TOWARDS THE SYNTHESIS OF FLUORO-THROMBOXANE A_2 ANALOGUES

THESIS

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Ian D Collier

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Chemistry Department

University of Stirling

ABSTRACT

Firstly, the biosynthesis and physiological role of thromboxanes and the potential for a thromboxane A_2 antagonist in cardiovascular therapy are discussed. Secondly, the importance of fluorine in drug design is outlined and the syntheses of a series of thromboxane A_2 structural analogues are reviewed. This is concluded by proposing how fluorine substitution should allow synthesis of a stable structural analogue of thromboxane A_2 which may have antagonistic properties.

Finally, the synthetic conversion of a carbohydrate precursor, levoglucosan, into a suitable precursor to 10-α-fluorothromboxane A_2 is discussed in detail.

ACKNOWLEDGEMENTS

I take this opportunity to express my gratitude to the many people who have helped me throughout the past four years.

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C O N T E N T S

CHAPTER ONE

INTRODUCTION

INTRODUCTION

The subject matter of this thesis deals with the synthetic approaches to a fluorinated analogue of a potent, biologically active compound, namely thromboxane A_2. In considering the synthesis of such a compound, a researcher is confronted with a wealth of diverse chemical literature. This encompasses the biological significance of the thromboxanes and prostaglandins and the synthetic methodology required for both the preparation of the parent compounds and for the introduction of fluorine.

With respect to the thromboxanes and prostaglandins[1,2] this diversity includes their isolation, characterisation and the subsequent elucidation of their biosynthesis and physiological role. Over and above this are the innumerable syntheses of the naturally-occurring compounds themselves, together with a myriad of structural analogues. Associated with these analogues are the exciting changes in biological profile that often result.

Overlaying these considerations is the important facet of fluorine chemistry whose literature contains a rapidly expanding number of synthetic methods for the introduction of fluorine into organic molecules.[3,4] This is complemented by the extensive range and quantity of biological molecules in which fluorine has been incorporated along with the profound changes in activity that occurs.[5-7]

In introducing the work embodied in this thesis only selective areas of the above topics are reviewed.

The first of these outlines the discovery and elucidation of the arachidonic acid cascade from a historical perspective. The potential

for thromboxane A_2 antagonists in cardiovascular therapy is also briefly discussed.

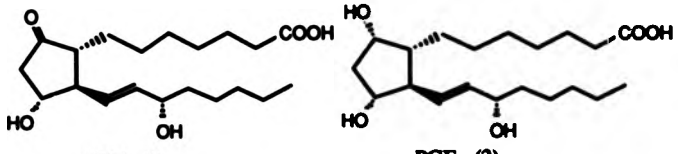
The second demonstrates why the unique physical properties of fluorine have made it invaluable in biomedicinal research. This is illustrated by using selected examples from a diverse range of compounds to highlight each particular property.

Finally, the synthetic approaches to a series of thromboxane A_2 structural analogues are reviewed emphasising the particular problems connected with their preparation. This section is concluded by briefly discussing why fluorine substitution may provide a method of stabilising the thromboxane A_2 molecule and thereby yield an analogue with an interesting biological profile.

1.1 Arachidonic acid cascade

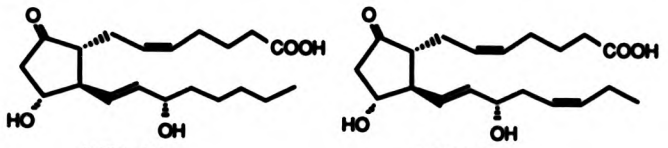
Some of the physiological responses caused by prostaglandins were first observed and recorded in the 1930's. Two research groups independently noted the smooth muscle stimulating activity of human seminal fluid. Furthermore, a hypotensive or vasodepressor activity was also noted in extracts from both seminal fluid and from the vesicular glands of sheep. von Euler showed that this activity was due to lipid soluble acidic compounds and he called these substances prostaglandins, believing that they originated from the prostate gland.[8-15]

The first pure, crystalline and biologically active prostaglandins, PGE_1 (1) and $PGF_{1\alpha}$ (2) were isolated in 1957.[16] The structural identity of these was first deduced using gas liquid chromatography/mass spectrometry studies on the oxidative degradation products. Later X-ray crystallography on the tris p-bromobenzoate derivative of $PGF_{1\alpha}$ (2) confirmed the structure and provided the stereochemical features of the molecule.



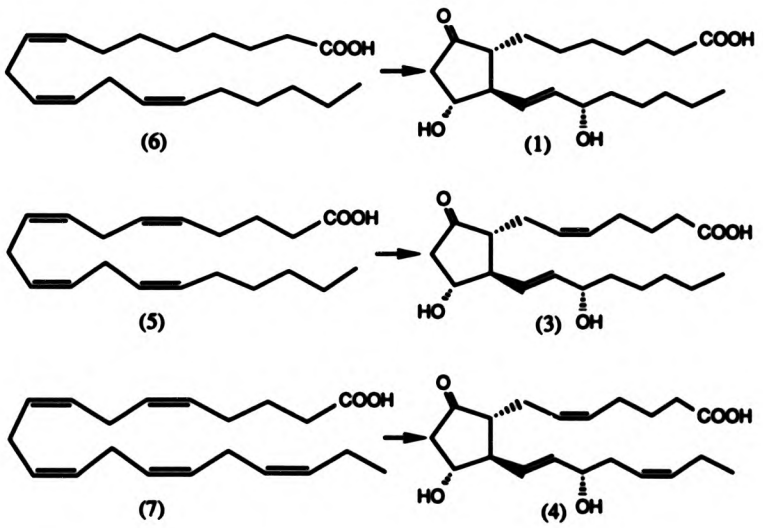
PGE_1 (1) $PGF_{1\alpha}$(2)

Shortly afterwards, PGE_2 (3) and PGE_3 (4) were isolated from the same tissue extracts. The pattern of unsaturation in these compounds suggested that the prostaglandins may be metabolites of the polyunsaturated C-20 carboxylic acids.



PGE_2 (3) PGE_3 (4)

It was then demonstrated that arachidonic acid (5) could be converted to PGE_2 (3) by homogenates of sheep vesicular gland. Similarly it was shown that homo-γ-linolenic acid (6) and all cis-eicosa-5,8,11,14,17-pentaenoic acid (7) were the precursors of PGE_1 (1) and PGE_3 (4) respectively.[17]



Since the discovery of the first two structural types of prostaglandins PGE_1 and $PGF_{1\alpha}$, many more have been isolated and are summarised in Figure 1.

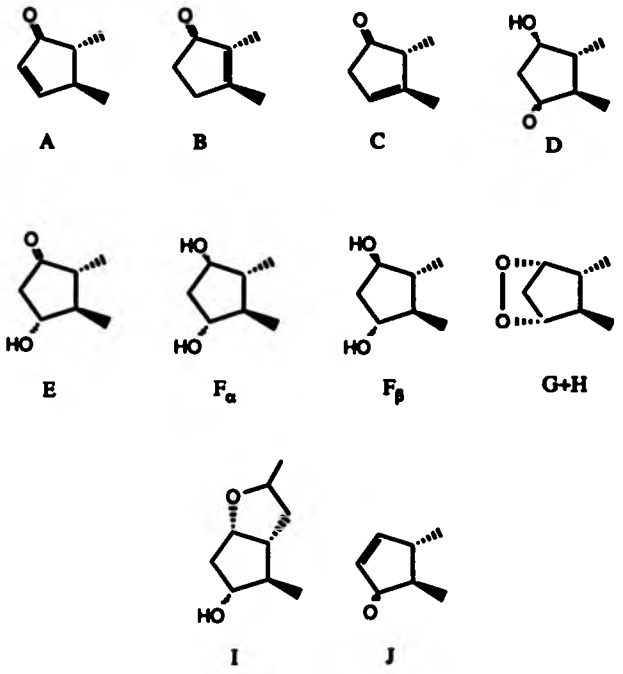
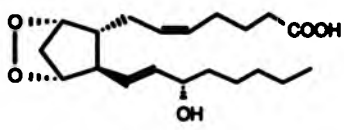


Figure 1

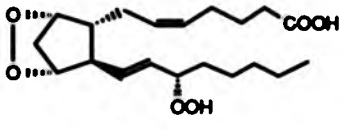
Similar biosynthetic studies revealed that the oxygen atoms at C-9

and C-11 in the prostaglandin skeleton were derived from the same molecule of oxygen.[18,19] From this it was concluded that an endoperoxide would be a likely intermediate between the fatty acids and the prostaglandins. The same experiments also revealed that the oxygen atom at C-15 was derived from molecular oxygen. This therefore implied the presence of a 15-peroxy intermediate, although it was shown that 15-peroxy fatty acids were not themselves biosynthesised to prostaglandins. This result indicates that incorporation of oxygen at C-15 occurs after oxygen incorporation at C-9 and C-11.

These ideas were confirmed seven years later by the isolation of the unstable endoperoxide intermediates PGH_2 (8) and PGG_2 (9) in 1973.[20]



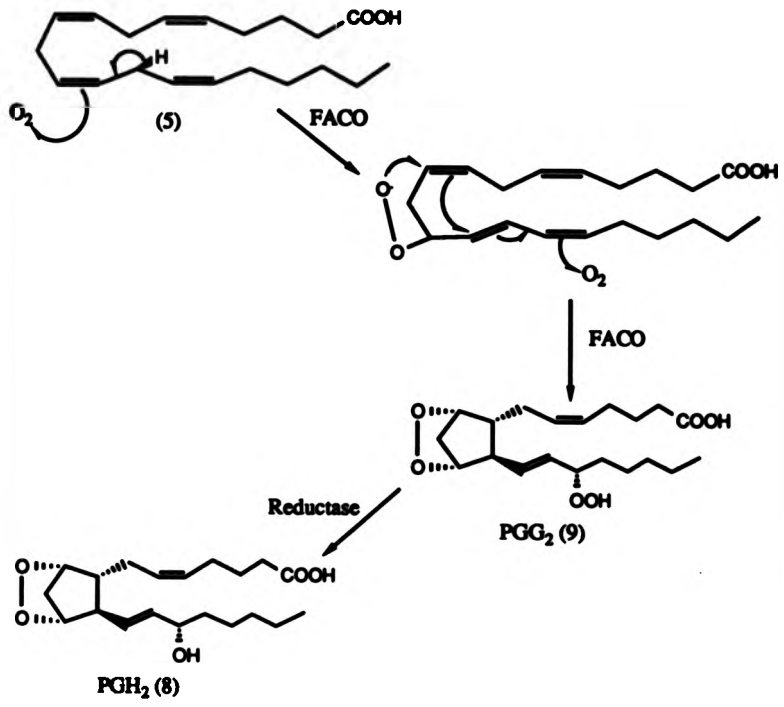
PGH_2 (8)



PGG_2 (9)

Interestingly, these 'intermediates' were shown to have potent biological activities in their own right. These were a smooth muscle stimulating activity greater than PGE_2 and a significant aggregatory activity on blood platelets.[21,22]

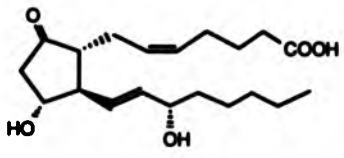
It has since been established that arachidonic acid (5) is metabolised by the enzyme PGH synthetase. This enzyme has two different activities; the first being fatty acid cyclooxygenase (FACO) activity, which catalyses the addition of two molecules of oxygen to produce PGG_2 (9)[23,24] Scheme 1. The second is a hydroperoxide activity which reduces PGG_2 (9) to PGH_2 (8).



