 mL) and acetic anhydride (19.2 mL). The resulting reaction mixture was heated at reflux overnight. Upon completion indicated by TLC analysis, the reaction was quenched slowly with water (15 mL), followed by extraction with EtOAc (× 3). The combined organic layer was washed with water, brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/heptane) to afford the desired product **4.44** (430 mg, 0.887 mmol, 91%) and diacetylated compound **4.42** (7 mg, 0.013 mmol, 1.4%) as solids.

4.44, **Formula** $C_{27}H_{42}F_2O_5$; **MW** 484.62; **IR** (neat) 3526 (br. w), 2946 (m), 2925 (m), 2868 (m), 1716 (s), 1356 (m), 1250 (s), 1017 (s), 960 (m), 899 (s), 727 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.58 (1H, tt, *J* 11.5, 4.5 Hz, $H_{3\beta}$), 3.86 - 3.95 (1H, m, $H_{7\beta}$), 3.68 (3H, s, $C_{24}OCH_3$), 2.03 (3H, s, CH_3COOC_3), 1.01 (3H, dd, $^3J_{H20\text{-}H21}$ 6.6 Hz, $^6J_{F12\text{-}H21}$ 2.0 Hz [confirmed by ^{1}H {^{19}F} NMR], H_{21}), 0.93 (3H, s, H_{19}), 0.84 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.6 (s, C_{24}), 170.7 (s, CH_3COOC_3), 126.1 (dd, *J* 250.3, 247.6 Hz, C_{12}), 74.0 (s, C_3), 67.5 (d, $^5J_{F12\text{-}C7}$ 1.7 Hz, C_7), 51.5 (s, $C_{24}OCH_3$), 49.0 (dd, *J* 20.9, 20.0 Hz, C_{13}), 47.6 (dd, *J* 3.1, 1.1 Hz, *C*H), 46.8 (d, *J* 7.2 Hz, *C*H), 40.8 (s, *C*H), 38.2 (d, *J* 1.3 Hz, *C*H), 35.1 (s, CH_2), 34.8 (s, CH_2), 34.7 (s, C_{10}), 34.5 (s, CH_2), 33.2 (s, *C*H), 31.5 (s, CH_2), 31.0 (dd, *J* 26.5, 25.7 Hz, C_{11}), 30.39 (d, *J* 9.4 Hz, *C*H), 30.37 (br. s, CH_2), 26.5 (s, CH_2), 26.2 (s, CH_2), 22.6 (d, *J* 1.5 Hz, CH_2), 22.4 (s, C_{19}), 21.4 (s, CH_3COOC_3), 18.9 (d, $^5J_{F12\text{-}C21}$ 10.8 Hz, C_{21}), 10.4 (dd, *J* 5.3, 1.5 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -91.4 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 229.9 Hz, $F_{12\beta}$), -111.9 (1F, ddd, $^2J_{F12\beta\text{-}F12\alpha}$ 230.9 Hz, $^3J_{H11\beta\text{-}F12\alpha}$ 35.4 Hz, $^3J_{H11\alpha\text{-}F12\alpha}$ 11.9 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -91.4 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 230.2 Hz, $F_{12\beta}$), -111.9 (1F, d, $^2J_{F12\beta\text{-}F12\alpha}$ 230.6 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 507.4 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{27}H_{42}F_2NaO_5$ [M+Na]$^+$, calcd 507.2893, found 507.2897 (-0.8 ppm error); **Other analysis** ^{1}H {^{19}F} NMR experiment.

Synthesis of methyl 3α-acetoxy-7-keto-12,12-difluoro-5β-cholan-24-oate (4.45)



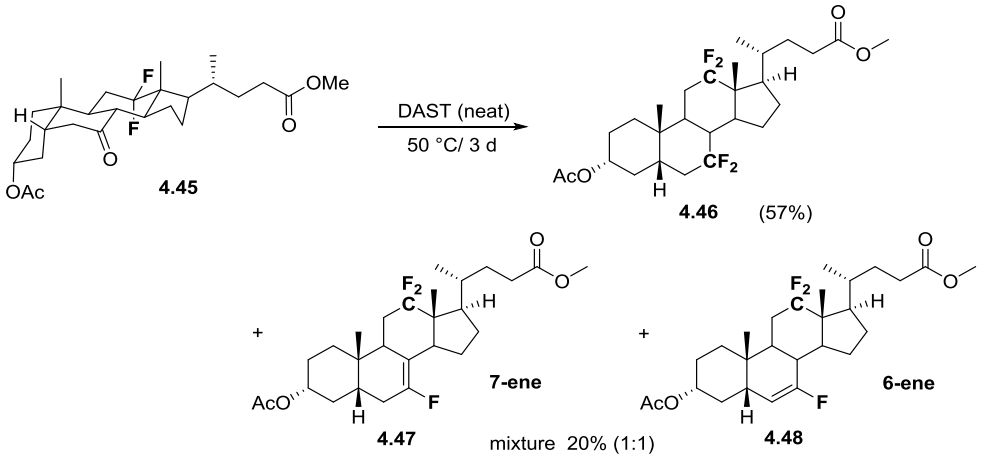
To a round-bottom flask were added **4.44** (330 mg, 0.68 mmol, 1.0 eq), DCM (10 mL), and then DMP (462 mg, 1.09 mmol, 1.6 eq) portionwise at 0 °C. The reaction mixture was then warmed to room temperature and stirred overnight. Upon completion indicated by TLC analysis, the reaction mixture was diluted with DCM, and quenched with a 10% $Na_2S_2O_3$/2% $NaHCO_3$ aq. solution, followed by extraction with DCM (× 3). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/heptane) to afford the desired product **4.45** (300 mg, 0.622 mmol, 91%) as a off-white solid.

4.45, **Formula** $C_{27}H_{40}F_2O_5$; **MW** 482.61; **IR** (neat) 2952 (s), 2873 (m), 1736 (s), 1709 (s), 1461 (m), 1437 (m), 1366 (m), 1315 (w), 1237 (s), 1170 (m), 1146 (m), 1030 (s), 1008 (s), 969 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.69 (1H, tt, *J* 11.0, 4.5 Hz, $H_{3\beta}$), 3.68 (3H, s, $C_{24}OCH_3$), 2.86 (1H, dd, *J* 13.1, 6.4 Hz, $H_{6\beta}$), 2.02 (3H, s, CH_3COOC_3), 1.23 (3H, s, H_{19}), 0.99 (3H, dd, $^3J_{H20\text{-}H12}$ 6.6 Hz, $^6J_{F12\text{-}H12}$ 1.9 Hz, H_{21}), 0.85 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 209.7 (d, $^5J_{F12\text{-}C7}$ 1.8 Hz, C_7), 174.5 (s, C_{24}), 170.4 (s, CH_3COOC_3), 125.3 (dd, *J* 250.5, 246.9 Hz, C_{12}), 72.4 (s, C_3), 51.4 (s, $C_{24}OCH_3$), 48.9 (dd, *J* 21.5, 19.8 Hz, C_{13}), 47.9 (d, *J* 1.1 Hz, *C*H), 46.9 (dd, *J* 2.8, 1.1 Hz, *C*H), 45.5 (d, *J* 7.2 Hz, *C*H), 45.3 (d, *J* 0.9 Hz, *C*H), 45.0 (s, CH_2), 39.6 (d, *J* 9.7 Hz, *C*H), 34.7 (s, C_{10}), 33.6 (s, CH_2), 33.0 (s, CH_2), 32.7 (s, *C*H), 31.7 (t, *J* 26.6 Hz, C_{11}), 31.5 (s, CH_2), 30.2 (br. s, CH_2), 25.84 (s, CH_2), 25.77 (s, CH_2), 23.6 (d, *J* 0.9 Hz, CH_2), 22.6

(s, C_{19}), 21.2 (s, CH_3COOC_3), 19.1 (d, $^5J_{F21\text{-}C21}$ 10.3 Hz, C_{21}), 10.6 (dd, J 5.5, 1.5 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -93.1 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 233.3 Hz, $F_{12\beta}$), -112.7 (1F, ddd, $^2J_{F12\beta\text{-}F12\alpha}$ 232.3 Hz, $^3J_{H11\beta\text{-}F12\alpha}$ 31.7 Hz, $^3J_{H11\alpha\text{-}F12\alpha}$ 14.3 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -93.1 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 233.0 Hz, $F_{12\beta}$), -112.7 (1F, d, $^2J_{F12\beta\text{-}F12\alpha}$ 232.6 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 505.3 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{27}H_{40}F_2NaO_5$ [M+Na]$^+$, calcd 505.2736, found 505.2743 (-1.4 ppm error); **Other analysis** ^{1}H {^{19}F} NMR experiment.

Synthesis of methyl 3α-acetoxy-7,7,12,12-tetrafluoro-5β-cholan-24-oate (4.46), methyl 3α-acetoxy-7-fluoro-12,12-difluoro-5β-cholan-7-en-24-oate (4.47), and methyl 3α-acetoxy-7-fluoro-12,12-difluoro-5β-cholan-6-en-24-oate (4.48, not separable from **4.47)**

To a round-bottom flask were added **4.45** (50 mg, 0.104 mmol) and DAST (1 mL). The reaction solution was heated behind a blast shield at 50 °C for 3 d. Upon completion indicated by TLC analysis, the reaction mixture was diluted with anhydrous DCM, and then slowly quenched with sat. aq. $NaHCO_3$ solution, followed by extraction with DCM (× 3). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/heptane) to afford the desired product **4.46** (30 mg, 0.059 mmol, 57%), and inseparable fluoroalkenes **4.47/4.48** (10 mg, 0.021 mmol, 20%).

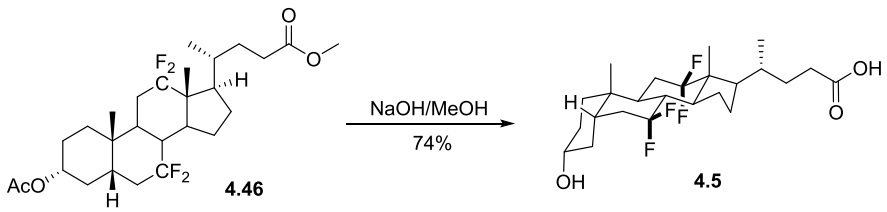


4.46, **Formula** $C_{27}H_{40}F_4O_4$; **MW** 504.61; **IR** (neat) 2950 (w), 2876 (w), 1728 (m), 1442 (w), 1364 (w), 1238 (m), 1123 (w), 1046 (w), 1025 (w), 903 (s), 723 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.64 (1H, tt, J 10.4, 5.1 Hz, $H_{3\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.39 (1H, ddd, J 15.7, 10.0, 5.2 Hz, H_{23}), 2.27 (1H, ddd, J 16.0, 9.3, 6.9 Hz, H_{23}'), 2.04 (3H, s, CH_3COOC_3), 1.00 (3H, dd, $^3J_{H20\text{-}H21}$ 6.8 Hz, $^6J_{F12\text{-}H21}$ 2.1 Hz, H_{21}), 0.99 (3H, s, H_{19}), 0.86 (3H, s, H_{18}) ppm; **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.6 (s, C_{24}), 170.6 (s, CH_3COOC_3), 125.4 (dd, J 250.8, 247.0 Hz, C_{12}), 123.9 (ddd, J 246.0, 244.1, 2.1 Hz, C_7), 73.0 (s, C_3), 51.5 (s, $C_{24}OCH_3$), 49.4 (dd, J 21.2, 20.0 Hz, C_{13}), 46.7 (dd, J 3.3, 1.2 Hz, CH), 45.2 (dd, J 6.8, 4.4 Hz, CH), 40.7 (br. d, J 10.7 Hz, CH), 40.7 (ddd, J 23.4, 20.0, 1.0 Hz, C_8), 35.9 (t, J 23.4 Hz, CH_2), 34.18 (s, CH_2), 34.17 (t, J 9.5 Hz, CH), 34.0 (d, J 1.0 Hz, C_{10}), 33.0 (s, CH), 32.8 (d, J 4.8 Hz, CH_2), 31.6 (s, CH_2), 31.1 (t, J 26.7 Hz, CH_2), 30.3 (s, CH_2), 26.1 (s, CH_2), 26.0 (d, J 1.7 Hz, CH_2), 24.1 (d, J 4.1 Hz, CH_2), 22.3 (s,

C_{19}), 21.3 (s, CH_3COOC_3), 19.1 (d, $^5J_{F12\text{-}C21}$ 10.3 Hz, C_{21}), 10.5 (dd, J 5.1, 1.5 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -85.3 (1F, d, $^2J_{F7\alpha\text{-}F7\beta}$ 243.5 Hz, $F_{7\beta}$), -93.1 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 233.0 Hz, $F_{12\beta}$), -101.4 (1F, dddd, $^2J_{F7\beta\text{-}F7\alpha}$ 243.2 Hz, $^3J_{H8\beta \text{ or } H6\beta\text{-}F7\alpha}$ 38.5 Hz, $^3J_{H6\beta \text{ or } H8\beta\text{-}F7\alpha}$ 24.9 Hz, $^3J_{H6\alpha\text{-}F7\alpha}$ 14.0 Hz, $F_{7\alpha}$), -111.8 (1F, ddd, $^2J_{F12\beta\text{-}F12\alpha}$ 233.0 Hz, $^3J_{H11\beta\text{-}F12\alpha}$ 34.1 Hz, $^3J_{H11\alpha\text{-}F12\alpha}$ 12.6 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -85.3 (1F, d, $^2J_{F7\alpha\text{-}F7\beta}$ 243.2 Hz, $F_{7\beta}$), -93.1 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 233.0 Hz, $F_{12\beta}$), -101.4 (1F, d, $^2J_{F7\beta\text{-}F7\alpha}$ 243.2 Hz, $F_{7\alpha}$), -111.8 (1F, d, $^2J_{F12\beta\text{-}F12\alpha}$ 232.6 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 527.3 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{27}H_{40}F_4NaO_4$ [M+Na]$^+$, calcd 527.2755, found 527.2741 (2.6 ppm error); **Other analysis** ^{1}H {^{19}F} NMR experiment.

4.47/4.48, **Formula** $C_{27}H_{39}F_3O_4$; **MW** 484.60; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.13 (1H, ddd, $^3J_{F7\text{-}H6}$ 18.1 Hz [confirmed by ^{1}H {^{19}F} NMR analysis], J_{HH} 5.9 Hz, J_{HH} 2.1 Hz, H_6 of 6-ene), 4.72 (1H, tt, J 11.4, 5.1 Hz, $H_{3\beta}$ of 6-ene or 7-ene), 4.66 (1H, tt, J 11.2, 4.2 Hz, $H_{3\beta}$ of 7-ene or 6-ene), 3.68 (3H, s, $C_{24}OCH_3$ of 6-ene or 7-ene), 3.68 (3H, s, $C_{24}OCH_3$ of 7-ene or 6-ene), 2.03 (3H, s, CH_3COOC_3 of 6-ene or 7-ene), 2.02 (3H, s, CH_3COOC_3 of 7-ene or 6-ene), 1.01 (3H, dd, $^2J_{H20\text{-}H21}$ 6.7 Hz, $^6J_{F12\text{-}H21}$ 2.1 Hz, H_{21} of 6-ene or 7-ene), 0.99 (3H, dd, $^2J_{H20\text{-}H21}$ 6.9 Hz, $^6J_{F12\text{-}H21}$ 2.8 Hz, H_{21} of 7-ene or 6-ene), 0.91 (3H, s, H_{19} or H_{18} of 6-ene or 7-ene), 0.90 (3H, s, H_{19} or H_{18} of 7-ene or 6-ene), 0.89 (3H, s, H_{18} or H_{19} of 6-ene or 7-ene), 0.85 (3H, s, H_{18} or H_{19} of 7-ene or 7-ene) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ N/A not provided; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -93.1 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 232.3 Hz, $F_{12\beta}$ of 6-ene or 7-ene), -94.4 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 231.3 Hz, $F_{12\beta}$ of 7-ene or 6-ene), -107.7 (1F, br. d, $^3J_{H6\text{-}F7}$ 18.1 Hz, F_7 of 6-ene), -108.9 - -108.7 (1F, m, F_7 of 7-ene), -110.9 (1F, ddd, $^2J_{F12\beta\text{-}F12\alpha}$ 233.3 Hz, $^3J_{H11\beta\text{-}F12\alpha}$ 33.4 Hz, $^3J_{H11\alpha\text{-}F12\alpha}$ 12.9 Hz, $F_{12\alpha}$ of 6-ene or 7-ene), -114.0 (1F, ddd, $^2J_{F12\beta\text{-}F12\alpha}$ 230.6 Hz, $^3J_{H11\beta\text{-}F12\alpha}$ 34.4 Hz, $^3J_{H11\alpha\text{-}F12\alpha}$ 12.9 Hz, $F_{12\alpha}$ of 7-ene or 6-ene) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -93.1 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 233.0 Hz, $F_{12\beta}$ of 6-ene or 7-ene), -94.4 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 230.6 Hz, $F_{12\beta}$ of 7-ene or 6-ene), -107.7 (1F, s, F_7 of 6-ene), -108.8 (1F, s, F_7 of 7-ene), -110.9 (1F, d, $^2J_{F12\beta\text{-}F12\alpha}$ 233.3 Hz, $F_{12\alpha}$ of 6-ene or 7-ene), -114.0 (1 F, d, $^2J_{F12\beta\text{-}F12\alpha}$ 230.9 Hz, $F_{12\alpha}$ of 7-ene or 6-ene) ppm; **MS** (ESI+) *m/z* RT: 5.89 mins 507.4 ([M+Na]$^+$, 100%); RT 5.95 mins 507.4 ([M+Na]$^+$, 55%); **HRMS** (ESI+) for $C_{27}H_{39}F_3NaO_4$ [M+Na]$^+$, calcd 507.2693, found 507.2699 (-1.2 ppm error); **Other analysis** ^{1}H {^{19}F} NMR experiment.

Synthesis of 7,7,12,12-tetrafluoro lithocholic acid (4.5)

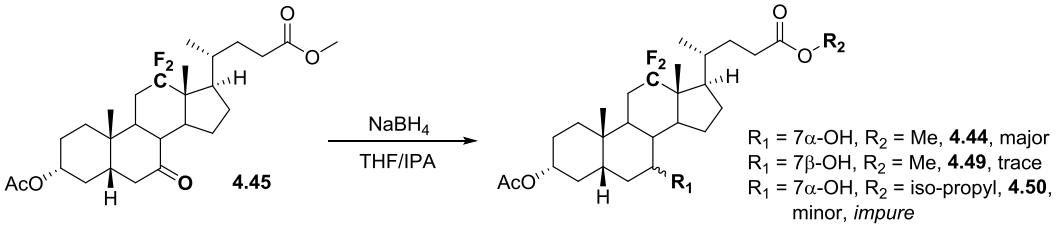


To a round-bottom flask were added **4.46** (15 mg, 0.0297 mmol), NaOH (400 mg) and MeOH (4 mL, HPLC grade). The reaction mixture was stirred at room temperature overnight. Upon completion indicated by TLC analysis, the solvent was removed under reduced pressure. The residue was acidified with aq. 2 M HCl solution, followed by extraction with EtOAc (× 3). The combined organic

layer was washed with brine, dried over Na_2SO_4 and concentrated to afford the desired product **4.5** (10 mg, 0.022 mmol, 74%).

4.5, **Formula** $C_{24}H_{36}F_4O_3$; **MW** 448.54; **^{1}H NMR** (500 MHz, CD_3OD) δ 3.42 - 3.53 (1H, m, $H_{3\beta}$), 1.00 (3H, s, H_{19}), 1.01 (3H, dd, *J* 6.4, 2.0 Hz, H_{21}), 0.90 (3H, s, H_{18}) ppm; **^{13}C NMR** (126 MHz, CD3OD) δ 178.1 (s, C_{24}), 127.0 (dd, *J* 249.6, 245.3 Hz, C_{12}), 125.6 (ddd, *J* 245.3, 243.9, 2.3 Hz, C_7), 71.8 (s, C_3), 50.9 (dd, *J* 21.2, 20.3 Hz, C_{13}), 48.4 (dd, *J* 3.1, 1.0 Hz, *C*H), 47.0 (dd, *J* 6.9, 5.0 Hz, *C*H), 42.4 (d, *J* 10.7 Hz, *C*H), 42.1 (dd, *J* 22.4, 21.0 Hz, *C*H), 38.1 (d, *J* 4.3 Hz, CH_2), 37.3 (t, *J* 23.1 Hz, CH_2), 35.8 (t, *J* 9.7 Hz, *C*H), 35.6 (d, *J* 1.2 Hz, CH_2), 35.2 (d, *J* 1.0 Hz, C_{10}), 34.4 (s, *C*H), 32.7 (s, CH_2), 32.3 (t, *J* 26.9 Hz, CH_2), 31.8 (d, *J* 0.6 Hz, CH_2), 30.9 (s, CH_2), 27.2 (d, *J* 1.4 Hz, CH_2), 25.4 (d, *J* 4.1 Hz, CH_2), 22.7 (s, C_{19}), 19.8 (d, $^5J_{F12\text{-}C21}$ 10.3 Hz, C_{21}), 10.9 (dd, *J* 5.1, 1.5 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ -85.9 (1F, d, $^2J_{F7\alpha\text{-}F7\beta}$ 242.3 Hz, $F_{7\beta}$), -94.1 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 233.2 Hz, $F_{12\beta}$), -102.5 (1F, dddd, $^2J_{F7\beta\text{-}F7\alpha}$ 242.8 Hz, $^3J_{H8\beta \text{ or } H6\beta\text{-}F7\alpha}$ 39.0 Hz, $^3J_{H6\beta \text{ or } H8\beta\text{-}F7\alpha}$ 25.6 Hz, $^3J_{H6\alpha\text{-}F7\alpha}$ 14.3 Hz, $F_{7\alpha}$), -113.4 (1F, ddd, $^2J_{F12\beta\text{-}F12\alpha}$ 233.2 Hz, $^3J_{H11\beta\text{-}F12\alpha}$ 33.4 Hz, $^3J_{H11\alpha\text{-}F12\alpha}$ 13.0 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, CD_3OD) δ -85.9 (1F, d, $^2J_{F7\alpha\text{-}F7\beta}$ 242.8 Hz, $F_{7\beta}$), -94.1 (1F, d, $^2J_{F12\alpha\text{-}F12\beta}$ 233.2 Hz, $F_{12\beta}$), -102.5 (1F, d, $^2J_{F7\beta\text{-}F7\alpha}$ 242.3 Hz, $F_{7\alpha}$), -113.4 (1F, d, $^2J_{F12\beta\text{-}F12\alpha}$ 233.2 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 409.3 ([M-2×HF+H]$^+$, 100%); **HRMS** (ESI+) for $C_{24}H_{36}F_4NaO_3$ [M+Na]$^+$, calcd 471.2493, found 471.2495 (-0.5 ppm error).

Synthesis of isopropyl 3α-acetoxy-7α-hydroxy-12,12-difluoro-5β-cholan-24-oate (4.50, impure**)**

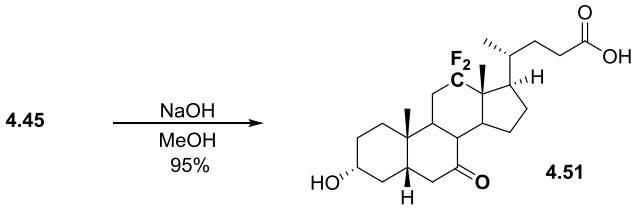


To a round-bottom flask were added **4.45** (60 mg, 0.124 mmol, 1.0 eq), THF (4 mL), IPA* (0.5 mL, *IPA was added to accelerate the reduction process) and $NaBH_4$ (7.1 mg, 0.186 mmol, 1.5 eq). The resulting mixture was stirred at room temperature overnight. Upon completion, the reaction was quenched with water, followed by extraction with EtOAc (× 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/petroleum ether) to afford **4.44** (major) and **4.50** (minor, *impure*). Only trace amount of the desired product **4.49** was formed, based on ^{19}F NMR analysis.

4.50, **Formula** $C_{29}H_{46}F_2O_5$; **MW** 512.68; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.01 (1H, spt, *J* 6.2 Hz, C*H* of isopropyl), 4.58 (1H, tt, *J* 11.4, 4.3 Hz, $H_{3\beta}$), 3.87 - 3.94 (1H, m, $H_{7\beta}$), 2.02 (3H, s, CH_3COOC_3), 1.24 (3H, s, CH_3 of isopropyl), 1.23 (3H, s, CH_3' of isopropyl), 1.01 (3H, dd, *J* 6.6, 1.8 Hz, H_{21}), 0.93 (3H, s, H_{19}), 0.84 (3H, s, H_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -91.2 (1F, d, *J* 230.6 Hz, $F_{12\beta}$), -111.9 (1F, ddd, *J* 230.2, 35.1, 12.1 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -91.2 (1F, d, *J* 230.6 Hz, $F_{12\beta}$),

-111.9 (1F, d, *J* 230.2 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 535.4 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{29}H_{46}F_2NaO_5$ [M+Na]$^+$, calcd 535.3206, found 535.3211 (-0.9 ppm error).

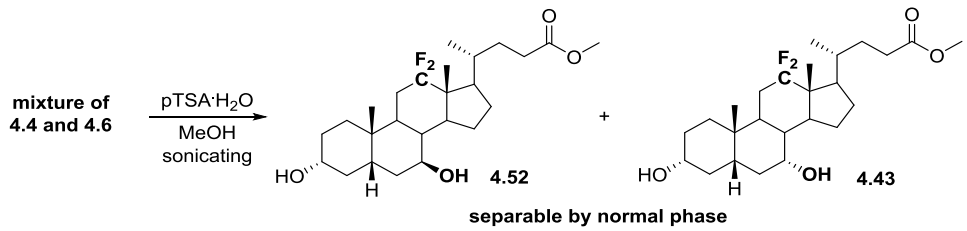
Synthesis of 12,12-difluoro-7-keto lithocholic acid (4.51)



To a round-bottom flask were added **4.45** (81 mg), NaOH (600 mg) and MeOH (6 mL). The resulting reaction mixture was stirred at room temperature overnight. Upon completion indicated by TLC analysis, the solvent was removed under reduced pressure. The residue was acidified with aq. 6 M HCl solution, followed by extraction with EtOAc (× 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated to afford the desired product **4.51** (95%).

4.51, **Formula** $C_{24}H_{36}F_2O_4$; **MW** 426.54; **^{1}H NMR** (400 MHz, CD_3OD) δ 3.54 (1H, tt, *J* 10.6, 4.6 Hz, $H_{3\beta}$), 3.01 (1H, dd, *J* 12.5, 5.9 Hz, $H_{6\beta}$), 2.61 (1H, t, *J* 10.8 Hz, $H_{8\beta}$), 1.25 (3H, s, H_{19}), 1.00 (3H, dd, *J* 6.6, 1.5 Hz, H_{21}), 0.88 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 213.1 (d, $^5J_{F12\text{-}C7}$ 1.8 Hz, C_7), 178.0 (s, C_{24}), 127.0 (dd, *J* 250.2, 245.0 Hz, C_{12}), 71.3 (s, C_3), 50.4 (dd, *J* 21.5, 19.8 Hz, C_{13}), 49.1 (d, *J* 0.7 Hz, *C*H), 48.6 (m, *C*H), 47.4 (d, *J* 7.2 Hz, *C*H), 47.1 (d, *J* 0.7 Hz, *C*H), 46.3 (s, CH_2), 41.4 (d, *J* 9.7 Hz, *C*H), 38.3 (s, CH_2), 36.0 (s, C_{10}), 35.1 (s, CH_2), 34.2 (s, *C*H), 32.9 (t, *J* 26.4 Hz, CH_2), 32.7 (s, CH_2), 31.7 (s, CH_2), 30.6 (s, CH_2), 27.1 (s, CH_2), 24.8 (s, CH_2), 23.2 (s, C_{19}), 19.9 (d, $^5J_{F12\text{-}C21}$ 10.3 Hz, C_{21}), 11.3 (dd, *J* 5.5, 1.3 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ -93.9 (1F, br. d, *J* 232.8 Hz, $F_{12\beta}$), -114.1 (1F, ddd, *J* 233.2, 33.8, 12.6 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H}** NMR (376 MHz, CD_3OD) δ -93.9 (1F, d, *J* 232.8 Hz, $F_{12\beta}$), -114.1 (1F, d, *J* 232.8 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 449.3 ([M+Na]$^+$, 5%); **HRMS** (ESI+) for $C_{24}H_{36}F_2NaO_4$ [M+Na]$^+$, calcd 449.2474, found 449.2480 (-1.4 ppm error).

Synthesis of methyl 3α,7β-dihydroxy-12,12-difluoro-5β-cholan-24-oate (4.52)

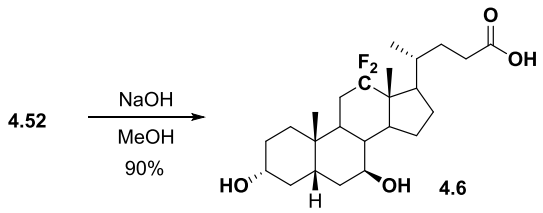


Following general procedure B, a mixture of 12,12-difluoro CDCA/UDCA (**4.4/4.6**, *not separable*) was esterified by sonication. The crude mixture was purified by using a Biotage apparatus (acetone/petroleum ether 12:88 – 32:68) to afford the desired product **4.52**, and also **4.43** as gummy solids.

4.52, **Formula** $C_{25}H_{40}F_2O_4$; **MW** 442.59; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.67 (3H, s, $C_{24}OCH_3$), 3.54 - 3.64 (2H, m, $H_{3\beta}$ + $H_{7\alpha}$), 2.39 (1H, ddd, *J* 15.7, 9.8, 5.3 Hz, H_{23}), 2.26 (1H, ddd, *J* 16.5, 9.7, 6.7 Hz, H_{23}'), 0.99 (3H, dd, *J* 6.7, 1.9 Hz, H_{21}), 0.96 (3H, s, H_{19}), 0.85 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.6 (s, C_{24}), 126.0 (dd, *J* 250.5, 245.6 Hz, C_{12}), 71.1 (s, C_3), 70.5 (d, $^5J_{F12\text{-}C7}$ 2.2 Hz, C_7), 51.9 (d, *J* 7.2 Hz, *C*H), 51.5 (s, $C_{24}OCH_3$), 49.9 (dd, *J* 21.3, 19.4 Hz, C_{13}), 46.5 (dd, *J* 3.6, 1.2 Hz, *C*H), 42.4 (d, *J* 1.3 Hz, *C*H), 42.1 (d, *J* 1.1 Hz, *C*H), 37.1 (s, CH_2), 37.0 (s, CH_2), 35.8 (d, *J* 9.5 Hz, *C*H), 34.7 (s, CH_2), 33.7 (s, C_{10}), 32.9 (s, *C*H), 31.7 (s, CH_2), 31.3 (dd, *J* 26.4, 25.5 Hz, C_{11}), 30.3 (d, *J* 0.6 Hz, CH_2), 30.1 (s, CH_2), 26.1 (s, CH_2), 25.7 (d, *J* 1.3 Hz, CH_2), 23.0 (s, C_{19}), 19.2 (d, $^5J_{F12\text{-}C21}$ 9.9 Hz, C_{21}), 10.7 (dd, *J* 5.0, 1.9 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -92.9 (1F, br. d, *J* 230.6 Hz, $F_{12\beta}$), -111.8 (1F, ddd, *J* 231.0, 33.4, 12.6 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -92.9 (1F, d, *J* 230.6 Hz, $F_{12\beta}$), -111.8 (1F, d, *J* 231.1 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 465.4 ([M+Na]$^+$, 47%); **HRMS** (ESI+) for $C_{25}H_{40}F_2NaO_4$ [M+Na]$^+$, calcd 465.2787, found 465.2781 (1.3 ppm error).

Synthesis of 12,12-difluoro ursodeoxycholic acid (4.6)

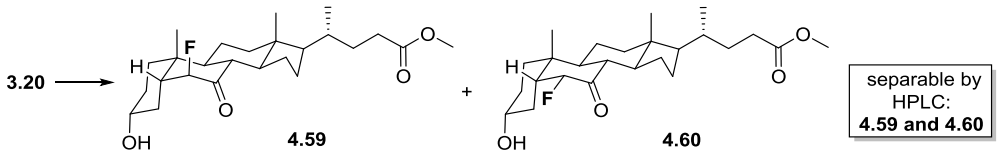
To a round-bottom flask were added **4.52** (17 mg, 0.0384 mmol), NaOH (400 mg) and MeOH (4 mL). The resulting reaction mixture was stirred at room temperature overnight. Upon completion indicated by TLC analysis, the solvent was removed under reduced pressure. The residue was acidified with aq. 2 M HCl solution, followed by extraction with EtOAc (× 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated to afford the desired product **4.6** (14.8 mg, 0.0345 mmol, 90%).



4.6, **Formula** $C_{24}H_{38}F_2O_4$; **MW** 428.56; **^{1}H NMR** (400 MHz, CD_3OD) δ 3.42 - 3.56 (2H, m, $H_{3\beta}$ + $H_{7\alpha}$), 2.35 (1H, ddd, *J* 15.2, 9.7, 5.3 Hz, H_{23}), 2.22 (1H, ddd, *J* 16.0, 9.0, 7.0 Hz, H_{23}'), 0.96 - 1.03 (6H, m, H_{21} + H_{19}), 0.88 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 178.2 (s, C_{24}), 127.5 (dd, *J* 250.0, 245.0 Hz, C_{12}), 72.0 (s, C_3), 71.3 (d, $^5J_{F12\text{-}C7}$ 2.0 Hz, C_7), 53.9 (d, *J* 7.2 Hz, *C*H), 51.3 (dd, *J* 21.3, 19.3 Hz, C_{13}), 48.2 (m, *C*H), 43.8 (d, *J* 0.7 Hz, *C*H), 43.3 (d, *J* 0.7 Hz, *C*H), 38.6 (s, CH_2), 38.0 (s, CH_2), 37.5 (d, *J* 9.4 Hz, *C*H), 35.9 (s, CH_2), 34.9 (s, C_{10}), 34.4 (s, *C*H), 32.8 (s, CH_2), 32.7 (dd, *J* 26.0, 25.1 Hz, C_{11}), 31.8 (s, CH_2), 31.0 (s, CH_2), 27.3 (s, CH_2), 26.8 (d, *J* 0.6 Hz, CH_2), 23.6 (s, C_{19}), 19.9 (d, $^5J_{F12\text{-}C21}$ 10.3 Hz, C_{21}), 11.3 (dd, *J* 5.1, 1.8 Hz, C_{18}) ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ -93.6 (1F, d, *J* 231.5 Hz, $F_{12\beta}$), -113.2 (1F, ddd, *J* 231.5, 32.9, 8.7 Hz, $F_{12\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, CD_3OD) δ -93.6 (1F, d, *J* 231.9 Hz, $F_{12\beta}$), -113.2 (1F, d, *J* 231.5 Hz, $F_{12\alpha}$) ppm; **MS** (ESI+) *m/z* 451.2 ([M+Na]$^+$, 8%); **HRMS** (ESI+) for $C_{24}H_{38}F_2NaO_4$ [M+Na]$^+$, calcd 451.2630, found 451.2625 (1.1 ppm error).

