

School of Chemistry

Synthesis and biological testing of 5-methyl-2’-deoxycytidine analogues

and

Perfluoroalkylation reactions of amines

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i) Abstract

Chapter one describes efficient syntheses of an array of 5-methyl-2'-deoxycytidine nucleoside analogues, designed to target the TET family of enzymes. Prepared compounds were tested on a cytotoxicity assay against several different cell lines and a highly toxic difluorinated compound was discovered. One compound was additionally prepared in the phosphoramidite form for solid phase DNA synthesis.

Chapter two explores the synthesis of perfluoroalkyl amines. A highly successful catalyst-free method of fluoroalkylation was achieved mediated by phenylsilane and is able to prepare primary, secondary and tertiary 1,1-dihydroperfluoroalkylamines, imines and anilines in a catalyst free procedure with a generalised set of conditions, to prepare compounds containing between five and twenty-three fluorine atoms.

ii) Abbreviations

2OG – 2-Oxoglutarate/α-ketoglutarate

5cadC – 5-Carboxyl-2'-deoxycytidine

5fdC – 5-Formyl-2'-deoxycytidine

5fmdC – 5-Fluoromethyl-2'-deoxycytidine

5hmdC – 5-Hydroxymethyl-2'-deoxycytidine

5mC – 5-Methylcytosine

5mdC – 5-Methyl-2'-deoxycytidine

5vdC – 5-Vinyl-2'-deoxycytidine

A – Adenine

ADK – Adenylate kinase

AdoHcy – *S*-Adenosyl-L-homocysteine

AdoMet – *S*-Adenosyl-L-methionine

ADP – Adenosine diphosphate

ADSL – Adenylosuccinase lyase

AID – Activation-induced deaminase

AML – Acute myeloid leukaemia

AMP – Adenosine monophosphate

APOBEC – Apolipoprotein B mRNA editing enzyme

Asp – Aspartate

ATase – Amidophosphoribosyl transferase

ATCase – Aspartate carbamoyltransferase

ATP – Adenosine triphosphate

ATR – Attenuated total reflectance

BER – Base excision repair

C – Cytosine

cLogP – Calculated distribution coefficient between 1-octanol and water

CMML – Chronic myelomonocytic leukaemia

COSY – Correlation spectroscopy

CpG – Cytosine-phosphate-guanine

DAST – Diethylaminosulfur trifluoride

DEPT – Distortionless enhancement by polarisation transfer

DIPEA – *N*,*N*-Diisopropylethylamine

DMDO – Dimethyldioxirane

DMEM – Dulbecco's modified Eagle's medium

DMOG – Dimethyloxalylglycine

DMTr – 4,4'-Dimethoxytrityl

DNA – Deoxyribonucleic acid

DNA MTase – DNA methyltransferases

DNMTase 1 – DNA methyltransferase 1

DSBH – Double stranded β helix

E. coli – Escherichia coli

G – Guanine

GAR TFase – Phosphoribosylglycinamide formyltransferase

GARS – Glycinamide ribonucleotide synthetase

Gln – Glutamine

Glu – Glutamate

GMP – Guanosine monophosphate

His – Histidine

HMBC – Heteronuclear multiple bond correlation spectroscopy

HPLC – High performance liquid chromatography

HSQC – Heteronuclear single quantum coherence spectroscopy

IBX – 2-Iodoxybenzoic acid

ihMSC – Induced human mesenchymal stem cells

IMP – Inosine monophosphate

LCMS – Liquid chromatography-mass spectrometry

MDS – Myelodysplastic syndromes

mRNA – Messenger RNA

NDPKs – Nucleoside disphosphate kinases

NMR – Nuclear magnetic resonance

NOG – *N*-Oxalylglycine

OMP – Orotidine monophosphate

OMP decarboxylase – Orotidine 5'-phosphate decarboxylase

OPRTase – Orotate phosphoribosyltransferase

PEMB – 5-Ethyl-2-methylpyridine borane complex

PET – Positron emission tomography

PFAS – Phosphoribosylformylglycinamide synthase

P_i – Inorganic phosphate

PMB – *para*-Methoxybenzyl

PMHS – Polymethylhydrosiloxane

PRPP – Phosphoribosyl pyrophosphate

PURH – Bifunctional purine biosynthesis protein

PVDF – Polyvinylidene fluoride

R_f – Retention factor

RNA – Ribonucleic acid

STAB – Sodium triacetoxyborohydride

T – Thymine

TAAR1 – Trace amine-associated receptor 1

TBAF – Tetrabutylammonium fluoride

TBDMS – *tert*-Butyldimethylsilyl

TDG – Thymine-DNA glycosylase

TET – Ten-eleven translocation methyl dioxygenase

TFA – Trifluoroacetic acid

THF – Tetrahydrofolate (biology) or Tetrahydrofuran (chemistry)

TIPS-Cl – 2,4,6-Triisopropylbenzenesulfonyl chloride

TLC – Thin layer chromatography

TLC-MS – Thin layer chromatography-mass spectrometry

TMDS – 1,1,3,3-Tetramethyldisiloxane

tRNA – Transfer RNA

U – Uracil

UCK2 – Uridine-cytidine kinase 2

UMP – Uridine monophosphate

UMP/CMP kinase – Uridinemonophosphate/cytidinemonophosphate kinase

UTR – Untranslated region

WHO – World Health Organisation

iii) Acknowledgments

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Chapter 1

Synthesis and biological testing of 5-methyl-2’-deoxycytidine analogues

1) Introduction

1.1) DNA structure and function

Deoxyribonucleic acid (DNA) is present in all living organisms and several viruses. This self-replicating material is unique to an individual and contains all the information required for development. DNA is a long chain macromolecular polymer found in the cell nucleus, comprised of two chains organised in a double helix structure, comprised of units called nucleotides. A nucleotide is a monomer of a DNA strand containing a phosphate group, a 2'-deoxyribose sugar and a nitrogenous base (**Fig. 1**). The corresponding nucleoside only consists of the ribose and base units. There are four nitrogenous nucleobases found in DNA – the pyrimidine bases thymine (T) and cytosine (C), and the purine bases adenine (A) and guanine (G). Ribonucleic acid (RNA) is a similar, single stranded nucleic acid, using the base uracil (U) in place of thymine and ribose instead of 2'-deoxyribose.

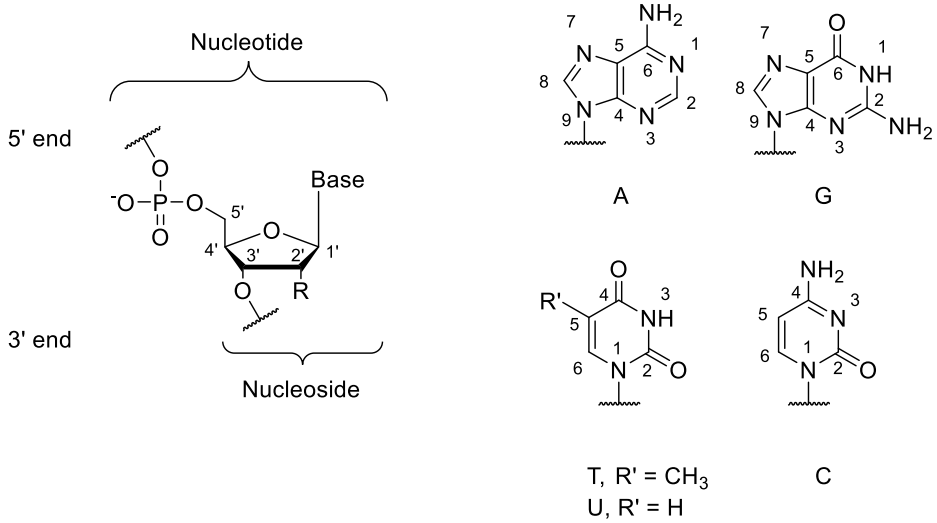


***Figure 1**: General structure of nucleotide and nucleoside (left) and nitrogenous bases (right), with numbering conventions. In DNA, R = H, in RNA, R = OH.*

A strand of DNA is held together by a phosphate backbone linking the 2'-deoxyribose units, and the two chains are held together by a series of hydrogen bonds formed by the nitrogenous bases in their complementary pairs – A to T and G to C.

DNA has directionality with the two strands running in opposite directions, indicated by the 3' and 5' ends – they are antiparallel. The double helix structure is tightly wound in a compact structure known as the supercoil. The degree of coiling changes

with the environment it exists in[1], due to interruption of the two primary stabilising forces of hydrogen bonding between each DNA strand (**Fig. 2**), and π-π stacking interactions on the same chain.

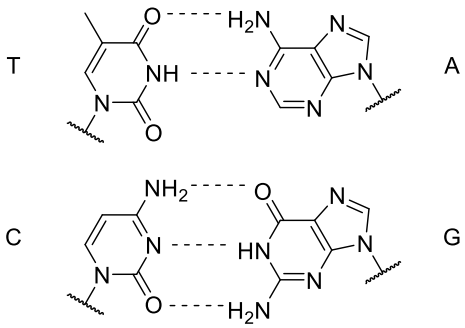


***Figure 2**: Watson-Crick base pairing between complementary nucleobases.*

Information on the structure of DNA came from the combined efforts of Watson, Crick, Franklin and Wilson using X-ray diffraction data[2]. This discovery earned the Nobel Prize for physiology or medicine in 1962, with several related prizes in physiology or medicine as well as chemistry awarded for nucleic acids research[3]. Whilst the X-ray crystal structure of DNA was not available at this time, information from diffraction patterns was enough to propose a structure and suggest a mechanism for encoding and copying of genetic information. A single crystal was not successfully prepared until nearly twenty years later, with new crystal structures being reported as recently as 2005[4,5].

DNA is the molecular code that governs many aspects of human development, in conjunction with environmental factors. DNA is a stable molecule as would be required for any compound fundamental for development of a successful organism. Having evolved from a widely accepted RNA precursor world[6–9], development over such lengths of time has led to complex systems with layers of formation.

The base sequence is the primary mechanism of information transfer, which no two organisms share outside of cloning. A sequence of DNA which codes for a product is known as a “gene”, and this product can be either a protein or RNA. The base sequence and variation between individuals determines the “genotype” of an organism. Synthesis of a product using information from a gene is known as gene

expression and is used by all known life to produce macromolecular components required for life.

The process of information decoding and gene expression proceeds *via* transcription and translation. The double stranded DNA in the nucleus unwinds from its supercoiled state and is transcribed into messenger RNA (mRNA) which exits the nucleus of the cell and enters a ribosome. Each three base sequence triplet is known as a codon, and codes for a specific amino acid. Transfer RNA (tRNA) is recruited to the ribosome and possesses an anticodon and an amino acid. The anticodon is comprised of three complementary bases, and using base pairing specific amino acids can be assembled in a precise order in correlation with the instructions contained in the original DNA, producing a protein product (translation)[10].

Not all of the DNA strand is transcribed and translated with every replication event, with control mechanisms in place to prevent wasteful formation of unnecessary products. The structure of a gene is comprised of several sections of which only some code for a protein. A regulatory sequence exists prior to the section which codes for a protein, known as the open reading frame. A second regulatory section exists at the end of a gene known as the terminator, to end the sequence[11] (**Fig. 3**).

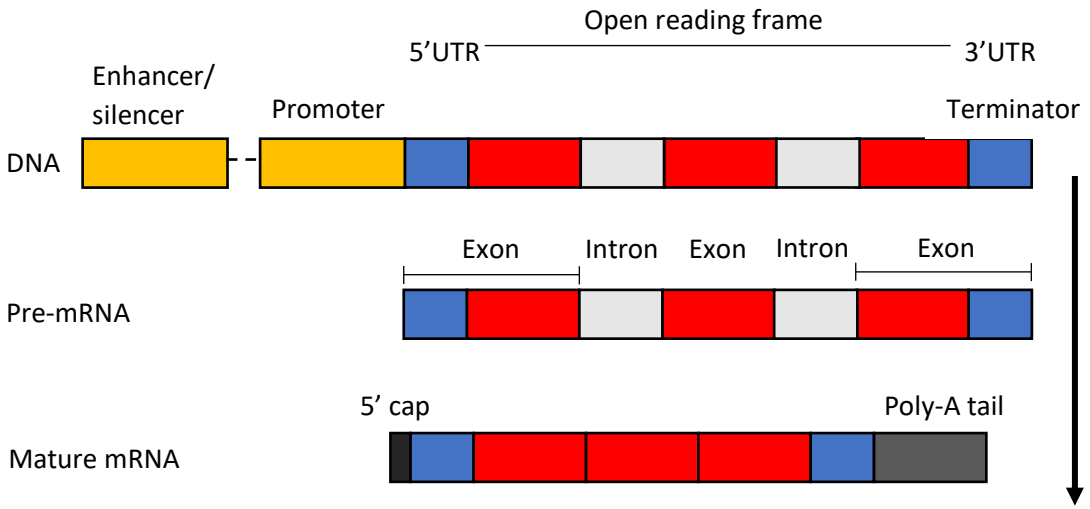


***Figure 3**: Structure of a gene, and its conversion to mRNA through pre-mRNA.*

The initial regulatory sequence contains an enhancer or silencer region, which transcription factors may bind to. These are proteins which increase or decrease the likelihood that transcription of the following gene will occur. The promoter region is

where RNA polymerase binds, which separates the two strands of the DNA helix and after a short untranslated region (UTR) adds complementary RNA nucleotides to form an RNA-DNA helix. The hydrogen bonds between complementary base pairs break and the pre-mRNA separates before post-transcriptional modification for removal of regulatory sequences and non-coding introns. The protein coding section with appropriate caps and poly-A tail is a mature mRNA which now exits the nucleus to the cytoplasm to undergo translation in the ribosome[11–14].

Gene regulation, an example of which is the enhancer or silencer domain, can occur at any stage of gene expression. Stored DNA is compacted and is wrapped around proteins called histones. Modulation of histones can facilitate or prevent access of enzymes to the DNA[15]. Once accessible, the enhancer and silencer regions provide control over transcription and the mRNA product can undergo post-transcriptional modification creating another layer of control[16]. Subsequent transport of the mRNA, translation in the ribosome and degradation of the mRNA are also all valid sites for gene regulation.

A notable example of gene regulation is the lac operon, which allows *E. coli* to effectively digest lactose when the preferable carbon source glucose is unavailable[17]. This event is not a heritable change and only affects that transcription-translation event, and proteins produced on that occasion. Later cycles or events are not necessarily affected.

A mammalian example of gene regulation involves cytosine-phosphate-guanine islands (CpG). The promoter region in mammalian genes contains high numbers of these sites[18] and is often used to aid identification of the start of a gene. Cytosines in CpG islands can be methylated to form 5-methylcytosine, and the presence of multiple methylated CpG sites in promoter regions of genes causes silencing of the affected genes[19] (**Fig. 4**).

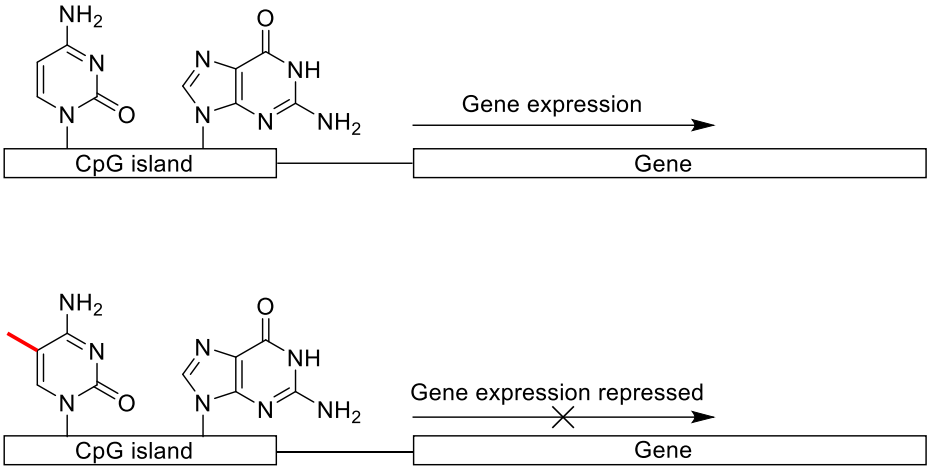


***Figure 4**: Effects of CpG methylation on gene expression, preventing transcription.*

Approximately 70% of human promoter regions contain CpG islands, indicating considerable levels of control over gene expression. Studies have identified around 29,000 CpG islands in humans, and methylation and demethylation of CpG sites is important in the development of cancers where hundreds of genes can be either activated or silenced[19].

Gene regulation such as the lac operon is not heritable, however the location of methylcytosine in the genome is. A key feature of methylcytosine in CpG islands is that it is symmetric and affects both chains in double stranded DNA[20]. The enzyme primarily responsible for cytosine methylation, DNA methyltransferase 1 (DNMTase 1), has an affinity for hemimethylated sites[21], and will methylate a newly produced strand of DNA[20]. This permits transfer of an additional layer of information to a daughter cell, without changing the base sequence. This heritability factor differentiates it from some forms of gene regulation, and is known as epigenetic regulation.

1.2) Epigenetics

Epigenetics is the field of studying heritable traits that cannot be explained by changes in the DNA base sequence. From the Greek prefix επί- meaning "over" or "around", this field often refers to changes in the expression of DNA rather than what it codes for – a change in phenotype rather than genotype. Whilst environmental factors can alter how the genetic code is expressed and gene regulation is common, epigenetics requires that any change is heritable[22–24], and may last through cell division or multiple generations.

There are numerous identified modifications to both DNA and associated replication proteins that are considered epigenetic. Histones are post translationally modified proteins which in clusters of eight help package DNA, and modifications such as methylation, acetylation and phosphorylation on the histones are known which can transcriptionally activate or deactivate sections of DNA, altering gene expression without changing the base sequence[25].

Methylation of the nitrogenous bases is another important epigenetic modification (**Fig. 5**). Of particular note is 5-methylcytosine (5mC) but many others have been identified, with over 100 modified nucleotides found in RNA[26].

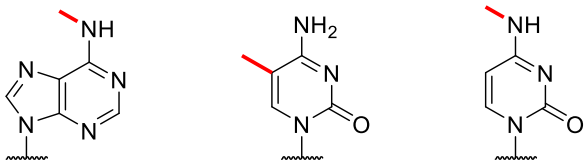


***Figure 5**: Modified nitrogenous bases, N^6-methyladenine (left), C^5-methylcytosine (5mC) (centre) and N^4-methylcytosine (right).*

Whilst 5mC has long been known[27], its function in gene expression is a more recent discovery[28,29]. Additional modified bases continue to be found such as N^6-methyladenosine and N^4-methylcytosine[30,31]. With advancing discovery and identification of new modifications, their functions can also be uncovered, offering the potential for advances in medicine[32].

A key example of epigenetics comes from cellular differentiation in embryonic development. During development, totipotent stem cells become pluripotent and

differentiate into specialised cells found in multicellular organisms[33]. This is controlled by gene silencing where a repressor protein will attach to silencer regions of the DNA resulting in that section of DNA not being expressed. The DNA methylation pattern in mammals is largely removed and re-established between generations. Demethylation occurs initially in the zygote, and methylation reoccurs during the implantation stage of the embryo. Post implantation, methylation patterns are tissue specific and wide ranging demethylation occurs again in the germline but not in somatic cells[34–36] (**Fig. 6**). Direct evidence for the importance of DNA methylation and demethylation comes from experiments performed on methyltransferase deficient mice, for which embryos died at the 5-20 somite stage and did not proceed to full term[37].

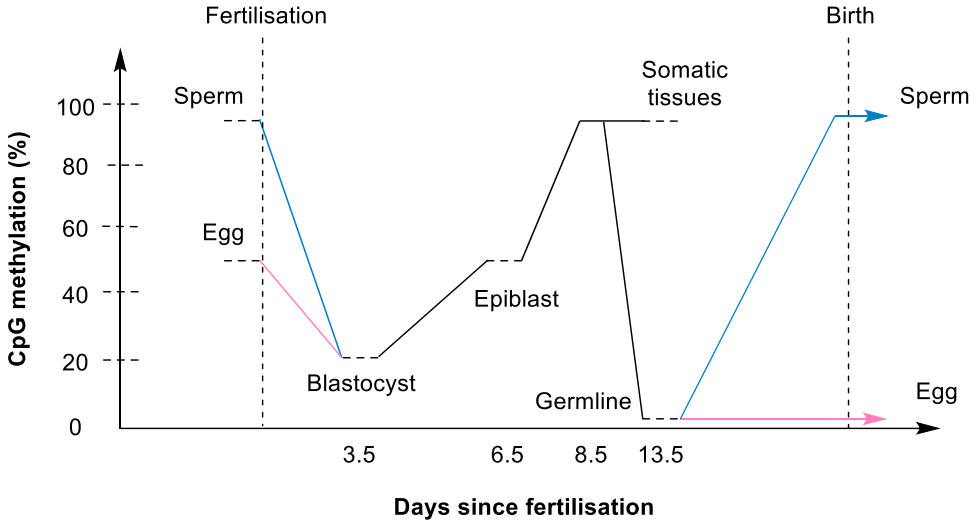


***Figure 6**: Depiction of levels of CpG methylation during embryonic development, with large scale demethylation events at the blastocyst stage during formation of the germline[38].*

1.3) DNA methylation

A key epigenetic modification is 5-methylcytosine. Often found at CpG islands, this nucleobase occurs for approximately 1% of all DNA bases in human somatic cells, and is found in approximately 70-80% of all CpG dinucleotides in the genome[39]. The degree of cytosine methylation varies dramatically between species, with between 0.03% and 14% of cytosines modified[40]. This modified nucleobase behaves like its unmethylated counterpart and will base pair with guanine in double-stranded DNA, but genes with considerable methylation of cytosine in promoter regions are significantly less transcriptionally active and considered silenced[41–46].

DNA methyltransferases (DNA MTase) catalyse the transfer of a methyl group to DNA using *S*-adenosyl-L-methionine (AdoMet) as a methyl donor, producing *S*-adenosyl-L-homocysteine as a by-product (**Fig. 7**). They can be subdivided into three groups – m6A, m4C and m5C, for those that generate N^6-methyladenine, N^4-methylcytosine and C^5-methylcytosine respectively.

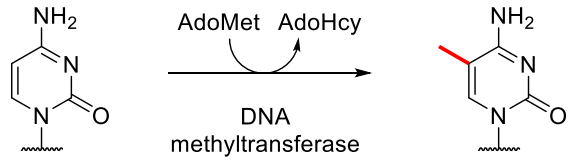


***Figure 7**: General scheme for cytosine methylation in vivo with AdoMet.*

In the case of m5C enzymes, a cysteine thiolate residue present in the active site of the enzyme nucleophilicly attacks the C6 position of cytosine, and following protonation by an adjacent glutamate residue facilitates transfer of a methyl group to C5[47,48]. Subsquent β-elimination releases the enzyme from the newly methylated base (**Fig. 8**).

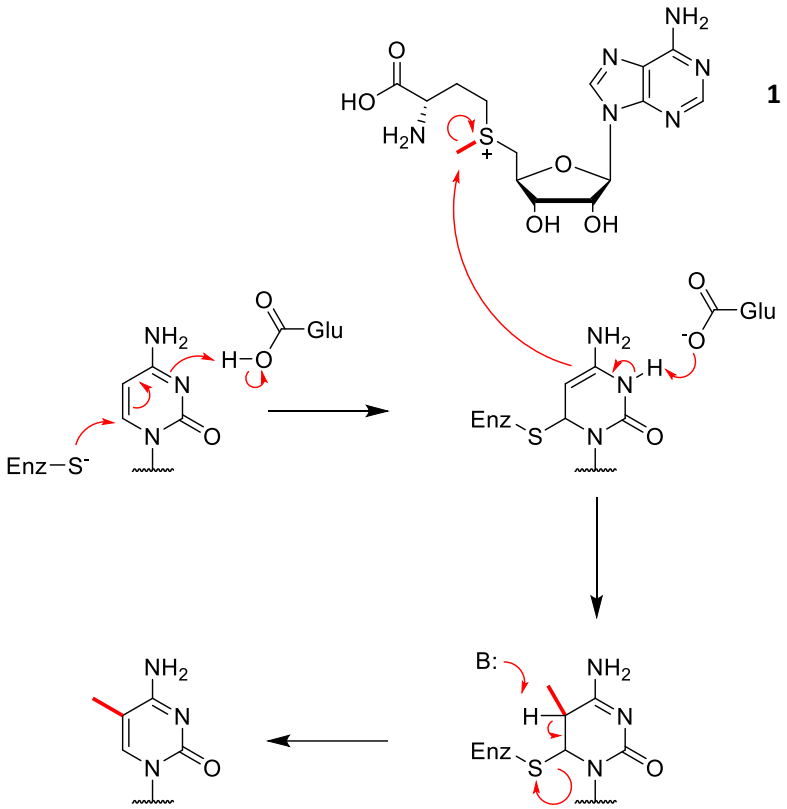


***Figure 8**: Mechanism of cytosine methylation by DNMTases and cosubstrate AdoMet (**1**).*

DNA methylation is described as either *de novo* or as maintenance. A *de novo* DNA MTase performs new methylation on cytosines and are found in early embryonic development and sets up the pattern of DNA methylation. Maintenance methyltransferases will only methylate when one strand of the DNA has already been methylated and do not perform new methylations, and maintain the pattern of methylation established by the *de novo* methyltransferases after DNA replication[49].

Three active DNA methyltransferases have been identified in mammals – DNMT1, DNMT3a and DNMT3b. DNMT1 is the primary maintenance methyltransferase in mammals and methylates CpG dinucleotides in the genome. Predominantly functioning on hemimethylated DNA[21], it is also capable of *de novo* methylation at a reduced rate[50]. This substrate promiscuity permits a wide range of repair functions and is important in tumour suppressor genes[50,51].

The DNMT3 family of DNA MTases are also capable of both maintenance and *de novo* methylation and perform both functions at the same rate. Important in gene repression, DNMT3a prefers CpG methylation to CpA, CpT and CpC and methylates CpG sites at a rate slower than DNMT1 but greater than DNMT3b[52,53]. Both enzymes have been found to interact with DNMT1 which may be a cooperative event during DNA methylation[54].

1.4) DNA demethylation

With DNA methylation being an important regulator of gene transcription, its role in carcinogenesis has been extensively studied. Sections of the genome have been identified in tumours as both hypermethylated and hypomethylated, with tumour suppressor genes being highly methylated and transcription control sequences grossly undermethylated[55].

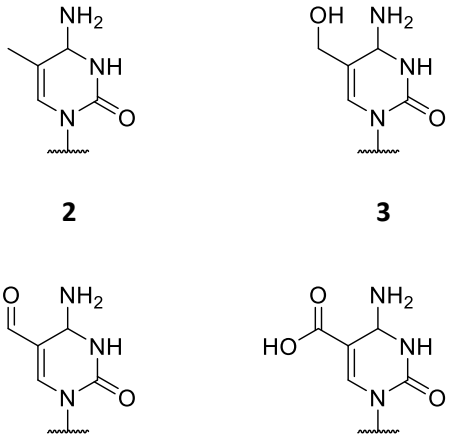
Pathways for demethylation must occur otherwise all changes are permanent. DNA demethylation may be passive or active – passive occurring through lack of methylated bases being incorporated into a DNA strand, or active *via* direct removal of either the methyl group or the DNA base entirely.

DNA demethylation can be global or at specific locations. Global demethylation is seen during embryonic development, and specific demethylation requires stimulation[56]. It has been demonstrated that oxidative enzymes are implicated in this process, specifically the ten-eleven translocation methyl dioxygenase (TET) family of enzymes.

1.5) TET enzmyes

The TET enzymes are a family coded for by the TET genes, and are examples of ferrous 2-oxoglutarate (2OG) dependent dioxygenases. Linked with DNA demethylation and gene regulation, research is ongoing into their biological function[57].

These enzymes have been demonstrated to oxidise 5-methyl-2'-deoxycytidine (5mdC) to 5-hydroxymethyl-2'-deoxycytidine (5hmdC), 5-formyl-2'-deoxycytidine (5fdC) and 5-carboxyl-2'-deoxycytidine (5cadC)[58,59]. Of note is 5hmdC (**3**) (**Fig. 9**) which is found in large quantities in primordial germ cells and neuronal cells, suggesting it may have another purpose beyond being an intermediate on a demethylation pathway, such as protein interactions.



2 **3**

4 **5**

Figure 9*: Structures of various cytosine forms: 5-methylcytosine (**2**), 5-hydroxymethylcytosine (**3**), 5-formylcytosine (**4**) and 5-carboxylcytosine (**5**).*

Of the most established proposals for cytosine demethylation, successive oxidation of 5mdC by TET enzymes to the formyl and carboxyl forms permits excision of the base by Thymine-DNA glycosylase (TDG), allowing for a base excision repair (BER) pathway to occur for active demethylation[60–62]. An alternative pathway involves direct deformylation or decarboxylation, and this direct route would yield the unmodified cytosine without the risk of DNA strand breaks associated with the BER pathway (**Fig. 10**). Deamination pathways of 5hmdC have previously been described using activation-induced deaminase (AID)/apolipoprotein B mRNA editing enzyme (APOBEC) however recent results have failed to detect any AID/APOBEC functionality on 5hmdC and severely reduced activity on 5mdC[63–65].

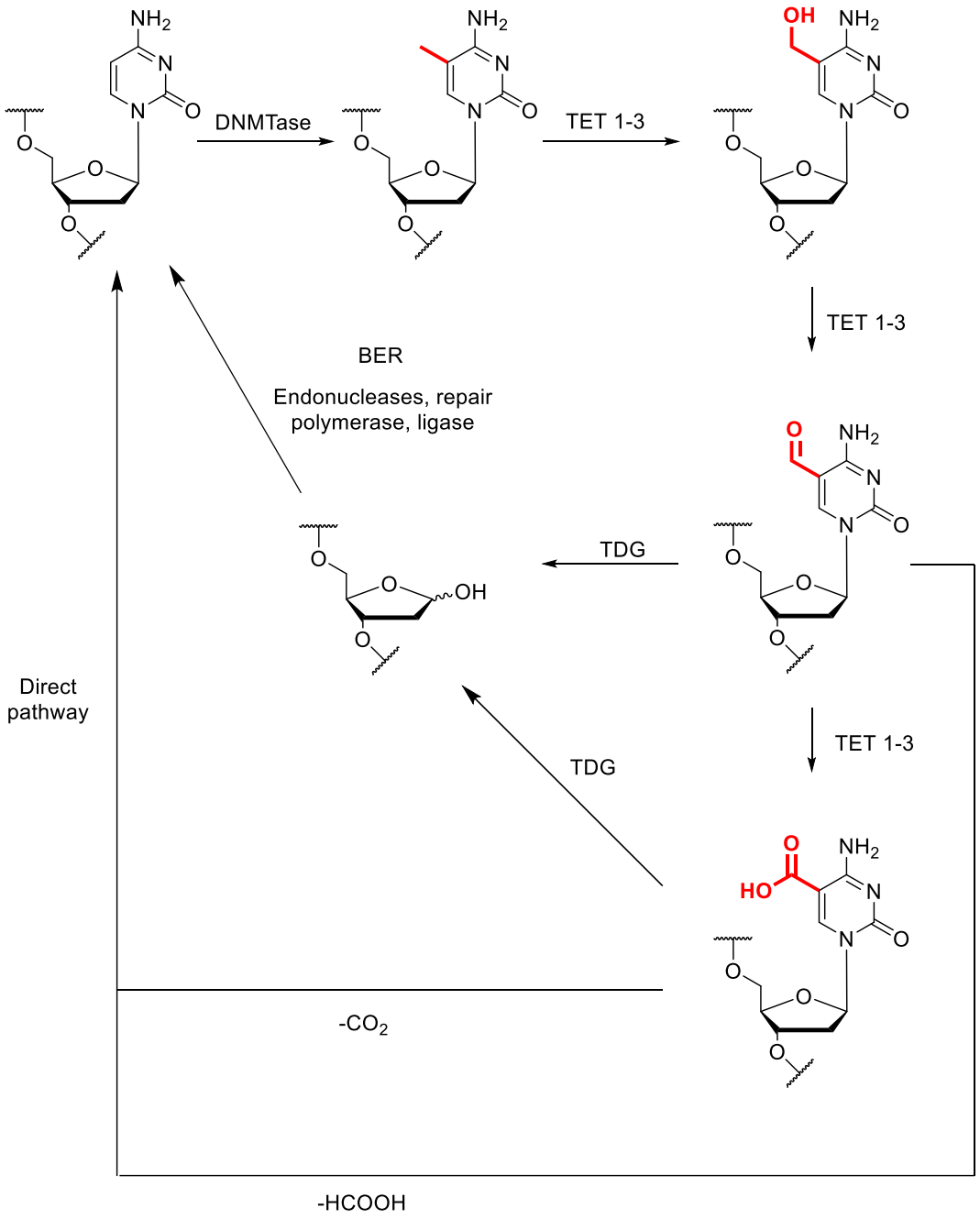


***Figure 10**: Pathways of demethylation by direct deformylation and decarboxylation or the base excision repair pathway, mediated by TET enzymes.*

Recently it has been demonstrated through radiolabelling and mass spectrometric methods that direct deformylation by C-C bond cleavage does occur *in vivo*, likely after incorporation of the modified nucleosides into the genome[66] (**Fig. 11**).

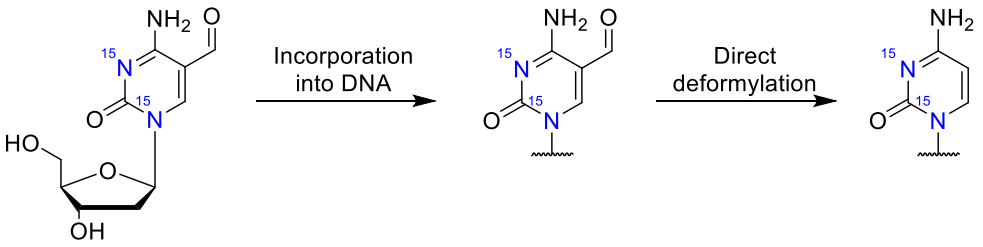


***Figure 11**: Isotopic labelling studies which demonstrated in vivo direct deformylation.*

This study additionally noted that neither DNA methyltransferases nor TET enzymes are required for the deformylation reaction to proceed. The 5fdC nucleoside has been demonstrated to be stable or at least semi-stable, and whilst deformylation and decarboxylation of 5dfC and 5cadC respectively are known, they were induced by chemical methods. The mechanism of deformylation *in vivo* is believed to proceed *via* nucleophilic attack at the C^6 position, much like in chemical methods[67].

Regulation of the production of TET enzymes has been shown as relevant in several cancers[68–71]. Three TET enzymes have been identified – 1, 2 and 3 – and knockout of the gene coding for TET1 correlates with increased quantities of methylcytosine, indicating a role in active demethylation[72]. TET1 and TET2 are generally found in embryonic stem cells, and TET3 in the germ line. The gene for TET2 is often found to be mutated in cancerous tissue rendering it inactive, notably in chronic myelomonocytic leukaemia (CMML), acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS)[73–80]. TET1 downregulation *via* mutation has been linked with colon and breast cancers, and suppression of enzyme production leads to methylation of the promotor regions of genes coding for WNT repressors, thus activating the WNT pathway and leading to tumour growth[81].

Currently, only a small number of patients with high risk CMML, AML and MDS respond to treatment with DNA hypomethylation agents such as decitabine and azacitidine[82]. The theorized mode of action of these drugs involves upregulation of the TET enzymes, in turn leading to demethylation of tumour suppressor genes which has been demonstrated as important for tissue regeneration[83,84].

1.6) 2OG enzymes

Ferrous iron and 2OG dependent dioxygenases are a large family of enzymes with an estimated 80 types found in humans alone[85] and more are predicted to exist in some species of plants[86]. Using 2OG as a cosubstrate produced by the Krebs cycle, they catalyse a variety of oxidative reactions through two-electron oxidation to generate carbon dioxide and succinate[87,88] (**Fig. 12**).

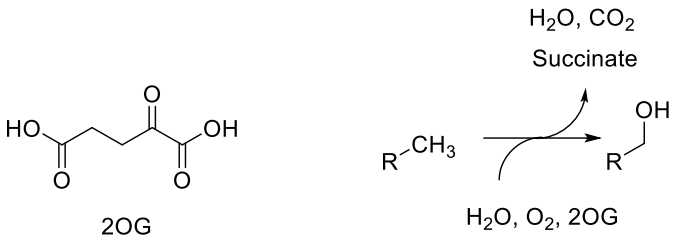


Figure 12*: Structure of 2-oxoglutarate (left) and general scheme for hydroxylation by a 2OG dependent enzyme (right).*

First discovered in collagen biosynthesis[89], this near ubiquitous family of non-haem iron (II) enzymes may catalyse cyclisations, ring fragmentations, C-C bond cleavage, epimerization, desaturation, the hydroxylation of aromatic rings and also chlorination reactions[90,91]. Examples of 2OG enzymes are found in DNA demethylation, carnitine biosynthesis, hypoxic sensing, fatty acid metabolism and signalling protein hydroxylation[92]. More recently, a discovery concerning the role of these 2OG oxygenase enzymes with the accuracy of ribosomal protein synthesis was found[93].

All identified 2OG dependent oxygenases possess a double stranded β helix (DSBH) core fold, containing eight β-strands. It is often referred to as a "jelly roll" and is common to many metalloenzymes that possess divalent metals (e.g. Fe (II), Zn (II))[94–96]. Crystal structures indicate the presence of a "facial triad" at the active site of the enzymes (**Fig. 13**) consisting of two histidine residues and one carboxylate residue (commonly aspartate, occasionally glutamate)[97].

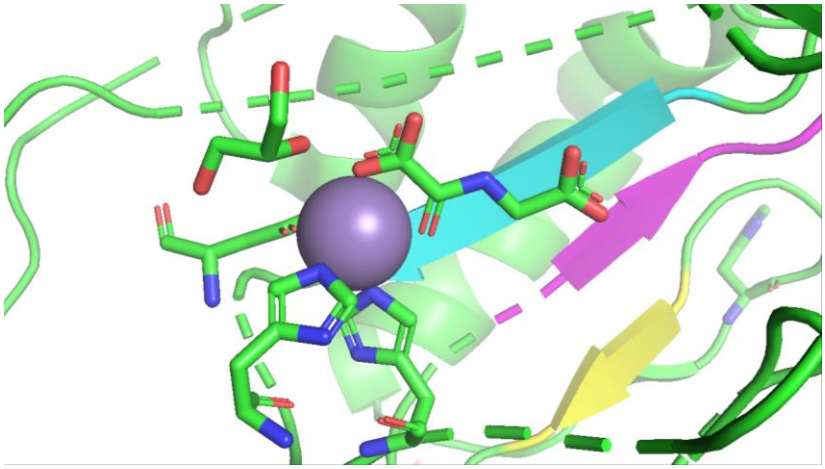


***Figure 13**: Crystal structure of active site of human OGFOD1, in complex with N-oxalylglycine (NOG, 2OG enzyme inhibitor). Facial triad of His218 (distal), His155 (proximal) and Asp157 (partially obscured) around Fe^{II} center. Tridentate compound (top left) is residual glycerol. Image created using PyMol. PDB ID: 4NHX.*

The remaining three binding sites on Fe (II) are occupied by labile water molecules generating an octahedral shape at the metal centre. The catalytic mechanism involves binding of the cosubstrate (2OG) to the metal centre, removing two of the water ligands. The substrate proceeds to bind to the active site of the enzyme (but does not directly coordinate to the metal centre), thus displacing the third and final water molecule. This generates a binding site for O_2 generating an Fe (III) species. The distal oxygen proceeds to attack 2OG creating a bicyclic intermediate which undergoes an oxidative decarboxylation to form CO_2 and an Fe (IV) oxo species – the ferryl intermediate – with a bound succinate molecule formed from decarboxylation of 2OG. The ferryl species abstracts a hydrogen from the substrate bound in the active site to form Fe (III)-OH and a substrate radical followed by oxidation of the substrate *via* a radical rebound mechanism, dissociation of the now oxidised substrate from the active site of the enzyme, succinate from the metal centre and binding of three water molecules[98,99] (**Fig. 14**).

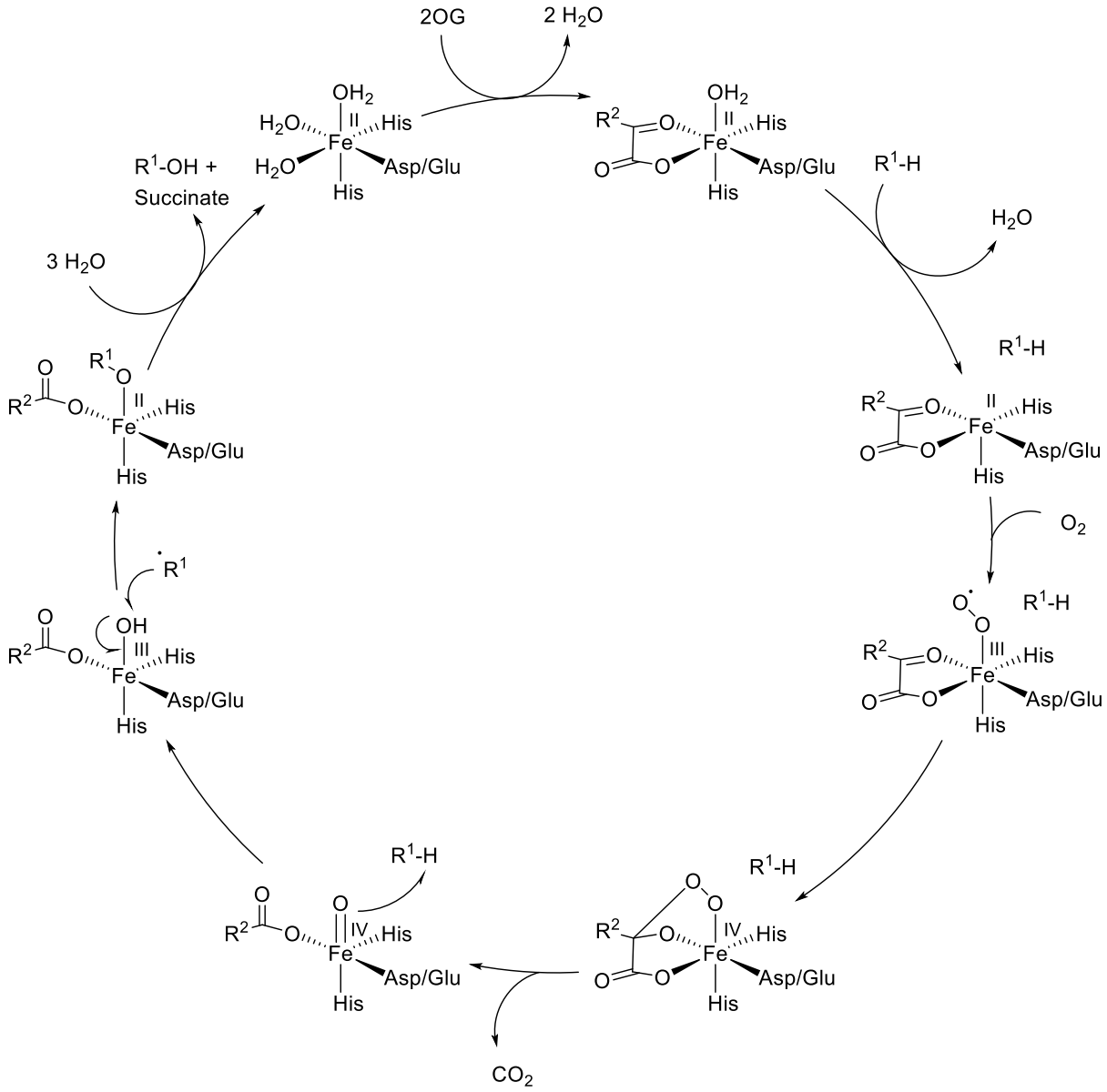


***Figure 14**: Catalytic system for mechanism of action of a 2OG dependent oxygenase.*

R^1 = substrate. R^2 = -$CH_2CH_2COO^-$.

An alternative proposal with an expanded mechanism instead involves deprotonation of the Fe (III)-OH species followed by formation of an Fe (II)-alkoxo species prior to dissociation to products has also been suggested[99] (**Fig. 15**).

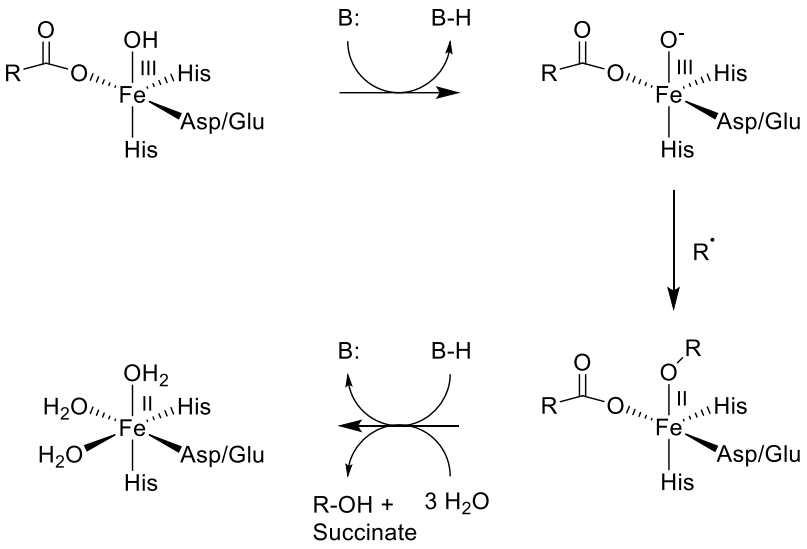


***Figure 15**: Expanded mechanism for function of 2OG enzymes with initial deprotonation prior to radical attack.*

Reports of evidence for this alternative mechanism are few but was determined by Mössbauer spectroscopy for the TauD protein domain at low temperature, and computational studies have predicted these intermediates. Experimental studies with several other 2OG enzymes have so far failed to produce evidence of this pathway[99].

1.7) Nucleosides as disease treatment

Some modified nucleosides are found commercially for disease treatment[100,101] (**Fig. 16**), and the list of analogues for use in research is vast. Due to high potential for functionalisation, the scope of possible modifications is large.

Some unnatural modifications to the DNA bases *in vivo* from environmental factors are known, from sources such as pollution. Examples include vinyl chloride, benzene and polycyclic aromatic hydrocarbons such as benzo[α]pyrene[102,103]. Where modifications to DNA can be exploited as medicines, they are also potentially the start of cancerous growths. Compounds such as cisplatin form DNA adducts leading to cell death, and are used as a cancer treatment[104].

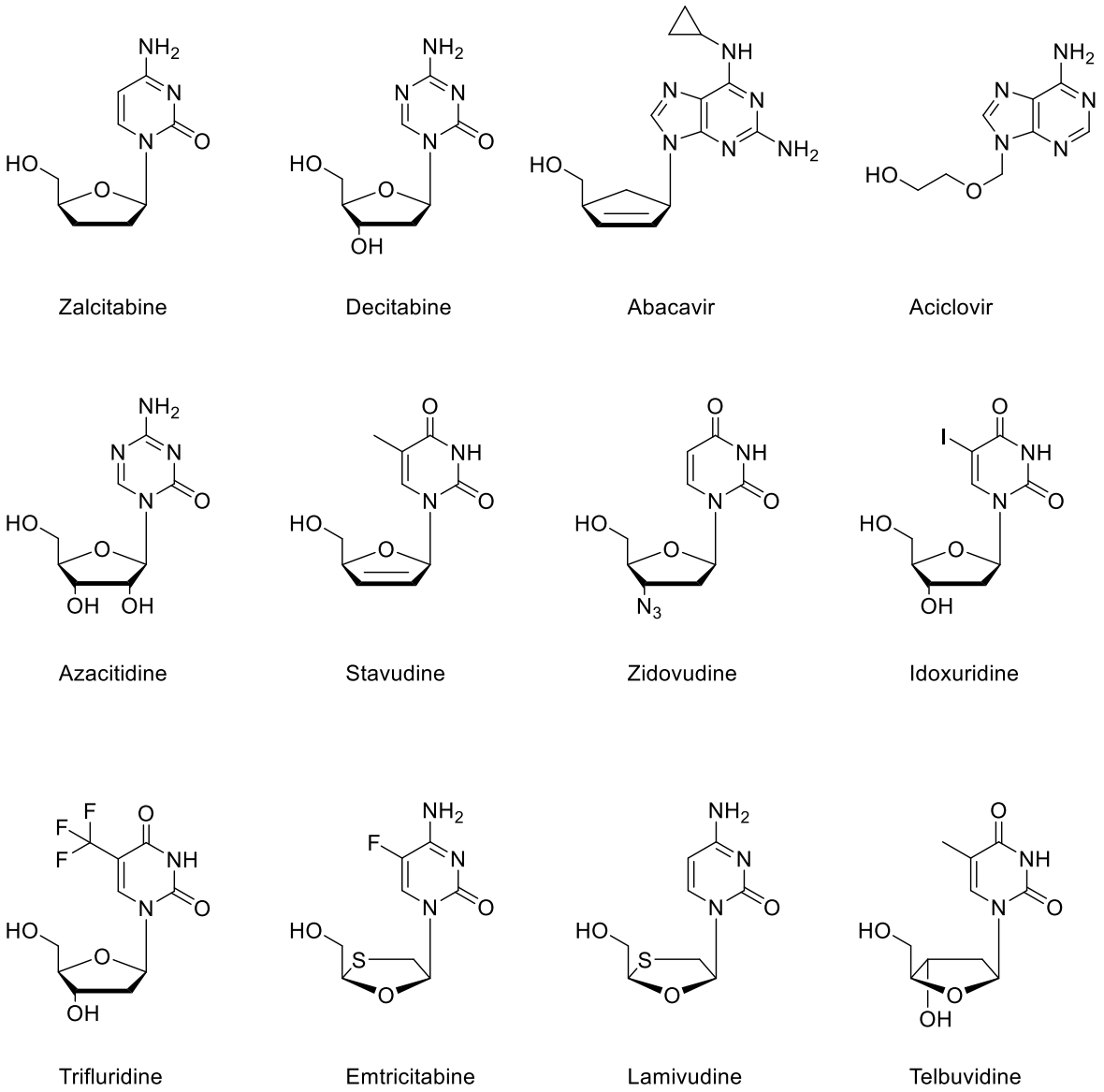


***Figure 16**: Representive selection of nucleoside analogues used as commercial drugs.*

Modifications to the ribose component of the nucleoside are common, with minor alterations to the nitrogenous base. Decitabine and azacitidine are currently approved for treatment of myelodysplastic syndromes, chronic myelomonocytic leukemia and acute myeloid leukemia[105,106]. Both function as hypomethylation agents, and function by irreversibly inhibiting the DNA methyltransferase enzymes[107]. Several modified nucleosides are found on the list of essential medicines provided by the World Health Organisation (WHO)[108].

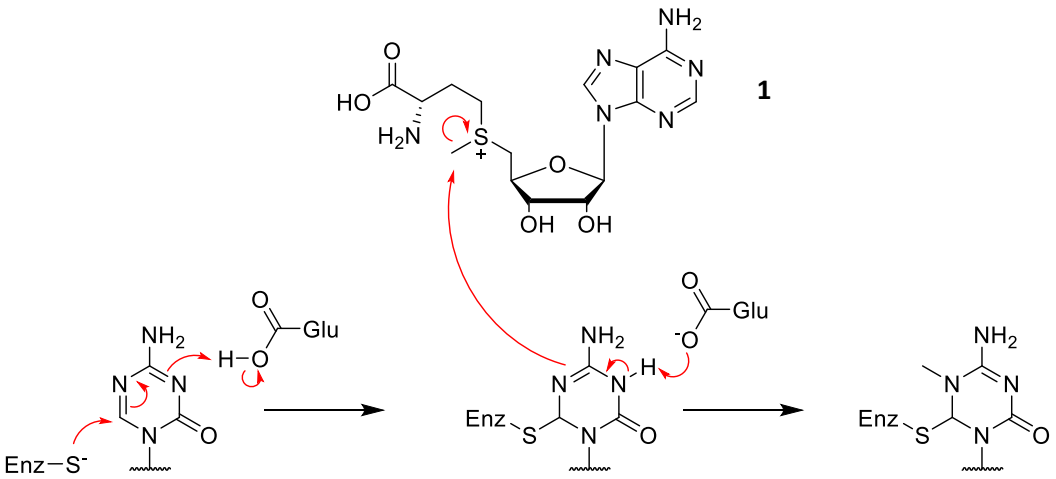


***Figure 17**: Mechanism of DNMTase inhibition by decitabine and azacitidine.*

In the case of the hypomethylation agents decitabine and azacitidine which are incorporated into the DNA and RNA respectively, irreversible inhibition occurs by blocking the β-elimination mechanism used to release the enzyme (**Fig. 17**).

Other nucleoside analogues such as abacavir and aciclovir function as antiviral agents and are used in treatment of HIV/AIDS and herpes simplex virus. Both are converted to the respective triphosphates and integrated into the viral DNA. Abacavir is a guanosine analogue and is capable of inhibiting HIV reverse transcriptase[109], and aciclovir specifically inhibits herpes simplex virus specified DNA polymerases[110], preventing viral replication without altering normal cellular processes.

1.8) Synthesis of nucleotides

1.8.1) Enzymatic synthesis

Nucleotides are synthesised *in vivo* by either the salvage pathway or *de novo*.

Some tissues are not able to perform *de novo* synthesis and so an alternative route is required. The salvage pathway recovers bases and nucleosides that are formed during degradation of DNA and RNA.

In the case of a free uracil base (**6**), uridine phosphorylase adds a ribose 1-phosphate (**7**) group and through a series of enzymatic reactions forms uridine triphosphate (**10**), utilising uridine kinase[111], uridinemonophosphate/cytidinemonophosphate kinase (UMP/CMP kinase)[112] and nucleoside diphosphate kinases (NDKPs) to perform the subsequent reactions to add the first, second and third phosphate groups sequentially[113]. An alternative pathway uses uracil phosphoribosyltransferase to combine the uracil free base and phosphoribosylpyrophosphate to form the uridine monophosphate. Thymidine is recovered following the same strategy, using thymidine phosphorylase, thymidine kinase, thymidylate kinase and NDPKs to yield the thymidine triphosphate[113–116] (**Fig. 18**).

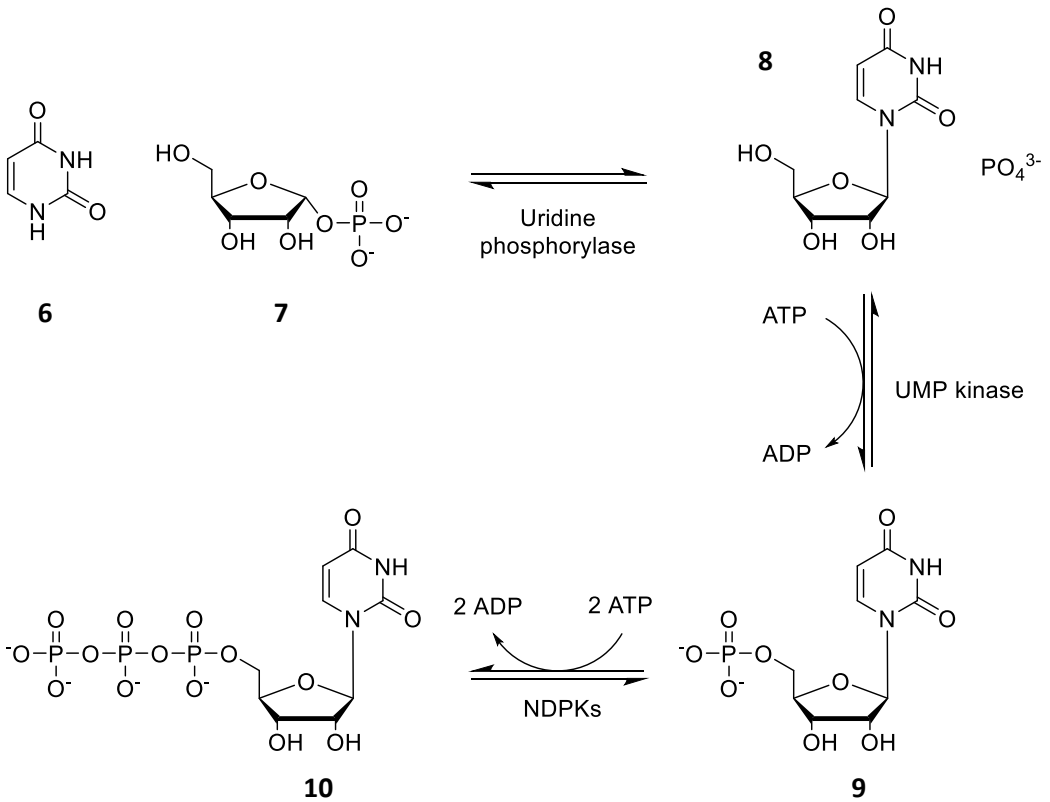


***Figure 18**: Salvage pathway to recover and repurpose uracil bases.*

Cytidine and deoxycytidine can be salvaged *via* the uracil pathway after deamination with cytidine deaminase, converting the compounds to uridine and deoxyuridine respectively. An alternative route utilises a separate set of enzymes – uridine-cytidine kinase, UMP/CMP kinase[112] and NDPKs to prepare cytidine triphosphate or deoxycytidine triphosphate[113].

Purine salvage pathways follow similar patterns to those of pyrimidines, with phosphoribosyltransferases adding a phosphoribosyl pyrophosphate (**12**, PRPP) to a free nucleobase to prepare the monophosphate. The enzymes adenine phosphoribosyltransferase (APRTase) and hypoxanthine-guanine phosphoribosyl transferase perform these reactions to prepare the monophosphates[117,118]. Conversion to the triphosphates is performed sequentially by NDPKs, or additionally by adenylate kinase (ADK) and ATP synthase specifically for ATP synthesis (**Fig. 19**).

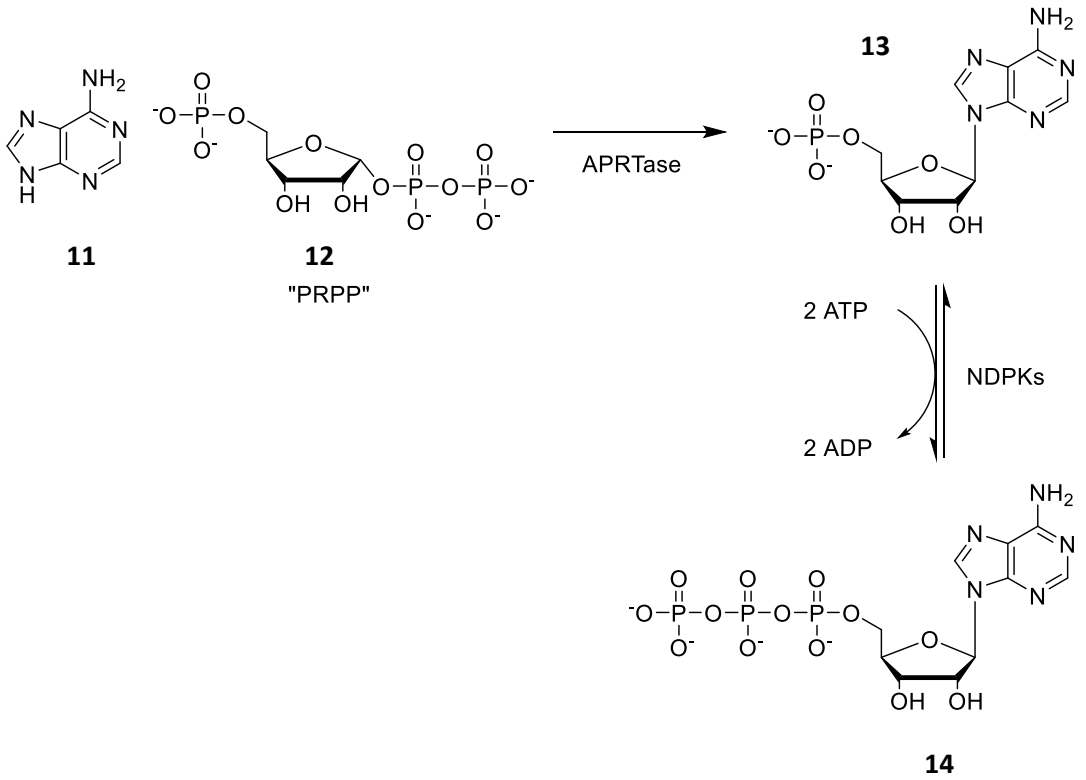


***Figure 19**: Salvage pathway for the recovery of adenine (**11**) to prepare adenosine triphosphate (**14**).*

The liver is the primary source for *de novo* synthesis of nucleotides, with different pathways for purine and pyrimidine bases. Both are prepared from biosynthetic precursors of carbohydrate and amino acid metabolism, carbon dioxide and ammonia.

Pyrimidine synthesis[119] occurs in the cytoplasm and ammonia derived from glutamine, CO_2 and a phosphate group are combined to form carbamoyl phosphate (**15**), performed by carbamoyl phosphate synthetase. Aspartate carbamoyltransferase (ATCase) catalyses the condensation of this with aspartate to form carbamoyl aspartic acid (**16**) which is enzymatically cyclised by dihydroorotase to 4,5-dihydroorotic acid (**17**). Dihydroorotate oxidase converts this to orotate (**18**). Orotate is linked to PRPP unit by orotate phosphoribosyltransferase (OPRTase) to form orotidine monophosphate (**19**, OMP), and subsequent decarboxylation to uridine monophosphate (**9**, UMP) by orotidine 5'-phosphate decarboxylase (OMP decarboxylase). From UMP, other pyrimidine nucleotides are derived by sequential phosphorylation to the triphosphate by uridine-cytidine kinase 2 (UCK2)[120] and NDPKs[113] (**Fig. 20**), and may be aminated to cytosine by CTP synthetase using glutamine as the amine donor[121].

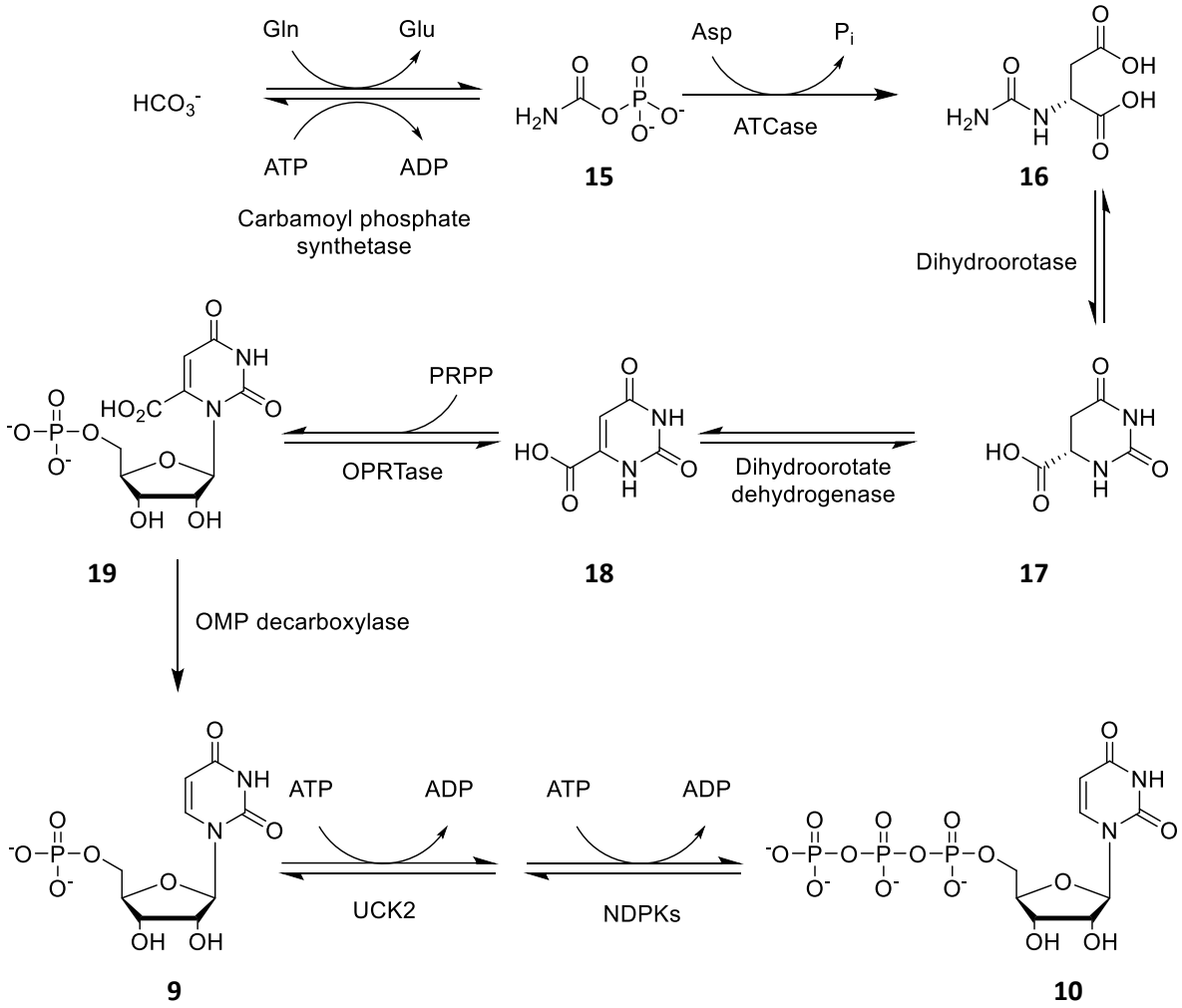


***Figure 20**: Enzymatic pathway for uridine triphosphate synthesis from amino acid and carbohydrate metabolism products.*

Purine synthesis is more complex and over ten steps uses ten enzymes to form the common intermediate inosine monophosphate (IMP, **29**) which through two separate pathways can be converted to adenosine and guanosine monophosphate.

Ribose 5-phosphate produced from the pentose phosphate pathway[122] is phosphorylated at the 1 position to form PRPP (**12**)[123], then the diphosphate is displaced with an amide nitrogen from glutamine by amidophosphoribosyltransferase (ATase), also causing inversion of the stereocentre[124]. A glycinamide ribonucleotide synthetase (GARS) enzyme catalyses amide formation with glycine, then phosphoribosylglycinamide formyltransferase (GAR TFase) enzyme catalyses amide formation and formylation by folic acid coenzyme N_{10}-formyl-THF[124] before a phosphoribosylformylglycinamidine synthase

(PFAS) uses a glutamine for conversion to the imine (**23**)[125], and AIR synthetase catalyses the cyclisation to the imidazole (**24**)[124] (**Fig. 21**).

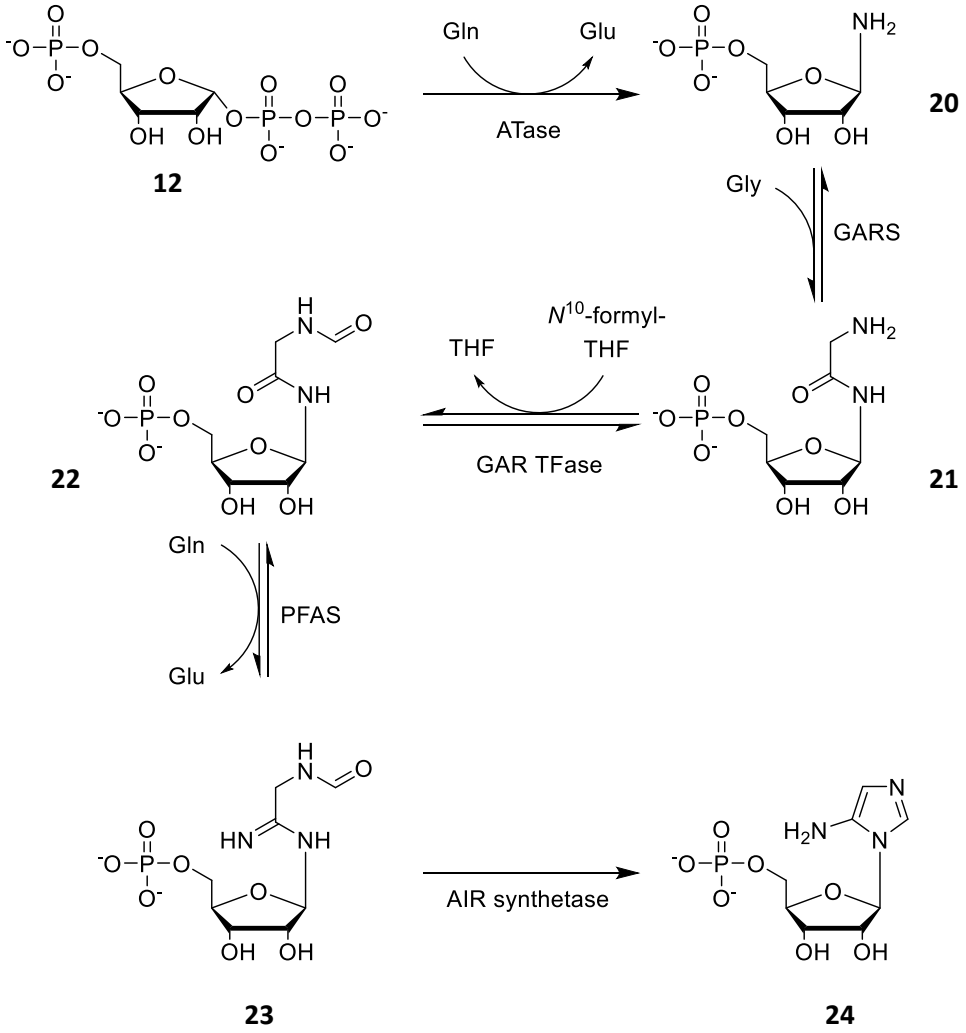


***Figure 21**: Enzymatic pathway for in vivo synthesis of the imidazole intermediate on the purine synthesis pathway. THF is tetrahydrofolate in this context.*

Carboxylation is performed by AIR carboxylase, and amidation by SAICAR synthase[126]. The adenylosuccinase lyase (ADSL) enzyme cleaves fumarate[127], and formylation by N_{10}-formyl-THF occurs a second time by AICAR transformylase, before bifunctional purine biosynthesis protein (PURH) catalyses the cyclisation to yield IMP (**29**) from the aminoimidazole carboxamide ribonucleotide[128] (**Fig. 22**).

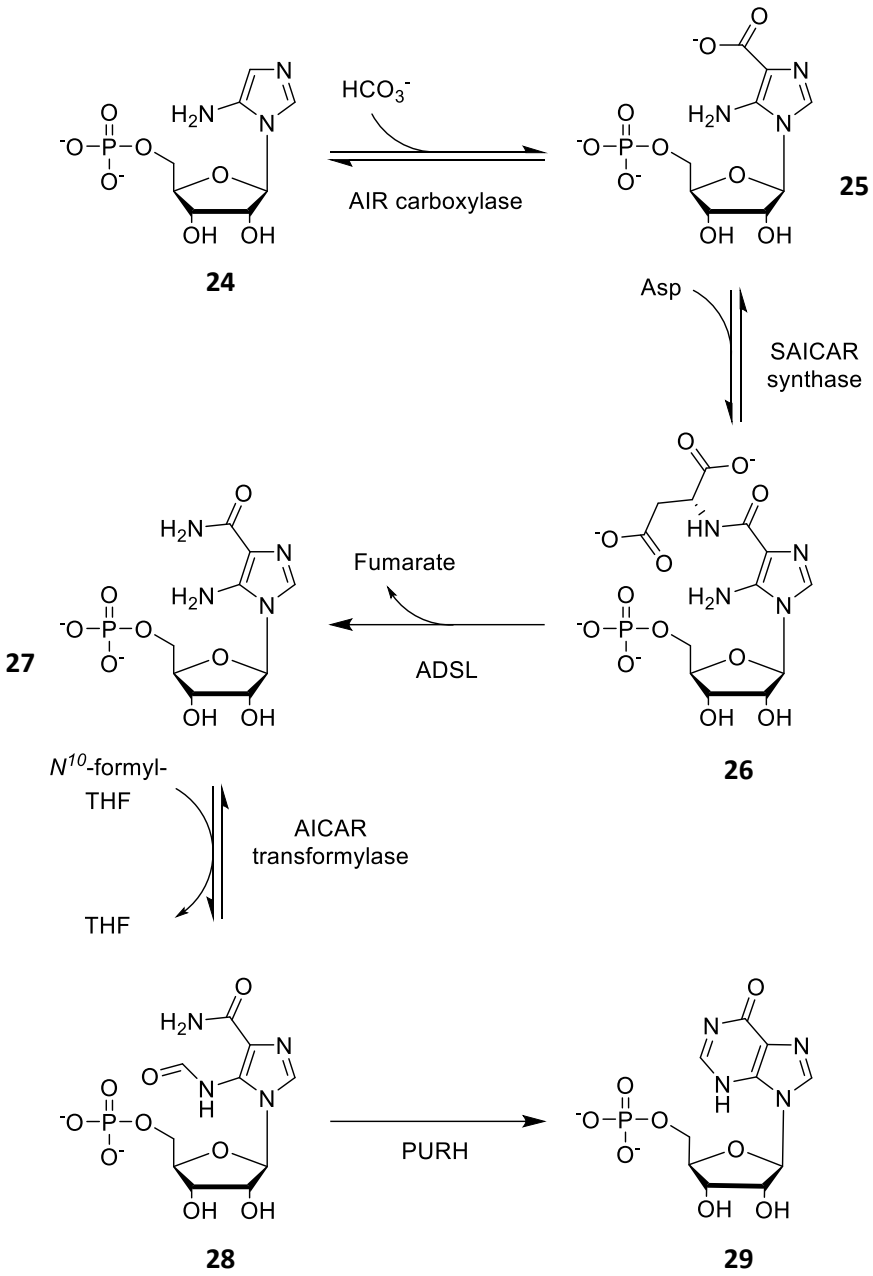


***Figure 22**: Enzymatic synthesis in vivo of common intermediate inosine monophosphate on the purine synthesis pathway, from imidazole intermediate.*

Conversion of IMP (**29**) to adenosine or guanosine monophosphate follows two branching pathways. Adenosine monophosphate (**31**, AMP) is made by formation of adenylsuccinate by adenylosuccinate synthase, substituting a glutamine at the amide carbonyl, before cleavage of fumarate by ADSL to furnish the desired product[127,129] (**Fig. 23**). The process is reversible by deamination with AMP deaminase.

