Radiofluorination of Squalenoylated Drug Analogues

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Declaration of Authorship

The work presented in this thesis was conducted at the Chemistry Research Laboratory at the University of Oxford under the supervision of Prof. Véronique Gouverneur between Hilary Term 2014 and Michaelmas Term 2017. All the work is my own, except where otherwise stated, and has not been submitted for any other degree at this or any other university.

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Abstract

The conjugation of drugs with squalene derivatives has found wide spread application due to the ability of the conjugates to form nanoparticles in aqueous solutions. The conjugated drugs exhibit increased bioavailability and effectiveness against the targeted disease. Although *in vitro* experiments revealed the mechanism of action at a cellular level, *in vivo* whole-body distribution studies have not been performed yet. The aim of this work was to develop a radioactive analogue of a squalenoylated drug to determine its pharmacokinetic and -dynamic properties through Positron Emission Tomography (PET).

In Chapter 1, a general introduction about the physical and biological properties of squalenoylated drugs is given alongside introductions to PET and previously employed strategies to radiolabel nanoparticles with [^{18}F]fluoride. The chapter will conclude in a proposed radiolabelling strategy for squalenoylated drugs on the example of squalenoyl gemcitabine.

Chapter 2 discusses the successful synthesis of a fluorinated analogue of squalenoyl gemcitabine (**F-Sq-Gem**). The nanoparticles of the novel conjugate were characterised with Dynamic Light Scattering (DLS) and exhibit similar properties to squalenoyl gemcitabine nanoparticles. The modification in the squalenoyl chain was well tolerated and the radiolabelled compound can be expected to behave similar to the native compound *in vivo*.

In the third chapter, different radiofluorination methods are reviewed and analysed for their potential to radiolabel vinyl boronic esters. The copper-mediated radiofluorination of aryl boronic esters offered the potential to be applied to vinylic substrates and therefore for the radiolabelling of squalenoylated drugs. Finally, the synthesis of a terpenoid based vinyl boronic ester precursor is presented.

In Chapter 4, the radiofluorination of vinyl boronic esters is discussed. The reaction required a Lewis basic moiety in proximity of the vinyl boronic ester to afford the desired products, which was not present in the envisaged precursors for squalenoylated drugs. Several attempts to adapt the target synthesis for this requirement are described. The most promising results were obtained using an allylic ether, which can be removed post-labelling.

In Memoriam of Gregor K. Cremosnik

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List of Abbreviations

5’-NT	5’-Nucleotidase
Å	Ångström
B_2pin_2	Bis(pinacolato)diboron
bipy	2,2’-Bipyridine
Bq	Bequerel
CDI	1,1’-Carbonyldiimidazole
CT	Computerised tomography
DAST	(Diethylamino)sulfur trifluoride
DBCO	Dibenzocyclooctyne
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
dCK	Deoxycytidine kinase
DCM	Dichloromethane
DDQ	Dichlorodicyanobenzoquinone
DHP	Dihydropyran
DIBAL-H	Diisobutylaluminum hydride
DLS	Dynamic Light Scattering
DMA	*N,N*-Dimethylacetamide
DMF	Dimethylformamide
DMI	*N,N'*-Dimethylethyleneurea

DMPU	1,3-Dimethyltetrahydropyrimidin-2(1*H*)-one
DMSO	Dimethylsulfoxide
EDC	3-(Ethyliminomethyleneamino)-N,N-dimethylpropan-1-amine
EPR	Enhanced permeability and retention
EtO	Ethyl ether
FDG	Fluorodeoxyglucose
HFIP	Hexafluoroisopropanol
HMPA	Hexamethylphosphoramide
HOBt	Benzotriazol-1-ol
HPMA	*N*(2-Hydroxypropyl)methacrylamide
*i*PrOBpin	2-Isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane
KHMDS	Potassium bis(trimethylsilyl)amide
LC	Liquid chromatography
LDA	Lithium diisopropylamide
LiHMDS	Lithium bis(trimethylsilyl)amide
LTMP	Lithium tetramethylpiperidide
*m*CPBA	3-Chloroperbenzoic acid
Me	Methyl
MRI	Magnetic resonance imaging
MS	Masspectrometry
MSN	Mesoporous silica nanoparticles

NA	Nanoassembly
NaHMDS	Sodium bis(trimethylsilyl)amide
NBS	*N*-Bromosuccinimide
*n*BuLi	*n*-Butyllithium
NHC	*N*-Heterocyclic carbene
NMR	Nuclear magnetic resonance
NRT	Nucleoside-analogue reverse transcriptase
PDI	Polydispersity Index
PEG	Polyethylene glycol
PET	Positron Emission Tomography
PET 40-60	Petroleum ether 40-60
PIDA	Iodobenzene diacetate
PPTS	Pyridinium p-toluenesulfonate
PT-sulfone	1-Phenyl-1*H*-tetrazol-5-yl sulfone
RCC	Radiochemical conversion
RCY	Radiochemical yield
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TBDPSCl	*tert*-Butyldiphenylchlorosilane
*t*Bu	*tert*-Butyl
*t*Bu-bipy	4,4′-Di-*tert*-butyl-2,2′-dipyridyl

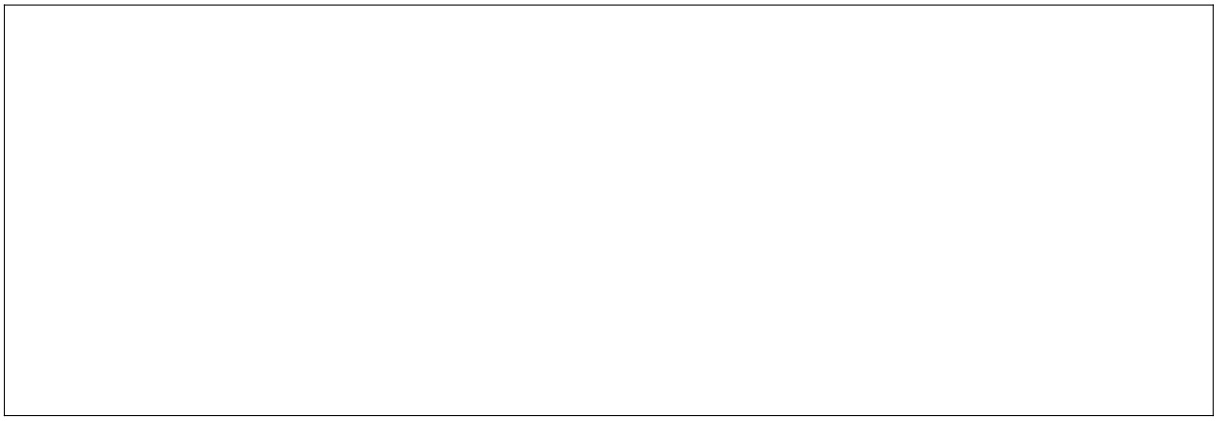
TEA	Triethylamine
THF	Tetrahydrofuran
TMSCl	Trimethylsilyl chloride

CHAPTER 1

General Introduction

1.1 Squalenoylation of Drugs

The concept of squalenoylation of drugs was first introduced in 2006. Couvreur and co-workers observed that derivatisation of the anticancer drug gemcitabine with 1,1',2-trisnorsqualenic acid enabled nanoparticle formation in aqueous solutions (Scheme 1.1).[1] Those nanoparticles exhibited improved cytotoxicity compared to gemcitabine *in vitro* and *in vivo* (Section 1.2).



Scheme 1.1: Synthesis of **Sq-Gem** nanoparticles *Adapted with permission from Nano Lett., Vol. 6, No. 11, 2006, 2544-2548. Copyright (2006) American Chemical Society.*

Squalenoylation of several different drug molecules highlighted the generality of nanoparticle formation, which was usually beneficial for drug efficiency. Besides anticancer drugs[1–4], Couvreur and co-workers investigated nucleoside-analogue reverse transcriptase (NRT) inhibitors[1], anti-parasitic drugs[5] and antibiotics[6] (Figure 1.1). Multimodal nanoparticles, containing a drug (**Sq-Gem**), an affinity ligand (**Sq-Biotin**) and a fluorescent marker (**Sq-Rhodamine**) were obtained by co-precipitation.[7]

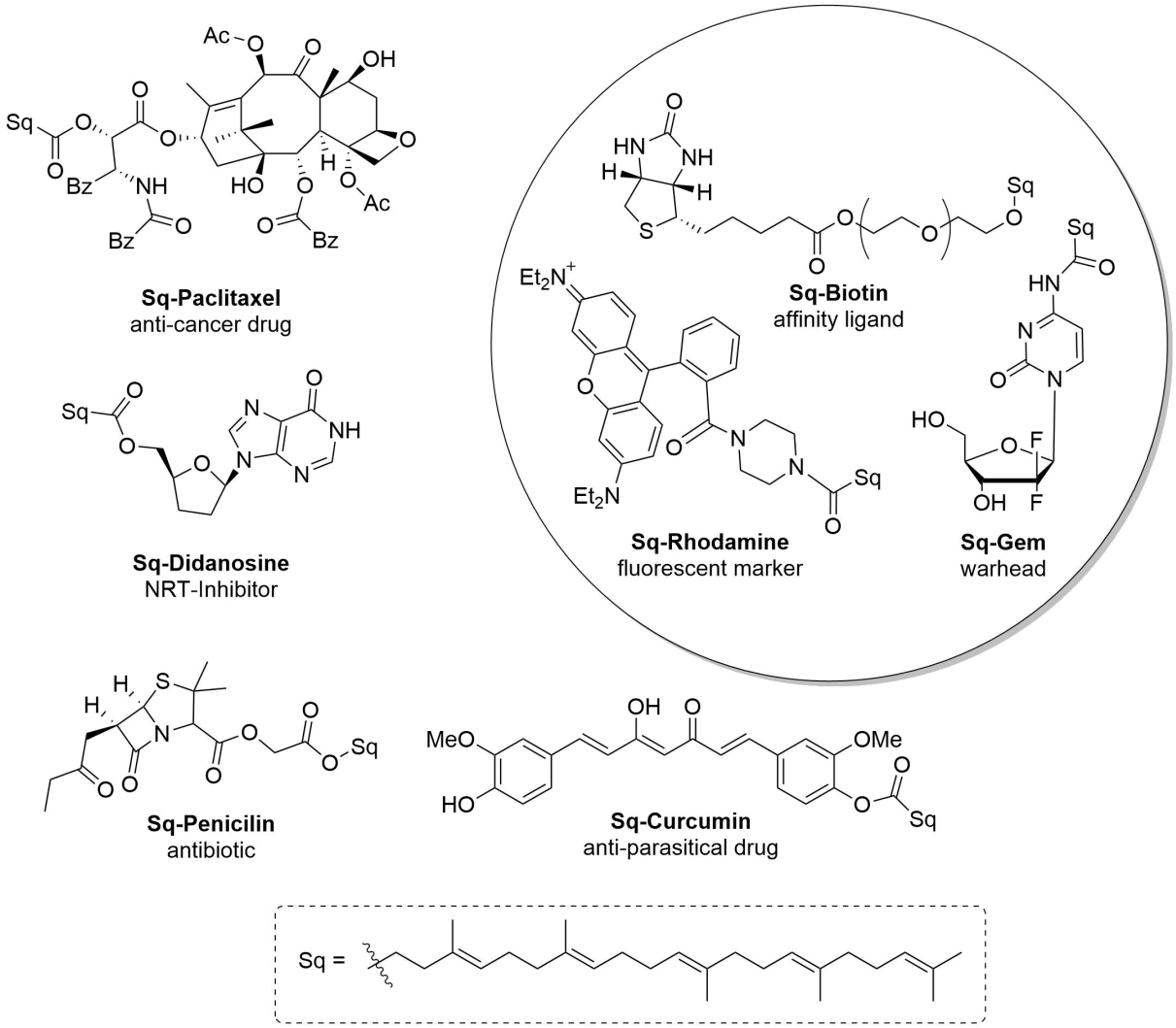


Figure 1.1: Selection of squalenoylated drugs

The particles were investigated for their physical properties and behaviour in solution. An overview over the crucial particle parameters is given in Table 1.1. The mean diameter describes the average particle size as measured by Dynamic Light Scattering (DLS) and the polydispersity index (PDI) specifies the particle size distribution. The zeta-potential (ζ) measures the surface charge of the particles, which is associated with particle stability and aggregation speed. Squalenoylated drugs form nanoparticles in the range of 70 nm to 200 nm with a generally low PDI. An exception

is **Sq-Didanosine**, which shows larger particle sizes as well as a wider size distribution. Couvreur and co-workers did not comment on this observation. Squalenoyl nanoparticles exhibit a negative surface charge. Commonly particles with zeta-potentials in the range of ±20 mV to ±30 mV are considered moderately to highly stable and aggregate only slowly.[8] This is an important characteristic to ensure proper *in vivo* uptake as large particles (>200 nm) are removed from the blood stream more quickly by cells of the immune system.[9]

Sq-Drug	mean diameter [nm]	PDI	ζ [mV]
Sq-Gem	140 ± 33	0.14	-27 ± 1
Sq-Paclitaxel	117 ± 7	0.08	-36
Sq-Didanosine	280 ± 13	0.45	N.M.
Sq-Penicillin	191	0.14	N.M.
Sq-Curcumin	71 ± 1	0.22	N.M.
Multimodal nanoparticle	149 ± 3	0.15	-25 ± 3

Table 1.1: Physical properties of different squalenoyl nanoparticles; N.M.: not measured

The nanoparticles were tested *in vitro* against their intended targets and exhibited increased cytotoxicity compared to the unconjugated drug. For several drugs, e.g. **Sq-Gem**, *in vivo* mice or rat studies were performed, and animals which received the squalenoylated drug exhibited better survivability compared to those treated with the native drug. The mechanisms of uptake and metabolism of squalenoylated drugs were explored on a cellular level by *in vitro* experiments but the *in vivo*

pharmacokinetic properties are unknown. For this purpose, the development of a radioactive analogue for Positron Emission Tomography (PET) studies was desirable. PET has been shown to be a valuable tool for the determination of *in vivo* pharmacokinetics and -dynamics and has previously been used to characterise other nanoparticles.[10]

The aim of this thesis was to develop and execute a radiolabelling strategy for squalenoylated drugs using **Sq-Gem** as an example. In the following sections, overviews over the properties of **Sq-Gem** (Section 1.2), PET (Section 1.3) and the radiolabelling of nanoparticles (Section 1.4) are given.

1.2 Properties of Sq-Gem

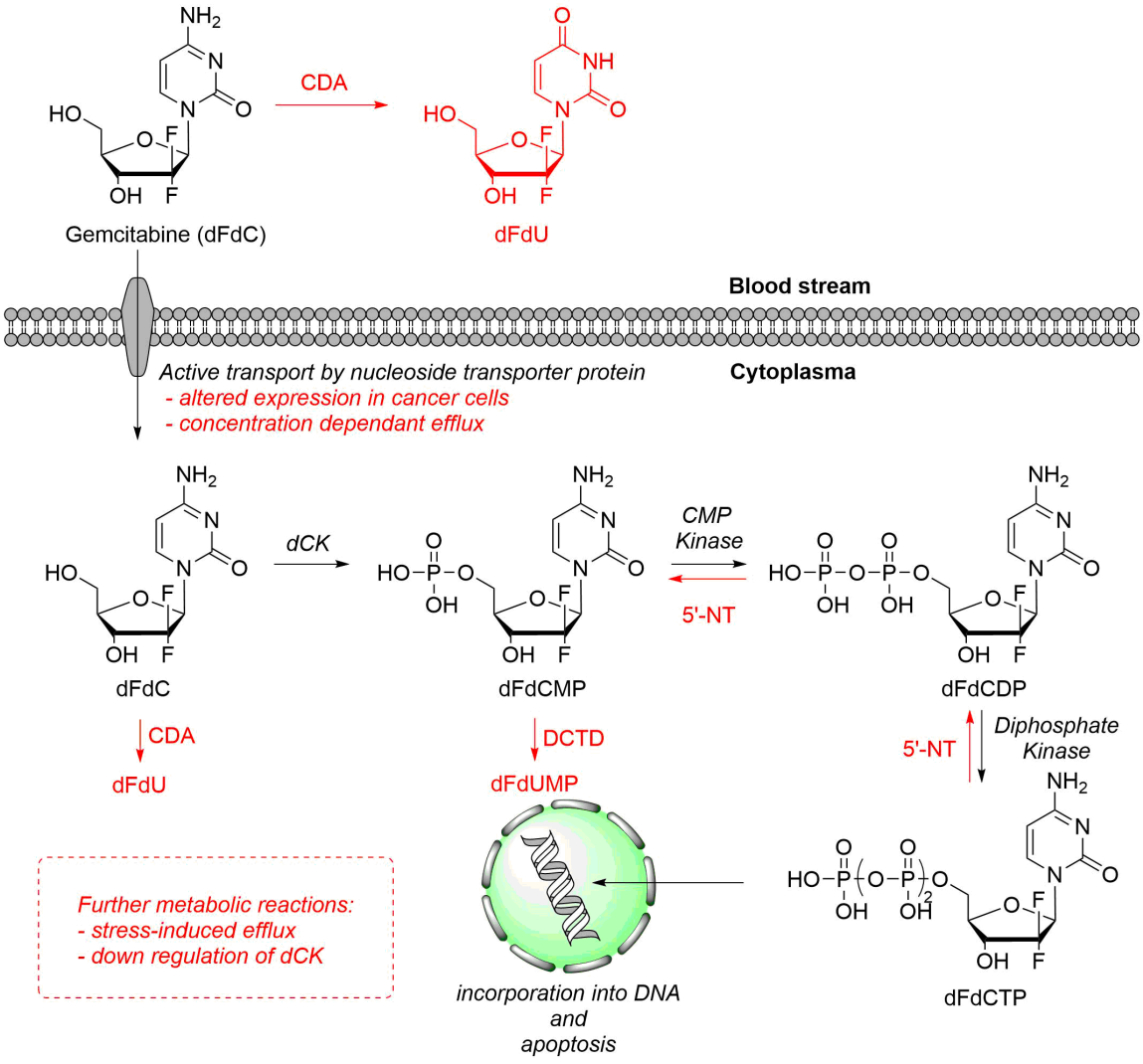
In this work, **Sq-Gem** will be used as the target molecule to develop a radiolabelling strategy, which could also be applied to other squalenoylated drugs. In contrast to other squalenoylated drugs, gemcitabine contains fluorine, which could also be radiolabelled, enabling an *in vivo* investigation of both fragments separately. In the following section, an overview of the anticancer drug gemcitabine and the benefits of squalenoylation will be given.

1.2.1 Gemcitabine

Gemcitabine is a last resort anticancer drug, which is used in the treatment of non-small cell lung cancer and pancreatic cancer.[11] The drug is marketed under the name Gemzar by Eli Lilly and had a market value of 1.15 billion dollars in 2010.[11] Gemcitabine (dFdC) must overcome several metabolic hurdles before it exhibits its cytotoxicity. The free amine at the 4-position of the cytosine base readily undergoes hydrolytic deamination in the blood plasma to 2',2'-difluorodeoxyuridine (dFdU), which is inactive. A half-life time of 8 minutes has been reported.[12] As a nucleoside analogue, gemcitabine relies on nucleoside transporters to cross the plasma membrane and enter the cell (Scheme 1.2). Five transporter proteins have been identified to transport gemcitabine. The main transporter hENT1 is an equilibrative nucleoside transporter, which reacts to a concentration gradient.[11] This can lead an undesired efflux of gemcitabine, if the concentration in the cell is too high. It was further demonstrated that nucleoside transporters can have altered expression in cancer cells.

Once within the cell, the 5'-hydroxy group must undergo three enzyme-catalysed phosphorylations before it can enter the cell nucleus and be incorporated into DNA. The phosphorylation steps are reversible by 5'-nucleotidases (5'-NT) and the crucial monophosphorylated intermediate (dFdCMP) can still undergo deamination to dFdUMP, which is not active. The triphosphorylated product (dFdCTP) is incorporated

into the DNA during replication. The DNA polymerases add another deoxynucleotide, after which the polymerases are unable to proceed. The drug is locked in the DNA strand as proof-reading exonucleases are unable to remove it from the penultimate position. This mechanism is called ”masked chain termination”. The faulty and incomplete DNA replication led to apoptosis. A high gemcitabine concentration was shown to trigger metabolic stress responses from the cell which leads to efflux of the drug and a down-regulation of deoxycytidine kinase (dCK).[11]



Scheme 1.2: Overview of Gemcitabine metabolism; red = reactions hindering drug efficiency; CDA = cytidine deaminase, DCTD = deoxycytidylate deaminase

The large variety of possible decomposition or inhibition pathways reduces the potential potency and effectiveness of the drug. To address the discussed shortcomings, a variety of pro-drugs have been synthesised.[11] Amongst them, **Sq-Gem** is unique in its ability to form well-defined nanoparticles. Couvreur and co-workers investigated the metabolism of **Sq-Gem** in depth and the results are summarised in the next section.

1.2.2 Biological Properties of Sq-Gem

Masking of the 4-amino group through the squalenoyl derivative successfully avoids the deamination reaction in the blood plasma; and 80% of **Sq-Gem** was retained after 24 hours. By introducing a tritium label on the 5'-position of gemcitabine, Couvreur and co-workers were able to observe the uptake and metabolism of **Sq-Gem** *in vitro*. Cell compartment distribution results were obtained from microautoradiography and metabolism products quantified by LC/MS-MS. Due to its apolar nature **Sq-Gem** enters the cell through the membrane without the need for active transport proteins (Figure 1.2).[1,13] Couvreur and co-workers were able to demonstrate that the individual **Sq-Gem** molecules and nanoparticles exist in an equilibrium. When the tumour cells were separated from the **Sq-Gem** nanoparticles by a size-exclusion membrane, the cytotoxicity was retained.[1] It is therefore unlikely that the particle diffuses through the cell membrane but rather individual **Sq-Gem** molecules. Once within the cell, the amide bond between the drug and the squalenoyl tail is cleaved by

lysosomal enzymes. The free gemcitabine (dFdC) follows the previously discussed metabolism pathway and induces apoptosis. Alternatively, **Sq-Gem** can accumulate within lipophilic cell compartments (e.g. endoplasmic reticulum). The slow release from those compartments was shown to avoid metabolic reactions the cell usually activates as a reaction to increased drug concentrations. This caused the cell to be exposed to the drug for longer, increasing the probability of the crucial phosphorylation.

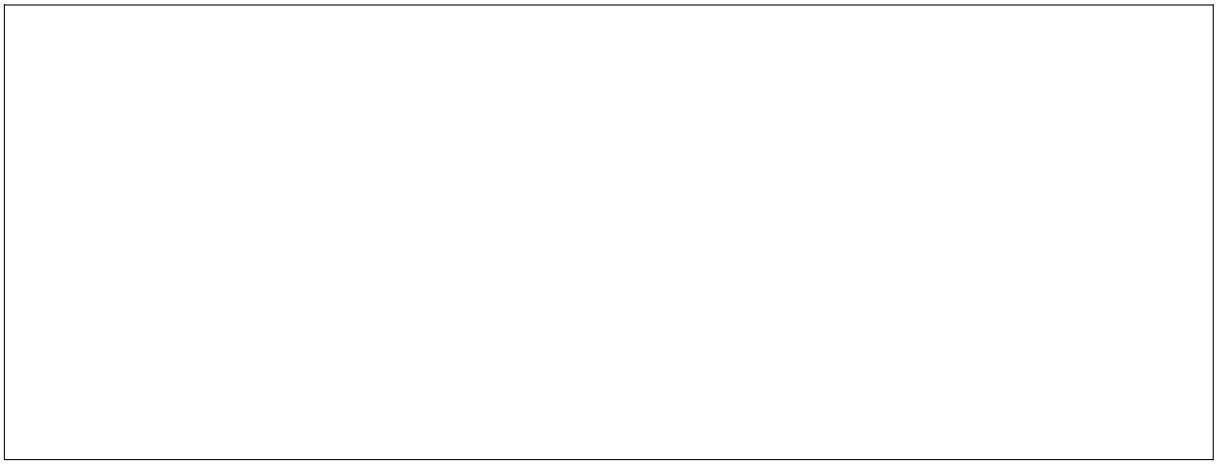


Figure 1.2: Intracellular metabolism of **Sq-Gem**. [1]

The cytotoxicity of **Sq-Gem** nanoparticles was tested *in vitro* against breast cancer (MCF-7) and leukaemia (KB3-1) cell lines (Table 1.2). **Sq-Gem** exhibited a higher cytotoxicity than gemcitabine against all tested cancer cell lines.[1,13] Noteworthy are the results for the leukaemia cell lines L1210 10K and CEM/ARAC8C (Entry 3 and 4) because both are considered gemcitabine resistant. In L1210 10K, deoxycytidine

[1] Reprinted from Journal of Controlled Release, 147, P. Couvreur et. al., Transmembrane diffusion of gemcitabine by a nanoparticulate squalenoyl prodrug: an original drug delivery pathway., 163 - 170, Copyright (2010), with permission from Elsevier.

kinase (dCK), which is required for the phosphorylation of gemcitabine to enable internalisation into the DNA, is downregulated. The CEM/ARAC8C cell line underexpresses the membrane transport proteins needed for the active transport of gemcitabine into the cell. **Sq-Gem** was more cytotoxic towards both cell lines, due to the alternative pathways for uptake into the cell and prolonged exposure of the cell to the drug.

Entry	Cell line	Gemcitabine IC_{50} [μM]	**Sq-Gem** IC_{50}[μM]
1	MCF-7	29 ± 5.8	4.8 ± 3.9
2	KB3-1	50.8 ± 9.2	8.8 ± 4.1
3	L1210 10K	18.8 ± 6.8	5.0 ± 8.4
4	CEM/ARAC8C	4.2 ± 0.6	0.14 ± 0.01

Table 1.2: Comparison of IC_{50} values for Gemcitabine and **Sq-Gem**

Sq-Gem was tested *in vivo* on leukaemia-bearing mice and rats (Figure 1.3).[1] The drug conjugate showed enhanced survivability of the animals compared to gemcitabine after intravenous and oral treatment.

Figure 1.3: A Survival of the mice bearing P388 leukaemia after treatment. Untreated (solid line), gemcitabine 5 mg kg^{-1} (dashed line), **Sq-Gem** NA equivalent to 5mg kg^{-1} gemcitabine (dotted dashed line), Gemcitabine 15 mg kg^{-1} (lightface dotted line), **Sq-Gem** NA equivalent to 15 mg kg^{-1} gemcitabine (boldface dotted line); **B** Survival of F344 Fischer rats bearing RNK-16 LGL leukaemia after oral treatment with 15 mg kg^{-1} gemcitabine and its equivalent as **Sq-Gem** NA. Untreated (solid line), gemcitabine (dotted line), SQdFdC NA (dotted dashed line). *Adapted with permission from Nano Lett., Vol. 6, No. 11, 2006, 2544-2548. Copyright (2006) American Chemical Society.*

In summary, Couvreur and co-workers were able to demonstrate that **Sq-Gem** overcomes many problems associated with gemcitabine on a cellular level. *In vivo* studies on mice and rats confirmed the potential of **Sq-Gem** as a drug, but so far no whole body biodistribution studies have been performed and the fate of the squalenoyl tail is unknown. To promote the squalenoylation methodology further, it is of interest to develop an approach for the radiolabelling of squalenoylated drugs with a positron emitting radioisotope. The radiolabelled particles could be used in *in vivo* PET studies and help to understand the pharmacokinetics of the drug.

1.2.3 Physical Properties of Sq-Gem

For the development of a radiolabelled squalenoylated drug, it was important that the shape and size of the obtained nanoparticles were not different from those of the native compound. In the following section, the physical properties of **Sq-Gem** are reviewed and discussed.

The formation of well-defined nanoparticles was observed by Couvreur and co-workers, when **Sq-Gem** in acetone was added into an aqueous dextrose solution.[1] The particles were characterised by Dynamic Light Scattering (DLS) and were found to have a mean diameter of 130 $\pm$ 8 nm. The polydispersity index (PDI), which describes particle size distribution, was 0.12.[1] The critical concentration for nanoparticle formation was observed to be 4.6 μM. Cryogenic Transmission Electron Microscopy (cryoTEM) revealed nanoparticles of hexagonal shape, surrounded by an external shell (Figure 1.4 **A**).[14] Combining the data from cryoTEM and X-ray diffraction studies, Couvreur and co-workers proposed tube-like substructures with interlocking squalenoyl chains (**B**). The polar gemcitabine was situated in a water channel in the middle of the tubes (**C**).[14] The terpenoid chain length was found to be crucial for the stability and shape of the nanoparticles.[15] Shorter terpenoid chains would lead to rapid precipitation of the compound without nanoparticle formation.

From the proposed packing of the **Sq-Gem** particles and the ability to co-precipitate several squalenoylated molecules, it was hypothesised that modification of the

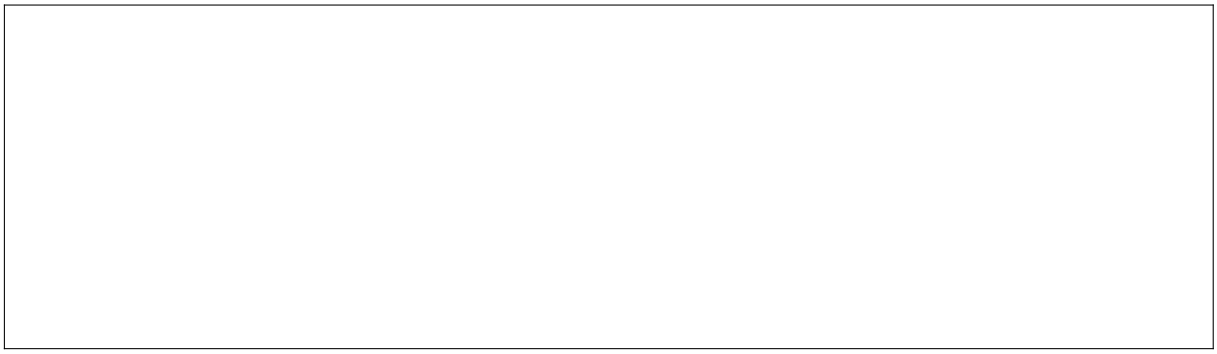


Figure 1.4: Structural data of **Sq-Gem**[14]: **A** cryoTEM image; **B** Schematic drawing of the hexagonal(HII) packing of amphiphilic molecules; **C** Packing of individual subunits. *Reproduced with the permission of ©2008 Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim*

squalenoyl tail could be tolerated. To obtain a complete understanding on how squalenoylated drugs behave *in vivo*, three different modes of labelling must be investigated (Figure 1.5): 1) Drug nanoparticles with a radiolabelled squalenoyl tail (e.g. **^{18}F-Sq-Gem**), 2) radiolabelling on the drug (e.g. Sq-[^{18}F]Gemcitabine) and 3) both components separately from each other (e.g. ^{18}F-squalenic acid and [^{18}F]Gemcitabine). 1 and 2 will give direct information about the behaviour of the nanoparticle, while 3 can be used to identify possible metabolic pathways. The fluorine-19 analogues must be synthesised to investigate the influence of the modification on the nanoparticle formation.

In the following sections, an overview of PET and the radiolabelling of nanoparticles will be given. The discussion is focused on determining the optimal radionuclide for the radiolabelling of squalenoylated drugs and methodologies to achieve this goal.

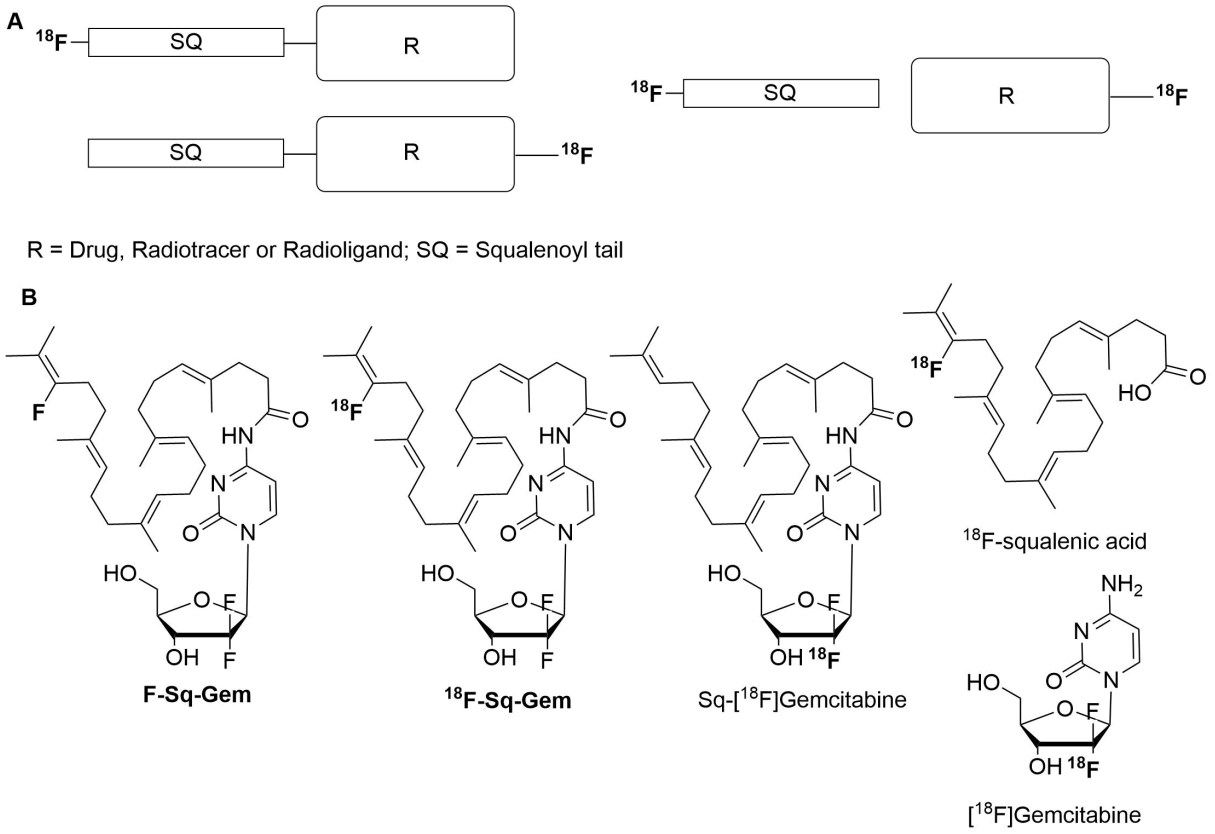


Figure 1.5: A Different modes of radiolabelling of squalenoylated drugs and controls; **B** Targets to investigate **Sq-Gem** nanoparticles

1.3 Introduction to Positron Emission Tomography

Positron Emission Tomography (PET) is a non-invasive medical nuclear imaging modality. This technology relies on the detection of γ-radiation generated in the annihilation of positrons (β^+ particle, Figure 1.6). The positron is emitted by the decay of a radionuclide and annihilates upon collision with an electron. This process emits two γ-ray photons of 511 keV, which travel at 180° from each other. Detecting those γ-photons allows the site of annihilation to be determined and the site of decay to be approximated.[16–18]

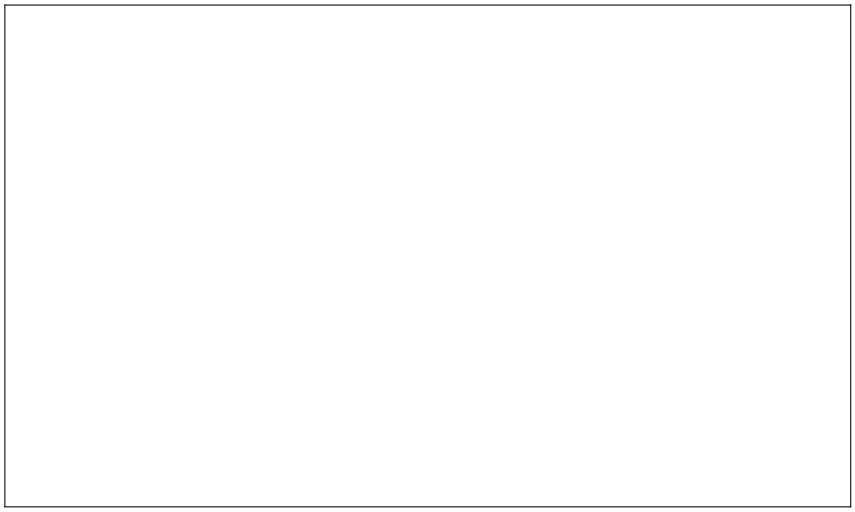


Figure 1.6: Schematic representation of the principle behind PET showing the positron decay and annihilation which produces two γ quanta. *Reproduced with the permission of ©2008 Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim*

The resolution of the obtained image depends on the size of the detectors, the collinearity of the photons and the range of the positrons.[17] The accurate detection of single annihilation events gives rise to quantitative data. PET only shows the site of accumulation but does not give structural information. To correlate the site of annihilation to biological structures, PET is combined with structural imaging techniques, like CT or MRI.

The choice of radionuclide is dependent on the target and its expected metabolism. Small, rapidly-metabolised, organic molecules are preferentially labelled with carbon-11 as it does not change the structure or electronics of the radioligand. Fluorine containing molecules can be labelled with fluorine-18. The advantage of fluorine-18 over carbon-11 is the longer half-life, which allows for longer imaging time. Large macromolecules, like antibodies or nanoparticles, with long circulation times

benefit from copper-64 labelling *via* chelation. The characteristics of the most employed PET radionuclides are shown in Table 1.3.

Radionuclide	Half-life [mins]	Decay product
^{15}O	2.04	^{15}N
^{13}N	9.97	^{13}C
^{11}C	20.4	^{11}B
^{18}F	110	^{18}O
^{64}Cu	762	^{64}Ni

Table 1.3: Frequently used positron emitting radionuclides

1.3.1 Choice for Radionuclide for Radiolabelling Squalenoylated Drugs

The ideal choice for the radiolabelling of squalenoylated drugs would be carbon-11, since this would ensure, that the structure and *in vivo* characteristics of the drug conjugates do not change. Previous studies on cellular uptake of nanoparticles showed that the enhanced permeability and retention (EPR) effect plays an important role.[19–21] The EPR effect arises from openings in endothelial layer of blood vessels in tumour tissues and reduced lymphatic drainage. Those defects allow macromolecules and nanoparticles to excessively leak into the tumour tissue.[22] The EPR effect has been described as a slow process, uptake concentrations peaking between 24 and 48 hours.[23] Carbon-11 and other radionuclei with shorter half-life times (^{15}O, ^{13}N) would

not offer a large enough detection window to observe uptake. Fluorine-18 ($T_{1/2}$ 110 mins) offers a much larger detection window (up to 6 hours post-injection) and has previously been used for nanoparticle labelling.[10,24] Positron-emitting metal isotopes (e.g. ^{64}Cu) were not considered as they would require heavy modification of the squalenoylated drug to allow for metal coordination.

Fluorine-18 has been previously used for biodistribution studies of nanoparticles (discussed in Section 1.4) and was therefore chosen for this investigation. As a consequence, the behaviour of fluorine-19 modified **Sq-Gem** has to be investigated and compared to **Sq-Gem**. In the following section, a short review over previous approaches to radiofluorination of nanoparticles is given and their adaptability for squalenoylated drugs discussed.

1.4 ^{18}F-labelling of Nanoparticles

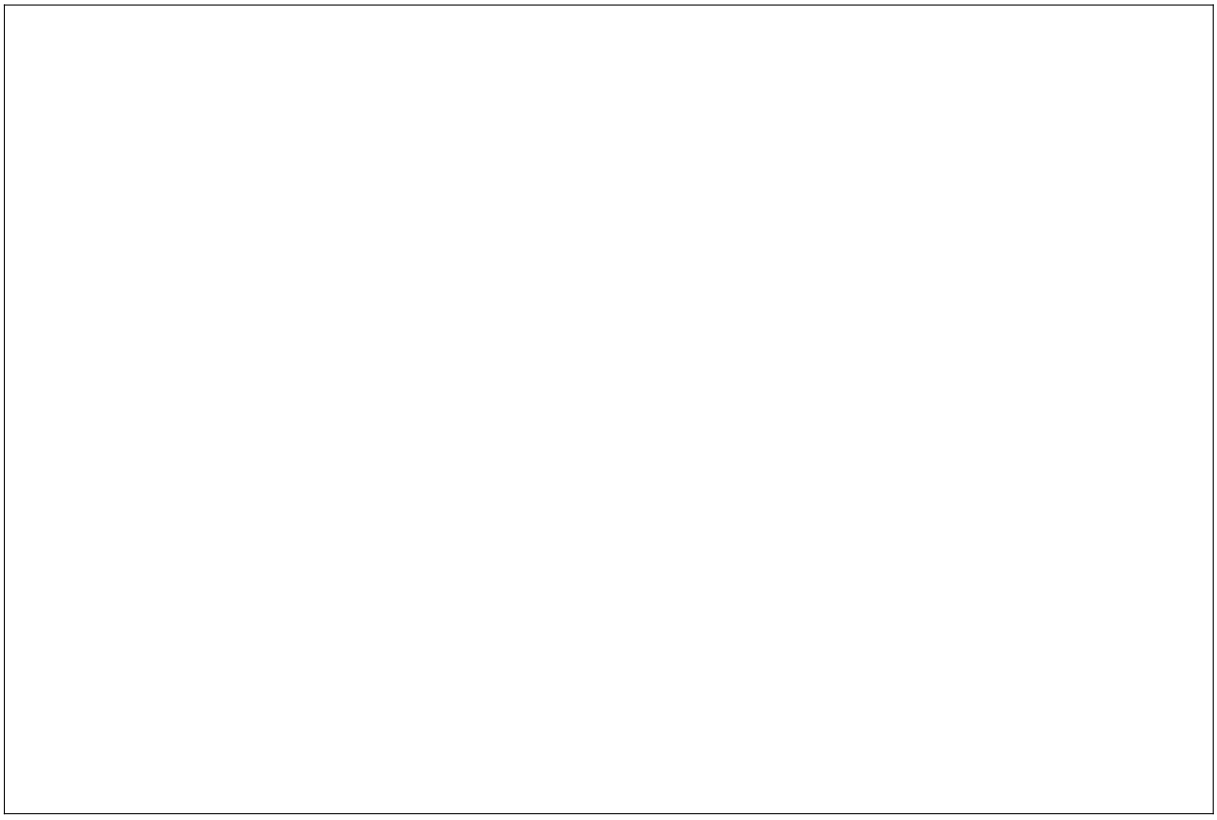
Fluorine-18 has been used in the past to investigate the biodistribution of nanoparticles. The utilised methodologies can be divided in three subgroups: 1) Hadronic activation by irradiation, 2) complexation of [^{18}F]fluoride to metals and 3) radiofluorination of an organic residue. While the first two subgroups are not viable for the radiofluorination of squalenoylated drugs, they are discussed to highlight the applicability of [^{18}F]fluoride in nanoparticle labelling. A few selected examples will be used to highlight the differences in biodistribution of nanoparticles and their application in PET imaging.

1.4.1 Hadronic Activation of Nanoparticles

Hadronic activation refers to the process of inducing a nuclear reaction through particle bombardment.[25] In this process, the target material is placed in a cyclotron target chamber and bombarded with neutrons or protons to induce nuclear reactions. This strategy has been exploited to induce nuclear reactions of metals due to the high natural abundances of suitable isotopes. For example, ^{197}Au is the only natural occurring isotope of gold and can undergo the nuclear reaction ^{197}Au(n,γ)^{198}Au in high yields.[25] Hadronic activation is especially suited for nanoparticles without surface modifications as the high energies likely destroy organic residues. If thermal rearrangements within the metal core can be avoided, the labelled particle should display the same properties as before the bombardment.[25]

[^{18}F]Fluoride is produced through the nuclear reaction ^{18}O(p,n)^{18}F, but the natural occurrence of ^{18}O is very low (0.2%).[26] Llop and co-workers developed a procedure to produce isotopically enriched $Al_2^{18}O_3$ (98%) nanoparticles. Upon irradiation of the particles with protons, they observed the desired nuclear reaction and obtained ^{18}F-labelled Al_2O_3 particles. Transmission-electron microscopy, powder X-ray diffraction (XRD) and dynamic light scattering (DLS) revealed no changes in the particle structure. Stability tests in rat serum revealed no leaking of [^{18}F]fluoride out of the particle.

The experimental setup for hadronic activation is shown in Scheme 1.3 with ^{18}O-enriched TiO_2 nanoparticles.[27]



Scheme 1.3: Preparation of ^{18}O-enriched TiO_2 NP and subsequent proton activation. NPs were synthesized by nanoprecipitation, purified by centrifugation, and dried in an inert gas atmosphere. After introduction in an appropriate aluminium holder, NPs were irradiated using high-energy protons in a specially designed target coupled to a biomedical cyclotron. *Reproduced with the permission of ©2013 Wiley-VCH Verlag GmbH&Co. KGaA, Weinheim*

Llop and co-workers observed several nuclear side reactions, when the methodology was applied to ^{18}O-enriched TiO_2 nanoparticles. The exposure of TiO_2 nanoparticles to atmospheric oxygen resulted in a large amount of ^{13}N ($T_{1/2}$ 9.9 mins) *via* the $^{16}O(p,\alpha)^{13}N$ reaction. Side reactions also occurred from natural titanium isotopes. At the end of bombardment (EOB), the following ratios of isotopes were obtained: ^{13}N (73.7%):^{47}V (7.6%):^{18}F (17.6%):^{44g}Sc (0.61%):^{48}V (0.37%). Due to the large differences in half-life times of the three major isotopes, ^{13}N ($T_{1/2}$ 9.9 mins) and ^{47}V ($T_{1/2}$ 32.6 mins) were eliminated by simple decay. Two hours after the end of bombardment, 85% of the

radiation came from ^{18}F decay. For biodistribution studies it was important to have a high percentage of one emitter to obtain accurate time activity curves. The authors note that the nanoparticles remain longer in the body than detection by PET was possible, due to the half-life of [^{18}F]fluoride.

Although organic molecules often contain oxygen bearing functional groups, which could be isotopically enriched, hadronic bombardment is not viable as this process would decompose the molecule.

1.4.2 Radiolabelling of Fluoride-Metal Complexes

The ability of fluoride to form strong metal complexes was exploited in the radiofluorination of rare-earth nanoparticles (Table 1.4).[28] The nanoparticles were suspended in aqueous [^{18}F]fluoride solution to allow coordination of the anion to the highly Lewis acidic metal atoms. The labelled nanoparticles were obtained in high radiochemical yields. Stability tests in PBS buffer showed a slow dissociation of [^{18}F]fluoride from the particles. It was proposed that part of the [^{18}F]fluoride was not coordinated but loosely absorbed onto the surface and could be released. Noteworthy is the successful labelling of a rare-earth upconverting nanophosphor particle (UCNP), $NaYF_4$(Yb,Tm). The $NaYF_4$ core was doped with ytterbium and thulium, which are able to emit visible light after excitation with near-infrared light.

Entry	Sample	Radiochemical Yield [%]	Dissociation rate after 0 h [%][b]	Dissociation rate after 2 h [%]
1	Y_2O_3	92.4 ± 0.9	11.6 ± 1.4	7.5 ± 0.9
2	$NaYF_4$	90.1 ± 1.5	5.3 ± 1.5	9.8 ± 1.5
3	$NaYF_4(Yb, Tm)$	88.6 ± 1.0	5.1 ± 0.5	11.3 ± 1.0
4	$Y(OH)_3$	97.3 ± 0.8	2.5 ± 1.5	2.6 ± 1.2
5	$Gd(OH)_3$	96.4 ± 1.2	1.3 ± 1.2	3.5 ± 1.2

Table 1.4: Radiofluorination of rare-earth nanoparticles[28]: [b] Dissociation rate was calculated by the following formula: [1 - (solid / total activity)] * 100%

Those particles offer multi-modal detection by PET and upconverting luminescence (UPL). Li and co-workers further doped UCNP's with gadolinium enabling a tri-modal detection by UPL, MRI and PET.[29,30] Functionalisation of the nanoparticles with organic residues increased their biocompatibility and gave a targeting function. The particles were investigated for their use in lymphatic imaging.[28] Radiofluorinated $NaYF_4$(Yb,Tm) nanoparticles were subcutaneously injected into mice. Distribution through the lymphatic system was observed after 30 minutes, leaving little residual activity at the injection point (Figure 1.7).

As an alternative to gadolinium doping of $NaYF_4$ particles, MRI activity was also obtained when $NaYF_4$ was encapsulated in iron oxides.[31] The defined core-shell particle Fe_3O_4@$NaYF_4$(Yb, Tm) and $Co_{0.16}Fe_{2.84}O_4$@$NaYF_4$(Yb, Er) were coated with

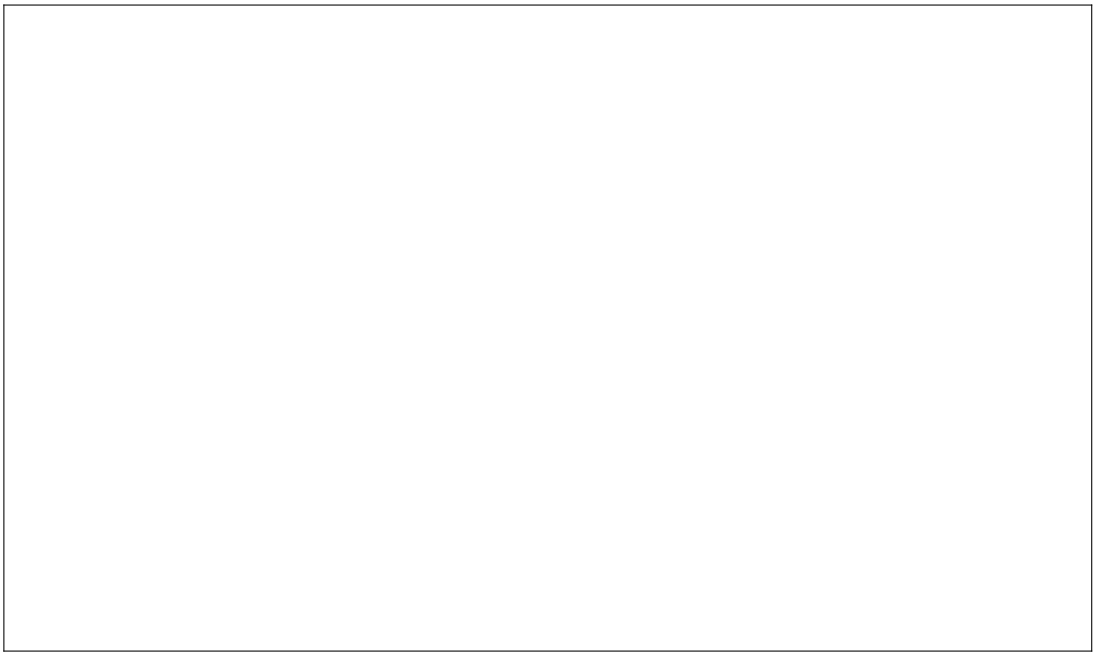


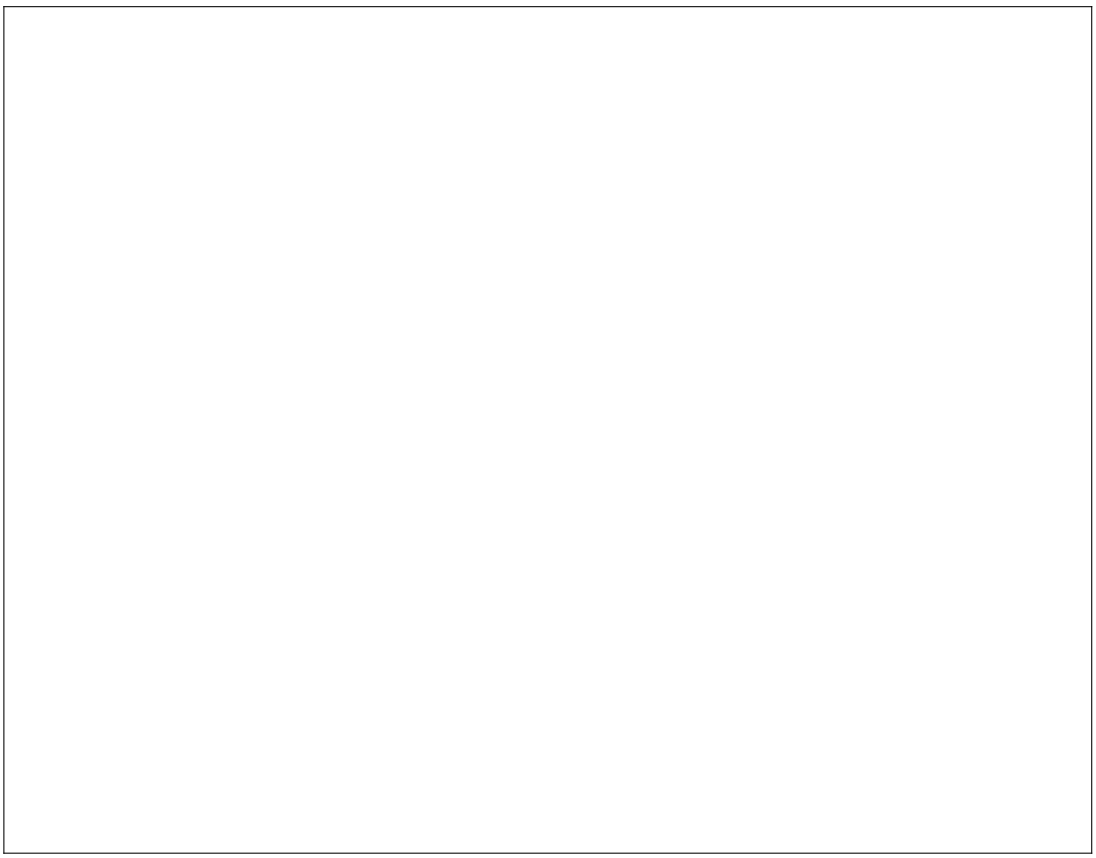
Figure 1.7: PET imaging (a) and PET/CT imaging (b) of lymph node 30 min after subcutaneous injection of ^{18}F-UCNPs. 30 min after subcutaneous injection of 740 kBq/0.05 mL ^{18}F-UCNPs into the left paw footpad, signal in lymph node reached the peak intensity and maintained it to 60 min post-injection. While as control free $^{18}F^-$ ions injected into the right paw showed no lymphatic imaging ability. [2]

bisphosphonate polyethylene glycol conjugates (BP-PEG) of different lengths. The nanoparticles were labelled with [^{18}F]fluoride. The labelling efficiency was comparatively low (38%) but the particles showed good stability with little fluoride release. *In vivo* PET/MRI studies demonstrated good uptake into the lymphatic system.

Chelation of radiometals is a popular approach for the radiolabelling of large proteins and antibodies. [^{18}F]Fluoride is coordinated to aluminium(III) and subsequently chelated to the desired biomolecule.[32–34] This principle was applied to functionalised iron-oxide nanoparticles (such as NOTA-IONPS, Scheme 1.4).[35] The nanoparticle

[2]Reprinted from Biomaterials, 32, F. Li et. al., Fluorine-18 labelled rare-earth nanoparticles for positron emission tomography (PET) imaging of sentinel lymph node, 2999 - 3007, Copyright (2011), with permission from Elsevier

was coated with a surfactant, which provided strong interactions with an amphiphilic polymer. The polymer contained the NOTA-chelator, which binds strongly to aluminium. Radioactive nanoparticles were obtained in 40% radiochemical yield.

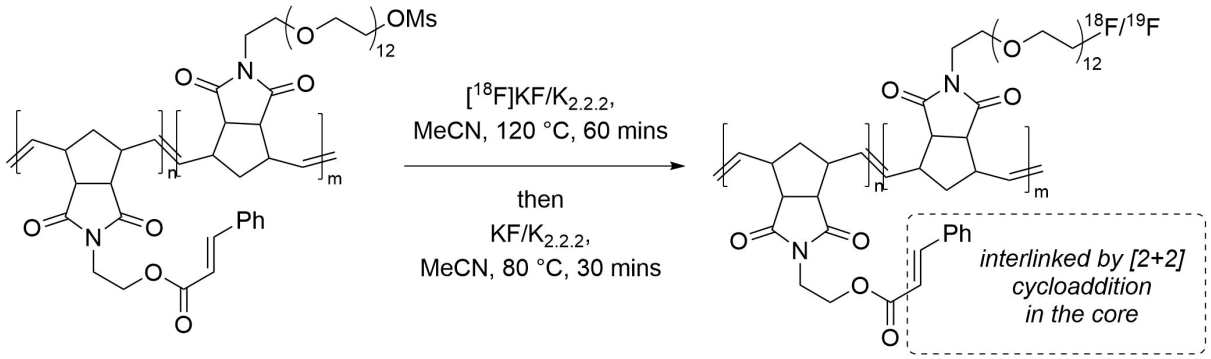


Scheme 1.4: Schematic illustration of the preparation and radiolabelling of NOTA-COBP-IONPs. (a) The branched COBP was synthesized from polyacrylic acid and oleylamine. (b) Conjugation of branched COBP and NOTA via amide bonds. (c) Coating of IONPs (8 nm in diameter) by NOTA–COBP (d) Chelation of ^{18}F-aluminum fluoride ([^{18}F]AlF) ions to the NOTA–COBP-IONPs. The balls represent the [^{18}F]AlF ions. *Z. Chen et. al. Nanoscale, 2016, 8, 19644[35] - Published by The Royal Society of Chemistry*

1.4.3 Radiofluorination of Organic Residues on Nanoparticles

The alternative to coordinating [^{18}F]fluoride to the metal core of a nanoparticle or chelating [^{18}F]AlF, is the labelling of the organic shell or polymer residues.

The first example of a radiofluorinated organic nanoparticle was presented by Grubbs and co-workers.[36] A norbornene based co-block polymer was developed, which formed nanoparticles upon cross-linking by [2+2] cycloadditions (Scheme 1.5). The mesylate residues were radiofluorinated with [^{18}F]fluoride. Unlabelled mesyl residues were capped with fluoride to avoid undesired *in vivo* reactions. The desired polymeric nanoparticles were obtained in 61% radiochemical conversion. The authors did not isolate the particles nor publish *in vivo* data.



Scheme 1.5: ^{18}F-Labelling of norbornene based co-block polymers

Many nanoparticles are coated with organic residues to increase their biocompatibility and circulation times. Those residues can be functionalised to allow for radiofluorination. A common strategy is to synthesise a radiofluorinated small molecule, which is then attached to the nanoparticle. This approach is advantageous as it does not expose the nanoparticle to the harsh conditions of the radiofluorination and thermally induced structure changes in the metal-core can be avoided. In the following section, several strategies for residue radiofluorination will be described and discussed. An overview of commonly used radioactive fragments is shown in Figure 1.8.

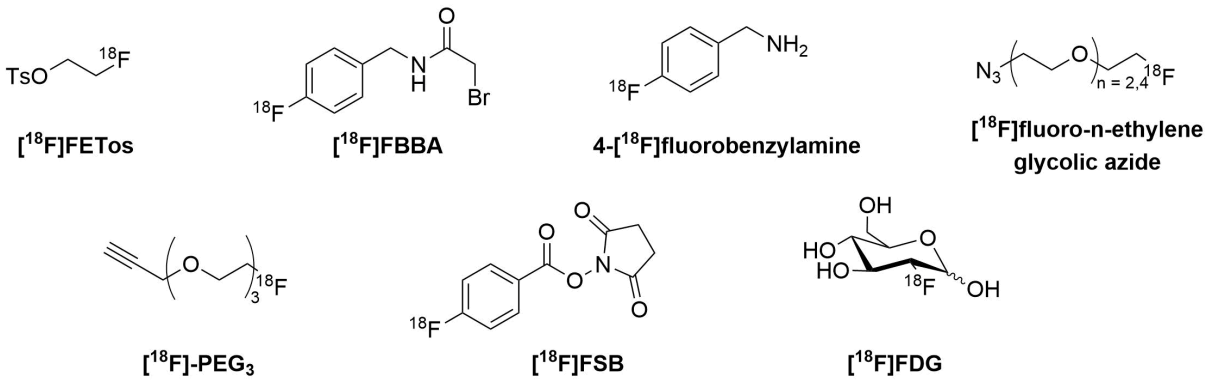
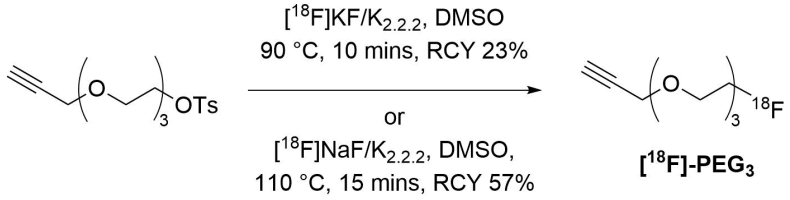


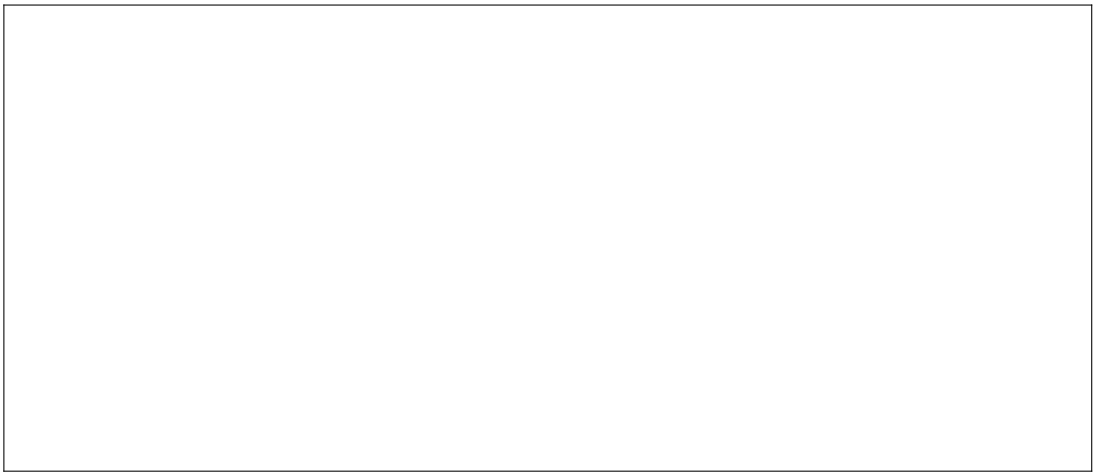
Figure 1.8: ^{18}F-Labelled fragments used for organic-shell nanoparticle labelling

One of the most exploited reactions is the alkyne-azide click reaction. The fragments are based on a short polyethylene glycol (PEG) chain with either an azide or alkyne functionality. Weissleder and co-workers demonstrated the radiolabelling of **[^{18}F]-PEG$_3$** from the corresponding tosylates with [^{18}F]KF or [^{18}F]NaF (Scheme 1.6).[37,38] **[^{18}F]-PEG$_3$** was obtained in acceptable radiochemical yields (RCY).



Scheme 1.6: Radiosynthesis of **[^{18}F]-PEG$_3$**

Subsequently, the resulting alkyne was used in a copper-mediated click reaction with an azide functionalised nanoparticle. The first example was an iron-oxide core (red, Scheme 1.7) particle which was coated with a polysaccharide shell (green).[37] The resulting radiolabelled nanoparticles were obtained in 58% RCY and high radiochemical purity (>98%). The total synthesis time was 120 minutes from the azeotropic drying of [^{18}F]fluoride.



Scheme 1.7: Radiolabelling of iron-oxide core nanoparticles *Adapted with permission from R. Weissleder et. al., Bioconjugate Chem. 2009, 20, 397–401. Copyright (2009) American Chemical Society.*

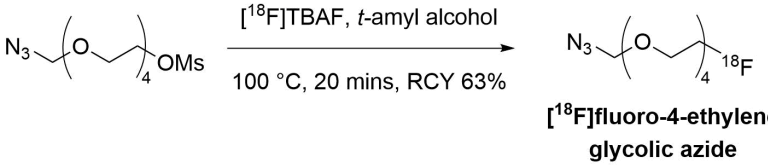
In depth biological tests were performed on a different nanoparticle: macrins are lysine-crosslinked carboxymethyl polyglucose polymers of low molecular weight.[38] Their small size of 5 nm should allow excretion over the renal pathway rather than accumulation in the liver.[38] The free amine residues were capped with azido-acetic *N*-hydroxysuccinimidyl ester to enable the click reaction (Scheme 1.8). Two different procedures for the click reaction were utilised depending on the amount of radiation. The microwave assisted click reaction afforded the product in 37% RCY and was applied to a starting activity of 3 GBq. On larger scales (up to 37 GBq), the click reaction was performed in a sealed vial and heated to 65 °C. This afforded Macroflor in 13% RCY.

Tests with fluorescent macrin nanoparticles suggested a rapid uptake of the particles by macrophages.[38] The authors were able to detect accumulation of macrophages labelled with Marcoflor in inflamed tissue.

Scheme 1.8: Radiolabelling of Macroflor nanoparticles *Adapted from R. Weissleder et. al., Nat. Commun. 8, 14064*

The fast renal clearance enabled the detection of macrophage accumulation in mice with ischaemic heart disease, which is difficult for particles with long blood half-life times.

Kim and co-workers exploited the ability to perform strain-promoted alkyne-azide cycloaddition (SPAAC) reactions *in vivo*.[39] [^{18}F]Fluoro-4-ethylene glycolic azide was synthesised from its tosylate in good radiochemical yields (Scheme 1.9).



Scheme 1.9: Radiosynthesis of [^{18}F]fluoro-4-ethylene glycolic azide

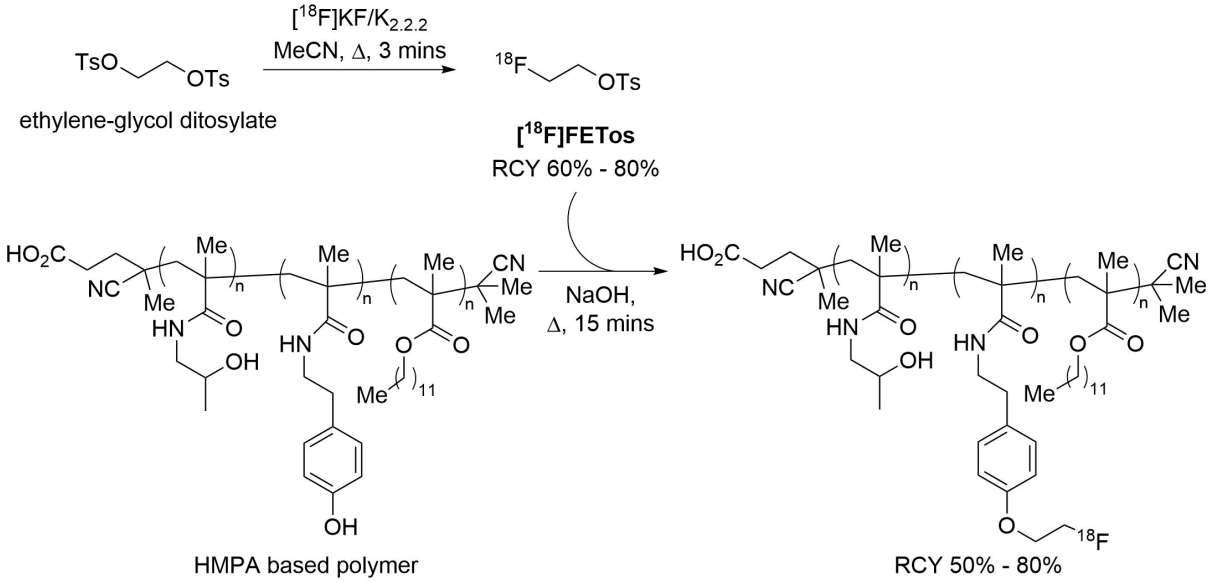
[^{18}F]Fluoro-4-ethylene glycolic azide was directly injected into tumour bearing mice which had been injected with mesoporous silica nanoparticles (MSN) 24 hours earlier (Scheme 1.10). The MSNs were functionalised with different PEG chains to increase bioavailability and a dibenzocyclooctyne (DBCO) containing linker which acts as an azide acceptor. The authors demonstrated the uptake and retention of the MSNs in cancerous tissue as well as rapid accumulation of radioactivity after administration of

[^{18}F]fluoro-4-ethylene glycolic azide. This pretargeting method allows to observe particles with slow tissue accumulation times with short-lived isotopes.

Scheme 1.10: Pretargeting PET imaging study by bioorthogonal covalent ^{18}F-labelling. A) The procedure for the in situ synthesis of ^{18}F-DBCOT-PEGMSNs in a living specimen by a bioorthogonal SPAAC reaction for the DBCO-PEG-MSN-pretargeting PET-imaging study. B),C) Three-dimensional reconstruction (upper) and transverse section (lower) combined PET-CT images of ^{18}F-labelled azide (2.6 MBq) in a U87 MG tumour-bearing mouse given only [^{18}F]fluoro-4-ethylene glycolic azide alone (non-pretargeted; B) or a mouse given DBCO-PEG-MSNs 24 h earlier (pretargeted; C) recorded at 15, 30, 60, and 120 min after injection of [^{18}F]fluoro-4-ethylene glycolic azide. T=tumour, K=kidneys *Reprinted with permission from D.W. Kim et. al., Angew. Chem. Int. Ed. 2013, 52, 10549 –10552. Copyright (2013) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim*

For the radiolabelling of nucleophilic polymer residues, **[^{18}F]FETos** was synthesised from ethylene glycol ditosylate by nucleophilic substitution (Scheme 1.11). Roesch and co-workers used this method to study the biological fate of *N*-(2-hydroxypropyl)methacrylamide (HPMA) based polymers.[40–42] A range of block polymers were synthesised and radiolabelled. The precise introduction of tyramine (4-(2-aminoethyl)phenol) into the hydrophilic block of the polymer offered a labelling position without influencing the particle structure.[40] The substitution reaction with

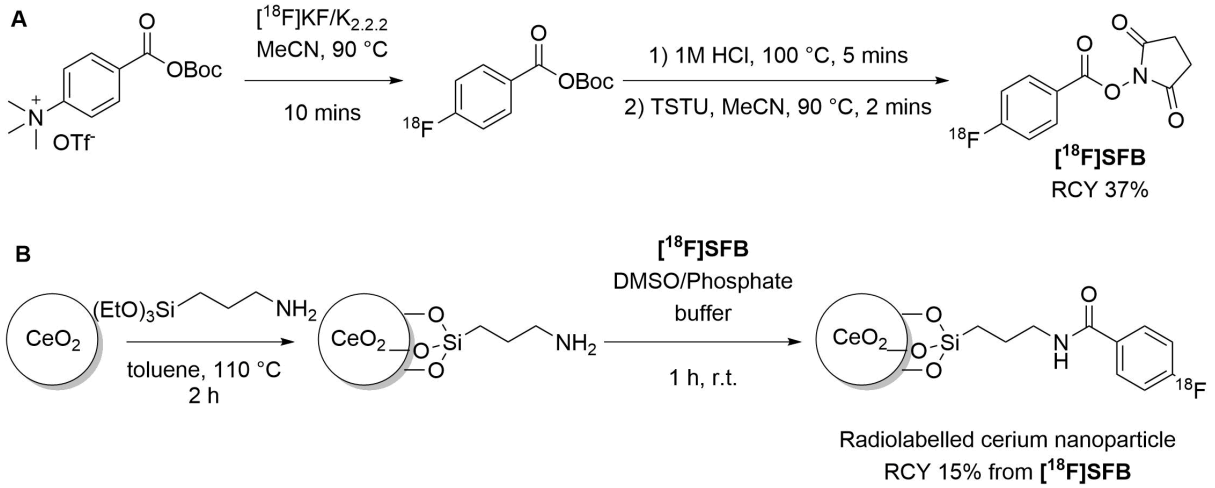
[^{18}F]FETos could be performed at low temperatures, allowing compatibility with thermally labile polymer residues. The RCY of the polymer labelling was found to be size dependent with higher molecular weight particles reducing the RCY.[40]



Scheme 1.11: Radiosynthesis of **[^{18}F]FETos** and labelling of an HPMA based block polymer

Roesch and co-workers investigated the biodistribution of different polymer particle sizes.[41] While small polymer nanoparticles were excreted by the renal pathway, larger particles were retained and probably taken up by macrophages. The authors further demonstrated that PEG side-chains had an influence on aggregate formation and non-specific protein binding within the bloodstream.[42] Higher PEG content was found to lead to better *in vivo* characteristics and increased tumour uptake in a rat model.

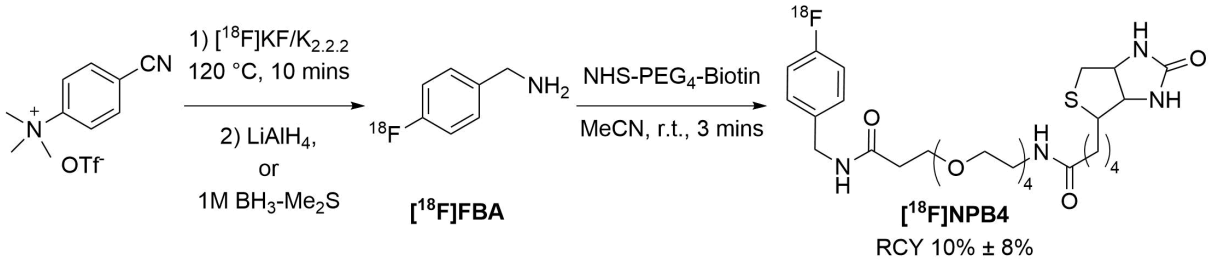
Cerium oxide nanoparticles have been investigated as therapeutics because of their ability to quench reactive oxygen species.[43] Herace and co-workers labelled cerium nanoparticles with **[^{18}F]SFB** (Scheme 1.12). The particle surface was modified with 3-(aminopropyl)triethoxysilane. **[^{18}F]SFB** was synthesised from 4-(tert-butoxycarbonylmethyl)phenyl trimethylammonium trifluoromethanesulfonate by SN_{Ar} (Scheme 1.12 **A**). The ester was hydrolysed with 1M HCl and the obtained acid activated with *N,N,N′,N′*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU). **[^{18}F]SFB** was obtained in 37% RCY. The functionalised cerium nanoparticles were treated with **[^{18}F]SFB** in DMSO and phosphate buffer for one hour. Purification by centrifugation afforded the radiolabelled nanoparticles in 15% RCY (Scheme 1.12 **B**).



Scheme 1.12: A Radiosynthesis of **[^{18}F]SFB**; **B** labelling of cerium oxide nanoparticles

In vivo, cerium oxide nanoparticles were observed to rapidly absorb into lungs, liver and spleen.[43] It was suspected that phagocytis was responsible for the rapid blood clearance. Time activity relations indicated a permanent internalisation into macrophages and the vascular network.

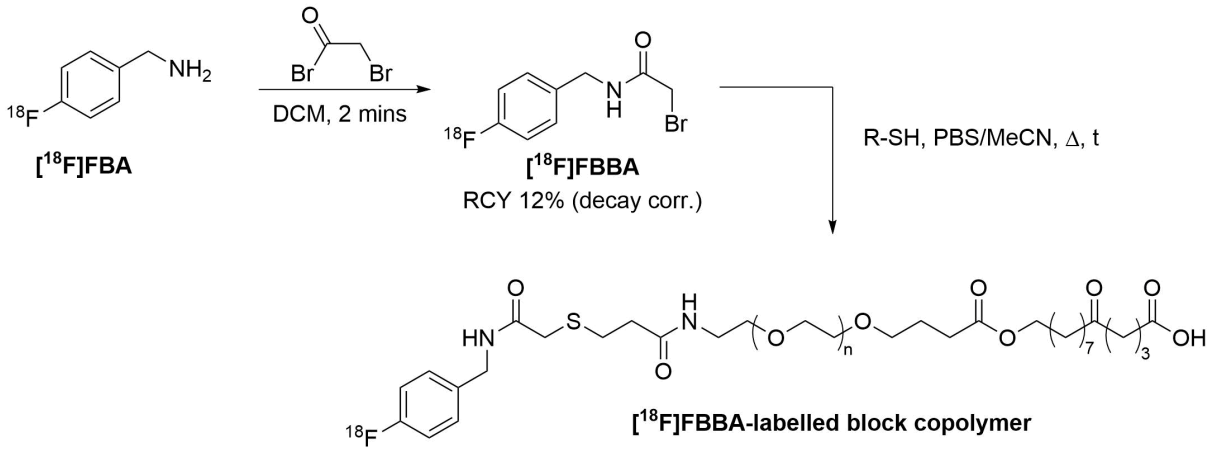
The synthesis of 4-[^{18}F]fluorobenzylamine (**[^{18}F]FBA**) and 2-bromo-*N*-(4-[^{18}F]fluorobenzyl)acetamide (**[^{18}F]FBBA**) employed the same chemistry as introduced for **[^{18}F]SFB**. SN_{Ar} of 4-cyano-*N,N,N*-trimethylanilinium trifluoromethanesulfonate with [^{18}F]fluoride afforded the radiolabelled nitrile (Scheme 1.13). Reduction of the nitrile was reported with lithium aluminium hydride or $BH_3 - Me_2S$.[44,45] Both reagents require anhydrous conditions for optimal results, which was achieved by solid phase extraction[45] or *in situ* addition of the reagent[44]. Amidation of **[^{18}F]FBA** with a PEG-biotin conjugate afforded **[^{18}F]NPB4**.[44]



Scheme 1.13: Radiosynthesis of **[^{18}F]FBA** and **[^{18}F]NPB4**

The resulting biotin conjugate was coordinated to an avitidin-modified Poly(lactid-co-glycolid) nanoparticles (PLGA). PLGA nanoparticles have the ability to incorporate drugs and active reagents and release them in a target tissue.[44] The aim of Huang's study was to observe the behaviour of those nanoparticles after direct injection into the brain by Convection Enhanced Delivery (CED). They observed that the distribution through the brain was size dependant, smaller particles (avg. diameter 71 nm) diffusing faster than larger ones (avg. diameter 147 nm). Larger nanoparticles also behaved differently during uptake and excretion. The authors concluded that there might be additional barrier mechanism within the brain, which nanomedicines need to overcome.