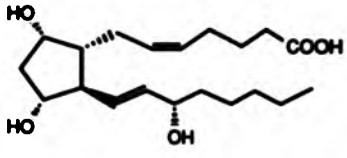
PGH synthetase activity

Scheme 1

In 1969 the release of a novel and potent compound from guinea pig lungs perfused with an antigen was observed by Piper and Vane.[25] This substance was distinct from PGE_2 (3) and $PGF_{2\alpha}$ (10) and was called



PGE_2 (3)



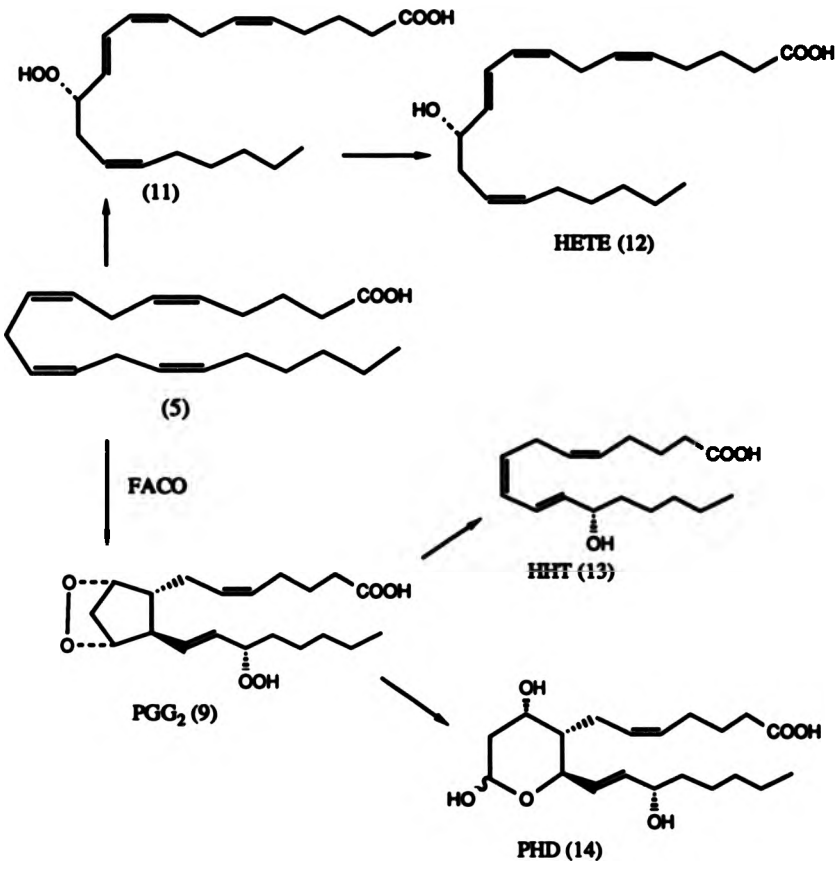
$PGF_{2\alpha}$ (10)

rabbit aorta contracting substance (RCS). The name was derived from the biological assay with which it was detected.

The substance RCS was shown to be very unstable and its release from tissues was stimulated by arachidonic acid. RCS was also detected during platelet aggregation that had been induced by collagen and arachidonic acid. Finally, the release of RCS was shown to be inhibited by anti-inflamatory drugs such as aspirin (PACO inhibitors), as was the synthesis of prostaglandins.[26,27]

These results indicated that RCS was a fatty acid cyclooxygenase-derived metabolite of arachidonic acid. Consequently, it was thought that RCS might be the endoperoxide intermediate proposed in the biosynthesis of prostaglandins.[20,21,26] However, once PGH_2 (8) and PGG_2 (9) had been isolated it was demonstrated that RCS was a mixture of two components having similar activities. One component was indeed the endoperoxide (8, 9), the other was as yet unknown. This unknown factor was shown to be far more potent in similar assays and to be much less stable.[28]

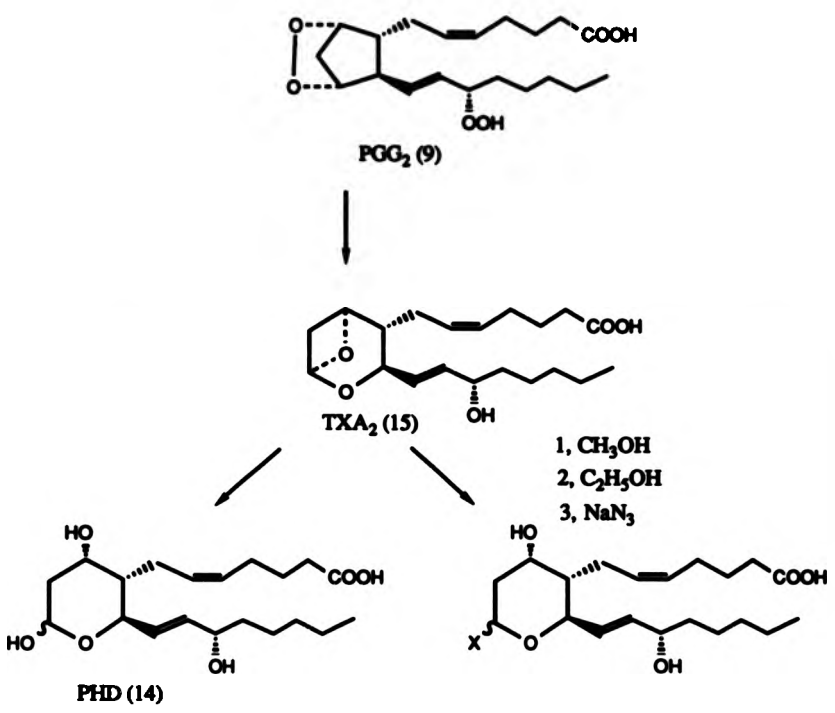
Incubation of human platelets with arachidonic acid (5) revealed that it was converted to three compounds.[29,30] The first of these was 12-S-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE) (12). This was shown to have a 12-hydroperoxy precursor (11) and its biosynthesis was not inhibited by aspirin. However, biosynthesis of the remaining two, 12-S-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (13) and 8-(1-hydroxy-3-oxo propyl)-9,12-S-dihydroxy-5,10-heptadecatrienoic acid (PHD) (14) was inhibited by aspirin. This implied that PGG_2 (9), a fatty acid cyclooxygenase product was a likely precursor of HHT (13) and PHD (14). To account for these observations the metabolic pathway in Scheme 2 was proposed.[29,30]



Metabolites of arachidonic acid

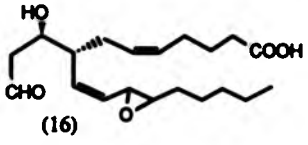
Scheme 2

Further work by the same group[31] showed that there was a highly unstable intermediate between PGG_2 (9) and PHD (14). This compound was called thromboxane A_2 (TXA_2) and the novel oxetane structure (15) was proposed from the results of various trapping experiments, Scheme 3. The compound PHD (14) was then renamed as thromboxane B_2 (TXB_2).



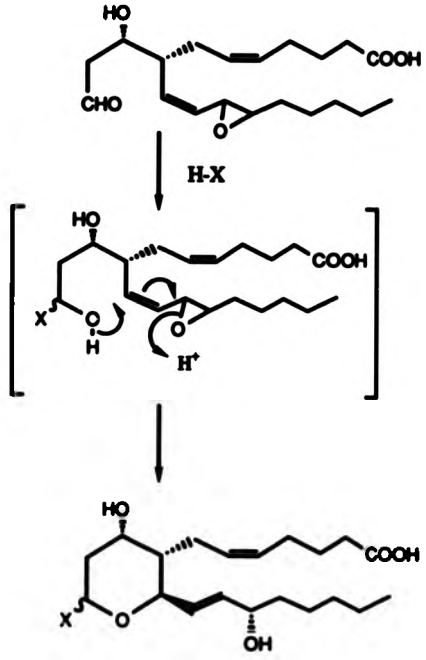
Conversion of PGG_2 to TXB_2 derivatives via TXA_2

Scheme 3



The structure (16) was proposed by Baldwin[32] as a possible alternative to the bicyclic oxetane (15). It was believed that this would also react with nucleophiles to yield thromboxane B_2 type

structures, Scheme 4.



Scheme 4

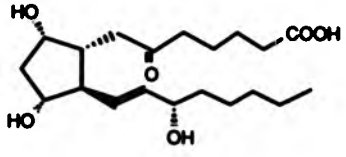
However, chemical synthesis of (16) by Kelly et al[32] showed it to be chemically and biologically distinct from natural TXA_2. Further evidence for the oxetane structure has been obtained from both the agonistic behaviour of structural analogues[93-108] and the similar reactivity of some simple oxetanes[33] with methanol or azide. However, more compelling evidence has recently been obtained by the total synthesis of the dioxabicyclic structure (15).[34] in vivo tests showed this to be indistinguishable from natural TXA_2. However, it was noted that this was still not entirely conclusive.

Thromboxane A_2 (15) has a very short half life ~30 seconds under physiological conditions. It was also shown to be a very potent

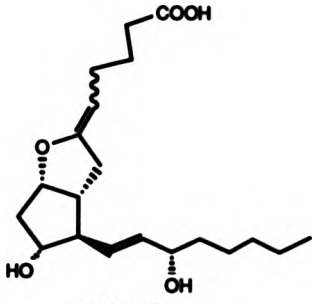
vasoconstrictor and platelet aggregator. On the basis of its powerful biological activity and its instability it was concluded that thromboxane A_2 was the unknown factor present in RCS.[31]

The discovery of thromboxane A_2 and its role in platelet aggregation was soon complemented by the observation of another prostaglandin endoperoxide metabolite.[35] Vane et al demonstrated that an enzyme isolated from blood vessels converted prostaglandin endoperoxides (8,9) into an unstable substance called PGX. This was shown to inhibit platelet aggregation and to disrupt preformed thrombi. It was also shown to relax certain smooth muscle tissues, particularly blood vessels.

Another new prostaglandin, 6-keto $PGF_{1\alpha}$ (17), had recently been isolated from several tissues. It was also detected in assay solutions which had previously exhibited PGX activity. This discovery prompted the suggestion that PGX may have the labile enol ether structure (18).[36,37]



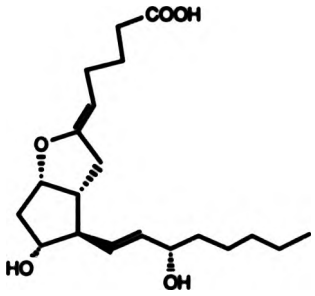
6-keto $PGF_{1\alpha}$(17)



PGX (18)

Radiolabelling experiments soon confirmed the enol ether structure.[37] Total synthesis then proved the Z configuration for the $\Delta^{5,6}$ double bond.[38-40] The trivial name prostacyclin was adopted

and it was classified as PGI_2 (18) under the prostaglandin nomenclature, Figure 1.