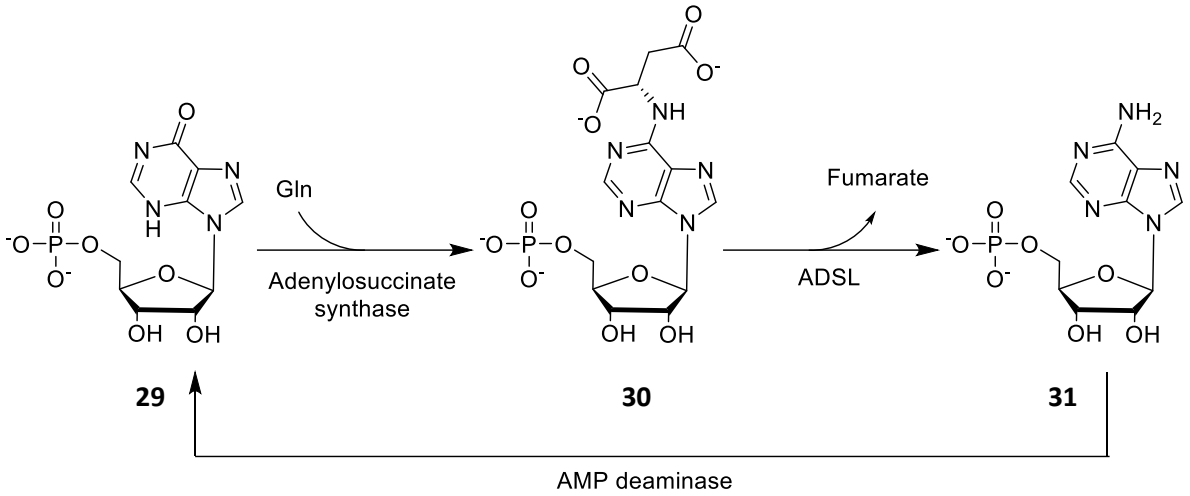


***Figure 23**: Enzymatic conversion of IMP (**29**) to AMP (**31**) and reverse reaction by deamination.*

The guanosine version for guanosine monophosphate (**33**, GMP) first requires oxidation of IMP (**29**) to the corresponding xanthine by IMP dehydrogenase, then GMP synthetase uses a glutamine residue for the final amination reaction (**Fig. 24**). The reverse can be performed with GMP reductase[130].

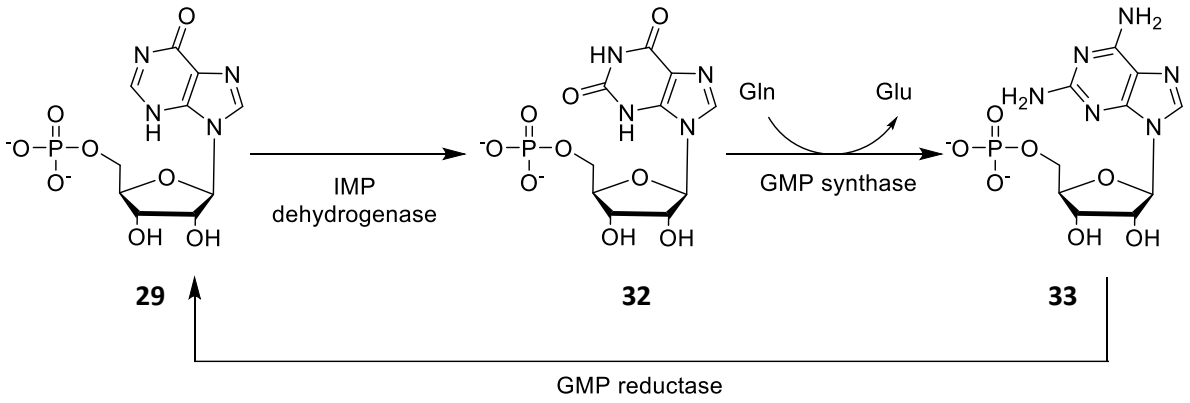


***Figure 24**: Enzymatic conversion of IMP (**29**) to GMP (**33**) and reverse reaction.*

The biosynthetic pathways shown produce nucleotides with 2'-hydroxyl groups, making them components of RNA. Conversion to the corresponding 2'-deoxyribonucleotides as components of DNA is performed by ribonucleotide reductase *via* radical mechanism and a series of cysteine residues[131,132].

1.8.2) Chemical synthesis of nucleotides

Unlike the biological synthesis, laboratory methods for nucleotide synthesis first prepare the corresponding nucleoside and phosphorylation occurs after. In a convergent synthesis, the nucleophilic base component is often prepared separately and coupled to an electrophilic sugar.

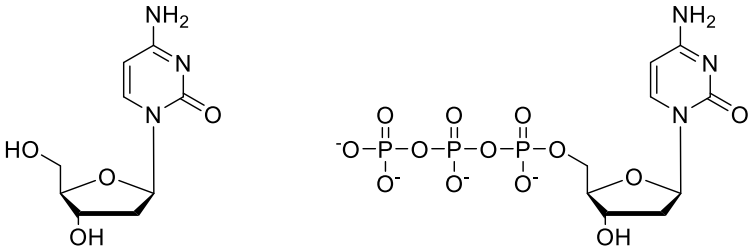
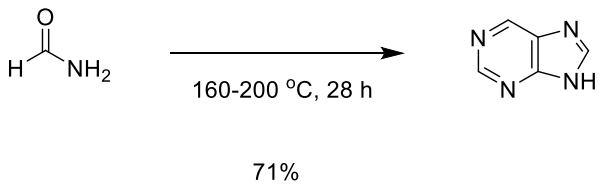


***Figure 25**: 2'-Deoxycytidine nucleoside (left) and 2'-deoxycytidine nucleotide triphosphate (right).*

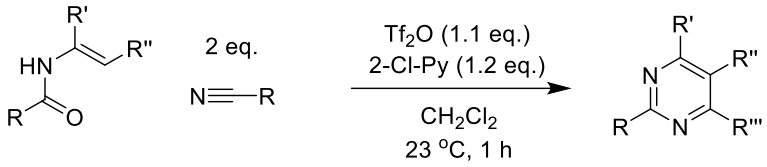
The basic purine skeleton can be obtained through a simple reaction by heating formamide in an open vessel (**Scheme 1)**. This reaction is of note in the context of the origins of life, preparing a complex compound from simple starting material[133].



***Scheme 1**: Synthesis of purine by heating formamide.*

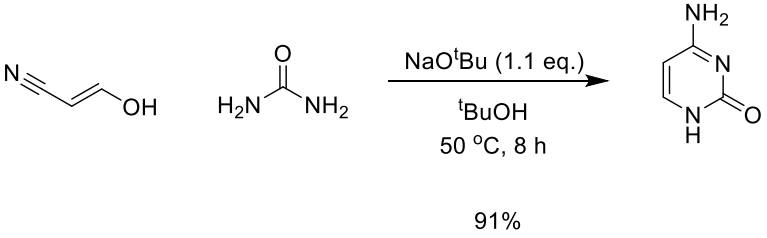
Further still, it has been shown that four molecules of HCN can tetramerize to form diaminomaleodinitrile, which can be converted into almost all naturally occurring purines. Five molecules of HCN will condense to form adenine, the addition of water and additional HCN produces xanthine and guanine, and fulminate yields hypoxanthine[134–137].

The Traube purine synthesis is a classic example of purine synthesis, combining an aminopyrimidine and formic acid[138]. Modern examples condense with aldehydes and use catalytic acid[139] to provide a wider range of products.



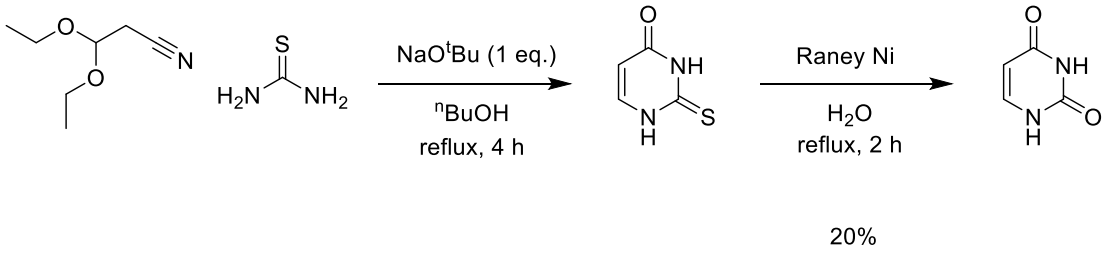
***Scheme 2**: Example of purine synthesis by condensation.*

Synthesis of pyrimidines typically proceeds *via* condensation reactions, and removal of functional groups from derivatives in order to generate the desired products. For example, cytosine can be produced by reaction of 3-hydroxyacrylic nitrile with urea in the presence of a strong base.[140] (**Scheme 3)**.



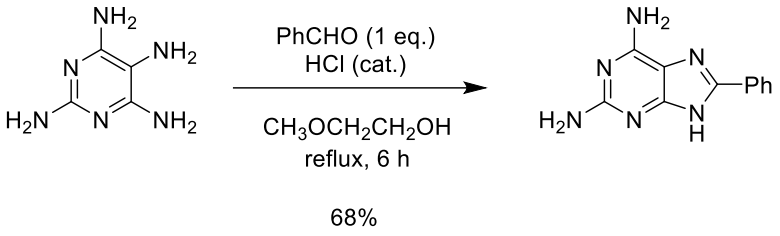
***Scheme 3**: Preparation of cytosine via condensation of urea and a nitrile.*

A method to produce uracil follows a similar route with condensation of a cyano compound, using thiourea followed by reduction to give the desired compound[141] (**Scheme 4**).



***Scheme 4**: Synthesis of uracil by condensation and reduction of thiourea.*

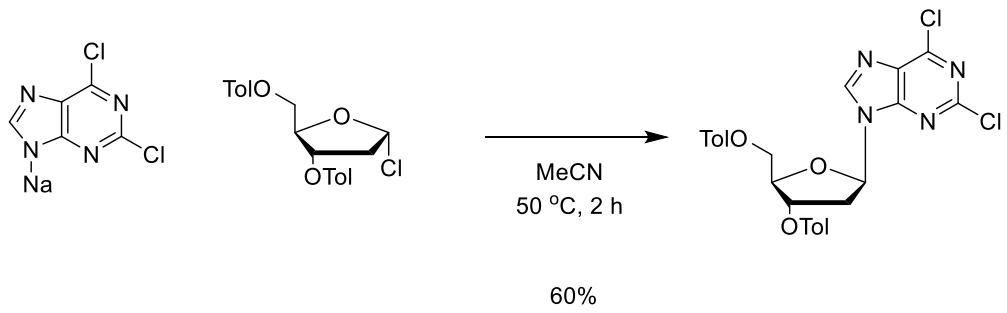
Highly substituted pyrimidines can also be prepared by using more complex starting materials. Reaction of substituted cyanic acids with vinyl amides permits for the synthesis of pyrimidines with heteroatom substituents (**Scheme 5**), useful for further derivatization[142].



***Scheme 5**: Synthesis of highly substituted pyrimidines promoted by a strong electrophile.*

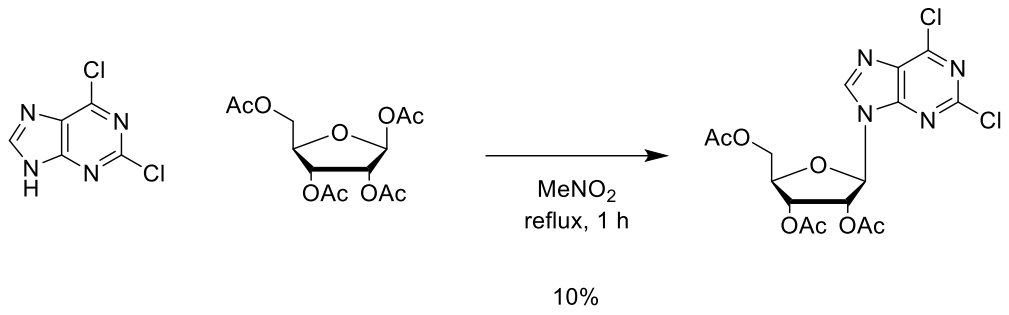
For further investigation into nucleic acids, prepared purine and pyrimidine compounds require coupling to a ribose unit. Three methods are commonly used to prepare nucleosides from nucleophilic nitrogenous bases and an electrophilic sugar.

The metal salt method combines a heterocycle metal salt with a protected sugar halide (**Scheme 6**). Modern methods use sodium salts[143], however original procedures utilised silver salts[143] or mercury mediated migration of a ribose unit from one nitrogen to another[144].



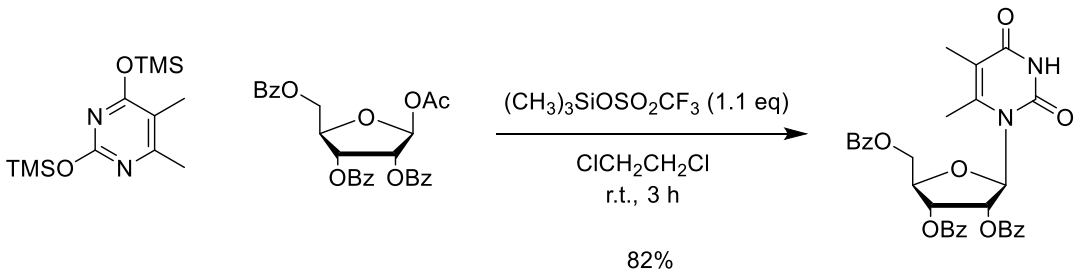
***Scheme 6**: Metal salt method of nucleoside synthesis.*

Another common procedure is the fusion method, where the nucleophilic base and a protected ribose are heated at high temperature (**Scheme 7**). This method however is limited to purines with strong electron withdrawing groups, and yields may be low[145,146].



***Scheme 7**: Fusion method of nucleoside synthesis.*

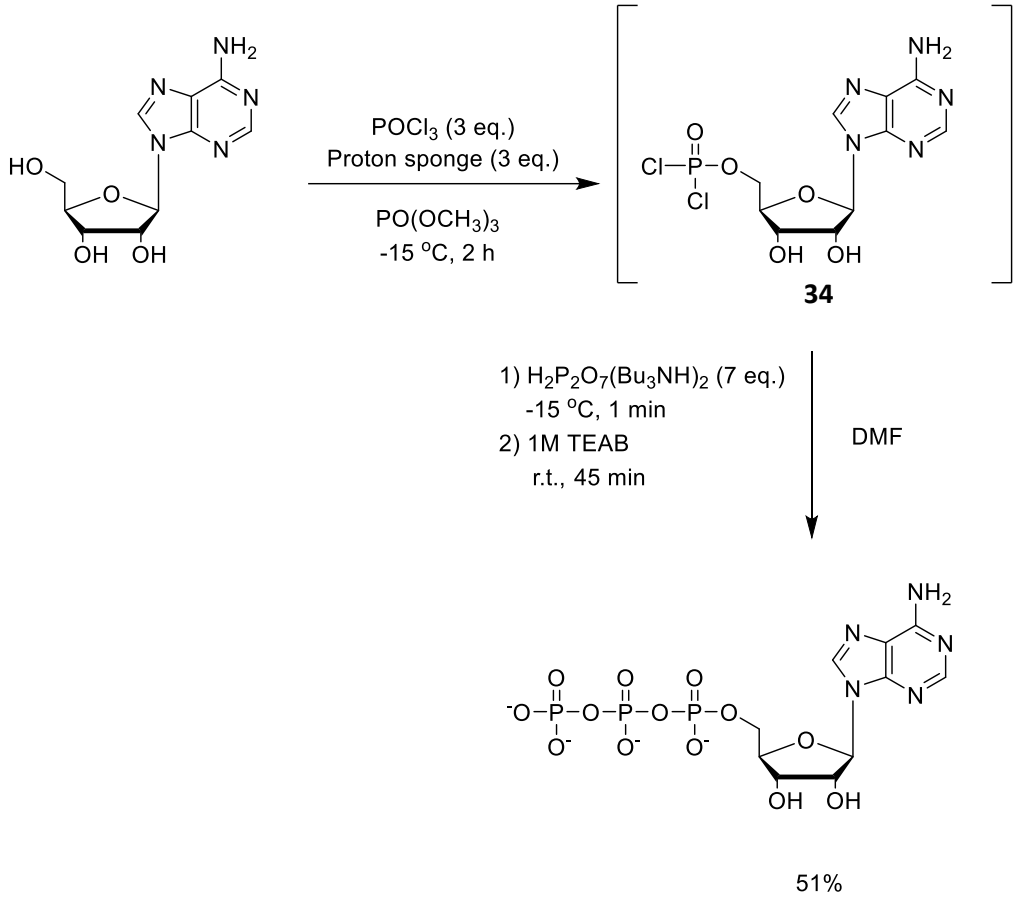
The mildest and most common method is the silyl-Hilbert-Johnson reaction, also known as the Vorbrüggen condensation[147] (**Scheme 8**). A silylated heterocycle and protected sugar in the presence of a base condense to generate nucleosides in high yield without resorting to harsh reagents such as metal alkoxides or high temperatures[148].



***Scheme 8**: Silyl-Hilbert-Johnson reaction to form a nucleoside.*

Synthesis of nucleotides is difficult compared to nucleosides due to their poor solubility in organic solvents. Whilst organic synthesis in water is becoming increasingly common, the use of water often limits some chemistry such as the use of metal alkoxides and reactive halide species.

A route to preparing the triphosphate from a nucleoside using phosphoryl chloride and tributylammonium pyrophosphate is able to produce the triphosphate in a one pot-three step procedure (**Scheme 9**), first preparing a dichlorophosphoridate (**34**) which reacts with the pyrophosphate to form a cyclic intermediate, which is hydrolysed to the desired triphosphate compound[149].

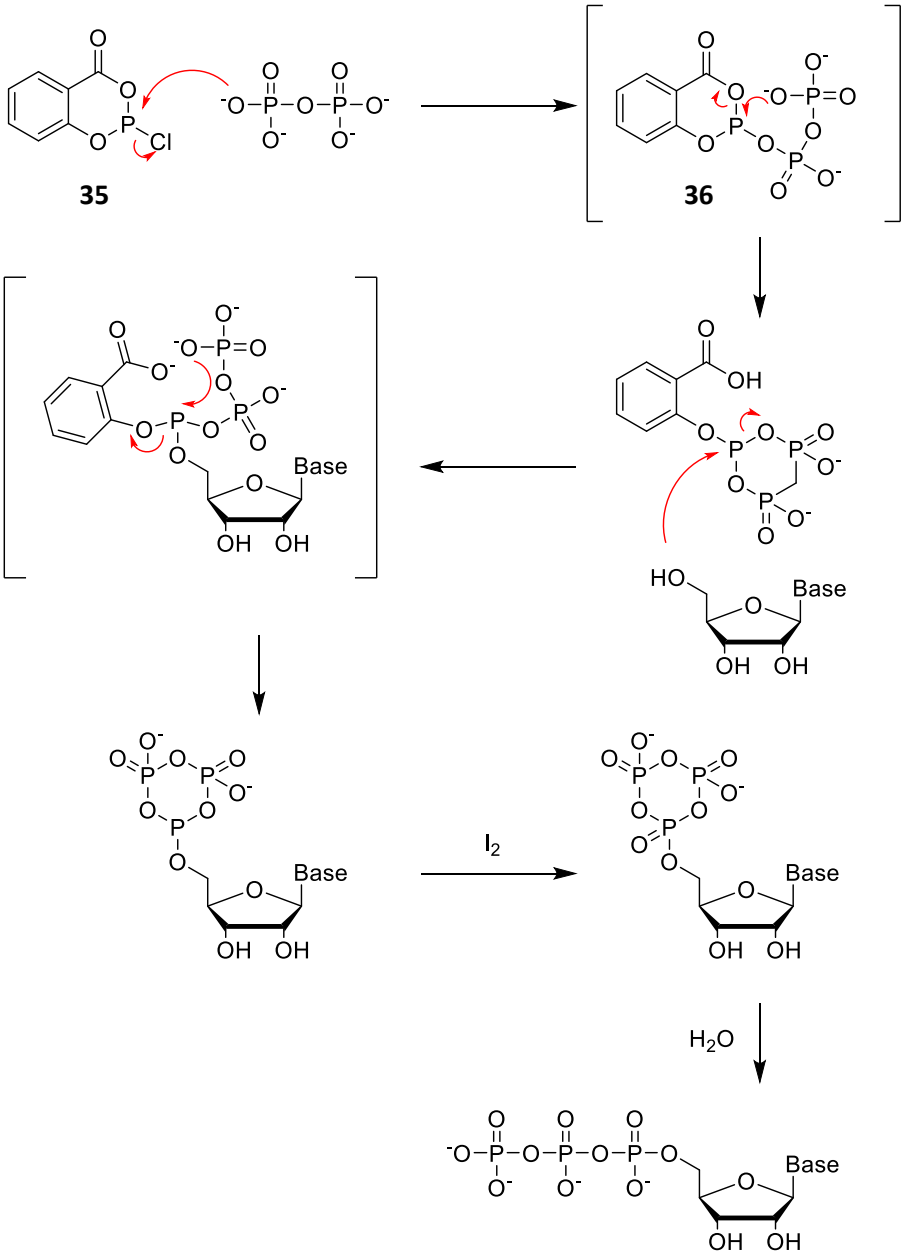


***Scheme 9**: Nucleotide triphosphate synthesis using $POCl_3$ and pyrophosphate.*

Whilst additional protection of the ribose not being required is a significant advantage to this approach, the moderate yield and numerous side products are problematic. Quantities of the monophosphate at both the 2' and 3' position on the ribose are recovered, along with a bridging phosphate between 2' and 3' as well as the cyclic triphosphate compound from reaction with itself.

An alteration to avoid these side products uses salicyl phosphorochloridite (**35**) to generate a mild phosphitylating reagent *in situ* as an alternative to phosphoryl chloride (**Scheme 10**). Reaction of the salicyl phosphorochloridite with

tributylammonium pyrophosphate forms a cyclic intermediate (**36**), and nucleophilic attack by the 5' alcohol of the ribose of the nucleoside opens the ring structure, which subsequently cyclises again and eliminates salicylate. Oxidation and hydrolysis of this compound affords the desired nucleotide triphosphate[150].



***Scheme 10**: Nucleotide triphosphate synthesis by formation of a mild phosphitylating reagent in situ.*

1.8.3) Chemical synthesis of DNA

Laboratory methods exist to prepare DNA of a chosen sequence. Proceeding *via* solid phase synthesis, the Nobel prize for chemistry was awarded in 1984 due to its importance. Analogous to methods in peptide synthesis, oligonucleotide synthesis can be achieved by broadly similar means. The most common method uses phosphoramidites, with a 4,4'-dimethoxytrityl group on the 5' alcohol and a phosphityl group on the 3' alcohol which forms the phosphate backbone[151] (**Fig. 26**).

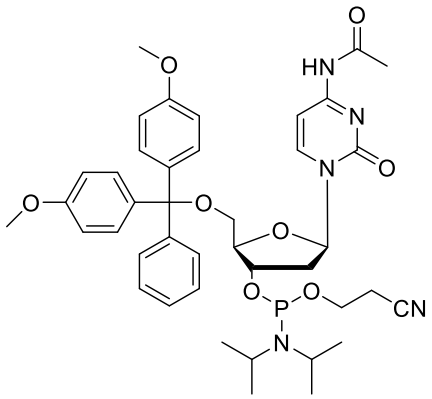


Figure 26*: Example cytosine phosphoramidite prepared for solid phase DNA synthesis.*

Solid phase synthesis is performed on columns allowing reagents and solvents to pass through them. In comparison to sequential solution based methods, large excesses of reagents can be used to rapidly advance a reaction towards completion, impurities are washed away preventing the need for time consuming purification, and the process is able to be automated *via* computer controlled systems. An oligonucleotide may be 50 DNA bases long, and so high yields for each coupling reaction are required. A 50mer with a coupling efficiency of 90% would only achieve a 0.6% yield of the desired oligonucleotide, and so the advantages conveyed by solid phase methods in terms of yield and time are of great importance.

DNA synthesis *in vivo* occurs in a 5' to 3' direction, but in solid phase synthesis proceeds from 3' to 5'. A repeated series of steps are performed and each cycle incorporates one nucleotide into the oligonucleotide. Solid supports with desired nucleotides attached are commercially available, and the first step is detritylation to yield the free 5' hydroxyl group. The subsequent desired phosphoramidite is added and coupled, adding the next unit to the oligonucleotide. Oxidation with iodine from

phosphorus (III) to phosphorus (V) is performed, then deprotection of the trityl group with an acidic solution (commonly trichloroacetic acid). This reveals a 5' hydroxyl group on ribose, to which another nucleotide can be added and the cycle repeats. Once the sequence is complete, cleavage of the oligonucleotide from the solid support can be performed with a basic solution to yield the oligonucleotide (**Fig. 27**)

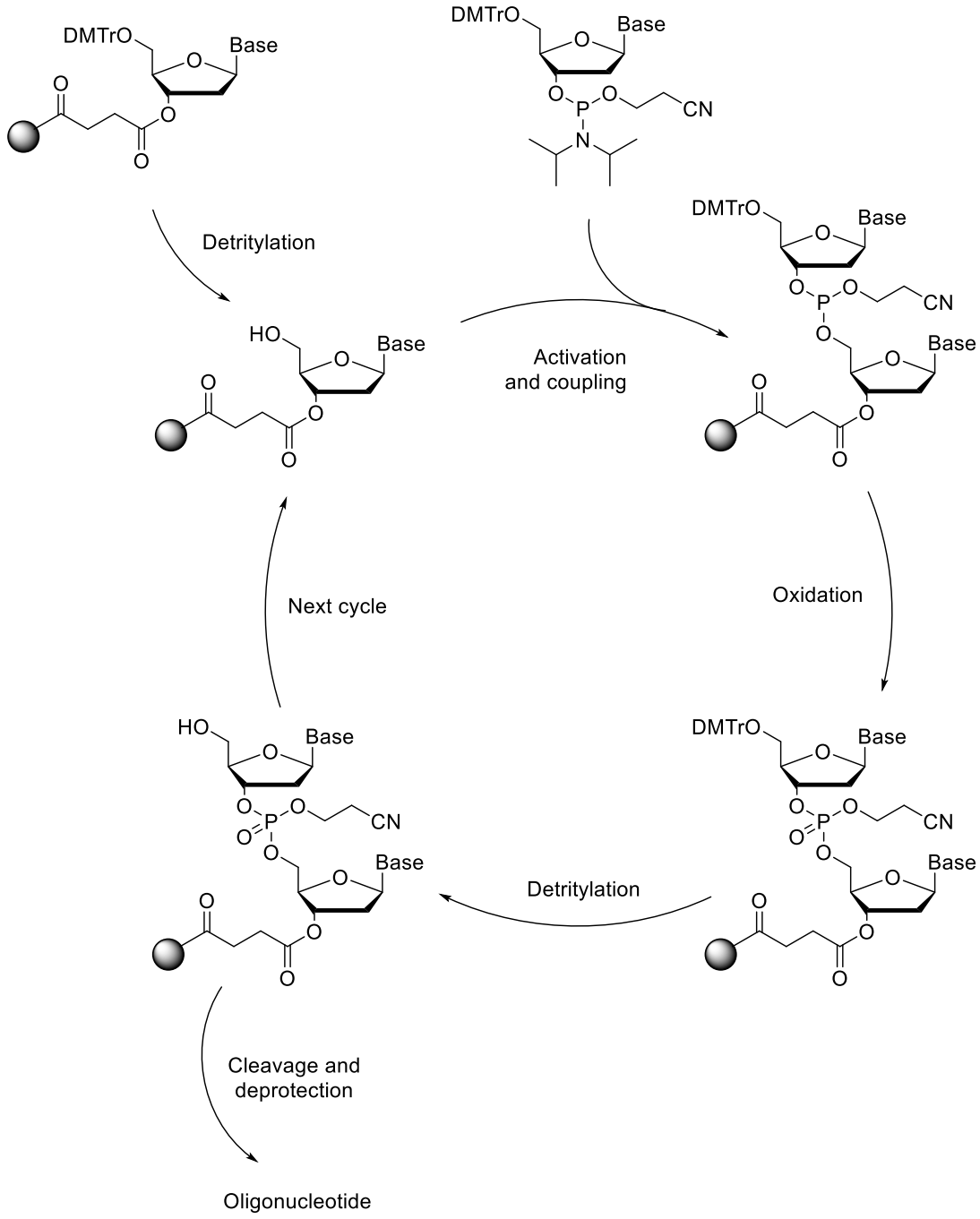


***Figure 27**: General procedure of solid phase DNA synthesis to prepare an oligonucleotide from phosphoramidite building blocks.*

The nitrogenous bases commonly possess protecting groups, and are usually base sensitive. Further heating in basic solution (often ammonium hydroxide) may be performed to ensure complete removal of protecting groups such as acetyl, benzoyl or isobutyryl amides (**Fig. 28**). The cyanoethyl chain on phosphorus is also removed under these basic conditions.

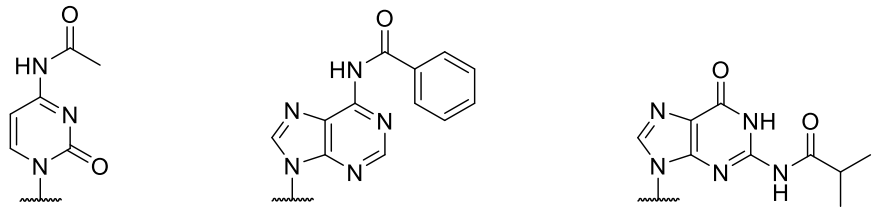


***Figure 28**: Example protecting groups used in solid phase DNA synthesis. Left to right: N^4-acetylcytosine, N^6-benzoyladenosine, N^2-isobutyrylguanine.*

Even with high yields achieved by excess of reagent, the reactions do not proceed to 100% completion. Any remaining 5' hydroxyl groups which did not react could lead to a base deletion in the sequence, and over a sequence of 50 bases would lead to numerous different oligonucleotides obtained after synthesis is complete.

This is solved through usage of a capping solution (**Fig. 29**). After each coupling step, a solution containing acetic anhydride and *N*-methylimidazole is flushed through the column. This mixture generates a highly electrophilic reagent which rapidly acetylates alcohols, preventing the need for complex purification of a range of oligonucleotides.

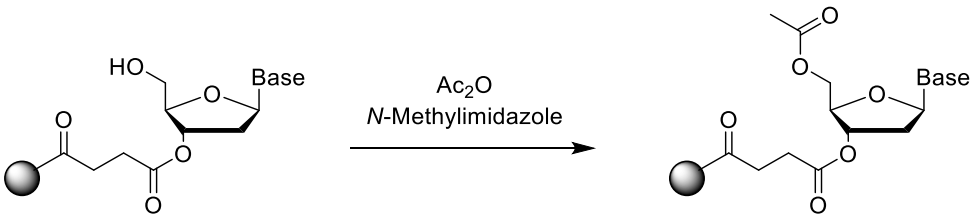
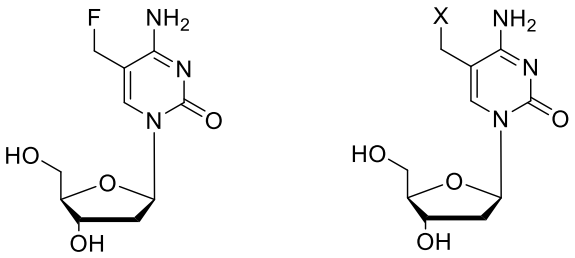


***Figure 29**: General procedure for capping of unreacted nucleotides, leading to chain termination.*

2) Results and discussion

2.1) Aim

The primary aim of the project was to produce a 5-fluoromethyl-2'-deoxycytidine nucleoside (5fmdC, **37**), which could be used to develop a high throughput fluorescence assay to study human TET enzymes. Several modified 2'-deoxycytidine nucleosides were also to be synthesised and tested on the assay as potential TET enzyme inhibitors (**Fig. 30**). All prepared compounds were to be tested on a cytotoxicity assay to assess their effects on cells.



37

***Figure 30**: Fluorinated 2'-deoxycytidine nucleoside (**37**) and general structure of a 5-modified example (right).*

A secondary aim was to perform further synthesis to prepare a phosphoramidite for solid phase DNA synthesis of the most interesting nucleosides from the cytotoxicity studies (**Fig. 31**), and therefore it is imperative that any synthetic routes developed are efficient and high yielding.

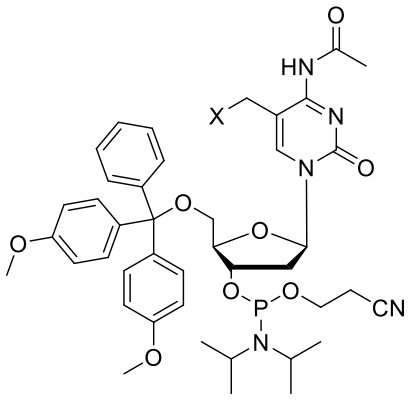


***Figure 31**: Example of 5-substituted 2'-deoxycytidine phosphoramidite for solid phase DNA synthesis.*

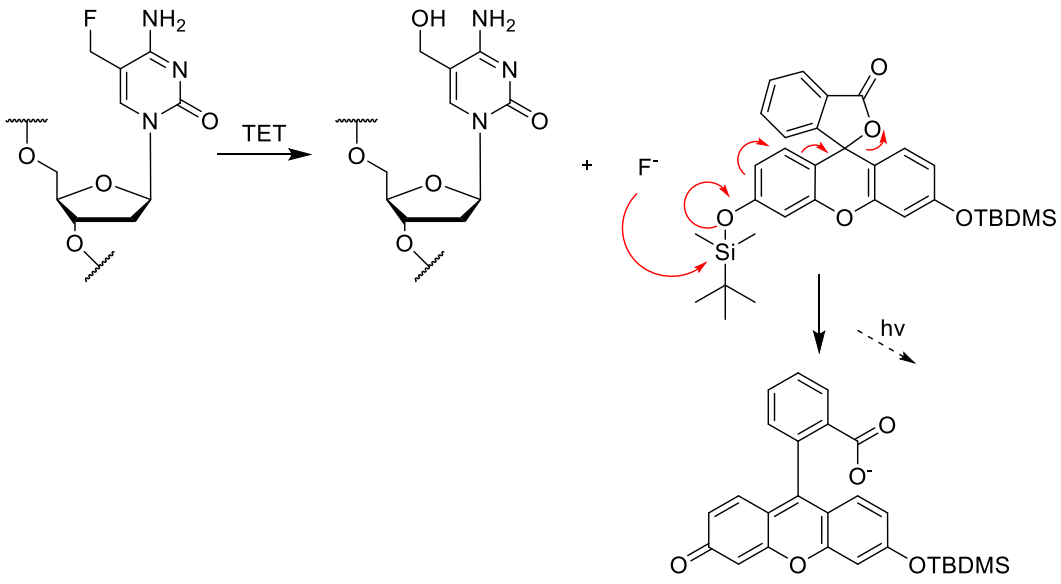


***Figure 32**: Hypothesised function of the assay utilising the fluorinated nucleoside.*

The fluorescence assay selected[152] utilises a silylated fluorescein compound. Upon cleavage of the silyl groups by fluoride, a fluorescence signal can be measured. The intensity of this signal is proportional to fluoride concentration and from this, the enzyme activity can be calculated. It is hypothesised that the designed 5fmdC nucleoside will be oxidised by the TET enzymes, releasing a fluoride anion to trigger the assay (**Fig. 32**).

Should an inhibitor of the TET enzymes be included in the assay then fluoride release would be suppressed, and the decrease of enzyme activity can be measured. In combination with the fluorinated nucleoside, this would function as the first high throughput assay suitable for measuring efficacy of compounds against TET enzymes. Compounds determined to be inhibitors of these enzymes can be investigated further as potential disease treatments.

2.2) Synthetic route

A retrosynthetic analysis was performed on the 5fmdC nucleoside to identify a route (**Fig. 33**). The key steps were functionalisation of the methyl group and fluorination.

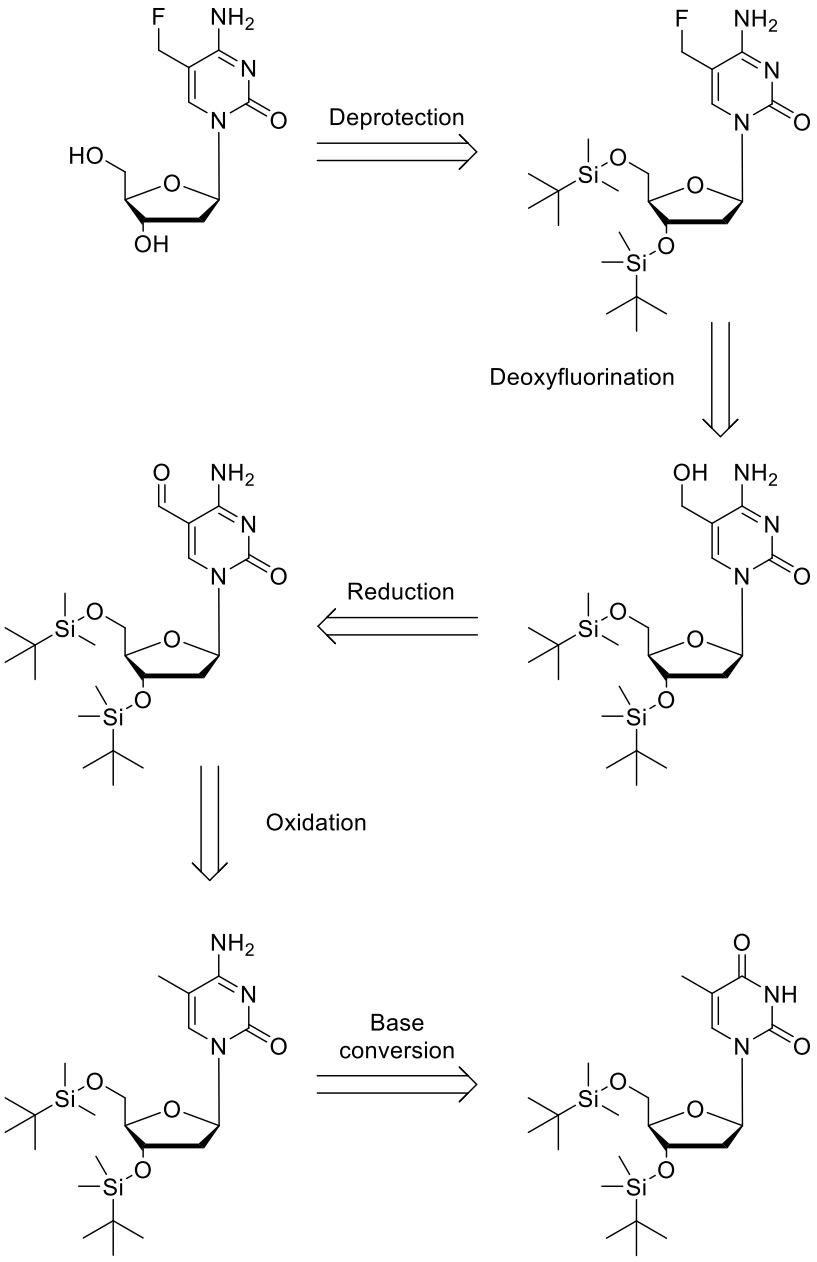


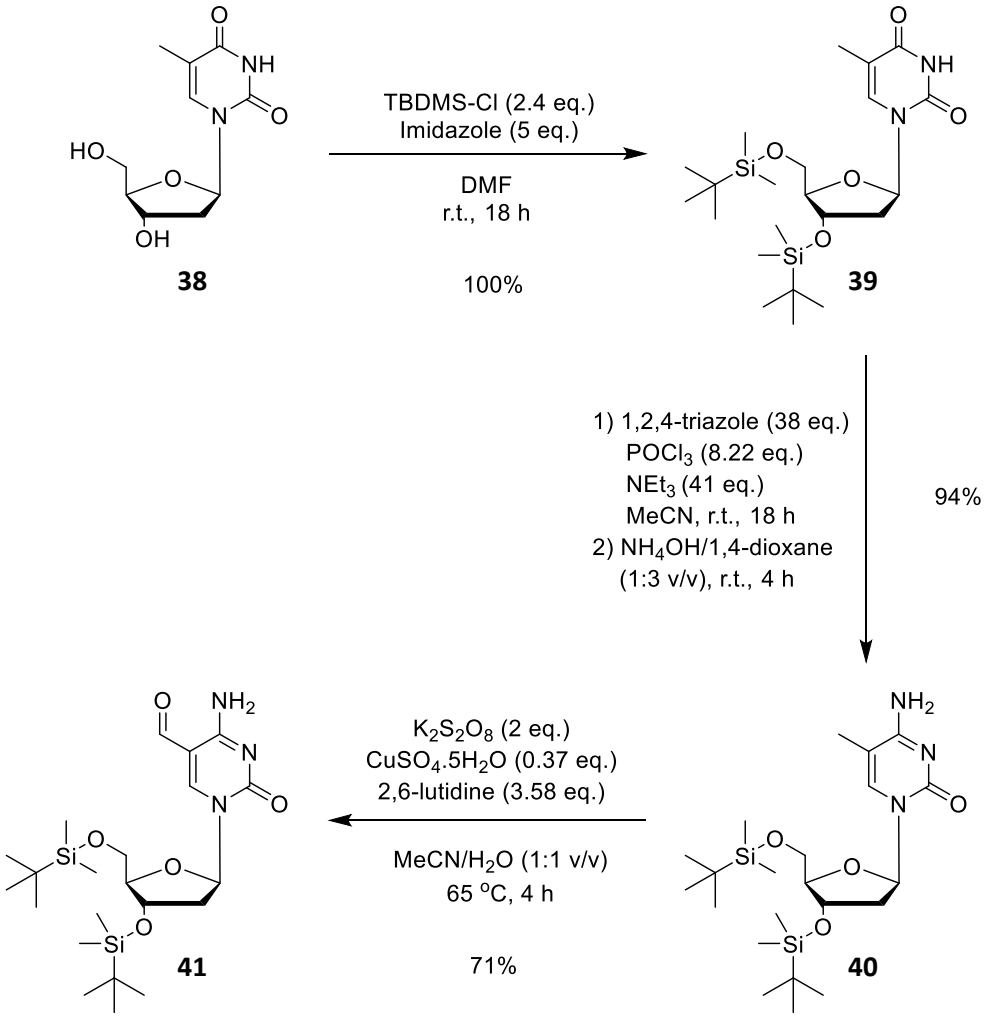
***Figure 33**: Retrosynthesis of the 5-fluoromethyl-2'-deoxycytidine nucleoside.*

Deoxyfluorination could be performed to convert a hydroxyl group to a fluorine moiety, and the hydroxyl group prepared by reduction of an aldehyde. Benzylic oxidation would prepare an aldehyde, and literature methods could be employed to convert the cheaper thymidine nucleoside (£1.31/mmol, Alfa Aesar) to the 5mdC

(£12.68/mmol, Alfa Aesar), as this contains a pre-installed methyl group. A silyl protecting group strategy common for nucleosides was to be used as these groups are stable to all conditions throughout the synthesis.

2.3) Synthesis of 5-fluoromethyl-2'-deoxycytidine

An efficient and high yielding route towards the protected 5-formyl-2'-deoxycytidine nucleoside (**41**) (**Scheme 11**) was desired as this key intermediate was important for synthesis of the fluoromethyl nucleoside, but also as an intermediate where the aldehyde can be used as a handle for further analogue synthesis.



***Scheme 11**: Silylation, base conversion and oxidation to prepare **41**.*

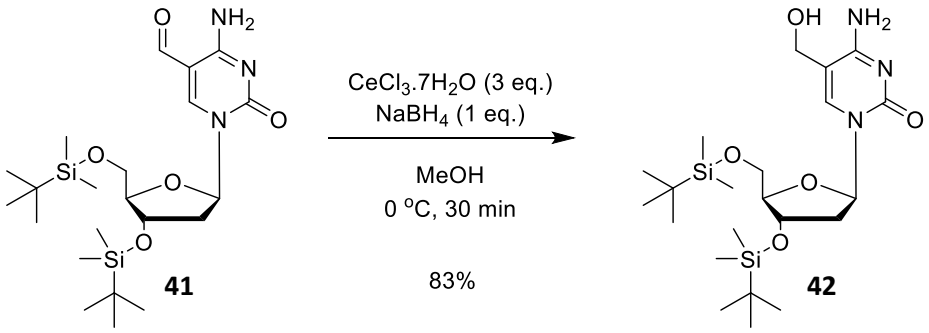
Following the retrosynthesis, *tert*-butyldimethylsilyl (TBDMS) protection of thymidine (**38**) using conditions established by Corey *et al.*[153] was performed and

proceeded in quantitative yield. Subsequent conversion from the protected thymidine **39** to cytidine **40** was performed with a method reported by Pirrung[154], proceeding in high yield without the requirement of chromatographic purification.

This method requires great excess of reagent to achieve such a high yield, and alternatives were sought. Methods developed by Hansen[155] and Matsuda[156] utilised TIPS-Cl (2,4,6-triisopropylbenzenesulfonyl chloride) to form a reactive intermediate which undergoes aminolysis, however yields were poor and additional purification steps were required.

A benzylic oxidation method demonstrated by Bhatt using $K_2S_2O_8$ with a Cu^{2+} catalyst[157] and improved for a nucleoside system by Matsuda[158] proved successful, furnishing the desired aldehyde **41** in good yield. No over-oxidation to the carboxylic acid product was detected, and it is hypothesised that there is a stabilising influence from the adjacent amine forming an intramolecular hydrogen bond. Alternative methods with catalytic TEMPO and NaOCl[159], or IBX (2-iodoxybenzoic acid)[160] were explored but were unable to prepare the desired aldehyde with only starting material recovered.

These procedures were able to produce **41** in a 67% yield across three steps from commercially available thymidine, at a lower cost than purchasing and oxidising 5-methyl-2'-deoxycytidine.

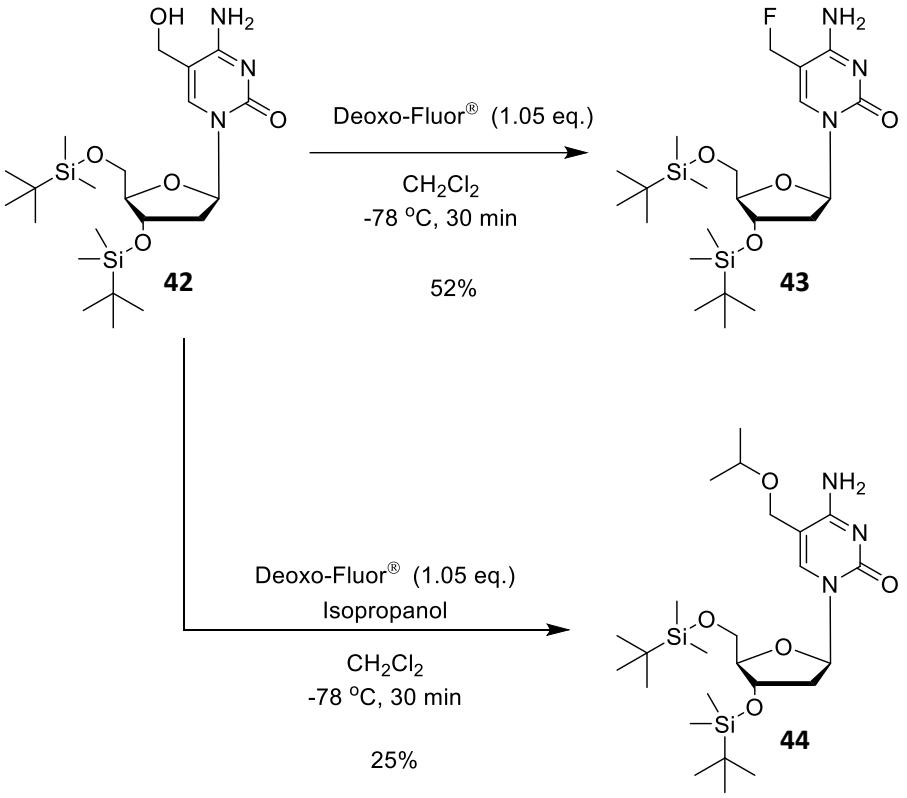


***Scheme 12**: Reduction of protected 5fdC to protected 5hmdC* via *the Luche method.*

The Luche method was selected for reduction of the aldehyde (**Scheme 12**) due to its 1,2-selectivity over the competing 1,4-reduction[161]. Additional steps to prevent 1,4-

reduction such as cooling the reaction further were unsuccessful, and alternative procedures such as the Meerwein-Ponndorf-Verly[162] or using activated alumina[163] as the Lewis acid were unable to selectively generate the desired product. With a yield of 83% *via* the initial method, further optimisation was not performed.

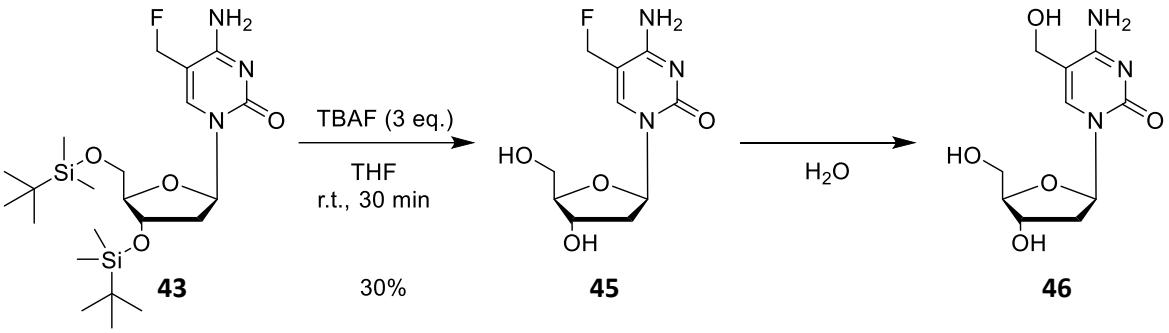
Deoxyfluorination of **42** in the presence of silyl protecting groups was performed with Deoxo-Fluor® as the reagent. The competing silyl cleavage reaction was of concern but it was anticipated that low temperatures may alleviate any issues.



***Scheme 13**: Deoxyfluorination of **42** and unexpected isopropoxy product **44**.*

In previous work performed on this project the reaction was quenched with methanol, and no fluorinated product was obtained except a 5-methoxy substituted nucleoside[164]. Isopropanol was selected as an alternative for quenching and as a chromatography solvent due to its lower nucleophilicity, however the isopropoxy substituted compound **44** was obtained in low yield (**Scheme 13**), and retained as an additional analogue for cellular testing. Quenching of the reaction was abandoned and the desired fluorinated compound **43** was obtained in moderate yield.

Cleavage of silyl ether groups is commonly performed by fluoride ions or under acidic conditions. A common source of fluoride ions for this reaction is tetrabutylammonium fluoride (TBAF) and was chosen as a milder method than treatment with acid.



***Scheme 14**: Deprotection and subsequent decomposition of compound **43**.*

The deprotection step rapidly proceeded to completion, however isolation of the product proved difficult. Chromatographic purification failed to easily separate the deprotected nucleoside from the tetrabutylammonium cation, and as the free nucleoside is preferentially soluble in aqueous solvent it could not be washed out. It was noted that the alcohol was also recovered in significant quantity and with each subsequent purification the quantity of alcohol increased and fluorinated compound decreased, which suggested decomposition by hydrolysis (**Scheme 14**).

Alternative deprotection reagents such as NaF, CsF and NH_4F were trialled to avoid the tetrabutylammonium cation, but due to limited solubility were unable to cleave the silyl protecting groups and starting material was recovered in each instance. A solvent change to an alcohol to aid solubility was deemed unsuitable due to the prior displacement of fluoride by isopropanol. An acidic method of 1% HCl in ^{i}PrOH was trialled yet complete decomposition to the alcohol was seen after purification.

That the compound decomposes is not inherently problematic, but it must have a lifetime under physiological conditions long enough to be incorporated into DNA and for the desired TET enzyme activity to occur. The rate of decomposition must be slow enough that enzyme activity could be distinguished from noise on the assay from

natural decomposition. Should the mechanism be understood then steps can be taken to reduce the impact.

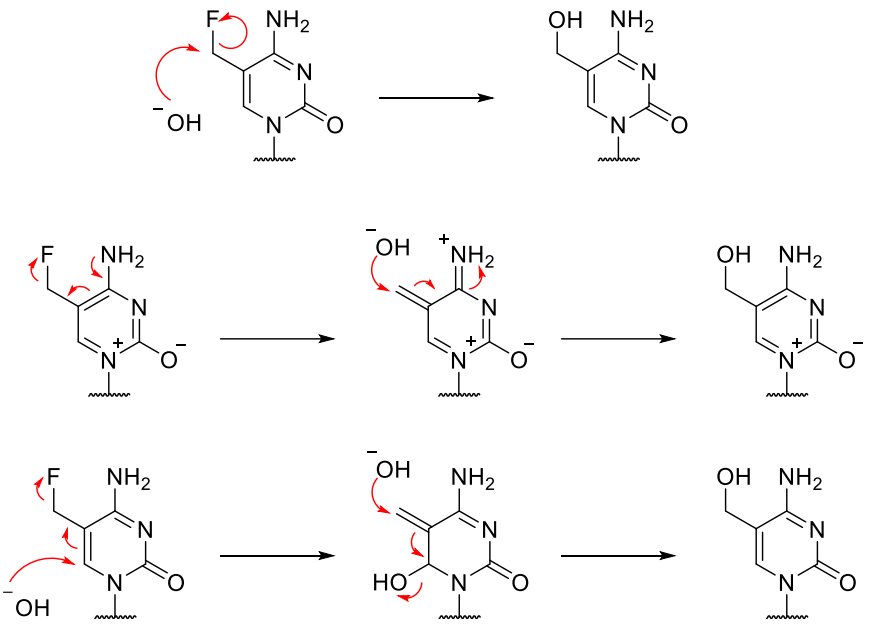
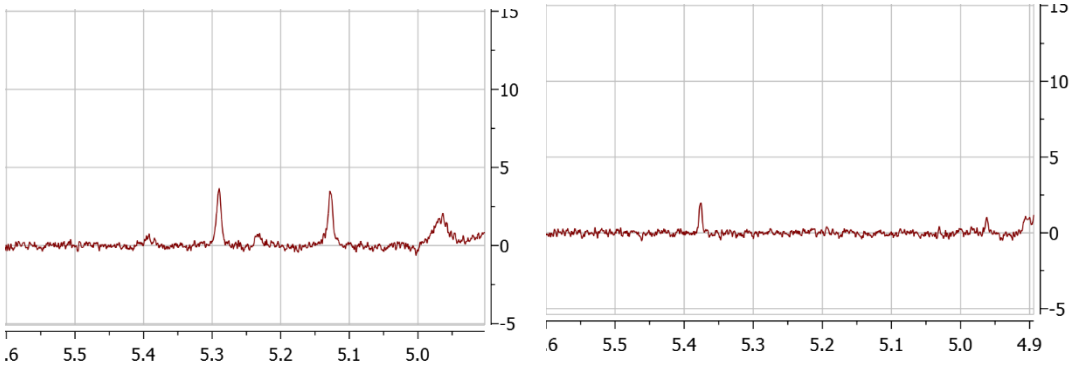


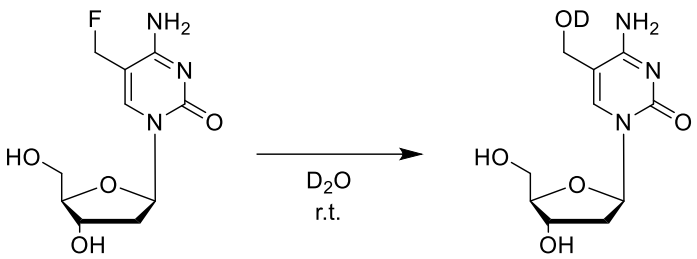
***Figure 34**: Hypothesised mechanisms for defluorination.*

We propose three mechanisms for defluorination (**Fig. 34**). The first would proceed *via* direct displacement of fluoride by a nucleophile and seems the most likely explanation, with formation of the isopropoxy compound **44** on as compelling evidence towards this. A second mechanism considers the electron donating nature of the N^4 amine first eliminating fluoride then undergoing nucleophilic attack to form the alcohol. A third decomposition mechanism could proceed *via* nucleophilic attack of a hydroxide ion at the C^6 position, as is well known for uracil, thymine and cytosine bases *in vivo*[165].

2.3.1) NMR decomposition studies

As decomposition of **45** in aqueous media was confirmed, an investigation into how and why this phenomenon occurs was undertaken. A deprotection reaction was performed with TBAF and upon completion the crude reaction mixture taken up in D_2O and analysed by ^1H NMR.





45 **46**

***Figure 35**: Section of the ^1H NMR spectra of **45** in D_2O. Left = 5 minutes. Right = 22 hours. Doublet at δ_H 5.20 shows CH_2F, and lack of peak after 22 hours showing decomposition.*

It was observed that the distinctive doublet at approximately δ_H 5.20 which is indicative of the -CH_2F peak had completely disappeared, with analysis of the remainder of the spectra indicating complete decomposition to the alcohol (**Fig. 35**). It is thought that significant decomposition of the sample had already occurred in the short period of time between addition of the NMR solvent and analysis of the sample.

Should aqueous conditions be responsible for decomposition then in an anhydrous environment the sample should not decompose. The deprotection reaction was repeated and the solvent was removed under reduced pressure and the residue taken up in DMSO-d_6 (**Fig. 36**).

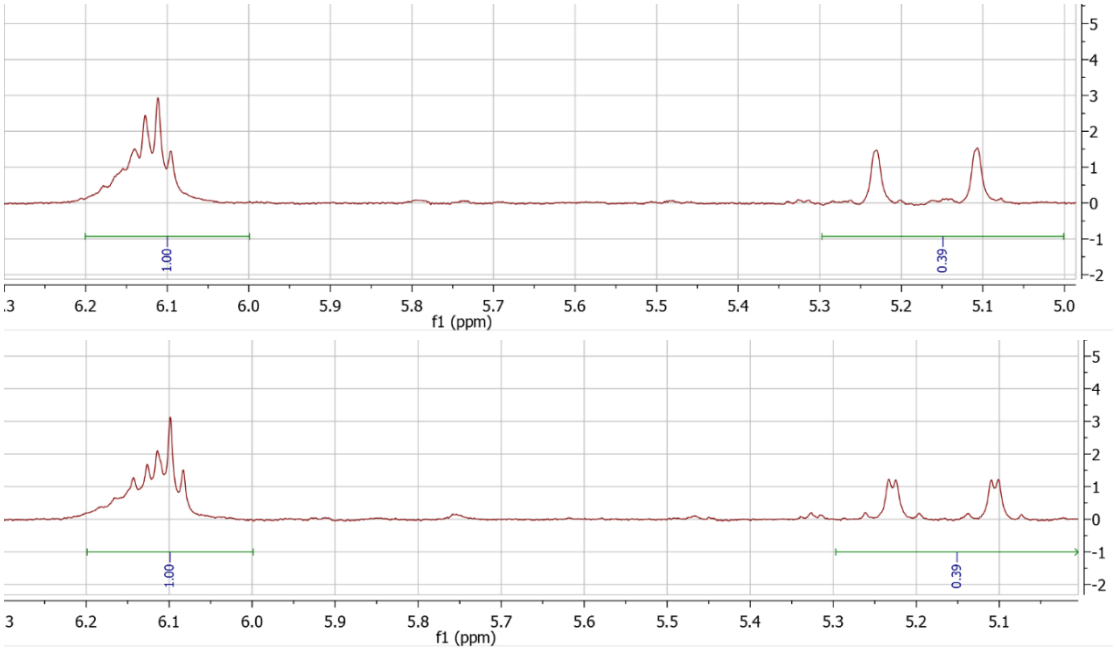


***Figure 36**: Section of the ^{1}H NMR spectra of deprotected fluorous nucleoside **45** in DMSO-d_6 indicating no decomposition. Top = 30 minutes. Bottom = 9 days. Multiplet at δ_H 6.20-6.08 is the 1' CH of ribose of both compound **45** and **46** and doublet at δ_H 5.16 indicates the CH_2F peak.*

Pleasingly, this hypothesis was vindicated with a sample in DMSO exposed to ambient conditions not markedly changing over nine days, confirmed by ^{1}H and ^{19}F NMR. This confirmed that decomposition was due to the presence of water rather than an inherent instability of the highly desired 5-fluoromethyl-2'-deoxycytidine **45** compound.

Any biological testing must be performed in aqueous media, and so an investigation into the lifetime of the nucleoside was undertaken. Decomposition of the compound under physiological conditions is acceptable provided the rate is low enough that accurate measurements can be made.

An experiment was performed with a sample of deprotected nucleoside in DMSO-d_6 and diluted to a 1:1 (v/v) ratio with D_2O, and monitored by ^{1}H NMR with a spectra recorded every five minutes for 150 minutes (**Fig. 37**).

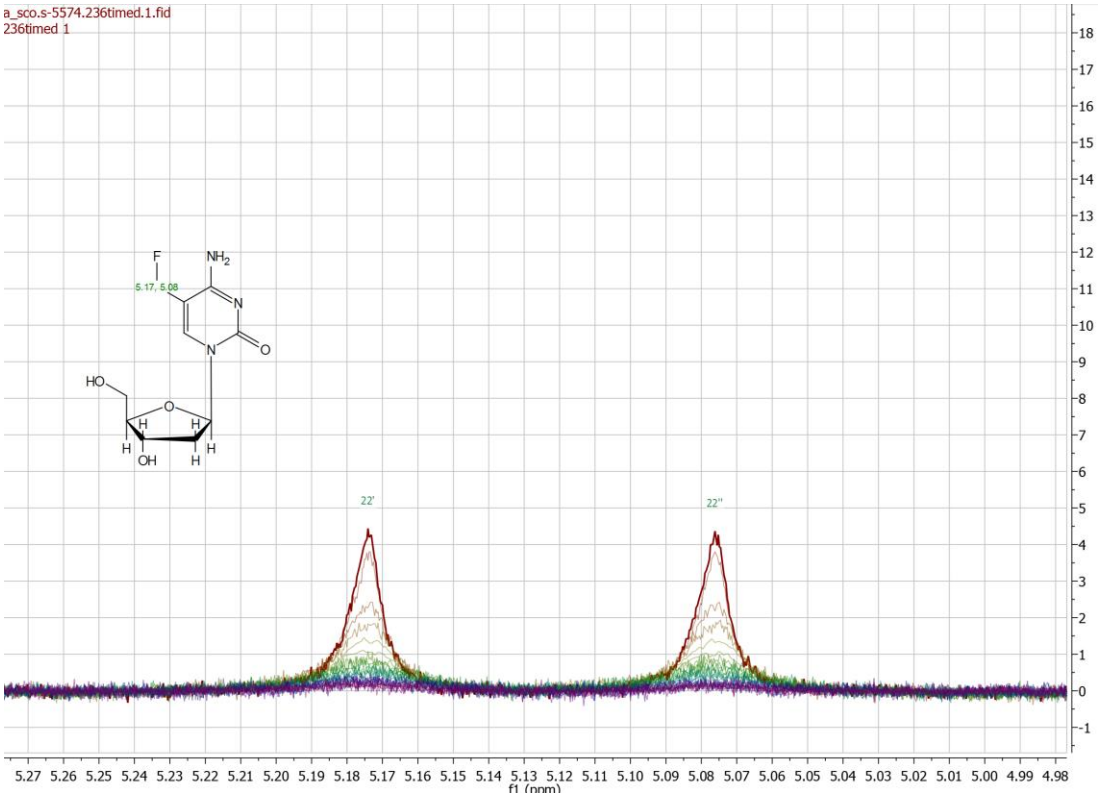


***Figure 37**: Section of twenty-five ^{1}H NMR experiments superimposed showing the -CH_2F peak, illustrating decomposition in aqueous media. Each subsequent line indicates a five-minute interval. Solvent system 1:1 (v/v) DMSO-d_6:D_2O.*

Under these conditions, after approximately thirty minutes the decomposition is significant enough that to distinguish the signal from noise becomes difficult. It could not be claimed with much certainty that any fluorinated nucleoside remains after this time period. At this point it was determined that the 5fmdC nucleoside was not appropriate for cellular testing as the rate of decomposition was too rapid, and collection of accurate data would not be successful. However, this decomposition was unexpected and so continued investigation into the mechanism was pursued.

As part of a further attempt to elucidate a mechanism, the pH dependency on rate of decomposition was investigated. A sample of D_2O was acidified with HCl (37% w/w) to a pH of 3, and the sample monitored by ^{19}F NMR (**Fig. 38**).

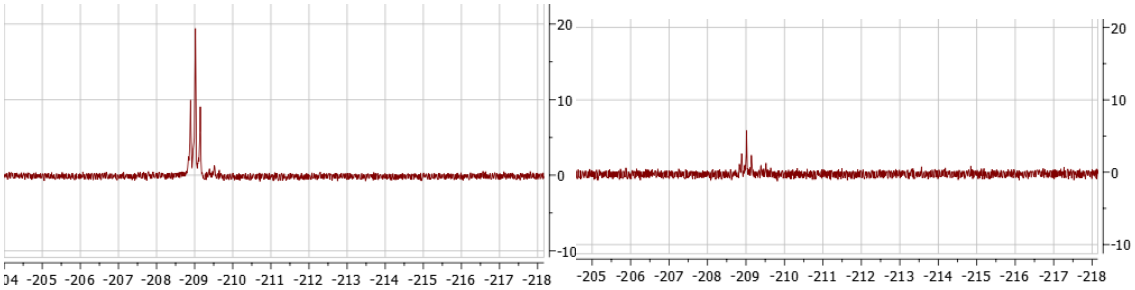


***Figure 38**: Section of ^{19}F NMR spectra of compound **45** in a pH 3 environment. Left = 1 hour. Right = 3 days. Triplet at δ_F -209.00 indicates the CH_2F peak.*

In the acidic media, the lifetime of the fluorinated nucleoside increased dramatically from minutes to several days, lending some credibility to the previously hypothesised mechanisms. The primary amine of cytosine is strongly electron donating to the ring system, however the ammonium cation that would exist under these acidic conditions is strongly electron withdrawing. A second experiment was performed using a sample of D_2O basified with NaOH to a pH of 11, and the sample monitored by ^{19}F NMR. No fluorinated nucleoside could be detected, indicating complete decomposition during the time elapsed to test the sample.

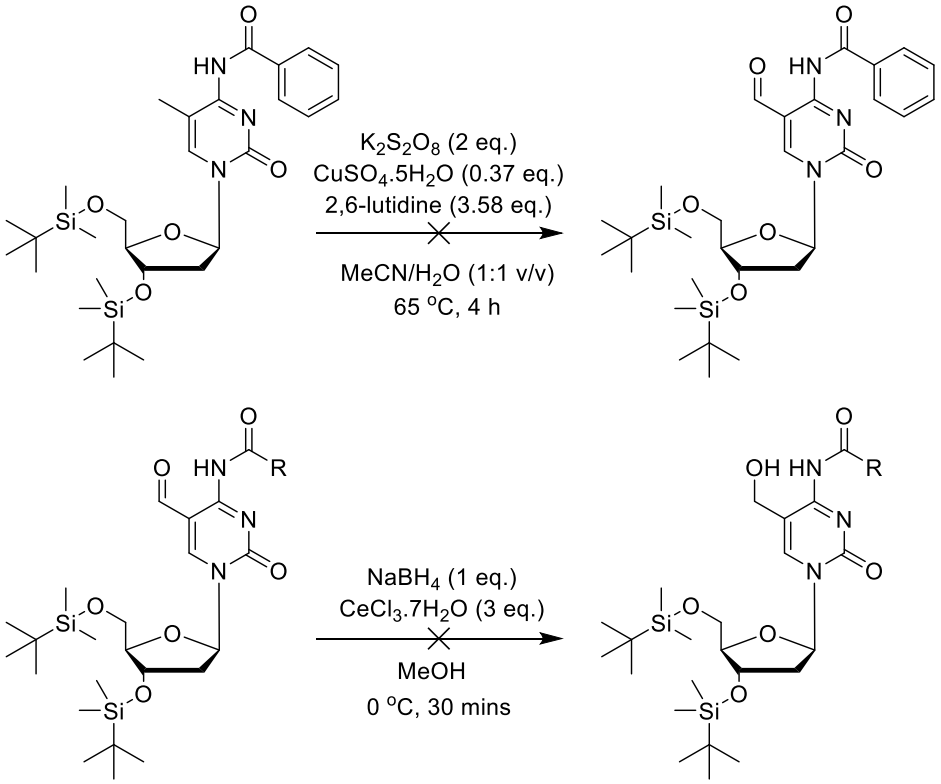
2.3.2) Potential mechanism investigation

A protecting group on the amine may prevent the second pathway from occurring. The additional problem of purification from the tetrabutylammonium cation persists, and a protecting group on the amine will render the desilylated compounds soluble in the organic phase aiding purification, should defluorination be prevented.

Three protecting groups were trialled – acetyl, benzoyl and pivaloyl – and could be incorporated either prior or post oxidation to 5fdC. With the lone pair of nitrogen incorporated into an amide bond, it is less available and so the hypothesised mechanism of defluorination becomes far less favourable (**Scheme 15**).

Surprisingly, upon protection neither of the following steps of oxidation or reduction were successful. In the case of oxidation, starting materials were recovered and for the reduction step only 1,4-reduction was seen. A potential explanation lies in

deactivation of the 5-methyl group due to a weaker electron donating effect from the amide, compared to the amine.



***Scheme 15**: Failed reactions after N^4 amine protection. R = CH_3, $C(CH_3)_3$ or Ph, used as amide protecting groups for the N^4 nitrogen of silyl 5fdC.*

These results were disappointing as they halted work towards preparing a protected form of the fluorinated nucleoside. Whilst they indicate that reactivity at the methyl position is significantly reduced, it was not possible to produce direct evidence for a more stable fluorine at this position *via* this experimental approach.

The final hypothesised mechanism considered attack at the C^6 position on the ring. It was hypothesised that preparation of an epoxide at the C^5 position would allow for introduction of a nucleophile to open it. Nucleophilic attack will primarily occur at the least substituted position, on the terminal carbon. Alternatively, should nucleophilic attack occur at the C^6 position then a different, distinct product would be obtained (**Fig. 39**). This would allow determination of whether this mechanism of defluorination was viable.

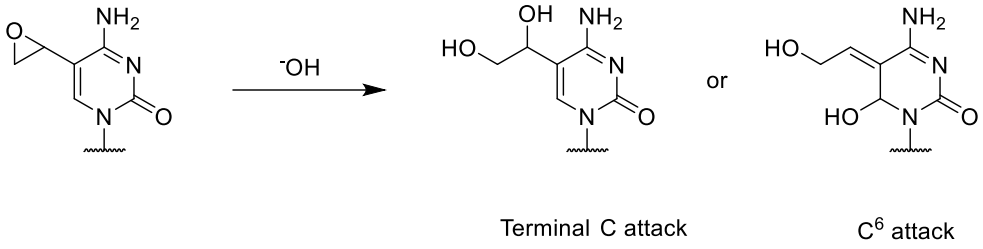


***Figure 39**: Possible products obtained from nucleophilic attack on the epoxide nucleoside.*

Should this mechanism be occurring *via* C^6 attack, steps to prevent this are limited. One possibility would be introduction of a nitrogen atom in the ring at the C^6 position to prevent nucleophilic attack (**Fig. 40**). Both 6-azathymine and 6-azauracil are commercially available, and preparation of the nucleosides could be performed by literature methods.

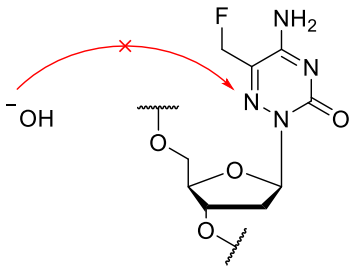


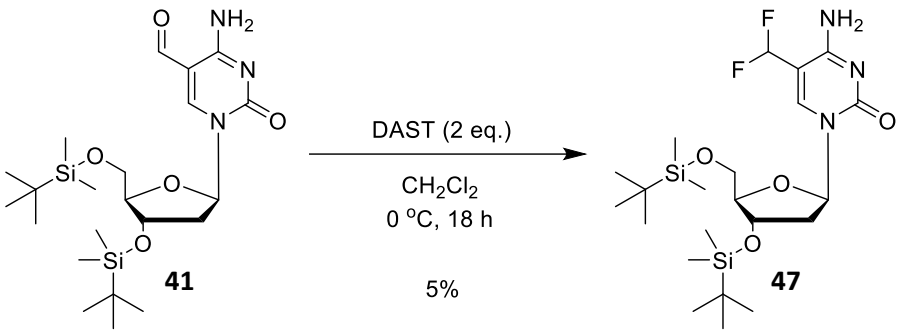
***Figure 40**: Example 6-azacytosine in DNA and blocked nucleophilic attack by hydroxide at the C^6 position due to nitrogen incorporation.*

The experimental results showing lack of reactivity once protection of the amine has occurred, combined with the theoretical explanations, highlight that the epoxide would be a compound of great interest. Electron rich benzyl fluorides are susceptible to fluoride elimination and the presence of a nucleophile may be sufficient to cause rapid compound degradation. After preparation and study of degradation of the epoxide, further synthesis of compounds containing a C^6 nitrogen may be pertinent.

2.4) Synthesis of 5-difluoromethyl-2'-deoxycytidine

With the initial target of 5-fluoromethyl-2'-deoxycytidine **45** established as unsuitable for further investigation due to decomposition in aqueous media, attention was turned to the difluorinated nucleoside as this may also be a suitable substrate for TET enzymes. Fluoride would still be released upon oxidation of this substrate and thus it would be a suitable substrate for the assay.

A geminal difluoride can be prepared by deoxyfluorination of an aldehyde or ketone, and with an efficient and scalable route to protected 5fdC (**41**) established only optimisation of a fluorination step was required, and was performed with DAST (**Scheme 16**).