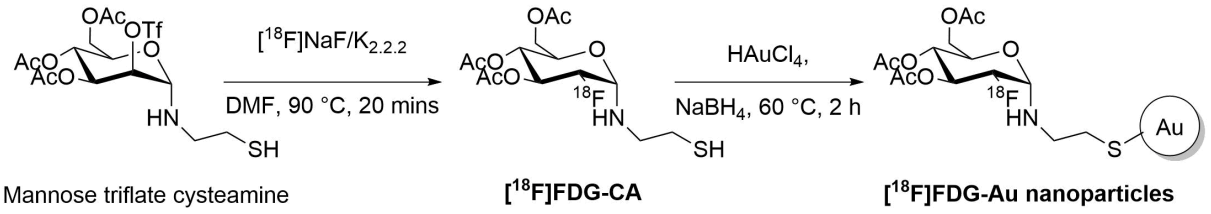
Llop and co-workers reacted **[^{18}F]FBA** with bromoacetyl bromide to obtain **[^{18}F]FBBA** in 12% decay-corrected RCY and a synthesis time of 90 minutes (Scheme 1.14).[45] The new prosthetic group was condensed with various block copolymers and pre-formed polymer-based nanoparticles to determine the most efficient radiolabelling strategy for polymer-based nanoparticles.



Scheme 1.14: Radiosynthesis of **[^{18}F]FBBA** and a radiolabelled block co-polymer

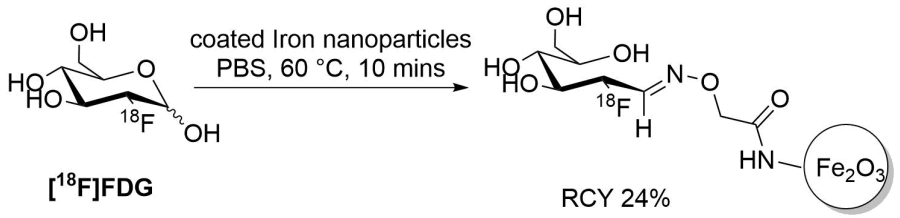
The overall yield of the nanoparticle formation was between 0.1% and 1.2% from [^{18}F]fluoride and a total synthesis time of 150 to 230 minutes. The fastest reaction times and highest radiochemical yields were observed when **[^{18}F]FBBA** was directly internalised into a preformed nanoparticle.

[^{18}F]FDG is one of the most widely-employed radiotracers and has been used for the radiolabelling of nanoparticles in two examples. Timur and co-workers described the radiolabelling of mannose triflate, which has been previously modified with cysteamine.[46] After the successful radiofluorination, **[^{18}F]FDG**-modified gold nanoparticles were formed from $HAuCl_4$ with subsequent reduction in two hours.



Scheme 1.15: Radiosynthesis of **[^{18}F]FDG** labelled gold nanoparticles

Salvadori and co-workers linked **[^{18}F]FDG** to dextran coated iron oxide nanoparticles through oxime formation (Scheme 1.16).[47] The nanoparticles were obtained in 24% RCY with a total synthesis time of 40 minutes, and were characterised by FT-IR and TEM. Stability testing revealed only a limited stability of the oxime in plasma, after 60 minutes only 35% of the intact nanoparticles were retained. Most notably, the authors were able to demonstrate targeted tissue uptake by external magnetic fields using the particles magnetic core.



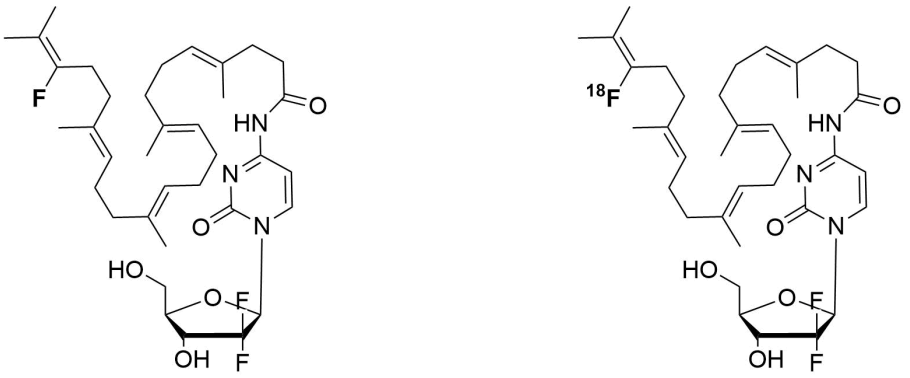
Scheme 1.16: Radiosynthesis of **[^{18}F]FDG** labelled iron oxide nanoparticles

1.5 Radiolabelling Strategy for Squalenoylated Drugs

In the past, organic residues of nanoparticles have predominantly been radiofluorinated by conjugating a prosthetic group to the outer shell coating. In the context of squalenoylated drugs, it was expected that modification of the squalenoyl tail might heavily influence the nanoprecipitation process. So far, the presented nanoparticles all consisted of a metal core or covalently linked polymer fragments but squalenoylated drugs form nanoparticles through intermolecular interactions. This is advantageous as single molecules can be radiolabelled and purified instead of particles with multiple reaction sites. Substitution of single atoms or methyl groups on the squalenoyl tail has not been investigated so far. It was uncertain how the change in electronic or steric properties would influence the molecular packing. Fluorine and hydrogen have similar van der Waals radii (H 1.20 Å[48], F 1.47 Å[48]) and the steric change could be well tolerated. Further, the *in vivo* metabolism of the heteroatom has to be considered. It was hypothesised that allylic fluorides metabolise similarly to benzylic fluorides, for which radiodefluorination has been reported *in vivo*.[49] Substitution of a vinylic hydrogen could avoid undesired decomposition. The terminal double bond was chosen to minimise conformational changes in the terpenoid chain.

In this thesis, the focus was set on the radiosynthesis of the ^{18}**F-Sq-Gem** conjugate (Figure 1.9). For this project several goals were set: 1) Synthesis of a fluorinated analogue of **Sq-Gem** (**F-Sq-Gem**) and investigate the formation of nanoparticles

(Chapter 2), 2) development of a suitable precursor for the radiolabelling of squalenoylated drugs (Chapter 3), and 3) validate the radiofluorination methodology and nanoprecipitation protocol in a radiochemical setting on **^{18}F-Sq-Gem** (Chapter 4).



F-Sq-Gem **^{18}F-Sq-Gem**

Figure 1.9: Target molecules for this thesis

Literature

[1] P. Couvreur, B. Stella, L. H. Reddy, H. Hillaireau, C. Dubernet, D. Desmaële, S. Lepêtre-Mouelhi, F. Rocco, N. Dereuddre-Bosquet, P. Clayette, V. Rosilio, V. Marsaud, J.-M. Renoir, L. Cattel, *Nano Lett.* **2006**, *6*, 2544–2548.

[2] F. Dosio, L. Reddy, A. Ferrero, B. Stell, L. Cattel, P. Couvreur, *Bioconjug. Chem.* **2010**, *21*, 1349–1361.

[3] E. Buchy, S. Valetti, S. Mura, J. Mougin, C. Troufflard, P. Couvreur, D. Desmaële, *Eur. J. Org. Chem.* **2015**, *2015*, 202–212.

[4] L. Kotelevets, E. Chastre, J. Caron, J. Mougin, G. Bastian, A. Pineau, F. Walker, T. Lehy, D. Desmaële, P. Couvreur, *Cancer Res.* **2017**, *77*, 2964–2975.

[5] Z. Cheikh-Ali, J. Caron, S. Cojean, C. Bories, P. Couvreur, P. M. Loiseau, D. Desmaële, E. Poupon, P. Champy, *ChemMedChem* **2014**, *10*, 411–418.

[6] N. Abed, F. Saïd-Hassane, F. Zouhiri, J. Mougin, V. Nicolas, D. Desmaële, R. Gref, P. Couvreur, *Sci. Rep.* **2015**, *5*, 1–14.

[7] D. T. Bui, J. Nicolas, A. Maksimenko, D. Desmaële, P. Couvreur, *Chem. Commun.* **2014**, *50*, 5336–5338.

[8] S. Bhattacharjee, *J. Control. Release* **2016**, *235*, 337–351.

[9] N. Hoshyar, S. Gray, H. Han, G. Bao, *Nanomedicine* **2016**, *11*, 673–692.

[10] X. Sun, W. Cai, X. Chen, *Acc. Chem. Res.* **2015**, *48*, 286–294.

[11] E. Moysan, G. Bastiat, J. Benoit, *Mol. Pharm.* **2013**, *10*, 430–444.

[12] J. L. Abbruzzese, R. Grunewald, E. a. Weeks, D. Gravel, T. Adams, B. Nowak, S. Mineishi, P. Tarassoff, W. Satterlee, M. N. Raber, *J. Clin. Oncol.* **1991**, *9*, 491–498.

[13] L. Bildstein, C. Dubernet, V. Marsaud, H. Chacun, V. Nicolas, C. Gueutin, A. Sarasin, H. Bénech, S. Lepêtre-Mouelhi, D. Desmaële, P. Couvreur, *J. Control. Release* **2010**, *147*, 163–170.

[14] P. Couvreur, L. H. Reddy, S. Mangenot, J. H. Poupaert, D. Desmaële, S. Lepêtre-Mouelhi, B. Pili, C. Bourgaux, H. Amenitsch, M. Ollivon, *Small* **2008**, *4*, 247–253.

[15] E. Lepeltier, C. Bourgaux, A. Maksimenko, F. Meneau, V. Rosilio, E. Sliwinski, F. Zouhiri, D. Desmaële, P. Couvreur, *Langmuir* **2014**, *30*, 6348–6357.

[16] A. M. J. Paans, A. Van Waarde, P. H. Elsinga, A. T. M. Willemsen, W. Vaalburg, *Methods* **2002**, *27*, 195–207.

[17] A. Shukla, U. Kumar, *J. Med. Phys.* **2006**, *31*, 13–21.

[18] P. W. Miller, N. J. Long, R. Vilar, A. D. Gee, *Angew. Chemie Int. Ed.* **2008**, *47*, 8998–9033.

[19] R. Duncan, *Nat. Rev. Drug Discov.* **2003**, *2*, 347–360.

[20] A. K. Iyer, G. Khaled, J. Fang, H. Maeda, *Drug Discov. Today* **2006**, *11*, 812–818.

[21] K. Stockhofe, J. M. Postema, H. Schieferstein, T. L. Ross, *Pharmaceuticals* **2014**, *7*, 392–418.

[22] Y. Matsumura, H. Maeda, *Cancer Res.* **1986**, *46*, 6387–6392.

[23] F. Kiessling, M. E. Mertens, J. Grimm, T. Lammers, *Radiology* **2014**, *273*, 10–28.

[24] B. R. Smith, S. S. Gambhir, *Chem. Rev.* **2017**, *117*, 901–986.

[25] N. Gibson, U. Holzwarth, K. Abbas, F. Simonelli, J. Kozempel, I. Cydzik, G. Cotogno, A. Bulgheroni, D. Gilliland, J. Ponti, F. Franchini, P. Marmorato, H. Stamm, W. Kreyling, A. Wenk, M. Semmler-Behnke, S. Buono, L. Maclocco, N. Burgio, *Arch. Toxicol.* **2011**, *85*, 751–773.

[26] C. Pérez-Campaña, V. Gómez-Vallejo, A. Martin, E. San Sebastián, S. E. Moya, T. Reese, R. F. Ziolo, J. Llop, *Analyst* **2012**, *137*, 4902.

[27] C. Pérez-Campaña, F. Sansaloni, V. Gõmez-Vallejo, Z. Baz, A. Martin, S. E. Moya, J. I. Lagares, R. F. Ziolo, J. Llop, *Part. Part. Syst. Charact.* **2014**, *31*, 134–142.

[28] Y. Sun, M. Yu, S. Liang, Y. Zhang, C. Li, T. Mou, W. Yang, X. Zhang, B. Li, C. Huang, F. Li, *Biomaterials* **2011**, *32*, 2999–3007.

[29] J. Zhou, M. Yu, Y. Sun, X. Zhang, X. Zhu, Z. Wu, D. Wu, F. Li, *Biomaterials* **2011**, *32*, 1148–1156.

[30] Q. Liu, Y. Sun, C. Li, J. Zhou, C. Li, T. Yang, X. Zhang, T. Yi, D. Wu, F. Li, *ACS Nano* **2011**, *5*, 3146–3157.

[31] X. Cui, D. Mathe, N. Kovács, I. Horváth, M. Jauregui-Osoro, R. Torres Martin De Rosales, G. E. Mullen, W. Wong, Y. Yan, D. Krüger, A. N. Khlobystov, M. Gimenez-Lopez, M. Semjeni, K. Szigeti, D. S. Veres, H. Lu, I. Hernández, W. P. Gillin, A. Protti, K. K. Petik, M. A. Green, P. J. Blower, *Bioconjug. Chem.* **2016**, *27*, 319–328.

[32] W. J. McBride, R. M. Sharkey, H. Karacay, C. A. D'Souza, E. A. Rossi, P. Laverman, C.-H. Chang, O. C. Boerman, D. M. Goldenberg, *J. Nucl. Med.* **2009**, *50*, 991–998.

[33] P. Laverman, W. J. McBride, R. M. Sharkey, A. Eek, L. Joosten, W. J. Oyen, D. M. Goldenberg, O. C. Boerman, *J. Nucl. Med.* **2010**, *51*, 454–461.

[34] S. Liu, H. Liu, H. Jiang, Y. Xu, H. Zhang, Z. Cheng, *Eur. J. Nucl. Med. Mol. Imaging* **2011**, *38*, 1732–1741.

[35] Z. Sun, K. Cheng, F. Wu, H. Liu, X. Ma, X. Su, Y. Liu, L. Xia, Z. Cheng, *Nanoscale* **2016**, *8*, 19644–19653.

[36] J. B. Matson, R. H. Grubbs, *J. Am. Chem. Soc.* **2008**, *130*, 6731–6733.

[37] N. K. Devaraj, E. J. Keliher, G. M. Thurber, M. Nahrendorf, R. Weissleder, *Bioconjug. Chem.* **2009**, *20*, 397–401.

[38] E. J. Keliher, Y. X. Ye, G. R. Wojtkiewicz, A. D. Aguirre, B. Tricot, M. L. Senders, H. Groenen, F. Fay, C. Perez-Medina, C. Calcagno, G. Carlucci, T. Reiner, Y. Sun, G. Courties, Y. Iwamoto, H. Y. Kim, C. Wang, J. W. Chen, F. K. Swirski, H. Y. Wey, J. Hooker, Z. A. Fayad, W. J. Mulder, R. Weissleder, M. Nahrendorf, *Nat. Commun.* **2017**, *8*, 1–12.

[39] S. B. Lee, H. L. Kim, H. J. Jeong, S. T. Lim, M. H. Sohn, D. W. Kim, *Angew. Chemie - Int. Ed.* **2013**, *52*, 10549–10552.

[40] M. M. Herth, M. Barz, D. Moderegger, M. Allmeroth, M. Jahn, O. Thews, R. Zentel, F. Roesch, *Biomacromolecules* **2009**, *10*, 1697–1703.

[41] M. Allmeroth, D. Moderegger, B. Biesalski, K. Koynov, F. Roesch, O. Thews, R. Zentel, *Biomacromolecules* **2011**, *12*, 2841–2849.

[42] M. Allmeroth, D. Moderegger, D. Guendel, H. G. Buchholz, N. Mohr, K. Koynov, F. Roesch, O. Thews, R. Zentel, *J. Control. Release* **2013**, *172*, 77–85.

[43] S. Rojas, J. D. Gispert, S. Abad, M. Buaki-Sogo, V. M. Victor, H. Garcia, J. R. Herance, *Mol. Pharm.* **2012**, *9*, 3543–3550.

[44] R. W. Sirianni, M. Q. Zheng, T. R. Patel, T. Shafbauer, J. Zhou, W. M. Saltzman, R. E. Carson, Y. Huang, *Bioconjug. Chem.* **2014**, *25*, 2157–2165.

[45] P. P. Di Mauro, V. Gómez-Vallejo, Z. Baz Maldonado, J. Llop Roig, S. Borrós, *Bioconjug. Chem.* **2015**, *26*, 582–592.

[46] G. Unak, F. Ozkaya, E. Ilker Medine, O. Kozgus, S. Sakarya, R. Bekis, P. Unak, S. Timur, *Colloids Surfaces B Biointerfaces* **2012**, *90*, 217–226.

[47] M. De Simone, D. Panetta, E. Bramanti, C. Giordano, M. C. Salvatici, L. Gherardini, A. Menciassi, S. Burchielli, C. Cinti, P. A. Salvadori, *Contrast Media Mol. Imaging* **2016**, *11*, 561–571.

[48] W. K. Hagmann, *J. Med. Chem.* **2008**, *51*, 4359–4368.

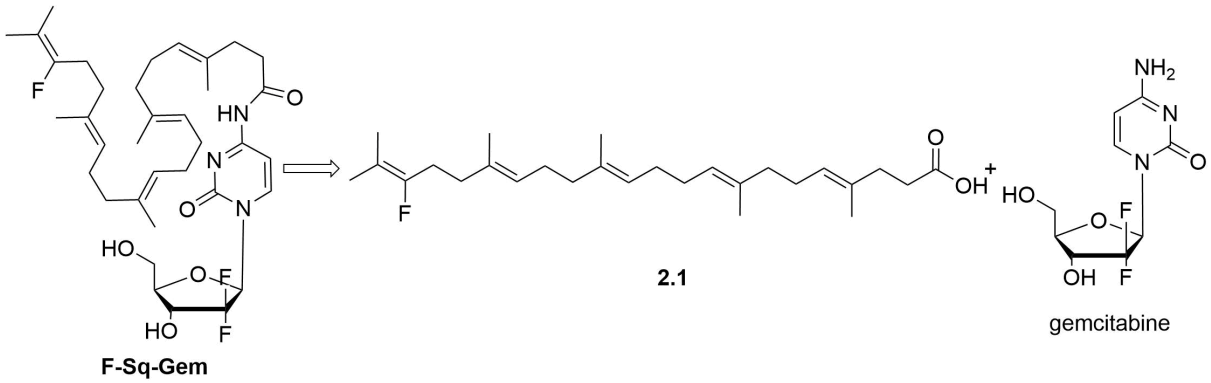
[49] M. Kuchar, C. Mamat, *Molecules* **2015**, *20*, 16186–16220.

CHAPTER 2

Synthesis of Fluoro-Squalenoyl Gemcitabine

2.1 Introduction

The first aim of this study was to synthesise a fluorinated analogue of squalenoyl gemcitabine (**F-Sq-Gem**) and investigate if **F-Sq-Gem** forms nanoparticles. The retrosynthetic analysis of the target compound presented two key fragments: the commercial drug gemcitabine and the fluorinated terpenoid **2.1** (Scheme 2.1).



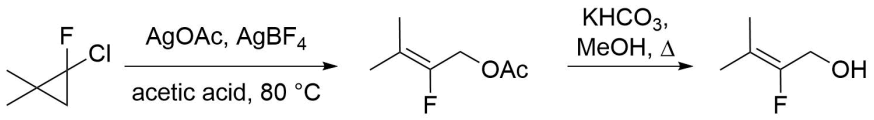
Scheme 2.1: Retrosynthesis of **F-Sq-Gem**

Due to the importance of fluorinated terpenoids in the elucidation of biosynthetic pathways, several different synthetic methodologies have already been reported. The high electronegativity of fluorine influences the electronics of the substrate and the stability of carbocationic intermediates in enzymatic reactions.[1] These electronic changes lead to an interruption in the reaction cascade thus allowing for the isolation and identification of intermediates and reaction pathways respectively.[1–9]

In this chapter, methods to synthesise fluorinated terpenoid derivatives are discussed followed by the successful synthesis of terpenoid **2.1** and **F-Sq-Gem**.

2.1.1 Synthesis of fluorinated terpenoids

Schlosser and co-workers, in 1974, published the synthesis of 2-fluoro-prenol (**2.2**) from cyclopropane **2.3** (Scheme 2.2).[10] Treatment of 1-chloro-1-fluoro-2,2-dimethylcyclopropane (**2.3**) with silver acetate and a catalytic amount of silver tetrafluoroborate in acetic acid afforded 2-fluoro-3-methyl-but-2-en-1-yl-acetate (**2.4**). Acetate **2.4** was then hydrolysed to form 2-fluoro-prenol. Cyclopropane **2.3** was prepared from gaseous 2-methyl-1-propene and dichlorofluoromethane (Freon R-21), a banned chlorofluorocarbon (CFC).

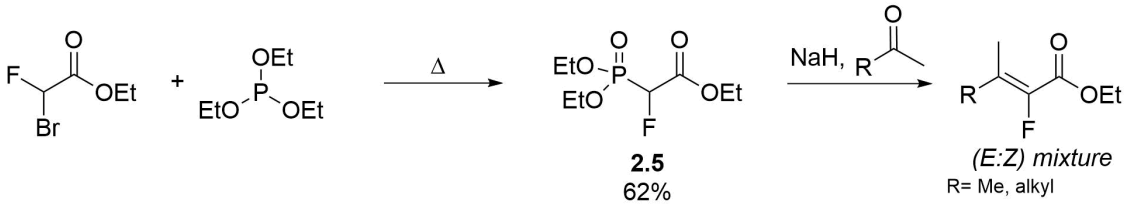


2.3 **2.4** 61% **2.2** 53%

Scheme 2.2: Synthesis of 2-fluoro-prenol **2.2** by Schlosser

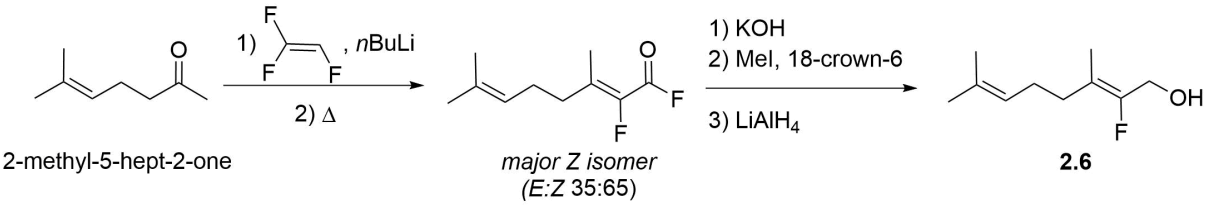
Machleidt and Wessendorf reported the synthesis of ethyl 2-(diethoxyphosphoryl)-2-fluoroacetate (**2.5**) from ethyl fluorobromoacetate (Scheme 2.3). The fluorinated Horner-Wadsworth-Emmons reagent **2.5** was reacted with aldehydes and ketones to afford 2-fluoro-terpenoids.[11] The resulting esters were reduced to the corresponding alcohols. The fluorinated Horner-Wadsworth-Emmons reagent is commercially available and the aforementioned reaction with ketones the most popular method to construct 2-fluoro-terpenoids.

2-Fluoro-geraniol, 2-fluoro-farnesol and their corresponding esters have been prepared in good yields.[4–6,8,11] The drawback of this method is the lack of stereoselectivity of the Horner-Wadsworth-Emmons reaction. Typically, 1:1 mixtures of *E*- and *Z*-isomers are obtained and have to be separated.



Scheme 2.3: Preparation of phosphonate **2.5** and subsequent Horner-Wadsworth-Emmons reaction to synthesise 2-fluoro-terpenes

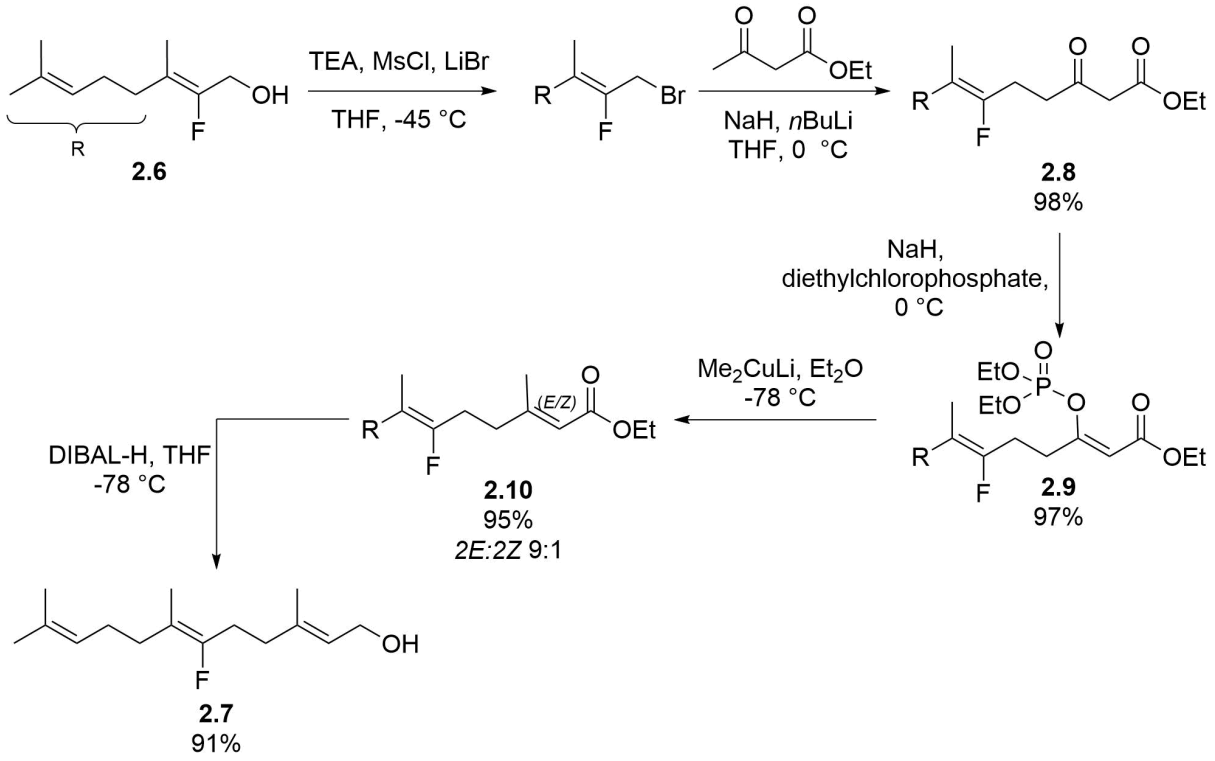
The synthesis of 2-fluoro-geraniol (**2.6**) from 6-methyl-5-heptene-2-one with trifluorovinyl lithium was reported by Poulter and co-workers (Scheme 2.4).[2] The reaction showed an increased *E:Z* ratio of 35:65 but included the handling of gaseous ethylene trifluoride.



Scheme 2.4: Synthesis of 2-fluoro-geraniol by Poulter

Coats and Allemann published the synthesis of 6-fluoro-farnesol (**2.7**) from 2-fluoro-geraniol (**2.6**) using Weiler's isoprenoid chain extension method (Scheme 2.5).[12–14] Bromination of the primary alcohol furnished a leaving group for the nucleophilic attack of the double enolate of ethyl acetonate.

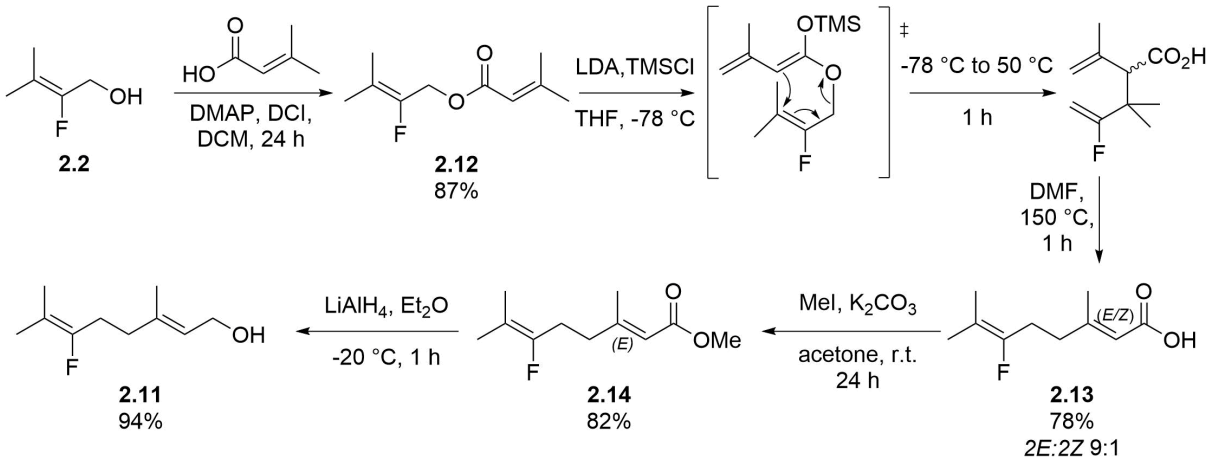
The resulting β-keto ester **2.8** was enolised to selectively form the *trans*-enol phosphate **2.9**. Methylation with dimethyl cuprate afforded ester **2.10** in a 9:1 *2E:2Z* ratio due to the addition-elimination-mechanism. The ester was reduced to the desired 6-fluoro-farnesol (**2.7**). Coats and co-workers synthesised 6-fluoro-geranyl geraniol from 2-fluoro-farnesol using the same methodology.[6]



Scheme 2.5: Synthesis of 6-fluoro-farnesol by Allemann

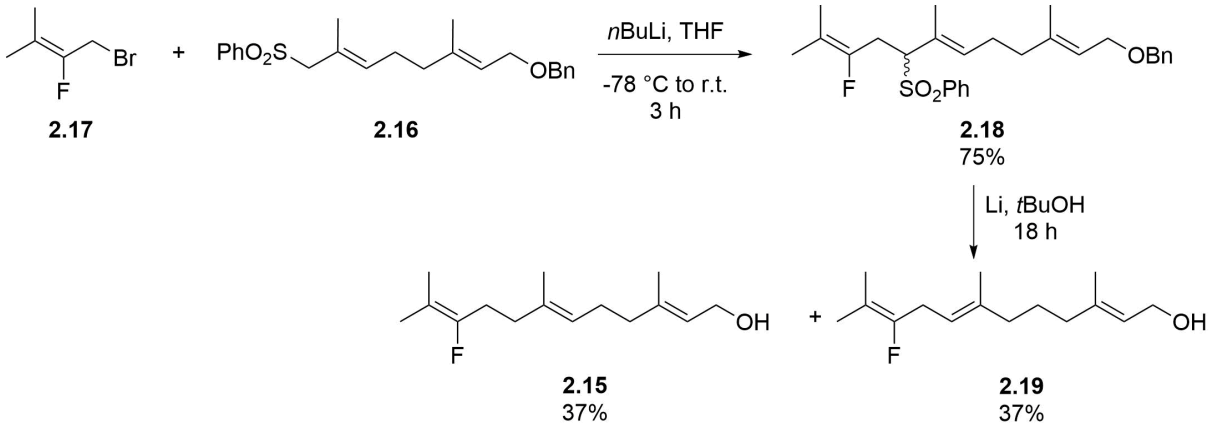
Camps and co-workers present a very elegant way to synthesise 6-fluoro-geraniol (**2.11**) by rearrangement (Scheme 2.6).[15] Esterification of 2-fluoro-prenol with 3,3-dimethylacrylic acid afforded ester **2.12**, which can undergo a Claisen-Cope cascade rearrangement to acid **2.13**. This one-pot procedure is very convenient with acid **2.13** reported in good yields and good stereoselectivity (*2E*:*2Z* 9:1).

Separation of the two isomers was achieved after esterification to **2.14**. Ester **2.14** could be readily reduced to 6-fluoro-geraniol (**2.11**).



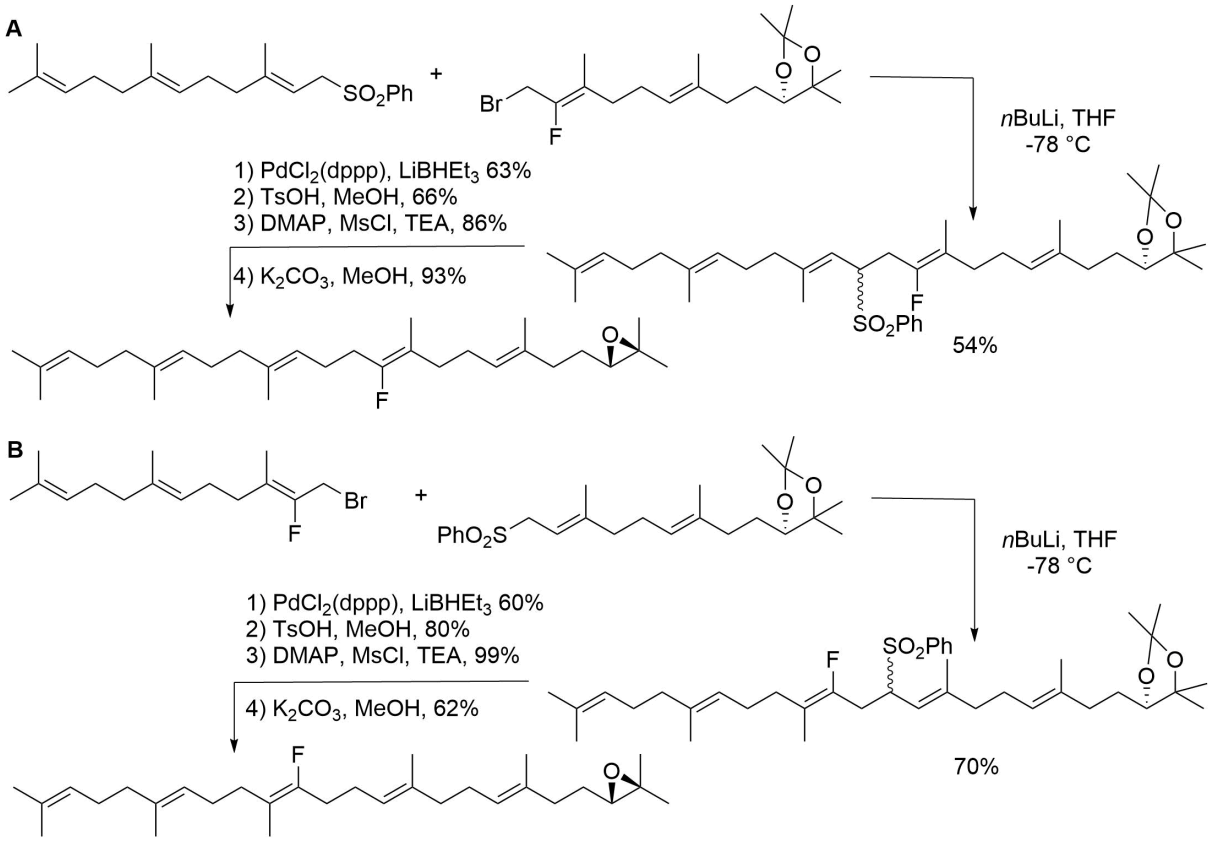
Scheme 2.6: Synthesis of 6-fluoro-geraniol by Camps and co-workers

For the synthesis of 10-fluoro-farnesol or longer terpenoid chains, a fragment-based synthesis strategy was developed based on sulfone chemistry.[16] The sulfonyl anion is a good nucleophile and readily attacks allyl-bromides in an S_N2 fashion to form a C-C bond. The resulting allylic sulfone can be reduced by several methods.[17–20] Cane and co-workers reported the synthesis of 10-fluoro-farnesol (**2.15**) by reacting sulfone **2.16** with hemiterpenoid **2.17** (Scheme 2.7).[16] The resulting sulfone **2.18** was reduced with lithium in *tert*-butanol to afford 10-fluoro-farnesol (**2.15**) and the undesired isomerisation product **2.19**, which could be separated by $AgNO_3$-impregnated silica.



Scheme 2.7: Synthesis of 10-fluoro-farnesol

The advantage of the fragment-based synthesis is the high diversity of structures which can be accessed. Fluorinated squalene derivatives were synthesised by Prestwich and co-workers. 2-Fluoro-farnesol analogues were linked with terminally functionalised sesquiterpernoids to obtain fluorinated oxidosqualenes (Scheme 2.8).[4,5] The sulfone reduction was achieved with a palladium-dppp complex in combination with $LiBHEt_3$ without double bond isomerisation. The two oxidosqualene analogues were used to investigate the mechanism of squalene-hopene cyclase.



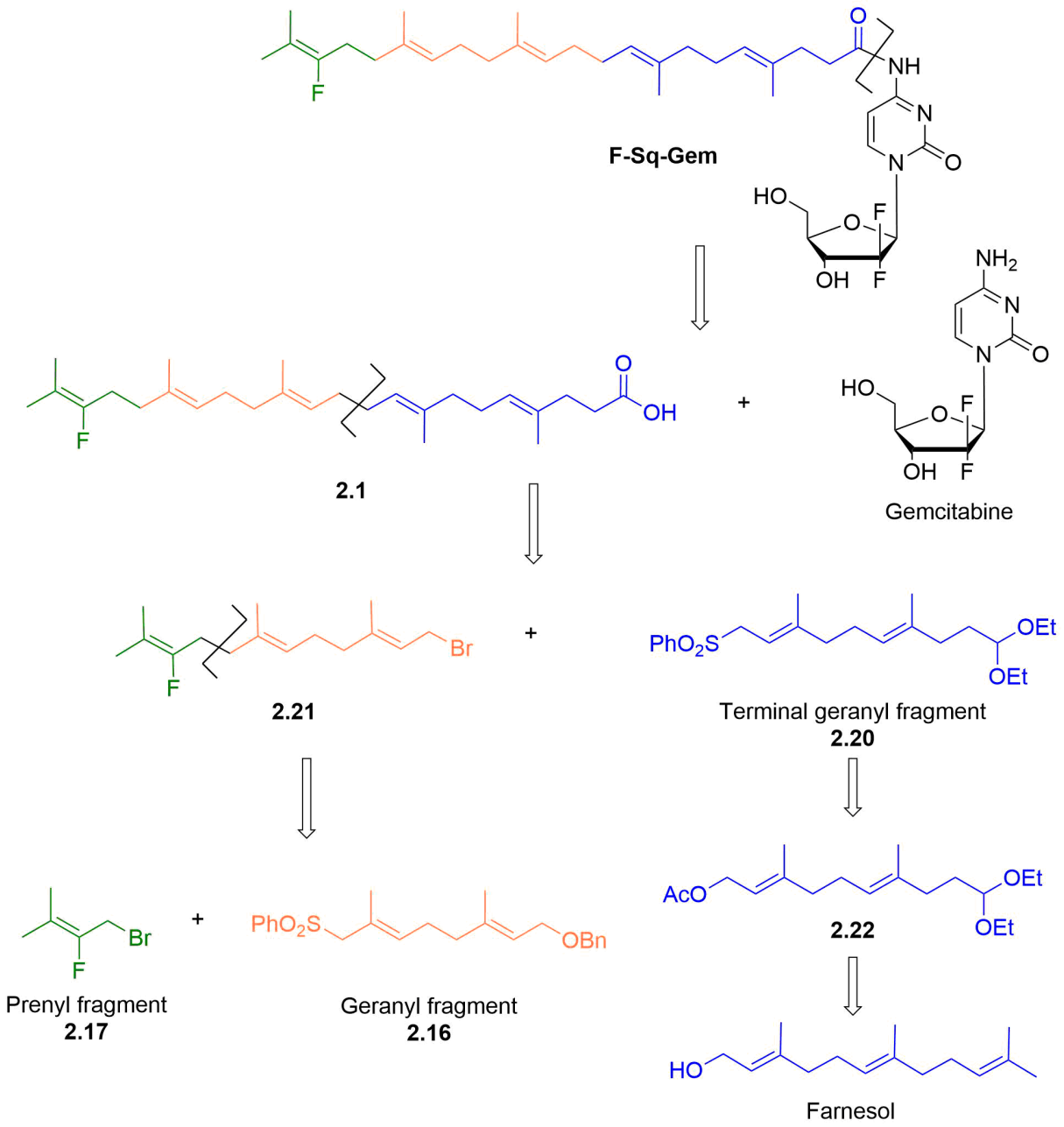
Scheme 2.8: Synthesis of fluorinated oxidosqualene analogues

Based on these literature reports a retrosynthetic plan for **F-Sq-Gem** was conceived.

2.1.2 Synthesis Plan for F-Sq-Gem

The retrosynthetic analysis of **F-Sq-Gem** revealed four individual fragments (Scheme 2.9): the commercial drug gemcitabine, the terminal geranyl fragment **2.20**, a linking geranyl fragment **2.16** and the fluorinated prenol derivative **2.17**. Gemcitabine would be amidated to the complete sesterterpenoid **2.1** at the final stage of the synthesis to avoid protecting groups.[21] The terpenoid chain could be assembled from two

fragments: a derivative of 10-fluoro-farnesol **2.21** and the terminal geranyl fragment **2.20**. Sulfone **2.20** would be accessed through palladium-catalysed allylic substitution from acetate **2.22**, which could be synthesised from farnesol.[22] The synthesis of 10-fluoro-farnesol has been discussed in Section 2.1.1.[16] The vinyl fluoride moiety would be introduced through a Horner-Wadsworth-Emmons reaction between ethyl 2-(diethoxyphosphoryl)-2-fluoroacetate and acetone. Subsequent reduction and bromination would afford prenyl fragment **2.17**. Geranyl fragment **2.16** could be synthesised from geraniol through allylic oxidation chemistry.

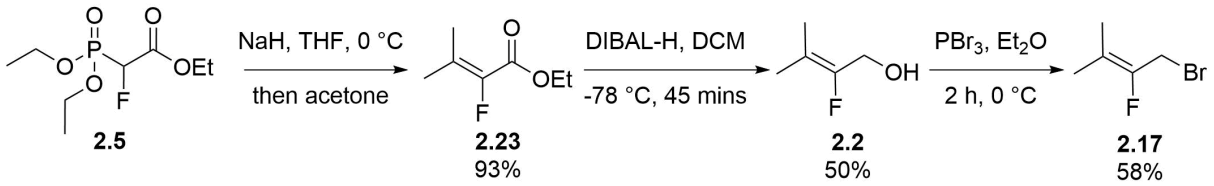


Scheme 2.9: Retrosynthetic analysis of **F-Sq-Gem**

2.2 Results and Discussion

2.2.1 Synthesis of 10-Fluoro-Farnesol Bromide 2.21

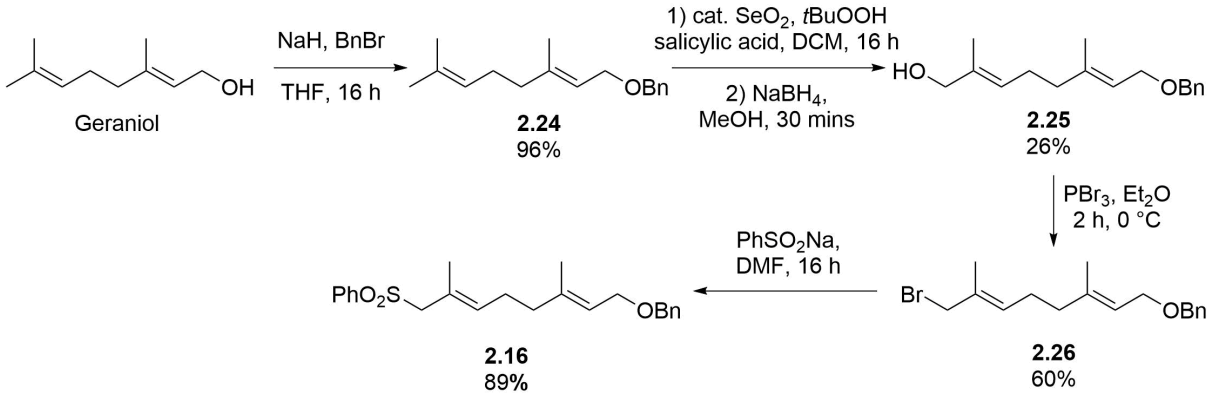
Horner-Wadsworth-Emmons reaction between phosphonate **2.5** and acetone afforded ester **2.23** in excellent yields (Scheme 2.10).[23] The resulting ester **2.23** was reduced using DIBAL-H in DCM to alcohol **2.2**, which is highly volatile and has to be handled with great care, therefore the yields vary greatly. Treatment of alcohol **2.2** with PBr_3 in diethyl ether afforded bromide **2.17** in good yields. The reactions were scaled up to 40 mmol without the loss of yield.



Scheme 2.10: Synthesis of the terminal prenyl fragment **2.17**

To synthesise the linking geraniol fragment **2.16**, geraniol was protected with benzyl bromide (Scheme 2.11).[23] Allylic oxidation of ether **2.24** using catalytic amounts of selenium dioxide and *tert*-butyl hydroperoxide as co-oxidant afforded alcohol **2.25** in low yields but crucially as a single regioisomer. Overoxidation to the aldehyde is well documented and the oxidation step was followed directly by reduction with sodium borohydride.[24] Stoichiometric amounts of selenium dioxide with or without *tert*-butyl hydroperoxide could have been employed to improve yields.[24]

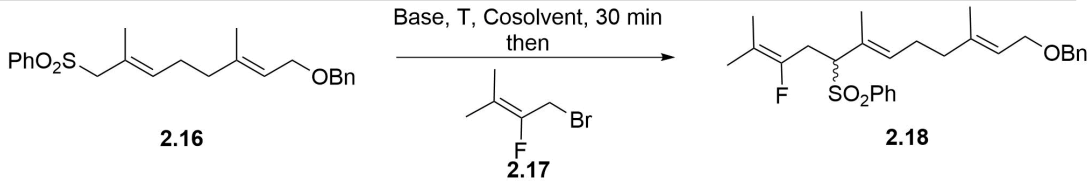
As the starting material **2.24** is easily accessible however, it was decided to keep toxic waste to a minimum. Alcohol **2.25** was brominated using PBr_3. The resulting bromide **2.26** was treated with benzene sulfinic acid sodium salt in DMF overnight to afford sulfone **2.16** in good yields.



Scheme 2.11: Synthesis of geranyl fragment **2.16**

The carbon-carbon bond formation between sulfone **2.16** and bromide **2.17** relies on a selective deprotonation of the sulfone, followed by an S_N2 reaction. Both Cane[16] and Christianson[23] report *n*-butyllithium as their base of choice. The suggested reaction conditions afforded a complex mixture of products. Given this result, the base was changed to the milder LDA with DMPU as an additive (Table 2.1), which improved the yield.[22] Changing from DMPU to HMPA whilst decreasing the overall concentration of the reaction improved the yield further, although it was later found that higher concentrations in this case showed better results. Dilution after organolithium formation (Entry 4) or change in the order of addition (Entry 5) did not give any reaction. Only after elevating the reaction temperature from -78 °C to -41 °C and concentrating the reaction to 2M were higher yields obtained (Entry 6).

The optimised reaction conditions (LDA in THF/HMPA at -41 °C) afforded sulfone **2.18** reliably even on gram-scale (1 gram).

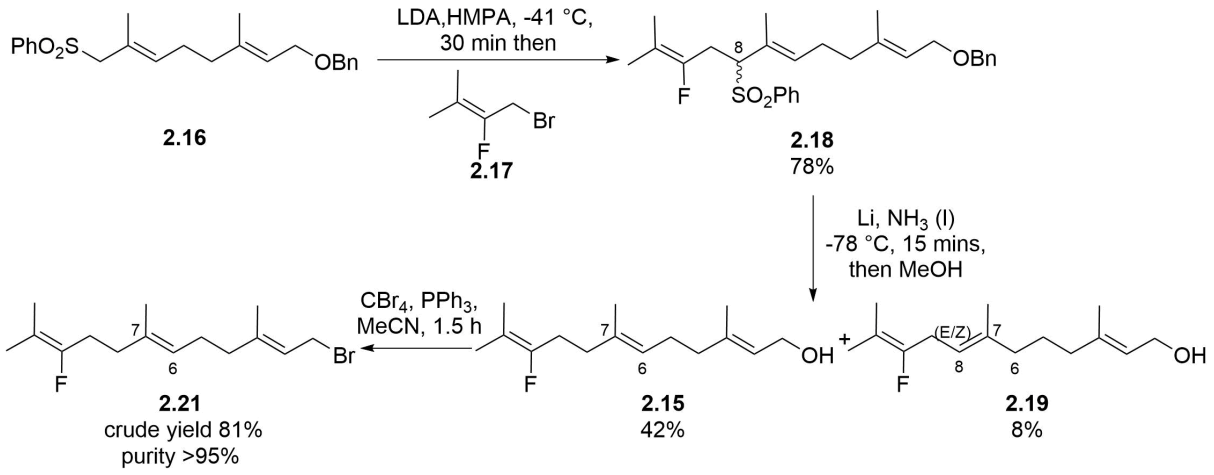


Entry	T [°C]	conc. [M]	Base[a]	Cosolvent	Yield[b]
1	-78	0.26	*n*BuLi	-	10%
2	-78	0.26	LDA	DMPU	24%
3	-78	0.1	LDA	HMPA	38%
4 [c]	-78	0.1	LDA	HMPA	no rxn.
5 [d]	-78	0.1	LDA	HMPA	no rxn.
6	-41	1	LDA	HMPA	64%
7	-41	2	LDA	HMPA	73%

Table 2.1: Optimisation of the coupling reaction: reactions performed on a 130 μmol scale, 30 mins at the indicated temperature then further 30 mins at room temperature; [a] *n*BuLi (2.5M in hexanes) and LDA (1M in THF, freshly prepared); [b] isolated yields; [c] diluted before addition; [d] Organolithium solution added to a solution of bromide **2.17**

The final step to 10-fluoro-farnesol (**2.15**) was the simultaneous reduction of the sulfone and deprotection of the alcohol. Previous syntheses used lithium in *tert*-butanol to achieve the reaction.[16,23] The reported yields are moderate (37%[16] and 28%[23]) and the formation of the C6-C7-regioisomers in a 1:1 ratio.[16] It was hypothesised that the reaction occurred over a radical intermediate on C8, which can isomerise. Harsher reaction conditions, e.g. Birch[17] or Benkser reduction[25], and

lower temperatures decreased the amount of isomerised product. The overall yield was improved to 50% when lithium in liquid ammonia was used as reductant (Scheme 2.12). Isomerisation of the C6-double bond still occurred to afford 8% of the 7-(*E/Z*)-isomer **2.19** as determined by ^{1}H-NMR integration (C6:C7 5:1). The two regioisomers were identified by the characteristic ^{1}H-NMR shifts of the methylene group on C9 (2.28 ppm for C6-isomer, 2.69 ppm for C7-isomer). The separation of the regioisomers by $AgNO_3$-impregnated silica was not successful at this point, due to high detection levels and improper staining of treated TLC plates. Bromination of the C6:C7 mixture of alcohol **2.15** was achieved using PBr_3 or the Appel reaction. The resulting bromide **2.21**[1] was unstable on silica and difficult to purify for the next step. It was found that the Appel reaction generally gives higher yields and the impurities, mainly triphenyl phosphine oxide, could be removed by precipitation with pentane from diethyl ether. The purity of bromide **2.21** was crucial for the following reaction and a purity greater than 95% had to be achieved by repeated precipitations.

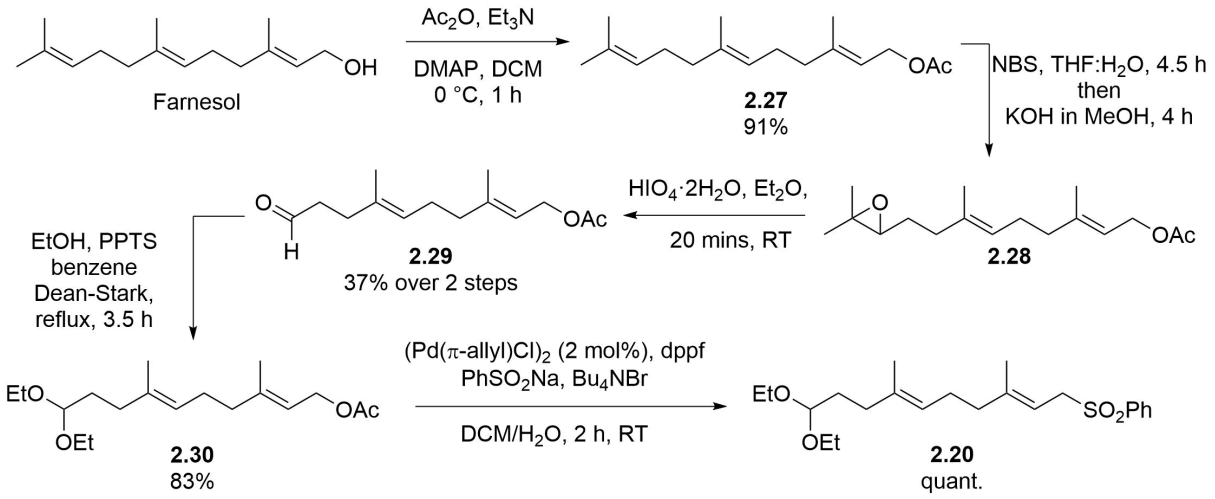


Scheme 2.12: Synthesis of bromide **2.21**

[1]The mixture of regioisomers was taken forward but for clarity only the major isomer is shown.

2.2.2 Synthesis of the terminal geranyl fragment 2.20

The synthesis of the terminal geranyl fragment **2.20** started from farnesol (Scheme 2.13). Farnesol was acetylated to afford ester **2.27** in excellent yields. The terminal bromohydrin was formed *in situ* in a THF-water mixture by the addition of NBS. After addition of a methanolic solution of potassium hydroxide, epoxide **2.28** was obtained. Earlier reports of one-pot epoxidations of **2.27** using potassium carbonate mentioned the cleavage of the acetate protecting group and the need for subsequent reprotection.[26–28] This was avoided by using one equivalent of hydroxide. The crude epoxide **2.28** was submitted to oxidative cleavage by periodic acid to afford aldehyde **2.29**. No purification was needed between the epoxidation and the cleavage. Aldehyde **2.29** was isolated in 37% yield over two steps. Refluxing aldehyde **2.29** in ethanol and benzene under Dean-Stark conditions afforded diethoxy-acetal **2.30** in good yields. The acetate was substituted by palladium catalysed allylic substitution under phase transfer conditions to afford sulfone **2.20** in quantitative yields. The synthesis was performed on such a scale so as to allow for the isolation of 5.2 mmol of sulfone **2.20**.

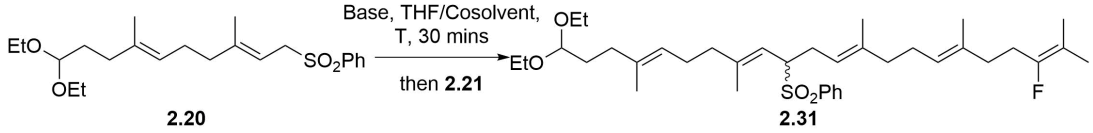


Scheme 2.13: Synthesis of terminal geranyl fragment **2.20**

2.2.3 Synthesis of Fluoro-Squalenoyl Gemcitabine

The two fragments **2.20** and **2.21** were subsequently linked to assemble the complete terpenoid backbone. When sulfone **2.20** was submitted to the same reaction conditions as developed in Section 2.2.1, acetal **2.31** was obtained in low yields (Table 2.2 Entry 1). Changing the additive from HMPA to the less toxic DMPU was not detrimental to the yield (Entry 2). It was previously observed that concentration has an influence on the reaction. In this case, lowering it to 1M was slightly beneficial (Entry 4), lowering it further to 0.3M led to a decrease in yield (Entry 3). Elongating the reaction time (Entry 5) was not beneficial for the reaction and produced more undesired side products. When both reactants were premixed and then treated with LDA, an even lower yield was observed (Entry 6). It was decided to scale up with the best conditions at hand, but only 28% yield was obtained (Entry 7) instead of the expected 45%. The yield was improved

when the base was changed to potassium *tert*-butoxide with DMF as an additive[29] (Entry 8). Under those conditions, both high yields and reproducibility was observed.

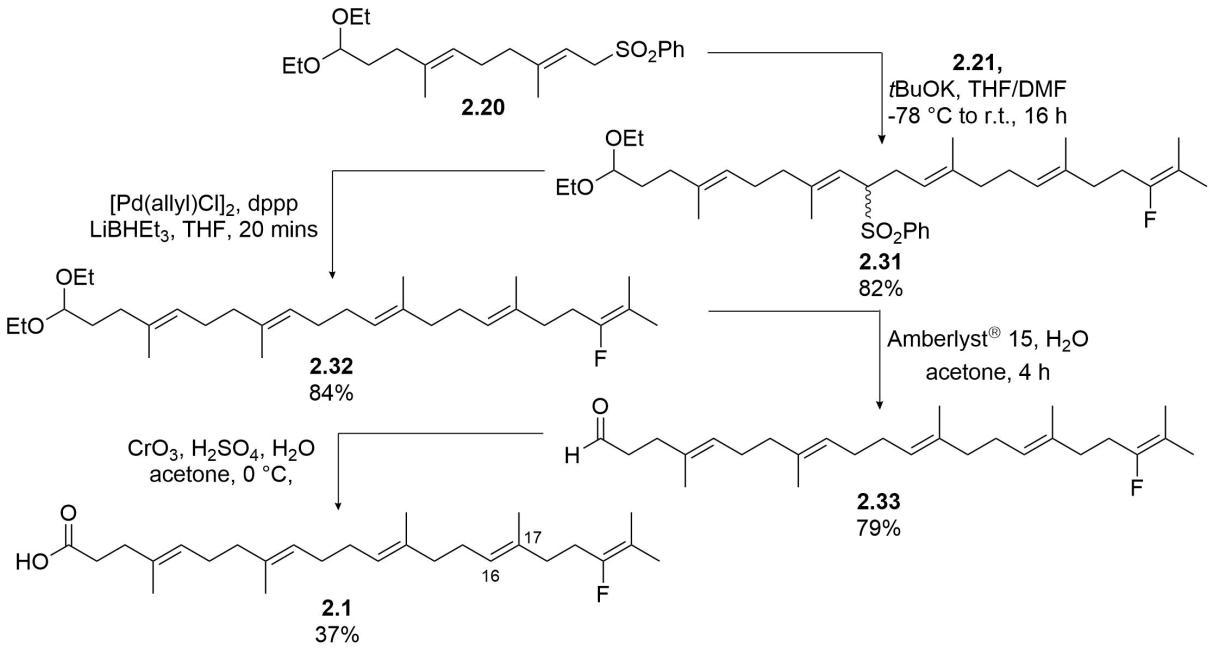


Entry	T [°C]	conc. [M]	Base[a]	Cosolvent	Yield[b]
1	-41	2	LDA	HMPA	41%
2	-41	2	LDA	DMPU	41%
3	-41	0.3	LDA	DMPU	28%
4	-41	1	LDA	DMPU	45%
5 [c]	-41	2	LDA	DMPU	30%
6 [d]	-41	2	LDA	DMPU	21%
7 [e]	-41	1	LDA	DMPU	28%
8	-78	0.42	*t*BuOK	DMF	82%

Table 2.2: Optimisation of the coupling reaction: reactions performed on a 137 μmol scale, bromide **21** added after deprotonation, 30 mins at the indicated temperature then further 30 mins at room temperature; [a] *n*BuLi (2.5M in hexanes), LDA (1M in THF, freshly prepared), *t*BuOK (1M in THF); [b] isolated yields; [c] lithiation time elongated; [d] Sulfone **2.20** and bromide **2.21** mixed before the base addition; [e] reaction scaled up to 1.92 mmol

Reduction of sulfone **2.31** was achieved using $LiBHEt_3$ and a palladium-allyl-complex (Scheme 2.14). The resulting acetal **2.32** was hydrolysed using Amberlyst® 15 resin in acetone to afford aldehyde **2.33**. The reaction proceeded very cleanly and no purification of the product was necessary. Aldehyde **2.33** was oxidised with the Jones reagent to acid **2.1**. The overall yield of the oxidation was 75% but a part of the

product was lost during the separation of regioisomers (yield of **2.1**: 37%). At this stage, $AgNO_3$-impregnated silica was able to separate the desired 16(*E*)-isomer from the 17(*E/Z*)-regioisomers. Literature precedent shows that polyene derivatives readily cyclise under Lewis or Brønsted acidic conditions.[30–34] When acid **2.1** was dissolved with $AgNO_3$ in dichloromethane, the sample immediately turned black. ^{1}H-NMR suggested the formation of several new alkyl species and the loss of vinylic protons. Aside from the aforementioned side reactions, Zhong and co-workers had reported that squalene coordinates readily to $AgNO_3$, therefore some retention on the column could be expected.[35] Nevertheless, up to 150 mg of acid **2.1** could be obtained.

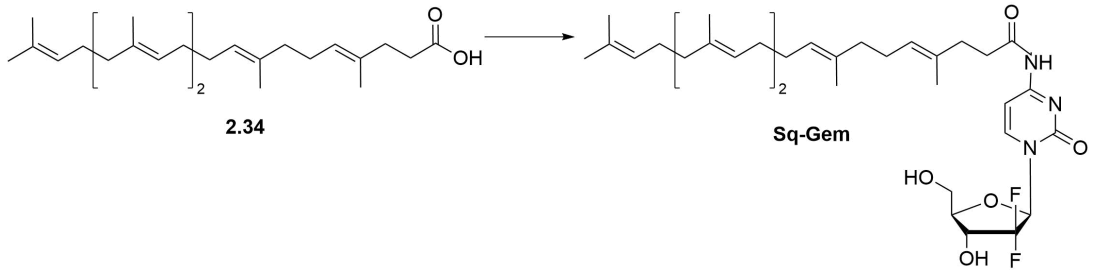


Scheme 2.14: Synthesis of acid **2.1**

In the final step of the synthesis acid **2.1** was amidated with commercially available gemcitabine· HCl. The suggested amidation method of Couvreur[21], which relies on the

formation of a mixed anhydride with ethyl chloroformate, only afforded 31% of fluoro-squalenoly gemcitabine (**F-Sq-Gem**) and required a reaction time of three days.

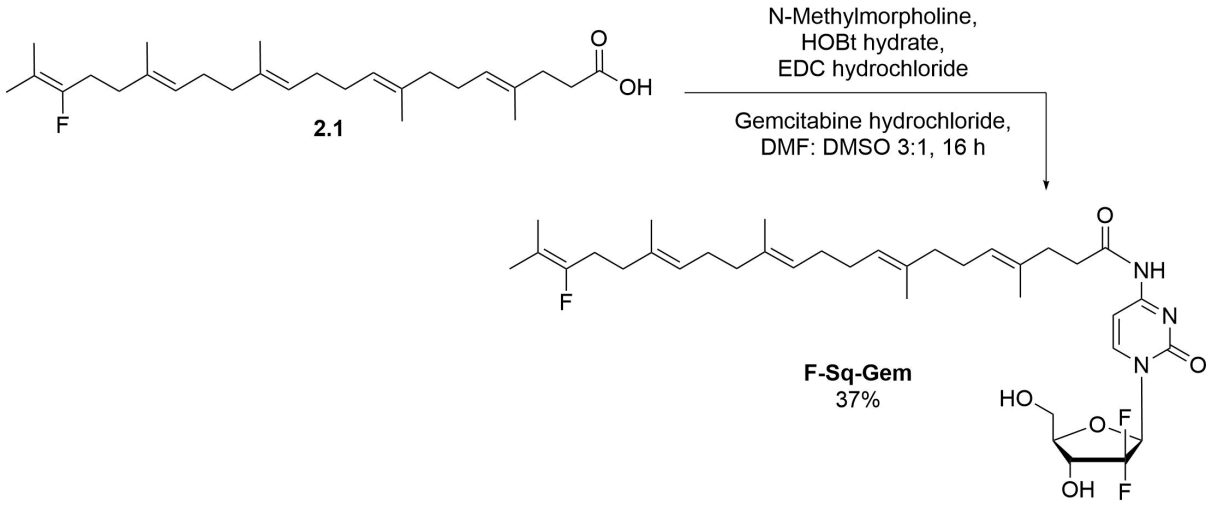
The reaction was optimised using trisnorsqualenic acid **2.34** as a readily available model (Table 2.3). Amidation *via* mixed anhydride formation, was revisited and quenched after 24, 48 and 72 hours. The reaction was found to be slow and benefited from long reaction times. 1,1'-Carbonyldiimidazole (CDI) was used to activate acid **2.34** for the amidation of *in situ* protected gemcitabine with TMSCl.[36] After 40 hours, the yield was lower than at a similar time point with ethyl chloroformate activation. Finally, a combination of 3-(ethyliminomethyleneamino)-*N,N*-dimethylpropan-1-amine (EDC) and benzotriazol-1-ol (HOBt) hydrate with *N*-morpholine were found to be the most effective amidation reagents. The reaction was observed to be complete after 16 hours, affording **Sq-Gem** in 45% yield without using any protecting groups.



Entry	Reagent	Time [h]	Yield
1	Ethyl chloroformate, TEA	24	16%
2	Ethyl chloroformate, TEA	48	21%
3	Ethyl chloroformate, TEA	72	34%
4 [a]	CDI	40	13%
5	N-methylmorpholine, HOBt, EDC	16	45%

Table 2.3: Optimisation of the amidation reaction: [a] *in situ* TMS protection with pyridine and TMSCl

The above discussed optimised amidation conditions were applied to acid **2.1** and **F-Sq-Gem** was obtained in a slightly improved yield (Scheme 2.15).



Scheme 2.15: Amidation of acid **2.1** to afford **F-Sq-Gem**

F-Sq-Gem was successfully synthesised in 11 steps in 2% overall yield (30 mg) from farnesol (longest linear sequence). The waxy solid was fully characterised and the compound was investigated for its ability to form nanoparticles.

2.2.4 Nanoprecipitation of F-Sq-Gem

The successful synthesis of **F-Sq-Gem** enabled an investigation of its colloidal properties. **F-Sq-Gem** was dissolved in acetone and added dropwise to a well stirred dextrose solution (5 wt%). The solution turned turbid during the addition. After complete addition, acetone was evaporated under reduced pressure. The solution showed the characteristic Tyndall-effect upon irradiation with light. The colloidal solution was analysed with Dynamic Light Scattering (DLS), which was used to

determine average particle diameter, size distribution (Polydispersity Index, PDI) and surface charge (ζ-potential). The measurements confirmed that nanoassemblies were formed (Table 2.4 Entry 1), although the average diameter was larger than reported for **Sq-Gem** (130 nm[21]). As a control **Sq-Gem** was precipitated at the same concentration. The observed diameters were similar to **F-Sq-Gem** nanoassemblies (Entry 2). The size of the particles changed when the solution was diluted: ten-fold dilution decreased the particle size of **F-Sq-Gem** to 130 nm. The polydispersity index (PDI) was similar for both compounds as was the ζ-potential. The measured ζ-potentials indicated good long-term stability with little aggregation.

Entry	Compound	avg. diameter [nm]	PDI	ζ [mV]
1	**F-Sq-Gem**	233.0 $\pm$ 7	0.15 $\pm$ 0.02	-28.5 $\pm$ 1.3
2	**Sq-Gem**	207.0 $\pm$ 1.5	0.14 $\pm$ 0.04	-27.0 $\pm$ 0.9
3	**F-Sq-Gem :** **Sq-Gem** 1:1	261.5 $\pm$ 1.7	0.24 $\pm$ 0.07	-38.6 $\pm$ 1.9
4	**F-Sq-Gem :** **Sq-Gem** 1:100	363.4 $\pm$ 2.7	0.35 $\pm$ 0.07	-34.7 $\pm$ 0.9

Table 2.4: DLS Measurements for **F-Sq-Gem** at 4.9 mM: PDI: Polydispersity Index, ζ: Zeta potential

In a radiochemical setting it is unlikely to achieve the required concentration for nanoprecipitation.[2] It was therefore important to see if **F-Sq-Gem** could be co-precipitated with **Sq-Gem**. Entry 3 and 4 of Table 2.4 present the measured data

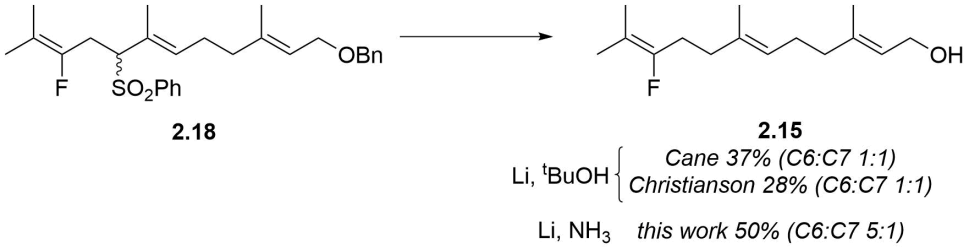
[2]Couvreur reported 4.6 μM for **Sq-Gem**.[21]

for mixtures of the two compounds in different ratios. In both cases, nanoprecipitation was readily observed although the particle size and PDI increased. Couvreur and co-workers reported that residual organic solvent has an effect on particle size.[37] It was suspected that the samples contained residual acetone from the nanoprecipitation, although great care was taken to evaporate the acetone.

In conclusion, it was shown that **F-Sq-Gem** readily forms nanoassemblies in aqueous solutions and readily co-precipitate with **Sq-Gem**. The particle size for both compounds were similar. From the ζ-potentials, it can be expected that the nanoassemblies behave the same way as discussed by Couvreur and co-workers[21] *in vivo*. The used precipitation method has the potential to be translated into a radiochemical setting, although great care has to be taken to evaporate all traces of organic solvents to avoid particle swelling.

2.2.5 Attempts to improve the synthesis of F-Sq-Gem

The overall yield of the synthesis of **F-Sq-Gem** suffers mainly in the reduction of sulfone **2.18** and the simultaneous protecting group removal. It was suspected that the harsh reaction conditions of the Birch reduction lead to several side reactions. Simultaneously, the observed isomerisation of the C6-double bond is not desirable.

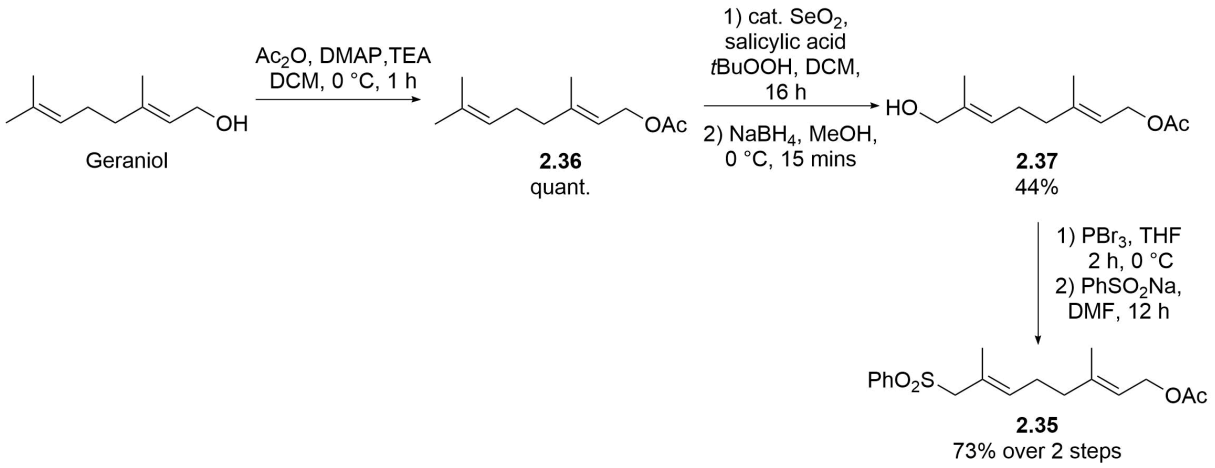


Scheme 2.16: Overview of reported methods to reduce sulfone **2.18** to alcohol **2.15**

The reduction as well as the benzyl deprotection are usually reported in high yields. The aim was to develop a synthesis which allows for two successive reactions. The first attempt was to change the hydroxy protecting group. The new protecting group should be able to be cleaved under mild conditions and not interfere with sulfone chemistry. To this end, different methods to reduce alkyl sulfones were investigated.

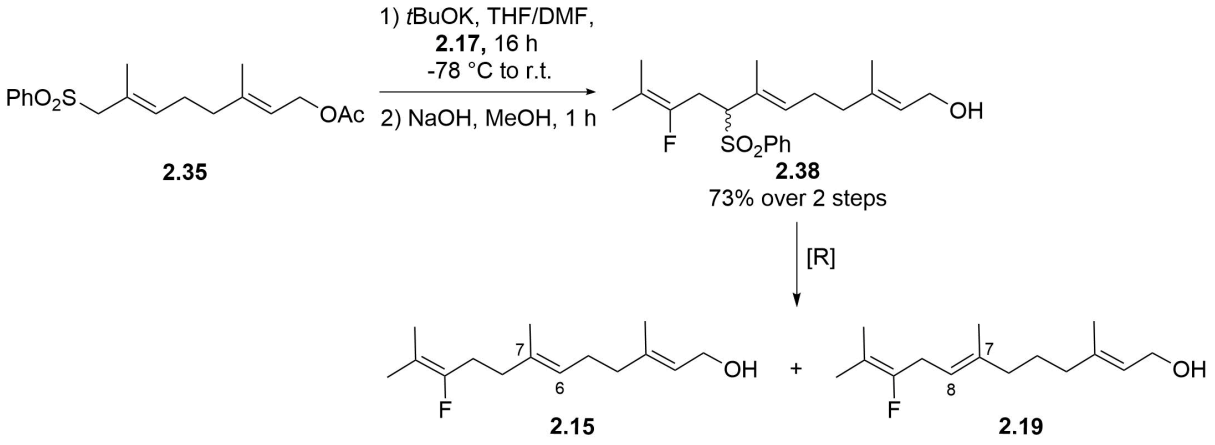
2.2.5.1 Alternative Protecting Groups

Acetate protecting groups were found to be stable to the necessary reaction conditions and sulfone **2.35** was obtained in good yields (Scheme 2.17). Geraniol was acetylated in quantitative yields to ester **2.36**. Stereoselective allylic oxidation afforded alcohol **2.37** in acceptable yields. A closer investigation of the bromination step revealed THF as a superior solvent to diethyl ether when phosphorus tribromide was employed. The reaction proceeded cleanly and no purification was necessary before the sulfonylation. Sulfone **2.35** was obtained in 64% yield over two steps.



Scheme 2.17: Synthesis of sulfone **2.35**

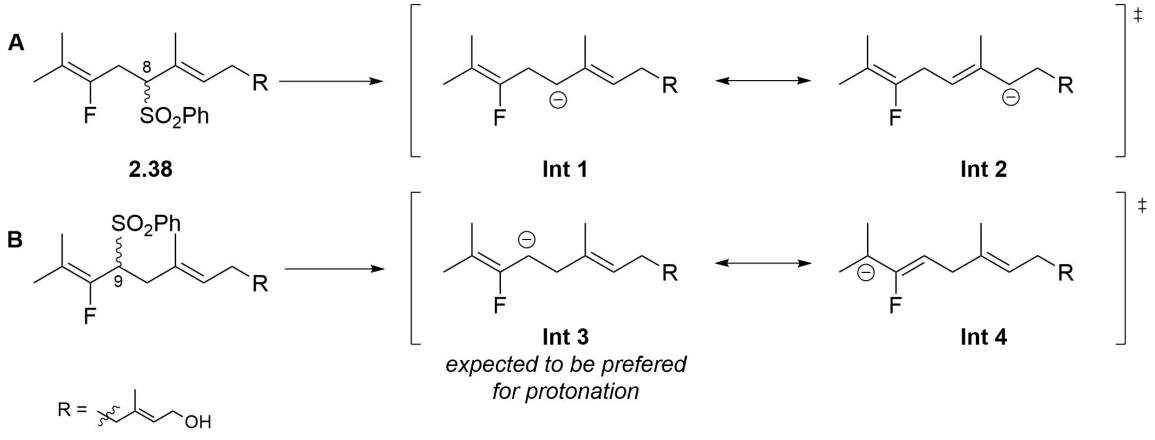
Potassium *tert*-butoxide was used to deprotonate sulfone **2.35**. The reaction of the sulfanion with bromide **2.17** afforded the desired sulfone. The reaction proceeded well but purification of the obtained sulfone proved challenging, therefore the protecting group was hydrolysed without prior purification. Alcohol **2.38** could be readily purified and was isolated in 73% yield (Scheme 2.18).



Scheme 2.18: Synthesis of sulfone **2.38**

For the reduction of sulfone **2.38**, several methodologies were investigated. The reduction using $LiBHEt_3$ and a palladium catalyst, described previously (Section 2.2.3), was found to be low yielding, likely due to the unprotected alcohol quenching the reagent. The reaction afforded **2.15** and **2.19** in a 3:1 ratio. This was the best ratio observed apart from the original protocol (lithium in ammonia). Alternatively, sodium amalgam[18] and magnesium in methanol[19,20] were able to reduce allylic sulfone **2.38** in good yields (generally >60%). In both cases, heavy isomerisation across C7 was observed with **2.15** and **2.19** obtained in a 1:1 mixture.

The reduction of sulfone **2.38** likely proceeds through radical and anionic intermediates on C8, which are in resonance with the adjacent double bond (Scheme 2.19 **A**). It was hypothesised, that during the reduction of a sulfone on C9 (**B**), protonation would predominantly take place on **Int 3**, due to the higher substitution of the double bond and the sterically less hindered environment.[38]

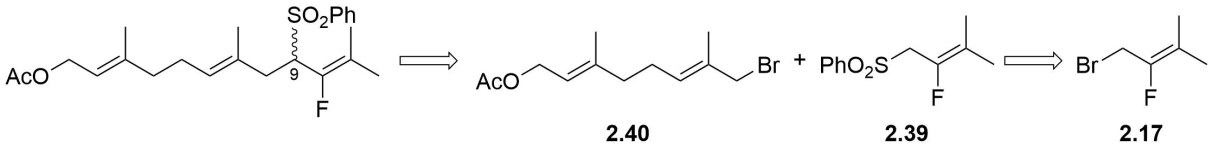


Scheme 2.19: Considerations on the intermediate anions

New fragments were designed to test the above outlined hypothesis and synthesise a C9 sulfone.

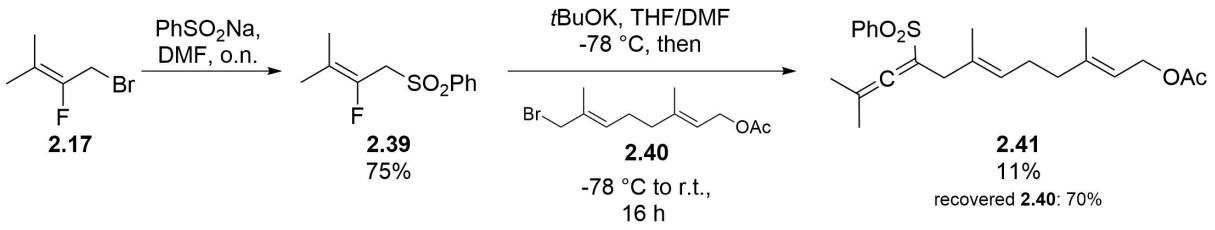
2.2.5.2 Synthesis of a C9-sulfone

The required fragments for a C9-sulfone could be accessed from already synthesised intermediates. Sulfone **2.39** could be synthesised from bromide **2.17** in one step. Electrophile **2.40** was synthesised previously as discussed in section 2.2.1.