7.4.2 Synthesis of C-6 fluorinated B-ring analogues

Synthesis of methyl 3α-hydroxy-6β-fluoro-7-keto-5β-cholan-24-oate (4.59), and methyl 3α-hydroxy-6α-fluoro-7-keto-5β-cholan-24-oate (4.60)

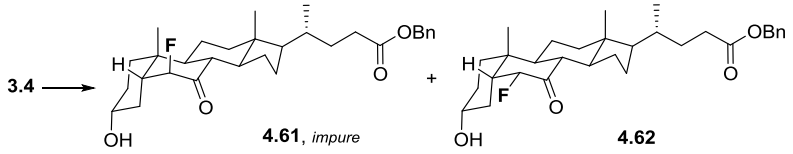


Following general procedure F, silyl enol ether **3.20** was treated with Selectfluor in DMF, leading to the formation of fluoroketones **4.59** and **4.60**. The crude mixture was purified by flash chromatography (acetone/petroleum ether 15:85 – 30:70 – 100:0) and by HPLC (acetone/hexane 20:80).

4.59, **Formula** $C_{25}H_{39}FO_4$; **MW** 422.58; $\mathbf{R_f}$ 0.26 (acetone/hexane 20:80); **IR** (neat) 3392 (br. w), 2935 (s), 2872 (s), 1716 (s), 1448 (m), 1381 (w), 1258 (m), 1175 (s), 1033 (s), 1002 (s), 915 (m), 726 (s), 639 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.29 (1H, dd, *J* 51.8, 3.1 Hz, $H_{6\alpha}$), 3.67 (3H, s, $C_{24}OCH_3$), 3.59 - 3.65 (1H, m, $H_{3\beta}$), 2.99 (1H, td, *J* 11.3, 5.2 Hz, $H_{8\beta}$), 1.26 (3H, d, *J* 3.4 Hz, H_{19}), 0.93 (3H, d, *J* 6.4 Hz, H_{21}), 0.70 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 208.2 (d, *J* 21.6 Hz, C_7), 174.6 (s, C_{24}), 97.9 (d, *J* 184.1 Hz, C_6), 70.2 (d, *J* 2.9 Hz, C_3), 54.8 (*C*H), 51.5 ($C_{24}OCH_3$), 49.7 (d, *J* 18.7 Hz, *C*H), 48.1 (s, *C*H), 45.7 (d, *J* 2.9 Hz, *C*H), 44.2 (s, *C*H), 42.4 (s, *C*), 38.8 (s, CH_2), 35.2 (s, *C*H), 34.9 (d, *J* 1.7 Hz, *C*), 34.5 (s, CH_2), 32.3 (d, *J* 9.7 Hz, CH_2), 31.0 (s, CH_2), 30.9 (s, CH_2), 29.7 (s, CH_2), 28.1 (s, CH_2), 24.8 (d, *J* 6.2 Hz, C_{19}), 24.2 (s, CH_2), 21.7 (s, CH_2), 18.3 (s, C_{21}), 12.0 (s, C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -177.9 (1F, dd, *J* 51.6, 13.4 Hz, $F_{6\beta}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -177.9 (1F, s, $F_{6\beta}$) ppm; **MS** (ESI+) 423.3 ($[M+H]^+$, 19%); **HRMS** (ESI+) for $C_{25}H_{39}FNaO_4$ $[M+Na]^+$, calcd 445.2725, found 445.2730 (-1.3 ppm error).

4.60, **Formula** $C_{25}H_{39}FO_4$; **MW** 422.58; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.20 (1H, dd, *J* 49.6, 6.4 Hz, $H_{6\beta}$), 3.64 (3H, s, $C_{24}OCH_3$), 3.57 (1H, tt, *J* 10.4, 5.4 Hz, $H_{3\beta}$), 1.20 (3H, s, H_{19}), 0.90 (3H, d, *J* 6.3 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 205.7 (d, *J* 13.8 Hz, C_7), 174.6 (s, C_{24}), 91.8 (d, *J* 193.7 Hz, C_6), 70.0 (s, C_3), 54.6 (s, *C*H), 51.4 (s, $C_{24}OCH_3$), 50.8 (d, *J* 15.4 Hz, *C*H), 48.4 (d, *J* 1.5 Hz, *C*H), 47.6 (s, *C*H), 42.6 (s, C_{13}), 42.5 (d, *J* 0.6 Hz, *C*H), 38.6 (s, CH_2), 35.9 (d, *J* 7.0 Hz, C_{10}), 35.1 (s, *C*H), 34.2 (d, *J* 1.1 Hz, CH_2), 31.0 (s, CH_2), 30.9 (s, CH_2), 30.0 (d, *J* 5.3 Hz, CH_2), 29.4 (s, CH_2), 28.1 (s, CH_2), 24.4 (s, CH_2), 23.2 (s, C_{19}), 21.5 (s, CH_2), 18.3 (s, C_{21}), 11.9 (s, C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -201.7 (1F, br. d, *J* 49.9 Hz, $F_{6\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -201.7 (1F, s, $F_{6\alpha}$) ppm; **MS** (ESI+) 445.4 ([M+Na]+, 50%); **HRMS** (ESI+) for $C_{25}H_{39}FNaO_4$ $[M+Na]^+$, calcd 445.2725, found 445.2718 (1.5 ppm error); ^{1}H NMR data was consistent with the reported data in the literature.[131]

Synthesis of benzyl 3α-hydroxy-6β-fluoro-7-keto-5β-cholan-24-oate (4.61), and benzyl 3α-hydroxy-6α-fluoro-7-keto-5β-cholan-24-oate (4.62)

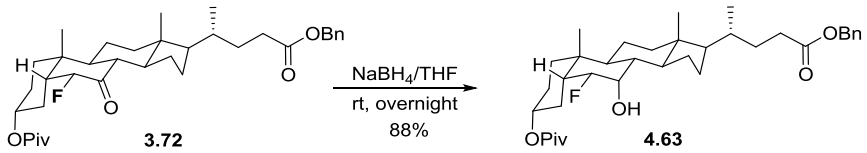


Following general procedure F, **3.4** was treated with Selectfluor, and the crude mixture was purified by flash chromatography (acetone/petroleum ether) and HPLC (acetone/hexane) to afford **4.61** (not pure, difficult to isolate from **4.62**) and **4.62**. The complete separation was facilitated by pivaloylation of the 3α-OH group.

4.61, **Formula** $C_{31}H_{43}FO_4$; **MW** 498.67; $\mathbf{R_f}$ 0.22 (acetone/hexane 20:80); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.29 - 7.41 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2), 5.09 (1H, d, *J* 12.3 Hz, PhCH_2), 4.30 (1H, dd, *J* 51.8, 2.9 Hz, $H_{6\alpha}$), 3.47 - 3.74 (1H, m, $H_{3\beta}$), 2.98 (1H, td, *J* 11.2, 5.1 Hz, $H_{8\beta}$), 2.35 - 2.48 (1H, m), 1.26 (3H, d, *J* 3.3 Hz, H_{19}), 0.92 (3H, d, *J* 6.2 Hz, H_{21}), 0.68 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 208.3 (d, *J* 21.6 Hz, C_7), 174.0 (C_{24}), 136.1 (CH_{Ar}), 128.5 (C_{Ar}), 128.2 (C_{Ar}), 128.2 (C_{Ar}), 97.9 (d, *J* 184.4 Hz, C_6), 70.2 (d, *J* 2.5 Hz, C_3), 66.1 (PhCH_2), 54.8, 49.7 (d, *J* 18.5 Hz), 48.1, 45.7 (d, *J* 2.8 Hz), 44.2, 42.4, 38.8, 35.1, 34.8, 34.5, 32.2 (d, *J* 9.7 Hz), 31.2, 30.9, 29.6, 28.1, 24.8 (d, *J* 6.1 Hz), 24.2, 21.6, 18.3, 11.9 ppm; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -178.1 (1F, dd, *J* 51.6, 14.0 Hz, $F_{6\beta}$) ppm; **MS** (ESI+) *m/z* 1019.7 $[2M+Na]^+$; **HRMS** (ESI+) for $C_{31}H_{43}FNaO_4$ $[M+Na]^+$ calcd 521.3038, found 521.3051 (-2.6 ppm error).

4.62, **Formula** $C_{31}H_{43}FO_4$; **MW** 498.67; $\mathbf{R_f}$ 0.36 (acetone/hexane 30:70); **IR** (neat) 3442 (br. w), 2937 (m), 2868 (m), 1728 (s), 1454 (w), 1381 (w), 1260 (w), 1162 (m), 1063 (m), 1016 (m), 979 (w), 742 (m), 694 (m) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.28 - 7.44 (5H, m, H_{Ar}), 5.22 (1H, dd, *J* 49.5, 6.4 Hz, $H_{6\beta}$), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2), 5.09 (1H, d, *J* 12.3 Hz, PhCH_2), 3.49 - 3.70 (1H, m, $H_{3\beta}$), 2.35 - 2.49 (1H, m), 1.22 (3H, s, H_{19}), 0.92 (3H, d, *J* 6.1 Hz, H_{21}), 0.63 (3H, s, H_{18}) ppm; **^{13}C NMR** (75 MHz, $CDCl_3$) δ 205.6 (d, *J* 13.5 Hz, C_7), 173.9 (C_{24}), 136.1 (C_{Ar}), 128.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.2 (CH_{Ar}), 91.8 (d, *J* 193.2 Hz, C_6), 70.1 (C_3), 66.1 (PhCH_2), 54.6 (CH), 50.8 (d, *J* 15.2 Hz, CH), 48.4 (CH), 47.6 (CH), 42.6 (C), 42.5 (CH), 38.6 (CH_2), 35.9 (d, *J* 6.9 Hz, C), 35.1 (CH), 34.2 (CH_2), 31.2 (CH_2), 30.9 (CH_2), 30.1 (d, *J* 5.3 Hz, CH_2), 29.5 (CH_2), 28.2 (CH_2), 24.4 (CH_2), 23.3 (CH_3), 21.6 (CH_2), 18.3 (CH_3), 12.0 (CH_3) ppm; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -201.9 (1F, br. d, *J* 50.5 Hz, $F_{6\alpha}$) ppm; **MS** (ESI+) *m/z* 1019.8 ($[2M+Na]^+$, 100%); **HRMS** (ESI+) for $C_{31}H_{43}FNaO_4$ $[M+Na]^+$ calcd 521.3038, found 521.3037 (0.0 ppm error).

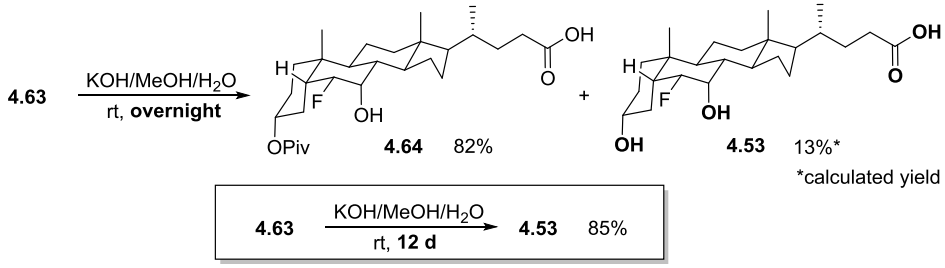
Synthesis of benzyl 3α-pivaloyloxy-6α-fluoro-7α-hydroxy-5β-cholan-24-oate (4.63)



Following general procedure G, compound **3.72** was reduced by $NaBH_4$ diastereoselectively to the desired compound **4.63**. The crude mixture was purified by flash chromatography (acetone/petroleum ether 8:92-15:85) to afford **4.63** (245 mg, 0.42 mmol, 88%*, *isolated yield) as gummy solid.

4.63, **Formula** $C_{36}H_{53}FO_5$; **MW** 584.80; **$[\alpha]_D$** +12.2 (c 1.8, $CHCl_3$, 21 °C); **R_f** 0.34 (acetone/hexane 10:90); **IR** (neat) 3508(w), 2943(s), 2870(m), 1719(s), 1457(m), 1377(m), 1289(m), 1165(s), 1031(m), 998(m), 757(w), 699(w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.25 - 7.31 (5H, m, H_{Ar}), 5.07 (1H, d, *J* 12.4 Hz, PhCH_2), 5.03 (1H, d, *J* 12.4 Hz, PhCH_2), 4.61 (1H, ddd, *J* 45.3, 5.2, 3.7 Hz, $H_{6\beta}$), 4.47 (1H, tt, *J* 11.1, 4.3 Hz, $H_{3\beta}$), 3.97 (1H, br. s, $H_{7\beta}$), 1.11 (9H, s, $(CH_3)_3C$), 0.86 (3H, s, H_{19}), 0.86 (3H, d, *J* 6.8 Hz, H_{21}), 0.58 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 177.9 (^{t}Bu*C*OO), 173.8 (BnO*C*O), 136.0 (C_{Ar}), 128.4 (C_{Ar}), 128.05 (C_{Ar}), 127.99 (C_{Ar}), 91.4 (d, *J* 178.6 Hz, C_6), 73.2 (C_3), 69.8 (d, *J* 16.1 Hz, C_7), 65.9, 55.6, 49.7, 45.9 (d, *J* 16.1 Hz), 42.5, 39.1, 38.4, 37.8 (d, *J* 4.4 Hz), 36.1 (d, *J* 7.3 Hz), 35.1, 35.0, 32.5, 31.1, 30.8, 28.2 (d, *J* 4.4 Hz), 27.9, 27.0, 26.5, 23.3, 22.8, 20.5, 18.1, 11.5 ppm; **^{19}F NMR** (282 MHz, $CDCl_3$) δ -197.1 (1F, br. d, *J* 45.1 Hz, $F_{6\alpha}$) ppm; **MS** (ESI+) *m/z* 607.2 $[M+Na]^+$, 1191.7 $[2M+Na]^+$; **HRMS** (ESI+) for $C_{36}H_{53}FNaO_5$ $[M+Na]^+$ calcd 607.3769, found 607.3765 (0.7 ppm error).

Synthesis of 3α-pivaloyloxy-6α-fluoro-7α-hydroxy-5β-cholanic acid (4.64), and 6α-fluoro chenodeoxycholic acid (4.53)

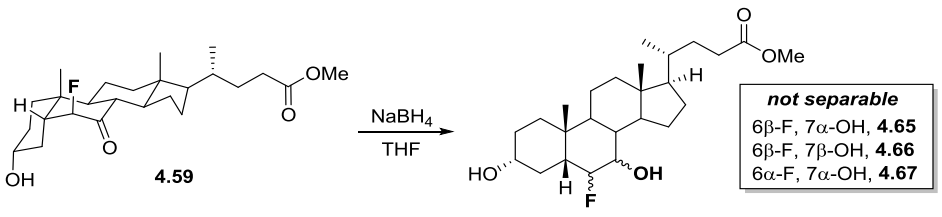


To a round-bottom flask were added benzyl 3α-pivaloyloxy-6α-fluoro-7α-hydroxy-5β-cholan-24-oate **4.63** (68 mg, 0.116 mmol, 1.0 eq), KOH (190 mg, 3.39 mmol, 29.2 eq), MeOH (8 mL) and H_2O (2.5 mL). The reaction mixture was stirred at room temperature overnight, followed by the removal of solvent. The residue was purified by flash chromatography (acetone/petroleum ether 10:90 – 15:85; MeOH/DCM 10:90 – 30:70) to afford **4.64** (47 mg, 0.095 mmol, 82%) and **4.53** (*impure*, 13%*, *calculated yield) as white solids. The full conversion of **4.63** to **4.53** was achieved by stirring at room temperature for 12 d with good yield (85%).

4.64, **Formula** $C_{29}H_{47}FO_5$; **MW** 494.68; **$[\alpha]_D$** +22.4 (c 0.4, MeOH, 20 °C); **IR** (neat) 3498 (br. w), 2932 (m), 2867 (m), 1728 (s), 1710 (s), 1456 (m), 1376 (m), 1285 (m), 1158 (s), 1035 (m), 995 (m), 687 (m) cm^{-1}; **^{1}H NMR** (400 MHz, CD_3OD) δ 4.64 (1H, ddd, *J* 45.0, 5.6, 3.5 Hz, $H_{6\beta}$), 4.49 (1H, tt, *J* 11.4, 4.4 Hz, $H_{3\beta}$), 3.92 - 4.02 (1H, m, $H_{7\beta}$), 1.17 (9H, s, $(CH_3)_3C$), 0.97 (3H, d, *J* 6.9 Hz, H_{21}),0.96 (3H, s, H_{19}), 0.70 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 179.9, 179.2, 92.6 (d, *J* 180.0 Hz, C_6), 75.6 (C_3), 70.9 (d, *J* 17.6 Hz, C_7), 57.4, 51.2, 47.9 (d, *J* 16.1 Hz), 43.8, 40.9, 39.8, 39.6 (d, *J* 5.9 Hz), 37.5 (d, *J* 7.3 Hz), 36.9, 36.3, 34.1, 32.6, 32.6 (overlapping of CH_2 x 2, confirmed by DEPT 135 integration and HSQC), 29.8 (d, *J* 4.4 Hz), 29.3, 27.9, 27.7, 24.6, 23.7, 21.9, 19.0, 12.3 ppm; **^{19}F NMR** (282 MHz, CD_3OD) δ -196.5 (1F, br. d, *J* 45.1 Hz, $F_{6\alpha}$) ppm; **MS** (ESI-) *m/z* 529.3 ([M+^{35}Cl]$^-$, 100%); **HRMS** (ESI+) for $C_{29}H_{47}FNaO_5$ $[M+Na]^+$ calcd 517.3300, found 517.3303 (-0.6 ppm error).

4.53, **Formula** $C_{24}H_{39}FO_4$; **MW** 410.56; **IR** (neat) 3417 (br. w), 2942 (s), 2870 (s), 1709 (s), 1472 (w), 1447 (w), 1378 (w), 1040 (m), 1015 (m), 907 (s), 731 (s) cm^{-1}; **^{1}H NMR** (400 MHz, CD_3OD) δ 4.62 (1H, ddd, *J* 45.3, 5.6, 3.5 Hz, $H_{6\beta}$), 3.90 - 4.01 (1H, m, $H_{7\beta}$), 3.32 - 3.40 (1H, m, $H_{3\beta}$), 2.27 - 2.41 (1H, m), 0.96 (3H, d, *J* 6.5 Hz, H_{21}), 0.93 (3H, s, H_{19}), 0.70 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, CD_3OD) δ 178.8 (C_{24}), 92.9 (d, *J* 179.0 Hz, C_6), 72.4 (C_3), 70.9 (d, *J* 16.7 Hz, C_7), 57.4 (*C*H), 51.2 (*C*H), 48.1 (d, *J* 16.3 Hz, *C*H), 43.8 (*C*), 40.9 (CH_2), 39.6 (d, *J* 6.1 Hz, *C*H), 37.4 (d, *J* 7.9 Hz, *C*), 36.9 (*C*H), 36.8 (CH_2), 34.1 (*C*H), 33.4 (d, *J* 3.7 Hz, CH_2), 32.5 (CH_2), 32.4 (CH_2), 31.4 (CH_2), 29.3 (CH_2), 24.6 (CH_2), 23.8 (C_{19}), 21.9 (CH_2), 19.0 (C_{21}), 12.3 (C_{18}) ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ -196.4 (1F, br. d, *J* 45.5 Hz, $F_{6\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, CD_3OD) δ -196.4 (1F, s, $F_{6\alpha}$) ppm; **MS** (ESI-) *m/z* 445.3 [M+^{35}Cl]$^-$; **HRMS** (ESI+) for $C_{24}H_{39}FNaO_4$ $[M+Na]^+$ calcd 433.2725, found 433.2725 (-0.1 ppm error).

Synthesis of methyl 3α,7α-dihydroxy-6β-fluoro-5β-cholan-24-oate (4.65), methyl 3α,7β-dihydroxy-6β-fluoro-5β-cholan-24-oate (4.66), and methyl 3α,7α-dihydroxy-6α-fluoro-5β-cholan-24-oate (4.67)

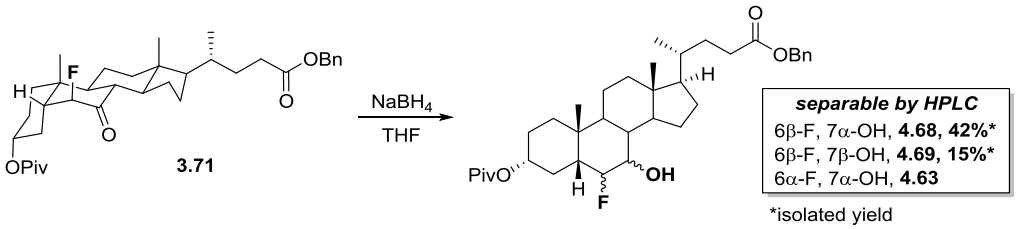


Following general procedure G, fluoroketone **4.59** (340 mg) was treated with $NaBH_4$. This led to three isomers **4.65-4.67** (*not separable*).

4.65/4.66/4.67, **Formula** $C_{25}H_{41}FO_4$; **MW** 424.60; **^{1}H NMR** (400 MHz, $CDCl_3$) δ *characteristic peaks only:* 4.69 (ddd, *J* 45.2, 5.7, 3.5 Hz, $H_{6\beta}$ of **4.67**), 4.51 (dt, *J* 50.1, 2.8 Hz, $H_{6\alpha}$ of **4.66**), 4.44 (dt, *J* 44.5, 2.2 Hz, $H_{6\alpha}$ of **4.65**) ppm[assignment based on their 3α-pivaloyloxy derivatives]; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -172.4 (dt, *J* 44.2, 13.4 Hz, $F_{6\beta}$ of **4.65**), -188.0 (ddd, *J* 46.4, 29.9, 14.7 Hz, $F_{6\beta}$ of **4.66**), -197.2

(br. d, *J* 45.5 Hz, $F_{6\alpha}$ of **4.67**) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -172.4 (s, $F_{6\beta}$ of **4.65**), -188.0 (s, $F_{6\beta}$ of **4.66**), -197.2 (s, $F_{6\alpha}$ of **4.67**) ppm; **MS** (ESI+) 447.5 $[M+Na]^+$ can be observed.

Synthesis of benzyl 3α-pivaloyloxy-6β-fluoro-7α-hydroxy-5β-cholan-24-oate (4.68), and benzyl 3α-pivaloyloxy-6β-fluoro-7β-hydroxy-5β-cholan-24-oate (4.69)

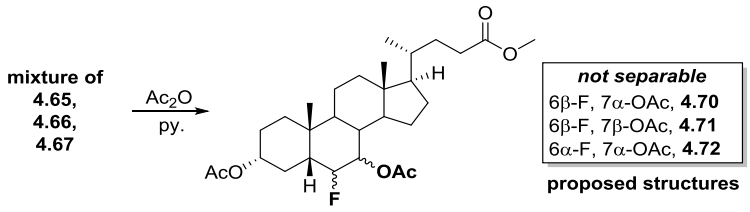


Following general procedure G, fluoroketone intermediate **3.71** was reduced by $NaBH_4$. The crude mixture was purified by flash chromatography (acetone/petroleum ether 5:95) to afford **4.68** (33 mg, 42%*) and **4.69** (12 mg, 15%*, *isolated yield) as gummy solids. By-product **4.63** was also formed when this reaction was repeated on a larger scale, and separation between three isomers were achieved by HPLC purification.

4.68, **Formula** $C_{36}H_{53}FO_5$; **MW** 584.80; **$[\alpha]_D$** +19.4 (c 0.5, $CHCl_3$, 21 °C); **R_f** 0.30 (acetone/hexane 10:90); **IR** (neat) 3529(w), 2941(s), 2873(m), 1722(s), 1457(m), 1288(m), 1163(s), 984(m), 701(w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 - 7.42 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, PhCH_2), 5.10 (1H, d, *J* 12.4 Hz, PhCH_2), 4.58 (1H, tt, *J* 10.9, 4.5 Hz, $H_{3\beta}$), 4.41 (1H, dt, *J* 44.5, 2.0 Hz, $H_{6\alpha}$), 3.88 (1H, br. d, *J* 9.6 Hz, $H_{7\beta}$), 2.35 - 2.46 (1H, m), 2.23 - 2.35 (1H, m), 1.17 (9H, s, (CH_3)$_3$C), 1.02 (3H, d, *J* 3.0 Hz, H_{19}), 0.94 (3H, d, *J* 6.4 Hz, H_{21}), 0.68 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 178.2 (^{t}Bu*C*OO), 174.0 (BnO*C*O), 136.1 (C_{Ar}), 128.5 (C_{Ar}), 128.22 (C_{Ar}), 128.18 (C_{Ar}), 95.2 (d, *J* 172.7 Hz, C_6), 73.3 (C_3), 69.6 (d, *J* 30.7 Hz, C_7), 66.1, 55.8, 49.7, 45.2 (d, *J* 19.0 Hz), 42.7, 39.4, 38.6, 35.4, 35.3, 35.1, 34.4, 32.6, 31.3, 31.0, 30.1 (d, *J* 11.7 Hz), 28.1, 27.1, 26.3, 24.5 (d, *J* 5.9 Hz), 23.5, 20.3, 18.2, 11.7 ppm; **^{19}F NMR** (282 MHz, *CDCl$_3$*) δ -172.7 (1F, dt, *J* 45.1, 11.8 Hz, $F_{6\beta}$) ppm; **MS** (ESI+) *m/z* 607.3 $[M+Na]^+$, 1191.9 $[2M+Na]^+$; **HRMS** (ESI+) for $C_{36}H_{53}FNaO_5$ $[M+Na]^+$ calcd 607.3769, found 607.3778 (-1.4 ppm error).

4.69, **Formula** $C_{36}H_{53}FO_5$; **MW** 584.80; **R_f** 0.27 (acetone/hexane 10:90); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.32 - 7.39 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2), 5.10 (1H, d, *J* 12.3 Hz, PhCH_2), 4.59 - 4.74 (1H, m, $H_{3\beta}$), 4.49 (1H, dt, *J* 50.0, 2.7 Hz, $H_{6\alpha}$), 3.48 (1H, dtd, $^3J_{F6\beta\text{-}H7\alpha}$ 28.8, $^3J_{H8\beta\text{-}H7\alpha}$ 10.1, $^3J_{H(OH)\text{-}H7\alpha}$ 10.1 [confirmed by ^{1}H NMR after D_2O shake: 3.47 (1H, ddd, *J* 29.4, 10.2, 2.8 Hz, $H_{7\alpha}$)], $^3J_{H6\alpha\text{-}H7\alpha}$ 3.2 Hz, $H_{7\alpha}$), 2.35 - 2.48 (1H, m), 2.22 - 2.35 (1H, m), 1.18 (9H, s, (CH_3)$_3$C), 1.03 (3H, d, *J* 3.8 Hz, H_{19}), 0.93 (3H, d, *J* 6.1 Hz, H_{21}), 0.68 (3H, s, H_{18}) ppm; **^{19}F NMR** (282 MHz, *CDCl$_3$*) δ -188.4 (1F, ddd, *J* 49.0, 29.0, 14.0 Hz, $F_{6\beta}$) ppm; **MS** (ESI+) *m/z* 607.6 ($[M+Na]^+$, 79%); **HRMS** (ESI+) for $C_{36}H_{53}FNaO_5$ $[M+Na]^+$, calcd 607.3769, found 607.3779 (-1.6 ppm error); **Other analysis** ^{1}H NMR after D_2O-shake.

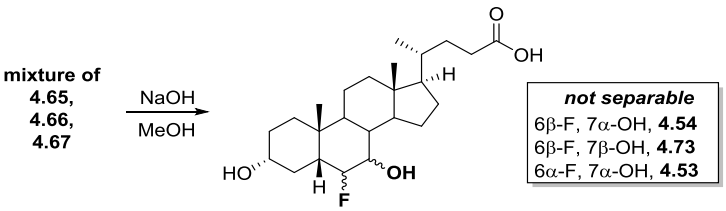
Synthesis of methyl 3α,7α-diacetoxy-6β-fluoro-5β-cholan-24-oate (4.70), methyl 3α,7β-diacetoxy-6β-fluoro-5β-cholan-24-oate (4.71), and methyl 3α,7α-diacetoxy-6α-fluoro-5β-cholan-24-oate (4.72)



A mixture of isomers **4.65/4.66/4.67** (10 mg) was acetylated by using acetic anhydride (0.5 mL) in pyridine (2 mL). The reaction was completed after stirring at room temperature overnight. The reaction was quenched by adding water, followed by extraction with EtOAc (× 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated to afford a mixture of **4.70-4.72** (not separable).

4.70/4.71/4.72, proposed structures based on NMR analysis: **^{1}H NMR** indicated formation of 3 pairs of acetate methyl peaks at 2.13, 2.103, 2.096, 2.06, 2.05, and 2.03 ppm; $H_{3\beta}$ and $H_{7\alpha/\beta}$ moved from lower than 4 ppm to higher chemical shift range; **^{19}F NMR** indicated formation of three major new fluorine peaks at -173.1, -186.8 and -197.0 ppm, with similar multiplicity compared to their 3,7-dihydroxy derivatives;

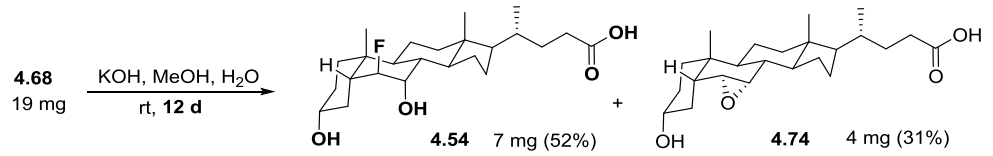
Attempted synthesis of 6β-fluoro CDCA (4.54), 6β-fluoro UDCA(4.73), and 6α-fluoro CDCA(4.53)



A mixture of isomers **4.65-4.67** (40 mg) was hydrolysed in a solution of NaOH (200 mg) in MeOH (2 mL). Upon completion, the solvent was removed under reduced pressure, and acidified by aq. 2 M HCl solution, followed by extraction with EtOAc (× 3). The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated to afford a mixture of compounds **4.54**, **4.73** and **4.53** (*not separable*).

4.54/**4.73**/**4.53**, **Selected ^{1}H NMR** (400 MHz, CD_3OD) δ *characteristic peaks only:* 4.62 (ddd, *J* 45.4, 5.5, 3.4 Hz, $H_{6\beta}$ of **4.53**), 4.39 (dt, J 49.8, 2.5 Hz, $H_{6\alpha}$ of **4.73**), 4.36 (dt, *J* 45.5, 1.9 Hz, $H_{6\alpha}$ of **4.54**) ppm [assignment based on their 3α-pivaloyloxy derivatives]; **^{19}F NMR** (376 MHz, CD_3OD) δ -172.4 (ddd, *J* 44.6, 15.6, 10.0 Hz, $F_{6\beta}$ of **4.54**), -187.0 (ddd, J 47.7, 31.2, 14.3 Hz, $F_{6\beta}$ of **4.73**), -197.0 (d, *J* 45.5 Hz, $F_{6\alpha}$ of **4.53**) ppm.

Synthesis of 6β-fluoro chenodeoxycholic acid (4.54), and 3α-hydroxy-6α,7α-epoxy-5β-cholanic acid (4.74)

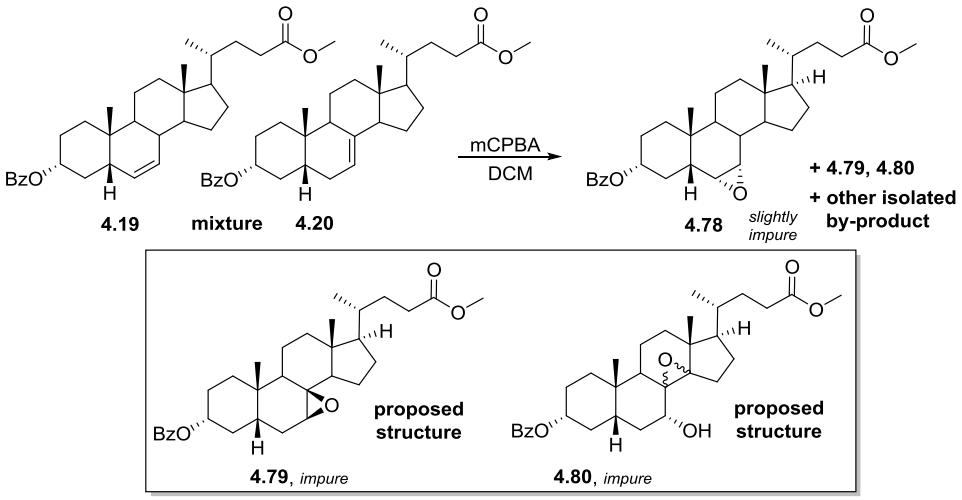


To a round-bottom flask were added benzyl 3α-pivaloyloxy-6β-fluoro-7α-hydroxy-5β-cholan-24-oate **4.68** (19 mg, 0.0325 mmol), KOH (50 mg), MeOH (6 mL) and H_2O (2 mL). The reaction mixture was stirred at room temperature for 12 d, followed by the removal of solvent. The residue was purified by flash chromatography (DCM/MeOH 95:5-90:10-70:30) to afford the desired product **4.54** (7 mg, 0.017 mmol, 52%) and by-product **4.74** (4 mg, 0.01 mmol, 31%).