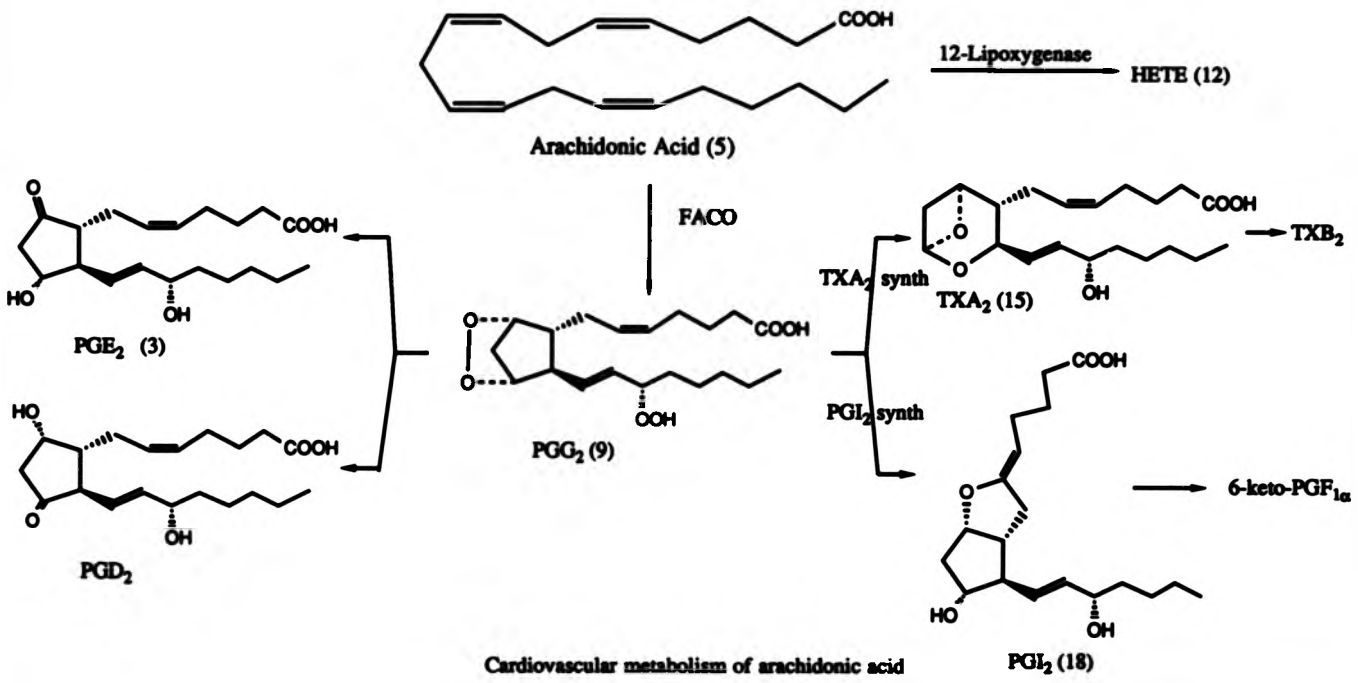


PGI_2 (18)

Scheme 5 shows the various possible outcomes from prostaglandin endoperoxide metabolism. It is now clear that the differential production of prostaglandins, prostacyclin and thromboxanes depends on the tissue in which they are biosynthesised. For instance the main pathway within the kidney is the production of PGE_2 (3) and $PGF_{2\alpha}$ (10). Production of TXA_2 (15) is the most predominant pathway in platelets as is prostacyclin in vascular tissue.

Arachidonic acid (5) and the endoperoxides (8,9) are therefore common intermediates of a range of compounds which have diverse biological functions. It must therefore be concluded that biological control is exerted through the terminal enzymes present in individual tissues, for instance thromboxane or prostacyclin synthetase.[41]

This concept is particularly relevant to Vane's proposal for the control of vascular tone.[42-45] It is known that one function of the



Cardiovascular metabolism of arachidonic acid

Scheme 5

endoperoxides (8,9) is to promote platelet aggregation. This is most probably due to their conversion to the physiologically more potent thromboxane A_2 (15). For many years it remained a mystery why platelets did not adhere to healthy vascular tissue. The discovery that this tissue can convert endoperoxides to prostacyclin, a potent anti-aggregatory substance, provided the answer. Vane et al have shown that vascular tissue does not have significant cyclooxygenase activity and therefore it does not produce endoperoxides. It seems likely that the tissue is 'fed' endoperoxides produced by platelets.

In a normal blood vessel, platelets will produce the endoperoxides (8,9), in the vicinity of the vessel wall. These will be converted to prostacyclin, thus preventing adhesion or thrombus formation. This provides a delicate mechanism whereby blood vessels are protected from the harmful deposition of platelet aggregates. However, in the case of damage to the vessel wall, this balance will be upset allowing aggregation to occur and maintaining the vascular integrity. Obviously, imbalances or faults in this mechanism are likely to cause problems within the cardiovascular system. For example, inhibition of prostacyclin synthesis has been implicated in atherosclerosis. Similarly, excessive levels of thromboxane A_2 have been shown to cause acute myocardial infarction or in less severe cases, angina pectoris responses. The role of thromboxane A_2 and prostacyclin in thrombus formation may be critical in the cause or prevention of heart attacks and strokes.

Naturally, a means of controlling the balance of TXA_2 and PGI_2 would be therapeutically valuable. The prevention or treatment of cardiovascular diseases may be possible by blocking or inhibiting the potentially harmful effects of thromboxane A_2. There are several ways

in which this may be achieved.[46]

Inhibition of the fatty acid cyclooxygenase or PGH synthetase would block the production of TXA_2. Anti-inflamatory drugs such as aspirin inhibit this enzyme and have been used in the treatment of some cardiovascular disorders. However, this would also prevent biosynthesis of PGI_2 and other prostaglandins and is therefore not completely satisfactory. The synthesis of more stable PGI_2 derivatives with agonist properties would be useful. An agonist has a biological profile similar to the compound it mimics. Compounds of this nature would increase the apparent levels of PGI_2, hence lowering blood pressure and preventing thrombus formation. Selective inhibition of thromboxane synthetase, the enzyme which converts PGG_2 (9) to TXA_2 (15) may also be useful. This could be achieved in principle by the synthesis of stable endoperoxide analogues which should act as substrate mimics for the enzyme. Finally, synthesis of thromboxane A_2 antagonists would be therapeutically useful. An antagonistic compound would compete at the receptor level with natural TXA_2 but have the opposite biological effect. Blocking these receptors would prevent the potentially harmful effects of TXA_2 without preventing its production by enzymes. Prevention of TXA_2 production by enzyme inhibition may cause unwanted side effects due to overproduction of either prostacyclin or other prostaglandins.

Receptor antagonists are usually compounds that are structurally similar to but more stable than the natural agonist. In this way they can fit receptors, but hopefully do not trigger the natural physiological response. Previous syntheses of a series of thromboxane A_2 analogues are discussed in a later section.

1.2 Properties of fluorine

The introduction of fluorine into organic compounds can cause a variety of very significant changes in their chemistry. As a result, organofluorine compounds have a diverse range of properties and uses. This can be seen in their use as coolants, aerosols, artificial blood substitutes and chemically resistant polymers. This diversity is also further reflected in the vast number and range of biomedicinal organofluorine compounds. The unique physical properties of fluorine make it particularly useful in this area and these are outlined below.[47,5]

i) The strength of the carbon fluorine bond exceeds that of the carbon hydrogen bond. This often results in increased thermal and oxidative stability in organofluorine compounds.

X	H	F	Cl	Br	I [48]
Bond energy CH_3- X (kcal mol^{-1})	104	109	84	70	56

ii) Fluorine is the second smallest substituent and consequently closely mimics hydrogen, with respect to steric requirement at enzyme receptor sites.

Van der Waals radii, Fluorine 1.35A[48]

Hydrogen 1.2A

iii) The high electronegativity of fluorine (4.0 Pauling scale) frequently alters the electronic properties and thereby the chemical reactivity of the compounds concerned.

iv) The introduction of fluorine into organic molecules usually increases their lipid solubility, thereby enhancing the rates of absorption and transport of drugs *in vivo*.

Naturally, any changes in the biological properties of a compound resulting from the introduction of fluorine will normally be due to a combination of these effects. However, the examples presented below have been chosen in an attempt to highlight these individual characteristics.

"The strength of the carbon fluorine bond exceeds that of the carbon hydrogen bond. This difference often results in increased thermal and oxidative stability in organofluorine compounds".

Although not clinically useful, fluoroacetic acid (19) is interesting both from a historical perspective and because it is a naturally occurring toxin.

FCH_2COOH (19)

Fluoroacetic acid (19) was first prepared in 1896[49] and was shown to contain a stable carbon fluorine bond being resistant to hot concentrated sulphuric acid. Further interest developed in 1943 when it was discovered to be the toxic constituent of a South African plant Dichapetatum cymosum[50]. Since then fluoroacetic acid and fluoro fatty acids have been found in a variety of other plants.[51-54]

However, serious research into the toxicity and chemistry of fluoroacetic acid (19) and related compounds was only initiated during the second world war when Saunders and co-workers prepared and tested a large number of fluorinated compounds. Some of these were toxic (in rabbits LD_{50} by intravenous injection was 0.25 mg/kg of body weight)[55] while others, although closely related were curiously non-toxic.

The first compound to be synthesised and investigated was methyl fluoroacetate (20).[55] This was a mobile liquid bpt 104°C and was prepared from methylchloroacetate and potassium fluoride.

FCH_2COOCH_3 (20)

This was also shown to have a stable fluorine substituent, being inert to both refluxing 20% alcoholic potassium hydroxide and to boiling concentrated sulphuric acid. Methyl fluoroacetate (20) and the related ethyl, n-propyl and isopropyl esters were all shown to have similar toxicity. However, methyl α-fluoropropionate (21) and methyl α-fluoroisobutyrate (22) were non-toxic.

$FCHMeCOOCH_3$ (21)

$FCMe_2COOCH_3$ (22)

Further work showed that 2-fluoroethanol (23), fluoroacetyl chloride (24) and fluoroacetyl fluoride (25) were all toxic, whereas chloroacetyl fluoride (26) was non-toxic.

FCH_2CH_2OH (23)

FCH_2COCl (24)

FCH_2COF (25)

$ClCH_2COF$ (26)

This series of experiments suggested that the toxicity was due to 'FCH_2CO' group. Further evidence was the non-toxicity of ethyl fluoroformate (27) and the enhanced toxicity of 2-fluoroethyl fluoro acetate (28).[56]

$FCOOC_2H_5$ (27)

$FCH_2COO\ CH_2CH_2F$ (28)

Similarly, the series of amides, Table 1, were also all toxic.[57]

Table 1

$FCH_2CONHCH_3$

$FCH_2CON(CH_3)_2$

$FCH_2CONHCH_2CH_2OH$

$FCH_2CONHCH_2CH_2Cl$

$FCH_2CON\ (CH_2CH_2Cl)_2$

These results clearly showed that if a compound was easily hydrolysed or oxidised to fluoroacetic acid, then it would be toxic. A particularly elegant example was the demonstration of the alternating toxicity within a series of w-fluoro carboxylic acids[58,59] Table 2.

Table 2

$FCH_2\ COOR$	Toxic	$F(CH_2)_5\ COOR$	Toxic
FCH_2CH_2COOR	non-Toxic	$F(CH_2)_7\ COOR$	Toxic
$F(CH_2)_3\ COOR$	Toxic	$F(CH_2)_{10}\ COOR$	non-Toxic
$F(CH_2)_4\ COOR$	non-Toxic	$F(CH_2)_{11}\ COOR$	Toxic

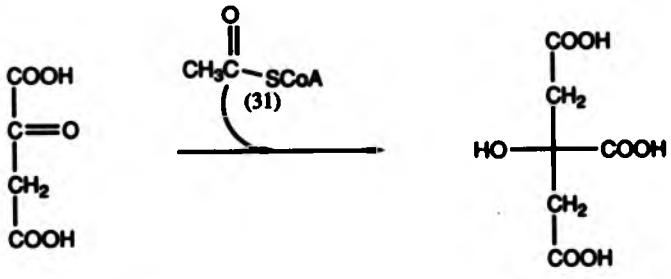
The metabolsim of long chain fatty acids occurs via the so called β-scission pathway. Here fatty acids are broken down in two carbon atom units owing to oxidation at the β-carbon atom.

β-Oxidation of acids with an even number of carbon atoms would ultimately result in the formation of fluoroacetic acid (19), and hence exhibit toxicity. Similar oxidation of the odd numbered homologues would lead to either non-toxic fluoropropionic acid (29) or fluoroformic acid (30).