Scheme 2.20: Proposed synthesis for the C9-sulfone

Sulfone **2.39** was synthesised from bromide **2.17** in good yields (Scheme 2.21). Deprotonation of sulfone **2.39** with potassium *tert*-butoxide and treatment with bromide **2.40** afforded an unexpected product however. The new product was identified as allene **2.41** by its characteristic quaternary ^{13}C shift and absence of fluoride. It was suspected that after the initial S_N2 reaction, the excess of base (0.1 eq.) deprotonated the formed sulfone, leading to an elimination of basic fluoride, which could autocatalytically drive the reaction further. Sulfone **39** was completely consumed in the reaction while a large portion of bromide **2.40** could be recovered. The fate of sulfone **2.39** is unknown but it is likely that it decomposed due to elimination of the fluoride.



Scheme 2.21: Synthesis of sulfone **2.39** and reaction with bromide **2.40**

The observed reaction prevented further investigation of the approach and no improved synthesis could be developed.

2.3 Summary

In this chapter, the successful synthesis of **F-Sq-Gem** from three fragments was described. The new squalenoyl drug analogue forms nanoassemblies in aqueous solutions. The obtained nanoassemblies were characterised by DLS and resemble those of **Sq-Gem**. It was shown that **Sq-Gem** and **F-Sq-Gem** can be co-precipitated, which is imperative for the translation of the technology into radiochemistry.

Two attempts to improve the synthesis of **F-Sq-Gem** were described. Acetate was found to be a suitable alternative to benzyl protecting groups and can be removed under mild conditions. No improved methodology for the reduction of sulfones was found. Double bond isomerisation could not be avoided. Changing the fragments led to the elimination of fluoride and the formation of an allene.

Literature

[1] J. A. Faraldos, D. J. Miller, V. González, Z. Yoosuf-Aly, O. Cascón, A. Li, R. K. Allemann, *J. Am. Chem. Soc.* **2012**, *134*, 5900–5908.

[2] C. D. Poulter, J. C. Argyle, E. A. Mash, *J. Am. Chem. Soc.* **1977**, *99*, 957–959.

[3] C. D. Poulter, H. C. Rilling, *Acc. Chem. Res.* **1978**, *11*, 307–313.

[4] B. Robustell, A. Ikuro, G. D. Prestwich, *Tetrahedron Lett.* **1998**, *39*, 9385–9388.

[5] B. Robustell, A. Ikuro, G. D. Prestwich, *Tetrahedron Lett.* **1998**, *39*, 957–960.

[6] Y. Jin, D. C. Williams, R. Croteau, R. M. Coates, *J. Am. Chem. Soc.* **2005**, *127*, 7834–7842.

[7] F. Yu, D. J. Miller, R. K. Allemann, *Chem. Commun.* **2007**, 4155.

[8] D. J. Miller, F. Yu, R. K. Allemann, *ChemBioChem* **2007**, *8*, 1819–1825.

[9] E. Y. Shishova, F. Yu, D. J. Miller, J. A. Faraldos, Y. Zhao, R. M. Coates, R. K. Allemann, D. E. Cane, D. W. Christianson, *J. Biol. Chem.* **2008**, *283*, 15431–15439.

[10] C. Le Van, M. Schlosser, *Synthesis (Stuttg).* **1974**, *1974*, 115–116.

[11] H. Machleidt, R. Wessendorf, *Justus Liebigs Ann. Chem.* **1963**, *674*, 1–21.

[12] J. A. Faraldos, Y. Zhao, P. E. O'Maille, J. P. Noel, R. M. Coates, *ChemBioChem* **2007**, *8*, 1826–1833.

[13] D. J. Miller, F. Yu, D. W. Knight, R. K. Allemann, *Org. Biomol. Chem.* **2009**, *7*, 962.

[14] F. W. Sum, L. Weiler, *Tetrahedron* **1981**, *37*, 303–317.

[15] F. Camps, A. Messeguer, F. J. Sánchez, *Tetrahedron* **1988**, *44*, 5161–5167.

[16] D. E. Cane, G. Yang, Q. Xue, J. H. Shim, *Biochemistry* **1995**, *34*, 2471–2479.

[17] A. Birch, H. Smith, *Q. Rev. Chem. Soc.* **1958**, *12*, 17–33.

[18] G. H. Posner, D. J. Brunelle, *J. Org. Chem.* **1973**, *38*, 2747–2756.

[19] A. C. Brown, L. A. Carpino, *J. Org. Chem.* **1985**, *50*, 1749–1750.

[20] N. K. Yee, R. M. Coates, *J. Org. Chem.* **1992**, *57*, 4598–4608.

[21] P. Couvreur, B. Stella, L. H. Reddy, H. Hillaireau, C. Dubernet, D. Desmaële, S. Lepêtre-Mouelhi, F. Rocco, N. Dereuddre-Bosquet, P. Clayette, V. Rosilio, V. Marsaud, J.-M. Renoir, L. Cattel, *Nano Lett.* **2006**, *6*, 2544–2548.

[22] B. M. Trost, M. R. Machacek, H. C. Tsui, *J. Am. Chem. Soc.* **2005**, *127*, 7014–7024.

[23] H. A. Gennadios, V. Gonzalez, L. Di Costanzo, A. Li, F. Yu, D. J. Miller, R. K. Allemann, D. W. Christianson, *Biochemistry* **2009**, *48*, 6175–6183.

[24] M. A. Umbreit, K. B. Sharpless, *J. Am. Chem. Soc.* **1977**, *99*, 5526–5528.

[25] E. M. Kaiser, A Comparison of Methods Using Lithium/Amine and Birch Reduction Systems, **1972**.

[26] S. A. Snyder, E. J. Corey, *J. Am. Chem. Soc.* **2006**, *128*, 740–742.

[27] M. Uyanik, K. Ishihara, H. Yamamoto, *Org. Lett.* **2006**, *8*, 5649–5652.

[28] G. R. Labadie, R. Viswanathan, C. D. Poulter, *J. Org. Chem.* **2007**, *72*, 9291–9297.

[29] A. Baj, P. Wałejko, A. Kutner, Ł. Kaczmarek, J. W. Morzycki, S. Witkowski, *Org. Process Res. Dev.* **2016**, *20*, 1026–1033.

[30] G. Gamboni, H. Schinz, A. Eschenmoser, *Helv. Chim. Acta* **1954**, *37*, 964–971.

[31] G. Stork, A. W. Burgstahler, *J. Am. Chem. Soc.* **1955**, *77*, 5068–5077.

[32] P. V. Fish, W. S. Johnson, *J. Org. Chem.* **1994**, *59*, 2324–2335.

[33] K. Ishihara, S. Nakamura, H. Yamamoto, *J. Am. Chem. Soc.* **1999**, *121*, 4906–4907.

[34] S. G. Sethofer, T. Mayer, F. D. Toste, *J. Am. Chem. Soc.* **2010**, *132*, 8276–8277.

[35] H. Xiao, Z. Yao, Q. Peng, F. Ni, Y. Sun, C. X. Zhang, Z. X. Zhong, *Sep. Purif. Technol.* **2016**, *169*, 196–201.

[36] D. M. Bender, J. Bao, A. H. Dantzig, W. D. Diseroad, K. L. Law, N. A. Magnus, J. A. Peterson, E. J. Perkins, Y. J. Pu, S. M. Reutzel-Edens, D. M. Remick, J. J. Starling, G. A. Stephenson, R. K. Vaid, D. Zhang, J. R. McCarthy, *J. Med. Chem.* **2009**, *52*, 6958–6961.

[37] D. Saha, F. Testard, I. Grillo, F. Zouhiri, D. Desmaele, A. Radulescu, S. Desert, A. Brulet, P. Couvreur, O. Spalla, *Soft Matter* **2015**, *11*, 4173–4179.

[38] B. Braida, V. Prana, P. C. Hiberty, *Angew. Chemie - Int. Ed.* **2009**, *48*, 5724–5728.

CHAPTER 3

Precursor Synthesis for the Radiolabelling of Squalenoylated Drug Analogues

3.1 Introduction

In this chapter, the synthesis of a precursor for the radiofluorination of squalenoylated drugs is described. The first aim was to identify a nucleophilic radiofluorination method, which could be used to radiolabel squalenoylated drugs (e.g. **^{18}F-Sq-Gem**) as well as squalene derivatives for post-labelling conjugation (Figure 3.1). Subsequently, the synthesis of a suitable precursor had to be developed.

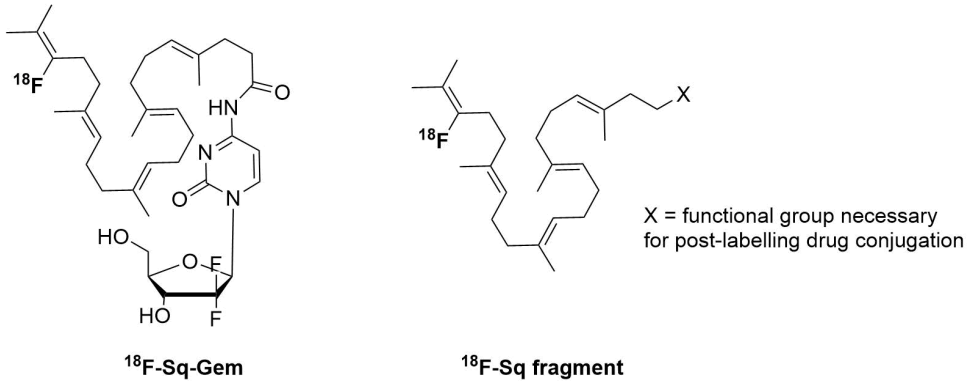


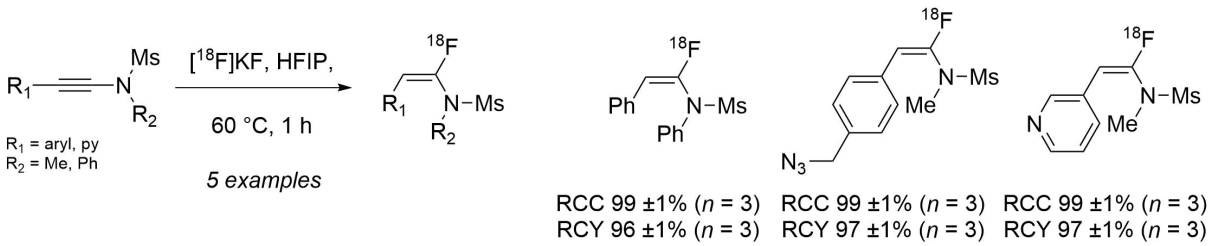
Figure 3.1: Radiofluorination targets

In section 3.1.1, different nucleophilic radiofluorination methods are reviewed and analysed for their potential to radiolabel squalene derivatives. In the second part, the synthesis of the precursor for radiolabelling is discussed.

3.1.1 Choice of Precursor for the Radiofluorination of Double Bonds

In the context of radiofluorination, fluoroalkenes have received little attention. Very recently, Xu, Hammond and co-workers published the first ^{18}F-hydrofluorination of alkynes.[1]

They presented the fluorination of electron-deficient ynamides with readily available [^{18}F]KF and hexafluoroisopropanol (HFIP) as proton donor (Scheme 3.1). The radiochemical conversions and isolated yields are high. The authors refrain from publishing molar activity values[1] making it difficult to judge the extent of isotopic exchange of the product with the solvent.



Scheme 3.1: Radiohydrofluorination of ynamines

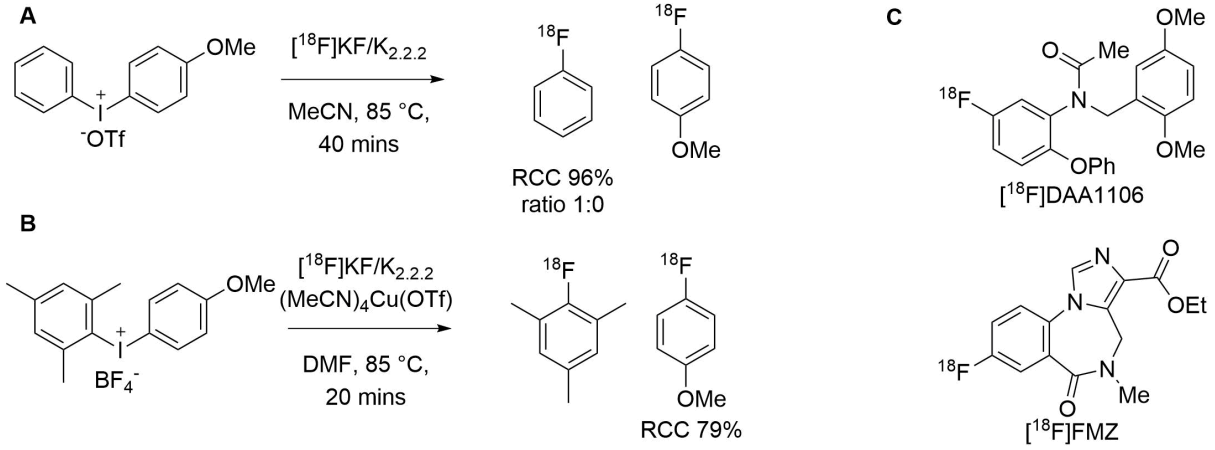
The above method afforded products which are not suitable for terpenoid synthesis. Inspiration was therefore found in the vast literature for the radiofluorination of arenes.[2] In the following section, different precursors and radiofluorination reactions are analysed for their ability to be translated onto double bonds.

3.1.1.1 Radiofluorination of Iodonium Salts

Diaryliodonium salts have been studied extensively for radiofluorination.[2] The electronic properties of the aryl substituents control the radiofluorination and the more electron-deficient aryl moiety is radiolabelled (Scheme 3.2 **A**). Thiophenes, *p*-methoxybenzene or *p*-toluene have been used as electron-rich partner substituents.[2] Sanford and co-workers demonstrated steric control of the substitution

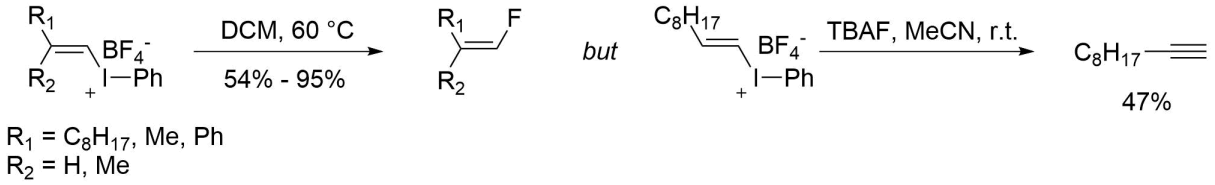
[1] The molar activity is the ratio of radiolabelled product to the complete mass of obtained product

under copper catalysis (Scheme 3.2 **B**).[3,4] Sterically controlled oxidative addition of the copper catalyst to the aryl iodonium salt resulted in the fluorination of the sterically less demanding aryl moiety. A range of complex biologically active molecules were successfully radiolabelled from diaryliodonium salts (for selected examples, see Scheme 3.2 **C**).[2] The methodology has been used in a microfluidic system and was automated on GE TRACElab FX_{FN} modules.[5,6]



Scheme 3.2: A) Radiofluorination of diaryliodonium salts; **B**) Radiofluorination of diaryliodonium salts under copper mediation; **C**) Examples of radiotracers synthesised from diaryliodonium salts

Vinyl(aryl)iodonium salts readily react with nucleophiles, preferentially giving access to functionalised double bonds.[7,8] Vinylic fluorination has been reported by thermolysis of vinyl(aryl)iodonium tetrafluoroborate salts (Scheme 3.3).[9,10] The authors reported an inversion of stereochemistry on the double bond. Nucleophilic fluoride, e.g. TBAF, was found to be too basic and afforded instead the elimination product.[7]



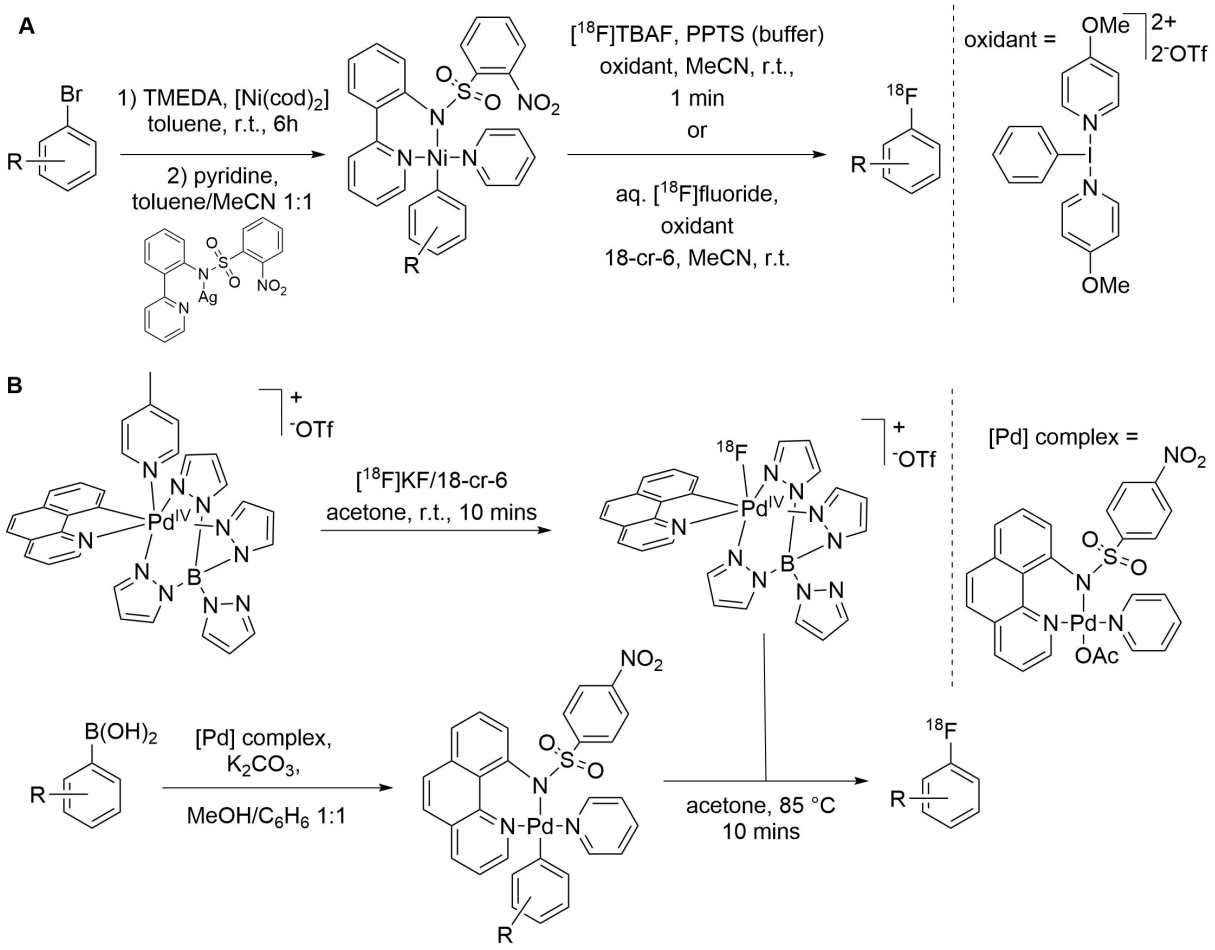
Scheme 3.3: Fluorination reactions of vinyl(phenyl)iodonium trifluoroborate salts

While diaryliodonium salts have already been shown to be very useful in radiolabelling, the potential of vinyl(aryl)iodonium salts to undergo radiofluorination is unknown. Reported fluorination methods used the counterion as the fluoride source, which would lead to low molar activities in radiochemistry. Further, nucleophilic fluoride led to undesirable elimination reactions. Reported syntheses of vinyl(aryl)iodonium salts start from vinyl boronic acids and esters,[7,11] which have been used as precursors for radiofluorination (discussed in Section 3.1.1.3). Vinyl(aryl)iodonium salts were therefore not considered as precursors for the synthesis of [^{18}F]vinyl fluorides.

3.1.1.2 Radiofluorination from Preformed Reactive Metal Complexes

Ritter and co-workers investigated the radiofluorination of pre-formed aryl palladium and nickel-complexes.[12,13] They demonstrated the formation of electrophilic metal-fluorine complexes from [^{18}F]fluoride (Scheme 3.4). While a single nickel complex was able to perform the umpolung of fluoride and the reductive elimination (Scheme 3.4 **A**), two different palladium complexes were required (**B**). The first complex captures the fluoride and transfers it to the substrate bound palladium species, which undergoes reductive elimination to the aryl fluoride.[13,14] Ritter and co-workers reported good functional group tolerance and were able to demonstrate the radiosynthesis of several radiotracers. Ortho-functionalisation and basic functional groups, e.g. tertiary amines, decreased the radiochemical conversion.

At the time, the described methods were revolutionary in their umpolung approach but the synthesis of the metal-substrate complexes adds complexity. The formation of a terpenoid palladium complex was deemed very difficult.

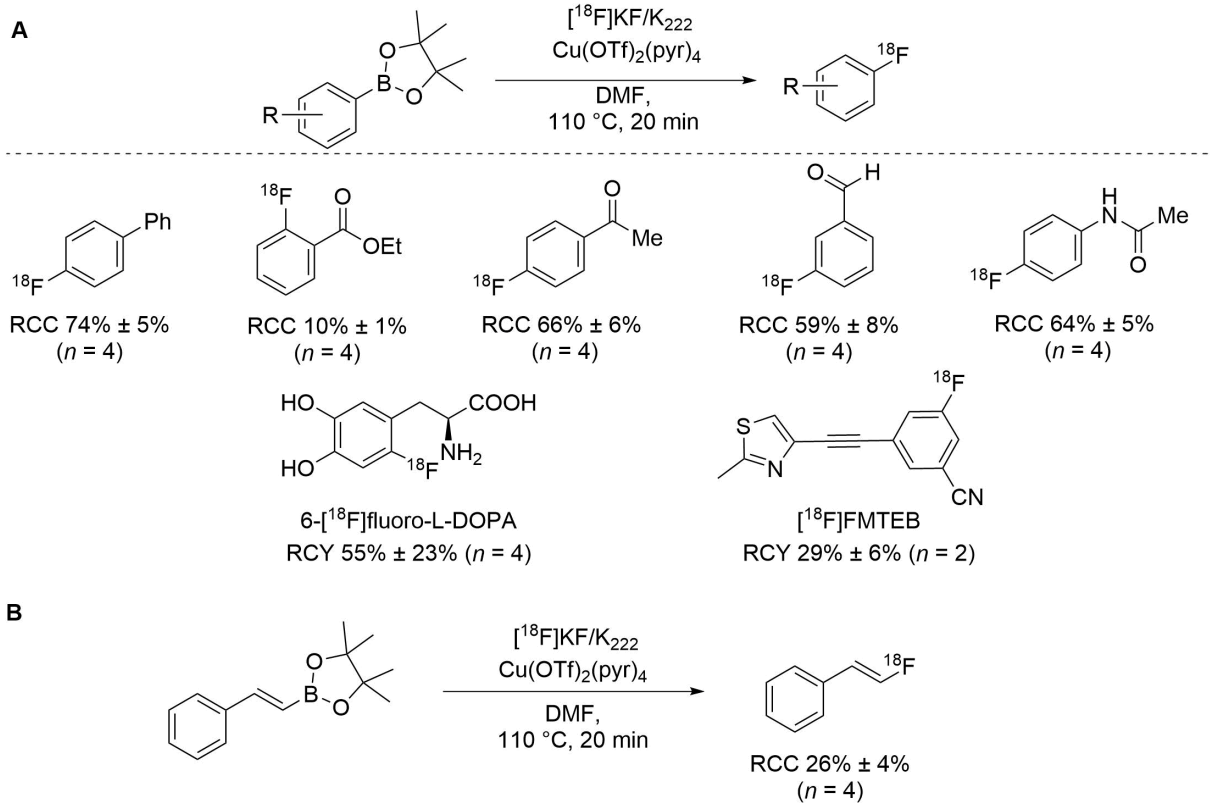


Scheme 3.4: Radiofluorination of aryl nickel (**A**) and palladium-complexes (**B**)

3.1.1.3 Radiofluorination of Boronic Acids and Esters

Gouverneur and co-workers published the radiofluorination of aryl boronic esters in 2014 (Scheme 3.5 **A**).[15] The copper-mediated reaction showed remarkable functional group tolerance and was employed to radiolabel several clinically-relevant drugs and radiotracers.[15,16]

It enabled the radiofluorination of electron-rich, -deficient and -neutral aromatics from readily available precursors. Gouverneur and co-workers also presented the successful radiofluorination of a styrene derived boronic ester, which was an encouraging result towards the labelling of vinyl boronic esters (Scheme 3.5 **B**).

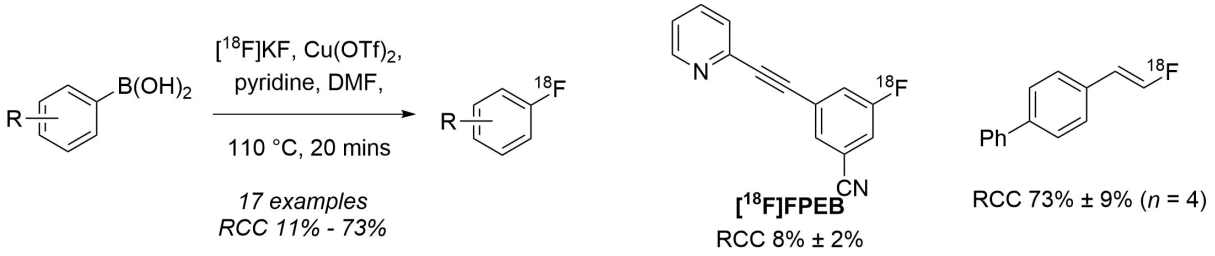


Scheme 3.5: Radiofluorination of aryl boronic esters: **A** Selected examples from the substrate scope and labelled radiopharmaceuticals; **B** Radiolabelling of a styrene based boronic ester; RCC = radiochemical conversion, RCY = radiochemical yield

Although the copper-mediated radiofluorination of aryl boronic esters displayed good functional group tolerance, some heterocycles were found to be problematic. To avoid long precursor synthesis without the guarantee of successful radiolabelling, Gouverneur and co-workers developed a de-risking strategy.[17] Fragments, whose influence on the radiolabelling reaction were unknown, were spiked into a model

reaction with known radiochemical conversion (RCC). The change in RCC gave a strong indication whether the radiolabelling of a molecule, containing the fragment in question, would be successful. Gouverneur and co-workers investigated a large number of heterocyclic motifs and confirmed the exceptional functional group tolerance of the method.[17] The spiking method could be employed to test for interactions of the squalenoylated drug with the labelling reaction.

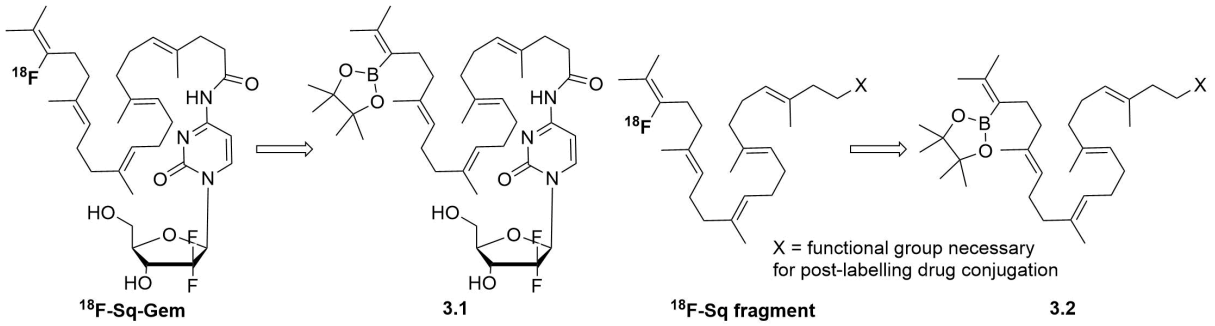
The copper-mediated radiofluorination methodology was later extended to aryl boronic acids by Sanford and co-workers (Scheme 3.6).[18] The reaction afforded aryl fluorides in moderate to high radiochemical conversions on arenes containing electron-neutral, -withdrawing or -donating substituents. The work was exemplified on the radiolabelling of [^{18}F]FPEB. Encouraging for the radiolabelling of vinylic boronic acids was the reported radiofluorination of a styrene derivative.



Scheme 3.6: Radiofluorination of aryl boronic acids

The successful radiolabelling of styrenes from boronic ester and acid precursors was considered a good starting point for the investigation of the radiofluorination of squalenoyl derivatives. Vinyl boronic esters **3.1** and **3.2** were identified as possible precursors (Scheme 3.7). The synthesis of vinyl boronic esters is well documented and has been investigated on various substitution patterns.

In the next section, several synthetic approaches to vinyl boronic esters will be discussed and evaluated for their applicability to the synthesis of squalenoyl vinyl boronic ester derivatives.



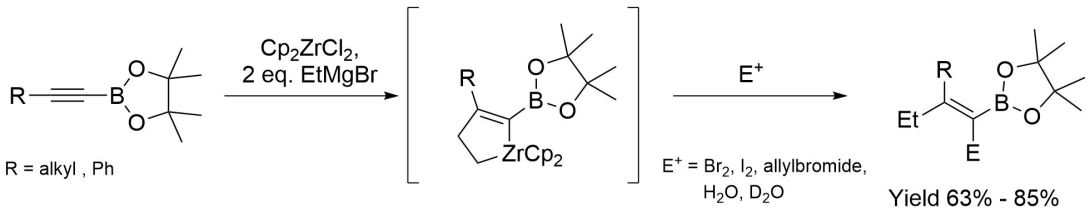
^{18}F-Sq-Gem 3.1 ^{18}F-Sq fragment 3.2

Scheme 3.7: Radiofluorination strategy for squalenoyl drug analogues

3.1.2 Synthesis of Vinyl Boronic Esters

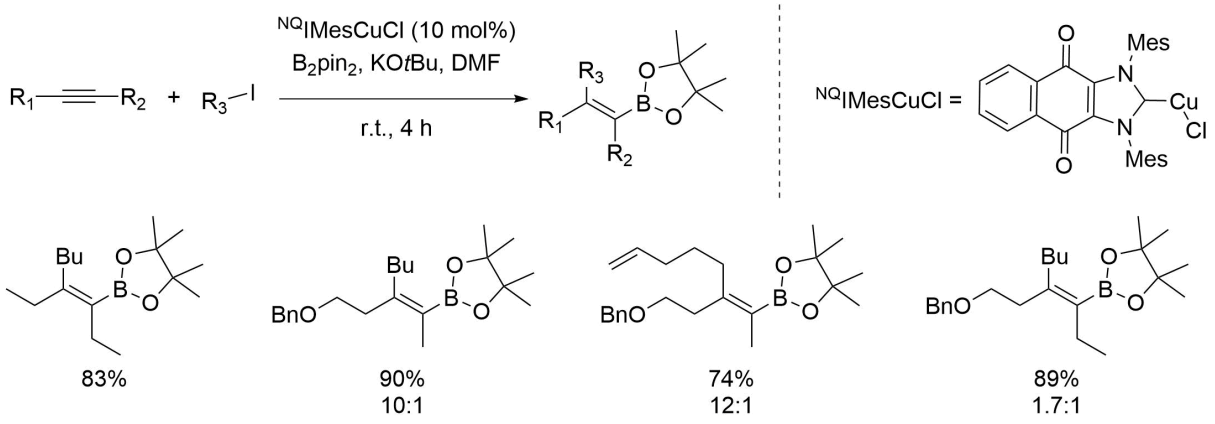
The synthesis of vinyl boronic esters has been a major research topic due to their synthetic utility in cross-coupling reactions.[19] While many methods have been developed to access di- and tri-substituted vinyl boronic esters, the synthesis of tetrasubstituted alkenes is still challenging. In the following section, several methods are presented and evaluated.

Carbo-zirconation of 1-alkynylboronic esters has been utilised to synthesise tri-alkyl substituted vinyl boronic esters (Scheme 3.8).[20] The formed cyclic zirconium species can be quenched with a range of electrophiles to yield highly substituted alkenes.



Scheme 3.8: Zirconation of alkenylboronic esters

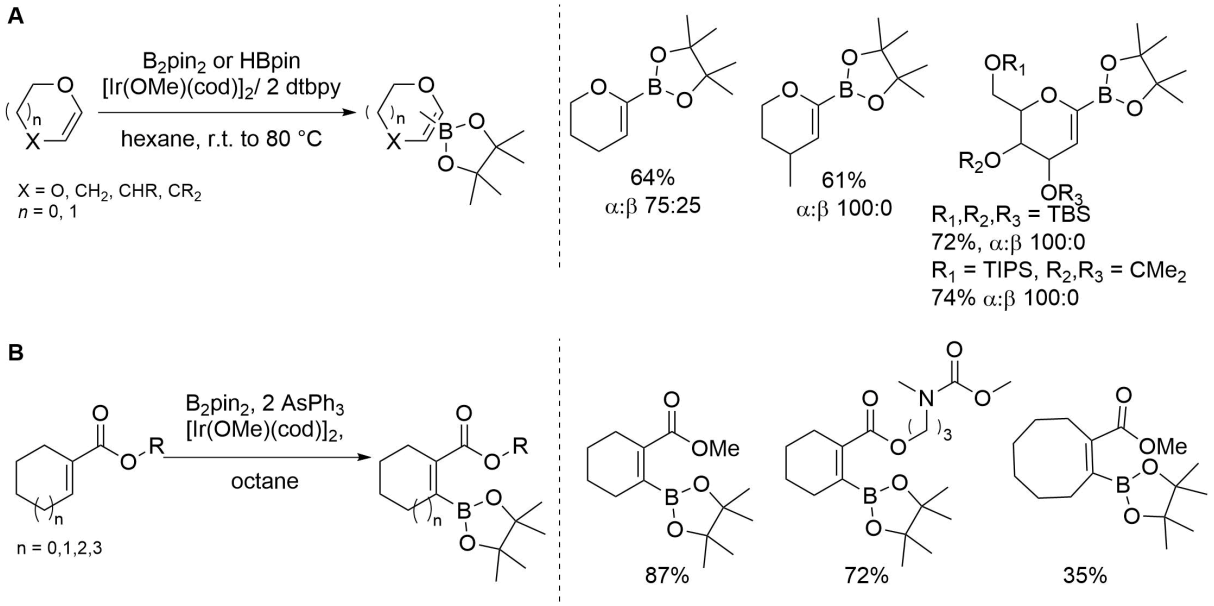
Copper-NHC complexes were utilised for the regioselective borylation of disubstituted alkynes (Scheme 3.9).[21] The choice of NHC ligand was found to be crucial for the regioselectivity of the reaction. This three-component reaction utilised bis(pinacolato)diboron (B_2pin_2) as the boronic ester source. The regiochemical outcome was heavily governed by the steric demand of the alkyne substituents with the sterically less hindered side preferred for borylation. It was suspected that a mixture of regioisomers would be obtained when the reaction was performed on a terpenoid derivative.



Scheme 3.9: Synthesis of tetrasubstituted vinyl boronic esters[21]

C-H borylation of alkenes has been achieved under iridium catalysis (Scheme 3.10 **A**).[22–24] Miyaura and co-workers first demonstrated the principle on cyclic vinyl ethers

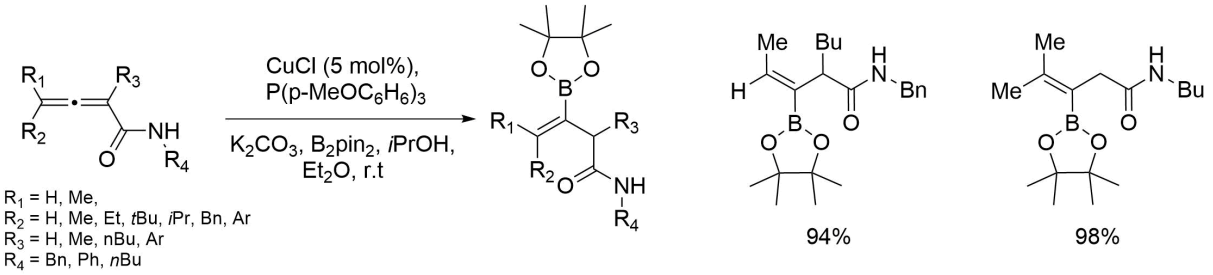
using bis(pinacolato)diboron as the boron source. Good regioselectivity was observed. Later, Ishiyama extended the principle to 1-cycloalkenecarboxylate esters (Scheme 3.10 **B**).[24]



Scheme 3.10: C-H borylation of **A**: cyclic vinyl ethers and **B** cycloalkenecarboxylates

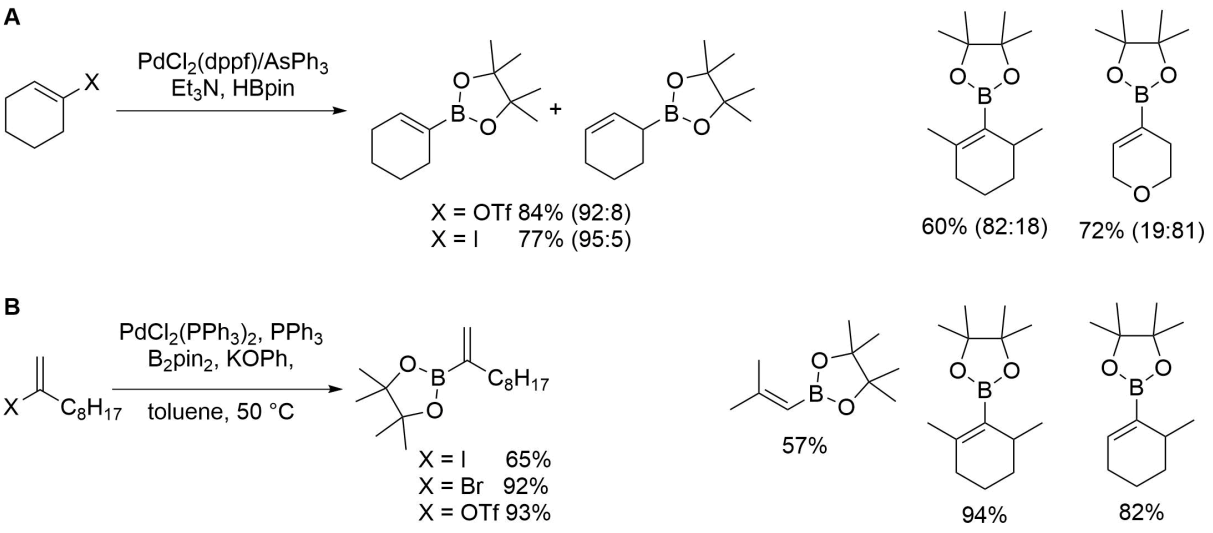
The discussed methods enabled the synthesis of a great variety of alkenes but failed to deliver *gem*-dimethyl alkenes.

Ma and co-workers reported the synthesis of *gem*-dimethyl vinyl boronic esters from substituted allenes (Scheme 3.11).[25] The formed copper-boronate species added regioselectively to 4,4-disubstituted alleneamides to afford tetrasubstituted alkenes. In asymmetrically substituted allenes the resulting vinyl boronic ester preferred *Z*-geometry in high selectivity.



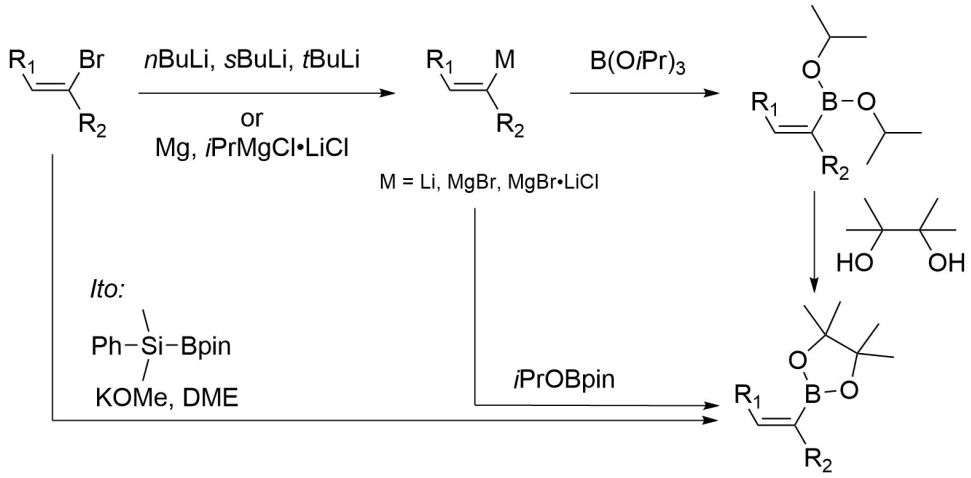
Scheme 3.11: Borylation of alleneamides

Borylation of double bonds was also described from vinyl halides and tosylates. Masuda and co-workers presented a system relying on palladium catalysis and pinacolborane (Scheme 3.12 **A**).[26] Pinacolborane is highly reactive and decomposes quickly; further isomerisation of the double bond was observed. Later, Miyaura and co-workers reported the use of the more stable bis(pinacolato)diboron for the borylation of alkenyl bromides and triflates (Scheme 3.12 **B**).[27] In contrast to the previous method, no isomerisation of the double bond was observed. Metal-catalysed borylations of vinyl halides were expected to be compatible with terpenoids.



Scheme 3.12: Palladium catalysed borylation of alkenyl iodides, bromides and triflates

Vinyl halides also were borylated through the formation of organolithium or Grignard reagents (Scheme 3.13).[28–30] The carbon nucleophile reacted with a range of different boron electrophiles of general structure $B(OR)_3$.[28] Depending on the reagent, pinacol boronic esters were obtained directly or after transesterification.[28,30,31] Ito and co-workers demonstrated that silylboranes are powerful borylation reagents, affording the desired products in excellent yields.[32]

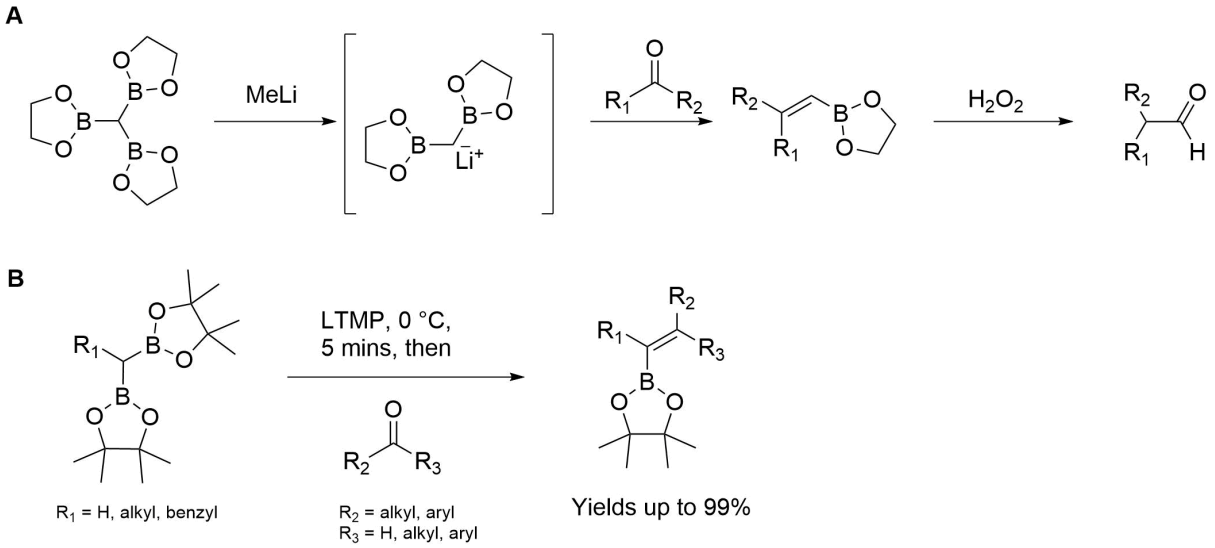


Scheme 3.13: Metal-halogen exchanged followed by borylation

Metal-catalysed borylations and borylations by lithium-halogen exchange both utilise vinyl halides as starting materials, and could complement each other in a synthesis.

An alternative approach to tri-alkyl substituted vinyl boronic esters was presented through the functionalisation of gem-diboronic esters. Matteson and co-workers reported the reaction between tris(ethylenedioxyboryl)methane and aldehydes, respectively ketones, to form terminal vinyl boronic esters, which were oxidised to

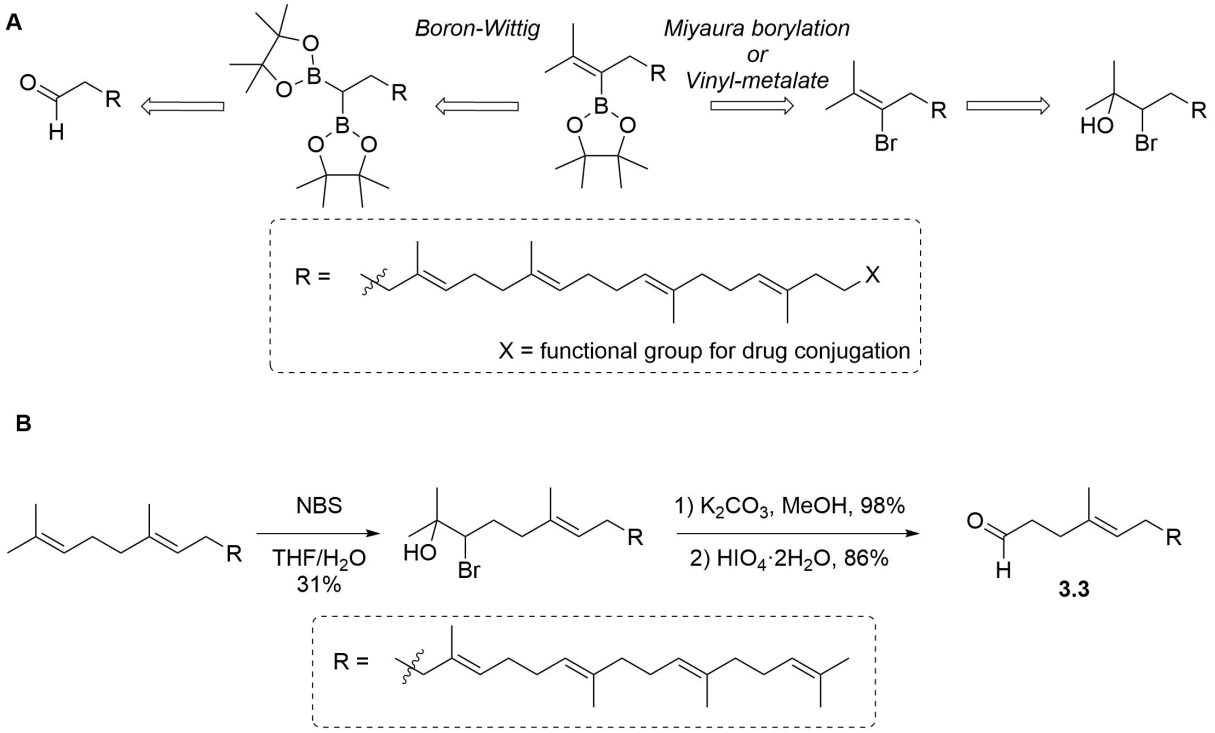
aldehydes, resulting in a one-carbon homologation (Scheme 3.14 **A**).[33] The methodology was extended by Shibata and co-workers to other 1,1-organodiboronates, which reacted with ketones to form tetrasubstituted vinyl boronic esters (Scheme 3.14 **B**). The reactions were named Boron-Wittig reactions due to their mechanistic similarity to classic Wittig reactions.[34] 1,1-Organodiboronates were deprotonated with lithium tetramethylpiperidide (LTMP) and the resulting carbanions attacked the ketone electrophiles. *Syn*-elimination of the intermediate afforded vinyl boronic esters. Good stereoselectivities were obtained with benzylic ketones and sterically demanding alkyl ketones. Later, Morken and co-workers extended the methodology to aldehydes with acceptable *E/Z* ratios.[35]



Scheme 3.14: A: Mattesons' one carbon homologation; **B** General Boron-Wittig reaction with ketones and aldehydes

Based on the discussed literature, two possible ways to access terpenoid derived vinyl boronic esters were identified (Scheme 3.15 **A**). The Boron-Wittig reaction of a

1,1-organodiboronate with acetone might give direct access to the desired functional group. 1,1-Organodiboronates have been synthesised from aldehydes[36], which is convenient as squalenealdehyde **3.3** can be accessed directly from squalene (Scheme 3.15 **B**).[37–41] The second synthetic approach relies on the borylation of a vinyl bromide. It was hypothesised that the vinyl bromide could be accessed through elimination reactions from bromohydrins, which are common intermediates in squalene functionalisation.



Scheme 3.15: A Envisaged synthesis plans for the synthesis of vinyl boronic ester; **B** Synthesis of squalenoylaldehyde **3.3**

The following goals were set: 1) reproduce the selective terminal bromohydrin formation and the synthesis of squalenealdehyde (Section 3.2.1), 2) confirm the Boron-Wittig reaction on a terpenoid model (Section 3.2.2) and 3) investigate the

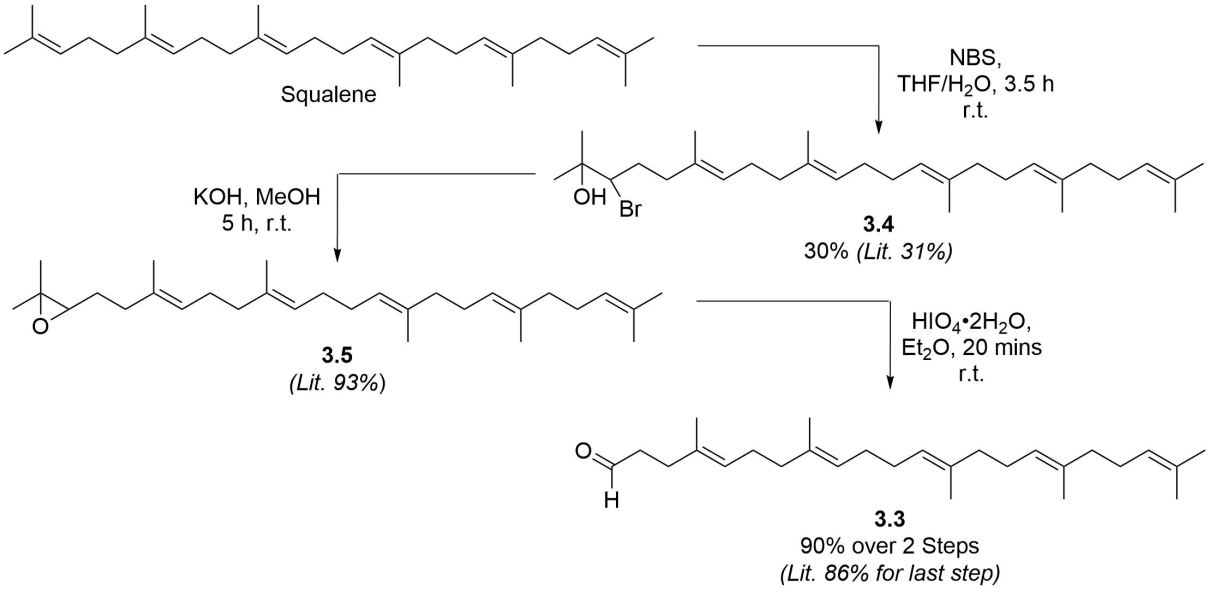
elimination of a bromohydrin to a vinyl bromide (Section 3.2.3). Based on the obtained results, a synthesis strategy for ^{18}F-squalenoylated drugs would subsequently be developed.

3.2 Results and Discussion

3.2.1 Selective Terminal Functionalisation of Squalene

Selective terminal functionalisation of squalene with *N*-bromosuccinimide (NBS) was achieved as described in the literature.[40,41] The unique solvent mixture of THF and water furnished exclusively the terminal bromohydrin **3.4** (Scheme 3.16). Bromohydrin **3.4** could be purified by column chromatography and was bench stable. Higher yields could be obtained when an excess of NBS was used but formation of doubly-functionalised squalene was observed, which increased the difficulty of purification. Epoxidation of bromohydrin **3.4** with potassium hydroxide in methanol afforded epoxide **3.5** in excellent purity; this was used further without purification. The oxidative cleavage of epoxide **3.5** to aldehyde **3.3** was achieved using periodic acid in diethyl ether. Previous reports dissolved periodic acid in a dioxane/water mixture prior to the reaction.[40,41] Higher yields were observed when periodic acid was pre-stirred for an hour in diethyl ether before the addition of crude epoxide **3.5**. A clean cleavage was observed in only 20 minutes and aldehyde **3.3** was isolated in excellent yields.

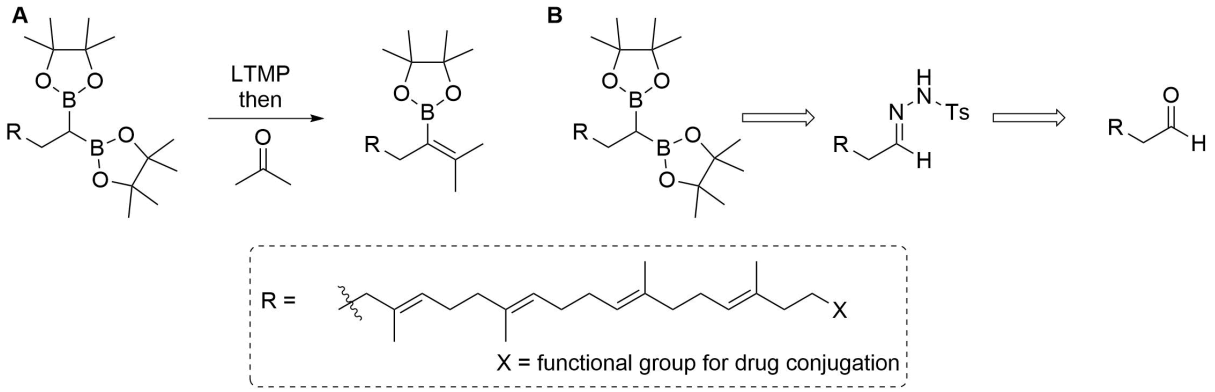
The synthesis of squalenealdehyde **3.3** was successfully reproduced. It was demonstrated that the required aldehyde and bromohydrin for the planned Boron-Wittig and elimination reactions could be accessed from squalene. Following this initial success, the synthesis of terpenoid derived boronic esters was investigated on model substrates.



Scheme 3.16: Synthesis of aldehyde **3.3**, literature yields from Ref.[40]

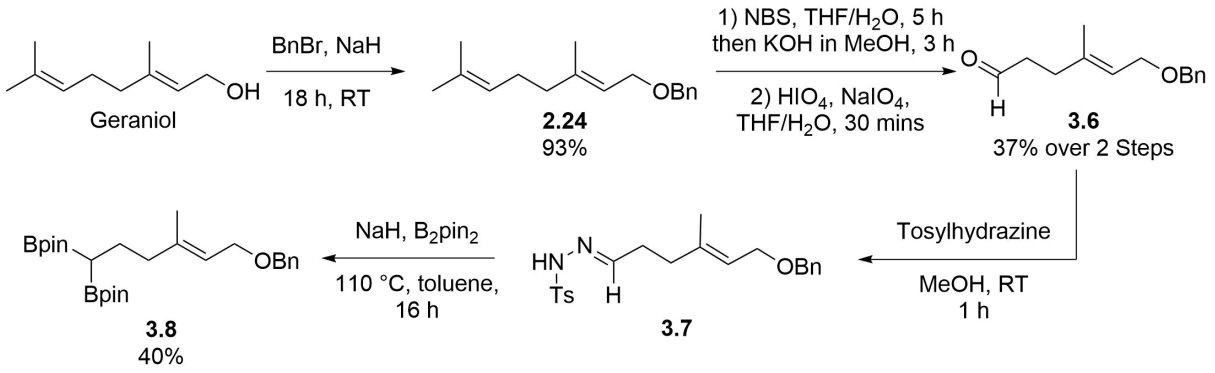
3.2.2 Synthesis of Vinyl Boronic Esters through the Boron-Wittig Reaction

The Boron-Wittig reaction offers a direct approach to vinyl boronic esters from geminal diboronic esters and ketones (Scheme 3.17 **A**). The required *gem*-diboronic ester can be accessed from aldehydes via tosylhydrazones (Scheme 3.17 **B**).[36] The aldehyde would be readily accessible from squalene derivatives, as described earlier.



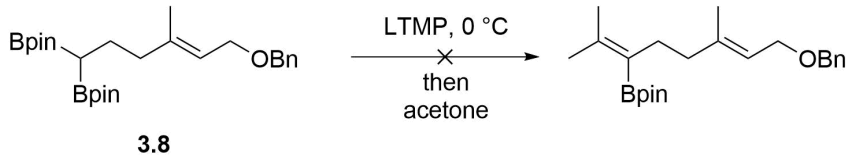
Scheme 3.17: A Boron-Wittig reaction with acetone, **B** retrosynthesis for *gem*-diboronic esters

Before the synthesis of squalenoyl *gem*-diboronic esters was attempted, the viability of the Boron-Wittig reaction was investigated on a geraniol model substrate. Benzyl protection of geraniol afforded ether **2.4** (Scheme 3.18). Terminal epoxidation via the *in situ* generated bromohydrin was followed by oxidative cleavage to aldehyde **3.6**. Hydrazone **3.7** was prepared by treating aldehyde **3.6** with *p*-toluenesulfonyl hydrazine in methanol. Precipitation of the resulting hydrazone afforded unsatisfactory yields but purification by column chromatography led to complete hydrolysis. Deprotonation of crude hydrazone **3.7** with sodium hydride and treatment with bis(pinacolato)diboron under reflux afforded *gem*-diboronic ester **3.8** in low yields. The conversion of the reaction was generally high as judged by ^{1}H-NMR, but multiple purification steps were necessary to remove the remaining bis(pinacolato)diboron, decreasing the isolated yield.



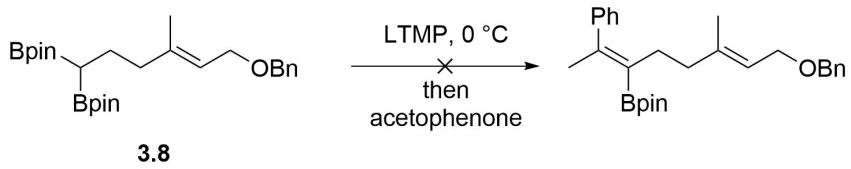
Scheme 3.18: Synthesis of *gem*-diboronic ester **3.8**

For the following Boron-Wittig reaction, acetone was dried by distillation over calcium chloride. Deprotonation of boronic ester **3.8** followed by treatment with dried acetone at room temperature did not yield the desired product (Scheme 3.19).



Scheme 3.19: Attempted Boron-Wittig reaction between boronic ester **3.8** and acetone

A new species lacking the expected *gem*-dimethyl moiety was observed but could not be identified. Varying reaction conditions did not yield the desired product. It was suspected that acetone was not electrophilic enough for the reaction. The hypothesis was tested by replacing acetone with acetophenone, which was used by Shibata and co-workers in the initial publication (Scheme 3.20). The desired reaction was not observed. The obtained product contained the expected aromatic functional groups as observed by NMR. The carbon of the ketone was not observed, which indicated an addition of the organolithium to the ketone, although the crucial elimination might not have occurred.



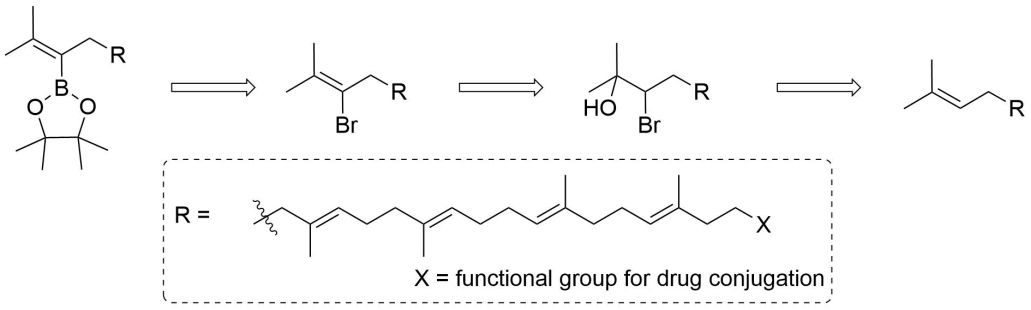
3.8

Scheme 3.20: Attempted Boron-Wittig reaction between boronic ester **3.8** and acetophenone

In conclusion, diboronic ester **3.8** was successfully synthesised from a geraniol derivative, but the desired Boron-Wittig reaction with acetone or acetophenone could not be achieved. The focus was therefore shifted to the synthesis of vinyl bromide terpenoid derivatives, which could undergo borylation.

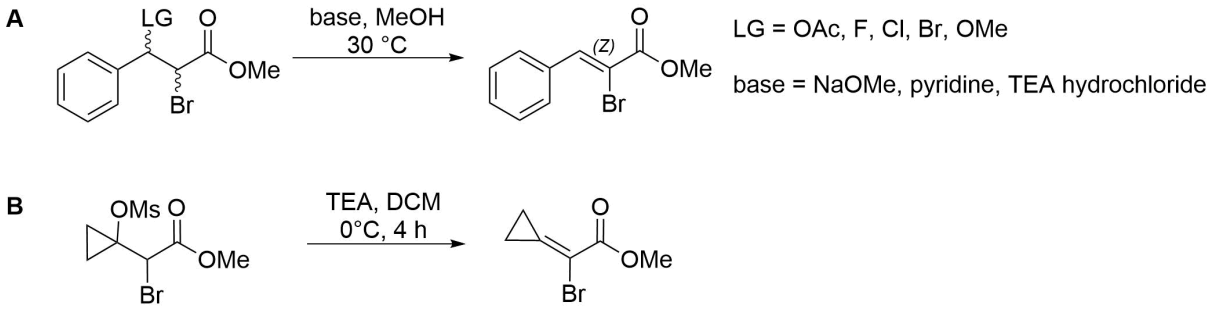
3.2.3 Synthesis of Vinyl Bromides from Bromohydrin Analogues

The second synthetic approach was based on the formation of a vinyl bromide from a bromohydrin *via* elimination (Scheme 3.21). The synthesis of bromohydrins from squalene was discussed in Section 3.2.1. It was expected that vinyl bromides would undergo borylation either under palladium catalysis or by lithium-halogen exchange and electrophilic borylation.



Scheme 3.21: Selective terminal functionalisation of squalene and synthesis strategy to vinyl boronic esters.

Elimination reactions from bromohydrin derivatives have been studied extensively in the past utilising various leaving groups. Cabalairo and Garay reported the successful elimination of methyl 3-acetoxy-2-bromo-3-phenylpropanoate to the corresponding vinyl bromide utilising sodium methoxide (Scheme 3.22 **A**).[42] Later, the investigation was expanded to other leaving groups (Cl, Br, F and OMe) using amine bases.[43] Dehydromesylation of a quaternary carbon to yield a trisubstituted vinyl bromides was published by de Meijere and co-workers on the example of cyclopropylidene synthesis (Scheme 3.22 **B**).[44] The elimination was achieved by amine bases.

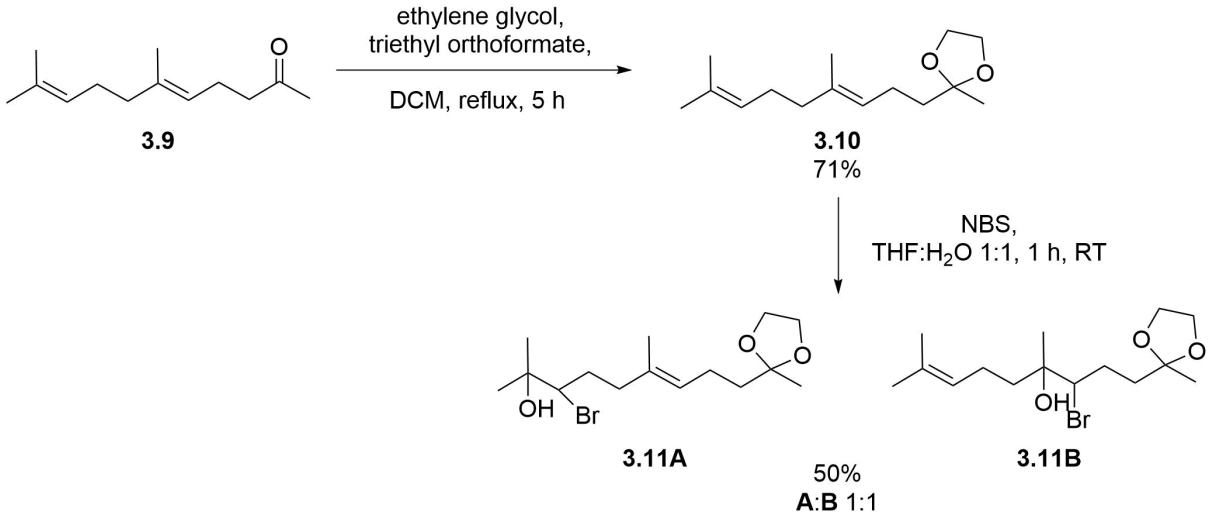


Scheme 3.22: Previous examples of vinyl bromide synthesis from bromohydrin derivatives[43,44]

Notably, previously published substrates benefit from increased acidity of the proton through the electron-withdrawing effect of the carbonyl. The following challenges were identified: 1) a stable precursor must be prepared and 2) stronger bases or harsher reaction conditions might be necessary and lead to undesired side products.

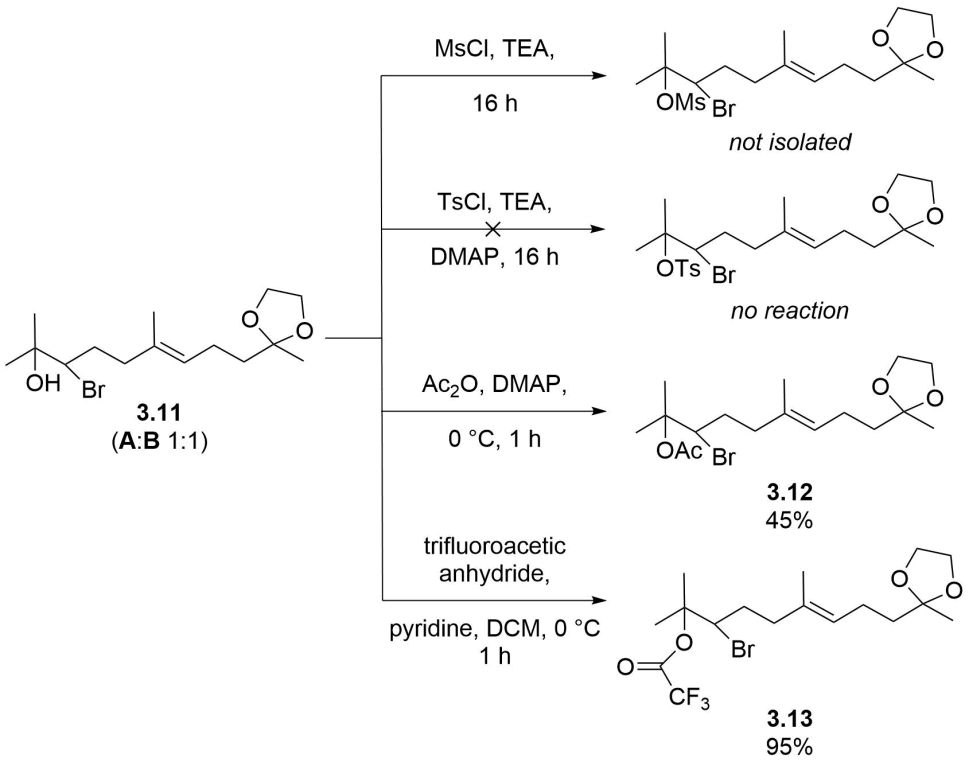
Geranyl ketone **3.9** was chosen as a model compound and protected as a ketal to avoid undesired keto-enol tautomerism (Scheme 3.23). The bromohydrin was formed by treating ketal **3.10** with NBS in a THF-water mixture. The reaction did not exhibit any site selectivity and both double bonds were functionalised.

The obtained bromohydrins **3.11A** and **3.11B** could not be separated and the mixture was taken forward.



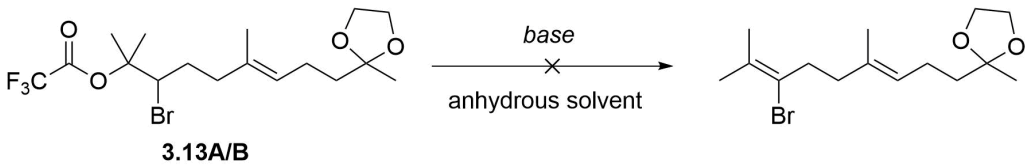
Scheme 3.23: Synthesis of model compound **3.11**

Mesyl, tosyl and acetate leaving groups were chosen for the initial investigation of the elimination reaction (Scheme 3.24). Mesylation of bromohydrin **3.11** with methanesulfonyl chloride was readily observed but the product rapidly hydrolysed upon isolation. A stable starting material for the elimination was preferable and mesylation was not further pursued. No tosylation of bromohydrin **3.11** was observed. It was suspected that the steric demand of the bromide and the *gem*-dimethyl moiety hindered the reaction. Bromohydrin **3.11** was acetylated in neat acetic anhydride and the acetate **3.12** was successfully isolated.



Scheme 3.24: Functionalisation of bromohydrin **3.11** *Note: Both regioisomers of **3.11** were used but only one is shown for clarity.*

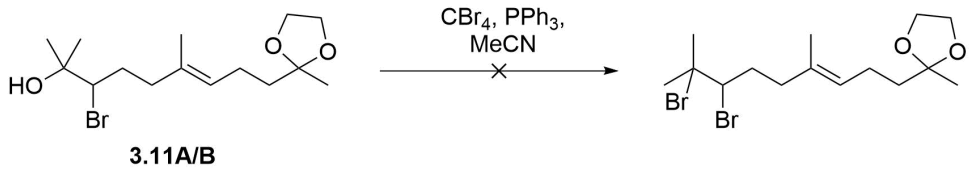
The resulting acetate **3.12** was treated with a range of non-nucleophilic bases (NaH, LDA, LiHMDS, LTMP, DBU) in anhydrous organic solvents but no elimination was observed. To increase the leaving group ability, trifluoroacetate **3.13** was synthesised from bromohydrin **3.11** in high yields. No elimination was observed when trifluoroacetate **3.13** was treated with the aforementioned bases (Scheme 3.25).



base = NaH, LDA, LiHMPA, LTMP, DBU

Scheme 3.25: Attempted elimination reactions under anhydrous organic conditions; *Note: Both regioisomers of **3.13** were used but only one is shown for clarity.*

It was suspected that a better leaving group than trifluoroacetate was necessary to allow elimination to the vinyl bromide. Halides would be expected to eliminate more readily and reactions of 1,2-dibromides to afford vinyl bromides have been reported under a wide variety of conditions. When bromohydrin **3.11** was treated with phosphorus tribromide, dehydroxybromination was observed alongside hydrolysis of the ketal. To avoid deprotection of the ketone, pyridine was added and the ketal was retained. The derived dibromide was obtained in poor yields alongside unreacted starting material. Attempting a milder bromination procedure, bromohydrin **3.11** was treated with carbon tetrabromide and triphenylphosphine, but no reaction was observed (Scheme 3.26).

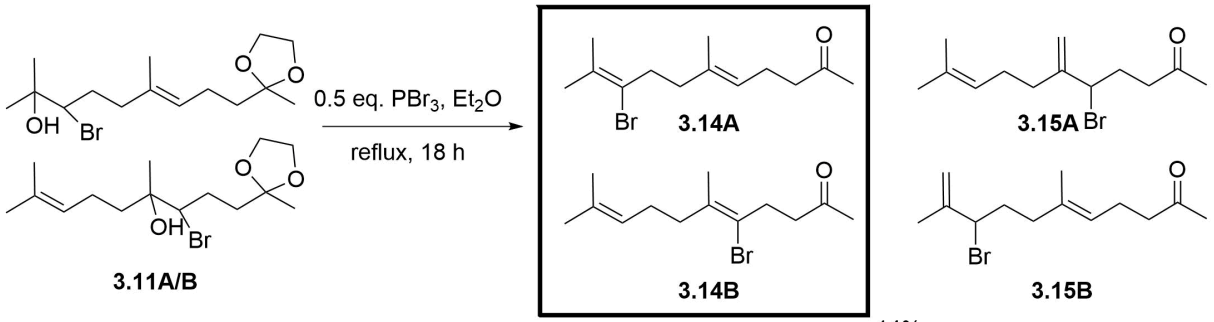


3.11A/B

Scheme 3.26: Attempted bromination of bromohydrin **3.11**

When bromohydrin **3.11** was refluxed in diethyl ether with phosphorus tribromide, complete conversion of the starting material was observed after 7 hours. The desired 1,2-dibromide was observed alongside elimination products. Elongating the reaction time afforded exclusively elimination products (Scheme 3.27). The different elimination products were distinguishable by their characteristic vinylic proton shifts (^{1}H-shifts in ppm: for vinylic bromide species **3.14A/B** 5.03 or 5.00 respectively; for allylic bromide species **3.15A/B** 4.97 and 5.12). A ratio of 4:1 in favour of the vinylic bromide **3.14A/B** was observed. The presence of **3.15A/B** indicate an alternative E1

elimination pathway of either the tertiary bromide or a phosphite intermediate of the bromination.



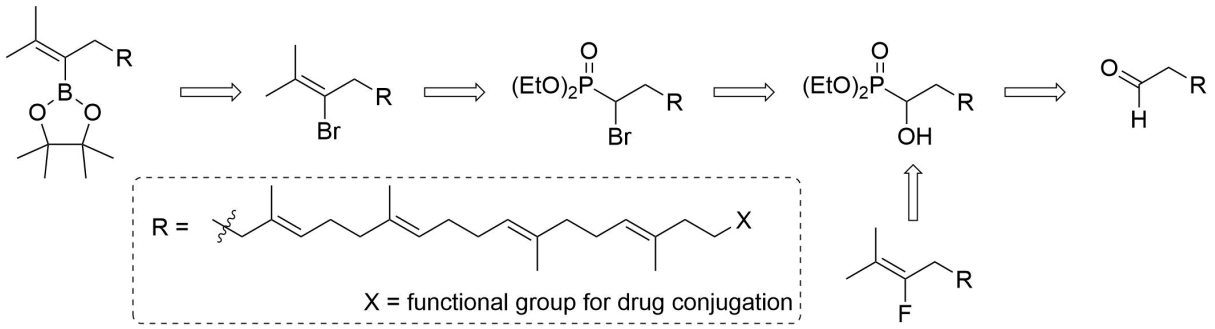
Scheme 3.27: Elimination of bromohydrin **3.11** with PBr_3

The approach was not pursued further because, since squalene cyclisations are known to proceed through tertiary carbocationic intermediates,[38] further side reactions were anticipated when translating the method to a squalenoyl backbone. Therefore the focus was shifted to different synthetic approaches.

3.2.4 Vinyl Bromide Synthesis through Horner-Wadsworth-Emmons Olefination

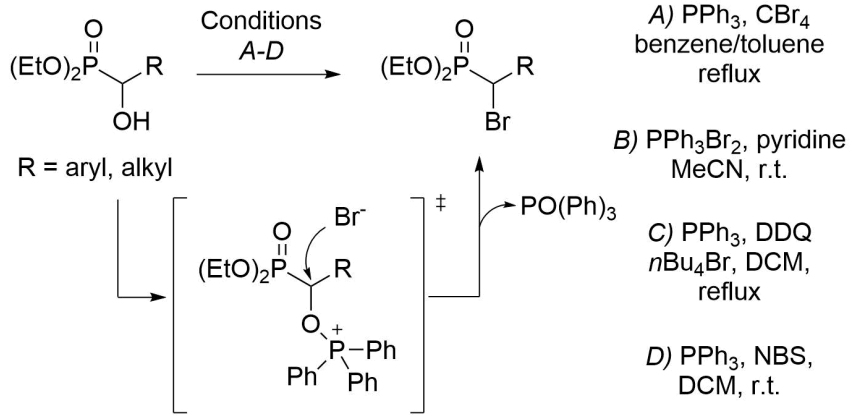
Direct access to the desired vinyl boronic ester through functionalisation of the squalenoyl backbone proved to be challenging. The Boron-Wittig reaction described in Section 3.2.2, did not afford the desired vinyl boronic ester. It was hypothesised that acetone was not electrophilic enough or the intermediate did not undergo the key *syn*-elimination. It was anticipated that a traditional Wittig reaction, or a modification

thereof, would not suffer from the same restriction due to the formation of a strong P–O bond. A synthetic strategy was developed for a Horner-Wadworth-Emmons olefination between an α-bromo-phosphonate and acetone (Scheme 3.28). The proposed synthesis also offered an alternative approach to fluorinated squalenoyl analogues, through deoxyfluorination of the α-hydroxy-phosphonate.



Scheme 3.28: Vinyl bromide synthesis through Horner-Wadsworth-Emmons olefination

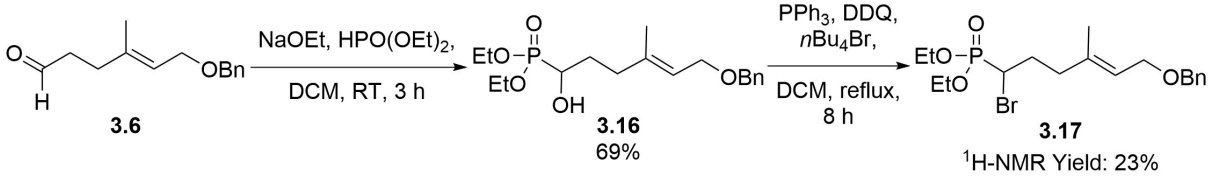
Deoxybromination of α-hydroxy-phosphonates has been previously described (Scheme 3.29).[45–48] The reported procedures rely on activation of the hydroxy group through triphenylphosphine, either on its own or by co-activation with DDQ, followed by S_N2 substitution by bromide. Traditional Appel conditions have been used for the deoxybromination, as well as preformed triphenylphosphine bromine complexes.