4.54, **Formula** $C_{24}H_{39}FO_4$; **MW** 410.56; **^{1}H NMR** (300 MHz, CD_3OD) δ 4.36 (1H, dt, *J* 45.2, 2.4 Hz, $H_{6\alpha}$), 3.73 (1H, br. d, *J* 10.0 Hz, $H_{7\beta}$), 3.36 - 3.47 (1H, m, $H_{3\beta}$), 2.25 - 2.39 (1H, m), 0.94 - 1.00 (6H, m, H_{19} + H_{21}), 0.72 (3H, s, H_{18}) ppm; **^{13}C NMR** N/A sample (7 mg) not enough for good ^{13}C NMR spectrum in CD_3OD; **^{19}F NMR** (282 MHz, CD_3OD) δ -172.0 (1F, ddd, *J* 45.1, 16.1, 11.8 Hz, $F_{6\beta}$) ppm; **MS** (ESI-) *m/z* 445.3 ([M+^{35}Cl]$^-$, 100%); **HRMS** (ESI+) for $C_{24}H_{39}FNaO_4$ [M+Na]$^+$ calcd 433.2725, found 433.2721 (0.7 ppm error).

4.74, **Formula** $C_{24}H_{38}O_4$; **MW** 390.56; **IR** (neat) 3414 (br. w), 2922 (s), 2868 (s), 1714 (s), 1455 (m), 1374 (w), 1257 (w), 1058 (w), 917 (w), 740 (m) cm^{-1}; **^{1}H NMR** (300 MHz, CD_3OD) δ 3.44 - 3.61 (1H, m, $H_{3\beta}$), 3.05 - 3.20 (2H, m, $H_{6\beta}$ + $H_{7\beta}$), 2.27 - 2.43 (1H, m), 0.96 (3H, d, *J* 6.5 Hz, H_{21}), 0.85 (3H, s, H_{19}), 0.75 (3H, s, H_{18}) ppm; **^{13}C NMR** N/A sample (4 mg) not enough for good ^{13}C NMR spectrum in CD_3OD; **MS** (ESI-) *m/z* 425.3 ([M+^{35}Cl]$^-$, 100%); **HRMS** (ESI+) for $C_{24}H_{38}NaO_4$ [M+Na]$^+$ calcd 413.2662, found 413.2659 (0.8 ppm error); **Other analysis** X-ray analysis included in the Appendices.

Synthesis of methyl 3α-benzoyloxy-6α,7α-epoxy-5β-cholan-24-oate (4.78), methyl 3α-benzoyloxy-7β,8β-epoxy-5β-cholan-24-oate (4.79), and by-product 01 (4.80)



To a round-bottom flask were added a mixture of **4.19/4.20** (188 mg, 0.382 mmol, 1.0 eq), DCM (5 mL), and then a solution of mCPBA (≤ 77%, 94 mg, 0.42 mmol, 1.1 eq) in DCM (5 mL) at 5 °C. The reaction mixture was then warmed to room temperature and stirred overnight. Upon completion, the reaction mixture was washed with sat. aq. $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated. The crude mixture (more 7 spots by TLC analysis) was purified by flash chromatography (acetone/petroleum ether 10:90 – 15:85 – 20:80 – 30:70 – 100:0) to afford *impure* compounds **4.78**, **4.79** and **4.80**, as well as other unidentified by-products.

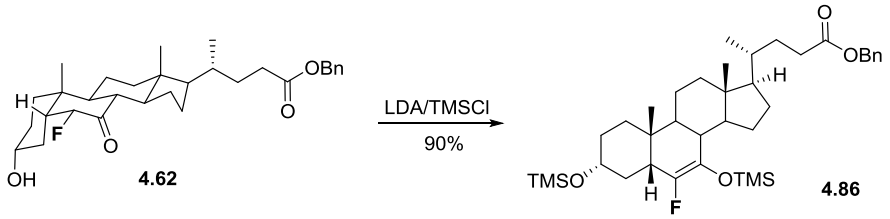
4.78, **Formula** $C_{32}H_{44}O_5$; **MW** 508.70; **IR** (neat) 3069 (w), 2939 (s), 2868 (m), 1736 (s), 1716 (s), 1603 (w), 1585 (w), 1448 (m), 1317 (m), 1270 (s), 1179 (m), 1108 (m), 1022 (w), 714 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.04 (2H, d, *J* 7.2 Hz, H_{Ar}), 7.52 - 7.57 (1H, m, H_{Ar}), 7.40 - 7.47 (2H, m, H_{Ar}), 4.97 (1H, tt, *J* 11.2, 4.2 Hz, $H_{3\beta}$), 3.68 (3H, s, $C_{24}OCH_3$), 3.09 - 3.16 (2H, m, $H_{6\beta}$ + $H_{7\beta}$), 2.37 (1H, ddd, *J* 15.4, 10.1, 5.2 Hz, H_{23}), 2.23 (1H, ddd, *J* 16.0, 9.4, 6.5 Hz, H_{23}'), 0.94 (3H, d, *J* 6.3 Hz, H_{21}), 0.88 (3H, s, H_{19}), 0.72 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (C_{24}), 166.1 (Ph*C*O), 132.7 (CH_{Ar}), 130.7 (C_{Ar}), 129.5 (CH_{Ar}), 128.2 (CH_{Ar}), 73.4 (C_3), 55.6 (*C*H), 55.3 (C_6 or C_7), 54.1 (C_7 or C_6), 51.8 (*C*H), 51.5 ($C_{24}OCH_3$), 43.2 (*C*), 40.2 (*C*H), 39.6 (CH_2), 35.8 (*C*H), 35.4 (*C*H), 34.8 (*C*H), 33.9 (CH_2), 32.6 (*C*), 31.1 (CH_2), 31.0 (CH_2), 29.6 (CH_2), 28.3 (CH_2), 26.4 (CH_2), 23.9 (C_{19}), 23.5 (CH_2), 20.2 (CH_2), 18.2 (C_{21}), 11.9 (C_{18}) ppm; **MS** (ESI+) 531.4 ($[M+Na]^+$, 56%); **HRMS** (ESI+) for $C_{32}H_{44}NaO_5$ $[M+Na]^+$, calcd 531.3081, found 531.3093 (-2.2 ppm error).

4.79, **Formula** $C_{32}H_{44}O_5$; **MW** 508.70; **IR** (neat) 2947 (m), 2872 (m), 1732 (s), 1712 (s), 1603 (w), 1583 (w), 1448 (m), 1314 (w), 1278 (s), 1171 (m), 1112 (m), 1033 (w), 903 (s), 730 (s), 718 (s), 659 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.05 (2H, d, *J* 7.5 Hz, H_{Ar}), 7.52 - 7.59 (1H, m, H_{Ar}), 7.39 - 7.48 (2H, m, H_{Ar}), 4.94 (1H, tt, *J* 11.1, 5.1 Hz, $H_{3\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.79 (1H, d, *J* 5.7 Hz, $H_{7\alpha}$), 1.01 (3H, s, H_{19}), 0.94 (3H, d, *J* 6.4 Hz, H_{21}), 0.85 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.7 (C_{24}), 166.0 (Ph*C*O), 132.8 (CH_{Ar}), 130.7 (C_{Ar}), 129.5 (CH_{Ar}), 128.2 (CH_{Ar}), 73.9 (C_3), 62.1 (C_8), 56.1 (*C*H), 52.7 (*C*H), 51.5 ($C_{24}OCH_3$), 51.3 (C_7), 45.4 (*C*), 40.5 (CH_2), 39.2 (*C*H), 36.7 (*C*H), 35.5 (CH_2), 35.0 (*C*H), 34.6 (CH_2), 33.3 (*C*), 31.0 (CH_2), 30.8 (CH_2), 28.7 (CH_2), 27.6 (CH_2), 26.9 (CH_2), 23.5 (C_{19}), 20.1 (CH_2), 19.0 (CH_2), 18.2 (C_{21}), 12.8 (C_{18}) ppm; **MS** (ESI+) 531.4 ($[M+Na]^+$, 100%); **HRMS** (ESI+) for $C_{32}H_{44}NaO_5$ $[M+Na]^+$, calcd 531.3081, found 531.3078 (0.6 ppm error).

4.80, **Formula** $C_{32}H_{44}O_6$; **MW** 524.70; **IR** (neat) 3501 (br. w), 2938 (s), 2864 (m), 1732 (s), 1716 (s), 1603 (w), 1583 (w), 1434 (m), 1323 (m), 1274 (s), 1176 (m), 1115 (m), 1086 (m), 894 (s), 731 (s), 711 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.96 (2H, d, *J* 7.2 Hz, H_{Ar}), 7.42 - 7.49 (1H, m, H_{Ar}), 7.31 - 7.39 (2H, m, H_{Ar}), 4.82 (1H, tt, *J* 11.4, 4.5 Hz, $H_{3\beta}$), 3.60 (3H, s, $C_{24}OCH_3$), 3.54 (1H, d, *J* 2.8 Hz, $H_{7\beta}$), 0.95 (3H, s, H_{19}), 0.87 (3H, s, H_{18}), 0.86 (3H, d, *J* 6.5 Hz, H_{21}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5 (C_{24}), 166.1 (Ph*C*O), 132.6 (CH_{Ar}), 130.8 (C_{Ar}), 129.5 (CH_{Ar}), 128.2 (CH_{Ar}), 83.1 (*C*), 74.3 (*C*H), 68.6 (*C*), 67.1 (*C*H), 52.9 (*C*H), 51.5 ($C_{24}OCH_3$), 42.6 (*C*), 40.6 (*C*H), 37.0 (*C*), 34.5 (*C*H), 34.4 (CH_2), 34.2 (CH_2), 31.6 (CH_2), 31.4 (CH_2), 30.9 (CH_2), 30.5 (CH_2), 29.2 (*C*H), 27.0 (CH_2), 26.3 (CH_2), 26.0 (CH_2), 25.5 (CH_3),

18.32 (*C*H_2), 18.26 (*C*H_3), 14.4 (*C*H_3) ppm; **MS** (ESI+) 547.4 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{32}H_{44}NaO_6$ [M+Na]$^+$, calcd 547.3030, found 547.3026 (0.8 ppm error);

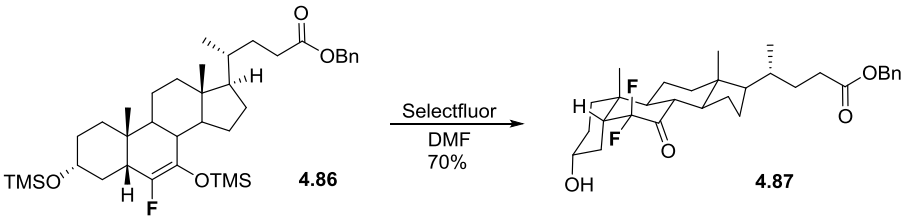
Synthesis of benzyl 3α,7-bis(trimethylsilyloxy)-6-fluoro-5β-cholan-6-en-24-oate (4.86)



Following general procedure C, intermediate **4.86** was prepared from fluoroketone **4.62** with a good yield (90%), and used directly for next step.

4.86, **Formula** $C_{37}H_{59}FO_4Si_2$; **MW** 643.03; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.30 - 7.40 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2), 5.10 (1H, d, *J* 12.3 Hz, PhCH_2), 3.54 (1H, tt, *J* 10.9, 4.2 Hz, $H_{3\beta}$), 2.36 - 2.47 (1H, m), 2.23 - 2.35 (1H, m), 0.92 (3H, d, *J* 6.3 Hz, H_{21}), 0.86 (3H, s, H_{19}), 0.66 (3H, s, H_{18}), 0.17 (9H, d, $^6J_{F\text{-}H}$ 1.7 Hz, CF=COSi(CH_3)$_3$), 0.12 (9H, s, CHOSi(CH_3)$_3$) ppm; **Selected ^{13}C NMR** (101 MHz, $CDCl_3$) δ 0.5 (d, $^5J_{F\text{-}C}$ 3.5 Hz, CF=COSi(*C*H_3)$_3$) ppm; **^{19}F NMR** (282 MHz, CDCl3) δ -135.2 (1F, br. s, C=C*F*) ppm; **^{19}F {^{1}H} NMR** (282 MHz, CDCl3) δ -135.2 (1F, s, C=C*F*) ppm.

Synthesis of benzyl 3α-hydroxy-6,6-difluoro-7-keto-5β-cholan-24-oate (4.87)

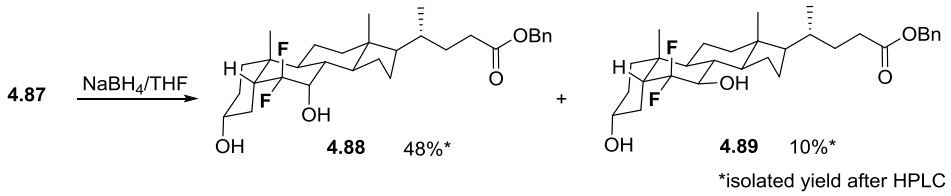


Following general procedure F, intermediate **4.86** was fluorinated by Selectfluor. The crude mixture was purified by flash chromatography (acetone/petroleum ether 15:85-20:80) and HPLC (acetone/hexane 20:80) to afford **4.87** (70%) as a white solid.

4.87, **Formula** $C_{31}H_{42}F_2O_4$; **MW** 516.66; **R_f** 0.21 (acetone/hexane 20:80); **IR** (neat) 3412 (br. w), 2935 (s), 2870 (m), 1747 (s), 1462 (m), 1383 (w), 1253 (m), 1195 (m), 1148 (s), 1061 (s), 733 (m), 704 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.28 - 7.43 (5H, m, H_{Ar}), 5.13 (1H, d, *J* 12.2 Hz, PhCH_2), 5.09 (1H, d, *J* 12.3 Hz, PhCH_2), 3.51 - 3.69 (1H, m, $H_{3\beta}$), 2.82 (1H, td, *J* 11.4, 5.4 Hz, $H_{8\beta}$), 2.35 - 2.47 (1H, m), 1.24 (3H, d, *J* 4.2 Hz, H_{19}), 0.92 (2H, d, *J* 6.3 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 200.0 (dd, *J* 28.2, 22.0 Hz, C_7), 173.9 (C_{24}), 136.0 (C_{Ar}), 128.5 (*C*H$_{Ar}$), 128.2 (*C*H$_{Ar}$), 128.1 (*C*H$_{Ar}$), 117.4 (dd, *J* 260.2, 249.4 Hz, C_6), 69.5 (C_3), 66.1 (Ph*C*H$_2$), 54.6, 52.1 (dd, *J* 19.7, 17.1 Hz), 48.2, 46.4, 42.9, 42.5, 38.5, 35.5 (d, *J* 5.5 Hz), 35.0, 34.5, 31.2, 30.8, 30.5 (t, *J* 5.5 Hz), 29.4, 28.0, 24.2, 24.1 (d, *J* 7.0 Hz, C_{19}), 21.4, 18.2 (C_{21}), 11.9 (C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -101.0 - -100.0 (1F, m [d, *J* 248.8 Hz observed], $F_{6\beta}$), -117.4 (1F, br. d, *J* 248.4 Hz, $F_{6\alpha}$) ppm; **^{19}F {^{1}H} NMR** (282 MHz, $CDCl_3$) δ -

100.6 (1F, d, *J* 249.3 Hz, $F_{6\beta}$), -117.5 (1F, d, *J* 248.8 Hz, $F_{6\alpha}$) ppm; **MS** (ESI+) *m/z* 1033.6 ([2M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{31}H_{46}F_2NO_4$ [M+NH_4]$^+$ calcd 534.3389, found 534.3387 (0.4 ppm error);

Synthesis of benzyl 3α,7α-dihydroxy-6,6-difluoro-5β-cholan-24-oate (4.88), and benzyl 3α,7β-dihydroxy-6,6-difluoro-5β-cholan-24-oate (4.89)



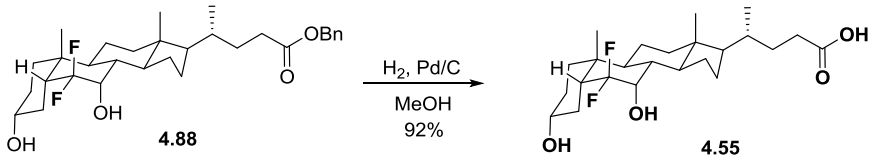
Following general procedure G, fluoroketone **4.87** was reduced by $NaBH_4$. The crude mixture was purified by flash chromatography (actone/petroleum ether 18:82-20:80-35:65) and HPLC (acetone/hexane 22:78) to afford **4.88** (48%*) and **4.89** (10%*, *isolated yield after HPLC).

4.88, **Formula** $C_{31}H_{44}F_2O_4$; **MW** 518.68; **R_f** 0.25 (acetone/hexane 25:75); **IR** (neat) 3427 (br. w), 2940 (m), 2875 (w), 1721 (m), 1458 (w), 1379 (w), 1260 (w), 1166 (m), 1047 (s), 1022 (m), 906 (s), 730 (s), 643 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.28 - 7.43 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.4 Hz, PhCH_2O), 5.09 (1H, d, *J* 12.3 Hz, PhCH_2O), 3.62 - 3.79 (1H, m, $H_{7\beta}$), 3.48 (1H, tt, *J* 10.9, 4.3 Hz, $H_{3\beta}$), 2.60 - 2.72 (1H, m), 2.35 - 2.47 (1H, m), 2.23 - 2.34 (1H, m), 0.95 (3H, d, *J* 4.3 Hz, H_{19}), 0.93 (3H, d, *J* 6.4 Hz, H_{21}), 0.65 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.1 (C_{24}), 136.0 (C_{Ar}), 128.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.1 (CH_{Ar}), 122.7 (dd, *J* 249.8, 245.6 Hz, C_6), 71.0 (dd, *J* 36.1, 22.4 Hz, C_7), 70.7 (C_3), 66.1 (CH_2Ph), 55.6 (*C*H), 49.4 (*C*H), 48.7 (t, *J* 19.2 Hz, *C*H), 42.5 (*C*), 39.1 (CH_2), 36.5 (d, *J* 5.0 Hz, *C*H), 35.7 (d, *J* 6.2 Hz, *C*), 35.3 (CH_2), 35.2 (*C*H), 32.5 (*C*H), 32.0 (dd, *J* 7.3, 4.0 Hz, CH_2), 31.2 (CH_2), 30.9 (CH_2), 30.2 (CH_2), 28.0 (CH_2), 24.2 (d, *J* 6.6 Hz, C_{19}), 23.3 (CH_2), 20.4 (CH_2), 18.2 (C_{21}), 11.6 (C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -95.1 (1F, br. d, *J* 258.4 Hz, $F_{6\beta}$), -104.7 (1F, br. d, *J* 257.5 Hz, $F_{6\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -95.1 (1F, d, *J* 257.9 Hz, $F_{6\beta}$), -104.7 (1F, d, *J* 257.9 Hz, $F_{6\alpha}$) ppm; **MS** (ESI+) *m/z* 519.3 ([M+H]$^+$, 27%); 1037.6 ([2M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{31}H_{48}F_2NO_4$ [M+NH_4]$^+$ calcd 536.3546, found 536.3558 (-2.3 ppm error).

4.89, **Formula** $C_{31}H_{44}F_2O_4$; **MW** 518.68; **R_f** 0.22 (acetone/hexane 25:75); **IR** (neat) 3379 (br. m), 2943 (s), 2862 (s), 1733 (s), 1457 (m), 1167 (s), 1122 (s), 1042 (s), 919 (m), 741 (s), 692 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 - 7.41 (5H, m, H_{Ar}), 5.14 (1H, d, *J* 12.3 Hz, PhCH_2), 5.10 (1H, d, *J* 12.4 Hz, PhCH_2), 3.46 - 3.76 (2H, m, $H_{7\alpha}$ + $H_{3\beta}$), 2.35 - 2.47 (1H, m), 2.22 - 2.35 (1H, m), 0.99 (3H, d, *J* 4.8 Hz, H_{19}), 0.92 (3H, d, *J* 6.3 Hz, H_{21}), 0.66 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.0 (C_{24}), 136.1 (C_{Ar}), 128.5 (CH_{Ar}), 128.2 (CH_{Ar}), 128.2 (CH_{Ar}), 123.3 (t, *J* 246.1 Hz, C_6), 73.1 (t, *J* 21.6 Hz, C_7), 70.2 (C_3), 66.1 (PhCH_2), 55.4 (*C*H), 54.9 (*C*H), 49.0 (dd, *J* 20.4, 18.2 Hz, *C*H), 43.7 (*C*), 40.7 (d, *J* 6.1 Hz, *C*H), 39.7 (CH_2), 39.3 (*C*H), 35.2 (*C*H), 35.11 (*C*), 35.07 (CH_2), 32.4 (dd, *J* 7.3, 4.4 Hz, CH_2), 31.3 (CH_2), 30.9 (CH_2), 29.9 (CH_2), 28.4 (CH_2), 26.8 (CH_2), 24.3 (d, *J* 7.5 Hz, C_{19}), 20.7 (CH_2), 18.3 (C_{21}), 12.0 (C_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -106.6 (1F, br. d, *J* 244.1 Hz, $F_{6\alpha}$), -108.2 (1F, dt, *J* 243.2, 14.7 Hz, $F_{6\beta}$) ppm;

^{19}F NMR (376 MHz, $CDCl_3$) δ -106.6 (1F, d, *J* 243.6 Hz, $F_{6\alpha}$), -108.2 (1 F, d, *J* 243.2 Hz, $F_{6\beta}$) ppm; **MS** (ESI+) *m/z* 1037.6 ([2M+H]$^+$, 100%); **HRMS** (ESI+) for $C_{31}H_{48}F_2NO_4$ [M+NH_4]$^+$ calcd 536.3546, 536.3552 (-1.2 ppm error).

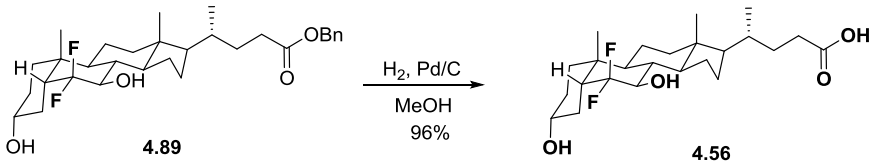
Synthesis of 6,6-difluoro chenodeoxycholic acid (4.55)



Following general procedure E, benzyl group of intermediate **4.88** was cleaved by hydrogenolysis to afford the desired compound **4.55** (92%).

4.55, **Formula** $C_{24}H_{38}F_2O_4$; **MW** 428.55; **IR** (neat) 3384 (br. w), 2941 (s), 2862 (s), 1710 (s), 1469 (w), 1454 (w), 1382 (w), 1296 (w), 1076 (w), 1047 (s), 1019 (m), 633 (w) cm^{-1}; **^{1}H NMR** (500 MHz, CD_3OD) δ 3.57 - 3.64 (1H, m, $H_{7\beta}$), 3.39 (1H, tt, *J* 11.3, 3.8 Hz, $H_{3\beta}$), 2.27 - 2.40 (1H, m), 2.08 - 2.26 (2H, m), 2.03 (1H, dt, *J* 12.5, 3.5 Hz), 0.97 (3H, d, *J* 6.6 Hz, H_{21}), 0.95 (3H, d, *J* 4.3 Hz, H_{19}), 0.71 (3H, s, H_{18}) ppm; **^{13}C NMR** (126 MHz, CD_3OD) δ 178.5 (C_{24}), 124.1 (dd, *J* 252.3, 242.7 Hz, C_6), 71.8 (dd, *J* 35.9, 21.8 Hz, C_7), 71.7 (C_3), 57.4 (*C*H), 50.9 (*C*H), 50.6 (t, *J* 19.4 Hz, *C*H), 43.7 (*C*), 40.8 (*C*H_2), 38.4 (d, *J* 5.4 Hz, *C*H), 37.0 (d, *J* 6.1 Hz, *C*), 36.9 (*C*H), 36.7 (*C*H_2), 34.0 (*C*H), 33.2 (dd, *J* 7.5, 4.1 Hz, *C*H_2), 32.5 (*C*H_2), 32.3 (*C*H_2), 31.2 (*C*H_2), 29.3 (*C*H_2), 25.1 (d, *J* 6.8 Hz, C_{19}), 24.5 (*C*H_2), 21.7 (*C*H_2), 18.9 (C_{21}), 12.2 (C_{18}) ppm; **^{19}F NMR** (376 MHz, CD_3OD) δ -95.1 (1F, br. d, *J* 256.6 Hz, $F_{6\beta}$), -105.2 (1F, d, *J* 255.8 Hz, $F_{6\alpha}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, CD_3OD) δ -95.1 (1F, d, *J* 256.6 Hz, $F_{6\beta}$), -105.2 (1F, d, *J* 255.8 Hz, $F_{6\alpha}$) ppm; **MS** (ESI+) *m/z* 857.4 ([2M+H]$^+$, 100%); **HRMS** (ESI+) $C_{24}H_{42}F_2NO_4$ [M+NH_4]$^+$ calcd 446.3076, found 446.3076 (0.2 ppm error).

Synthesis of 6,6-difluoro ursodeoxycholic acid (4.56)



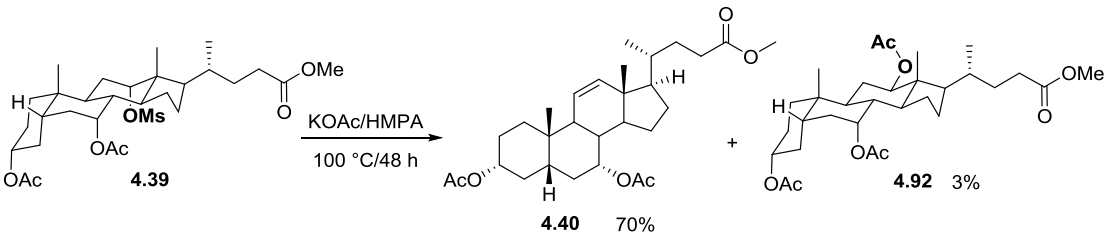
Following general procedure E, benzyl group of intermediate **4.89** was cleaved by hydrogenolysis to afford the desired compound **4.56** (96%).

4.56, **Formula** $C_{24}H_{38}F_2O_4$; **MW** 428.56; **IR** (neat) 3362 (br. w), 2933 (s), 2868 (s), 2492 (br. w), 1708 (s), 1450 (w), 1381 (w), 1283 (w), 1115 (s), 1042 (s), 1013 (m), 972 (m), 702 (m) cm^{-1}; **^{1}H NMR** (500 MHz, CD_3OD) δ 3.45 - 3.65 (2H, m, $H_{7\alpha}$ + $H_{3\beta}$), 2.33 (1H, ddd, *J* 15.2, 9.9, 5.2 Hz, H_{23}), 2.20 (1H, ddd, *J* 15.7, 9.3, 6.6 Hz, H_{23}'), 2.06 (1H, dt, *J* 12.6, 3.1 Hz), 0.99 (3H, d, $^5J_{F6\text{-}H19}$ 4.6 Hz, H_{19}), 0.97 (3H, d, *J* 6.6 Hz, H_{21}), 0.73 (3H, s, H_{18}) ppm; **^{1}H {^{19}F} NMR** (500 MHz, CD_3OD) δ 3.58 (1H, dd, $^3J_{H8\beta\text{-}H7\alpha}$ 10.3 Hz, $^3J_{H(OH)\text{-}H7\alpha}$ 3.7 Hz, $H_{7\alpha}$), 3.51 (1H, tt, *J* 11.0, 4.3 Hz, $H_{3\beta}$), 2.33 (1H, ddd, *J* 15.2, 9.9, 5.2 Hz, H_{23}), 2.20

(1H, ddd, *J* 15.8, 9.2, 6.7 Hz, H_{23}'), 2.06 (1H, dt, *J* 12.6, 3.1 Hz), 0.99 (3H, s, H_{19}), 0.97 (3H, d, *J* 6.5 Hz, H_{21}), 0.73 (3H, s, H_{18}) ppm; **^{13}C NMR** (126 MHz, CD_3OD) δ 178.7 (C_{24}), 124.7 (t, *J* 245.6 Hz, C_6), 73.8 (t, *J* 21.2 Hz, C_7), 71.1 (C_3), 57.1 (*C*H), 56.6 (*C*H), 50.8 (t, *J* 18.4 Hz, *C*H), 45.0 (*C*), 41.7 (d, *J* 6.0 Hz, *C*H), 41.3 (CH_2), 41.0 (*C*H), 36.8 (*C*H), 36.4 (CH_2), 36.4 (*C*, overlapped with another CH_2 [confirmed by full NMR analysis and ^{13}C NMR integration]), 33.5 (dd, *J* 7.4, 4.1 Hz, CH_2), 32.6 (CH_2), 32.5 (CH_2), 30.9 (CH_2), 29.7 (CH_2), 27.9 (CH_2), 25.0 (d, *J* 7.4 Hz, C_{19}), 22.2 (CH_2), 19.1 (C_{21}), 12.7 (C_{18}) ppm; **^{19}F NMR** (471 MHz, CD_3OD) δ -106.9 (1F, br. d, $^2J_{F6\beta\text{-}F6\alpha}$ 245.9 Hz, $F_{6\alpha}$), -108.6 - -107.6 (1F, m [a doublet with $^2J_{F6\alpha\text{-}F6\beta}$ 245.9 Hz can be observed], $F_{6\beta}$) ppm; **^{19}F {^{1}H} NMR** (471 MHz, CD_3OD) δ -106.9 (1F, d, $^2J_{F6\beta\text{-}F6\alpha}$ 246.4 Hz, $F_{6\alpha}$), -108.0 (1F, d, $^2J_{F6\alpha\text{-}F6\beta}$ 246.1 Hz, $F_{6\beta}$) ppm; **MS** (ESI-) *m/z* 427.6 ($[M-H]^-$, 36%), 856.2 ($[2M-H]^-$, 16%); **HRMS** (ESI-) for $C_{24}H_{38}ClF_2O_4$ $[M+Cl]^-$ calcd 463.2432, found 463.2431 (0.3 ppm error).

7.4.3 Towards the synthesis of C-11 fluorinated C-ring BA analogues

Synthesis of methyl 3α,7α,12β-triacetoxy-5β-cholan-24-oate (4.92)

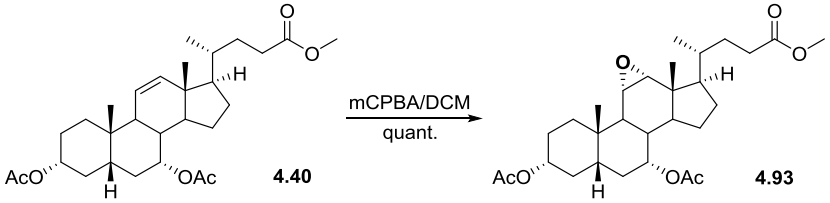


To a round-bottom flask were added **4.39** (1.78 g, 3.04 mmol, 1.0 eq), KOAc (7.12 g, 24 eq) and HMPA (18 mL, 6 mL/mmol). The reaction mixture was heat at 100 °C under argon for 48 h. Upon completion, the mixture was poured into icy water (200 mL) and left at 0 °C for product(s) to solidify, followed by filtration and rinsing with water. The crude mixture was purified by using a Biotage apparatus (acetone/heptane) to afford the desired product **4.40** (1.04 g, 2.13 mmol, 70%, white solid), and by-product **4.92** (50 mg, 0.091 mmol, 3%, gummy solid).

4.92, **Formula** $C_{31}H_{48}O_8$; **MW** 548.72; **IR** (neat) 2958 (m), 2876 (w), 1716 (s), 1434 (w), 1356 (m), 1238 (s), 1172 (w), 1066 (w), 1021 (m), 976 (w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.89 (1H, q, *J* 2.7 Hz, $H_{7\beta}$), 4.69 (1H, dd, *J* 11.1, 4.9 Hz, $H_{12\alpha}$), 4.56 (1H, tt, *J* 11.3, 4.5 Hz, $H_{3\beta}$), 3.65 (3H, s, $C_{24}OCH_3$), 2.14 - 2.43 (2H, H_{23} + H_{23}'), 2.04 (3H, s, CH_3COOC_3 or CH_3COOC_7 or CH_3COOC_{12}), 2.02 (3H, s, CH_3COOC_3 or CH_3COOC_7 or CH_3COOC_{12}), 2.01 (3H, s, CH_3COOC_3 or CH_3COOC_7 or CH_3COOC_{12}), 0.93 (3H, s, H_{19}), 0.87 (3H, d, *J* 6.7 Hz, H_{21}), 0.78 (3H, s, H_{18}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.3 (C_{24}), 170.6 (CH_3COOC_3 or CH_3COOC_7 or CH_3COOC_{12}), 170.5 (CH_3COOC_3 or CH_3COOC_7 or CH_3COOC_{12}), 170.2 (CH_3COOC_3 or CH_3COOC_7 or CH_3COOC_{12}), 80.7 (C_{12}), 73.7 (C_3), 70.6 (C_7), 56.4 (*C*H), 51.4 ($C_{24}OCH_3$), 48.7 (*C*H), 46.3 (*C*), 40.6 (*C*H), 36.7 (*C*H), 34.8 (*C*, overlapped with CH_2, confirmed by full NMR analysis), 34.8 (CH_2), 34.5 (CH_2), 33.0 (*C*H), 32.6 (*C*H), 32.2 (CH_2), 31.2 (CH_2), 29.5 (CH_2), 26.74 (CH_2),

26.66 (*C*H$_2$), 24.0 (*C*H$_2$), 23.0 (*C*H$_2$), 22.4 (*C*$_{19}$), 21.47 (*C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$ or *C*H$_3$COOC$_{12}$), 21.45 (*C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$ or *C*H$_3$COOC$_{12}$), 21.38 (*C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$ or *C*H$_3$COOC$_{12}$), 20.6 (*C*$_{21}$), 8.5 (*C*$_{18}$) ppm; **MS** (ESI+) *m/z* 571.4 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{31}H_{48}NaO_8$ [M+Na]$^+$, calcd 571.3241, found 571.3233 (1.4 ppm error).

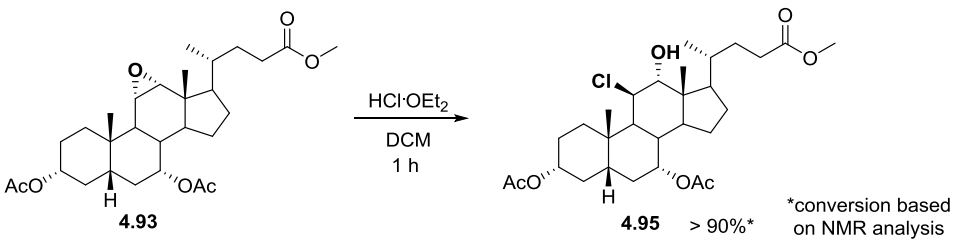
Synthesis of methyl 3α,7α-diacetoxy-11α,12α-epoxy-5β-cholan-24-oate (4.93)



To a round-bottom flask were added **4.40** (1.0 g, 2.05 mmol, 1.0 eq), DCM (15 mL), and mCPBA (≤70.9%, 548 mg, 2.25 mmol, 1.1 eq) at 0 °C. The resulting reaction mixture was warmed to room temperature and stirred overnight. Upon completion, sat. aq. NaHCO$_3$ solution (20 mL) was added, followed by extraction with DCM (25 mL × 3). The combined organic layer was washed with brine, dried over MgSO$_4$ and concentrated to afford the desired product **4.93** quantitatively.