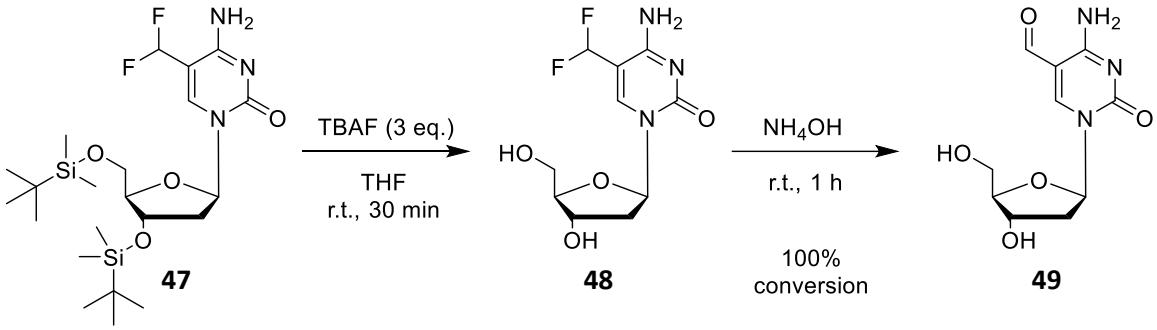


***Scheme 16**: Deoxyfluorination of **41** to form protected difluorinated nucleoside **47**.*

A variety of conditions were trialled but only poor yields were achieved. Different stoichiometries, temperatures and reaction times failed to improve the reaction and only very minor quantities of difluorinated product were obtained, with large quantities of starting material recovered and significant loss of organic material from the organic phase, likely due to desilylation and subsequent loss during aqueous workup or chromatographic purification. Deoxyfluorination to form a *gem*-difluoride is difficult and requires HF to add across the carbonyl prior to further reaction, however in the presence of silyl protecting groups the competing deprotection reaction can lead to lower yields.

The monofluorinated nucleoside 5fmdC decomposed in aqueous media, so tests were performed to determine whether the difluorous compound was also

susceptible. A sample was deprotected by the same method as previously, and upon completion the solvent was removed under reduced pressure and concentrated, dissolved in D_2O and analysed.

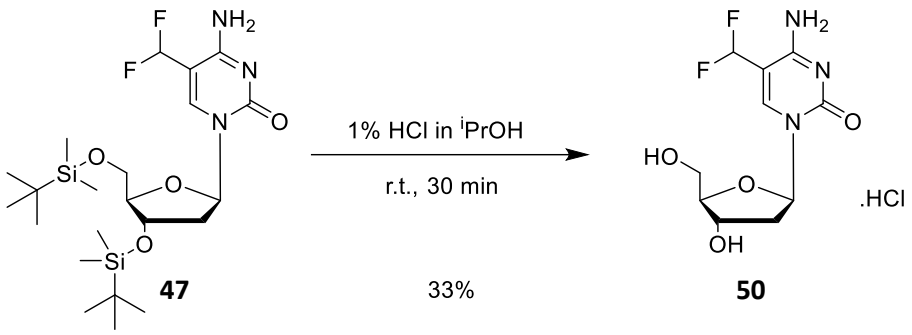


***Scheme 17**: Deprotection and decomposition study of the difluorinated nucleoside.*

Pleasingly, the difluorinated nucleoside **48** was found to be stable in aqueous media at neutral pH. Whilst minor decomposition may have occurred, ^{1}H and ^{19}F NMR analysis after 27 hours demonstrated no significant alteration in the composition of the sample. A sample stored in D_2O in the freezer for two weeks did not indicate any decomposition either, and was determined to be resistant to hydrolysis.

The sample was recovered and stirred in NH_4OH for one hour to assess stability to strongly basic conditions (**Scheme 17**). No fluorine could be detected by ^{19}F NMR and the ^{1}H NMR indicated the complete conversion to aldehyde **49**. Under strongly basic conditions, hydrolysis is possible but water alone was not sufficient for this process to occur.

With stability to neutral conditions established, the difluorinated nucleoside was deemed suitable for cellular testing and deprotection was performed. Due to purification concerns when using TBAF, an alternative acid-mediated TBDMS cleavage was instead employed using dilute HCl in isopropanol (**Scheme 18**).



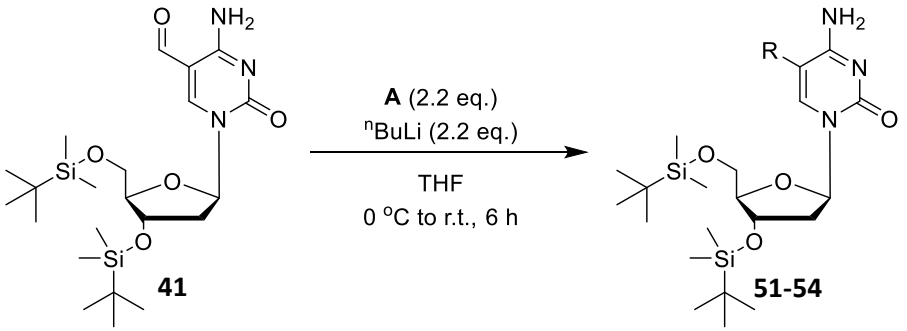
***Scheme 18**: Acidic deprotection of **47** to the deprotected difluorinated nucleoside.*

Acidic deprotection and purification *via* aqueous workup gave the final product as an HCl salt in moderate yield. With a disappointing 2% yield of the deprotected nucleoside over two steps from aldehyde **41**, this route was suitable for cellular testing experiments but an improved route was sought for further biological experiments.

2.5) Alkenyl analogue synthesis

With synthesis of the mono- and difluorinated cytidine nucleosides complete, intermediates on their synthesis routes can be used to prepare a variety of alternative, non-fluorous nucleosides which may exhibit biological activity. Whilst the fluorinated nucleosides were unstable to basic conditions and may not be suitable for solid phase DNA synthesis and incorporation into oligonucleotides, this may not be the case for alternative substituents.

The Wittig reaction was selected to generate analogues from the common starting material protected 5fdC (**41**). Many phosphonium salts are commercially available and facile preparation of others allows for a great variety of analogues to be rapidly prepared.



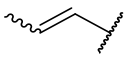
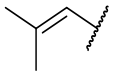
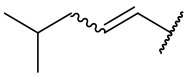
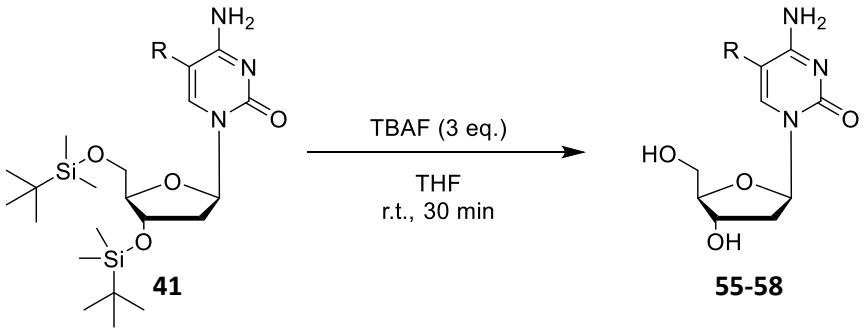
Compound number	**A**	R	Yield (%)	*E*/*Z* ratio
51	Ph_3PCH_3I		50	-
52	$Ph_3PCH_2CH_3I$		30	1:4
53	$Ph_3PCH_2CH(CH_3)_2I$		36	-
54	$Ph_3PCH_2CH_2CH(CH_3)_2Br$		42	1:2

***Table 1**: Results for derivatisation via Wittig reaction of **41** to prepare alkenyl analogues.*

Preparation of four alkenyl nucleosides was successful, and whilst yields were moderate only small quantities were required for cellular testing, and starting materials were not prohibitively expensive. No steps to control *E*/*Z* isomerism of

compounds **52** and **54** were pursued and compounds were taken forward as a mixture of isomers. Should interesting results be obtained from cellular testing then these compounds could be readily prepared again and separated into their constituent isomers.

Deprotection of these compounds was facile in comparison to fluorous analogues. Decomposition through aqueous or basic means was not a concern, treatment with TBAF followed by purification on Dowex ion exchange resin was selected.



41 **55-58**

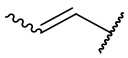
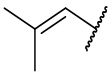
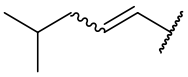
Compound number	R	Yield (%)
55		81
56		48
57		54
58		24

***Table 2**: Results for silyl cleavage of the alkenyl nucleoside analogues.*

Whilst yields were varied, all were in appropriate quantities for cellular testing. All nucleosides could potentially be hydrogenated to prepare alkyl variants instead of alkenyl, offering a further line of compounds.

Of particular interest is 5-vinyl-2'-deoxycytidine (5vdC, **55**) which was designed such that it may be enzymatically epoxidized *in vivo* (**Fig. 41**). Due to interest, this nucleoside was prepared on greater scale so that it may be prepared for solid phase DNA synthesis.

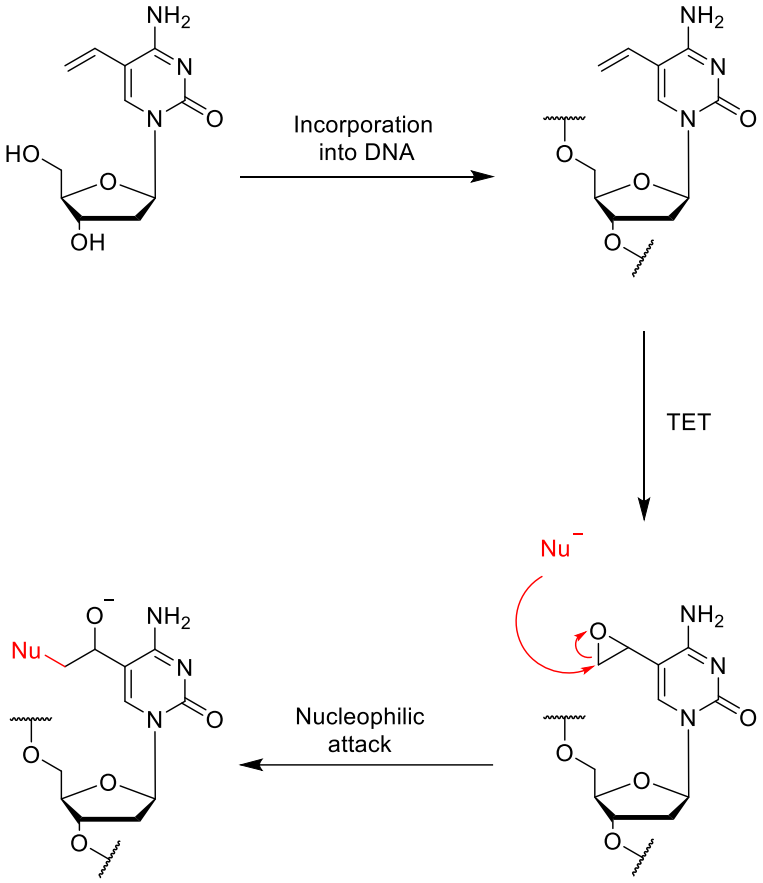


***Figure 41**: Hypothesised mechanism of action of TET enzymes on 5vdC (**55**). After oxidation to the epoxide, nucleophilic ring opening by amines on DNA bases may generate a lesion.*

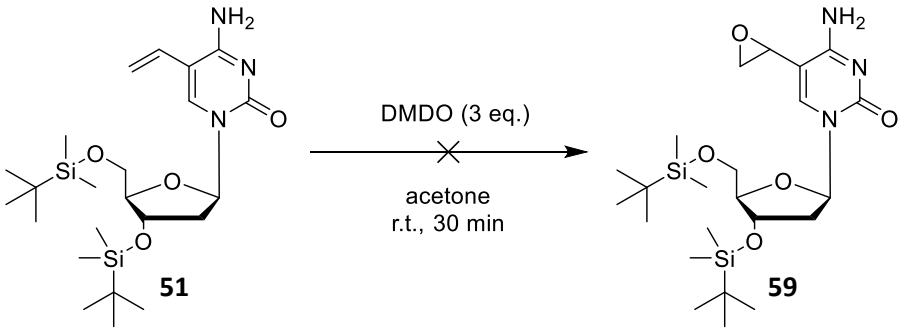
Additional information could be gathered from whether the amine at the N^4 position was nucleophilic enough to open the amine, as this may provide insight as to the potential lesions that may occur *in vivo* should the TET enzyme form the epoxide from the 5vdC nucleoside.

2.6) Epoxide synthesis

With successful preparation of the 5vdC nucleoside **55**, it was now possible to investigate the mechanism of defluorination further.

Initial attempts at preparing the epoxide used mCPBA and peractic acid, however the reactions did not proceed to completion with excess reagent. Purification proved unsuccessful and only small quantities of starting material was recovered. It is possible that any epoxide formed was opened on the silica column and lost. TLC analysis had demonstrated multiple new products formed, however ^{1}H NMR analysis of the crude reaction mixture was unable to identify the epoxide with a large variety of unknown products formed, possibly from crosslinking of compounds from the weak epoxide, or benzoate and acetate ions performing ring opening.

Analysis of a crude reaction mixture of the epoxide where the products can be identified would suffice rather than preparing a pure sample, however an alternative route without nucleophilic by-products from the epoxidation reaction would be required. Dimethyldioxirane (DMDO), also known as Murray's reagent was prepared[166], as epoxidation with this reagent produces acetone as a by-product rather than a nucleophile. The epoxidation reaction could be performed in acetone and as the only by-product is acetone any destruction of the epoxide product should occur from cross linking with itself.



***Scheme 19**: Failed oxidation of 5vdC with DMDO.*

An excess of DMDO was used (**Scheme 19**) and whilst full consumption of starting material was seen, it was not possible to identify the epoxide as the product by ^{1}H

NMR spectroscopy. Several products appeared to be formed, indicating oxidation and possible subsequent cross linking.

In the chemistry of 2-methylquinolines, a direct conversion of the methyl group to a vinyl group has been demonstrated[167], through a minor resonance state of an exocyclic double bond and a Mannich reaction (**Fig. 42**).

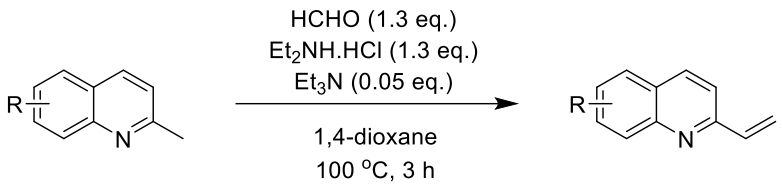
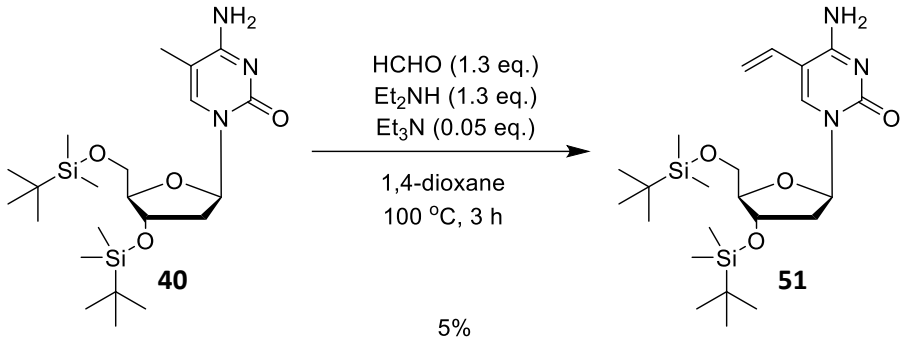


***Figure 42**: Direct vinylation of aromatic methyl group via Mannich reaction.*

As the exocyclic double bond is a hypothetical intermediate for two mechanisms of defluorination, if this reaction succeeds it would indicate a minor component of the exocyclic double bond resonance state in stabilising the compound. The protected 5mdC nucleoside **40** was subjected to the conditions for the vinylation procedure (**Scheme 20**).



***Scheme 20**: Direct vinylation of protected 5mdC. Conversion by ^{1}H NMR.*

The free base of diethylamine was used instead of the hydrochloride salt, but the desired product of protected 5vdC (**51**) was prepared in low yield. This result was pleasing as it does indicate a small component of the hypothesised exocyclic alkene resonance state contributing to the stability of this compound. The same reaction

was performed with TBDMS-thymidine and no vinylation was seen, as would be expected as this resonance state cannot exist for the thymine nucleobase.

This confirmed the hypothesis of the exocyclic double bond character but not by which mechanism it is formed, or which mechanism contributes the most to this form. It does not rule out an S_N2 mechanism for decomposition of the fluorinated nucleoside but does suggest a γ-elimination contributing component (**Fig. 43**).

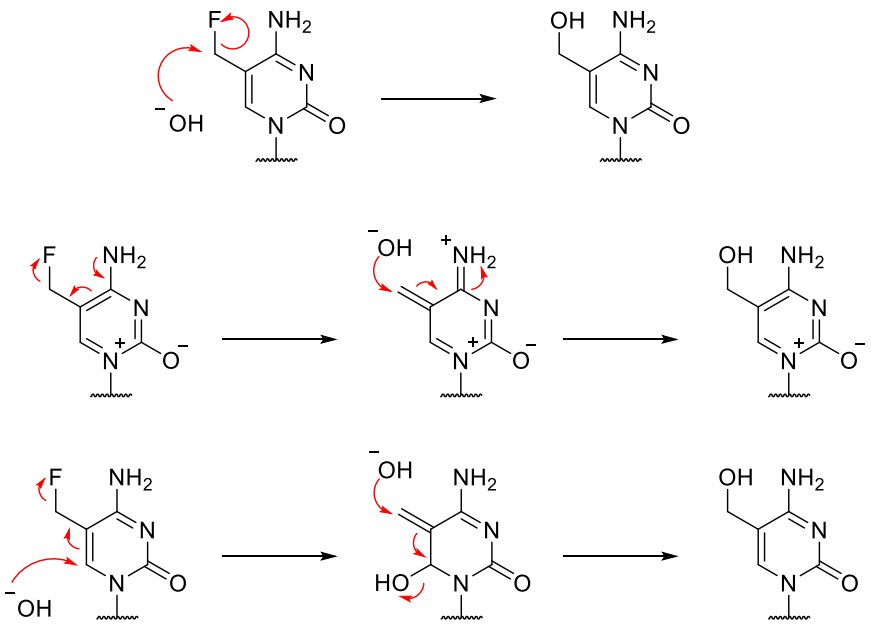
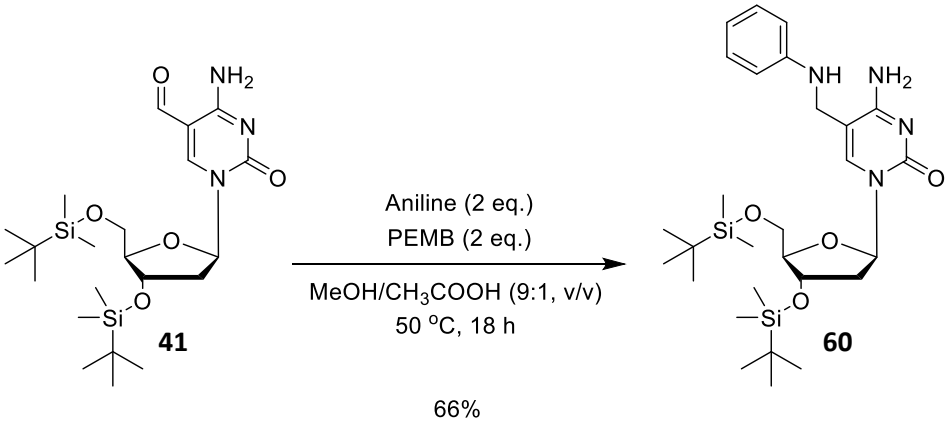


***Figure 43**: Hypothesised mechanisms for defluorination.*

2.7) Further analogue synthesis

It is hypothesised that 5-formylcytosine and 5-hydroxymethylcytosine may be involved in protein interactions *in vivo*, hence their unusually high abundance as opposed to merely being intermediates on the pathway of cytosine demethylation. A lysine adduct of 5-formylcytosine was detected both *in vivo* and *in vitro* as part of an experiment to discover further function of these oxidised cytosine bases[168].

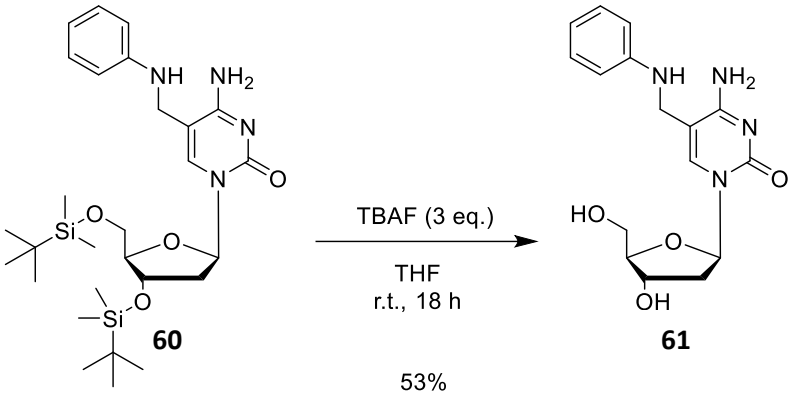
With imine formation determined as possible, preparation of amine substituted compounds *via* reductive amination was considered. Aniline was trialled as a test substrate to function as an example of a bulky amine (**Scheme 21**).



***Scheme 21**: Reductive amination of protected 5fdC with aniline.*

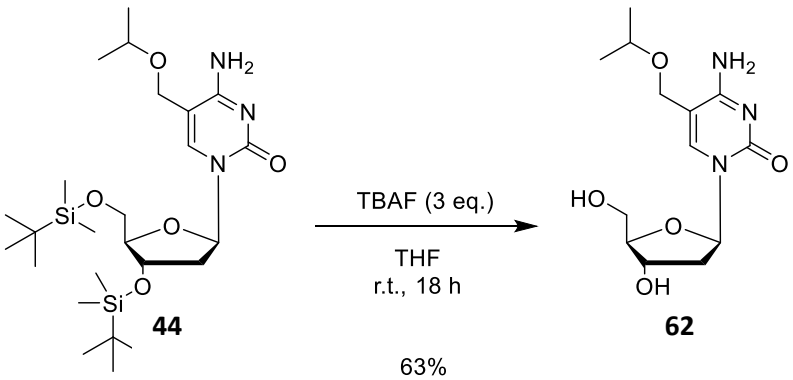
Reductive amination proceeded in good yield and limited 1,4-reduction was seen using the 5-ethyl-2-methylpyridine borane complex (PEMB) reagent[169]. This underused reagent is stable and effective in aqueous conditions and lower order alcohols unlike sodium triacetoxyborohydride (STAB) and avoids the toxic by-products generated by cyanoborohydrides.

Deprotection of **60** proceeded *via* methodology developed in this project, using TBAF as the fluoride source and Dowex ion exchange resin for purification (**Scheme 22**).



***Scheme 22**: Deprotection reaction of aniline analogue.*

The yield of the deprotection step was good and purification facile, providing an additional compound for cellular testing with an unusual and bulky substituent. Additionally, the isopropoxy substituted compound **44** that was recovered during preparation of the monofluorinated nucleoside was deprotected to prepare another analogue (**Scheme 23**).



Scheme 23**: Deprotection of isopropoxy compound **44.

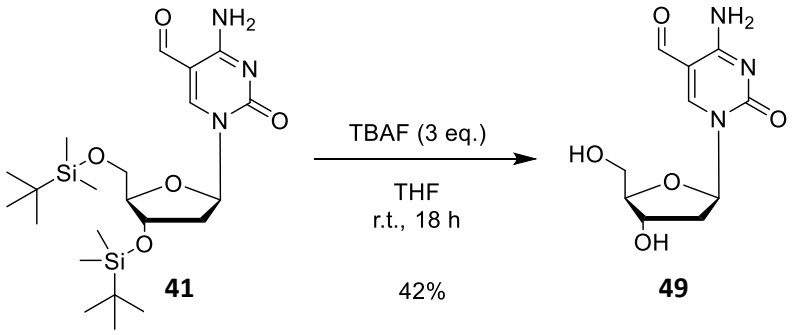
Deprotection proceeded smoothly generating the desired compound in good yield. Whilst the preparation of this compound was accidental, as it was unusual and only small quantities are required for cytotoxicity assays it was taken forward to gain insight into the effects of bulkier substituents.

2.8) Control compound synthesis

For an effective assay, control compounds are required to validate the results. The hypothesised products of enzymatic metabolism of tested compounds were required as controls, alongside the possible decomposition products. The naturally occurring base 5fdC was required and also the further oxidised product 5cadC.

5-Formyl-2'-deoxycytidine

In synthetic routes developed from commercially available thymidine, the silylated form of 5fdC had already been prepared on multigram scale as an intermediate towards other highly desired compounds. Deprotection was performed according to the developed procedure and proceeded in good yield (**Scheme 24**).

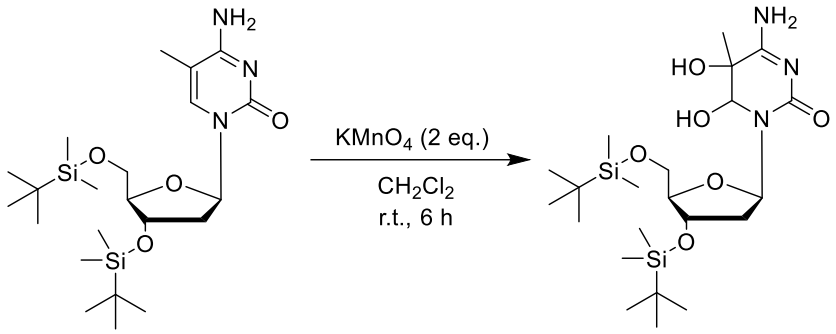


***Scheme 24**: Silyl cleavage to prepare 5fdC (**49**) for biological experiments.*

5-Carboxyl-2'-deoxycytidine and ester synthesis

A synthetic route was planned involving oxidation of 5fdC to 5cadC followed by deprotection. Due to concerns over cellular permeability with the free carboxylic acid, the methyl ester of 5cadC was designed to bypass this.

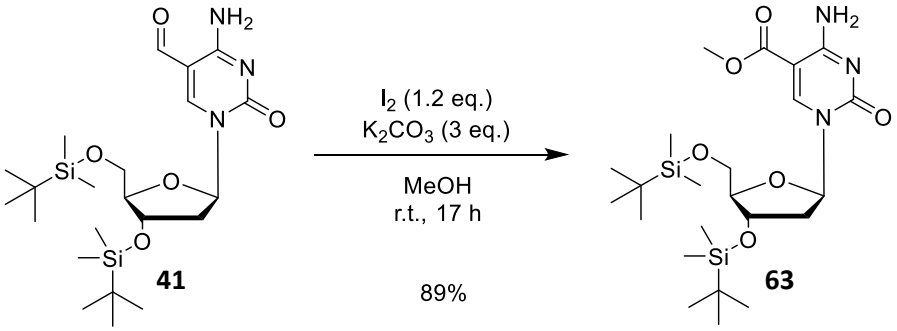
Initial reactions sought to extend the previously successful benzylic oxidation and continue oxidising to the carboxylic acid. Using $KMnO_4$ on 5mdC, interestingly no oxidation was seen at the benzylic position and the hydrate was identified as the major product (**Scheme 25**), and no starting material remained indicating the formation of heavily over-oxidised products.



***Scheme 25**: Failed oxidation to 5cadC and observed hydrate product.*

Alternative oxidation methods were trialled without success, on 5mdC and 5fdC. The Pinnick oxidation, further $KMnO_4$ treatment, hydrogen peroxide and oxone all failed to yield the desired product and starting material or unidentifiable oxidised compounds were recovered.

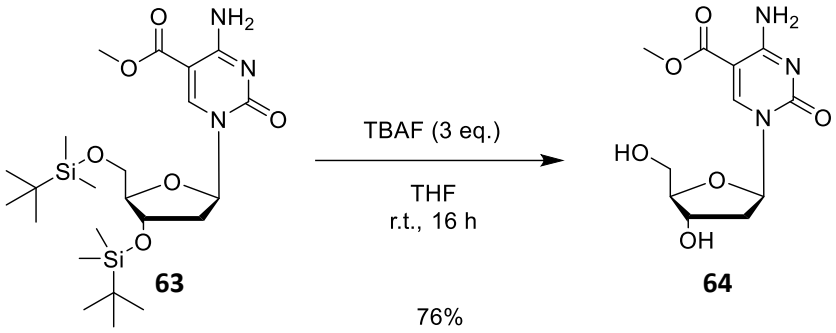
An alternative route was required and it was hypothesised that a reversal of steps may be successful, first performing oxidative esterification allowing a direct synthesis of the desired ester which could be deprotected to give the free acid[170].



***Scheme 26**: Oxidative esterification of 5fdC to generate the ester **63**.*

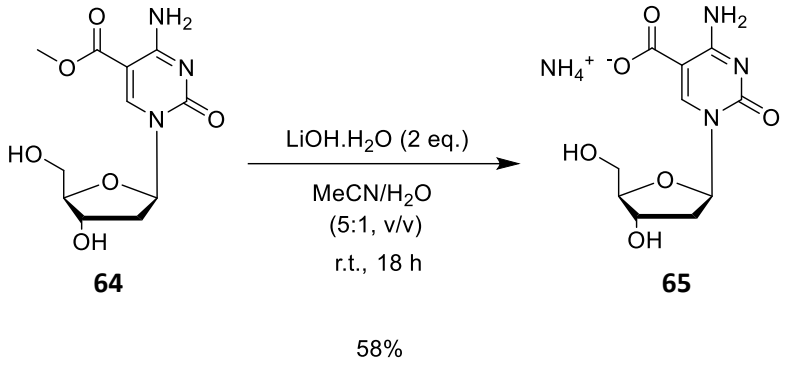
Modification of a literature procedure fashioned the methyl ester in good yield (**Scheme 26**), however attempts to prepare the ethyl and octyl esters *via* the same method failed. These compounds were desired as esterification was successful to such a degree that rapid preparation of analogues was considered possible, and longer alkyl chains would increase the lipophilicity and mayaid in cell permeability. Competitive condensation of the solvent with itself was favoured and in the case of 1-octanol as solvent, octyl octanoate was detected as a major product.

Desilylation of the ribose was performed using TBAF and established methods in this project and proceeded in good yield (**Scheme 27**).



***Scheme 27**: Removal of silyl protecting groups by fluoride to yield the free ribose.*

Hydrolysis of the ester was performed according to a literature procedure[171], and purification *via* the developed ion exchange method proved successful and provided the target compound as its ammonium salt in moderate yield (**Scheme 28**).



***Scheme 28**: Synthesis of 5cadC from the methyl ester via basic hydrolysis.*

A reversal of steps was trialled performing ester hydrolysis prior to silyl cleavage, however purification issues were encountered that were avoided by leaving ester hydrolysis until the last step. Once the acid is free, purification without the use of preparative reverse phase HPLC becomes difficult.

Current routes to prepare 5cadC use 2'-deoxycytidine as a starting material (£4.66/mmol, Alfa Aesar), with a yield of 14% over five steps using palladium chemistry, CO and HF.pyridine[171]. The route developed in this project generated a 24% yield over six steps from cheaper thymidine (£1.31/mmol, Alfa Aesar), utilising cheaper, safer reagents.

2.9) Cellular testing

2.9.1) Cytotoxicity assays – HeLa cells

With a range of 2'-deoxycytidine nucleoside analogues synthesised, cytotoxicity assays were performed to assess their effects on cells (**Fig. 44**). Some nucleosides (**B5**, **B6**) exist *in vivo* and were prepared to act as control compounds and validate results.

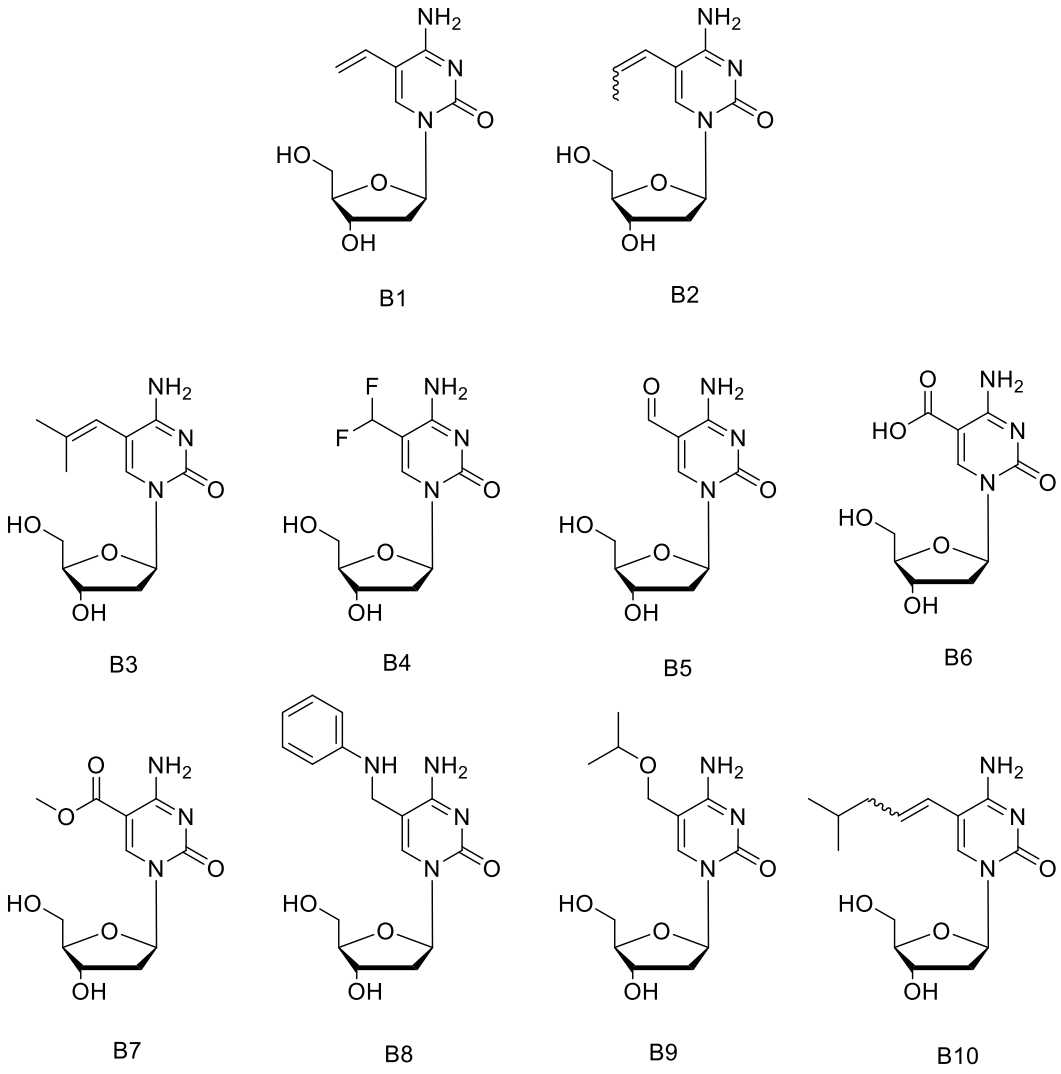


***Figure 44**: Nucleosides prepared and tested on cytotoxicity assay. Compounds numbered according to biological experiments, independent of previous numbering system. Compounds **B2** and **B10** a mixture of E/Z isomers (**B2**: 1:4, **B10**: 1:2).*

Two additional compounds were purchased, with the commercial anti-cancer drug decitabine used as the positive control. Decitabine (**B12**) is a drug used to treat myelodysplastic syndromes and functions as a hypomethylating agent by irreversibly binding to DNA methyltransferases, leading to cell death upon replication[172]. The non-toxic 2'-deoxycytidine (**B11)** was used as a negative control (**Fig. 45**).

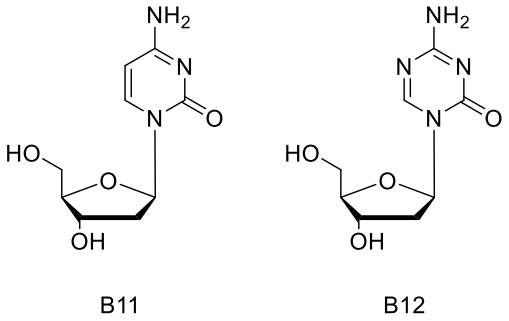


Figure 45*: Negative control 2'-deoxycytidine (left) and positive control decitabine (right).*

The assays were performed by Agata Mieżaniec. Stock solutions of 10 mM were prepared of 2'-deoxycytidine, decitabine and all prepared nucleosides, and dilution series performed to prepare sample concentrations of 100 µM, 10 µM, 1 µM and 0.1 µM. Cytotoxicity was measured by a colorimetric assay with a water soluble dye that changes its absorption spectra upon cellular reduction, where the absorption ratio change is directly proportional to the number of living cells. Cell viability is the proportion of live cells in a population, and was calculated for each compound at each concentration tested. Results over 100% may occur through random fluctuation or experimental error. A compound will be considered toxic if the viability is significantly reduced (< 60%) The experiments were performed according to biological procedure 1 (see Experimental section).

An initial experiment was performed with the vinyl nucleoside (5vdC, **B1**), the difluorinated nucleoside (**B4**) and 5fdC (**B5**) along with the two control compounds (**Fig. 46**). These were selected as the vinyl and difluorinated nucleosides were hypothesised to demonstrate interesting results, and the aldehyde as the hydrolysis product of the difluorinated nucleoside to determine whether any observed results were a result of the trialled compound or its hydrolysis product.

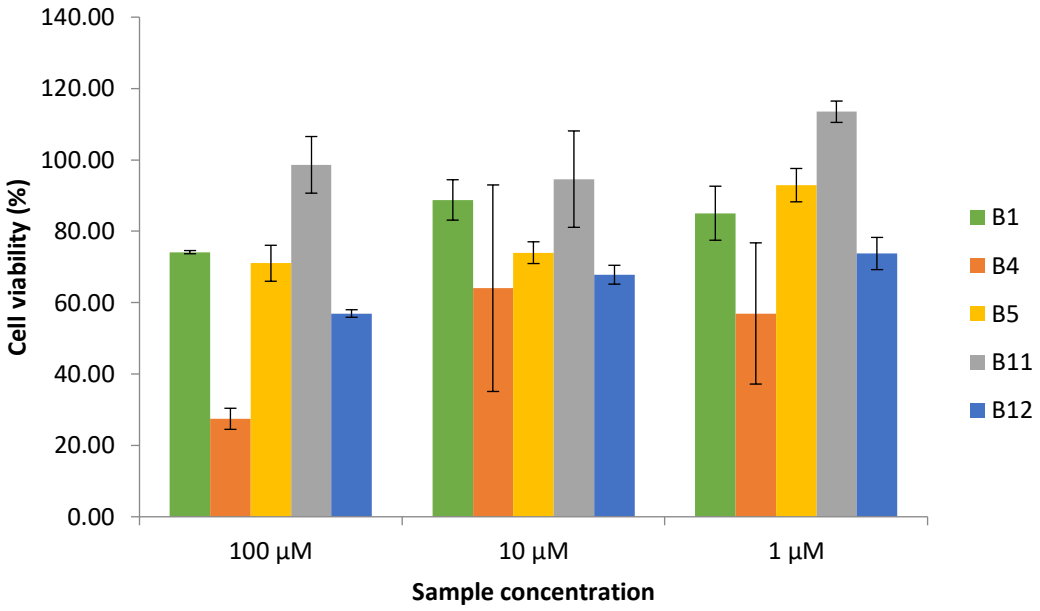


***Figure 46**: Cytotoxicity results for compounds **B1, B4** and **B5** with 2'-deoxycytidine and decitabine as controls, tested on HeLa cells over 72 hours.*

The most notable result was the high toxicity of the difluorinated nucleoside **B4** in comparison to the other trialled compounds. The naturally occurring 5fdC nucleoside **B5** had a greater cytotoxic effect than was anticipated, likely due to cross linking, however as this nucleoside occurs in such small quantities *in vivo* its observed cytotoxic effects are not expected to be reciprocated under normal conditions. There is a large difference between the two nucleosides, suggesting that the cytotoxic effect of the difluorinated compound is a result of the nucleoside itself rather than a hydrolysis product. Whilst some cytotoxic effects were seen from the vinyl nucleoside **B1**, they were not as pronounced as for the difluorinated nucleoside **B4** and are comparable to those of the naturally occurring 5fdC **B5**. The positive control of decitabine **B12** did not demonstrate a greatly cytotoxic effect, and as decitabine requires cell replication to be effective, longer incubation times may be required. Observation of the cells under microscope indicated that cell reproduction was halted in comparison to control cells for both **B1** and **B4**, and cells had jagged edges. These results suggest that the synthesised nucleosides may operate in a similar manner to decitabine by halting cell growth, but with a greater directly toxic effect to cells as evidenced by the lower cell viability for **B4**.

A second assay was performed to test the remaining compounds, using the same controls and cell preparation as the previous assay according to biological procedure 1 (**Fig. 47**). The experiment time was reduced from 72 hours to 48 hours to lessen the highly destructive effect of the difluorinated nucleoside and obtain clearer results.

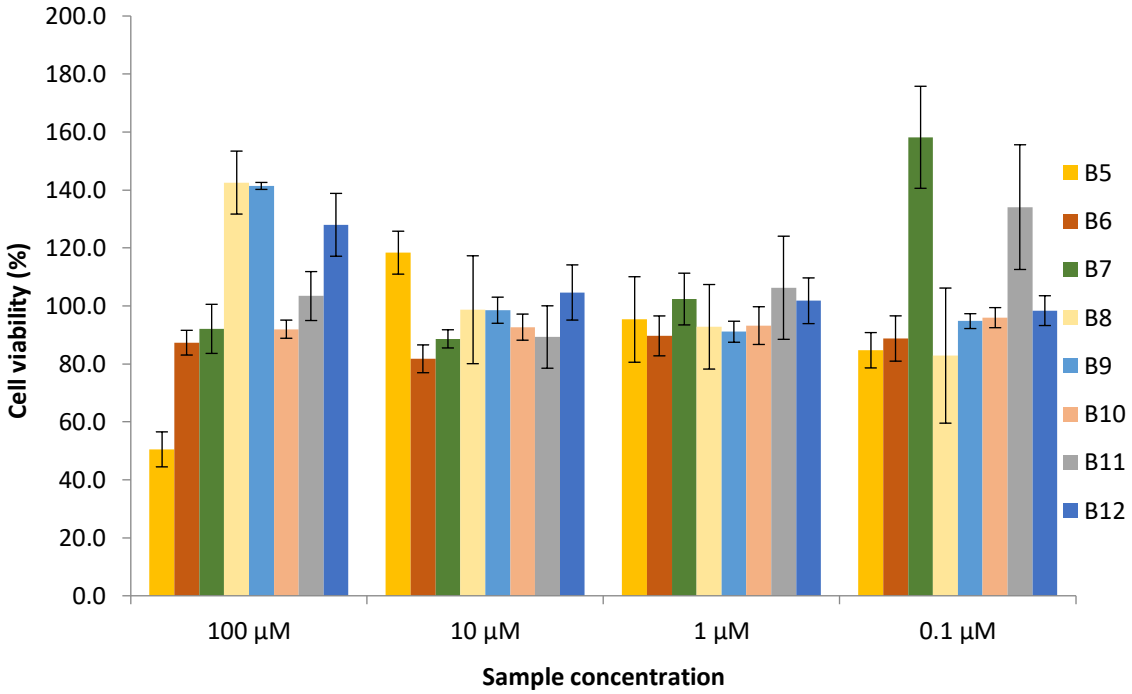


***Figure 47**: Cytotoxicity results for nucleosides **B5-B10** with 2'-deoxycytidine and decitabine as controls, tested on HeLa cells over 48 hours.*

Little effect was seen from any of the remaining compounds, with the retesting of naturally occurring nucleoside **B5** showing the largest cytotoxic effect, greater than any of the other naturally occurring or synthetic compounds. Several of these additional nucleosides are bulky (**B8**, aniline or **B9**, isopropoxy) and were not expected to fit into enzyme active sites at any stage in the process of DNA synthesis. It is not possible to say based off this data whether the nucleosides can enter the cells to begin any of the enzymatic reactions required to be incorporated into DNA. At low concentrations (1 µM and 0.1 µM) very little cytotoxic activity can be determined for any compound tested in this series. Error bars for this experiment are large and no high interest compounds are readily determinable.

A final experiment directly compared all previously tested nucleosides and control compounds on the same well plate (**Fig. 48**). As results at low concentrations had not shown any notable cytotoxic effects, only two concentrations of 100 µM and 10 µM were trialled. Experiment performed according to biological procedure 1.

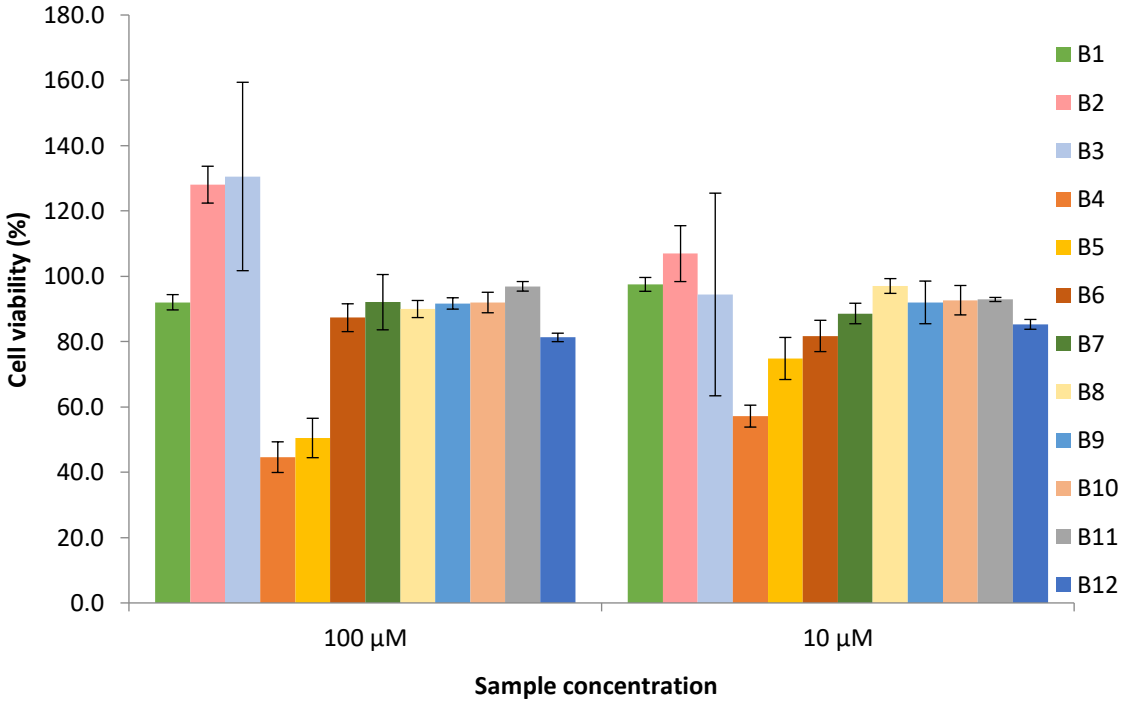


***Figure 48**: Cytotoxicity results for all prepared nucleosides with 2'-deoxycytidine and decitabine as controls, tested on HeLa cells over 48 hours.*

Of all compounds tested, only the difluorinated nucleoside **B4** and naturally occurring 5fdC **B5** were cytotoxic to any significant degree at the concentrations tested. Whilst many other nucleosides demonstrate some very minor cytotoxic results, effects were not substantial enough to warrant further investigation. The clear discrepancy between **B4** and **B5** that has been observed through numerous experiments does suggest an effect at least not entirely due to hydrolysis.

Significant cytotoxic effects were only seen at high concentration, and no determination of mechanism of action can be made from these results alone. Test compounds must be absorbed into cells and phosphorylated before they can be incorporated into DNA, and must be resistant to any degradation pathways for nucleic acids in cells. Once absorbed into the cells, all these steps require enzymes and there is no evidence that these enzymes can accept these compounds as substrates.

2.9.2) DMOG competition assay

The cytotoxicity assays performed do not demonstrate whether the nucleosides are interacting with TET enzymes as desired. It was hypothesised that a competition assay with a known 2OG enzyme inhibitor would be able to demonstrate whether the observed cytotoxic effects were due to the 2OG enzymes interacting with the substrates, or another component of the cell.

Dimethyloxalylglycine (DMOG) is a competitive 2OG inhibitor[173] and should prevent the binding of any introduced nucleosides to this family of enzymes, including TET. However, DMOG is cytotoxic itself due to the important functions and variety of 2OG enzymes found in cells. Should the difluorinated nucleoside be toxic to cells through 2OG inhibition then combination treatment with DMOG and nucleoside **B4** should exhibit a lesser cytotoxic effect in comparison to just the difluorinated nucleoside.

The assay was performed on HeLa cells, and a 5 mM solution of DMOG was prepared and a 10 µM solution of difluorinated nucleoside **B4**. Wells were prepared testing both DMOG and the difluorinated nucleoside separately and combined, using water as a control. Experiment performed according to biological procedure 1 (**Fig. 49**).

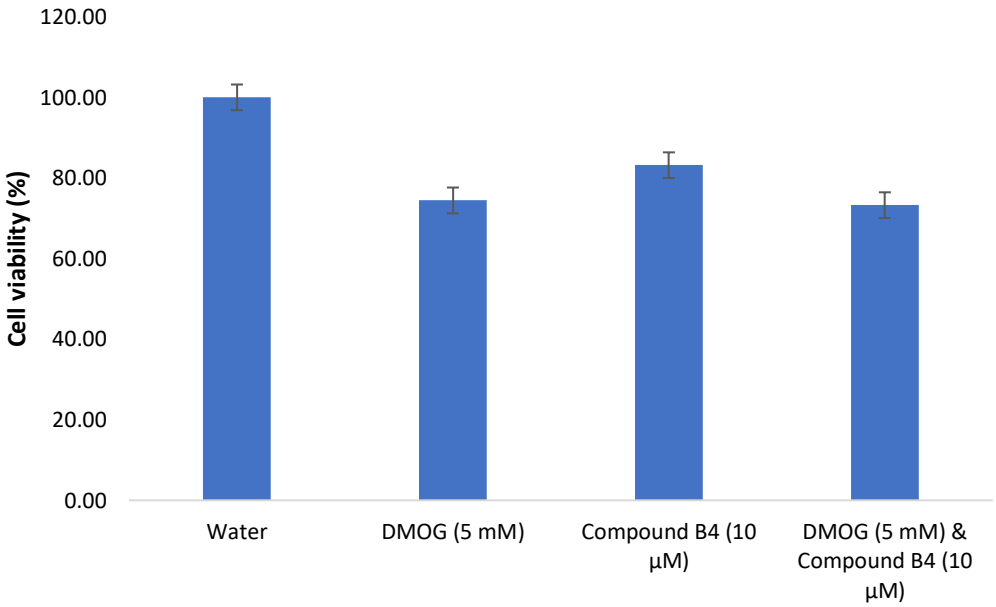


***Figure 49**: Results of the competition assay with N-oxalylglycine after 48 hours.*

Results were disappointing as at the concentration of the difluorinated nucleoside used in this experiment, it appears DMOG possesses a greater cytotoxicity. This is confirmed by combination treatment of both DMOG and the nucleoside demonstrating the same level of cytotoxicity as DMOG alone. This result cannot confirm whether the nucleoside is affecting 2OG enzymes of any sort, as the effect is overpowered by the effects of DMOG. Using a higher concentration of the difluorinated nucleoside (100 μM) where a far stronger cytotoxic effect is observed, may be able to determine whether 2OG activity is affected by this compound.

2.9.3) Alternative cell lines

Cytotoxicity assays have been performed on HeLa cells, and the nucleosides synthesised were designed to potentially interact with TET enzymes. As TET enzymes are involved with DNA demethylation and have been linked to various cancers, alternate cell lines including several cancer lines were tested to determine whether the cytotoxic effects were specific to HeLa or a more general effect. As the difluorinated nucleoside had shown large effects in comparison to all other nucleosides, only this compound was tested against the control compounds of 2'-deoxycytidine and decitabine.

Caco-2 is a cell line derived from epithelial colorectal adenocarcinoma and expresses enzymes such as peptidases, esterases and transporters for amino acids. The HT29 line is also derived from a colon adenocarcinoma and forms layers of undifferentiated cells which subsequently differentiate, simulating real colon tissues *in vitro*. HepG2 is a liver cancer cell line and produces many plasma proteins such as albumin and fibrinogen. Induced human mesenchymal stem cells (ihMSC) are pluripotent and can differentiate into bone, fat, cartilage and fibroblasts alongside their immunoregulatory functions and ability to secrete cytokines to promote tissue healing.

For all five cells lines, stock solutions of 10 mM were prepared of difluorinated nucleoside (**B4**), 2'-deoxycytidine (**B11**) and decitabine (**B12**), and dilution series performed to prepare sample concentrations of 100 µM, 10 µM and 1 µM. Experiments performed according to biological procedure 1.

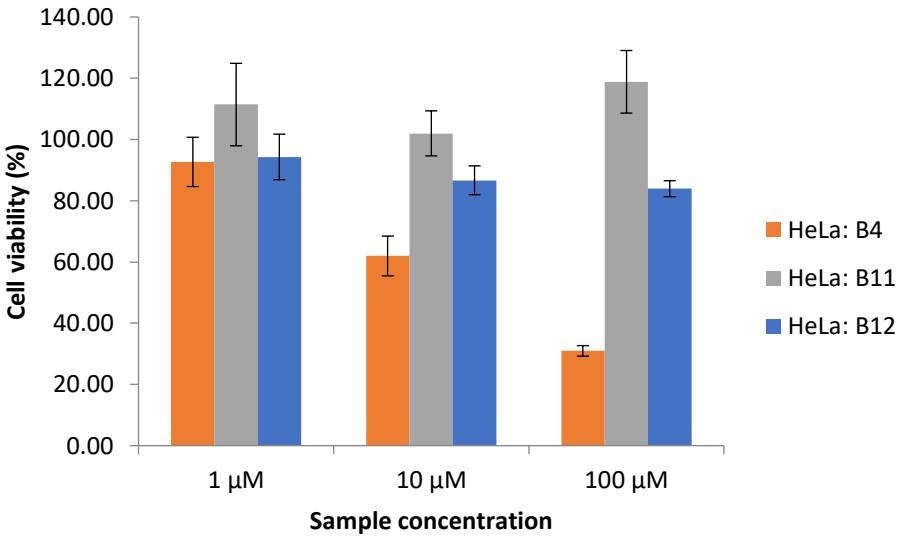


***Figure 50**: Cytotoxicity results using HeLa cells after 48 hours.*

Results for tests performed on HeLa cells were in line with previous experiments (**Fig. 50**), with significant cell death occurring at a concentration of 100 µM and some at 10 µM, but very little effect on the cells was seen at lower concentration.

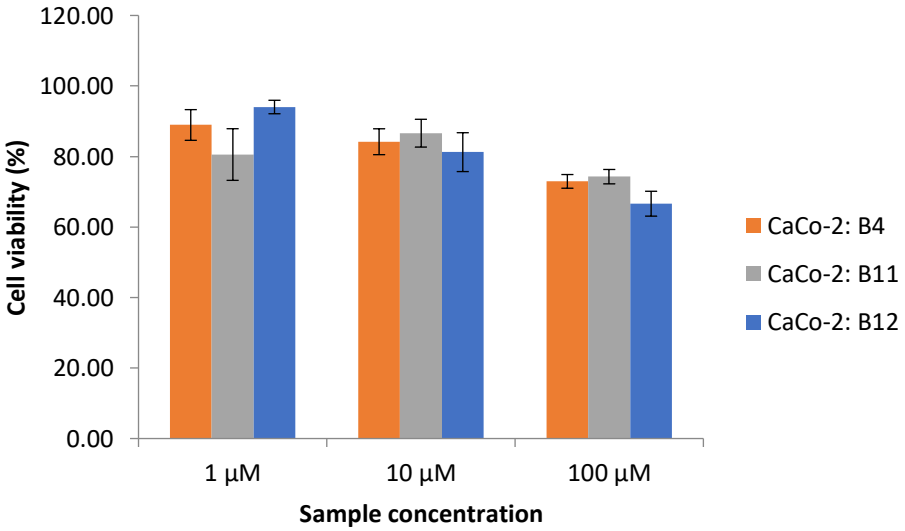


***Figure 51**: Cytotoxicity results using CaCo-2 cells after 48 hours.*

Different results were obtained from the CaCo-2 cell line (**Fig. 51**), with the difluorinated compound displaying no discernible difference from the control

compounds. A potential explanation comes from the nature of the cells, as CaCo-2 is commonly used as a monolayer to model the intestinal tract[174]. This layer is known to produce a physical and biochemical barrier, preventing passage of small molecules. Due to this, it is possible that the cells were not able to take up the difluorinated nucleoside.

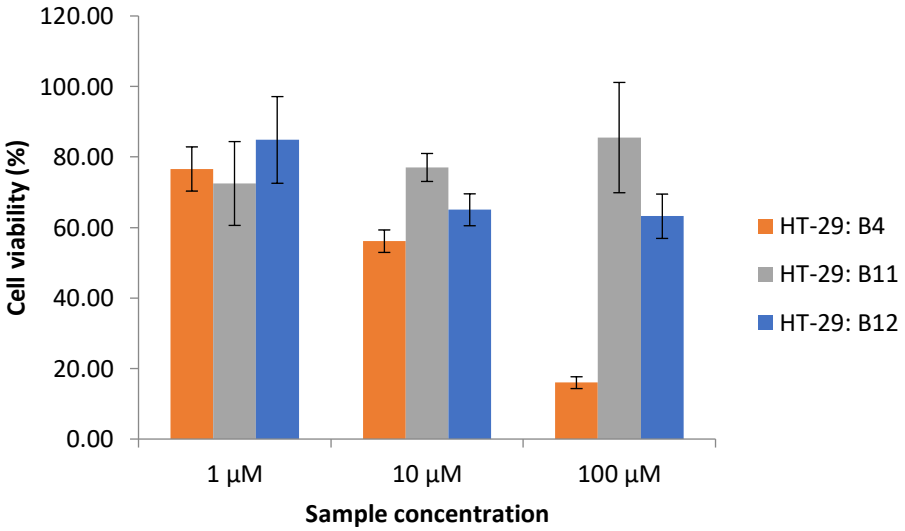


***Figure 52**: Cytotoxicity results using HT-29 cells after 48 hours.*

Contrary to the results from CaCo-2, tests on HT-29 cells demonstrated a greater cytotoxic effect at a concentration of 100 µM for the difluorinated nucleoside than against HeLa cells (**Fig. 50** & **Fig. 52**). Effects at lower concentrations were comparable to those observed for HeLa. In comparison to CaCo-2 cells, HT-29 also form a monolayer of epithelial cells and both resemble the enterocytes found in the human small intestine, but the cytotoxicity results were markedly different. It is possible that a degree of specificity has been demonstrated at these high concentrations, but is equally plausible that the nucleoside was able to pass any physical or biochemical barrier at high concentration for HT-29 but unable to do so for CaCo-2.

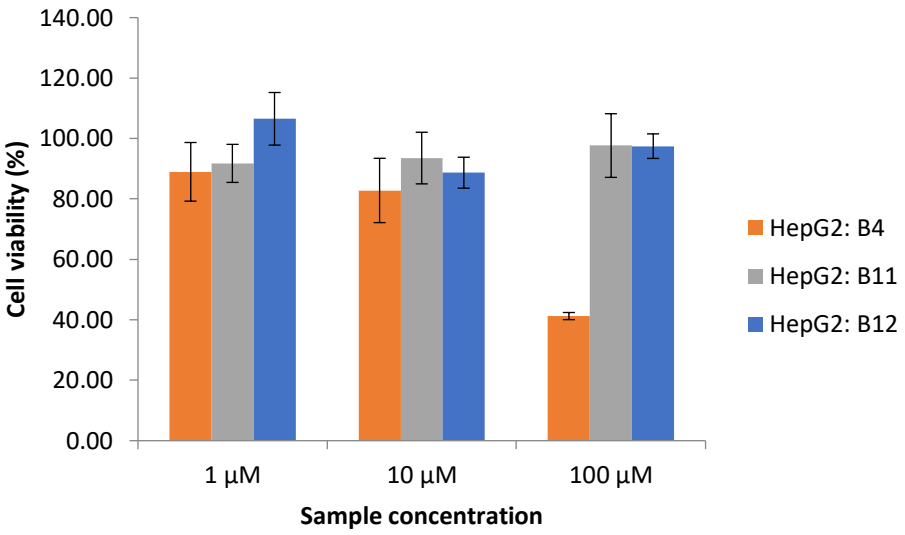


***Figure 53**: Cytotoxicity results using HepG2 cells after 48 hours.*

HepG2 demonstrated similar results again to HeLa cells, with significant cell death occurring at high concentration, but worse results at lower concentrations (**Fig. 50** & **Fig. 53**). A liver cancer cell line of epithelial morphology, these results are not particularly distinctive from the original results and do not present an interesting result in comparison to CaCo-2 and HT-29.

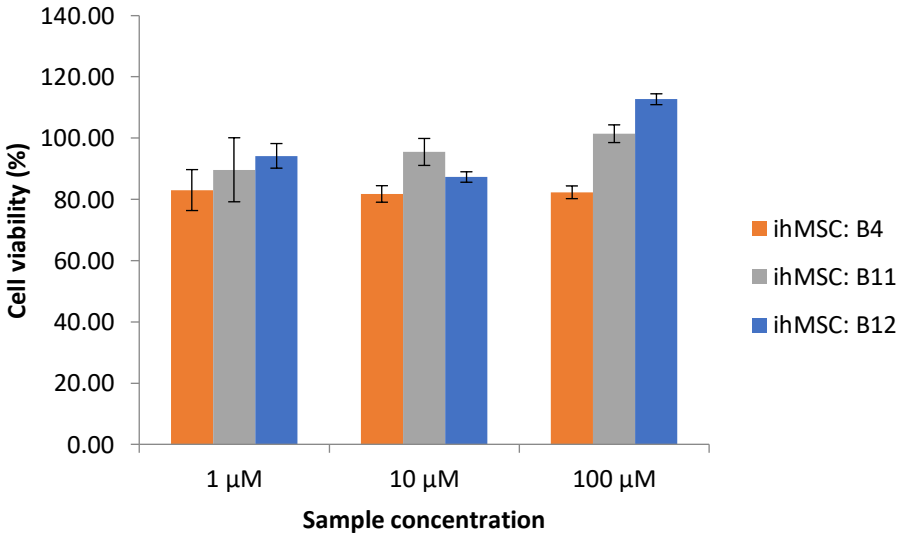


***Figure 54**: Cytotoxicity results using ihMSC cells after 48 hours.*

Results from tests on ihMSC cells were comparable to CaCo-2 where no effect was seen (**Fig. 51** & **Fig. 54**). These cells are markedly different from the others tested as these cells are stromal rather than epithelial. They have a high capacity for self renewal and whilst it is possible that any cell damage effected by the difluorinated

nucleoside may simply be repaired, it is equally plausible that the nucleoside was simply unable to enter the cells or was rendered inactive by some cellular process.

Results against different cell lines were largely disappointing, with effects only seen at high concentrations of the difluorinated nucleoside. Whilst it could be argued that some specificity was seen for HT-29 and HepG2 cells, the required concentrations were high and as the mechanism of action is currently unknown the effects cannot be directly and certainly attributed to the test compound.

2.10) Preparation for DNA synthesis

A nucleoside prepared for solid phase DNA synthesis usually possesses a protecting group on any amines (often benzoyl), a 4,4'-dimethyoxytrityl (DMTr) group for the 1° alcohol and a phosphoramidite to protect the 2° alcohol (**Fig. 55**), which will later form the phosphate backbone.

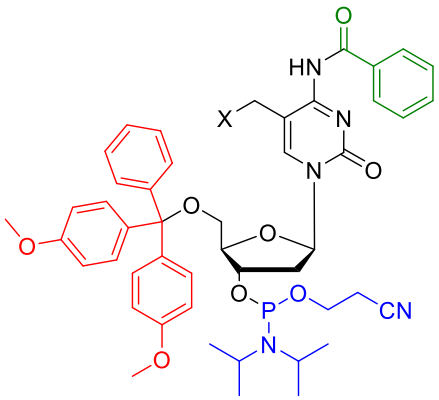
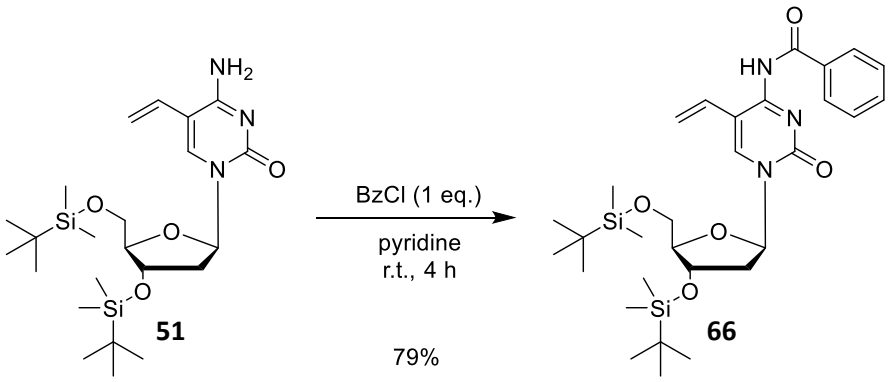


Figure 55: *General structure of a protected 2'-deoxycytidine nucleoside analogue prepared for solid phase DNA synthesis. X = substituent, Green = amine protecting group (benzoyl), Red = 4,4'-dimethoxytrityl, Blue = phosphoramidite.*

The 5vdC nucleoside was selected for phosphoramidite synthesis and incorporation into an oligonucleotide, as it was designed to possibly have an interesting interaction with TET enzymes and form an epoxide, leading to a lesion formed *in situ*.

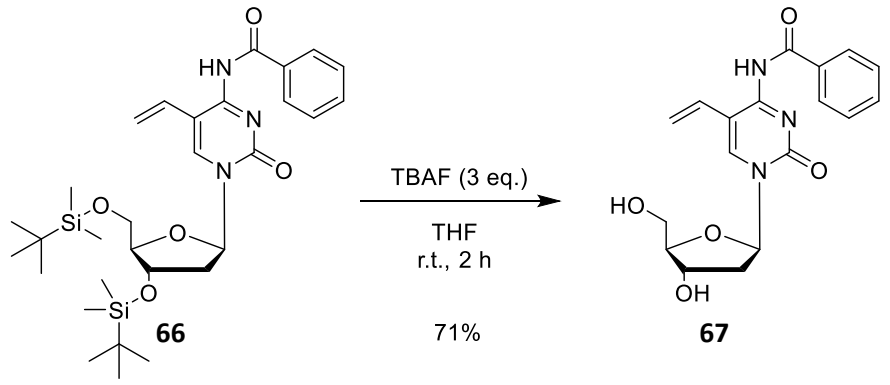
2.10.1) Benzoylation, deprotection and tritylation

Methods of solid phase DNA synthesis without protecting groups on amines are known[175] however most procedures continue to use one. Timing of incorporation of the protecting group is dependent on the impact on any further chemistry. Often performed prior to tritylation, it has been shown to be possible in either order[155]. Due to purification difficulties encountered after silyl cleavage and the potential for selectivity issues, benzoylation was performed as the first step.



Scheme 29: *Benzoyl protection of silyl 5vdC (**51**).*

Benzoylation proceeded in high yield (**Scheme 29**), with only a very minor quantity of dibenzoylated material recovered. The disubstituted compound proved difficult to separate chromatographically and was ultimately carried forward, and purification of this step eventually abandoned. Deprotection was performed by the previously established method with TBAF.

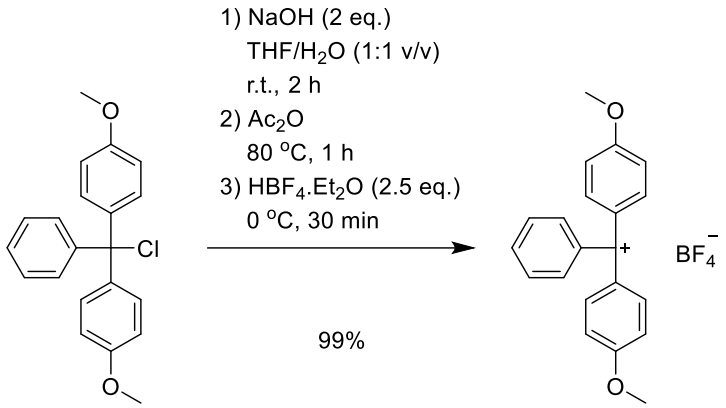


Scheme 30*: Deprotection of the silyl benzoyl 5vdC compound (**66**).*

Deprotection also proceeded in high yield (**Scheme 30**), and pleasingly it was noted that any dibenzoylated material carried forward from the previous step was hydrolysed to the amide under these conditions. Benzoylated nucleoside **67** is soluble in the organic phase, and any unreacted starting material from the previous step (**51**) after deprotection is aqueous soluble, allowing for a liquid-liquid phase separation purification.

A trityl group is capable of selectively protecting primary alcohols in the presence of secondary alcohols due to steric bulk. For this feature it is commonly used in nucleoside chemistry, and the 4,4'-dimethoxytrityl group is used over trityl due to increased ease of deprotection owing to the electronic effects of the methoxy groups.

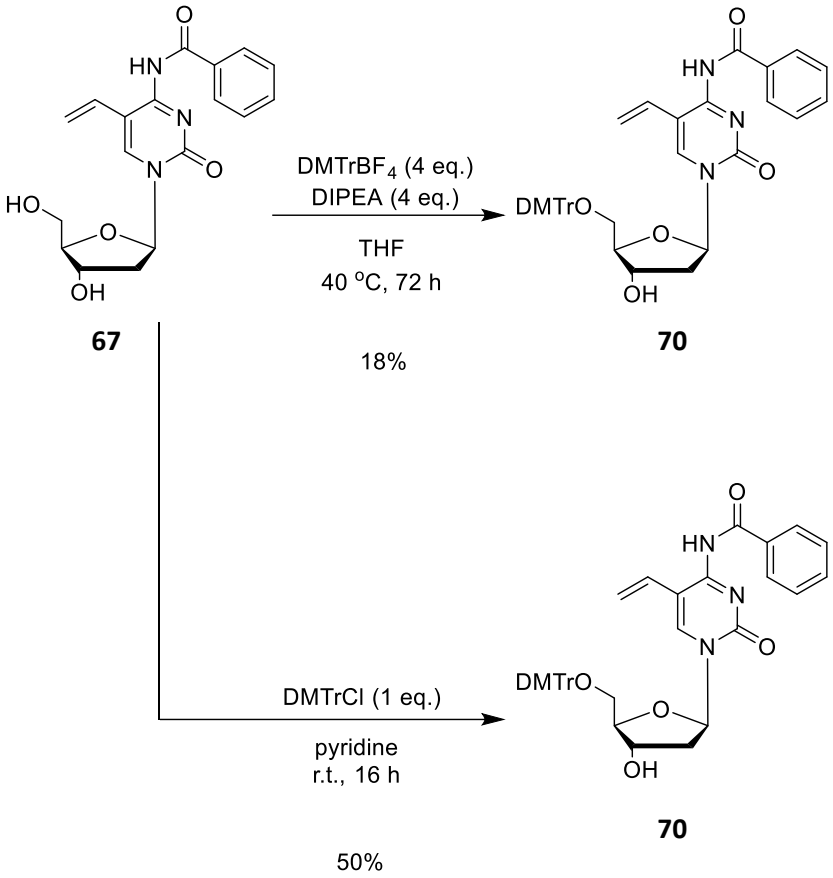
A common reagent for this reaction is 4,4'-dimethoxytrityl chloride (DMTrCl) but reactivity may be improved using a tetrafluoroborate counter ion ($DMTrBF_4$), converted by a literature procedure[176] (**Scheme 31**).



68 **69**

Scheme 31: *Preparation of $DMTrBF_4$ from DMTrCl.*

Yields for this reaction were excellent and the reaction was performed on multigram scale to prepare large quantities of material for subsequent tritylation reactions.

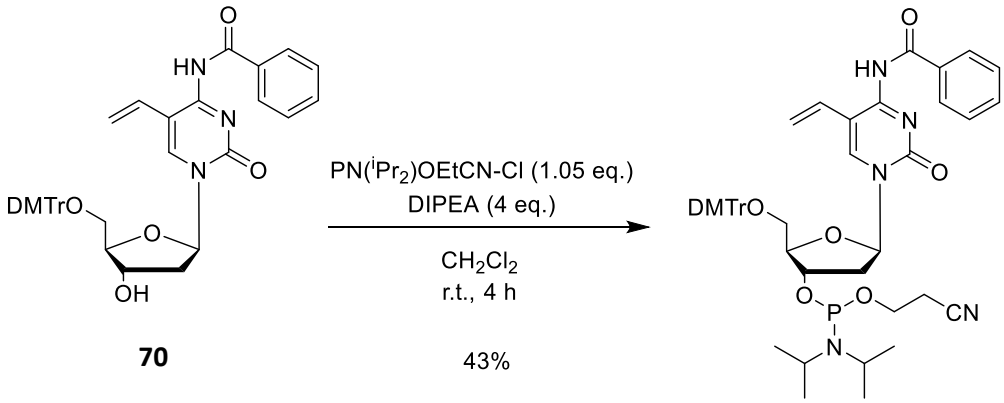


***Scheme 32**: Tritylation reactions of benzoyl 5vdC.*

Disappointingly, despite the increased reactivity of $DMTrBF_4$, tritylation proved difficult and only meagre yields of the desired compound were attained (**Scheme 32**), with large quantities of recovered starting material. Superstoichiometric quantities were required however no ditritylation was observed, but yields remained low, possibly due to solubility issues. The reverse was noted for DMTrCl in pyridine where additional equivalents lead to protection of the secondary alcohol. The prepared $DMTrBF_4$ was not found to be an improvement over DMTrCl, and whilst yields for this step were low, substitution at the C^5 position of nucleosides has been linked with reduced yields in tritylation reactions[177].