Scheme 3.29: Deoxybromination of α-hydroxy-phosphonates in literature

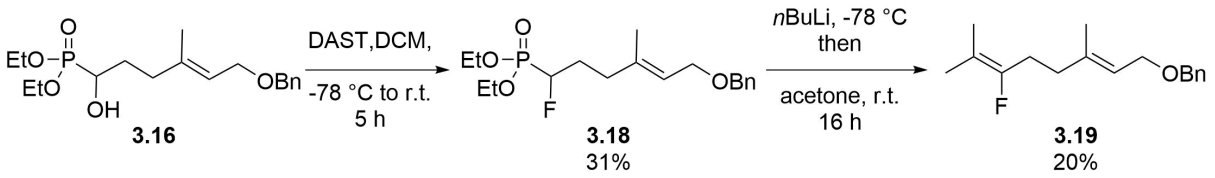
Horner-Wadsworth-Emmons reactions of α-bromo-phosphonates with ketones have been reported in the context of the synthesis of retinal chromophores[49], fungal anti-inflammatory natural products[50] and quinolines[51].

Phosphorylation of the previously described aldehyde **3.6** (Section 3.2.2) afforded α-hydroxy-phosphonate **3.16**. The combination of triphenylphosphine and DDQ activated phosphonate **3.16** to nucleophilic substitution with bromide (Scheme 3.30). Although the desired α-bromo-phosphonate **3.17** was observed, purification of the compound was challenging. The high polarity of the compound made it difficult to separate from residual triphenylphosphine and triphenylphosphine oxide. Competing side reactions made the optimisation tedious and the highest conversion was only 23% determined by ^{1}H-NMR. A lower yield, due to more side reactions, was anticipated when the methodology would be applied to longer terpenoid derivatives and the approach was therefore abandoned.



Scheme 3.30: Synthesis of α-bromo-phosphonate **3.17**

Deoxyfluorination of α-hydroxy-phosphonate **3.16** was successful using (diethylamino)sulfur trifluoride (DAST) (Scheme 3.31). The reaction showed a significant temperature dependence: at -78 °C only decomposition products were observed, but the reaction afforded phosphonate **3.18** when warmed to room temperature after the addition of DAST. The best yield obtained was 31%. In contrast to α-bromo-phosphonate **3.17**, α-fluoro-phosphonate **3.18** could be readily purified by column chromatography. The choice of base played an important role in the Horner-Wadsworth-Emmons olefination of phosphonate **3.18** with acetone. LiHMDS did not deprotonate α-fluoro-phosphonate **3.18** and the starting material was recovered unchanged. Sodium hydride also failed to afford the desired product either but in this case, no starting material was recovered. *n*-Butyllithium was able to deprotonate phosphonate **3.18** and a successful olefination reaction with acetone was observed. Ether **3.19** was obtained in low yields. Due to the excessive formation of side products, and low yields of the fluorination and olefination reactions, this strategy was not investigated further.



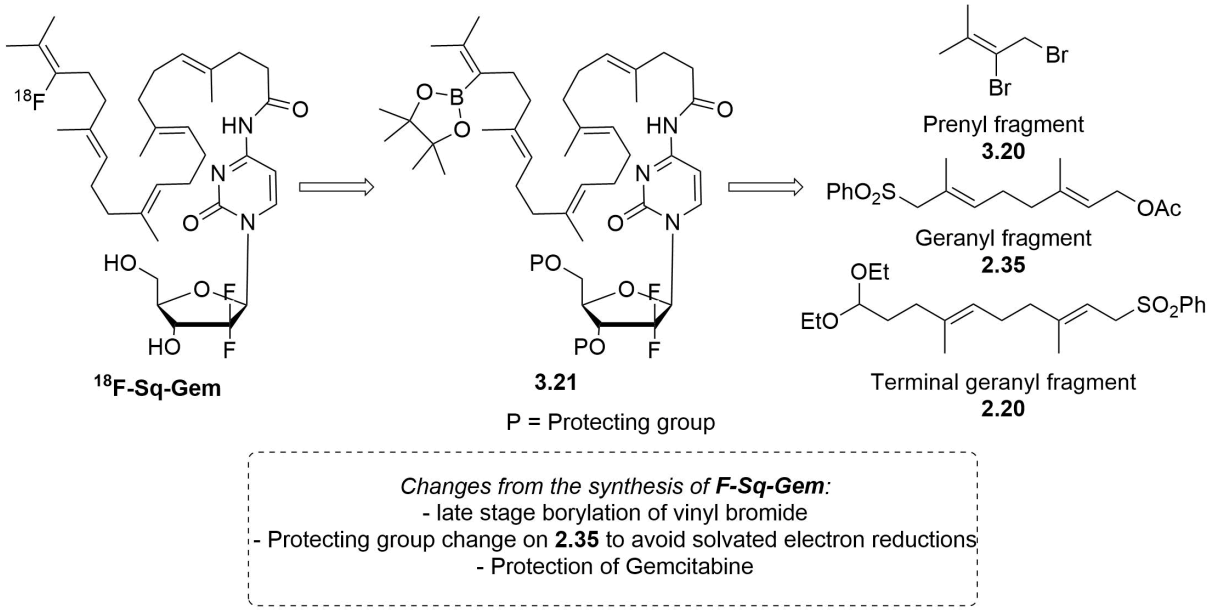
Scheme 3.31: Synthesis of α-fluoro-phosphonate **3.18** and ether **3.19**

In summary, α-hydroxy-phosphonate **3.16** was successfully prepared. Deoxybromination resulted in the desired α-bromo-phosphonate **3.17** but the purification difficulties did not encourage further studies. α-Fluoro-phosphonate **3.18** was synthesised from phosphonate **3.16** using DAST in low yields and underwent Horner-Wadsworth-Emmons olefination with acetone. This synthetic strategy was not further investigated due to the many observed side reactions and harsh reaction conditions.

3.2.5 Fragment-Based Synthesis

The aforementioned model studies failed to provide a practical synthetic route to vinyl boronic esters from squalene derivatives. The proposed key reactions either failed due to reagent incompatibility or required harsh reaction conditions leading to side reactions. It was decided to design a synthesis based on fragments similar to the ones applied in the **F-Sq-Gem** synthesis (Chapter 2). The terminal geranyl fragment (**2.20**) would be left unchanged (Scheme 3.32). The prenyl fragment (**3.20**) and the second geranyl fragment (**2.35**) had to be adapted to address several difficulties: 1) vinyl bromides are more easily reduced than vinyl fluorides and are not compatible with the solvated

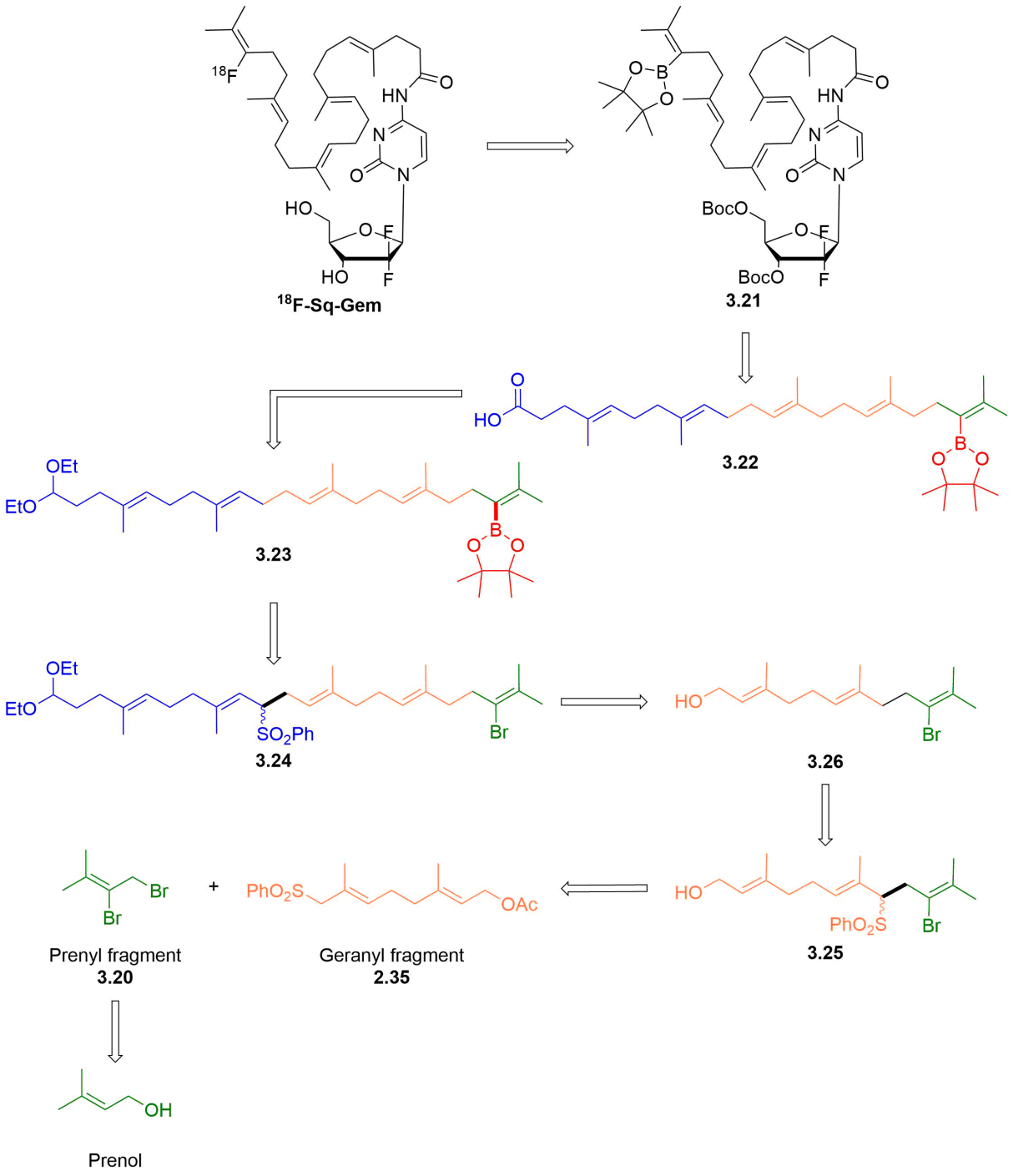
electron reductions used for the reduction of the benzyl protecting group and sulfones, 2) the stability of vinyl boronic esters is largely unknown and reports of compatible reactions are scarce, therefore a late stage borylation was preferred, 3) a methodology for the borylation of vinyl bromides had to be validated and 4) the hydroxy groups of gemcitabine have to be protected for the radiolabelling as the envisaged strategy does not tolerate acidic protons.



Scheme 3.32: Proposed fragment-based synthesis of ^{18}**F-Sq-Gem**

In order to avoid functional group incompatibilities during the radiofluorination, the alcohol groups of gemcitabine would be protected by *tert*-butyloxycarbonyl (Boc) protecting groups (**3.21**, Scheme 3.33). The Boc-protecting group has previously been successfully employed in the radiolabelling of aryl boronic esters and can be cleaved under acidic conditions retaining the amide bond of the squalenoyl drug conjugate.[15] The protected gemcitabine derivative would be amidated with acid **3.22**

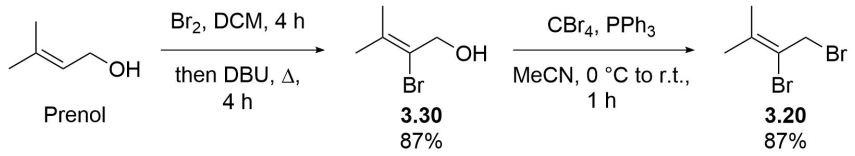
before the radiolabelling. Borylation after the complete assembly of the squalenoyl chain would afford acetal **3.23**. In the previous synthesis, reduction of sulfones was achieved by palladium catalysis or solvated electron reductions, which likely would also reduce the vinyl bromide of intermediates **3.24** and **3.25**. Alternative methods, like magnesium in methanol, were expected to be compatible but double bond isomerisation was expected for sulfone **3.25**. Finally, it would be necessary to change the protecting group on geranyl fragment **2.35** to acetate in order to enable deprotection by hydrolysis. Prenyl fragment **3.20** would be synthesised from prenol.



Scheme 3.33: Fragment-based synthesis of ^{18}**F-Sq-Gem**

3.2.5.1 Synthesis of 10-Bromo-Farnesol (3.26)

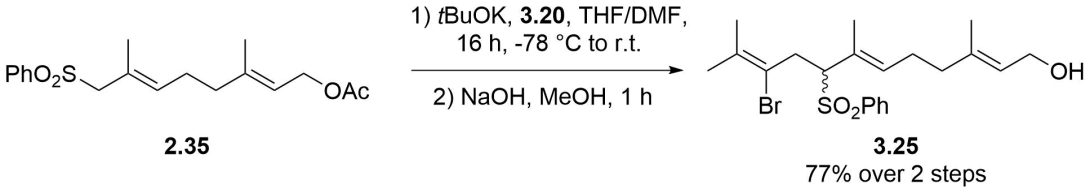
Vinyl bromide **3.20** was obtained in a one-pot bromination-elimination sequence from prenol, followed by deoxybromination of alcohol **3.27** under Appel conditions.[52]



Scheme 3.34: Synthesis of vinyl bromide **3.20**

Sulfone **2.35** was synthesised as described in Chapter 2.2.5. The coupling of sulfone **2.35** and bromide **3.20** was first attempted using LDA and HMPA. Those conditions were previously employed successfully to afford the vinyl fluoride analogue (Chapter 2.2.5). The desired brominated sulfone **3.25** was obtained in only 11% yield after direct deprotection of the alcohol. Sulfone **3.25** was readily separated from undesired side products by column chromatography. In previous studies, the concentration played an important role in the carbon-carbon bond formation. It was observed that dilution or concentration of the starting materials shut down the reaction completely and no product was observed. Changing the additive from HMPA to DMPU did not improve the yield, but more productive reactions were obtained when the base was changed to potassium *tert*-butoxide in THF with DMF as co-solvent (Scheme 3.35). Sulfone **3.25** was obtained in good yields. It was important to add vinyl bromide **3.20** in a controlled manner. From colour changes of the reaction mixture, it was judged that the reaction did not start at -78 °C but rather when warming the reaction mixture to room temperature.

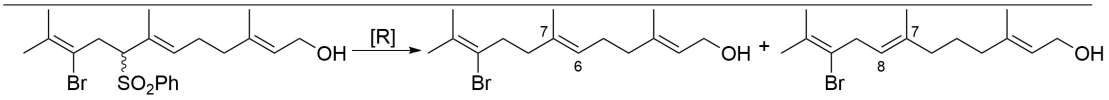
If bromide **3.20** was added too fast, the reaction mixture would turn from bright orange to dark brown and lower yields were obtained. Slow addition of bromide **3.20** would induce no or only slight colour change until the mixture was allowed to warm. The exact reaction temperature was not determined.



2.35 **3.25** 77% over 2 steps

Scheme 3.35: Synthesis of sulfone **3.25**

Key considerations for the reduction of sulfone **3.25** were the yield and the degree of double bond isomerisation across *C7*. The previous study on the vinyl fluoride analogue showed that most methods furnish 1:1 mixtures of the double bond isomers. The exceptions were reductions using the dissolved metal in ammonia (5:1) or $LiBHEt_3$ with a palladium catalyst (3:1) (Chapter 2.2.1).



3.25 **3.26**

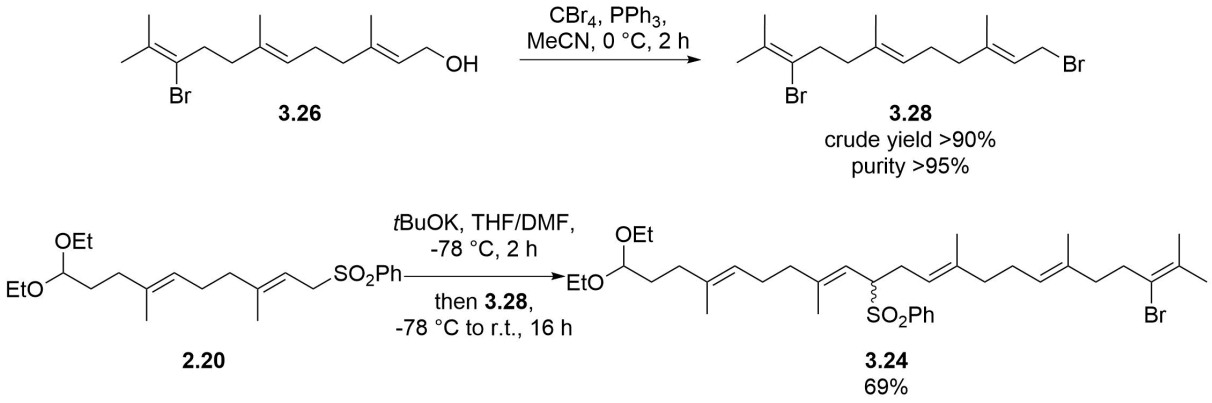
Entry	Reducing agent	Solvent	Additive	Yield	*C6:C7* ratio
1	Li	NH_3	-	decomp.	-
2	SmI_2	THF	HMPA	no rxn.	-
3	$LiBHEt_3$	THF	$[Pd(allyl)Cl_2]$	21%	1.1:1
4	Na(Hg) (5%)	MeOH	Na_2HPO_4	92%	1:1
5	Mg	MeOH	-	80%	1:1
6 [a]	Mg	MeOH	-	64%	1:1

Table 3.1: Optimisation of the sulfone reduction: reactions performed on a 46 μmol scale until complete consumption of starting material was observed; [a] reaction performed on 3.1 mmol scale

As expected, attempted reduction using lithium in ammonia did not yield any desired product (Table 3.1). Samarium iodide in THF did not reduce sulfone **3.25** and the starting material was recovered. Several side products were observed when sulfone **3.25** was treated with $LiBHEt_3$ and a palladium allyl complex. Nevertheless, **3.26** was isolated in low yields. The two regioisomers could be clearly distinguished by the ^{1}H-NMR shift of the protons on C9 (C6 isomer: 2.57 ppm, C7 isomer: 3.24 ppm). Contrary to the results discussed in Chapter 2.2.1, the reaction did not exhibit increased regioselectivity. Sodium amalgam (5% Na) reduced sulfone **3.25** in excellent yields but also afforded a 1:1 mixture of regioisomers. The same result was obtained when magnesium in methanol was used as reducing agent but the yields were slightly lower. Ecological considerations and availability of the reagents led to the decision to not pursue the sodium amalgam reduction further. It was decided to instead use magnesium in methanol for future reductions. A drop in yield was observed when the reduction of sulfone **3.25** was performed on larger scale (Entry 6). The reaction produced a significant amount of salts hampering the work-up procedure. It was suspected that some product was retained despite considerable extraction efforts. The larger amount of isomerised product enabled its detection on $AgNO_3$-impregnated silica and the two regioisomers were readily separated. 10-Bromo-farnesol (**3.26**) was obtained in high purity and the synthesis could be scaled up to a scale where over 6 mmol of product was isolated.

3.2.6 Synthesis of the Borylated Squalenoyl Aldehyde 3.32

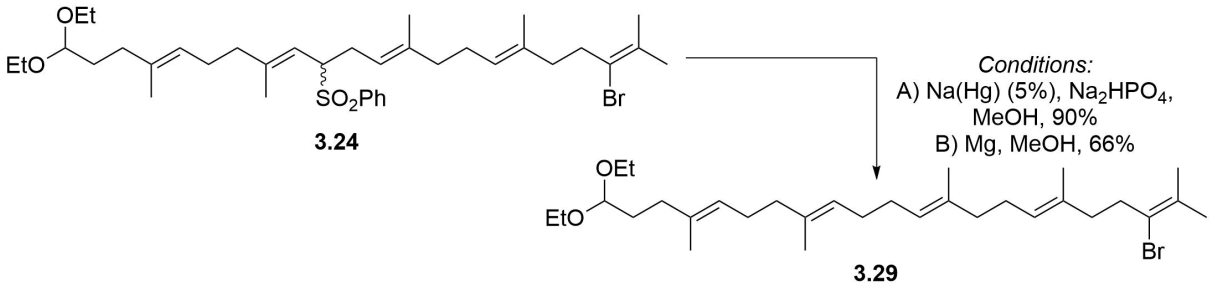
Alcohol **3.26** was brominated under Appel conditions and purified by repeated precipitation until a purity higher 95% was achieved (Scheme 3.36). Purification by precipitation was necessary as bromide **3.28** decomposed on silica. Sulfone **2.20** was deprotonated with potassium *tert*-butoxide in a THF/DMF mixture. Crude bromide **3.28** in THF was added and the reaction slowly warmed to room temperature. Sulfone **3.24** was obtained in good yields. The purity of bromide **3.28** was very important to the reaction and lower purity starting material was detrimental.



Scheme 3.36: Bromination of alcohol **3.26** and synthesis of sulfone **3.24**

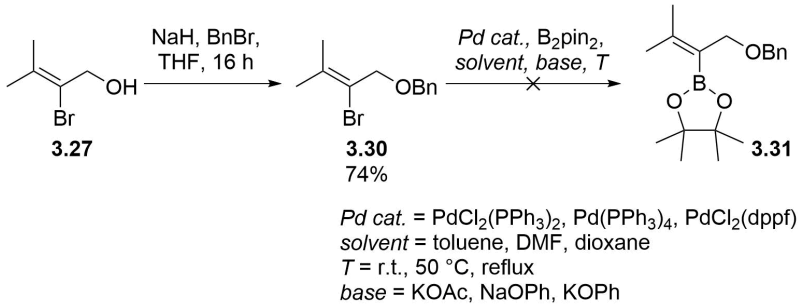
The reduction of sulfone **3.24** was achieved with sodium amalgam (Scheme 3.37 Conditions A) or magnesium in methanol (Conditions B). Sodium amalgam was the superior reagent affording acetal **3.29** in 90% yield. At this stage the work-up for the magnesium reduction became cumbersome, as strong aqueous acid would readily hydrolyse the acetal in addition to dissolving the magnesium salts. Alternatively, aqueous ammonium chloride could be used but the results were poor and large

quantities of acidic solution and organic solvents were necessary. The choice of reaction conditions was dependent on the scale of the reaction and the availability of sodium amalgam.



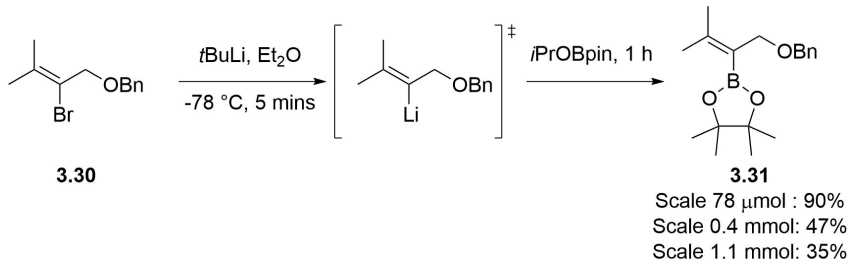
Scheme 3.37: Reduction of sulfone **3.24** to acetal **3.29**

The borylation of terpenoid derived vinyl bromides was first investigated on an easily accessible prenol model, which was prepared by benzylation of alcohol **3.27** (Scheme 3.38). Transition-metal catalysed borylation conditions were investigated first due to their good functional group tolerance. Miyaura proposed the use of $PdCl_2(PPh_3)_2$ with triphenylphosphine as an additional ligand.[27] Under standard conditions at 50 °C in toluene with potassium phenoxide as base, no reaction of bromide **3.30** was observed. Variations in temperature (room temperature and reflux), solvent (DMF, dioxane) or base (KOAc, NaOPh) did not yield the desired product. Decomposition of the starting material was observed when the catalyst was changed to $Pd(PPh_3)_4$ or the more bulky $PdCl_2(dppf)$. It was not clear whether the palladium catalyst underwent oxidative addition. Prolonged thermal exposure of the reaction mixtures led to decomposition.



Scheme 3.38: Attempted synthesis of vinyl boronic ester **3.31**

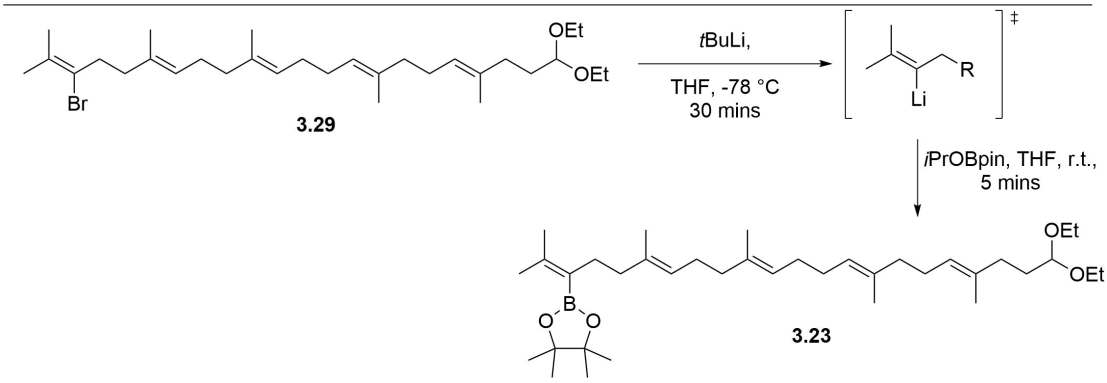
Lithium-halogen exchange chemistry was investigated next. Following early reports of vinyl-lithium formation by Schlosser and Seebach, ether **3.30** was treated with *n*-butyllithium solution in diethyl ether but no exchange was observed.[53,54] Seebach also proposed the use of *tert*-butyllithium for the lithium-halogen exchange of vinyl bromides.[54] The exchange was readily observed at -78 ◦C (Scheme 3.39). After 30 minutes, the organolithium solution was treated with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (*i*PrOBpin) and vinyl boronic ester **3.31** was isolated in low yields. Investigation of the lithium-halogen exchange revealed that the intermediate organolithium species readily decomposed even at low temperatures. Attempts to stabilise the organolithium species by using THF as solvent prevented the lithium-halogen exchange from occurring; the starting material **3.30** was recovered. As a result, the lithiation time was shortened to 5 minutes. The desired pinacol boronic ester **3.31s** was isolated in excellent yields on small scale (78 μmol) but only in moderate yields on synthetically useful scales (0.4 to 1.1 mmol).



Scheme 3.39: Lithium-halogen exchange and borylation of **3.31**

The optimised borylation method was applied to bromide **3.29** and a very slow change in colour was observed, which was interpreted as slow organolithium formation. No product was observed when the reaction was quenched with *i*PrOBpin after 5 minutes. Elongating the lithiation time, boronic ester **3.23** was isolated in 9% yield after 1 hour and 5% after 2 hours. Shorter reaction times did not improve the yield. Subsequently the solvent was changed to THF and rapid lithiation was observed. Quenching the reaction mixture after 15 minutes showed full conversion of the starting material. Attempts to quench the organolithium with excess *i*PrOBpin only afforded trace amounts of boronic ester **3.23**. The yield drastically improved when the organolithium solution was added slowly to a prepared solution of *i*PrOBpin (Table 3.2, Entry 1). Vinyl boronic ester **3.23** was isolated in 55% yield on a 37 μmol scale. As with the model substrate, the yield deteriorated when the reaction was scaled up (Entry 2). Adjusting the stoichiometry of reagents did not influence the outcome of the reaction (Entry 3). It was suspected that vinyl boronic ester **3.23** was unstable on silica but chromatography over neutral aluminium oxide did not afford more product (Entry 4). The yield improved when the organolithium solution was added rapidly to

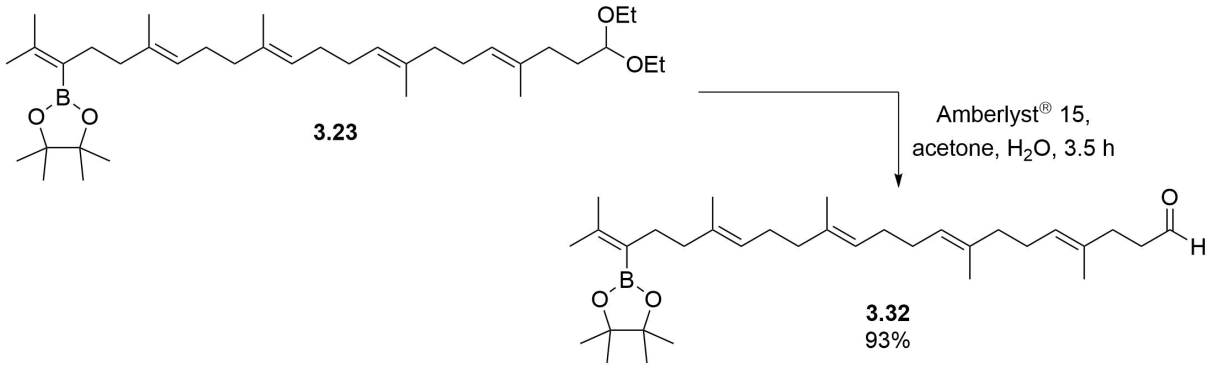
the boronate solution (Entry 5). Yields up to 91% were observed with a large excess of *i*PrOBpin (Entry 6). A decrease in yield was observed at larger scales (Entry 7). The speed of addition and the exact temperature of the solutions seemed to have an important influence on the reaction, which was difficult to control and would change considerably on different scales. The developed reaction afforded enough material to pursue the synthesis and no further optimisation was attempted.



Entry	**3.29** [μmol]	eq. *i*PrOBpin	Addition speed	Yield
1	37	1.5	dropwise	55%
2	558	1.5	dropwise	33%
3	102	3	dropwise	31%
4	670	1.5	dropwise	26% [a]
5	37	1.5	fast	64%
6	37	3	fast	91%
7	186	3	fast	47%

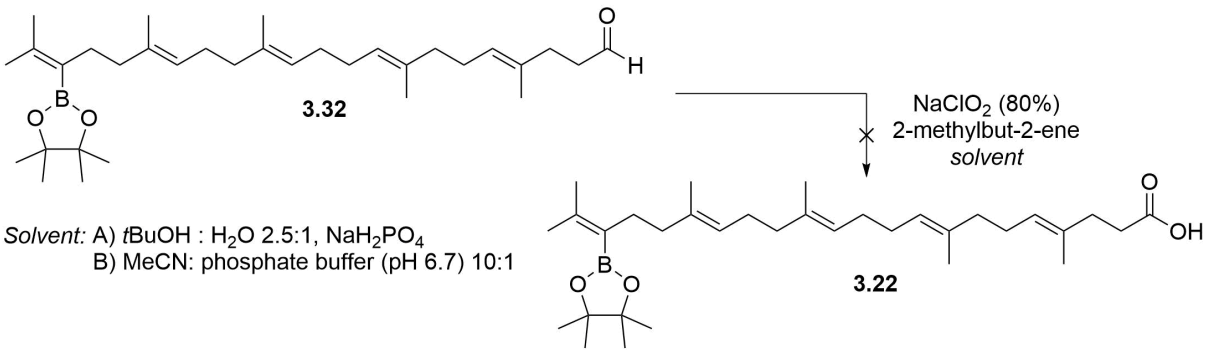
Table 3.2: Optimisation of the borylation of vinyl bromide **3.23**: Organolithium solution was added to a solution of *i*PrOBpin at different speeds. [a] purified over neutral aluminium oxide Brockman grade 1

Hydrolysis of acetal **3.23** was achieved using the acidic Amberlyst® 15 resin in acetone (Scheme 3.40). The vinyl boronic ester exhibited unexpected stability towards the acidic reaction conditions and aldehyde **3.32** was obtained in excellent yields.



Scheme 3.40: Hydrolysis of acetal **3.23**

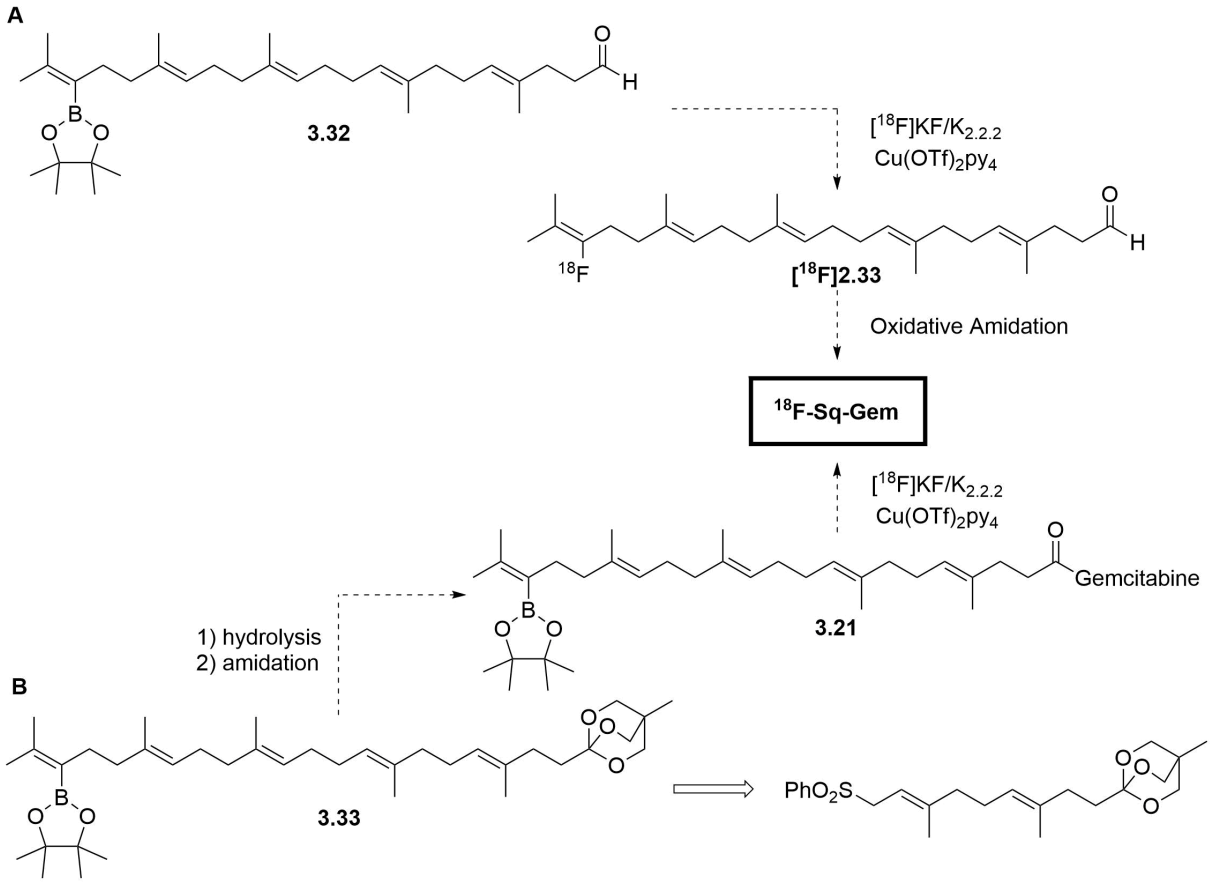
The stability of vinyl boronic esters to oxidation reactions was unknown and it was expected that the previously employed Jones oxidation would lead to protodeborylation due to the highly acidic conditions. So far, oxidation of an aldehyde was only reported with aryl boronic esters.[55] Boitrel and co-workers employed potassium permanganate as oxidising agent, which would also oxidise double bonds. The Pinnick oxidation offered a milder alternative at nearly neutral pH and if necessary in buffered solutions.[56–58] To avoid oxidation of the double bonds, 2-methylbut-2-ene was added as a scavenger. While aldehyde **3.32** was completely consumed in all attempted reactions, a loss of the pinacol ester was observed (Scheme 3.41). Whether the compound decomposed by protodeborylation or hydrolysed to the corresponding boronic acid was not clear and MS analysis was not conclusive. The oxidation was not further investigated.



Scheme 3.41: Attempted Pinnick oxidation of aldehyde **3.32**

The failure to oxidise aldehyde **3.32** demanded reconsideration of the radiolabelling strategy. The first route considered used aldehyde **3.32** as a radiolabelling precursor, which would afford aldehyde **[^{18}F]2.33** (Scheme 3.42 **A**). A post-labelling oxidative amidation would complete the synthesis of ^{18}**F-Sq-Gem**. Due to the limiting half-life time of fluorine-18 (approx. 110 mins), post-labelling steps have to be fast, chemoselective and be easy to purify. Alternatively, ortho-esters could be employed to mask the carboxylic acid for the borylation reaction (Route **B**). The stability of boronic ester **3.33** to the conditions required for hydrolysing the ortho-ester was unknown. In this case, the radiolabelling would be performed on the completely assembled squalenoyl drug conjugate **3.21**. The tolerance of the copper-mediated radiofluorination towards gemcitabine could be determined by spiking experiments (Chapter 4.2.4).

It was decided to investigate the post-labelling oxidative amidation approach first (Route **A**). In the following section, efforts to develop a suitable oxidative amidation protocol are described.



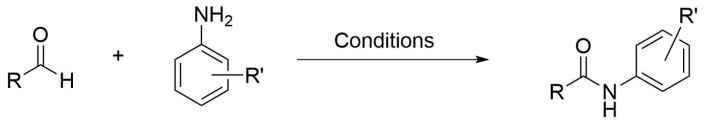
Scheme 3.42: Possible strategies to ^{18}**F-Sq-Gem**

3.2.7 Development of One-Pot Oxidation Procedures

Oxidative amidation of benzylic aldehydes was first reported using stoichiometric amounts of nickel peroxide.[59] Nakagawa and co-workers postulated a hemiacetal intermediate between ammonia and the aldehyde, which was then oxidised. Following this initial report, methods to access secondary and tertiary amides from aldehydes under palladium, nickel, rhodium, ruthenium and lanthanide catalysis have been reported.[60–64] Metal-free oxidative amidations were reported using NBS,

tert-butylhydroperoxide or sodium chlorite with different additives to mediate the reaction.[65–69] While most reactions accept a large variety of aldehydes as substrates, only a few are reported with anilines.[64,68,69] Tan and co-workers reported a Pinnick-type oxidation of benzyl and alkyl aldehydes with aniline using sodium chlorite as oxidant (Scheme 3.43).[68] The amidation does not require a metal-catalyst and reportedly works in a large variety of solvents, which is beneficial for translation into radiochemistry. Shah and co-workers reported oxidative amidation using *tert*-butylhydroperoxide and either pyridine or tetra-butylammonium iodide (TBAI).[69] Benzylic aldehydes were amidated with electron-rich and -neutral anilines in high yields. The choice of pyridine or TBAI depends on the desired reaction mechanism: while pyridine reportedly facilitates oxidation of enamine species, TBAI induces the radical oxidation of an acetal with *tert*-butylhydroperoxide. The third method, published by Dong and Whittaker, employed $[\mathrm{Ni(cod)_2}]$ with an NHC ligand (IPr) to catalyse the same reaction.[64] They reported a wide substrate scope of aromatic and aliphatic aldehydes with amines and electron-rich and -poor anilines. The reaction requires an organic hydrogen acceptor, which facilitates the nucleophilic attack of the amine. Due to the hygroscopic nature of the nickel catalyst, the reaction has to be performed in a glove box. This requirement excludes it from being translated into radiochemistry.

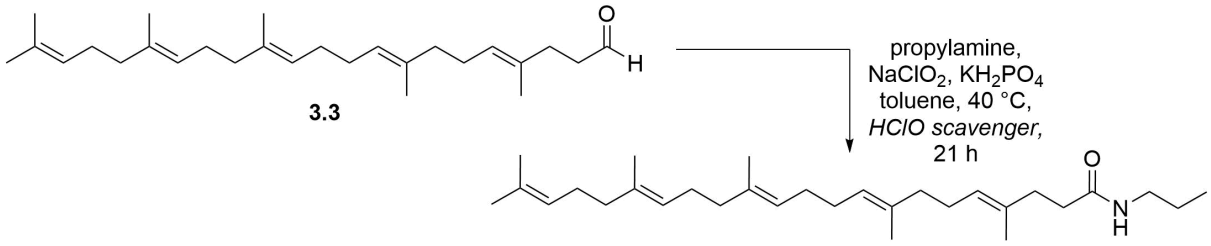
The initial efforts were focused on the oxidation with sodium chlorite.[68] Squalenealdehyde **3.3** was used as a readily available model and underwent



Tan *et. al*: $NaClO_2$, NaH_2PO_4, 2,3-dimethylbut-2-ene, r.t. or 40 °C
Shah *et. al.*: *t*BuOOH, TBAI, MeCN, 80 °C
Dong *et. al.*: [Ni(cod)$_2$], IPr, α,α,α-trifluoroaceto-phenone, dioxane, 30-40 °C

Scheme 3.43: Overview over published oxidative amidation reactions with anilines

oxidative amidation with propylamine in toluene at 40 °C (Scheme 3.44). Amide **3.34** was obtained in 45% yield. The low yield was attributed to inefficient scavenging of the hypochlorous acid and subsequent reactions with the double bonds of aldehyde **3.3**. The scavenger was subsequently changed to 2-methylbut-2-ene and conversions up to 99% by ^{1}H-NMR spectroscopy were observed.



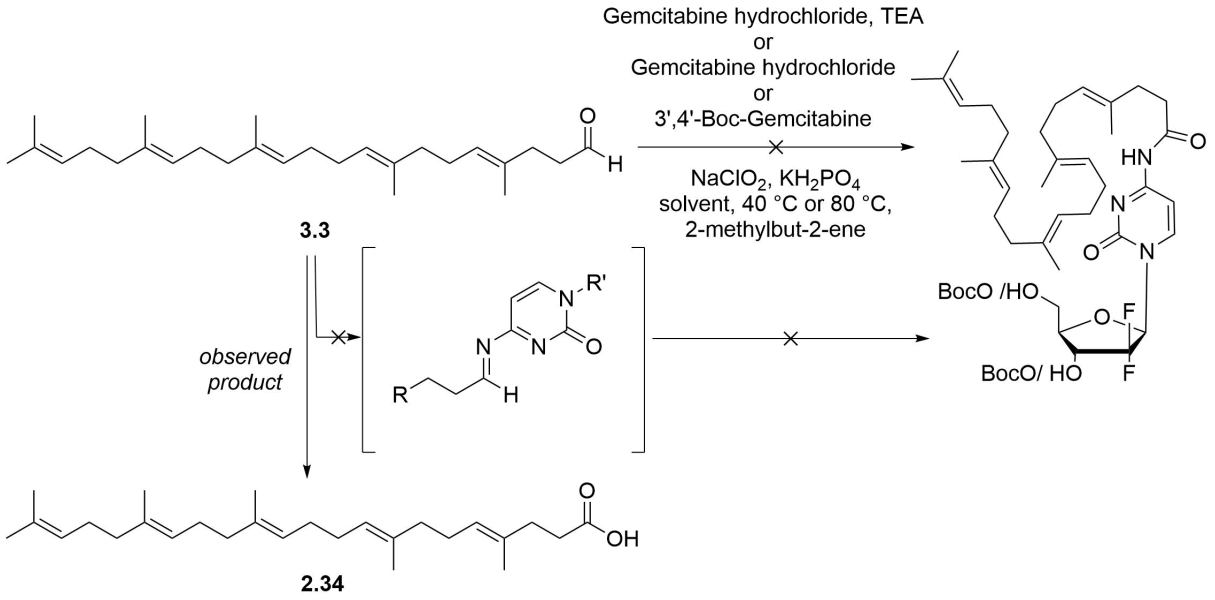
3.3

3.34
HClO scavenger:
2,3-dimethylbut-2-ene ^{1}H-NMR Yield 45%
2-methylbut-2-ene ^{1}H-NMR Yield 99%

Scheme 3.44: Oxidative amidation between squalenaldehyde **3.3** and propylamine

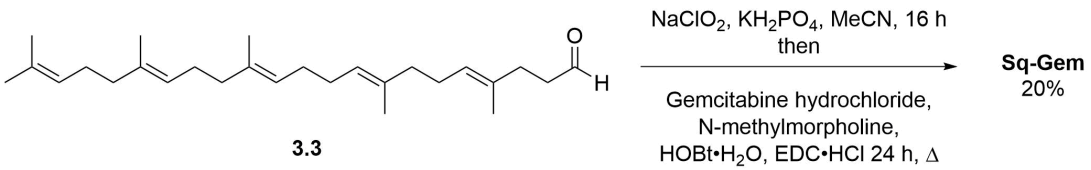
No reaction was observed between aniline and aldehyde **3.3** under the same reaction conditions, nevertheless the investigation continued with gemcitabine hydrochloride. The reaction was carried out in a variety of solvents (toluene, MeCN, *t*BuOH, DMF, DMSO, sulfolane) without any success. Elevating the temperature or pre-stirring gemcitabine hydrochloride with a base did not influence the reaction.

Further, no reaction was observed with the protected 3',4'-Boc-gemcitabine. It is likely that the crucial imine formation does not take place. Commonly, trisnorsqualenic acid **2.34** was observed as a side product and led to the hypothesis that a one-pot oxidation-amidation sequence could lead to the desired product.



Scheme 3.45: Attempted oxidative amidation between squalenaldehyde **3.3** and Gemcitabine

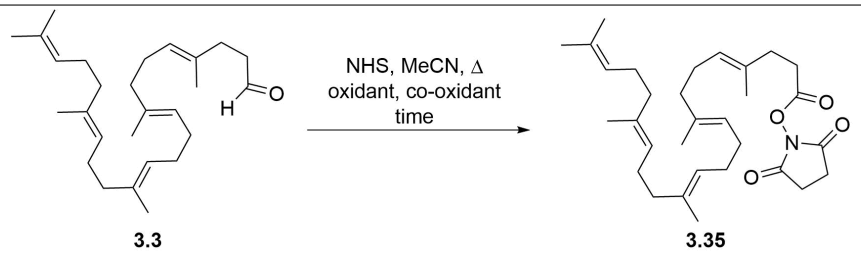
Complete oxidation of aldehyde **3.3** was observed after 16 hours at 40 °C. Elevating the temperature to 80 °C did not shorten the reaction time. At this point, Gemcitabine hydrochloride was added in a mixture of DMSO and DMF together with *N*-methylmorpholine, HOBt hydrate and EDC hydrochloride. Squalenoyl gemcitabine (**Sq-Gem**) was isolated in 20% overall yield after 24 hours. Although the reaction times were long, a successful one-pot oxidation amidation sequence was developed. Due to the large difference in stoichiometry of reagents in radiochemistry, shorter reaction times could be expected.



Scheme 3.46: Oxidation amidation sequence of squalenaldehyde **3.3** and Gemcitabine

Having failed to develop a direct oxidative amidation, it was hypothesised that an activated ester could be accessed from aldehyde **3.3**, which could shorten the amidation times. The successful amidation of gemcitabine with succinimide esters has been previously reported[70] and several methods are known to access these from aldehydes.[71,72]

Iodobenzene diacetate (PIDA) failed to oxidise aldehyde **3.3** but catalytic copper acetate with *tert*-butylhydroperoxide (*t*BuOOH) as co-oxidant delivered succinimide ester **3.35** (Table 3.3). As in the initial report, 0.5 equivalents of NHS were used and 40% of aldehyde **3.3** was consumed. Subsequently one equivalent of NHS was used and conversions around 50% were observed after 1.5 h hours. Increasing the amount of co-oxidant and elongating the reaction time somewhat was beneficial for the reaction. However, if the reaction time exceeded five hours, a reduction in conversion was observed. Increasing the catalyst loading to 30 mol% had no beneficial influence. The best reaction conditions were found to be 0.15 equivalents copper acetate, 2 equivalents of *tert*-butylhydroperoxide and NHS for 3.5 hours.

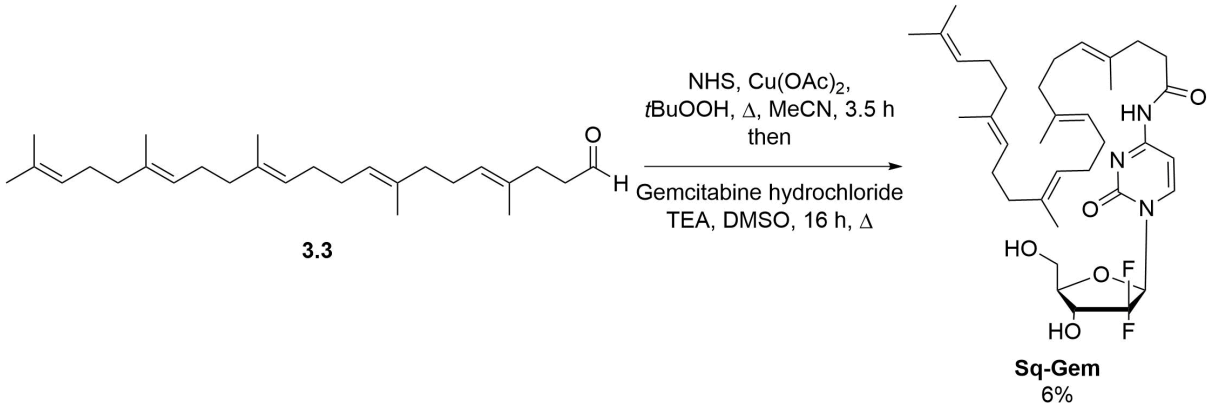


3.3 3.35

Entry	Oxidant (eq.)	co-oxidant (eq.)	eq. NHS	Time [h]	Conversion[a]
1	PIDA (1)	-	1	4	no rxn.
2	$Cu(OAc)_2$ (0.07)	*t*BuOOH (0.93)	0.5	0.5	40%
3	$Cu(OAc)_2$ (0.15)	*t*BuOOH (1)	1	1.5	50%
4	$Cu(OAc)_2$ (0.15)	*t*BuOOH (2)	1	3.5	70%
5	$Cu(OAc)_2$ (0.15)	*t*BuOOH (2)	2	7	57%
6	$Cu(OAc)_2$ (0.3)	*t*BuOOH (2)	1	5.5	65%

Table 3.3: Oxidation of aldehyde **3.3** to succinimide ester **3.35**: reaction carried out on a 130 μmol scale under reflux in acetonitrile for the indicated time. [a] Conversions determined by ^{1}H-NMR.

Subsequently, gemcitabine hydrochloride was added to the oxidation reaction but no reaction was observed, likely due to the low solubility of gemcitabine in acetonitrile. Successful amidation was observed when gemcitabine was added as a suspension in DMSO, although only in very low yields (Scheme 3.47). Filtering off remaining metal catalyst through celite before the addition of the drug did not improve the yield. The obtained results were nevertheless encouraging for a translation into radiochemistry, where the large excess of gemcitabine might give high amidation yields.



Scheme 3.47: One-pot oxidation amidation reaction

The two developed oxidation amidation sequences had the potential to be translated into a radiochemical setting. In both reactions, no purification was necessary between the oxidation and the amidation reaction. They could be performed in one pot, which would save valuable radiosynthesis time. Aldehyde **3.3** could be considered as a viable precursor for the synthesis of **^{18}F-Sq-Gem**.

3.3 Summary

In this chapter, the successful synthesis of a squalene-derived vinyl boronic ester is presented. A fragment-based synthesis for vinyl boronic esters was developed based on the synthesis of **F-Sq-Gem**. Changing the protection group strategy and reduction procedures afforded a highly robust synthesis. As in the synthesis of **F-Sq-Gem**, double bond isomers were encountered but could be separated by use of $AgNO_3$-impregnated silica. Adapting for the failed oxidation of aldehyde **3.32**, possible

post-labelling oxidation amidation sequences were investigated on squalenealdehyde **3.3** and gemcitabine. Two one-pot procedures were successfully developed. The radiolabelling of the obtained vinyl boronic ester will be discussed in the next chapter.

Literature

[1] X. Zeng, J. Li, C. K. Ng, G. B. Hammond, B. Xu, *Angew. Chemie - Int. Ed.* **2018**, 2924–2928.

[2] S. Preshlock, M. Tredwell, V. Gouverneur, *Chem. Rev.* **2016**, *116*, 719–766.

[3] N. Ichiishi, A. J. Canty, B. F. Yates, M. S. Sanford, *Org. Lett.* **2013**, *15*, 5134–5137.

[4] N. Ichiishi, A. F. Brooks, J. J. Topczewski, M. E. Rodnick, M. S. Sanford, P. J. H. Scott, *Org. Lett.* **2014**, *16*, 3224–3227.

[5] K. S. Jang, Y. W. Jung, G. Gu, R. A. Koeppe, P. S. Sherman, C. A. Quesada, D. M. Raffel, *J. Med. Chem.* **2013**, *56*, 7312–7323.

[6] J.-H. Chun, V. W. Pike, *Org. Biomol. Chem.* **2013**, *11*, 6300–6306.

[7] M. Ochiai, *J. Organomet. Chem.* **2000**, *611*, 494–508.

[8] V. V. Zhdankin, P. J. Stang, *Chem. Rev.* **2002**, *102*, 2523–2584.

[9] T. Okuyama, M. Fujita, R. Gronheid, G. Lodder, *Tetrahedron Lett.* **2000**, *41*, 5125–5129.

[10] R. Gronheid, G. Lodder, T. Okuyama, *J. Org. Chem.* **2002**, *67*, 693–702.

[11] M. Ochiai, M. Toyonari, T. Nagaoka, D. W. Chen, M. Kida, *Tetrahedron Lett.* **1997**, *38*, 6709–6712.

[12] E. Lee, A. S. Kamlet, D. C. Powers, C. N. Neumann, G. B. Boursalian, T. Furuya, D. C. Choi, J. M. Hooker, T. Ritter, *Science* **2011**, *334*, 639–642.

[13] E. Lee, J. M. Hooker, T. Ritter, *J. Am. Chem. Soc.* **2012**, *134*, 17456–17458.

[14] J. R. Brandt, E. Lee, G. B. Boursalian, T. Ritter, *Chem. Sci.* **2014**, *5*, 169–179.

[15] M. Tredwell, S. M. Preshlock, N. J. Taylor, S. Gruber, M. Huiban, J. Passchier, J. Mercier, C. Génicot, V. Gouverneur, *Angew. Chemie Int. Ed.* **2014**, *53*, 7751–7755.

[16] S. Preshlock, S. Calderwood, S. Verhoog, M. Tredwell, M. Huiban, A. Hienzsch, S. Gruber, T. C. Wilson, N. J. Taylor, T. Cailly, M. Schedler, T. L. Collier, J. Passchier, R. Smits, J. Mollitor, A. Hoepping, M. Mueller, C. Genicot, J. Mercier, V. Gouverneur, *Chem. Commun.* **2016**, *52*, 8361–8364.

[17] N. J. Taylor, E. Emer, S. Preshlock, M. Schedler, M. Tredwell, S. Verhoog, J. Mercier, C. Genicot, V. Gouverneur, *J. Am. Chem. Soc.* **2017**, *139*, 8267–8276.

[18] A. V. Mossine, A. F. Brooks, K. J. Makaravage, J. M. Miller, N. Ichiishi, M. S. Sanford, P. J. Scott, *Org. Lett.* **2015**, *17*, 5780–5783.

[19] N. Miyaura, A. Suzuki, *Chem. Rev.* **1995**, *95*, 2457–2483.

[20] S. Ben-Valid, A. A. A. Quntar, M. Srebnik, *J. Org. Chem.* **2005**, *70*, 3554–3559.

[21] T. Itoh, Y. Shimizu, M. Kanai, *J. Am. Chem. Soc.* **2016**, *138*, 7528–7531.

[22] T. Kikuchi, J. Takagi, T. Ishiyama, N. Miyaura, *Chem. Lett.* **2008**, *37*, 4–5.

[23] T. Kikuchi, J. Takagi, H. Isou, T. Ishiyama, N. Miyaura, *Chem. - An Asian J.* **2008**, *3*, 2082–2090.

[24] I. Sasaki, H. Doi, T. Hashimoto, T. Kikuchi, H. Ito, T. Ishiyama, *Chem. Commun.* **2013**, *49*, 7546.

[25] W. Yuan, X. Zhang, Y. Yu, S. Ma, *Chem. - A Eur. J.* **2013**, *19*, 7193–7202.

[26] M. Murata, T. Oyama, S. Watanabe, Y. Masuda, *Synthesis (Stuttg).* **2000**, *2000*, 778–780.

[27] J. Takagi, K. Takahashi, T. Ishiyama, N. Miyaura, *J. Am. Chem. Soc.* **2002**, *124*, 8001–8006.

[28] H. C. Brown, T. Imai, N. G. Bhat, *J. Org. Chem.* **1986**, *51*, 5277–5282.

[29] H. C. Brown, N. G. Bhat, *J. Org. Chem.* **1988**, *53*, 6009–6013.

[30] H. C. Brown, N. G. Bhat, *Tetrahedron Lett.* **1988**, *29*, 21–24.

[31] J. Wei, Y. Zhang, W.-X. Zhang, Z. Xi, *Org. Chem. Front.* **2014**, *1*, 983–987.

[32] E. Yamamoto, K. Izumi, Y. Horita, H. Ito, *J. Am. Chem. Soc.* **2012**, *134*, 19997–20000.

[33] D. S. Matteson, R. J. Moody, P. K. Jesthi, *J. Am. Chem. Soc.* **1975**, *97*, 5608–5609.

[34] K. Endo, M. Hirokami, T. Shibata, *J. Org. Chem.* **2010**, *75*, 3469–3472.

[35] J. R. Coombs, L. Zhang, J. P. Morken, *Org. Lett.* **2015**, *17*, 1708–1711.

[36] H. Li, X. Shangguan, Z. Zhang, S. Huang, Y. Zhang, J. Wang, *Org. Lett.* **2014**, *16*, 448–451.

[37] E. van Tamelen, T. Curphey, *Tetrahedron Lett.* **1962**, *3*, 121–124.

[38] E. van Tamelen, *Acc. Chem. Res.* **1968**, *1*, 111–120.

[39] S. E. Sen, G. D. Prestwich, *J. Am. Chem. Soc.* **1989**, *111*, 1508–1510.

[40] S. Borrelli, M. S. Christodoulou, I. Ficarra, A. Silvani, G. Cappelletti, D. Cartelli, G. Damia, F. Ricci, M. Zucchetti, F. Dosio, D. Passarella, *Eur. J. Med. Chem.* **2014**, *85*, 179–190.

[41] A. Maksimenko, F. Dosio, J. Mougin, A. Ferrero, S. Wack, L. H. Reddy, A.-A. Weyn, E. Lepeltier, C. Bourgaux, B. Stella, L. Cattel, P. Couvreur, *Proc. Natl. Acad. Sci.* **2014**, *111*, 217–226.

[42] M. C. Cabaleiro, R. Garay, *J. Chem. Soc. Perkin Trans. II* **1986**, 1091–1095.

[43] M. C. Cabaleiro, R. Garay, *J. Chem. Soc. Perkin Trans. II* **1987**, 1473–1476.

[44] M. Limbach, S. Dalai, A. De Meijere, *Adv. Synth. Catal.* **2004**, *346*, 760–766.

[45] W. P. Taylor, Z. Y. Zhang, T. S. Widlanski, *Bioorganic Med. Chem.* **1996**, *4*, 1515–1520.

[46] S. Kumar, B. Zhou, F. Liang, W.-Q. Wang, Z. Huang, Z.-Y. Zhang, *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 7943–7948.

[47] H. Firouzabadi, N. Iranpoor, S. Sobhani, *Tetrahedron* **2004**, *60*, 203–210.

[48] G. Jiang, Y. Xu, Y. Fujiwara, T. Tsukahara, R. Tsukahara, J. Gajewiak, G. Tigyi, G. D. Prestwich, *ChemMedChem* **2007**, *2*, 679–690.

[49] G. Bassolino, T. Sovdat, M. Liebel, C. Schnedermann, B. Odell, T. D. Claridge, P. Kukura, S. P. Fletcher, *J. Am. Chem. Soc.* **2014**, *136*, 2650–2658.

[50] K. Tanaka, K. Kobayashi, H. Kogen, *Org. Lett.* **2016**, *18*, 1920–1923.

[51] S. Zhao, Y. hong He, D. Wu, Z. Guan, *J. Fluor. Chem.* **2010**, *131*, 597–605.

[52] M. Gatti, E. Drinkel, L. Wu, I. Pusterla, F. Gaggia, R. Dorta, *J. Am. Chem. Soc.* **2010**, *132*, 15179–15181.

[53] K. S. Lau, M. Schlosser, *J. Org. Chem.* **1978**, *43*, 1595–1598.

[54] H. Neumann, D. Seebach, *Tetrahedron Lett.* **1976**, *17*, 4839–4842.

[55] S. Balieu, A. M. Bouraiou, B. Carboni, B. Boitrel, *J. Porphyr. Phthalocyanines* **2008**, *12*, 11–18.

[56] B. O. Lindgren, T. Nilsson, *Acta Chem. Scand.* **1973**, *27*, 888–890.

[57] B. S. Bal, W. E. Childers, H. W. Pinnick, *Tetrahedron* **1981**, *37*, 2091–2096.

[58] G. A. Kraus, M. J. Taschner, *J. Org. Chem.* **1980**, *45*, 1175–1176.

[59] K. Nakagawa, H. Onoue, K. Minami, *J. Chem. Soc. Chem. Commun.* **1966**, 17–18.

[60] A. Tillack, I. Rudloff, M. Beller, *European J. Org. Chem.* **2001**, *2001*, 523–528.

[61] J. W. W. Chang, P. W. H. Chan, *Angew. Chemie - Int. Ed.* **2008**, *47*, 1138–1140.

[62] Y. Suto, N. Yamagiwa, Y. Torisawa, *Tetrahedron Lett.* **2008**, *49*, 5732–5735.

[63] S. Seo, T. J. Marks, *Org. Lett.* **2008**, *10*, 317–319.

[64] A. M. Whittaker, V. M. Dong, *Angew. Chemie - Int. Ed.* **2015**, *54*, 1312–1315.

[65] I. E. Marko, A. Mekhalfia, *Tetrahedron Lett.* **1990**, *31*, 7237–7240.

[66] K. Rajender Reddy, C. Uma Maheswari, M. Venkateshwar, M. Lakshmi Kantam, *European J. Org. Chem.* **2008**, 3619–3622.

[67] K. Ekoue-Kovi, C. Wolf, *Org. Lett.* **2007**, *9*, 3429–3432.

[68] K. S. Goh, C.-H. Tan, *RSC Adv.* **2012**, *2*, 5536–5538.

[69] R. Deshidi, M. A. Rizvi, B. A. Shah, *RSC Adv.* **2015**, *5*, 90521–90524.

[70] G. Pasut, F. Canal, L. Dalla Via, S. Arpicco, F. M. Veronese, O. Schiavon, *J. Control. Release* **2008**, *127*, 239–248.

[71] M. Pilo, A. Porcheddu, L. De Luca, *Org. Biomol. Chem.* **2013**, *11*, 8241–8246.

[72] H. Yao, Y. Tang, K. Yamamoto, *Tetrahedron Lett.* **2012**, *53*, 5094–5098.

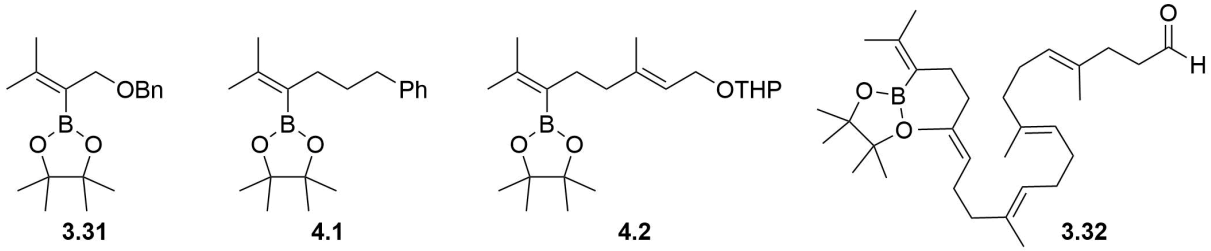
CHAPTER 4

Radiofluorination of Vinyl Boronic Esters

The synthetic work in the following chapter was performed by G.S. Cremosnik except noted otherwise . Radiofluorination experiments were carried out by Dr. S. Preshlock, T. Wilson, Dr. N. Taylor, Dr. S. Verhoog, Dr. N. Straathof and Dr. P. Isenegger

4.1 Radiofluorination of Terpenoid Derived Boronic Esters

The successful synthesis of prenol derivative **3.31** and aldehyde **3.32** (Scheme 4.1) allowed for the investigation of the copper-mediated radiofluorination of vinylboronic esters. To gain insight into the reaction without using the high value aldehyde **3.32**, a set of terpenoid derived vinyl boronic esters was prepared. Boronic ester **4.1** and geraniol derivative **4.2** were chosen as intermediates.



3.31 **4.1** **4.2** **3.32**

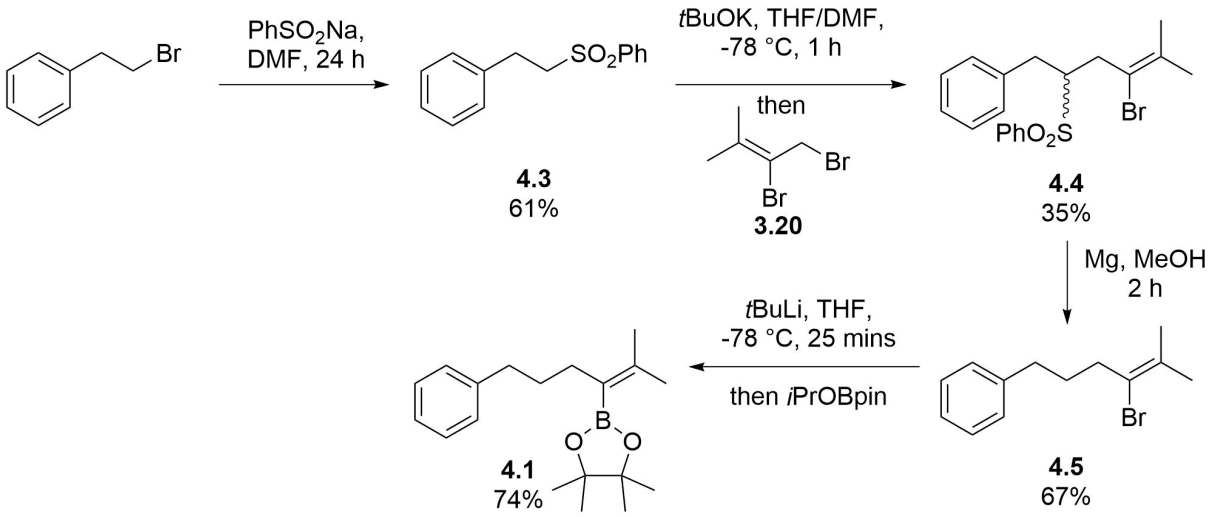
Scheme 4.1: Synthesised terpenoid based vinyl boronic esters

In the first part, the synthesis of the required boronic esters and fluorine-19 derivatives is presented (Section 4.1.1). In Section 4.1.2, the results of the radiolabelling are discussed.

4.1.1 Synthesis of Model Boronic Esters 4.1 and 4.2

4.1.1.1 Synthesis of Boronic Ester 4.1 and Fluorine-19 reference

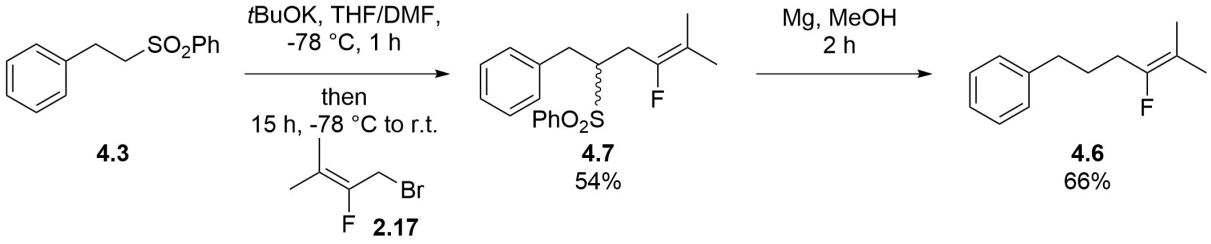
(2-Bromoethyl)benzene was treated with benzenesulfinic acid sodium salt in DMF to obtain sulfone **4.3**. Deprotonation of the sulfone with potassium *tert*-butoxide and subsequent nucleophilic substitution on bromide **3.20** afforded vinyl bromide **4.4** in low yields. Sulfone **4.4** was reduced with magnesium in methanol in good yields. The resulting bromide **4.5** was borylated with *i*PrOBpin, after lithium-halogen exchange.



Scheme 4.2: Synthesis of vinyl boronic ester **4.1**

The fluorinated reference compound **4.6** was synthesised from sulfonylanion of sulfone **4.3** by alkylation using vinyl fluoride **2.17** as the electrophile (Scheme 4.3). The yield of the sulfonylation reaction was higher than observed for the brominated electrophile. This observation is likely due to the electronic effects of the fluoride substituent on the

allylic position and the higher steric demand of bromide. Reduction of sulfone **4.7** with magnesium in methanol afforded vinyl fluoride **4.6** in good yields.

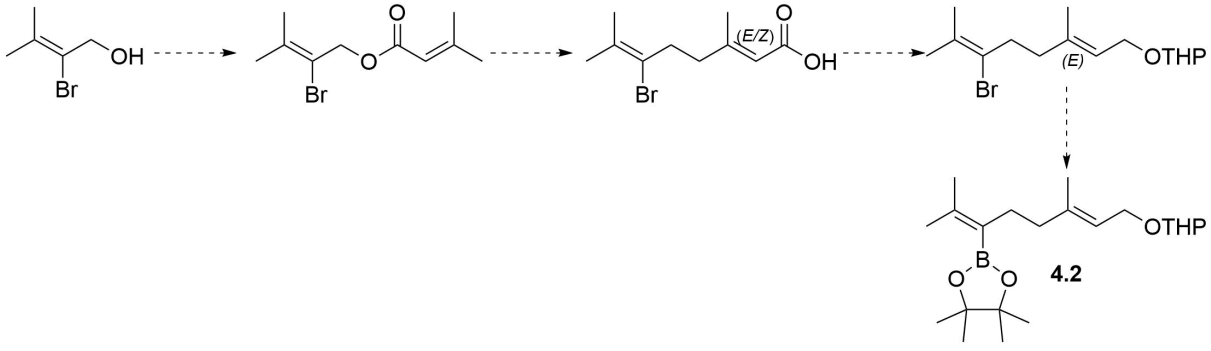


Scheme 4.3: Synthesis of vinyl fluoride **4.6**

The results of the radiolabelling reaction are presented in Section 4.1.2.

4.1.1.2 Synthesis of a Geraniol Derived Vinyl Boronic Ester 4.2 and its Fluoro-Analogue

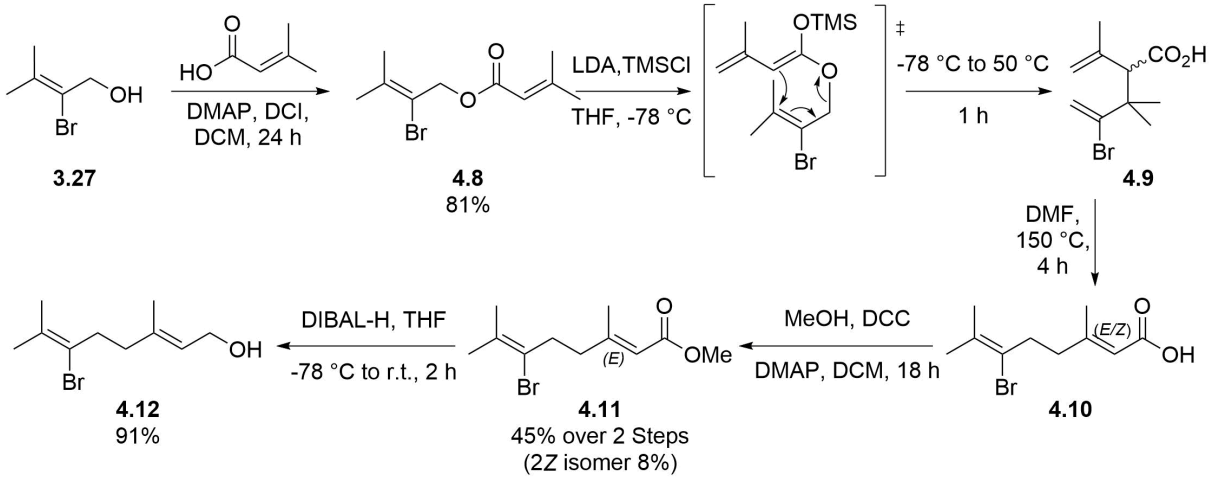
The synthesis of the geraniol derived vinyl boronic ester **4.2** was inspired by Camps' synthesis of 6-fluoro-geraniol (Scheme 4.4).[1] By substituting the fluorine for a bromine moiety, a late stage borylation could give access to the desired vinyl boronic ester **4.2**.



Scheme 4.4: Synthesis plan for a geraniol derived vinyl boronic ester

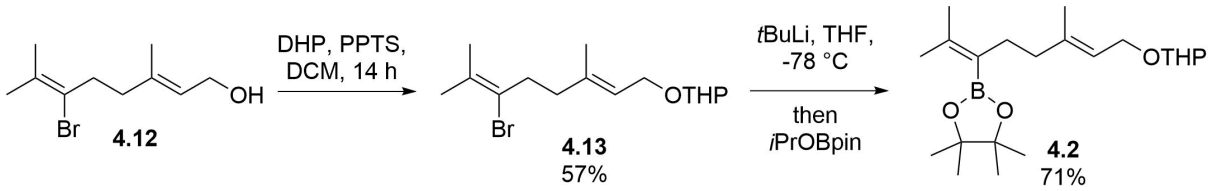
4.1.1.2.1 Synthesis of Vinyl Boronic Ester 4.2

Ester **4.8** was synthesised from alcohol **3.27** (Scheme 4.5). Claisen-Cope rearrangement was successfully achieved under the conditions published by Camps and co-workers.[1] *In situ* formation of a silyl enol ether and subsequent Claisen rearrangement at 50 °C afforded diene **4.9**, which was not isolated. After addition of DMF, the reaction mixture was gradually heated to 150 °C, while THF was removed by distillation. The resulting acid **4.10** was obtained as an inseparable mixture of *E/Z*-stereoisomers. Esterification of acid **4.10** with methanol allowed the separation of the stereoisomers by column chromatography. It is noteworthy, that the ratio of products changed depending on the scale of the reaction. On a 1.5 mmol scale a 2*E*:2*Z* ratio of 9:1 was observed after isolation, while the ratio dropped to 6.5:1 when the reaction was performed on 23 mmol. Reduction with DIBAL-H afforded alcohol **4.12** in excellent yields.



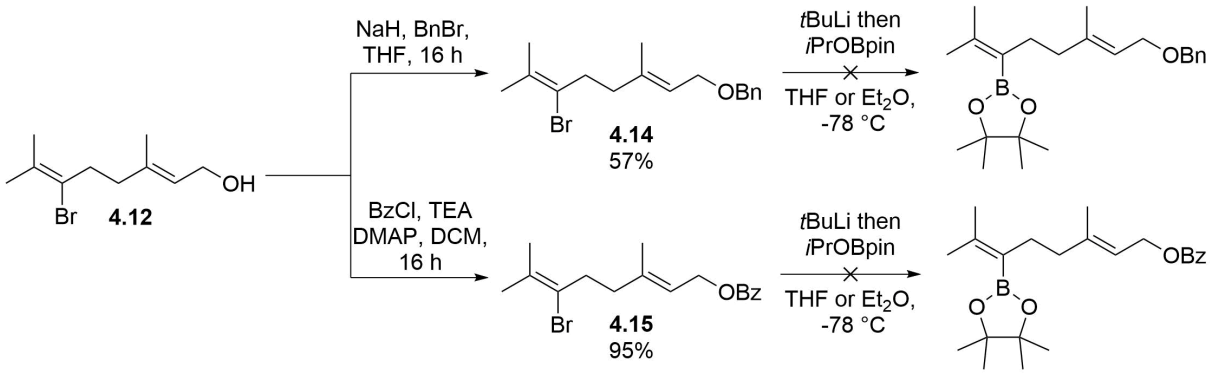
Scheme 4.5: Synthesis of 6-bromo-geraniol (*4.12*)

The obtained alcohol **4.12** was protected with dihydropyran (DHP) to afford the tetrahydropyranyl ether (THP ether) **4.13** (Scheme 4.6). Lithium-halogen exchange of bromide **4.13** followed by borylation with *i*PrOBpin afforded vinyl boronic ester **4.2** in good yields.



Scheme 4.6: Synthesis of vinyl boronic ester **4.2**

It was necessary to deviate from the preferred benzyl protecting group as the lithium halogen exchange resulted in the decomposition of ether **4.14** (Scheme 4.7). The same was observed for the benzoyl protected alcohol **4.15**.