4.93, **Formula** $C_{29}H_{44}O_7$; **MW** 504.66; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.86 (1H, q, *J* 2.1 Hz, *H*$_{7β}$), 4.61 (1H, tt, *J* 11.4, 4.5 Hz, *H*$_{3β}$), 3.67 (3H, s, C$_{24}$OC*H*$_3$), 3.16 (1H, d, *J* 4.0 Hz, *H*$_{11β}$ or *H*$_{12β}$), 2.99 (1H, d, *J* 4.1 Hz, *H*$_{11β}$ or *H*$_{12β}$), 2.04 (3H, s, C*H*$_3$COOC$_3$ or C*H*$_3$COOC$_7$), 2.03 (3H, s, C*H*$_3$COOC$_3$ or C*H*$_3$COOC$_7$), 1.05 (3H, d, *J* 6.1 Hz, *H*$_{21}$), 1.00 (3H, s, *H*$_{19}$), 0.79 (3H, s, *H*$_{18}$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.4 (*C*$_{24}$), 170.6 (CH$_3$*C*OOC$_3$ or CH$_3$*C*OOC$_7$), 170.3 (CH$_3$*C*OOC$_3$ or CH$_3$*C*OOC$_7$), 73.5 (*C*$_3$), 70.6 (*C*$_7$), 60.5 (*C*$_{12}$ or *C*$_{11}$), 53.2 (*C*$_{11}$ or *C*$_{12}$), 51.5 (C$_{24}$O*C*H$_3$), 50.1 (*C*H), 43.6 (*C*), 42.1 (*C*H), 40.5 (*C*H), 35.0 (*C*H), 35.0 (*C*H$_2$, overlapping confirmed by full DEPT analysis and ^{13}C NMR integration), 34.9 (*C*), 34.8 (*C*H), 34.7 (*C*H$_2$), 34.3 (*C*H), 31.5 (*C*H$_2$), 30.8 (*C*H$_2$), 30.6 (*C*H$_2$), 27.1 (*C*H$_2$), 26.8 (*C*H$_2$), 23.4 (*C*$_{19}$), 21.55 (*C*H$_2$), 21.50 (*C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$), 21.4 (*C*H$_3$COOC$_3$ or *C*H$_3$COOC$_7$), 18.2 (*C*$_{21}$), 11.8 (*C*$_{18}$) ppm; NMR data were consistent with the literature values.[138]

Synthesis of methyl 3α,7α-diacetoxy-11β-chloro-12α-hydroxy-5β-cholan-24-oate (4.95)

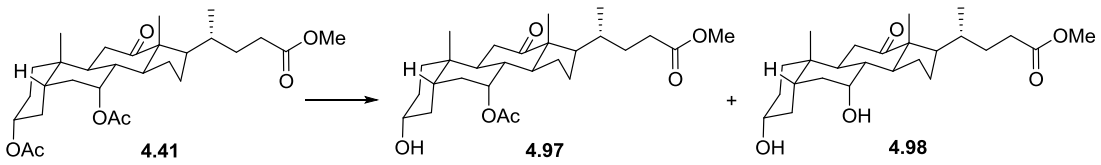


To a round-bottom flask were added **4.93** (20 mg, 0.04 mmol, 1 eq), DCM (1 mL) and HCl (2 M in Et$_2$O, 60 µL, 3 eq). The resulting mixture was stirred at room temperature for 1 h. Upon completion, the reaction was quenched with sat. aq. NaHCO$_3$ solution, followed by extraction with DCM (× 3).

The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated to afford compound **4.95** with high conversion (>90%) based on NMR analysis.

4.95, **Formula** $C_{29}H_{45}ClO_7$; **MW** 541.12; **IR** (neat) 3499 (br. w), 2947 (s), 2864 (m), 1732 (s), 1444 (w), 1377 (m), 1246 (s), 1053 (m), 1018 (m), 895 (m), 730 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.99 (1H, q, *J* 2.7 Hz, $H_{7\beta}$), 4.63 (2H, tt, *J* 11.3, 4.2 Hz, $H_{3\beta}$), 4.30 (1H, dd, *J* 4.5, 2.4 Hz, $H_{11\alpha}$), 4.22 (1H, br. s, $H_{12\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.66 (1H, dd, *J* 11.4, 4.5 Hz, $H_{9\alpha}$), 2.38 (1H, ddd, *J* 15.8, 10.0, 5.2 Hz, H_{23}), 2.25 (1H, ddd, *J* 16.3, 9.7, 6.8 Hz, H_{23}'), 2.06 (3H, s, CH_3COOC_3 or CH_3COOC_7), 2.04 (3H, s, CH_3COOC_3 or CH_3COOC_7), 1.25 (3H, s, H_{19} or H_{18}), 1.00 (3H, s, H_{19} or H_{18}), 0.997 (3H, d, *J* 6.3 Hz, H_{21}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.5 (C_{24}), 170.6 (CH_3COOC_3 or CH_3COOC_7), 170.2 (CH_3COOC_3 or CH_3COOC_7), 78.1 (C_{12}), 73.9 (C_3), 71.0 (C_7), 61.8 (C_{11}), 51.5 ($C_{24}OCH_3$), 48.4 (*C*H), 45.1 (*C*), 44.2 (*C*H), 42.7 (*C*H), 35.4 (*C*), 35.0 (*C*H), 34.7 (*C*H), 34.5 (CH_2), 34.0 (*C*H), 34.0 (CH_2, overlapping confirmed by full DEPT analysis and ^{13}C NMR integration), 31.0 (CH_2), 30.7 (CH_2), 30.6 (CH_2), 27.2 (CH_2), 26.8 (CH_2), 26.5 (CH_3), 23.0 (CH_2), 21.6 (CH_3COOC_3 or CH_3COOC_7), 21.4 (CH_3COOC_3 or CH_3COOC_7), 17.3 (CH_3), 13.8 (CH_3) ppm; **MS** (ESI+) *m/z* 563.4 ($[C_{29}H_{45}{}^{35}ClO_7 + Na]^+$, 100%), 565.4 ($[C_{29}H_{45}{}^{37}ClO_7 + Na]^+$, 32%); **HRMS** (ESI+) for $C_{29}H_{45}ClNaO_7$ $[M+Na]^+$, calcd 563.2746, found 563.2747 (-0.1 ppm error).

Synthesis of methyl 3α-hydroxy-7α-acetoxy-12-keto-5β-cholan-24-oate (4.97), and methyl 3α,7α-dihydroxy-12-keto-5β-cholan-24-oate (4.98)



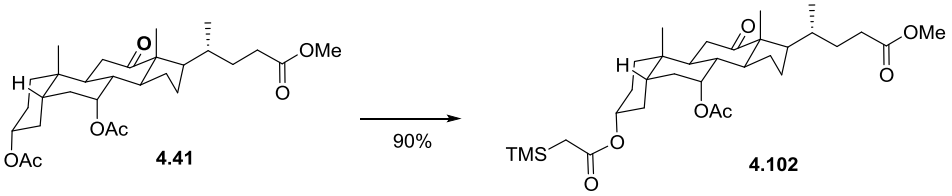
To a round-bottom flask were added **4.41** (5.94 mmol), MeOH (50 mL) and NaOMe (25% w/w in MeOH, 1.54 mL). The resulting mixture was stirred at room temperature over 4 d. Water was then added to the reaction mixture, followed by extraction with DCM (× 3). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/heptane 15% - 40%, 20 CV) to afford the desired product **4.98** (1.69 g, 4.02 mmol, 68%), and also compound **4.97** (0.4 g, 0.86 mmol, 15%) as white solids.

4.97, **Formula** $C_{27}H_{42}O_6$; **MW** 462.63; **IR** (neat) 3450 (br. w), 2929 (s), 2864 (m), 1732 (s), 1704 (s), 1458 (w), 1446 (m), 1381 (m), 1246 (s), 1225 (s), 1168 (m), 1078 (m), 1017 (m), 960 (w), 735 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.97 (1H, q, *J* 3.0 Hz, $H_{7\beta}$), 3.66 (3H, s, $C_{24}OCH_3$), 3.48 (1H, tt, *J* 11.0, 4.1 Hz, $H_{3\beta}$), 2.50 (1H, t, *J* 12.6 Hz), 2.02 (3H, s, CH_3COOC_7), 0.97 - 1.04 (6H, m, H_{18} + H_{19}), 0.85 (3H, d, *J* 6.6 Hz, H_{21}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 213.9 (C_{12}), 174.5 (C_{24}), 170.3 (CH_3COOC_7), 71.3 (C_3), 70.6 (C_7), 57.0 (C_{13}), 53.0 (*C*H), 51.4 ($C_{24}OCH_3$), 46.3 (*C*H), 40.6 (*C*H), 38.8 (CH_2), 37.9 (*C*H), 37.8 (*C*H), 37.6 (CH_2), 35.48 (*C*H), 35.46 (C_{10}), 35.2 (CH_2), 31.4 (CH_2), 31.2 (CH_2), 30.5 (CH_2), 30.4 (CH_2), 27.3 (CH_2), 23.7 (CH_2), 22.1 (C_{18} or C_{19}), 21.4 (CH_3COOC_7), 18.5 (C_{21}), 11.5 (C_{18} or C_{19}) ppm; **MS** (ESI+)

m/z 463.4 ([M+H]$^+$, 90%), 485.4 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{27}H_{42}NaO_6$ [M+Na]$^+$, calcd 485.2874, found 485.2885 (-2.3 ppm error).

4.98, **Formula** $C_{25}H_{40}O_5$; **MW** 420.59; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.91 - 3.98 (1H, m, $H_{7\beta}$), 3.67 (3H, s, OCH_3), 3.45 (1H, tt, *J* 11.1, 4.3 Hz, $H_{3\beta}$), 1.03 (3H, s, H_{18} or H_{19}), 1.00 (3H, s, H_{18} or H_{19}), 0.86 (3H, d, *J* 6.6 Hz, H_{21}) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 214.3 (C_{12}), 174.7 (C_{24}), 71.6 (C_3 or C_7), 68.0 (C_3 or C_7), 57.0 (C_{13}), 53.2 (*C*H), 51.5 (O*C*H$_3$), 46.3 (*C*H), 41.1 (*C*H), 39.7 (*C*H$_2$), 39.3 (*C*H), 37.7 (*C*H$_2$), 36.8 (*C*H), 35.8 (C_{10}), 35.6 (*C*H), 35.3 (*C*H$_2$), 35.0 (*C*H$_2$), 31.3 (*C*H$_2$), 30.6 (*C*H$_2$), 30.5 (*C*H$_2$), 27.5 (*C*H$_2$), 23.8 (*C*H$_2$), 22.2 (*C*H$_3$), 18.6 (*C*H$_3$), 11.5 (*C*H$_3$) ppm; NMR data were consistent with the literature values.[221],[222]

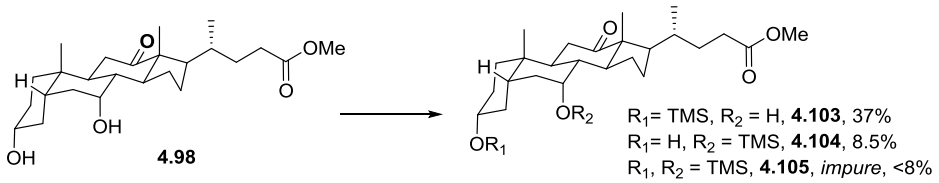
Synthesis of methyl 3α-trimethylsilylacetoxy-7α-acetoxy-12-keto-5β-cholan-24-oate (4.102)



To a round-bottom flask were added **4.41** (505 mg, 1.0 mmol, 1.0 eq), DCM (20 mL, 20 mL/mmol), TEA (0.28 mL, 2.0 mmol, 2.0 eq), and then TMSOTf (0.2 mL, 1.1 mmol, 1.1 eq) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 2 h. Upon completion, the reaction was quenched with sat. aq. $NaHCO_3$ solution (20 mL), followed by extraction with DCM (× 4). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/heptane) to afford only by-product **4.102** (90%) as a white solid, which was further recrystallised from actone/heptane solution. The desired silyl enol ether product was not obtained.

4.102, **Formula** $C_{32}H_{52}O_7Si$; **MW** 576.85; **IR** (neat) 2942 (m), 2860 (w), 1732 (s), 1708 (s), 1442 (w), 1377 (w), 1250 (s), 1234 (s), 1086 (s), 1009 (m), 845 (s), 727 (w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.94 (1H, q, *J* 2.7 Hz, $H_{7\beta}$), 4.52 (1H, tt, *J* 11.4, 3.9 Hz, $H_{3\beta}$), 3.61 (3H, s, C_{24}OCH_3), 2.47 (1H, t, *J* 12.7 Hz), 1.95 (3H, s, CH_3COOC_7), 1.83 (1H, d, *J* 11.5 Hz, TMSC*H*H'), 1.79 (1H, d, *J* 11.7 Hz, TMSCH*H*'), 0.987 (3H, s, H_{19} or H_{18}), 0.985 (3H, s, H_{19} or H_{18}), 0.81 (3H, d, *J* 6.5 Hz, H_{21}), 0.06 (9H, s, (CH_3)$_3$Si) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 213.8 (C_{12}), 174.3 (C_{24}), 172.2 (TMSCH$_2$*C*OO), 169.9 (CH3*C*OOC_7), 72.7 (C_3), 70.4 (C_7), 56.9 (C_{13}), 52.9 (*C*H), 51.3 (C_{24}O*C*H$_3$), 46.2 (*C*H), 40.4 (*C*H), 37.71 (*C*H), 37.70 (*C*H), 37.5 (*C*H$_2$), 35.4 (C_{10}), 35.3 (*C*H), 34.9 (*C*H$_2$), 34.8 (*C*H$_2$), 31.2 (*C*H$_2$), 31.0 (*C*H$_2$), 30.3 (*C*H$_2$), 27.2 (*C*H$_2$), 27.0 (*C*H$_2$), 26.7 (*C*H$_2$), 23.6 (*C*H$_2$), 22.0 (C_{18} or C_{19}), 21.2 (*C*H$_3$COOC_7), 18.4 (C_{21}), 11.3 (C_{18} or C_{19}), -1.4 ((*C*H$_3$)$_3$Si) ppm; **MS** (ESI+) *m/z* 599.4 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{32}H_{52}NaO_7Si$ [M+Na]$^+$, calcd 599.3375, found 599.3373 (0.3 ppm error); **Other analysis:** X-Ray structural analysis included in the Appendices.

Synthesis of methyl 3α-trimethylsilyloxy-7α-hydroxy-12-keto-5β-cholan-24-oate (4.103), methyl 3α-hydroxy-7α-trimethylsilyloxy-12-keto-5β-cholan-24-oate (4.104), methyl 3α,7α-bis(trimethylsilyloxy)-12-keto-5β-cholan-24-oate (4.105)



Following general procedure C, compound **4.98** (300 mg, 0.71 mmol) was treated with LDA and TMSCl. However, no desired silyl enol ether product was formed. Instead, by-products **4.103** (130 mg, 0.264 mmol, 37%), **4.104** (30 mg, 0.061 mmol, 8.5%), and **4.105** (30 mg, *impure*, <8%) were obtained as gummy solids.

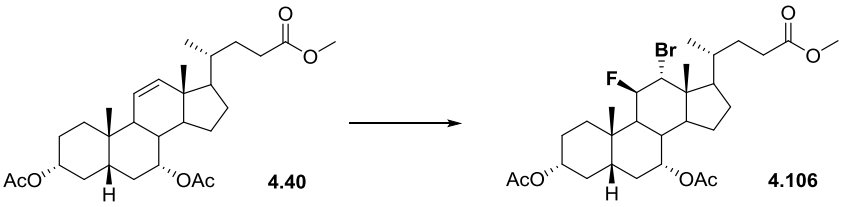
4.103, **Formula** $C_{28}H_{48}O_5Si$; **MW** 492.77; **IR** (neat) 3408 (br. w), 2931 (s), 2864 (s), 1743 (s), 1708 (s), 1432 (m), 1254 (m), 1175 (m), 1081 (s), 998 (m), 730 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.82 - 3.93 (1H, m, $H_{7\beta}$), 3.61 (3H, s, OCH_3), 3.38 (1H, tt, *J* 10.9, 4.4 Hz, $H_{3\beta}$), 0.97 (3H, s, H_{19} or H_{18}), 0.94 (3H, s, H_{19} or H_{18}), 0.81 (3H, d, *J* 6.6 Hz, H_{21}), 0.04 (9H, s, (CH_3)$_3$Si) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 213.9 (C_{12}), 174.5 (C_{24}), 72.0 (C_3), 67.8 (C_7), 56.8 (C_{13}), 53.0 (*C*H), 51.3 (O*C*H$_3$), 46.2 (*C*H), 41.1 (*C*H), 39.7 (*C*H$_2$), 39.2 (*C*H), 37.5 (*C*H$_2$), 36.6 (*C*H), 35.7 (C_{10}), 35.5 (*C*H), 35.4 (*C*H$_2$), 34.9 (*C*H$_2$), 31.2 (*C*H$_2$), 30.6 (*C*H$_2$), 30.4 (*C*H$_2$), 27.4 (*C*H$_2$), 23.7 (*C*H$_2$), 22.1 (C_{19} or C_{18}), 18.4 (C_{21}), 11.4 (C_{19} or C_{18}), 0.2 ((*C*H$_3$)$_3$Si) ppm; **MS** (ESI+) *m/z* 421.3 ([M-TMS+H+H]$^+$, 100%) [-OTMS not stable in acidic column of RP-HPLC]; **HRMS** (ESI+) for $C_{25}H_{40}NaO_5$ [M-TMS+H+Na]$^+$, calcd 443.2768, found 443.2775 (-1.7 ppm error) [-OTMS not stable in acidic column of RP-HPLC].

4.104, **Formula** $C_{28}H_{48}O_5Si$; **MW** 492.77; **IR** (neat) 3440 (br. w), 2962 (m), 2925 (m), 2872 (m), 1732 (s), 1708 (s), 1438 (m), 1377 (w), 1250 (s), 1164 (m), 1074 (s), 1009 (s), 939 (m), 833 (s), 723 (m) cm^{-1}; 3.89 (1H, q, *J* 2.8 Hz, $H_{7\beta}$), 3.66 (3H, s, OCH_3), 3.42 (1H, tt, *J* 11.0, 4.3 Hz, $H_{3\beta}$), 1.01 (3H, s, H_{19} or H_{18}), 0.99 (3H, s, H_{19} or H_{18}), 0.86 (3H, d, *J* 6.3 Hz, H_{21}), 0.09 (9H, s, (CH_3)$_3$Si) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 214.6 (C_{12}), 174.6 (C_{24}), 71.8 (C_3), 69.0 (C_7), 56.8 (C_{13}), 53.2 (*C*H), 51.4 (OCH_3), 46.2 (*C*H), 41.4 (*C*H), 40.1 (*C*H), 40.0 (*C*H$_2$), 37.7 (*C*H$_2$), 36.8 (*C*H), 35.8 (C_{10}), 35.6 (*C*H), 35.5 (*C*H$_2$), 34.9 (*C*H$_2$), 31.3 (*C*H$_2$), 30.5 (*C*H$_2$), 30.5 (*C*H$_2$, overlapped with another *C*H$_2$ at 30.5 ppm and confirmed by ^{13}C NMR integration as well as comparision with its 3α-trimethylsilyloxy regioisomer and unsilyated 7α-OH analogue), 27.4 (*C*H$_2$), 24.0 (*C*H$_2$), 22.2 (C_{19} or C_{18}), 18.6 (C_{21}), 11.7 (C_{19} or C_{18}), 0.6 ((*C*H$_3$)$_3$Si) ppm; **MS** (ESI+) *m/z* 515.4 ([M+Na]$^+$, 100%); **HRMS** (ESI+) for $C_{28}H_{48}NaO_5Si$ [M+Na]$^+$, calcd 515.3163, found 515.3163 (-0.0 ppm error).

4.105, **Formula** $C_{31}H_{56}O_5Si_2$; **MW** 564.95; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.88 (1H, q, *J* 2.6 Hz, $H_{7\beta}$), 3.66 (3H, s, OCH_3), 3.40 (1H, tt, *J* 10.9, 4.6 Hz, $H_{3\beta}$), 0.99 (3H, s, H_{18} or H_{19}), 0.97 (3H, s, H_{18} or H_{19}), 0.85 (3H, d, *J* 6.6 Hz, H_{21}), 0.09 (9H, s, (CH_3)$_3$SiOC$_3$ or (CH_3)$_3$SiOC$_7$), 0.08 (9H, s, (CH_3)$_3$SiOC$_3$ or (CH_3)$_3$SiOC$_7$) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ [characteristic peaks only due to the presence of impurities]

214.4 (C_{12}), 174.6 (C_{24}), 72.2 (C_3), 69.0 (C_7), 56.7 (C_{13}), 53.3 (*C*H), 51.4 (O*C*H$_3$), 22.2 (C_{18} or C_{19}), 18.5 (C_{21}), 11.7 (C_{18} or C_{19}), 0.6 ((*C*H$_3$)$_3$SiOC$_3$ or (*C*H$_3$)$_3$SiOC$_7$), 0.3 ((*C*H$_3$)$_3$SiOC$_3$ or (*C*H$_3$)$_3$SiOC$_7$) ppm; **MS** (ESI+) *m/z* 515.4 ([M-TMS+H+Na]$^+$, 100%), 587.5 ([M+Na]$^+$, 100%) [-OTMS not stable in acidic column of RP-HPLC]; **HRMS** (ESI+) for $C_{28}H_{48}NaO_5Si$ [M-TMS+H+Na]$^+$, calcd 515.3163, found 515.3161 (0.4 ppm error) [-OTMS not stable in acidic column of RP-HPLC].

Synthesis of methyl 3α,7α-diacetoxy-11β-fluoro-12α-bromo-5β-cholan-24-oate (4.106)



To a round-bottom flask were added Et_2O (0.6 mL), HF.py (0.2 mL) and NBS (22 mg, 0.123 mmol, 1.21 eq). The mixture was stirred at room temperature for 5 min. A solution of **4.40** (50 mg, 0.1 mmol, 1.0 eq) in Et_2O (1 mL) was then dropwise added. After stirring for another 2 h, the reaction was quenched by adding sat. aq. $NaHCO_3$ solution, followed by extraction with DCM (× 3). The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude mixture was purified by using a Biotage apparatus (acetone/heptane) to afford the desired compound **4.106** (*not separable from impurities*).

4.106, **Formula** $C_{29}H_{44}BrFO_6$; **MW** 587.57; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.15 (1H, dt, $^2J_{F11\beta\text{-}H11\alpha}$ 48.2 Hz, $^3J_{H9\alpha\text{-}H11\alpha}$ 3.1 Hz, $^3J_{H12\beta\text{-}H11\alpha}$ 3.1 Hz, $H_{11\alpha}$ [**^{1}H {H$_{9\alpha}$} NMR** (500 MHz, $CDCl_3$) δ 5.15 (1H, dd, $^2J_{F11\beta\text{-}H11\alpha}$ 48.9 Hz, $^3J_{H12\beta\text{-}H11\alpha}$ 2.4 Hz)]), 4.96 - 5.03 (1H, m, $H_{7\beta}$), 4.59 - 4.66 (1H, m, $H_{3\beta}$), 4.57 (1H, dd, $^3J_{F11\beta\text{-}H12\beta}$ 11.9 Hz, $^3J_{H11\alpha\text{-}H12\beta}$ 2.8 Hz, $H_{12\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.62 (1H, ddd, $^3J_{F11\beta\text{-}H9\alpha}$ 43.9 Hz, $^3J_{H8\beta\text{-}H9\alpha}$ 11.6 Hz, $^3J_{H11\alpha\text{-}H9\alpha}$ 2.6 Hz, $H_{9\alpha}$), 2.17 - 2.46 (2H, m, H_{23} + H_{23}'), 2.09 (3H, s, C*H*$_3$COOC$_3$ or C*H*$_3$COOC$_7$), 2.05 (3H, s, C*H*$_3$COOC$_3$ or C*H*$_3$COOC$_7$), 1.08 (3H, d, $^5J_{F12\beta\text{-}H19}$ 4.0 Hz, H_{19}), 1.03 (3H, d, $^3J_{H20\text{-}H21}$ 6.3 Hz, H_{21}), 0.97 (3H, d, $^5J_{F12\beta\text{-}H18}$ 4.8 Hz, H_{18}) ppm; **^{1}H {^{19}F} NMR** (500 MHz, $CDCl_3$) δ 5.15 (1H, t, *J* 2.7 Hz, $H_{11\alpha}$), 4.98 - 5.03 (1H, m, $H_{7\beta}$), 4.58 - 4.65 (1H, m, $H_{3\beta}$), 4.57 (1H, d, *J* 2.7 Hz, $H_{12\beta}$), 3.67 (3H, s, $C_{24}OCH_3$), 2.62 (1H, dd, *J* 11.7, 2.8 Hz, $H_{9\alpha}$), 2.20 - 2.45 (2H, m, H_{23} + H_{23}'), 2.09 (3H, s, C*H*$_3$COOC$_3$ or C*H*$_3$COOC$_7$), 2.06 (3H, s, C*H*$_3$COOC$_3$ or C*H*$_3$COOC$_7$), 1.08 (3H, s, H_{19}), 1.03 (3H, d, *J* 6.5 Hz, H_{21}), 0.97 (3H, s, H_{18}) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -162.7 - -162.3 (1F, m [coupling constants of 47.7 Hz, 12.3 Hz and 4.4 Hz can be observed], $F_{11\beta}$) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -162.5 (1F, s, $F_{11\beta}$) ppm; **MS** (ESI+) *m/z* 609.3 ([$C_{29}H_{44}{}^{79}BrFNaO_6$]$^+$, 60%), 611.1 ([$C_{29}H_{44}{}^{81}BrFNaO_6$]$^+$, 77%); **HRMS** (ESI+) for $C_{29}H_{44}BrFNaO_6$ [M+Na]$^+$, calcd 609.2198, found 609.2180 (2.8 ppm error); NMR data were consistent with the reported values.[145]

Chapter 8: Experimental for Lipophilicity

8.1 Detailed log*P* determination protocols

1) Partitioning

To a 10 mL pear-shaped flask was added octanol (HPLC grade, ca. 2 mL), compound A (ca. 1–10 mg), reference compound R (ca. 1–10 mg, *primary reference 2,2,2-trifluoroethanol*) and water (HPLC grade, ca. 2 mL). The resulting biphasic mixture was stirred at 25 °C (temperature controlled by recirculating chiller) for 2 hours and then left to stand at 25 °C overnight to enable complete phase separation. In some cases the formation of a foam was observed, which resided at the octanol-water phase boundary. In this case, the vial was centrifuged for until disappearance of the foam.

2) Sample preparation

Using two 1 mL disposable syringes, ca. 0.7 – 0.85 mL was carefully taken from each layer. In particular for taking a water sample aliquot, ca. 0.05 mL of air was taken into syringe before putting the needle into the solution, and while moving through the upper octanol layer, the air was gently pushed out, in order to avoid the contamination of octanol phase, as very small amount of octanol phase may otherwise enter into the needle (which would cause a big error for lipophilic compounds). Upon reaching the water phase, all air bubbles are pushed out of the syringe, the aliquot takes, and the needle quickly removed from the solution. Then a small amount of water phase was discarded (to ensure all traces of octanol are out of the needle, leaving ca. 0.6 mL sample in the syringe. The needle was carefully wiped with dry tissue and then ca. 0.5 mL of sample was injected into the new NMR tube, followed by addition of ca. 0.1 mL acetone-d_6. The NMR tube was sealed (checking for leaks) using a blow torch, and inverted carefully for 20 times to obtain a homogenous solution for NMR measurement.

3) NMR measurement

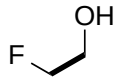
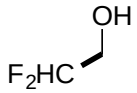
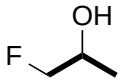
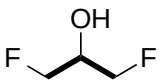
^{19}F $\{^{1}H\}$ NMR was firstly run with standard parameter conditions for chemical shift identification. The frequency offset point (O1P) is centered between the two diagnostic F signals. If T1 values had not been determined, a D1 of 30 sec for the octanol sample, and 60 sec for the water sample is chosen. The spectral width (SW) is left at 300 ppm (but can be reduced to obtain better SNR ratio is required (should be >250)). The number of transients (NS) is left at 64 (but can be increased if higher SNR is required).

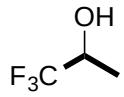
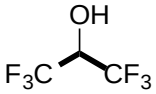
4) Data processing

Data were processed using ACD/Labs NMR software. The obtained FID file was reprocessed using following conditions: WFunction (LB 2 Exponential), Zero Filling (from 65536 to 262144) and then Fourier transform, followed by phasing with mouse and auto baseline correction. The integration ratio was obtained by manual integration (bias correction can be applied *via* adjusting tilt and slope if integral curve is not parallel to the baseline).

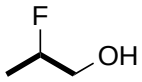
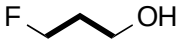
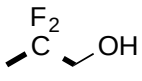
8.2 Detailed experimental data for the logP determinations

Unless indicated, the general procedure as detailed above was followed (O1P centered; SW: 300 ppm; D1: oct 30 sec; wat: 60 sec; SR: 295.14 ppm; NMR machine: 400-3). TFE was used as primary reference; 4,4,4-trifluorobutan-1-ol (**TB**), 2-fluoroethanol (**FE**) and 2-fluorophenol were also used as reference for some cases.

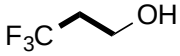
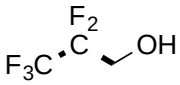
Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
8.2.1 Ethanol series						
	**6.2**	au0814zw2/ au0814zw3	0.0832/ 1.0625	-0.746	-0.75	-0.746 (±0.003)
		au2614zw8/ au2614zw9	0.1102/ 1.4167	-0.749		
		au2614zw10/ au2614zw11	0.1040/ 1.3137	-0.741		
	**6.20**	nv1014zw1/ nv1014zw2	0.4596/ 2.0203	-0.283	-0.29	+0.285 (±0.001)
		nv1014zw3/ nv1014zw4	0.4445/ 1.9680	-0.286		
		nv1014zw5/ nv1014zw6	0.3238/ 1.4323	-0.286		
8.2.2 2-Propanol series						
	**6.4**	nv2014zw3/ nv2014zw4	0.8682/ 4.4354	-0.348	-0.35	+0.346 (±0.004)
		nv2014zw5/ nv2014zw6	0.8193/ 4.1163	-0.341		
		nv2014zw7/ nv2014zw8	0.9956/ 5.0887	-0.349		
	**6.5**	nv1114zw1/ nv1114zw2	0.2925/ 1.7780	-0.424	-0.42	-0.421 (±0.003)
		nv1114zw3/ nv1114zw4	0.2469/ 1.4886	-0.420		
		nv1114zw5/ nv1114zw6	0.2773/ 1.6622	-0.418		

Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
	**6.34**	jy0315zw5/ jy0315zw6	1.2375/ 0.5507	+0.712	+0.71	+0.715 (±0.003)
		jy0315zw7/ jy0315zw8	1.2811/ 0.5620	+0.718		
		jy0315zw9/ jy0315zw10	1.2551/ 0.5551	+0.714		
	**6.35**	my3015zw5/ my3015zw6	5.8692/ 0.2765	+1.687	+1.69	+1.694 (±0.005)
		my3015zw7/ my3015zw8	4.5478/ 0.2081	+1.700		
		my3015zw9/ my3015zw10	4.6120/ 0.2136	+1.694		

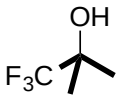
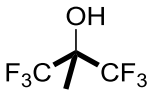
8.2.3 1-Propanol series

	**6.8**	nv2914zw13/ nv2914zw14	5.5538/ 1.9083	-0.286	-0.29[a]	-0.287 (±0.001)
		nv2914zw15/ nv2914zw16	3.3986/ 1.1753	-0.289		
		nv2914zw17/ nv2914zw18	3.3499/ 1.1540	-0.287		
	**6.7**	nv1414zw1/ nv1414zw2	3.3434/ 1.1035	-0.269	-0.26[a]	-0.260 (±0.007)
		nv1414zw3/ nv1414zw4	3.4050/ 1.0885	-0.255		
		nv1414zw5/ nv1414zw6	3.0305/ 0.9685	-0.255		
	**6.22**	ma1715zw3/ ma1715zw4	0.1111/ 0.2358	+0.033	+0.04	+0.038 (±0.004)
		ma1715zw5/ ma1715zw6	0.1112/ 0.2345	+0.036		
		ma1715zw7/ ma1715zw8	0.1128/ 0.2338	+0.043		
	**6.21**	nv1314zw1/ nv1314zw2	0.3455/ 0.6107	+0.113	+0.11	+0.110 (±0.003)
		nv1314zw3/ nv1314zw4	0.4166/ 0.7460	+0.107		
		nv1314zw5/ nv1314zw6	0.4318/ 0.7654	+0.111		

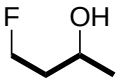
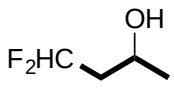
[a] Reference compound: FE

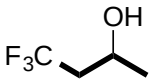
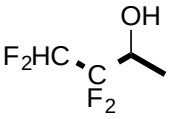
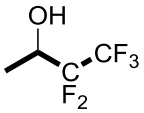
Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
	**6.28**	ma1115zw3/ ma1115zw4	1.0328/ 0.9138	+0.413	+0.41	+0.414 (±0.001)
		ma1115zw5/ ma1115zw6	1.3310/ 1.1738	+0.415		
		ma1115zw7/ ma1115zw8	1.5206/ 1.3375	+0.416		
	**6.40**	oc0214zw1/ oc0214zw2	2.1983/ 0.3163	+1.202	+1.20	1.200 (±0.001)
		oc0214zw3/ oc0214zw4	1.9761/ 0.2854	+1.200		
		oc0214zw5/ oc0214zw6	2.3148/ 0.3352	+1.199		

8.2.4 *tert*-Butanol series

	**6.37**	nv2314zw3/ nv2314zw4	5.9389/ 1.2568	+1.034	+1.03	+1.028 (±0.004)
		nv2314zw5/ nv2314zw6	9.1209/ 1.9688	+1.026		
		nv2314zw7/ nv2314zw8	5.4572/ 1.1808	+1.025		
	**6.38**	ju0515zw9/ ju0515zw10	5.7536/ 0.1164	+2.054	+2.08	+2.080 (±0.019)
		ju0515zw11/ ju0515zw12	5.8469/ 0.1069	+2.098		
		ju0515zw13/ ju0515zw14	6.0948/ 0.1137	+2.089		

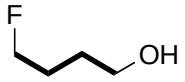
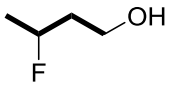
8.2.5 2-Butanol series

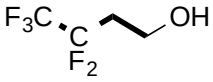
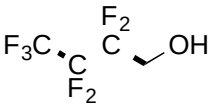
	**6.10**	ma1915zw1/ ma1915zw2	0.1211/ 0.2326	+0.077	+0.08	+0.084 (±0.007)
		ma1915zw3/ ma1915zw4	0.1237/ 0.2348	+0.082		
		ma1915zw5/ ma1915zw6	0.1282/ 0.2366	+0.094		
	**6.23**	ma2815zw3/ ma2815zw4	0.7442/ 0.6484	+0.420	+0.42	+0.417 (±0.002)
		ma2815zw5/ ma2815zw6	0.7388/ 0.6496	+0.416		
		ma2815zw7/ ma2815zw8	0.7412/ 0.6546	+0.414		

Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
	**6.29**	jy0315zw11/ jy0315zw12	0.7712/ 0.3327	+0.725	+0.72	+0.721 (±0.003)
		jy0315zw13/ jy0315zw14	0.7502/ 0.3265	+0.721		
		jy0315zw15/ jy0315zw16	0.7634/ 0.3353	+0.717		
	**6.41**	ja0615zw3/ ja0615zw4	2.2187/ 1.5831	+1.057	+1.05[a]	+1.054 (±0.003)
		ja0615zw5/ ja0615zw6	1.4520/ 1.0433	+1.054		
		ja0615zw7/ ja0615zw8	1.7014/ 1.2318	+1.050		
	**6.42**	ap2515zw5/ ap2515zw6	4.5737/ 0.3075	+1.532	+1.53	+1.530 (±0.002)
		ap2515zw7/ ap2515zw8	4.5465/ 0.3072	+1.530		
		ap2515zw9/ ap2515zw10	4.4489/ 0.3018	+1.529		

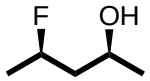
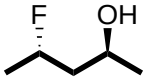
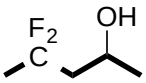
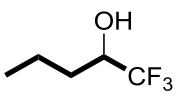
[a] Reference compound: 4,4,4-trifluoro-1-butanol (**TB**, logP: +0.91[EXP]).