2.10.2) Phosphitylation

After protection of the primary alcohol the final step may proceed without concerns about regioselectivity with respect to the alcohol groups. Numerous literature procedures exist and there are several commercially available phosphoramidites with which to perform the reaction. The chloro-phosphoramidite is the most common and was selected due to its high reactivity.



70 **71**

Scheme 33: *Phosphoramidation step completing 5vdC for solid phase synthesis.*

The target compound was recovered in modest yield but high purity, and in sufficient quantity for use in solid phase DNA synthesis (**Scheme 33**). Whilst further synthesis to prepare an oligonucleotide was not undertaken, the yields obtained demonstrate that the route is suitable for the preparation of 5-methyl substituted 2'-deoxycytidine phosphoramidites for the preparation of oligonucleotides.

2.11) Conclusions and future work

Development of synthetic routes to prepare the monofluorinated and difluorinated nucleosides was successful. Whilst neither of the compounds proved suitable for their initially intended uses due to instability in basic conditions, further studies on both revealed interesting information such as susceptibility to nucleophiles and strong cytotoxic properties.

A range of 5-methyl-2'-deoxycytidine analogues were prepared, branching out from a common intermediate. Rapid preparation of a diverse array of compounds is an advantage of this method, and uses the cheap start material thymidine as an alternative to the expensive 5-methyl-2'-deoxycytidine.

Further synthesis was completed to prepare a phosphoramidite suitable for solid phase DNA synthesis of the 5-vinyl-2'-deoxycytidine nucleoside, and further work could be performed to fashion an oligonucleotide. Expression and purification of TET enzymes could be performed and *in vitro* studies conducted on the oligonucleotides to observe TET activity, whether the vinyl group is oxidised by the enzymes, and if this inactivates the enzyme as anticipated.

Whilst additional nucleosides could be prepared and undergo testing on the cytotoxicity assay, an investigation into their delivery needs to precede further experiments. Results from the assays did not demonstrate strong effects outside of the difluorinated nucleoside, but the reason for this is unknown. It cannot be determined currently whether the nucleosides were absorbed into cells or were then suitable substrates for enzymes *in vivo*.

Preparation of the triphosphates of interesting nucleosides may help elucidate this, and *in vitro* studies with purified enzymes may aid in determining whether these compounds are tolerated substrates. If used as substrates for the polymerase chain reaction, these compounds may be incorporated into oligonucleotides and it could be determined if they are suitable substrates for Taq polymerase, and potentially for DNA polymerase enzymes found *in vivo*.

Chapter 2

Perfluoroalkylation reactions of amines

3) Introduction

3.1) Origins of fluorinated chemicals

The fluorochemicals market was valued at US$21.4 billion in 2018 and is expected to grow to US$29.8 billion by 2026, largely due to increased demand from developing countries in Asia, Africa and South America[178]. Fluorspar (also known as fluorite) is the main source of fluorine (**Fig. 56**) and the industry of mining it is growing.

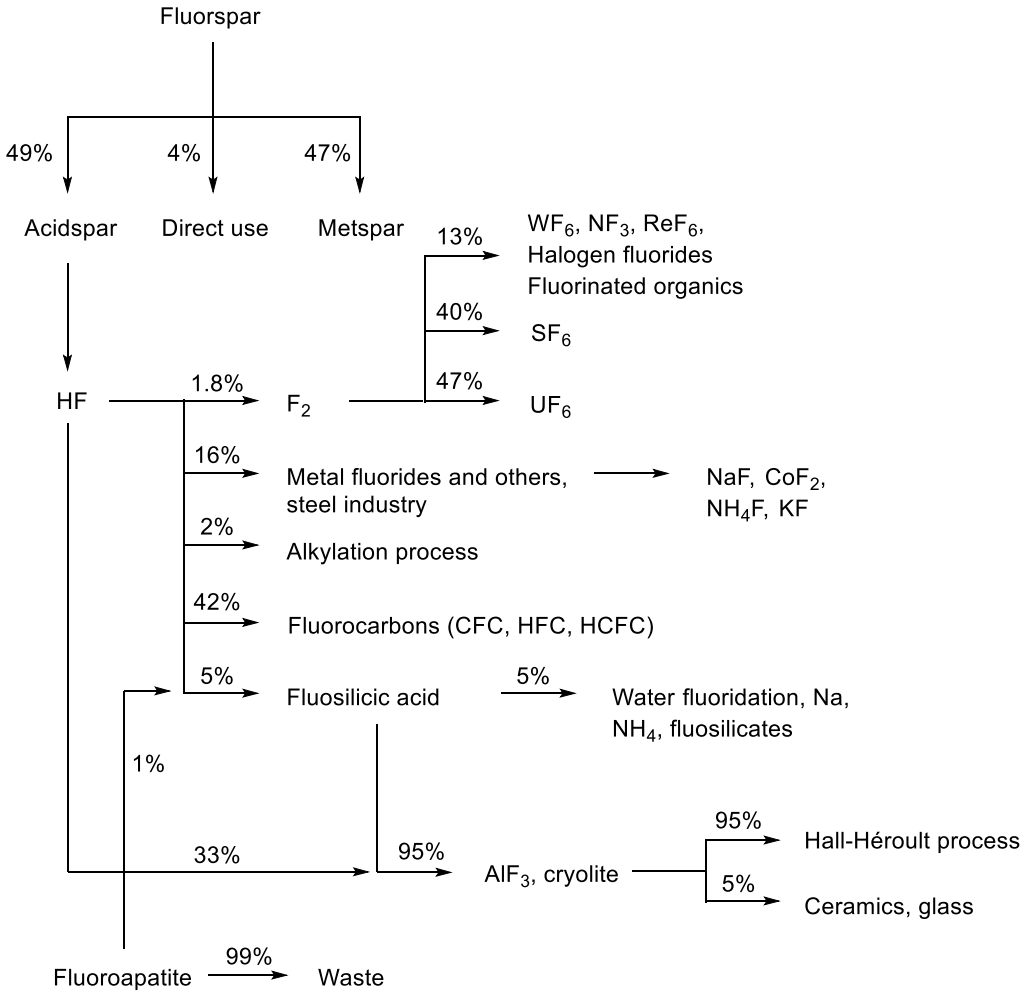


***Figure 56**: Fluorine industry supply chain from refinement of raw materials to commercial applications.*

Acidspar is a high quality mineral comprising approximately 97% CaF_2 and it is from this that fluorochemicals are primarily derived. Metspar is lower quality material and is commonly used for iron smelting[179,180]. This acidspar is treated with sulfuric acid to generate HF from which a wealth of compounds are derived, with a relatively small

amount going directly to fluorine gas which is largely consumed for uranium refinement and sulfur hexafluoride with wide industrial use[181].

Industrial production of HF is a critical process on which the majority of fluorination chemistry relies upon. Preparing such a toxic and corrosive material on scale relies upon stringent safety measures. Where possible it is not advised to use either HF or F_2 as fluorination reagents for both safety and selectivity reasons and is largely refined further before use, and a great number of fluorination reagents for general laboratory use have been developed.

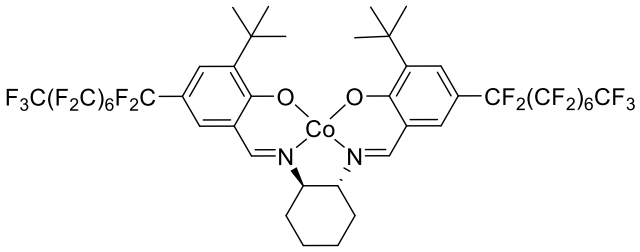
A large portion of prepared HF goes into preparation of fluorocarbon compounds which are used as refrigerants and for preparation of fluoropolymers such as Teflon™ or polyvinylidene fluoride (PVDF), and is key in preparation of aluminium fluoride and synthetic cryolite which are necessary for aluminium refinement.

3.2) Introduction to fluorous compounds

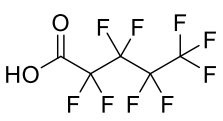
A fluorous compound is one where a significant quantity of C-F bonds are present. Heavily fluorinated compounds behave differently to their hydrocarbon counterparts and are not miscible with general organic solvents[182], and the presence of fluorine greatly affects the electronic properties of a compound which further changes their behaviour and properties.

Organofluorine compounds have extensive uses in pharmaceutical products, polymers and catalysts. Such materials are commonly found in laboratory, medical and domestic environments as surface coatings, due to the physicochemical properties imparted by the fluorine atoms, such as low reactivity and difficulty for bacteria to adhere to the surface[183].

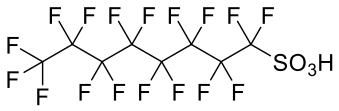
All the compounds shown in **Fig. 57** are considered fluorous as they predominantly contain C-F bonds. Most are also considered perfluorous as all C-H bonds are replaced with C-F, and some may consider even F-Co-Salen (**72**) to be perfluorous as it contains perfluorinated sections. Heteroatoms such as sulfur and oxygen may also be present and the fluorine may alter their behaviour.



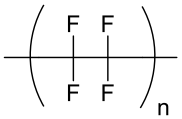
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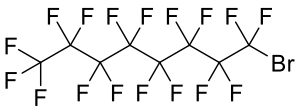
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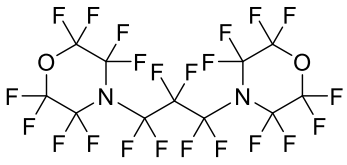
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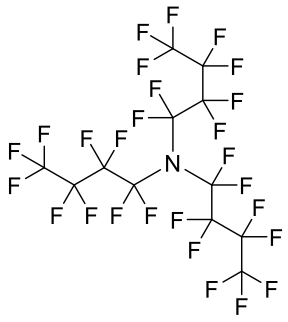
Figure 57*: Examples of commercially available fluorous compounds.*

3.2.1) Uses of fluorous compounds

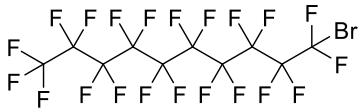
Polytetrafluoroethylene or Teflon™ (**75**) is an example of a fluoropolymer and is widely found in daily life due to its inert nature. Perfluorous acids such as perfluoropentanoic acid (**73**) and perfluorooctanesulfonic acid (**74**) exhibit surfactant behaviour due to their polar hydrophilic carboxylate and sulfonate sections but the alkyl chain is strongly hydrophobic, significantly more so than the hydrocarbon variants. The surfactants function in a superior manner to the hydrocarbon forms but pose significant environmental hazards with possible links to disease and strong bioaccumulative tendencies[184–186].

Of note are perflubron (**76**) and perfluorodimorpholinopropane (**77**) (**Fig. 58**). Heavily fluorinated compounds are capable of dissolving large quantities of oxygen and have been tested as components of artificial blood or as breathable liquids[187–190]. Research is ongoing in these fields primarily towards perfluorous amines for these interesting properties with medical uses, however environmental concerns and difficult

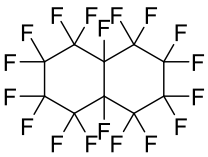
preparation have slowed efforts. Recent efforts to advance this field also explore these compounds as short term oxygen therapies rather than complete blood substitutes, for triage medicine and military purposes[191–193].



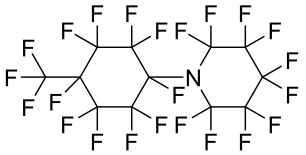
Perfluorotributylamine



Perfluorodecylbromide



Perfluorodecalin



Perfluorocyclohexylpiperidine

***Figure 58**: Fluorous components of artificial blood and oxygen therapies.*

Heavily fluorinated aliphatic compounds separate from hydrocarbons due to fluorine having the lowest polarizability of any atom, resulting in weak intermolecular forces granting simultaneous hydrophobicity and lipophobicity[194]. These perfluorinated compounds are capable of dissolving other fluorinated compounds in a separate phase from the organic and aqueous, known as the “fluorous” phase. This additional phase can become miscible with an organic phase upon heating before separating on cooling, allowing for a perfluorinated catalyst to be easily recovered from a reaction mixture[182] (**Fig. 59**).



***Figure 59**: General depiction of fluorous phase synthesis, permitting facile separation.*

This fluorous synthesis method can be exploited for facile purification of reaction mixtures. Should either by-products or reagents contain a perfluorous moiety then extraction of the desired compound(s) from by-products is readily achieved with a fluorous solid phase functioning offering a binary separation[195].

Protecting groups with perfluorous tags such as F-PMB-OH or F-Cbz-OSu (**Fig. 60**) for alcohol and amine protection are examples of such compounds. Protection and deprotection can be performed with traditional methods and do not require fluorous solvents. Utilising a fluorous solid phase such as FluoroFlash® silica gel, the desired fluorinated compound is readily separated from any by-products[195,196].

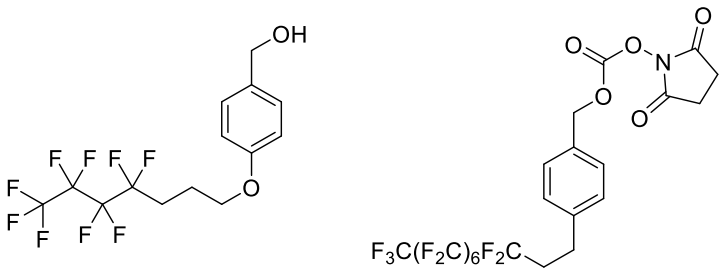


***Figure 60**: Fluorous protecting groups F-PMB-OH (left) and F-Cbz-OSu (right).*

This strategy can be reversed and applies fluorous tags to reagents, aiding removal of difficult impurities. Examples include fluorous tagged phosphines and azodicarboxylates (**Fig. 61**) for the Mitsunobu reaction, permitting a fluorous phase separation to wash out any undesired fluorinated compounds[197].

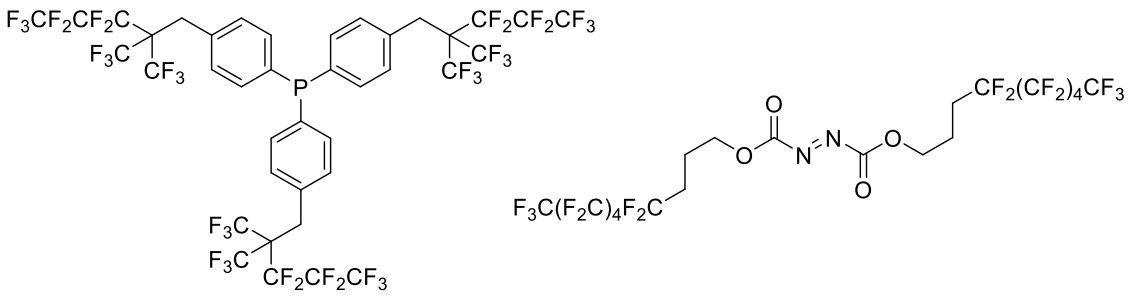
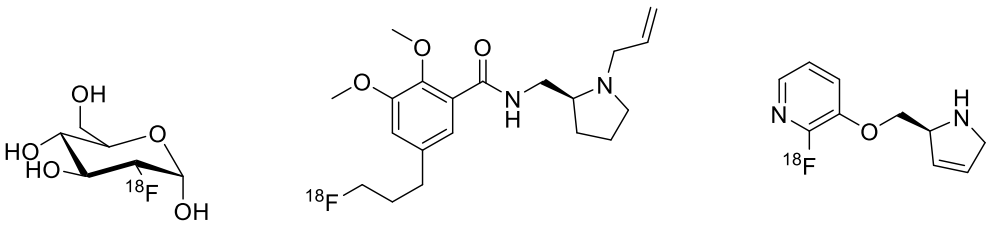


***Figure 61**: Fluorous tagged reagents for the Mitsunobu reaction. F-triphenylphosphine (left) and F-DIAD (right).*

This approach can be extended yet further to organometallic reagents which may be difficult to remove from reaction mixtures. Fluorous tagged tin hydride compounds or silanes are commercially available which can be readily converted to other species *in situ* before use and subsequent simplified purification.[197].

Organofluorine compounds are widespread in medicine as diagnostic tools or as drugs. Positron emission tomography (PET) measures gamma rays emitted by a positron emitting tracer, of which ^{18}F is a source. Fluorodeoxyglucose (**78**) is widely used in oncology[198] as it is taken up by glucose-using cells but cannot be metabolised, causing a concentration increase and the β^+ decay can be detected to map tissues. Other commonly used tracers are fallypride (**79**) for dopamine receptors[199], and nifine (**80**) for nicotinic acetylcholine receptors[200] (**Fig. 62**). The half-life of ^{18}F is 109.771 minutes before decomposing to ^{18}O at which point these compounds can be metabolised and excreted, preventing a harmful long-term bioaccumulation.



78 **79** **80**

***Figure 62**: ^{18}F labelled compounds for use in PET scans. Fluorodeoxyglucose (**78**), fallypride (**79**) and nifine (**80**).*

Additional interactions between a compound and a drug target can occur through selective positioning of fluorine. These interactions lead to a stronger binding and can generate a greater potency. This effect is compounded by the greater hydrophobicity in comparison to a C-H bond, as greater lipid solubility is a successful method of increasing potency.

Drug metabolism may be circumvented by addition of fluorine, by blocking pathways or reducing the efficacy of others. Aromatic rings or benzylic carbons are commonly hydroxylated during metabolism, and prevention of bioactivation can extend the half-life of compounds *in vivo*. Aromatic ethers are commonly found in pharmaceuticals, and replacement with fluoroethers can prevent dealkylation (**Fig. 63**) but also due to alteration of electronic properties of the ring system, slow down oxidation at other sites.

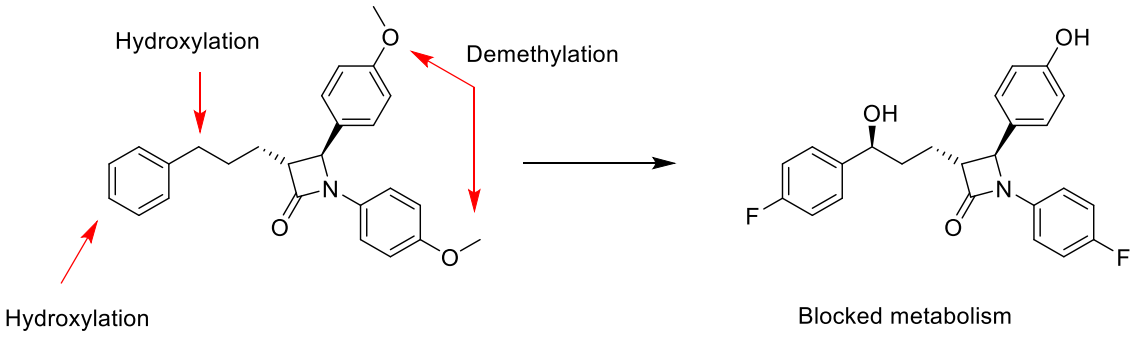


***Figure 63**: Targeted fluorination to prevent metabolic pathways. Potential metabolic sites highlighted (left) and final compound ezetimibe (right) with blocked metabolism due to fluorine incorporation.*

Due to these properties, C-F bonds have become a staple in pharmaceutical products, with over 20% of commercial drugs containing fluorine atoms. Present in treatments ranging from antidepressants to cancer therapies to statins (**Fig. 64**), fluorine has become an integral component of the medicinal chemist's toolkit[201].

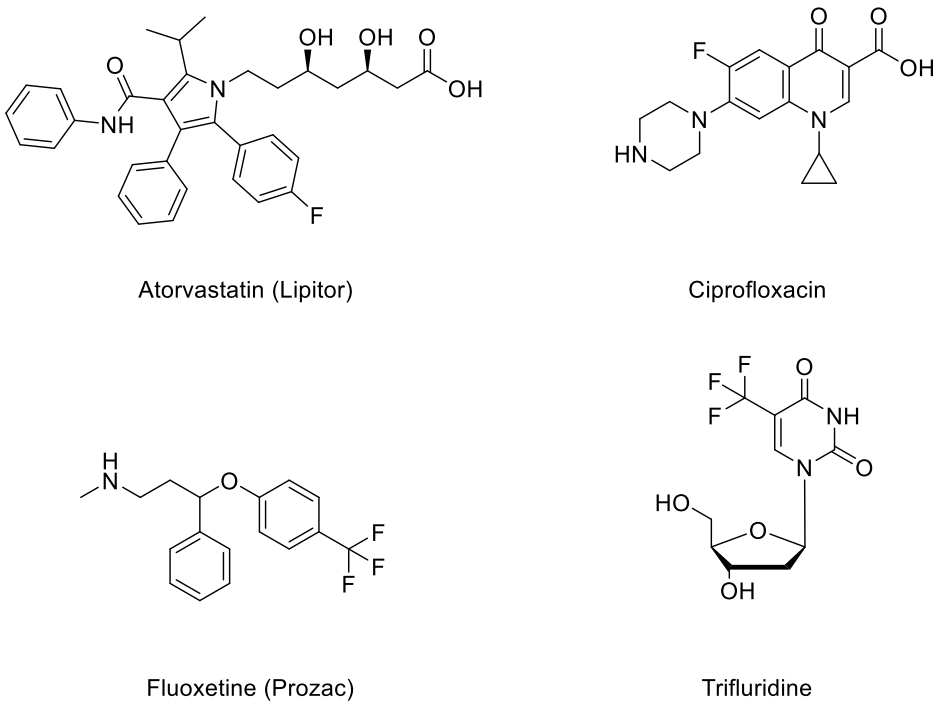


***Figure 64**: Representative selection of commercial drugs containing C-F bonds.*

Fluorinated moieties have shown promise for use as bioisosteres in medicinal chemistry. The concept of a bioisostere is a substituent with similar physical or chemical properties that exhibits similar biological properties to another[202,203]. Examples of bioisosteres are esters and amides, alcohols and amines, or carboxylic acids and tetrazoles[204]. Amide bonds can be hydrolysed *in vivo* and greater stability can be achieved through other functional groups. Replacement of the carbonyl with a trifluoroethyl group maintains the hydrogen bond donor character of the amide, and renders the amine non-basic[205,206]. Other replacements for the carbonyl component of an amide will either change the geometry or generate a basic amine (**Fig. 65**).

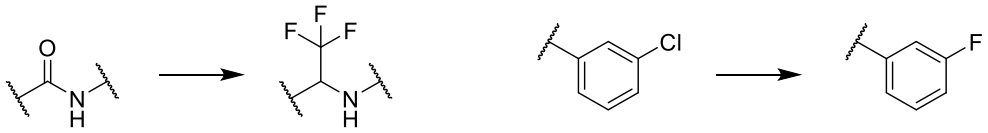


***Figure 65**: Fluorine containing bioisosteres. Trifluoroethyl amine (left) and aryl fluoride (right).*

3.2.2) Physicochemical properties of fluorinated compounds

Fluorine has the highest electronegativity of any element and the C-F bond has a high dipole moment, significantly impacting the electronics, acidity and basicity of a system.

The C-H and C-F bonds are similar in all aspects other than polarity which may be exploited when designing compounds. The C-F bond is marginally longer, the bond strength is higher and the van der Waals radius is slightly larger. This concentrated polarity opposite to a C-H bond makes it attractive and can be used to probe interactions. Despite being a highly polar bond, incorporation of fluorine into a compound increases the cLogP (calculated distribution coefficient between octanol and water) giving fluorine the unique capability of introducing "polar hydrophobicity".

	C-H	C-F
Bond length (Å)	1.09	1.35
Bond strength (kcal.mol^{-1})	98.8	105.4
Atomic radius (pm)	120	147
Carbon polarity	negative	positive

***Table 3**: Comparison of average C-H and C-F bonds[207].*

Fluorination can also lead to alterations in pK_aH values due to its strongly electron withdrawing nature. These effects can be calculated and follow a logical trend with additional fluorines reducing the pK_aH further (approx. 1.6 per F) and proximity to the functional group increasing pK_aH as the chain length increases[208].

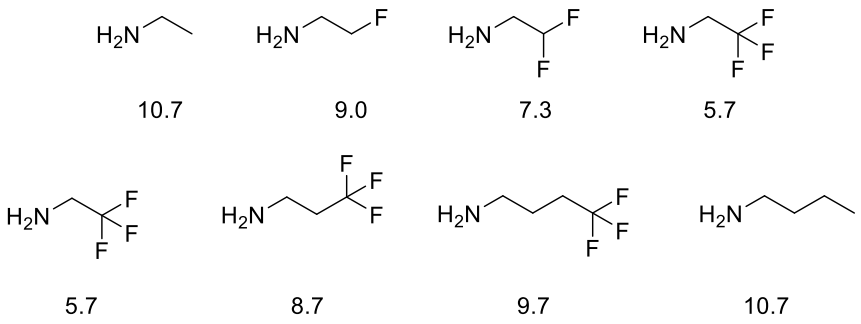


***Figure 66**: pK_aH effects of fluorine on alkyl amines in water.*

Not limited to amines and basicity, the acidity of adjacent functional groups can also be influenced as demonstrated by the comparison of acetic acid and trifluoroacetic acid, and the second ionisation comparing an alkylphosphate and fluoroalkylphosphonates. Incorporation of fluorine also leads to conformational effects due to the high electronegativity – bond angles are impacted, and with an increasing quantity of fluorine a geometry closer to that of phosphate is adopted by a difluorophosphonate[207] (**Fig. 67**).

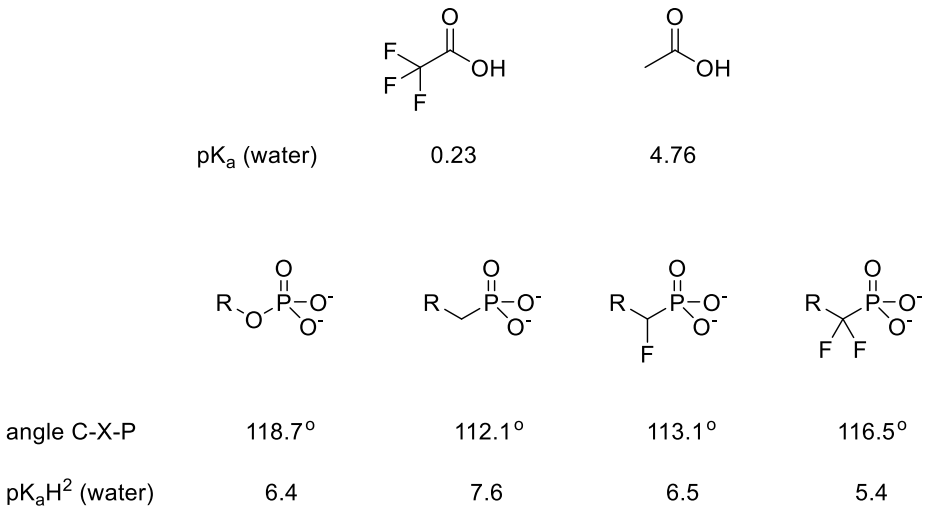


***Figure 67**: Comparison of dissociation constant values and bond angles for carboxylic acids and phosphates versus phosphonates.*

Due to hyperconjugation and the electron poor C-F σ^* orbital, the gauche conformation of vicinal fluorines is energetically favoured. This is not limited to vicinal fluorines and nearby charged or polar functional groups can lead to profound effects with strong attraction or repulsion forces influencing the ground state conformation[207] (**Fig. 68**).

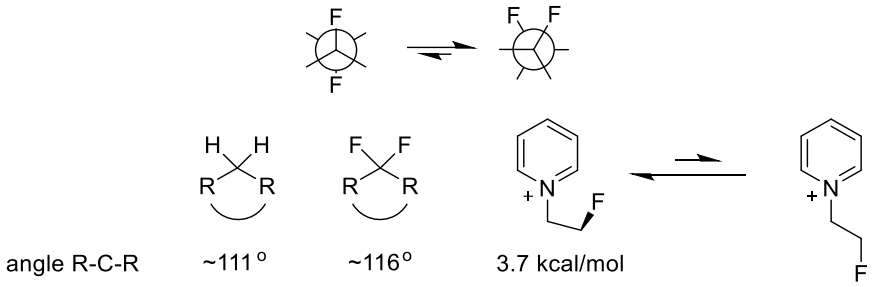


***Figure 68**: Impact of fluorine on molecular conformation.*

3.3) Fluorine in nature

Despite abundance in synthetic chemistry, organofluorine compounds in nature are rare with only five being identified to date[209]. Large numbers of organochlorine and bromine compounds have been identified in nature and despite fluoride being more abundant in seawater than iodide, approximately 182 natural products containing iodine have been identified[210].

Fluoroacetic acid is a rare example of this and is widely used as a pesticide[211], but is also found in several species of plant spanning the globe[212]. This highly toxic material is metabolised to (2*R*, 3*R*)-fluorocitrate (**Fig. 69**) which is a potent inhibitor of aconitase, halting the citric acid cycle[213,214]. The effects of this compound are well studied and crystal structures obtained of the enzyme-inhibitor complex[215].

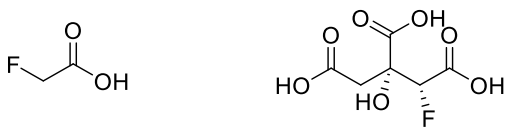
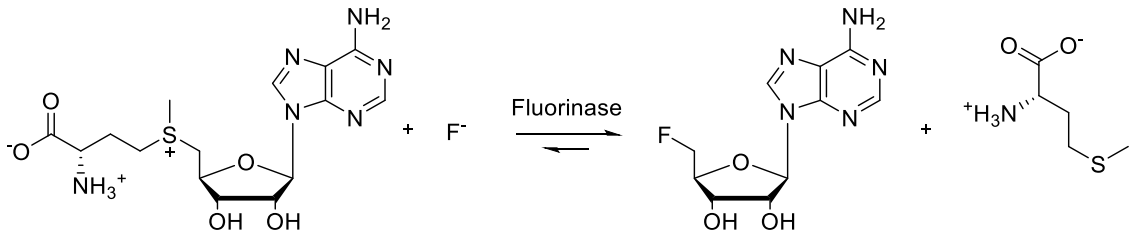


***Figure 69**: Fluoroacetic acid (left) and its metabolite (2R, 3R)-fluorocitrate (right).*

Enzymatic synthesis of organohalides first requires desolvation and oxidation to the X^+ species through chloro-, bromo- and iodoperoxidase enzymes but this is not possible for fluoride due to hydrogen peroxide having a higher oxidation potential (-1.80 eV vs -2.87 eV)[216].

Only one fluorinase enzyme has been identified and was isolated from *Streptomyces cattleya* and catalyses the reaction between a fluoride ion and AdoMet (*S*-adenosyl-L-methionine, **1**)[217]. Several homologues have been characterised and crystal structures show a greater than 80% homology to the originally discovered fluorinase[217]. This is the only known example of an enzyme capable of catalysing the formation of a C-F bond (**Scheme 34**).

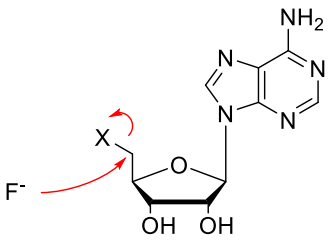


1 **81** **82**

***Scheme 34**: Action of the fluorinase enzyme, catalysing the reaction between fluoride and AdoMet (**1**) to generate 5'-fluoro-5'-deoxyadenosine (**81**) and L-methionine (**82**).*

The biosynthetic pathway for fluoroacetate production in plants is currently unknown, but has been determined for *S. cattleya* through a series of reactions breaking down 5'-fluoro-5'-deoxyadenosine into fluoroacetate and 4-fluorothreonine[218].

Research continues into this extraordinary enzyme, with recent work demonstrating that the enzyme can catalyse a Finkelstein reaction with 5'-halodeoxyadenosine in the absence of L-methionine (**Fig. 70**), and biological engineering has incorporated the enzyme into a modified *E. coli* strain[219,220].



X = Br >> I > Cl
(rate of fluorination)

***Figure 70**: Fluorinase mediated Finkelstein reaction.*

Fluorine is the thirteenth most abundant element in the earth's crust as a component of various minerals[221]. Organofluorine compounds have been detected in trace quantities in volcanic eruptions and geothermal vents[222].

Despite the quantity of fluorine present in the earth's crust, fluorine gas was not thought to occur naturally due to its highly reactive nature. Recently, evidence of small quantities trapped inside antozonite minerals[223,224] has been unearthed, possibly generated by small amounts of uranium present irradiating the fluoride minerals causing decomposition.

3.4) Amines in biology and medicinal chemistry

Amines are a diverse class of compounds of which many are biologically important and are produced by living organisms primarily through decarboxylation of amino acids such as phenylalanine, histidine, tyrosine and tryptophan[225].

Monoamine neurotransmitters are involved in regulation processes such as memory and emotion[226,227], and are removed from biological systems by monoamine oxidase enzymes[228,229]. The classical five biologically important monoamine neurotransmitters are histamine (**83**), serotonin (**84**), dopamine (**85**), noradrenaline (**86**), and adrenaline (**87**) (**Fig. 71**).

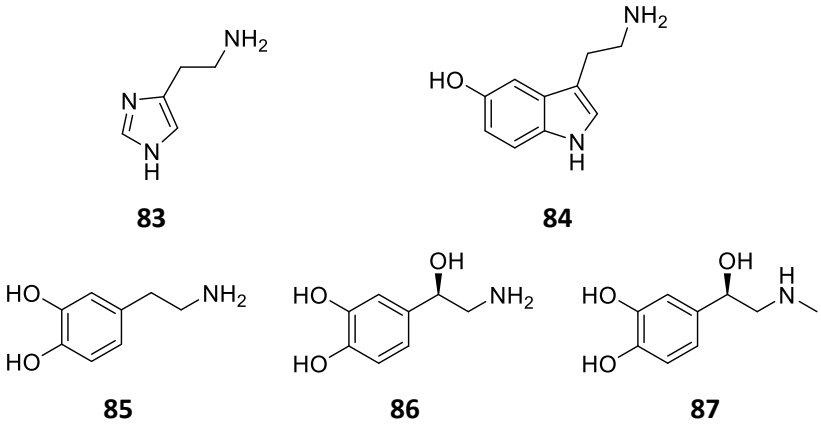


***Figure 71**: Five classical biogenic amine neurotransmitters.*

In addition to these monoamine neurotransmitters, there are a variety of "trace amines" that bind to the human TAAR1 receptor (trace amine-associated receptor 1) such as phenethylamine, phenylethanolamine, *meta*- and *para*-tyramine, synephrine and tryptamine[230]. Other amines such as trimethylamine, melatonin and polyamines such as cadaverine, putrescine and spermidine also have biological functions such as sleep mediation, plant growth regulation and avoidance responses[231–235]. Interference with the action of these natural amine neurotransmitters is an attractive drug target, with many developed such as antihistamines for general allergies, opiate analgesics, decongestants, sedatives and antidepressants (**Fig. 72**).

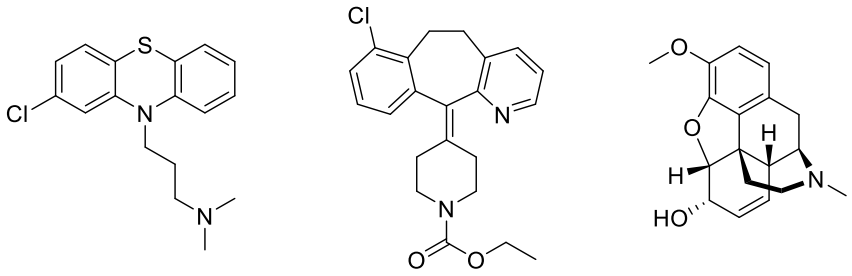


***Figure 72**: Common amine containing drugs – (left to right) Chlorpromazine, Loratidine and Codeine.*

The addition of fluorine can fine tune the properties of amines through electronic means, and with the abundance of amines in biological systems, drugs containing amine functional groups with nearby fluorine are common (**Fig. 73**). Primarily aryl fluorides or aryl trifluorides, the presence of these groups provide large electronic

effects which cascade through the compound. In the case of sitagliptin a trifluoromethyl group is attached to a nitrogenous heterocycle, greatly affecting the basicity of the nitrogen.

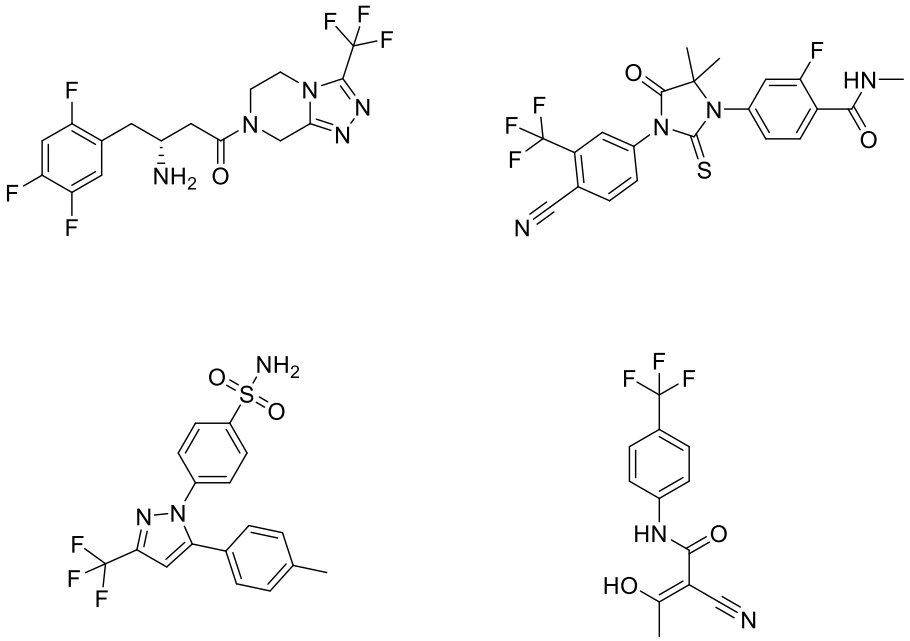


***Figure 73**: Examples of commercially available fluorous amines. Clockwise from top left: sitagliptin, enzalutamide, teriflunomide, celecoxib.*

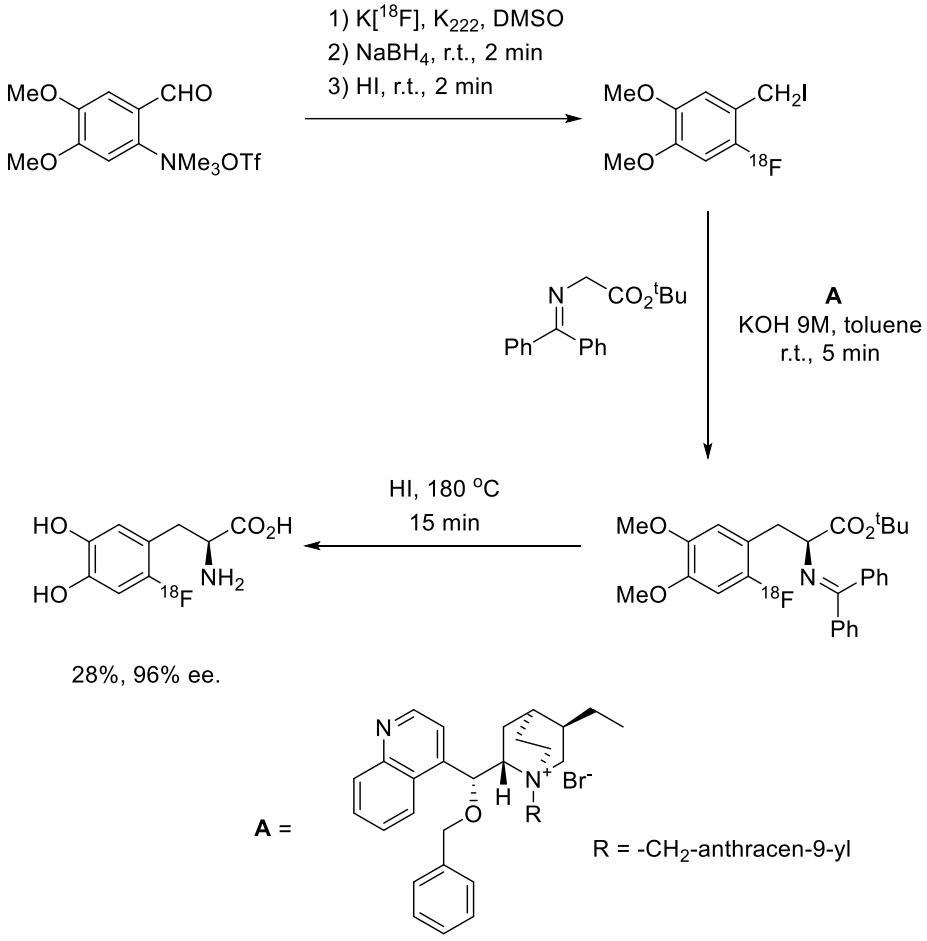
3.5) Synthesis of fluorinated compounds

A significant portion of HF is refined into metal fluorides such as KF, NaF and CsF which are commonly used as a fluoride source for synthesis. Other methods utilise organofluorine reagents and are prepared on an industrial scale for laboratory usage. Fluorination methods can be largely subdivided into nucleophilic, electrophilic and radical reactions.

Nucleophilic fluorination

Fluoride salts have traditionally been the source of F^- for nucleophilic methods and are effective at displacing suitable leaving groups to install fluorine. Fluoride simultaneously functions as a nucleophile and a base and is highly hygroscopic, a

disadvantage as fluoride has a low nucleophilicity when solvated. Poor solubility can hinder use, though AgF, CsF and KF are widely successful and commonplace. Such salts are convenient for installation of ^{18}F for PET scanning (**Scheme 35**), and a variety of conditions have been developed to aid solubility and nucleophilicity[236,237].



***Scheme 35**: ^{18}F labelling for PET scanning with K[^{18}F]. Radiosynthesis of 6-[^{18}F]fluoro-L-DOPA.*

Deoxyfluorination is a common method of nucleophilic fluorination, with reagents converting a hydroxyl group into an appropriate leaving group. A variety of aminosulfur trifluorides are often used, typically DAST (**88**) or Deoxo-Fluor® (**89**), but suffer from thermal stability issues which can lead to runaway reactions and generate HF. These reagents are also capable of converting a carbonyl to a geminal difluoro group[238,239]. The XtalFluor range (**90**) does not generate free HF and is far more thermally stable but is not reactive enough to deoxyfluorinate carbonyl groups[240].

Deoxytrifluorination of carboxylic acids is difficult, with SF_4 being the traditional reagent[241], however recent advances have allowed for a more facile synthesis with Fluolead[242] (**91**). Other methods of deoxyfluorination use Yarovenko's reagent[243] (**92**), the Ishikawa reagent[244] (**93**), tetraalkylammonium fluorides or Alkylfluor[245] (**94**). Deoxyfluorination of phenols is more challenging but can be achieved with reagents such as Phenofluor[246] (**95**) (**Fig. 74**).

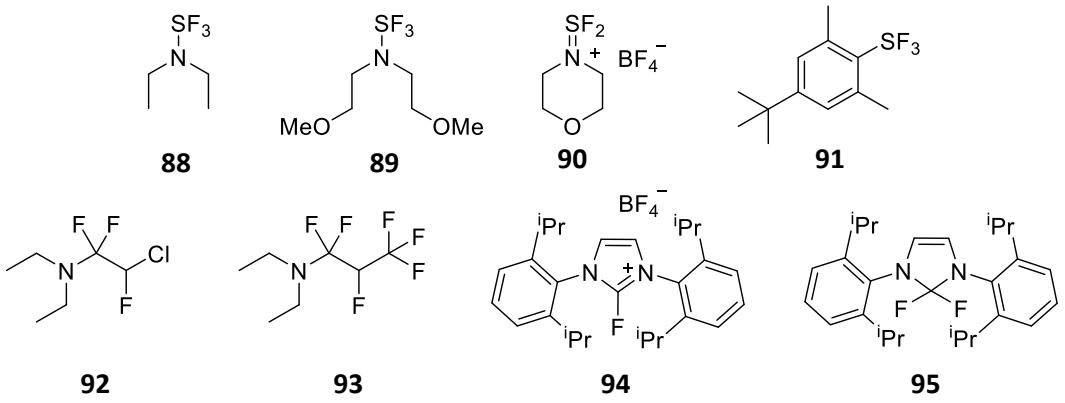
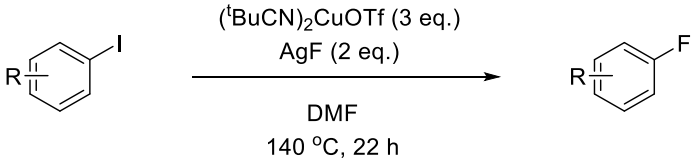


***Figure 74**: Selection of deoxyfluorination reagents. DAST (**88**), Deoxo-Fluor® (**89**), XTAL-M (**90**), Fluolead (**91**), Yarovenko's reagent (**92**), Ishikawa's reagent (**93**), Alkylfluor (**94**), Phenofluor (**95**).*

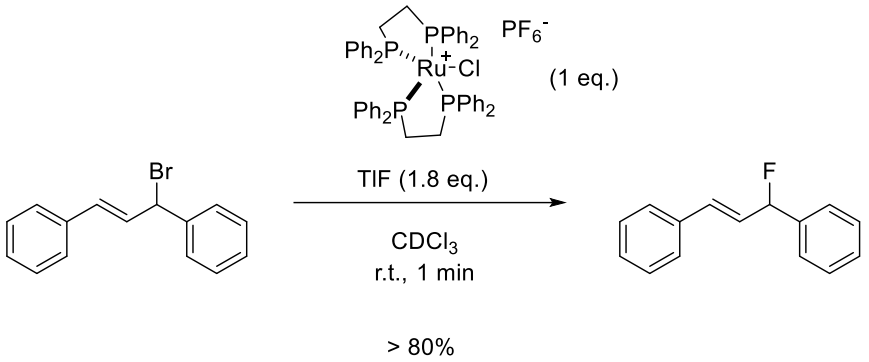
Transition metal-catalysed fluorinations have been developed but many require harsh conditions such high temperatures. Addition of phosphorous ligands leads to generation of P-F side products, and there is a high thermodynamic barrier to Ar-F formation. Both copper mediated[247] and copper catalysed[248] methods of fluorination of aryl iodides have been developed (**Scheme 36**), in addition to Pd catalysed methods[249].



***Scheme 36**: Copper mediated fluorination of aryl iodides.*

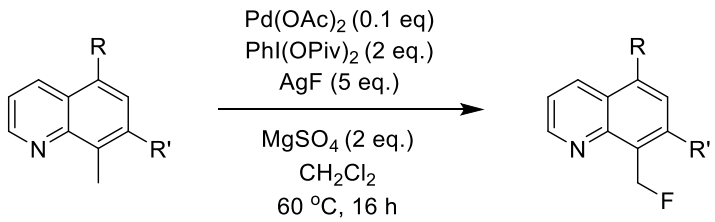
Allylic fluorination has been demonstrated through palladium, ruthenium and copper mediated methods, and stereochemistry can be directed by addition of chiral ligands. Combination ruthenium and thallium (I) fluoride methods have been demonstrated

but due to high cost and toxicity of thallium compounds, this method is highly undesirable[250–253] (**Scheme 37**).



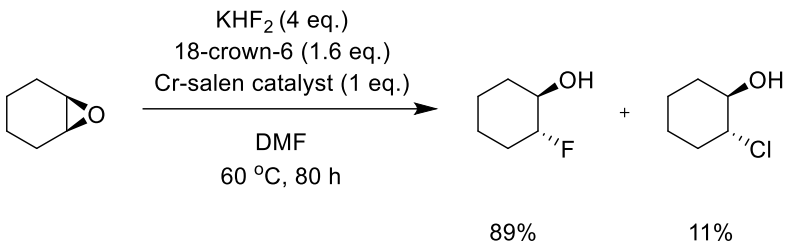
***Scheme 37**: Allylic fluorination with a ruthenium-thallium catalyst system.*

Palladium-catalysed reactions have been demonstrated with both nucleophilic and electrophilic sources of fluorine. Using an external oxidant to generate a Pd(IV) species, a fluoride source provides a ligand which can participate in a reductive elimination reaction[254] (**Scheme 38**).



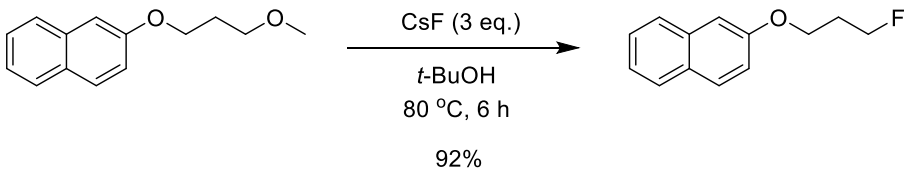
***Scheme 38**: Catalytic palladium C-H activation-fluorination reactions.*

Similar to the use of fluoride salts, reagents such as the bifluoride anion (HF_2^-) are nucleophilic and have been widely demonstrated to be applicable for the opening of epoxides through nucleophilic fluoride action. Addition of a crown ether or cryptand to sequester cationic species and increase the fluoride nucleophilicity has been demonstrated to be an effective strategy[255] (**Scheme 39**).



***Scheme 39**: Hydrofluorination with the bifluoride anion.*

Polar aprotic solvents such as MeCN have long been considered essential for nucleophilic fluorination, with protic solvents such as water and alcohols unsuitable due to the hydrogen bond network rendering the fluoride anion non-nucleophilic. The fluorinase enzyme functions in water (after removing all coordinated water molecules on fluoride in its active site), and successful S_N2 fluorination with a *t*-BuOH-TBAF complex[256–258] (**Scheme 40**) suggested that careful control of the hydrogen bond network could enable and facilitate nucleophilic fluorination in protic solvents[259] (**Fig. 75**).



***Scheme 40**: Nucleophilic fluorination with alkali fluoride in tertiary alcohol solvent.*

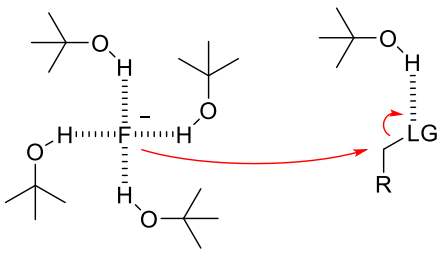


***Figure 75**: Solvent effect on aliphatic nucleophilic fluorination.*

Electrophilic fluorination

Elemental fluorine or compounds containing an oxygen-fluorine bond are the traditional electrophilic sources of "F^+". Due to high reactivity and danger, potential for unwanted side reactions through radicals, strong oxidising power and lack of selectivity, these methods have been largely abandoned. They have been replaced with compounds containing nitrogen-fluorine bonds such as Selectfluor (**96**, **97**) and NFSI[260] (**99**) (**Fig. 76**). The mechanism of fluorination is unclear but highly effective and stereoselective methods have been developed, though many use a chiral auxillary[261]. Synthesis of these reagents still requires use of F_2, but the developed reagents are far safer and easier to handle and reactions can often be performed in traditional glassware.

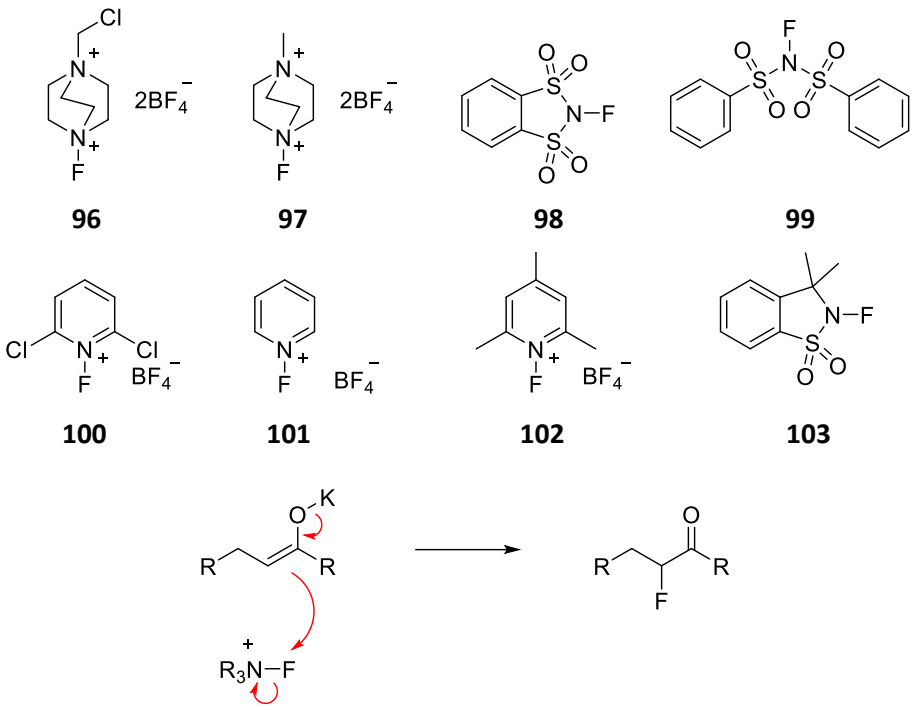


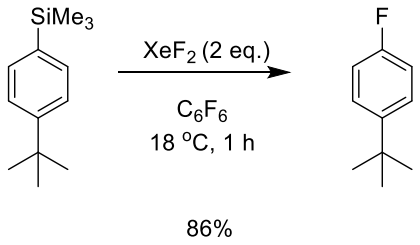
***Figure 76**: Selection of electrophilic fluorination reagents with hypothesised mechanism (below). Selectfluor I (**96**), Selectfluor II (**97**), N-fluoro-o-benzenedisulfonimide (NFOBS) (**98**), N-fluorobenzenesulfonimide (NFSI) (**99**), 2,6-dichloro1-fluoropyridinium tetrafluoroborate (**100**), 1-fluoropyridinium tetrafluoroborate (**101**), 1-fluoro-2,4,6-trimethylpyridinium tetrafluoroborate (**102**), N-fluoro-2,α-cumenesultam (**103**).*

Radical fluorination

Radical fluorination complements electrophilic and nucleophilic fluorination and combines a carbon centered radical with an atomic fluorine source to generate the C-F bond. Traditional fluorine sources were fluorine gas which can also behave as an electrophilic source, hypofluorites and xenon difluoride. Due to danger in handling each of these reagents and high occurrence of side products, usage was limited until electrophilic fluorination reagents were demonstrated to have a dual usage and partake in radical mechanisms[262,263].

Usage of F_2 and hypofluorites continues but is confined to specialist procedures. Elemental fluorine is non-selective and its reputation for danger well reported, its tendency for overreaction limiting it to perfluorination reactions. The weak O-F bond present in hypofluorites has lead to similar drop in use but is useful in polymerization reactions[264].

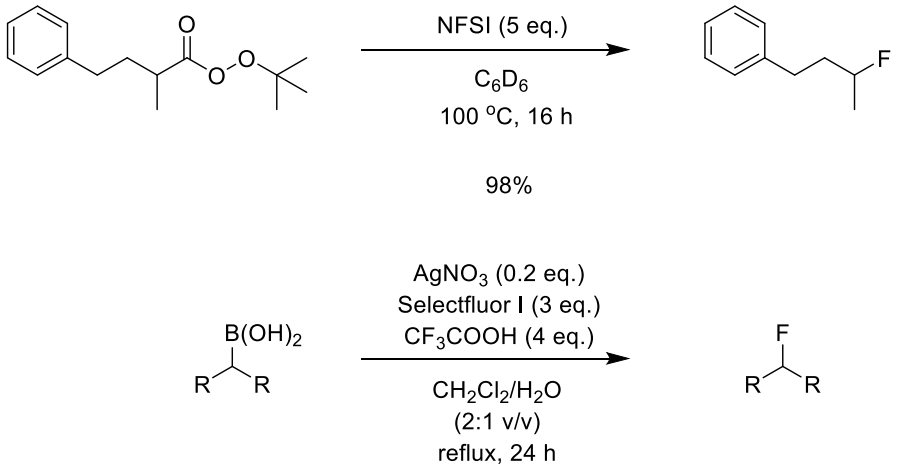
Xenon difluoride remains somewhat common, but has a high cost and difficult storage as it decomposes on exposure to air, water or light. Used in a fluorination version of the Hunsdiecker reaction and also for aryl fluoride synthesis from aryl silanes[265] (**Scheme 41**), it is capable of generating the carbon radical and providing the fluorine source[266,267]. Weak Xe-F bonds and an entropic driving force makes this reagent highly reactive.



***Scheme 41**: Example of radical fluorination with XeF_2.*

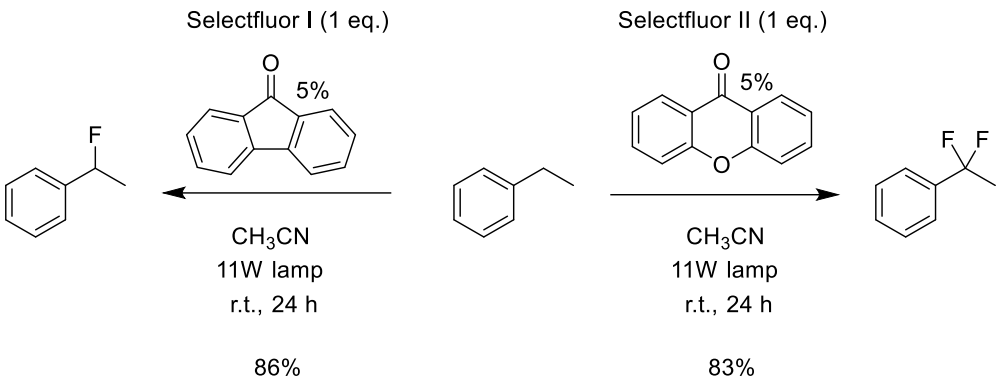
Xenon difluoride performs decarboxylative fluorination but cheaper and safer electrophilic reagents have been shown to perform the same reaction by radical mechanisms. The most common reagents for this are Selectfluor and NFSI[262,263]. Use of peresters to generate a radical or catalysis by metal salts such as silver[268] have

proven effective and also photochemistry[269,270]. Silver salts are additionally capable of fluorination of boronic acids[271]. Surprisingly, aqueous solvents are utilized for many of these reactions. Unsuitable for nucleophilic methods due to the basicity of fluoride, the radical transfer mechanism bypasses this and adherence to dry conditions is not required as is usual for much fluorination chemistry (**Scheme 42**).



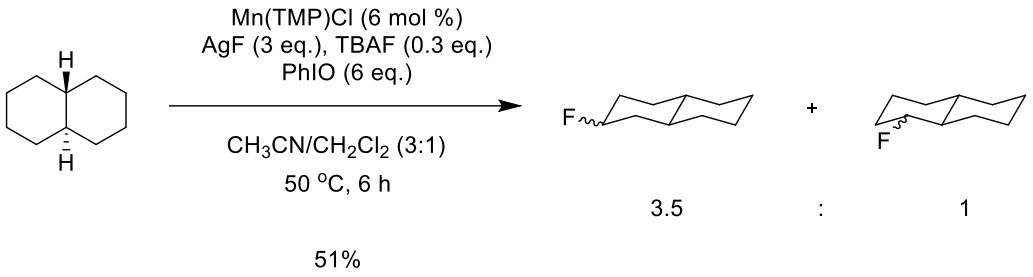
***Scheme 42**: Radical fluorination of a perester using an N-F fluorinating reagent, and radical fluorination of boronic acids under aqueous conditions.*

An advantage to radical fluorination not afforded to other methods is reaction at unactivated bonds. Simple alkenes have been targeted through a range of radical generators[272–275], and sp^3 C-H bonds have been successfully fluorinated with transition metal catalysts[276,277] and some metal free variants[278,279], reliant on an external radical generator. Benzylic positions are more reactive than simple alkane chains and methods to selectively fluorinate at this position are established. It has been demonstrated using photochemistry that mono- or difluorination at this benzylic site can be selectively achieved using different catalysts[280] (**Scheme 43**).



***Scheme 43**: Selective metal free mono- and difluorination using a photoinitiated radical.*

With advances in C-H activation chemistry, formation of alkyl fluorides *via* this method has followed. Requiring an external oxidant, manganese-porphyrin methods for fluorination at unactivated C-H bonds have been demonstrated[281] (**Scheme 44**).



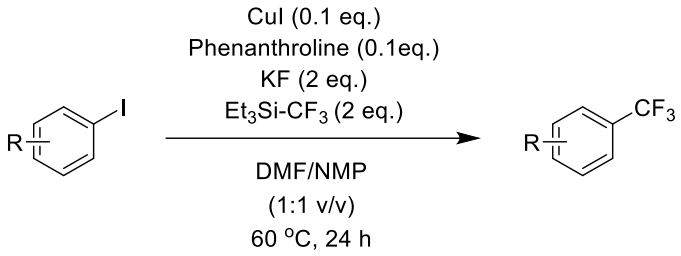
***Scheme 44**: C-H activation for fluorination with a manganese complex.*

Fluoroalkylation

Despite a variety of methods, fluorination is difficult and multiple fluorinations to di- or tri-fluoro groups are uncommon. Few reagents are capable of multiple fluorinations, with DAST being suitable for synthesis of *gem*-difluorides from aldehydes or ketones[238], and preparation of -CF_3 groups is usually performed by treatment of carboxylic acids with SF_4[241] or more recently Fluolead[242].

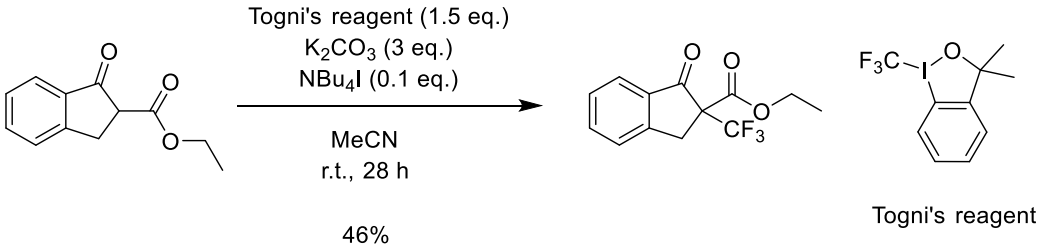
An alternative method involves transfer of prefluorinated carbon, for which many reagents are commercially available or can be readily synthesised. Introduction of an additional carbon is not always desirable, but a variety of methods with electrophilic, nucleophilic and radical mechanisms are known.

Aromatic coupling-based methods are known to achieve trifluoromethylation of arenes, often from the aryl iodide (**Scheme 45**). Copper, zinc and palladium methods have been used including catalytic versions and recently aryl boronic acids have proven suitable substrates[282–285].



***Scheme 45**: Trifluoromethylation of aryl iodide exploiting a "$CuCF_3$" reagent.*

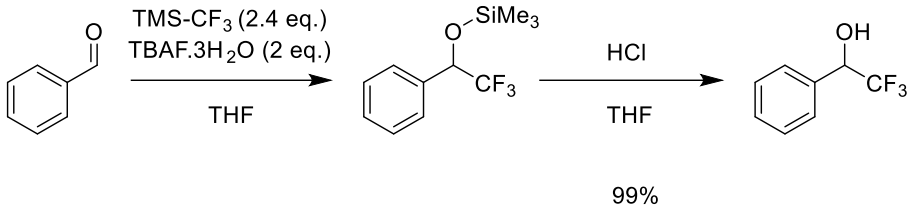
For electrophilic trifluoromethylation the CF_3^+ species is required. This uncommon species is difficult to produce and few examples are present in the literature. Benzothiophenium salts have been used[286] but the most common example is Togni's reagent, a hypervalent iodine reagent capable of reacting with many nucleophiles such as alcohols, thiols and alkenes[287–290] (**Scheme 46**).



***Scheme 46**: Electrophilic trifluoromethylation with Togni's reagent.*

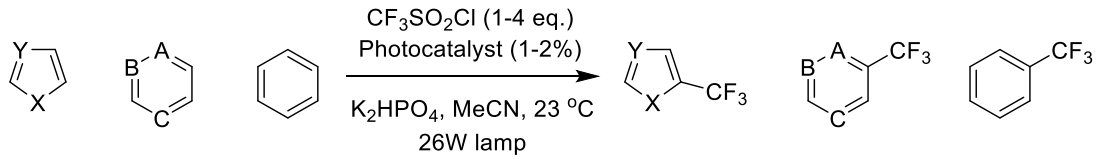
Ruppert's reagent is one of the most famous examples of a trifluoromethylation reagent, and is capable of trifluoromethylation of carbonyl compounds. Work by Prakash extended the use of this reagent and isolated the CF_3^- ion as the active

species[291–293]. This anion can also be generated from fluoroform or methyl fluorosulfonyldifluoroacetate and stabilised with copper to form a "$CuCF_3$" species, commonly generated and exploited in a number of trifluoromethylation strategies[294].



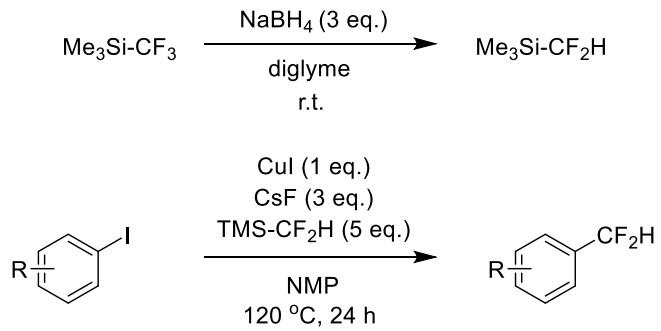
***Scheme 47**: Trifluoromethylation with Ruppert's reagent.*

Radical trifluoromethylation is known to be effective for both aromatic and alkenyl systems. Trifluoroiodomethane has been used over haloform due to the Montreal protocol[295], or sodium trifluoromethanesulfinate as demonstrated by Langlois[296]. A light generated radical from trifluoromethanesulfonyl chloride offers a mild method tolerant of a range of functional groups[297] (**Scheme 48**).



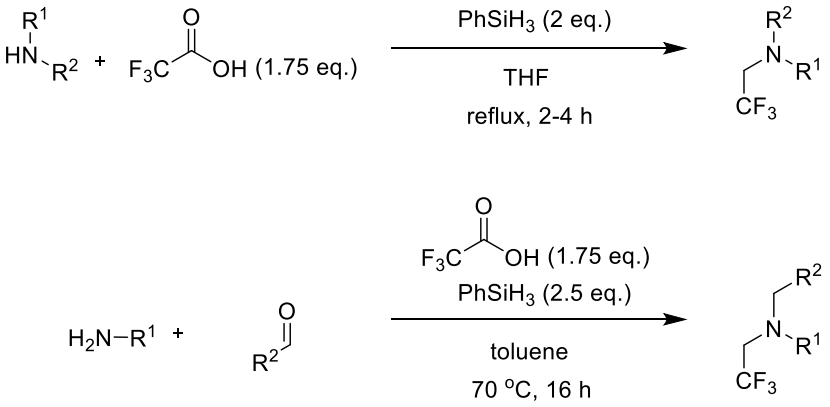
***Scheme 48**: Scope for trifluoromethylation reaction of arenes by photoredox catalysis.*

The difluoromethyl group has received less attention than trifluoromethyl, but proceeds by broadly similar methods. Preparation of difluoromethylating reagents often begins with the respective trifluoromethyl form[298], and difluoromethylation of aryl and vinyl iodides has been reported by the Hartwig group after preparation of difluoromethyltrimethylsilane from Ruppert's reagent[299] (**Scheme 49**). Difluoromethyl ethers have also drawn attention and can be prepared from phenols using difluoromethyltriflate[300]. Alternative methods often require the use of ozone depleting gaseous reagents and are undesirable[301].



***Scheme 49**: Difluoromethylation of aryl iodides with TMS-CF_2H.*

An alternative strategy not suitable for methyl groups but for longer carbon chains was demonstrated by the Denton group, with the single step and catalyst free reaction between an amine, trifluoroacetic acid and phenylsilane. This method allows for efficient trifluoroethylation of secondary amines, and a three component reaction of a primary amine, aldehyde and trifluoroacetic acid can generate complex tertiary trifluoroethyl amines in a one pot procedure[302] (**Scheme 50**).



***Scheme 50**: Trifluoroethylation of amines with trifluoroacetic acid and phenylsilane.*

Synthesis of perfluorous compounds

Fluorite is a major source of HF which is in turn converted into fluorine gas, fluorocarbons and other materials. The added difficulty of working with HF or F_2 thus constrains fluorination chemistry. Whilst fluorination can be achieved with other simple and safer reagents such as CsF, KF and TBAF, preparation of perfluorous compounds is not readily achievable *via* these methods.

The two prominent methods of perfluorination are electrochemical or telomerisation. For electrochemical fluorination the hydrocarbon compound is fluorinated using a (commonly) nickel anode and anhydrous HF as solvent and reagent (**Fig. 77**). This destructive and poorly controlled method leads to only a low yield of the desired product, with a variety of rearrangements, fragmentations, couplings and partial fluorinations occurring[303,304].

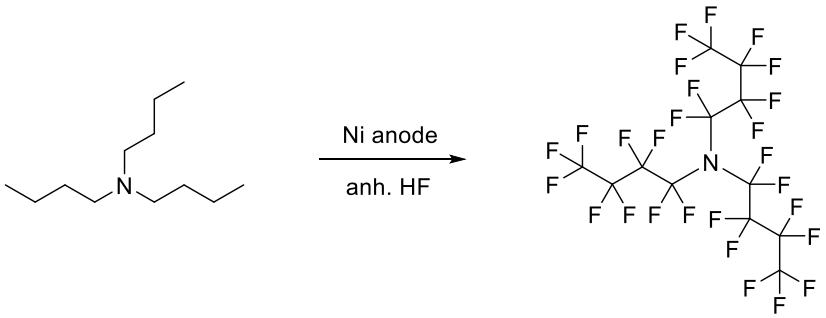


***Figure 77**: Electrochemical fluorination to prepare perfluorotributylamine.*

An alternative method more commonly found in industry is telomerisation; radical polymerisation of heavily fluorinated building blocks (**Fig. 78**). These small compounds are prepared *via* electrochemical fluorination but due to their simplicity are less valuable and so the destructive nature of electrochemical methods are financially acceptable. Whilst difficult to control chain length, compounds are all linear and can be purified by distillation and products do not have to be extracted from anhydrous HF[305,306].

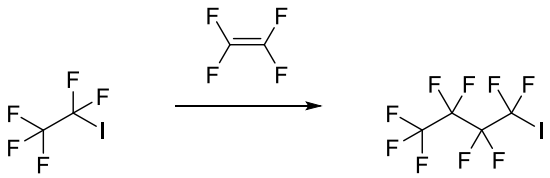


***Figure 78**: Telomerisation method of perfluorous compound preparation.*

Such methods lack the finesse and control desired in research laboratories and to prepare a chosen heavily fluorous compound is not facile *via* these methods. Fluorinated building blocks are commercially available to fill this niche (**Fig. 79**), with many small compounds ready for further synthesis purchasable, negating the requirement for chemists to perform the fluorination themselves[307–309]. An improvement in safety and time, this method is commonplace in research with an incredibly diverse array of compounds ready for purchase for synthetic use, analogous to the variety of boronic acids commercially available for coupling reactions.

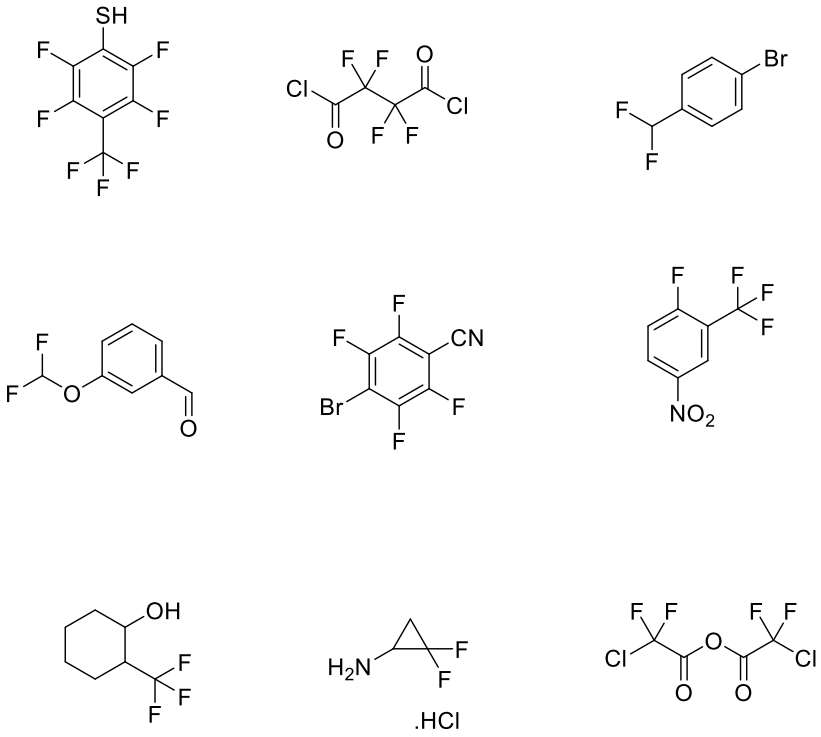


***Figure 79**: Selection of commercially available fluorinated building blocks for use in further synthetic procedures.*

Synthesis of perfluoroalkylamines

Despite the abundance of nitrogen in pharmaceutical compounds and the ability of fluorine to carefully tune physicochemical properties, preparations of perfluoroalkylamines are limited and commonly utilise perfluorinated building blocks for their synthesis.

Of interest are 1,1-dihydroperfluoroalkylamines for which only a few methods have been reported for their synthesis (**Fig. 80**). Such methods may use pyrophoric or aggressive reagents which requires additional safety measures, can limit scale up and wide scale applicability to compounds with sensitive functional groups. Additional complications such as quantity or nature of waste are prevalent.