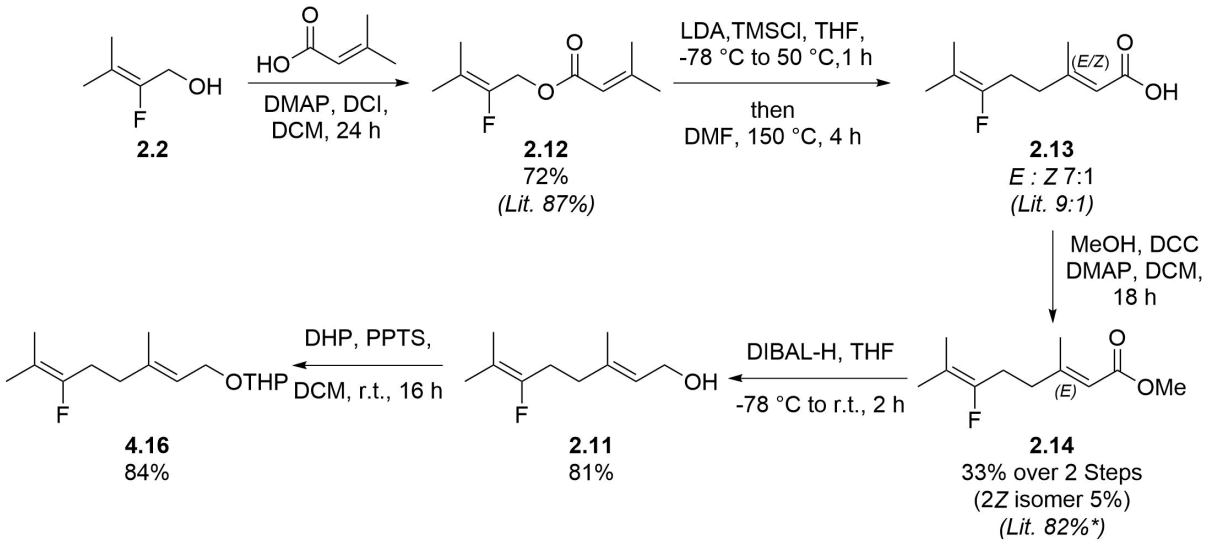


Scheme 4.7: Protection of 6-bromo-geraniol and attempted borylations

4.1.1.2.2 Synthesis of THP-protected 6-Fluoro-Geraniol

The synthesis of 6-fluoro-geraniol was achieved as previously published by Camps and co-workers.[1] Alcohol **2.2** was prepared as described in Chapter 2 and underwent

esterification with 3,3-dimethylacrylic acid to afford ester **2.12** (Scheme 4.8). After *in situ* formation of the silyl enol ether and subsequent Claisen rearrangement at 50 °C, the expected diene was obtained. Adding DMF to the reaction mixture and heating to 150 °C afforded acid **2.13**. Both rearrangements were performed in one-pot by mounting a distillation bridge after completion of the Claisen rearrangement. Acid **2.13** was obtained as mixture of stereoisomers (2*E*:2*Z* 7:1 by ^{19}F-NMR). The reported 9:1 ratio of stereoisomers was not obtained.[1] Esterification of the crude acid **2.13** with methanol allowed 2*E*-ester **2.14** to be separated by column chromatography. Reduction using DIBAL-H afforded 6-fluoro-geraniol (**2.11**) in high yields. The primary alcohol was protected with DHP resulting in the desired ether **4.16**.



Scheme 4.8: Synthesis of THP-protected 6-fluoro-geraniol (**4.16**), Literature values from Ref[1]: *alternative methylation procedure used (original: MeI, K_2CO_3 in acetone)

The procedure of Camps was successfully reproduced albeit in lower yields and stereoselectivity. The altered methylation procedure allowed direct esterification of acid **2.13** without purification.

4.1.2 Radiofluorination of Vinyl Boronic Esters

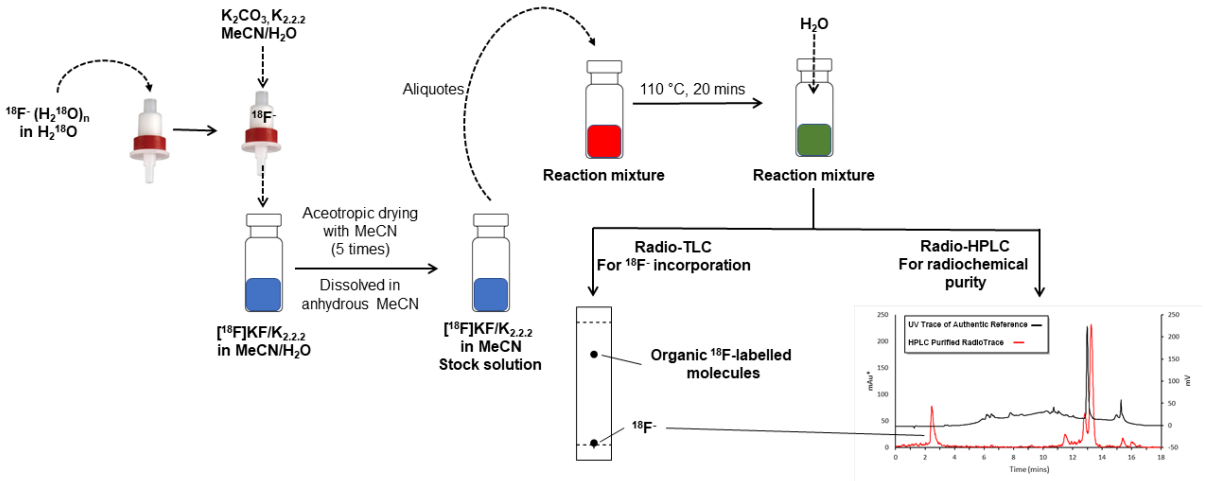
With the required vinyl boronic esters at hand the radiofluorination could be investigated. In the following section, the general procedure of a radiofluorination reaction will be discussed first (Section 4.1.2.1), then the results for the terpenoid derived precursors are presented (Section 4.1.2.2).

4.1.2.1 General Procedure of Copper-Mediated Radiofluorinations

The radiofluorination reactions of vinyl boronic esters were performed and analysed as outlined below.

[^{18}F]Fluoride was received from a commercial cyclotron operator in [^{18}O]-enriched water (Scheme 4.9). As most radiochemical reactions can not be performed in water, a standardised procedure was followed to dehydrate fluoride-18. The solution was passed through a strong anion exchange cartridge trapping the [^{18}F]fluoride. The radioisotope was then eluted with a solution of potassium carbonate and Kryptofix 222 in acetonitrile/water. The [^{18}F]KF/$K_{2.2.2}$ salt was azeotropically dried under a stream of nitrogen at 105 °C. The drying cycle was repeated four times with anhydrous acetonitrile. Finally, a stock solution in acetonitrile was prepared. Aliquots from the [^{18}F]KF/$K_{2.2.2}$ solution (20-30 MBq) were added to a mixture of [$Cu(OTf)_2py_4$] and the substrate (usually 20 μmol) in the desired solvent. Solvents frequently used were *N,N*-dimethylacetamide (DMA) and 1,3-Dimethyl-2-imidazolidinone (DMI).

The reaction vessels were flushed with air (30 ml) and the mixture was heated to 110 °C for 20 minutes before quenching with water. Radio-TLC was employed to measure [^{18}F]fluoride incorporation. Radio-HPLC was used to identify the radioactive species, by comparing retention times to those of the fluorine-19 analogues. Integration of the obtained HPLC signals provided the radiochemical purity. The radiochemical conversion (RCC) was calculated by multiplying the radiochemical purity with the amount of incorporated [^{18}F]fluoride from the radio-TLC. The experiments were performed typically in duplicate.

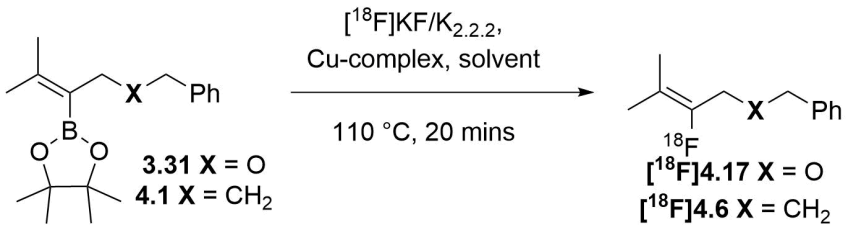


Scheme 4.9: General procedure of a copper mediated radiofluorination

4.1.2.2 Results of Radiolabelling

The radiofluorination of prenol derived vinyl boronic ester **3.31** was investigated first. An excess of $[Cu(OTf)_2py_4]$ (1.5 eq. with respect to vinyl boronic ester **3.31**) afforded the desired radiofluorinated product **[^{18}F]4.17** in 92% radiochemical conversion (RCC) (Table 4.1, Entry 1). Using substoichiometric amounts of copper-mediator (0.08 eq.)

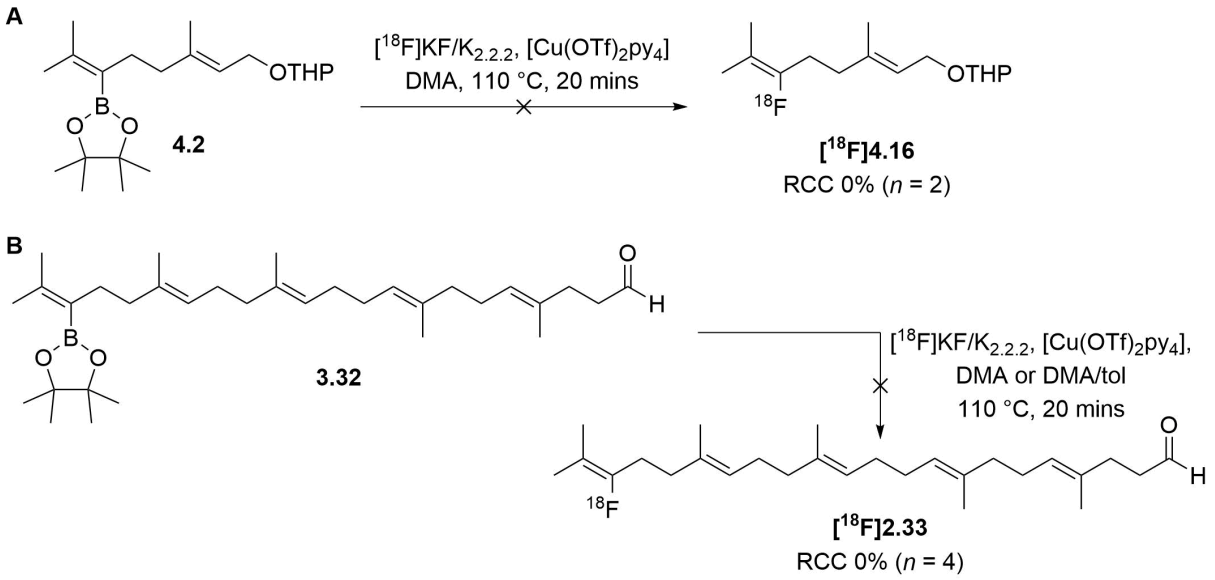
decreased the RCC to 69% (Entry 2). Surprisingly, only 4% RCC was obtained when vinyl boronic ester **4.1** was used under the same conditions (Entry 3). Changing the solvent from DMA to DMI did reduce the RCC further (Entry 4). No [^{18}F]fluoride incorporation was observed when the metal catalyst was changed to $[Cu(OTf)_2(impy)_4]$ (impy = imidazo[1,2-*b*]pyridazine) in either solvent (Entry 5 and 6).



Entry	**X**	Cu source	Eq. [Cu][a]	Solvent	RCC
1	O	$[Cu(OTf)_2py_4]$	1.5	DMA	92% ± 1%(*n* = 2)
2	O	$[Cu(OTf)_2py_4]$	0.08	DMA	69% ± 2%(*n* = 2)
3	CH_2	$[Cu(OTf)_2py_4]$	1.5	DMA	4% ± 1% (*n* = 4)
4	CH_2	$[Cu(OTf)_2py_4]$	1.5	DMI	2% ± 0% (*n* = 2)
5	CH_2	$[Cu(OTf)_2(impy)_4]$	1.5	DMA	0% (*n* = 2)
6	CH_2	$[Cu(OTf)_2(impy)_4]$	1.5	DMI	0% (*n* = 2)

Table 4.1: Investigation of the radiofluorination of vinyl boronic ester **3.31** and **4.1**: starting activity approx. 30 MBq of [^{18}F]KF/$K_{2.2.2}$, *n* = number of experiments done, [a] with respect to the vinyl boronic ester

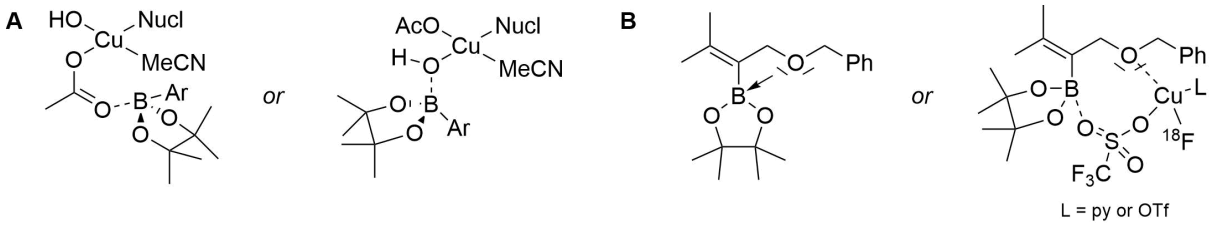
The optimal reaction conditions for prenol **3.31** (1.5 eq. $[Cu(OTf)_2py_4]$, DMA, 110 °C, 20 mins) were then applied to the geraniol derived boronic ester **4.2** and aldehyde **3.32** (Scheme 4.10 **A** and **B**). In both reactions, no incorporation of [^{18}F]fluoride was observed.



Scheme 4.10: A Attempted radiofluorination of vinyl boronic ester **4.2**; **B** Attempted radiofluorination of aldehyde **3.32**

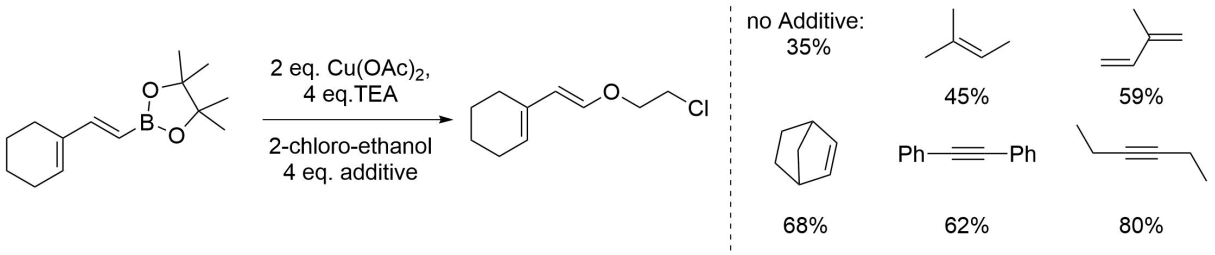
The obtained data demonstrate the possibility to radiofluorinate vinyl boronic esters through copper mediation under certain conditions. Prenol derived ether **3.31** underwent successful [^{18}F]fluorination in high radiochemical conversions. The allylic ether functionality played an important role in promoting the reaction.

Recent mechanistic investigations of Watson and co-workers on Chan-Lam reactions of aryl boronic esters indicated the necessity for the formation of a pre-transmetallation boronate complex (Scheme 4.11 **A**).[2] It was hypothesised that the allylic ether in vinyl boronic ester **3.31** could act as a Lewis base and facilitate the transmetallation by either interacting with the boronic ester or the copper complex (**B**).



Scheme 4.11: A Pre-transmetallation transition states proposed by Watson and co-workers[2]; **B** Possible interactions of the oxygen lone pairs onto the radiolabelling reaction of ether **3.31**

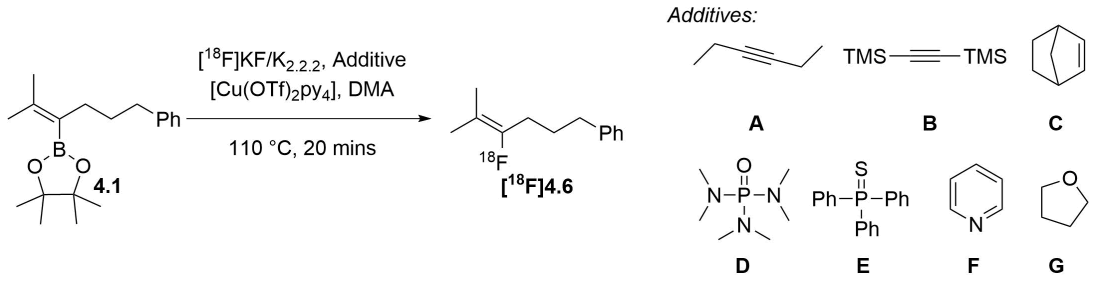
To this date, little is known about Chan-Lam reactions of vinyl boronic esters. Merlic and co-workers reported the first and only examples on terminal vinyl boronic esters.[3,4] Addition of an alkene or alkyne to the reaction mixture was found to be beneficial for the Chan-Lam reaction and higher yields were obtained (Scheme 4.12).[4] Alkenes and alkynes have been shown to act as π-Lewis bases to copper, which could explain the reaction promoting effect.[5,6]



Scheme 4.12: Lewis base promoted Chan-Lam coupling[4]

Although vinyl boronic ester **3.32** and **4.2** contain proximal double bonds, no radiofluorination reaction was observed. They likely did not interact with the copper-mediator due to conformational restrictions of the terpenoid backbone. It was decided to test a range of alkenes, alkynes and other Lewis basic additives in a radiofluorination reaction. Vinyl boronic ester **4.1** was chosen because conversion

was observed without an additive and the effect of those would display directly in the change of RCC (Table 4.2). For the reactions, one equivalent of additive was added to the reaction mixture before the addition of [^{18}F]KF/$K_{2.2.2}$. None of the additives had an influence on the radiochemical conversion. Whether a different set of additives would promote the radiofluorination or if an intramolecular activation is necessary, was not clear.



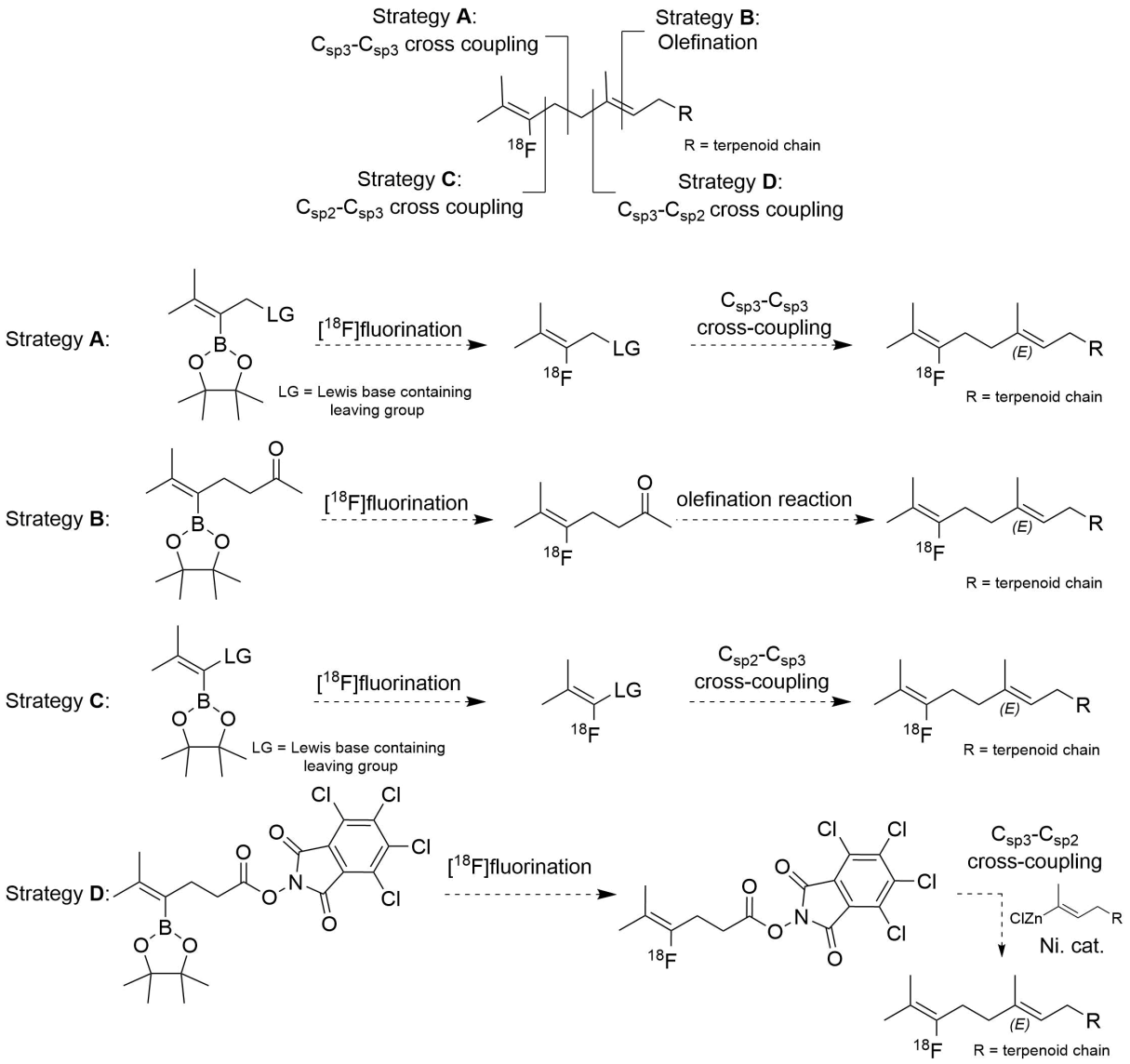
Entry	Additive	RCC
1	None	4% ± 1% (*n* = 4)
2	**A**	3% ± 1%(*n* = 2)
3	**B**	4% ± 0% (*n* = 2)
4	**C**	3% ± 1% (*n* = 2)
5	**D**	3% ± 1% (*n* = 2)
6	**E**	3% ± 1% (*n* = 2)
7	**F**	3% ± 1% (*n* = 2)
8	**G**	3% ± 1% (*n* = 2)

Table 4.2: Effect of Lewis base additives on the radiolabelling of vinyl boronic ester **4.1**: Reactions carried out with 0.02 mmol of **4.1**, 1 eq. of additive, 1.5 eq. of $[Cu(OTf)_2py_4]$

In conclusion, the feasibility of a copper mediated radiofluorination of vinyl boronic esters was demonstrated. An intramolecular activation through a Lewis base was found to be necessary. Intermolecular activation of the radiolabelling reaction with Lewis basic additives was not successful. The focus of the project was subsequently shifted to explore new ways to combine the intramolecular activation with **[^{18}F]F-Sq-Gem** synthesis. Several new synthesis strategies were developed and are presented in Section 4.2.

4.2 Alternative Synthesis of ^{18}F-Sq-Gem

The radiolabelling of vinyl boronic esters required a Lewis basic group close to the reaction site. With this restriction in mind, new approaches for ^{18}**F-Sq-Gem** were conceived. The plan will be to radiolabel a small fragment containing a Lewis basic functional group, which is connected post-labelling to the remaining terpenoid chain. Post-labelling reactions are frequently encountered in radiotracer synthesis to remove protecting groups or form carbon-heteroatom bonds. The terpenoid backbone was analysed and possible disconnections were identified (Scheme 4.13).



Scheme 4.13: Possible post-labelling carbon-carbon bond forming reactions

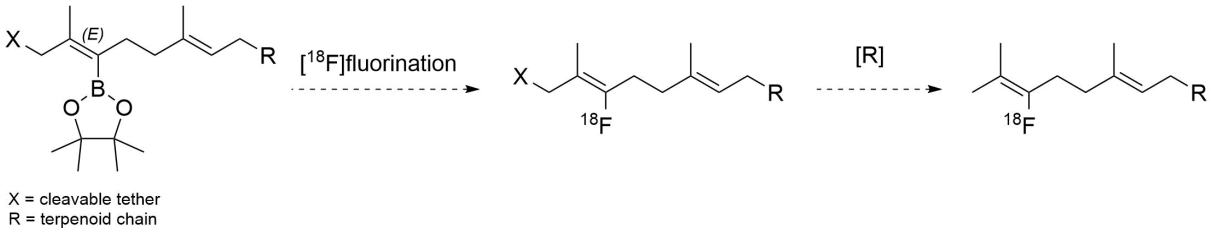
Of the aforementioned strategies, **A** and **C** were excluded as they would require the handling of highly volatile radiofluorinated fragments.

Wittig-type olefinations (Strategy **B**) have been developed in large varieties and were investigated in depth. This approach was considered to be low risk - high reward due to the facile synthesis of model compounds and possible precursors. The carbonyl

functionality could act as the Lewis base, enabling the radiofluorination. A challenge will be the stereoselective formation of the desired *E*-double bond.

The recently published C_{sp3}-C_{sp2} decarboxylative cross couplings by Baran and co-workers, was expected to be a suitable methodology for the post-labelling assembly of terpenoid backbones (Strategy **D**).[7] The redox-active ester would provide the required Lewis basic activation and undergo cross-coupling with a vinyl zinc reagent post-labelling furnishing the terpenoid backbone. Such a cross-coupling has not been attempted in a radiochemical environment before. For a successful translation it will be required to investigate the reaction for its sensitivity to excess reagents and non-anhydrous conditions.

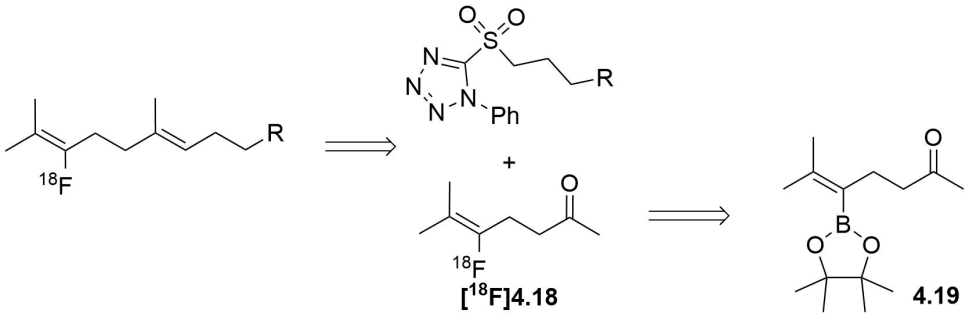
Proximity of the activating functional group to the vinyl boronic ester was expected to be crucial. Alternatively to the presented constructive post-labelling reactions, a strategy was considered where the activating group would be removed after the radiolabelling (Scheme 4.14). A functional group X could be placed on the terminal methyl group, which would facilitate the radiofluorination reaction. Afterwards, the tether would be chemoselectively reduced affording the desired terpenoid derivative.



Scheme 4.14: Tether approach to radiofluorination

4.2.1 Strategy B: Double Bond Forming Reactions

Wittig reactions and modifications thereof are reliable tools for organic chemists. The main challenge when applied to terpenoid synthesis is the control of double bond geometry. It was expected that phosphonium ylides would preferably afford *Z*-geometry.[8] Sulfones, as employed in the Julia-Kocienski reaction, show higher *E*-selectivity in electronically unbiased systems.[9] It was decided to use 1-phenyl-1*H*-tetrazol-5-yl sulfones (PT) for the coupling to the radiofluorinated ketone **[^{18}F]4.18**, which is synthesised from vinyl boronic ester **4.19**.

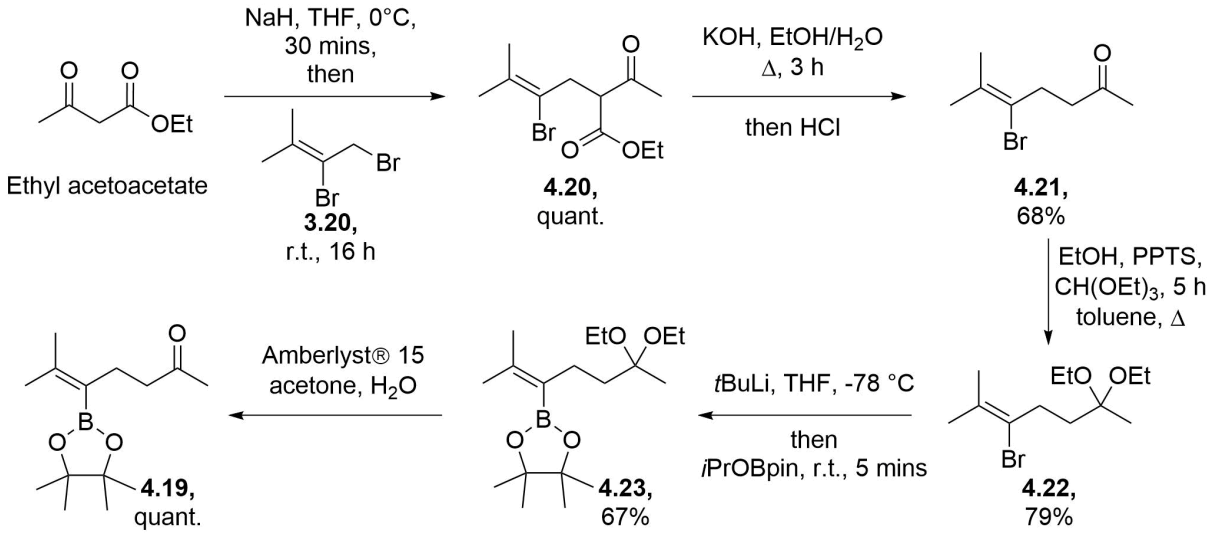


Scheme 4.15: Retrosynthetic analysis for post-labelling Julia-Kocienski reaction

4.2.1.1 Synthesis of Ketone [^{18}F]4.18

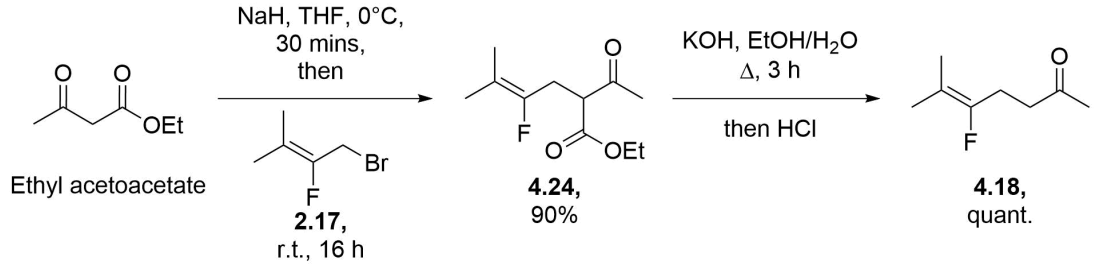
The required vinyl boronic ester precursor **4.19** was synthesised from bromide **3.20** (Scheme 4.16). Nucleophilic attack of ethyl acetoacetate, after deprotonation with sodium hydride, on bromide **3.20** afforded β-keto ester **4.20** in quantitative yields. Decarboxylation to ketone **4.21** was achieved under aqueous basic conditions and reflux. To avoid carbanion addition during the borylation reaction, the ketone was

protected as acetal **4.22**. Lithium-halogen exchange was achieved with *tert*-butyllithium and the formed organolithium was quenched with *i*PrOBpin affording vinyl boronic ester **4.23** in good yields. Finally, the acetal was hydrolysed to ketone **4.19** in quantitative yields.



Scheme 4.16: Synthesis of vinyl boronic ester **4.19**

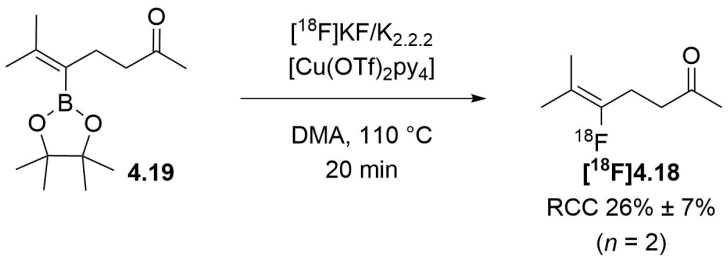
The required ^{19}F-reference **4.18** was synthesised from bromide **2.17** (see Scheme 4.17) in two steps with excellent overall yield.



Scheme 4.17: Synthesis of vinyl fluoride **4.18**

Radiofluorination of vinyl boronic ester **4.19** was achieved under the same conditions as discussed earlier ([^{18}F]KF/$K_{2.2.2}$, 1.5 eq. [$Cu(OTf)_2py_4$], DMA, 110 °C, 20 mins) and

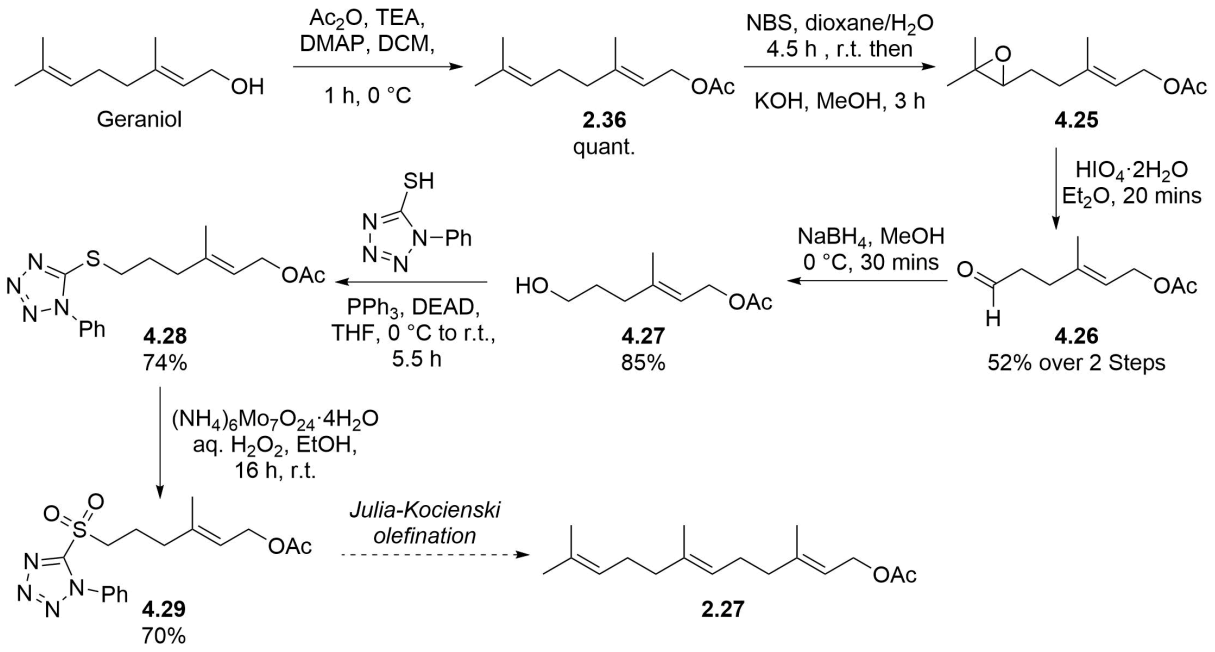
afforded the desired **[^{18}F]4.18** in 26% RCC (Scheme 4.18). The obtained RCC allows to produce enough **[^{18}F]4.18** to investigate the post-labelling olefination. The ability of ketones to promote the radiofluorination of vinyl boronic esters was encouraging for other strategies utilising carbonyl compounds.



Scheme 4.18: Radiosynthesis of **[^{18}F]4.18**

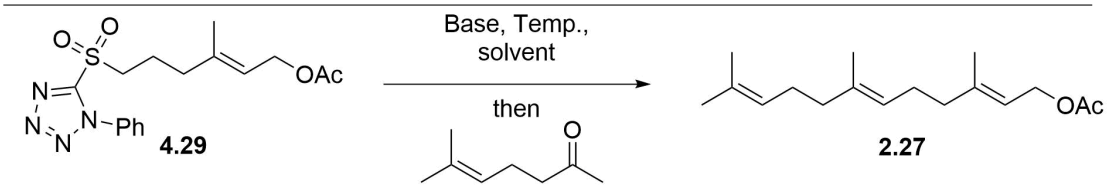
4.2.1.2 Julia-Kocienski Reaction for Terpenoid Synthesis

The Julia-Kocienski reaction was first investigated on model substrate to afford farnesol acetate (**2.27**, Scheme 4.19). The required sulfone was synthesised from geraniol. Acetylation of geraniol afforded ester **2.36** in quantitative yields. The terminal double bond was selectively epoxidised by forming the bromohydrin and *in situ* elimination of bromine. The crude epoxide **4.25** underwent oxidative cleavage to aldehyde **4.26**. Reduction with sodium borohydride resulted in alcohol **4.27**. Mitsunobu reaction of alcohol **4.27** with 1-phenyl-1*H*-tetrazol-5-thiol afforded thioether **4.28**, which was oxidised to PT-sulfone **4.29** under mild conditions.



Scheme 4.19: Synthesis of PT-sulfone **4.29**

Increasing stereoselectivity towards the *trans*-product was reported with bulky bases and bigger counter ions (KHMDS > NaHMDS > LiHMDS).[9] Deprotonation of sulfone **4.29** with KHMDS in dry diethyl ether, followed by treatment with 6-methyl-5-hepten-2-one did not afford the desired product (Table 4.3, Entry 1). Changing the solvent to THF afforded trace amounts of the desired farnesyl acetate **2.27** (Entry 2). NaHMDS afforded 9% of acetate **2.27**, while LiHMDS did not undergo the desired reaction (Entry 3 and 4). It was observed that the deprotonation reaction requires temperatures as low as -78 °C to avoid decomposition (Entry 5).

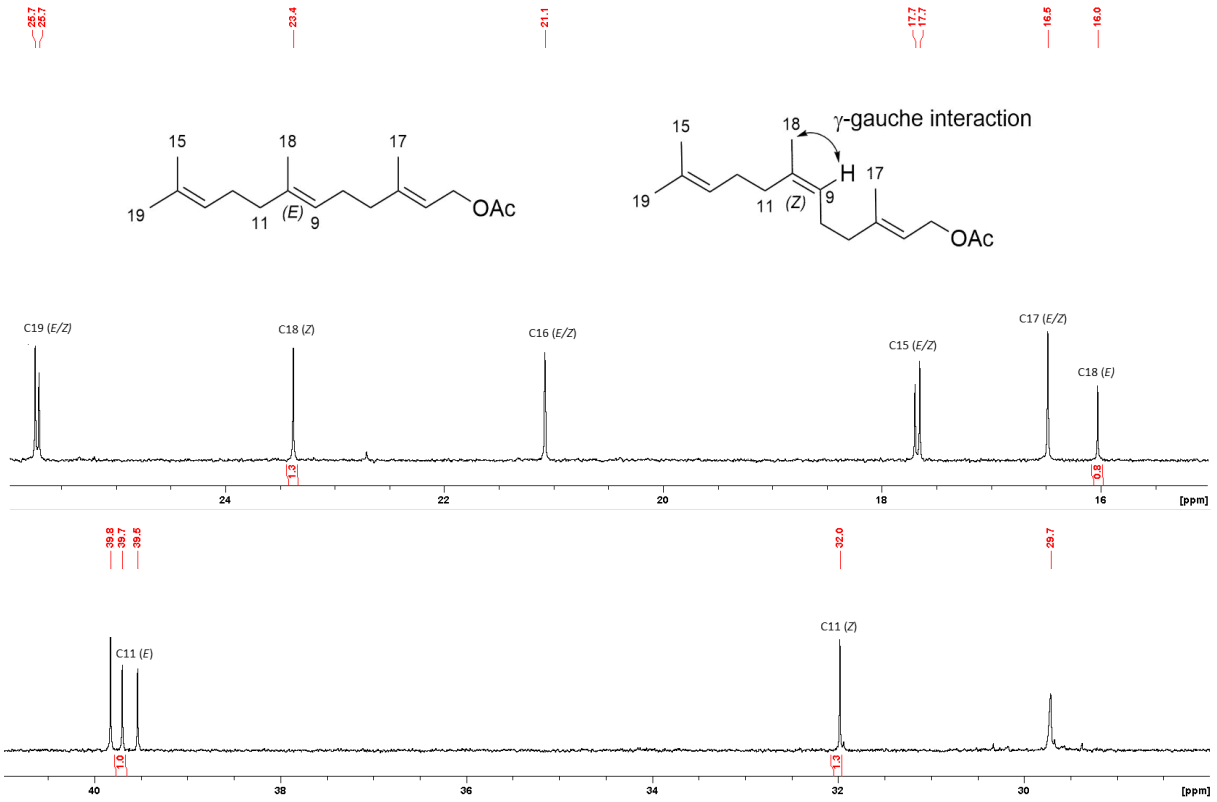


Entry	Base	Solvent	Temperature	Yield
1	KHMDS	Et_2O	-78 °C	N.A.
2	KHMDS	THF	-78 °C	trace
3	NaHMDS	THF	-78 °C	9%
4	LiHMDS	THF	-78 °C	N.A.
5	NaHMDS	THF	-10 °C	N.A.

Table 4.3: Julia-Olefination of PT-sulfone **4.29**; reaction carried out on a 54 μmol scale with 1.2 eq. base

The ratio of stereoisomers was determined by ^{13}C-NMR spectroscopy (Scheme 4.20). The γ-gauche interaction of the protons on C18 and C9 in the *Z*-isomer shifted the C18 signal downfield (*Z* isomer 23.4 ppm vs. *E* isomer 15.0 ppm). This observation enabled the assignment of the two stereoisomers by HMBC, HSQC and COSY.[1] Integration of the C11 peaks (*Z*-isomer 32.0 ppm and *E*-isomer 39.7 ppm) revealed an *E:Z*-ratio of 1:1.3.

[1]Data acquisition and interpretation performed by Dr. N. Amin, NMR Facility, Oxford University



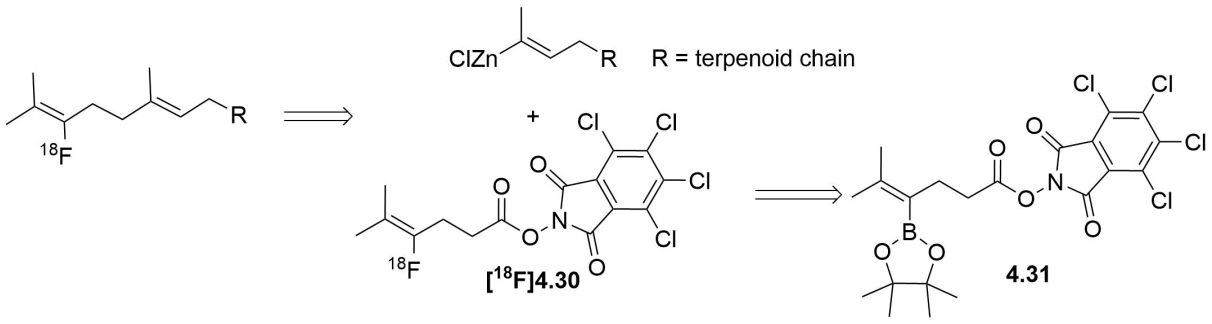
Scheme 4.20: ^{13}C-NMR of *E:Z* mixture of acetate **2.27**

The NMR investigation showed that the reaction preferentially forms the undesired *Z*-isomer. While KHMDS likely would have improved the ratio in favour of the *E*-isomer, it was not expected that a synthetically useful ratios could be obtained. The reaction required low temperatures (-78 °C) to afford the desired product, which can not be achieved with the available radiochemical instrumentation.

In conclusion, the successful radiofluorination of a vinyl boronic ester using a ketone, as reaction promoting functional group, was demonstrated. The planned post-labelling Julia-Kocienski reaction afforded low yields and an unfavourable *E:Z*-ratio on a model substrate. Therefore the olefination was not translated into a radiochemical setting and the approach was abandoned.

4.2.2 Strategy D: Post-labelling $C_{sp3} - C_{sp2}$ coupling reactions

In the previous section, it was demonstrated that ketones were able to mediate the radiofluorination reaction. It was expected that other carbonyl functional groups would behave similarly. A radiolabelled redox-active ester (**[^{18}F]4.30**) could be used to construct the terpenoid backbone post-labelling, through decarboxylative alkenylation reactions (Scheme 4.21). The elegant reaction presented by Baran and co-workers was shown to work under various conditions.[7] It was decided to explore this methodology for post-labelling modification and the following aims were set: 1) successful cross-coupling of the fluorinated fragment (**4.30**) to a model substrate, 2) translation of the cross-coupling onto a terpenoid backbone and 3) successful radiofluorination of a redox-active ester (**4.31**).

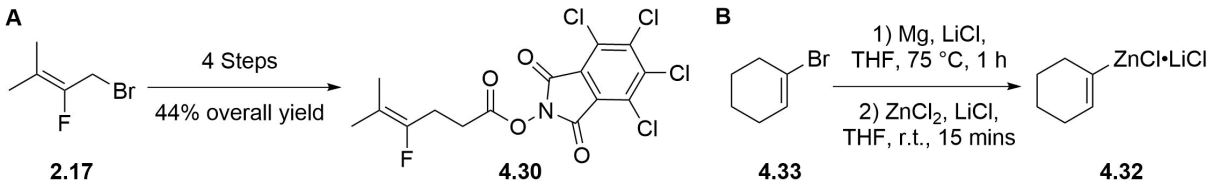


Scheme 4.21: Retrosynthetic plan for post-labelling decarboxylative cross couplings

4.2.2.1 Decarboxylative Alkenylation for Terpenoid Synthesis

The decarboxylative alkenylation was first investigated on vinyl fluoride **4.30** and organozinc **4.32** (Scheme 4.22). The fluorinated redox-active ester **4.30** was

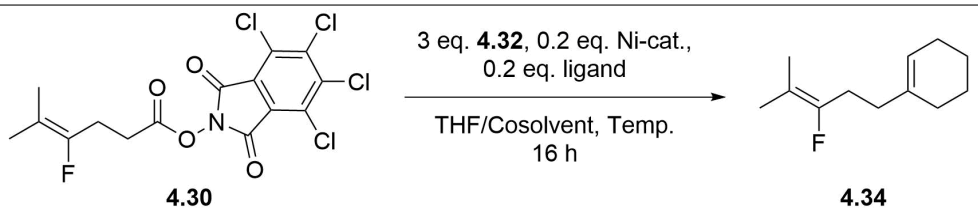
synthesised from bromide **2.17** in 4 steps.[2] Organozinc **4.32** was freshly prepared from vinyl bromide **4.33**.



Scheme 4.22: A Synthesis of ester **4.30**; **B** Synthesis of vinyl zinc **4.32**

The investigation of the cross-coupling was carried out according to Table 4.4. Baran's standard conditions using DMF as a co-solvent (Entry 1) did not afford any product. The solvent was changed to acetonitrile and the product was obtained in 25% yield (Entry 2). Increasing the temperature did not improve the yield (Entry 3) nor did changing the ligand to the more bulky *t*Bu-Bipy (Entry 4). An improvement was observed when the solvent was changed to NMP (Entry 5). In a radiochemical setting, organozinc **4.32** as well as the catalyst and ligand would be in large excess compared to ester **4.30**. When one equivalent of catalyst and ligand were employed an improvement in yield was observed (Entry 6), which became more prominent at elevated temperature (Entry 7). A large excess of vinyl zinc **4.32** and stoichiometric amounts of catalyst and ligand afforded the product in similar yields (Entry 8). The obtained results were encouraging for the translation of the methodology into a radiochemical setting, therefore the attention was shifted towards the radiofluorination of the redox-active ester.

[2]Synthesis and cross-coupling investigation performed by Z. Chen (DPhil student, University of Oxford)



4.30 **4.34**

Entry	Ni catalyst	Ligand	Cosolvent	Temperature	Yield [a]
1	$Ni(acac)_2$	Bipy	DMF	r.t.	N.A.
2	$Ni(acac)_2$	Bipy	MeCN	r.t.	25%
3	$Ni(acac)_2$	Bipy	MeCN	60 °C	22%
4	$Ni(acac)_2$	*t*Bu-Bipy	MeCN	60 °C	22 %
5	$Ni(acac)_2$	Bipy	NMP	r.t.	27%
6[b]	$Ni(acac)_2$	Bipy	NMP	r.t.	40%
7[b]	$Ni(acac)_2$	Bipy	NMP	60 °C	47%
8[c]	$Ni(acac)_2$	Bipy	NMP	r.t.	45%

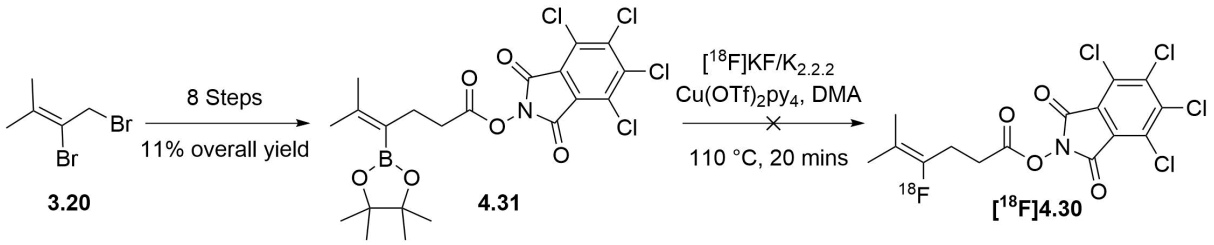
Table 4.4: Optimisation of decarboxylative alkenylation (*Data from Z. Chen*): reactions were carried out on a 36 μmol scale with 0.2 eq. of catalyst, 0.2 eq. ligand and 3 eq. organozinc **4.32**. [a] Yields determined by ^{19}F-NMR against an internal standard; [b] 1 eq. of catalyst and ligand used; [c] 1 eq. of catalyst and ligand, 5 eq. of vinyl zinc used

4.2.2.2 Radiofluorination of Redox-Active Ester 4.31

To investigate the radiofluorination of redox-active esters, vinyl boronic ester **4.31** was synthesised from bromide **3.20** in eight steps (Scheme 4.23).[3] The copper mediated radiofluorination failed to afford the desired redox-active ester **[^{18}F]4.30** under standard conditions ([^{18}F]KF/$K_{2.2.2}$, 1.5 eq. [$Cu(OTf)_2py_4$], DMA, 110 °C, 20 mins).

[3]Synthesis performed by Z. Chen (DPhil student, University of Oxford)

No incorporation of [^{18}F]fluoride was observed. The absence of other radiofluorinated products indicated no competing side reactions. It was hypothesised that the ester does not undergo the same coordinative interactions with the boronic ester or the copper catalyst as the ketone in the previous example.



Scheme 4.23: Synthesis of boronic ester **4.31** and failed radiolabelling

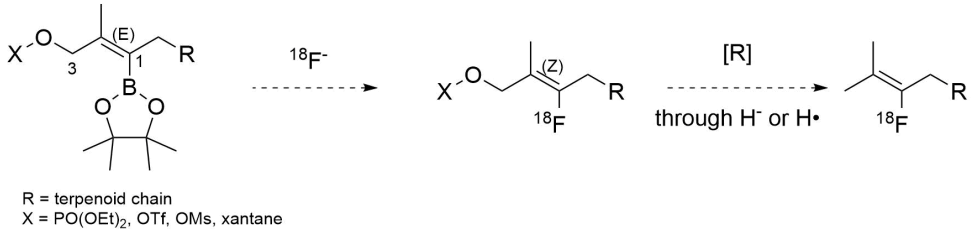
In conclusion, Baran's decarboxylative alkenylation offers great potential for post-labelling modification but a suitable activation group for the radiofluorination needs to be found.[4]

4.2.3 Activation through Cleavable Tether

In previous approaches, the terpenoid chain was assembled after the radiolabelling step through carbon-carbon bond forming reactions. The nature and proximity of the activating electron donor were important to the radiofluorination. The highest radiochemical conversions were obtained with ethers close to the vinyl boronic ester moiety. After difficulties were experienced with carbon-carbon bond forming reactions, a synthesis was considered where the activating group is chemoselectively cleaved

[4]Ongoing work of Z. Chen (DPhil Student, University of Oxford)

post-labelling. Hypothetically, a terminal ether moiety would be in close proximity to the boronic ester, enabling radiofluorination, and could be removed through deoxygenation reactions (Scheme 4.24).

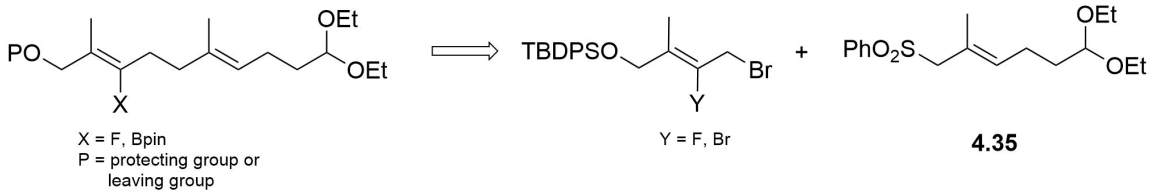


Scheme 4.24: Possible post-labelling deoxygenation

Reduction of allylic mesylates, tosylates and phosphates has been achieved by metal hydrides like lithium aluminium hydride or $LiBHEt_3$.[10–12] The use of those reducing agents could potentially lead to the undesired reduction of the amide bond in ^{18}**F-Sq-Gem**. A post-labelling amidation would circumvent that problem (Section 3.2.7). Although xanthates are likely to facilitate the radiofluorination of vinyl boronic esters, the radical mechanism of the Barton-McCombie deoxygenation could lead to undesired double bond isomerisation.[13,14] Xanthates were therefore not considered in this study.

A model substrate based on two isoprene units was developed to investigate the radiofluorination and deoxygenation (Scheme 4.25). The acetal masks the required aldehyde for the post-labelling oxidative amidation. It was expected that the positioning of the ether group would be important and only the *Z*-stereoisomer would undergo radiofluorination. It was left open, which hydroxide protecting group or leaving group should be employed in the radiolabelling and the deoxygenation.

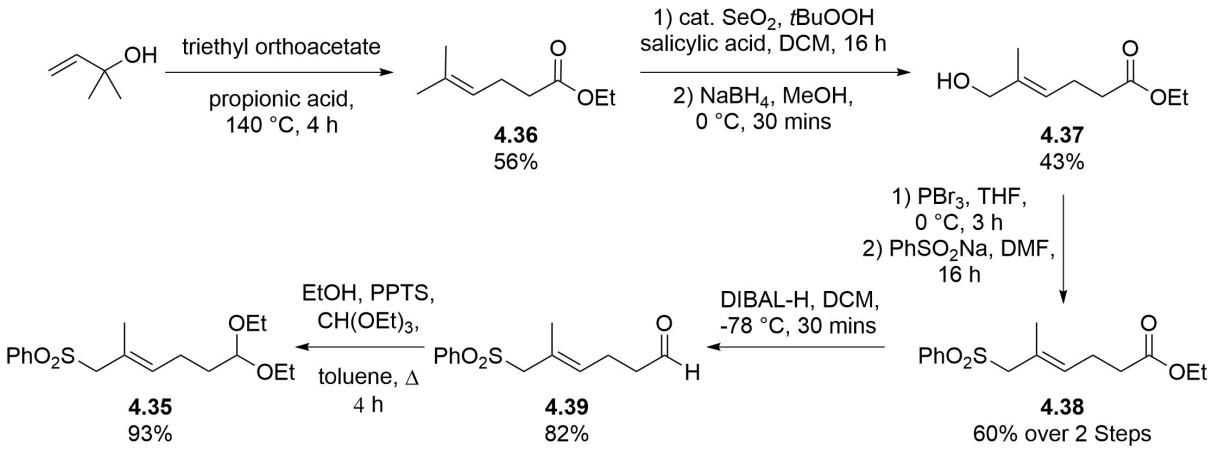
The synthesis plan relied on two fragments which could be combined by sulfone chemistry. At an early stage of the synthesis, the hydroxy group will be protected by a TBDPS group, which was judged to be easily removable at a later stage to introduce the leaving group.



Scheme 4.25: Precursor and reference synthesis for post-labelling deoxygenation

4.2.3.1 Preparation of Sulfone 4.35

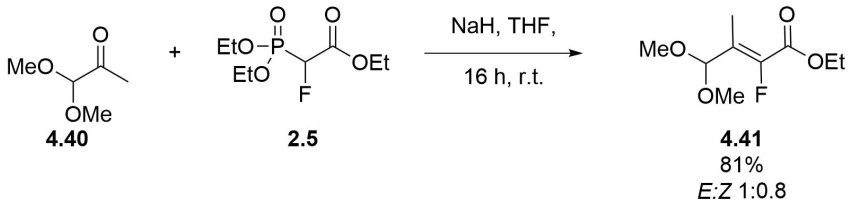
Ester **4.36** was synthesised by Johnson-Claisen rearrangement between triethyl-orthoacetate and 2-methyl-3-buten-2-ol (Scheme 4.26). Allylic oxidation with catalytic selenium dioxide and subsequent reduction with sodium borohydride afforded alcohol **4.37**. Phosphorus tribromide deoxybrominated alcohol **4.37** in high purity and the sulfonylation was carried out without prior purification. Sulfone **4.38** was obtained in good yields. Reduction of the ester with DIBAL-H afforded aldehyde **4.39**, which was protected to acetal **4.35** in excellent yields.



Scheme 4.26: Synthesis of sulfone **4.35**

4.2.3.2 Stereoselective Synthesis of 4.49

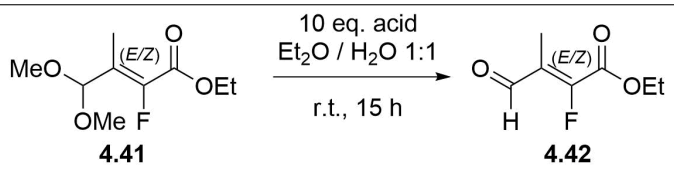
Horner-Wadsworth-Emmons reaction between 1,1-dimethoxyacetone (**4.40**) and phosphonate **2.5** furnished vinyl fluoride **4.41** in good yields and a *E:Z*-ratio of 1:0.8 (Scheme 4.27), after the same reaction using protected α-hydroxy acetone failed. At this stage, the two stereoisomers were not separable.



Scheme 4.27: Horner-Wadsworth-Emmons reaction to vinyl fluoride **4.41**

Several acids were investigated for the hydrolysis of the acetal (Table 4.5). The reactions were slow and required a large excess of acid likely due to the biphasic reaction conditions. It was observed that the *E:Z*-ratio changed depending on the

choice of acid. Reaction control by ^{19}F-NMR showed that the hydrolysis rate was higher for the undesired *E*-isomer than the *Z*-isomer and the product likely decomposed upon prolonged exposure to strong acids. The most consistent results were obtained with trifluoroacetic acid and the *E:Z*-ratio (Entry 4) was retained.

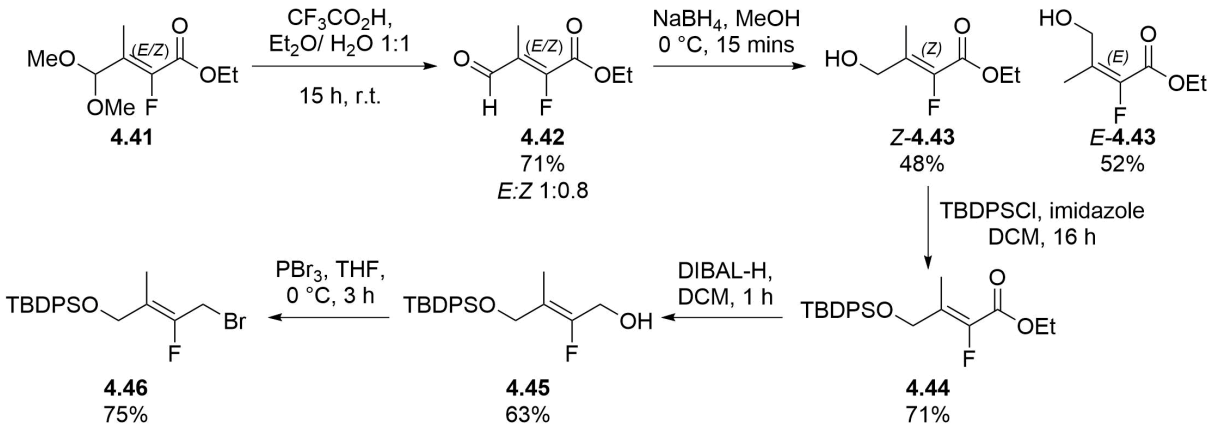


4.41 **4.42**

Entry	Acid	pKa	Yield	*E:Z*-ratio
1	HCl	-8.0	95%	1:0.6
2	H_2SO_4	-3.0	<5%	1:1.5
3 [a]	H_2SO_4	-3.0	68%	1:1
4	CF_3CO_2H	-0.25	80%	1:0.9
5	H_3PO_4	2.1	85%	1:0.8
6	TsOH	2.1	43%	1:21
7	CH_3CO_2H	4.7	N.A[b]	1:0.4

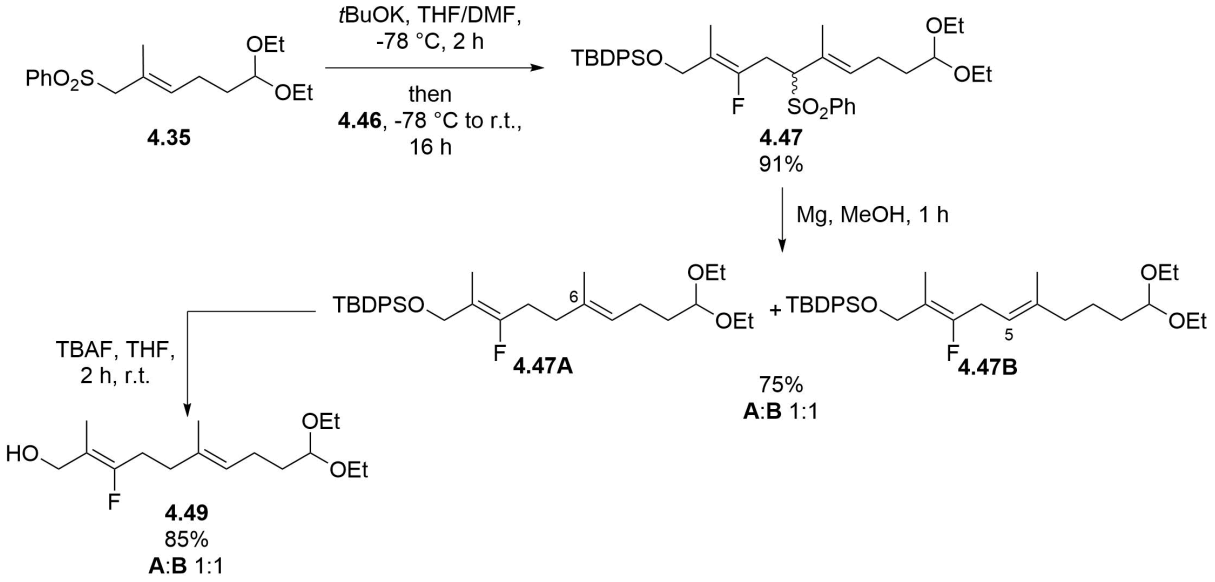
Table 4.5: Hydrolysis of acetal **4.41**. Reactions were carried out on a 0.24 mmol scale; [a]: 1 eq. H_2SO_4 used; [b]: Reaction was not complete after 15 hours

The resulting aldehyde (**4.42**) was fully reduced by sodium borohydride to the alcohol. Chromatography over silica separated the two stereoisomers *E*-**4.43** and *Z*-**4.43**. The stereochemical assignment was done by ^{19}F-^{1}H-HOESY. Silylation of *Z*-alkene **4.43** with TBDPSCl afforded ester **4.44**, which was reduced to alcohol **4.45** in good yields. The hydroxy group was brominated with tribromophosphine to bromide **4.46**.



Scheme 4.28: Synthesis of bromide **4.46**

Nucleophilic substitution of bromide **4.46** with the anion of sulfone **4.35** resulted in acetal **4.47** in exceptionally high yields (Scheme 4.29). The reduction of the sulfone moiety with magnesium in methanol afforded acetal **4.48A** and its regioisomer **4.48B** in a 1:1 ratio. The two isomers could not be separated at this point or at a later stage.[5] Finally, the terminal silyl ether was cleaved with TBAF to afford the desired alcohol **4.49**.

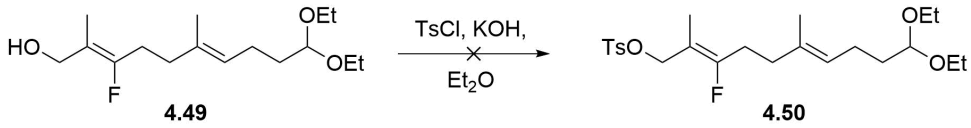


Scheme 4.29: Synthesis of alcohol **4.49**

[5]For a clearer overview only one regioisomer will be shown in future.

4.2.3.2.1 Investigation of Deoxygenation Reactions

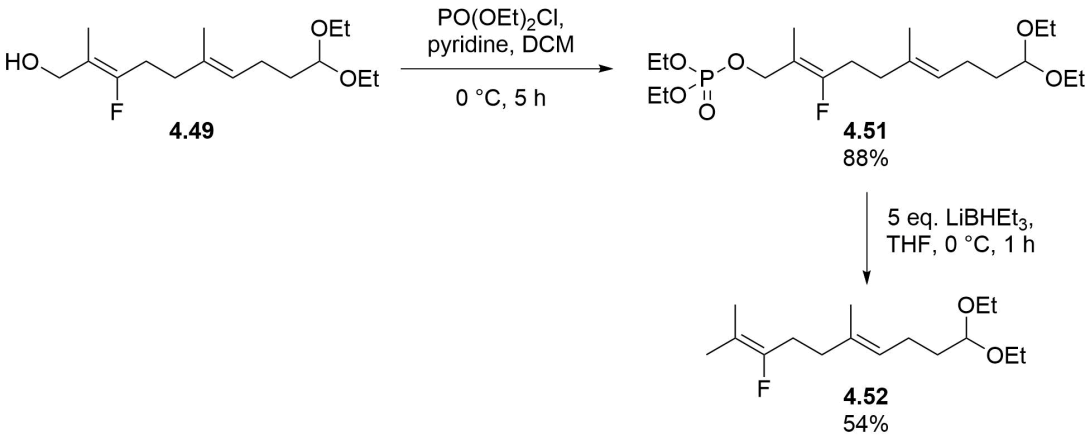
With alcohol **4.49** at hand, different means for the dehydroxylations were investigated. Difficulties were experienced preparing tosylate **4.50**. Previous reports indicate an incompatibility of the system with amine bases and used potassium hydroxide (Scheme 4.30).[12] The observed conversion was poor and the product would quickly decompose to black tars upon isolation from the reaction. Sulfonates were not investigated further.



4.49 **4.50**

Scheme 4.30: Attempted synthesis of tosylate **4.50**

Phosphorylation of alcohol **4.49** proceeded cleanly in just 5 hours (Scheme 4.31). The obtained phosphate **4.51** was stable and could be stored in the freezer for several months without decomposition. The conditions for the deoxygenation respectively reduction of the phosphate were chosen to emulate the conditions found in radiochemistry: the reaction was only marginally cooled in an ice bath and the reducing agent was used in 5-fold excess, which was still underestimated. Heavy gas evolution was observed during the reaction, but acetal **4.52** was isolated in good yields.

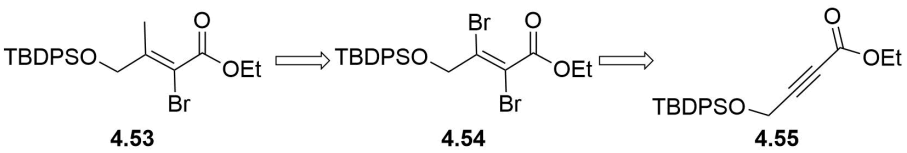


Scheme 4.31: Synthesis of phosphate **4.51** and reductive deoxygenation

The feasibility of a post-labelling deoxygenation was demonstrated. It is still unclear if the phosphate might interfere with the radiolabelling reaction or undergo nucleophilic substitution with [^{18}F]fluoride. In the next section the synthesis of the corresponding vinyl boronic ester is discussed.

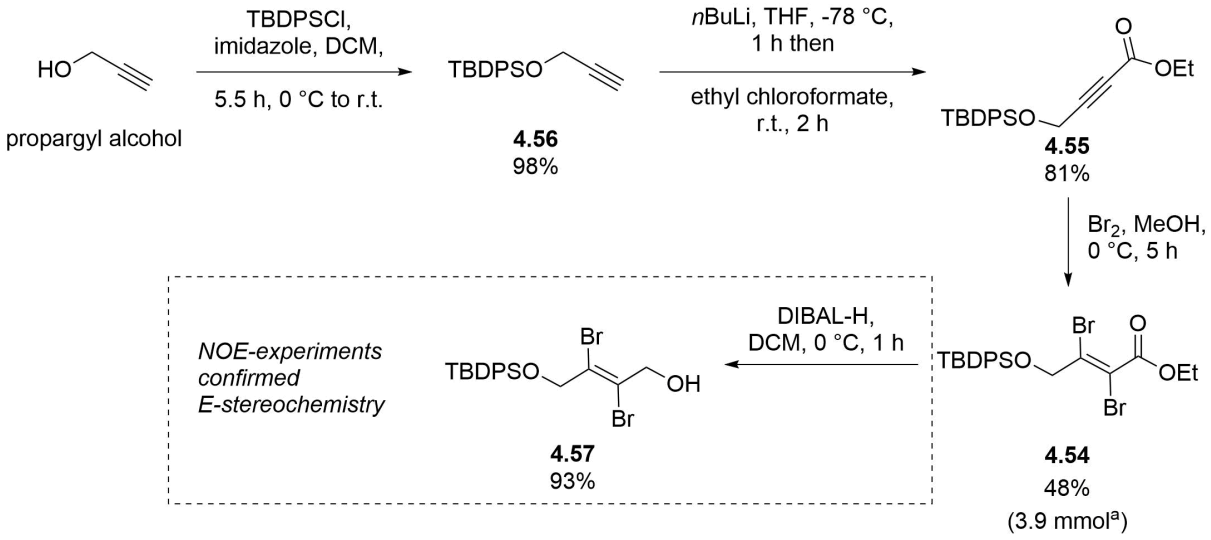
4.2.3.3 Synthesis of a *(Z)*-1,3-Bromo-Ether

Initially, the plan was to synthesise the vinyl bromide fragment (**4.53**) by a Horner-Wadsworth-Emmons reaction as described for the fluoro-analogue (Section 4.2.3.2) but no reactivity of the brominated phosphonate towards the ketone was observed. An alternative synthesis was developed based on the site-selective methylation of vinyl dibromide **4.54** (Scheme 4.32). The ester functionality should provide an electronic bias, which directs the methylation reaction. Dibromide **4.54** could be accessed by bromination of alkyne **4.55**.



Scheme 4.32: Retrosynthetic analysis for **4.53** *via* methylation reaction

Silylation of propargyl alcohol provided alkyne **4.56** in high yields (Scheme 4.33). Alkyne **4.56** was acetylated with ethyl chloroformate to afford ester **4.55**. Treatment of ester **4.55** with bromine in methanol resulted in the desired dibromide **4.54**. The yield of the bromination reaction varied significantly from batch to batch. On small scale (under 1 mmol), yields up to 60% were obtained but on larger scale the yield deteriorated. No effort was made to improve the yield as enough ester **4.54** could be obtained. To confirm the stereochemistry of the dibromide, ester **4.54** was reduced to alcohol **4.57**. NOE-experiments showed only *E*-product.



Scheme 4.33: Synthesis of dibromide **4.54**; [a] obtained amount of product

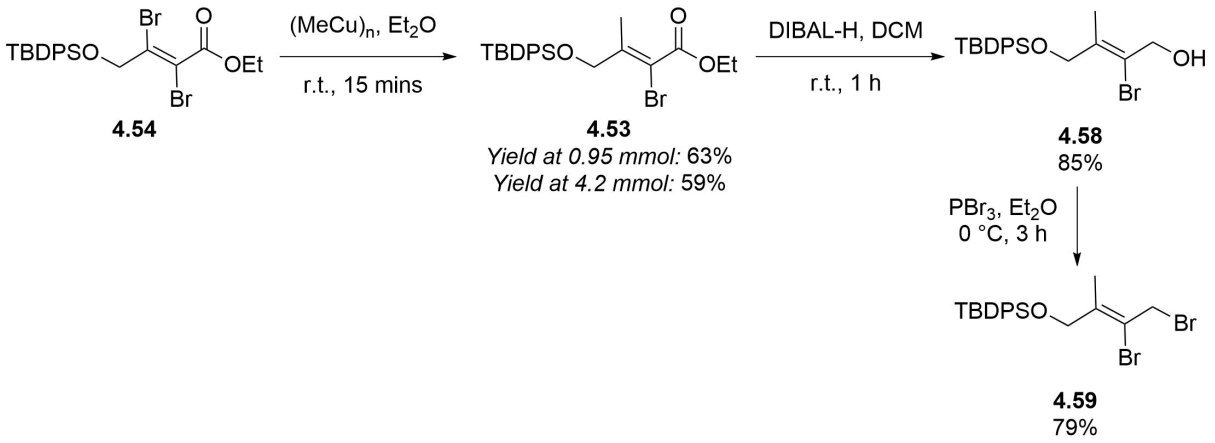
Methylation of dibromides has been described in the literature by lithium halogen exchange with a methylating agent or by utilising dimethyl cuprates.[15,16] It was not clear, if the electron withdrawing effect of the neighbouring ester group in **4.54** would provide a bias for a completely selective lithium-halogen exchange. But coordination of said carbonyl group to cuprates should direct the methylation. When dibromide **4.54** was treated with dimethyl cuprate several products were observed, where the main product was double methylated. The reagent was changed to the less reactive methyl copper ($(MeCu)_n$) and the desired vinyl bromide **4.53** was observed alongside double methylated and debromohydrogenated products (Table 4.6 Entry 1). When the reaction was performed at room temperature, increased reactivity and complete conversion were observed after 30 minutes (Entry 3 and 4). Using a more coordinating solvent, like THF, did slow down the reaction noticeably and full conversion was observed after 120 minutes (Entry 6). The reaction afforded mainly proteodebrominated side products (X = H) which were difficult to separate from vinyl bromide **4.53**. Cyanocuprates were reported to be more stable than simple organocuprates.[17] The use of MeCu(CN)Li did not afford full conversion and interestingly the regioisomer of **4.53** was observed (Entry 8, **4.53** : isomer 3.3:1). Increasing the amount of methyl copper to account for thermal decomposition,[17] increased the overall conversion and the optimal reaction time was determined to be 15 minutes (Entry 10). The observed side-products were readily separated from the desired product by column chromatography.

4.54 **4.53** *identified side products X = Me, H*

Entry	eq. $(MeCu)_n$	solvent	Temp. [°C]	time [mins]	**4.53**	remaining **4.54**	side products[a]
1	1.1	Et_2O	0	10	37	47	16
2	1.1	Et_2O	0	90	39	33	28
3	1.1	Et_2O	r.t.	10	55	10	35
4	1.1	Et_2O	r.t.	30	66	n.d	34
5	1.1[b]	Et_2O	r.t.	30	34	56	10
6	1.1	THF	r.t.	120	56	n.d	44
7	1.1	Et_2O	r.t.	30	43	n.d	57
8	1.1[c]	Et_2O	r.t.	30	28[d]	32	40
9	1.5	Et_2O	r.t.	5	43	28	29
10	1.5	Et_2O	r.t.	15	69	n.d	31

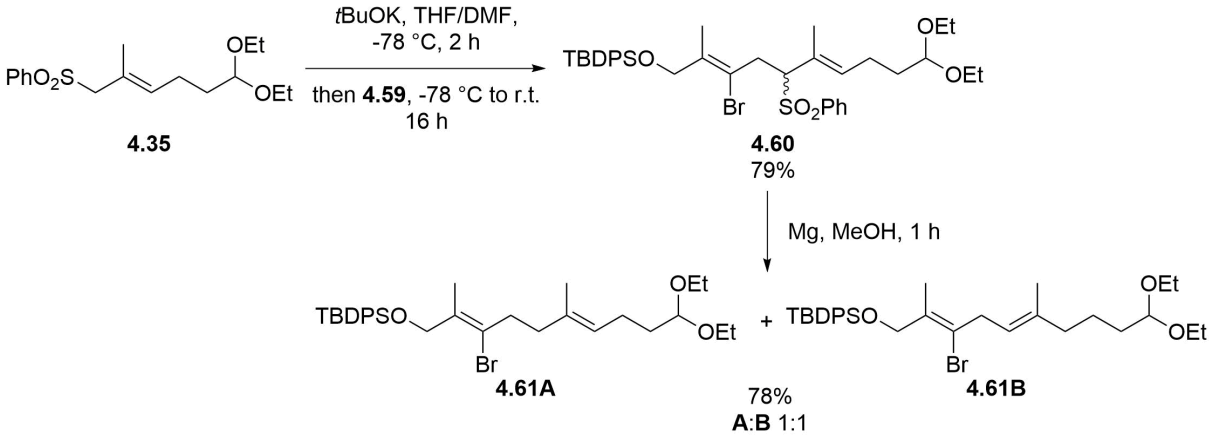
Table 4.6: Optimisation of the site-selective methylation of dibromide **4.54**; reactions were performed on a 38 μmol scale under anhydrous conditions, analysis was performed by GC-MS integration. [a]: identified and unknown side products; [b] reagent added by syringe pump; [c] MeCu(CN)Li used; [d] regioisomer observed

Optimal results for the methylation were achieved with 1.5 equivalents of methyl copper and only 15 minutes reaction time. The reaction was scaled up without significant loss of yield (Scheme 4.34). Vinyl bromide **5.53** was reduced using DIBAL-H in good yields. Bromination of the resulting alcohol **4.58** afforded bromide **4.59**.



Scheme 4.34: Synthesis of bromide **4.59**

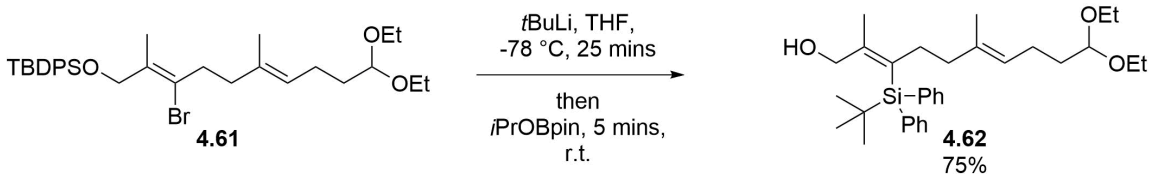
Sulfone **4.35** was deprotonated with potassium *tert*-butoxide and then treated with bromide **4.59**. Silyl ether **4.60** was obtained in good yields. The sulfone moiety was reduced using magnesium in methanol. As before, double bond isomerisation across the quaternary carbon was observed and acetals **4.61A** and **4.61B** were obtained in a 1:1 ratio.[6]



Scheme 4.35: Synthesis of acetal **4.61**

[6] **4.61A** and **4.61B** were not separable. For clarity only one isomer will be shown from here on.