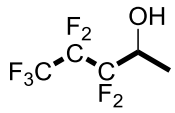
8.2.6 1-Butanol series

	**6.13**	nv1714zw3/ nv1714zw4	1.5512/ 2.8742	+0.092	+0.09	+0.090 (±0.002)
		nv1714zw5/ nv1714zw6	1.4427/ 2.6988	+0.088		
		nv1714zw7/ nv1714zw8	1.5758/ 2.9372	+0.090		
	**6.12**	my3015zw11/ my3015zw12	0.4122/ 0.6449	+0.166	+0.17	+0.173 (±0.006)
		my3015zw13/ my3015zw14	0.4144/ 0.6279	+0.180		
		my3015zw15/ my3015zw16	0.4146/ 0.6379	+0.173		
	**6.30**	nv2314zw9/ nv2314zw10	0.8687/ 0.2406	+0.918	+0.91	+0.910 (±0.005)
		nv2314zw11/ nv2314zw12	1.0628/ 0.3035	+0.904		
		nv2314zw13/ nv2314zw14	0.9520/ 0.2691	+0.909		
		nv2314zw15/ nv2314zw16	1.4533/ 0.4124	+0.907		

Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
	**6.43**	nv2614zw1/ nv2614zw2	7.2581/ 0.8504	+1.291	+1.30	+1.298 (0.005)
		nv2614zw3/ nv2614zw4	9.8785/ 1.1251	+1.303		
		nv2614zw5/ nv2614zw6	9.7072/ 1.1159	+1.299		
	**6.44**	nv2214zw9/ nv2214zw10	15.3820/ 0.3601	+1.991	+1.98	+1.976 (±0.015)
		nv2214zw11/ nv2214zw12	26.3642/ 0.6361	+1.977		
		nv2214zw13/ nv2214zw14	30.1285/ 0.7556	+1.961		

8.2.7 2-Pentanol series

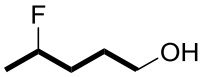
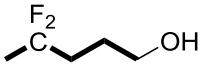
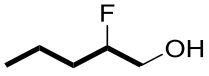
	**6.53**	dc1614zw3/ dc1614zw4	0.3660/ 0.2804	+0.476	+0.48	+0.477 (±0.001)
		dc1614zw5/ dc1614zw6	0.4450/ 0.3407	+0.476		
		dc1614zw7/ dc1614zw8	0.4663/ 0.3556	+0.478		
	**6.52**	dc1414zw1/ dc1414zw2	0.6245/ 0.3847	+0.570	+0.57	+0.569 (±0.003)
		dc1414zw3/ dc1414zw4	0.4612/ 0.2880	+0.564		
		dc1414zw5/ dc1414zw6	0.7161/ 0.4398	+0.572		
	**6.25**	ma2215zw1/ ma2215zw2	0.6733/ 0.3383	+0.659	+0.66	+0.662 (±0.003)
		ma2315zw1/ ma2315zw2	0.6715/ 0.3316	+0.666		
		ma2315zw3/ ma2315zw4	0.6782/ 0.3394	+0.661		
	**6.39**	my1615zw5/ my1615zw6	1.5849/ 0.0535	+1.832	+1.82	+1.817 (±0.011)
		my1615zw7/ my1615zw8	1.5285/ 0.0538	+1.813		
		my1615zw9/ my1615zw10	1.5499/ 0.0557	+1.804		

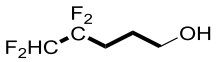
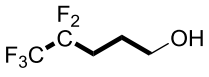
Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
	**6.45**	ap2515zw23/ ap2515zw24	2.7176/ 0.02402	+2.414	+2.41	+2.409 (±0.012)
		ap2515zw25/ ap2515zw26	2.8090/ 0.02438	+2.422		
		ap2515zw27/ ap2515zw28	2.7523/ 0.02553	+2.393		

8.2.8 Neopentyl alcohol series

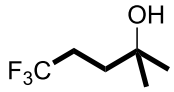
	**6.15**	ma2715zw7/ ma2715zw8	0.3694/ 0.1264	+0.826	+0.80	+0.796 (±0.021)
		ma2715zw9/ ma2715zw10	0.2208/ 0.0828	+0.786		
		ma2715zw11/ ma2715zw12	0.3175/ 0.1217	+0.776		

8.2.9 1-Pentanol series

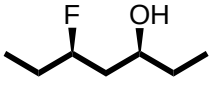
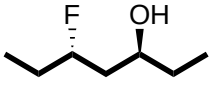
	**6.19**	my3115zw3/ my3115zw4	1.3428/ 0.9210	+0.524	+0.52	+0.524 (±0.002)
		my3115zw5/ my3115zw6	1.3407/ 0.9133	+0.527		
		my3115zw7/ my3115zw8	1.3500/ 0.9303	+0.522		
	**6.18**	my2315zw5/ my2315zw6	0.5138/ 0.3580	+0.517	+0.52	+0.522 (±0.004)
		my2315zw7/ my2315zw8	0.5189/ 0.3573	+0.522		
		my2315zw9/ my2315zw10	0.5237/ 0.3575	+0.526		
	**6.26**	my0915zw5/ my0915zw6	0.4653/ 0.2071	+0.712	+0.71	+0.708 (±0.005)
		my0915zw7/ my0915zw8	0.4598/ 0.2098	+0.701		
		my0915zw9/ my0915zw10	0.4674/ 0.2078	+0.712		
	**6.17**	ju1215zw9/ ju1215zw10	0.7883/ 0.2427	+0.872	+0.87	+0.868 (±0.003)
		ju1215zw11/ ju1215zw12	0.8108/ 0.2512	+0.869		
		ju1215zw13/ ju1215zw14	0.7916/ 0.2479	+0.864		

Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
	**6.47**	ju0515zw21/ ju0515zw22	0.5401/ 0.1322	+0.971	+0.97	+0.974 (±0.002)
		ju0515zw23/ ju0515zw24	0.5476/ 0.1326	+0.976		
		ju0515zw25/ ju0515zw26	0.5373/ 0.1307	+0.974		
	**6.31**	nv2214zw3/ nv2214zw4	4.4606/ 0.6154	+1.220	+1.22	+1.222 (0.004)
		nv2214zw5/ nv2214zw6	4.2519/ 0.5881	+1.219		
		nv2214zw7/ nv2214zw8	3.0699/ 0.4159	+1.228		
	**6.46**	ap2515zw11/ ap2515zw12	3.3028/ 0.1425	+1.725	+1.72	+1.720 (±0.004)
		ap2515zw13/ ap2515zw14	3.1018/ 0.1356	+1.719		
		ap2515zw15/ ap2515zw16	3.3643/ 0.1480	+1.717		

8.2.10 2-Methyl-2-pentanol series

	**6.33**	ap2515zw17/ ap2515zw18	2.7049/ 0.1875	+1.519	+1.52	+1.519 (±0.002)
		ap2515zw19/ ap2515zw20	2.6849/ 0.1848	+1.522		
		ap2515zw21/ ap2515zw22	2.7060/ 0.1887	+1.517		

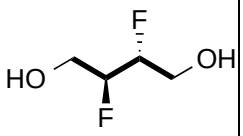
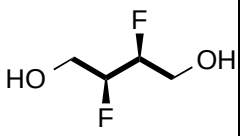
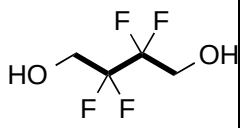
8.2.11 3-Heptanol series

	**6.56**	dc0814zw3/ dc0814zw4	0.3801/ 0.0808	+1.582	+1.58[a]	+1.584 (±0.005)
		dc0814zw5/ dc0814zw6	0.5037/ 0.1049	+1.591		
		dc0814zw7/ dc0814zw8	0.4058/ 0.0867	+1.580		
	**6.55**	dc1114zw1/ dc1114zw2	1.0099/ 0.1686	+1.687	+1.71[a]	+1.710 (±0.017)
		dc1114zw3/ dc1114zw4	0.6804/ 0.1051	+1.721		
		dc1114zw5/ dc1114zw6	0.6872/ 0.1056	+1.723		

[a] Reference compound: 4,4,4-trifluoro-1-butanol (**TB**, logP: +0.91[EXP])

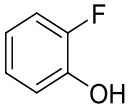
Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error

8.2.12 1,4-Butanediol series

	**6.50**	nv2714zw1/ nv2714zw2	1.5892/ 2.8679	-1.006	-1.01[a]	-1.009 (±0.003)
		nv2714zw3/ nv2714zw4	1.4013/ 2.5419	-1.009		
		nv2714zw5/ nv2714zw6	0.7770/ 1.4206	-1.012		
	**6.49**	nv2914zw3/ nv2914zw4	1.5649/ 2.5229	-0.957	-0.97[a]	-0.968 (±0.009)
		nv2914zw5/ nv2914zw6	1.3398/ 2.2144	-0.968		
		nv2914zw7/ nv2914zw8	1.0521/ 1.7864	-0.980		
	**6.51**	dc0314zw1/ dc0314zw2	1.1289/ 3.4162	-0.121	-0.11	-0.109 (±0.009)
		dc0314zw3/ dc0314zw4	1.6429/ 4.8079	-0.106		
		dc0314zw5/ dc0314zw6	0.8021/ 2.3130	-0.100		

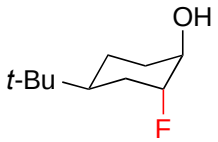
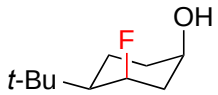
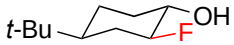
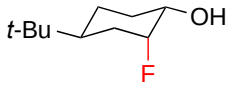
[a] Reference compound: **FE**.

8.2.13 Additional compound: *o*-fluorophenol

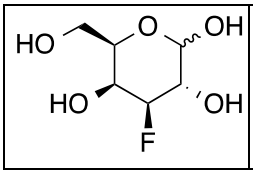
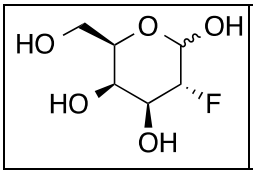
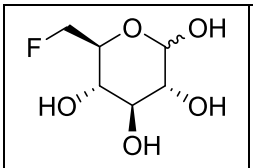
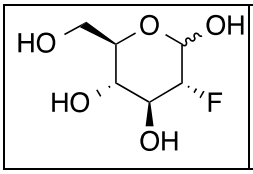
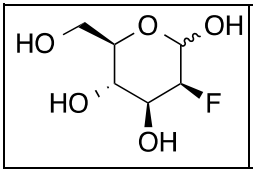
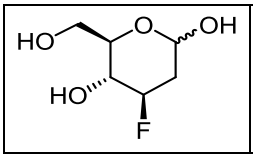
	**exp1**	ju1215zw15/ ju1215zw16	1.2149/ 0.0554	+1.701	+1.70	+1.696 (±0.004)
		ju1215zw17/ ju1215zw18	1.1761/ 0.0544	+1.695		
		ju1215zw19/ ju1215zw20	1.2282/ 0.0571	+1.693		

Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error

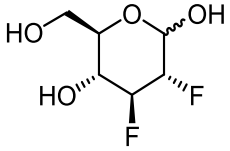
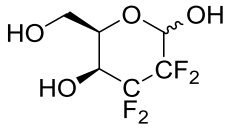
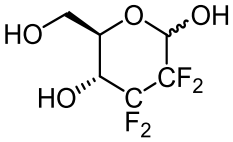
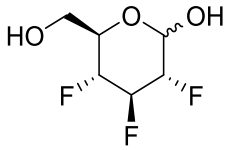
8.2.14 4-*tert*-Butylcyclohexanol series[a]

	**6.61**	ma2415zw3/[b] ap0215njwzw1[c]	0.8521/ 0.02657	+3.206	-	-
	**6.62**	ap0115zw3/[d] ap0115zw4	2.2293/ 0.6523	+2.234	+2.23	+2.229 (±0.005)
		ap0115zw5/ ap0115zw6	2.1797/ 0.6533	+2.223		
		ap0115zw3/ my0115njwzw1[e]	2.2293/ 0.6582	+2.230		
	**6.59**	ma2715njwzw1/[f] ma2715njwzw2	2.1788/ 0.2689	+2.609	+2.61	+2.608 (±0.009)
		ma3115zw3/ ma3115zw4	2.2142/ 0.2666	+2.619		
		ma2815zw9/ ma2815zw10	2.0216/ 0.2563	+2.597		
	**6.58**	nv1214zw3/ nv1214zw4	1.4736/ 0.1738	+2.628	+2.63	+2.630 (±0.002)
		nv1214zw3/ ja3015njwzw1[g]	1.4736/ 0.1726	+2.631		

[a] Reference: 2-fluorophenol (logP: +1.70^{EXP});
[b] NS: 64; D1: 30 sec; SW: 100 ppm; centered O1P; NMR: 400-3;
[c] NS: 32768; D1: 15 sec; SW: 100 ppm; centered O1P; NMR: 400-2;
[d] SW for ap0115zw3-6 and my0115njwzw1: 100 ppm;
[e] NMR tube of ap0115zw4 was re-run on NMR machine 400-2 with extended NS of 4096;
[f] NMR tube of ma3115zw3/4 were re-run on NMR machine 400-2 with extended NS of 4096; SW for ma2715njwzw1/2 and ma3115zw3/4: 100 ppm;
[g] NMR tube of nv1214zw4 was re-run on NMR machine 400-2 with extended NS of 4096 and SW 100 ppm.

Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
	**6.63**	ju2614njwzw5/ ju2614njwzw6[22]	0.0371/ 1.9271[23]	-2.465	-	-
	**6.64**	ju2614njwzw3/ ju2614njwzw4[24]	0.0550/ 2.3122[25]	-2.374	-	-
	**6.65**	se1115njwzw2/ se1115njwzw1[26]	0.0992/ 3.9888	-2.354	-2.36	-2.355 (±0.001)
		oc0215njwzw1/ se3015njwzw1[27]	0.1182/ 4.7741	-2.356		
	**6.66**	ju2614njwzw1/[28] ju2614njwzw2[29]	0.0792/ 2.2992	-2.213	-	-
	**6.67**	ju2614njwzw7/ ju2614njwzw8[30]	0.1208/ 2.7976	-2.114	-	-
	**6.68**	ju22z4zw3/ ju2214zw4[31]	0.0755/ 4.8445	-1.447	-	-

[22] For both oct and wat sample: D1: 15 sec; O1P: -150 ppm; NS: oct 3296; wat 2329; reference: **FE**;
[23] For data reprocessing of ju2614njwzw6: no zero-filling and LB value 0.15 for WF (exponential) as it was difficult to obtain good baseline with standard settings (doublezero-filling and LB value 2);
[24] For both oct and wat sample: D1: 15 sec; O1P: -150 ppm; NS: oct 3296; wat 2687; reference: **FE**;
[25] For data reprocessing of ju2614njwzw4: no zero-filling and LB value 0.15 for WF (exponential) as it was difficult to obtain good baseline with standard settings (doublezero-filling and LB value 2);
[26] For both oct and wat sample: D1: 15 sec; O1P: -230 ppm; NS: oct 4647; wat 512; Commercial compound was purified by flash chromatography and then used for log*P* partition. The sample after partition equilibrium was centrifuged for 4 times with each duration of 1 hr at 500 rpm to obtain clear water and octanol layer for NMR sample preparation; reference: **FE**;
[27] For both oct and wat sample: D1: 15 sec; O1P: -230 ppm; NS: oct 9216; wat 512; reference: **FE**;
[28] For both oct and wat sample: D1: 15 sec; O1P: -150 ppm; NS: oct 3296; wat 1744; reference: **FE**;
[29] For data reprocessing of ju2614njwzw2: no zero-filling and LB value 0.15 for WF (exponential) as it was difficult to obtain good baseline with standard settings (doublezero-filling and LB value 2);
[30] For both oct and wat sample: D1: 15 sec; O1P: -150 ppm; NS: oct 3296; wat 427; reference: **FE**;
[31] For both oct and wat sample: D1: 50 sec; O1P -100 ppm; reference: **TFE**;

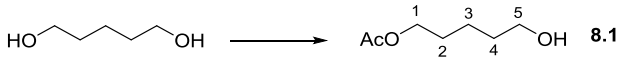
Compound	Nr	Experiment (octanol/water)	r_o/r_w	log*P*	Average log*P*	error
	**6.69**	jy0814zw4/ jy0814zw5[32]	0.8113/ 1.8759	-1.114	-	-
	**6.70**	my0915zw11/[33] my0915zw12	0.1232/ 1.0571	-0.574	-0.58	-0.576 (±0.005)
		my0915zw13/ my0915zw14	0.1186/ 1.0156	-0.573		
		my0915zw15/ my0915zw16	0.1270/ 1.1141	-0.583		
	**6.71**	ju2314zw1/[34] ju2314zw2	1.9975/ 9.4638	-0.316	-0.32	-
	**6.72**	nv2514zw1/ nv2514zw2	1.2117/ 0.3190	-0.170	-0.17[35]	-

[32] For both oct and wat sample D1: 50 sec; O1P: -100 ppm; reference: **FE**.
[33] Reference: **TFE**;
[34] O1P: -100 ppm; D1: 50 sec for both oct and wat sample; reference: **TFE**;
[35] Very low S/N ratios due to the fluorine coupling; reference: **FE**;

8.3 Synthesis of fluorinated alkanols

8.3.1 Synthesis of 5-fluoro-1-pentanol (6.19)

8.3.1.1 Synthesis of 5-hydroxypentyl acetate (8.1)

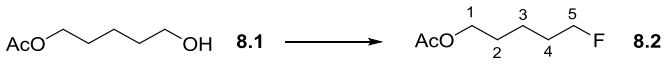


8.1

To a round-bottom flask were added 1,5-pentanediol (10 mL, 95.4 mmol, 1 eq), DCM (200 mL, 2 mL/mmol), pyridine (9.78 mL, 120.9 mmol, 1.27 eq), 4-DMAP (1.40 g, 11.5 mmol, 0.12 eq) and acetic anhydride (9.02 mL, 95.4 mmol, 1 eq). The resulting reaction solution was stirred at 0°C for 45 min and then quenched with water (150 mL), followed by extraction with DCM (150 mL× 3). The combined organic layer was washed with aq. HCl solution (1M, 150 mL), dried over $MgSO_4$, filtered and concentrated. The crude was purified by flash chromatography (acetone/petroleum ether 10:90 - 40:60) to afford 6.65 g (45.5 mmol, 48%) of the desired monoacetate **8.1** as a colorless oil.

8.1, **Formula** $C_7H_{14}O_3$; **MW** 146.19; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.00 (2H, t, *J* 6.7 Hz, H_1), 3.55 (2H, t, *J* 6.5 Hz, H_5), 3.09 (1H, br. s, -O*H*), 1.97 (3H, s, CH_3COO), 1.43 - 1.69 (4H, m, H_2 + H_4), 1.29 - 1.42 (2H, m, H_3) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.2, 64.3, 62.2, 32.0, 28.2, 22.0, 20.8 ppm; NMR data were consistent with the reported data.[223]

8.3.1.1.1 Synthesis of 5-fluoropentyl acetate (8.2)

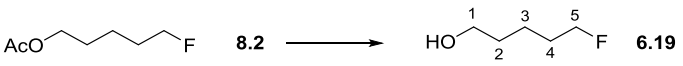


8.2

To a round-bottom flask were added 5-hydroxypentyl acetate **8.1** (6.65 g, 45.5 mmol, 1 eq), DCM (100 mL) and DAST (9.02 mL, 68.2 mmol, 1.5 eq). The resulting reaction mixture was stirred at room temperature overnight and then quenched by sat. aq. $NaHCO_3$ solution (150 mL), followed by extraction with DCM (150 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mixture was purified by flash chromatography (DCM/pentane 20:80, then neat acetone) to give 5.0* g (33.7* mmol, 74%*, *calculated based on ^{1}H NMR) of the desired 5-fluoropentyl acetate **8.2**. Due to the presumed volatility of this fluorinated pentyl acetate, this compound (containing eluting solvents) was used directly for next step without further removal of the solvent residue.

8.2, **Formula** $C_7H_{13}FO_2$; **MW** 148.18; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.46 (2H, dt, $^2J_{F5\text{-}H5}$ 47.9 Hz, $^3J_{H4\text{-}H5}$ 6.3 Hz, H_5), 4.09 (2H, t, *J* 6.2 Hz, H_1), 2.06 (3H, s, CH_3COOC_1), 1.62 - 1.85 (4H, m, H_4 + H_2), 1.41 - 1.60 (2H, m, H_3) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -218.7 (1F, tt, $^2J_{H5\text{-}F5}$ 46.7 Hz, $^3J_{H4\text{-}F5}$ 26.4 Hz, F_5) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -218.7 (1F, s, F_5) ppm;

8.3.1.1.2 Synthesis of 5-fluoro-1-pentanol (6.19)

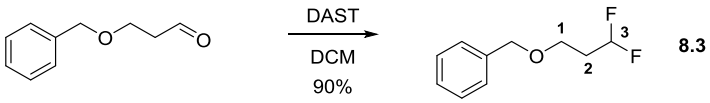


To a round-bottom flask were added 5-fluoropentyl acetate **8.2** (5.0 g, 33.7 mmol, 1 eq), diethyl ether (50 mL) and NaOMe solution (25% w/w in MeOH, 15.4 mL, 67.4 mmol, 2 eq). The resulting reaction mixture was stirred at room temperature overnight and then neutralized with aq. HCl solution (1M) up to ca. pH 7, followed by extraction with DCM (100 mL × 3). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The crude mixture was purified by flash chromatography (diethyl ether/DCM 4:94 - 20:80) to afford **6.19** (2.16 g, 20.35 mmol, 60%) as a colorless oil.

6.19, **Formula** $C_5H_{11}FO$; **MW** 106.14; $\mathbf{R_f}$ 0.25 (Et_2O/DCM 8:92); **IR** (neat) 3334 (br., w), 2939 (m), 1391, (w), 1055 (s), 1029 (s), 1006 (m), 990 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.45 (2H, dt, $^2J_{F5\text{-}H5}$ 47.4 Hz, $^3J_{H4\text{-}H5}$ 6.1 Hz, H_5), 3.65 (2H, t, J 6.2 Hz, H_1), 1.55 - 1.81 (5H, m, H_4 + -OH + H_2), 1.42 - 1.54 (2H, m, H_3) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 84.0 (d, $^1J_{F5\text{-}C5}$ 164.7 Hz, C_5), 62.6 (C_1), 32.2 (C_2), 30.1 (d, $^2J_{F5\text{-}C4}$ 19.6 Hz, C_4), 21.5 (d, $^3J_{F5\text{-}C3}$ 5.5 Hz, C_3) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -218.4 (1F, tt, $^2J_{H5\text{-}F5}$ 48.1 Hz, $^3J_{H4\text{-}F5}$ 25.1 Hz, F_5) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -218.4 (1F, s, F_5) ppm; **HRMS** (EI) for $C_5H_{11}FO$ [$M^{\cdot+}$], calcd 106.0788, found 106.0774.

8.3.2 Synthesis of 3,3-difluoro-1-propanol (6.22)

8.3.2.1 Synthesis of benzyl 3,3-difluoropropyl ether (8.3)

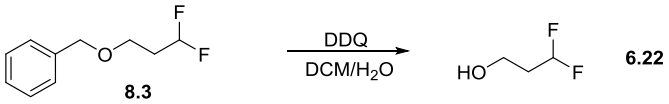


To a round-bottom flask were added 3-benzyloxypropionaldehyde (1.00 g, 6.09 mmol, 1.0 eq), dichloromethane (20 mL) and diethylaminosulfur trifluoride (1.61 mL, 12.18 mmol, 2.0 eq). The resulting solution was stirred at room temperature overnight. The reaction mixture was quenched with aq. sat. $NaHCO_3$ solution (40 mL) and extracted with dichloromethane (3 × 60 mL). The combined organic layer was washed with brine (100 mL), dried over $MgSO_4$, filtered and concentrated. The crude was purified by flash chromatography (Et_2O/pentane 3:97 – 10/90) to afford the desired compound benzyl 3,3-difluoropropyl ether (**8.3**) as a colourless oil (1.02 g, 5.48 mmol, 90%).

8.3, **Formula**: $C_{10}H_{12}F_2O$; **MW**: 186.20; **IR** (neat) 3087 (w), 3059 (w), 3028 (w), 2970 (w), 2939 (w), 2866 (w), 2798 (w), 1492 (w), 1454 (m), 1395 (m), 1364 (m), 1095 (s), 1023 (s), 975 (s), 906 (m), 733 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.28-7.42 (5H, m, H_{Ar}), 6.02 (1H, tt, $^2J_{F3\text{-}H3}$ 56.9 Hz, $^3J_{H2\text{-}H3}$ 4.8 Hz, H_3), 4.53 (2H, s, CH_2Ph), 3.64 (2H, t, $^3J_{H2\text{-}H1}$ 6.1 Hz, H_1), 2.15 (2H, ttd, $^3J_{F3\text{-}H2}$ 16.7 Hz, $^3J_{H1\text{-}H2}$ 6.1 Hz, $^3J_{H3\text{-}H2}$ 4.9 Hz, H_2) ppm; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.8 (C_{Ar}), 128.4 (m-C_{Ar}H × 2), 127.8 (p-C_{Ar}H), 127.6 (o-C_{Ar}H × 2), 115.9 (t, $^1J_{F3\text{-}C3}$ 237.7 Hz, C_3), 73.2 (CH$_2$Ph), 64.0 (t, $^3J_{F3\text{-}C1}$ 6.9 Hz, C_1), 34.8 (t, $^2J_{F3\text{-}C2}$ 21.8

Hz, C_2) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -117.8 (2F, dt, $^2J_{H3\text{-}F3}$ 57.2 Hz, $^3J_{H2\text{-}F3}$ 16.5 Hz, F_3) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -117.8 (2F, s, F_3) ppm; **MS** (EI) *m/z* 186.2 ($M^{\cdot+}$, 8%); **HRMS** (EI) for $C_{10}H_{12}F_2O$ [$M^{\cdot+}$] calcd 186.08507, found 186.08476 (-0.31 ppm error).

8.3.2.1.1 Synthesis of 3,3-difluoro-1-propanol (6.22)



8.3 **6.22**

To a round-bottom flask were added benzyl 3,3-difluoropropyl ether **8.3** (360 mg, 1.93 mmol, 1 eq), DCM (22.20 mL), water (2.50 mL) and DDQ (1.74 g, 7.72 mmol, 4 eq). The resulting reaction mixture was stirred under reflux overnight. After the completion of the reaction indicated by TLC analysis, the reaction mixture was directly purified by flash chromatography (pentane/diethyl ether 70:30) and then followed by re-purification over column (DCM 100%) to afford the desired product 3,3-difluoro-1-propanol (**6.22**) as a colourless oil (isolated yield: 30 mg, 0.31 mmol, 16%).

6.22, **Formula**: $C_3H_6F_2O$; **MW**: 96.08; **IR** (neat) 3350 (br, w), 2958 (w), 2913 (m), 2852 (w), 1430 (w), 1401 (w), 1381 (w), 1119 (s), 1070 (s), 1054 (s), 964 (s), 805 (w) cm^{-1}; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.03 (1H, tt, $^2J_{F3\text{-}H3}$ 57.0 Hz, $^3J_{H2\text{-}H3}$ 4.1 Hz, H_3), 3.87 (2H, td, $^3J_{H2\text{-}H1}$ 5.9 Hz, $^3J_{H(OH)\text{-}H1}$ 5.1 Hz, H_1), 2.12 (2H, ttd, $^3J_{F3\text{-}H2}$ 17.0 Hz, $^3J_{H1\text{-}H2}$ 6.0 Hz, $^3J_{H3\text{-}H2}$ 4.6 Hz, H_2), 1.45 (1H, tt, $^3J_{H1\text{-}H(OH)}$ 5.1, $^{1h}J_{F3\text{-}H(OH)}$ 0.4 Hz, O*H*) ppm; **^{1}H {^{19}F} NMR** (500 MHz, $CDCl_3$) δ 6.03 (1H, t, $^3J_{H2\text{-}H3}$ 4.6 Hz, H_3), 3.87 (2H, td, $^3J_{H2\text{-}H1}$ 5.9 Hz, $^3J_{H(OH)\text{-}H1}$ 5.2 Hz, H_1), 2.12 (2H, td, $^3J_{H1\text{-}H2}$ 6.0 Hz, $^3J_{H3\text{-}H2}$ 4.6 Hz, H_2), 1.45 (1H, t, $^3J_{H1\text{-}H(OH)}$ 5.1 Hz, O*H*) ppm; **^{13}C NMR** (126 MHz, $CDCl_3$) δ 116.1 (t, $^1J_{F3\text{-}C3}$ 239.4 Hz, C_3), 56.8 (t, $^3J_{F3\text{-}C1}$ 6.8 Hz, C_1), 36.9 (t, $^2J_{F3\text{-}C2}$ 20.3 Hz, C_2) ppm; **^{19}F NMR** (376 MHz, $CDCl_3$) δ -117.8 (2F, dt, $^2J_{H3\text{-}F3}$ 56.8 Hz, $^3J_{H2\text{-}F3}$ 16.9 Hz, F_3) ppm; **^{19}F {^{1}H} NMR** (376 MHz, $CDCl_3$) δ -117.6 (2F, s, F_3) ppm; **HRMS** (EI) for $C_3H_6F_2O$ [$M^{\cdot+}$], calcd 96.03812, found 96.03807 (-0.05 ppm error).