FCH_2CH_2 COOH (29)

FCOOH (30)

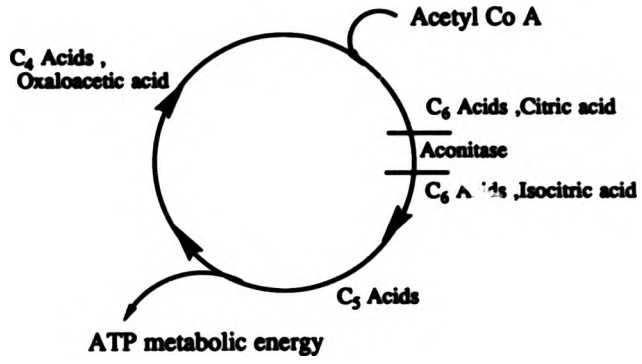
The biochemical mechanism for fluoroacetic acid poisoning was elucidated by Peters et al.[60-62] The normal product of fatty acid oxidation is a two carbon acetyl unit bound to co-enzyme A, a biochemical leaving group. This compound, collectively referred to as acetyl-CoA (31) is taken into the mitochondria and incorporated into the tricarboxylic acid (TCA) cycle. Here acetyl-CoA is condensed with oxaloacetic acid (32) to form citric acid (33), Scheme 6.



Oxaloacetic acid (32) Citric acid (33)

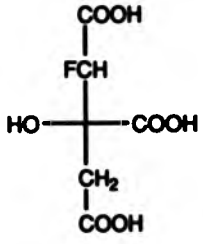
Scheme 6

Citric acid is then isomerised by the enzyme aconitase to isocitric acid. Subsequent metabolism of isocitric acid provides the energy required to produce ATP. The cycle is finally completed with the regeneration of oxaloacetic acid (32), Scheme 7.



Scheme 7

The small size of the fluorine atom allows fluoroacetate to mimic acetate. It is therefore readily bound to co-enzyme A and condensed with oxaloacetic acid (Scheme 6) to form fluorocitric acid (34).



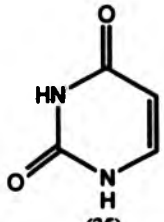
(34)

However, the stability of the carbon fluorine bond prevents further metabolism. Fluorocitric acid (34) is therefore an effective inhibitor of the enzyme aconitase. Inhibition of this enzyme breaks down the TCA cycle due to accumulation of citric acid and an inability to regenerate

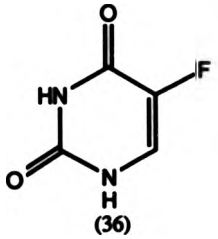
oxaloacetic acid. Fluoroacetic acid poisoning then, is caused by blocking the TCA cycle which prevents the biosynthesis of the metabolic energy source ATP.

The term 'lethal synthesis'[63] was coined by Peters to describe this type of mechanism. That is, the biosynthesis of an enzyme inhibitor such as fluorocitrate from a precursor, in this case fluoroacetate.

Another fascinating illustration of 'lethal synthesis' is the use of fluoropyrimidines as anti-cancer drugs, these created a major breakthrough in cancer chemotherapy in the late 1950's. This was initiated by the discovery that the biosynthesis of DNA by tumour cells had an enhanced demand for uracil (35).[64,65] Subsequently several uracil analogues were prepared among which was 5-fluoro uracil (36).[66,67] This was shown to have a remarkable tumour inhibiting activity. Since then, 5-fluoro uracil or its derivatives have been widely used throughout the world for the treatment of cancer.



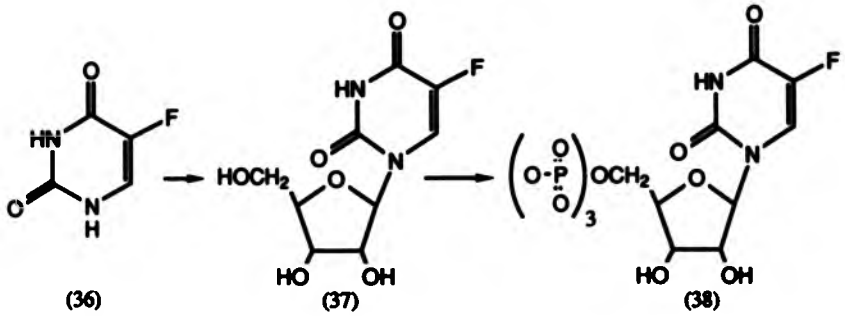
(35)



(36)

There are two different mechanisms for the cytotoxicity of 5-fluoro-uracil (36). As with fluoroacetic acid, the size of the fluorine atom allows the fluoro compound to mimic uracil (35) itself. Consequently, it is enzymatically converted to the nucleotide 5-fluoro uridine (37) and then to 5-fluoro uridine mono, di and triphosphate (38) Scheme 8.

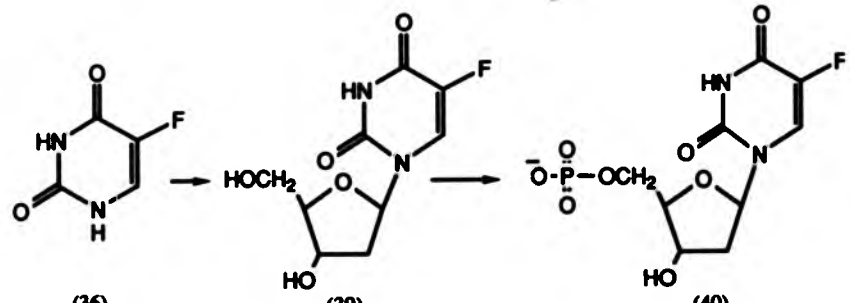
Finally, the triphosphate (38) becomes incorporated into RNA. Once incorporated into RNA it is believed to cause transcriptional errors within the developing tumour cells.[68]



(36) (37) (38)

Scheme 8

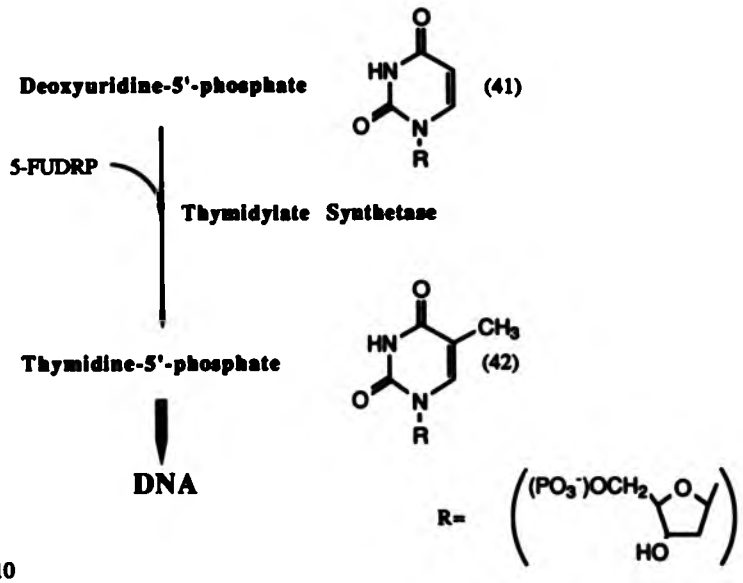
More clearly understood, however, is the inhibition of the enzyme thymidylate synthetase.[69-73] As before 5-fluoro uracil (36) is converted to a nucleotide, 5-fluoro-2'-deoxy-β-uridine (39). Interestingly, this compound has subsequently proved to be clinically more effective and less toxic than 5-fluoro uracil (36) itself. The nucleotide is then converted to 5-fluoro-2'-deoxyuridine-5'-phosphate (40) (5-FUDRP) Scheme 9.



(36) (39) (40)

Scheme 9

5-FUDRP is a competitive inhibitor of thymidylate synthetase. This enzyme normally converts 2'-deoxyuridine-5'-phosphate (41) to thymidine-5'-phosphate (42), an essenial component of DNA, Scheme 10.

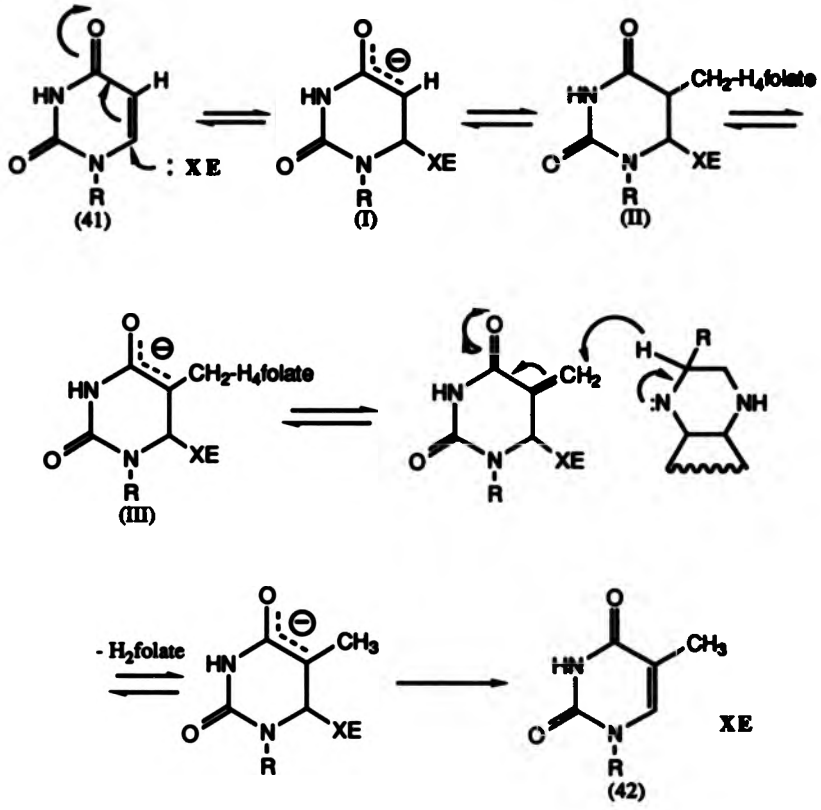


Scheme 10

As in the case of fluorocitrate, inhibition of this enzyme is due to the stability of the carbon fluorine bond. Scheme 11 shows the mechanistic details of the normal enzymatic reaction.

2'-Deoxyuridine-5'-phosphate (41) reacts with a cysteine residue within the enzyme to form the negatively charged complex (I). This then reacts with the folate cofactor (CH_2-H_4 folate), a biochemical methylating reagent, to form complex (II). The proton on C-5 is then removed to form (III). This then detaches from folate with abstraction of a further proton, to yield ultimately thymidine and H_2 folate. The fluorinated derivative proceeds as far as complex (II) where a stable C-F

bond is present instead of the normal carbon hydrogen bond.



Scheme 11

This naturally interupts the reaction sequence causing the complex to accumulate and therefore inhibit the enzyme. Inhibition of this enzyme prevents the incorporation of thymine into DNA, resulting in a so called 'thymineless death' for the tumour cells.

"Fluorine is the second smallest substituent 1.35A and consequently closely mimics hydrogen with respect to steric requirements at enzyme sites."

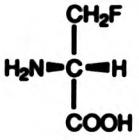
This ability of fluorine to mimic hydrogen has already been seen with respect to both fluoroacetic acid and 5-fluorouracil. Both these compounds were readily accepted by enzymes which converted them into compounds which then inhibited a different enzyme. Although not directly responsible for enzyme inhibition, the size of the fluorine atom was crucial to the activity of both these compounds.