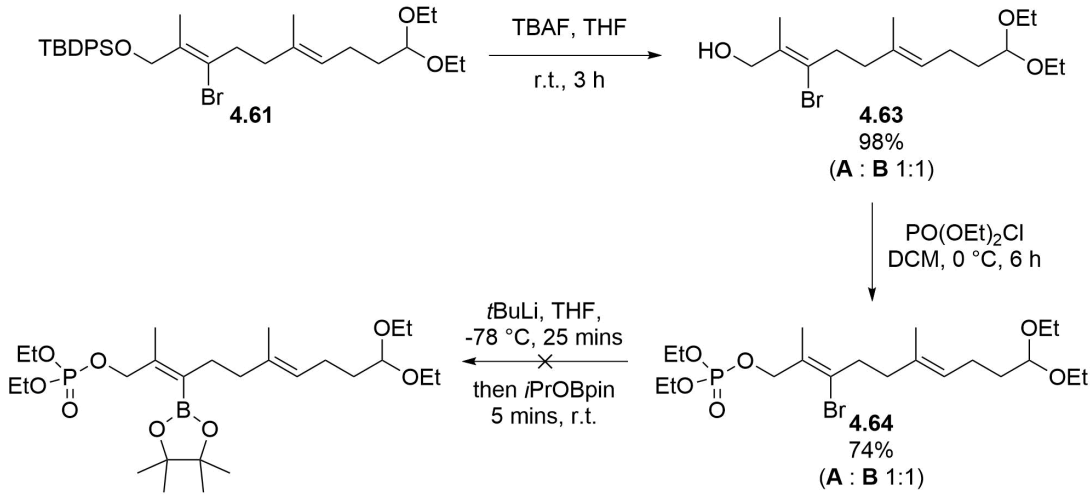
It was expected that vinyl boronic esters would be stable to desilylation with TBAF and derivatisation would become easier if the borylation was performed on acetal **4.61**. But instead of observing the desired vinyl boronic ester, acetal **4.61** underwent retro-Brook rearrangement to silane **4.62** (Scheme 4.36).[18,19]



Scheme 4.36: Observed retro-Brook rearrangement of acetal **4.61** to silane **4.62**

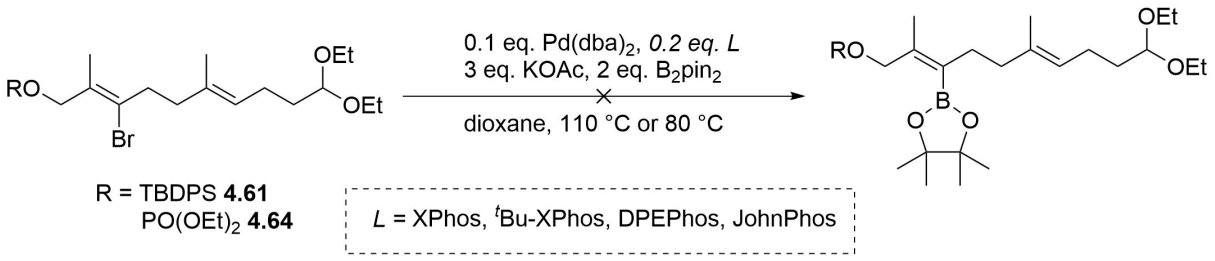
Alternative borylation attempts *via* the formation of Grignard reagents of acetal **4.61** with magnesium or *i*PrMgCl· LiCl failed. It was decided to synthesise the corresponding terminal phosphate and revisit the borylation later.

Acetal **4.61** was desilylated with TBAF in excellent yields (Scheme 4.37). The obtained alcohol **4.63** was phosphorylated to afford phosphate **4.64**. The attempted borylation with *tert*-butyllithium only afforded decomposition products and a loss of the phosphate moiety.



Scheme 4.37: Synthesis of phosphate **4.64** and attempted borylation

As a final option, palladium-catalysed borylations were investigated. Buchwald and others showed that bulky phosphine ligands improve the Myiaura borylation of challenging substrates.[20,21] A set of sterically demanding ligands were screened on vinyl bromides **4.61** and **4.64** without success (Scheme 4.38). The compounds underwent thermal decomposition without interacting with the palladium catalyst.



Scheme 4.38: Attempted Miyaura borylations of acetal **4.61** and **4.64**

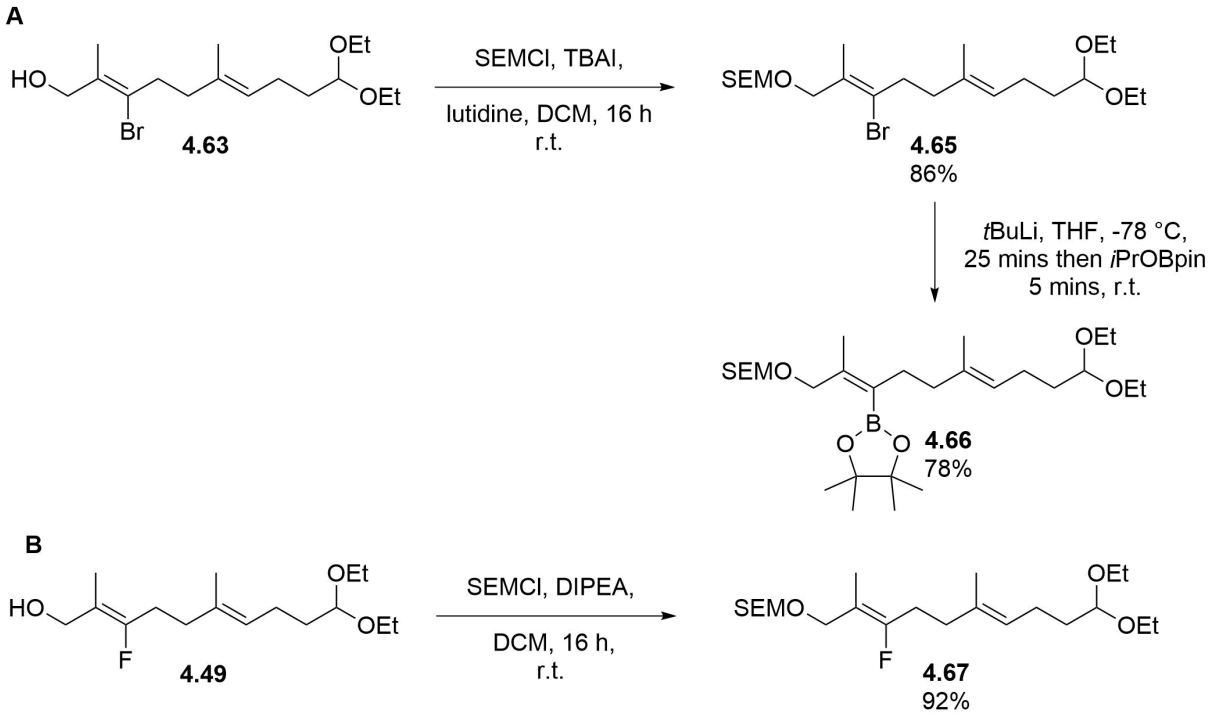
As the desired vinyl boronic esters could not be obtained, it was decided to investigate the radiofluorination of this motif with a stable protecting group and later revisit the synthesis of a precursor with a cleavable ether group.

4.2.3.4 SEM-protection of Alcohol 4.63 and Radiochemical Investigation

Despite containing silicon, the SEM protecting group has shown no involvement in the radiofluorination of aryl boronic esters. The protecting group can be cleaved by a number of conditions, most notably anhydrous TBAF and Lewis acids, which could be compatible with a vinyl boronic ester functionality and the acetal protecting group.

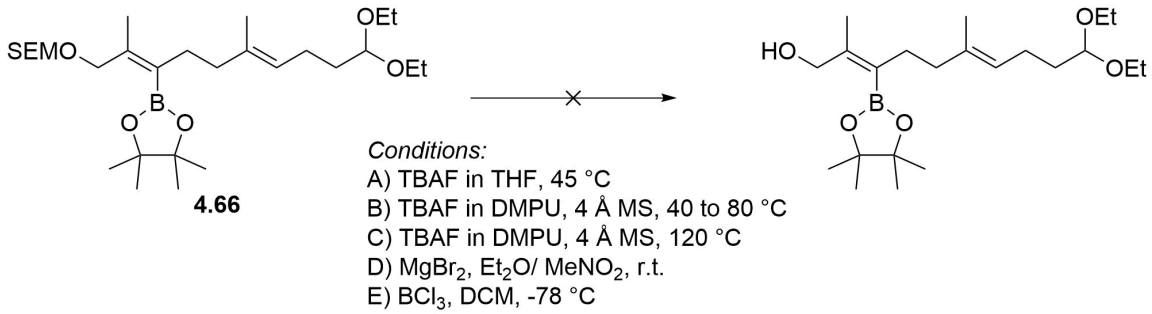
Alcohol **4.63** was treated with SEMCl, TBAI and lutidine to afford acetal **4.65** (Scheme 4.39 **A**). The compound readily underwent lithium-halogen exchange and borylation

with *i*PrOBpin. Vinyl boronic ester **4.66** was isolated in good yields. The required vinyl fluoride reference was synthesised from alcohol **4.49** in a similar procedure (Scheme 4.39 **B**).



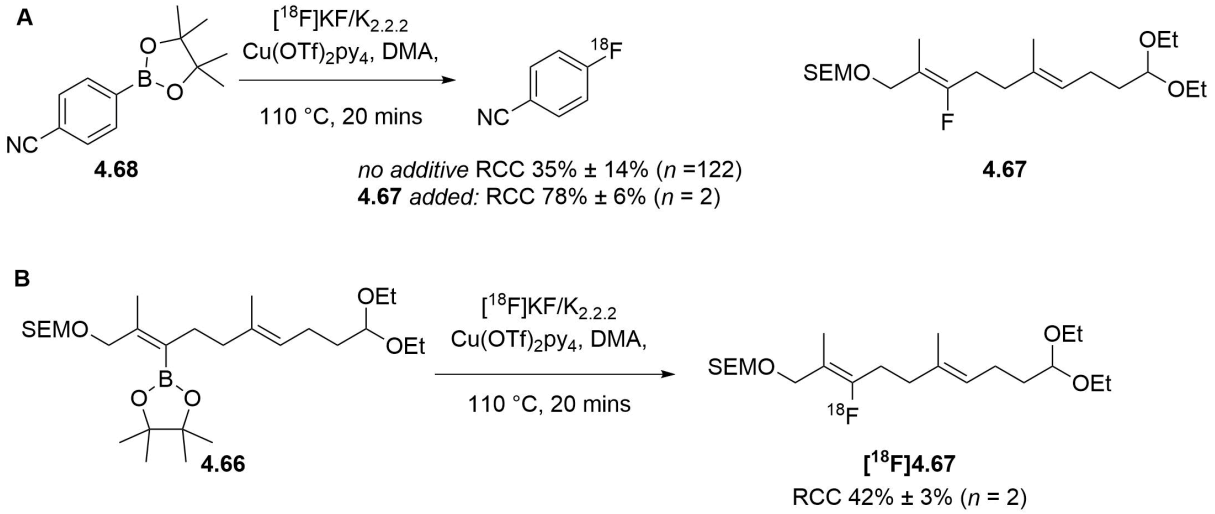
Scheme 4.39: A SEM-protection and borylation of vinyl bromide **4.63**; **B** SEM-protection of vinyl fluoride **4.49**

Attempts to cleave the SEM group after the borylation were unsuccessful (Scheme 4.40). TBAF in THF or DMPU was not able to cleave the protecting group.[22,23] It was observed that **4.66** was stable over an extended period of time at temperatures up to 120 °C before it thermally decomposed. Magnesium bromide and boron trichloride decomposed acetal **4.66** instantaneously.[24]



Scheme 4.40: Deprotection attempts of acetal **4.66**

To ensure that the SEM protection of a primary alcohol does not influence the radiolabelling reaction, the copper mediated radiofluorination of benzonitrile **4.68** was spiked with vinyl fluoride **4.67** (Scheme 4.41 **A**). The radiochemical conversion increased indicating a good tolerance for the present functional groups and the SEM-protecting group. When vinyl boronic ester **4.66** was submitted to the same reaction conditions, incorporation of [^{18}F]fluoride was observed. Ether **[^{18}F]4.67** was obtained in 42% RCC. The radio-HPLC trace showed several side products. It was not clear whether the sideproducts were decomposition products from the radiochemical reaction or if hydrolysis of the acetal moiety was observed on the HPLC column.



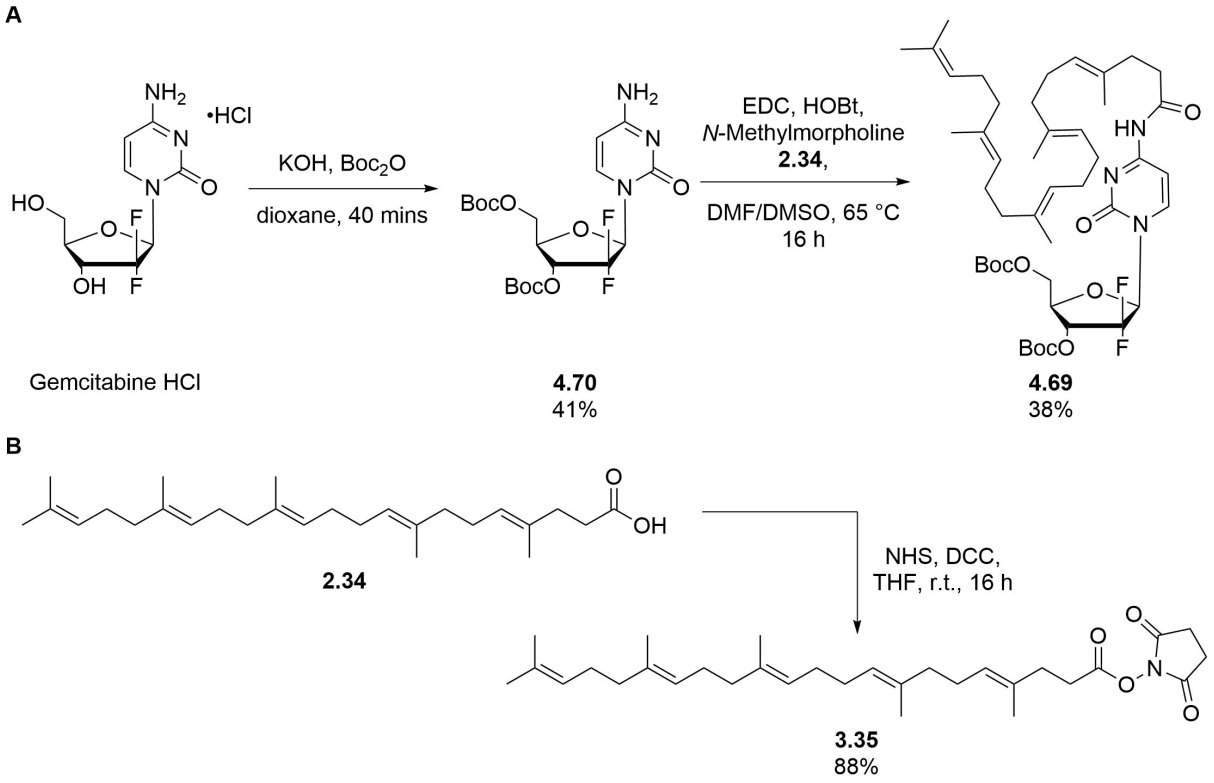
Scheme 4.41: A Spiking of vinyl fluoride **4.67** to a standard reaction; **B** Radiofluorination of vinyl boronic ester **4.66**

The obtained radiolabelling result was highly encouraging and demands further investigation of the allylic ether moiety for mediating the radiofluorination of vinyl boronic esters. For future investigations, a more suitable protecting group for the precursor synthesis has to be found, which can be easily cleaved after the borylation enabling derivatisation and ultimately deoxygenation.

4.2.4 Testing for Functional Group Tolerance

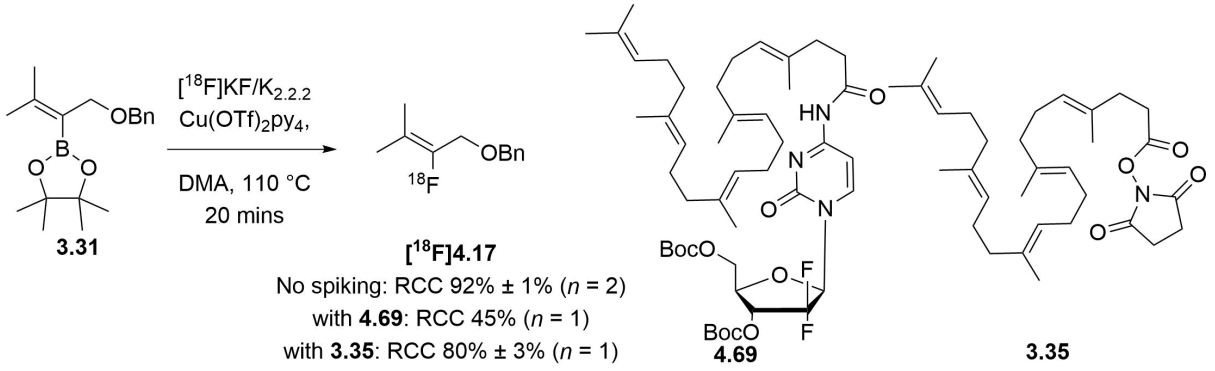
The ability of terminal ethers to mediate the radiofluorination of vinyl boronic esters (discussed in Section 4.2.3.4) enabled the radiolabelling of assembled squalenoyl drug conjugates. For future synthesis plans it was therefore important to know which functional groups are tolerated and if a post-labelling conjugation would be necessary.

Boc-protected squalenoyl gemcitabine (**4.69**) will indicate the tolerance of the radiofluorination to the cytosyl heterocycle. Succinimide esters (**3.35**) offer an alternative post-labelling amidation procedure to the already investigated oxidation-amidation sequence from aldehydes (Section 3.2.7). The two required compounds were prepared from gemcitabine and carboxylic acid **2.34** (Scheme 4.42). Gemcitabine was first protected using di-*tert*-butyl dicarbonate (Boc_2O) followed by amidation with carboxylic acid **2.34**. Succinimide ester **3.35** was obtained in one step from acid **2.34**.



Scheme 4.42: A Synthesis of Boc-protected squalenoyl gemcitabine **4.69**; **B** Synthesis of succinimide ester **3.35**

One equivalent of amide **4.69** or ester **3.35** was added to the radiolabelling reaction of vinyl boronic ester **3.31** and the obtained radiochemical conversions compared (Scheme 4.43). Addition of amide **4.69** reduced the RCC significantly from 92% to 45%. Boc-protected squalenoyl gemcitabine conjugate (**4.69**) clearly interacted with the radiolabelling reaction but did not inhibit it completely. A late stage radiofluorination of the assembled drug squalenoyl conjugate could be successful. Succinimide ester **3.35** influenced the radiolabelling of vinyl boronic ester **3.31** less. The RCC was only slightly decreased to 80% indicating a good tolerance of the reaction for succinimide esters.



Scheme 4.43: Spiking experiments

Both molecules are tolerated in spiking experiments and it will be important for future approaches to determine whether radiolabelling on the conjugate or on the succinimide ester followed by post-labelling amidation, will produce higher yields. Both approaches are likely to afford the desired product.

4.3 Summary

In this chapter, the successful radiolabelling of vinyl boronic esters was presented. It was shown that neighbouring Lewis basic functional groups have to activate vinyl boronic esters for the copper-mediated radiofluorination. An attempt to use external Lewis bases for the radiolabelling of inactivated vinyl boronic esters was not successful. This restriction led to a redesign of the synthesis of ^{18}**F-Sq-Gem**. Post-labelling modifications, namely Julia-Kocienski olefinations, decarboxylative alkenylations and deoxygenations, were investigated for their compatibility in a radiochemical setting. Ketones and allylic ethers were confirmed to mediate the radiolabelling reaction. Further, a method for the terminal deoxygenation of allylic phosphates, with the potential to be translated into a radiochemical setting, was presented. The feasibility of a late-stage radiofluorination of a squalenoyl drug conjugate or a squalenoyl succinimide ester was confirmed through spiking experiments.

4.4 Outlook

In the second chapter of this thesis the successful synthesis of a fluorinated analogue of **Sq-Gem** was reported. Subsequently, it was demonstrated that **F-Sq-Gem** forms nanoparticles in aqueous solutions similar to those of the parent compound, which was important for future *in vivo* studies. A strategy was conceived to synthesise **^{18}F-Sq-Gem** from a vinyl boronic ester. The investigation of the radiolabelling of vinyl boronic esters by copper-mediation revealed the necessity for a proximate heteroatom. Based on this observation new synthesis strategies were developed. The most promising results were obtained, when an ether moiety was introduced at a terminal methyl group. Radiofluorination of a SEM-protected analogue was readily observed but the synthesis of a molecule which could undergo deoxygenation was not achieved.

Future investigations on this topic will have to focus on finding a robust synthetic strategy to incorporate the vinyl boronic ester with a cleavable tether. During this work, borylation reactions required heavy optimisation and many functional groups were found not to be compatible with the employed lithium-halogen exchange strategy. A successful deoxygenation was demonstrated on an allylic phosphate with hydride but it would be beneficial to investigate radical reactions as well. Alternatively, a new radiofluorination method could be developed based on strategies for the radiosynthesis of aryl fluorides. Iodonium salts or pre-formed metal complexes could be used as alternative precursors. The last option to mention would be to change the

site of radiofluorination. In this case, the molecule will have to be tested for its ability to form nanoparticles and possible metabolic radiodefluorination.

Literature

[1] F. Camps, A. Messeguer, F. J. Sánchez, *Tetrahedron* **1988**, *44*, 5161–5167.

[2] J. C. Vantourout, H. N. Miras, A. Isidro-Llobet, S. Sproules, A. J. Watson, *J. Am. Chem. Soc.* **2017**, *139*, 4769–4779.

[3] R. E. Shade, A. M. Hyde, J. C. Olsen, C. A. Merlic, *J. Am. Chem. Soc.* **2010**, *132*, 1202–1203.

[4] D. J. Winternheimer, C. A. Merlic, *Org. Lett.* **2010**, *12*, 2508–2510.

[5] J. B. Johnson, T. Rovis, *Angew. Chemie - Int. Ed.* **2008**, *47*, 840–871.

[6] G. Frenking, N. Fröhlich, *Chem. Rev.* **2000**, *100*, 717–774.

[7] J. T. Edwards, R. R. Merchant, K. S. McClymont, K. W. Knouse, T. Qin, L. R. Malins, B. Vokits, S. A. Shaw, D. H. Bao, F. L. Wei, T. Zhou, M. D. Eastgate, P. S. Baran, *Nature* **2017**, *545*, 213–218.

[8] B. E. Maryanoff, A. B. Reitz, *Chem. Rev.* **1989**, *89*, 863–927.

[9] P. R. Blakemore, W. J. Cole, P. J. Kocieński, A. Morley, *Synlett* **1998**, 26–28.

[10] M. L. Wise, H. J. Pyun, G. Helms, B. Assink, R. M. Coates, R. B. Croteau, *Tetrahedron* **2001**, *57*, 5327–5334.

[11] M. Stratakis, C. Raptis, N. Sofikiti, C. Tsangarakis, G. Kosmas, I. P. Zaravinos, D. Kalaitzakis, D. Stavroulakis, C. Baskakis, A. Stathoulopoulou, *Tetrahedron* **2006**, *62*, 10623–10632.

[12] A. F. Thomas, *Chem. Commun.* **1968**, 1657–1659.

[13] W. Hartwig, *Tetrahedron* **1983**, *39*, 2609–2645.

[14] J. M. Herrmann, B. König, *European J. Org. Chem.* **2013**, 7017–7027.

[15] C. Yip, S. Handerson, G. K. Tranmer, W. Tam, *J. Org. Chem.* **2001**, *66*, 276–286.

[16] R. S. Orugunty, D. L. Wright, M. A. Battiste, R. J. Helmich, K. Abboud, *J. Org. Chem.* **2004**, *69*, 406–416.

[17] S. H. Bertz, G. Dabbagh, *J. Chem. Soc. Chem. Commun.* **1982**, 1030–1032.

[18] R. West, R. Lowe, H. F. Stewart, A. Wright, *J. Am. Chem. Soc.* **1971**, *93*, 282–283.

[19] A. Wright, R. West, *J. Am. Chem. Soc.* **1974**, *96*, 3214–3222.

[20] K. L. Billingsley, T. E. Barder, S. L. Buchwald, *Angew. Chemie - Int. Ed.* **2007**, *46*, 5359–5363.

[21] P. B. Dzhevakov, M. A. Topchiy, D. A. Zharkova, O. S. Morozov, A. F. Asachenko, M. S. Nechaev, *Adv. Synth. Catal.* **2016**, *358*, 977–983.

[22] B. H. Lipshutz, J. J. Pegram, *Tetrahedron Lett.* **1980**, *21*, 3343–3346.

[23] B. H. Lipshutz, T. A. Miller, *Tetrahedron Lett.* **1989**, *30*, 7149–7152.

[24] L. E. Overman, D. V. Paone, *J. Am. Chem. Soc.* **2001**, *123*, 9465–9467.

Supporting Information

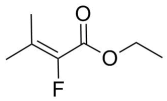
General

All NMR spectra were recorded on BrukerDPX200, DPX250, AVIII400, AVC500, AVB500 and DRX500 spectrometers. NMR spectral data are reported as chemical shifts (δ) in parts per million (ppm) relative to the solvent peak using the Bruker internal referencing procedure. Coupling constants (*J*) are reported to the nearest 0.1 hertz (Hz). The following abbreviations are used to describe multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Assignments were made by reference to the relevant 2D spectra (COSY, HSQC and HMBC). High-resolution mass spectra (HRMS, *m/z*) were recorded on a Bruker MicroTof spectrometer using electrospray ionisation (ESI), a Micromass GCT spectrometer using field ionisation (FI) or a Agilent 7200 QTOF spectrometer fitted with a direct insertion probe (Scientific Instruments Manufacturer GmbH) for electron ionisation (EI) or chemical ionisation (CI). Infrared spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer. Absorptions were measured in wavenumbers (cm^{-1}). Melting points of solids were measured on a Griffin apparatus and are reported uncorrected. Unless otherwise specified, all reactions were performed in flame dried glass apparatus with magnetic stirring under an inert atmosphere of nitrogen or argon. The reactions were followed by thin layer chromatography (TLC), using Merck aluminium-foil backed plates precoated with Kieselgel 60 F254. The products were visualised using UV fluorescence (254 nm, 365 nm) and an appropriate staining agent. Flash column

chromatography was performed on Merck silica gel (40-60 μm). Unless otherwise stated, reagents were obtained from commercial suppliers and used without further purification. Solvents were dried by expression through an activated alumina column built according to the procedures described by Pangborn and Grubbs, except for reactions involving water.

Experimental Procedures for Chapter 2

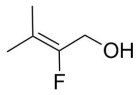
Ethyl 2-fluoro-3-methylbut-2-enoate (2.23)



NaH (1.38 g, 34.5 mmol) was suspended in dry THF (60 ml) and cooled in an ice bath. Ethyl 2-(diethoxyphosphoryl)-2-fluoroacetate (6.63 ml, 31.3 mmol) was added dropwise and the mixture was stirred for 30 mins. Then acetone (2.8 ml, 37.6 mmol) was slowly added and a large exotherm was observed. The reaction was stirred at room temperature over night and quenched with NH_4Cl. The mixture was extracted three times with diethyl ether. The combined organic phases were washed with brine and dried over Na_2SO4. The solvent was carefully removed under reduced pressure (min. 240 mbar, 40°C). The product was purified by column chromatography (pentane/diethyl ether 1:1, R_f 0.67). This procedure afforded pure ester **2.23** (4.29 g, 29.4 mmol, 93%) as a clear oil.

The analytical data were in accordance with the literature.[1] **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 4.27 (q, *J*=7.1, 2H), 2.11 (d, *J*=3.3, 3H), 1.87 (d, *J*=4.2, 3H), 1.33 (t, *J*=7.1, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -128.14 (hept, *J*=3.6).

Ethyl 2-fluoro-3-methylbut-2-en-1-ol (2.2)

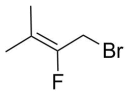


Ester **2.23** (4.29 g, 29.4 mmol) was dissolved in dry DCM (50 ml) and cooled to -78°C. DIBAL-H (1 M in DCM, 58.8 ml, 58.8 mmol) was slowly added. After 45 mins, sat. Rochelle salt solution (50 ml) was added and the solution allowed to warm to room temperature. The organic phase was separated and the aqueous phase was washed three times with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent was carefully evaporated under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 1:1, R_f 0.42). This procedure afforded pure alcohol **2.2** (1.55 g, 14.9 mmol, 50%) as clear oil.

Note: The product is highly volatile! The pressure should not fall below 680 mbar at 40°C.

The analytical data were in accordance with the literature.[2] **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 4.25 (d, *J*=22.5, 1H), 4.23 (d, *J*=22.4, 1H), 1.69 (d, *J*=2.7, 3H), 1.68 (d, *J*=2.3, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm):-121.38 (dd, *J*=22.3, 19.1).

1-Bromo-2-fluoro-3-methylbut-2-ene (2.17)

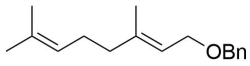


The synthesis was performed according to literature procedure.[2]

Alcohol **2.2** (1.55 g, 14.9 mmol) was dissolved in dry diethyl ether (15 ml) and cooled in an ice bath. PBr_3 (1.40 ml, 14.9 mmol) was added dropwise. After 2 hours, water was carefully added. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were washed with sat. $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was carefully evaporated under reduced pressure. The product was purified by column chromatography (pentane, R_f 0.45). This procedure afforded pure bromide **2.17** (1.46 g, 8.77 mmol, 58%) as a clear oil.

The analytical data were in accordance with the literature.[2] **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 4.09 (d, *J*=22.9, 2H), 1.71 (d, *J*=3.4, 3H), 1.69 (d, *J*=3.0, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -115.78 - -116.00 (m).

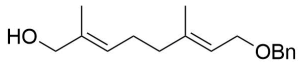
(*E*)-(((3,7-Dimethylocta-2,6-dien-1-yl)oxy)methyl)benzene (2.24)



NaH (2.59 g, 64.8 mmol, 60% in mineral oil) was suspended in dry THF (250 ml). Geraniol (11.39 ml, 64.8 mmol) was slowly added while cooling the reaction flask in a water bath. The mixture was stirred for one hour at room temperature and then benzylbromide (7.71 ml, 64.8 mmol) was added. The reaction was stirred for 16 hours. NH_4Cl was slowly added to quench the reaction. Diethyl ether was added and the aqueous phase extracted three times. The combined organic phases were washed with $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (Petroleum ether/ diethyl ether 2:1). This procedure afforded pure ether **2.24** (15.19 g, 62.1 mmol, 96%) as a clear oil.

The analytical data were in accordance with the literature.[3] **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 7.45-7.29 (m, 5H), 5.41 (t, *J*=6.8 Hz, 1H), 5.19-5.04 (m, 1H), 4.51 (s, 2H), 4.04 (d, *J*=6.6 Hz, 2H), 2.23-1.94 (m, 4H), 1.74-1.55 (m, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 140.3, 138.5, 131.6, 128.3 (2C), 127.8 (2C), 127.4, 123.9, 120.8, 71.9, 66.5, 39.5, 26.3, 25.6, 17.6, 16.4;

(2*E*,6*E*)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dien-1-ol (2.25)



The synthesis was modified from a literature procedure.[1]

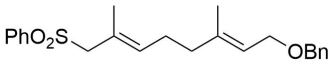
SeO_2 (137 mg, 1.24 mmol) and salicylic acid (857 mg, 6.21 mmol) were dissolved in dry DCM (55 ml) and *t*BuOOH (6 M in decane, 37.2 ml, 223.5 mmol) was added. Ether **2.24** was added subsequently and the mixture was stirred over night. The mixture was concentrated as far as possible then diethyl ether was added. The organic phase was washed with sat. $NaHCO_3$ and brine and concentrated again. Methanol (120 ml) was added and the solution cooled in an ice bath. $NaBH_4$ (469 mg, 12.4 mmol) was added in small portions and the mixture stirred for an additional 15 mins. Acetone was added and the solution concentrated under reduced pressure. The remains were taken up into EtOAc and washed with sat. NH_4Cl and brine. The organic phase was dried over Na_2SO4 and the solvent evaporated under reduced pressure as far as possible. The product was purified by column chromatography (PET 40-60/EtOAc 2:1, R_f 0.3). This procedure afforded pure alcohol **17** (4.20 g, 16.13 mmol, 26%) as a clear oil. In a typical experiment, starting ether **2.25** (5.88 g, 24.0 mmol, 38%) was recovered.

The analytical data were in accordance with the literature.[1]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.39 - 7.26 (m, 5H), 5.45 - 5.35 (m, 2H), 4.51 (s, 2H), 4.02 (d, *J*=6.7, 2H), 3.98 (s, 2H), 2.22 - 2.14 (m, 2H), 2.12 - 2.04 (m, 2H), 1.66

(s, 3H), 1.65 (s, 3H).; **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 140.1, 138.6, 135.2, 128.5 (2C), 128.0 (2C), 127.7, 125.7, 121.2, 72.2, 69.0, 66.7, 39.3, 25.9, 16.6, 13.8. *The stereochemistry of the terminal double bond was confirmed by NOE-experiments.*

((((2*E*,6*E*)-8-(Benzyloxy)-2,6-dimethylocta-2,6-dien-1-yl) sulfonyl)benzene (2.16)



Alcohol **2.25** (3.68 g, 14.1 mmol) was dissolved in dry diethyl ether (14 ml) and cooled in an ice bath. PBr_3 (1.32 ml, 14.1 mmol) was dropwise added. After 2 hours, water was carefully added. The mixture was extracted three times with diethyl ether. The combined organic phases were washed with sat. $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 9:1, R_f 0.36). This procedure afforded pure bromide **2.26** (2.76 g, 8.55 mmol, 60%) as a yellowish oil.

Bromide **2.26** (2.80 g, 8.68 mmol) was dissolved in dry DMF (8.5 ml). $PhSO_2Na$ (2.85 g, 17.3 mmol) was added and the mixture stirred over night. Then diethyl ether was added and washed with water. The aqueous phase was extracted two times with diethyl ether. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The product was

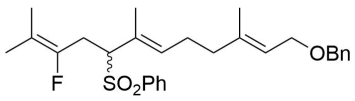
purified by column chromatography (PET 40-60/diethyl ether 1:1, R_f 0.27). This procedure afforded pure sulfone **2.16** (2.99 g, 7.79 mmol, 89%) as a clear to yellowish oil.

Note: The product decomposes at room temperature within two weeks but can be stored in a freezer for several month.

The analytical data were in accordance with the literature.[1]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.86 - 7.81 (m, 2H), 7.65 - 7.59 (m, 1H), 7.56 - 7.48 (m, 2H), 7.36 - 7.27 (m, 5H), 5.30 (tq, *J*=6.7, 1.3, 1H), 5.04 (td, *J*=7.0, 1.5, 1H), 4.49 (s, 2H), 3.99 (d, *J*=6.5, 2H), 3.71 (s, 2H), 2.14 - 2.02 (m, 2H), 1.93 - 1.84 (m, 2H), 1.76 (s, 3H), 1.58 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 139.4, 138.5, 138.5, 135.6, 133.5, 128.9, 128.5, 128.4, 127.8, 127.6, 127.6, 123.6, 121.3, 72.3, 66.5, 66.2, 38.5, 26.6, 16.7, 16.4;

(((6E,10E)-12-(Benzyloxy)-3-fluoro-2,6,10-trimethyldodeca-2,6,10-trien-5-yl)sulfonyl)benzene (2.18)



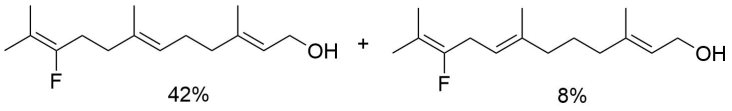
Sulfone **2.16** (1.00 g, 2.60 mmol) was dissolved in dry THF (1.3 ml) and HMPA (0.8 ml). The mixture was cooled to -41°C and LDA (1M solution in THF, 2.60 ml, 2.60 mmol) was added dropwise. A quick change in colour to orange was observed. After 30 mins,

bromide **2.17** (521 mg, 3.12 mmol) was added dropwise. The mixture was stirred for 30 mins and then allowed to warm to room temperature for 30 mins. The reaction was quenched with sat. NH_4Cl. The solution was extracted three times with diethyl ether. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 2:1, R_f 0.3). This procedure afforded pure sulfone **2.18** (0.95 g, 2.03 mmol, 78%) as a clear oil.

The analytical data were in accordance with the literature.[1]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.84 - 7.79 (m, 2H), 7.65 - 7.58 (m, 1H), 7.54 - 7.48 (m, 2H), 7.37 - 7.26 (m, 5H), 5.29 (tq, *J*=6.7, 1.3, 1H), 5.12 (td, *J*=7.1, 1.6, 1H), 4.49 (s, 2H), 4.01 - 3.96 (m, 2H), 3.78 (dd, *J*=10.8, 4.3, 1H), 3.06 - 2.81 (m, 2H), 2.12 - 1.91 (m, 2H), 1.83 - 1.75 (m, 2H), 1.66 (d, *J*=1.3, 3H), 1.58 - 1.54 (m, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 149.47 (d, J_{CF}=241.2), 139.6, 138.6, 138.0, 135.7, 133.6, 128.9, 128.9, 128.5, 128.0, 127.7, 126.5, 121.3, 110.81 (d, J_{CF}=17.0), 72.4, 71.1, 66.7, 38.5, 26.6, 25.7, 25.4, 17.94 (d, J_{CF}=5.4), 16.5, 15.79 (d, J_{CF}=9.2), 13.9; **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -114.55 - -114.81 (m); **IR** (cm^{-1}): 2921, 2860, 2358, 1447, 1305, 1144, 1085, 736, 689, 610; **HRMS** (CI): *m/z*: 471.2349 $[M + H]^+$ (calcd.: *m/z*: 471.2369 $[M + H]^+$).

(2*E*,6*E*)-10-Fluoro-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (2.15)



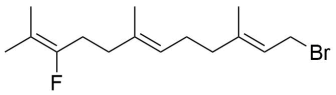
A three necked round-bottom flask was equipped with a gas condenser, nitrogen inlet and oil bubbler. NH_3 (21 ml) was condensed at -78° and lithium (129 mg, 18.6 mmol) was added to give a dark blue solution. The mixture was stirred for 30 mins to ensure complete dissolution of the sodium. Then sulfone **2.18** (875 mg, 1.85 mmol) was dissolved in dry THF (2.1 ml) and added dropwise. After 15 minutes, the reaction was quenched by the careful addition of methanol. The NH_3 was evaporated at room temperature and the remains taken up in water. The aqueous phase was extracted three times with EtOAc. The combined organic phases were washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 2:1, R_f 0.3). This procedure afforded alcohols **2.15** (224 mg, 931 μmol, 42%) with approx. 8% of the 7*E/Z*-isomer (**2.19**) as clear oils.

The analytical data were in accordance with the literature.[1]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.46 - 5.36 (m, 1H), 5.13 (td, *J*=6.9, 1.3, 1H), 4.15 (d, *J*=7.0, 2H), 2.29 (dt, J_{HF}=23.5,*J*=7.8, 2H), 2.17 - 2.07 (m, 4H), 2.06 - 2.01 (m, 2H), 1.68 (d, *J*=1.3, 3H), 1.63 - 1.60 (m, 6H), 1.56 (d, *J*=2.7, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 153.9 (d, J_{CF}=241.4) , 139.7, 134.4, 124.5, 123.4, 107.2 (d, J_{CF}=17.9), 59.4,

39.4, 36.58 (d, J_{CF}=0.9), 27.63 (d, J_{CF}=29.4), 26.3, 17.6 (d, J_{CF}=6.0), 16.3, 15.9, 15.5 (d, J_{CF}=9.4); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.76 - -112.98 (m); **IR** (cm^{-1}): 3338, 2920, 2861, 2359, 1715, 1668, 1449, 1383, 1155, 1001, 620; **HRMS** (CI): *m/z*: 258.2221 $[M + NH_4]^+$ (calcd.: *m/z*: 258.2233 $[M + NH_4]^+$).

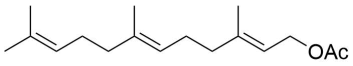
(2*E*,6*E*)-1-Bromo-10-fluoro-3,7,11-trimethyldodeca-2,6,10-triene (2.21)



Alcohol **2.15** (0.76 g, 3.15 mmol) was dissolved in MeCN (12.6 ml) and cooled in an ice bath. CBr_4 (1.36 mg, 4.09 mol) was added followed by PPh_3 (1.07 mg, 4.09 mmol) in small portions. The reaction was stirred for 1.5 hours and then water was added. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The remains were taken up in diethyl ether (4 ml). pentane (20 ml) was carefully layered on top. The solution turned cloudy and was set into in a freezer. The solution was filtered and the remaining solid washed with ice-cold pentane several times. The combined filtrate was concentrated under reduced pressure affording bromide **2.21** (0.815 g, 2.55 mmol, purity 95%, 81%) as a brown oil.

^{19}F-NMR (377 MHz, $CDCl_3$, δ/ppm): -112.71 - -112.92 (m); **HRMS** (CI): *m/z*: 320.1385 $[M + NH_4]^+$ (calcd.: *m/z*: 320.1389 $[M + NH_4]^+$).

((2*E*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl acetate (2.27)

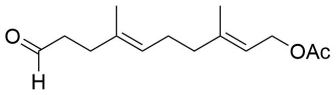


Farnesol (7.12 ml, 28.4 mmol) was dissolved in dry DCM (50 ml) and cooled in an ice bath. Et_3N (5.91 ml, 42.6 mmol) and DMAP (69.0 mg, 568 μmol) were subsequently added followed by the slow addition of acetic anhydride (3.22 ml, 34.1 mmol). After 1 hour, the reaction was poured into water and extracted three times with DCM. The combined organic phases were washed with 1M HCl, sat. $NaHCO_3$ and brine, then dried over Na_2SO_4. The solvent was removed under reduced pressure. This procedure afforded acetate **2.27** (6.87 g, 25.9 mmol, 91%) as a clear oil, which was used without further purification.

The analytical data were in accordance with the literature.[4]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.34 (tq, *J*=7.1, 1.3, 1H), 5.14 - 5.05 (m, 2H), 4.58 (d, *J*=7.1, 2H), 2.18 - 2.02 (m, 9H), 2.00 - 1.93 (m, 2H), 1.70 (s, 3H), 1.68 (s, 4H), 1.59 (s, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 171.2, 142.4, 135.6, 131.4, 124.4, 123.7, 118.4, 61.5, 39.8, 39.6, 26.8, 26.3, 25.8, 21.2, 17.8, 16.6, 16.1.

((2*E*,6*E*)-3,7-Dimethyl-10-oxodeca-2,6-dien-1-yl acetate (2.29)



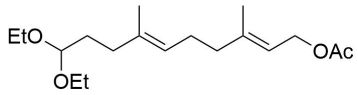
Acetate **2.27** (6.87 g, 25.9 mmol) was dissolved in THF (100 ml) and water (40 ml) under vigorous stirring. The mixture was cooled in an ice bath and NBS (3.05 g, 25.9 mmol) was added portionwise over the course of 30 minutes. When the solution cleared more water was added. After the complete addition of NBS the reaction was warmed to room temperature and stirred for 4.5 hours. Then KOH (1.45 g, 25.9 mmol) was dissolved in MeOH (12 ml) and added to the reaction. After 4 hours, diethyl ether was added. The solution was washed twice with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. The remains were taken up in diethyl ether (10 ml) and added to a solution of $HIO_4 \cdot H_2O$ (8.85 g, 38.8 mmol) in diethyl ether (280 ml, pre-stirred for 1 hour). After 20 mins, the reaction was poured into water and the organic phase washed twice with brine, then dried over Na_2SO_3. The solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 8:2). This procedure afforded pure aldehyde **2.29** (2.35 g, 9.80 mmol, 37% over 3 steps) as a clear oil.

The analytical data were in accordance with the literature.[5]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.74 (t, *J*=1.9, 1H), 5.31 (tq, *J*=7.1, 1.3, 1H), 5.12 (tq, *J*=6.9, 1.3, 1H), 4.57 (d, *J*=7.1, 2H), 2.50 (td, *J*=7.6, 1.9, 2H), 2.31 (t, *J*=7.5, 2H),

2.15 - 2.07 (m, 2H), 2.07 - 2.01 (m, 5H), 1.68 (s, 3H), 1.61 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 202.7, 171.3, 142.0, 133.6, 124.9, 118.6, 61.5, 42.2, 39.4, 31.9, 26.2, 21.2, 16.6, 16.2.

((2*E*,6*E*)-10,10-Diethoxy-3,7-dimethyldeca-2,6-dien-1-yl acetate (2.30)

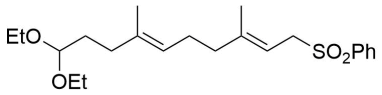


Aldehyde **2.29** (1.5 g, 6.29 mmol) was dissolved in a mixture of pyridinium p-toluenesulfonate (158 mg, 629 μmol) and benzene (10 ml). The reaction was heated to reflux under Dean-Stark conditions. After 3.5 hours, the reaction was cooled to room temperature and diethyl ether was added. The resulting organic phase was washed with sat. $NaHCO_3$ and dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 9:1, R_f 0.2). This yielded in pure acetal **2.30** (1.63 g, 5.22 mmol, 83%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.32 (t, *J*=7.2, 1H), 5.10 (t, *J*=7.0, 1H), 4.57 (d, *J*=7.1, 2H), 4.45 (t, *J*=5.7, 1H), 3.63 (q, *J*=7.0, 2H), 3.47 (q, *J*=7.1, 2H), 2.15 - 1.97 (m, 9H), 1.74 - 1.65 (m, 5H), 1.59 (d, *J*=1.2, 3H), 1.19 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 171.3, 142.3, 135.0, 123.9, 118.4, 102.7, 61.5, 61.0 (2C), 39.6, 34.8,

32.0, 26.3, 21.2, 16.6, 16.2, 15.5 (2C); **IR** (cm^{-1}): 2974, 2932, 2359, 1737, 1444, 1372, 1230, 1123, 1058, 1024, 955, 875; **HRMS** (ESI): *m/z*: 335.2192 $[M + Na]^+$ (calcd.: *m/z*: 335.2192 $[M + Na]^+$).

(((2*E*,6*E*)-10,10-Diethoxy-3,7-dimethyldeca-2,6-dien-1-yl) sulfonyl)benzene (2.20)

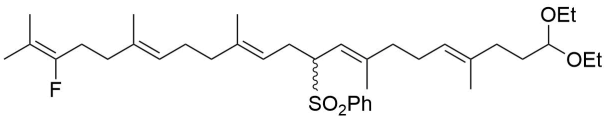


Note: DCM and water were thoroughly degassed under an argon stream prior to the reaction. Acetal **2.30** (1.63 g, 5.22 mmol) was dissolved in DCM (12 ml) and water (14 ml). $PhSO_2Na$ (1.45 g, 8.84 mmol) and tetraethylammonium bromide (109 mg, 522 μmol) were subsequently added. A solution of $(Pd(\pi - allyl)Cl)_2$ (41.1 mg, 104 μmol) and dppf (170 mg, 313 μmol) in DCM (2 ml) were added via cannula. A quick colour change from yellow to organe to red was observed. After 2 hours, the aqueous phase was separated and extracted two times with DCM. The combined organic phases were dried over $MgSO_4$ and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 1:1, R_f 0.27). This procedure afforded pure sulfone **2.20** (2.06 g, 5.22 mmol, quant.) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.86 (d, nmrj 7.5, 1H), 7.63 (t, *J*=7.3, 1H), 7.53 (t, *J*=7.8, 2H), 5.24 - 5.14 (m, 1H), 5.12 - 5.01 (m, 1H), 4.45 (t, *J*=5.7, 1H), 3.80 (d,

J=8.0, 2H), 3.70 - 3.58 (m, 2H), 3.55 - 3.42 (m, 2H), 2.08 - 1.95 (m, 6H), 1.76 - 1.61 (m, 2H), 1.58 (s, 3H), 1.30 (d, *J*=1.3, 3H), 1.20 (t, *J*=7.0, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 146.5, 138.8, 135.3, 133.7, 129.1 (2C), 128.7 (2C) , 123.6, 110.4, 102.7, 61.1 (2C) , 56.2, 39.8, 34.8, 32.0, 26.3, 16.3, 16.2, 15.5 (2C); **HRMS** (ESI): *m/z*: 417.2062 $[M + Na]^+$ (calcd.: *m/z*: 417.2070 $[M + Na]^+$).

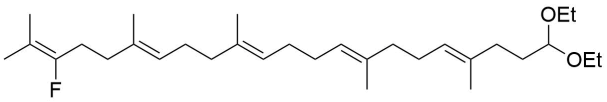
(((4*E*,8*E*,12*E*,16*E*)-1,1-Diethoxy-20-fluoro-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaen-10-yl)sulfonyl)benzene (2.31)



Sulfone **2.20** (0.911 g, 2.31 mmol) was dissolved in dry THF (5.5 ml) and DMF (1.8 ml). The solution was cooled to -78 °C, then *t*BuOK (1M in THF, 2.55 ml, 2.55 mmol) was added dropwise. A sharp colour change to orange was observed. After 2 hours, bromide **2.21** (0.815 g, 2.55 mmol, 95% pure) was added dropwise and the solution was stirred for further 2 hours at -78 °C. Then the reaction was allowed to warm to room temperature over night. Sat. NH_4Cl solution was added and extracted three times with diethyl ether. The combined organic phases were washed with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 4:1, R_f 0.12). This procedure afforded pure acetal **2.31** (1.18 mmol, 82%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.90 - 7.80 (m, 2H), 7.65 - 7.57 (m, 1H), 7.55 - 7.47 (m, 2H), 5.11 - 5.05 (m, 2H), 5.02 - 4.93 (m, 2H), 4.45 (t, *J*=5.7, 1H), 3.72 (td, *J*=10.6, 3.3, 1H), 3.70 - 3.57 (m, 2H), 3.54 - 3.44 (m, 2H), 2.93 - 2.83 (m, 1H), 2.42 - 2.20 (m, 2H), 2.10 (dd, *J*=9.2, 6.3, 1H), 2.06 - 1.85 (m, 8H), 1.73 - 1.50 (m, 18H), 1.20 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 154.06 (d, J_{CF}=241.5), 145.3, 138.7, 138.3, 135.2, 134.3, 133.4, 129.3 (2C), 128.8 (2C), 124.7, 123.7, 118.8, 117.1,107.31 (d, J_{CF}=17.9), 102.7, 68.1, 65.0, 61.0, 39.9, 39.82 (d, J_{CF}=14.8), 34.8, 32.1, 27.9, 27.6, 26.7, 26.6, 26.5, 17.8, 17.73 (d, J_{CF}=5.9), 16.5, 16.1, 16.0, 15.5 (2C); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.72 - -112.93 (m) ; **IR** (cm^{-1}): 2979, 2929, 1715, 1664, 1446, 1382, 1304, 1145, 1084, 1062, 739, 717, 689, 608; **HRMS** (CI): *m/z*: 588.3830 $[M - C_2H_5 + H]^+$ (calcd.: *m/z*: 588.3649 $[M - C_2H_5 + H]^+$).

(6*E*,10*E*,14*E*,18*E*)-22,22-Diethoxy-3-fluoro-2,6,10,15,19-pentamethyldocosa-2,6,10,14,18-pentaene (2.32)

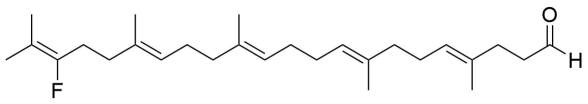


Sulfone **2.31** (0.311 g, 0.536 mmol) was dissolved in dry THF (5.3 ml). A mixture of $[Pd(allyl)Cl]_2$ (19.6 mg, 53.6 μmol) and dppp (66.3 mg, 160 μmol) were added followed by $LiBHEt_2$ solution (1.34 ml, 1.34 mmol, 1M in THF). A quick colour change was observed from yellow to red and finally brown. After 2 hours, the mixture was

cooled in an ice bath and sat. NH_4Cl solution carefully added. The mixture was extracted three times with diethyl ether and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product was purified by column chromatography (PET 40-60/diethyl ether 96:4, R_f 0.32). This procedure afforded pure acetal **2.32** (215 mg, 0.450 mmol, 84%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.21 - 5.07 (m, 4H), 4.46 (t, *J*=5.8, 1H), 3.64 (dq, *J*=9.4, 7.0, 2H), 3.49 (dq, *J*=9.4, 7.0, 2H), 2.42 - 2.20 (m, 2H), 2.19 - 1.90 (m, 15H), 1.77 - 1.66 (m, 3H), 1.63 - 1.55 (m, 18H), 1.20 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 154.1 (d, J_{CF}=241.7), 135.2, 135.2, 134.4, 134.1, 125.1, 124.6, 124.5, 124.5, 124.4, 119.1, 107.2 (d, J_{CF}=17.9), 102.8, 77.4, 61.1 (2C), 39.8 (d, J_{CF}=6.2), 36.8, 34.8, 32.1, 28.4, 28.0, 27.7, 26.8, 26.8, 17.8, 17.7 (d, *J*=5.9), 16.2, 16.1, 15.5 (2C); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.82 (ttq, *J*=26.3, 5.8, 3.0); **IR** (cm^{-1}): 2973, 2926, 2360, 1715, 1668, 1446, 1375, 1260, 1189, 1120, 1063, 915, 846, 612; **HRMS** (ESI): *m/z*: 499.3919 $[M+Na]^+$ (calcd.: *m/z*: 499.3921 $[M+Na]^+$).

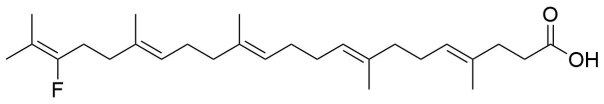
(4*E*,8*E*,12*E*,16*E*)-20-fluoro-4,8,13,17,21-pentamethyldocosa -4,8,12,16,20-pentaenal (2.33)



Acetal **2.32** (215 mg, 0.450 mmol) was dissolved in acetone (1.4 ml) and 6 drops of water were added. Amberlyst® 15 resin (400 mg) were added and the mixture stirred until TLC showed complete consumption of starting material. The mixture was filtered through celite and dried over Na_2SO_4. The solvent was removed under reduced pressure. This procedure afforded pure aldehyde **2.33** (148 mg, 0.355 mmol, 79%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.75 (t, *J*=2.0, 1H), 5.25 - 4.99 (m, 4H), 2.50 (td, *J*=7.5, 2.0, 2H), 2.38 - 2.29 (m, 3H), 2.26 (dd, *J*=9.4, 6.4, 1H), 2.19 - 1.85 (m, 14H), 1.66 - 1.53 (m, 18H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 202.8, 154.1 (d, J_{CF}=241.5), 135.2, 134.9, 134.1, 133.0, 125.6, 125.1, 124.6, 124.4, 107.2 (d, J_{CF}=17.9), 42.3, 39.7 (d, J_{CF}=12.0), 36.8, 32.0, 28.4, 28.0, 27.7, 26.8, 26.7,17.7 (d, J_{CF}=6.0), 16.2, 16.2, 16.1, 16.1, 15.7, 15.6; **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.67 - -112.87 (m); **IR** (cm^{-1}): 2922, 2855, 2713, 2360, 1727, 1447, 1384, 1260, 1187, 1154, 1113, 914, 846, 743; **HRMS** (ESI): *m/z*: 425.3187 $[M+Na]^+$ (calcd.: 425.3190 *m/z*: $[M + Na]^+$).

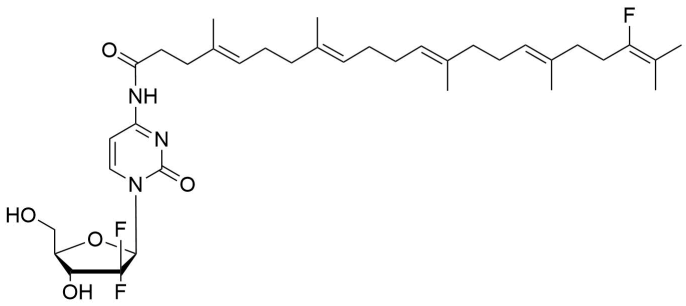
(4*E*,8*E*,12*E*,16*E*)-20-Fluoro-4,8,13,17,21-pentamethyldocosa -4,8,12,16,20-pentaenoic acid (2.1)



Aldehyde **2.33** (148 mg, 0.355 mmol) was dissolved in acetone (3.4 ml) and cooled in an ice bath. Jones Reagent was added dropwise until the red colour persisted. Excess of Jones Reagent was quenched with *i*PrOH. Brine and diethyl ether were added. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography and the desired regioisomer was separated by column chromatography over $AgNO_3$-infused silica gel (PET 40-60/EtOAc/MeOH 8:1.5:0.5). This procedure afforded pure acid **2.1** (56 mg, 133 μmol, 37%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.38 - 5.12 (m, 4H), 2.45 (t, *J*=7.4, 2H), 2.38 - 2.21 (m, 4H), 2.21 - 2.00 (m, 14H), 1.69 - 1.64 (m, 12H), 1.60 (d, *J*=3.3, 3H), 1.55 (d, *J*=2.7, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 179.8, 154.1 (d, J_{CF}=241.6), 135.2, 135.0, 134.1, 133.0, 125.5, 125.1, 124.6, 124.4, 107.2 (d, J_{CF}=18.2), 39.7 (d, J_{CF}=10.6), 36.8, 34.4, 33.1, 28.4, 28.0, 27.7, 26.8, 26.8, 17.7 (d, J_{CF}=6.1), 16.2, 16.1, 15.7, 15.6; **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.77 (t, J_{HF}=26.6); **IR** (cm^{-1}): 2917, 2857, 1709, 1447, 1384, 1287, 1211, 1154, 1098, 1036, 917, 848, 704; **HRMS** (ESI): *m/z*: 441.3141 $[M+Na]^+$ (calcd. *m/z*: 441.3139 $[M+Na]^+$).

Synthesis of Fluoro-squalenoyl Gemcitabine (F-Sq-Gem)



Gemcitabine HCl (36.4 mg, 121 μmol) was suspended in dry DMF (1 ml) and DMSO (0.5 ml). N-methylmorpholine (14.8 μl, 133 μmol) and HOBt hydrate (18 mg, 133 μmol) were added sequentially. Carboxylic acid **2.1** (56 mg, 133 μmol) was dissolved in DMF (0.5 ml) and DMSO (0.1 ml) and added. Finally, EDC HCl (30 mg, 158 μmol) was added and the mixture was heated to 65 °C. After 16 hours, the mixture was cooled to room temperature and EtOAc and brine were added. The aqueous phase was extracted two more times with EtOAc. The combined organic phases were washed with sat. $NaHCO_3$ and brine, then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The product was purified by column chromatography (DCM/MeOH 96:4). This procedure afforded pure **F-Sq-Gem** (30 mg, 45.2 μmol, 37%) as a clear waxy solid.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 8.83 (s, 1H), 8.04 (d, *J*=7.6, 1H), 7.48 (d, *J*=7.6, 1H), 6.21 (t, *J*=7.5, 1H), 5.26 - 5.04 (m, 4H), 4.65 - 4.44 (m, 1H), 4.06 (d, *J*=11.2, 2H), 4.00 - 3.90 (m, 1H), 2.53 (dd, *J*=9.0, 6.4, 2H), 2.39 - 2.23 (m, 4H), 2.17 - 1.92 (m, 14H),

1.65 - 1.57 (m, 18H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 173.5, 162.9, 155.6, 154.0 (d, J_{CF}=241.4), 153.1, 145.3, 135.1, 134.9, 134.0, 132.6, 125.9, 125.0, 124.4, 124.3 119.6 (d, J_{CF}=165.4), 107.1 (d, J_{CF}=18.0), 97.6, 81.6, 69.16 (t, J_{CF}=22.8), 59.8, 39.6 (d, J_{CF}=13.0), 36.6, 36.5, 34.4, 28.3, 28.3, 27.8, 27.6, 26.8, 26.7, 17.6 (d, J_{CF}=6.0), 16.1, 16.1, 15.9, 15.9, 15.5 (d, J_{CF}=9.3); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm):-112.54 - -112.96 (m, 1F), -117.74 (br, 2F); **IR** (cm^{-1}): 3309, 2919, 2857, 2360, 2341, 1715, 1661, 1620, 1559, 1492, 1437, 1386, 1323, 1275, 1196, 1129, 1081, 915, 814, 814, 787, 743, 668; **HRMS** (ESI): *m/z*: 664.3927 $[M + H]^+$ (calcd. *m/z*: 664.3931 $[M + H]^+$).

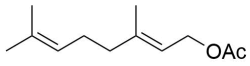
F-Sq-Gem Nanoparticle formation and characterisation

A stock solution of **F-Sq-Gem** (5 mg) in acetone (1 ml) was prepared. An aliquote of the stock solution (0.5 ml) was added dropwise to 5% dextrose solution (2.5 ml) while vigorously stirring (500 rpm). The solution became turbid and the characteristic Tyndall effect of colloidal solutions was well visible. After complete addition, acetone was evaporated under reduced pressure at 40 °C. This resulted in a solution of **F-Sq-Gem** nanoparticles (1 mg/ml).

The nanoparticles were characterised by Dynamic Light Scattering (DLS) on a Malvern Zetasizer Nano ZS at 25 °C. Zeta-potentials were measured on the same instrument applying the Smoluchowski equation. All measurements were performed three times.

F-Sq-Gem and **Sq-Gem** co-nanoparticles were prepared by mixing the stock solutions in the desired ratio and following the nanoprecipitation procedure explained above.

(*E*)-3,7-Dimethylocta-2,6-dien-1-yl acetate (2.36)



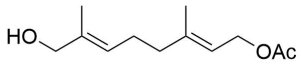
The synthesis was performed according to literature procedure.[6]

Geraniol (5 ml, 28.4 mmol), DMAP (69.0 mg, 0.568 mmol) and triethylamin (5.9 ml, 42.6 mmol) were dissolved in dry DCM (60 ml) and cooled to 0 °C. Acetic anhydride (3.2 ml, 34.1 mmol) was slowly added. After 40 minutes, the reaction mixture was poured into water and extracted three times with DCM. The combined organic phases were washed with 1m HCl, sat. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. This procedure afforded pure **2.36** (5.5 g, 28.4 mmol, quant.) as a clear oil.

The analytical data were in accordance with the literature.[4]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.35 - 5.28 (m, 1H), 5.09 - 5.01 (m, 1H), 4.55 (d, *J*=7.1 Hz, 2H), 2.15 - 1.97 (m, 7H), 1.67 (s, 3H), 1.65 (d, *J*=1.0 Hz, 3H), 1.57 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 171.0, 142.2, 131.8, 123.8, 118.3, 61.4, 39.5, 26.3, 25.6, 21.0, 17.7, 16.4.

(2*E*,6*E*)-8-Hydroxy-3,7-dimethylocta-2,6-dien-1-yl acetate (2.37)

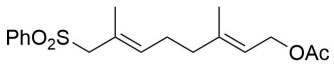


Ester **2.36** (12.6 g, 64.4 mmol) were dissolved in DCM (80 ml). *t*BuOOH (70% in water, 26.4 ml, 193 mmol), salicylic acid (0.889 g, 6.44 mmol) and selenium dioxide (0.714 g, 6.44 mmol) were added. The mixture was stirred over night. The aqueous phase was separated and extracted twice with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The remains were dissolved in methanol (120 ml) and cooled to 0°C. $NaBH_4$ (0.609 g, 16.1 mmol) was added portionwise. The mixture was stirred for 15 mins., then sat. $NaHCO_3$ was added carefully. The solution was extracted three times with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 1:1, R_f0.25). This procedure afforded pure alcohol **2.37** (6.11 g, 28.7 mmol, 44%) as a clear oil.

The analytical data were in accordance with the literature.[4]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.41 - 5.27 (m, 2H), 4.62 - 4.52 (m, 2H), 3.98 (d, *J*=1.2, 2H), 2.20 - 2.13 (m, 2H), 2.11 - 2.04 (m, 5H), 1.70 (s, 3H), 1.66 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 171.2, 141.7, 135.2, 125.2, 118.7, 68.7, 61.3, 39.0, 25.6, 21.0, 16.3, 13.6;

(2*E*,6*E*)-3,7-Dimethyl-8-(phenylsulfonyl)octa-2,6-dien-1-yl acetate (2.35)



Alcohol **2.37** (6.11 g, 28.7 mmol) was dissolved in dry THF (6.5 ml) and cooled to 0°C. PBr_3 (1.34 ml, 14.3 mmol) was added dropwise. The mixture was stirred for 3 hours, then ice cold water was carefully added. The solution was extracted three times with diethyl ether. The combined organic phases were washed with sat. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure.

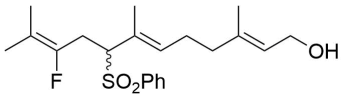
NMR data for intermediate bromide **2.40**: **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 5.55 (t, *J*=6.6, 1H), 5.33 (t, *J*=7.1, 1H), 4.57 (d, *J*=7.1, 2H), 3.95 (s, 2H), 2.15 (dd, *J*=8.6, 6.4, 2H), 2.08 (d, *J*=8.0, 2H), 2.05 (s, 3H), 1.74 (s, 3H), 1.69 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 171.2, 141.4, 132.5, 130.5, 118.9, 61.4, 41.7, 38.6, 26.5, 21.1, 16.5, 14.8.

The crude bromide **2.40** was dissolved in dry DMF (28 ml) and benzene sulfinic acid sodium salt (9.25 g, 57.4 mmol) was added. The suspension was stirred for 16 hours, then diethyl ether was added. The organic phase was washed with water and brine, then dried over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by column chromatography (PET 40-60/diethyl ether 1:1, R_f 0.3). This

procedure afforded pure sulfone **2.35** (7.38 g, 20.1 mmol, 70%) as a clear to yellowish oil.

The analytical data were in accordance with the literature.[7] **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 7.88 - 7.80 (m, 2H), 7.69 - 7.59 (m, 1H), 7.58 - 7.49 (m, 2H), 5.24 (tq, *J*=7.1, 1.3, 1H), 5.03 (t, *J*=6.6, 1H), 4.55 (d, *J*=7.1, 2H), 3.72 (s, 2H), 2.11 - 2.02 (m, 5H), 1.91 - 1.85 (m, 2H), 1.76 (d, *J*=1.3, 3H), 1.64 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 171.1, 141.2, 138.4, 135.4, 133.5, 128.9 (2C), 128.5 (2C), 123.7, 118.7, 66.1, 61.2, 38.4, 26.4, 21.1, 16.8, 16.4.

(2*E*,6*E*)-10-Fluoro-3,7,11-trimethyl-8-(phenylsulfonyl)dodeca-2,6,10-trien-1-ol (2.38)

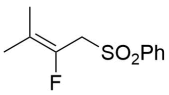


Sulfone **2.35** (2.23 g, 6.65 mmol) was dissolved in dry THF (18 ml) and dry DMF (4.6 ml). The solution was cooled to -78°C and *t*BuOK solution (1M in THF, 7.31 ml, 7.31 mmol) was added dropwise. A sharp colour change to orange was observed. After 2 hours, bromide **2.17** (1.22 g, 7.31 mmol) in dry THF (4.6 ml) was added dropwise. The mixture was stirred for 2 hours at -78°C and then allowed to warm to room temperature overnight. The reaction was quenched with sat. NH_4Cl and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent

evaporated under reduced pressure. The remains were dissolved in methanol (23 ml) and 1 M aqueous NaOH solution was added until pH was approximately 12. Then brine was added and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (PET 40-60/EtOAc 1:1, R_f 0.3). This yielded pure alcohol **2.38** (1.84 g, 4.85 mmol, 73%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.79 - 7.75 (m, 2H), 7.59 - 7.53 (m, 1H), 7.47 (dd, *J*=8.4, 7.0, 2H), 5.27 (t, *J*=7.0, 1H), 5.12 (t, *J*=7.1, 1H), 4.05 (d, *J*=7.1, 2H), 3.75 - 3.67 (m, 1H), 2.90 - 2.78 (m, 2H), 2.07 - 1.91 (m, 5H), 1.80 (t, *J*=7.4, 2H), 1.61 (s, 3H), 1.54 (s, 3H), 1.49 (s, 6H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm):-114.75 - -114.95 (m); **IR** (cm^{-1}): 3017, 2935, 2860, 1446, 1304, 1195, 1143, 1112, 1084, 1024, 905, 759, 735, 689; **HRMS** (CI): *m/z*: 398.2160 $[M + NH_4]^+$ (calcd. *m/z*: 398.2165 $[M + NH_4]^+$).

((2-Fluoro-3-methylbut-2-en-1-yl)sulfonyl)benzene (2.39)



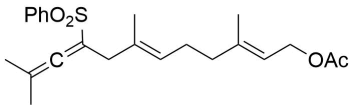
Bromide **2.17** (0.5 g, 2.99 mmol) was dissolved in dry DMF (3 ml). Benzene sulfinic acid sodium salt (0.964 g, 5.98 mmol) was added and the reaction stirred for 16 hours. Water was added and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The

product was purified by column chromatography (PET 40-60/diethyl ether 2:1, R_f 0.22). This procedure afforded pure sulfone **2.39** (0.516 g, 2.26 mmol, 75%) as a colourless solid.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.93 - 7.88 (m, 2H), 7.70 - 7.63 (m, 1H), 7.60 - 7.52 (m, 2H), 4.03 (d, *J*=20.4, 2H), 1.64 (d, *J*=3.4, 3H), 1.38 (d, *J*=3.1, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 142.8 (d, J_{CF}=240.1), 138.5, 134.0, 129.2, 129.2, 129.2, 128.5, 118.4 (d, J_{CF}=15.2), 57.0 (d, J_{CF}=32.0), 17.6 (d, J_{CF}=4.2), 16.0 (d, J_{CF}=8.0); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -110.26 - -110.44 (m); **IR** (cm^{-1}): 2360, 1708, 1447, 1320, 1162, 1139, 1083, 943, 868, 756, 687, 607. *HRMS could not be obtained for this compound with the available methods.*

(2*E*,6*E*)-3,7,11-Trimethyl-9-(phenylsulfonyl)dodeca-2,6,9,10-tetraen-1-yl acetate

(2.41)



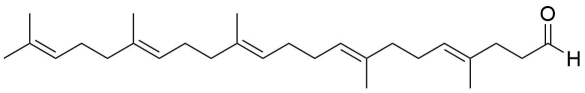
Sulfone **2.39** (0.1 g, 0.438 mmol) was dissolved in dry THF (1 ml) and DMF (0.3 ml). The mixture was cooled to -78 ◦ C and *t*BuOK (1M in THF, 0.48 ml, 0.48 mmol) was added dropwise. After 2 hours, bromide **2.40** (0.144 g, 0.525 mmol) in dry THF (0.3 ml) was slowly added. The reaction was warmed to room temperature after 2 hours,

and stirred overnight. Sat. aq. NH_4Cl was added and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 2:1, R_f 0.18). This procedure afforded allene **2.41** (20 mg, 49.7 μmol, 11%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.90 - 7.81 (m, 2H), 7.61 - 7.56 (m, 1H), 7.54 - 7.48 (m, 2H), 5.31 (t, *J*=7.1, 1.3, 1H), 5.15 (tq, *J*=7.0, 1.3, 1H), 4.57 (d, *J*=7.0, 2H), 2.95 (s, 2H), 2.05 (s, 3H), 2.04 – 1.94 (m, 4H), 1.71 (s, 6H), 1.69 (s, 3H), 1.43 (s, 3H) ; **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 202.8, 171.1, 141.9, 141.0, 133.0, 130.5, 128.9, 128.8, 128.0, 127.9, 127.5, 118.4, 109.4, 106.4, 61.3, 39.1, 37.5, 26.3, 21.0, 19.5, 19.5, 16.4, 15.2.

Experimental Procedures for Chapter 3

(4*E*,8*E*,12*E*,16*E*)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaenal (3.3)



The reaction was performed according to literature procedure.[8]

Squalene (11.6 g, 24.3 mmol) was dissolved in dry THF (90 ml) and water was added until the mixture turned opaque. The reaction was cooled in an ice bath and NBS (4.3 g, 36.5 mmol) was slowly added. When the solution started to become clear, more water was added. After complete addition the reaction was allowed to warm to room temperature and stirred for 5 hours. PET 40-60 was added and the aqueous phase separated. The organic phase was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 9:1). This procedure afforded pure bromohydrin **3.4** (3.90 g, 7.68 mmol, 31%) as a clear oil.

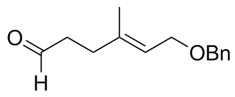
Bromohydrin **3.4** (3.76 g, 7.4 mmol) was dissolved in methanol (160 ml) and a methanolic solution of KOH (0.83 g, 14.8 mmol) was added. The reaction was stirred for 2.5 hours, then poured into PET 40-60. The organic phase was washed with brine.

The obtained aqueous phase was extracted twice with PET 40-60. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. This afforded crude epoxide **3.5**.

Periodic acid (3.37 g, 14.8 mmol) was suspended in diethyl ether (1.1 l) and stirred vigorously for one hour. A solution of epoxide **3.5** was added at room temperature. After 20 minutes, the reaction was decanted into brine. The phases were separated and the organic phase was washed two more times with brine before drying over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 9:1). This procedure afforded pure aldehyde **3.3** (2.57 g, 6.68 mmol, 90% over 2 steps) as a clear oil.

The analytical data were in accordance with the literature.[8] **^{1}H-NMR** (200 MHz, $CDCl_3$, δ/ppm): 9.75 (t, *J*=1.8 Hz, 1H), 5.38-4.96 (m, 5H), 2.59-2.44 (m, 2H), 2.32 (t, *J*=7.2 Hz, 2H), 2.16-1.90 (m, 16H), 1.75-1.54 (m, 18H); **MS** (ESI): *m/z*: 407.3 $[M+Na]^+$ (calcd.: *m/z*: 407.3 $[M+Na]^+$).

(E)-6-(Benzyloxy)-4-methylhex-4-enal (3.6)



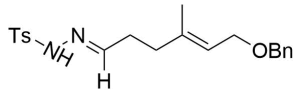
Ether **2.24** (3.7 g, 15.4 mmol) was dissolved in dioxane (20 ml) and water (1.6 ml), then cooled in an ice bath. NBS (1.8 g, 15.4 mmol) was added in three portions over

15 minutes, before the reaction was allowed to warm to room temperature and stirred for 5 hours. KOH (0.8 g, 15.4 mmol) was dissolved in 3 ml methanol and added to the reaction mixture. After 3 hours, water was added and the aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure.

The remaining oil was dissolved in THF (20 ml) and water (1.6 ml). $HIO_4 \cdot 2H_2O$ (4.2 g, 18.4 mmol) and $NaIO_4$ (1.6 g, 7.7 mmol) were subsequently added. After 30 minutes the reaction was quenched with sat. Na_2SO_4 and extracted three times with ethyl acetate. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the product was purified by column chromatography (Petroleum ether 40-60/ diethyl ether 2:1). This procedure afforded pure aldehyde **3.6** (1.2 g, 5.6 mmol, 37% over 2 steps) as a clear oil.

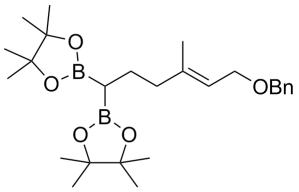
The analytical data were in accordance with the literature.[9] **^{1}H-NMR** (200 MHz, $CDCl_3$, δ/ppm): 9.78 (t, *J*=1.6 Hz, 1H), 7.53-7.28 (m, 5H), 5.42 (d, *J*=6.7 Hz, 1H), 4.51 (s, 2H), 4.03 (d, *J*=6.7 Hz, 2H), 2.70-2.49 (m, 2H), 2.49-2.27 (m, 2H), 1.66 (s, 3H); **MS** (ESI): *m/z*: 241.1 $[M+Na]^+$ (calcd.: *m/z*: 241.1 $[M+Na]^+$).

N'-((1*E*,4*E*)-6-(Benzyloxy)-4-methylhex-4-en-1-ylidene)-4-methylbenzenesulfonohydrazide (3.7)



Aldehyde **3.6** (50.0 mg, 0.229 mmol) was dissolved in dry methanol (1 ml) and tosylhydrazin (46 mg, 0.252 mmol) was added. The reaction was stirred at room temperature for 1 hour. The solvent was removed *in vacuo*. The product was used in the next step without purification.

(E)-2,2'-(6-(Benzyloxy)-4-methylhex-4-ene-1,1-diyl)bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (3.8)

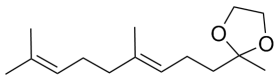


Hydrazone **3.7** (0.229 mmol) was dissolved in dry toluene (1 ml) and sodium hydride (11.0 mg, 0.275 mmol, 60% in mineral oil) was added. After 1 hour, bis(pinacolato)diboron (69.8 mg, 0.275 mmol) was added. The mixture was heated to 110°C and stirred overnight. After cooling to room temperature diethyl ether and water

(5 ml each) were added, the aqueous phase was extracted twice with diethyl ether and the combined organic phases were washed with brine. The organic phase was dried over Na_2SO_4 and the solvent removed under vacuum. The product was columned twice (PET 40-60/diethyl ether 4:1). This procedure afforded ether **3.8** (41.7 mg, 91.6 μmol, 40%).

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.51-7.15 (m, 5H), 5.38 (t, *J*=6.8 Hz, 1H), 4.50 (s, 2H), 4.01 (d, *J*=6.7 Hz, 2H), 2.02 (dd, *J*=9.5, 6.0 Hz, 2H), 1.80-1.58 (m, 5H), 1.32-1.11 (m, 24H), 0.72 (t, *J*=7.8 Hz, 1H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 140.9, 138.6, 128.4, 127.9, 127.5, 121.0, 72.0, 66.7, 42.5, 25.1, 25.0, 24.2, 16.4; **HRMS** (FI): *m/z*: 456.3051 $[M]^+$ (calcd.: *m/z*: 456.3218 $[M]^+$).

(E)-2-(4,8-Dimethylnona-3,7-dien-1-yl)-2-methyl-1,3-dioxolane (3.10)

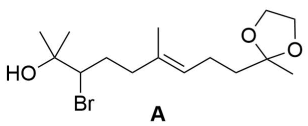


(E)-6,10-Dimethylundeca-5,9-diene-2-one (*3.9*, 5.0 g, 25.7 mmol,) was dissolved in dry DCM (20 ml). Ethylene glycol (4.3 ml, 77.1 mmol), triethyl orthoformate (12.8 ml, 77.1 mmol) and p-toluene sulfonic acid monohydrate (1.4 g, 7.7 mmol) were added subsequently. The mixture was heated to reflux and stirred for 5 hours. Then solid $NaHCO_3$ was added and the mixture cooled to room temperature. The organic phase

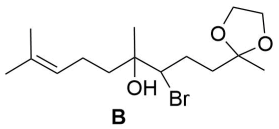
was washed with sat. $NaHCO_3$, water and brine. It was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 9:1). This procedure afforded pure acetal **3.10** (4.4 g, 18.5 mmol, 72%) as a clear oil.