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Appendices

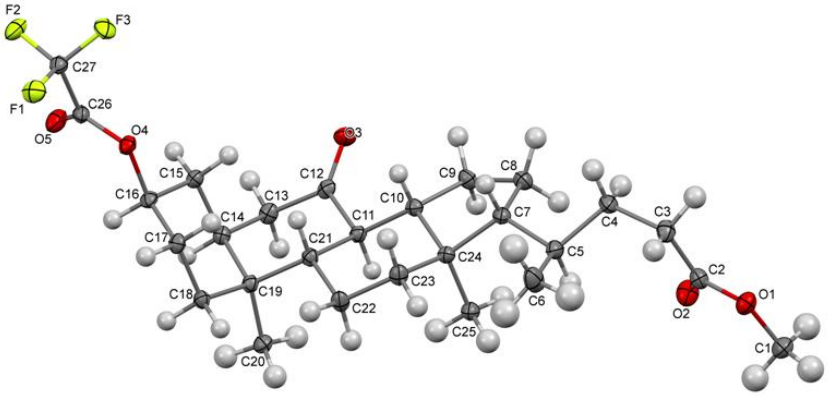
Appendix A Bile acids

A.1 Molecular structure of compound 3.45

Crystal data and structure refinement details

Identification code	**2013ncs0653 (ZW6826-85A / 2013sot0087)**	
Empirical formula	$C_{27}H_{39}F_3O_5$	
Formula weight	500.58	
Temperature	100(2) K	
Wavelength	0.71075 Å	
Crystal system	Orthorhombic	
Space group	*P*212121	
Unit cell dimensions	*a* = 7.6016(5) Å	α = 90°
	b = 16.2236(11) Å	β = 90°
	c = 20.2419(14) Å	γ = 90°
Volume	2496.3(3) Å^3	
Z	4	
Density (calculated)	1.332 Mg / m^3	
Absorption coefficient	0.104 mm^{-1}	
F(000)	1072	
Crystal	Cut Block; Colorless	
Crystal size	0.190 × 0.160 × 0.120 mm^3	
θ range for data collection	2.959 – 27.484°	
Index ranges	$-9 \le h \le 9$, $-21 \le k \le 21$, $-26 \le l \le 26$	
Reflections collected	17988	
Independent reflections	5695 [R_{int} = 0.0392]	
Completeness to θ = 25.242°	99.5 %	
Absorption correction	Semi–empirical from equivalents	
Max. and min. transmission	1.000 and 0.739	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5695 / 0 / 320	
Goodness-of-fit on F^2	1.024	
Final *R* indices [$F^2 > 2\sigma(F^2)$]	*R1* = 0.0288, *wR2* = 0.0753	
R indices (all data)	*R1* = 0.0304, *wR2* = 0.0763	
Absolute structure parameter	–0.1(2)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.258 and –0.153 e Å^{-3}	

Diffractometer: *Rigaku AFC12* goniometer equipped with an enhanced sensitivity (HG) *Saturn724+* detector mounted at the window of an *FR-E+ SuperBright* molybdenum rotating anode generator with HF *Varimax* optics (100µm focus). **Cell determination and data collection**: *CrystalClear-SM Expert 2.0 r11* (Rigaku, 2011). **Data reduction, cell refinement and absorption correction**: *CrystalClear-SM Expert 2.0 r13* (Rigaku, 2011). **Structure solution**: *SHELXS-2013* (Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122). **Structure refinement**: *SHELXL-2013* (Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122). **Special details**: Absolute configuration cannot be assign reliably based on anomalous dispersion effect due to lack of heavy element the crystal structure (>Si). However, the relative configuration may be assigned. [Displacement ellipsoids – 50% probability]

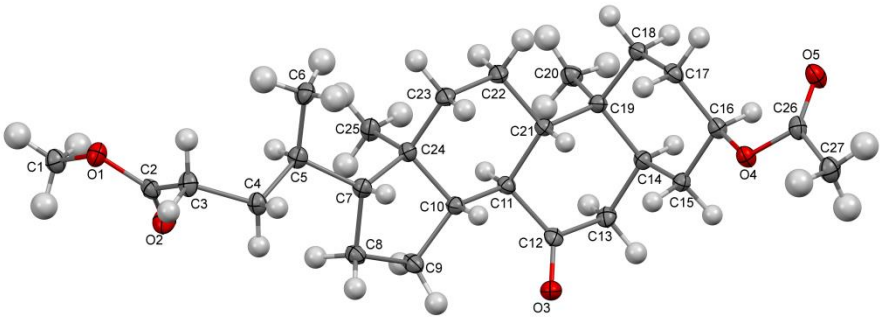


A.2 Molecular structure of compound 3.50

Crystal data and structure refinement details

Identification code	**2013ncs0662 (ZW6826-42P/2013SOT0078)**	
Empirical formula	$C_{27}H_{42}O_5$	
Formula weight	446.60	
Temperature	100 K	
Wavelength	0.71075 Å	
Crystal system	Orthorhombic	
Space group	*P*212121	
Unit cell dimensions	a = 7.7190(3) Å	α = 90°
	b = 16.0365(6) Å	β = 90°
	c = 19.4360(13) Å	γ = 90°
Volume	2405.9(2) Å^3	
Z	4	
Density (calculated)	1.233 Mg / m^3	
Absorption coefficient	0.083 mm^{-1}	
F(000)	976	
Crystal	Column; Colorless	
Crystal size	0.180 × 0.050 × 0.040 mm^3	
θ range for data collection	3.111 – 27.480°	
Index ranges	$-10 \le h \le 9$, $-20 \le k \le 19$, $-25 \le l \le 22$	
Reflections collected	17512	
Independent reflections	5498 [R_{int} = 0.0286]	
Completeness to θ = 25.242°	99.7 %	
Absorption correction	Semi–empirical from equivalents	
Max. and min. transmission	1.000 and 0.812	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5498 / 0 / 294	
Goodness-of-fit on F^2	1.045	
Final R indices [$F^2 > 2\sigma(F^2)$]	$R1$ = 0.0354, $wR2$ = 0.0848	
R indices (all data)	$R1$ = 0.0390, $wR2$ = 0.0863	
Absolute structure parameter	–0.5(4)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.311 and –0.155 e Å^{-3}	

Diffractometer: *Rigaku AFC12* goniometer equipped with an enhanced sensitivity (HG) *Saturn724+* detector mounted at the window of an *FR-E+ SuperBright* molybdenum rotating anode generator with HF *Varimax* optics (100µm focus). **Cell determination and data collection**: *CrystalClear-SM Expert 2.0 r11* (Rigaku, 2011). **Data reduction, cell refinement and absorption correction**: *CrystalClear-SM Expert 2.0 r13* (Rigaku, 2011). **Structure solution**: *SHELXS-2013* (Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122). **Structure refinement**: *SHELXL-2013* (Sheldrick, G.M. (2008). Acta Cryst. A64, 112-122). **Special details**: The absolute configuration based on anomalous dispersion effect can not be assigned accurately, due to lack of presence in the structure heavy element (>Si). However, stereochemistry may be assigned on the basis of unchanging stereocenters during reaction. [Displacement ellipsoids – 50% probability]

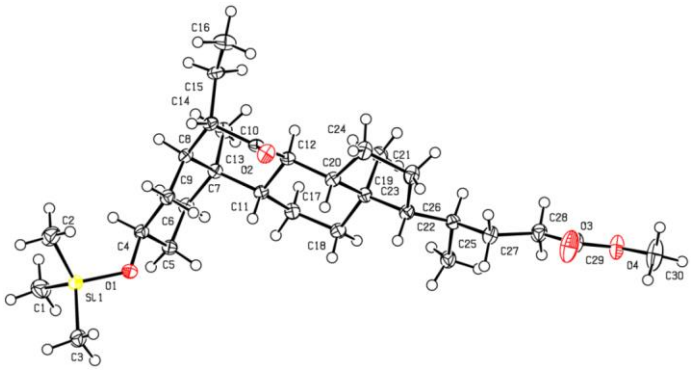


A.3 Molecular structure of compound 3.60

Crystal data and structure refinement details

Identification code	2014sot0015
Empirical formula	$C_{30}H_{52}O_4Si$
Formula weight	504.80
Temperature/K	100(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	6.4325(16)
b/Å	19.471(5)
c/Å	23.765(6)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2976.5(13)
Z	4
ρ_{calc}mg/mm^3	1.126
m/mm^{-1}	0.110
F(000)	1112.0
Crystal size/mm^3	0.18 × 0.03 × 0.02
Radiation	MoKα (λ = 0.71075)
2Θ range for data collection	5.552 to 55.08°
Index ranges	-6 ≤ h ≤ 8, -25 ≤ k ≤ 25, -30 ≤ l ≤ 30
Reflections collected	19206
Independent reflections	6638 [R_{int} = 0.0461, R_{sigma} = 0.0576]
Data/restraints/parameters	6638/0/324
Goodness-of-fit on F^2	1.025
Final R indexes [I>=2σ (I)]	R_1 = 0.0444, wR_2 = 0.0925
Final R indexes [all data]	R_1 = 0.0625, wR_2 = 0.1025
Largest diff. peak/hole / e Å^{-3}	0.22/-0.20
Flack parameter	0.07(8)

Experimental: A suitable crystal was selected and analyzed on a Rigaku Saturn724+ (2x2 bin mode) diffractometer. The crystal was kept at 100(2) K during data collection. Using Olex2 [J. Appl. Cryst. 42, 339-341], the structure was solved with the ShelXS [Acta Cryst. A64, 112-122] structure solution program using Direct Methods and refined with the ShelXL [Acta Cryst. A64, 112-122] refinement package using Least Squares minimisation. **Crystal structure determination of [2014sot0015]: Crystal Data** for $C_{30}H_{52}O_4Si$ (*M* =504.80): orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 6.4325(16) Å, *b* = 19.471(5) Å, *c* = 23.765(6) Å, *V* = 2976.5(13) Å^3, *Z* = 4, *T* = 100(2) K, μ(MoKα) = 0.110 mm^{-1}, *Dcalc* = 1.126 g/mm^3, 19206 reflections measured (5.552 ≤ 2Θ ≤ 55.08), 6638 unique (R_{int} = 0.0461, R_{sigma} = 0.0576) which were used in all calculations. The final R_1 was 0.0444 (I > 2σ(I)) and wR_2 was 0.1025 (all data). [Displacement ellipsoids – 50% probability]

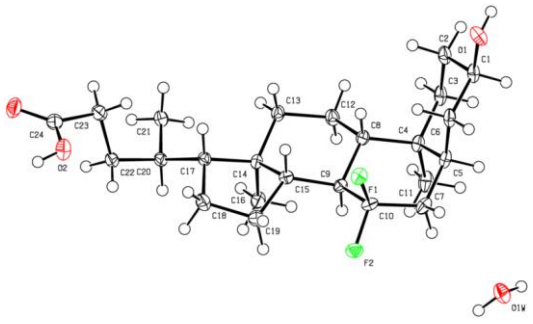


A.4 Molecular structure of compound 4.1

Crystal data and structure refinement details

Identification code	2014sot0007
Empirical formula	$C_{24}H_{40}F_2O_4$
Formula weight	430.56
Temperature/K	100(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.552(11)
b/Å	9.45(3)
c/Å	31.29(5)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2233(9)
Z	4
ρ_{calc}mg/mm^3	1.281
m/mm^{-1}	0.096
F(000)	936.0
Crystal size/mm^3	0.12 × 0.03 × 0.03
Radiation	MoKα (λ = 0.71075)
2Θ range for data collection	5.99 to 52.742°
Index ranges	-5 ≤ h ≤ 9, -6 ≤ k ≤ 11, -38 ≤ l ≤ 39
Reflections collected	10344
Independent reflections	4516 [R_{int} = 0.0309, R_{sigma} = 0.0449]
Data/restraints/parameters	4516/0/279
Goodness-of-fit on F^2	1.043
Final R indexes [I>=2σ (I)]	R_1 = 0.0386, wR_2 = 0.0824
Final R indexes [all data]	R_1 = 0.0492, wR_2 = 0.0867
Largest diff. peak/hole / e Å^{-3}	0.21/-0.19
Flack parameter	0.3(4)

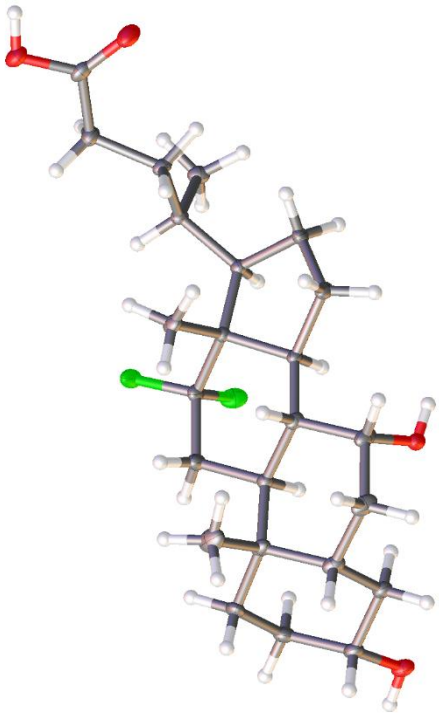
Experimental: A suitable crystal was selected and analyzed on a Rigaku Saturn724+ (2x2 bin mode) diffractometer. The crystal was kept at 100(2) K during data collection. Using Olex2 [J. Appl. Cryst. 42, 339-341], the structure was solved with the olex2.solve [Puschmann, H. (2013). in preparation] structure solution program using Charge Flipping and refined with the ShelXL [Acta Cryst. A64, 112-122] refinement package using Least Squares minimisation. **Crystal structure determination of [2014sot0007]: Crystal Data** for $C_{24}H_{40}F_2O_4$ (*M* =430.56): orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 7.552(11) Å, *b* = 9.45(3) Å, *c* = 31.29(5) Å, *V* = 2233(9) Å^3, *Z* = 4, *T* = 100(2) K, μ(MoKα) = 0.096 mm^{-1}, *Dcalc* = 1.281 g/mm^3, 10344 reflections measured (5.99 ≤ 2Θ ≤ 52.742), 4516 unique (R_{int} = 0.0309, R_{sigma} = 0.0449) which were used in all calculations. The final R_1 was 0.0386 (I > 2σ(I)) and wR_2 was 0.0867 (all data). [Displacement ellipsoids – 50% probability]



A.5 Molecular structure of compound 4.4

Experimental. Single clear colourless block-shaped crystals of (**2015sot0051-R-100K**) were recrystallised from a mixture of methanol and water by slow evaporation. A suitable crystal (0.26×0.12×0.07) was selected and mounted on a MITIGEN holder in perfluoroether oil on a Rigaku AFC12 FRE-VHF 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 Direct Methods solution method. The model was refined with version of **ShelXL** (Sheldrick, 2008) using Least Squares minimisation.

Crystal Data. $C_{24}H_{38}F_2O_4$, M_r = 428.54, orthorhombic, $P2_12_12_1$ (No. 19), a = 6.2699(2) Å, b = 11.2427(3) Å, c = 31.3635(6) Å, $\alpha = \beta = \gamma = 90^\circ$, V = 2210.85(10) Å^3, T = 100(2) K, Z = 4, Z' = 1, μ(MoK$_\alpha$) = 0.096, 12773 reflections measured, 5698 unique (R_{int} = 0.0168) which were used in all calculations. The final wR_2 was 0.0912 (all data) and R_1 was 0.0356 (I > 2(I)).



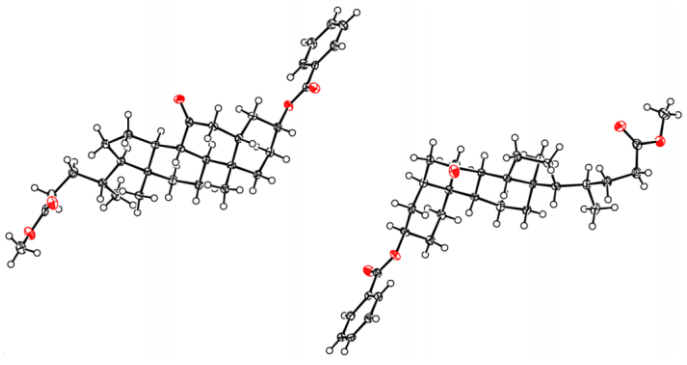
Compound	**2015sot0051-R-100K**
Formula	$C_{24}H_{38}F_2O_4$
$D_{calc.}$/ g cm^{-3}	1.287
μ/mm^{-1}	0.096
Formula Weight	428.54
Colour	clear colourless
Shape	block
Max Size/mm	0.26
Mid Size/mm	0.12
Min Size/mm	0.07
T/K	100(2)
Crystal System	orthorhombic
Flack Parameter	-0.2(2)
Hooft Parameter	-0.24(14)
Space Group	$P2_12_12_1$
a/Å	6.2699(2)
b/Å	11.2427(3)
c/Å	31.3635(6)
α/°	90
β/°	90
γ/°	90
V/Å^3	2210.85(10)
Z	4
Z'	1
Θ_{min}/°	3.168
Θ_{max}/°	28.691
Measured Refl.	12773
Independent Refl.	5698
Reflections Used	5445
R_{int}	0.0168
Parameters	286
Restraints	0
Largest Peak	0.319
Deepest Hole	-0.164
GooF	1.033
wR_2 (all data)	0.0912
wR_2	0.0898
R_1 (all data)	0.0379
R_1	0.0356

A.6 Molecular structure of compound 4.7

Crystal data and structure refinement details

Identification code	2014sot0006
Empirical formula	$C_{32}H_{44}O_5$
Formula weight	508.70
Temperature/K	100(2)
Crystal system	monoclinic
Space group	$P2_1$
a/Å	19.279(3)
b/Å	7.7036(8)
c/Å	20.162(3)
α/°	90
β/°	114.609(2)
γ/°	90
Volume/Å^3	2722.4(7)
Z	4
ρ_{calc}mg/mm^3	1.241
m/mm^{-1}	0.082
F(000)	1104.0
Crystal size/mm^3	0.3 × 0.06 × 0.06
Radiation	MoKα (λ = 0.71075)
2Θ range for data collection	6.08 to 50.052°
Index ranges	-15 ≤ h ≤ 22, -9 ≤ k ≤ 8, -23 ≤ l ≤ 20
Reflections collected	21864
Independent reflections	8995 [R_{int} = 0.0231, R_{sigma} = 0.0290]
Data/restraints/parameters	8995/1/675
Goodness-of-fit on F^2	1.047
Final R indexes [I>=2σ (I)]	R_1 = 0.0332, wR_2 = 0.0821
Final R indexes [all data]	R_1 = 0.0381, wR_2 = 0.0842
Largest diff. peak/hole / e Å^{-3}	0.22/-0.16
Flack parameter	-0.7(3)

Experimental: A suitable crystal was selected and analyzed on a Rigaku Saturn724+ (2x2 bin mode) diffractometer. The crystal was kept at 100(2) K during data collection. Using Olex2 [J. Appl. Cryst. 42, 339-34], the structure was solved with the olex2.solve [Puschmann, H. (2013). in preparation] structure solution program using Charge Flipping and refined with the ShelXL [Acta Cryst. A64, 112-122] refinement package using Least Squares minimisation. **Crystal structure determination of [2014sot0006]: Crystal Data** for $C_{32}H_{44}O_5$ (*M* =508.70): monoclinic, space group $P2_1$ (no. 4), *a* = 19.279(3) Å, *b* = 7.7036(8) Å, *c* = 20.162(3) Å, *β* = 114.609(2)°, *V* = 2722.4(7) Å^3, *Z* = 4, *T* = 100(2) K, μ(MoKα) = 0.082 mm^{-1}, *Dcalc* = 1.241 g/mm^3, 21864 reflections measured (6.08 ≤ 2Θ ≤ 50.052), 8995 unique (R_{int} = 0.0231, R_{sigma} = 0.0290) which were used in all calculations. The final R_1 was 0.0332 (I > 2σ(I)) and wR_2 was 0.0842 (all data). [Displacement ellipsoids – 50% probability]

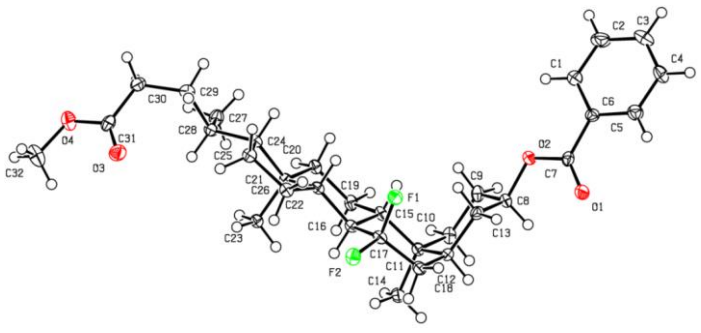


A.7 Molecular structure of compound 4.9

Crystal data and structure refinement details

Identification code	2014sot0008
Empirical formula	$C_{32}H_{44}F_2O_4$
Formula weight	530.69
Temperature/K	100(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.976(5)
b/Å	16.418(8)
c/Å	21.117(9)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2765(2)
Z	4
ρ_{calc}mg/mm^3	1.275
m/mm^{-1}	0.091
F(000)	1144.0
Crystal size/mm^3	0.12 × 0.1 × 0.08
Radiation	MoKα (λ = 0.71075)
2Θ range for data collection	5.998 to 52.728°
Index ranges	-9 ≤ h ≤ 8, -13 ≤ k ≤ 20, -26 ≤ l ≤ 24
Reflections collected	16053
Independent reflections	5576 [R_{int} = 0.0322, R_{sigma} = 0.0405]
Data/restraints/parameters	5576/0/347
Goodness-of-fit on F^2	1.073
Final R indexes [I>=2σ (I)]	R_1 = 0.0393, wR_2 = 0.0838
Final R indexes [all data]	R_1 = 0.0485, wR_2 = 0.0880
Largest diff. peak/hole / e Å^{-3}	0.27/-0.19
Flack parameter	-0.2(4)

Experimental: A suitable crystal was selected and analyzed on a Rigaku Saturn724+ (2x2 bin mode) diffractometer. The crystal was kept at 100(2) K during data collection. Using Olex2 [J. Appl. Cryst. 42, 339-341], the structure was solved with the olex2.solve [Puschmann, H. (2013). in preparation] structure solution program using Charge Flipping and refined with the ShelXL [Acta Cryst. A64, 112-122] refinement package using Least Squares minimisation. **Crystal structure determination of [2014sot0008]: Crystal Data** for $C_{32}H_{44}F_2O_4$ (*M* =530.69): orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 7.976(5) Å, *b* = 16.418(8) Å, *c* = 21.117(9) Å, *V* = 2765(2) Å^3, *Z* = 4, *T* = 100(2) K, μ(MoKα) = 0.091 mm^{-1}, *Dcalc* = 1.275 g/mm^3, 16053 reflections measured (5.998 ≤ 2Θ ≤ 52.728), 5576 unique (R_{int} = 0.0322, R_{sigma} = 0.0405) which were used in all calculations. The final R_1 was 0.0393 (I > 2σ(I)) and wR_2 was 0.0880 (all data). [Displacement ellipsoids – 50% probability]

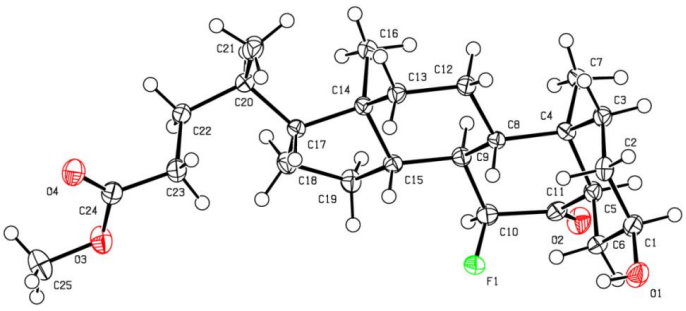


A.8 Molecular structure of compound 4.27

Crystal data and structure refinement details

Identification code	2014sot0020
Empirical formula	$C_{25}H_{39}FO_4$
Formula weight	422.56
Temperature/K	100(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.1958(9)
b/Å	10.4500(13)
c/Å	29.868(4)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2246.0(5)
Z	4
ρ_{calc}mg/mm^3	1.250
m/mm^{-1}	0.088
F(000)	920.0
Crystal size/mm^3	0.2 × 0.2 × 0.01
Radiation	MoKα (λ = 0.71075)
2Θ range for data collection	4.758 to 55.094°
Index ranges	-9 ≤ h ≤ 9, -13 ≤ k ≤ 13, -38 ≤ l ≤ 38
Reflections collected	17029
Independent reflections	5148 [R_{int} = 0.0495, R_{sigma} = 0.0451]
Data/restraints/parameters	5148/0/276
Goodness-of-fit on F^2	0.979
Final R indexes [I>=2σ (I)]	R_1 = 0.0350, wR_2 = 0.0842
Final R indexes [all data]	R_1 = 0.0438, wR_2 = 0.0882
Largest diff. peak/hole / e Å^{-3}	0.20/-0.17
Flack parameter	0.1(3)

Experimental: A suitable crystal was selected and analyzed on a Rigaku Saturn724+ (2x2 bin mode) diffractometer. The crystal was kept at 100(2) K during data collection. Using Olex2 [J. Appl. Cryst. 42, 339-341], the structure was solved with the ShelXS [Acta Cryst. A64, 112-122] structure solution program using Direct Methods and refined with the ShelXL [Acta Cryst. A64, 112-122] refinement package using Least Squares minimisation. **Crystal structure determination of [2014sot0020]: Crystal Data** for $C_{25}H_{39}FO_4$ (*M* =422.56): orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 7.1958(9) Å, *b* = 10.4500(13) Å, *c* = 29.868(4) Å, *V* = 2246.0(5) Å^3, *Z* = 4, *T* = 100(2) K, μ(MoKα) = 0.088 mm^{-1}, *Dcalc* = 1.250 g/mm^3, 17029 reflections measured (4.758 ≤ 2Θ ≤ 55.094), 5148 unique (R_{int} = 0.0495, R_{sigma} = 0.0451) which were used in all calculations. The final R_1 was 0.0350 (I > 2σ(I)) and wR_2 was 0.0882 (all data). [Displacement ellipsoids – 50% probability]

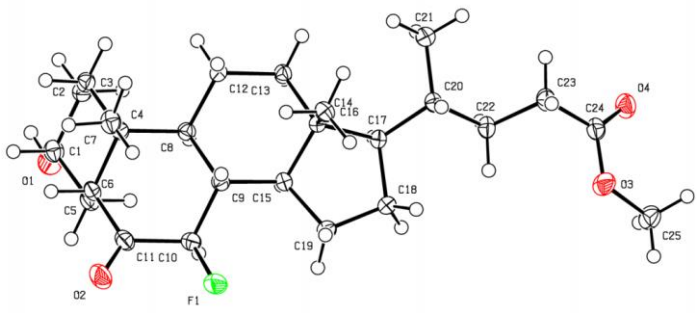


A.9 Molecular structure of compound 4.28

Crystal data and structure refinement details

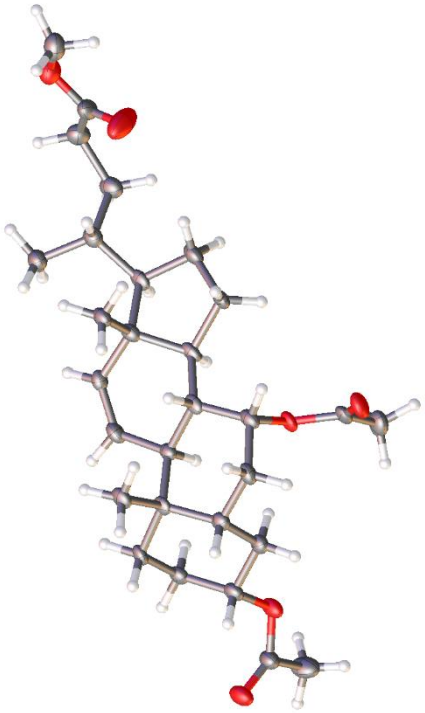
Identification code	2014sot0009
Empirical formula	$C_{25}H_{39}FO_4$
Formula weight	422.58
Temperature/K	100(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	7.0562(11)
b/Å	12.224(2)
c/Å	26.010(4)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2243.5(6)
Z	4
ρ_{calc}mg/mm^3	1.251
m/mm^{-1}	0.088
F(000)	920.0
Crystal size/mm^3	0.24 × 0.08 × 0.08
Radiation	MoKα (λ = 0.71075)
2Θ range for data collection	5.982 to 54.97°
Index ranges	-9 ≤ h ≤ 9, -15 ≤ k ≤ 15, -33 ≤ l ≤ 25
Reflections collected	13685
Independent reflections	5059 [R_{int} = 0.0277, R_{sigma} = 0.0289]
Data/restraints/parameters	5059/0/279
Goodness-of-fit on F^2	1.099
Final R indexes [I>=2σ (I)]	R_1 = 0.0403, wR_2 = 0.1320
Final R indexes [all data]	R_1 = 0.0417, wR_2 = 0.1349
Largest diff. peak/hole / e Å^{-3}	0.22/-0.25
Flack parameter	-0.1(3)

Experimental: A suitable crystal was selected and analyzed on a Rigaku Saturn724+ (2x2 bin mode) diffractometer. The crystal was kept at 100(2) K during data collection. Using Olex2 [J. Appl. Cryst. 42, 339-341], the structure was solved with the ShelXS [Acta Cryst. A64, 112-122] structure solution program using Direct Methods and refined with the ShelXL [Acta Cryst. A64, 112-122] refinement package using Least Squares minimisation. **Crystal structure determination of [2014sot0009]: Crystal Data** for $C_{25}H_{39}FO_4$ (*M* =422.58): orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 7.0562(11) Å, *b* = 12.224(2) Å, *c* = 26.010(4) Å, *V* = 2243.5(6) Å^3, *Z* = 4, *T* = 100(2) K, μ(MoKα) = 0.088 mm^{-1}, *Dcalc* = 1.251 g/mm^3, 13685 reflections measured (5.982 ≤ 2Θ ≤ 54.97), 5059 unique (R_{int} = 0.0277, R_{sigma} = 0.0289) which were used in all calculations. The final R_1 was 0.0403 (I > 2σ(I)) and wR_2 was 0.1349 (all data). [Displacement ellipsoids – 50% probability]



A.10 Molecular structure of compound 4.40

Experimental. Single clear colourless rod-shaped crystals of (**2015sot0057-R-100K**) were recrystallised from acetone by slow evaporation. A suitable crystal (0.11×0.04×0.01) was selected and mounted on a MITIGEN holder in perfluoroether oil on a Rigaku AFC12 FRE-VHF 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 Direct Methods solution method. The model was refined with **ShelXL** (Sheldrick, 2008) using Least Squares minimisation.
Crystal Data. $C_{29}H_{44}O_6$, M_r = 488.64, triclinic, P1 (No. 1), a = 6.1535(4) Å, b = 14.5788(8) Å, c = 16.2542(8) Å, α = 72.227(5)°, β = 81.659(5)°, γ = 77.853(5)°, V = 1352.37(14) Å^3, T = 100(2) K, Z = 2, Z' = 2, μ(MoK$_\alpha$) = 0.082, 24704 reflections measured, 13507 unique (R_{int} = 0.0967) which were used in all calculations. The final wR_2 was 0.1929 (all data) and R_1 was 0.0808 (I > 2(I)).

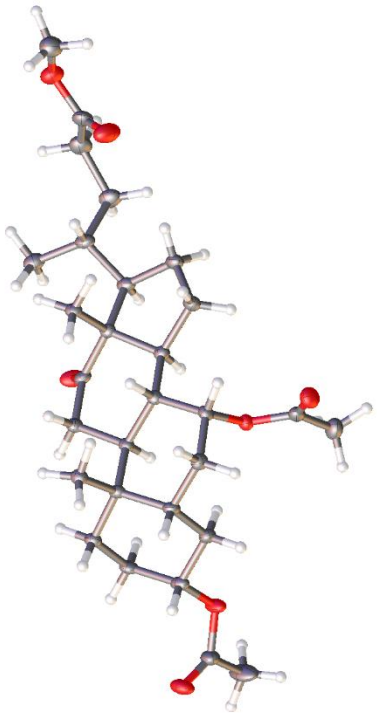


Compound	**2015sot0057-R-100K**
Formula	$C_{29}H_{44}O_6$
$D_{calc.}$/ g cm^{-3}	1.200
μ/mm^{-1}	0.082
Formula Weight	488.64
Colour	clear colourless
Shape	rod
Max Size/mm	0.11
Mid Size/mm	0.04
Min Size/mm	0.01
T/K	100(2)
Crystal System	triclinic
Flack Parameter	-2.0(10)
Hooft Parameter	-0.7(8)
Space Group	P1
a/Å	6.1535(4)
b/Å	14.5788(8)
c/Å	16.2542(8)
α/°	72.227(5)
β/°	81.659(5)
γ/°	77.853(5)
V/Å^3	1352.37(14)
Z	2
Z'	2
Θ_{min}/°	2.981
Θ_{max}/°	28.699
Measured Refl.	24704
Independent Refl.	13507
Reflections Used	7424
R_{int}	0.0967
Parameters	643
Restraints	3
Largest Peak	0.510
Deepest Hole	-0.287
GooF	0.968
wR_2 (all data)	0.1929
wR_2	0.1585
R_1 (all data)	0.1674
R_1	0.0808

A.11 Molecular structure of compound 4.41

Experimental. Single clear colourless block-shaped crystals of (**2015sot0050-R-100K**) were recrystallised from a mixture of DCM and THF by slow evaporation. A suitable crystal (0.12×0.04×0.02) was selected and mounted on a MITIGEN holder in perfluoroether oil on a Rigaku AFC12 FRE-VHF 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 Direct Methods solution method. The model was refined with version of **ShelXL** (Sheldrick, 2008) using Least Squares minimisation.

Crystal Data. $C_{29}H_{44}O_7$, M_r = 504.64, monoclinic, I2 (No. 5), a = 24.0277(8) Å, b = 6.2858(2) Å, c = 18.1418(5) Å, β = 97.807(3)°, $\alpha = \gamma = 90°$, V = 2714.63(16) Å^3, T = 100(2) K, Z = 4, Z' = 1, μ(MoK$_\alpha$) = 0.087, 13133 reflections measured, 6963 unique (R_{int} = 0.0274) which were used in all calculations. The final wR_2 was 0.1075 (all data) and R_1 was 0.0491 (I > 2(I)).

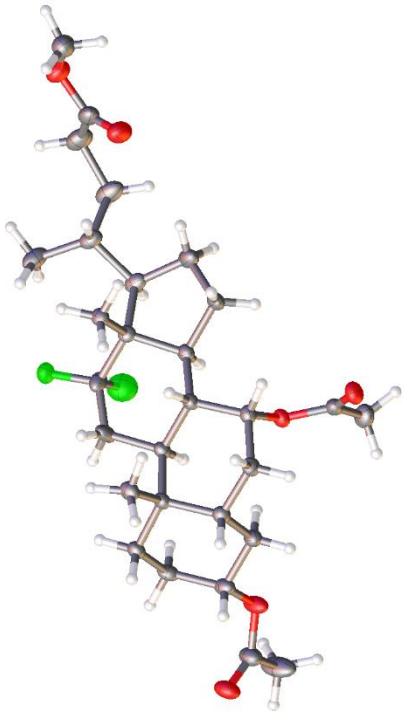


Compound	**2015sot0050-R-100K**
Formula	$C_{29}H_{44}O_7$
$D_{calc.}$/ g cm^{-3}	1.235
μ/mm^{-1}	0.087
Formula Weight	504.64
Colour	clear colourless
Shape	block
Max Size/mm	0.12
Mid Size/mm	0.04
Min Size/mm	0.02
T/K	100(2)
Crystal System	monoclinic
Flack Parameter	-1.2(5)
Hooft Parameter	-1.1(4)
Space Group	I2
a/Å	24.0277(8)
b/Å	6.2858(2)
c/Å	18.1418(5)
α/°	90
β/°	97.807(3)
γ/°	90
V/Å^3	2714.63(16)
Z	4
Z'	1
Θ_{min}/°	2.944
Θ_{max}/°	28.698
Measured Refl.	13133
Independent Refl.	6963
Reflections Used	5689
R_{int}	0.0274
Parameters	331
Restraints	1
Largest Peak	0.252
Deepest Hole	-0.208
GooF	1.026
wR_2 (all data)	0.1075
wR_2	0.1005
R_1 (all data)	0.0660
R_1	0.0491

A.12 Molecular structure of compound 4.42

Experimental. Single clear colourless block-shaped crystals of (**2015sot0048-K-100K**) were recrystallised from a mixture of DCM and Water by slow evaporation. A suitable crystal (0.25×0.12×0.10) was selected and mounted on a MITIGEN holder in perfluoroether oil on a Rigaku AFC12 FRE-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 Direct Methods solution method. The model was refined with version of **ShelXL** (Sheldrick, 2008) using Least Squares minimisation.