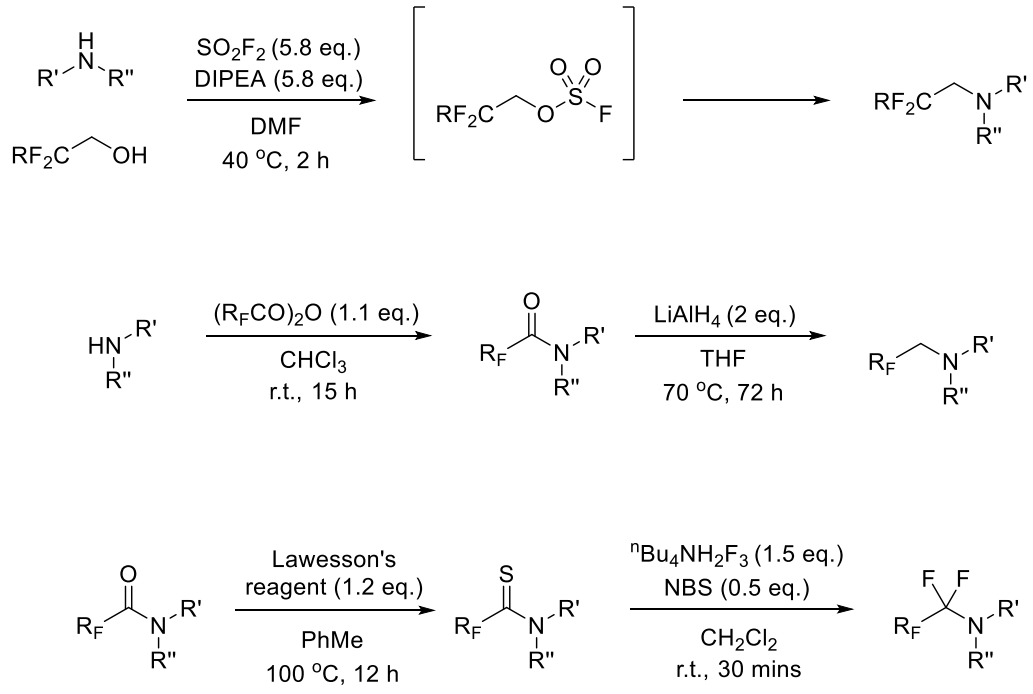
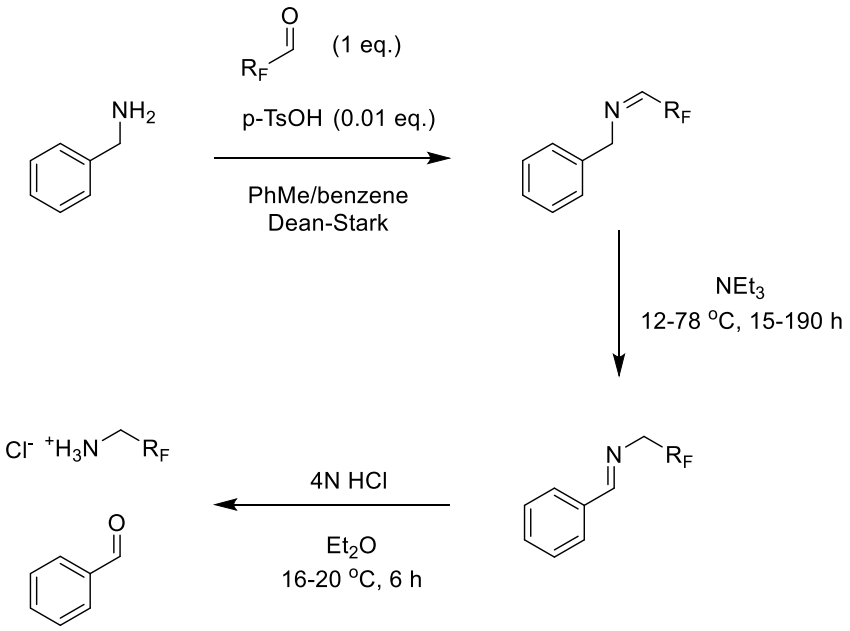


***Figure 80**: Top to bottom. 1) Reaction of an amine with a fluorous alcohol derived intermediate compound to form fluoroalkylamine. 2) Anhydride preparation of fluorous amide and subsequent reduction. 3) Fluorous amide conversion to thioamide prior to further fluorination.*

Examples of such preparations include use of sulfuryl fluoride to facilitate an S_N2 reaction[310]. Whilst the reaction proceeds well and uses readily available starting materials, the fluorous alcohols are expensive and sulfuryl fluoride must be prepared *in situ* and is a potent neurotoxin and greenhouse gas. Other more traditional methods first prepare the fluorous amide from the anhydride resulting in one equivalent waste of fluorous carboxylic acid, and subsequently reduce with $LiAlH_4$ which can greatly limit substrate scope[311]. An alternative method for a complete perfluoroalkyl amine involves conversion to the thioamide before further fluorination to the desired product[312].

These methods are only applicable for the preparation of secondary and tertiary fluorous amines, however a biomimetic method by Soloshonok[313] inspired by pyridoxal (a form of vitamin B_6) can prepare the primary amine (**Scheme 51**). Utilising the fluorous aldehyde and condensing to form the imine, subsequent rearrangement and hydrolysis affords the primary 1,1-dihydroperfluoroalkylamine under generally less harsh conditions than other examples in the literature. The major concern with this method however is the cost and instability of the fluorous aldehydes.



***Scheme 51**: Preparation of primary perfluoroalkylamine through biomimetic synthesis using fluorous aldehydes.*

4) Results and discussion

New methods to prepare perfluoroalkyl amines have been developed.

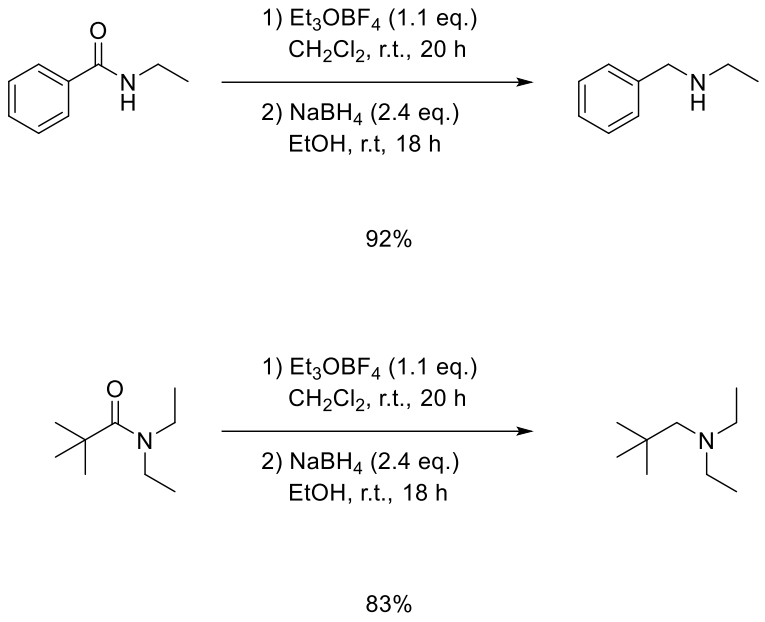
An investigation into using Meerwein salts to activate an amide followed by reduction with sodium borohydride was performed. Activation and reduction of amides is a common procedure however an examination of the literature failed to find evidence of this method used to prepare fluoroalkyl amines. The fluoroalkyl chain significantly changes the electronics of the system compared to the hydrocarbon variant which may complicate matters, however such a mild process is desirable and with such a simple procedure much experimentation and improvement may be possible should difficulties be encountered.

A second approach sought to use silanes, without resorting to development of a metal catalyst-reductant system of which numerous examples are known in the literature. Expansion and improvement of previous work for a one pot fluoroalkylation procedure with phenylsilane was selected as a route to perfluoroalkyl amines, using longer chain perfluorous carboxylic acids rather than trifluoroacetic acid, and attempting to produce a range of products beyond tertiary amines. The possibility of preparing primary and secondary amines is attractive as these were previously unattainable *via* similar methods, or complex tertiary amines without requiring an aldehyde as a third component in the reaction.

4.1) Meerwein salt activation of amides

Triethyloxonium tetrafluoroborate is also known as Meerwein's Salt or the Meerwein Reagent, and is an example of a strong alkylating agent[314]. A variety are commercially available with different length alkyl chains and counterions which impact solubility.

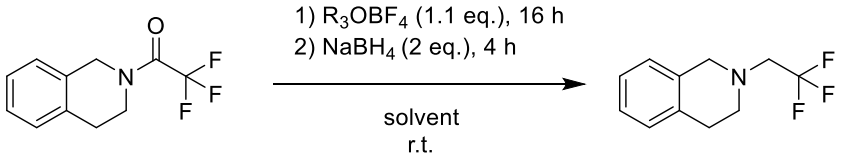
Commonly used for alkylation of carbonyls, in combination with a reducing agent, reduction of amides has been previously reported[315] (**Scheme 52**). Further still, selective formation of esters can be performed in the presence of base[316].



***Scheme 52**: Examples of amide reduction to amines with $NaBH_4$ via Meerwein salt activation.*

It was hypothesised that reduction of an amide derived from trifluoroacetic acid or a longer fluorous acid could be achieved in a similar manner. To our knowledge there are no examples in the literature involving reduction of a fluoroalkyl amide utilising Meerwein salts, but hydrocarbon variants of both secondary and tertiary amides are known.

Efforts to explore the potential for reduction of a fluorous amide began with a tertiary trifluoroacetamide prepared from 1,2,3,4-tetrahydroisoquinoline and trifluoroacetic anhydride. A series of conditions were then trialled for a two step-one pot reaction.



104 **105**

Entry	R	Solvent	Yield (%)
1	Me	CH_3CN	39
2	Et	CH_3CN	55
3	Me	CH_2Cl_2 then EtOH	51
4	Et	CH_2Cl_2 then EtOH	51
5	Me ($MgSO_4$ 1 eq.)	CH_3CN	8
6	Et ($MgSO_4$ 1 eq.)	CH_3CN	8

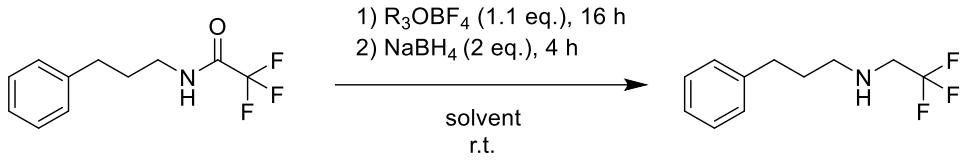
***Table 4**: Conditions screened for amide reduction. ^1H NMR yields, using 1,1,2,2-tetrachloroethane as an internal standard.*

Two Meerwein salts were trialled, but only mediocre yields were achieved and large quantities of starting material were observed. Alternative solvents were trialled using CH_2Cl_2 for the alkylation and EtOH for the reduction, but no significant improvement was seen. It was hypothesised that hydrolysis may be a factor and so an equivalent of $MgSO_4$ was added as a desiccant, however this decreased the yield of the reaction significantly, possibly due to interaction with the sulfate anion.

Meerwein salts are strong alkylating agents and could alkylate even the weakly basic acetonitrile solvent[317], which may explain the lower yield for the more reactive methyl variant over the ethyl in this instance. Acetonitrile was selected over the more usual combination of CH_2Cl_2 and EtOH as the subsequent reduction could readily be performed in MeCN and not require a solvent change, but the results demonstrate that the solvent choice was not the limiting factor in these reactions as yields were poor independent of solvent selection.

Poor results were obtained thus far with tertiary amides but the reason for their failure was unclear. To assess whether this was a substrate issue or simply that this

is an unfavourable process, the same reactions were trialled with secondary amides to determine whether this would be viable method for fluoroamide reduction.



106 **107**

Entry	R	Solvent	Yield (%)
1	Me	CH_3CN	25
2	Et	CH_3CN	0
3	Me	CH_2Cl_2 then EtOH	0
4	Et	CH_2Cl_2 then EtOH	0
5	Me ($MgSO_4$ 1 eq.)	CH_3CN	0
6	Et ($MgSO_4$ 1 eq.)	CH_3CN	0

***Table 5**: Conditions screened for amide reduction. ^1H NMR yields, using 1,1,2,2-tetrachloroethane as an internal standard.*

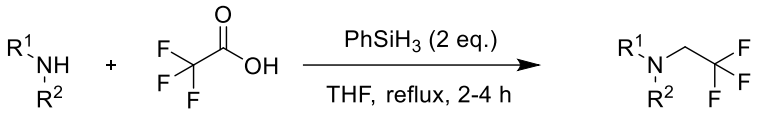
Disappointingly, results with the secondary amide were significantly worse than the tertiary amide results. With one exception, no product was detected under any conditions, and only starting material was recovered. Electronic factors are the likely explanation, with secondary amides being less reactive towards electrophiles due to the lack of additional electron donating groups leading to a relatively electron poor carbonyl. This combined with the electron withdrawing nature of the $-CF_3$ group could explain the poor yields obtained.

This indicates that the electron withdrawing effect of the fluorous group is the problem that may render this method unsuitable for the reliable preparation of fluoroalkyl amines. The stage of the reaction which is failing cannot be determined from these experiments, and it remains uncertain whether the electrophilic alkylation is the problem or the subsequent reduction. Further experimentation and monitoring of the reactions by ^{19}F NMR may indicate whether alkylation is occurring, to determine the failure point of the reaction.

4.2) Catalyst free perfluoroalkylation of amines

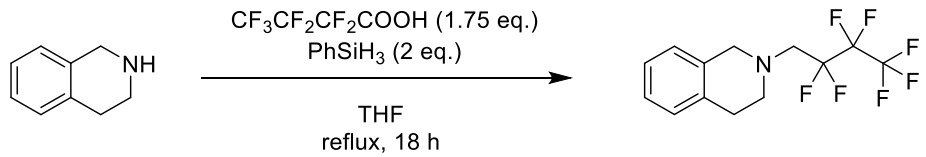
Background and discovery

Previous work on the fluoroalkylation of amines in the Denton group was performed in a catalyst free manner with trifluoroacetic acid and phenylsilane (**Scheme 53**). The reaction proceeds *via* generation of a silyl acetal and condensation with an amine, before reduction of the iminium ion by additional silane. Additionally, a three component coupling was demonstrated between a primary amine, an aldehyde and trifluoroacetic acid to generate complex tertiary amines[302].



***Scheme 53**: Catalyst free trifluoroethylation of secondary amines.*

An initial experiment towards improving the scope of this reaction concerned trialling longer chain perfluorous carboxylic acids. The test substrate chosen was 1,2,3,4-tetrahydroisoquinoline and successful perfluoroalkylation was demonstrated with heptafluorobutyric acid (**Scheme 54**).

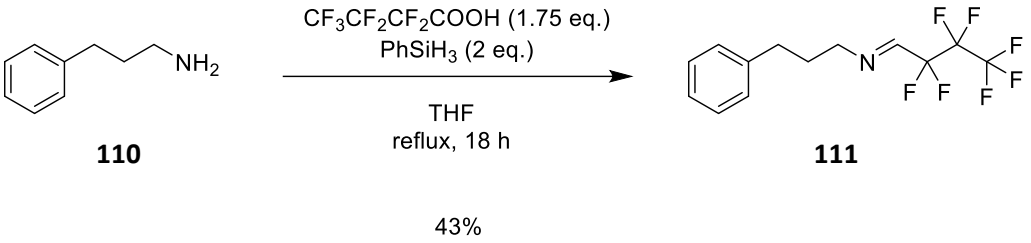


108 **109**

39%

***Scheme 54**: Heptafluorobutylation of a secondary amine.*

With a successful result for the reaction proceeding with an alternative fluorous acid, a primary amine was trialled (**Scheme 55**). Whilst the desired product of the secondary amine was not detected, interestingly however the major product formed was the imine.



***Scheme 55**: Synthesis of a perfluoroalkyl imine.*

This curious result was confirmed by ^{1}H NMR with a triplet seen at δ_H 7.72 (J = 6.1 Hz) and two missing protons upfield of the aromatic region. No distinctive carbonyl or amide N-H peaks could be found *via* infrared spectroscopy and mass spectrometry detected a singly positively charged ion with a mass equal to that of the imine plus a proton as a major product. Further confirmation was obtained by reduction with $NaBH_4$ which successfully generated a secondary amine product.

This result is surprising due to the excess of silane reducing agent, which in the presence of the strong perfluorous acid should reduce an iminium ion. Hydrosilanes are not intrinsically nucleophilic and require activation of a substrate in order to react, such as a Lewis acid or protonation[318] (**Fig. 81**).

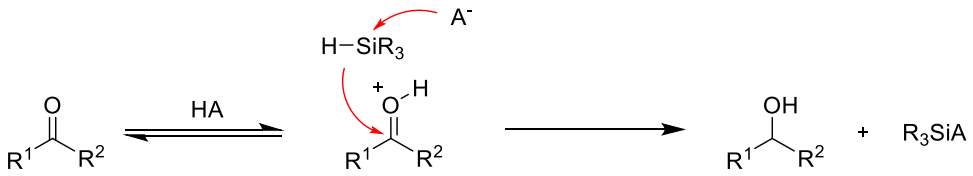
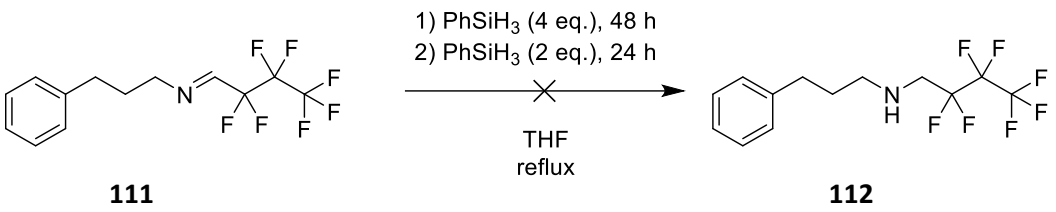


***Figure 81**: Hypothesised mechanism of reduction by a hydrosilane in a Brønsted acid, requiring activation of the substrate prior to reduction.*

Due to the strong electron withdrawing effect of fluorine, the lone pair on the nitrogen may not be readily protonated, and so reduction with the silane reagent is not possible. To investigate this hypothesis, the reaction was trialled for an extended period with additional silane in order to determine whether it is possible to reduce the imine using only phenylsilane (**Scheme 56**).



***Scheme 56**: Additional silane experiment assessing further reduction.*

After 48 hours an aliquot was removed and an NMR experiment performed to determine the ratio of imine to amine. A clear peak was seen by ^{1}H NMR at δ_H 7.72 corresponding to the proton at the base of the imine and no amine was detected by NMR, infrared or mass spectrometry methods. Additional phenylsilane was added and after a further 24 hours no reaction was seen, confirming that phenylsilane alone is not sufficient to reduce the imine.

Previous mechanistic work on the trifluoroethylation of secondary amines performed in the Denton group established the reaction proceeds *via* formation of a silyl acetal in the presence of excess acid, which forms a highly reactive iminium ion which is subsequently reduced by silanes to form the secondary amine products[302] (**Fig. 82**).

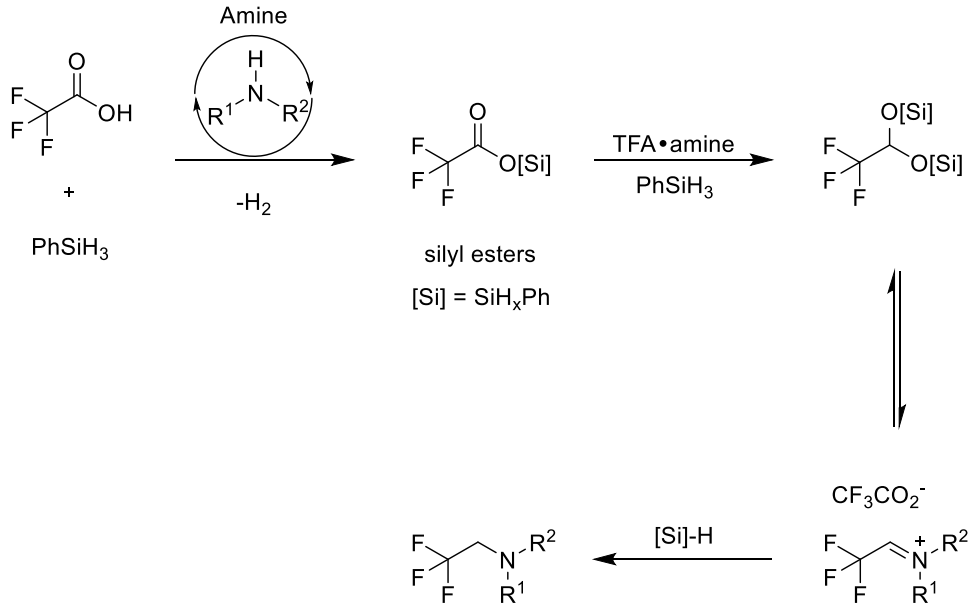


***Figure 82**: Proposed pathway for trifluoroethylation of secondary amines via silyl acetals.*

Due to the electronic effects of fluorine on adjacent carbons drawing electron density away from the CH of the imine, the C-C σ* orbital becomes a good electron acceptor.

In the case of the secondary amine, the lone pair of nitrogen is unavailable, a stable imine cannot be formed and the iminium ion is reduced to the tertiary amine. Conversely for the primary amine the lone pair of the nitrogen is available and can form a stable imine, functioning via a stabilising interaction with the C-C σ* orbital, halting the reaction prior to reduction (**Fig. 83**).

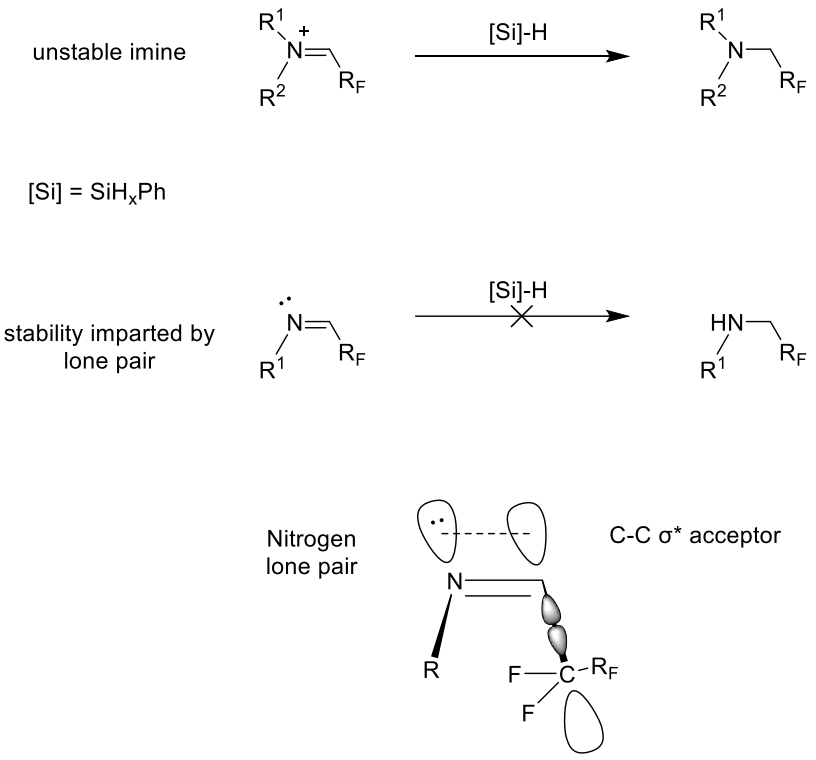
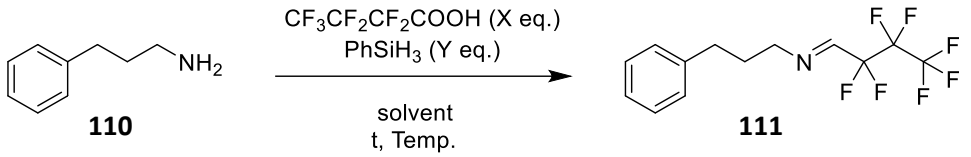


***Figure 83**: Illustration of the stabilising influence of the available nitrogen lone pair on the formed imines, halting the reaction prior to reduction to the amine.*

Reaction optimisation

With these results, a screen was performed to determine the optimal reaction conditions for formation of perfluorinated imine **111**.



110 **111**

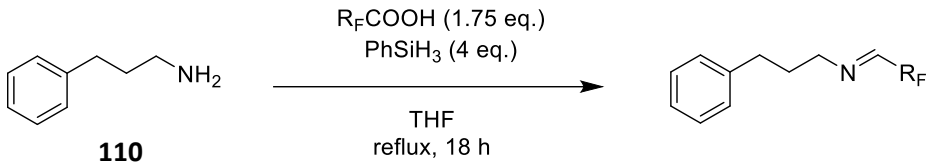
Entry	Y (eq.)	X (eq.)	Solvent (Temp, °C)	Time (h)	Yield (%)
1	2.00	1.75	THF (70)	18	43
2	2.00	3.00	THF (70)	18	8
3	4.00	1.75	THF (70)	18	63
4	2.00	1.75	PhMe (115)	18	43
5	2.00	1.75	THF (70)	42	15
6	4.00	1.75	THF (70)	42	32
7	2.00	1.75	PhMe (115)	42	26
8	4.00	1.75	PhMe (115)	18	39

***Table 6**: Optimisation results from the imine formation screen.*

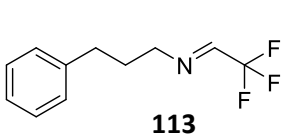
All reactions performed in THF solely generated the imine. However, reactions in toluene lead to formation of a small quantity of the secondary amine (< 5%). This suggests that further reduction to the amine is only possible at higher temperatures, due to the stability of the imine. It may be possible to preferentially generate the amine, however the yields were minimal and conversion to the amine is simple enough *via* other means to not warrant further investigation.

The best result was achieved by maintaining the same quantity of acid as previously optimised for trifluoroethylation, though an increase of phenylsilane equivalents improved the yield significantly. Solvent and temperature changes merely hindered results and elongated reaction times were also unable to improve on yields achieved under less forcing conditions.

With an optimised procedure, a brief scope of fluorous acids was performed to investigate how general the reaction is.

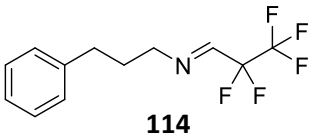


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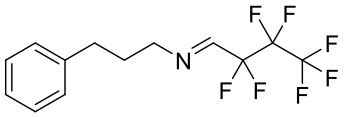
113

0% [a]



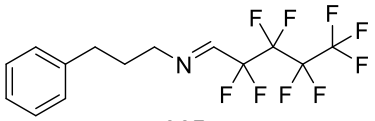
114

25%



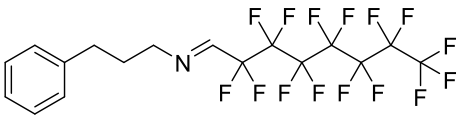
111

48%



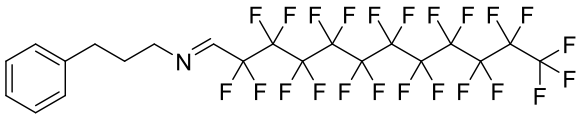
115

69%



116

72%



117

45%

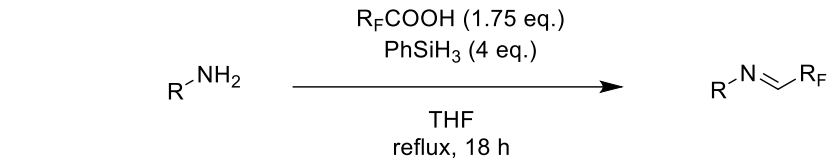
[a]: Reaction performed with and without presence of $MgSO_4$ (2 eq.). Yield of 0% obtained each time.

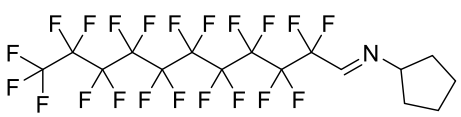
***Table 7**: Results from preparation of imines via developed methodology.*

A trend of higher yields is seen corresponding to elongation of the carbon chain, but even pentafluoropropionic acid gave a moderate yield. A lower yield for the longest chain with twenty three fluorine atoms is likely an isolation issue, due to the surfactant qualities of reagents and products. Experiments were performed with trifluoroacetic acid (TFA) to ensure that the imine products had not been overlooked in previous work, and an additional reaction with TFA and a desiccant ($MgSO_4$, 2 eq.)

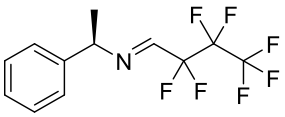
was performed to ensure hydrolysis was not occurring *in situ*. Despite these efforts, only starting materials were recovered in each instance with no desired product detected.

An additional two imines were prepared *via* the same methodology in order to ensure these results were not unique to this substrate.





118
80%



119
86%

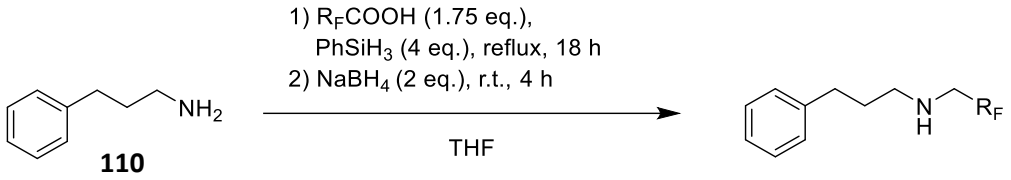
***Table 8**: Perfluoroalkylation to form imines from alternative amine substrates.*

Yields from these alternative amines were very good, significantly higher than for the 3-phenylpropylamine trial substrate. Possibly due to a more nucleophilic amine due to greater electron density from the proximity of the cyclic group or the benzylic nature of the nitrogen.

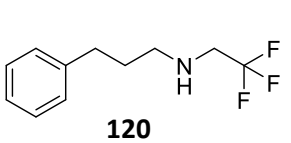
These results demonstrate that imines with a variety of lengths of fluorous tail can be prepared, with yields ranging from moderate to very high. Trifluoroacetic acid remains unsuccessful, possibly due to not being electron withdrawing enough to allow a stable intermediate to form, but additional fluorine permits such reactions to take place.

4.3) Secondary Amines

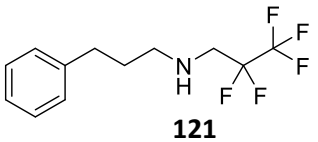
With imines determined as the product of the perfluoroalkylation reactions performed on primary amines, efforts towards a one pot procedure to form secondary amines were pursued. After the initial imine formation step was complete, the reaction mixture was allowed to cool and $NaBH_4$ added directly.



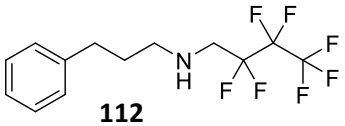
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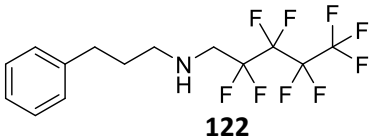
120
0%



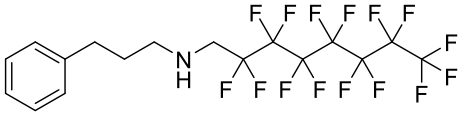
121
86%



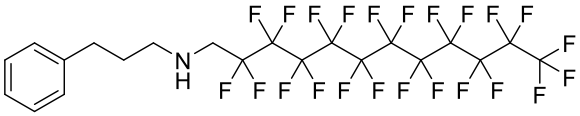
112
59%



122
83%



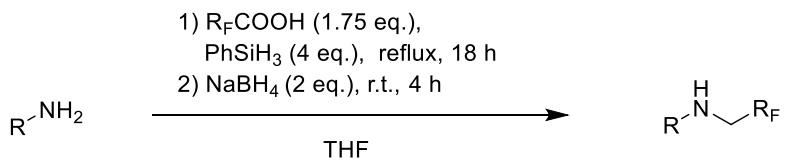
123
95%



124
39%

***Table 9**: Results of one pot formations of secondary perfluoroalkylamines.*

Trifluoroacetic acid was trialled mirroring the imine experiments and remained unsuccessful, suggesting that the imine is either not formed or hydrolyses in the reaction mixture, rather than during purification. With successful conditions determined and previous evidence of the imine formation step functioning on alternative amines to 3-phenylpropylamine, an investigation to prepare a variety of secondary amines *via* a two step-one pot process was performed.



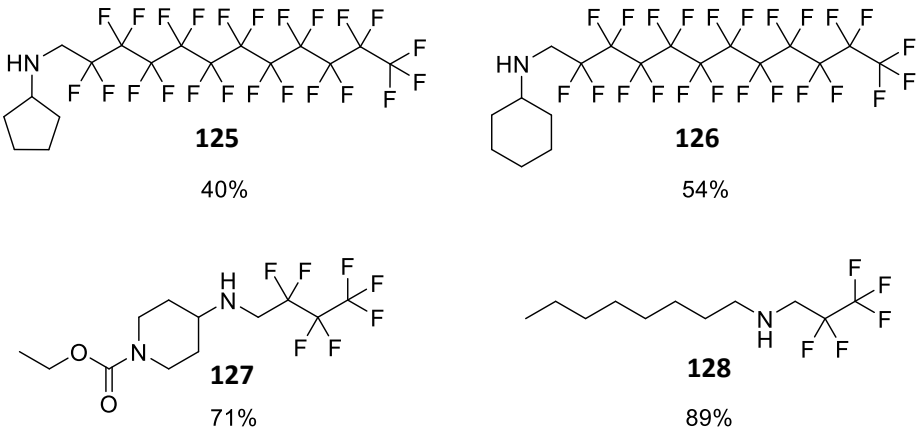
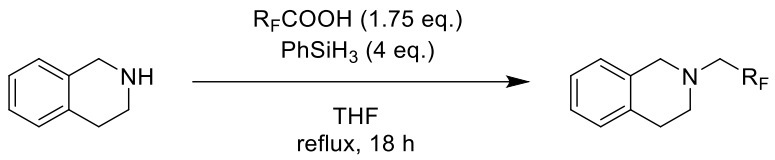


***Table 10**: Results from preparation of alternative perfluoroalkyl secondary amines.*

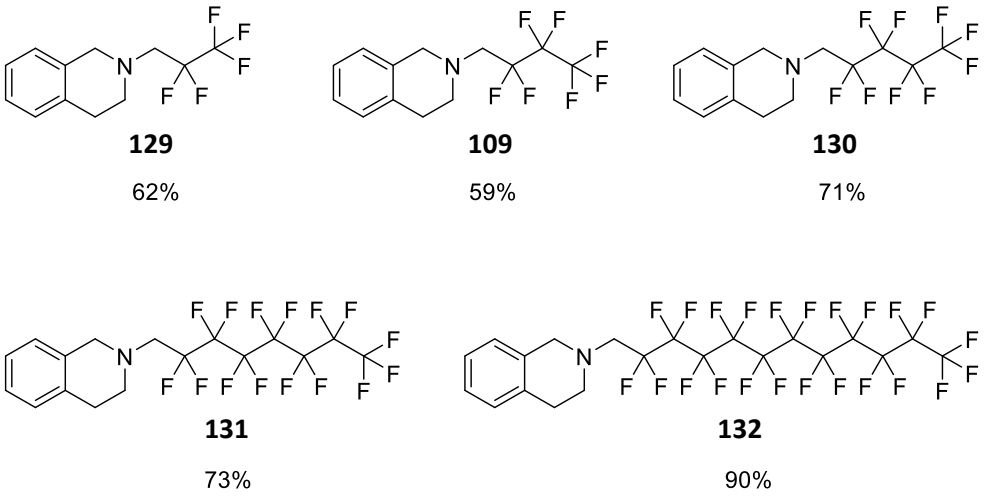
Yields were generally higher for the secondary amines than for the imines. This is possibly due to decomposition of the imine products during purification, either hydrolysis during workup or instability on silica. A variety of secondary amines with varying lengths of perfluorous tail were prepared, with a common test substrate and additionally some cyclic compounds, aliphatic compounds and interestingly one compound with an ethyl carbamate protecting group, **127**. This indicates that some acid labile protecting groups may be stable to the reaction conditions thus making this reaction widely applicable, likely due to the excess of phenylsilane rendering reaction conditions non-acidic, as anticipated when using perfluorous carboxylic acids due to an initial reaction that yields hydrogen gas.

4.4) Tertiary amines

The optimised conditions for perfluoroalkylation of primary amines were subsequently performed on secondary amines, to extend the previous work of trifluoroethylation and to give a general set of conditions capable of producing a wide variety of compounds.



108



129	**109**	**130**
62%	59%	71%

131	**132**
73%	90%

Table 11*: Results from preparation of perfluoroalkylamine derivatives of 1,2,3,4-tetrahydroisoquinoline.*

As the perfluorous chain grows in length, yields improved, a similar trend to previously prepared compounds. Additionally, these compounds were not plagued by isolation issues suffered by the imines or secondary amines and so yields for the perfluorooctanoic and perfluorododecanoic acid derived compounds remained high.

With positive results over the range of perfluorous carboxylic acids for the test amine, a small substrate scope was performed to generate an array of perfluoroalkyl tertiary amines.

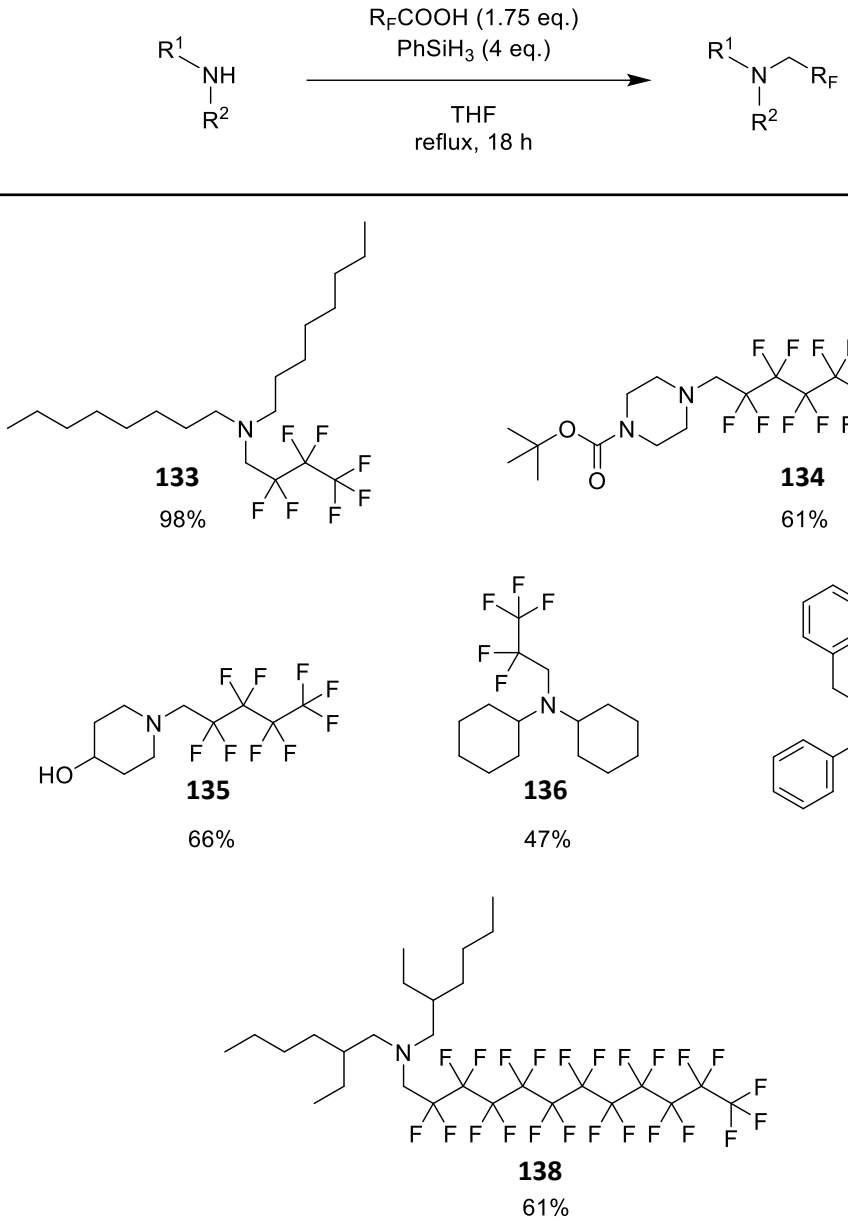


***Table 12**: Results from perfluoroalkylation of secondary amines.*

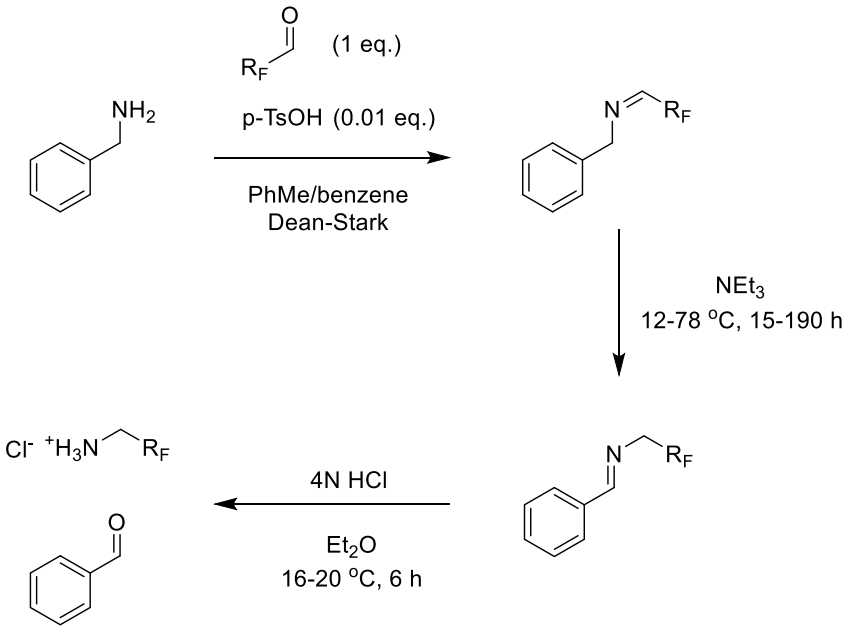
Of interest was the success of the dibenzylamine derived compound **137** as previous efforts to prepare compounds from benzylamine had proven ineffective. Yields were lower as steric bulk increased around the nitrogen and cyclic systems factored into this. This can be offset by using longer chain carboxylic acids which had proven to give superior yields from previous experiments, and yields of more congested nitrogens such as in **138** can provide a good yield. Of particular use are compounds **134** and **135**, as the Boc protecting group was stable to the reaction conditions and

the piperazine and hydroxy piperidine motifs are useful in medicinal chemistry[319,320]. The carbamate protecting group was previously determined stable under these conditions during the secondary amine substrate scope.

4.5) Primary perfluoroalkylamines

With successful preparation of secondary and tertiary 1,1-dihydroperfluoroalkylamines, efforts towards synthesis of primary amine variants was pursued. Whilst it is possible to use a source of ammonia to produce a primary amine, conditions are often forcing or low yielding[321–324].

The Soloshonok group published a preparation of 1,1-dihydroperfluoroalkyl primary amines (**Scheme 57**), condensing a fluorous aldehyde with benzylamine followed by a formal [1,3]-proton shift and subsequent hydrolysis in a biomimetic fashion for amine transfer[313].

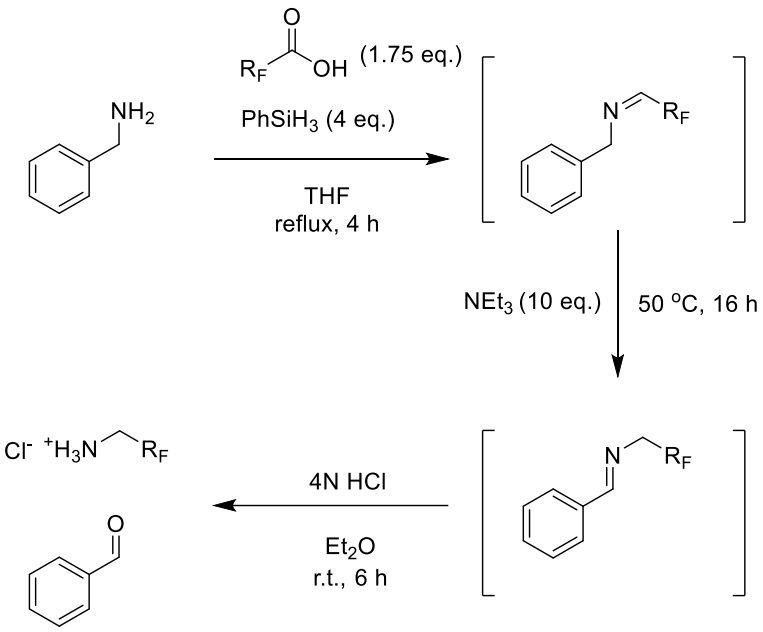


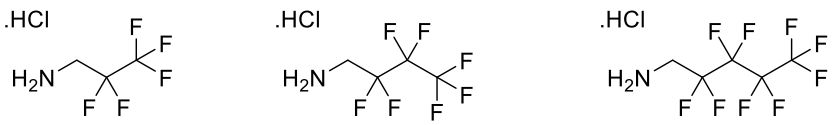
***Scheme 57**: Biomimetic synthesis of primary perfluoroalkylamines*[313].

Through methods developed in this project it was thought it may be possible to improve this synthesis using the more readily available perfluorous carboxylic acids in a reductive manner. Heptafluorobutyric acid (£0.51/mmol, Sigma-Aldrich) is

significantly cheaper than heptafluorobutyraldehyde (£34.60/mmol, Sigma-Aldrich), and is easier to store and work with due the highly reactive nature of the fluorous aldehydes, thus the use of a carboxylic acids could be a significant improvement.

It was determined that the benzylamine-imine was not easily isolable from this reaction mixture and the conversion benefitted from shorter reaction times. Conditions used by Soloshonok[313] were then adapted for the formal [1,3] proton shift and hydrolysis, with no purification of any intermediate and only concentration of the reaction mixture performed between each step.





139	**140**	**141**
34%	54%	53%

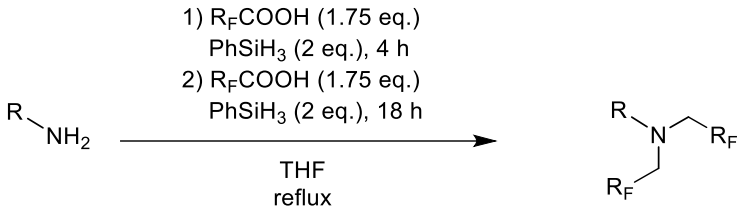
***Table 13**: Results for the preparation of primary 1,1-dihydroperfluoroalkylamines with primary perfluoroalkylamine products and yields.*

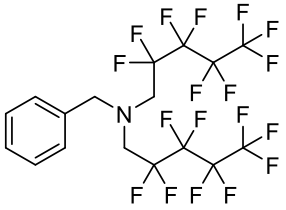
Purification of these compounds was facile with only a recrystallisation required and yields were pleasing after a three step-one pot procedure. Attempts to prepare the primary amine from perfluorooctanoic acid and perfluorododecanoic acid were unsuccessful due to purification issues. As perfluorous carboxylic acids behave like surfactants, this greatly complicated procedures and removal of excess acid from the desired amine was not possible for chains longer than perfluoropentanoic acid.

4.6) Double alkylation

For the fluorous imines prepared, ^{1}H NMR analysis of the crude material occasionally showed a minor quantity of doubly alkylated material. Selective preparation of either material could be another perk of this chemistry, permitting a series of yet greater fluorinated materials. Converting a hydrocarbon amine into a tertiary amine with two fluorous tails fills a niche between completely perfluorous compounds and the singly fluoroalkylated compounds prepared thus far.

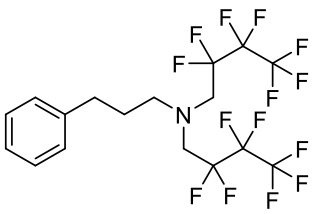
After numerous trials and optimisation it was found that portioned addition of both perfluorous carboxylic acid and phenylsilane yielded the best results.





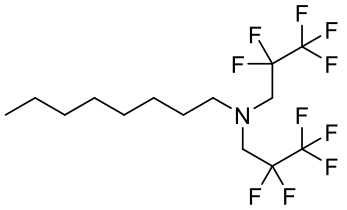
142

19%



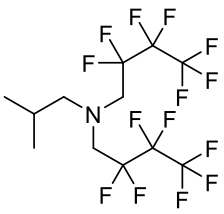
143

64%



144

64%



145

61%

Table 14*: General scheme for preparation of doubly alkylated compounds with products and yields shown.*

Yields were modest but consistent, with the exception of the benzylamine derived compound **142**. A lower yield was anticipated for this compound due to the previously observed instability of the derived imine to the reaction conditions. Despite this, for a two step-one pot procedure to prepare some highly unusual compounds selectively over the monosubstituted forms is highly pleasing.

During optimisation it was determined that simply increasing the quantity of reagents was insufficient to drive the formation of doubly alkylated material, with addition of 3.5 eq. of perfluorous acid and 4 eq. of phenylsilane in one portion solely generating the imine. By staggering addition in two portions it was found possible to use a relatively lower phenylsilane loading by ratio to the perfluorous acid in comparison to earlier results.

This observation implies that the imine is formed first and then under the reaction conditions is either reduced to the amine for further reaction, or the imine is directly alkylated a second time by nucleophilic attack of the nitrogen lone pair onto a second silyl acetal (**Fig. 84**). However, no secondary amine product was recovered from any experiment but unreacted imine was, suggesting that reduction to the secondary amine does not occur.

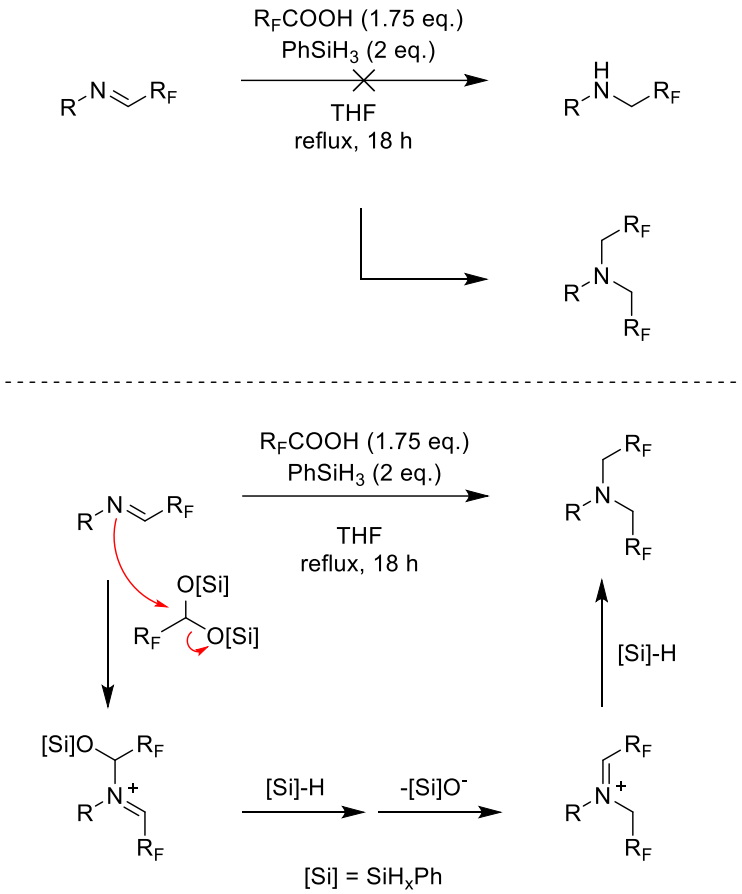


***Figure 84**: Observed products from the double alkylation procedure and proposed pathway.*

This proposed pathway and products observed are in accordance with the mechanistic study performed for trifluoroethylation of secondary amines, where a silyl acetal is formed which is a good electrophile[302], then nucleophilic attack forms an unstable iminium ion which is readily reduced. Whilst the nucleophilic character of the imine nitrogen lone pair will be diminished by the nearby fluorine, this could proceed to nucleophilically attack a second silyl acetal.

The primary amine would be a better nucleophile than the fluorous imine due to the availability of the nitrogen lone pair, as a result of the heavily electron withdrawing nature of adjacent fluorines. Therefore, the rate of reaction for the first alkylation would be significantly faster than for reductive alkylation of the imine. Under the reaction conditions, the silane compounds formed *in situ* will polymerise, and any silanes responsible for reduction may be inactivated. This would explain why portioned addition is required to prepare the dialkylated materials, as the extended

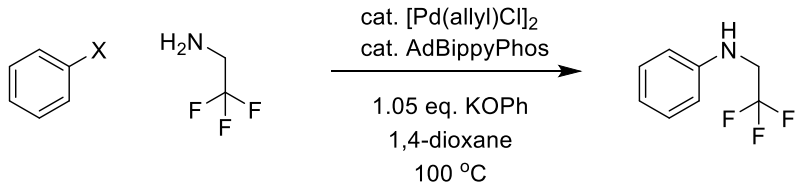
reaction time would lead to polymerisation of the silanes, and freshly prepared silyl acetals are required for a successful reaction due to loss of hydride.

4.7) Anilines

Preparation of primary, secondary and tertiary amines, imines and double alkylations have been achieved *via* the presented methodology, but aromatic amines are a remaining class of compound which could hypothetically be prepared.

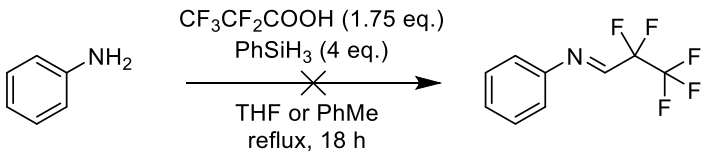
Anilines are a challenging substrate for this chemistry due to partial delocalisation of the lone pair into the π system of the aromatic ring. This renders them less nucleophilic and less reactive towards electrophiles such as the silyl acetal generated upon combination of the phenylsilane and the perfluorous carboxylic acid.

Very limited methods exist to prepare such compounds, and those which do often require metal catalysts or begin with an aryl halide and couple a fluorous amine, rather than beginning from an aniline derivative[325] (**Scheme 58**).



***Scheme 58**: Preparation of secondary fluorous aniline by palladium cross-coupling.*

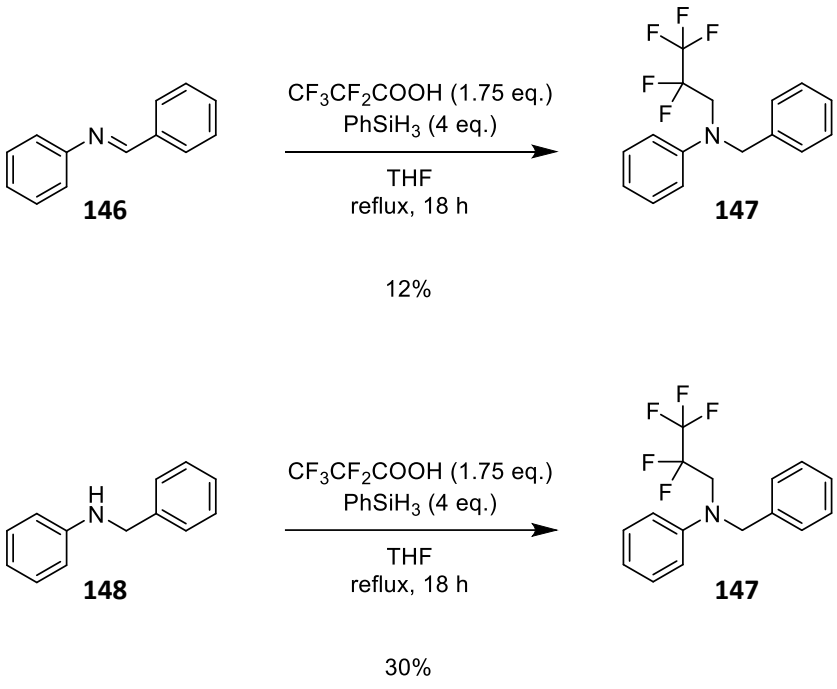
Initial experiments used aniline as a substrate and sought to prepare either the secondary amine or the imine *via* previous methods, but were unsuccessful (**Scheme 59**), with starting material and unknown decomposition products obtained.



***Scheme 59**: Initial experiment performed with aniline as a test substrate.*

Additional experiments that involved not isolating the imine were performed and a reducing agent was added in attempts to prepare the secondary amine, but in no instance was either the imine or amine product detected and analysis of recovered organics indicated a complex mixture.

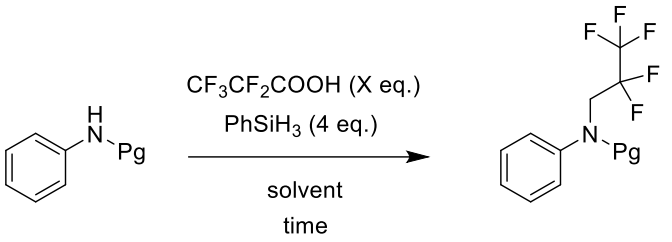
It was determined that a protecting group might be required in order to successfully prepare an aniline derivative. To minimise reduction in availability of the lone pair on nitrogen, common protecting groups for amines such as amides and carbamates were discounted. A benzyl group was selected and conditions from previous reactions were employed. The protected aniline was prepared *via* reductive amination, and both the imine and amine forms were trialled as substrates due to the positive results of double alkylation experiments (**Scheme 60**).



***Scheme 60**: Initial reactions for perfluoroalkylation of a secondary aniline.*

Moderate yields of the product were obtained along with significant recovery of starting material. It was noted that the organic recovery was lower than expected, between 70-80%, possibly due to deprotection of the aniline and loss through workup or chromatographic purification. Regardless, this was considered a pleasing result due to this being the first example of this methodology functioning on an

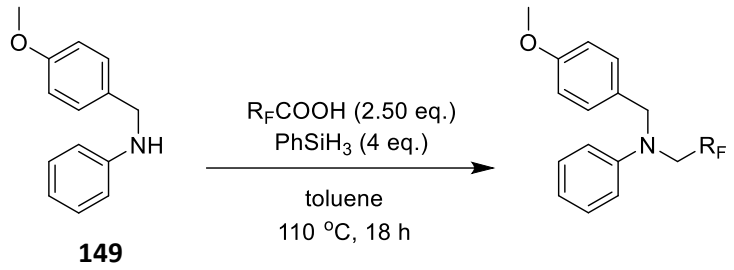
aromatic amine. That a sizeable quantity of starting material was recovered allowed for investigation into optimisation of the reaction conditions. In addition to benzyl as a protecting group, a *para*-methoxybenzyl group (PMB) was trialled due to easier deprotection. Both protecting groups were introduced by reductive amination.



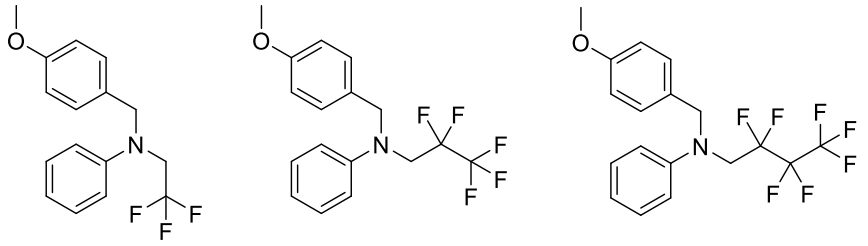
Entry	Pg	Solvent (Temp, °C)	X (eq.)	Time (h)	Yield (%)
1	benzyl	THF (70)	1.75	18	30
2	benzyl	THF (70)	1.75	42	20
3	benzyl	toluene (110)	1.75	18	36
4	benzyl	THF (70)	2.50	18	27
5	benzyl	toluene (110)	2.50	18	53
6	PMB	toluene (110)	2.50	18	53
7	PMB	toluene (110)	1.75	18	41

***Table 15**: Conditions trialled for perfluoroalkylation of protected anilines.*

Efforts to improve the yield without increasing the quantity of acid were unsuccessful and neither extended reaction times nor more forcing conditions lead to improvement. A combination of harsher conditions and excess reagent gave the most significant improvement, and whilst the yield remains moderate and large excess of reagents were employed, anilines are difficult substrates and significant advancements were made. With this successful result a screen of perfluorous acids was performed.



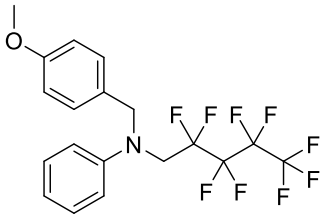
149



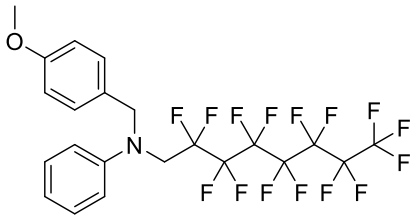
150
0%

151
53%

152
55%



153
53%



154
37%

***Table 16**: Scope with respect to the perfluorous carboxylic acid on aniline substrates.*

Whilst yields were only moderate, they were consistent across all trialled lengths of the perfluorous tail. These compounds were previously unattainable *via* this methodology and so is a vast improvement. Starting material was recovered in each instance indicating that there is scope for further improvement of this reaction. Trifluoroacetic acid was once again trialled; as these compounds are secondary anilines it was hypothesised that the reactions should proceed, however disappointingly no desired product was obtained.

There are numerous methods in the literature for PMB deprotection. Both oxidative and acidic measures were employed such as TFA, DDQ and CAN[326–328] however none were successful in generating the desired secondary aniline and decomposition occurred.

4.8) Failure of trifluoroacetic acid

Previous work demonstrated that TFA is viable for trifluoroethylation of secondary amines, however throughout this work on other classes of amine it was unable to produce the desired products, and starting materials were recovered. This suggests the imine was never formed, otherwise it would be readily reduced or remain at the imine stage.

It is anticipated that the longer chain perfluorous carboxylic acids proceed *via* the same silyl acetal intermediates as TFA, and the corresponding acetals should have a greater electrophilicity due to additional fluorine content. This suggests that the nucleophilicity of amines is an important factor, and that the TFA acetal may not be electrophilic enough for a primary amine to attack. It has been experimentally determined that the pK_aH of an amine is a poor measure of its nucleophilicity. In polar solvents such as MeCN and water, secondary amines are more nucleophilic than primary amines[329–331] (**Fig. 85**).

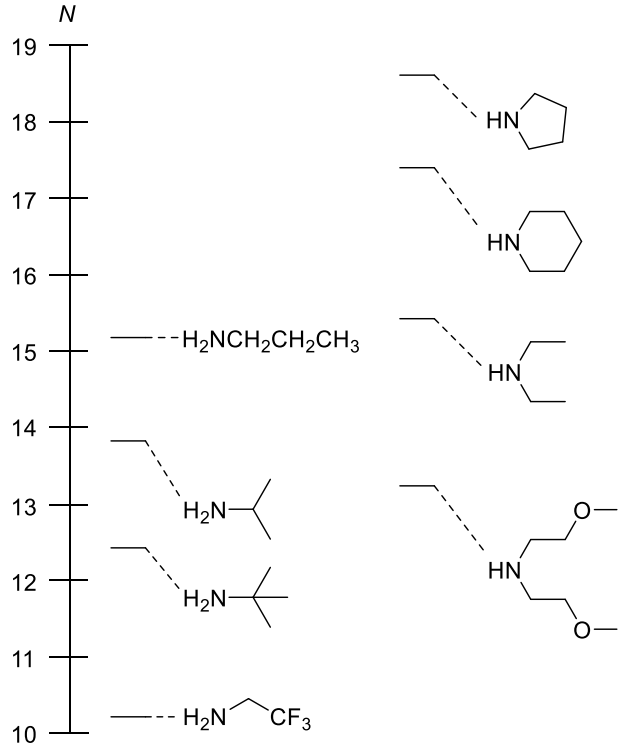


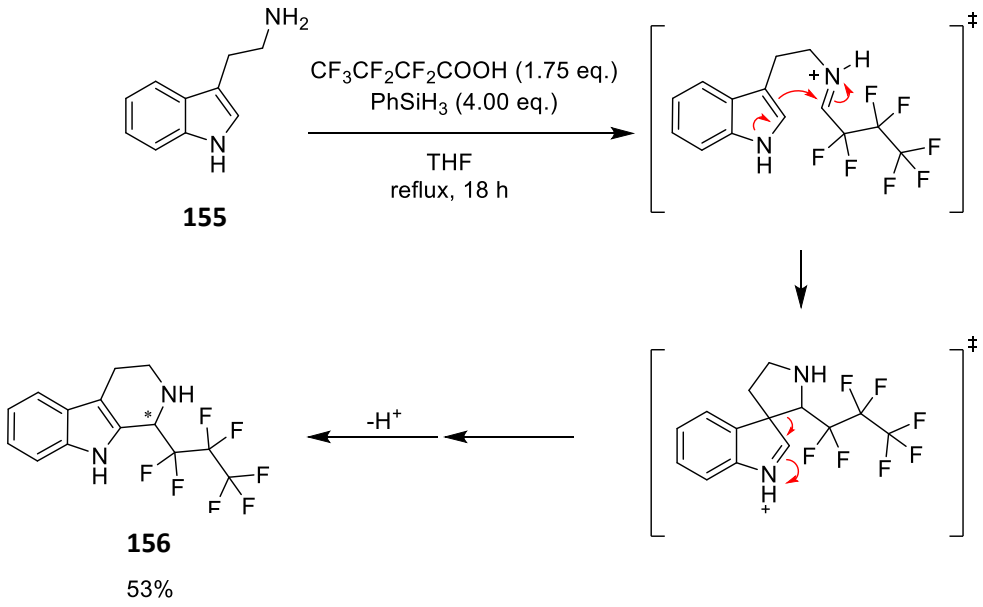
***Figure 85**: Comparison of the nucleophilic reactivities of amines in MeCN. N is a nucleophilicity parameter determined from the linear free energy relationship*[329].

This would suggest that a threshold is passed where a primary amine is nucleophilic enough to attack the silyl acetal upon increase of the fluorine content beyond TFA. This would also explain why the yields generally rise in accordance with greater quantities of fluorine as the strength of the electrophile is increased.

4.9) Cascade perfluoroalkylation-cyclisation reactions

The Pictet-Spengler reaction concerns condensation of an aldehyde with a β-arylethylamine and the resulting imine is involved in a ring closing reaction[332]. An industrially useful reaction, this is often performed under acidic conditions but is not required[333]. The driving force is the formation of the electrophilic imine from the condensation reaction.

It was hypothesised that as these heavily fluorinated imines would be highly electrophilic, the cyclisation should be facile under the reaction conditions with an excess of acid. Tryptamine (**155**) is a common substrate for the Pictet-Spengler reaction and was subjected to the general conditions.

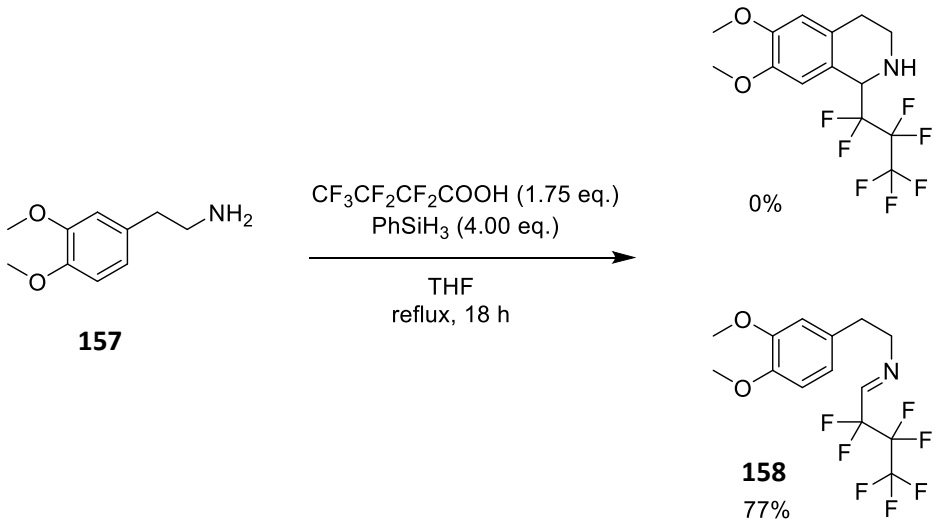


***Scheme 61**: Initial cascade Pictet-Spengler reaction with tryptamine.*

The tryptamine reaction proceeded well and in moderate yield, granting access to this cyclic fluoroalkyl motif **156** with an adjacent and functionalisable nitrogen.

Cyclisation occurred spontaneously (**Scheme 61**) and hypothetically a series of compounds of this general structure could be readily prepared with a fluoroalkyl chain in a position that would otherwise be difficult to synthesise.

Another common substrate is 3,4-dimethoxyphenylethylamine (**157**) which under harsher acidic conditions than the tryptamine is known to undergo Pictet-Spengler cyclisation to form the 1,2,3,4-tetrahydroisoquinoline. Strong acids added to the reaction mixture include hydrochloric acid and trifluoroacetic acid[334,335], and with the excess of perfluorous carboxylic acid present it was hypothesised that the reaction may proceed (**Scheme 62**).

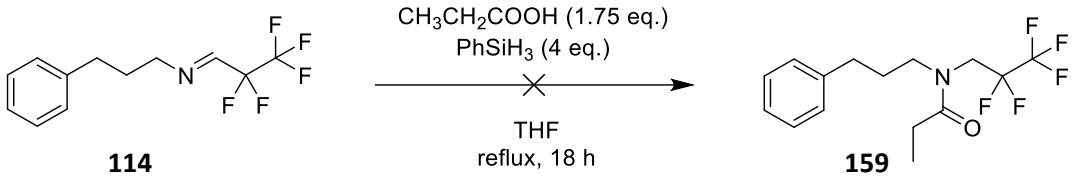


***Scheme 62**: Attempted Pictet-Spengler reaction of 3,4-dimethoxyphenylethylamine.*

Disappointingly none of the desired tetrahydroisoquinoline product was detected, and the intermediate imine was recovered in good yield. This is likely due to the reduced nucleophilic character of the aromatic ring in comparison to tryptamine. It is possible that altering the stoichiometry of the reaction may permit a one pot procedure or doping with a second, stronger acid, however as the imine was recovered in good yield and purification was facile conversion to the desired product should be simple following well established procedures.

4.10) Reductive alkylations of amides

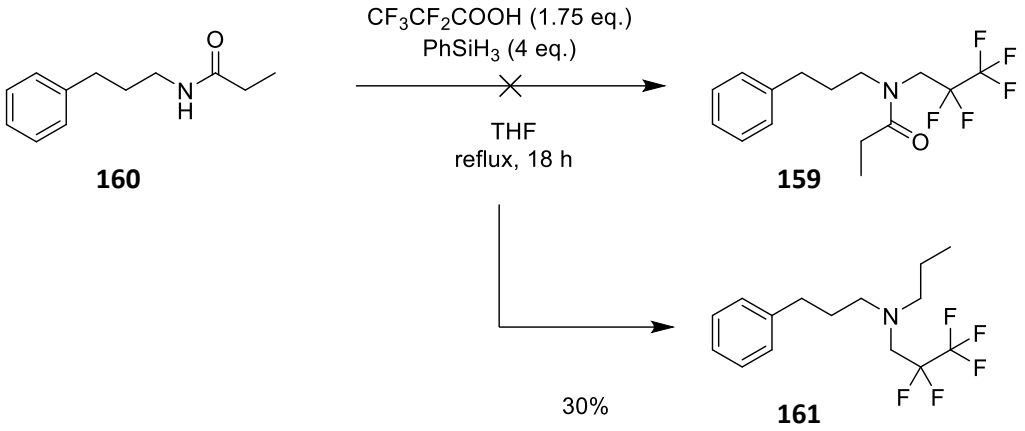
Whilst exploring reactions for the dialkylation procedure, an investigation into using non-fluorous carboxylic acids was undertaken. The desire was to expand current work to a one pot procedure to prepare complex tertiary partially fluorinated amines and amides without the use of an aldehyde. The first experiment prepared the imine and attempted to add an alkyl chain with propionic acid and either the tertiary amine or amide would be considered desired products (**Scheme 63**).



***Scheme 63**: Initial reaction trialling propionic acid to facilitate reduction.*

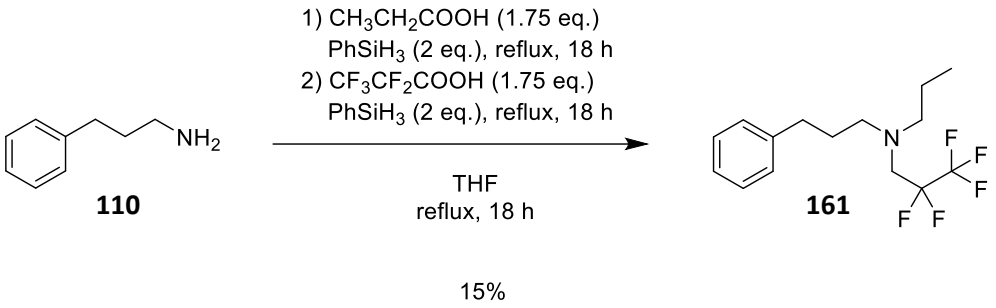
This reaction was unsuccessful and only imine starting material was recovered. The reaction failed due to the mechanism of addition of a fluorous chain being different to that of a hydrocarbon variant, with the mechanism requiring the formation of a fluorous silyl ester which undergoes nucleophilic attack from the amine[302].

A different approach was explored by first forming the amide and then inserting the fluorous chain, and it was hypothesised that whilst the lone pair on the nitrogen in an amide is delocalized in the system and poorly available, due to the strength of the electrophile it might be possible to generate complex tertiary amides. An initial experiment trialled propionic acid and pentafluoropropionic acid (**Scheme 64**), with an intermediate workup after formation of the amide.



***Scheme 64**: Attempted formation of tertiary amide and obtained tertiary amine product. ^{1}H NMR yield, using 1,1,2,2-tetrachloroethane as an internal standard.*

Surprisingly, the tertiary amide was not detected but rather the tertiary amine in moderate yield of 30%, with significant quantities of amide starting material recovered. This reaction was repeated in a one pot procedure starting with 3-phenylpropylamine and propionic acid, using $PhSiH_3$ as an amide coupling reagent, before additional $PhSiH_3$ and pentafluoropropionic acid were added with no workup or purification of the intermediate (**Scheme 65**).



***Scheme 65**: One pot-two step procedure to prepare a tertiary amine from a primary amine. ^{1}H NMR yield, using 1,1,2,2-tetrachloroethane as an internal standard.*

Whilst the yield was reduced and a small amount of primary amine starting material was recovered, large quantities of secondary amide intermediate **160** were recovered and no tertiary amide could be detected.