This particular property of fluorine was used to demonstrate the concept of 'drug design' in the early 1970's.[74] That is, the preparation of a compound with desired biological activity by conceptual design instead of empirical search. This approach resulted in the design and synthesis of a new antibacterial drug, which was the first of a new class of compounds now known as suicide substrate enzyme inactivators.

The original thinking behind the 'design' approach[75] was to select a crucial metabolite of a key biological process which was also of special importance to a 'disease process'. Having identified a suitable metabolite the next step would be to prepare synthetically an 'antimetabolite'; that is to prepare a compound that resembled the metabolite very closely, but be different enough to block or inhibit the selected biological process. To accommodate this paradoxical idea the term 'isogeometric modification' was used to describe the required chemical change. Naturally, the similarity in size between hydrogen and fluorine but their drastically different chemical properties made the use of fluorine an obvious choice for 'isogeometric modification'.

These ideas led Kollonitsch and co-workers to synthesise

3-fluoro-D-alanine (43), as an 'anti metabolite'. The D-amino acids, D-alanine and D-glutamate are important constituents of bacterial cell walls. They are normally prepared by enzymic racemisation from the more common L-amino acids.

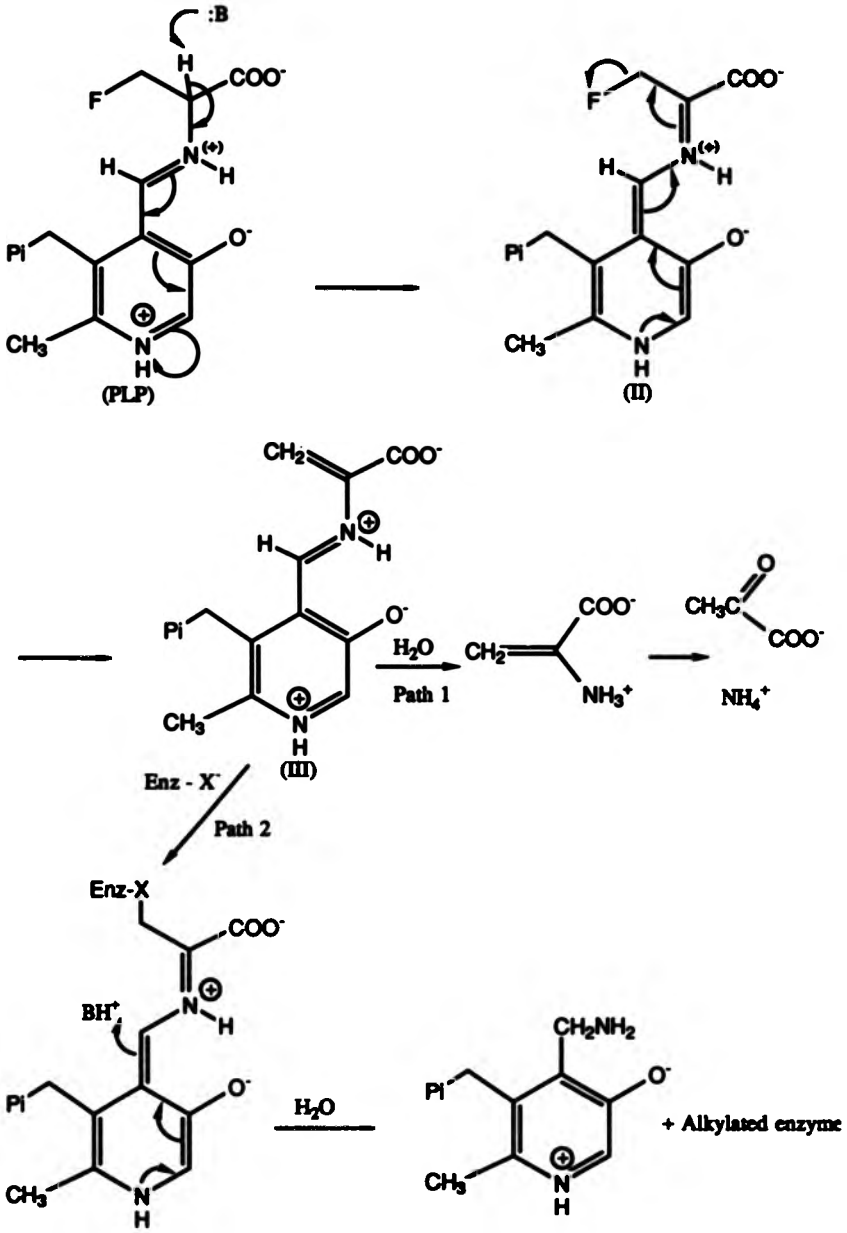


3-fluoro-D-alanine

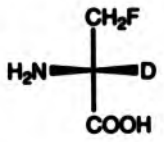
(43)

The fluoro compound (43) proved to have a wide spectrum of antibacterial activity both <u>in vitro</u> and <u>in vivo</u>. Subsequently it was proved that fluoroalanine (43) did indeed act as an antimetabolite by being an irreversible inhibitor of the enzyme alanine racemase.[76] The mechanism for this enzyme inhibition is shown in Scheme 12.[77]

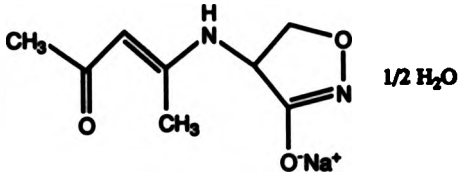
Normally the enzyme will bind the amino acid L-alanine through the pyridoxal phosphate (PLP) moiety. This would then be followed by proton abstraction to form the complex (II), and racemisation would normally occur by reprotonation of this complex. However, for the fluoro analogue loss of fluoride is faster than reprotonation, hence generating the 'eneimino' complex (III). This common complex then has a choice of two routes. The first is hydrolysis to pyruvate and the ammonium ion (Path 1), while the second involves Michael attack on the complex by an enzyme bound nucleophile (eg terminal amino group of a lysine residue). This would then result in an alkylated and hence irreversibly inhibited enzyme.


:B
F
COO⁻
H
N(+)H
H
Pi
O⁻
CH₃
N
H
(PLP)
(II)
CH₂
COO⁻
(III)
H₂O
Path 1
NH₃⁺
CH₃C
O
NH₄⁺
Enz - X⁻
Path 2
Enz-X
BH⁺
CH₂NH₂
+ Alkylated enzyme

Scheme 12



(44)

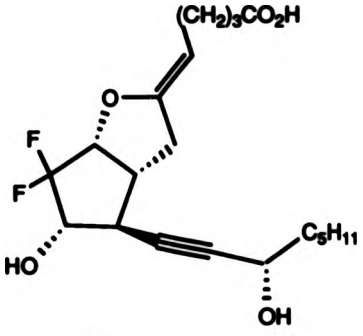


(45)

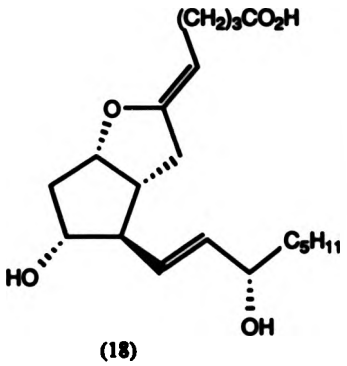
Subsequent research has shown that the deuterated analogue[78] (44) in combination with the previously known natural antibiotic cycloserine (45),[79] has enhanced antibacterial activity. This activity is a result of a sequential blockade of cell wall biosynthesis.

"Fluorine with its very high electronegativity frequently alters electronic effects and thereby chemical reactivity."

A particularly striking example of a change in chemical reactivity caused by the introduction of fluorine is the stability of the difluoro prostacyclin analogue[80] (46).



(46)



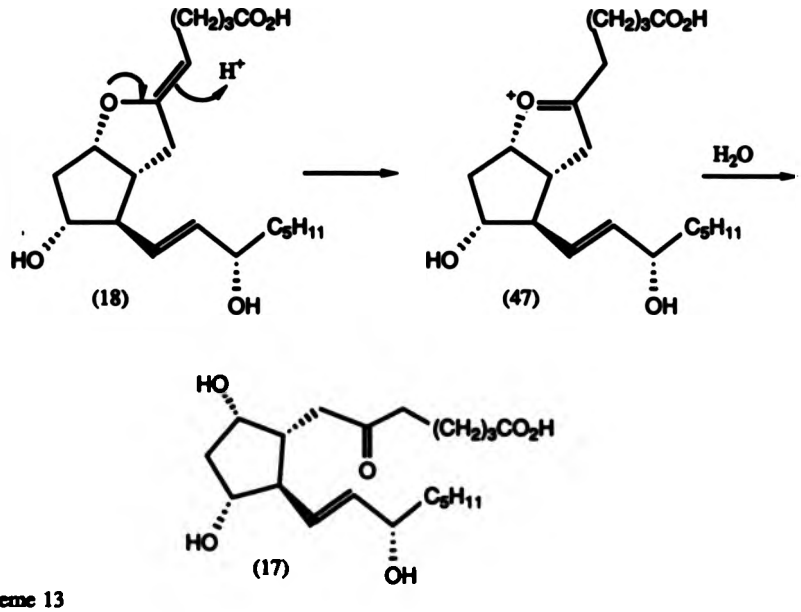
(18)

The natural compound, prostacyclin (18) is a powerful vasodilator and anticoagulant, with a relatively short half-life $t_{1/2}$ ~3 mins. These properties are normally used to counter the effects of thromboxane A_2 within a healthy blood vessel. However, compounds with these properties may be therapeutically useful in their own right. It is therefore likely that a more stable derivative of prostacyclin may provide a useful drug.

The normal hydrolysis of prostacyclin (18) proceeds through the intermediate oxonium ion (47) Scheme 13. It was reasoned that the powerful electron withdrawing properties of fluorine would destabilise this intermediate and thus stabilise the original enol-ether function.

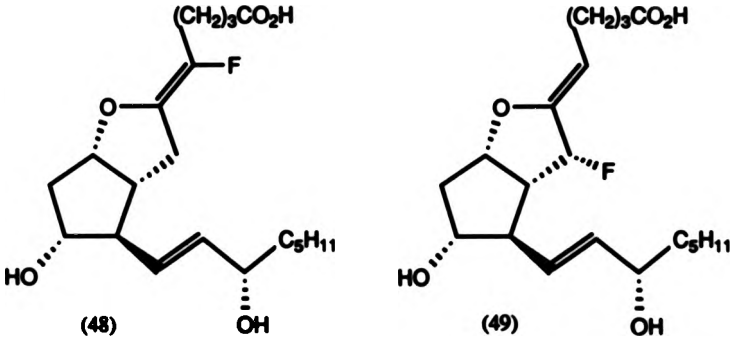
The difluoro PGI_2 analogue (46) was shown to be one hundred times more stable to hydrolysis. More significantly, it was equal in potency

to natural prostacyclin in lowering blood pressure.

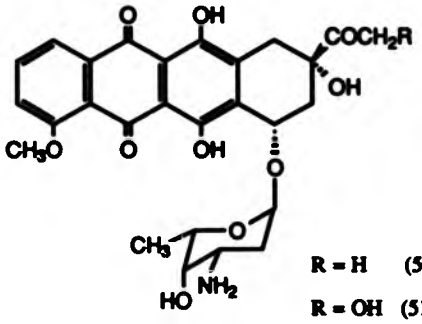


Scheme 13

Similar effects of enhancing the hydrolytic stability have been observed for the 5-fluoro (48) and 7-fluoro (49) PGI_2 derivatives[81] both of which had prostacyclin agonist activity.



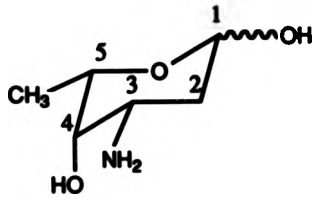
Another example concerns the naturally occurring anti-cancer agents daunomycin (50) and adriamycin (51).