Crystal Data. $C_{29}H_{44}F_2O_6$, M_r = 526.64, monoclinic, I2 (No. 5), a = 24.0450(5) Å, b = 6.2780(2) Å, c = 18.1990(4) Å, β = 97.245(2)°, $\alpha = \gamma = 90°$, V = 2725.27(12) Å^3, T = 100(2) K, Z = 4, Z' = 1, μ(MoK$_\alpha$) = 0.097, 14999 reflections measured, 6287 unique (R_{int} = 0.0189) which were used in all calculations. The final wR_2 was 0.1377 (all data) and R_1 was 0.0466 (I > 2(I)).



Atom F1 has unrealistically large ADP values, if the occupancy is allowed to refine a value of ca. 0.63 is obtained in conjunction with more reasonable ADPs. Pending additional corroborative chemical evidence for a partial occupancy it has been

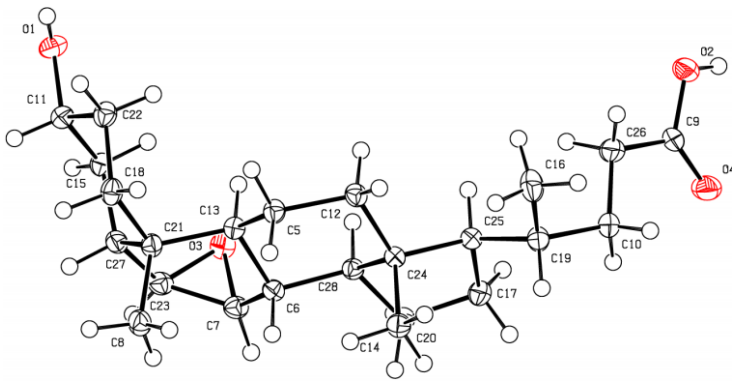
Compound	**2015sot0048-K-100K**
Formula	$C_{29}H_{44}F_2O_6$
$D_{calc.}$/ g cm^{-3}	1.284
μ/mm^{-1}	0.097
Formula Weight	526.64
Colour	clear colourless
Shape	block
Max Size/mm	0.25
Mid Size/mm	0.12
Min Size/mm	0.10
T/K	100(2)
Crystal System	monoclinic
Flack Parameter	-0.01(18)
Hooft Parameter	0.04(15)
Space Group	I2
a/Å	24.0450(5)
b/Å	6.2780(2)
c/Å	18.1990(4)
α/°	90
β/°	97.245(2)
γ/°	90
V/Å^3	2725.27(12)
Z	4
Z'	1
Θ_{min}/°	2.997
Θ_{max}/°	28.695
Measured Refl.	14999
Independent Refl.	6287
Reflections Used	5950
R_{int}	0.0189
Parameters	340
Restraints	1
Largest Peak	0.314
Deepest Hole	-0.665
GooF	1.082
wR_2 (all data)	0.1377
wR_2	0.1345
R_1 (all data)	0.0492
R_1	0.0466

A.13 Molecular structure of compound 4.74

Crystal data and structure refinement details

Identification code	2014sot0017
Empirical formula	$C_{24}H_{38}O_4$
Formula weight	390.54
Temperature/K	100(2)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	6.6731(5)
b/Å	11.9888(10)
c/Å	26.858(2)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	2148.7(3)
Z	4
ρ_{calc}mg/mm^3	1.207
m/mm^{-1}	0.080
F(000)	856.0
Crystal size/mm^3	0.21 × 0.2 × 0.02
Radiation	MoKα (λ = 0.71075)
2Θ range for data collection	4.554 to 55.03°
Index ranges	-5 ≤ h ≤ 8, -15 ≤ k ≤ 15, -34 ≤ l ≤ 34
Reflections collected	11037
Independent reflections	4799 [R_{int} = 0.0306, R_{sigma} = 0.0366]
Data/restraints/parameters	4799/0/258
Goodness-of-fit on F^2	1.005
Final R indexes [I>=2σ (I)]	R_1 = 0.0315, wR_2 = 0.0809
Final R indexes [all data]	R_1 = 0.0334, wR_2 = 0.0817
Largest diff. peak/hole / e Å^{-3}	0.26/-0.17
Flack parameter	-0.3(4)

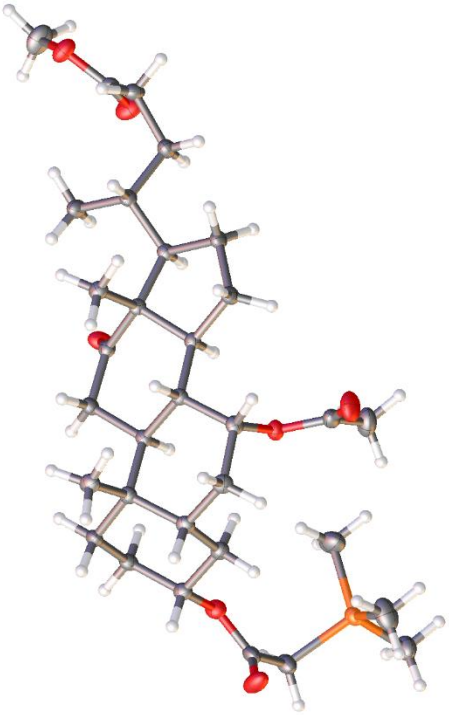
Experimental: A suitable crystal was selected and analyzed on a Rigaku Saturn724+ (2x2 bin mode) diffractometer. The crystal was kept at 100(2) K during data collection. Using Olex2 [J. Appl. Cryst. 2009, 42, 339-341], the structure was solved with the ShelXS [Acta Cryst. 2008, A64, 112-122] structure solution program using Direct Methods and refined with the ShelXL [Acta Cryst. 2008, A64, 112-122] refinement package using Least Squares minimisation. **Crystal structure determination of [2014sot0017]: Crystal Data** for $C_{24}H_{38}O_4$ (*M* =390.54): orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 6.6731(5) Å, *b* = 11.9888(10) Å, *c* = 26.858(2) Å, *V* = 2148.7(3) Å^3, *Z* = 4, *T* = 100(2) K, μ(MoKα) = 0.080 mm^{-1}, *Dcalc* = 1.207 g/mm^3, 11037 reflections measured (4.554 ≤ 2Θ ≤ 55.03), 4799 unique (R_{int} = 0.0306, R_{sigma} = 0.0366) which were used in all calculations. The final R_1 was 0.0315 (I > 2σ(I)) and wR_2 was 0.0817 (all data). [Displacement ellipsoids – 50% probability]



A.14 Molecular structure of compound 4.102

Experimental. Single clear colourless rod-shaped crystals of (**2015sot0052-R2-100K**) were recrystallised from a mixture of TEA and DCM by slow evaporation. A suitable crystal (0.23×0.03×0.02) was selected and mounted on a mylar loop in perfluoroether oil on a Rigaku AFC12 FRE-VHF 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 Direct Methods solution method. The model was refined with version of **ShelXL** (Sheldrick, 2008) using Least Squares minimisation.

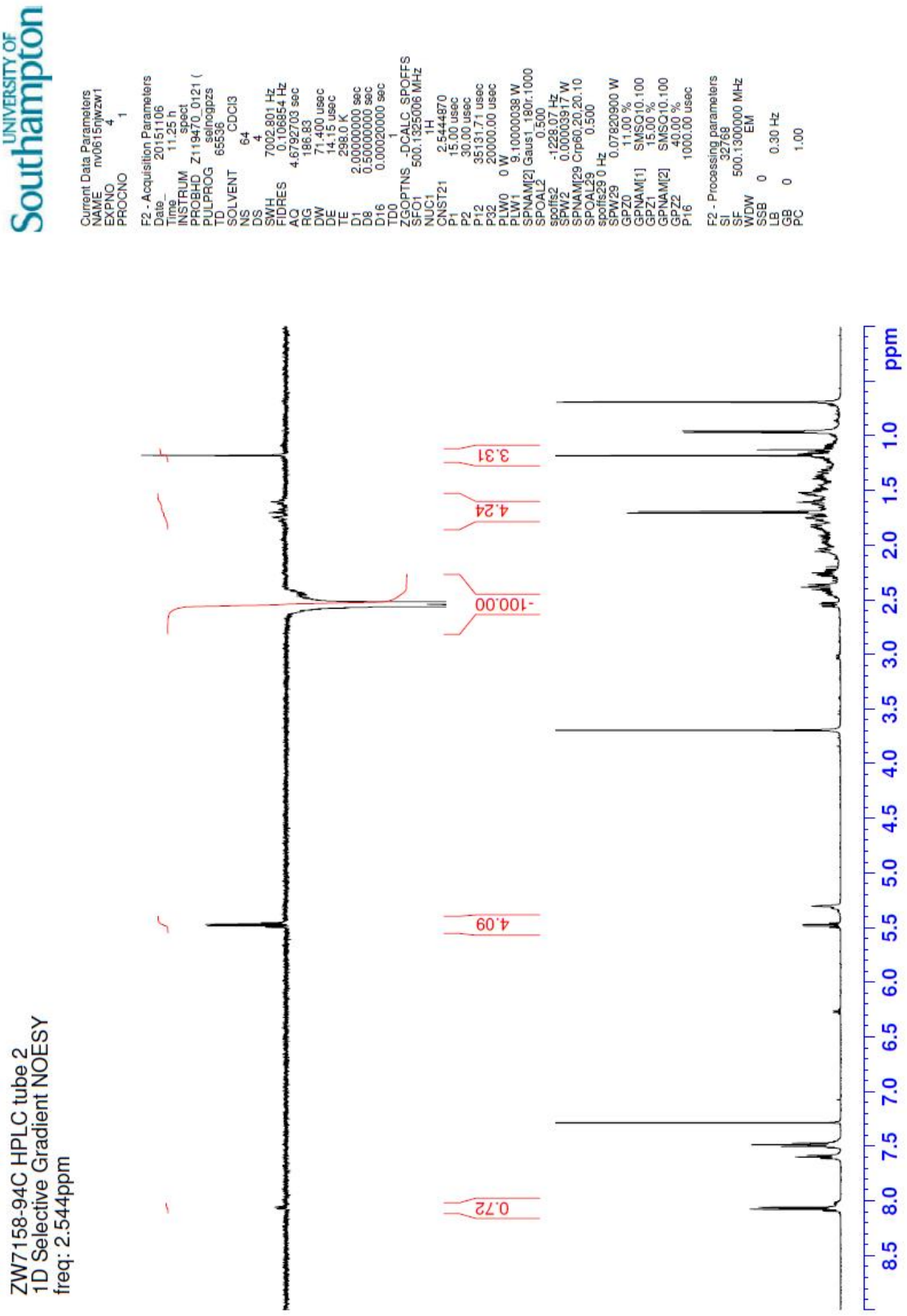
Crystal Data. $C_{32}H_{52}O_7Si$, M_r = 576.82, monoclinic, $P2_1$ (No. 4), a = 15.5875(8) Å, b = 6.5293(2) Å, c = 17.5341(9) Å, β = 114.190(6)°, $\alpha = \gamma = 90°$, V = 1627.84(15) Å^3, T = 100(2) K, Z = 2, Z' = 1, μ(MoK$_\alpha$) = 0.115, 16912 reflections measured, 6487 unique (R_{int} = 0.0321) which were used in all calculations. The final wR_2 was 0.0910 (all data) and R_1 was 0.0386 (I > 2(I)).

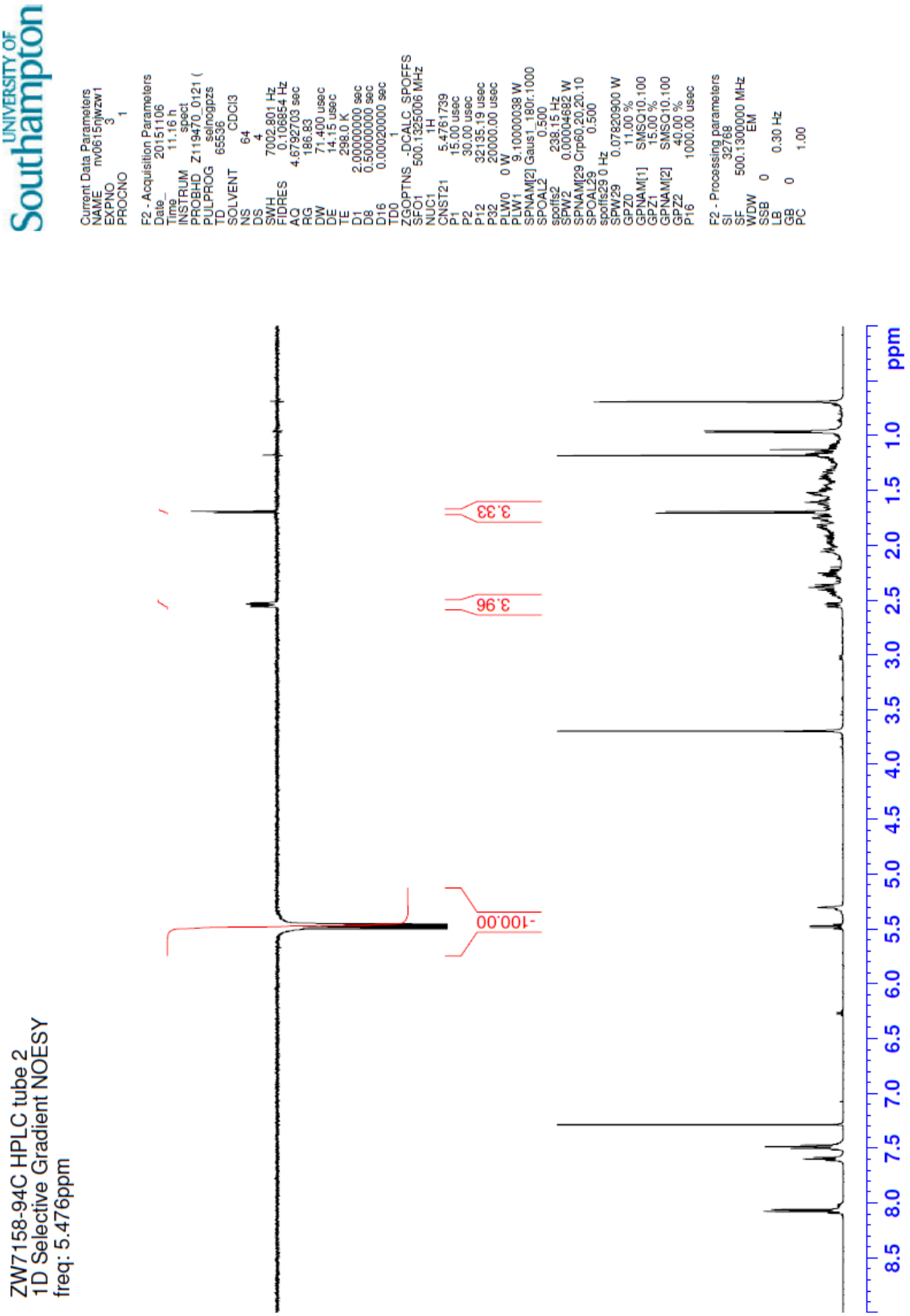


Compound	**2015sot0052-R2-100K**
Formula	$C_{32}H_{52}O_7Si$
$D_{calc.}$/ g cm^{-3}	1.177
μ/mm^{-1}	0.115
Formula Weight	576.82
Colour	clear colourless
Shape	rod
Max Size/mm	0.23
Mid Size/mm	0.03
Min Size/mm	0.02
T/K	100(2)
Crystal System	monoclinic
Flack Parameter	0.06(8)
Hooft Parameter	0.10(5)
Space Group	$P2_1$
a/Å	15.5875(8)
b/Å	6.5293(2)
c/Å	17.5341(9)
α/°	90
β/°	114.190(6)
γ/°	90
V/Å^3	1627.84(15)
Z	2
Z'	1
Θ_{min}/°	2.952
Θ_{max}/°	28.699
Measured Refl.	16912
Independent Refl.	6487
Reflections Used	5693
R_{int}	0.0321
Parameters	369
Restraints	1
Largest Peak	0.286
Deepest Hole	-0.187
GooF	1.038
wR_2 (all data)	0.0910
wR_2	0.0870
R_1 (all data)	0.0480
R_1	0.0386

A.15 NOE and COSY Spectra

A.15.1 NOE sepctra of compound 3.65




UNIVERSITY OF Southampton
Current Data Parameters
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PROCNO 1
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Time_ 11.16 h
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SOLVENT CDCl3
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SWH 7002.801 Hz
FIDRES 0.106854 Hz
AQ 4.6792703 sec
RG 186.83
DW 71.400 usec
DE 14.15 usec
TE 298.0 K
D1 2.00000000 sec
D8 0.50000000 sec
D16 0.00020000 sec
TD0 1
ZGOPTNS -DCALC_SPOFFS
SFO1 500.1325006 MHz
NUC1 1H
CNST21 5.47761739
P1 15.00 usec
P2 30.00 usec
P12 32135.19 usec
P32 20000.00 usec
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PLW1 9.10000038 W
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spoffs2 238.15 Hz
SPW2 0.00004682 W
SPNAM[29 Crp60,20,20.10
SPOAL29 0.500
spoffs29 0 Hz
SPW29 0.07820900 W
GPZ0 11.00 %
GPNAM[1] SMSQ10.100
GPZ1 15.00 %
GPNAM[2] SMSQ10.100
GPZ2 40.00 %
P16 1000.00 usec
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LB 0.30 Hz
GB 0
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3.5
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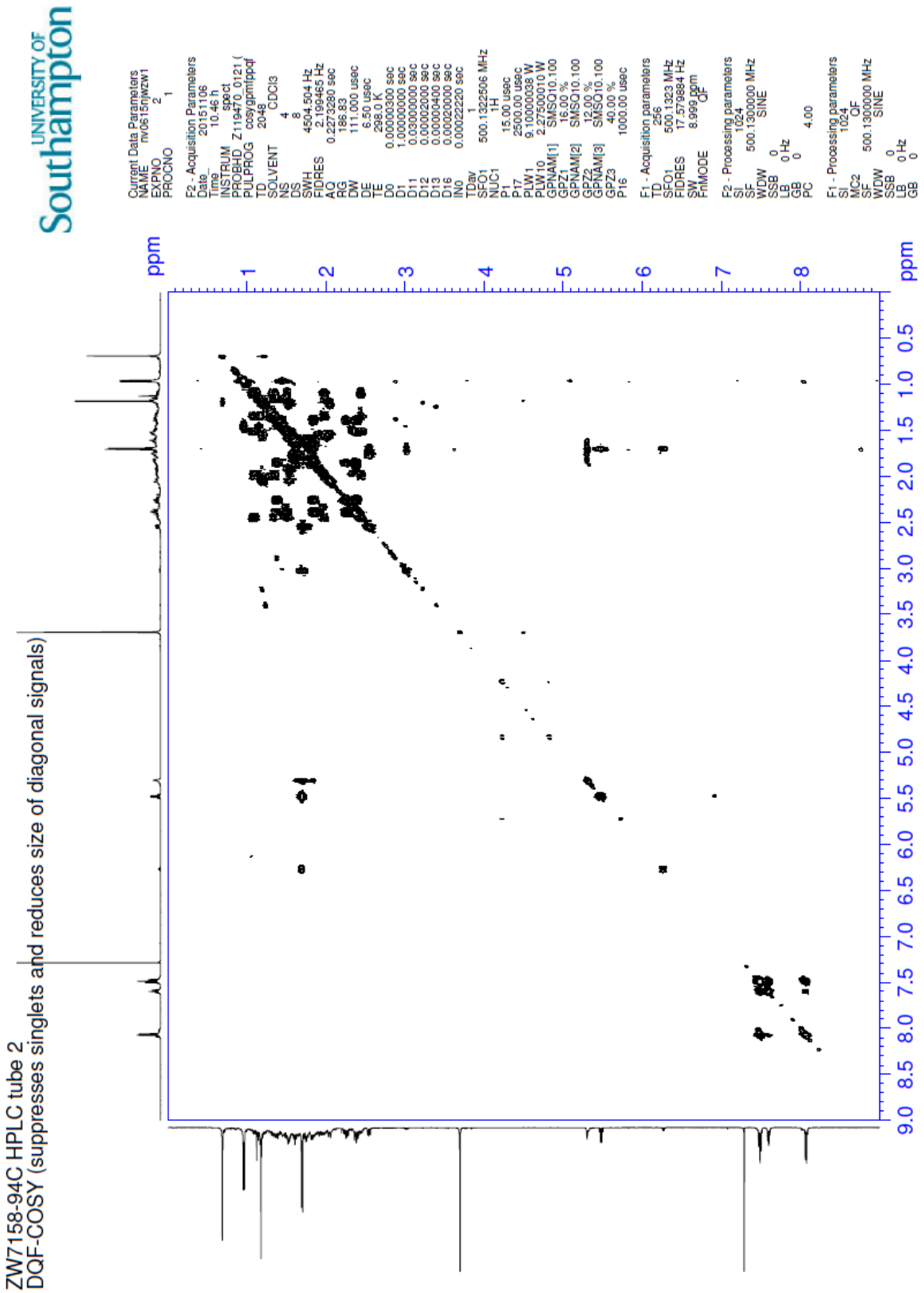
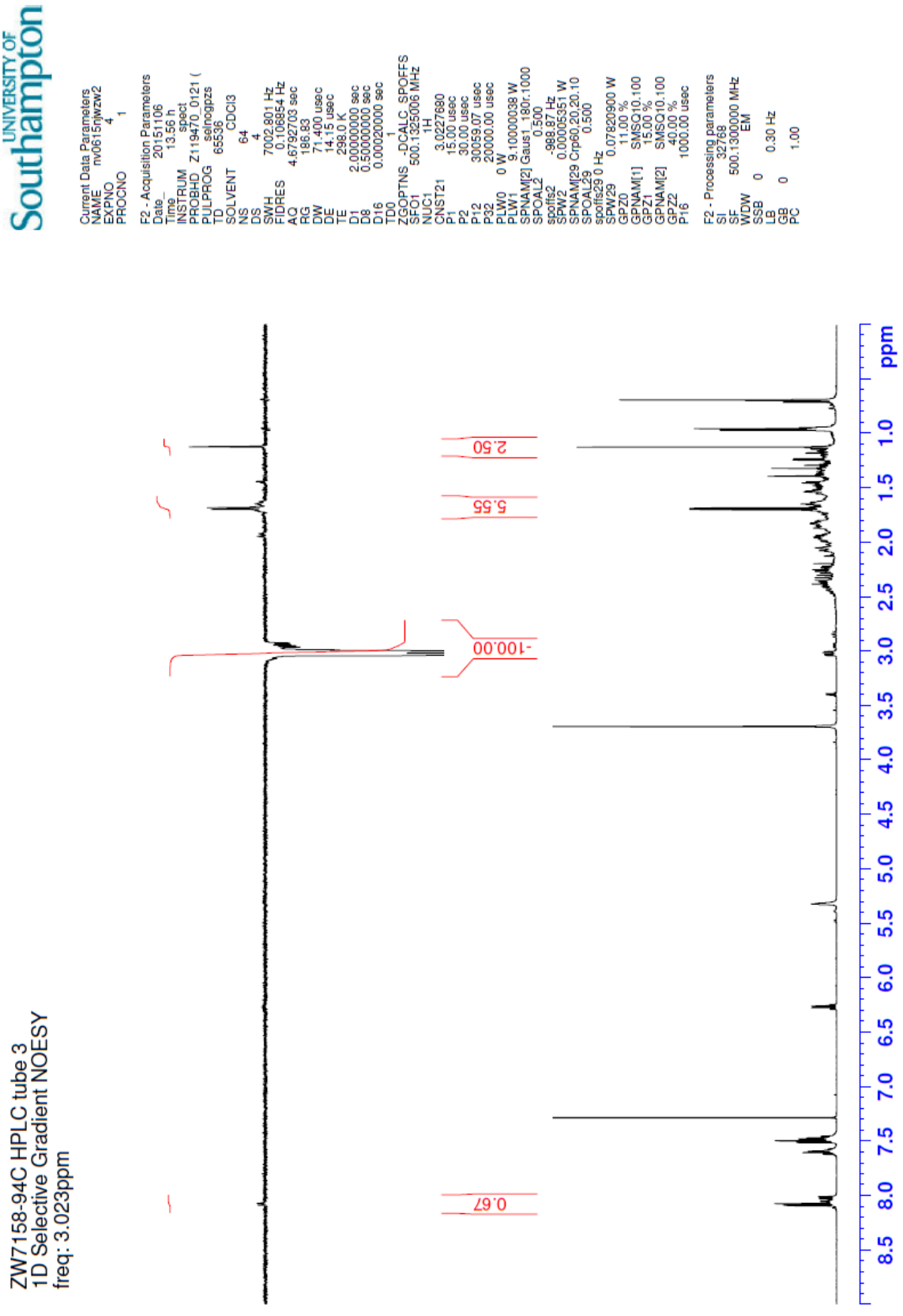
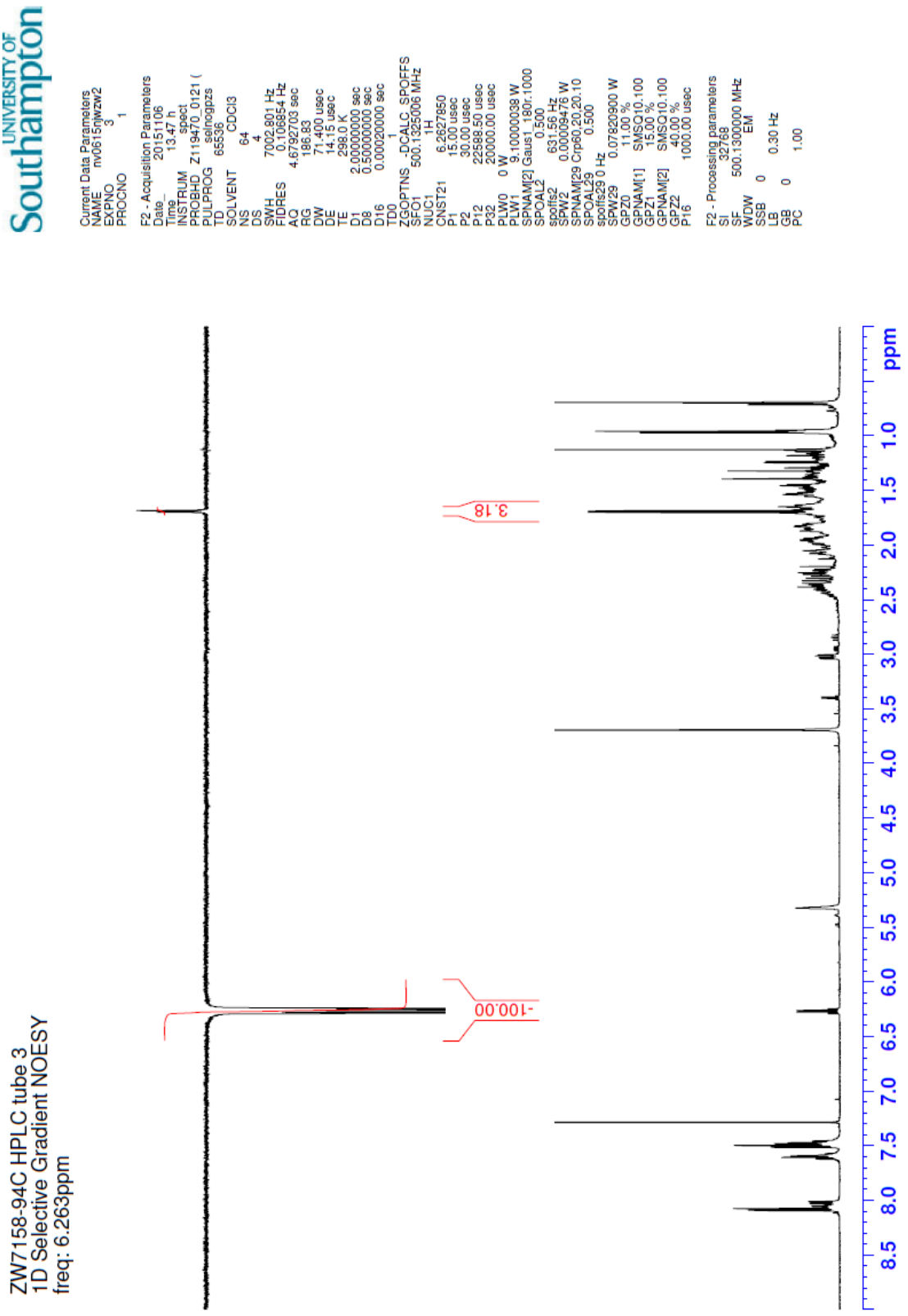
2.5
2.0
1.5
1.0
ppm

A.15.2 COSY spectrum of compound 3.65



A.15.3 NOE spectra of compound 3.66




UNIVERSITY OF Southampton
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EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
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Time 13.47 h
INSTRUM spect
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PULPROG selnogpzs
TD 65536
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NS 64
DS 4
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FIDRES 0.106854 Hz
AQ 4.6792703 sec
RG 186.83
DW 71.400 usec
DE 14.15 usec
TE 298.0 K
D1 2.00000000 sec
D8 0.50000000 sec
D16 0.00020000 sec
TD0 1
ZGOPTNS -DCALC_SPOFFS
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NUC1 1H
CNST21 6.2627850
P1 15.00 usec
P2 30.00 usec
P12 22588.50 usec
P32 20000.00 usec
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PLW1 9.10000038 W
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SPOAL2 0.500
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SPW2 0.000089476 W
SPNAM[29 Crp60,20,20.10
SPOAL29 0.500
spoffs29 0 Hz
SPW29 0.07820900 W
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GPNAM[2] SMSQ10.100
GPZ2 40.00 %
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F2 - Processing parameters
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LB 0.30 Hz
GB 0
PC 1.00
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6.5
6.0
5.5
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2.5
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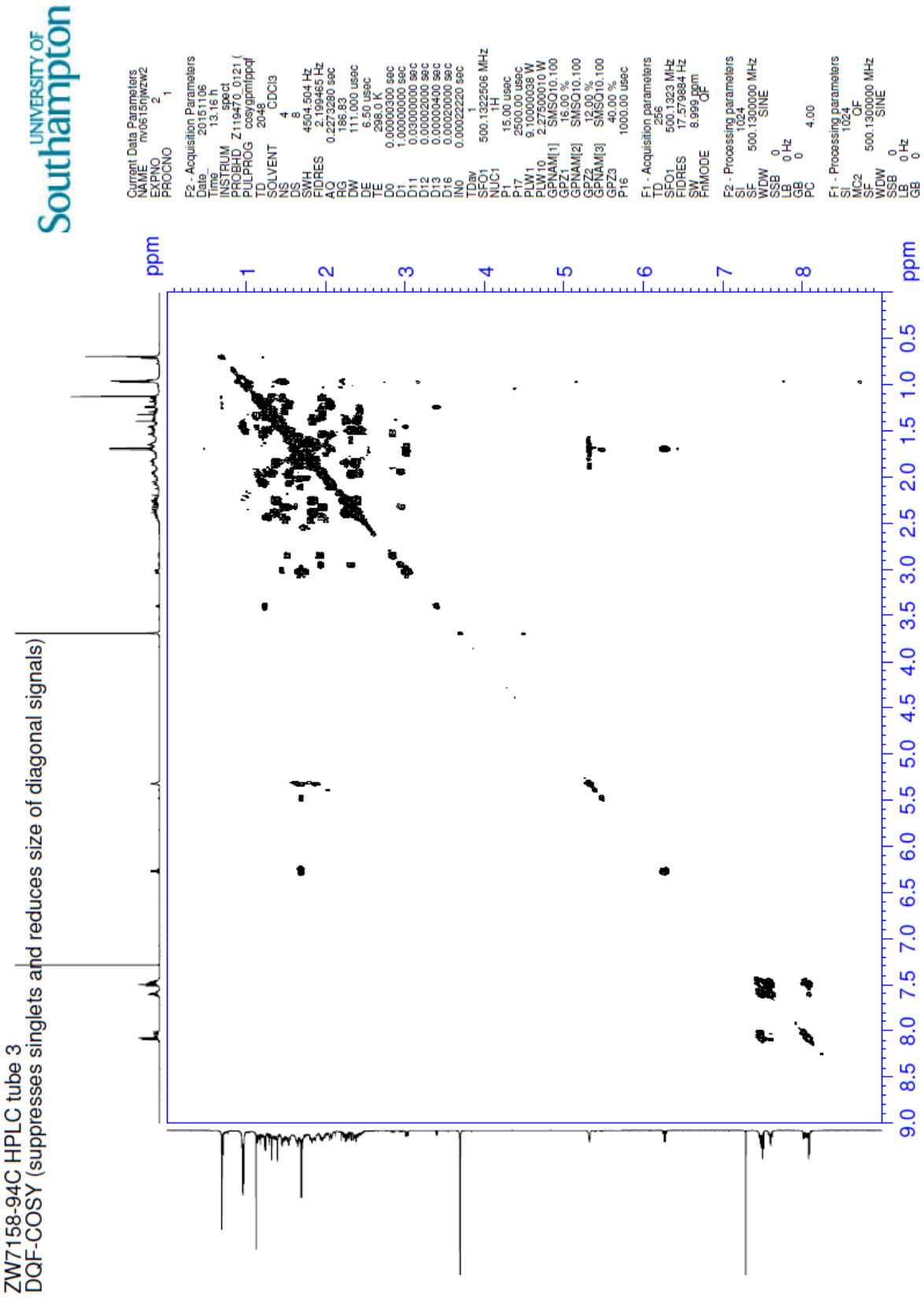
1.0
ppm

A.15.4 COSY spectrum of compound 3.66

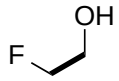
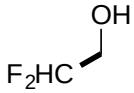
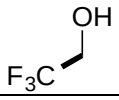
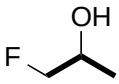
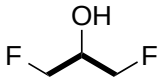
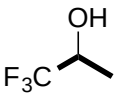
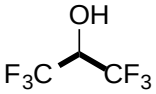
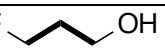
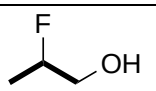
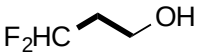
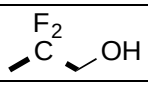
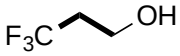
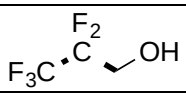
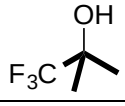


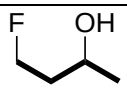
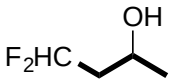
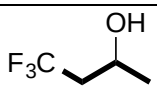
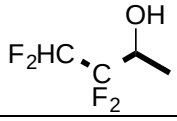
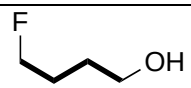
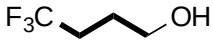
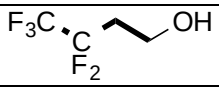
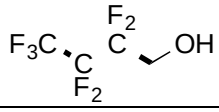
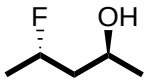
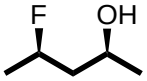
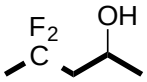
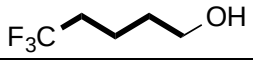
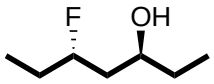
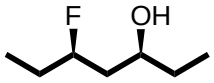
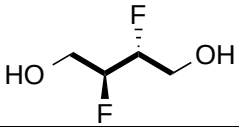
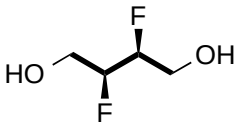
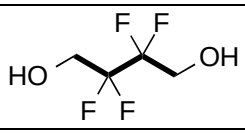
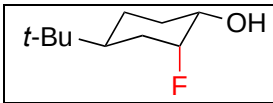
Appendix B Lipophilicity

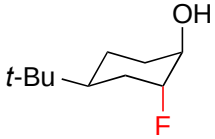
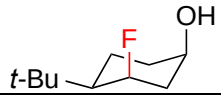
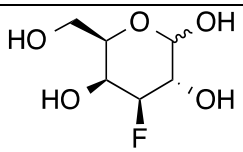
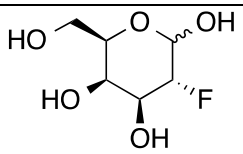
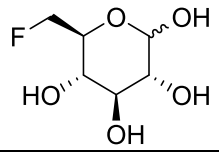
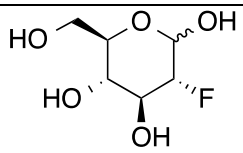
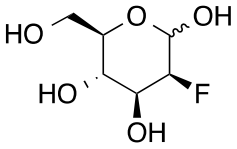
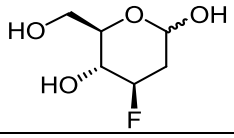
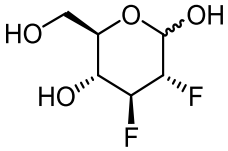
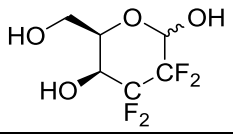
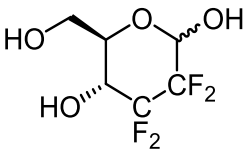
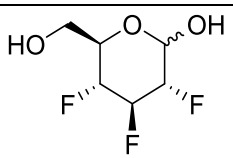
B.1 Comparison of clog*P* with experimental log*P*

The log*P* values obtained by our method are compared with two calculated log*P* values.