The analytical data were in accordance with the literature.[10] **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 5.21-5.03 (m, 1H), 4.02-3.86 (m, 4H), 2.15-1.92 (m, 6H), 1.73-1.55 (m, 12H), 1.38-1.26 (m, 3H); **HRMS** (ESI): *m/z*: 313.0765 $[M+Na]^+$ (calcd.: *m/z*: 313.0773 $[M+Na]^+$).

(E)-3-Bromo-2,6-dimethyl-9-(2-methyl-1,3-dioxolan-2-yl)non-6-en-2-ol (3.11A) and 3-bromo-4,8-dimethyl-1-(2-methyl-1,3-dioxolan-2-yl)non-7-en-4-ol (3.11B)



A



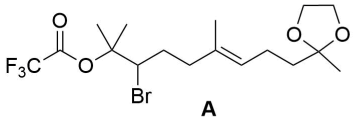
B

Acetal **3.10** (3.7 g, 15.4 mmol) was dissolved in THF:H_2O 1:1 (280 ml) and cooled to 0°C. Crystalline *N*-bromosuccinimide (2.2 g, 18.5 mmol) was added slowly while stirring vigorously. When the solution began to clear more water was added. After complete addition of NBS the solution was stirred for another hour. The reaction was quenched by adding sat. $NaHCO_3$. The mixture was extracted three times with diethyl ether.

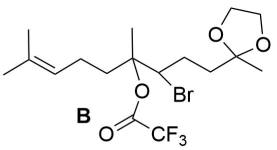
The combined organic phases were washed with sat. $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by column chromatography (PET 40-60/diethyl ether 1:1). This procedure afforded the regioisomers **3.11A** and **B** (2.6 g, 7.8 mmmol, 50%) in a inseparable 1:1 mixture as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.31-4.98 (m, 1H), 4.09-3.78 (m, 4H), 2.31-2.18 (m, 1H), 2.18-2.11 (m, 1H), 2.11-1.99 (m, 3H), 1.99-1.85 (m, 1H), 1.78-1.64 (m, 1H), 1.64-1.52 (m, 4H), 1.31-1.22 (m, 8H); **^{13}C-NMR** (101 MHz,$CDCl_3$, δ/ppm): 133.5, 133.2, 126.5, 125.6, 109.8, 72.4, 70.8, 70.7, 64.6, 39.3, 38.9, 32.3, 32.0, 30.4, 26.5, 26.5, 25.9, 25.8, 23.8, 23.8, 23.2, 22.7, 22.6, 15.8; **IR** (cm^{-1}): 2971, 1708, 1365, 1217, 1051, 626. **HRMS** (ESI): *m/z*: 335.12054 $[M+H]^+$ (calcd.: *m/z*: 335.12163 $[M+H]^+$).

(E)-3-Bromo-2,6-dimethyl-9-(2-methyl-1,3-dioxolan-2-yl)non-6-en-2-yl 2,2,2-trifluoroacetate (3.13A) and *(E)*-3-bromo-2,6-dimethyl-9-(2-methyl-1,3-dioxolan-2-yl)non-6-en-2-yl 2,2,2-trifluoroacetate (3.13B)



A

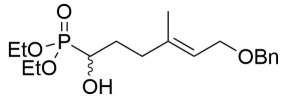


B

Bromohydrin **3.11** (mixture of regioisomers, 0.4 g, 1.2 mmol) was dissolved in dry DCM (10 ml) and cooled to 0°C. Pyridine (0.4 ml, 4.8 mmol) and trifluoroacetic anhydride (0.3 ml, 2.4 mmol) were added and the solution stirred for 10 minutes, then it was allowed to warm to room temperature and stirred for another hour. The reaction was quenched with sat. $NaHCO_3$. The organic phase was further washed with sat. $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. This afforded regioisomers bromohydrin **3.13A** and **B** (485.0 mg, 1.1 mmol, 93%) in an inseparable 1:1 mixture as a yellow oil. The product was sufficiently pure for further use without additional purification.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.22 (t, *J*=7.1 Hz, 2x 1H), 4.36 (dd, *J*=8.2, 1.8 Hz, 1H), 4.33 (dd, *J*=8.2, 1.8 Hz, 1H), 3.99-3.85 (m, 2x 4H), 2.37-2.19 (m, 2x 1H), 2.17-2.04 (m, 2x 2H), 2.01-1.86 (m, 2x 1H), 1.81-1.55 (m, 2x 13H), 1.31 (s, 2x 3H); **^{13}C-NMR** (101 MHz,$CDCl_3$, δ/ppm): 155.9 (q, *J*=41.9 Hz), 155.8 (q, *J*=41.8 Hz), 133.0, 132.8, 127.2, 126.4, 115.7, 112.9, 109.9, 109.9, 89.4, 89.4, 64.7, 64.7, 59.7, 59.4, 39.4, 39.0, 37.5, 31.5, 31.2, 29.9, 24.1, 24.0, 23.9, 23.9, 23.1, 22.8, 22.7, 22.4, 22.2, 15.6; **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -75.63 (s); **IR** (cm^{-1}): 2984, 2880, 1780, 1449, 1369, 1219, 1162, 1116, 1057, 947, 865, 775, 730, 634. **HRMS** (ESI): *m/z*: 430.0958 $[M+H]^+$ (calcd.: *m/z*: 430.0966 $[M+H]^+$).

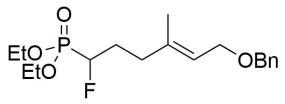
Diethyl *(E)*-(6-(benzyloxy)-1-hydroxy-4-methylhex-4-en-1-yl) phosphonate) (3.16)



Sodium (782.4 mg, 34.3 mmol) was dissolved in dry ethanol (approx. 20 ml). Excess solvent was evaporated *in vacuo*. The remaining solid was suspended in dry DCM (60 ml) and diethyl phosphonate (3.2 ml, 25.1 mmol) was added. The mixture was stirred for 30 minutes during which it turned clear. Aldehyde **3.6** (5.0 g, 22.9 mmol) in dry DCM (10 ml) was added to the mixture and stirred for 3 hours at room temperature. The reaction was quenched with 1M aq. HCl and EtOAc (100 ml) were added. The organic phase was separated, dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (EtOAc). This procedure afforded pure phosphonate **3.16** (5.8 g, 16.3 mmol, 71%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.34-7.20 (m, 5H), 5.48-5.33 (m, 1H), 4.43 (s, *J*=5.5 Hz, 2H), 4.19-4.03 (m, 4H), 3.96 (d, *J*=6.7 Hz, 2H), 3.84-3.72 (m, 1H), 2.32-2.23 (m, 1H), 2.18-2.04 (m, 1H), 1.92-1.79 (m, 1H), 1.79-1.67 (m, 1H), 1.59 (s, 3H), 1.27 (td, *J*=7.1, 2.6 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 139.2, 138.4, 128.3, 127.8, 127.5, 121.8, 72.1, 67.3 (d, J_{CP}=161.1 Hz), 62.6, 35.4 (d, J_{CP}=14.0 Hz), 29.3, 16.5 (d, J_{CP}=5.6 Hz); **^{31}P-NMR** (162 MHz, $CDCl_3$, δ/ppm): 24.96 (s); **IR** (cm^{-1}): 2981, 2929, 2858, 1668, 1453, 1391, 1367, 1229, 1051, 1025, 966, 792, 738, 698; **HRMS** (ESI): *m/z*: 379.1634 $[M+Na]^+$ (calcd.: *m/z*: 379.1644 $[M+Na]^+$).

Diethyl *(E)*-(6-(benzyloxy)-1-fluoro-4-methylhex-4-en-1-yl) phosphonate (3.18)

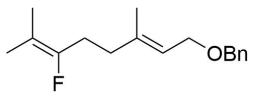


Phosphonate **3.16** (0.5 g, 1.4 mmol) was dissolved in dry DCM (10 ml) and cooled to-78 °C. Diethylaminosulfur trifluoride (DAST, 222 μl, 1.6 mmol) was added dropwise to the solution. After 1 hour, the mixture was allowed to warm to room temperature and was stirred for 2 more hours. The reaction was quenched by adding sat. $NaHCO_3$ until no more gas evolved. The aqueous phase was extracted three times with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (diethyl ether). This procedure afforded pure phosphonate **3.18** (114.0 mg, 0.3 mmol, 23%) as a yellowish oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.34-7.18 (m, 5H), 5.39 (td, *J*=6.7, 1.1 Hz, 1H), 4.62 (dm, J_{HF}=46.2 Hz, 1H), 4.43 (s, 2H), 4.21-4.05 (m, 4H), 3.96 (d, *J*=6.7 Hz, 2H), 2.32-2.19 (m, 1H), 2.17-2.05 (m, 1H), 2.03-1.86 (m, 2H), 1.59 (s, 3H), 1.27 (q, *J*=6.9 Hz, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 138.4, 138.2, 128.3, 127.8, 127.6, 122.4, 88.1 (dd, J_{CF}=180.4, J_{CP}=170.4 Hz), 72.2, 66.4, 63.0 (dd, J_{CF}=36.2, J_{CP}=6.7 Hz), 34.8 (dd, J_{CF}=13.3,J_{CP}=3.3 Hz), 28.3, 28.1, 16.5 (d, J_{CP}=4.1 Hz), 16.45 (d, J_{CP}=4.1 Hz), 16.3 ; **^{31}P-NMR** (162 MHz, $CDCl_3$, δ/ppm): 18.1 (d, J_{PF}=75.3 Hz); **^{19}F-NMR** (337 MHz,

$CDCl_3$, δ/ppm):-209.6- -210.2 (m); **IR** (cm^{-1}): 2983, 2918, 1717, 1415, 1393, 1245, 1023, 976, 716; **MS** (ESI): *m/z*: 381.0 $[M+Na]^+$ (calcd.: *m/z*: 381.1 $[M+Na]^+$).

(*E*)-(((6-Fluoro-3,7-dimethylocta-2,6-dien-1-yl)oxy)methyl)-benzene(3.19)

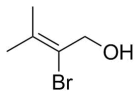


Phosphonate **3.18** (0.2 g, 0.6 mmol) was dissolved in dry THF (2 ml) and cooled to -78 °C. *n*BuLi (0.4 ml, 0.6 mmol, 1.6 M in hexanes) was added dropwise. Subsequently, acetone (143 μl, 1.9 mmol) was added. After 30 minutes the solution was allowed to warm to room temperature and stirred for another 2.5 hours. The reaction was quenched by adding sat. NH_4Cl and the aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 95:5). This yielded in pure **3.19** (31.0 mg, 114.3 μmol, 20 %) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.35-7.20 (m, 5H), 5.35 (td, *J*=6.8, 1.2 Hz, 1H), 4.43 (s, 2H), 3.96 (d, *J*=6.6 Hz, 2H), 2.34-2.21 (m, 2H), 2.16-2.10 (m, 2H), 1.59 (s, 3H), 1.54 (d, J_{HF}=3.1 Hz, 3H), 1.50 (d, J_{HF}=2.6 Hz, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 153.6 (d, J_{CF}=172 Hz), 140.1, 138.6, 128.3, 127.8, 127.5, 121.7, 72.1, 66.7, 36.5, 27.1,

17.6, 16.4, 15.5; **^{19}F-NMR** (337 MHz, $CDCl_3$, δ/ppm): -112.8 - -113.0 (m). *HRMS could not be obtained for this compound with the available methods.*

2-Bromo-3-methylbut-2-en-1-ol (3.27)

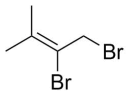


The synthesis was performed according to a literature procedure.[11]

3-Methyl-2-buten-1-ol (3.53 ml, 34.8 mmol) was dissolved in dry DCM (60 ml) and cooled in an ice bath. Bromine (1.87 ml, 36.5 mmol) was dissolved in DMC (6 ml) and added dropwise. After one hour the reaction was allowed to warm to room temperature and stirred for further 3 hours. Then DBU (7.79 ml, 52.2 mmol) was added dropwise and the reaction heated to reflux. After 4 hours, the reaction was cooled to room temperature and quenched with sat. $Na_2S_2O_3$ solution until colourless. Diethyl ether and 1M HCl solution were added. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were washed with sat. $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 1:1, R_f 0.4). This procedure afforded in pure alcohol **3.27** (5.00 g, 30.3 mmol, 87%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 4.37 (s, 2H), 1.99 (br, 1H), 1.90 (s, 3H), 1.86 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 134.8, 121.2, 64.8, 25.5, 20.7.

1,2-Dibromo-3-methylbut-2-ene (3.20)

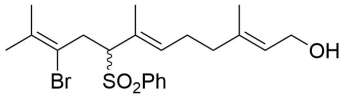


Alcohol **3.27** (5.00 g, 30.3 mmol) was dissolved in dry MeCN (60 ml) and cooled in an ice bath. CBr_4 (13.0 g, 39.3 mmol) was added then PPh_3 (10.3 g, 39.3 mmol) was added portionwise. After 5 minutes, the reaction was allowed to warm to room temperature and was stirred for an additional hour. Water was added and the mixture was extracted three times with DCM. The solvent was removed under reduced pressure. The remains were taken up into a minimal amount of DCM and evaporated onto silica gel. The product was purified by flash column chromatography (PET 40-60, R_f 0.46). This procedure afforded pure bromide **3.20** (6.03 g, 26.4 mmol, 87%) as a yellow oil.

The analytical data were in accordance with the literature.[11]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 4.38 (s, 2H), 1.92 (s, 3H), 1.86 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 138.7, 115.7, 36.5, 25.8, 20.6.

(2*E*,6*E*)-10-Bromo-3,7,11-trimethyl-8-(phenylsulfonyl)dodeca-2,6,10-trien-1-ol (3.25)

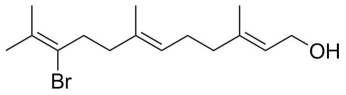


Sulfone **2.35** (6.03 g, 17.9 mmol) was dissolved in dry THF (50 ml) and dry DMF (12 ml). The solution was cooled to -78 °C and *t*BuOK solution (1M in THF, 19.6 ml, 19.6 mmol) was added dropwise. A sharp colour change to orange was observed. After 2 hours, bromide **3.20** (4.48 g, 19.6 mmol) in dry THF (4.6 ml) was added dropwise. The mixture was stirred for 2 hours at -78 °C and then allowed to warm to room temperature overnight. The reaction was quenched with sat. NH_4Cl and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The remains were dissolved in methanol (23 ml) and 1M aqueous NaOH solution was added until pH was approximately 12. Then brine was added and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (PET 40-60/EtOAc 1:1, R_f 0.3). This procedure afforded pure alcohol **3.25** (6.17 g, 13.9 mmol, 77%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.86 - 7.82 (m, 2H), 7.66 - 7.60 (m, 1H), 7.57 - 7.50 (m, 2H), 5.34 (tq, *J*=6.9, 1.3, 1H), 5.26 (tq, *J*=7.2, 1.6, 1H), 4.11 (d, *J*=6.9, 2H), 3.97 (dd, *J*=10.5, 4.0, 1H), 3.20 (dd, *J*=14.7, 10.5, 1H), 3.03 - 2.93 (m, 1H), 2.20 - 1.98 (m, 2H),

1.88 (t, *J*=7.6, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.68 (d, *J*=1.3, 3H), 1.61 (d, *J*=1.3, 3H) ; **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 138.5, 138.3, 135.7, 133.9, 133.6, 129.0 (2C), 128.8 (2C), 126.0, 124.2, 116.6, 72.4, 59.4, 38.4, 34.0, 26.3, 25.6, 21.0, 16.2, 14.9; **IR** (cm^{-1}): 3523, 2919, 2360, 1668, 1446, 1383, 1303, 1221, 1144, 1083, 1019, 753, 720, 689, 630; *HRMS could not be obtained for this compound with the available methods.*

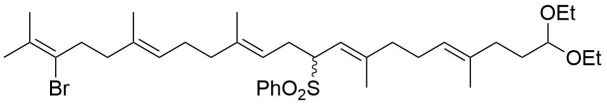
(2*E*,6*E*)-10-Bromo-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (3.26)



Magnesium turnings (0.755 g, 31.1 mmol) were suspended in dry THF (6.8 ml) and cooled to 0 °C. Sulfone **3.25** (1.33 g, 3.11 mmol) was dissolved in dry methanol (26.5 ml) and slowly added to the suspension. Small bubbles rising from the turnings confirmed the start of the reaction. If the reaction did not start spontaneously, a small crystall of iodine was added. The progress of the reaction was monitored by TLC (usual reaction time 1 hour). After completion, 1M aq. HCl was added and the mixture extracted 3 times with diethyl ether. The combined organic phases were washed with sat. $NaHCO_3$ and brine. The organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. This yielded a 1:1 mixture of the desired (6*E*)- and (7*E*)-alcohol. The two isomers were separated by $AgNO_3$-infused silica (PET 40-60/diethyl ether 2:3, $R_{f:6E}$ 0.56 $R_{f:7E}$ 0.61). This procedure afforded the desired (6*E*)-alcohol **3.26** (0.304 g, 1.00 mmol, 32 %) as clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.41 (t, *J*=7.0, 0H), 5.13 (t, *J*=6.1, 0H), 4.14 (d, *J*=7.0, 2H), 2.55 (dd, *J*=9.1, 6.4, 2H), 2.21 - 2.14 (m, 2H), 2.14 - 2.06 (m, 2H), 2.02 (dd, *J*=9.2, 5.8, 2H), 1.84 (s, 3H), 1.73 (s, 2H), 1.67 (s, 2H), 1.61 (s, 2H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 139.7, 134.2, 130.2, 124.8, 123.5, 121.7, 59.5, 39.5, 38.2, 38.2, 36.9, 26.4, 25.4, 20.4, 16.4, 16.3; **IR** (cm^{-1}): 3322, 2916, 2855, 2360, 2341, 1667, 1444, 1382, 1222, 1176, 1125, 1087, 998, 828, 745, 668, 640; **HRMS** (CI): *m/z*: 318.1413 $[M + NH_4]^+$ (calcd. *m/z*: 318.1433 $[M + NH_4]^+$).

(((4*E*,8*E*,12*E*,16*E*)-20-Bromo-1,1-diethoxy-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaen-10-yl)sulfonyl)benzene (3.24)



Alcohol **3.26** (0.541 g, 1.79 mmol) was dissolved in dry MeCN (7.1 ml) and cooled to 0 °C. CBr_4 (0.774 g, 2.33 mmol) was added followed by a slow addition of PPh_3 (0.612 g, 2.33 mmol). After 2 hours, water was added to the reaction and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The remains were dissolved in diethyl ether and layered with pentane (ratio 1:5). The precipitate was filtered off and washed thoroughly with pentane. The solvent was evaporated under reduced pressure. This

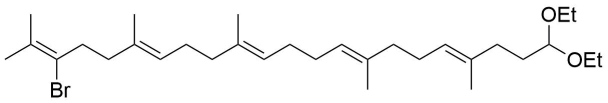
was repeated until less than 5% triphenylphosphineoxide was observed by NMR. This resulted in crude bromide **3.28** (>90% crude yield, purity >95%).

Sulfone **2.20** (0.564 g, 1.43 mmol) was dissolved in dry THF (3.4 ml) and dry DMF (1.14 ml) and cooled to -78 °C. *t*BuOK solution (1M in THF, 1.57 ml, 1.57 mmol) was added dropwise. A sharp colour change to orange was observed. After 2 hours a solution of bromide **3.28** in dry THF (1.14 ml) was added dropwise. After 2 hours, the solution was allowed to warm to room temperature overnight. The reaction was quenched by adding sat. aq. NH_4Cl and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 4:1, R_f 0.19). This procedure afforded pure sulfone **3.24** (0.676 g, 0.997 mmol, 69 %) as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.87 - 7.81 (m, 2H), 7.64 - 7.57 (m, 1H), 7.51 (dd, *J*=8.3, 7.0, 2H), 5.12 - 5.03 (m, 2H), 5.03 - 4.93 (m, 2H), 4.45 (t, *J*=5.7, 1H), 3.73 (td, *J*=10.5, 3.4, 1H), 3.68 - 3.59 (m, 2H), 3.53 - 3.43 (m, 2H), 2.88 (ddd, *J*=14.1, 7.4, 3.3, 1H), 2.35 (ddd, *J*=14.0, 10.6, 7.0, 1H), 2.15 (dd, *J*=9.2, 6.4, 2H), 2.56 - 2.50 (m, 2H), 2.08 - 1.97 (m, 4H), 1.97 - 1.93 (m, 5H), 1.84 (s, 3H), 1.75 - 1.66 (m, 5H), 1.63 - 1.56 (m, 12H), 1.22 - 1.18 (m, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 145.3, 138.7, 138.3, 135.1, 134.1, 133.4, 130.2, 129.3 (2C), 128.8(2C), 124.9, 123.7, 121.7, 118.8, 117.1, 102.7, 64.9, 61.0 (2C), 39.9, 39.7, 38.3, 36.9, 34.8, 32.1, 26.7, 26.6, 26.5, 25.4, 20.4, 16.7, 16.5, 16.2, 16.1, 15.5 (2C); **IR** (cm^{-1}): 2974, 2926, 2360, 1662, 1446, 1382, 1304,

1145, 1084, 1062, 1062, 738, 689, 609; **HRMS** (CI): *m/z*: 630.2751 $[M - C_2H_5O]^+$ (calcd. *m/z*: 630.2731 $[M - C_2H_5O]^+$).

(6*E*,10*E*,14*E*,18*E*)-3-bromo-22,22-diethoxy-2,6,10,15,19-pentamethyldocosa-2,6,10,14,18-pentaene (3.29)

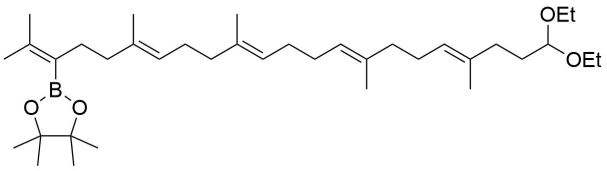


Sulfone **3.24** (0.579 g, 0.854 mmol) was dissolved in dry methanol (8.5 ml) and cooled in an ice bath. Na_2HPO_4 (0.242 g, 1.78 mmol) was added followed by sodium amalgam beads (5% Na, 3.81 g, 17.0 mmol). The mixture was stirred for 30 mins then filtered through celite into sat. NH_4Cl. The aqueous solution was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (PET 40-60/diethyl ether 96:4, R_f 0.26). This procedure afforded pure acetal **3.29** (0.416 g, 0.773 mmol, 90%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.22 - 5.07 (m, 4H), 4.46 (t, *J*=5.8, 1H), 3.64 (q, *J*=7.1, 2H), 3.48 (q, *J*=7.1, 2H), 2.55 (dd, *J*=9.2, 6.4, 2H), 2.18 (dd, *J*=9.2, 6.4, 2H), 2.13 - 1.89 (m, 14H), 1.85 (s, 3H), 1.76 - 1.68 (m, 5H), 1.62 - 1.58 (m, 13H), 1.20 (t, *J*=7.0, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 136.2, 135.2, 135.1, 134.3, 134.1, 133.8, 130.2, 127.2, 125.3, 124.6, 124.4, 124.4, 122.7, 102.7, 61.0, 39.8, 39.8, 39.7,

38.3, 37.0, 34.8, 32.0, 28.4, 26.8, 26.8, 25.8, 25.4, 20.4, 16.3, 16.1, 16.1, 15.5; **HRMS** (CI): *m/z*: 491.2879 $[M - C_2H_5O]^+$ (calcd. *m/z*: 491.2883 $[M - C_2H_5O]^+$).

2-((6*E*,10*E*,14*E*,18*E*)-22,22-Diethoxy-2,6,10,15,19-pentamethyldocosa-2,6,10,14,18-pentaen-3-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.23)

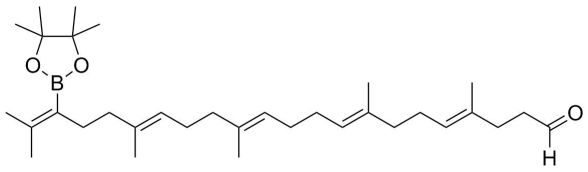


Acetal **3.29** (300 mg, 557 μmol) was dissolved in dry THF (2.7 μl) and cooled to -78 °C. *t*BuLi solution (1.9M in pentane, 587 μl, 1.11 mmol) was added dropwise. After 30 minutes, the organolithium solution was added quickly to a solution of *i*PrOBpin (170 μl, 836 μmol) in dry THF (540 μl) at room temperature. The mixture was stirred for 5 minutes, then water was added. The aqueous mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 96:4, R_f 0.15). This procedure afforded pure boronic ester **3.23** (108 mg, 184 μmol, 33%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.18 - 5.05 (m, 4H), 4.46 (t, *J*=5.7, 1H), 3.72 - 3.57 (m, 2H), 3.49 (q, *J*=7.1, 2H), 2.23 - 2.15 (m, 2H), 2.10 - 1.87 (m, 14H), 1.73 - 1.68 (m,

5H), 1.62 (s, 3H), 1.60 (s, 6H), 1.56 (s, 3H), 1.27 (s, 12H), 1.20 (t, *J*=7.0, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 146.7 (2C), 135.7, 135.4, 135.1, 134.4, 124.6, 124.5, 124.3, 124.0, 102.8, 82.8, 61.0 (2C), 40.4, 39.9, 39.8, 34.8, 32.1, 30.5, 28.4, 26.9, 26.8, 25.8, 25.0, 24.8, 20.8, 16.2 (2C), 16.2 (2C), 15.5 (2C); **IR** (cm^{-1}): 2975, 2926, 2360, 2341, 1742, 1627, 1444, 1372, 1351, 1291, 1220, 1146, 1062, 965, 933, 883, 853, 704, 669; *HRMS could not be obtained for this compound with the available methods.*

(4*E*,8*E*,12*E*,16*E*)-4,8,13,17,21-Pentamethyl-20-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)docosa-4,8,12,16,20-pentaenal (3.32)

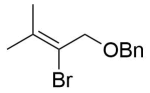


Acetal **3.23** (102 mg, 174 μmol) was dissolved in acetone (1 ml) and 4 drops of water were added. Amberlyst® 15 resin (100 mg) was added and the reaction was stirred until complete consumption of starting material was observed by TLC. The resin was filtered off and the organic phase dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by flash column chromatography (PET 40-60/diethyl ether 9:1, R_f 0.3). This procedure afforded pure boronic ester **3.32** (83 mg, 162 μmol, 93%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.75 (t, *J*=1.8, 1H), 5.23 - 5.07 (m, 4H), 2.50 (td, *J*=7.6, 1.9, 2H), 2.37 - 2.26 (m, 2H), 2.12 - 1.86 (m, 15H), 1.75 - 1.71 (m, 3H), 1.66 -

1.55 (m, 14H), 1.51 - 1.40 (m, 1H), 1.29 - 1.25 (m, 13H, 2x pinacol CH_3 +1)); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 202.7, 146.6, 146.4, 135.9, 135.6, 135.3, 134.8, 132.8, 125.4, 124.5, 124.2, 123.7, 42.2, 40.2, 39.8, 39.5, 31.9, 28.3, 28.3, 26.8, 26.6, 25.7, 24.8, 24.7, 20.7, 16.1, 16.1, 16.0, 16.0; **IR** (cm^{-1}): 2977, 2924, 2856, 2716, 2361, 1727, 1627, 1445, 1371, 1350, 1292, 1220, 1145, 1106, 1058, 965, 878, 852, 704, 670; *HRMS could not be obtained for this compound with the available methods.*

(((2-Bromo-3-methylbut-2-en-1-yl)oxy)methyl)benzene (3.30)

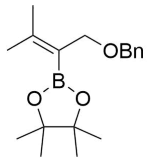


Sodium hydride (60%, 0.727 g, 18.1 mmol) was suspended in dry THF (72 ml) and cooled in a water bath. Alcohol **3.27** (3 g, 18.1 mmol) was slowly added and stirred for 30 minutes. Benzyl bromide (2.16 ml, 18.1 mmol) was added to the suspension and stirred overnight. The reaction was quenched by carefully adding sat. NH_4Cl and extracted three times with diethyl ether. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by flash column chromatography (PET 40-60/diethyl ether 98:2). This procedure afforded pure ester **3.30** (3.48 g, 13.5 mmol, 74 %) as clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.41 - 7.27 (m, 5H), 4.54 (s, 2H), 4.31 (s, 2H), 1.94 (s, 3H), 1.81 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 138.1, 137.1, 128.5, 128.0,

127.8, 117.5, 71.6, 71.4, 25.7, 20.9; **IR** (cm^{-1}): 3030, 2916, 2857, 1651, 1495, 1453, 1382, 1245, 1203, 1088, 1073, 1028, 1008, 937, 905, 816, 736, 697; **HRMS** (ESI): *m/z*: 277.0198 $[M + Na]^+$ (calcd. *m/z*: 277.0198 $[M + Na]^+$).

2-(1-(benzyloxy)-3-methylbut-2-en-2-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.31)



Ether **3.30** (0.1 g, 391 μmol) was dissolved in dry diethyl ether (1.5 ml) and cooled to -78 °C. *t*BuLi solution (1.9 M in pentane, 226 μl, 431 μmol) was added. A sharp colour change to yellow was observed. After 5 minutes, *i*PrOBpin (159 μl, 783 μmol) was added. After 1 hour, the mixture was allowed to warm to room temperature and water was added. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 95:5, R_f 0.25). This procedure afforded pure boronic ester **3.31** (56 mg, 185 μmol, 47%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.39 - 7.22 (m, 5H), 4.50 (s, 2H), 4.13 (s, 2H), 1.99 (d, J=0.9, 3H), 1.78 (s, 3H), 1.26 (s, 12H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm):152.1,

139.2, 128.2, 127.9, 127.3, 83.1, 72.1, 69.2, 24.9, 21.4; **IR** (cm^{-1}): 2977, 2927, 2360, 1627, 1453, 1372, 1352, 1294, 1225, 1146, 1086, 1065, 1028, 965, 879, 852, 734, 697, 671; **HRMS** (ESI): *m/z*: 303.2129 $[M+H]^+$ (calcd. *m/z*: 303.2126 $[M+H]^+$).

Synthesis of Sq-Gem by one-pot oxidation amidation sequence

via Pinnick oxidation

Aldehyde **3.3** (50 mg, 129 μmol) was dissolved in dry MeCN (0.5 ml). 2-Methyl-2-butene (68 μl, 649 μmol), $NaClO_2$ (46 mg, 405 μmol) and KH_2PO_4 (62 mg, 454 μmol) were subsequently added. The reaction was heated to 40 °C for 16 hours. Then a solution of gemcitabine hydrochloride (39 mg, 130 μmol) in DMSO (1 ml) and DMF (0.16 ml) was added followed by *N*-methyl morpholine (16 μl, 143 μmol), HOBt hydrate (19 mg, 142 μmol) and EDC hydrochloride (32 mg, 168 μmol). The reaction mixture was heated to 65 °C for 24 hours. The reaction was cooled to room temperature and EtOAc was added. The organic phase was washed with sat. $NaHCO_3$, then dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (DCM/MeOH 98:2 to 96:4). This procedure afforded pure **Sq-Gem** (17 mg, 26 μmol, 20%) as a waxy solid.

***via* succinimide ester**

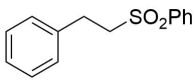
Aldehyde **3.3** (50 mg, 129 μmol), *N*-hydroxysuccinimide (15 mg, 130 μmol), $Cu(OAc)_2$ (3.4 mg, 19.5 μmol) and *t*BuOOH (70% in water, 36 μl, 260 μmol) were dissolved in MeCN (0.5 ml) and heated to reflux. After 3.5 hours, a solution of gemcitabine hydrochloride (39 mg, 130 μmol) was added. The reaction was stirred for 16 hours at reflux. After cooling to room temperature, water and EtOAc were added. The organic phase was separated and dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (DCM/MeOH 98:2 to 96:4). This procedure afforded pure **Sq-Gem** (5 mg, 7.7 μmol, 6%) as a waxy solid.

The analytical data were in accordance with the literature.[12]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.05 (br, 1H), 8.08 (d, *J*=7.6 Hz, 1H), 7.48 (d, *J*=7.6 Hz, 1H), 6.19 (t, *J*=7.0 Hz, 1H), 5.29-4.93 (m, 5H), 4.50 (dd, *J*=16.5, 8.2 Hz, 1H), 4.21-3.77 (m, 3H), 2.69-2.45 (m, 2H), 2.43-2.23 (m, 2H), 2.17-1.89 (m, 16H), 1.84 (s, 3H), 1.68 (s, 3H), 1.65-1.54 (m, 12H); **MS** (ESI): *m/z*: 668.3 $[M+Na]^+$ (calcd.: *m/z*: 668.3 $[M+Na]^+$).

Experimental Procedures for Chapter 4

(Phenethylsulfonyl)benzene (4.3)

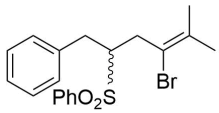


(2-Bromoethyl)benzene (1 g, 5.4 mmol) was dissolved in dry DMF (10 ml) and $PhSO_2Na$ was added. The suspension was stirred for 24 hours then diethyl ether was added. The organic phase was washed two times with water then with brine. The organic phase was dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 8:2 to 6:4, $R_{f8:2}$ 0.08). This procedure afforded pure sulfone **4.3** (0.81 g, 3.3 mmol, 61%) as a colourless solid.

The analytical data were in accordance with the literature.[13]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.90 - 7.85 (m, 2H), 7.63 - 7.57 (m, 1H), 7.55 - 7.47 (m, 2H), 7.24 - 7.10 (m, 3H), 7.08 - 7.02 (m, 2H), 3.34 - 3.26 (m, 2H), 3.02 - 2.93 (m, 2H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 139.0, 137.5, 133.9, 129.5 (2C), 128.9(2C), 128.4(2C), 128.2(2C), 127.0, 57.6, 28.8;

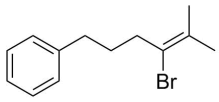
((4-Bromo-5-methyl-1-phenylhex-4-en-2-yl)sulfonyl)benzene (4.4)



Sulfone **4.3** (1 g, 4.05 mmol) was dissolved in dry THF (11 ml) and DMF (3 ml). The solution was cooled to -78 °C and *t*BuOK (1M in THF, 4.46 ml, 4.46 mmol) was added dropwise. The solution was stirred for 2 hours, then a solution of bromide **3.20** (0.57 g, 4.46 mmol) in THF (3 ml) was dropwise added. After another 2 hours, the reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched with NH_4Cl and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 8:2, R_f 0.25). This procedure afforded pure sulfone **4.4** (0.56 g, 1.43 mmol, 35%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.86 - 7.81 (m, 2H), 7.61 - 7.55 (m, 1H), 7.52 - 7.44 (m, 2H), 7.23 - 7.11 (m, 3H), 7.11 - 7.06 (m, 2H), 3.95 (tt, *J*=7.8, 5.7, 1H), 3.35 (dd, *J*=14.7, 5.7, 1H), 3.08 - 2.98 (m, 1H), 2.83 (ddd, *J*=15.1, 7.8, 2.9, 2H), 1.75 (s, 3H), 1.65 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 138.6, 137.0, 134.5, 133.6, 129.0 (2C), 128.4, 128.4, 126.7, 116.8, 63.6, 35.9, 33.5, 25.5, 21.1; **IR** (cm^{-1}): 1585, 1496, 1446, 1304, 1144, 1085, 1027, 745, 691, 624; **HRMS** (CI): *m/z*: 393.0521 $[M+H]^+$ (calcd. *m/z*: 393.0518 $[M+H]^+$).

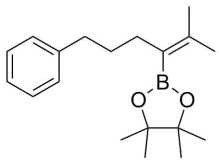
(4-Bromo-5-methylhex-4-en-1-yl)benzene (4.5)



Magnesium turnings (0.34 g, 14.3 mmol) were stirred in a flame-dried round bottom flasks with several drops of dibromoethane. Sulfone **4.4** (0.56 g, 1.43 mmol) in dry methanol (14 ml) was added. After 2 hours, the liquid was carefully decanted into 1M HCl. The remaining metal was washed several times with methanol. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were washed with brine. The organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane, R_f 0.25). This procedure afforded pure bromide **4.5** (0.24 g, 0.96 mmol, 67%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.21 (t, *J*=7.7, 2H), 7.12 (d, *J*=7.5, 3H), 2.54 (t, *J*=7.9, 2H), 2.45 (t, *J*=7.5, 2H), 1.88 - 1.76 (m, 5H), 1.63 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 142.2, 130.5, 128.5(2C), 128.4(2C), 125.9, 121.7, 37.1, 34.8, 30.0, 25.5, 20.5; **IR** (cm^{-1}): 1496, 1453, 12221, 1123, 1032, 744, 698; **HRMS** (CI): *m/z*: 252.0513 $[M+H]^+$ (calcd. *m/z*: 252.0508 $[M+H]^+$).

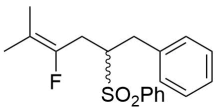
4,4,5,5-Tetramethyl-2-(2-methyl-6-phenylhex-2-en-3-yl)-1,3,2-dioxaborolane (4.1)



Bromide **4.5** (0.1 g, 0.394 mmol) was dissolved in dry THF (0.9 ml) and cooled to -78 °C. *t*BuLi (1.9 M in pentane, 0.436 ml, 0.829 mmol) was added dropwise. After 25 minutes, *i*PrOBpin (0.24 ml, 1.18 mmol) was added. The reaction mixture was stirred for 1.5 hours, then warmed to room temperature and water was added. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 99:1, R_f 0.13). This procedure afforded pure boronic ester **4.1** (88 mg, 0.293 mmol, 74%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm):7.24 - 7.15 (m, 2H), 7.15 - 7.03 (m, 3H), 2.62 - 2.47 (m, 2H), 2.18 - 2.06 (m, 2H), 1.88 (s, 3H), 1.61 (s, 3H), 1.60 - 1.52 (m, 2H), 1.19 (s, 12H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 147.0, 143.1, 128.5 (2C), 128.2(2C), 125.5, 82.9(2C), 36.0, 31.9, 31.0, 24.9(4C), 24.8, 20.9; **IR** (cm^{-1}): 1626, 1452, 1398, 1371, 1289, 1143, 698; **HRMS** (CI): *m/z*: 301.2324 $[M+H]^+$ (calcd. *m/z*: 301.2337 $[M+H]^+$).

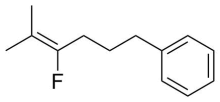
((4-Fluoro-5-methyl-1-phenylhex-4-en-2-yl)sulfonyl)benzene (4.7)



Sulfone **4.3** (0.5 g, 2.02 mmol) was dissolved in dry THF (5.5 ml) and DMF (1.5 ml). The solution was cooled to -78 °C and *t*BuOK (1M in THF, 2.23 ml, 2.23 mmol) was added dropwise. After one hour, a solution of bromide **2.17** (0.37 g, 2.23 mmol) in dry THF (1.5 ml) was added dropwise. The mixture was allowed to warm to room temperature after further 30 minutes and stirred overnight. The reaction was quenched with sat. aq. NH_4Cl solution and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 8:2, R_f 0.23). This procedure afforded pure sulfone **4.7** (0.36 g, 1.09 mmol, 54%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.91 - 7.84 (m, 2H), 7.65 - 7.58 (m, 1H), 7.52 (dd, *J*=8.5, 7.0, 2H), 7.25 - 7.13 (m, 3H), 7.10 - 7.00 (m, 2H), 3.74 - 3.62 (m, 1H), 3.38 (dd, *J*=14.6, 4.9, 1H), 2.89 - 2.73 (m, 2H), 2.57 (ddd, *J*=23.3, 15.2, 8.0, 1H), 1.47 (d, *J*=2.7, 3H), 1.36 (d, *J*=3.3, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -113.52 (dddt, J=23.4, 17.6, 5.9, 2.9); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 148.6 (d, J_{CF}=240.1), 138.2, 137.0, 133.7, 129.1 (2C), 128.9 (2C), 128.6 (2C), 128.5 (2C), 126.8, 111.3 (d, J_{CF}=16.7), 62.1, 33.8, 27.5 (d, J_{CF}=28.8),17.8 (d, J_{CF}=5.5), 15.5 (d, J_{CF}=9.0); **IR** (cm^{-1}): 2970, 1466, 1378, 1307, 1160, 1128, 950, 816; **HRMS** (CI): *m/z*: 333.1328 $[M+H]^+$ (calcd. *m/z*: 333.1319 $[M+H]^+$).

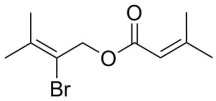
(4-Fluoro-5-methylhex-4-en-1-yl)benzene (4.6)



Magnesium turnings (0.25 g, 10.3 mmol) were stirred in a flame-dried round bottom flask with a several drops of dibromoethane. The roundbottom flask was placed in an ice bath and solution of sulfone **4.7** (0.344 g, 1.03 mmol) in dry methanol (10 ml) was added. After 1 hour, the reaction was carefully decanted into 1M HCl solution. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane, R_f 0.5). This procedure afforded pure fluoride **4.6** (0.132 g, 0.68 mmol, 66%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.36 - 7.28 (m, 2H), 7.26 - 7.17 (m, 3H), 2.73 - 2.62 (m, 2H), 2.29 (dt, *J*=23.4, 7.4, 2H), 1.94 - 1.82 (m, 2H), 1.67 (d, *J*=3.2, 3H), 1.58 (d, *J*=2.8, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.80 (tt, *J*=23.6, 3.2); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 153.9 (d, J_{CF}=241.5), 142.1, 128.5 (2C), 128.4 (2C), 125.9, 107.6 (d, J_{CF}=17.6), 35.2, 28.4, 28.0 (d, J_{CF}=29.5), 17.7 (d, J_{CF}=6.2), 15.6 (d, J_{CF}=9.5); **IR** (cm^{-1}): 2541, 2340, 2214, 2138, 2048, 1996, 1963, 1716, 1455, 1154, 1105, 921, 742, 697, 667, 615; *HRMS could not be obtained for this compound with the available methods.*

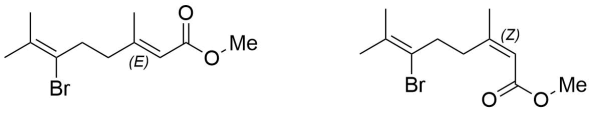
2-bromo-3-methylbut-2-en-1-yl 3-methylbut-2-enoate (4.8)



Alcohol **3.27** (4.73 g, 28.7 mmol) was dissolved in dry DCM (70 ml). 3,3-Dimethylacrylic acid (3.73 g, 37.3 mmol), DCC (7.99 g, 38.7 mmol) and DMAP (0.35 g, 2.87 mmol) were added. The reaction was stirred at room temperature for 18 hours then filtered through a plug of celite. The solvent was removed under reduced pressure. The product was purified by by column chromatography (PET 40-60/diethyl ether 95:5, R_f 0.38). This procedure afforded pure ester **4.8** (5.77 g, 23.3 mmol, 81%) as clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm):5.74 (sept, *J*=1.4, 1H), 4.90 (s, 2H), 2.18 (d, *J*=1.3, 3H), 1.93 (s, 3H), 1.91 (s, 3H), 1.90 (d, *J*=1.3, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 166.3, 157.8, 138.2, 115.7, 114.9, 65.3, 27.6, 25.6, 20.9, 20.4; **IR** (cm^{-1}): 2914, 2361, 1720, 1650, 1442, 1379, 1343, 1268, 1221, 1139, 1077, 1059, 984, 850; **HRMS** (CI): *m/z*: 247.0336 $[M+H]^+$ (calcd. *m/z*: 247.0334 $[M+H]^+$).

Methyl (*E*)-6-bromo-3,7-dimethylocta-2,6-dienoate (4.11)



Diisopropyl amine (3.59 ml, 25.6 mmol) was dissolved in dry THF (37.4 ml) and cooled to 0 °C. *n*BuLi (2.5 M, 10.2 ml, 25.6 mmol) was added dropwise. The reaction was stirred for 30 minutes and then cooled to -78 °C. Ester **4.8** (5.77 g, 23.3 mmol) in dry THF (6.1 ml) was added dropwise. After 10 minutes, TMSCl (3.25 ml, 25.6 mmol) was added and the reaction allowed to warm to room temperature. After 50 minutes, the reaction was heated to 50 °C. After another hour, the reaction flask was mounted with a destillation bridge and DMF (30 ml) was added. The reaction was heated to 150 °C and the THF was allowed to distil off. After 4 hours, the reaction was cooled to room temperature. 1M aq. NaOH was added and extracted with diethyl ether. The aqueous phase was acidified with 1M HCl and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. This procedure afforded crude acid **4.10** as brown solid of sufficient purity to be used without further purification.

The residue was dissolved in dry DCM (50 ml) and methanol (2.23 ml, 55.2 mmol), DCC (4.95 g, 23.9 mmol) and DMAP (0.224 g, 1.84 mmol) were added. The reaction was stirred overnight and then filtered through a plug of celite. The solvent was evaporated under reduced pressure and the product purified by column chromatography (PET 40-

60/diethyl ether 95:5). This procedure afforded the desired *E*-isomer **4.11** (2.77 g, 10.6 mmol, 45%, R_f 0.22) and the **Z**-isomer (0.504 g, 1.92 mmol, 8%, R_f 0.29) as clear oils.

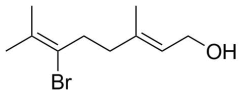
Analytical data for *E*-isomer:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.68 (q, *J*=1.2, 1H), 3.68 (s, 3H), 2.68 - 2.59 (m, 2H), 2.42 - 2.30 (m, 2H), 2.17 (d, *J*=1.2, 3H), 1.85 (s, 3H), 1.74 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 167.2, 158.8, 131.4, 120.1, 116.0, 51.0, 39.5, 36.0, 25.5, 20.4, 19.1; **IR** (cm^{-1}): 2994, 2949, 2916, 1719, 1648, 1434, 1384, 1358, 1332, 1281, 1224, 1148, 1065, 1020, 921, 866, 821, 741; **HRMS** (CI): *m/z*: 261.0487 $[M+H]^+$ (calcd. *m/z*: 261.0490 $[M+H]^+$).

Analytical data for *Z*-isomer:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.64 (q, *J*=1.4, 1H), 3.64 (s, 4H), 2.80 - 2.70 (m, 3H), 2.66 - 2.55 (m, 3H), 1.87 (d, *J*=1.4, 4H), 1.82 (s, 4H), 1.76 (s, 4H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 166.7, 159.4, 131.4, 120.8, 116.6, 51.0, 36.8, 32.5, 25.8, 25.5, 20.4;

(*E*)-6-Bromo-3,7-dimethylocta-2,6-dien-1-ol (4.12)



Ester **4.11** (2.77 g, 10.6 mmol) was dissolved in dry THF (21 ml) and cooled to -78 °C. DIBAL-H (1M in DCM, 21.2 ml, 21.2 mmol) was added slowly. After 20 minutes the

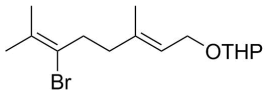
reaction was allowed to warm to room temperature. After a further hour, sat. Rochelle salt solution was added and vigorously stirred overnight. The aqueous solution was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/EtOAc 8:2, R_f 0.14). This procedure afforded pure alcohol **4.12** (2.25 g, 9.65 mmol, 91%) as a clear oil.

The analytical data were in accordance with the literature.[14]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.43 (tq, *J*=7.0, 1.3, 1H), 4.15 (d, *J*=6.9, 2H), 2.59 (t, *J*=9.5, 2H), 2.23 (t, *J*=9.4, 2H), 1.85 (s, 3H), 1.75 (s, 3H), 1.70 (d, *J*=1.2, 3H);

^{13}C-NMR (101 MHz, $CDCl_3$, δ/ppm): 138.7, 130.6, 124.2, 121.1, 59.5, 38.1, 36.5, 25.4, 20.4, 16.5;

(*E*)-2-((6-Bromo-3,7-dimethylocta-2,6-dien-1-yl)oxy)tetrahydro-2*H*-pyran (4.13)

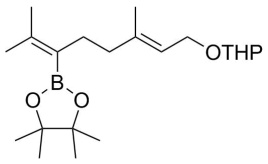


Alcohol **4.12** (0.233 g, 1 mmol) was dissolved in dry DCM (0.18 ml). DHP (0.136 ml, 1.5 mmol) and PPTS (25.1 mg, 0.1 mmol) were added. The mixture was stirred overnight. The reaction was diluted with DCM and washed three times with sat. $NaHCO_3$. The organic phase was dried over Na_2SO_4 and the solvent evaporated

under reduced pressure. The product was purified by column chormatography (PET 40-60/diethyl ether 9:1, R_f 0.25). This procedure afforded pure ester **4.13** (0.183 g, 0.576 mmol, 57%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.37 - 5.25 (m, 1H), 4.55 (dd, *J*=4.3, 2.8, 1H), 4.23 - 4.11 (m, 1H), 4.02 - 3.90 (m, 1H), 3.86 - 3.76 (m, 1H), 3.51 - 3.38 (m, 1H), 2.52 (dd, *J*=9.4, 6.3, 2H), 2.17 (dd, *J*=9.4, 6.5, 2H), 1.78 (s, 4H), 1.67 (s, 4H), 1.65 - 1.59 (m, 3H), 1.58 - 1.41 (m, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 139.1, 130.6, 129.2, 126.0, 121.5, 98.0, 63.7, 62.4, 38.1, 36.5, 30.8, 25.6, 25.5, 20.4, 19.7, 16.7; **IR** (cm^{-1}): 2939, 2869, 2360, 2341, 1718, 1676, 1441, 1383, 1353, 1321, 1261, 1222, 1200, 1022, 905, 869, 814; *HRMS could not be obtained for this compound with the available methods.*

(*E*)-2-(2,6-Dimethyl-8-((tetrahydro-2H-pyran-2-yl)oxy)octa-2,6-dien-3-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (4.2)

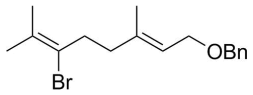


Ether **4.13** (0.141 g, 0.444 mmol) was dissolved in dry THF (1.33 ml) and cooled to -78 °C. *t*BuLi (1.9 M in pentane, 0.491 ml, 0.933 mmol) was added dropwise. After 30 minutes, *i*PrOBpin (0.272 ml, 1.33 mmol) was added. After further 10 minutes water was added to the reaction and the mixture allowed to warm to room temperature. The

mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and solvent evaporated under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 9:1, R_f 0.17). This procedure afforded pure boronic ester **4.2** (0.115 g, 0.315 mmol, 71%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.35 (dd, *J*=7.7, 6.6, 1H), 4.63 (dd, *J*=4.4, 2.9, 1H), 4.32 - 4.18 (m, 1H), 4.00 (dd, *J*=11.8, 7.4, 1H), 3.89 (ddd, *J*=11.1, 7.6, 3.2, 1H), 3.58 - 3.46 (m, 2H), 2.28 - 2.17 (m, 2H), 2.07 - 1.91 (m, 5H), 1.90 - 1.77 (m, 1H), 1.72 (s, 3H), 1.70 (s, 3H), 1.64 - 1.49 (m, 3H), 1.26 (s, 12H) ; **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 147.3, 141.1, 120.2, 98.0, 82.8, 63.9, 62.4, 40.2, 30.8, 30.0, 25.6, 24.9 (2C), 24.8, 20.8, 19.7, 16.6; **IR** (cm^{-1}): 2939, 1627, 1371, 1351, 1291, 1145, 1023, 965, 869, 704; *HRMS could not be obtained for this compound with the available methods.*

(*E*)-(((6-Bromo-3,7-dimethylocta-2,6-dien-1-yl)oxy)methyl)benzene (4.14)

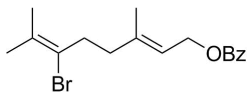


Sodium hydride (60 % in mineral oil, 22.3 mg, 0.557 mmol) was suspended in dry THF (2.2 ml). Alcohol **4.12** (130 mg, 0.557 mmol) in dry THF (0.1 ml) was added slowly. After one hour, benzyl bromide (66.3 μl, 0.557 mmol) was added. The reaction was stirred overnight, then quenched with sat. NH_4Cl. The mixture was extracted three

times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 96:4, R_f 0.32). This procedure afforded pure ether **4.14** (104 mg, 0.321 mmol, 57%) as clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.37 - 7.26 (m, 5H), 5.43 (tq, *J*=6.7, 1.3, 1H), 4.50 (s, 2H), 4.03 (d, *J*=6.7, 2H), 2.68 - 2.57 (m, 2H), 2.31 - 2.21 (m, 2H), 1.85 (s, 3H), 1.75 (s, 3H), 1.67 (d, *J*=1.3, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 139.2, 138.5, 130.5, 128.4 (2C), 127.8 (2C), 127.6, 121.6, 121.2, 72.1, 66.6, 38.1, 36.4, 25.4, 20.3, 16.7; **IR** (cm^{-1}): 2915, 2854, 1495, 1364, 1068, 942, 902, 827, 734, 697; *HRMS could not be obtained for this compound with the available methods.*

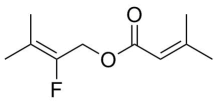
(*E*)-6-Bromo-3,7-dimethylocta-2,6-dien-1-yl benzoate (4.15)



Alcohol **4.12** (0.233 g, 1 mmol) was dissolved in dry DCM (3 ml) and cooled in an ice bath. DMAP (24.4 mg, 0.2 mmol), triethylamine (0.209 ml, 1.5 mmol) and benzoyl chloride (0.139 ml, 1.2 mmol) were successively added. The reaction was allowed to warm to room temperature and stirred over night. The reaction was concentrated directly onto silica gel. The product was eluted with PET 40-60/diethyl ether 96:4 (R_f 0.21). The solvent was evaporated under reduced pressure. This yielded pure ester **4.15** (0.32 g, 0.948 mmol, 95%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 8.09 - 8.01 (m, 2H), 7.61 - 7.51 (m, 1H), 7.48 - 7.39 (m, 2H), 5.50 (t, *J*=7.0, 1H), 4.83 (d, *J*=7.0, 2H), 2.66 - 2.59 (m, 2H), 2.28 (dd, *J*=9.1, 6.4, 2H), 1.84 (s, 3H), 1.79 (s, 3H), 1.74 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 166.6, 141.1, 132.9, 130.7, 130.4, 129.6 (2C), 128.3 (2C), 120.9, 119.2, 61.8, 37.9, 36.3, 25.3, 20.3, 16.8 ; **IR** (cm^{-1}): 1717, 1450, 1268, 1109, 1069, 1026, 710; *HRMS could not be obtained for this compound with the available methods.*

2-Fluoro-3-methylbut-2-en-1-yl 3-methylbut-2-enoate (2.12)

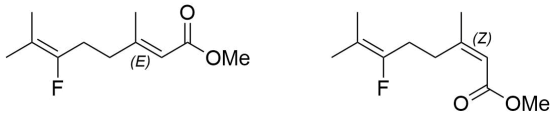


The synthesis was performed according to a literature procedure.[15]

Alcohol **2.2** (2.50 g, 24.02 mmol), 3-methyl-2-butenoic acid (3.12 g, 31.22 mmol), DCC (6.69 g, 32.42 mmol) and DMAP (0.29 g, 2.40 mmol) were dissolved in dry DCM (60 ml). The reaction was stirred for 24 hours, then formic acid (0.86 ml) was added. After a further 5 minutes, the reaction mixture was filtered over celite and the solvent evaporated under reduced pressure. The product was purified by column chromatography (PET/diethyl ether 9:1). This procedure afforded pure ester **2.12** (3.24 g, 17.4 mmol, 72%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.72 (d, *J*=1.3, 1H), 4.71 (d, *J*=22.7, 2H), 2.17 (d, *J*=1.3, 3H), 1.90 (d, *J*=1.4, 4H), 1.72 (d, *J*=3.0, 3H), 1.70 (d, *J*=3.5, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -118.16 (tsep, *J*=25.9, 3.2).

Methyl (*E*)-6-fluoro-3,7-dimethylocta-2,6-dienoate (2.14)



The synthesis was performed according to a literature procedure.[15]

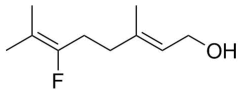
Diisopropyl amine (2.26 ml, 16.1 mmol) was dissolved in dry THF (23.5 ml) and cooled in an ice bath. *n*BuLi (2.5M, 6.44 mmol, 16.1 mmol) was added dropwise and the mixture stirred for 30 minutes. Ester **2.12** (2.73 g, 14.6 mmol) was dissolved in dry THF (5.5 ml) and added dropwise to the prepared LDA solution. The reaction was stirred for 10 minutes, then TMSCl (2.04 ml, 16.1 mmol) was added and the reaction warmed to room temperature. After one hour, the reaction was heated to 50 °C. The mixture was stirred for 1.5 hours, then DMF (80 ml) was added and a distillation apparatus mounted. The reaction was heated to 150 °C and the THF distilled off. After 4 hours, the reaction was cooled to room temperature. 1M aq. NaOH was added and the reaction was extracted three times with diethyl ether. The combined organic phases were discarded. The aqueous phase was acidified with 1M HCl and extracted three times with diethyl ether. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. The crude oil was dissolved in dry DCM (32 ml) and dry methanol (1.46 ml, 36.2 mmol), DCC (3.24 g, 15.7 mmol) and DMAP (0.147 g, 1.20 mmol) were added. The reaction was stirred for 18 hours then formic acid (0.42 ml) was added. After 5 minutes, the mixture was filtered over

celite and the solvent evaporated under reduced pressure. The product was purified by column chromatography (hexanes/diethyl ether 95:5) and the *E*- and *Z*-isomers were separated. This procedure afforded the desired *E*-isomer **2.14** (0.973g, 4.86 mmol, 33%) and the *Z*-isomer (0.155 g, 0.776 mmol, 5%) as clear oils.

Analytical data for *E*-isomer: **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 5.68 (q, *J*=1.3, 1H), 3.69 (s, 3H), 2.45 - 2.28 (m, 4H), 2.17 (d, *J*=1.3, 3H), 1.62 (d, *J*=3.2, 3H), 1.57 (d, *J*=2.7, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -113.53 - -113.72 (m).

Analytical data for *Z*-isomer: **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 5.69 (d, *J*=1.6, 1H), 3.68 (s, 3H), 2.75 (t, *J*=7.7, 2H), 2.41 (dt, *J*=23.8, 7.7, 2H), 1.90 (d, *J*=1.4, 3H), 1.62 (d, *J*=3.0, 3H), 1.59 (d, *J*=2.9, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.47 - -112.69 (m).

(*E*)-6-Fluoro-3,7-dimethylocta-2,6-dien-1-ol (2.11)



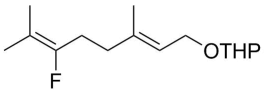
Ester **2.14** (0.847 g, 4.23 mmol) was dissolved in dry THF (8.5 ml) and cooled to -78 °C. DIBAL-H (1M in toluene, 8.46 ml, 8.46 mmol) was added dropwise. After 1 hour, the reaction was warmed to room temperature and stirred for another hour. Rochelle salt solution was added to quench the reaction and the mixture was stirred vigorously overnight. The reaction was extracted three times with diethyl ether. The combined

organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/EtOAc 8:2, R_f 0.16). This procedure afforded pure alcohol **2.11** (0.596 g, 3.46 mmol, 81%) as a clear oil.

The analytical data were in accordance with the literature.[15]

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.43 (t, *J*=6.9, 1H), 4.20 - 4.11 (m, 2H), 2.34 (dt, *J*=22.8, 7.9, 2H), 2.23 - 2.14 (m, 2H), 1.69 (s, 3H), 1.62 (d, *J*=3.2, 4H), 1.57 (d, *J*=2.7, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.83 - -113.05 (m).

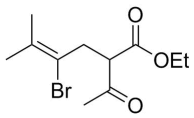
(*E*)-2-((6-fluoro-3,7-dimethylocta-2,6-dien-1-yl)oxy)-tetrahydro-2*H*-pyran (4.16)



Alcohol **2.11** (0.1 g, 0.58 mmol) was dissolved in dry DCM (0.58 ml). DHP (79.1 μl, 0.87 mmol) and PPTS (14.5 mg, 58.0 μmol) were added. The reaction was stirred overnight. $NaHCO_3$ was added and the mixture was extracted three times with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 96:4, R_f 0.19). This procedure afforded pure ester **4.16** (0.126 g, 0.491 mmol, 84%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.43 - 5.33 (m, 1H), 4.62 (t, *J*=3.6, 1H), 4.23 (dd, *J*=12.0, 6.4, 1H), 4.02 (dd, *J*=12.0, 7.4, 1H), 3.89 (ddd, *J*=11.0, 7.6, 3.2, 1H), 3.61 - 3.43 (m, 1H), 2.33 (ddd, *J*=22.8, 9.4, 6.2, 2H), 2.19 (dd, *J*=9.4, 6.2, 2H), 1.83 (dp, *J*=11.6, 4.6, 3.9, 1H), 1.77 - 1.66 (m, 4H), 1.64 - 1.48 (m, 11H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -113.00 (ddp, *J*=23.1, 20.2, 3.0); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 153.6 (d, J_{CF}=241.4), 139.3, 121.2, 107.5, 107.4, 97.8, 63.6, 62.3, 36.5, 30.7, 27.23 (d, J_{CF}=29.4), 25.5, 19.6, 17.6 (d, J_{CF}=5.6), 16.4, 15.5 (d, J_{CF}=9.5); **IR** (cm^{-1}): 2938, 2867, 2360, 1715, 1452, 1384, 1352, 1260, 1200, 1184, 1155, 1116, 1076, 1052, 1021, 972, 905, 869, 814; **HRMS** (CI): *m/z*: 261.0487 $[M+H]^+$ (calcd. *m/z*: 261.0490 $[M+H]^+$).

Ethyl 2-acetyl-4-bromo-5-methylhex-4-enoate (4.20)



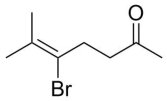
The synthesis was performed according to a literature procedure.[16]

Sodium hydride (60% in mineral oil, 0.512 g, 12.8 mmol) was suspended in dry THF (14.5 ml) at 0 °C. Ethyl acetoacetate (1.62 ml, 12.8 mmol) was added dropwise. After 30 minutes, bromide **3.20** (3.21 g, 14.1 mmol) in dry THF (9.7 ml) was added and the reaction warmed to room temperature. The reaction was stirred overnight, then quenched with 1M HCl solution. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed

under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 8:2, R_f 0.28). This procedure afforded pure ester **4.20** (2.90 g, 12.8 mmol, quant.) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 4.27 - 4.12 (m, 2H), 4.02 (t, *J*=7.3, 1H), 3.06 - 2.95 (m, 2H), 2.28 (s, 3H), 1.84 (s, 3H), 1.80 (s, 3H), 1.26 (t, *J*=7.1, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 202.5, 169.0, 133.9, 117.4, 61.7, 58.0, 35.6, 30.3, 25.7, 20.8, 14.2.

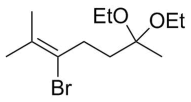
5-Bromo-6-methylhept-5-en-2-one (4.21)



Ester **4.20** (3.19 g, 14.0 mmol) was dissolved in ethanol (28 ml). KOH (1.57 g, 28.0 mmol) was dissolved in water (9.3 ml) and added. The mixture was heated to reflux for 3 hours. After cooling to room temperature the reaction was poured into 1M HCl and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 8:2, R_f 0.35). This procedure afforded pure ketone **4.21** (1.97 g, 9.61 mmol, 68%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 2.74 (s, 4H), 2.20 (s, 3H), 1.84 (s, 3H), 1.78 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 211.0, 131.8, 119.6, 42.4, 31.6, 30.3, 25.5, 20.5; **IR** (cm^{-1}): 2920, 1719, 1431, 1362, 1279, 1221, 1161, 1055, 1007, 808; **HRMS** (CI): *m/z*: 205.0229 $[M+H]^+$ (calcd. *m/z*: 205.0228 $[M+H]^+$).

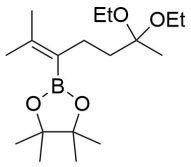
3-Bromo-6,6-diethoxy-2-methylhept-2-ene (4.22)



Ketone **4.21** (1.97 g, 9.61 mmol) was dissolved in dry toluene (20 ml). Ethanol (6.73 ml, 115.3 mmol), PPTS (0.241 g, 0.961 mmol) and triethyl orthoformate (4.79 ml, 28.8 mmol) were added and the mixture heated to reflux. After 5 hours, the reaction was cooled to room temperature and diluted with diethyl ether. The organic phase was washed with sat. $NaHCO_3$ and brine, then dried over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by column chromatography (PET 40-60/ diethyl ether 96:4, R_f 0.25). This procedure afforded pure acetal **4.22** (2.12 g, 7.60 mmol, 79%) as a clear oil.

^{1}H-NMR (400 MHz, DCM-d2, δ/ppm): 3.36 (q, *J*=7.1, 4H), 2.46 - 2.38 (m, 2H), 1.76 (s, 3H), 1.75 - 1.70 (m, 2H), 1.69 (s, 3H), 1.18 (s, 3H), 1.05 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, DCM-d2, δ/ppm): 130.1, 121.3, 100.6, 55.5 (2C), 36.0, 33.0, 25.0, 21.8, 19.9, 15.2 (2C); **IR** (cm^{-1}): 2974, 1443, 1375, 1270, 1252, 1223, 1210, 1144, 1127, 1089, 1070, 1055, 1014, 936, 851, 823; *HRMS could not be obtained for this compound with the available methods.*

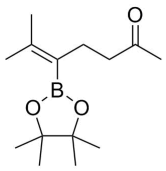
2-(6,6-diethoxy-2-methylhept-2-en-3-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (4.23)



Acetal **4.22** (279 mg, 1 mmol) was dissolved in dry THF (3 ml) and cooled to -78 °. *t*BuLi (1.9 M in pentane, 1.10 ml, 2.10 mmol) was added dropwise and a sharp colour change to yellow was observed. After 30 minutes, *i*PrOBpin (0.61 ml, 3 mmol) was added and the cooling bath removed. The reaction was quenched with sat. NH_4Cl. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 9:1, R_f 0.26). This procedure afforded pure boronic ester **4.23** (219 mg, 0.67 mmol, 67%) as a clear oil.

^{1}H-NMR (400 MHz, DCM – d2, δ/ppm): 3.54 - 3.35 (m, 4H), 2.15 - 2.02 (m, 2H), 1.93 (s, 3H), 1.73 (s, 3H), 1.56 - 1.48 (m, 2H), 1.27 (s, 3H), 1.24 (s, 12H), 1.13 (t, *J*=7.0, 6H); **^{13}C-NMR** (101 MHz, DCM – d2, δ/ppm): 147.8, 101.7, 83.0 (2C), 55.7 (2C), 37.7, 26.2, 25.0 (4C), 24.6, 22.1, 20.9, 15.7 (2C) ; **IR** (cm^{-1}): 2975, 2929, 1628, 1444, 1372, 1351, 1291, 1268, 1252, 1213, 1147, 1108, 1073, 1057, 964, 936, 882, 845, 707, 672; *HRMS could not be obtained for this compound with the available methods.*

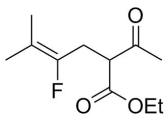
6-Methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hept-5-en-2-one (4.19)



Acetal **4.23** (200 mg, 0.61 mmol) was dissolved in acetone (1.2 ml). 4 drops of water and Amberlyst® 15 resin (200 mg) were added. The solution was stirred until complete conversion was observed on TLC. The resin was filtered off and the solvent evaporated. The product was purified by flash column chromatography (pentane/ diethyl ether 8:2, R_f 0.2). This procedure afforded pure ketone **4.19** (0.15 mg, 0.61 mmol, quant.) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 2.48 - 2.30 (m, 4H), 2.13 (s, 3H), 1.95 (s, 3H), 1.73 (s, 3H), 1.27 (s, 12H). **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 209.6, 148.6, 83.0 (2C), 44.3, 29.9, 25.6, 24.9 (4C), 24.7, 21.0; **IR** (cm^{-1}): 2977, 2926, 1715, 1628, 1445, 1371, 1351, 1293, 1220, 1144, 1108, 1021, 966, 877, 851, 701, 671; **HRMS** (CI): *m/z*: 252.1971 $[M+H]^+$ (calcd. *m/z*: 253.1970 $[M+H]^+$).

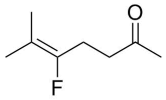
Ethyl 2-acetyl-4-fluoro-5-methylhex-4-enoate (4.24)



Sodium hydride (51 mg, 1.27 mmol) was suspended in dry THF (1.5 ml) and cooled in an ice bath. Acetylacetic acid ethyl ester (0.16 ml, 1.27 mmol) was added dropwise. After 30 minutes, bromide **2.17** (0.23 g, 1.40 mmol) in dry THF (1 ml) was added. The reaction was stirred overnight and quenched with 1M HCl solution. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 8:2). This procedure afforded ester **4.24** (0.23 g, 1.07 mmol, approx. 90% pure) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 4.19 (q, *J*=7.1, 3H), 3.76 (t, *J*=7.4, 1H), 2.79 (dd, *J*=21.9, 7.0, 2H), 2.26 (s, 3H), 1.60 (t, *J*=3.6, 6H), 1.26 (t, *J*=7.1, 3H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -114.78 (ddp, *J*=24.9, 18.7, 3.1); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 202.2, 168.9, 150.2 (d, J_{CF}=239.2), 128.2, 110.3 (d, J_{CF}=16.7), 61.6, 56.3, 29.6, 27.1 (d, J_{CF}=28.6), 17.6 (d, J_{CF}=5.6), 15.6 (d, J_{CF}=8.9), 14.1; **IR** (cm^{-1}): 2987, 2923, 1741, 1717, 1643, 1448, 1359, 1247, 1227, 1180, 1155, 1112, 1062, 1018, 858; **HRMS** (CI): *m/z*: 217.1227 $[M+H]^+$ (calcd. *m/z*: 217.1234 $[M+H]^+$).

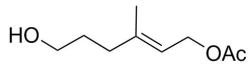
5-Fluoro-6-methylhept-5-en-2-one (4.18)



Ester **4.24** (0.17 g, 0.81 mmol) was dissolved in ethanol (1.6 ml). KOH (0.09 g, 1.62 mmol) was dissolved in water (0.54 ml) and added. The mixture was heated to reflux for 3 hours. After cooling to room temperature 1M HCl was added and the mixture extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 8:2, R_f 0.27). This procedure afforded pure ketone **4.18** (118 mg, 0.81 mmol, quant.) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 2.63 (t, *J*=7.3, 2H), 2.50 (dt, *J*=22.2, 7.3, 2H), 2.16 (s, 3H), 1.65 - 1.55 (m, 6H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -114.69 (ddp, *J*=22.0, 19.0, 3.0); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 207.7, 152.5 (d, *J*=240.4), 108.3, 40.2, 30.0, 22.6 (d, *J*=29.8), 17.5, 15.5 (d, *J*=9.1); **IR** (cm^{-1}):2924, 2856, 2358, 1720, 1457, 1361, 1210, 1161, 1115, 1018, 667, 611; **HRMS** (CI): *m/z*: 145.1024 $[M+H]^+$ (calcd. *m/z*: 145.1023 $[M+H]^+$).

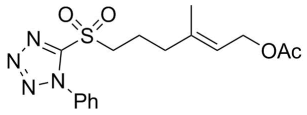
(*E*)-6-Hydroxy-3-methylhex-2-en-1-yl acetate (4.27)



Aldehyde **4.26** (1.70 g, 10 mmol) was dissolved in methanol (20 ml) and cooled in an ice bath. Sodium borohydride (0.11 g, 3.0 mmol) was added in small portions. After finishing the addition the reaction was stirred for 30 minutes, then quenched with sat. $NaHCO_3$. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/EtOAc 6:4, R_f 0.26). This procedure afforded pure alcohol **4.27** (1.47 g, 8.55 mmol, 85%) as clear oil.

The analytical data were in accordance with the literature.[17] **^{1}H-NMR** (400 MHz, $CDCl_3$, δ/ppm): 5.37 (tq, *J*=7.1, 1.4, 1H), 4.64 - 4.52 (m, 2H), 3.64 (t, *J*=6.5, 2H), 2.17 - 2.08 (m, 2H), 2.05 (s, 3H), 1.74 - 1.64 (m, 5H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 171.3, 142.0, 118.7, 62.6, 61.4, 35.9, 30.5, 21.2, 16.5.

(*E*)-3-Methyl-6-((1-phenyl-1H-tetrazol-5-yl)sulfonyl)hex-2-en-1-yl acetate (4.29)

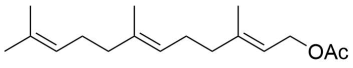


Alcohol **4.27** (0.1 g, 0.58 mmol) was dissolved in dry THF (5 ml) and cooled in an ice bath. Triphenylphosphine (167 mg, 0.63 mmol) and 1-phenyl-1*H*-tetrazol-5-thiol (113 mg, 0.63 mmol) were added sequentially. DEAD (0.1 ml, 0.63 mmol) was added dropwise. After the addition the reaction was warmed to room temperature and stirred for 5.5 hours. Sat. $NaHCO_3$ was added and extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/EtOAc 9:1, R_f 0.15). This procedure afforded pure acetate **4.28** (144 mg, 0.43 mmol, 74%) as a clear oil.

The obtained oil was dissolved in EtOH (5 ml) and cooled in an ice bath. Ammonium molybdate tetrahydrate (26 mg, 0.02 mmol) was added followed by aq. hydrogen peroxide (30% w/v., 0.76 ml, 7.44 mmol). The reaction was warmed to room temperature and stirred overnight. Sat. $NaHCO_3$ was added and extracted three times with EtOAc. The combined organic phases were dried over Na_2SO_3 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (pentane/EtOAc 8:2, R_f 0.15). This yielded pure sulfone **4.29** (111 mg, 0.30 mmol, 70%) as a clear oil.

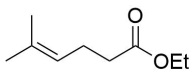
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.72 - 7.66 (m, 2H), 7.65 - 7.57 (m, 3H), 5.40 (t, *J*=7.0, 1H), 4.59 (d, *J*=6.9, 2H), 3.76 - 3.63 (m, 2H), 2.25 (t, *J*=7.3, 2H), 2.18 - 2.08 (m, 2H), 2.06 (s, 3H), 1.72 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 171.1, 153.4, 139.0, 131.5, 129.8, 125.0, 120.9, 61.0, 55.2, 37.4, 21.0, 19.9, 16.1; **IR** (cm^{-1}): 3065, 2936, 2361, 1732, 1595, 1497, 1460, 1340, 1233, 1154, 1104, 1076, 1024, 957, 919, 833, 764, 734, 690, 629; *HRMS could not be obtained for this compound with the available methods.*

Synthesis of farnesol acetate (2.27) by Julia-Kocienski Olefination



Sulfone **4.29** (0.1 g, 0.27 mmol) and 6-methyl-5-hepten-2-one (36 μl, 0.25 mmol) were dissolved in dry THF (1 ml). The mixture was cooled to -78 °C. NaHMDS (1M in THF, 0.29 ml, 0.29 mmol) was added dropwise. The reaction was allowed to warm to room temperature overnight. The reaction was quenched with 1M HCl and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 96:4, R_f 0.36). This procedure afforded pure acetate **2.27** (6 mg, 22 μmol, 9%, *6E:6Z* 1:1.3).

Ethyl 5-methylhex-4-enoate (4.36)

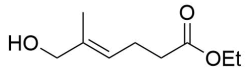


2-Methyl-3-buten-2-ol (12.1 ml, 116 mmol), triethyl orthoacetate (100 ml) and propionic acid (0.5 ml) were heated to 140 °C for 4 hours. After cooling to room temperature, 1M HCl was added and the mixture extracted three times with EtOAc. The combined organic phases were washed with sat. $NaHCO_3$ and brine, then dried over Na_2SO_4. The solvent was removed under reduced pressure and the product purified by flash column chromatography (pentane/diethyl ether 95:5, R_f 0.5). This procedure afforded pure ester **4.36** (10.2 g, 65.4 mmol, 56%) as a clear oil.

The analytical data were in accordance with the literature.[18]

^{1}H-NMR (200 MHz, $CDCl_3$, δ/ppm): 5.17 - 4.99 (m, 1H), 4.12 (q, *J*=7.1, 2H), 2.34 - 2.27 (m, 4H), 1.68 (s, 2H), 1.62 (s, 2H), 1.25 (t, *J*=7.1, 4H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 173.5, 133.0, 122.5, 60.2, 34.6, 25.7, 23.7, 17.7, 14.3.

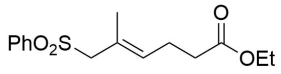
Ethyl (*E*)-6-hydroxy-5-methylhex-4-enoate (4.37)



Ester **4.36** (10.2 g, 65.4 mmol) was dissolved in DCM (100 ml). *t*BuOOH (70% in water, 26.9 ml, 96.4 mmol), salicylic acid (0.904 g, 6.50 mmol) and selenium dioxide (0.726 g, 6.5 mmol) were added successively. The reaction was stirred overnight then sat. $NaHCO_3$ was added. The aqueous phase was extracted three times with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The remaining oil was dissolved in methanol (100 ml) and cooled in an ice bath. Sodium borohydride (0.618 g, 16.3 mmol) was added in small portions. The reaction was stirred for 30 minutes then sat. $NaHCO_3$ was added and diluted with water. The mixture was extracted three times with EtOAc. The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 1:1, R_f 0.15). This procedure afforded pure alcohol **4.37** (4.81 g, 27.9 mmol, 43%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.37 (t, *J*=6.5, 1H), 4.11 (q, *J*=7.1, 2H), 3.97 (s, 2H), 2.40 - 2.31 (m, 4H), 1.66 (s, 4H), 1.24 (t, *J*=7.1, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 173.4, 136.3, 123.7, 68.6, 60.5, 34.2, 23.2, 14.3, 13.7; **IR** (cm^{-1}): 3408, 2981, 2916, 2359, 1732, 1446, 1372, 1346, 1261, 1184, 1149, 1095, 1039, 1010, 856, 771; *HRMS could not be obtained for this compound with the available methods.*

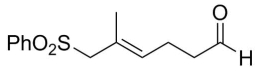
Ethyl (*E*)-5-methyl-6-(phenylsulfonyl)hex-4-enoate (4.38)



Alcohol **4.37** (4.81 g, 27.9 mmol) was dissolved in dry THF (30 ml) and cooled in an ice bath. PBr_3 (1.31 ml, 13.9 mmol) was added dropwise. The reaction was stirred for 3 hours at 0 °, then ice cold water was added. The mixture was extracted three times with diethyl ether. The combined organic phases were washed with sat. $NaHCO_3$, water and brine. The solvent was removed under reduced pressure. The remaining oil was dissolved in dry DMF (20 ml) and benzene sulfinic acid sodium salt (9.0 g, 55.8 mmol) was added. The reaction was stirred overnight then diluted with diethyl ether. The mixture was washed twice with water then with brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 1:1, R_f 0.25). This procedure afforded pure sulfone **4.38** (4.99 g, 16.8 mmol, 60%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.83 (d, *J*=7.2, 2H), 7.63 (t, *J*=7.3, 1H), 7.54 (t, *J*=7.6, 2H), 5.02 (t, *J*=7.2, 1H), 4.09 (t, *J*=7.1, 2H), 3.71 (s, 2H), 2.30 - 2.21 (m, 2H), 2.15 (t, *J*=7.8, 2H), 1.77 (s, 3H), 1.24 (t, *J*=7.1, 3H) ; **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 172.8, 138.4, 134.1, 133.7, 129.0 (2C), 128.6 (2C), 125.0, 66.1, 60.6, 33.5, 23.8, 16.9, 14.3.; **IR** (cm^{-1}): 2981, 1729, 1446, 1373, 1349, 1306, 1254, 1148, 1133, 1085, 1020, 889, 768, 742, 727, 689, 614; **HRMS** (CI): *m/z*: 297.1163 $[M+H]^+$ (calcd. *m/z*: 297.1155 $[M+H]^+$).