R = H (50)

R = OH (51)

Although clinically useful, these compounds have some serious side effects.[82] In attempts to improve the therapeutic value, attention has recently focused on fluorination of the amino sugar moiety, daunosamine (52).[83-88]

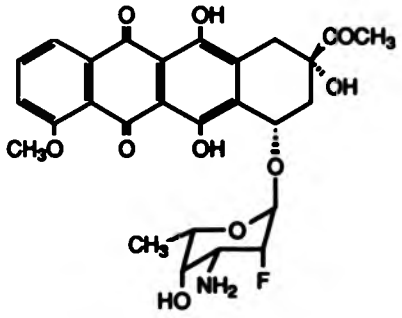


(52)

Fluorination at C-2 will increase the hydrolytic stability of the glycosidic linkage in the parent compound. As before, this is because the electron withdrawing properties of fluorine will destabilise the oxonium ion intermediate.

The synthesis, and preliminary biological evaluation of

2'-C-fluoro-β-daunomycin (53) has been reported. These results showed a similar activity to daunomycin (50) in vitro. However, in vivo the range of active doses was considerably wider. Presumably this is a result of the increased hydrolytic stability, as this would mean that more of the 'active' compound is likely to reach the target cells, hence lowering the required dosage.

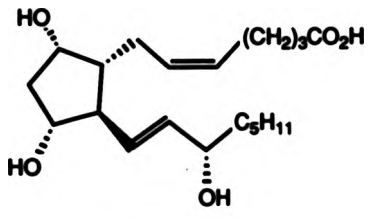


(53)

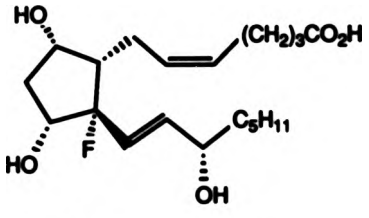
"The introduction of fluorine into organic molecules usually increases lipid solubility, thereby enhancing the rates of absorption and transport of drugs in vivo."

The introduction of fluorine into organic molecules often results in profound changes in chemical or biological activity. Consequently this technique has been used to develop many classes of drugs ranging from prostaglandins and steroids to the psychopharmacological or central nervous system (CNS) agents.

Some of the changes in chemical behaviour resulting from fluorination have already been demonstrated. Unfortunately, these changes are often not so predictable and a trial and error approach has to be used to optimise potency and selectivity in drugs. In many of these cases no obvious chemical mechanism can explain the changes in activity observed. However, it is believed that increased lipophilicity may be the significant factor. A simple illustration is the change resulting from substitution at C-12 in $PGF_{2\alpha}$ (10). Relative to the parent compound, 12-fluoro $PGF_{2\alpha}$ (54) has enhanced antifertility activity and diminished smooth muscle contractile activity.[89]



$PGF_{2\alpha}$ (10)

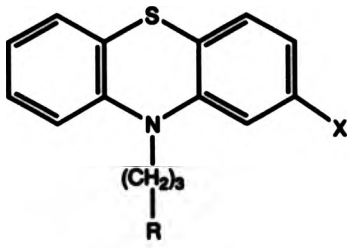


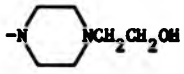
12-Fluoro $PGF_{2\alpha}$ (54)

More interesting are the subtle changes in structure activity

relationships evident in some classes of the CNS agents.[90] A series of therapeutically important phenothiazines are presented in Table 3. These belong to a class of compounds called neuroleptics which are used in the treatment of schizophrenia.

Table 3



X	R	Compound
Cl	$-N(CH_3)_2$	(55)
CF_3	$-N(CH_3)_2$	(56)
CF_3		(57)
CF_3		(58)

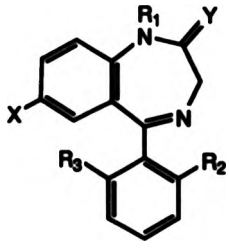
The parent compound chloropromazine (55) was first introduced in 1954 and subsequent research has resulted in the introduction of many others. Substitution of chlorine with trifluoromethyl (56) led to a five fold increase in potency. Manipulation of the amine substituents

then resulted in trifluoroperazine (57) and fluphenazine (58) which are fifty and one hundred times more potent respectively.

An experiment attempting to increase the potency of fluphenazine (58) led to the replacement of the 2-trifluoromethyl group with more electronegative groups. The 2-nitro derivative was three times more potent whereas the 2-cyano derivative was one half as potent. The cyano group has electron withdrawing properties between that of trifluoromethyl and nitro. It was therefore concluded that differential lipid-water solubility was probably an important factor in determining the potency.[91]

Another series of clinically important drugs are the 1,4-benzodiazepines shown in Table 4.[90] The parent compound diazepan (59) is used to treat anxiety and tension in patients with neuroses and depressive states.

Table 4



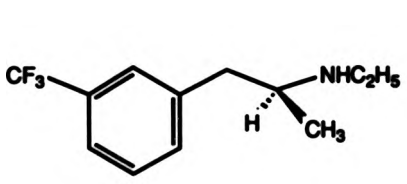
Compound No	X	R_1	R_2	R_3	Y
59	Cl	CH_3	H	H	O
60	Cl	$(CH_2)_2N(Et)_3$	F	H	O
61	Cl	CH_2CF_3	H	H	O
62	Cl	CH_2CF_3	F	H	S
63	NO_2	CH_3	F	H	O
64	Cl	H	F	F	O
65	Cl	CH_2-cyclopropyl	F	H	O
66	Cl	CH_2CF_3	F	H	H_2

Subsequent structure activity work has resulted in the introduction of fluazepam (60) and flunitrazepam (63) as hypnotic agents. Flunitrazepam (63) has also been used as an intravenous anaesthetic. Both flurazepam (60) and quazepam (62) have found use in the treatment of insomnia. Compound (64) is an extremely potent anticonvulsant agent, whereas compounds (65) and (66) show both muscle relaxant and anticonvulsant activity.

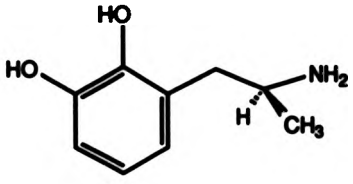
Both the 1,4-benzodiazepines and phenothiazines are CNS agents. Therefore, their mode of action will be either agonistic or antagonistic at various neuro-transmitter receptor sites. It is easy to see that in both these cases structural changes have generally resulted in unpredictable changes in selectivity and potency. Presumably this is a consequence of the complexity of chemical interaction within the synapse.

However, the increased lipid solubility resulting from introduction of fluorine must be an important factor in determining potency because an increased lipophilicity will increase the rate of absorption across the blood brain barrier and hence into the central nervous system.

Finally, an example of particular interest is fenfluramine (67).



(67)

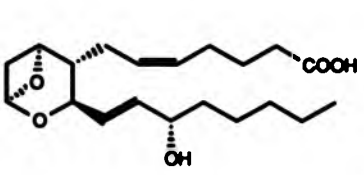


(68)

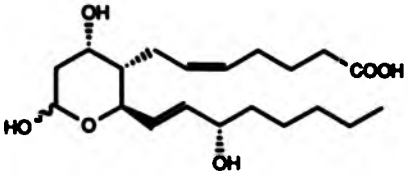
This compound has the same absolute configuration as amphetamine (68). However, the fluoro compound causes a diminuation of the hyperactivity caused by amphetamine administration. Clinically fenfluramine (67) is used as an appetite repressant or anorectic for the treatment of obesity.[92]

1.3 Synthesis of analogues

Thromboxane A_2 (15) has extremely potent vasoconstricting and platelet aggregatory properties, whereas thromboxane B_2 (14) is biologically inactive.



TXA_2 (15)

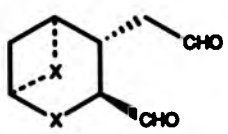


TXB_2 (14)

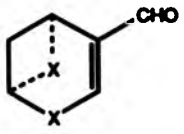
It must therefore be concluded that the bicyclic structural unit within thromboxane A_2 is vital for its biological activity. The biological activity is in turn dependent on receptor binding properties. Consequently, any analogue, either agonist or antagonist, which is required to act at a receptor, must have a similar structural unit.

The dioxa bicyclo[3,1,1] heptane unit in TXA_2 (15) is very labile. This has made both total synthesis of the natural product and of close structural analogues difficult. However, many analogues with much more stable bicyclic units have been prepared. This has generally been achieved by substitution of one or both of the oxygen atoms with either carbon or other hetero atoms.

There are two problems associated with the synthesis of TXA_2 analogues. These are the construction of the bicyclic unit and the establishment of the trans stereochemistry of the prostanoid sidechains. The common strategy adopted has been to prepare intermediates such as (69) or (70).



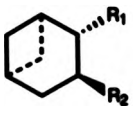
(69)



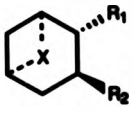
(70)

From these, established methodology is available for the introduction of the prostanoid sidechains.

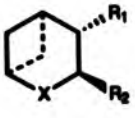
For the ease of discussion the syntheses of the various TXA_2 analogues have been subdivided into three sections. These are the all carbon analogues (71), the carbon, hetero atom analogues (72) or (73) and finally the hetero atom analogues (74) or (75).



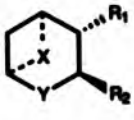
(71)



(72)



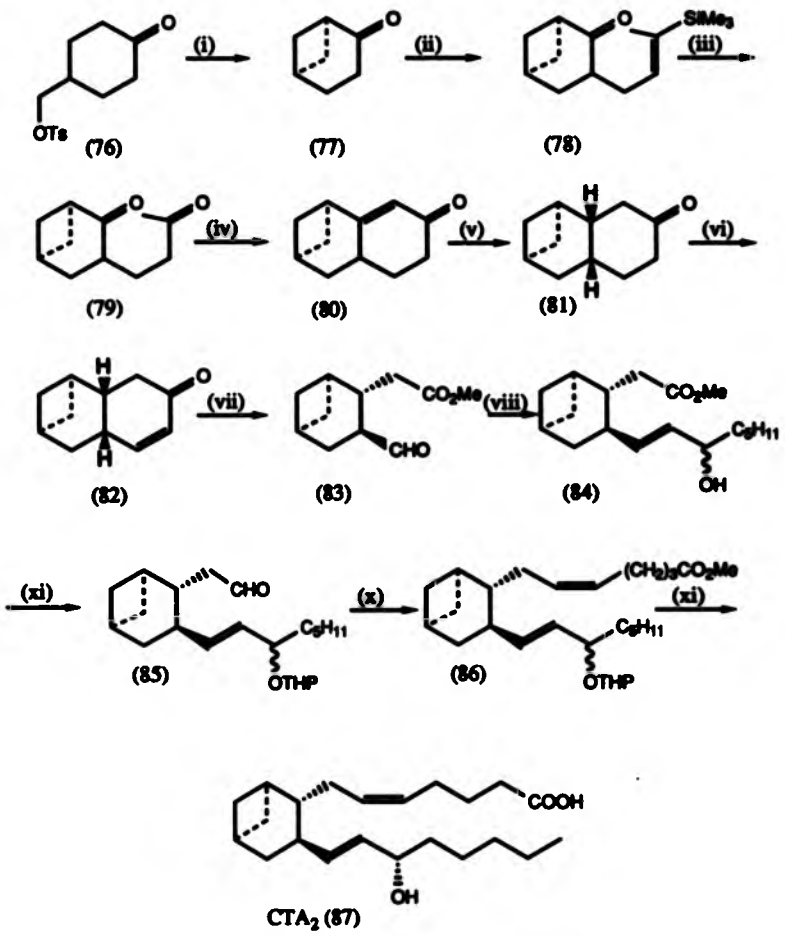
(73)



X=Y (74)
X≠Y (75)

<u>All carbon analogues</u>

Three independent syntheses of the carbon analogue (87), CTA_2, have now been published. The first of these is presented in Scheme 14.[93]



Scheme 14

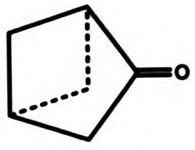
(i) $NaN(SiMe_3)_2$ (2eq), C_6H_6, 80°, 5h (57%).
(ii) LDA (2eq), THF, -78°. 2h, then $ICH_2CH{=}C(Me)SiMe_3$, HMPA, -78°, 2h, then 25°, 1h (48%).
(iii) MCPBA (1.5 eq), CH_2Cl_2, 0°, 3h then HCO_2H, CH_2Cl_2, 25°, 30 min (87%).
(iv) 10% aq KOH, MeOH, reflux 2h (85%).
(v) Li-NH_3 (liq) - Bu^tOH, -78°, 10 min, then Jones oxidation (51%).
(vi) $Ph_3P^+CH_2CH_2CO_2H\ Br_3^-$, THF, 0°, 30 min, then LiBr, Li_2CO_3, DMF, 125°, 1h (53%).
(vii) OsO_4, pyridine, 25°, 2h, then $NaHSO_3$, then $Pb(OAc)_4$, (3eq), in MeOH-benzene, 25°, 12h (62%).