The mechanism for this reaction was uncertain and could proceed by two methods: formation of the tertiary amide, and the electron withdrawing nature of the fluorous

tail may be significant enough to facilitate amide reduction, or by reduction of the secondary amide followed by alkylation of the secondary amine (**Fig. 86**).

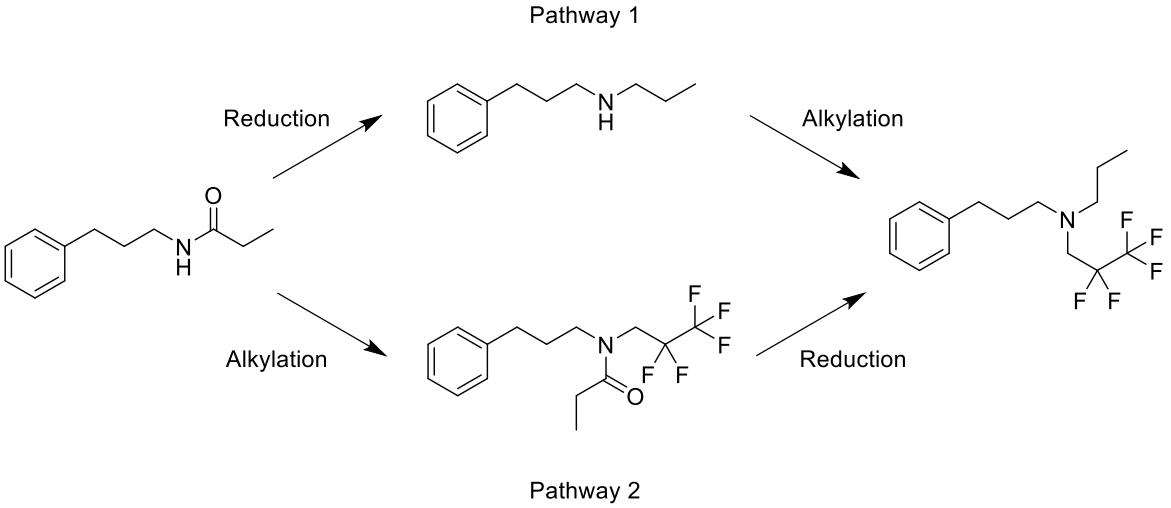
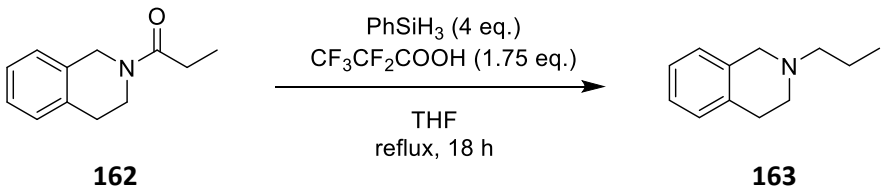


Figure 86: *Possible pathways towards formation of tertiary amines from secondary amide.*

In order to determine which pathway was prevalent, a tertiary amide was prepared and subjected to the reaction conditions (**Scheme 66**). Should the tertiary amide be recovered then this would suggest that alkylation occurs first, followed by reduction. A simple amide was prepared and subjected to the same reaction conditions as before.



162 **163**

28%

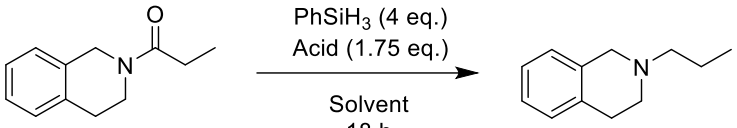
Scheme 66*: Reduction of tertiary amide with silane and perfluoropropionic acid.* ^{1}H *NMR yield, using 1,1,2,2-tetrachloroethane as an internal standard.*

This result was pleasing, achieving a moderate yield of the desired amine and a large quantity of recovered amide, indicating significant scope for optimisation. This was also a surprising result, as metal catalysts are commonly required for similar silane reductions. This reaction is known to be acid catalysed, and so this result would

suggest that the perfluorous acid is strong enough that the silyl ester formed is reactive enough that it can reduce the amide without an added catalyst.

The result of the tertiary amide reduction lead to the hypothesis that reduction occurs first and alkylation second, but cannot exclude the possibility of a second pathway.

This procedure is of interest as silane mediated reductions of amides currently employ metal catalysts. Phenylsilane is additionally capable of performing the amide coupling[336], opening up the possibility of a one pot procedure to progress from amine through amide back to higher order amine, using a sacrificial acid to replace the metal catalyst. A screen of various acids was performed to determine the most suitable system.



162 **163**

Entry	Acid	pKa (H_2O)	Solvent (Temp, °C)	Yield (%)
1	Perfluoropropionic	0.18	THF (70)	34
2	Perfluorobutyric	0.18	THF (70)	48
3	HBF_4	-0.44	THF (70)	0
4	Methanesulfonic	-1.9	THF (70)	35
5	Tosic	-2.8	THF (70)	39
6	Benzenesulfonic	-2.8	THF (70)	53
7	Triflic	-14.7	THF (70)	-
8	Perfluoropentanoic	0.18	1,4-dioxane (105)	37
9	Perfluorobutyric	0.18	1,4-dioxane (105)	33
10	Benzenesulfonic	-2.8	1,4-dioxane (105)	77
11	Benzenesulfonic	-2.8	PhMe (110)	63
12	Triflic	-14.7	PhMe (110)	91

***Table 17**: Conditions and acids screened during trial reactions. ^1H NMR yields, using 1,1,2,2-tetrachloroethane as an internal standard.*

Yields improved as the temperature of the reaction was increased, with a polar solvent such as 1,4-dioxane being more successful than toluene for benzenesulfonic acid, likely due to increased dissociation of the acid. A concern with using cyclic ethers as solvents and these strong acids was ring opening and polymerisation of the parent alcohols, and occurred in significant quantities for triflic acid in THF leading to inability to determine an NMR yield for this reaction. Some polymerisation was seen for HBF_4 but significantly less than with triflic acid. The reaction with HBF_4 was unable to form any product and suggests that the $PhSiH_3$ plus a proton is not sufficient to effect the desired transformation. This also suggest that the hydrosilane is not the active reducing agent, and that the silyl esters might be instead.

The most pleasing results were those of benzenesulfonic acid and triflic acid, able to produce the desired tertiary amide in 77% and 91% yield respectively after solvent and temperature changes. Use of both of these acids is widespread and so both were taken forward for further development towards a one pot reaction for reductive alkylation of amides.

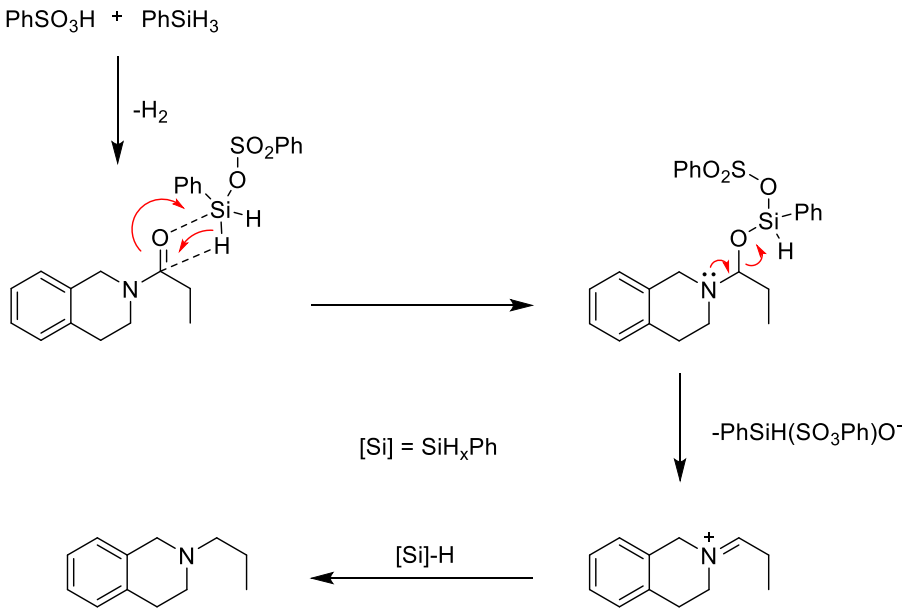


***Figure 87**: Proposed mechanism for tertiary amide reduction by developed methodology.*

Reduction must occur *via* activation of the silane or activation of the carbonyl. It has been shown in the literature that reaction of a strong acid with a hydrosilane forms

a silyl ester and produces hydrogen[337]. A plausible mechanism first forms the silyl ester and the carbonyl of the amide coordinates to silicon, facilitating the transfer of a hydride to the amide during a four membered transition state. The lone pair of nitrogen aids in eliminating the silicate and the unstable iminium ion is subsequently reduced by a currently undetermined hydrosilane species (**Fig. 87**).

The reduction of an existing amide sets this reaction apart from the previous perfluoroalkylation reactions, where work had demonstrated that an exogenous amide was not reduced, and determined that an activated reductant to reduce an amide was not formed[302] (**Fig. 88**).

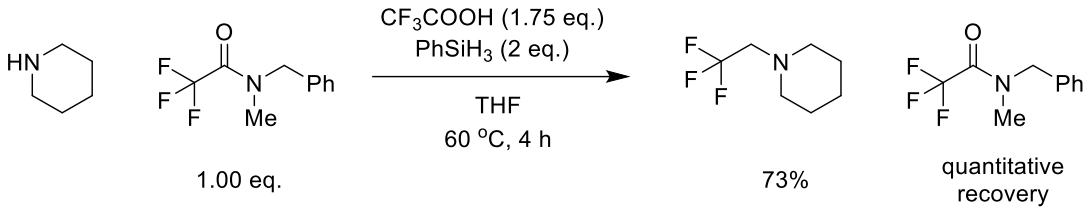


***Figure 88**: Trifluoroethylation of secondary amide in the presence of an exogenous amide, with complete recovery and no evidence of reduction.*

During comparison of the NMR spectra of the trial reactions, it was clear that a large number of side products were formed when using the perfluorous carboxylic acids. Reactions performed with the sulfonic acids were clean with minimal impurities, which in combination with higher yields makes those reactions significantly more favourable.

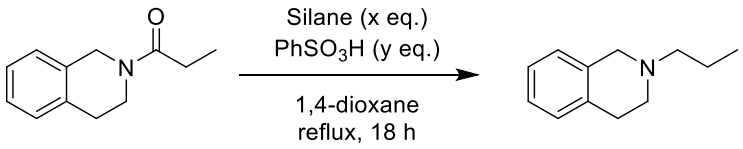
Alternative silanes

Phenylsilane was selected for the initial reactions as it is capable of performing amide coupling[336], which would allow for both the preparation and reduction of an amide without the requirement of a second silane.

Using an alternative silane may lose the amide coupling component of the reaction, however a metal and catalyst free amide reduction could be developed, using a cheaper or greener silane as the reducing agent, in place of $PhSiH_3$. To use a strong

acid to activate a low-cost silane, or one that is the by-product of an industrial process, would be a worthwhile procedure to develop.

With a series of acids screened, alternative silanes were trialled for a potential second reaction to be developed. Benzenesulfonic acid was selected as the acid to use throughout these trials as this reagent would be likely more applicable for a wide range of amides than triflic acid.



162 **163**

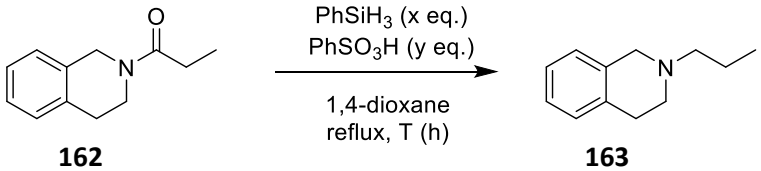
Entry	X (eq.)	Y (eq.)	Yield (%)
1	PMHS (4)	1.75	0
2	TMDS (2)	1.75	0
3	Ph_2SiH_2 (4)	1.75	41
4	Ph_3SiH (4)	1.75	0

***Table 18**: Conditions trialled for alternative silanes in the reduction of tertiary amides. ^1H NMR yields using 1,1,2,2-tetrachloroethane as an internal standard.*

Disappointingly, no improvement over $PhSiH_3$ was found for alternative silanes. A moderate yield was achieved with diphenylsilane but no product was detected when using triphenylsilane. This observation follows the trend of a reduced yield as the hydride equivalents are lowered from $PhSiH_3$ > Ph_2SiH_2 > Ph_3SiH. Upon reaction with the acid a hydride equivalent is lost, leaving products prepared from Ph_3SiH with no hydride equivalents and unable to perform the reduction. It was hoped that polymethylhydrosiloxane (PMHS) would be an effective reagent due to its low cost, however only amide starting material was recovered. Similarly, tetramethyldisiloxane (TMDS) did not generate the product and starting materials were recovered, suggesting that the electron donating properties of the phenyl groups are important for reduction to occur.

Optimisation of conditions for reduction of amides

With both triflic acid and benzenesulfonic acid identified as highly suitable for the reaction, attempts to improve the conditions by reducing the quantity of silane and acid were performed. Initially the benzenesulfonic acid conditions were optimized.



162 **163**

Entry	X (eq.)	Y (eq.)	T (h)	Yield (%)
1	2.00	1.75	18	85
2	2.00	1.75	4	61
3	2.00	1.75	6	61
4	1.00	1.00	18	42
5	1.50	1.25	18	56
6	1.75	1.50	18	67
7	2.00	0.50	18	39
8	0.50	1.75	18	0
9	0.00	1.75	18	0
10	2.00	0.00	18	0

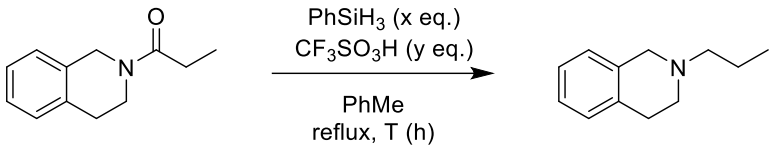
***Table 19**: Conditions screened during trial reactions. ^{1}H NMR yields using 1,1,2,2-tetrachloroethane as an internal standard.*

It was chosen to optimise the benzenesulfonic acid conditions despite a lower yield in the initial screen, as a reflux in the presence of triflic acid are extremely harsh conditions and may not prove suitable for a wide variety of substrates.

The initial reaction lowered the quantity of silane and acid and gave a slight improvement in the yield. Reducing the reaction time was unsuccessful and an overnight reflux proved critical, and lowering the quantity of silane and acid further whilst maintaining a similar ratio only led to decreased yields. Final reactions looked to see if the acid or silane could be used in a substoichiometric quantity. It is hypothesised that the silyl ester formed between acid and silane is the active

reducing agent, so a reduced quantity of acid but similar silane loading may allow for multiple reductions per silyl ester. Similarly, the silane is not one hydride equivalent but three and so a lower quantity with excess acid may suffice. It was found that with a substoichiometric quantity of acid the reaction still proceeded but in lower yield. For a reduced quantity of silane the reaction ceased to proceed at all and only amide starting material was recovered.

A second series of conditions was optimized using triflic acid.



162 **163**

Entry	X (eq.)	Y (eq.)	T (h)	Yield (%)
1	2.00	1.75	6	79
2	2.00	1.75	18	>99
3	1.75	1.50	18	98
4	1.50	1.25	18	94
5	1.25	1.00	18	74
6	1.00	1.00	18	81
7	1.00	0.50	18	56
8	0.50	1.00	18	11
9	0.00	1.25	18	0
10	2.00	0.00	18	0

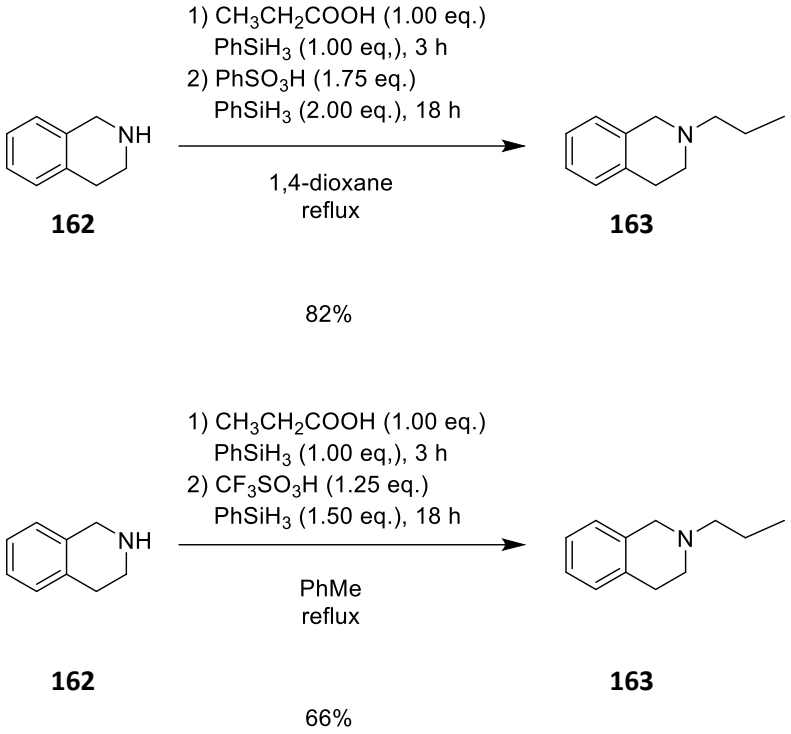
Table 20: *Conditions screened during trial reactions. ^{1}H NMR yields using 1,1,2,2-tetrachloroethane as an internal standard.*

The results of the triflic acid screen show that very high yields can be achieved with significantly less reagent than for the analogous $PhSO_3H$ reactions. The reaction time could be significantly shortened and yield maintained by nearly doubling the quantity of reagents, and a yield of over 94% was attained with only 1.50 eq. of silane and 1.25 eq. of acid. This yield is exceptional but even a minor increase in reagent quantity can push the yield yet higher, which may be useful in the case of a difficult

substrate. Yields for reactions with substoichiometric quantities of acid and silane once again demonstrated reduced yields but were noticeable improvements over those for benzenesulfonic acid, a trend present for all comparable entries in the optimisation tables.

One pot reactions

With optimised conditions achieved for the test substrate for two different acids, a one pot reaction for *N*-alkylation of secondary amines was trialled. Two one pot reactions were performed with an initial amide coupling step followed by addition of further reagents to effect the full transformation (**Scheme 67**).



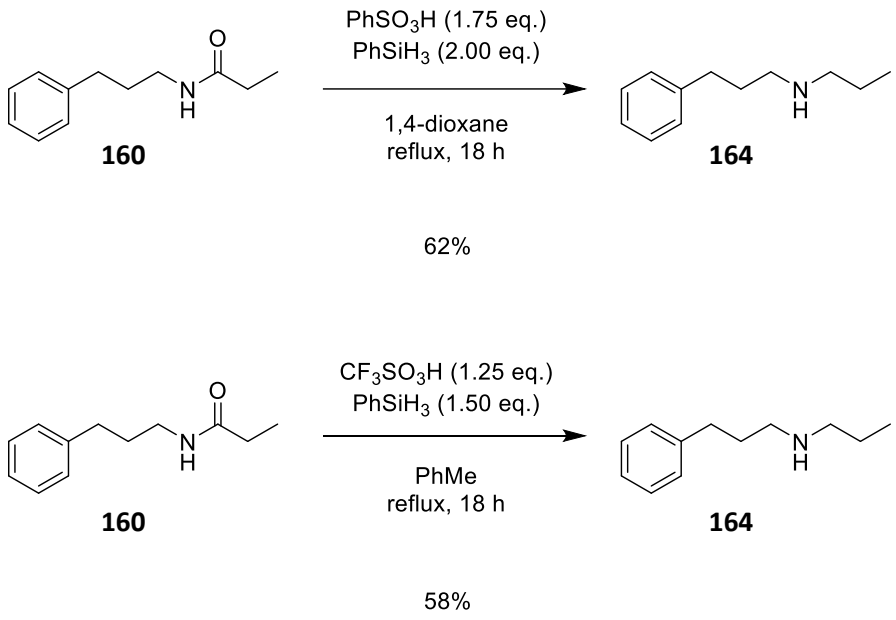
***Scheme 67**: Two step-one pot metal free alkylations of secondary amines. ^{1}H NMR yields, using 1,1,2,2-tetrachloroethane as an internal standard.*

The yields from these reactions were pleasing, especially in the case of $PhSO_3H$ which over two steps maintained a similar yield as was achieved for the one reaction of solely reducing an amide. The result for triflic acid however was disappointing and a sizeable quantity of amide was recovered. During optimisation it was noted that as

the silane loading increased in relation to the quantity of acid, the yield dropped significantly. Under these one pot conditions the concentration of silanes and related by-products from the amide coupling is significantly higher than for the single transformation of the amide reduction, and this in combination with an increase in number of steps may explain the drop in yield.

Reduction of secondary amides

For a general method of reducing amides, reduction of a secondary amide as well as a tertiary amide must be achieved. A similar amide was prepared and subjected to the optimised reaction conditions as determined for the tertiary amides (**Scheme 68**).



***Scheme 68**: Reduction of secondary amides by developed methodology. ^{1}H NMR yields, using 1,1,2,2-tetrachloroethane as an internal standard.*

Pleasingly, in each case the reaction was successful and with moderate yields. An initial reason for pursuing the use of sulfonic acids instead of fluorous acids was that unlike the carboxylic acids which incorporated into the compounds to form tertiary amines, no evidence could be found for sulfonamide formation. In neither instance of using $PhSO_3H$ or triflic acid was any sulfonamide detected which would indicate a different pathway occurring.

4.11) Conclusions and future work

Foundation work on Meerwein salt assisted synthesis of fluorous amines from amides was performed, with minor success achieved for the preparation of tertiary amines and a proof of concept for secondary amines. With the difficulties determined it may now be possible through further work to overcome them and a simple and mild process may be developed.

One pot fluoroalkylation of amines was highly successful, expanding previous work from trifluoroethylation of secondary amines using one general method to encompass primary and secondary amines, anilines, imines and multiple fluoroalkyl chains. Further work could include tuning the procedure for each substrate with minor alterations on a common system for greater yields.

A new line of investigation relating to additions to the imines could generate complex tertiary amine products both fluorous and non-fluorous. An initial foray into this led to the discovery of combining an acid and phenylsilane as a reducing system without the use of metal catalysts. The two optimal reducing systems identified with triflic acid and benzenesulfonic acid are promising, and future experiments should investigate the possibility of a microwave assisted synthesis to achieve higher temperatures and shorten reaction times.

The Pictet-Spengler reactions demonstrated how electrophilic the fluorous imines are, and so reactions requiring strong electrophiles are potential targets for expansion of these methods. Further cyclisations both electrophilic and radical are attractive targets and the preparation of complex, cyclic fluorous compounds through such facile means would be a great achievement enabling the rapid production of a range of compounds otherwise inaccessible due to synthetic difficulty or cost.

5) Experimental

General experimental and equipment

NMR spectra were recorded on a Bruker AV-500 (500 MHz), Bruker DPX-400 (400 MHz), Bruker AV-400 (400 MHz) or Bruker DPX-300 (300 MHz) spectrometer as dilute solutions at ambient temperature and referenced to the residual solvent peak[338]. The chemical shifts are quoted in ppm and the multiplicity denoted by: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), hept (heptet), m (multiplet) or a combination of the above. Coupling constants (*J*) are quoted in Hertz and assignments done on the basis of chemical shift, distortionless enhancement by polarization transfer (DEPT), and 2D NMR spectra – COSY (correlation spectroscopy), HSQC (heteronuclear single quantum coherence spectroscopy) and HMBC (heteronuclear multiple bond correlation spectroscopy).

Infrared spectra were recorded on a PIKE technologies GladiATR as an ATR (attenuated total reflectance) and peaks are quoted as wavenumber (cm^{-1}).

Mass spectra were recorded on a Bruker MicroTOF system using electrospray ionisation techniques operating in positive and negative ion mode.

Liquid chromatography-mass spectrometry (LCMS) analysis was performed using an Agilent 1260 Infinity HPLC with a 6120 Quadrupole mass spectrometer. Chromatography conditions: Low pH – Waters Sunfire C18 3.5µm 2.1 x 30 mm column. Mobile phase A: 0.1% formic acid in water, mobile phase B: 0.1% formic acid in acetonitrile. High pH – Waters XBridge C18 3.5µm 2.1 x 30mm column. Mobile phase A: 0.1% ammonia in water, mobile phase B: acetonitrile. Method run at 40 °C with a flow rate of 0.8 mL/min, gradient of 5-95% mobile phase B over 3.5 minutes with UV detection at 210 – 400 nm reported at 254 nm.

Reactions were monitored by thin layer chromatography (TLC) using aluminium plates precoated with Merck silica gel 60F254 and visualized using ultraviolet light (λ_{max} = 254 nm), potassium permanganate, vanillin, ninhydrin or iodine on silica stains.

Thin layer chromatography-Mass spectrometry (TLC-MS) was performed on Advion Expression mass spec using electrospray ionisation with CAMAG TLC interface.

Flash chromatography was performed on Fluorochem silica gel 60Å, grade 43-60µ and eluted with specified solvents.

Solvents were used as provided without distillation. Anhydrous solvents, amines or alcohols were used as purchased or prepared by the following methods: Anhydrous *N,N*-diisopropylethylamine (DIPEA) and triethylamine (NEt_3) were distilled over KOH and stored over KOH in an amber glass bottle. Aniline was distilled under vacuum over KOH and stored over KOH in an amber glass bottle. Anhydrous acetonitrile (MeCN) was distilled and stored over 4 Å molecular sieves. Anhydrous 1,4-dioxane and ethyl acetate (EtOAc) were prepared by storage over 4 Å molecular sieves. Anhydrous toluene and diethyl ether (Et_2O) were obtained from solvent drying towers, passing solvent over activated alumina. Anhydrous tetrahydrofuran (THF), dichloromethane (CH_2Cl_2) and dimethylformamide (DMF) were used as purchased.

General procedure for Dowex ion exchange resin

Dowex ion exchange resin – 50WX2, 50WX4, 50WX8

Dowex resin was packed in a glass column with water and washed until the eluate was colourless. The packed resin was washed with 1M HCl solution until the eluate was colourless, then with water until colourless, and then with 2M NH_4OH solution until colourless. The resin was subsequently washed with water, activated by 1M HCl solution and washed with water until the eluate was pH neutral. Compounds were loaded onto the Dowex column as a solution in 1M HCl and washed with water until the pH was neutral. Compounds were eluted with 2M NH_4OH solution and the solvent removed to give the desired product.

Biological procedure 1

Cells cultured and assays run by Agata Mieżaniec.

Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% Fetal Bovine Serum, 1% Antibiotic-Antimycotic, 1% L-glutamine and 1% Non-essential Amino Acids at 37 °C in a humidified incubator with 5% CO_2. A 96 well plate containing the cells and growth medium was prepared 24 hours before the testing was carried out.

Stock solutions of test compounds and control compounds were prepared using sterile water and equipment (autoclaved). A dilution series was performed to prepare stock solutions with concentrations of 1 mM, 100 µM and 10 µM. For all compounds, 5 µL of each stock solution was added to a well resulting in the test concentrations of 100 nM, 1 µM, 10 µM and 100 µM for each compound. Cell growth was observed under a microscope, and then stained with the dye provided as a part of ab112118 Cell Cytotoxicity Assay Kit – Colorimetric, provided by abcam. The cells were incubated at the above conditions for a further 3 hours and then absorbance measured on a plate reader at 570 nm and 605 nm.

Cytotoxicity assays – HeLa cells

HeLa cells were seeded at 5000/well in 96 well plate. Photos were taken after 24 hours and 48 hours exposure to tested compounds. Cytotoxicity was measured after 48 or 72 hours of exposure to tested compounds and 3 hours of incubation with cytotoxicity dye. All experiments carried out in triplicate and results averaged.

DMOG assay

HeLa cells were seeded at 5000/well in 96 well plate. A 5 mM solution of DMOG was prepared and a 10 µM solution of difluorinated nucleoside **B4** (**50**). Wells were prepared testing both DMOG and the difluorinated nucleoside separately and combined, using water as a control. Cytotoxicity was measured after 48 hours of

exposure to tested compounds and 3 hours of incubation with cytotoxicity dye. All experiments carried out in triplicate and results averaged.

Alternate cell lines assay

Cells were seeded at 5000/well in 96 well plate. Cytotoxicity was measured after 48 hours of exposure to tested compounds and 3 hours of incubation with cytotoxicity dye. All experiments carried out in triplicate and results averaged.

General procedure to determine yields by ^{1}H NMR spectroscopy

The crude reaction mixture was dissolved in $CDCl_3$ and to this solution was added 1,1,2,2-tetrachloroethane (0.25 mmol, 42 mg or 0.50 mmol, 84 mg) and the ^{1}H NMR spectra recorded. The percentage yield was calculated by the following equation:

$$yield\ (\%) = \left(\frac{integral\ (product)}{integral\ (standard)}\right) * \left(\frac{no.nuclei\ (standard)}{no.nuclei\ (product)}\right)$$

For compounds where the amine or amide starting material was commercially purchased, an additional purity term was included for a greater accuracy, and was measured as the percentage purity of the 1,1,2,2-tetrachloroethane standard and the percentage purity of the starting material.

$$yield\ (\%) = \left(\frac{integral\ (product)}{integral\ (standard)}\right) * \left(\frac{no.nuclei\ (standard)}{no.nuclei\ (product)}\right) * \left(\frac{purity\ (standard)}{purity\ (starting\ material)}\right)$$

NMR assignments for phosphoramidites and precursor compounds

Assignments of NMR peaks was attempted for all nucleosides, however despite the collection of 2D data, due to overlap of peaks it was not always possible to differentiate the signals. Assignments have been made for nuclei with a certain identity, and other peaks simply listed. Examples in the literature of phosphoramidites in particular occasionally only perform ^{31}P NMR analysis due to

instability of the compounds, and identity is confirmed by successful incorporation into DNA.

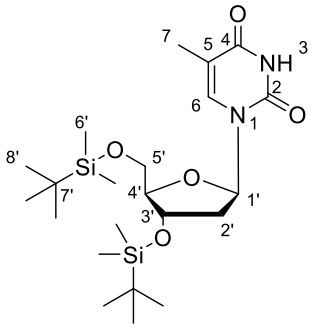
NMR assignments for heavily fluorous compounds

NMR methods were developed to aid assignments of the heavily fluorous compounds. Coupling of fluorine to carbon rendered the $^{13}C\{^{1}H\}$ spectra difficult to analyse and determination of centre points and multiplicities was not always possible without access to a triple resonance probe capable of performing ^{19}F experiments.

A series of $^{13}C\{^{19}F\}$ experiments were used to aid in multiplet determination of the $^{13}C\{^{1}H\}$ spectra. A ^{13}C-^{19}F HSQC experiment was developed and in combination with $^{13}C\{^{1}H\}$, $^{13}C\{^{19}F\}$ and ^{19}F data some assignments could be made. Due to similar chemical shifts and low resolution of the developed method, it was not always possible to make accurate assignments, and peaks are simply listed. For compounds derived from perfluorooctanoic and perfluorododecanoic acid, the spectra were too complex to differentiate all but the terminal carbon units.

5.1) Chapter 1

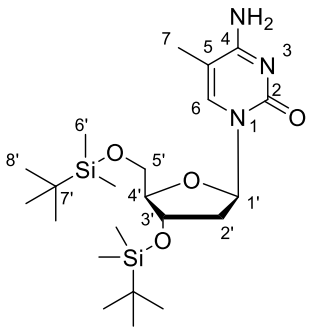
3',5'-(O-*tert*-Butyldimethylsilyl)-thymidine (39)[339]



To a solution of imidazole (7.02 g, 0.103 mol) in anhydrous dimethylformamide (45 mL) was added thymidine (5.00 g, 20.6 mmol) and *tert*-butyldimethylsilyl chloride (7.47 g, 49.5 mmol) and stirred at room temperature under nitrogen balloon. After 18 hours, reaction diluted with diethyl ether (150 mL), separated, and the dimethylformamide layer was extracted with diethyl ether (50 mL). The combined organics washed with water (5 x 150 mL) and brine (150 mL), dried ($MgSO_4$) and the solvent removed under reduced pressure to give the Title Compound as a white solid (9.70 g, quantitative).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.6. **Mp:** 121-123 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.49-7.47 (m, 1H, H-6), 6.37-6.34 (m, 1H, H-1'), 4.43-4.39 (m, 1H, H-3'), 3.95-3.93 (m, 1H, H-4'), 3.88 (dd, *J* = 11.4, 2.5 Hz, 1H, H-5'), 3.77 (dd, *J* = 11.4, 2.5 Hz, 1H, H-5'), 2.26 (ddd, *J* = 13.1, 5.8, 2.6 Hz, 1H, H-2'), 2.05-1.97 (m, 1H, H-2'), 1.93-1.91 (m, 3H, H-7), 0.94 (s, 9H, H-8'), 0.90 (s, 9H, H-8'), 0.13-0.11 (m, 6H, H-6'), 0.09 (s, 3H, H-6'), 0.08 (s, 3H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 164.0 (C, C-4), 150.4 (C, C-2), 135.4 (CH, C-6), 110.8 (C, C-5), 87.8 (CH, C-4'), 84.8 (CH, C-1'), 72.2 (CH, C-3'), 63.0 (CH_2, C-5'), 41.4 (CH_2, C-2'), 25.9 (CH_3, C-8'), 25.8 (CH_3, C-8'), 25.7 (2 x CH_3, C-8'), 18.4 (C, C-7'), 18.1 (C, C-7'), 18.0 (C, C-7'), 12.5 (CH_3, C-7), -3.0 (CH_3, C-6'), -3.6 (CH_3, C-6'), -4.6 (2 x CH_3, C-6'), -4.8 (CH_3, C-6'), -5.4 (CH_3, C-6'), -5.5 (CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3152, 3026, 2952, 2929, 2866, 2857, 1703, 1664,1471, 1426, 1401. **HRMS** (ESI^+): Calc. for $C_{22}H_{43}N_2O_5Si_2$ ($M+H^+$) 471.2705. Found 471.2708. Calc. for $C_{22}H_{42}N_2NaO_5Si_2$ ($M+Na^+$) 493.2524. Found 493.2535. Calc. for $C_{25}H_{47}N_4O_5Si_2$ ($M+C_3H_5N_2^+$) 539.3080. Found 539.3090.

5-Methyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (40)[340]

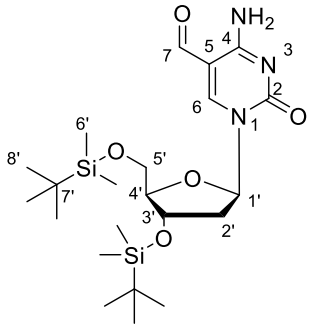


To a solution of 1,2,4-triazole (27.9 g, 0.404 mol) in acetonitrile (80 mL) was added triethylamine (60.8 mL, 0.436 mol). Phosphorous (V) oxychloride (8.15 mL, 87.4 mmol) was added dropwise at 0 °C and the resultant slurry stirred for 30 minutes. To this slurry was added a solution of 3',5'-(O-*tert*-butyldimethylsilyl)-thymidine (**39**) (5.00 g, 10.6 mmol) in acetonitrile (60 mL) over 30 minutes, then the reaction mixture was allowed to warm to room temperature and stirred under nitrogen balloon. After 18 hours, diluted with CH_2Cl_2 (300 mL) and washed with saturated sodium hydrogen carbonate solution (300 mL) and brine (300 mL). Organics separated, dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the triazoloyl intermediate as an orange solid, and immediately treated with 1:3 (v/v) NH_4OH/1,4-dioxane solution (200 mL) and allowed to stir at room temperature under nitrogen balloon. After 4 hours, diluted with Et_2O (300 mL) and washed with saturated sodium hydrogen carbonate solution (300 mL) and brine (300 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure to give the Title Compound as a white solid (4.71 g, 94%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.6. **Mp**: 182-184 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.55 (s, 1H, H-6), 6.32 (app. t, *J* = 6.5 Hz, 1H, H-1'), 4.39-4.35 (m, 1H, H-3'), 3.95-3.93 (m, 1H, H-4'), 3.89 (dd, *J* = 11.3, 2.8 Hz, 1H, H-5'), 3.77 (dd, *J* = 11.3, 2.8 Hz, 1H, H-5'), 2.41 (ddd, *J* = 13.2, 6.0, 3.5 Hz, 1H, H-2'), 2.01-1.93 (m, 1H, H-2'), 1.91 (s, 3H, H-7), 0.93 (s, 9H, H-8'), 0.89 (s, 9H, H-8'), 0.12 (s, 3H, H-6'), 0.11 (s, 3H, H-6'), 0.08 (s, 3H, H-6'), 0.07 (s, 3H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 165.7 (C, C-4), 155.9 (C, C-2), 137.9 (CH, C-6), 101.3 (C, C-5), 87.6 (CH, C-4'), 85.8 (CH, C-1'), 71.8 (CH, C-3'), 62.8 (CH_2, C-5'), 42.1 (CH_2, C-2'), 25.9 (CH_3, C-8'), 25.7 (CH_3, C-8'), 18.4 (C, C-7'), 18.0 (C, C-7'), 13.3 (CH_3, C-

7), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.4 (2 x CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3507, 3408, 3065, 2951, 2929, 2886, 2857, 1673, 1633, 1515, 1485. **HRMS** (ESI^+): Calc. for $C_{22}H_{44}N_3O_4Si_2$ ($M+H^+$) 470.2865. Found 470.2863. Calc. for $C_{22}H_{43}N_3NaO_4Si_2$ ($M+Na^+$) 492.2684. Found 492.2681.

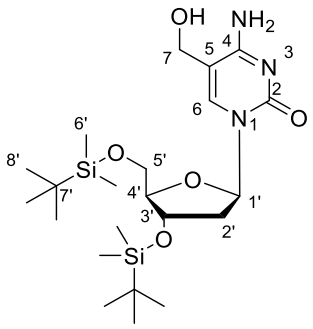
5-Formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (41)[341]



To a solution of 3',5'-(O-*tert*-butyldimethylsilyl)-thymidine (**40**) (3.00 g, 6.39 mmol) in acetonitrile:water (150 mL, 1:1 v/v) was dissolved copper sulfate pentahydrate (590 mg, 2.37 mmol), 2,6-lutidine (2.63 mL, 22.9 mmol) and potassium persulfate (3.46 g, 12.8 mmol). The resultant solution was stirred at 65 °C. After 4 hours, diluted with EtOAc (200 mL) and washed with 10% EDTA disodium salt solution (70 mL), 0.5 M HCl (100 mL), saturated sodium hydrogen carbonate solution (100 ml) and brine (100 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2 + 0.1% NEt_3) to give the Title Compound as a yellow solid (2.19 g, 64%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.4. **Mp**: 147-149 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 9.48 (s, 1H, H-7), 8.51 (s, 1H, H-6), 8.19 (s, 1H, NH_2), 7.63 (br, 1H, NH_2), 6.19 (app. t, *J* = 6.1 Hz, 1H, H-1'), 4.38-4.32 (m, 1H, H-3'), 4.07-4.02 (m, 1H, H-4'), 3.95 (dd, *J* = 11.6, 2.7 Hz, 1H , H-5'), 3.78 (dd, *J* = 11.6, 2.7 Hz, 1H, H-5'), 2.60 (ddd, *J* = 13.5, 6.2, 3.8 Hz, 1H, H-2'), 2.12-2.03 (m, 1H, H-2'), 0.89 (s, 9H, H-8'), 0.88 (s, 9H, H-8'), 0.10 (s, 3H, H-6'), 0.08 (2 x s, 6H, H-6'), 0.07 (s, 3H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 187.1 (CH, C-7), 162.8 (C, C-4), 153.3 (CH, C-6), 152.9 (C, C-2), 105.1 (C, C-5), 88.6 (CH, C-4'), 87.7 (CH, C-1'), 71.6 (CH, C-3'), 62.6 (CH_2, C-5'), 42.9 (CH_2, C-2'), 25.9 (CH_3, C-8'), 25.7 (CH_3, C-8'), 18.4 (C, C-7'), 18.0 (C, C-7'), -4.5 (CH_3, C-6'), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.3 (CH_3, C-6'), -5.4 (CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3397, 2953, 2929, 2886, 2857, 2161, 2020, 1967, 1662, 1508, 1498, 1421. **HRMS** (ESI^+): Calc. for $C_{22}H_{42}N_3O_5Si_2$ ($M+H^+$) 484.2658. Found 484.2662. Calc. for $C_{22}H_{41}N_3NaO_5Si_2$ ($M+Na^+$) 506.2477. Found 506.2470.

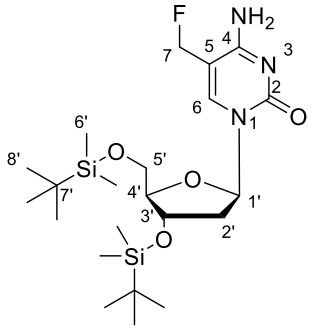
5-Hydroxymethyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (42)[342]



To a solution of 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (737 mg, 1.53 mmol) in MeOH (15 mL) was added cerium chloride heptahydrate (1.70 g, 4.58 mmol), and $NaBH_4$ (58.0 mg, 1.53 mmol) was added slowly over 5 minutes at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred under nitrogen balloon. After 4 hours, silica was added and purified by flash column chromatography (dry load, 5:95 CH_3OH/CH_2Cl_2) to give the Title Compound as a cream solid (616 mg, 83%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.2. **Mp:** 188-190 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.62 (s, 1H, H-6), 6.18 (app. t, *J* = 6.5 Hz, 1H, H-1'), 4.40 (m, 2H, H-7), 4.37-4.32 (m, 1H, H-3'), 3.95-3.89 (m, 1H, H-4'), 3.88-3.81 (m, 1H, H-5'), 3.77-3.71 (m, 1H, H-5'), 2.42-2.33 (m, 1H, H-2'), 2.02-1.91 (m, 1H, H-2'), 0.91 (s, 9H, H-8'), 0.89 (s, 9H, H-8'), 0.10 (s, 3H, H-6') 0.09 (s, 3H, H-6'), 0.07 (s, 3H, H-6'), 0.06 (s, 3H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 165.2 (C, C-4), 156.2 (C, C-2), 138.7 (CH, C-6), 106.3 (C, C-5), 87.8 (CH, C-4'), 86.1 (CH, C-1'), 71.8 (CH, C-3'), 62.7 (CH_2, C-5'), 59.2 (CH_2, C-7), 42.1 (CH_2, C-2'), 26.0 (CH_3, C-8'), 25.8 (CH_3, C-8'), 18.4 (C, C-7'), 18.0 (C, C-7'), -4.6 (2 x CH_3, C-6'), -4.9 (CH_3, C-6'), -5.3 (CH_3, C-6'), -5.4 (2 x CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3344, 3214, 2953, 2929, 2865, 2857, 1658, 1606, 1517, 1486, 1462, 1408. **HRMS** (ESI^+): Calc. for $C_{22}H_{44}N_3O_5Si_2$ ($M+H^+$) 486.2814. Found 486.2822. Calc. for $C_{22}H_{43}N_3NaO_5Si_2$ ($M+Na^+$) 508.2633. Found 508.2647.

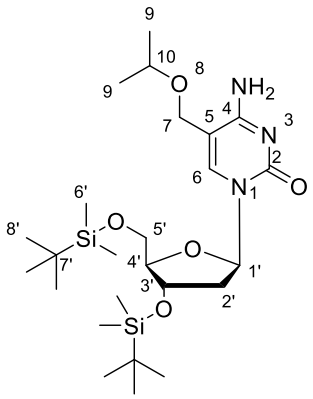
5-Fluoromethyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (43)



To a solution of 5-hydroxymethyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**42**) (636 mg, 1.31 mmol) in anhydrous CH_2Cl_2 (8 mL) was added Deoxo-Fluor® solution (50% in THF, 2.76 mL, 1.38 mmol) at -78°C under nitrogen. After 30 minutes, washed with water (10 mL) and brine (10 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (1:9 $^{i}PrOH/CH_2Cl_2$) to produce the Title Compound as a yellow-green solid (332 mg, 33%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.7. **Mp:** 113-115 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.94-7.92 (m, 1H, H-6), 6.25 (app. t, *J* = 6.3 Hz, 1H, H-1'), 5.14 (dq, *J* = 49.1, 11.4 Hz, 2H, H-7), 4.38-4.34 (m, 1H, H-3'), 4.00-3.96 (m, 1H, H-4'), 3.91 (dd, *J* = 11.4, 2.5 Hz, 1H, H-5'), 3.78 (dd, *J* = 11.4, 2.5 Hz, 1H, H-5'), 2.49 (ddd, *J* = 13.3, 6.0, 3.8 Hz, 1H, H-2'), 2.05-1.97 (m, 1H, H-2'), 0.93 (s, 9H, H-8'), 0.90 (s, 9H, H-8'), 0.12 (s, 3H, H-6'), 0.11 (s, 3H, H-6'), 0.09 (s, 3H, H-6'), 0.08 (s, 3H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 164.6 (C, C-4), 155.3 (C, C-2), 142.1 (CH, C-6), 100.9 (C, C-5), 88.0 (CH, C-4'), 86.5 (CH, C-1'), 80.4 (CH_2, C-7), 71.6 (CH, C-3'), 62.7 (CH_2, C-5'), 42.5 (CH_2, C-2'), 25.9 (CH_3, C-8'), 25.7 (CH_3, C-8'), 25.4 (CH_3, C-8'), 18.4 (C, C-7'), 18.0 (C, C-8'), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.4 (CH_3, C-6'). **^{19}F NMR** (376 MHz, $CDCl_3$) δ_F -205.16 (t, *J* = 49.2 Hz). **IR** ν_{max}/cm^{-1} (ATR): 2972, 2859, 1715, 1680, 1596, 1561, 1464, 1412, 1389, 1098, 1055, 1033, 1017. **HRMS** (ESI^+): Calc. for $C_{22}H_{43}FN_3O_4Si_2$ ($M+H^+$)488.2771. Found 488.2774. Calc. for $C_{22}H_{42}FN_3NaO_4Si_2$ ($M+Na^+$) 510.2590. Found 510.2589.

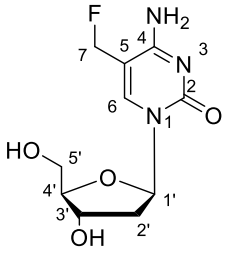
5-Isopropoxy-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (44)



To a solution of 5-hydroxymethyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**42**) (312 mg, 0.642 mmol) in anhydrous CH_2Cl_2 (5 mL) was added Deoxo-Fluor® solution (50% in THF, 1.35 mL, 0.674 mmol) at -78 °C under nitrogen. After 30 minutes, quenched by addition of ice cold isopropanol (8 mL) and washed with water (15 mL), saturated sodium hydrogen carbonate solution (15 mL) and brine (15 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (95:5 CH_2Cl_2/^{i}PrOH) and the solvent removed under reduced pressure to afford the Title Compound a pale yellow-green solid (86 mg, 25%).

R_f (5:95 ^{i}PrOH /CH_2Cl_2) = 0.3. **Mp** 84-86 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.72 (s, 1H, H-6), 6.30 (app. t, *J* = 6.4 Hz, 1H, H-1'), 4.38-4.33 (m, 1H, H-3'), 4.27 (s, 2H, H-7), 3.96-3.93 (m, 1H, H-4'), 3.89 (dd, *J* = 11.3, 2.8 Hz, 1H, H-5'), 3.77 (dd, *J* = 11.3, 2.8 Hz, 1H, H-5'), 3.67 (hept, *J* = 6.1 Hz, 1H, H-10), 2.45 (ddd, *J* = 13.3, 6.1, 3.6 Hz, 1H, H-2'), 2.02-1.94 (m, 1H, H-2'), 1.22 (d, *J* = 2.7 Hz, 3H, H-9), 1.20 (d, *J* = 2.7 Hz, 3H, H-9), 0.94 (s, 9H, H-8'), 0.90 (s, 9H, H-8'), 0.12 (2 x s, 6H, H-6'), 0.08 (s, 3H, H-6'), 0.07 (s, 3H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 165.2 (C, C-4), 157.8 (C, C-2), 139.3 (CH, C-6), 102.8 (C, C-5), 87.7 (CH, C-4'), 86.1 (CH, C-1'), 71.6 (CH, C-10), 71.0 (CH, C-3'), 63.4 (CH_2, C-7), 62.7 (CH_2, C-5'), 42.3 (CH_2, C-2'), 26.0 (CH_3, C-8'), 25.8 (CH_3, C-8'), 22.1 (CH_3, C-9), 21.8 (CH_3, C-9), 18.4 (C, C-7'), 18.0 (C, C-7'), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.3 (CH_3, C-6'), -5.4 (CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 2953, 2928, 2885, 2857, 1663, 1513, 1471, 1408, 1387, 1361, 1330, 1295. **HRMS** (ESI^{+}): Calc. for $C_{25}H_{50}N_3O_5Si_2$ ($M+H^{+}$) 528.3284. Found 528.3289. Calc. for $C_{25}H_{49}N_3NaO_5Si_2$ ($M+Na^{+}$) 550.3103. Found 550.3100.

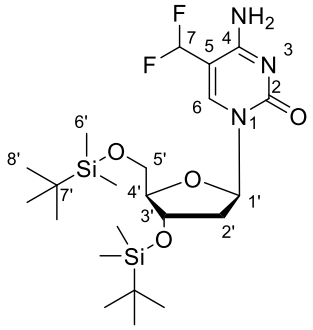
5-Fluoromethyl-2'-deoxycytidine (45)



To a solution of 5-fluoromethyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**43**) (33 mg, 0.070 mmol) in anhydrous THF (2 mL) was added tetrabutylammonium fluoride solution (0.20 mL, 0.20 mmol of a 1M solution in THF) and the solution stirred at room temperature under nitrogen balloon. After 30 minutes, the solvent was removed under reduced pressure and residue dissolved in isopropanol. Residue placed on silica column and washed with hexanes (200 mL). Compound eluted with EtOAc:^{i}PrOH:H_2O:NH_4OH (4:2:1:0.01) and the solvent removed under reduced pressure to yield the Title Compound as a white solid (5 mg, 30%)

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O) = 0.3. **^{1}H NMR** (300 MHz; D_2O) δ_H 7.91 (s, 1H, H-6), 6.17 (app. t, *J* = 6.4 Hz, 1H, H-1'), 5.21 (d, *J* = 48.5 Hz, 2H, H-7), 4.39-4.25 (m, 1H, H-3'), 4.02-3.96 (m, 1H, H-4'), 3.79 (m, 1H, H-5'), 3.69 (m, 1H, H-5'), 2.42.2.20 (m, 2H, H-2').**^{19}F NMR** (376 MHz; DMSO-d_6) δ_F -119.15 (t, *J* = 48.9 Hz). **HRMS** (ESI^+): Calc. for $C_{10}H_{14}FN_3NaO_4$ ($M+Na^+$) 282.0861. Found 282.0851.

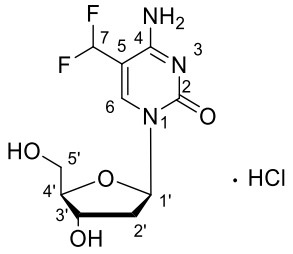
5-Difluoromethyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (47)



To a solution of 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (100 mg, 0.207 mmol) in anhydrous CH_2Cl_2 (4 mL) was added diethylaminosulfur trifluoride (54 µL, 0.41 mmol) dropwise at 0 °C under nitrogen. The reaction mixture was allowed to warm to room temperature and stir under nitrogen balloon. After 18 hours, diluted with CH_2Cl_2 (4 mL) and washed with water (4 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2) to give the Title Compound as a white solid (5 mg, 5%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.4. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 8.29 (s, 1H, H-6), 6.96 (t, *J* = 53.8 Hz, 1H, H-7), 6.21 (app. t, *J* = 6.2 Hz, 1H, H-1'), 4.47-4.43 (m, 1H, H-3'), 4.10-4.06 (m, 1H, H-4'), 3.92 (dd, *J* = 11.6, 2.5 Hz, 1H, H-5'), 3.79 (dd, *J* = 11.6, 2.5 Hz, 1H, H-5'), 2.57-2.48 (m, 1H, H-2'), 2.10-2.01 (m, 1H, H-2'), 0.93-0.90 (m, 18H, H-8'), 0.12-0.09 (m, 12H, H-6'). **^{19}F NMR** (376 MHz, $CDCl_3$) δ_F -115.50 (dd, *J* = 53.1, 12.9 Hz, 2F, F-7). **HRMS** (ESI^+): Calc. for $C_{22}H_{42}F_2N_3O_4Si_2$ ($M+H^+$) 506.2676. Found 506.2672. Calc. for $C_{22}H_{41}F_2N_3NaO_4Si_2$ ($M+Na^+$) 528.2496. Found 528.2497. Calc. for $C_{25}H_{46}F_2N_5O_4Si_2$ ($M+C_3H_5N_2$) 574.3051. Found 574.3059.

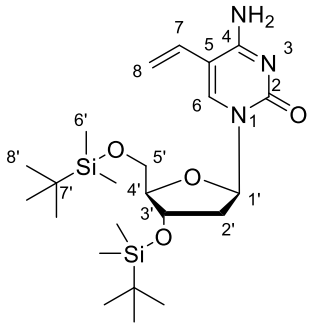
5-Difluoromethyl-2'-deoxycytidine hydrochloride (50)



A solution of 5-difluoromethyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**47**) (5 mg, 0.01 mmol) in HCl:isopropanol (2.5 mL of a 1:99 solution) was allowed to stir at room temperature under nitrogen balloon. After 30 minutes, diluted with CH_2Cl_2 (10 mL) and water (5 mL). Organics separated and aqueous extracted with CH_2Cl_2 (3 x 10 mL). Aqueous layer separated and solvent removed under reduced pressure to give the Title Compound as a white solid (1 mg, 33%).

^{1}H NMR (400 MHz; D_2O) δ_H 8.49 (s, 1H, H-6), 6.73 (t, *J* = 53.2 Hz, 1H, H-7), 6.17-6.12 (m, 1H, H-1'), 4.37 (m, 1H, H-3'), 4.06-4.02 (m, 1H, H-4'), 3.82 (dd, *J* = 12.7, 3.2 Hz, 1H, H-5'), 3.70 (m, 1H, H-5'), 2.49-2.41 (m, 1H, H-2'), 2.40-2.25 (m, 1H, H-2'). **^{19}F NMR** (376 MHz; D_2O) δ_F -115.44 (dd, *J* = 53.0, 14.0 Hz, 2F, F-7). **HRMS** (ESI^+): Calc. for $C_{10}H_{13}F_2N_3NaO_4$ ($M+Na^+$) 300.0766. Found 300.0767.

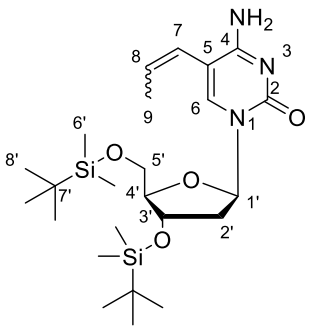
5-Vinyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (51)



To methyltriphenylphosphonium iodide (3.63 g, 8.97 mmol) stirred in anhydrous THF (15 mL) was added n-butyllithium (3.59 mL, 8.97 mmol of a 2.5M solution in hexanes) dropwise at 0 °C under nitrogen. The resulting solution was stirred for 30 minutes and 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (1.97 g, 4.08 mmol) added dropwise as a solution in anhydrous THF (4 mL). The reaction mixture was allowed to warm to room temperature and stirred for 5 hours, then quenched with water (5 mL) and the THF removed under reduced pressure. The solution was diluted with Et_2O (50 mL) and washed with water (3 x 50 mL), brine (50 mL) and the combined dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2) to give the Title Compound as an off white solid (978 mg, 50%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.5. **Mp:** 91-93 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.76 (s, 1H, H-6), 6.40-6.28 (m, 1H, H-7), 6.29 (app. t, *J* = 6.5 Hz, 1H, H-1'), 5.44 (d, *J* = 17.4 Hz, 1H, H-8), 5.29 (d, *J* = 10.9 Hz 1H, H-8), 4.40-4.35 (m, 1H, H-3'), 4.00-3.96 (m, 1H, H-4'), 3.87 (dd, *J* = 11.3, 3.0 Hz, 1H, H-5'), 3.76 (dd, *J* = 11.3, 3.0 Hz, 1H, H-5'), 2.45 (ddd, *J* = 13.4, 6.0, 3.2 Hz, 1H, H-2'), 2.03-1.95 (m, 1H, H-2'), 0.91 (s, 9H, H-8') 0.90 (s, 9H, H-8'), 0.10-0.07 (m, 12H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 163.9 (C, C-4), 155.2 (C, C-2), 138.2 (CH, C-6), 128.3 (CH, C-7), 117.2 (CH_2, C-8), 105.7 (C, C-5), 87.9 (CH, C-4'), 86.4 (CH, C-1'), 72.05 (CH, C-3'), 62.9 (CH_2, C-5'), 42.3 (CH_2, C-2'), 26.0 (CH_3, C-8'), 25.8 (CH_3, C-8'), 18.4 (C, C-7'), 18.0 (C, C-7'), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.4 (2 x CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3184, 2953, 2929, 2886, 2856, 2380, 2350, 2162, 2022, 1982, 1642, 1574, 1555, 1502, 1470, 1405, 1388. **HRMS** (ESI^+): Calc. for $C_{23}H_{44}N_3O_4Si_2$ ($M+H^+$) 482.2865. Found 482.2871. Calc. for $C_{23}H_{43}N_3NaO_4Si_2$ ($M+Na^+$) 504.2684. Found 504.2692.

5-(Prop-1-en-1-yl)-3',5'-(O-*tert*-butyldimethylsilyl)-2'-deoxycytidine (52)

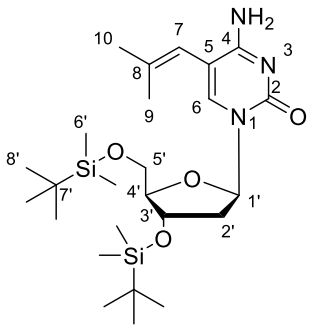


To ethyltriphenylphosphonium iodide (380 mg, 0.909 mmol) stirred in anhydrous THF (6 mL) was added n-butyllithium (365 µL, 0.909 mmol of a 2.5M solution in hexanes) dropwise at 0 °C under nitrogen. The resulting solution was stirred for 30 minutes and 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (200 mg, 0.413 mmol) added dropwise as a solution in anhydrous THF (2 mL). The reaction mixture was allowed to warm to room temperature and stirred for 5 hours, then quenched with water (5 mL) and the THF removed under reduced pressure. The solution was diluted with Et_2O (30 mL) and washed with water (3 x 30 mL), brine (30 mL) and the combined organics dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2) to afford the Title Compound as a yellow solid (62 mg, 30%). NMR analysis indicates *E*/*Z* ratio of 1:4.

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.3. **Mp**: 121-123 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.67 (s, 1H, H-6, *Z*, major), 7.60 (s, 1H, H-6, *E*, minor), 6.40-6.28 (m, 1H, H-1', *E* & *Z*), 6.05-5.79 (m, 2H, H-7 & H-8, *E* & *Z*), 4.40-4.35 (m, 1H, H-3'), 3.98-3.94 (m, 1H, H-4'), 3.91-3.73 (m, 2H, H-5'), 2.48-2.40 (m, 1H, H-2'), 2.03-1.94 (m, 1H, H-2'), 1.85-1.82 (m, 3H, H-9, *Z*, major), 1.70 (d, *J* = 4.9 Hz, 3H, H-9, *E*, minor), 0.91 (s, 9H, H-8'), 0.90 (s, 9H, H-8'), 0.09 (m, 6H, H-6'), 0.08 (s, 3H, H-6'), 0.07 (s, 3H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 164.1 (C, C-4), 155.4 (C, C-2), 137.4 (CH, C-6), 130.3 (CH, C-8), 121.9 (CH, C-7), 105.8 (C, C-5), 87.8 (CH, C-4'), 86.1 (CH, C-1'), 71.9 (CH, C-3'), 62.8 (CH_2, C-5'), 42.3 (CH_2, C-2'), 25.9 (CH_3, C-8'), 25.8 (CH3, C-8') 18.5 (C, C-7'), 18.4 (C, C-7'), 18.0 (C, C-7'), 14.5 (CH_3, C-9), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.4 (CH_3, C-6'), -5.5 (CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3336, 3191, 2952, 2928, 2856, 2366, 2349, 2157, 2023, 1976, 1639,1503,

1471, 1407. **HRMS** (ESI^+): Calc. for $C_{24}H_{46}N_3O_4Si_2$ ($M+H^+$) 496.3021. Found 496.3026. Calc. for $C_{24}H_{45}N_3NaO_4Si_2$ ($M+Na^+$) 518.2841. Found 518.2836.

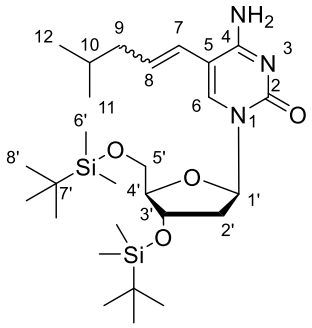
5-(2-Methylprop-1-en-1-yl)-3',5'-(O-*tert*-butyldimethylsilyl)-2'-deoxycytidine (53)



To isopropyltriphenylphosphonium iodide (393 mg, 0.909 mmol) stirred in anhydrous THF (6 mL) was added n-butyllithium (365 µL, 0.909 mmol of a 2.5M solution in hexanes) dropwise at 0 °C under nitrogen. The resulting solution was stirred for 30 minutes and 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (200 mg, 0.413 mmol) added dropwise as a solution in anhydrous THF (2 mL). The reaction mixture was allowed to warm to room temperature and stirred for 5 hours, then quenched with water (5 mL) and the THF removed under reduced pressure. The solution was diluted with Et_2O (30 mL) and washed with water (3 x 30 mL), brine (30 mL) and the combined organics dried ($MgSO_4$) and the solvent removed under reduced. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2) to afford the Title Compound as a yellow solid (76 mg, 36%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.3. **Mp**: 79-81 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.55 (s, 1H, H-6), 6.35 (app. t, *J* = 6.4 Hz, 1H, H-1'), 5.70 (s, 1H, H-7), 4.39-4.35 (m, 1H, H-3'), 3.94-3.90 (m, 1H, H-4'), 3.85 (dd, *J* = 11.3, 2.7 Hz, 1H, H-5'), 3.75 (dd, *J* = 11.3, 2.7 Hz, 1H, H-5'), 2.41 (ddd, *J* = 13.2, 6.0, 3.8 Hz, 1H, H-2'), 2.02-1.93 (m, 1H, H-2'), 1.86 (s, 3H, H-10), 1.66 (s, 3H, H-9), 0.90 (s, 9H, H-8'), 0.89 (s, 9H, H-8'), 0.09-0.06 (m, 12H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 164.7 (C, C-4), 155.5 (C, C-2), 142.1 (CH, C-6), 138.9 (C, C-8), 115.2 (CH, C-7), 104.5 (C, C-5), 87.5 (CH, C-4'), 85.9 (CH, C-1'), 71.7 (CH, C-3'), 62.7 (CH_2, C-5'), 42.2 (CH_2, C-2'), 25.9 (CH_3, C-8'), 25.8 (CH_3, C-8'), 25.5 (CH_3, C-8'), 19.4 (2 x CH_3, C-9 & C-10), 18.4 (C, C-7'), 18.0 (C, C-7'), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.6 (2 x CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3337, 2952, 2928, 2856, 2182, 2024, 1974, 1642, 1502, 1471, 1406. **HRMS** (ESI^+): Calc. for $C_{25}H_{48}N_3O_4Si_2$ ($M+H^+$) 510.3178. Found 510.3186. Calc. for $C_{25}H_{47}N_3NaO_4Si_2$ ($M+Na^+$) 532.2997. Found 532.3005.

5-(4-Methylpent-1-en-1-yl)-3',5'-(O-*tert*-butyldimethylsilyl)-2'-deoxycytidine (54)

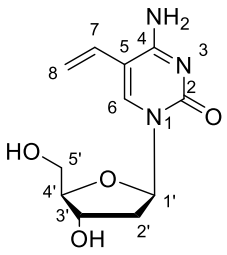


To (3-methylbutyl)triphenylphosphonium bromide (378 mg, 0.909 mmol) stirred in anhydrous THF (6 mL) was added n-butyllithium (365 µL, 0.909 mmol of a 2.5M solution in hexanes) dropwise at 0 °C under nitrogen. The resulting solution was stirred for 30 minutes and 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (200 mg, 0.413 mmol) added dropwise as a solution in anhydrous THF (2 mL). The reaction mixture was allowed to warm to room temperature and stirred for 5 hours, then quenched with water (5 mL) and the THF removed under reduced pressure. The solution was diluted with Et_2O (30 mL) and washed with water (3 x 30 mL) and brine (30 mL) and the combined organics dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2) to afford the Title Compound as a yellow solid (93 mg, 42%). NMR analysis indicates an *E*/*Z* ratio of 1:2.

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.3. **Mp**: 61-63 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.64 (s, 1H, H-6, *Z*, major), 7.53 (s, 1H, H-6, *E*, minor), 6.36-6.27 (m, 1H, H-1'), 6.01-5.82 (m, 2H, H-7 & H-8), 4.39-4.34 (m, 1H, H-3'), 3.98-3.93 (m, 1H, H-4'), 3.87-3.72 (m, 2H, H-5'), 2.44 (ddd, *J* = 13.2, 5.9, 3.1 Hz, 1H, H-2'), 2.06-1.92 (m, 3H, H-2' & H-9), 1.74-1.62 (m, 1H, H-10), 0.95-0.87 (m, 24H, H-8', H-11 & H-12), 0.09-0.07 (m, 12H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 164.2 (C, C-4), 155.3 (C, C-2), 137.3 (CH, C-6), 134.0 (CH, C-8), 121.7 (CH, C-7), 105.8 (C, C-5), 87.8 (CH, C-4'), 86.3 (CH, C-1'), 72.1 (CH, C-3'), 62.9 (CH_2, C-5'), 42.5 (CH_2, C-2'), 37.5 (CH_2, C-9) 28.3 (CH, C-10), 26.0 (CH_3, C-8'), 25.8 (CH_3, C-8'), 22.4 (CH_3, C-11 & C-12), 18.4 (C, C-7'), 18.0 (C, C-7'), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.5 (CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3336, 3186, 2953, 2928, 2896, 2857, 2165, 2021, 1641, 1503, 1470, 1438, 1407. **HRMS** (ESI^+): Calc. for $C_{27}H_{52}N_3O_4Si_2$ ($M+H^+$)

538.3491. Found 538.3498. Calc. for $C_{27}H_{51}N_3NaO_4Si_2$ ($M+Na^+$) 560.3310. Found 560.3332.

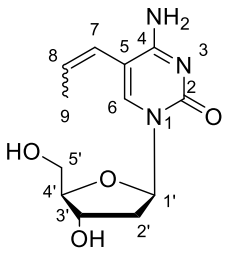
5-Vinyl-2'-deoxycytidine (55)



To a solution of 5-vinyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**51**) (50 mg, 0.10 mmol) in anhydrous tetrahydrofuran (4 mL) was added tetrabutylammonium fluoride solution (0.30 mL, 0.30 mmol of a 1M solution in THF) and the solution stirred at room temperature under nitrogen. After 30 minutes, solvent was removed under reduced pressure and the resultant solids dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX4 ion exchange resin to give the Title Compound as a white solid (21 mg, 81%).

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O) = 0.4. **Mp:** decomp. 220 °C. **^{1}H NMR** (400 MHz; D_2O) δ_H 7.94 (s, 1H, H-6), 6.37 (dd, *J* = 17.3, 11.0 Hz, 1H, H-7), 6.16 (app. t, *J* = 6.4 Hz, 1H, H-1'), 5.48 (d, *J* = 17.2 Hz, 1H, H-8), 5.27 (d, *J* = 11.0 Hz, 1H, H-8), 4.42-4.33 (m, 1H, H-3'), 4.00-3.96 (m, 1H, H-4'), 3.80 (dd, *J* = 12.5, 3.4 Hz, 1H, H-5'), 3.70 (dd, *J* = 12.5, 4.6 Hz, 1H, H-5'), 2.44-2.42 (m, 1H, H-2'), 2.29-2.18 (m, 1H, H-2'). **^{13}C NMR** (101 MHz; D_2O) δ_C 163.9 (C, C-4), 156.8 (C, C-2), 138.1 (CH, C-6), 126.8 (CH, C-7), 117.5 (CH_2, C-8), 107.6 (C, C-5), 86.6 (CH, C-4'), 86.3 (CH, C-1'), 70.0 (CH, C-3'), 60.8 (CH_2, C-5'), 39.6 (CH_2, C-2'). **IR** ν_{max}/cm^{-1} (ATR): 3185, 3069, 2927, 1713, 1643, 1608, 1557, 1471, 1436, 1044. **HRMS** (ESI$^+$): Calc. for $C_{11}H_{16}N_3O_4$ (M+H$^+$) 254.1135. Found 254.1143. Calc. for $C_{11}H_{15}N_3NaO_4$ (M+Na$^+$) 276.0955. Found 276.0953.

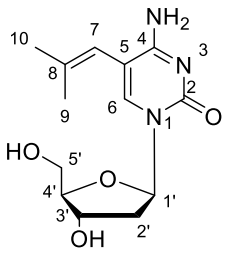
5-(Prop-1-en-1-yl)-2'-deoxycytidine (**56**)



To a solution of 5-(prop-1-en-1-yl)-3',5'-(O-*tert*-butyldimethylsilyl)-2'-deoxycytidine (**52**) (50 mg, 0.10 mmol) in anhydrous tetrahydrofuran (5 mL) was added tetrabutylammonium fluoride solution (0.30 mL, 0.30 mmol of a 1M solution in THF) and the solution stirred at room temperature under nitrogen for 30 minutes. The solvent was removed under reduced pressure and the resultant solids dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX4 ion exchange resin to give the Title Compound as a white solid (13 mg, 48%). NMR analysis indicates *E*/*Z* ratio of 1:4.

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O) = 0.5. **Mp:** 158-160 °C. **^{1}H NMR** (400 MHz; D_2O) δ_H 7.80 (s, 1H, H-6, *Z*, major), 7.69 (s, 1H, H-6, *E*, minor), 6.24-6.15 (m, 1H, H-1'), 6.05-5.86 (m, 2H, H-7 & H-8, *E* & *Z*), 4.41-4.33 (m, 1H, H-3'), 4.00-3.95 (m, 1H, H-4'), 3.79 (dd, *J* = 12.5, 3.4 Hz, 1H, H-5'), 3.69 (dd, *J* = 12.5, 4.6 Hz, 1H, H-5'), 2.41-3.32 (m, 1H, H-2'), 2.26-2.18 (m, 1H, H-2'), 1.75 (d, *J* = 5.5 Hz, 3H, H-9, *Z*, major), 1.62 (d, *J* = 5.6 Hz, 1H, H-9, *E*, minor). **^{13}C NMR** (101 MHz; D_2O) δ_C 163.7 (C, C-4), 156.3 (C, C-2), 137.4 (CH, C-6), 131.3 (CH, C-8), 119.8 (CH, C-7) 107.9 (C, C-5), 86.6 (CH, C-4'), 86.1 (CH, C-1'), 70.1 (CH, C-3'), 60.9 (CH_2, C-5'), 39.5 (CH_2, C-2'), 17.7 (CH_3, C-9). **IR** ν_{max}/cm^{-1} (ATR): 3323, 2195, 2925, 2350, 2312, 2164, 2138, 2091, 2027, 2014, 1978, 1718, 1636, 1591, 1506, 1472. **HRMS** (ESI$^+$): Calc. for $C_{12}H_{18}N_3O_4$ ($M+H^+$) 268.1292. Found 268.1286. Calc. for $C_{12}H_{17}N_3NaO_4$ ($M+Na^+$) 290.1111. Found 290.1114.

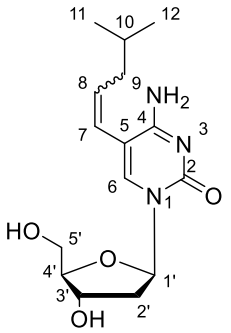
5-(2-Methylprop-1-en-1-yl)-2'-deoxycytidine (57)



To a solution of 5-(2-methyl-prop-1-en-1-yl)-3',5'-(O-*tert*-butyldimethylsilyl)-2'-deoxycytidine (**53**) (50 mg, 0.10 mmol) in anhydrous tetrahydrofuran (5 mL) was added tetrabutylammonium fluoride solution (0.30 mL, 0.30 mmol of a 1M solution in THF) and the solution stirred at room temperature under nitrogen for 30 minutes. The solvent was removed under reduced pressure and the resultant solids dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX4 ion exchange resin to give the Title Compound as a white solid (15 mg, 54%).

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O) = 0.5. **Mp:** 130-132 °C. **^{1}H NMR** (400 MHz; D_2O) δ_H 7.59 (s, 1H, H-6), 6.21 (app. t, *J* = 6.5 Hz, 1H, H-1'), 5.64 (s, 1H, H-7), 4.40-4.30 (m, 1H, H-3'), 3.99-3.94 (m, 1H, H-4'), 3.75 (dd, *J* = 12.4, 3.5 Hz, 1H, H-5'), 3.66 (dd, *J* = 12.4, 4.7 Hz, 1H, H-5'), 2.40-2.32 (m, 1H, H-2'), 2.26-2.12 (m, 1H, H-2'), 1.80 (s, 3H, H-10), 1.60 (s, 3H, H-9). **^{13}C NMR** (101 MHz; D_2O) δ_C 164.8 (C, C-4), 156.4 (C, C-2), 142.9 (CH, C-6), 139.6 (C, C-8), 113.1 (CH, C-7), 106.7 (C, C-5), 86.6 (CH, C-4'), 86.0 (CH, C-1'), 70.4 (CH, C-3'), 61.1 (CH_2, C-5'), 39.5 (CH_2, C-2'), 24.8 (CH_3, C-10), 18.5 (CH_3, C-9). **IR** ν_{max}/cm^{-1} (ATR): 3314, 3196, 3064, 2969, 2916, 2872, 2355, 2014, 1717, 1636, 1591, 1503, 1471. **HRMS** (ESI$^+$): Calc. for $C_{13}H_{20}N_3O_4$ ($M+H^+$) 282.1448. Found 282.1451.