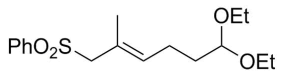
(*E*)-5-Methyl-6-(phenylsulfonyl)hex-4-enal (4.39)



Sulfone **4.38** (4.99 g, 16.8 mmol) was dissolved in dry DCM (50 ml) and cooled to -78 °C. DIBAL-H (1M in hexanes, 21.8 ml, 21.8 mmol) was added dropwise. After 30 minutes, sat. Rochelle salt solution (50 ml) was added. The suspension was allowed to warm to room temperature and stirred vigorously overnight. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 2:8, R_f 0.22). This procedure afforded pure aldehyde **4.39** (3.49 g, 13.8 mmol, 82%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 9.63 (d, *J*=1.3, 1H), 7.84 - 7.78 (m, 2H), 7.67 - 7.58 (m, 1H), 7.52 (dd, *J*=8.4, 7.0, 2H), 5.01 (t, *J*=7.1, 1H), 3.69 (s, 2H), 2.34 - 2.28 (m, 2H), 2.27 - 2.19 (m, 2H), 1.75 (s, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 201.2, 138.3, 133.8, 133.7, 129.0 (2C), 128.5 (2C), 125.1, 66.0, 42.9, 20.9, 16.8; **IR** (cm^{-1}): 2981, 1729, 1446, 1373, 1349, 1306, 1254, 1148, 1133, 1085, 1020, 889, 768, 742, 727, 689, 614; **HRMS** (CI): *m/z*: 270.1167 $[M + NH_4]^+$ (calcd. *m/z*: 270.1158 $[M + NH_4]^+$).

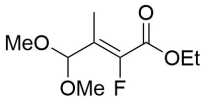
(*E*)-((6,6-Diethoxy-2-methylhex-2-en-1-yl)sulfonyl)benzene (4.35)



Aldehyde **4.39** (3.49 g, 13.8 mmol) was dissolved in dry toluene (27.6 ml). Dry ethanol (9.71 ml, 166 mmol), PPTS (0.348 g, 1.38 mmol) and triethyl orthoformate (5.13 ml, 34.6 mmol) were added. The reaction was heated to reflux for 4 hours. After cooling to room temperature, diethyl ether was added. The organic phase was washed with water and brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 1:1, R_f 0.35). This procedure afforded pure acetal **4.35** (4.22 g, 12.9 mmol, 93%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.85 (d, *J*=8.4, 2H), 7.63 (t, *J*=6.7, 1H), 7.54 (t, *J*=6.9, 2H), 5.07 (t, *J*=7.5, 1H), 4.36 (t, *J*=5.6, 1H), 3.72 (s, 2H), 3.67 - 3.55 (m, 2H), 3.50 - 3.39 (m, 2H), 2.06 - 1.96 (m, 2H), 1.76 (s, 3H), 1.53 - 1.43 (m, 2H), 1.19 (t, *J*=7.0, 6H) ; **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 138.5, 135.5, 133.5, 128.9 (2C), 128.5 (2C), 123.8, 102.1, 66.2, 61.1, 32.7, 23.7, 16.7, 15.4 ; **IR** (cm^{-1}): 2974, 2361, 1146, 1376, 1310, 1255, 1132, 1085, 1060, 883, 742, 689, 615; **HRMS** (CI): *m/z*: 344.1901 $[M + NH_4]^+$ (calcd. *m/z*: 344.189 $[M + NH_4]^+$).

Ethyl (*E/Z*)-2-fluoro-4,4-dimethoxy-3-methylbut-2-enoate (4.41)



E:Z 1.2:1

Sodium hydride (0.908 g, 22.7 mmol) was suspended in dry THF (40 ml) in an ice bath. Triethyl 2-fluoro-2-phosphonoacetate (5 g, 20.6 mmol) was added dropwise. The suspension was stirred for 30 minutes, then 1,1-dimethoxyacetone (2.99 ml, 24.7 mmol) was added. The reaction was allowed to warm to room temperature and stirred over night. The reaction was quenched with sat. NH_4Cl and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 96:4, R_f 0.23). This procedure afforded pure acetal **4.41** as a mixture of isomers (3.44 g, 16.7 mmol, 81%, *E:Z* 1.2:1) as a clear oil. The assignment of stereochemistry was achieved by ^{19}F-^{1}H-HOESY.

Analytical data for *Z*-isomer:

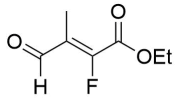
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.80 (d, *J*=2.4, 1H), 4.29 (qd, *J*=7.1, 1.7, 2H), 3.39 (s, 3H), 1.82 (d, *J*=4.5, 3H), 1.34 (td, *J*=7.1, 2.9, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 160.6 (d, J_{CF}=34.7), 145.8 (d, J_{CF}=258.9), 129.6 (d, J_{CF}=12.3),100.9 (d, J_{CF}=9.4), 61.7, 55.5 (2C), 14.2, 9.04 (d, J_{CF}=7.9); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -122.05 - -122.44 (m, 1F); **IR** (cm^{-1}): 2936, 2833, 1726, 1671, 1446, 1396, 1291, 1230, 1212, 1188, 1135, 1099, 1064, 961, 929, 864, 775, 726, 677; *HRMS*

could not be obtained for this compound with the available methods.

Analytical data for *E*-isomer:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.16 (d, *J*=1.5, 1H), 4.29 (qd, *J*=7.1, 1.7, 2H), 3.38 (s, 6H), 2.00 (d, *J*=3.2, 3H), 1.34 (td, *J*=.1, 2.9, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 161.0 (d, J_{CF}=35.4), 145.0 (d, J_{CF}=254.3), 128.2 (d, J_{CF}=8.1) , 100.4 (d, J_{CF}=13.2) , 61.6, 55.2 (2C), 14.2, 9.6 (d, J_{CF}=1.7); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -127.29 - -127.79 (m).

Ethyl (*E/Z*)-2-fluoro-3-methyl-4-oxobut-2-enoate (4.42)



E:Z 1.2:1

Acetal **4.41** (3.24 g, 15.7 mmol) was dissolved in diethyl ether (30 ml). Water (30 ml) and trifluoroacetic acid (12.0 ml, 157.4 mmol) were added. The reaction was stirred vigorously overnight. Solid $NaHCO_3$ was added until the pH was slightly basic. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 94:6, R_f 0.3). This procedure afforded pure aldehyde **4.42** as a mixture of isomers (1.76 g, 11.0 mmol, 71%), *E:Z* 1.2:1) as a clear oil. The assignment of stereochemistry was achieved by ^{19}F-^{1}H-HOESY.

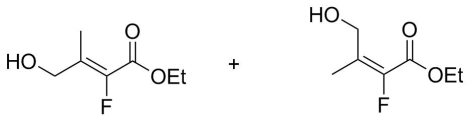
Analytical data for *Z*-isomer:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 10.56 (d, *J*=1.7, 1H), 4.44 - 4.33 (m, 2H), 1.90 (dd, *J*=4.6, 1.7, 3H), 1.39 (td, *J*=7.2, 1.4, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 191.52 (d, J_{CF}=10.6), 160.6 (d, J_{CF}=34.8), 156.1 (d, J_{CF}=279.7), 125.6, 62.5, 14.0, 8.6; **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -101.13 - -103.11 (m); **IR** (cm^{-1}): 2361, 1732, 1686, 1646, 1447, 1400, 1371, 1324, 1298, 1224, 1144, 1060, 1015, 923, 861, 776, 713; *HRMS could not be obtained for this compound with the available methods.*

Analytical data for *E*-isomer:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 10.30 (d, *J*=1.6, 1H), 4.44 - 4.33 (m, 2H), 2.05 (dd, *J*=3.6, 1.7, 3H), 1.39 (t, *J*=7.2, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 189.6 (d, J_{CF}=17.6), 160.6 (d, J_{CF}=34.8), 156.1 (d, J_{CF}=279.7), 125.6, 62.5, 14.0, 8.6; **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -123.48 (t, *J*=3.7).

Ethyl (*Z*)-2-fluoro-4-hydroxy-3-methylbut-2-enoate (*Z*-4.43) and ethyl (*E*)-2-fluoro-4-hydroxy-3-methylbut-2-enoate (*E*-4.43)



Aldehyde **4.42** (1.76 g, 11.0 mmol) was dissolved in MeOH (22 ml) and cooled in an ice bath. Sodium borohydride (0.208 g, 5.5 mmol) was added portionwise. After 15 minutes, the reaction was quenched with 1M HCl and extracted three times with

diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The desired *Z*-isomer and the *E*-isomer were separated by column chromatography (pentane/diethyl ether 1:1, $R_{f:Z}$ 0.35 and $R_{f:E}$0.2). This procedure afforded pure ester *Z*-**4.43** (0.643 g, 3.96 mmol, 48%) and *E*-**4.43** (0.702 g, 4.32 mmol, 52%) as clear oils. Assignment of stereochemistry was achieved by ^{19}F-^{1}H-HOESY.

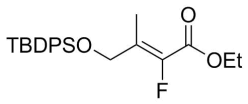
Analytical data for *Z*-isomer:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 4.37 - 4.32 (m, 2H), 4.28 (q, *J*=7.1, 2H), 2.16 (d, *J*=3.3, 3H), 1.33 (t, *J*=7.1, 3H) ; **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 161.2 (d, J_{CF}=35.0), 143.7 (d, J_{CF}=250.3), 130.9 (d, J_{CF}=11.0), 61.5, 60.5 (d, J_{CF}=11.6), 14.2, 13.7 (d, J_{CF}=1.4); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -126.22 - -127.13 (m); **IR** (cm^{-1}): 3400, 2986, 123, 1664, 1446, 1370, 1306, 1227, 1132, 1227, 1132, 1082, 1021, 926, 863, 774, 653; **HRMS** (CI): *m/z*: 163.0771 $[M+H]^+$ (calcd.: *m/z*: 163.0765 $[M+H]^+$).

Analytical data for *E*-isomer:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 4.69 (d, *J*=5.4, 2H), 3.46 (q, *J*=7.0, 2H), 2.08 - 1.98 (m, 3H), 1.19 (t, *J*=7.0, 3H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 165.3 (d, J_{CF}=31.3), 144.1 (d, J_{CF}=269.4), 134.8 (d, J_{CF}=7.8), 68.7, 68.7, 65.9, 15.3, 9.4 (d, J_{CF}=2.2); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -151.45 - -152.77 (m); **IR** (cm^{-1}): 3400, 2986, 123, 1664, 1446, 1370, 1306, 1227, 1132, 1227, 1132, 1082, 1021, 926, 863, 774, 653; **HRMS** (CI): *m/z*: 163.0771 $[M+H]^+$ (calcd.: *m/z*: 163.0765 $[M+H]^+$).

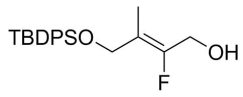
Ethyl (*Z*)-4-((*Tert*-butyldiphenylsilyl)oxy)-2-fluoro-3-methylbut-2-enoate (4.44)



Alcohol *Z*-**4.43** (1.06 g, 6.54 mmol) was dissolved in dry DCM (6.5 ml) and cooled in a water bath. Imidazole (0.489 g, 7.19 mmol) and TBDPSCl (1.87 ml, 7.19 mmol) were added. The reaction was stirred at room temperature overnight, then diluted with diethyl ether. The organic phase was washed with water and brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 96:4, R_f 0.22). This procedure afforded pure ester **4.44** (1.88 g, 4.67 mmol, 71%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.69 - 7.64 (m, 4H), 7.48 - 7.36 (m, 6H), 4.41 (d, *J*=3.5, 2H), 4.28 (q, *J*=7.1, 2H), 2.22 (d, *J*=3.1, 3H), 1.34 (t, *J*=7.1, 3H), 1.07 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 161.2 (d, J_{CF}=34.6), 142.7 (d, J_{CF}=249.8), 135.7 (4C), 133.2, 131.6 (d, J_{CF}=10.8), 129.9 (2C), 127.9 (4C), 61.3,61.1 (d, J_{CF}=12.3), 26.9 (3C), 19.4, 14.3, 13.3 (d, J_{CF}=1.8); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -126.48 - -126.54 (m); **IR** (cm^{-1}):2932, 2858, 2360, 1726, 1668, 1472, 1428, 1389, 1368, 1308, 1257, 1231, 1112, 1063, 936, 824, 773, 740, 701, 613; **HRMS** (CI): *m/z*: 401.1941 $[M]^+$ (calcd.: *m/z*: 401.1943 $[M+H]^+$).

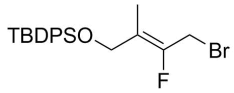
(*Z*)-4-((*Tert*-butyldiphenylsilyl)oxy)-2-fluoro-3-methylbut-2-en-1-ol (4.45)



Ester **4.44** (1.88 g, 4.67 mmol) was dissolved in dry DCM (8 ml) and cooled in an ice bath. DIBAL-H (1M in hexanes, 9.8 ml, 9.8 mmol) was added. After 1 hour, sat. Rochelle salt solution was added and the mixture stirred vigorously overnight. The mixture was diluted with diethyl ether and the organic phase separated. The aqueous phase was extracted two more times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 2:1, R_f 0.22). This procedure afforded pure alcohol **4.45** (1.06 g, 2.97 mmol, 63%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.71 - 7.65 (m, 4H), 7.47 - 7.35 (m, 6H), 4.33 (d, *J*=3.1, 2H), 4.19 (dd, *J*=21.9, 6.1, 2H), 1.79 (d, *J*=2.9, 3H), 1.06 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 152.5 (d, J_{CF}=246.6), 135.7 (4C), 133.7, 129.8 (2C), 127.8 (4C), 115.7 (d, J_{CF}=12.8), 60.3 (d, J_{CF}=11.0), 58.1 (d, J_{CF}=30.9), 27.0 (3C), 19.4, 12.8 (d, J_{CF}=4.2); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -119.13 - -119.31 (m); **IR** (cm^{-1}): 3329, 2957, 2857, 1707, 1589, 1471, 1427, 1388, 1361, 1264, 1158, 1111, 1069, 1008, 938, 906, 823, 766, 739, 700, 612; **HRMS** (CI): *m/z*: 376.2114 $[M + NH_4]^+$ (calcd.: *m/z*: 376.2103 $[M + NH_4]^+$).

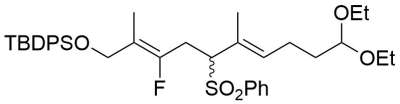
(*Z*)-((4-Bromo-3-fluoro-2-methylbut-2-en-1-yl)oxy)(*tert*-butyl) diphenylsilane (4.46)



Alcohol **4.45** (1.06 g, 2.97 mmol) was dissolved in dry THF (6 ml) and cooled in an ice bath. PBr_3 (0.14 ml, 1.48 mmol) was added dropwise. The reaction was stirred for 3 hours at 0 °C, then quenched with ice cold water. The mixture was extracted three times with diethyl ether. The combined organic phases were washed with sat. $NaHCO_3$ and brine, then dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 96:4, R_f 0.61). This procedure afforded pure bromide **4.45** (0.946 g, 2.24 mmol, 75%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.72 - 7.62 (m, 4H), 7.49 - 7.35 (m, 6H), 4.31 (d, *J*=3.0, 2H), 4.01 (d, *J*=22.9, 2H), 1.80 (d, *J*=2.9, 3H), 1.06 (s, 10H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 149.6 (d, J_{CF}=243.5), 135.7 (4C), 133.5, 129.8 (2C), 127.8(4C), 118.1 (d, J_{CF}=13.3), 60.4 (d, J_{CF}=9.8), 26.9 (3C), 25.3 (d, J_{CF}=34.2), 19.4, 13.2 (d, J_{CF}=3.8); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -113.38 - -113.58 (m); **IR** (cm^{-1}): 3071, 2931, 2857, 1696, 1589, 1472, 1427, 1387, 1263, 1210, 1180, 1152, 1111, 1065, 1007, 938, 859, 823, 739, 701, 613; **HRMS** (CI): *m/z*: 421.0984 $[M + H]^+$ (calcd.: *m/z*: 421.0993 $[M + H]^+$).

Tert-butyl(((2*Z*,6*E*)-10,10-diethoxy-3-fluoro-2,6-dimethyl-5-(phenylsulfonyl)deca-2,6-dien-1-yl)oxy)diphenylsilane (4.47)

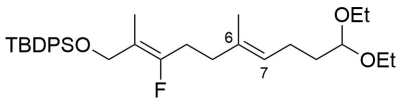


Sulfone **4.35** (0.666 g, 2.04 mmol) was dissolved in dry THF (4.8 ml) and dry DMF (1.5 ml), then cooled to -78 °C. *t*BuOK (1M in THF, 2.24 ml, 2.24 mmol) was added dropwise. The reaction was stirred for 2 hours, then a solution of bromide **4.46** (0.946 g, 2.24 mmol) in dry THF (5.1 ml) was added dropwise. After another 2 hours, the reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched with sat. NH_4Cl. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 2:1, R_f 0.26). This procedure afforded pure acetal **4.47** (1.24 g, 1.85 mmol, 90%) as a clear oil.

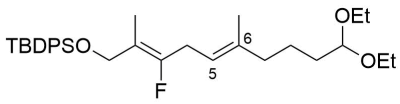
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.85 - 7.79 (m, 2H), 7.68 - 7.58 (m, 5H), 7.52 (t, nmrj 7.6, 2H), 7.45 - 7.32 (m, 6H), 5.14 (t, *J*=7.3, 1H), 4.27 (t, *J*=5.7, 1H), 4.19 (qd, *J*=11.8, 3.0, 2H), 3.75 (dd, *J*=10.8, 4.4, 1H), 3.61 - 3.49 (m, 2H), 3.47 - 3.33 (m, 2H), 3.03 - 2.82 (m, 2H), 2.00 - 1.90 (m, 2H), 1.70 (d, *J*=2.5, 3H), 1.66 (s, 3H), 1.39 (td, *J*=7.7, 5.7, 2H), 1.15 (td, *J*=7.1, 5.1, 6H), 1.03 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 150.2 (d, J_{CF}=246.5), 137.8, 135.6 (4C), 133.7, 133.6 , 129.7 (2C), 129.0 (2C),

128.9 (2C), 127.7 (4C), 126.5, 114.8 (d, J_{CF}=13.5), 102.2, 70.7, 61.3, 61.1, 60.2 (d, J_{CF}=11.2), 32.7, 26.9 (3C), 25.7 (d, J_{CF}=28.5), 23.6, 19.4, 15.4 (2C), 14.0, 13.4 (d, J_{CF}=4.7); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.12 - -112.38 (m); **IR** (cm^{-1}): 2930, 2857, 1710, 1472, 1446, 1428, 1389, 1305, 1143, 1111, 1083, 821, 742, 702, 689, 609. **HRMS** (APCI): *m/z*: 701.2907 $[M+Cl]^-$ (calcd.: *m/z*: 701.2904 $[M+Cl]^-$).

Tert-butyl(((2*Z*,6*E*)-10,10-diethoxy-3-fluoro-2,6-dimethyldeca-2,6-dien-1-yl)oxy)diphenylsilane (4.48A) and *tert*-butyl(((2*Z*,5*E*)-10,10-diethoxy-3-fluoro-2,6-dimethyldeca-2,5-dien-1-yl)oxy)diphenylsilane (4.48B)



A



B

Magnesium turnings (1.35 g, 55.5 mmol) were suspended in dry THF (4 ml). A small crystal of iodine was added and the mixture stirred for 15 minutes. The reaction was placed in a cold water bath and sulfone **4.47** (1.23 g, 1.85 mmol) in dry THF (4 ml) and dry methanol (16 ml) was added slowly. Heavy gas evolution indicated the start of the reaction. After 1 hour, the remaining turnings were decanted and sat. aq. NH_4Cl (50 ml) and water (50 ml) were added. The resulting mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4

and the solvent removed under reduced pressure. The product was purified immediately by flash column chromatography (pentane/diethyl ether 9:1, R_f 0.5). This procedure afforded pure acetal **4.48A** and **4.48B** in an inseparable 1:1 mixture (0.737 g, 1.39 mmol, 75%) as a clear oil.

Analytical data for **4.48A**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.70 - 7.65 (m, 4H), 7.45 - 7.32 (m, 6H), 5.18 - 5.11 (m, 1H), 4.51 - 4.43 (m, 1H), 4.31 - 4.26 (m, 2H), 3.68 - 3.58 (m, 2H), 3.53 - 3.42 (m, 2H), 2.35 - 2.21 (m, 2H), 2.16 - 2.10 (m, 2H), 2.09 - 2.02 (m, 2H), 1.71 (d, *J*=2.8, 3H), 1.66 - 1.54 (m, 5H), 1.20 (t, *J*=7.1, 6H), 1.05 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 154.8 (d, J_{CF}=247.6), 135.7 (4C), 134.0 (2C), 129.6 (2C), 127.7 (4C), 124.7, 111.5 (d, J_{CF}=14.1), 102.5, 61.0 (2C), 60.5 (d, J_{CF}=11.7), 36.4, 33.6, 33.5, 27.7 (d, J_{CF}=16.9), 26.9, 23.4, 16.0, 15.5 (2C), 14.2, 13.1 (d, J_{CF}=3.3); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -110.28 (tq, *J*=23.2, 2.8); **IR** (cm^{-1}): 2929, 2857, 1707, 1472, 1428, 1385, 1261, 1111, 1060, 822, 739, 701, 612; **HRMS** (ESI): *m/z*: 549.3161 $[M + Na]^+$ (calcd.: *m/z*: 549.3176 $[M + Na]^+$).

Analytical data for **4.48B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.70 - 7.65 (m, 4H), 7.45 - 7.32 (m, 6H), 5.18 - 5.11 (m, 1H), 4.51 - 4.43 (m, 1H), 4.31 - 4.26 (m, 2H), 3.68 - 3.58 (m, 2H), 3.53 - 3.42 (m, 2H), 2.90 (dd, *J*=23.7, 7.1, 2H), 2.08 - 1.97 (m, 2H), 1.69 (d, *J*=2.6, 3H), 1.65 - 1.54 (m, 5H), 1.53 - 1.40 (m, 2H), 1.20 (t, *J*=7.1, 6H), 1.05 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 154.0 (d, *J*=248.4), 135.7 (4C), 134.0 (2C), 129.6 (2C), 127.7 (4C),

118.7, 111.5 (d, *J*=14.1), 103.0, 61.0 (2C), 60.6 (d, *J*=11.7), 39.4, 33.6, 28.1 (d, *J*=9.7), 27.0, 23.0, 16.1, 15.5 (2C), 14.2, 13.2 (d, *J*=4.0); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -108.78 - -109.11 (m).

(2*Z*,6*E*)-10,10-Diethoxy-3-fluoro-2,6-dimethyldeca-2,6-dien-1-ol (4.49A) and (2Z,5E/Z)-10,10-diethoxy-3-fluoro-2,6-dimethyldeca-2,5-dien-1-ol (4.49B)

Acetal **4.48** (0.5 g, 0.949 mmol) was dissolved in dry THF (9.4 ml) and cooled in an ice bath. TBAF (1M in THF, 1.89 ml, 1.89 mmol) was added and the mixture warmed to room temperature. After 2 hours, EtOAc was added and the mixture washed with water and brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 6:4, R_f 0.2). This procedure afforded pure alcohols **4.49A** and **4.49B** in an inseparable 1:1 mixture (0.233 g, 0.807 mmol, 85%) as a clear oil.

Analytical data for **4.49A**:

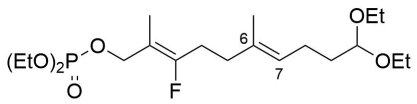
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.20 - 5.10 (m, 1H), 4.50 - 4.42 (m, 1H), 4.14 (d,

J=3.0, 2H), 3.67 - 3.56 (m, 2H), 3.53 - 3.40 (m, 2H), 2.31 (dt, *J*=23.0, 7.5, 2H), 2.15 (t, *J*=7.5, 2H), 2.08 - 1.98 (m, 2H), 1.68 (s, 3H), 1.66 - 1.61 (m, 5H), 1.17 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 155.74 (d, J_{CF}=248.1), 134.1, 118.4, 112.1 (d, J_{CF}=14.1), 102.0, 60.7 (2C), 59.6 (d, J_{CF}=11.5), 36.1, 33.3, 27.5 (d, J_{CF}=28.8), 23.3, 15.7, 15.4 (2C), 13.5 (d, J_{CF}=5.1); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -111.00 - -111.22 (m); **IR** (cm^{-1}): 3404, 2974, 2928, 1705, 1444, 1375, 1346, 1127, 1057, 1010, 897, 744; **HRMS** (APCI): *m/z*: 311.1989 $[M+Na]^+$ (calcd.: *m/z*: 311.1998 $[M+Na]^+$).

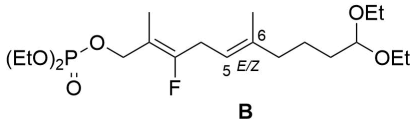
Analytical data for **4.49B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.20 - 5.10 (m, 1H), 4.50 - 4.42 (m, 1H), 4.14 (d, *J*=3.0, 2H), 3.67 - 3.56 (m, 2H), 3.53 - 3.40 (m, 2H), 2.92 (dd, *J*=23.7, 7.1, 2H), 1.68 (s, 3H), 1.66 - 1.59 (m, 5H), 1.59 - 1.52 (m, 2H), 1.50 - 1.40 (m, 2H), 1.17 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 155.5 (d, J_{CF}=249.2), 138.2, 124.9, 111.2 (d, J_{CF}=14.1), 102.8, 60.9 (2C), 59.8 (d, J_{CF}=11.0), 39.4, 33.1, 27.9 (d, J_{CF}=29.0), 23.0, 15.7, 15.4 (2C), 13.5 (d, J_{CF}=5.2); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -109.01 - -109.22 (m, 0.58 F), -109.29 - -109.48 (m, 0.33 F).

(2*Z*,6*E*)-10,10-Diethoxy-3-fluoro-2,6-dimethyldeca-2,6-dien-1-yl diethyl phosphate (4.51A) and (2Z,5E/Z)-10,10-diethoxy-3-fluoro-2,6-dimethyldeca-2,5-dien-1-yl diethyl phosphate (4.51B)



A



B

Alcohol **4.49** (50 mg, 173 μmol) was dissolved in dry DCM (0.5 ml) and cooled to 0°C. Pyridine (23 μl, 249 μmol) and diethyl chlorophosphae (35 μl, 242 μmol) were added. The reaction was stirred for 5 hours at 0°C, then was diluted with diethyl ether. The organic phase was washed twice with water, sat. $NaHCO_3$ and brine, then dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (pentane/EtOAc 6:4 with 1% TEA, R_f 0.15). This procedure afforded pure phosphates **4.51A** and **4.51B** in an inseparable 1:1 mixture (65 mg, 153 μmol, 88%) as a clear oil.

Analytical data for **4.51A**:

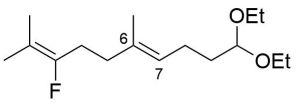
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.17 - 5.10 (m, 1H), 4.57 (dd, *J*=6.9, 2.7, 2H), 4.48 - 4.41 (m, 1H), 4.15 - 4.03 (m, 4H), 3.67 - 3.56 (m, 2H), 3.52 - 3.40 (m, 2H), 2.31 (dt, *J*=23.2, 7.8, 2H), 2.19 - 2.09 (m, 2H), 2.07 - 1.97 (m, 2H), 1.65 (d, *J*=2.8, 3H), 1.64 - 1.58 (m, 5H), 1.31 (t, *J*=7.1, 6H), 1.17 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm):

157.9 (d, J_{CF}=253.3), 134.1, 117.7, 107.82 (dd, J_{CF}=13.4, J_{CP}=7.5), 102.4, 64.25 (dt, J_{CF}=12.6, J_{CP}=6.1), 63.7 (d, J_{CP}=5.8, 2C), 61.0 (2C), 36.0, 33.5, 27.8 (d, J_{CF}=28.1), 23.3, 23.3, 16.2 (d, J_{CP}=6.8, 2C), 15.9, 15.4 (2C), 13.3 (d, J_{CF}=4.1); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -106.54 - -106.73 (m); **^{31}P-NMR** (162 MHz, $CDCl_3$, δ/ppm): -0.75 (sept, *J*=7.8); **IR** (cm^{-1}): 2977, 2360, 1707, 1445, 1373, 1272, 1128, 1005, 868, 817, 748; **HRMS** (APCI): *m/z*: 447.2277 $[M+Na]^+$ (calcd.: *m/z*: 447.2287 $[M+Na]^+$).

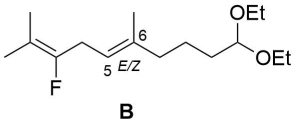
Analytical data for **4.51B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.17 - 5.10 (m, 1H), 4.57 (dd, *J*=6.9, 2.7, 2H), 4.48 - 4.41 (m, 1H), 4.15 - 4.03 (m, 4H), 3.67 - 3.56 (m, 2H), 3.52 - 3.40 (m, 2H), 2.93 (dd, *J*=23.6, 7.1, 2H), 2.08 - 1.92 (m, 2H), 1.65 (d, *J*=2.8, 3H), 1.60 (s, 3H), 1.58 - 1.51 (m, 2H), 1.48 - 1.41 (m, 2H), 1.31 (t, *J*=7.1, 6H), 1.18 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 157.1 (d, J_{CF}=254.0), 138.2, 124.9, 107.6 - 107.0 (m), 102.9, 102.8,64.2 (dt, J_{CF}=12.6, J_{CP}=6.1), 63.8 (d, J_{CP}=5.8, 2C), 61.0 (2C), 39.3, 33.2, 28.0 (d, J_{CF}=28.5), 22.8, 16.2 (d,J_{CP}=6.8, 2C), 15.9, 15.4 (2C), 13.3 (d, J_{CF}=4.1); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -105.22 - -105.53 (m); **^{31}P-NMR** (162 MHz, $CDCl_3$, δ/ppm): -0.75 (sept, *J*=7.8).

(*E*)-10,10-diethoxy-3-fluoro-2,6-dimethyldeca-2,6-diene (4.52A) and (*E*)-10,10-diethoxy-3-fluoro-2,6-dimethyldeca-2,5-diene (4.52B)



A



B

Phosphate **4.51** (20 mg, 47.1 μmol) was dissolved in dry THF (0.2 ml) and cooled in an ice bath. $LiBHEt_3$ (1M in THF, 235 μl, 235 μmol) was added slowly. Heavy gas evolution was observed. After 1 hour, water was carefully added and the mixture extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 9:1, R_f 0.8). This procedure afforded pure acetal **4.52A** and **4.52B** in an inseparable 1:1 mixture (7 mg, 25.7 μmol, 54%) as a clear oil.

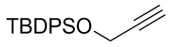
Analytical data for **4.52A**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.23 - 5.11 (m, 1H), 4.52 - 4.43 (m, 1H), 3.69 - 3.58 (m, 2H), 3.54 - 3.44 (m, 2H), 2.37 - 2.23 (m, 2H), 2.17 - 2.11 (m, 2H), 2.07 (dd, *J*=9.1, 5.8, 2H), 1.67 - 1.54 (m, 11H), 1.20 (t, *J*=7.0, 6H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -112.68 - -112.87 (m) ; **IR** (cm^{-1}): 2975, 2927, 1715, 1446, 1374, 1345, 1128, 1062, 898; **HRMS** (APCI): *m/z*: 295.2043 $[M + Na]^+$ (calcd.: *m/z*: 295.2049 $[M + Na]^+$).

Analytical data for **4.52B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.22 - 5.10 (m, 1H), 4.53 - 4.43 (m, 1H), 3.70 - 3.57 (m, 2H), 3.55 - 3.42 (m, 2H), 2.92 (dd, *J*=23.7, 7.1, 2H), 2.10 - 1.99 (m, 2H), 1.67 - 1.54 (m, 11H), 1.52 - 1.44 (m, 2H), 1.20 (t, *J*=7.0, 6H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -111.32 - -111.67 (m) ;

Tert-butyldiphenyl(prop-2-yn-1-yloxy)silane (4.56)

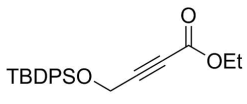


The synthesis was performed according to literature proccedure.[19]

TBDPSCl (9.15 ml, 35.2 mmol) and imidazole (2.39 g, 35.2 mmol) were dissolved in dry DCM (30 ml) and cooled in a water bath. Propargyl alcohol (1.85 ml, 32.0 mmol) was dissolved in dry DCM (10 ml) and slowly added. An exothermic reaction was observed. After 5.5 hours, diethyl ether was added and washed twice with brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 99:1, R_f 0.6). This procedure afforded pure silane **4.56** (9.29 g, 31.5 mmol, 98 %) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.75 - 7.70 (m, 4H), 7.49 - 7.37 (m, 6H), 4.32 (d, *J*=2.1, 2H), 2.40 (t, *J*=2.3, 1H), 1.08 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 135.6 (4C), 132.9 (2C), 129.9 (2C), 127.8 (4C), 82.0, 73.0, 52.5, 26.7 (3C), 19.2.

Ethyl 4-((*tert*-butyldiphenylsilyl)oxy)but-2-ynoate (4.55)

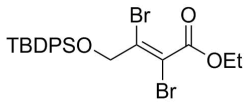


The synthesis was performed according to literature proccedure.[19]

Silane **4.56** (9.10 g, 30.9 mmol) was dissolved in dry THF (150 ml) and cooled to -78 °C. *n*BuLi (2.5M in hexanes, 13.6 ml, 34.0 mmol) was added dropwise. The reaction was stirred for 1 hour, then ethyl chloroformate (3.25 ml, 34.0 mmol) was added and the reaction allowed to warmed to room temperature. The reaction was stirred for further 2 hours, then quenched with sat. NH_4Cl. The aqueous phase was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/DCM 8:2 to 6:4, $R_{f8:2}$0.6). This procedure afforded pure ester **4.55** (9.21 g, 25.1 mmol, 81%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.69 (d, *J*=7.9, 4H), 7.49 - 7.36 (m, 6H), 4.41 (s, 2H), 4.23 (q, *J*=7.2, 2H), 1.32 (t, *J*=7.1, 3H), 1.06 (s, 9H) ; **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 153.4, 135.6 (4C), 132.4 (2C), 130.0 (2C), 127.9 (4C), 85.3, 62.0, 52.3, 26.6 (3C), 19.2, 14.0.

Ethyl (*E*)-2,3-dibromo-4-((*tert*-butyldiphenylsilyl)oxy)but-2-enoate (4.54)

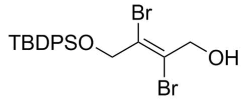


Ester **4.55** (3.0 g, 8.18 mmol) was dissolved in MeOH (16 ml) and cooled to 0 °C. Bromine (0.46 ml, 9.00 mmol) was added dropwise. After 5 hours, diethyl ether was added and the mixture washed with sat. aq. Na_2SO_3 and twice with brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 98:2, R_f 0.26). This procedure afforded pure ester **4.54** (2.07 g, 3.93 mmol, 48%) as a clear oil.

Note: The reaction could be performed with up to 60% yield at smaller scale (0.81 mmol). Upscaling the reaction further led to deterioration of yield.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.74 - 7.68 (m, 4H), 7.49 - 7.38 (m, 6H), 4.52 (s, 2H), 4.33 (q, *J*=7.1, 2H), 1.37 (t, *J*=7.1, 3H), 1.09 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 163.5, 135.8 (4C), 132.7 (2C), 130.1 (2C), 128.0(4C), 126.3, 107.1, 66.3, 62.8, 26.8 (3C), 19.5, 14.0; **IR** (cm^{-1}): 3071, 2931, 2858, 2363, 1736, 1589, 1472, 1428, 1390, 1365, 1268, 1239, 1113, 1075, 1030, 823, 740, 702, 615; *HRMS could not be obtained for this compound with the available methods.*

(*E*)-2,3-Dibromo-4-((*tert*-butyldiphenylsilyl)oxy)but-2-en-1-ol (4.57)

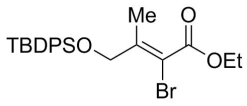


Ester **4.54** (50 mg, 94.9 μmol) was dissolved in dry DCM (0.4 ml) and cooled in an ice bath. DIBAL-H (1M in hexanes, 0.208 ml, 0.208 mmol) was added dropwise. After 1 hour, sat. Rochelle salt solution was added and the mixture stirred vigorously overnight. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 8:2, R_f 0.26). This procedure afforded pure alcohol **4.57** (43 mg, 88.7 μmol, 93%) as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.78 - 7.67 (m, 5H), 7.52 - 7.37 (m, 6H), 4.56 (s, 2H), 4.50 (d, *J*=7.0, 2H), 1.09 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 135.8 (4C), 133.0 (2C), 130.0 (2C), 127.9 (4C), 123.1, 121.4, 67.6, 67.4, 26.9 (3C), 19.5; **IR** (cm^{-1}): 3343, 3070, 2930, 2857, 2359, 1589, 1471, 1427, 1362, 1112, 1006, 939, 823, 740, 700, 613; **HRMS** (APCI): *m/z*: 516.9600 $[M+Cl]^-$ (calcd.: *m/z*: 516.9606 $[M+Cl]^-$).

The absence of any NOE between the methylene groups confirms the (E)-stereochemistry of the double bond.

Ethyl (*Z*)-2-bromo-4-((*tert*-butyldiphenylsilyl)oxy)-3-methylbut-2-enoate (4.53)

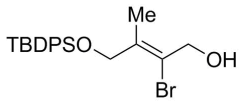


A 0.5M stock solution of $(MeCu)_n$ in dry diethyl ether was prepared: CuI (1.42 g, 7.5 mmol) was suspended in dry diethyl ether (10.32 ml) and cooled in an ice bath. MeLi solution (1.6M in diethyl ether, 4.68 ml, 7.5 mmol) was added dropwise. The yellow suspension was stirred for 15 minutes.

Ester **4.54** (2.20 g, 4.19 mmol) was dissolved in dry diethyl ether (8.4 ml) and $(MeCu)_n$ solution (12.6 ml, 6.28 mmol) was slowly added. The suspension was stirred for 15 minutes after complete addition, then filtered over celite with diethyl ether. The solvent was evaporated under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 98:2, R_f 0.36). This procedure afforded pure ester **4.53** (1.14 g, 2.47 mmol, 59%) as a clear oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.69 - 7.64 (m, 4H), 7.47 - 7.37 (m, 6H), 4.42 (s, 2H), 4.27 (q, *J*=7.1, 2H), 2.24 (s, 2H), 1.34 (t, *J*=7.1, 3H), 1.08 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 163.8, 150.0, 135.5 (4C), 133.0 (2C), 129.9 (2C), 127.8 (4C), 106.6, 67.9, 62.0, 26.8 (3C), 19.3, 18.1, 14.1; **IR** (cm^{-1}): 2931, 2857, 1715, 1589, 1472, 1427, 1364, 1243, 1218, 1112, 1061, 823, 740, 701, 623; **HRMS** (EI): *m/z*: 403.0294 $[M - C_4H_9]^+$ (calcd.: *m/z*: 403.0360 $[M - C_4H_9]^+$).

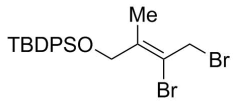
(*Z*)-2-Bromo-4-((*tert*-butyldiphenylsilyl)oxy)-3-methylbut-2-en-1-ol (4.58)



Ester **4.53** (1.14 g, 2.47 mmol) was dissolved dry DCM (5.2 ml) and cooled in an ice bath. DIBAL-H (1M in hexanes, 5.2 ml, 5.2 mmol) was added slowly. After 1 hour, sat. Rochelle salt solution was added and stirred vigorously overnight. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 2:1, R_f 0.19). This procedure afforded pure alcohol **4.58** (0.888 g, 2.11 mmol, 85 %) as a yellowish oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.70 - 7.66 (m, 4H), 7.48 - 7.36 (m, 7H), 4.38 (s, 2H), 4.35 (d, *J*=6.6, 2H), 1.98 (s, 3H), 1.07 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 137.4, 135.7 (4C), 133.4 (2C), 129.9 (2C), 127.8 (4C), 120.0, 67.9, 64.8, 27.(3C)0, 19.4, 16.2; **IR** (cm^{-1}): 3343, 3070, 2932, 2857, 1467, 1427, 1385, 1189, 1111, 1075, 1005, 822, 740, 702, 615; **HRMS** (APCI): *m/z*: 441.0853 $[M+Na]^+$ (calcd.: *m/z*: 441.0861 $[M+Na]^+$).

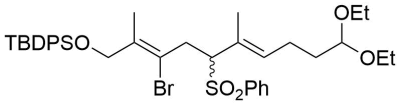
(*Z*)-*Tert*-butyl((3,4-dibromo-2-methylbut-2-en-1-yl)oxy) diphenylsilane (4.59)



Alcohol **4.58** (0.88 g, 2.11 mmol) was dissolved in dry THF (3 ml) and cooled in an ice bath. PBr_3 (98 μl, 1.05 mmol) was added dropwise. The reaction was stirred at 0°C for 3 hours, then ice cold water was added. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 98:2, R_f 0.63). This procedure afforded pure silane **4.59** (0.809 g, 1.67 mmol, 79 %) as yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.73 - 7.61 (m, 4H), 7.48 - 7.34 (m, 6H), 4.35 (s, 2H), 4.33 (s, 2H), 1.97 (s, 3H), 1.07 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 141.0, 135.6 (4C), 133.3 (2C), 129.9 (2C), 127.9 (4C), 114.6, 67.9, 36.2, 26.9 (3C), 19.4, 16.1; **IR** (cm^{-1}): 2930, 2856, 1589, 1471, 1427, 1377, 1205, 1112, 1077, 1006, 938, 907, 823, 739, 701, 606; **HRMS** (APCI): *m/z*: 503.0005 $[M + Na]^+$ (calcd.: *m/z*: 503.0017 $[M + Na]^+$).

(((2*Z*,6*E*)-3-Bromo-10,10-diethoxy-2,6-dimethyl-5-(phenylsulfonyl) deca-2,6-dien-1-yl)oxy)(*tert*-butyl)diphenylsilane (4.60)

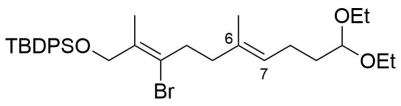


Sulfone **4.35** (0.495 g, 1.51 mmol) was dissolved in dry THF (3.5 ml) and dry DMF (1.1 ml). The solution was cooled to -78 °C and *t*BuOK (1M in THF, 1.67 ml, 1.67 mmol) was added dropwise. The reaction was stirred for 2 hours, then a solution of bromide **4.59** (0.809 g, 1.67 mmol) in dry THF (5.5 ml) was added dropwise. After 2 hours, the reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched with sat. NH_4Cl and diethyl ether. The aqueous phase was washed two times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 2:1, R_f 0.26). This procedure afforded pure sulfone **4.60** (0.867 g, 1.19 mmol, 79%) as a yellowish oil.

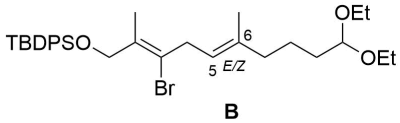
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.86 - 7.81 (m, 2H), 7.66 - 7.61 (m, 5H), 7.56 - 7.49 (m, 2H), 7.44 - 7.33 (m, 6H), 5.25 - 5.19 (m, 1H), 4.33 - 4.26 (m, 3H), 3.95 (dd, *J*=10.7, 3.9, 1H), 3.61 - 3.52 (m, 2H), 3.45 - 3.36 (m, 2H), 3.25 (dd, *J*=14.7, 10.7, 1H), 3.05 (ddd, *J*=14.5, 4.1, 1.5, 1H), 2.02 - 1.93 (m, 2H), 1.90 (s, 3H), 1.67 (s, 3H), 1.92 - 1.89 (m, 3H), 1.16 (t, *J*=7.0, 6H), 1.04 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 138.0, 136.9, 135.7, 135.5, 133.5 (4C), 133.4, 133.3, 129.7 (2C), 128.8 (2C), 128.8

(2C), 127.7 (4C), 126.1, 116.0, 102.1, 72.3, 68.0, 61.2, 61.1, 33.6, 32.6, 26.8 (3C), 23.5, 22.3, 19.3, 16.6, 15.4 (2C), 14.5, 14.1; **IR** (cm^{-1}): 2930, 2857, 2358, 1446, 1428, 1375, 1306, 1147, 1113, 1065, 824, 742, 703, 689. *HRMS could not be obtained for this compound with the available methods.*

(((2*Z*,6*E*)-3-Bromo-10,10-diethoxy-2,6-dimethyldeca-2,6-dien-1-yl)oxy)(*tert*-butyl)diphenylsilane (4.61A) and (((2*Z*,5*E*)-3-bromo-10,10-diethoxy-2,6-dimethyldeca-2,5-dien-1-yl)oxy)(*tert*-butyl)diphenylsilane (4.61B)



A



B

Magnesium turnings (0.984 g, 40.5 mmol) were suspended in dry THF (2.9 ml) and a small crystal of iodine was added. After 15 minutes, the reaction was placed in a cold water bath and a solution of sulfone **4.60** (0.982 g, 1.35 mmol) in dry THF (2.9 ml) and dry MeOH (11.5 ml) was slowly added. Heavy gas evolution indicated a successful start of the reaction. After 1 hour, the liquid was decanted into sat. NH_4Cl and the remaining solids washed with MeOH. The aqueous mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column

chromatography (pentane/diethyl ether 9:1, R_f 0.43). This procedure afforded pure acetal **4.61A** and **4.61B** in an inseparable 1:1 mixture (0.62 g, 1.05 mmol, 78%) as a clear oil.

Analytical data for **4.61A**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.71 - 7.65 (m, 4H), 7.48 - 7.35 (m, 4H), 5.19 - 5.14 (m, 1H), 4.52 - 4.44 (m, 1H), 4.36 (s, 2H), 3.69 - 3.58 (m, 2H), 3.54 - 3.43 (m, 2H), 2.59 - 2.52 (m, 2H), 2.19 - 2.12 (m, 2H), 2.11 - 2.01 (m, 2H), 1.86 (s, 3H), 1.68 - 1.56 (m, 5H), 1.24 - 1.16 (m, 6H), 1.06 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 135.6 (4C), 134.3, 133.6(2C), 133.2, 129.6 (2C), 127.7 (4C), 124.6, 121.1, 102.4, 68.0, 60.9 (2C), 37.8, 37.0, 33.5, 26.9 (3C), 23.3, 19.3, 16.1, 15.9, 15.4 (2C); **IR** (cm^{-1}): 2930, 2857, 1428, 1376, 1112, 1063, 1007, 824, 739, 701, 614; **HRMS** (APCI): *m/z*: 609.2365 $[M + Na]^+$ (calcd.: *m/z*: 609.2375 $[M + Na]^+$).

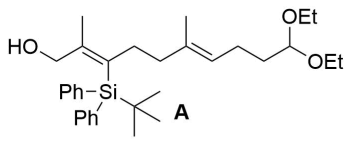
Analytical data for **4.61B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.71 - 7.65 (m, 4H), 7.48 - 7.35 (m, 6H), 5.12 - 5.08 (m, 1H), 4.52 - 4.44 (m, 1H), 4.36 (s, 2H), 3.69 - 3.58 (m, 2H), 3.54 - 3.43 (m, 2H), 3.22 (d, *J*=6.9, 2H), 2.11 - 2.00 (m, 2H), 1.89 (s, 3H), 1.68 - 1.56 (m, 5H), 1.52 - 1.45 (m, 2H), 1.24 - 1.16 (m, 6H), 1.06 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 137.0, 135.6 (4C), 134.3, 133.6 (2C), 129.6 (2C), 127.7 (4C), 121.1, 120.2, 102.8, 68.1, 60.9 (2C), 39.4, 36.7, 33.5, 26.9 (3C), 22.9, 19.3, 16.2, 15.9, 15.4 (2C).

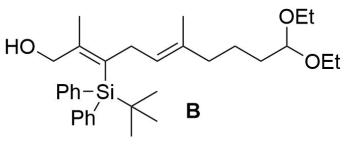
Identification of retro-Brook rearrangement products

(2*Z*,6*E*)-3-(*tert*-butyldiphenylsilyl)-10,10-diethoxy-2,6-dimethyldeca-2,6-dien-1-ol (4.62A) and

(2*Z*,5*E*)-3-(*tert*-butyldiphenylsilyl)-10,10-diethoxy-2,6-dimethyldeca -2,5-dien-1-ol (4.62B)



A



B

Acetal **4.61** (20 mg, 34 μmol) was dissolved in dry THF (0.1 ml) and cooled to -78 °C. *t*BuLi (1.9M in pentane, 38 μl, 71 μmol) was added dropwise. After 25 minutes, *i*PrOBpin (21 μl, 102 μmol) was added and the mixture warmed to room temperature. Water was added and extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 8:2, R_f 0.3). This procedure afforded acetal **4.62A** and **4.62B** in an inseparable 1:1 mixture (13 mg, 25.5 μmol, 75%) as a clear oil.

Analytical data for **4.62A**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.66 (td, *J*=4.2, 1.8, 2H), 7.64 - 7.59 (m, 2H), 7.40 - 7.31 (m, 6H), 4.86 (t, *J*=7.2, 1H), 4.44 (t, *J*=5.8, 1H), 3.72 (s, 2H), 3.70 - 3.57 (m, 4H), 3.55 – 3.42 (m, 4H), 2.46 - 2.37 (m, 2H), 2.02 - 1.94 (m, 5H), 1.85 - 1.78 (m, 2H),

1.64 - 1.53 (m, 2H), 1.46 (s, 3H), 1.24 - 1.17 (m, 6H), 1.14 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 144.6, 137.5, 135.9 (2C), 135.8 (2C), 133.9, 129.1 (2C), 127.8 (4C), 123.4, 102.7, 61.0 (2C), 39.4, 39.3, 32.3, 32.1, 28.9 (3C), 19.3, 16.6, 15.7, 15.4 (2C).

Analytical data for **4.62B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 7.66 (td, *J*=4.2, 1.8, 2H), 7.64 - 7.59 (m, 2H), 7.40 - 7.31 (m, 6H), 5.03 - 4.95 (m, 1H), 4.51 - 4.46 (m, 1H), 3.72 (s, 2H), 3.70 - 3.57 (m, 4H), 3.55 - 3.42 (m, 4H), 3.10 (d, *J*=5.9, 2H), 2.02 - 1.94 (m, 7H), 1.64 - 1.53 (m, 4H), 1.46 (s, 3H), 1.24 - 1.17 (m, 6H), 1.08 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 144.6, 135.9 (2C), 135.8 (2C), 127.8 (2C), 137.5, 133.9, 129.1 (2C), 123.9, 103.1, 68.4, 61.0 (2C), 39.3, 33.1, 31.2, 28.9 (3C), 23.4, 19.3, 15.7, 15.4 (2C).

(((2*Z*,6*E*)-3-Bromo-10,10-diethoxy-2,6-dimethyldeca-2,6-dien-1-ol (4.63A) and (((2*Z*,5*E*)-3-bromo-10,10-diethoxy-2,6-dimethyldeca-2,5-dien-1-ol (4.63B)

Silane **4.61** (0.3 g, 0.510 mmol) was dissolved in dry THF (5.1 ml) and cooled in an ice bath. TBAF (1M in THF, 1.02 ml, 1.02 mmol) was added and the reaction stirred at

room temperature for 3 hours. The reaction was diluted with EtOAc and the organic phase washed with water and brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by columnn chromatography (pentane/diethyl ether 6:4, R_f 0.2). This procedure afforded pure acetal **4.63A** and **4.63B** in an inseparable 1:1 mixture (0.175 g, 0.501 mmol, 98%) as a clear oil.

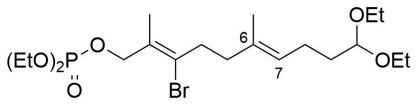
Analytical data for **4.63A**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.22 - 5.10 (m, 1H), 4.49 (t, *J*=5.6, 1H), 4.27 (d, *J*=3.3, 2H), 3.69 - 3.57 (m, 2H), 3.57 - 3.44 (m, 2H), 2.60 (t, *J*=7.4, 2H), 2.23 (t, *J*=7.4, 2H), 2.11 - 2.00 (m, 2H), 1.83 (s, 3H), 1.65 - 1.57 (m, 5H), 1.20 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 133.8, 125.1, 123.5, 120.1, 102.8, 67.6, 67.6, 60.5 (2C), 37.5, 36.2, 33.2, 23.2, 16.7, 16.0, 15.3 (2C); **HRMS** (APCI): *m/z*: 371.1190 $[M + Na]^+$ (calcd.: *m/z*: 371.1197 $[M + Na]^+$).

Analytical data for **4.63B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.23 - 5.10 (m, 1H), 4.48 (t, *J*=5.8, 1H), 4.25 (d, *J*=3.5, 2H), 3.70 - 3.58 (m, 2H), 3.54 - 3.44 (m, 2H), 3.24 (t, *J*=5.6, 2H), 2.12 - 2.00 (m, 2H), 1.92 - 1.86 (m, 3H), 1.67 - 1.56 (m, 5H), 1.54 - 1.44 (m, 2H), 1.20 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 137.4, 123.5, 120.6, 120.1, 101.9, 67.6, 60.5 (2C), 39.4, 36.7, 33.2, 23.0, 16.9, 16.0, 15.3 (2C).

(2Z,6E)-3-Bromo-10,10-diethoxy-2,6-dimethyldeca-2,6-dien-1-yl diethyl phosphate (4.64A) and (((2*Z*,5*E*)-3-bromo-10,10-diethoxy-2,6-dimethyldeca-2,5-dien-1-yl diethyl phosphate (4.64B)



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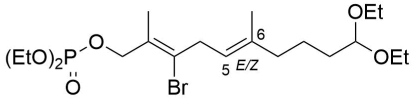


B

Alcohol **4.63** (150 mg, 0.429 mmol) was dissolved in dry DCM (1.24 ml) and cooled in an ice bath. Pyridine (59 μl, 0.73 mmol) and diethyl chlorophosphate (86 μl, 0.601 mmol) were added. The reaction was stirred at 0°C for 6 hours, then diluted with diethyl ether. The organic phase was washed twice with water, sat. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/EtOAc 6:1 with 1% TEA, R_f 0.22). This procedure afforded pure acetal **4.64A** and **4.64B** in an inseparable 1:1 mixture (154 mg, 317 μmol, 74 %) as a clear oil.

Analytical data for **4.64A**:

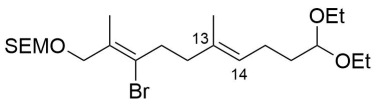
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.16 (t, *J*=6.5, 1H), 4.69 (d, *J*=6.7, 2H), 4.50 - 4.44 (m, 1H), 4.13 (dt, *J*=7.3, 4H), 3.68 - 3.57 (m, 2H), 3.54 - 3.43 (m, 2H), 2.64 - 2.55 (m, 2H), 2.24 - 2.14 (m, 2H), 2.11 - 2.00 (m, 2H), 1.82 (s, 3H), 1.67 - 1.56 (m, 5H), 1.34 (t, *J*=7.1, 6H), 1.20 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 133.9, 129.3

(d, J_{CP}=8.1), 125.4, 124.9, 102.4, 71.3 (d, J_{CP}=5.2), 71.2, 63.8 (d, J_{CP}=5.8, 2C), 60.9 (2C), 37.7, 37.0, 33.4, 23.3, 16.2, 16.1, 16.1, 15.4 (2C); **^{31}P-NMR** (162 MHz, $CDCl_3$, δ/ppm): -0.97 (hept, *J*=7.5); **IR** (cm^{-1}): 2975, 2930, 1649, 1444, 1372, 1267, 1165, 1127, 1099, 1010, 978, 867, 818, 750; **HRMS** (APCI): *m/z*: 507.1480 $[M+Na]^+$ (calcd.: *m/z*: 507.1487 $[M+Na]^+$).

Analytical data for **4.64B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.10 (t, *J*=6.3, 1H), 4.69 (d, *J*=6.7, 1H), 4.50 - 4.44 (m, 1H), 4.13 (dt, *J*=7.3, 4H), 3.68 - 3.57 (m, 2H), 3.54 - 3.43 (m, 2H), 3.25 (d, *J*=6.9, 2H), 2.11 - 2.00 (m, 2H), 1.82 (s, 3H), 1.67 - 1.56 (m, 5H), 1.52 - 1.43 (m, 2H), 1.34 (t, *J*=7.1, 6H), 1.20 (t, *J*=7.1, 6H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 137.8, 128.8 (d, J_{CP}=8.0), 125.4, 119.6, 102.8,71.2 (d, J_{CP}=5.1),63.8 (d, J_{CP}=5.8, 2C), 60.9 (2C), 39.4, 36.8, 33.1, 23.3, 16.2, 16.1, 16.1, 15.4 (2C).

(9Z,13E)-10-Bromo-17-ethoxy-2,2,9,13-tetramethyl-5,7,18-trioxa-2-silaicosa-9,13-diene (4.65A) and (9*Z*,12*E*)-10-bromo-17-ethoxy-2,2,9,13-tetramethyl-5,7,18-trioxa-2-silaicosa-9,12-diene (4.65B)



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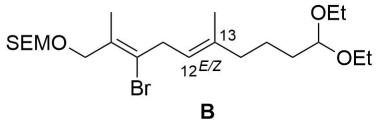


B

Alcohol **4.63** (0.14 g, 0.4 mmol) was dissolved in dry DCM (4 ml) and cooled in an ice bath. TBAI (0.162 g, 0.440 mmol), lutidine (93 μl, 0.8 mmol) and SEMCl (106 μl, 0.6 mmol) were subsequently added. The reaction was stirred at room temperature overnight, then quenched with sat. NH_4Cl. The aqueous phase was extracted three times with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified immediately by column chromatography (pentane/diethyl ether 9:1, R_f 0.25). This procedure afforded pure acetal **4.65A** and **4.65B** in an inseparable 1:1 mixture (0.166 g, 0.346 mmol, 86 %) as a clear oil.

Analytical data for **4.65A**:

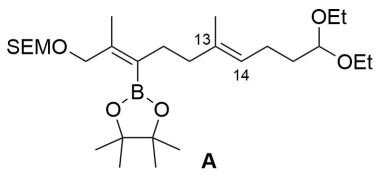
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.19 - 5.11 (m, 1H), 4.68 (s, 1H), 4.52 - 4.44 (m, 1H), 4.22 (s, 2H), 3.69 - 3.58 (m, 4H), 3.55 - 3.44 (m, 2H), 2.64 - 2.55 (m, 2H), 2.25 - 2.16 (m, 2H), 2.13 - 1.99 (m, 2H), 1.80 (s, 3H), 1.69 - 1.56 (m, 5H), 1.20 (t, *J*=7.1, 6H), 1.00

- 0.93 (m, 2H), 0.02 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 134.2, 130.9, 124.9, 120.8, 102.5, 94.5, 71.9, 65.3, 61.0 (2C), 37.9, 37.2, 33.6, 23.4, 18.3, 16.9, 16.3, 15.5 (2C), -1.3 (3C); **IR** (cm^{-1}): 2951, 1444, 1375, 1248, 1102, 1057, 937, 859, 835, 693; **HRMS** (APCI): *m/z*: 392.1617 $[M - C_2H_6O]^+$ (calcd.: *m/z*: 392.1615 $[M - C_2H_6O]^+$).

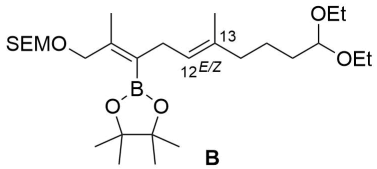
Analytical data for **4.65B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.19 - 5.11 (m, 1H), 4.68 (s, 1H), 4.52 - 4.44 (m, 1H), 4.22 (s, 2H), 3.69 - 3.58 (m, 4H), 3.55 - 3.44 (m, 2H), 3.26 (d, *J*=6.9, 2H), 2.11 - 2.00 (m, 2H), 1.84 (s, 2H), 1.68 - 1.55 (m, 5H), 1.53 - 1.42 (m, 2H), 1.20 (t, *J*=7.1, 6H), 1.00 - 0.93 (m, 2H), 0.02 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 137.4, 134.2, 120.8, 120.3, 102.9, 94.4, 71.9, 65.3, 61.0 (2C), 39.5, 36.9, 33.2, 23.0, 18.3, 16.8, 16.3, 15.5 (2C), -1.3 (3C).

(9Z,13E)-17-Ethoxy-2,2,9,13-tetramethyl-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,7,18-trioxa-2-silaicosa-9,13-diene (4.66A) and (9*Z*,12*E*)-17-ethoxy-2,2,9,13-tetramethyl-10-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,7,18-trioxa-2-silaicosa-9,12-diene (4.66B)



A



B

Bromide **4.65** (35 mg, 73.0 μmol) was dissolved in dry THF (220 μl) and cooled to -78°C. *t*BuLi (1.7M in pentane, 90 μl, 153 μmol) was added dropwise. After 30 minutes, *i*PrOBpin (45 μl, 219 μmol) was added. The reaction was stirred for further 5 minutes, then quenched with sat. NH_4Cl. The mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (pentane/diethyl ether 9:1, R_f 0.15). This procedure afforded pure acetal **4.66A** and **4.66B** in an inseparable 1:1 mixture (30 mg, 57 μmol, 78 %) as a clear oil.

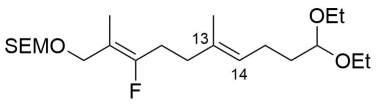
Analytical data for **4.66A**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.16 - 5.09 (m, 1H), 4.67 (s, 1H), 4.51 - 4.43 (m, 1H), 4.22 (s, 2H), 3.69 - 3.57 (m, 4H), 3.54 - 3.43 (m, 2H), 2.25 - 2.19 (m, 2H), 2.13 - 2.02 (m, 2H), 1.96 (t, *J*=8.0, 2H), 1.77 (s, 3H), 1.69 - 1.58 (m, 5H), 1.26 (s, 6H), 1.24 (s, 6H), 1.22 - 1.17 (m, 6H), 0.98 - 0.91 (m, 2H), 0.01 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 136.1, 134.7, 123.5, 102.6, 83.1 (2C), 71.7, 65.1, 61.0 (2C), 39.6, 33.7, 30.4, 24.9 (4C), 23.4, 18.2, 16.3, 16.1, 15.5 (2C), -1.3 (3C); **IR** (cm^{-1}): 2975, 1630, 1445, 1373, 1348, 1300, 1249, 1145, 1101, 1057, 965, 936, 857, 835, 694; **HRMS** (ESI): *m/z*: 548.3787 $[M+Na]^+$ (calcd.: *m/z*: 548.3789 $[M+Na]^+$).

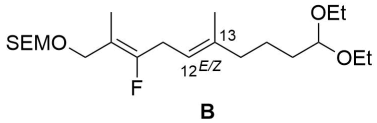
Analytical data for **4.66B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.07 - 5.00 (m, 1H), 4.67 (s, 1H), 4.51 - 4.43 (m, 1H), 4.22 (s, 2H), 3.69 - 3.57 (m, 4H), 3.54 - 3.43 (m, 2H), 2.86 (d, *J*=7.1, 2H), 2.13 - 2.02 (m, 2H), 1.77 (s, 3H), 1.69 - 1.55 (m, 5H), 1.50 - 1.40 (m, 2H), 1.26 (s, 6H), 1.24 (s, 6H), 1.22 - 1.17 (m, 6H), 0.98 - 0.91 (m, 2H), 0.01 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 144.7, 136.1, 123.1, 103.0, 94.4, 83.1 (2C), 71.7, 65.1, 61.0 (2C), 33.7, 33.3, 30.0, 25.0 (4C), 23.2, 18.2, 16.7, 16.1, 15.5 (2C), -1.3 (3C).

(9*Z*,13*E*)-17-ethoxy-10-fluoro-2,2,9,13-tetramethyl-5,7,18-trioxa-2-silaicosa-9,13-diene (4.67A) and (9*Z*,12*E/Z*)-17-ethoxy-10-fluoro-2,2,9,13-tetramethyl-5,7,18-trioxa-2-silaicosa-9,12-diene (4.67B)



A



B

Alcohol **4.49** (30 mg, 104 μmol) was dissolved in dry DCM (1 ml) and cooled in an ice bath. DIPEA (36 μl, 206 μmol) and SEMCl (27 μl, 152 μmol) were added. The reaction was stirred overnight at room temperature, then quenched with sat. NH_4Cl. The aqueous phase was extracted three times with DCM. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (pentane/diethyl ether 9:1, R_f 0.27). This procedure afforded pure acetal **4.67A** and **4.67B** in an inseparable 1:1 mixture (40 mg, 95.5 μmol, 92%) as a clear oil.

Analytical data for **4.67A**:

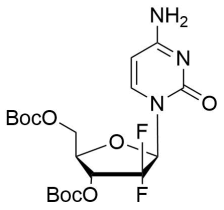
^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.21 - 5.11 (m, 1H), 4.65 (d, *J*=3.7, 2H), 4.52 - 4.42 (m, 1H), 4.11 (d, *J*=3.0, 2H), 3.69 - 3.57 (m, 4H), 3.55 - 3.43 (m, 2H), 2.32 (dt, *J*=23.1, 7.8, 2H), 2.16 (t, *J*=7.7, 2H), 2.10 - 1.98 (m, 2H), 1.68 - 1.60 (m, 8H), 1.19 (t, *J*=7.1, 6H), 0.99 - 0.91 (m, 2H), 0.01 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 157.2 (d,

J_{CF}=249.9), 134.4, 118.4, 108.8 (d, J_{CF}=14.3), 102.5, 94.1, 65.2, 64.0 (d, J_{CF}=11.0), 61.0 (2C), 36.3, 33.6, 33.5, 27.9 (d, J_{CF}=28.7), 23.4, 18.2, 15.5 (2C), 13.9 (d, J_{CF}=5.0), -1.3 (3C); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -108.80 (tq, *J*=23.2, 3.0); **IR** (cm^{-1}): 2951, 1708, 1445, 1375, 1248, 1101, 1055, 937, 858, 835, 757, 693; **HRMS** (APCI): *m/z*: 441.2803 $[M+Na]^+$ (calcd.: *m/z*: 441.2812 $[M+Na]^+$).

Analytical data for **4.67B**:

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 5.21 - 5.11 (m, 1H), 4.65 (d, *J*=3.7, 2H), 4.52 - 4.42 (m, 1H), 4.11 (d, *J*=3.0, 2H), 3.69 - 3.57 (m, 4H), 3.55 - 3.43 (m, 2H), 2.95 (dd, *J*=23.6, 7.1, 2H), 2.10 - 1.98 (m, 2H), 1.68 - 1.60 (m, 6H), 1.60 - 1.52 (m, 2H), 1.51 - 1.41 (m, 2H), 1.19 (t, *J*=7.1, 6H), 0.99 - 0.91 (m, 2H), 0.01 (s, 9H); **^{13}C-NMR** (101 MHz, $CDCl_3$, δ/ppm): 156.5 (d, J_{CF}=250.9), 137.8, 124.7, 108.4 (d, J_{CF}=14.2), 102.9, 94.2, 65.2, 64.2, 64.1, 61.0 (2C), 39.4, 33.2, 28.0 (d, J_{CF}=29.1), 22.9, 18.2, 16.1 (2C), 15.5,13.9 (d, J_{CF}=5.0), -1.3 (3C); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -107.29 - -107.65 (m).

3',5'-*O*-Bis(*tert*-butoxycarbonyl)gemcitabine (4.70)

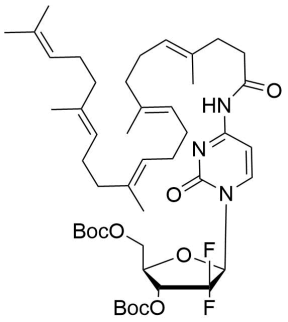


The synthesis was performed according to a literature procedure.[20]

Gemcitabine hydrochloride (0.3 g, 1 mmol) was dissolved in 20 ml of 1M aq. KOH and dioxane (20 ml). Di-*tert*-butyl dicarbonate (2.29 ml, 10 mmol) was added dropwise. After 30 minutes, the reaction mixture was extracted three times with EtOAc. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. The remains were dissolved in dioxane (20 ml) and di-*tert*-butyl dicarbonate (2.29 ml, 10 mmol) was added followed by 20 ml 1M KOH. The reaction mixture was stirred for 30 minutes, then extracted three times with EtOAc. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure. The product was purified by column chromatography (DCM/acetone 1:1, R_f 0.33). This procedure afforded pure ester **4.70** (0.194 g, 0.418 mmol, 41%) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm):7.50 - 7.44 (m, 1H), 6.45 - 6.36 (m, 1H), 5.79 (d, *J*=7.6, 1H), 5.15 - 5.04 (m, 1H), 4.48 - 4.26 (m, 3H), 1.51 (s, 9H), 1.49 (s, 9H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -115.28 - -115.57 (m), -115.95 - -116.15 (m);

4-*N*-Squalenoyl-3',5'-*O*-bis(*tert*-butoxycarbonyl)gemcitabine (4.69)

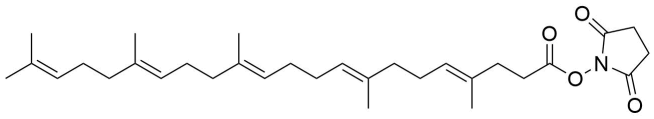


Ester **4.70** (100 mg, 215 μmol) was dissolved in a mixture of DMF and DMSO (3:1, 4 ml). Subsequently, *N*-methylmorpholine (26 μl, 237 μmol), HOBt hydrate (32 mg, 237 μmol), acid **2.34** (95 mg, 237 μmol) and EDC hydrochloride (54 mg, 280 μmol) were added. The reaction mixture was heated to 65 °C and stirred for 16 hours. After cooling to room temperature, brine was added. The aqueous phase was extracted three times with EtOAc. The combined organic phases were dried over Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by column chromatography (DCM/MeOH 96:4, R_f 0.48). This procedure afforded pure amide **4.69** (69 mg, 81 μmol, 38%) as a waxy solid.

^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): 8.52 (s, 1H), 7.83 (d, *J*=7.7, 1H), 7.47 (d, *J*=7.6, 1H), 6.44 (dd, *J*=10.2, 6.6, 1H), 5.25 - 5.05 (m, 5H), 4.52 - 4.32 (m, 3H), 2.57 - 2.49 (m, 2H), 2.35 (t, *J*=7.7, 2H), 2.11 - 1.92 (m, 16H), 1.67 (s 3H), 1.64 (s, 3H), 1.63 - 1.61 (m, 3H), 1.59 (s, 9H), 1.51 (s, 9H), 1.50 (s, 9H); **^{19}F-NMR** (377 MHz, $CDCl_3$, δ/ppm): -115.36 (t, *J*=11.6), -116.01 (t, *J*=11.6); **^{13}C-NMR** (126 MHz, $CDCl_3$, δ/ppm): 173.6,

163.0, 155.7, 155.1, 153.1, 145.5, 135.3, 135.1, 135.1, 135.0, 134.0, 132.7, 126.1, 126.0, 125.9, 125.9, 125.0, 125.0, 124.6, 124.5, 124.5, 124.4, 124.4, 124.3, 122.4, 119.7 (d, J_{CF}=165.4), 107.2 (d, J_{CF}=18.0), 97.6, 81.7, 69.2 (t, J_{CF}=22.8), 39.7, 39.6, 36.7, 36.6, 34.5, 28.4, 28.4, 27.9, 27.7, 26.9, 26.8, 17.7, 17.7, 16.1, 16.1, 16.0, 16.0, 15.6, 15.6; **IR** (cm^{-1}): 2979, 2920, 2360, 1750, 1680, 1490, 1278, 1253, 1158, 1136, 1104, 856, 788; *HRMS could not be obtained for this compound with the available methods.*

2,5-Dioxopyrrolidin-1-yl (4*E*,8*E*,12*E*,16*E*)-4,8,13,17,21-pentamethyldocosa-4,8,12,16,20-pentaenoate (3.35)



Acid **2.34** (50 mg, 125 μmol) and *N*-hydroxysuccinimide (17.2 mg, 150 μmol) were dissolved in dry THF (1.2 ml). The mixture was cooled in an ice bath and DCC (26.7 mg, 125 μmol) was added. The reaction was allowed to warm to room temperature and stirred for 16 hours. The precipitate was filtered off and washed with diethyl ether. The solvent was removed under reduced pressure. The product was purified by column chromatography (PET 40-60/diethyl ether 1:1, R_f 0.24). This procedure afforded pure ester **3.35** (55 mg, 110 μmol, 88%) as a clear oil.

The analytical data were in accordance with the literature.[21] **^{1}H-NMR** (400 MHz, $CDCl_3$,

δ/ppm): 5.24 - 5.18 (m, 1H), 5.17 - 5.05 (m, 4H), 2.83 (s, 4H), 2.73 - 2.66 (m, 2H), 2.40 (t, *J*=7.7, 2H), 2.12 - 1.94 (m, 16H), 1.68 (s, 3H), 1.63 (s, 3H), 1.60 (s 12H); **HRMS** (CI): *m/z*: 515.3844 $[M + NH_4]^+$ (calcd. *m/z*: 515.3849 $[M + NH_4]^+$).

Literature

[1] H. A. Gennadios, V. Gonzalez, L. Di Costanzo, A. Li, F. Yu, D. J. Miller, R. K. Allemann, D. W. Christianson, *Biochemistry* **2009**, *48*, 6175–6183.

[2] D. E. Cane, G. Yang, Q. Xue, J. H. Shim, *Biochemistry* **1995**, *34*, 2471–2479.

[3] F. L. Wu, B. P. Ross, R. P. McGeary, *European J. Org. Chem.* **2010**, 1989–1998.

[4] Y. Suhara, Y. Hirota, K. Nakagawa, M. Kamao, N. Tsugawa, T. Okano, *Bioorganic Med. Chem.* **2008**, *16*, 3108–3117.

[5] G. R. Labadie, R. Viswanathan, C. D. Poulter, *J. Org. Chem.* **2007**, *72*, 9291–9297.

[6] B. M. Trost, M. R. Machacek, H. C. Tsui, *J. Am. Chem. Soc.* **2005**, *127*, 7014–7024.

[7] D. J. Miller, J. Gao, D. G. Truhlar, N. J. Young, V. Gonzalez, R. K. Allemann, *Org. Biomol. Chem.* **2008**, *6*, 2346.

[8] S. Borrelli, M. S. Christodoulou, I. Ficarra, A. Silvani, G. Cappelletti, D. Cartelli, G. Damia, F. Ricci, M. Zucchetti, F. Dosio, D. Passarella, *Eur. J. Med. Chem.* **2014**, *85*, 179–190.

[9] R. M. Coates, D. a. Ley, P. L. Cavendera, *J. Org. Chem.* **1978**, *43*, 4915–4922.

[10] C. F. Fávaro, R. A. Soldi, T. Ando, J. R. Aldrich, P. H. G. Zarbin, *Org. Lett.* **2013**, *15*, 1822–1825.

[11] M. Gatti, E. Drinkel, L. Wu, I. Pusterla, F. Gaggia, R. Dorta, *J. Am. Chem. Soc.* **2010**, *132*, 15179–15181.

[12] P. Couvreur, B. Stella, L. H. Reddy, H. Hillaireau, C. Dubernet, D. Desmaële, S. Lepêtre-Mouelhi, F. Rocco, N. Dereuddre-Bosquet, P. Clayette, V. Rosilio, V. Marsaud, J.-M. Renoir, L. Cattel, *Nano Lett.* **2006**, *6*, 2544–2548.

[13] C.-Z. Yao, Q.-Q. Li, M.-M. Wang, X.-S. Ning, Y.-B. Kang, *Chem. Commun.* **2015**, *51*, 7729–7732.

[14] D. Takayuki, J. Robertson, G. Stork, A. Yamashita, *Tetrahedron Lett.* **1994**, *35*, 1481–1484.

[15] F. Camps, A. Messeguer, F. J. Sánchez, *Tetrahedron* **1988**, *44*, 5161–5167.

[16] D. Schmidt, C. C. Malakar, U. Beifuss, *Org. Lett.* **2014**, *16*, 4862–4865.

[17] J. Mori, M. Iwashima, M. Takeuchi, H. Saito, *Chem. Pharm. Bull. (Tokyo).* **2006**, *54*, 391–6.

[18] P. Rabe, L. Barra, J. Rinkel, R. Riclea, C. A. Citron, T. A. Klapschinski, A. Janusko, J. S. Dickschat, *Angew. Chemie - Int. Ed.* **2015**, *54*, 13448–13451.

[19] I. Larrosa, M. I. Da Silva, P. M. Gómez, P. Hannen, E. Ko, S. R. Lenger, S. R. Linke, A. J. White, D. Wilton, A. G. Barrett, *J. Am. Chem. Soc.* **2006**, *128*, 14042–14043.

[20] Z. W. Guo, J. M. Gallo, *J. Org. Chem.* **1999**, *64*, 8319–8322.

[21] F. Dosio, L. Reddy, A. Ferrero, B. Stell, L. Cattel, P. Couvreur, *Bioconjug. Chem.* **2010**, *21*, 1349–1361.