(viii) $Bu_3P = CHCOC_5H_{11}$, ether, 25°, 17h, then $NaBH_4$, MeOH, -40° (80%).
(ix) DHP then Bu^i_2 AlH (3eq), then SO_3-pyridine, Et_3N, DMSO, 25°, 20 min (84%).
(x) $Ph_3P = CH(CH_2)_3CO_2Na$, DMSO, then CH_2N_2 (83%).
(xi) H^+, chromatography, then 5% aq NaOH, MeOH.

Scheme 14 Reagents

The tosylate (76) was treated with sodium bistrimethylsilylamide to yield the bicyclic ketone (77). With the construction of the bicyclic nucleus completed the problem of establishing the correct relative stereochemistry of the prostanoid sidechains was achieved by alkylation of the ketone (77) and annulation to yield the tricyclic enone (80). Reduction followed by oxidation of the intermediate alcohol gave the tricyclic ketone (81) with a trans ring junction. Selective bromination and dehydrobromination gave the enone (82) and oxidative cleavage of the double bond provided the key intermediate (83). This compound afforded a bicyclic nucleus with sidegroups of the appropriate stereochemistry and functionality, therefore allowing completion of the synthesis by established protocol.

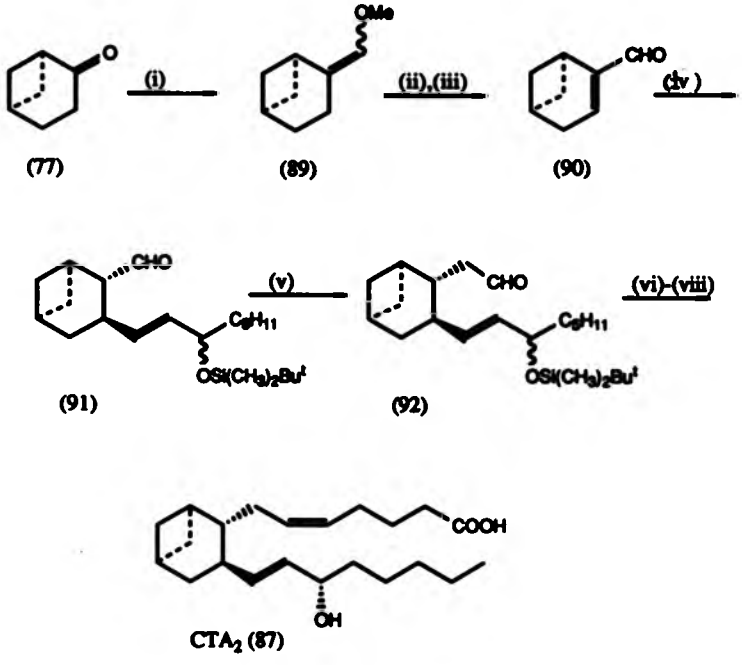
The second synthesis,[94] presented in Scheme 15 uses the same starting ketone (77) which had been constructed in two different ways, namely by cyclisation of the keto-tosylate (76) with dimsyl potassium as base or by ring expansion of ketone (88).



(88)

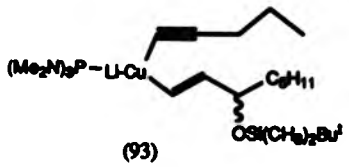
Elaboration of the bicyclic ketone (77) to CTA_2 (87) was then carried out by preparation of the α,β-unsaturated aldehyde (90). This was achieved by homologation with a Wittig reagent to yield the enol ether (89). Reaction with phenyl selenyl chloride gave the selenide and oxidation and elimination yielded the α,β-unsaturated aldehyde (90).

The bottom prostanoid sidechain was then added by conjugate addition of the appropriate mixed cuprate. This gave predominantly the trans isomer (91), the cis isomer being epimerised with potassium carbonate in methanol to yield exclusively the required trans stereochemistry. Further homologation and selective hydrolysis yielded the aldehyde (92) which was converted to CTA_2 (87) by Wittig reaction and removal of the protecting groups.



Scheme 15

Reagents (i) $Ph_3P{=}CHOMe$ (2eq), THF-toluene, 5h, 0°. (ii) PhSeCl (excess), K_2CO_3, CH_2Cl_2-toluene, -78°, 4h (62% from (77)). (iii) MCPBA, CH_2Cl_2, -78°, 30 min, then Pr^i_2NH (2.2 eq), warming to 25°C (88%). (iv) Cuprate (93), ether, -78°, 4h then after work up K_2CO_3, MeOH, room temp. 12h (53%). (v) $Ph_3P{=}CHOMe$ (1.5 eq) in toluene-THF, 0°, 30 min, then $Hg(OAc)_2$, H_2O-THF, 25°, 1h, then 7% aq KI (79%). (vi) $Ph_3P{=}CH(CH_2)_3CO_2Na$, DMSO, 25°, 1h, then CH_2N_2, 0°, (74%). (vii) AcOH-THF-H_2O (3:2:2), 45°, 10h, then chromatography (65% α-epimer, 33% β-epimer). (viii) 1M LiOH, THF-H_2O, 12h (95-97%).

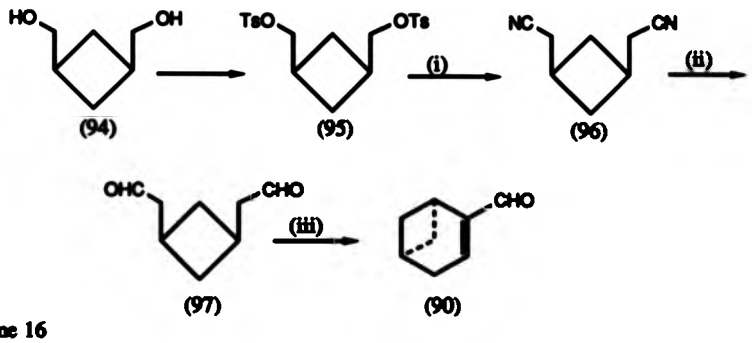


(93)

Scheme 15 Reagents

Finally, the third synthesis, outlined in Scheme 16,[95] started with the cyclobutane diol (94), the tosylate of which (95) was reacted with sodium cyanide to give (96). Reduction afforded the dialdehyde (97) which underwent efficient aldol cyclisation to the α,β-unsaturated aldehyde (90). Subsequent elaboration to CTA_2 was identical to that outlined in Scheme 15.

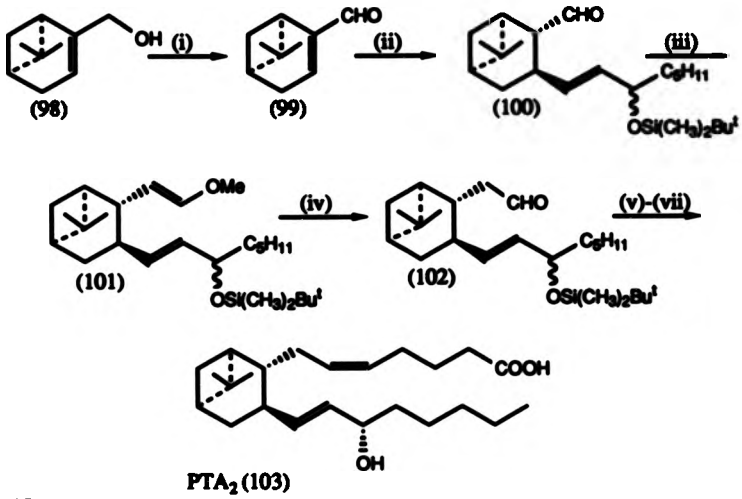
Other carbon analogues prepared have made use of the naturally occurring [3.1.1] bicyclic unit present in the pinane skeleton. Three syntheses of pinane thromboxane A_2 or PTA_2 (103) have appeared in the literature. The first of these (Scheme 17)[96] used (-)myrtenol (98), oxidation of which gave the α,β-unsaturated aldehyde (99). Once more conjugate addition was used to attach the lower sidechain with trans stereochemistry.



Scheme 16

(i) NaCN, DMSO, overnight at room temperature (70%). (ii) Bu^i_2AlH (85%). (iii) Piperidinium Acetate, C_6H_6 reflux, (70%).

Scheme 16 Reagents



PTA_2 (103)

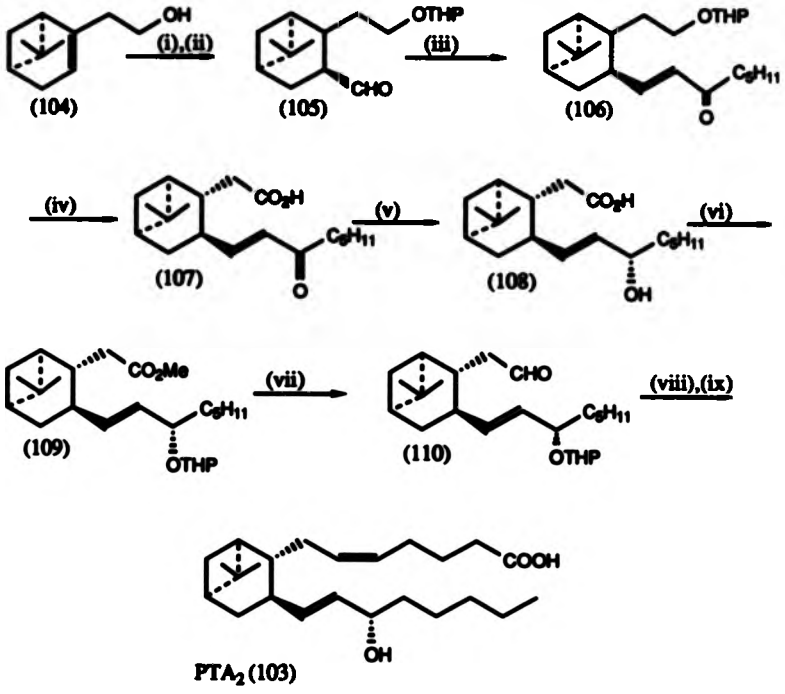
Scheme17

(i) MnO_2, CH_2Cl_2, 25°C, 48h (95%). (ii) Cuprate (93), ether, -78°, 4h then after w/u K_2CO_3, MeOH, rt, 12h (80%).
(iii) $Ph_3P{=}CHOMe$ (1.5 eq) in toluene-THF, 0°C (94%).
(iv) $[Hg(OAc)_2/KI]/H_2O$/THF, (95%-97%).
(v) $Ph_3P{=}CH(CH_2)_3CO_2Na$, DMSO, 25°, 1h, then CH_2N_2, 0°, (80%). (vi) AcOH-THF-H_2O (3:2:2) 45°, then chromatography (1:1 epimeric ratio). (vii) 1M LiOH, THF-H_2O, (95%-97%).