5-(4-Methylpent-1-en-1-yl)- 2'-deoxycytidine (58)



To a solution of 5-(4-methylpent-1-en-1-yl)-3',5'-(O-*tert*-butyldimethylsilyl)-2'-deoxycytidine (**54**) (50 mg, 0.09 mmol) in anhydrous tetrahydrofuran (2 mL) was added tetrabutylammonium fluoride solution (0.27 mL, 0.27 mmol of a 1M solution in THF) and the solution stirred at room temperature under nitrogen for 30 minutes. The solvent was removed under reduced pressure and the resultant solids dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX4 ion exchange resin to give the Title Compound as a white solid (7 mg, 24%). NMR analysis indicates an *E*/*Z* ratio of 1:2.

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O) = 0.6. **Mp:** 78-80 °C. **^{1}H NMR** (400 MHz; D_2O) δ_H 7.86 (s, 1H, H-6, *Z*, major), 7.63 (s, 1H, H-6, *E*, minor), 6.23 (t, *J* = 6.3 Hz, 1H, H-1', *E*, minor), 6.17 (app. t, *J* = 6.3 Hz, 1H, H-1', *Z*, major), 6.07-5.85 (m, 2H, H-7 & H-8), 4.42-4.33 (m, 1H, H-3'), 4.00-3.95 (m, 1H, H4'), 3.82-3.64 (m, 2H, H-5'), 2.43-2.34 (m, 1H, H-2'), 2.27-2.15 (m, 1H, H-2'), 2.00 (t, *J* = 6.5 Hz, 2H, H-9, *Z*, major), 1.91 (t, *J* = 6.5 Hz, 2H, H-9, *E*, minor), 1.67-1.52 (m, 1H, H-10), 0.82 (d, *J* = 6.7 Hz, 4H, H-11 & H-12), 0.78 (d, *J* = 6.6 Hz, 2H, H-11 & H-12). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 163.9 (C, C-4), 156.4 (C, C-2), 137.4 (CH, C-6), 135.6 (CH, C-8), 119.7 (CH, C-7), 105.7 (C, C-5), 86.6 (CH, C-4'), 86.1 (CH, C-1'), 69.9 (CH, C-3'), 60.6 (CH_2, C-5'), 41.6 (CH_2, C-2'), 39.6 (CH_2, C-9), 27.74 (CH, C-10), 21.5 (2 x CH_3, C-11 & C-12). **IR** ν_{max}/cm^{-1} (ATR): 3332, 3194, 2954, 2924, 2868, 2183, 2159, 2025, 1977, 1718, 1638, 1593, 1507. **HRMS** (ESI$^+$): Calc. for $C_{15}H_{24}N_3O_4$ ($M+H^+$) 310.1761. Found 310.1761. Calc. for $C_{15}H_{23}N_3NaO_4$ ($M+Na^+$) 332.1581. Found 332.1579.

3,3-Dimethyldioxirane (DMDO, Murray's reagent)[166]

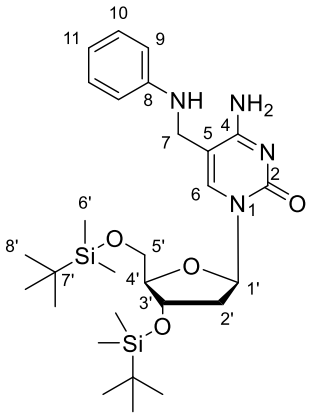


To a mixture of acetone (30 mL) and water (20 mL) was added sodium hydrogen carbonate (25 g) and stirred in an open flask at 0 °C for 1 hour. Oxone ™ (40 g) was added and the reaction left to stir for 20 minutes at 0 °C. Flask attached to a rotary evaporator with 100 mL splash guard in a dry ice/acetone bath and evaporated at 155 mmHg (207 mbar) for 15 minutes with water bath at room temperature. After 15 minutes, water bath heated to 40 °C and vacuum released. The Title Compound was recovered as a pale yellow liquid as a solution in acetone and transferred by glass pipette from splash guard to glass vial and stored over Na_2SO_4 in a freezer at -20 °C. Concentration determined by iodometric titration as 0.08 M.

Iodometric titration

To a solution of glacial acetic acid and acetone (2 mL, 3:2, v/v) was added DMDO (1 mL) and saturated potassium iodide solution (2 mL). Dry ice added to deoxygenate and sample left to stand in a dark cupboard for 10 minutes. Sample diluted with water (5 mL), and 1 mL aliquots removed and titrated three times against a sodium thiosulfate solution (0.1 M).

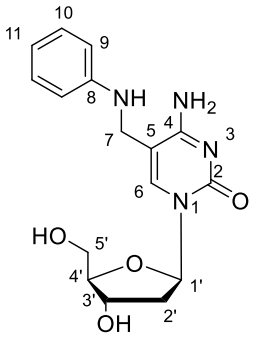
5-(Phenylamino)methyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (60)



To a solution of 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (50 mg, 0.10 mmol) in methanol/glacial acetic acid (2 mL, 9:1 v/v) was added aniline (0.02 mL, 0.20 mmol) and the solution stirred at room temperature for 10 minutes under nitrogen. To this solution was added 5-ethyl-2-methylpyridine borane complex (0.03 mL, 0.20 mmol) and the solution stirred at 50 °C. After 17 hours diluted with EtOAc (15 mL) and washed with saturated sodium hydrogen carbonate solution (15 mL) and brine (15 mL). The combined organics were dried ($MgSO_4$) and solvent removed under reduced pressure. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2) to give the Title Compound as a colourless solid (39 mg, 66%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.4. **Mp:** 199-202 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.83 (s, 1H, H-6), 7.27-7.22 (m, 2H, H-10), 6.85 (t, *J* = 7.4 Hz, 1H, H-11), 6.72 (d, *J* = 7.7 Hz, 2H, H-9), 6.32 (app. t, *J* = 6.4 Hz, 1H, H-1'), 4.38-4.34 (m, 1H, H-3'), 4.04 (s, 2H, H-7), 3.98-3.95 (m, 1H, H-4'), 3.86 (dd, *J* = 11.3, 2.9 Hz, H-5'), 3.76 (dd, *J* = 11.3, 2.9 Hz, 1H, H-5'), 2.47 (ddd, *J* = 13.3, 6.0, 3.6 Hz, 1H, H-2'), 2.04-1.95 (m, 1H, H-2'), 0.90 (s, 9H, H-8'), 0.89 (s, 9H, H-8'), 0.10-0.07 (m, 12H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 164.8 (C, C-4), 155.3 (C, C-2), 147.2 (C, C-8), 140.0 (CH, C-6), 129.4 (2 x CH, C-10), 119.4 (CH, C-11), 114.0 (2 x CH, C-9), 102.4 (C, C-5), 87.8 (CH, C-4'), 86.3 (CH, C-1'), 71.8 (CH, C-3'), 62.8 (CH_2, C-5'), 43.9 (CH_2, C-7), 42.2 (CH_2, C-2'), 25.9 (CH_3, C-8'), 25.8 (CH_3, C-8'), 18.4 (C, C-7'), 18.0 (C, C-7'), -4.5 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.3 (CH_3, C-6'), -5.4 (CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3437, 3285, 3057, 2929, 2896, 2856, 2323, 1675, 1625, 1601, 1514, 1498, 1487. **HRMS** (ESI^+): Calc. for $C_{28}H_{49}N_4O_4Si_2$ ($M+H^+$) 561.3287. Found 561.3297. Calc. for $C_{28}H_{48}N_4NaO_4Si_2$ ($M+Na^+$) 583.3106. Found 583.3114.

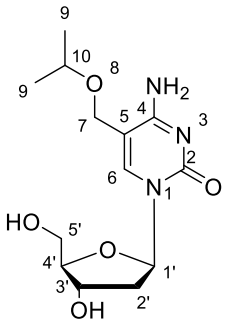
5-(Phenylamino)methyl-2'-deoxycytidine (61)



To a solution of (5-(phenylamino)methyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**60**) (20 mg, 0.04 mmol) in THF (2 mL) was added tetrabutylammonium fluoride solution (0.12 mL, 0.12 mmol of a 1M solution in THF) and the solution stirred at room temperature under nitrogen. After 19 hours the solvent was removed under reduced pressure. The resultant solids were dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX4 ion exchange resin to give the Title Compound as a white solid (6 mg, 53%).

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O) = 0.5. **^{1}H NMR** (400 MHz; D_2O) δ_H 7.55 (s, 1H, H-6), 7.23-7.18 (m, 2H, H-10), 6.78 (t, *J* = 7.4 Hz, 1H, H-11), 6.74-6.69 (m, 2H, H-9), 6.15 (app. t, *J* = 6.4 Hz, 1H, H-1'), 4.12-4.07 (m, 1H, H-3'), 4.05 (s, 2H, H-7), 3.92-3.84 (m, 1H, H-4'), 3.54 (dd, *J* = 12.4, 3.9 Hz, 1H, H-5'), 3.38 (dd, *J* = 12.4, 5.4 Hz, 1H, H-5'), 2.32-2.24 (m, 1H, H-2'), 2.05-1.96 (m, 1H, H-2'). **HRMS** (ESI$^+$): Calc. for $C_{16}H_{21}N_4O_4$ (M+H$^+$) 333.1557. Found 333.1569. Calc. for $C_{16}H_{20}N_4NaO_4$ (M+Na$^+$) 355.1377. Found 355.1371.

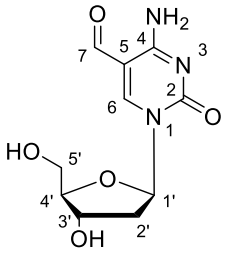
5-Isopropoxy-2'-deoxycytidine (62)



To a solution of 5-isopropoxy-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**44**) (25 mg, 0.047 mmol) in anhydrous tetrahydrofuran (2 mL) was added tetrabutylammonium fluoride solution (0.14 mL, 0.14 mmol of a 1M solution in THF) and the solution stirred at room temperature under nitrogen. After 30 minutes the solvent was removed under reduced pressure and the resultant solids dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX8 ion exchange resin to give the Title Compound as a white solid (9 mg, 63%).

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O) = 0.6. **^{1}H NMR** (400 MHz; D_2O) δ_H 7.88 (s, 1H, H-6), 6.16 (app. t, *J* = 6.4 Hz, 1H, H-1'), 4.40-4.35 (m, 1H, H-3'), 4.31 (s, 2H, H-7), 4.00-3.95 (m, 1H, H-4'), 3.85-3.62 (m, 3H, H-5' & H-10), 2.42-2.33 (m, 1H, H-2'), 2.27-2.19 (m, 1H, H-2'), 1.12 (d, *J* = 6.2 Hz, 6H, H-9). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 164.7 (C, C-4), 156.6 (C, C-2), 141.4 (CH, C-6), 104.3 (C, C-5), 86.7 (CH, C-4'), 86.2 (CH, C-1'), 71.8 (CH, C-10), 70.1 (CH, C-3'), 63.5 (CH_2, C-7), 60.9 (CH_2, C-5'), 39.5 (CH_2, C-2'), 20.9 (2 x CH_3, C-9). **HRMS** (ESI$^+$): Calc. for $C_{13}H_{22}N_3O_5$ (M+H$^+$) 300.1554. Found 300.1551. Calc. for $C_{13}H_{21}N_3NaO_5$ (M+Na$^+$) 322.1373. Found 322.1365.

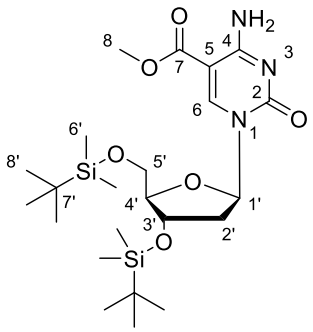
5-Formyl-2'-deoxycytidine (49)[340]



To a solution of 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (50 mg, 0.10 mmol) in THF (2 mL) was added tetrabutylammonium fluoride solution (0.30 mL, 0.30 mmol of a 1M solution in THF) and the solution stirred at room temperature. After 23 hours the solvent was removed under reduced pressure and the resultant solids were dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX8 ion exchange resin to give the Title Compound as an off white solid (11 mg, 42%).

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O) = 0.4. **Mp**: 192-194 °C. **^{1}H NMR** (400 MHz; D_2O) δ_H 9.44 (s, 1H, H-7), 8.73 (s, 1H, H-6), 6.12 (app. t, *J* = 6.1 Hz, 1H, H-1'), 4.38-4.33 (m, 1H, H-3'), 4.08-4.04 (m, 1H, H-4'), 3.84 (dd, *J* = 12.6, 3.4 Hz, 1H, H-5'), 3.72 (dd, *J* = 12.6, 4.9 Hz, 1H, H-5'), 2.54-2.46 (m, 1H, H-2'), 2.33-2.26 (m, 1H, H-2'). **^{13}C NMR** (101 MHz; D_2O) δ_C 190.4 (CHO, C-7), 160.2 (C, C-4), 155.3 (CH, C-6), 154.6 (C, C-2), 105.7 (C, C-5), 87.5 (CH, C-4'), 87.2 (CH, C-1'), 69.8 (CH, C-3'), 60.7 (CH_2, C-5'), 40.0 (CH_2, C-2'). **HRMS** (ESI$^+$): Calc. For $C_{10}H_{14}N_3O_5$ ($M+H^+$) 256.0933. Found 256.0897.

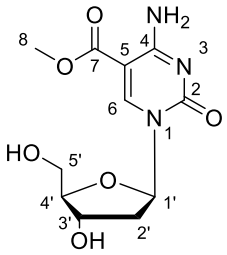
5-Methoxycarbonyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (63)[341]



To a solution of 5-formyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**41**) (300 mg, 0.620 mmol) in methanol (5 mL) was added potassium carbonate (257 mg, 1.86 mmol) and iodine (189 mg, 0.744 mmol) and the solution allowed to stir at room temperature. After 17 hours the reaction mixture was diluted with EtOAc (25 mL) and washed with water (25 mL) and sodium bisulfite (322 mg, 3.10 mmol), saturated sodium hydrogen carbonate solution (25 mL) and brine (25 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (1:9 CH_3OH/CH_2Cl_2) to give the Title Compound as an off white solid (285 mg, 89%).

R_f (1:9 CH_3OH/CH_2Cl_2) = 0.7. **Mp**: 127-129 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 8.65 (s, 1H, H-6), 6.22-6.17 (m, 1H, H-1'), 4.39-4.34 (m, 1H, H-3'), 4.09-4.05 (m, 1H, H-4'), 3.87 (dd, *J* = 11.2, 3.1 Hz, 1H, H-5'), 3.84 (s, 3H, H-8), 3.79 (dd, *J* = 11.2, 3.1 Hz, 1H, H-5'), 2.63 (ddd, *J* = 13.5, 5.8, 2.4 Hz, 1H, H-2'), 2.02-1.95 (m, 1H, H-2'), 0.92-0.88 (m, 18H, H-8'), 0.10-0.08 (m, 12H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 165.3 (C, C-4), 163.6 (C, C-7), 154.1 (C, C-2), 147.6 (CH, C-6), 95.1 (C, C-5), 88.8 (CH, C-4'), 87.9 (CH, C-1'), 72.8 (CH, C-3'), 70.6 (CH_2, C-5'), 63.1 (CH_3, C-8), 42.9 (CH_2, C-2'), 26.0 (CH_3, C-8'), 25.8 (2 x CH_3, C-8'), 25.7 (CH_3, C-8'), 18.3 (C, C-7'), 18.0 (C, C-7'), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.5 (CH_3, C-6'), -5.7 (CH_3, C-6'). **HRMS (ESI$^+$)**: Calc. for $C_{23}H_{44}N_3O_6Si_2$ ($M+H^+$) 514.2763. Found 514.2779.

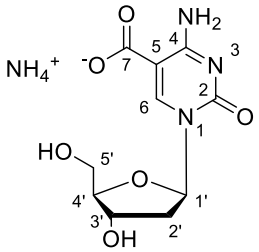
5-Methoxycarbonyl-2'-deoxycytidine (64)[171]



To a solution of 5-methoxycarbonyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**63**) (285 mg, 0.549 mmol) in anhydrous tetrahydrofuran (5 mL) was added tetrabutylammonium fluoride solution (1.65 mL, 1.65 mmol of a 1M solution in THF) and the solution allowed to stir at room temperature under nitrogen. After 16 hours, the solvent was removed under reduced pressure and the resultant solids were dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX8 ion exchange resin to give the Title Compound as an off white solid (120 mg, 76%).

R_f (4:2:1 EtOAc/^{i}PrOH/H_2O + 1% NEt_3) = 0.6. **Mp**: 162-164 °C **^{1}H NMR** (400 MHz; D_2O) δ_H 8.88 (s, 1H, H-6), 6.10-6.06 (m, 1H, H-1'), 4.38-4.31 (m, 1H, H-3'), 4.02-3.98 (m, 1H, H-4'), 3.84 (dd, *J* = 12.6, 3.3 Hz, 1H, H-5'), 3.77 (s, 3H, H-8), 3.71 (dd, *J* = 12.6, 4.3 Hz, 1H, H-5'), 2.48-2.41 (m, 1H, H-2'), 2.30-2.23 (m, 1H, H-2'). **^{13}C NMR** (101 MHz; D_2O) δ_C 165.9 (C, C-4), 163.4 (C, C-7), 155.8 (C, C-2), 148.8 (CH, C-6), 96.7 (C, C-5), 87.0 (CH, C-4'), 86.8 (CH, C-1'), 69.2 (CH, C-3'), 60.1 (CH_2, C-5'), 52.4 (CH_3, C-8) 40.0 (CH_2, C-2'). **HRMS (ESI$^+$)**: Calc. for $C_{11}H_{16}N_3O_6$ ($M+H^+$) 286.1034. Found 286.1027. Calc. for $C_{11}H_{15}N_3NaO_6$ ($M+Na^+$) 308.0853. Found 308.0848.

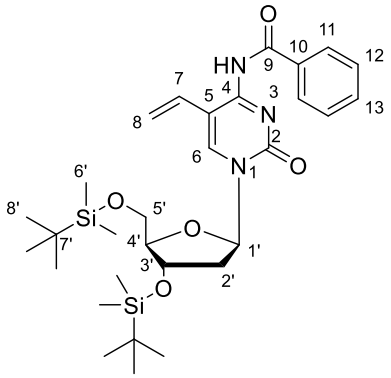
5-Carboxyl-2'-deoxycytidine ammonium salt (65)



To a solution of 5-methoxycarbonyl-2'-deoxycytidine (**64**) (100 mg, 0.351 mmol) in MeCN/H_2O (5 mL, 5:1 v/v) was added lithium hydroxide monohydrate (29 mg, 0.70 mmol) and the solution allowed to stir at room temperature. After 19 hours the solvent was removed under reduced pressure and the resultant solids were dissolved in 1M HCl (2 mL) and eluted on a column of Dowex 50WX4 ion exchange resin to give the Title Compound as a white solid (55 mg, 58%).

^{1}H NMR (400 MHz; D_2O) δ_H 8.51 (s, 1H, H-6), 6.19 (app. t, *J* = 6.5 Hz, 1H, H-1'), 4.40-4.36 (m, 1H, H-3'), 4.05-4.00 (m, 1H, H-4'), 3.80 (dd, *J* = 12.5, 3.5 Hz, 1H, H-5'), 3.72 (dd, *J* = 12.5, 5.1 Hz, 1H, H-5'), 2.43-2.36 (m, 1H, H-2'), 2.27-2.19 (m, 1H, H-2'). **^{13}C NMR** (101 MHz; D_2O) δ_C 170.7 (C, C-4), 164.8 (C. C-7), 156.6 (C, C-2) 146.3 (CH, C-6), 103.1 (C, C-5), 86.8 (CH, C-4'), 86.4 (CH, C-1'), 70.5 (CH, C-3'), 61.2 (CH_2, C-5'), 39.6 (CH_2, C-2'). **HRMS (ESI$^+$)**: Calc. for $C_{10}H_{14}N_3O_6$ ($M+H^+$) 272.0877. Found 272.0881. Calc. for $C_{10}H_{13}N_3NaO_6$ ($M+Na^+$) 294.0697. Found 294.0710.

4-Benzoyl-5-vinyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (66)

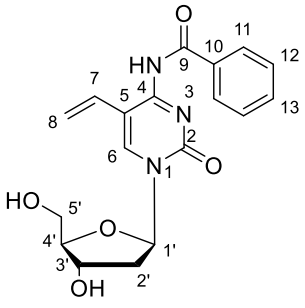


To a solution of 5-vinyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**51**) (978 mg, 2.03 mmol) in anhydrous pyridine (10 mL) was added benzoyl chloride (236 µL, 2.03 mmol) dropwise at 0°C. The resultant solution was allowed to stir at room temperature under nitrogen. After 4 hours the reaction mixture was quenched by addition of methanol (5 mL) and the solvent was removed under reduced pressure. The resultant oil was dissolved in CH_2Cl_2 (50 mL) and washed with water (2 x 50 mL), saturated sodium hydrogen carbonate solution (50 mL) and brine (50 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2 + 0.1% NEt_3) to give the Title Compound as a yellow oil (935 mg, 79%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.65. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.78 (s, 1H, H-6), 7.77-7.68 (m, 2H, H-11), 7.58-7.46 (m, 3H, H-12 & H-13), 6.41-6.28 (m, 2H, H-7 & H-1'), 5.44 (d, *J* = 17.4 Hz, 1H, H-8), 5.30 (d, *J* = 11.0 Hz, 1H, H-8), 4.41-4.30 (m, 1H, H-3'), 3.99-3.96 (m, 1H, H-4'), 3.88 (dd, *J* = 11.3, 2.9 Hz, 1H, H-5'), 3.77 (dd, *J* = 11.3, 2.9 Hz, 1H, H-5'), 2.46 (ddd, *J* = 13.3, 6.0, 3.2 Hz, 1H, H-2'), 2.01-1.91 (m, 1H, H-2'), 0.92-0.88 (m, 18H, H-8'), 0.10-0.06 (m, 12H, H-6'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 163.7 (C, C-4), 158.8 (C, C-9), 155.3 (C, C-2), 138.3 (C, C-6), 133.5 (C, C-10), 131.8 (CH, C-13), 130.5 (CH, C-12), 128.6 (CH, C-11), 128.3 (CH, C-7), 117.3 (CH_2, C-8), 105.7 (C, C-5), 87.9 (CH, C-4'), 86.4 (CH, C-1'), 72.0 (CH, C-3'), 62.9 (CH_2, C-5'), 42.3 (CH_2, C-2'), 26.0 (CH_3, C-8'), 25.8 (CH_3, C-8'), 18.4 (C, C-7'), 18.0 (C, C-7'), -4.6 (CH_3, C-6'), -4.9 (CH_3, C-6'), -5.4 (2 x CH_3, C-6'). **IR** ν_{max}/cm^{-1} (ATR): 3068, 2953, 2929, 2885, 2857, 1707, 1638, 1596,

1561, 1471, 1449. **HRMS** (ESI^+): Calc. for $C_{30}H_{48}N_3O_5Si_2$ ($M+H^+$) 586.3127. Found 586.3129. Calc. for $C_{30}H_{47}N_3NaO_5Si_2$ ($M+Na^+$) 608.2946. Found 608.2939.

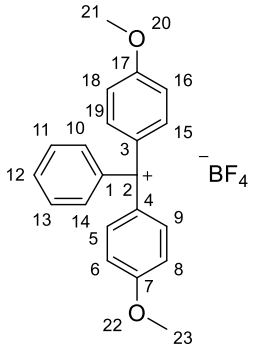
4-Benzoyl-5-vinyl-2'-deoxycytidine (67)



To a solution of 4-benzoyl-5-vinyl-3',5'-(O-*tert*-butyl-dimethylsilyl)-2'-deoxycytidine (**66**) (935 mg, 1.60 mmol) in anhydrous THF (8 mL) was added tetrabutylammonium fluoride solution (4.79 mL, 4.79 mmol of a 1M solution in THF) and the solution stirred at room temperature under nitrogen. After 2 hours the solvent was removed under reduced pressure and the residue dissolved in methanol. Silica gel (5g) was added and solvent removed under reduced pressure. Purified by flash column chromatography (dry load, 5:95 CH_3OH/CH_2Cl_2 + 0.1% NEt_3) to give the Title Compound as a white solid (405 mg, 71%).

R_f (5:95 CH_3OH/CH_2Cl_2) = 0.3. **Mp** 144-146 °C. **^{1}H NMR** (400 MHz; DMSO-d_6) δ_H 13.22 (s, 1H, NH), 8.63 (s, 1H, H-6), 8.17 (d, *J* = 7.5 Hz, 2H, H-11), 7.63-7.48 (m, 3H, H-12 & H-13), 6.17 (app. t, *J* = 6.1 Hz, 1H, H-1'), 5.34-5.23 (m, 3H, H-7 & H-8), 4.34-4.27 (m, 1H, H-3'), 3.89-3.85 (m, 1H, H-4'), 3.77-3.59 (m, 2H, H-5'), 2.30-2.24 (m, 2H, H-2'). **^{13}C NMR** (101 MHz; DMSO-d_6) δ_C 178.7 (C, C-4), 150.1 (C, C-9), 147.3 (C, C-2), 139.5 (CH, C-6), 137.2 (C, C-10), 133.0 (CH, C-13), 129.8 (CH, C-7), 128.8 (2 x CH, C-12), 128.4 (2 x CH, C11), 114.9 (CH_2, C-8), 111.5 (C, C-5), 88.3 (CH, C-4'), 86.1 (CH, C-1'), 69.8 (CH, C-3'), 60.9 (CH_2, C-5'), 41.0 (CH_2, C-2'). **IR** ν_{max}/cm^{-1} (ATR): 3516, 3054, 2921, 2855, 1904, 1673, 1634, 1591, 1549, 1482, 1464, 1447, 1413, 1390, 1366, 1318. **HRMS** (ESI^+): Calc. for $C_{18}H_{20}N_3O_5$ ($M+H^+$) 358.1397. Found 358.1403. Calc. for $C_{18}H_{19}N_3NaO_5$ ($M+Na^+$) 380.1217. Found 380.1220.

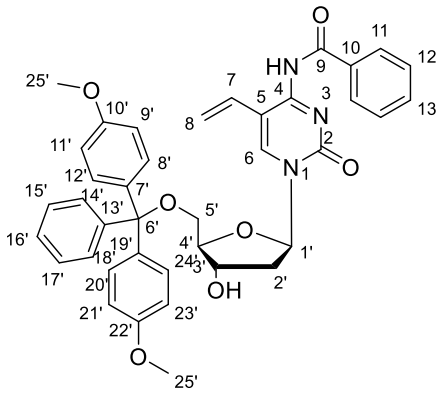
4,4'-Dimethoxytrityl tetrafluoroborate[176] (69)



To a solution of 4,4-dimethoxytrityl chloride (5.00 g, 14.8 mmol) in THF (15 mL) was added water (15 mL) and sodium hydroxide (1.18 g, 29.5 mmol) and the white emulsion formed stirred for 2 hours. Additional water (20 mL) was added and extracted with CH_2Cl_2 (3 x 20 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure to produce a colourless oil. The oil was placed in a vacuum desiccator overnight, then dissolved in acetic anhydride (27.9 mL, 0.295 mol) and heated at 80 °C for 60 minutes to form a pale yellow solution. The reaction mixture was cooled in an ice bath and tetrafluoroboric acid (50% in diethyl ether, 5.02 mL, 36.9 mmol) was added dropwise under nitrogen at 0 °C. The reaction mixture was stirred for 30 minutes and then diethyl ether (300 mL) added and the solution filtered to obtain the Title Compound as a red, crystalline solid (5.70 g, 99%).

Mp: 192-194 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 7.95 (t, *J* = 7.5 Hz, 1H, H-12), 7.72 (t, *J* = 7.9 Hz, 2H, H-11 & H-13), 7.65 (d, *J* = 8.7 Hz, 4H, H-5, H-9, H-15, H-19), 7.49-7.46 (m, 2H, H-10 & H-14), 7.36 (d, *J* = 9.1 Hz, 4H, H-6, H-8, H-16, H-18), 4.15 (s, 6H, H-21 & H-23). **^{13}C NMR** (75 MHz; $CDCl_3$) δ_C 194.6 (C, C-2), 172.5, 144.5, 139.0, 137.9, 137.6, 132.4, 129.5 117.4, 57.6 (2 x OCH_3, C-21 & C-23). **^{19}F NMR** (282 MHz) δ_F -153.3 (s, 4F). **IR** ν_{max}/cm^{-1} (ATR): 3060, 2971, 2936, 2845, 2733, 2640, 2349, 2080, 1991, 1940, 1609, 1576. **HRMS** (ESI^+): Calc. for $C_{21}H_{19}O_2$ ($M-BF_4$) 303.1380. Found 303.1383.

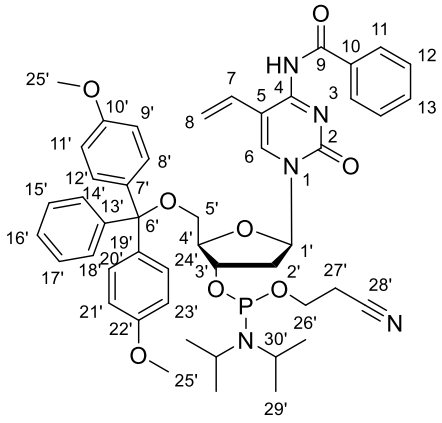
4-Benzoyl-5-vinyl-5'-(4,4'-dimethoxytrityl)-2'-deoxycytidine (70)



To a solution of 4-benzoyl-5-vinyl-2'-deoxycytidine (**67**) (300 mg, 0.840 mmol) in anhydrous pyridine (3 mL) was added 4,4'-dimethoxytritylchloride (284 mg, 0.840 mmol) as a solution in anhydrous pyridine (1.5 mL). The reaction mixture was stirred at room temperature under nitrogen. After 16 hours the solvent was removed under reduced pressure and diluted with CH_2Cl_2 (40 mL) and washed with saturated sodium hydrogen carbonate solution (40 mL) and brine (40 mL), the combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (5:95 CH_3OH/CH_2Cl_2 + 0.5% NEt_3) to give the Title Compound as an off white solid (277 mg, 50%).

R_f (5:95 CH_3OH/CH_2Cl_2 + 0.1% NEt_3) = 0.5. **Mp** 60-62 °C. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 8.30 (m, 2H, H-11), 7.93 (s, 1H, H-6), 7.57-7.52 (m, 1H, H-Ar), 7.49-7.41 (m, 5H, H-Ar), 7.35-7.29 (m, 6H, H-Ar), 6.86 (m, 4H, H-Ar), 6.50 (dd, *J* = 18.0, 11.3 Hz, 1H, H-7), 6.37 (app. t, *J* = 6.4 Hz, 1H, H-1'), 5.54 (dd, *J* = 17.7, 1.2 Hz, 1H, H-8), 5.04 (dd, *J* = 11.3, 1.1 Hz, 1H, H-8), 4.57-4.52 (m, 1H, H-3'), 4.15-4.12 (m, 1H, H-4'). 3.81 (s, 3H, H-25'), 3.67 (s, 3H, H-25'), 3.50 (dd, *J* = 10.5, 3.6 Hz, 1H, H-5'), 3.41 (dd, *J* = 10.5, 3.6 Hz, 1H, H-5'), 2.56-2.52 (m, 1H, H-2'), 2.37-2.29 (m, 1H, H-2'). **^{13}C NMR** (101 MHz; $CDCl_3$) δ_C 179.8, 178.1, 158.7, 147.3, 144.3, 137.0, 136.5, 135.3, 132.5, 130.0, 129.9, 128.2, 128.2, 128.1, 127.7, 127.2, 115.9 (CH_2, C-8), 113.3, 86.9 (C, C-6'), 86.3 (CH, C-4'), 85.8 (CH, C-1'), 72.2 (CH, C-3'), 63.3 (CH_2, C-5'), 55.3 (2 x CH_3, C-25'), 41.5 (CH_2, C-2'). **IR** ν_{max}/cm^{-1} (ATR): 3394, 3062, 2929, 2044, 1703, 1636, 1606, 1558, 1507, 1488. **HRMS** (ESI$^+$): Calc. for $C_{39}H_{38}N_3O_7$ ($M+H^+$) 660.2704. Found 660.2716. Calc. for $C_{39}H_{37}N_3NaO_7$ ($M+Na^+$) 682.2524. Found 682.2538.

4-Benzoyl-5-vinyl-3'-(Diisopropylcyanoethylphosphino)-5'-(4,4'dimethoxytrityl)-2'-deoxycytidine (71)



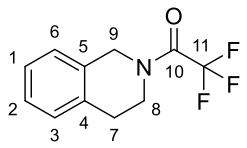
To a solution of 4-benzoyl-5-vinyl-5'-(4,4'-dimethoxytrityl)-2'-deoxycytidine (**70**) (267 mg, 0.405 mmol) in anhydrous CH_2Cl_2 (3 mL) was added anhydrous *N*,*N*-diisopropylethylamine (282 µL, 1.62 mmol) and 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (101 mg, 0.43 mmol) as a solution in anhydrous CH_2Cl_2 (1.5 mL). The resulting solution was stirred at room temperature under nitrogen. After 4 hours, diluted with EtOAc (50 mL) and washed with saturated sodium hydrogen carbonate solution (50 mL) and brine (50 mL). The combined organics were dried ($MgSO_4$) and the solvent removed under reduced pressure. Purified by flash column chromatography (1:1 EtOAc/hexane + 0.5% NEt_3) to give the Title Compound as a white solid (150 mg, 43%).

R_f (1:1 EtOAc/hexane + 0.5% NEt_3) = 0.8. **^{1}H NMR** (400 MHz; $CDCl_3$) δ_H 8.30 (d, *J* = 7.3 Hz, 2H, H-11), 7.99 (s, 0.5H, H-6). 7.94 (s, 0.5H, H-6), 7.57-7.53 (m, 1H, H-Ar), 7.48-7.41 (m, 5H, H-Ar), 7.35-7.29 (m, 6H, H-Ar), 6.88-6.83 (m, 4H, H-Ar), 6.48-6.36 (m, 2H, H-7, H-1'), 5.55-5.49 (m, 1H, H-8), 5.01-4.96 (m, 1H, H-8), 4.67-4.61 (m, 1H, H-3'), 4.28-4.21 (m, 1H, H-4'), 3.82-3.79 (m, 6H, H-25'), 3.69-3.49 (m, 5H), 3.38-3.31 (m, 1H, H-5'), 2.71-2.58 (m, 2H), 2.44 (t, *J* = 6.4 Hz, 1H), 2.36-2.30 (m, 1H, H-2'), 1.22-1.28 (m, 10H, H-29), 1.10-1.06 (m, 2H, H-29). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 179.9, 171.2, 158.4, 147.3, 142.2, 137.0, 136.5, 135.3, 132.5, 130.1, 129.9, 128.2, 128.0, 127.6, 127.1, 117.5, 116.0 (CH_2, C-8), 113.2, 86.9 (C, C-6'), 86.2 (CH, C-4'), 85.6 (CH, C-1'), 73.5 (dd, *J* = 54.2 Hz, CH, C-3'), 63.0 (2 x s, CH_2, C-5'), 58.2 (t, *J* = 19.1 Hz, CH_2), 55.3 (2 x CH_3, C-25'), 43.3 (t, *J* = 12.1 Hz), 24.6 (m, CH_3), 20.3 (dd, *J* = 28.7, 7.2 Hz, CH_2, C-28'). **^{31}P NMR**

(162 MHz; $CDCl_3$) δ_P 149.4-149.1 (m), 148.7-148.3 (m). **HRMS** (ESI^+): Calc. for $C_{48}H_{55}N_5O_8P$ ($M+H^+$) 860.3783. Found 860.3744. Calc. for $C_{48}H_{54}N_5NaO_8P$ ($M+Na^+$) 882.3602. Found 882.3567

5.2) Chapter 2

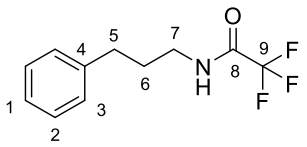
1-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-2,2,2-trifluoroethan-1-one (104)



To a solution of 1,2,3,4-tetrahydroisoquinoline (626 µL, 5.00 mmol) and *N,N*-diisopropylethylamine (1.74 mL, 10.0 mmol) in anhydrous EtOAc (5 mL) was added trifluoroacetic anhydride (695 µL, 5.00 mmol) at 0 °C. Solution allowed to warm to room temperature and stir under Ar balloon. After 18 hours, diluted with EtOAc (5 mL) and washed with brine (10 mL). The combined organics were dried ($MgSO_4$) and concentrated. Purified by flash column chromatography (1:1 Et_2O/petroleum ether) to give the Title Compound as a colourless oil (940 mg, 82%).

R_f (1:1 Et_2O/petroleum ether) = 0.7. **^{1}H NMR** (500 MHz; $CDCl_3$, mixture of two rotamers) δ_H 7.34-6.96 (m, 4H, H-1, H-2, H-3 & H-6), 4.82 (s, 1.3H, H-9, major), 4.77 (s, 0.7H, H-9, minor), 3.97-3.83 (m, 2H, H-8), 3.01-2.95 (m, 2H, H-7). **^{13}C NMR** (126 MHz; $CDCl_3$, mixture of two rotamers) δ_C 155.9 (q, *J* = 36.0 Hz, C, C-10), 134.0 (C), 133.3 (C), 131.5 (C), 131.1 (C), 128.9 (CH), 128.6 (CH), 127.5 (CH), 127.1 (CH), 126.9 (CH), 126.8 (CH), 126.0 (CH), 116.5 (q, *J* = 287.7 Hz, CF_3, C-11), 47.0 (CH_2), 45.6 (CH_2), 43.4 (CH_2), 41.9 (CH_2), 29.3 (CH_2), 27.9 (CH_2). **^{19}F NMR** (376 MHz; $CDCl_3$, mixture of two rotamers) δ_F -69.36 (s, 1F, F-11, minor), -69.41 (s, 2F, F-11, major). **IR** ν_{max}/cm^{-1} (ATR): 3029, 2941, 2904, 2851, 1685, 1499, 1459, 1373, 1305. **HRMS** (ESI$^+$): Calc. for $C_{11}H_{11}F_3NO$ ($M+H^+$) 230.0787. Found 230.0786. Calc. for $C_{11}H_{10}F_3NNaO$ ($M+Na^+$) 252.0607. Found 252.0608. Calc. for $C_{11}H_{14}F_3N_2O$ ($M+H_4N^+$) 247.1053. Found 247.1050.

2,2,2-Trifluoro-*N*-(3-phenylpropyl)acetamide (106)



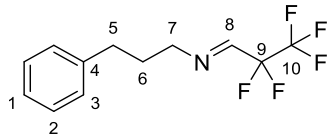
To a solution of 3-phenylpropylamine (711 µL, 5.00 mmol) and *N,N*-diisopropylethylamine (1.74 mL, 10.0 mmol) in anhydrous EtOAc (5 mL) was added trifluoroacetic anhydride (695 µL, 5.00 mmol) at 0 °C. Solution allowed to warm to room temperature and stir under Ar balloon. After 18 hours, diluted with EtOAc (5 mL) and washed with brine (10 mL). The combined organics were dried ($MgSO_4$) and concentrated. Purified by flash column chromatography (1:1 Et_2O/petroleum ether) to give the Title Compound as an off white solid (1.07 g, 93%).

R_f (1:1 Et_2O/petroleum ether) = 0.5. **Mp:** 41-43 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.36-7.29 (m, 2H, H-Ar), 7.27-7.12 (m, 3H, H-Ar), 6.45 (br, 1H, NH), 3.44-3.38 (m, 2H, H-7), 2.71 (t, *J* = 7.5 Hz, 2H, H-5), 2.01-1.91 (m, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 157.3 (q, *J* = 36.8 Hz, C, C-8), 140.6 (C, C-4), 128.7 (CH), 128.3 (CH), 126.4 (CH), 115.8 (q, *J* = 287.8 Hz, CF_3, C-9), 39.6 (CH_2, C-7), 33.1 (CH_2, C-5), 30.3 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -76.01 (s, 3F, F-9). **IR** ν_{max}/cm^{-1} (ATR): 3307, 3107, 3089, 3029, 2942, 2864, 1700, 1556, 1496. **HRMS** (ESI^+): Calc. for $C_{11}H_{13}F_3NO$ ($M+H^+$) 232.0944. Found 232.0947. Calc. for $C_{11}H_{12}F_3NNaO$ ($M+Na^+$) 254.0763. Found 254.0763. Calc. for $C_{11}H_{16}F_3N_2O$ ($M+H_4N^+$) 249.1209. Found 249.1210.

General procedure 1: Synthesis of 1,1-dihydroperfluoroalkyl imines

To a solution of primary amine (1.00 eq.) in anhydrous THF (2 mL) was added perfluorinated carboxylic acid (1.75 eq.) and phenylsilane (4.00 eq.). The solution was heated to reflux (70 °C) under Ar balloon. After 18 hours the solution was allowed to cool to room temperature and diluted with EtOAc (5 mL), washed with 1M NaOH solution (10 mL) and the combined organics dried ($MgSO_4$) and concentrated. Purified by flash column chromatography (dry load with NEt_3 treated silica gel) to give the perfluoroalkylated imine products.

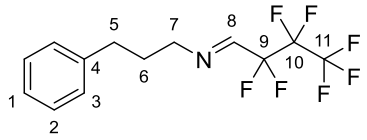
2,2,3,3-Pentafluoro-*N*-(3-phenylpropyl)propan-1-amine (114)



Prepared according to general procedure 1 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluoropropionic acid (92.0 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/pentane) to give the Title Compound as a colourless oil (33 mg, 25%).

R_f (5:95 EtOAc/pentane) = 0.7. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.72-7.69 (m, 1H, H-8), 7.37-7.30 (m, 2H, H-Ar), 7.27-7.19 (m, 3H, H-Ar), 3.71-3.65 (m, 2H, H-7), 2.80-2.62 (m, 2H, H-5), 2.08 (dt, *J* = 14.3, 6.9 Hz, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 150.1 (t, *J* = 28.1 Hz, CH, C-8), 141.1 (C, C-4), 128.5 (2 x CH), 126.1 (CH), 118.5 (qt, *J* = 286.1, 35.8 Hz, CF_3, C-10), 108.8 (tq, *J* = 253.0, 37.9 Hz, CF_2, C-9), 60.3 (CH_2, C-7), 33.0 (CH_2, C-5), 31.2 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -83.12 (app. s, 3F, F-10), -120.04- -120.09 (m, 2F, F-9). **IR** ν_{max}/cm^{-1} (ATR): 3029, 2934, 2858, 1682, 1604, 1497, 1455, 1344, 1192 1138. **HRMS** (ESI^+): Calc. for $C_{12}H_{13}F_5N$ ($M+H^+$) 266.0963. Found 266.0954.

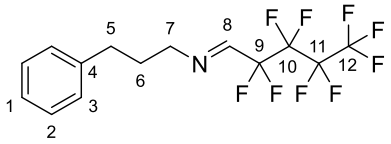
2,2,3,3,4,4,4-Heptafluoro-*N*-(3-phenylpropyl)butan-1-amine (111)



Prepared according to general procedure 1 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluorobutanoic acid (114 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 Et_2O/pentane) to give the Title Compound as a colourless oil (78 mg, 48%).

R_f (5:95 Et_2O/pentane) = 0.7. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.72 (t, *J* = 6.1 Hz, 2H, H-8), 7.38-7.31 (m, 2H, H-Ar), 7.27-7.19 (m, 3H, H-Ar), 3.72-3.68 (m, 2H, H-7), 2.75-2.68 (m, 2H, H-5), 2.09 (dt, *J* = 14.4, 6.9 Hz, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 150.0 (t, *J* = 26.9 Hz, CH, C-8), 141.1 (C, C-4), 128.5 (2 x CH), 126.1 (CH), 117.7 (qt, *J* = 287.4, 33.4 Hz, CF_3, C-11), 110.3 (tt, *J* = 255.2, 30.4 Hz, CF_2, C-9), 108.8 (tq, *J* = 265.3, 38.6 Hz, CF_2, C-10), 60.4 (CH_2, C-7), 33.0 (CH_2, C-5), 31.3 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.51 (t, *J* = 8.7 Hz, 3F, F-11), -118.37- -118.47(m, 2F, F-9), -127.56 (br, 2F, F-10). **IR** ν_{max}/cm^{-1} (ATR): 3029, 2932, 2859, 2018, 1679, 1604, 1497, 1455. **HRMS** (ESI^+): Calc. for $C_{14}H_{17}F_7NO$ ($M+CH_3OH+H^+$) 348.1198. Found 348.1195.

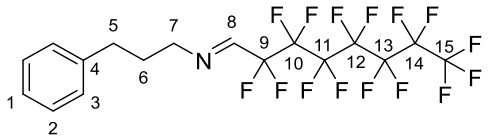
2,2,3,3,4,4,5,5,5-Nonafluoro-*N*-(3-phenylpropyl)pentan-1-amine (115)



Prepared according to general procedure 1 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluoropentanoic acid (135 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a colourless oil (127 mg, 69%).

R_f (5:95 EtOAc/petroleum ether + 0.5% NEt_3) = 0.7. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.73 (t, *J* = 6.1 Hz, 1H, H-8), 7.38-7.33 (m, 2H, H-Ar), 7.27-7.22 (m, 3H, H-Ar), 3.73-3.69 (m, 2H, H-7), 2.76-2.71 (m, 2H, H-5), 2.10 (dt, *J* = 14.4, 6.9 Hz, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 150.1 (t, *J* = 26.8 Hz, CH, C-8), 141.1 (C, C-4), 128.6 (CH), 128.5 (CH), 126.1 (CH), 117.4 (qt, *J* = 287.9, 33.2 Hz, CF_3, C-12), 113.3-105.9 (m, CF_2, C-10 & C-11), 110.8 (tt, *J* = 258.1, 31.7 Hz, CF_2, C-9), 60.4 (CH_2, C-7), 33.0 (CH_2, C-5), 31.3 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -81.07- -81.17 (m, 3F, F-12), -117.52- -117.83 (m, 2F, F-9), 124.02- -124.37 (m, 2F), -125.71- -125.85 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3030, 2938, 2858, 1679, 1604, 1497, 1455. **HRMS** (ESI^+): Calc. for $C_{14}H_{12}F_9NNa$ ($M+Na^+$) 388.0718. Found 388.0714.

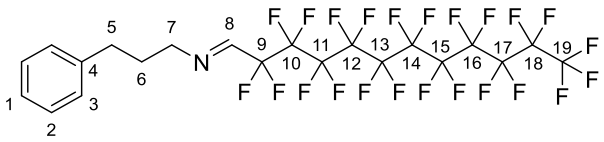
2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluoro-*N*-(3-phenylpropyl)octan-1-amine (116)



Prepared according to general procedure 1 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluorooctanoic acid (362 mg, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a colourless oil (185 mg, 72%).

R_f (5:95 EtOAc/petroleum ether + 0.5% NEt_3) = 0.8. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.73 (t, *J* = 6.2 Hz, 1H, H-8), 7.37-7.33 (m, 2H, H-Ar), 7.29-7.21 (m, 3H, H-Ar), 3.73-3.68 (m, 2H, H-7), 2.76-2.71 (m, 2H, H-5), 2.10 (dt, *J* = 14.2, 6.9 Hz, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 150.1 (t, *J* = 26.8 Hz, CH, C-8), 141.1 (C, C-4), 128.5 (CH), 128.4 (CH), 126.1 (CH), 117.2 (qt, *J* = 288.3, 33.2 Hz, CF_3, C-15), 113.5-105.7 (m, CF_2, C-9, C-10, C-11, C-12, C-13 & C-14), 60.3 (CH_2, C-7), 33.0 (CH_2, C-5), 31.3 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -81.05 (t, *J* = 10.0 Hz, 3F, F-15), -117.45 (br, 2F), -121.43 (br, 2F), -122.10 (br, 2F), -122.78 (br, 2F), -123.11- -123.25 (m, 2F), -126.23- -126.28 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3030, 2934, 2859, 1680, 1604, 1497, 1455, 1367, 1320, 1235, 1200. **HRMS** (ESI$^+$): Calc. for $C_{17}H_{12}F_{15}NNa$ (M+Na$^+$) 538.0622. Found 538.0627.

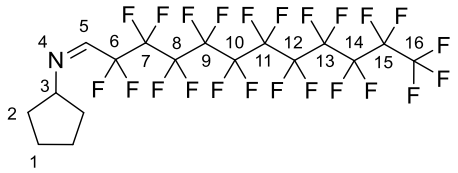
2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12- Tricosafluoro-*N*-(3-phenylpropyl)dodecan-1-amine (117)



Prepared according to general procedure 1 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluorododecanoic acid (537 mg, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a colouress solid (161 mg, 45%).

R_f (5:95 EtOAc/petroleum ether + 0.5% NEt_3) = 0.8. **Mp:** 51-53 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.71 (t, *J* = 6.1 Hz, 1H, H-8), 7.34-7.29 (m, 2H, H-Ar), 7.25-7.18 (m, 3H, H-Ar), 3.72-3.65 (m, 2H, H-7), 2.75-2.63 (m, 2H, H-5) 2.12-2.03 (m, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 150.1 (t, *J* = 26.7 Hz, CH, C-8), 141.1 (C, C-4), 128.5 (CH), 128.4 (CH), 126.1 (CH), 117.1 (qt, *J* = 288.3, 32.8 Hz, CF_3, C-19), 113.4-105.7 (m, CF_2, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17 & C-18), 60.4 (CH_2, C-7), 33.0 (CH_2, C-5), 31.3 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.75 (t, *J* = 9.9 Hz, 3F, F-19), -117.42 (br, 2F), -121.34 (br, 2F), -121.57- -121.97 (m, 10F), -122.68 (br, 2F), -123.11 (br, 2F), -126.08 (br, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3029, 2929, 2857, 1680, 1604, 1497, 1455, 1374, 1347, 1200. **HRMS** (ESI^+): Calc. for $C_{21}H_{12}F_{23}NNa$ ($M+Na^+$) 738.0495. Found 738.0492.

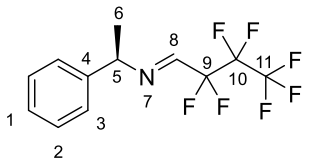
(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Tricosafluorododecyl)cyclopentylamine (118)



Prepared according to general procedure 1 from cyclopentylamine (49 µL, 0.50 mmol), perfluorododecanoic acid (537 mg, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 Et_2O/pentane) to give the Title Compound as a white solid (267 mg, 80%).

R_f (5:95 Et_2O/pentane) = 0.8. **Mp:** 36-38 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.67 (t, *J* = 6.3 Hz, 1H, H-5), 3.85 (p, *J* = 5.9 Hz, 1H, H-3), 1.95-1.82 (m, 4H, H-1), 1.76-1.64 (m, 4H, H-2). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 147.2 (t, *J* = 26.6 Hz, CH, C-5), 117.1 (qt, *J* = 288.6, 33.3 Hz, CF_3, C-16), 113.5-105.7 (m, CF_2, C-6, C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14 & C-15), 71.4 (CH, C-3), 33.9 (CH_2, C-2), 24.6 (CH_2, C-1). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -81.04 (t, *J* =10.1 Hz, 3F, F-16), -117.59 (br, 2F), -121.47 (br, 2F), -121.79- -122.17 (m, 10F), -122.88 (br, 2F), -123.45 (br, 2F), -126.32 (br, 2F). **IR** ν_{max}/cm^{-1} (ATR): 2958, 2871, 1673, 1450, 1376, 1349, 1316, 1199, 1146, 1100. **HRMS** (ESI^+): Calc. for $C_{18}H_{15}F_{23}NO$ ($M+CH_3OH+H^+$) 698.0786. Found 698.0782.

(*R*)-2,2,3,3,4,4,4-Heptafluoro-*N*-(1-phenylethyl)butan-1-amine (119)



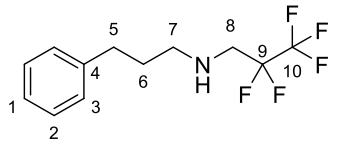
Prepared according to general procedure 1 from (*R*)-(-)-α-methylbenzylamine (258 µL, 2.00 mmol), perfluorobutanoic acid (455 µL, 3.50 mmol) and phenylsilane (988 µL, 8.00 mmol). Purified by flash column chromatography (1:9 Et_2O/pentane) to give the Title Compound as a colourless oil (520 mg, 86%).

R_f (1:9 Et_2O/pentane) = 0.8. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.74 (t, *J* = 6.1 Hz, 1H, H-8), 7.50-7.30 (m, 5H, H-1, H-2 & H-3), 4.69 (q, *J* = 6.8 Hz, 1H, H-5), 1.63 (d, *J* = 6.3 Hz, 3H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 148.5 (t, *J* = 27.1 Hz, CH, C-8), 142.2 (C, C-5), 128.8 (CH, C-2), 127.7 (CH, C-1), 126.5 (CH, C-3), 117.6 (app. qt, *J* = 287.4, 33.4 Hz, CF_3, C-11), 110.5 (app. tt, *J* = 255.5, 30.1 Hz, CF_2, C-9), 108.8 (app. tq, *J* = 265.5, 38.5 Hz, CF_2, C-10), 69.4 (CH, C-5), 24.0 (CH_3, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.53 (app. t, *J* = 8.5 Hz, 3F, F-11), -118.29- -118.45 (m, 2F, F-9), -127.60 (br, 2F, F-10). **IR** ν_{max}/cm^{-1} (ATR): 3032, 2981, 2872, 2104, 1678, 1604, 1495, 1453, 1376, 1353, 1279, 1221. **HRMS** (ESI^+): Calc. for $C_{12}H_{13}F_7N$ ($M+H^+$) 304.0931. Found 304.0922.

General procedure 2: Synthesis of 1,1-dihydroperfluoroalkyl secondary amines

To a solution of primary amine (1.00 eq.) in anhydrous THF (2 mL) was added perfluorinated carboxylic acid (1.75 eq.) and phenylsilane (4.00 eq.). The solution was heated to reflux (70 °C) under Ar balloon. After 18 hours the solution was allowed to cool to room temperature and sodium borohydride (2.00 eq.) added and stirred at room temperature under Ar balloon. After 4 hours, diluted with EtOAc (5 mL), washed with 1M NaOH solution (10 mL) and the combined organics dried ($MgSO_4$) and concentrated. Purified by flash column chromatography with specified solvents to give the perfluoroalkylated secondary amine products.

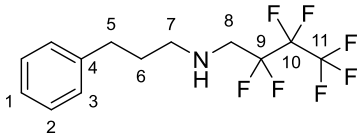
2,2,3,3,3-Pentafluoro-*N*-(3-phenylpropyl)propan-1-amine (121)



Prepared according to general procedure 2 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluoropropionic acid (92.0 µL, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (1:4 EtOAc/hexane + 0.5% NEt_3) to give the Title Compound as a colourless oil (114 mg, 86%).

R_f (1:4 EtOAc/hexane + 0.5% NEt_3) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.35-7.30 (m, 2H, H-Ar), 7.25-7.19 (m, 3H, H-Ar), 3.23 (t, *J* = 15.6 Hz, 2H, H-8), 2.79 (t, *J* = 7.0 Hz, 2H, H-7), 2.71 (t, *J* = 7.9 Hz, 2H, H-5), 1.85 (dt, *J* = 14.2, 7.3 Hz, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 141.8 (C, C-4), 128.4 (CH, C-2 & C-3), 125.9 (CH, C-1), 119.1 (qt, *J* = 285.9, 35.6 Hz, CF_3, C-10), 114.6 (tq, *J* = 253.1, 36.3 Hz, CF_2, C-9), 49.3 (CH_2, C-7), 48.7 (t, *J* = 22.6 Hz, CH_2, C-8), 33.2 (CH_2, C-5), 31.7 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -84.06 (app. s, 3F, F-10), -121.68 (t, *J* = 15.6 Hz, 2F, F-9). **IR** ν_{max}/cm^{-1} (ATR): 3065, 3028, 2931, 2859, 2308, 2074, 1957, 1724, 1603, 1496, 1474, 1455, 1343, 1187, 1129. **HRMS** (ESI^+): Calc. for $C_{12}H_{14}F_5NNa$ ($M+Na^+$) 290.0939. Found 290.0935.

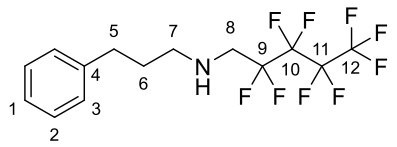
2,2,3,3,4,4,4-Heptafluoro-*N*-(3-phenylpropyl)butan-1-amine (112)



Prepared according to general procedure 2 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluorobutanoic acid (114 µL, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (1:9 EtOAc/pentane) to give the Title Compound as a colourless oil (88 mg, 56%).

R_f (1:9 EtOAc/pentane) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.36-7.29 (m, 2H, H-Ar), 7.25-7.19 (m, 3H, H-Ar), 3.27 (t, *J* = 16.2 Hz, 2H, H-8), 2.79 (t, *J* = 7.1 Hz, 2H, H-7), 2.75-2.68 (m, 2H, H-5), 1.85 (dt, *J* = 14.3, 7.2 Hz, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 141.8 (C, C-4), 128.4 (CH, C-2 & C-3), 125.9 (C-1), 117.8 (qt, *J* = 287.5, 34.2 Hz, CF_3, C-11), 116.4 (tt, *J* = 254.3, 30.0 Hz, CF_2, C-9), 109.0 (tq, *J* = 264.6, 38.0 Hz, CF_2, C-10), 49.3 (CH_2, C-7), 48.7 (t, *J* = 22.3 Hz, CH_2, C-8), 33.2 (CH_2, C-5), 31.7 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.81 (t, *J* = 9.4 Hz, 3F, F-11), -118.66- -119.16 (m, 2F, F-9), -127.74 (br, 2F, F-10). **IR** ν_{max}/cm^{-1} (ATR): 3029, 2932, 2860, 2397, 1604, 1496, 1474, 1455, 1352. **HRMS** (ESI^+): Calc. for $C_{13}H_{14}F_7NNa$ ($M+Na^+$) 340.0907. Found 340.0898.

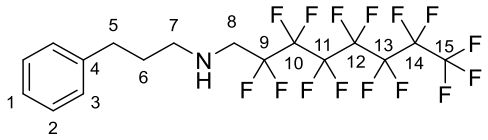
2,2,3,3,4,4,5,5,5-Nonafluoro-*N*-(3-phenylpropyl)pentan-1-amine (122)



Prepared according to general procedure 2 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluoropentanoic acid (135 µL, 0.875 mmol), phenysilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (1:9 EtOAc/pentane) to give the Title Compound as a colourless oil (152 mg, 83%).

R_f (1:9 EtOAc/pentane) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.35-7.29 (m, 2H, H-Ar), 7.25-7.19 (m, 3H, H-Ar), 3.29 (t, *J* = 16.3 Hz, 2H, H-8), 2.80 (t, *J* = 7.1 Hz, 2H, H-7), 2.76-2.68 (m, 2H, H-5), 1.90-1.81 (m, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 141.8 (C, C-4), 128.4 (CH, C-2 & C-3), 125.9 (CH, C-1), 117.4 (qt, *J* = 288.4, 33.9 Hz, CF_3, C-12), 116.9 (tt, *J* = 255.5, 30.5 Hz, CF_2, C-9), 113.1-106.1 (m, CF_2, C-10 & C-11), 49.3 (CH_2, C-7), 48.8 (t, *J* = 22.3 Hz, CH_2, C-8), 33.2 (CH_2, C-5), 31.8 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -81.03 (tt, *J* = 9.6, 3.2 Hz, 3F, F-12), -118.10- -118.20 (m, 2F, F-9), -124.35- -124.50 (m, 2F), -126.14- -126.3 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3029, 2935, 2860, 1604, 1496, 1474, 1455, 1355, 1219. **HRMS** (ESI$^+$): Calc. for $C_{14}H_{15}F_9N$ ($M+H^+$) 368.1055. Found 368.1059. Calc. for $C_{14}H_{14}F_9NNa$ ($M+Na^+$) 390.0872. Found 390.0875.

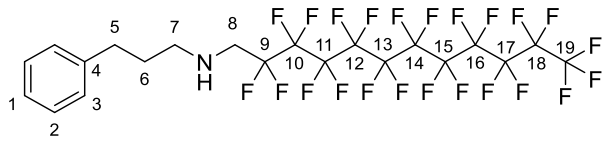
2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluoro-*N*-(3-phenylpropyl)octan-1-amine (123)



Prepared according to general procedure 2 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluorooctanoic acid (362 mg, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (1:9 EtOAc/hexane + 0.5% NEt_3) to give the Title Compound as a colourless oil (245 mg, 95%).

R_f (1:9 EtOAc/hexane + 0.5% NEt_3) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.35-7.29 (m, 2H, H-Ar), 7.26-7.20 (m, 3H, H-Ar), 3.29 (t, *J* = 16.3 Hz, 2H, H-8), 2.80 (t, *J* = 7.1 Hz, 2H, H-7), 2.71 (t, *J* = 7.6 Hz, 2H, H-5), 1.90-1.81 (m, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 141.8 (C, C-4), 128.4 (CH, C-2 & C-3), 125.9 (CH, C-1), 117.2 (qt, *J* = 288.6, 33.0 Hz, CF_3, C-15), 117.1 (tt, *J* = 256.1, 30.6 Hz, CF_2, C-9), 113.7-105.7 (m, CF_2, C-10, C-11, C-12, C-13 & C-14), 49.3 (CH_2, C-7), 48.9 (t, *J* = 22.3 Hz, CH_2, C-8), 33.2 (CH_2, C-5), 31.8 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.88 (t, *J* = 10.0 Hz, 3F, F-15), -117.90- -118.00 (m, 2F, F-9), -121.92 (br, 2F), -122.10 (br, 2F), -122.80 (br, 2F), -123.48 (br, 2F), -126.11- -126.26 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3029, 2932, 2860, 2325, 2181, 2113, 1981, 1604, 1496, 1475, 1455, 1365, 1323, 1236, 1201, 1143, 1129, 1103. **HRMS** (ESI$^+$): Calc. for $C_{17}H_{14}F_{15}NNa$ (M+Na$^+$) 540.0779. Found 540.0787.

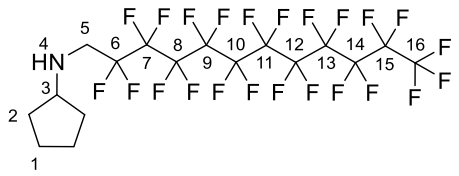
2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Tricosafluoro-*N*-(3-phenylpropyl)dodecan-1-amine (124)



Prepared according to general procedure 2 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluorododecanoic acid (537 mg, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (1:9 EtOAc/hexane + 0.5% NEt_3) to give the Title Compound as a white solid (140 mg, 39%).

R_f (1:9 EtOAc/hexane + 0.5% NEt_3) = 0.6. **Mp:** 62-64 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.36-7.29 (m, 2H, H-Ar), 7.25-7.18 (m, 3H, H-Ar), 3.28 (t, *J* = 16.3 Hz, CH_2, H-8), 2.79 (t, *J* = 7.1 Hz, 2H, H-7), 2.70 (t, *J* = 7.7 Hz, 2H, H-5), 1.89-1.79 (m, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 141.8 (C, C-4), 128.4 (CH, C-2 & C-3), 125.9 (CH, C-1), 117.1 (qt, *J* = 288.5, 32.4 Hz, CF_3, C-20), 117.0 (tt, *J* = 256.5, 31.0 Hz, CF_2, C-10), 113.7-107.7 (m, CF_2, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18 & C-19), 49.3 (CH_2, C-7), 48.9 (t, *J* = 22.3 Hz, CH_2, C-8), 33.2 (CH_2, C-5), 31.8 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.71 (t, *J* = 10.0 Hz, 3F, F-19), -117.98 (br, 2F, F-9), -121.45- -122.10 (m, 12F), -122.65 (br, 2F), -123.42 (br, 2F), -126.05 (br, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3027, 2929, 2858, 2164, 1980, 1604, 1497, 1455, 1374, 1348, 1315, 1200, 1148, 1101. **HRMS** (ESI^+): Calc. for $C_{21}H_{15}F_{23}N$ ($M+H^+$) 718.0832. Found 718.0838.

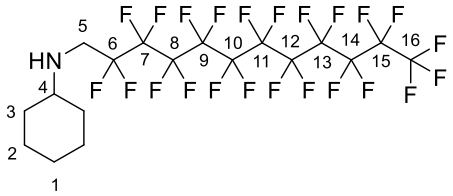
(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Tricosafluorododecyl)cyclopentylamine (125)



Prepared according to general procedure 2 from cyclopentylamine (49 µL, 0.50 mmol), perfluorododecanoic acid (537 mg, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (5:95 EtOAc/pentane) to give the Title Compound as a white solid (133 mg, 40%).

R_f (5:95 EtOAc/petroleum ether) = 0.6. **Mp:** 69-71 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.27 (t, *J* = 16.2 Hz, 2H, H-5), 3.22 (p, *J* = 6.4 Hz, 1H, H-3), 1.91-1.82 (m, 2H, H-2), 1.78-1.68 (m, 2H, H-1), 1.64-1.52 (m, 2H, H-1), 1.42-1.33 (m, 2H, H-2). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 117.1 (qt, *J* = 288.2, 32.9 Hz, CF_3, C-16), 117.0 (tt, *J* = 255.7, 30.5 Hz, CF_2, C-6), 113.6-107.7 (m, CF_2, C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14 & C-15), 59.6 (CH, C-3), 47.6 (t, *J* = 22.1 Hz, CH_2, C-5), 33.0 (CH_2, C-2), 23.8 (CH_2, C-1). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.92 (t, *J* = 9.9 Hz, 3F, F-16), -118.15 (br, 2F, F-6), -121.68- -122.08 (m, 12F), -122.80 (br, 2F), -123.43 (br, 2F), -126.23 (br, 2F). **IR** ν_{max}/cm^{-1} (ATR): 2957, 2869, 1469, 1375, 1348, 1199, 1148. **HRMS** (ESI$^+$): Calc. for $C_{17}H_{13}F_{23}N$ ($M+H^+$) 668.0675. Found 668.0679.

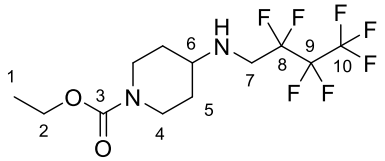
(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Tricosafluorododecyl)cyclohexylamine (126)



Prepared according to general procedure 2 from cyclohexylamine (57 µL, 0.50 mmol), perfluorododecanoic acid (537 mg, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (5:95 EtOAc/pentane) to give the Title Compound as a white solid (183 mg, 54%).

R_f (5:95 EtOAc/pentane) = 0.6. **Mp:** 66-68 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.30 (t, *J* = 16.1 Hz, 2H, H-5), 2.59-2.52 (m, 1H, H-4), 1.94-1.87 (m, 2H), 1.80-1.73 (m, 2H), 1.66-1.60 (m, 1H), 1.36-1.00 (m, 7H). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 117.1 (qt, *J* =288.0, 33.1 Hz. CF_3, C-16), 117.0 (tt, *J* = 255.9, 30.5 Hz, CF_2, C-6), 114.2-105.5 (m, CF_2, C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14 & C-15), 56.4 (CH, C-4), 46.0 (t, *J* = 22.2 Hz, CH_2, C-5), 33.3 (CH_2), 25.9 (CH_2), 24.8 (CH_2). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.92 (t, *J* = 9.9 Hz, 3F, F-16), -118.39 (br, 2F, F-6), -121.65- -122.10 (m, 12F), -122.80 (br, 2F), -123.42 (br, 2F), -126.22 (br 2F). **IR** ν_{max}/cm^{-1} (ATR): 2928, 2854, 1450, 1349, 1200, 1148, 1039. **HRMS** (ESI$^+$): Calc. for $C_{18}H_{14}F_{23}NNa$ (M+Na$^+$) 704.0651. Found 704.0642.

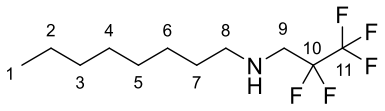
Ethyl 4-((2,2,3,3,4,4,4-heptafluorobutyl)amino)piperidine-1-carboxylate (127)



Prepared according to general procedure 2 from ethyl 4-aminopiperidine-1-carboxylate (86 µL, 0.50 mmol), perfluorobutanoic acid (114 µL, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (1:4 EtOAc/pentane + 0.5% NEt_3) to give the Title Compound as a colourless oil (126 mg, 71%).

R_f (1:4 EtOAc/pentane + 0.5% NEt_3) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 4.18-3.99 (m, 4H), 3.30 (t, *J* = 16.0 Hz, 2H, H-7), 2.96-2.85 (m, 2H), 2.80-2.72 (m, 1H, H-6), 1.91-1.81 (m, 2H), 1.32-1.22 (m, 5H). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 155.5 (C, C-3), 117.8 (qt, *J* = 287.2, 33.7 Hz, CF_3, C-10), 116.2 (tt, *J* = 254.4, 30.1 Hz, CF_2, C-8), 109.0 (qt, *J* = 264.0, 38.0 Hz, CF_2, C-9), 61.3 (CH_2, C-2), 54.3 (CH, C-6), 45.6 (t, *J* = 22.6 Hz, CH_2, C-7), 42.2 (CH_2), 32.2 (CH_2), 14.7 (CH_3, C-1). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.87 (t, *J* = 9.4 Hz, 3F, F-10), -119.01- -119.21 (m, 2F, F-8), -127.62 (app. s, 2F, F-9). **IR** ν_{max}/cm^{-1} (ATR): 3340, 2935, 2859, 1689, 1473, 1433, 1387, 1353, 1276, 1220, 1171, 1112. **HRMS** (ESI^+): Calc. for $C_{12}H_{18}F_7N_2O_2$ ($M+H^+$) 355.1251. Found 355.1256. Calc. for $C_{12}H_{17}F_7N_2NaO_2$ ($M+Na^+$) 377.1070. Found 377.1073.

***N*-(2,2,3,3,3-Pentafluoropropyl)octan-1-amine (128)**



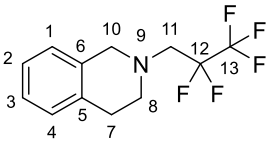
Prepared according to general procedure 2 from 1-octylamine (83 µL, 0.50 mmol), perfluoropropionic acid (92.0 µL, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol) and sodium borohydride (37.8 mg, 1.00 mmol). Purified by flash column chromatography (1:9 Et_2O/pentane) to give the Title Compound as a colourless oil (117 mg, 89%).

R_f (1:9 Et_2O/pentane) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.23 (t, *J* = 15.7 Hz, 2H, H-9), 2.73 (t, *J* = 7.2 Hz, 2H, H-8), 1.55-1.43 (m, 2H, H-7), 1.37-1.23 (m, 10H, H-2, H-3, H-4, H-5 & H-6), 0.90 (t, *J* = 6.8 Hz, 3H, H-1). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 119.0 (qt, *J* = 285.9, 35.6 Hz, CF_3, C-11), 114.6 (tq, *J* = 253.3, 36.3 Hz, CF_2, C-10), 50.0 (CH_2, C-8), 48.7 (t, *J* = 22.5 Hz, CH_2, C-9), 31.8 (CH_2), 30.1 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 27.0 (CH_2), 22.6 (CH_2), 14.0 (CH_3, C-1). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -84.21 (app. s, 3F, F-11), -121.76 (t, *J* = 15.7 Hz, 2F, F-10). **IR** ν_{max}/cm^{-1} (ATR): 2927, 2857, 2391, 1469, 1343, 1190. **HRMS** (ESI^+): Calc. for $C_{11}H_{21}F_5N$ ($M+H^+$) 262.1589. Found 262.1586.

General procedure 3: Synthesis of 1,1-dihydroperfluoroalkyl tertiary amines

To a solution of secondary amine (1.00 eq.) in anhydrous THF (2 mL) was added perfluorinated carboxylic acid (1.75 eq.) and phenylsilane (4.00 eq.). The solution was heated to reflux (70 °C) under Ar balloon. After 18 hours the solution was allowed to cool to room temperature and diluted with EtOAc (5 mL), washed with 1M NaOH solution (10 mL) and the combined organics dried ($MgSO_4$) and concentrated. Purified by flash column chromatography with specified solvents to give the perfluoroalkylated tertiary amine products.

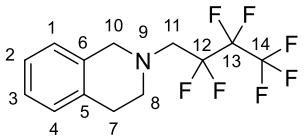
2-(2,2,3,3-Pentafluoropropyl)-1,2,3,4-tetrahydroisoquinoline (129)



Prepared according to general procedure 3 from 1,2,3,4-tetrahydroisoquinoline (67 µL, 0.50 mmol), pentafluoropropionic acid (92.0 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a colourless oil (68 mg, 52%).

R_f (5:95 EtOAc/petroleum ether + 0.5% NEt_3) = 0.5. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.22-7.12 (m, 3H, H-Ar), 7.08-7.01 (m, 1H, H-Ar), 3.91 (s, 2H, H-10), 3.20 (t, *J* = 14.9 Hz, 2H, H-11), 3.01 (t, *J* = 5.4 Hz, 2H, H-8), 2.94 (t, *J* = 5.8 Hz, 2H, H-7). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 134.0 (C), 133.8 (C), 128.8 (CH), 126.5 (CH), 126.4 (CH), 125.8 (CH), 119.1 (qt, *J* = 286.2, 35.3 Hz, CF_3, C-13), 114.8 (tq, *J* = 254.5, 36.1 Hz, CF_2, C-12), 56.6 (CH_2, C-10), 56.3 (t, *J* = 22.5 Hz, CH_2, C-11), 51.9 (CH_2), 28.7 (CH_2). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -83.83 (app. s, 3F, F-13), -119.15 (t, *J* = 15.3 Hz, 2F, F-12). **IR** ν_{max}/cm^{-1} (ATR): 3026, 2926, 2795, 2323, 2167, 2095, 1671, 1607, 1586, 1498, 1468, 1451, 1431, 1384, 1364, 1348, 1319, 1183, 1144. **HRMS** (ESI^+): Calc. for $C_{12}H_{13}F_5N$ ($M+H^+$) 266.0963. Found 266.0964.