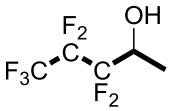
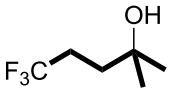
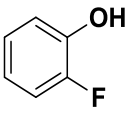
Difference of <0.10 log*P*
Difference 0.10–0.49 log*P*
Difference 0.50–0.75 log*P*
Difference >0.75 log*P*

Compound	Nr	log*P* exp	clog*P*[a]	Δlog*P*	milog*P*[b]	Δlog*P*
	**6.2**	-0.75	-0.32	***+0.43***	-0.02	***+0.73***
	**6.20**	-0.29	-0.05	***+0.24***	+0.25	***+0.54***
	**6.27**	+0.36	+0.44	***+0.08***	+0.61	***+0.25***
	**6.4**	-0.35	+0.10	***+0.45***	+0.34	***+0.69***
	**6.5**	-0.42	-0.06	***+0.36***	+0.26	***+0.68***
	**6.34**	+0.71	+0.85	***+0.14***	+0.97	***+0.26***
	**6.35**	+1.69	+1.45	***+0.24***	+1.52	***+0.17***
	**6.7**	-0.26	-0.26	***0.00***	+0.25	***+0.51***
	**6.8**	-0.29	+0.10	***+0.39***	+0.34	***+0.63***
	**6.22**	+0.04	+0.22	***+0.18***	+0.52	***+0.48***
	**6.21**	+0.11	+0.20	***+0.09***	+0.70	***+0.59***
	**6.28**	+0.41	+0.49	***+0.08***	+0.88	***+0.47***
	**6.40**	+1.20	+1.14	***-0.06***	+1.25	***+0.05***
	**6.37**	+1.03	+1.13	***+0.10***	+1.42	***+0.39***

	**6.10**	+0.08	+0.16	***+0.08***	+0.61	***+0.53***
	**6.23**	+0.42	+0.63	***+0.21***	+0.88	***+0.46***
	**6.29**	+0.72	+0.90	***+0.18***	+1.24	***+0.52***
	**6.41**	+1.05	+1.06	***+0.01***	+1.61	***+0.56***
	**6.13**	+0.09	+0.26	***+0.17***	+0.52	***+0.43***
	**6.30**	+0.91	+1.00	***+0.09***	+1.15	***+0.24***
	**6.43**	+1.30	+1.41	***+0.11***	+1.52	***+0.22***
	**6.44**	+1.98	+1.84	***-0.14***	+1.89	***-0.09***
	**6.52**	+0.57	+0.58	***+0.01***	+0.97	***+0.40***
	**6.53**	+0.48	+0.58	***+0.10***	+0.97	***+0.49***
	**6.25**	+0.66	+0.88	***+0.22***	+1.33	***+0.67***
	**6.31**	+1.22	+1.44	***+0.22***	+1.65	***+0.43***
	**6.55**	+1.71	+1.62	***-0.09***	+1.98	***+0.27***
	**6.56**	+1.58	+1.62	***+0.04***	+1.98	***+0.40***
	**6.50**	-1.01	-0.69	***+0.32***	-0.38	***+0.63***
	**6.49**	-0.97	-0.69	***+0.28***	-0.38	***+0.59***
	**6.51**	-0.11	+0.19	***+0.30***	+0.33	***+0.44***
	**6.58**	+2.63	+2.42	***-0.21***	+2.70	***+0.07***

	**6.59**	+2.61	+2.42	***-0.19***	+2.70	***-0.09***
	**6.61**	+3.21	+2.42	***-0.79***	+2.70	***-0.51***
	**6.62**	+2.23	+2.11	***-0.12***	+2.70	***-0.47***
	**6.63**	-2.47	-2.04	***+0.43***	-2.15	***+0.32***
	**6.64**	-2.37	-2.04	***+0.33***	-1.93	***+0.44***
	**6.65**	-2.36	-2.04	**+0.32**	-1.71	***+0.65***
	**6.66**	-2.21	-2.04	***+0.17***	-1.93	***+0.28***
	**6.67**	-2.11	-2.04	***+0.07***	-1.93	***-0.18***
	**6.68**	-1.45	-1.14	***-0.31***	-1.23	***-0.22***
	**6.69**	-1.11	-1.15	***+0.04***	-1.22	***+0.14***
	**6.70**	-0.58	-0.27	***+0.31***	-0.51	***+0.07***
	**6.71**	-0.32	-0.27	***+0.05***	-0.51	***-0.19***
	**6.72**	-0.17	-0.26	***-0.09***	-0.29	***-0.12***

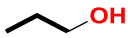
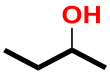
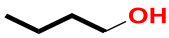
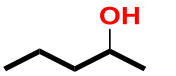
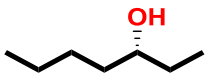
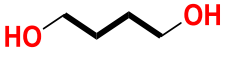
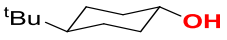
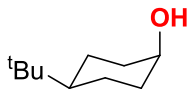
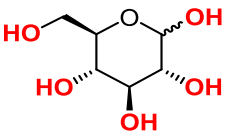
Appendix B

	**6.45**	+2.41	+2.25	***-0.19***	+2.25	***-0.19***
	**6.33**	+1.52	+1.69	***+0.17***	+1.96	***+0.44***
	**6.15**	+0.80	+0.71	***+0.09***	+1.14	***+0.34***
	**exp1**	+1.70	+1.81	***+0.11***	+1.83	***+0.13***

[a] Calculated log*P* using Marvin Sketch 14.8.4.0;
[b] milog*P* calculated log*P* using Molinspriation Cheminformatics on the Web: http://www.molinspiration.com/cgi-bin/properties

B.2 Literature logP values for non-fluorinated alcohols

The first value was used to compare with the log*P* of the fluorohydrins. In general, this is the log*P* value catalogued by U.S. National Library of Medicine (NIH).

Compound	Nr	log*P* (lit) (*T*, method, analysis method [if reported])
	**6.1**	**-0.30**[23]; -0.31[1]; -0.15[2]; -0.32[2] (pH 6.00)
	**6.3**	**+0.05**[1,2]
	**6.6**	**+0.30**[2]; +0.25[1];
	**6.36**	**+0.37**[2]; +0.35[1]
	**6.9**	**+0.76**[2]; +0.61[1]; 0.83[2]; 0.65[2];
	**6.11**	**+0.88**[1,2]; 0.89[2]; 0.32[2];
	**6.24**	**+1.19**[1] 1.16[2];
	**6.16**	**+1.51**[1] 1.40[2];
	**6.54**	**+2.24**[1];
	**6.48**	**-0.83**[1];
	**6.57**	**+3.09**[1];
	**6.60**	**+3.02**[1];
	**Glc**	**-3.24**[1]; -2.84[4] (5 °C, enzymatic) -2.82[4] (20 °C, enzymatic) -3.10[4] (25 °C, Radiochemical) -3.02[4] (LC) -3.00[4]/-3.24[4];

	**6.14**	**+1.31**[1]

[1]log*P* data retrieved from U.S. National Library of Medicine online database: http://chem.sis.nlm.nih.gov/chemidplus/; partition and analysis method as well as temperature for log*P* values are not provided from the database;
[2]Value in ref [36]. Partition and analysis method as well as temperature for logP values are not provided in this book;
[3] Value in ref [37]. Method used for partition: shake-flask (method of Hansch and Muir[38]); method used for analysis: gas chromatography (method developed by Bluestein and Posmanter[39]; temperature for logP measurement: not reported;
[4] Value in ref [40];

[36] C. Hansch, A. Leo, in *Substituent constants for correlation analysis in chemistry and biology*. Wiley, **1979**.
[37] E. O. Dillingham, R. W. Mast, G. E. Bass, J. Autian, *J. Pharm. Sci.* **1973**, *62*, 22-30.
[38] C. Hansch, R. M. Muir, T. Fujita, P. P. Maloney, F. Geiger, M. Streich, *J. Am. Chem. Soc.* **1963**, *85*, 2817-2824.
[39] C. Bluestein, H. N. Posmanter, *Anal. Chem.* 1966, *38*, 1865-1869.
[40] M. F. Mazzobre, M. V. Roman, A. F. Mourelle, H. R. Corti, *Carbohydrate research*, **2005**, *340*, 1207-1211

B.3 Ideal sample mass to minimize error for quantitative integration

For practical purpose, the ideal sample mass (or mass ratio) can be used to minimize error for optimal integration ratios in octanol and water layers. It is also helpful to predict integration outcome in both layers when calculated clog*P* value is used.

The parameters for the experiments are defined in the following table.

	mass	**MW**	**P**	**conc. in octanol**	**conc. in water**	**number of F**	**Intensity in octanol**	**Intensity in water**
Sample A	m_A	M_A	P^*_A	C_A	C'_A	N_A	I_A	I'_A
Reference R	m_R	M_R	P_R	C_R	C'_R	N_R	I_R	I'_R

*If P_A is unknown, the calculated cP_A can be used as P_A for the initial trial experiment.

Ratio ***r*** is defined as the ratio of partition coefficient between sample **A** and reference **R**.

$$\boldsymbol{r} = P_A / P_R$$

For simplification, sample **A** and reference **R** contain same number of fluorine atom(s), which is also generally the case for the favoured closer fluorine chemical shifts in the spectrum.

$$N_A = N_R$$

Therefore, $C_A/C_R = I_A/I_R$ and $C'_A/C'_R = I'_A/I'_R$.

If $\boldsymbol{r \sim 1}$ and $M_A/M_R \sim \mathbf{1}$, same or similar mass of sample **A** regarding reference **R** can be used, as long as the corresponding concentration can bring satisfactory S/N ratio. This is the ideal case by choosing a reference with similar **P** value compared to $\mathbf{P_A}$ or $\mathbf{cP_A}$ (*calculated value*).

If ***r*** and $\mathbf{M_A/M_R}$ values are not close to **1** (e.g., larger than **4** or even more), the sample mass used for the experiment should be properly considered, to minimize the error which can caused by largely different integral ratio (poor S/N ratio for minor peak) in octanol/water layer. Larger ratio value (e.g., 10/1 or even higher) in single layer can cause relatively bigger error from integration itself, compared to the integration error from lower ratio value (e.g., 3/1 or less), which is related directly to S/N ratios for all four fluorine peaks when lowest necessary or minimal amount of sample/reference is used.

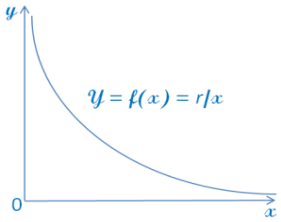
Appendix B

$$P_A / P_R = (C_A/C'_A) / (C_R/C'_R) = (C_A/C_R) / (C'_A/C'_R) = (I_A/I_R) / (I'_A/I'_R) = \boldsymbol{r}$$

Let I_A/I_R be a variable $\boldsymbol{x}$ and I'_R/I'_A be a variable $\boldsymbol{y}$,

then function $\boldsymbol{x} * \boldsymbol{y} = \boldsymbol{r}$ ($\boldsymbol{x}$, $\boldsymbol{y} > 0$; $\boldsymbol{r}$, any positive constant) can be derived.

Only when $\boldsymbol{x} = \boldsymbol{y} = \boldsymbol{r}^{0.5}$, $\boldsymbol{x}$ and $\boldsymbol{y}$, i.e., integral ratios (I_A/I_R and I'_R/I'_A), can have the lower values/ratios at the same time.



Optimal integration ratio in octanol layer: $I_A/I_R = \boldsymbol{r}^{0.5} / 1$

Optimal integration ratio in water layer: $I'_A/I'_R = 1 / \boldsymbol{r}^{0.5}$

$C_A/C_R = I_A/I_R = \boldsymbol{r}^{0.5} / 1$

- ⇨ $\{(m_A/ M_A) * [P_A / (1 + P_A)] /V_{oct}\} / \{(m_R/ M_R) * [P_R/ (1 + P_R)] /V_{oct}\} = \boldsymbol{r}^{0.5}$
- ⇨ $(m_A/ M_A) * [P_A / (1 + P_A)] = \boldsymbol{r}^{0.5} * \{(m_R/ M_R) * [P_R/ (1 + P_R)]\}$
- ⇨ $m_A = \boldsymbol{r}^{0.5} * \{(m_R/ M_R) * [P_R/ (1 + P_R)]\} / [P_A / (1 + P_A)] * M_A$ $\quad [\boldsymbol{r} = P_A / P_R]$
- ⇨ **$m_A / m_R = r^{-0.5} * (M_A / M_R) * [(1 + P_A) / (1 + P_R)]$**

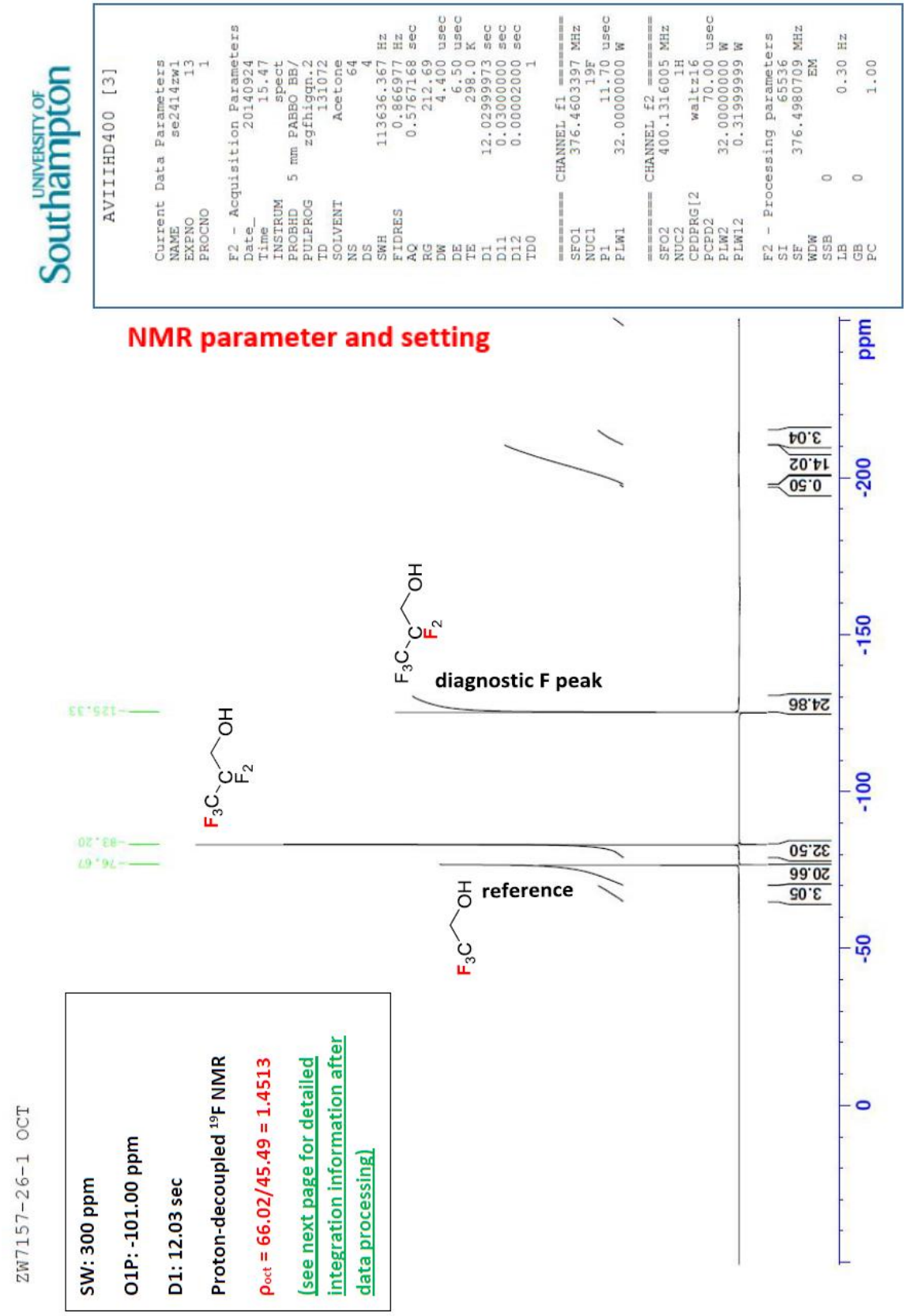
When $\boldsymbol{r} \sim 1$ ($P_A \sim P_R$) and $M_A \sim M_R$, m_A can be the same as m_R.

Otherwise, it would be better to use ideal sample mass ratio based on the equation mentioned above, to obtain optimal integration ratios in octanol/water layer for higher accuracy of quantitative integration.

If N_A is not equal to N_R, the mass ratio (m_A/m_R) can be simply divided by the N_A/N_R ratio for further use $\{m_A/m_R = r^{-0.5} * (M_A / M_R) * [(1 + P_A) / (1 + P_R)] / (N_A/N_R)\}$.

The equation can be applied directly in Excel and conveniently used for any calculation. Similar equations can be derived for integration ratio calculation/prediction, if optimal sample mass is not used.

B.4 Selected NMR spectra for log*P* determination



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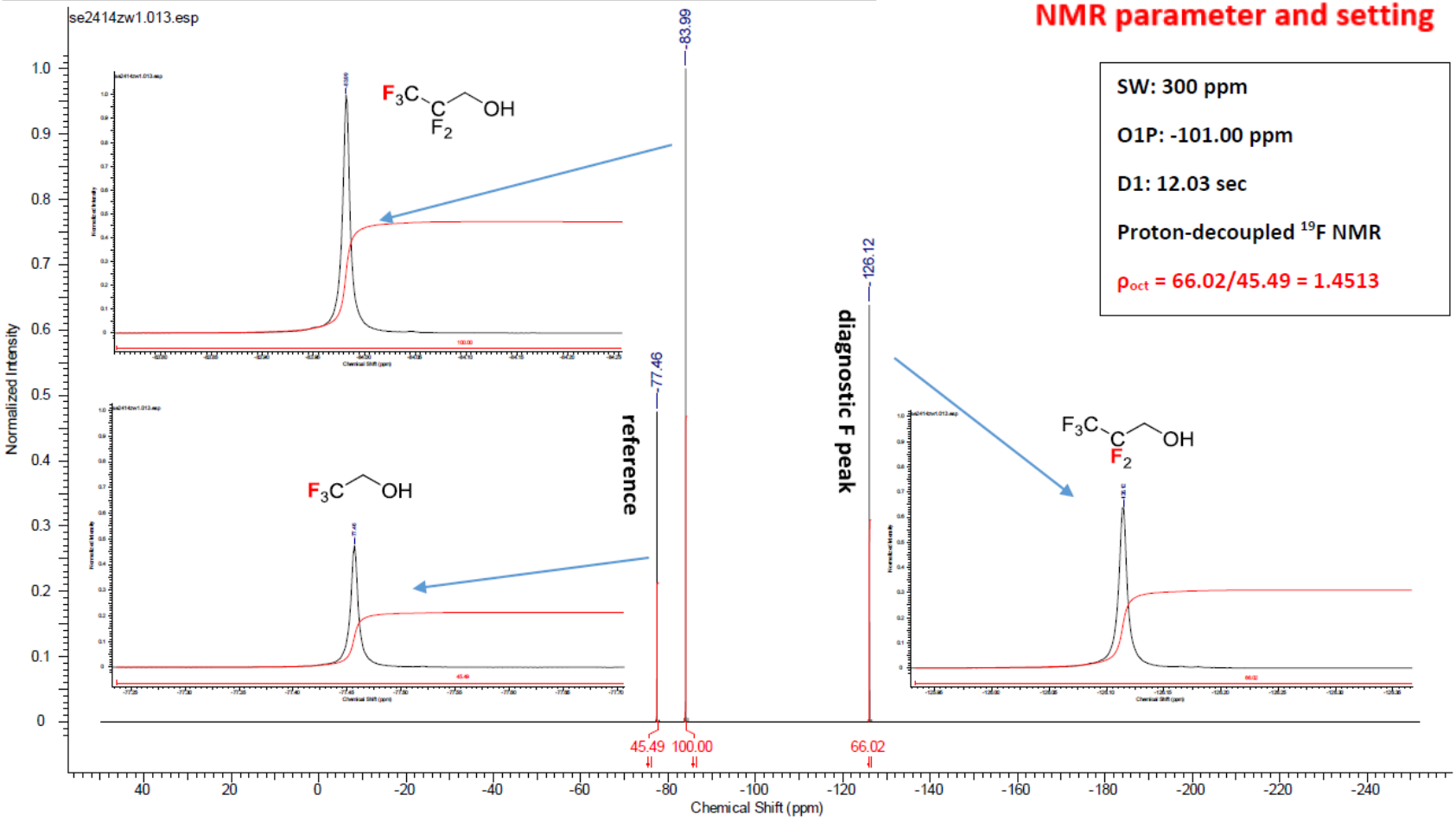
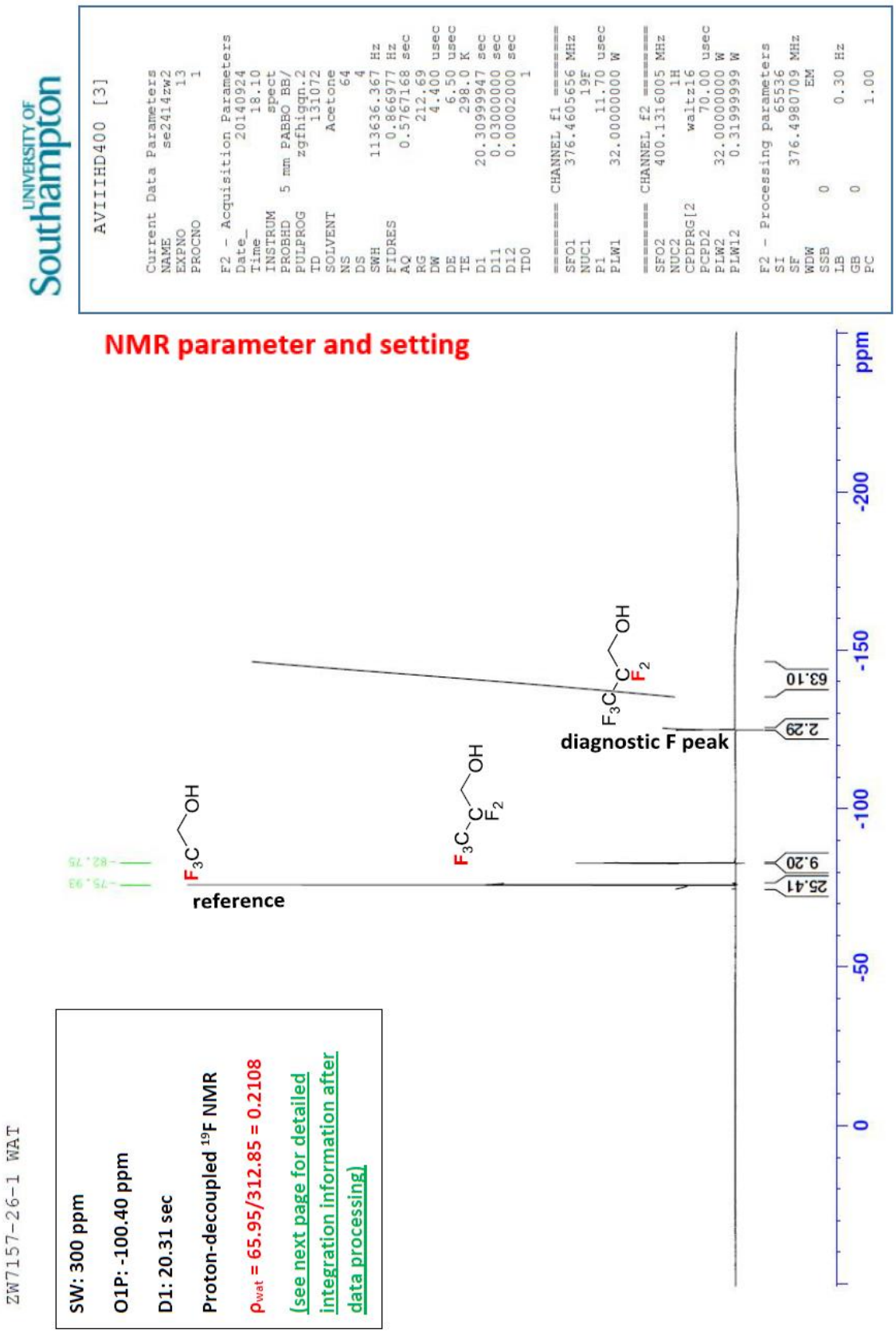
Frequency (MHz)	376.46	*Nucleus*	19F	*Number of Transients*	64	*Origin*	spect
Original Points Count	65536	*Owner*	nmr	*Points Count*	262144	*Pulse Sequence*	zgfhigqn.2
Receiver Gain	212.69	*SW(cyclical) (Hz)*	113636.36	*Solvent*	Acetone	*Spectrum Offset (Hz)*	-38026.2969
Spectrum Type	STANDARD	*Sweep Width (Hz)*	113635.93	*Temperature (degree C)*	24.999		




Southampton UNIVERSITY OF
AVIIIHD400 [3]
Current Data Parameters
NAME se2414zw2
EXPNO 13
PROCNO 1
F2 - Acquisition Parameters
Date_ 20140924
Time 18.10
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgfhigqn.2
TD 131072
SOLVENT Acetone
NS 64
DS 4
SWH 113636.367 Hz
FIDRES 0.866977 Hz
AQ 0.5767168 sec
RG 212.69
DW 4.400 usec
DE 6.50 usec
TE 298.0 K
D1 20.30999947 sec
D11 0.03000000 sec
D12 0.00002000 sec
TD0 1
======== CHANNEL f1 ========
SFO1 376.4605656 MHz
NUC1 19F
P1 11.70 usec
PLW1 32.00000000 W
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 70.00 usec
PLW2 32.00000000 W
PLW12 0.31999999 W
F2 - Processing parameters
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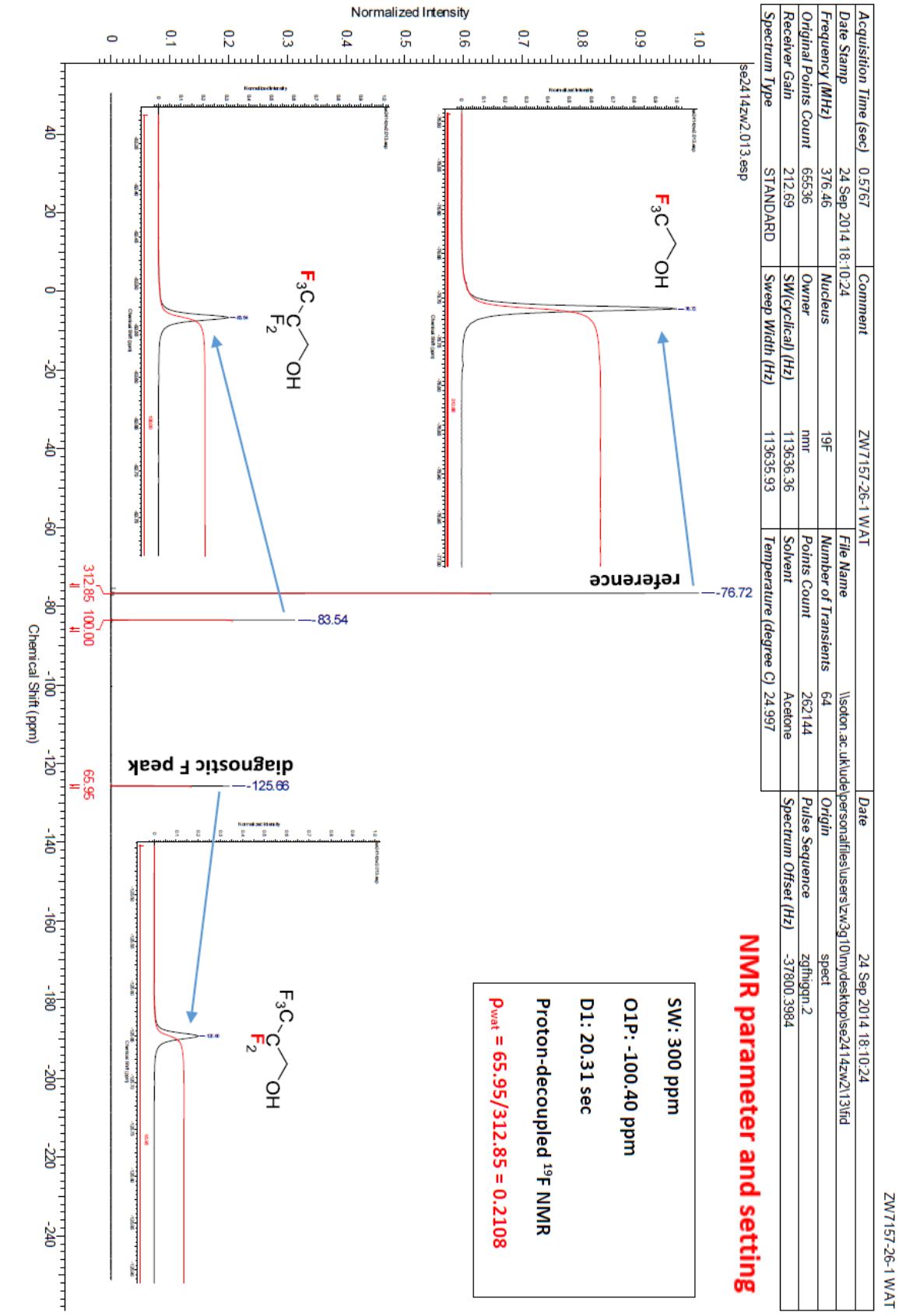
SF 376.4980709 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
NMR parameter and setting
ZW7157-26-1 WAT
SW: 300 ppm
O1P: -100.40 ppm
D1: 20.31 sec
Proton-decoupled 19F NMR
ρwat = 65.95/312.85 = 0.2108
(see next page for detailed integration information after data processing)
reference
diagnostic F peak
63.10
2.29
9.20
25.41
ppm
0
-50
-100
-150
-200

Appendix B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Acquisition Time (sec) 0.5767
Comment ZW7157-26-1 WAT
Date 24 Sep 2014 18:10:24
Date Stamp 24 Sep 2014 18:10:24
File Name \\soton.ac.uk\ude\personalfiles\users\zw3g10\mydesktop\se2414zw2\13\fid
Frequency (MHz) 376.46
Nucleus 19F
Number of Transients 64
Origin spect
Original Points Count 65536
Owner nmr
Points Count 262144
Pulse Sequence zgfhigqn.2
Receiver Gain 212.69
SW(cyclical) (Hz) 113636.36
Solvent Acetone
Spectrum Offset (Hz) -37800.3984
Spectrum Type STANDARD
Sweep Width (Hz) 113635.93
Temperature (degree C) 24.997
se2414zw2.013.esp
Normalized Intensity
Chemical Shift (ppm)
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diagnostic F peak
312.85
100.00
65.95
NMR parameter and setting
SW: 300 ppm
O1P: -100.40 ppm
D1: 20.31 sec
Proton-decoupled 19F NMR
ρwat = 65.95/312.85 = 0.2108

Appendix C Publications