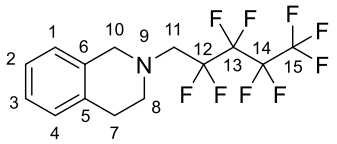
2-(2,2,3,3,4,4,4-Heptafluorobutyl)-1,2,3,4-tetrahydroisoquinoline (109)



Prepared according to general procedure 3 from 1,2,3,4-tetrahydroisoquinoline (67 µL, 0.50 mmol), perfluorobutanoic acid (114 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/pentane) to give the Title Compound as a colourless oil (92 mg, 59%).

R_f (5:95 EtOAc/petroleum ether + 0.5% NEt_3) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.22-7.14 (m, 3H, H-Ar), 7.07-7.04 (m, 1H, H-Ar), 3.94 (s, 2H, H-10), 3.26 (t, *J* = 15.9 Hz, 2H, H-11), 3.03 (t, *J* = 5.8 Hz, 2H, H-8), 2.96 (t, *J* = 5.9 Hz, 2H, H-7). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 134.1 (C), 133.8 (C), 128.8 (CH), 126.5 (CH), 126.4 (CH), 125.8 (CH), 117.9 (qt, *J* = 287.5, 33.8 Hz, CF_3, C-14), 116.7 (tt, *J* = 255.7, 29.7 Hz, CF_2, C-12), 109.1 (tq, *J* = 264.3, 37.8 Hz, CF_2, C-13), 56.6 (CH_2, C-10), 56.3 (t, *J* = 22.0 Hz, CH_2, C-11), 51.9 (CH_2, C-8), 28.7 (CH_2, C-7). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.69 (t, *J* = 9.6 Hz, 3F, F-14), -116.45- -116.65 (m, 2F, F-12), -127.41- -127.51 (m, 2F, F-13). **IR** ν_{max}/cm^{-1} (ATR): 3027, 2928, 2811, 2313, 2059, 1668, 1606, 1585, 1499, 1482, 1468, 1452, 1438, 1384, 1352, 1340, 1220, 1175, 1113. **HRMS** (ESI^+): Calc. for $C_{13}H_{13}F_7N$ ($M+H^+$) 316.0931. Found 316.0934. Calc. for $C_{13}H_{12}F_7NNa$ ($M+Na^+$) 338.0750. Found 338.0756.

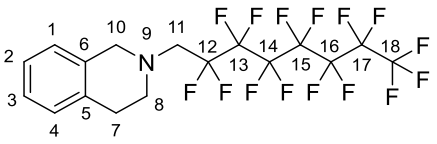
2-(2,2,3,3,4,4,5,5,5-Nonafluoropentyl)-1,2,3,4-tetrahydroisoquinoline (130)



Prepared according to general procedure 3 from 1,2,3,4-tetrahydroisoquinoline (67 µL, 0.50 mmol), perfluoropentanoic acid (135 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a colourless oil (168 mg, 92%).

R_f (5:95 EtOAc/petroleum ether + 0.5% NEt_3) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.24-7.15 (m, 3H, H-Ar), 7.09-7.04 (m, 1H, H-Ar), 3.96 (s, 2H, H-10), 3.29 (t, *J* = 16.0 Hz, 2H, H-11), 3.05 (t, *J* = 5.8 Hz, 2H, H-8), 2.97 (t, *J* = 5.9 Hz, 2H, H-7). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 133.9 (C), 133.9 (C), 128.8 (CH), 126.5 (2 x CH), 125.8 (CH), 117.5 (qt, *J* = 288.0, 33.4 Hz, CF_3, C-15), 117.3 (tt, *J* = 256.3, 30.3 Hz, CF_2, C-12), 113.5-105.9 (m, CF_2, C-13 & C-14), 56.6 (CH_2, C-10), 56.5 (t, *J* = 22.0 Hz, CH_2, C-11), 51.9 (CH_2, C-8), 28.7 (CH_2, C-7). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -81.02- -81.10 (m, 3F, F-15), -115.80- -116.00 (m, 2F, F-12), -124.15 (br, 2F), -126.06- -126.22 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3026, 2928, 2856, 2231, 2110, 1987, 1669, 1606, 1499, 1482, 1467, 1429, 1382, 1352, 1321, 1218, 1198, 1130. **HRMS** (ESI^+): Calc. for $C_{14}H_{13}F_9N$ ($M+H^+$) 366.0899. Found 366.0904. Calc. for $C_{14}H_{12}F_9NNa$ ($M+Na^+$) 388.0718. Found 388.0718.

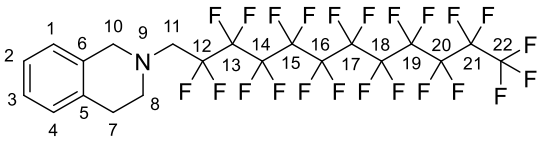
2-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluorooctyl)-1,2,3,4-tetrahydroisoquinoline (131)



Prepared according to general procedure 3 from 1,2,3,4-tetrahydroisoquinoline (67 µL, 0.50 mmol), perfluorooctanoic acid (362 mg, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a white solid (199 mg, 73%).

R_f (5:95 EtOAc/petroleum ether + 0.5% NEt_3) = 0.7. **Mp:** 50-52 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.19-7.11 (m, 3H, H-Ar), 7.07-7.03 (m, 1H, H-Ar), 3.94 (s, 2H, H-10), 3.27 (t, *J* = 15.9 Hz, 2H, H-11), 3.03 (t, *J* = 5.8 Hz, 2H, H-8), 2.95 (t, *J* = 5.9 Hz, 2H, H-7). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 134.1 (C), 133.8 (C), 128.8 (CH), 126.5 (CH), 126.4 (CH), 125.8 (CH), 117.4 (tt, *J* = 257.1, 30.2 Hz, CF_2, C-12), 117.2 (qt, *J* = 289.1, 33.5 Hz, CF_3, C-18), 113.6-105.7 (m, CF_2, C-13, C-14, C-15, C-16 & C-17), 56.8-56.3 (m, CH_2, C-10 & C-11), 51.9 (CH_2, C-8), 28.7 (CH_2, C-7). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.68- -80.80 (m, 3F, F-18), -115.55- -115.75 (m, 2F, F-12), -121.77 (br, 2F), -122.01 (br, 2F), -122.70 (br, 2F), -123.12 (br, 2F), -125.98- -126.18 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3067, 3045, 3027, 2964, 2932, 2900, 2829, 2790, 2747, 2069, 1984, 1585, 1499, 1466, 1424, 1370, 1325, 1195, 1142, 1128. **HRMS** (ESI^+): Calc. for $C_{17}H_{13}F_{15}N$ ($M+H^+$) 516.0803. Found 516.0834. Calc. for $C_{17}H_{12}F_{15}NNa$ ($M+Na^+$) 538.0622. Found 538.0662.

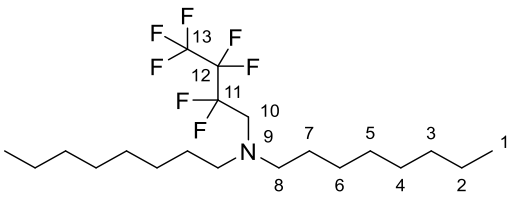
2-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Tricosafluorododecyl)-1,2,3,4-tetrahydroisoquinoline (132)



Prepared according to general procedure 3 from 1,2,3,4-tetrahydroisoquinoline (67 µL, 0.50 mmol), perfluorododecanoic acid (537 mg, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a white solid (323 mg, 90%).

R_f (5:95 EtOAc/petroleum ether + 0.5% NEt_3) = 0.8. **Mp:** 96-98 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.20-7.12 (m, 3H, H-Ar), 7.07-7.02 (m, 1H, H-Ar), 3.93 (s, 2H, H-10), 3.26 (t, *J* = 15.9 Hz, 2H, H-11), 3.02 (t, *J* = 5.8 Hz, 2H, H-8), 2.94 (t, *J* = 5.9 Hz, 2H, H-7). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 134.1 (C), 133.8 (C), 128.8 (CH), 126.5 (CH), 126.4 (CH), 125.8 (CH), 117.4 (tt, *J* = 257.4, 30.4 Hz, CF_2, C-12), 117.1 (qt, *J* = 287.9, 33.3 Hz, CF_3, C-22), 113.8-105.9 (m, CF_2, C-13, C-14, C-15, C-16, C-17, C-18, C-19, C-20 & C-21), 56.9-56.2 (m, CH_2, C-10 & C-11), 51.9 (CH_2, C-8), 28.7 (CH_2, C-7). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.71 (t, *J* = 9.9 Hz, 3F, F-22), -115.65 (br, 4F), -121.68 (br, 8F), -121.81 (br, 2F), -122.65 (br, 2F), -123.11 (br, 2F), -126.06 (br, 2F). **IR** ν_{max}/cm^{-1} (ATR): 2966, 2932, 2900, 2828, 2038, 1684, 1499, 1466, 1457, 1424, 1376, 1348, 1324, 1200, 1147, 1120. **HRMS** (ESI^+): Calc. for $C_{21}H_{13}F_{23}N$ ($M+H^+$) 716.0675. Found 716.0673.

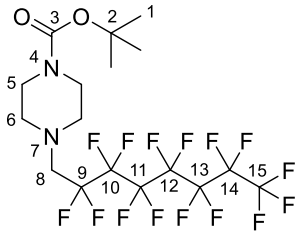
***N*-(2,2,3,3,4,4,4-Heptafluorobutyl)-*N*-octyloctan-1-amine (133)**



Prepared according to general procedure 3 from dioctylamine (151 µL, 0.50 mmol), perfluorobutanoic acid (114 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/hexane + 0.5% NEt_3) to give the Title Compound as a colourless oil (209 mg, 98%).

R_f (5:95 EtOAc/hexane + 0.5% NEt_3) = 0.9. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.11 (t, *J* = 16.7 Hz, 2H, H-10), 2.60 (t, *J* = 7.6 Hz, 4H, H-8), 1.48-1.43 (m, 4H, H-7), 1.36-1.24 (m, 20H, H-2, H-3, H-4, H-5 & H-6), 0.91 (t, *J* = 6.9 Hz, 6H, H-1). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 117.9 (qt, *J* = 287.5, 34.1 Hz, CF_3, C-13), 116.8 (tt, *J* = 254.8, 30.0 Hz, CF_2, C-11), 109.1 (tq, *J* = 263.3, 36.6 Hz, CF_2, C-12), 55.5 (CH_2, C-8), 53.3 (t, *J* = 20.9 Hz, CH_2, C-10), 31.9 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 22.7 (CH_2), 14.1 (CH_3, C-1). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.72 (t, *J* = 9.6 Hz, 3F, F-13), -117.49- -117.70 (m, 2F, F-11), -127.68- -127.78 (m, 2F, F-12). **IR** ν_{max}/cm^{-1} (ATR): 2928, 2855, 2289, 2080, 2020, 1594, 1501, 1465, 1453, 1388, 1347, 1312, 1277, 1178, 1118. **HRMS** (ESI^+): Calc. for $C_{20}H_{37}F_7N$ ($M+H^+$) 424.2809. Found 424.2809.

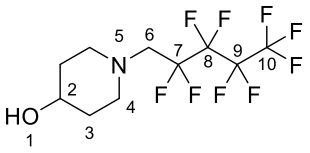
***tert*-Butyl 4-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctyl)piperazine-1-carboxylate (134)**



Prepared according to general procedure 3 from 1-boc-piperazine (93 mg, 0.50 mmol), perfluorooctanoic acid (362 mg, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (1:9 EtOAc/hexane + 0.5% NEt_3) to give the Title Compound as a white solid (172 mg, 61%).

R_f (1:9 EtOAc/hexane + 0.5% NEt_3) = 0.35. **Mp:** 59-61 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.46 (t, *J* = 9.8 Hz, 4H, H-5), 3.07 (t, *J* = 15.8 Hz, 2H, H-8), 2.64 (t, *J* = 9.7 Hz, 4H, H-6), 1.48 (s, 9H, H-1). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 154.7 (C, C-3), 121.2-113.2 (m, CF_3, C-15), 117.2 (tt, *J* = 257.3, 29.9 Hz, CF_2, C-9), 114.0-106.1 (m, CF_2, C-10, C-11, C-12, C-13 & C-14), 79.8 (C, C-2), 57.1 (CH_2, C-8), 53.9 (CH_2, C-5), 43.7 (br, CH_2, C-6), 28.4 (CH_3, C-1). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.72- -80.82 (m, 3F, F-15), -115.26- -115.49 (m, 2F), -121.81 (br, 2F), -122.04 (br, 2F), -122.73 (br, 2F), -123.10 (br, 2F), -126.03- -126.20 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 2983, 2970, 2919, 2889, 2864, 2808, 2083, 1687, 1480, 1454, 1426, 1396, 1365, 1323, 1294, 1283, 1244. **HRMS** (ESI$^+$): Calc. for $C_{17}H_{20}F_{15}N_2O_2$ (M+H$^+$) 569.1280. Found 569.1273. Calc. for $C_{17}H_{19}F_{15}N_2NaO_2$ (M+Na$^+$) 591.1099. Found 591.1090.

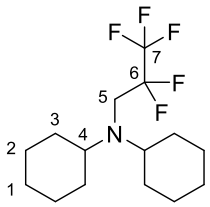
1-(2,2,3,3,4,4,5,5,5-Nonafluoropentyl)piperidin-4-ol (135)



Prepared according to general procedure 3 from 4-hydroxypiperidine (51 mg, 0.50 mmol), perfluoropentanoic acid (135 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (4:6 EtOAc/hexane + 0.5% NEt_3) to give the Title Compound as a colourless oil (110 mg, 66%).

R_f (4:6 EtOAc/hexane + 0.5% NEt_3) = 0.5. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.76-3.70 (m, 1H, H-2), 3.05 (t, *J* = 16.1 Hz, 2H, H-6), 2.94-2.87 (m, 2H), 2.60-2.46 (m, 2H), 1.93-1.87 (m, 2H), 1.66-1.58 (m, 2H). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 117.4 (qt, *J* = 288.0, 33.0 Hz, CF_3, C-10), 117.2 (tt, *J* = 256.8, 30.8 Hz, CF_2, C-7), 114.3-106.1 (m, CF_2, C-8 & C-9), 67.1 (CH, C-2), 56.6 (t, *J* = 22.0 Hz, CH_2, C-6), 52.0 (CH_2, C-4), 34.4 (CH_2, C-3). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -81.05 (tt, *J* = 9.7, 3.1 Hz, 3F, F-10), -115.05- -116.05 (m, 2F, F-7), -124.17- -124.30 (m, 2F), -126.08- -126.24 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3351, 2933, 2808, 2084, 1687, 1454, 1427, 1365, 1322, 1295, 1216, 1200, 1129. **HRMS** (ESI^+): Calc. for $C_{10}H_{13}F_9NO$ ($M+H^+$) 334.0848. Found 334.0864. Calc. for $C_{10}H_{12}F_9NNaO$ ($M+Na^+$) 356.0667. Found 356.0664.

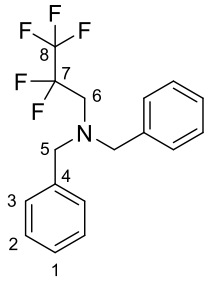
***N*-Cyclohexyl-*N*-(2,2,3,3,3-pentafluoropropyl)cyclohexanamine (136)**



Prepared according to general procedure 3 from dicyclohexylamine (91 µL, 0.50 mmol), pentafluoropropionic acid (92.0 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (1:9 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a white solid (73 mg, 47%).

R_f (1:9 EtOAc/petroleum ether + 0.5% NEt_3) = 0.9. **Mp:** 56-58 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.20 (t, *J* = 15.9 Hz, 2H, H-5), 2.60-2.51 (m, 2H, H-4), 1.82-1.75 (m, 4H), 1.75-1.69 (m, 4H), 1.66-1.58 (m, 2H), 1.33-1.15 (m, 8H), 1.12-1.10 (m, 2H). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 119.5 (qt, *J* = 286.7, 36.0 Hz, CF_3, C-7), 114.6 (tq, *J* = 252.4, 34.5 Hz, CF_2, C-6), 59.3 (CH, C-4) 46.2 (t, *J* = 21.8 Hz, CH_2), 32.1 (CH_2), 26.5 (CH_2), 26.0 (CH_2). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -83.71 (app. s, 3F, F-7), -120.27 (t, *J* = 15.9 Hz, 2F, F-6). **IR** ν_{max}/cm^{-1} (ATR): 2928, 2854, 2668, 2291, 2078, 1986, 1593, 1501, 1465, 1452, 1390, 1345, 1312, 1277, 1259, 1178, 1122. **HRMS** (ESI^+): Calc. for $C_{15}H_{25}F_5N$ ($M+H^+$) 314.1902. Found 314.1900.

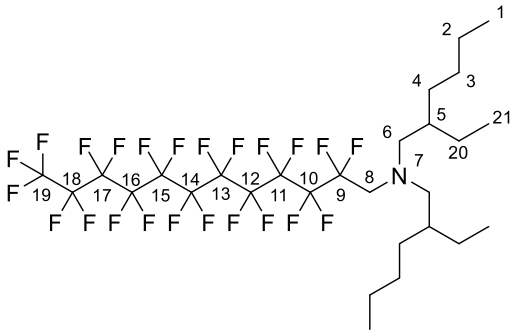
***N,N*-Dibenzyl-2,2,3,3,3-pentafluoropropan-1-amine (137)**



Prepared according to general procedure 3 from dibenzylamine (96 µL, 0.50 mmol), pentafluoropropionic acid (92.0 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (1:9 EtOAc/petroleum ether + 0.5% NEt_3) to give the Title Compound as a colourless oil (109 mg, 66%).

R_f (1:9 EtOAc/petroleum ether + 0.5% NEt_3) = 0.8. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.50-7.28 (m, 10H, H-1, H2- & H-3), 3.84 (s, 4H, H-5), 3.20 (t, *J* = 15.6 Hz, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 119.9 (qt, *J* = 286.6, 35.6 Hz, CF_3, C-8), 115.5 (tq, *J* = 254.6, 35.9 Hz, CF_2, C-7), 138.0 (C, C-4), 129.0 (CH), 128.4 (CH), 127.5 (CH), 58.8 (CH_2, C-5), 51.49 (t, *J* = 21.9 Hz, CF_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -84.11 (app. s, 3F, F-8), -118.12 (t, *J* = 15.6 Hz, 2F, F-7). **IR** ν_{max}/cm^{-1} (ATR): 3088, 3065, 3030, 2929, 2838, 2805, 2319, 2092, 1996, 1951, 1873, 1809, 1603, 1586, 1496, 1454, 1437, 1372, 1350, 1275, 1187, 1130. **HRMS** (ESI$^+$): Calc. for $C_{17}H_{17}F_5N$ ($M+H^+$) 330.1276. Found 330.1271. Calc. for $C_{17}H_{16}F_5NNa$ ($M+Na^+$) 352.1095. Found 352.1103.

***N,N*-Bis(2-ethylhexyl)-2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-tricosafluorododecan-1-amine (138)**



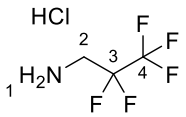
Prepared according to general procedure 3 from bis(2-ethylhexyl)amine (0.15 mL, 0.50 mmol), tricosafluorododecanoic acid (537 mg, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (5:95 EtOAc/hexane + 0.5% NEt_3) to give the Title Compound as a colourless oil (252 mg, 61%).

R_f (5:95 EtOAc/hexane + 0.5% NEt_3) = 0.9. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.09 (t, *J* = 17.2 Hz, 2H, H-8), 2.42 (d, *J* = 6.5 Hz, 4H, H-6), 1.49-1.19 (m, 18H, H-2, H-3, H-4, H-5 & H-20), 0.92 (t, *J* = 7.0 Hz, 6H, H-1), 0.90-0.84 (m, 6H, H-21). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 118.1 (tt, *J* = 258.4, 30.5 Hz, CF_2, C-9), 117.1 (qt, *J* = 288.7, 33.1 Hz, CF_3, C-19), 114.3-106.0 (m, CF_2, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17 & C-18), 60.6 (2 x CH_2, C-6), 54.0 (t, *J* = 19.8 Hz, CH_2, C-8), 37.7 (2 x CH, C-5), 30.8 (CH_2), 30.7 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 24.0 (CH_2), 23.9 (CH_2), 23.2 (2 x C-2), 14.1 (CH_3, C-1), 10.7 (CH_3, C-21), 10.6 (CH_3, C-21). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.88 (t, *J* = 9.9 Hz, 3F, F-19), -115.95 (br, 2F), -121.77 (br, 8F), -121.93 (br, 4F), -122.76 (br, 2F), -123.77 (br, 2F), -126.19 (br, 2F). **IR** ν_{max}/cm^{-1} (ATR): 2961, 2930, 2876, 2861, 2326, 2216, 2112, 1995, 1981, 1463, 1380, 1347, 1208, 1151, 1119. **HRMS** (ESI^+): Calc. for $C_{28}H_{37}F_{23}N$ ($M+H^+$) 824.2553. Found 824.2544.

General procedure 4: Synthesis of 1,1-dihydroperfluoroalkyl primary amine hydrochlorides

To a solution of benzylamine (1.00 eq.) in anhydrous THF (1 mL) was added perfluorous carboxylic acid (1.75 eq.) and phenylsilane (4.00 eq.). Solution heated to reflux (70 °C) under Ar balloon. After 4 hours solution concentrated under reduced pressure. The crude reaction mixture was dissolved in NEt_3 (20 eq.) and heated to 50 °C under Ar balloon. After 16 hours, solution concentrated under reduced pressure and dissolved in Et_2O (3 mL) and 4N HCl solution added (3 mL). Reaction mixture stirred at room temperature under Ar balloon. After 6 hours, the organics were separated and aqueous washed with Et_2O (5 mL) and the aqueous evaporated under reduced pressure. Resultant solids purified by recrystallisation to provide the amine hydrochloride salts.

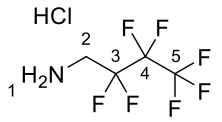
2,2,3,3,3-Pentafluoropropan-1-amine hydrochloride (139)



Prepared according to general procedure 4 from benzylamine (55 µL, 0.50 mmol), pentafluoropropionic acid (92.0 µL, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol, and triethylamine (1.39 mL, 10.0 mmol). Purified by recrystallization in hot MeCN to give the Title Compound as a white solid (32 mg, 34%).

Mp: Sublime 124-126 °C. **^{1}H NMR** (500 MHz; CD_3OD) δ_H 3.95 (t, *J* = 16.7 Hz, 2H, H-2). **^{13}C NMR** (126 MHz; CD_3OD) δ_C 118.1 (qt, *J* = 284.8, 33.9 Hz, C-4), 112.6 (tq, *J* = 255.5, 39.1 Hz, C-3), 38.3 (t, *J* = 22.3 Hz, C-2). **^{19}F NMR** (376 MHz; CD_3OD) δ_F -86.32 (app. s, 3F, F-4), -122.68 (t, *J* = 16.4 Hz, 2F, F-3). **IR** ν_{max}/cm^{-1} (ATR): 3018, 2982, 2866, 2778, 2619, 2564, 2480, 2164, 1937, 1608, 1585, 1528, 1463, 1411. **HRMS** (ESI^+): Calc. for $C_3H_5F_5N$ ($M+H^+$) 150.0337. Found 150.0338.

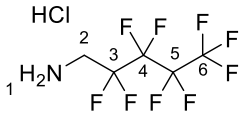
2,2,3,3,4,4,4-Heptafluorobutan-1-amine hydrochloride (140)



Prepared according to general procedure 4 from benzylamine (55 µL, 0.50 mmol), perfluorobutanoic acid (114 µL, 0.88 mmol), phenylsilane (246 µL, 2.00 mmol) and triethylamine (1.39 mL, 10.0 mmol). Purified by recrystallization in hot MeCN to give the Title Compound as a white solid (63 mg, 54%).

Mp: Sublime 156-158 °C. **^{1}H NMR** (500 MHz; CD_3OD) δ_H 3.99 (t, *J* = 16.8 Hz, 2H, H-2). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 117.4 (qt, *J* = 286.4, 33.3 Hz, CF_3, C-5), 114.6 (tt, *J* = 257.1, 31.9 Hz, CF_2, C-3), 110.9-105.4 (m, CF_2, C-4), 38.4 (t, *J* = 22.1 Hz, CH_2, C-2). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -82.20 (t, *J* = 9.5 Hz, 3F, F-5), -119.44- -119.65 (m, 2F, F-3), -129.95- -129.01 (m, 2F, F-4). **IR** ν_{max}/cm^{-1} (ATR): 3009, 2879, 2651, 2486, 2415, 1990, 1594, 1533, 1463, 1408. **HRMS** (ESI^+): Calc. for $C_4H_5F_7N$ ($M+H^+$) 200.0305. Found 200.0306.

2,2,3,3,4,4,5,5,5-Nonafluoropentan-1-amine (141)



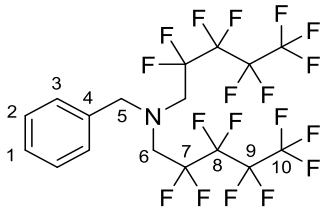
Prepared according to general procedure 4 from benzylamine (55 µL, 0.50 mmol), perfluoropentanoic acid (135 µL, 0.875 mmol), phenylsilane (246 µL, 2.00 mmol, and triethylamine (1.39 mL, 10.0 mmol). Purified by recrystallization in hot MeCN to give the Title Compound as a white solid (75 mg, 53%).

Mp: Sublime 180-182 °C. **^{1}H NMR** (500 MHz; CD_3OD) δ_H 4.01 (t, *J* = 16.8 Hz, 2H, H-2). **^{13}C NMR** (126 MHz; CD_3OD) δ_C 117.2 (qt, *J* =287.0, 33.0 Hz, CF_3, C-6), 115.2 (tt, *J* = 258.4, 32.3 Hz, CF_2, C-3), 113.4-105.9 (m, CF_2, C-4 & C-5), 38.6 (t, *J* = 22.1 Hz, CH_2, C-2). **^{19}F NMR** (376 MHz; CD_3OD) δ_F -82.53 (tt, *J* = 9.6, 3.0 Hz, 3F, F-8), -118.66- -118.86 (m, 2F, F-3), -125.45 (br, 2F), -127.20- -127.35 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3010, 2945, 2881, 2649, 2581, 2488, 2416, 2027, 1610, 1595, 1536, 1463, 1407. **HRMS** (ESI^+): Calc. for $C_5H_5F_9N$ ($M+H^+$) 250.0273. Found 250.0277.

General procedure 5: Synthesis of doubly alkylated materials

To a solution of amine (1.00 eq.) in THF (1 mL) was added perfluorous carboxylic acid (1.75 eq.) and phenylsilane (2.00 eq.) and heated to reflux (70 °C) under Ar balloon. After 4 hours, condenser briefly removed and second portions of perfluorous carboxylic acid (1.75 eq.) and phenylsilane (2.00 eq.) added and the solution heated at reflux (70 °C) under Ar balloon. After 18 hours, solution allowed to cool to room temperature and diluted with EtOAc (5 mL), washed with 1M NaOH solution (5 mL). The combined organics were dried ($MgSO_4$) and concentrated. Purified by flash column chromatography with specified solvents to give the Title Compounds.

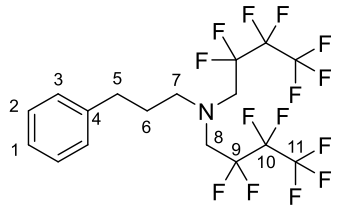
***N*-Benzyl-2,2,3,3,4,4,5,5,5-nonafluoro-*N*-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)pentan-1-amine (142)**



Prepared according to general procedure 5 from benzylamine (55 µL, 0.50 mmol), perfluoropentanoic acid (2 x 135 µL, 1.75 mmol) and phenylsilane (2 x 123 µL, 2.00 mmol). Purified by flash column chromatography (1:99 EtOAc/pentane) to give the Title Compound as a colourless oil (51 mg, 19%).

R_f (1:99 EtOAc/pentane) = 0.7. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.43-7.31 (m, 5H, H-1, H-2 & H-3), 4.12 (s, 2H, H-5), 3.46 (t, *J* = 16.2 Hz, 4H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 136.7 (C, C-4), 128.8 (CH), 128.6 (CH), 128.0 (CH), 117.8 (tt, *J* = 257.9, 31.4 Hz, CF_2, C-7), 117.3 (qt, *J* = 288.3, 33.2 Hz, CF_3, C-10), 113.0-105.7 (CF_2, C-8 & C-9), 59.5 (CH_2, C-5), 52.2 (t, *J* = 21.1 Hz, CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -81.02- -81.12 (m, 6F, F-10), -116.48- -116.70 (m, 4F, F-7), -124.69 (br, 4F), -126.05- -126.20 (m, 4F). **IR** ν_{max}/cm^{-1} (ATR): 3037, 2917, 2323, 2211, 2191, 2167, 2122, 2039, 2008, 1990, 1683, 1531, 1498, 1456. HRMS spectra could not be obtained due to high volatility of the compound.

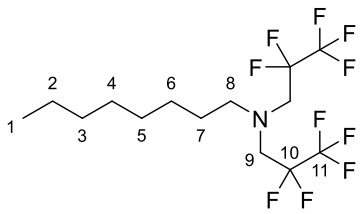
2,2,3,3,4,4,4-Heptafluoro-*N*-(2,2,3,3,4,4,4-heptafluorobutyl)-*N*-(3-phenylpropyl)butan-1-amine (143)



Prepared according to general procedure 5 from 3-phenylpropylamine (71 µL, 0.50 mmol), perfluorobutanoic acid (2 x 114 µL, 1.75 mmol) and phenylsilane (2 x 123 µL, 2.00 mmol). Purified by flash column chromatography (1:99 EtOAc/pentane) to give the Title Compound as a colourless oil (160 mg, 64%).

R_f (1:99 EtOAc/pentane) = 0.7. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.37-7.30 (m, 2H, H-Ar), 7.27-7.19 (m, 3H, H-Ar), 3.43 (t, *J* = 16.1 Hz, 4H, H-8), 2.96 (t, *J* = 7.3 Hz, 2H, H-7), 2.71-2.64 (m, 2H, H-5), 1.92-1.81 (m, 2H, H-6). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 141.6 (C, C-4), 128.4 (CH), 128.3 (CH), 126.0 (CH), 117.7 (qt, *J* = 287.3, 33.8 Hz, CF_3, C-11), 116.9 (tt, *J* = 256.4, 30.7 Hz, CF_2, C-9), 108.9 (tq, *J* = 263.7, 37.9 Hz, CF_2, C-10), 55.7 (CH_2, C-7), 53.3 (t, *J* = 21.2 Hz, CH_2, C-8), 32.9 (CH_2, C-5), 29.6 (CH_2, C-6). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.73 (t, *J* = 9.5 Hz, 3F, 6F, F-11), -117.70- -117.90 (m, 4F, F-9), -127.99- -128.05 (m, 4F, F-10). **IR** ν_{max}/cm^{-1} (ATR): 3031, 2943, 2865, 1604, 1497, 1455, 1351, 1289, 1217. **HRMS** (ESI^+): Calc. for $C_{17}H_{16}F_{14}N$ ($M+H^+$) 500.1054. Found 500.1048.

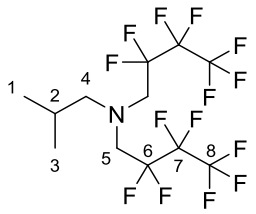
***N*,*N*-Bis(2,2,3,3,3-pentafluoropropyl)octan-1-amine (144)**



Prepared according to general procedure 5 from 1-octylamine (83 µL, 0.50 mmol), pentafluoropropionic acid (2 x 92.0 µL, 1.75 mmol) and phenylsilane (2 x 123 µL, 2.00 mmol). Purified by flash column chromatography (1:99 EtOAc/pentane) to give the Title Compound as a colourless oil (125 mg, 64%).

R_f (1:99 EtOAc/pentane) = 0.7. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.34 (t, *J* = 15.4 Hz, 2H, H-9), 2.87-2.83 (m, 2H, H-8), 1.56-1.44 (m, 2H, H-7), 1.38-1.21 (m, 10H, H-2, H-3, H-4, H-5 & H-6), 0.91 (t, *J* = 6.8 Hz, 3H, H-1). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 118.8 (qt, *J* = 286.2, 35.6, CF_3, C-11), 114.9 (tq, *J* = 255.1, 36.2, CF_2, C-10), 55.9 (CH_2), 53.3 (t, *J* = 22.6 Hz, CF_2, C-9), 31.8 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 27.3 (CH_2), 26.6 (CH_2), 22.6 (CH_2), 14.1 (CH_3, C-1). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -84.51 (app. s, 6F, F-11), -120.50 (t, *J* = 15.5 Hz, 4F, F-10). **IR** ν_{max}/cm^{-1} (ATR): 2929, 1344, 1184, 1097, 1017. HRMS spectra could not be obtained due to high volatility of the compound.

2,2,3,3,4,4,4-Heptafluoro-*N*-(2,2,3,3,4,4,4-heptafluorobutyl)-*N*-isobutylbutan-1-amine (145)



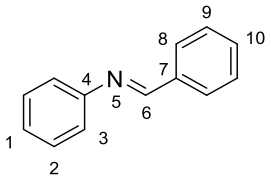
Prepared according to general procedure 5 from isobutylamine (50 µL, 0.50 mmol), perfluorobutanoic acid (2 x 114 µL, 1.75 mmol) and phenylsilane (2 x 123 µL, 2.00 mmol). Purified by flash column chromatography (1:99 Et_2O/pentane) to give the Title Compound as a colourless oil (133 mg, 61%).

R_f (1:99 Et_2O/pentane) = 0.9. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 3.40 (t, *J* =16.4 Hz, 4H, H-5), 2.67 (d, *J* =7.4 Hz, 2H, H-4), 1.79-1.75 (m, 1H, H-2), 0.96-0.91 (m, 6H, H-1 & H-3). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 117.7 (qt, *J* = 287.0, 33.6 Hz, CF_3, C-8), 117.03 (tt, *J* = 256.5, 30.6 Hz, CF_2, C-6), 108.32 (tq, *J* = 267.0, 38.2, 36.7 Hz, CF_2, C-7), 64.4 (CH, C-4), 53.4 (t, *J* =21.0 Hz, CH_2, C-5), 26.8 (CH, C-2), 19.9 (CH_3, C-1 & C-3). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.76 (t, *J* = 9.4 Hz, 6F, F-8), 117.54- -117.78 (m, 4F, F-6), -128.08- -128.16 (m, 4F, F-7). **IR** ν_{max}/cm^{-1} (ATR): 2965, 2878, 2106, 1719, 1495, 1474, 1452, 1386, 1352, 1277, 1216, 1166, 1115. HRMS spectra could not be obtained due to high volatility of the compound.

General procedure 6: Synthesis of 1,1-dihydroperfluoroalkyl anilines

To a solution of *N*-(4-methoxybenzyl)aniline aniline (1.00 eq.) in anhydrous toluene (1 mL) was added perfluorous carboxylic acid (2.50 eq.) and $PhSiH_3$ (4.00 eq.) and heated to reflux (110 °C) under Ar balloon. After 18 hours, solution allowed to cool to room temperature and diluted with EtOAc (5 mL) and washed with 1M NaOH solution (10 mL). The combined organics dried ($MgSO_4$) and concentrated. Purified by flash column chromatography to give the Title Compounds.

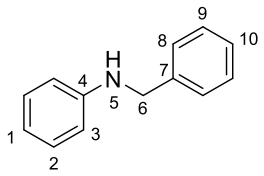
***N*-Diphenylethanimine (146)**



To a solution of aniline (501 µL, 5.50 mmol) in anhydrous THF (4 mL) was added benzaldehyde (508 µL, 5.00 mmol) and the solution left to stir at room temperature under Ar balloon. After 18 hours, silica gel added and purified by flash column chromatography (1:9 Et_2O/petroleum ether) to give the Title Compound as a pale yellow liquid (crystallised in air to off white solid) (789 mg, 87%).

R_f (1:9 Et_2O/petroleum ether) = 0.8. **Mp:** 48-50 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 8.50 (s, 1H, H-6), 7.97-7.94 (m, 2H, H-Ar), 7.54-7.50 (m, 3H, H-Ar), 7.47-7.41 (m, 2H, H-Ar), 7.30-7.25 (m, 3H, H-Ar). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 160.4 (CH, C-6), 152.1 (C), 136.3 (C), 131.4 (CH), 129.2 (CH), 128.8 (CH), 126.0 (CH), 120.9 (CH). **IR** ν_{max}/cm^{-1} (ATR):3060, 2889, 2119, 1701, 1625, 1589, 1577, 1483, 1450, 1366, 1313. **HRMS** (ESI^+): Calc. for $C_{13}H_{12}N$ ($M+H^+$) 182.0964. Found 182.0967. Calc. for $C_{13}H_{11}NNa$ ($M+Na^+$) 204.0784. Found 204.0782.

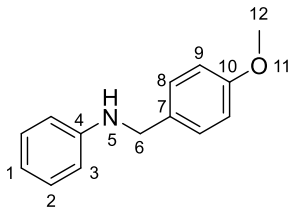
_N_-Benzylaniline (148)



To a solution of aniline (501 µL, 5.50 mmol) in anhydrous THF (4 mL) was added benzaldehyde (508 µL, 5.00 mmol) and the solution left to stir at room temperature under Ar balloon. After 18 hours, solvent removed under reduced pressure and crude compound redissolved in MeOH (5 mL), and sodium borohydride (283 mg, 7.50 mmol) added in one solid portion and solution stirred at room temperature under Ar balloon. After 18 hours, diluted with EtOAc (10 mL) and washed with brine (10 mL), the combined organics were dried ($MgSO_4$) and concentrated. Purified by flash column chromatography (1:9 Et_2O/petroleum ether) to give the Title Compound as a colourless liquid (crystallised in air to a white solid) (716 mg, 78%).

R_f (1:9 Et_2O/petroleum ether) = 0.4. **Mp:** 35-37 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.45-7.36 (m, 4H, H-Ar), 7.35-7.29 (m, 1H, H-Ar), 7.25-7.19 (m, 2H, H-Ar), 6.76 (t, *J* = 7.3 Hz, 1H, H-Ar), 6.68 (d, *J* = 7.7 Hz, 2H, H-Ar), 4.37 (s, 2H, H-6), 4.06 (br, 1H, H-5). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 148.2 (C), 139.5 (C), 129.3 (CH), 128.7 (CH), 127.5 (CH), 127.3 (CH), 117.6 (CH), 112.9 (CH), 48.4 (CH_2, C-6). **IR** ν_{max}/cm^{-1} (ATR): 3417, 3051, 3022, 2925, 1823, 1599, 1509, 1491, 1447, 1431, 1361, 1327, 1301. **HRMS** (ESI$^+$): Calc. for $C_{13}H_{14}N$ ($M+H^+$) 184.1121. Found 184.1117. Calc. for $C_{13}H_{13}NNa$ ($M+Na^+$) 206.0940. Found 206.0945.

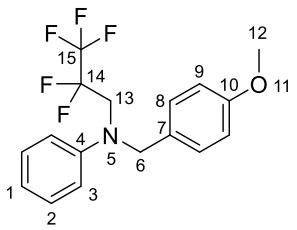
***N*-(4-Methoxybenzyl)aniline (149)**



To a solution of aniline (501 µL, 5.50 mmol) in anhydrous THF (4 mL) was added 4-anisaldehyde (608 µL, 5.00 mmol) and the solution left to stir at room temperature under Ar balloon. After 18 hours, solvent removed under reduced pressure and crude compound redissolved in MeOH (5 mL), and sodium borohydride (283 mg, 7.50 mmol) added in one solid portion and solution stirred at room temperature under Ar balloon. After 18 hours, diluted with EtOAc (10 mL) and washed with brine (10 mL), the combined organics were dried ($MgSO_4$) and concentrated. Purified by flash column chromatography (1:9 Et_2O/petroleum ether) to give the Title Compound as a colourless liquid (756 mg, 71%).

R_f (1:9 Et_2O/petroleum ether) = 0.4. **^{1}H NMR** (600 MHz; $CDCl_3$) δ_H 7.33 (d, *J* = 8.8 Hz, 2H, H-8), 7.22 (t, *J* = 7.9 Hz, 2H, H-2), 6.93 (d, *J* = 8.7 Hz, 2H, H-9), 6.76 (t, *J* = 7.3 Hz, 1H, H-1), 6.68 (d, *J* = 7.6 Hz, 2H, H-3), 4.29 (s, 2H, H-6), 3.98 (br, 1H, H-5), 3.85 (s, 3H, H-12). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 158.9 (C, C-10), 148.3 (C, C-4), 131.5 (C, C-7), 129.3 (2 x CH, C-2), 128.8 (2 x CH, C-8), 117.5 (CH, C-1), 114.1 (2 x CH, C-9), 112.9 (2 x CH, C-3), 55.3 (CH_3, C-12), 47.8 (CH_2, C-6). **IR** ν_{max}/cm^{-1} (ATR): 3414, 3050, 3018, 2999, 2916, 2834, 1602, 1504, 1463, 1429, 1360, 1319, 1301. **HRMS** (ESI$^+$): Calc. for $C_{14}H_{16}NO$ ($M+H^+$) 214.1226. Found 214.1225. Calc. for $C_{14}H_{15}NNaO$ ($M+Na^+$) 236.1046. Found 236.1046.

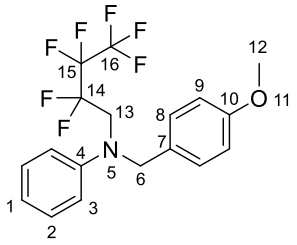
***N*-(4-Methoxybenzyl)-*N*-(2,2,3,3,3-pentafluoropropyl)aniline (151)**



Prepared according to general procedure 6 from *N*-(4-methoxybenzyl)aniline (107 mg, 0.500 mmol), pentafluoropropionic acid (131 µL, 1.25 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography on silica (1:9 Et_2O/petroleum ether) to yield the Title Compound as a colourless oil (crystallised in air to a white solid) (92 mg, 53%).

R_f (1:9 Et_2O/petroleum ether) = 0.6. **Mp**: 36-38 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.34-7.23 (m, 2H, H-Ar), 7.17 (d, *J* = 8.7 Hz, 2H, H-Ar), 6.99-6.79 (m, 5H, H -Ar), 4.70 (s, 2H, H-6), 4.06 (t, *J* = 15.7 Hz, 2H, H-13), 3.83 (s, 3H, H-12). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 158.8 (C), 148.3 (C), 129.3 (CH), 128.9 (C), 127.9 (CH), 119.1 (qt, *J* = 286.5, 35.1 Hz, CF_3, C-15), 118.7 (CH), 115.0 (tq, *J* = 256.0, 36.3 Hz, CF_3, C-14), 114.2 (CH), 113.7 (CH), 55.3 (CH_3, C-12), 54.6 (CH_2, C-6), 50.4 (t, *J* = 21.5 Hz, CH_2, C-13). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -84.43 (app. s, 3F, F-15), -119.81 (t, *J* = 15.7 Hz, 2F, F-14). **IR** ν_{max}/cm^{-1} (ATR): 3064, 3033, 3010, 2955, 2838, 1600, 1505, 1462, 1440, 1387, 1343. **HRMS** (ESI^+): Calc. for $C_{17}H_{17}F_5NO$ ($M+H^+$) 346.1225. Found 346.1234. Calc. for $C_{17}H_{16}F_5NNaO$ ($M+Na^+$) 368.1044. Found 368.1042.

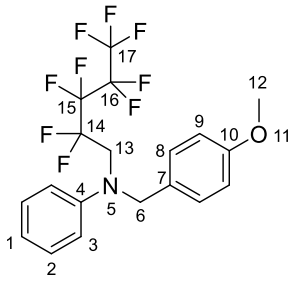
***N*-(4-Methoxybenzyl)-*N*-(2,2,3,3,4,4,4-heptafluorobutyl)aniline (152)**



Prepared according to general procedure 6 from *N*-(4-methoxybenzyl)aniline (107 mg, 0.500 mmol), heptafluorobutyric acid (163 µL, 1.25 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography on silica (1:9 Et_2O/petroleum ether) to yield the Title Compound as a colourless oil (crystallised in air to a white solid) (109 mg, 55%).

R_f (1:9 Et_2O/petroleum ether) = 0.6. **Mp:** 60-62 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.28-7.23 (m, 2H, H-Ar), 7.17 (d, *J* = 8.7 Hz, 2H, H-Ar), 6.92-6.82 (m, 5H, H-Ar), 4.69 (s, 2H, H-6), 4.10 (t, *J* = 16.3 Hz, 2H, H-13), 3.82 (s, 3H, H-12). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 158.8 (C), 148.3 (C), 129.3 (CH), 128.9 (C), 127.9 (CH), 118.6 (CH), 117.7 (qt, *J* = 287.2, 33.7 Hz, CF_3, C-16), 117.3 (tt, *J* = 256.8, 30.8 Hz, CF_2, C-14), 114.2 (CH), 113.7 (CH), 109.1 (tq, *J* = 263.8, 38.2 Hz, CF_2, C-15), 55.3 (CH_3, C-12), 54.7 (CH_2, C-6), 50.4 (t, *J* = 21.0 Hz, CH_2, C-13). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.51 (t, *J* = 9.5 Hz, 3F, F-16), -116.87- -117.09 (m, 2F, F-14), -127.86- -127.94 (m, 2F, F-15). **IR** ν_{max}/cm^{-1} (ATR): 3063, 2963, 2940, 2914, 2844, 1598, 1504, 1459, 1380, 1351. **HRMS** (ESI$^+$): Calc. for $C_{18}H_{17}F_7NO$ ($M+H^+$) 396.1193. Found 396.1195. Calc. for $C_{18}H_{16}F_7NNaO$ ($M+Na^+$) 418.1012. Found 418.1016.

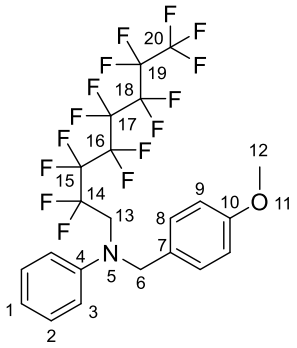
***N*-(4-Methoxybenzyl)-*N*-(2,2,3,3,4,4,5,5,5-nonafluoropentyl)aniline (153)**



Prepared according to general procedure 6 from *N*-(4-methoxybenzyl)aniline (107 mg, 0.500 mmol), perfluoropentanoic acid (193 µL, 1.25 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography on silica (1:9 Et_2O/petroleum ether) to yield the Title Compound as a colourless oil (crystallised in air to a white solid) (118 mg, 53%).

R_f (1:9 Et_2O/petroleum ether) = 0.6. **Mp:** 68-70 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.31-7.22 (m, 2H, H-Ar), 7.20-7.12 (m, 2H, H-Ar), 6.97-6.74 (m, 5H, H-Ar), 4.69 (s, 2H, H-6), 4.10 (t, *J* = 16.3 Hz, 2H, H-13), 3.82 (s, 3H, H-12). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 158.8 (C), 148.3 (C), 129.3 (CH), 128.9 (C), 127.9 (CH), 118.6 (CH), 117.6 (tt, *J* = 257.1, 31.2 Hz, CF_2, C-14), 117.4 (qt, *J* = 288.7, 33.4 Hz, CF_3, C-17), 114.0-107.7 (m, CF_2, C-15 & C-16), 114.2 (CH), 113.6 (CH), 55.3 (CH_3, C-12), 54.7 (CH_2, C-6), 50.5 (t, *J* = 20.9 Hz, CH_2, C-13). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.86- -81.00 (m, 3F, F-17), -116.20- -116.40 (m, 2F, F-14), -124.60 (br, 2F), -125.88- -126.01 (m, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3063, 3030, 2893, 2845, 2036, 1974, 1627, 1591, 1578, 1505, 1484, 1451, 1366. **HRMS** (ESI^+): Calc. for $C_{19}H_{17}F_9NO$ ($M+H^+$) 446.1161. Found 446.1162. Calc. for $C_{19}H_{16}F_9NNaO$ ($M+Na^+$) 468.0980. Found 468.1001.

***N*-(4-Methoxybenzyl)-*N*-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctyl)aniline (154)**



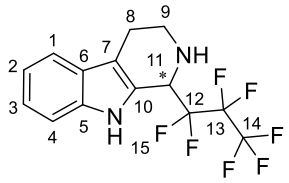
Prepared according to general procedure 6 from *N*-(4-methoxybenzyl)aniline (107 mg, 0.500 mmol), perfluorooctanoic acid (518 mg, 1.25 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography on silica (1:9 Et_2O/petroleum ether) to yield the Title Compound as a white solid (111 mg, 37%).

R_f (1:9 Et_2O/petroleum ether) = 0.7. **Mp:** 89-91 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.34-7.23 (m, 2H, H-Ar), 7.18 (d, *J* = 8.6 Hz, 2H, H-Ar), 6.96-6.83 (m, 5H, H-Ar), 4.70 (s, 2H, H-6), 4.12 (t, *J* = 16.2 Hz, 2H, H-13), 3.83 (s, 3H, H-12). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 158.8 (C), 148.3 (C), 129.2 (CH), 129.0 (C), 120.6-114.5 (m, CF_3, C-20), 118.6 (CH), 117.8 (tt, *J* = 259.2, 31.5 Hz, CF_2, C-14), 115.3-105.3 (m, CF_2, C-15, C-16, C-17, C-18 & C-19), 114.2 (CH), 113.6 (CH), 55.2 (CH_3, C-12), 54.7 (CH_2, C-6), 50.6 (t, *J* = 20.9 Hz, CH_2, C-13). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.83 (t, *J* = 10.0 Hz, 3F, F-20), -115.97- -116.20 (m, 2F), -121.66 (br, 2F), -122.03 (br, 2F), -122.73 (br, 2F), -123.63 (br, 2F), -126.13 (br, 2F). **IR** ν_{max}/cm^{-1} (ATR): 3063, 2963, 2941, 2914, 2844, 1597, 1505, 1458, 1393, 1369, 1353, 1326. **HRMS** (ESI$^+$): Calc. for $C_{22}H_{17}F_{15}NO$ ($M+H^+$) 596.1065. Found 596.1061. Calc. for $C_{22}H_{16}F_{15}NNaO$ ($M+Na^+$) 618.0885. Found 618.0900.

General procedure 7: Synthesis of imines for cascade perfluoroalkylation-cyclisation reactions

To a solution of amine (0.50 mmol) in THF (1 mL) was added perfluorous carboxylic acid (0.875 mmol) and phenylsilane (2.00 mmol), and heated to reflux (70°C) under argon balloon. After 18 hours, the solution was allowed to cool to room temperature and diluted with EtOAc (10 mL) and washed with 1M NaOH solution (10 mL). The combined organics were dried ($MgSO_4$) and concentrated. Purified by flash column chromatography (dry load with NEt_3 treated silica gel) to give the perfluoroalkylated amine products.

1-(Perfluoropropyl)-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole (156)

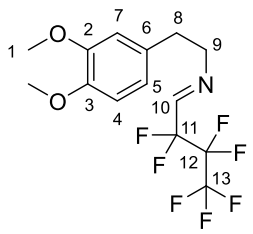


Prepared according to general procedure 7 from tryptamine (80 mg, 0.50 mmol), perfluorobutanoic acid (114 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (1:9 EtoAc/pentane + 0.5% NEt_3) to give the Title Compound as an off white solid (90 mg, 53%).

R_f (1:9 EtOAc/pentane + 0.5% NEt_3) = 0.3. **Mp:** 58-60 °C. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.59 (d, *J* = 7.8 Hz, 1H, H-1), 7.39 (d, *J* = 8.1 Hz, 1H, H-4), 7.28-7.25 (m, 1H, H-3), 7.20-7.15 (m, 1H, H-2), 4.80-4.71 (m, 1H, H-11), 3.38-3.13 (m, 2H, H-9), 2.92-2.69 (m, 2H, H-8). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 136.2 (C), 126.6 (C, C-10), 124.8 (C, C-6), 122.9 (CH, C-3), 119.7 (CH, C-2), 118.7-114.5 (m, CF_2, C-12), 118.5 (CH, C-1), 117.9 (app. qt, *J* = 288.1, 34.4 Hz, CF_3, C-14), 113.8 (C), 111.1 (CH, C-4), 109.7 (app. tq, *J* = 264.8, 38.0 Hz, CF_2, C-13), 53.1 (dd, *J* = 26.8, 22.2 Hz, CH, C-11), 40.8 (2 x s, CH_2, C-9), 22.0 (CH_2, C-8). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.68 (app. dd, *J* = 12.7, 7.7 Hz, 3F, F-14), -110.28--111.51 (m, 2F, F-12), -120.04- -121.05 (m, 2F, F-12), -122.97- -123.82 (m, 2F, F-13), -126.24- -127.06 (m, 2F, F-13). **IR** ν_{max}/cm^{-1} (ATR): 3418, 3364, 2948, 2903, 2838, 2287, 2162, 2115, 1895, 1782, 1607, 1463, 1436, 1368, 1335, 1292, 1269. **HRMS** (ESI^+): Calc.

for $C_{14}H_{12}F_7N_2$ ($M+H^+$) 341.0883. Found 341.0890. Calc. for $C_{14}H_{11}F_7N_2Na$ ($M+Na^+$) 363.0703. Found 363.0702.

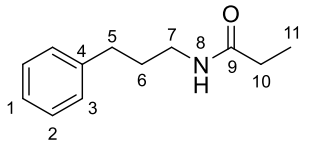
***N*-(3,4,-Dimethoxyphenethyl)-2,2,3,3,4,4,4-heptafluorobutan-1-imine (158)**



Prepared according to general procedure 7 from 2-(3,4-dimethoxyphenyl)ethan-1-amine (84 µL, 0.50 mmol), heptafluorobutyric acid (114 µL, 0.875 mmol) and phenylsilane (246 µL, 2.00 mmol). Purified by flash column chromatography (1:1 Et_2O/petroleum ether) to give the Title Compound as a colourless oil (144 mg, 80%).

R_f (1:1 Et_2O/petroleum ether) = 0.6. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.51 (t, *J* = 6.1 Hz, 1H, H-10), 6.82 (d, *J* = 8.0 Hz, 1H, H-Ar), 6.74-6.67 (m, 2H, H-Ar), 3.99-3.79 (m, 8H, H-1 & H-8), 2.98 (t, *J* = 6.9 Hz, 2H, H-9). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C 150.2 (t, *J* = 26.8 Hz, CH, C-10), 148.9 (C), 147.7 (C), 131.0 (C), 120.9 (CH), 117.6 (qt, *J* = 287.2, 33.5 Hz, CF_3, C-13), 112.3 (CH), 111.3 (CH), 110.2 (tt, *J* = 255.2, 30.6 Hz, CF_2, C-11), 108.7 (tq, *J* = 265.3. 38.4 Hz, CF_2, C-12), 62.7 (CH_2, C-9), 55.9 (CH_3, C-1), 55.7 (CH_3, C-1), 35.7 (CH_2, C-8). **^{19}F NMR** (376 MHz; $CDCl_3$) δ_F -80.61 (t, *J* = 8.8 Hz, 3F, F-13), -118.22- -118.35 (m, 2F, F-11), -127.58 (br, 2F, F-12). **IR** ν_{max}/cm^{-1} (ATR): 3006, 2940, 2916, 2839, 1677, 1593, 1516, 1466, 1353, 1224. **HRMS** (ESI$^+$): Calc. for $C_{14}H_{14}F_7NNaO_2$ ($M+Na^+$) 384.0805. Found 384.0804. Calc. for $C_{14}H_{18}F_7N_2O_2$ ($M+H_4N^+$) 379.1251. Found 379.1243.

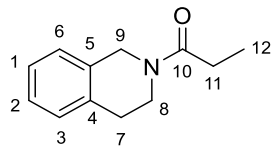
***N*-(3-Phenylpropyl)propionamide (160)**



To a solution of 3-phenylpropylamine (711 µL, 5.00 mmol) and *N,N*-diisopropylethylamine (1.74 mL, 10.0 mmol) in anhydrous EtOAc (5 mL) was added propionic acid (373 µL, 5.00 mmol) and propylphosphonic anhydride solution (3.57 mL, 6.00 mmol of a 50% solution in EtOAc) at 0 °C. Solution allowed to warm to room temperature and stirred under Ar balloon. After 18 hours, diluted with EtOAc (5 mL) and washed with brine (15 mL). The combined organics were dried ($MgSO_4$) and concentrated. Purified by flash column chromatography (1:1 Et_2O/petroleum ether) to give the Title Compound as a colourless oil (710 mg, 74%).

R_f (1:1 Et_2O/petroleum ether) = 0.4. **^{1}H NMR** (500 MHz; $CDCl_3$) δ_H 7.33-7.26 (m, 2H, H-Ar), 7.23-7.17 (m, 3H, H-Ar), 3.34-3.25 (m, 2H, H-7), 2.70-2.62 (m, 2H, H-5), 2.17 (q, *J* = 7.6 Hz, 2H, H-10), 1.88-1.82 (m, 2H, H-6), 1.14 (t, *J* = 7.6 Hz, 3H, H-11). **^{13}C NMR** (126 MHz; $CDCl_3$) δ_C173.9 (C, C-9), 141.5 (C, C-4), 128.5 (CH), 128.4 (CH), 126.0 (CH), 39.2 (CH_2, C-7), 33.4 (CH_2, C-5), 31.2 (CH_2, C-10), 29.7 (CH_2, C-6), 10.0 (CH_3, C-11). **IR** ν_{max}/cm^{-1} (ATR): 3291, 3085, 3026, 2973, 2937, 2875, 2861, 1640, 1546, 1496, 1453, 1372. **HRMS** (ESI$^+$): Calc. for $C_{12}H_{18}NO$ ($M+H^+$) 192.1383. Found 192.1384. Calc. for $C_{12}H_{17}NNaO$ ($M+Na^+$) 214.1202. Found 214.1201.

1-(3,4-Dihydroisoquinolin-2(1*H*)-yl)propan-1-one (162)



To a solution of 1,2,3,4-tetrahydroisoquinoline (626 µL, 5.00 mmol) and *N,N*-diisopropylethylamine (1.74 mL, 10.0 mmol) in anhydrous EtOAc (5 mL) was added propionic acid (373 µL, 5.00 mmol) and propylphosphonic anhydride solution (3.57 mL, 6.00 mmol of a 50% solution in EtOAc) at 0 °C. Solution allowed to warm to room temperature and stirred under Ar balloon. After 18 hours, diluted with EtOAc (5 mL) and washed with brine (15 mL). The combined organics were dried ($MgSO_4$) and concentrated. Purified by flash column chromatography (1:1 Et_2O/petroleum ether) to give the Title Compound as a colourless oil (701 mg, 74%).

R_f (1:1 Et_2O/petroleum ether) = 0.5. **^{1}H NMR** (500 MHz; $CDCl_3$, mixture of two rotamers) δ_H 7.25-7.09 (m, 4H, H-1, H-2, H-3 & H-6), 4.75 (s, 1.3H, H-9, major), 4.63 (s, 0.7H, H-9, minor), 3.85 (t, *J* = 6.0 Hz, 1.3H, H-8, major), 3.69 (t, *J* = 6.0 Hz, 0.7H, H-8, minor), 2.92 (t, *J* = 6.0 Hz, 1.3H, H-7, major), 2.86 (t, *J* = 6.0 Hz, 0.7H, H-7, minor), 2.49-2.41 (m, 2H, H-11), 1.24-1.17 (m, 3H, H-12). **^{13}C NMR** (126 MHz; $CDCl_3$, mixture of two rotamers) δ_C 172.8 (C, C-10), 172.7 (C, C-10), 135.2 (C), 134.1 (C), 133.7 (C), 132.7 (C), 129.0 (CH), 128.3 (CH), 126.9 (CH), 126.7 (CH), 126.6 (CH), 126.5 (CH), 126.3 (CH), 126.1 (CH), 47.2 (CH_2, C-9), 44.2 (CH_2, C-9), 43.1 (CH_2, C-8), 39.7 (CH_2, C-8), 29.5 (CH_2, C-7), 28.6 (CH_2, C-7), 27.0 (CH_2, C-11), 26.8 (CH_2, C-11), 9.5 (CH_3, C-12), 9.4 (CH_3, C-12). **IR** ν_{max}/cm^{-1} (ATR): 2977, 2936, 2874, 2839, 1636, 1425, 1371, 1344. **HRMS** (ESI$^+$): Calc. for $C_{12}H_{16}NO$ ($M+H^+$) 190.1226. Found 190.1228. Calc. for $C_{12}H_{15}NNaO$ ($M+Na^+$) 212.1046. Found 212.1047.

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7) Appendix

This appendix contains photographs of select biological experiments, showing the effects on cells of important compounds prepared in this work. The compounds selected were the difluorinated nucleoside **50** (**B4**) and the vinyl nucleoside **55** (**B1**), along with the two control compounds of 2'-deoxycytidine (**B11**) and decitabine (**B12**) for comparison purposes.

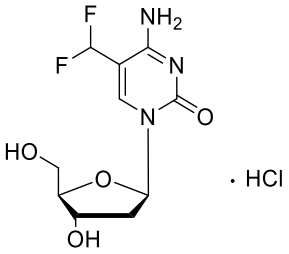
Two columns are present for each compound, showing pictures at the 24 and 48 hour stages for each of the four listed concentrations, demonstrating change over time.

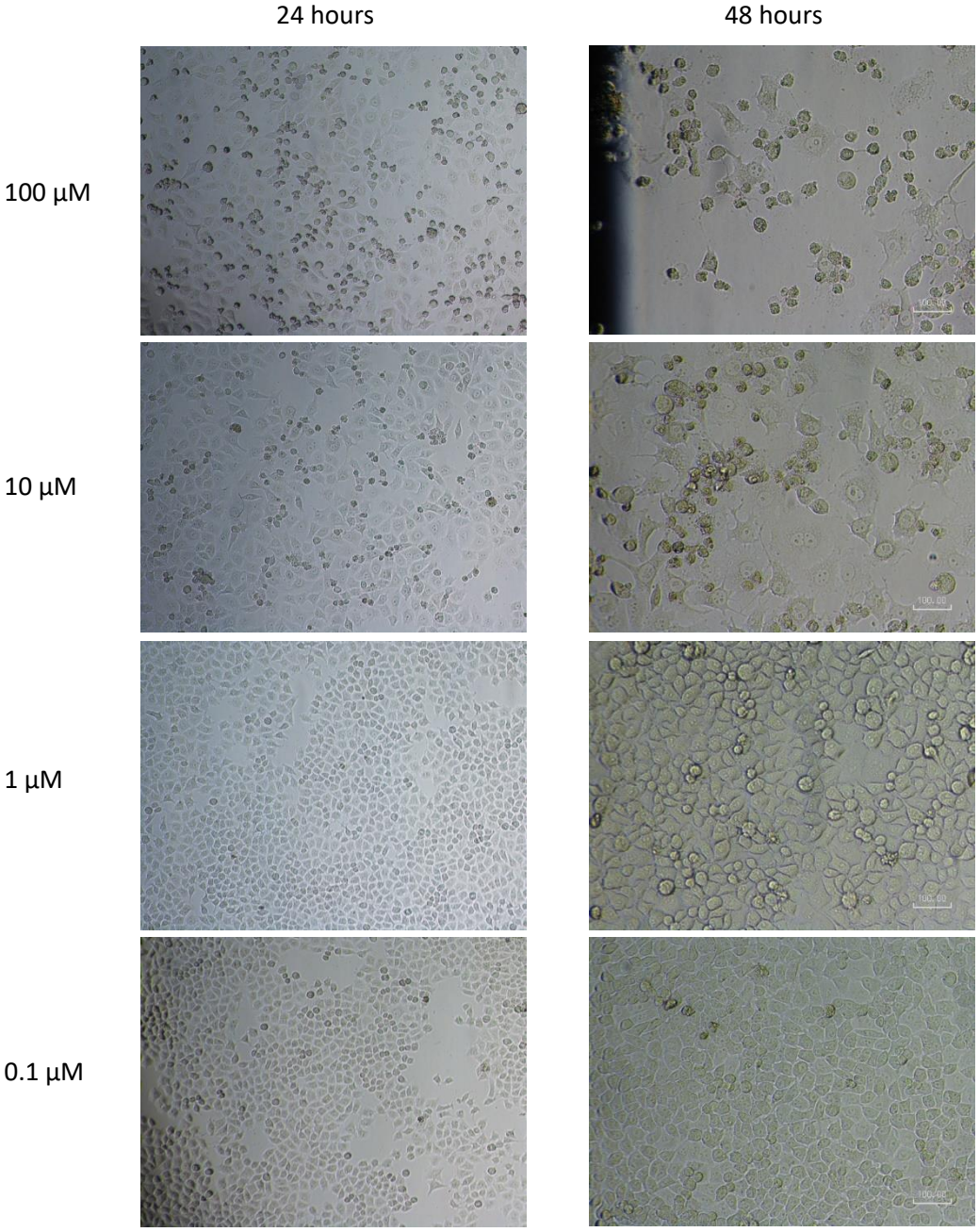
Healthy cells will appear in a variety of shapes, but will have mostly smooth edges. Cells that have been either killed or damaged beyond repair by compounds tested will have jagged edges with sharp corners.

The quantity of cells used at the start of each experiment was the same, and so any effects prohibiting cell division can be seen. For compounds which were not highly toxic to the cells, or for low concentrations of substrate, significantly more cells appear on pictures in the 48 hour column in comparison to the 24 hour. Particularly for the difluorinated nucleoside **50**, and for high concentrations of vinyl nucleoside **55**, it can be seen that little cell division has occurred and the cell cycle has been halted, preventing growth, indicating a strong effect by the test substrate. As the concentrations are lowered these effects lessen.

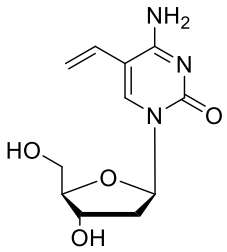
At all concentrations of the two control compounds, little effect is seen on the cells in terms of shape or effects on cell division. Decitabine is a commercial drug that functions by preventing DNA replication and thus cell division. Any effect that may be considered to be seen in the following images, when compared to difluorinated nucleoside **50**, does not mean it does not work at all but that it is not as actively toxic or destructive to the cells. This does not indicate a greater clinical efficacy however, as many compounds are highly destructive to cells and will cause such rapid cell death, but this does not translate perfectly to use as a therapeutic agent. Other considerations such as selectivity and the effect of such rapid, wide ranging cell death must be considered.

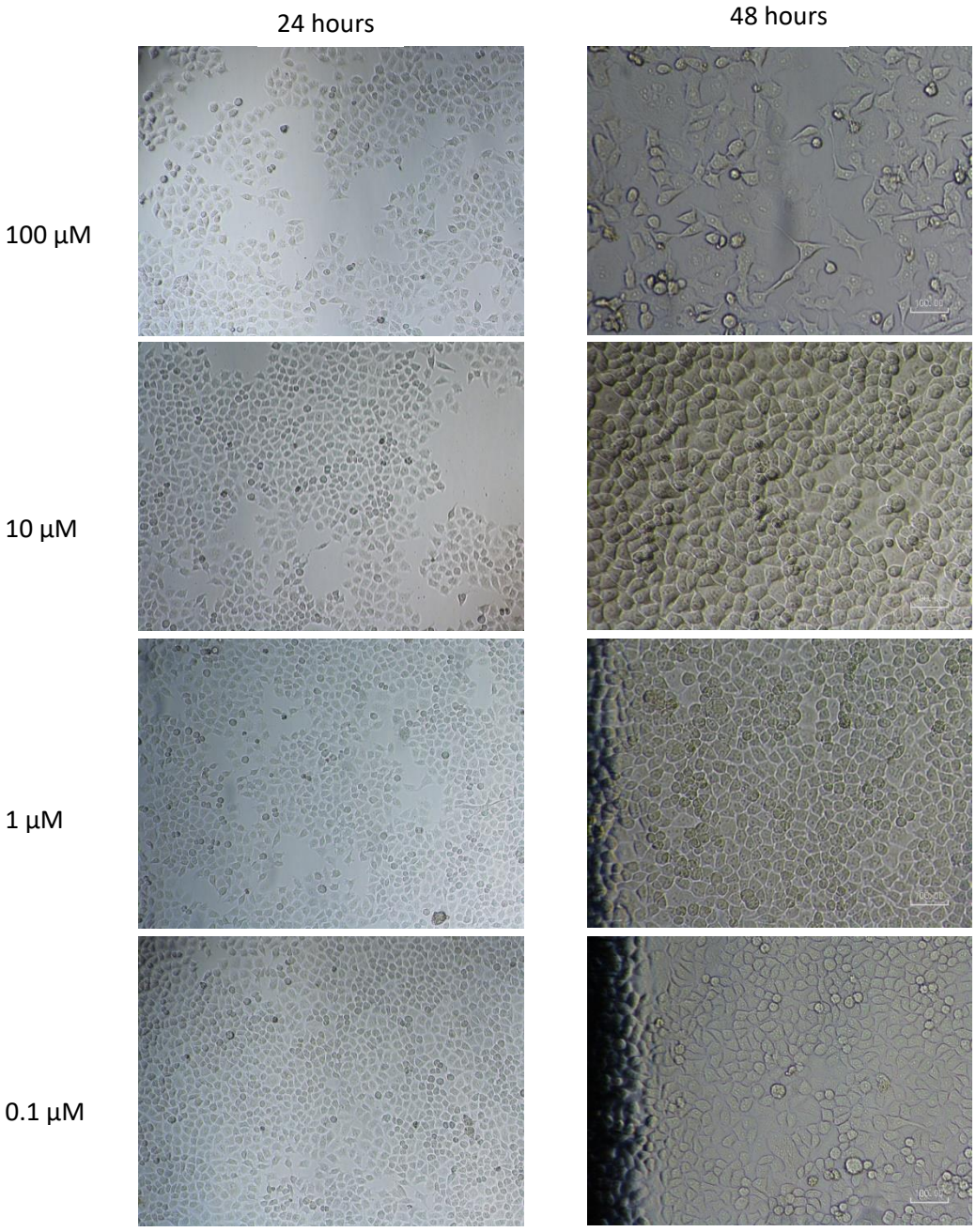
5-Difluoromethyl-2'-deoxycytidine hydrochloride (50) (B4)



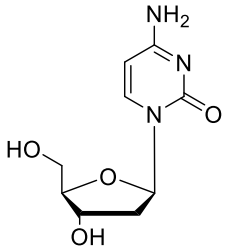


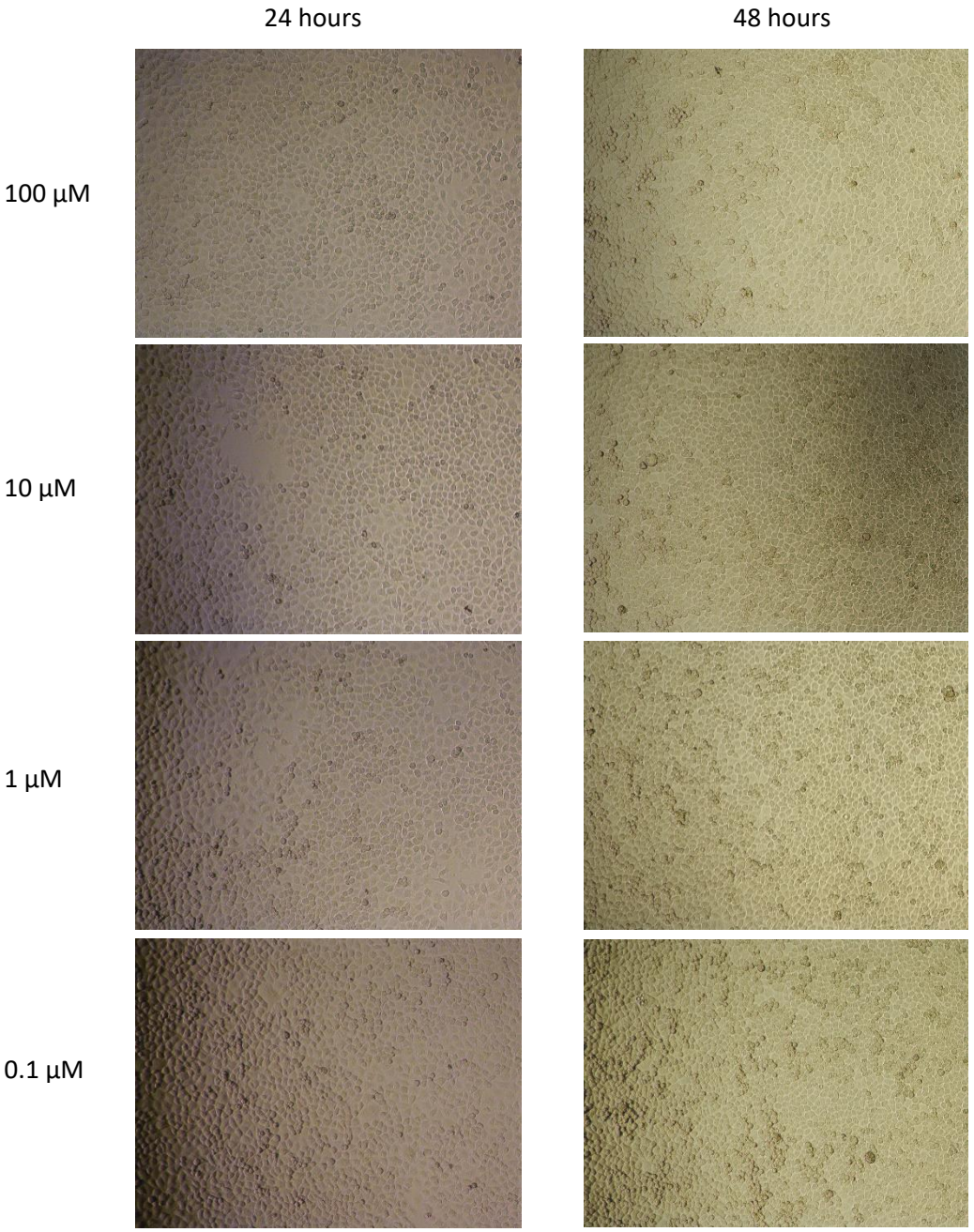
5-Vinyl-2'-deoxycytidine (55) (B1)





2'-Deoxycytidine (B11)





Decitabine (B12)