Scheme 17 Reagents

Standard protocol was followed to complete the synthesis.

The second of these syntheses (Scheme 18)[97] used the alcohol, (-)nopol (104). This was initially protected and the formyl group introduced by carbonylation with 9-borabicyclo(3.3.1) borane/trialkoxy lithium aluminium hydride and carbon monoxide. The stereochemistry of this reaction was controlled by addition of the bulky borane reagent on



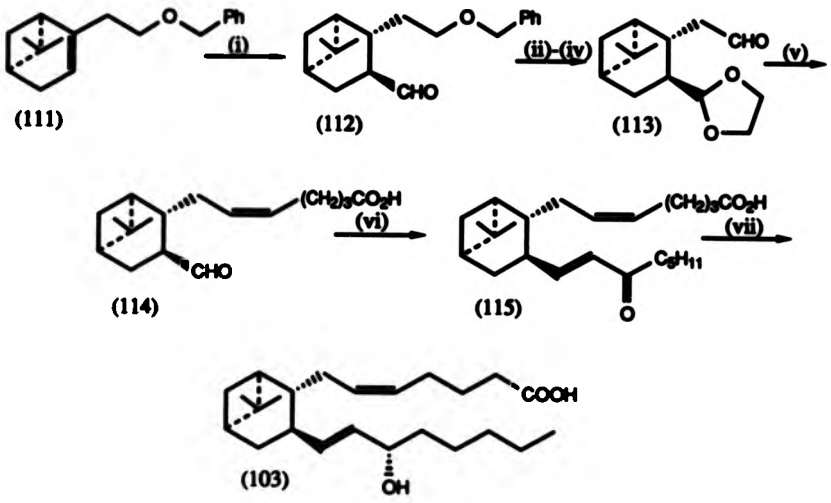
Scheme 18

(i) DHP. (ii) 9-BBN, then CO, $LiAlH(OBu^t)_3$, -35°C, then H_2O_2. (iii) $(MeO)_2P(O)CHCOC_5H_{11}$. (iv) AcOH-H_2O-THF (6:4:1), 40°, then CrO_3, conc H_2SO_4, DMF. (v) L-Selectride, THF, 0°. (vi) CH_2N_2, then DHP. (vii) $LiAlH_4$, then Pyridine chlorochromate. (viii) $Ph_3P = CH(CH_2)_3 CO_2K$, THF. (ix) AcOH-H_2O-THF.

Scheme 18 Reagents

the less hindered face of the molecule. Displacement of the borane then occurs with retention of configuration to yield the trans adduct (105). Having established the correct relative stereochemistry of the side groups normal procedures were followed to attach the prostanoid chains. The same research group[97] also presented a synthesis based on the α,β-unsaturated aldehyde (99); once again the methodology was similar to that previously shown in Scheme 17.

The third synthesis is outlined in Scheme 19.[98] This also started with the alcohol (-)nopol (104). Here the only difference is in the order of attachment of the prostanoid groups and will not be discussed in any further detail.



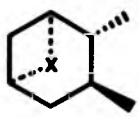
Scheme 19

(i) 9-BBN, $LiAlH(OMe)_3$. CO, THF, then H_2O_2. (ii) glycol/H^+. (iii) NH_3/Na, ether. (iv) PDC, CH_2Cl_2. (v) $Ph_3P = CH(CH_2)_3CO_2Li$, DMSO then H^+/H_2O. (vi) $(MeO)_2 P(O)CHCOC_5H_{11}$. THF. (vii) $Al(OPr^i)_3$, toluene, then chromatography.

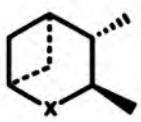
Scheme 19 Reagents

Carbon hetero atom analogues

This series consists of analogues of the type (72) or (73) in which a bicyclic (3.1.1) heptane skeleton incorporates a hetero atom either at position 2 (type 72) or position 6 (type 73).



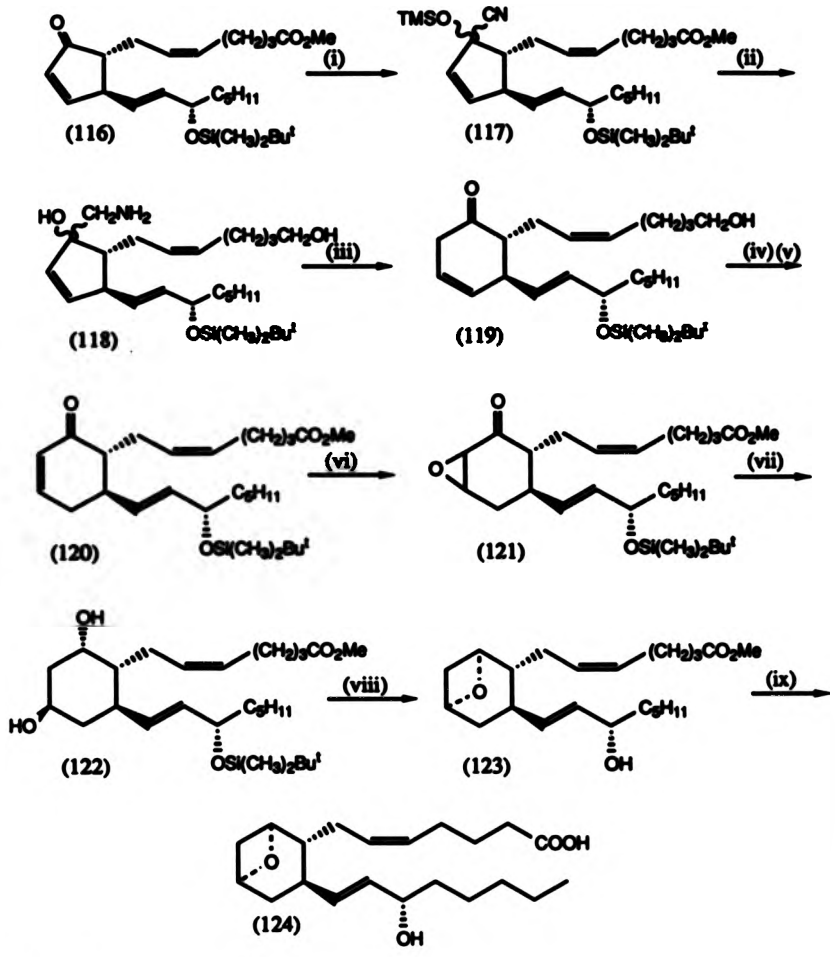
(72)



(73)

Two different synthetic strategies have been developed for the preparation of each type of bicyclic nucleus. Compounds of type (73) have been prepared by using a cyclobutane template to build in the appropriate functionality. Cyclisation of these compounds then created the bicyclic unit and established the stereochemistry of the side groups. Compounds of type (72) have been prepared by adjusting functional groups on a cyclohexane template prior to formation of the bicyclic compound.

The first of the type (72) compounds was prepared by ring expansion of a prostaglandin, as shown in Scheme 20.[99] Prostaglandin A_2 methyl ester (116) was reacted with trimethyl silyl cyanide to yield the protected cyanohydrin (117). Hydride reduction produced the amino alcohol (118) which yielded the ring expanded ketone (119) on treatment with nitrous acid. This then provided the cyclohexane framework with prostanoid sidechains of the correct relative and absolute stereochemistry. Subsequent functional group transformation then furnished the diol (122). This was cyclised to the 9α-11α oxetane (123)



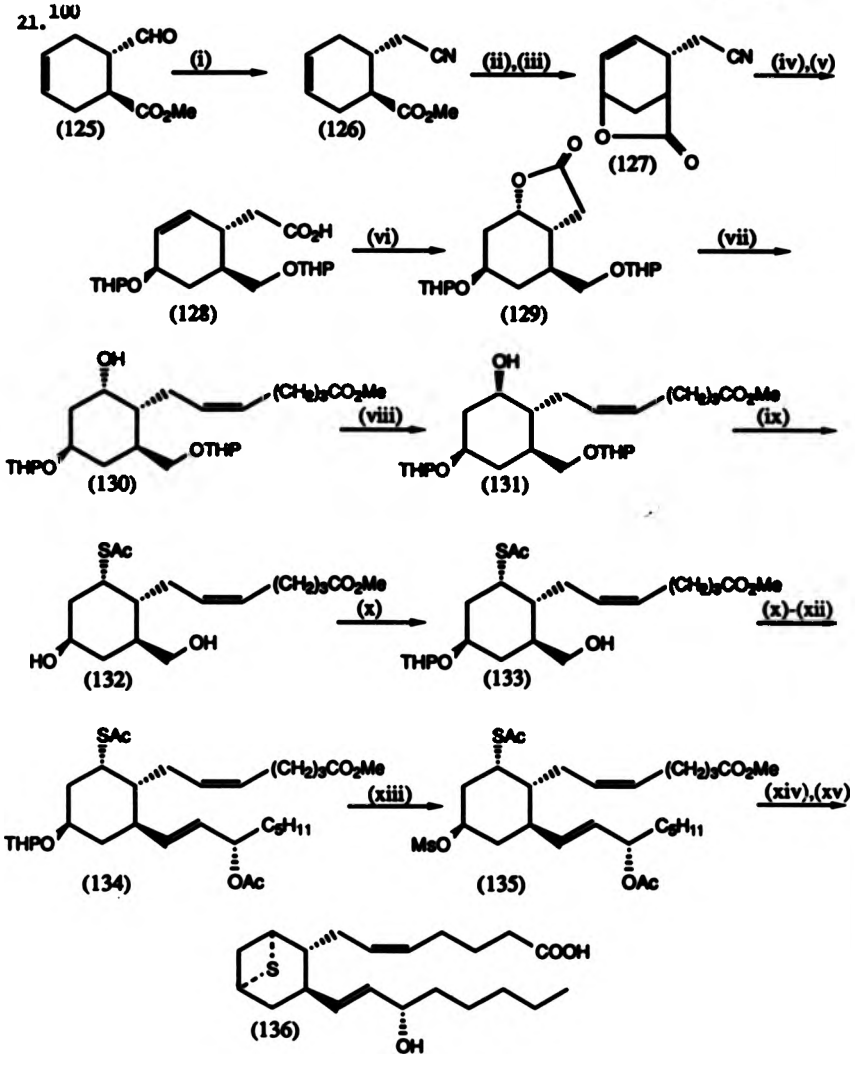
Scheme 20

(i) $(CH_3)_3SiCN$, CH_2Cl_2, (50%). (ii) $LiAlH_4$. (iii) HNO_2, 25°, 10 min (28%). (iv) Basic alumina, THF, 25°, 18h. (v) Jones oxidation, then CH_2N_2, (40%). (vi) H_2O_2, OH^- (2:1 ratio epoxides), chromatographic separation. (vii) Minor epoxide (121), aluminium amalgam, then L-selectride. (viii) $(CF_3SO_2)_2O$ (1 eq), CH_2Cl_2, -78° (25%). (ix) LiOH, THF-H_2O (2:1), 25°, 3h.

Scheme 20 Reagents

in low (20%) but reproducible yields by reaction with trifluoromethane sulphonic anhydride. Deprotection then gave 11-*e*-carbothromboxane A_2 (124).

The synthesis of the sulphur analogue (136) is outlined in Scheme 21.[100]



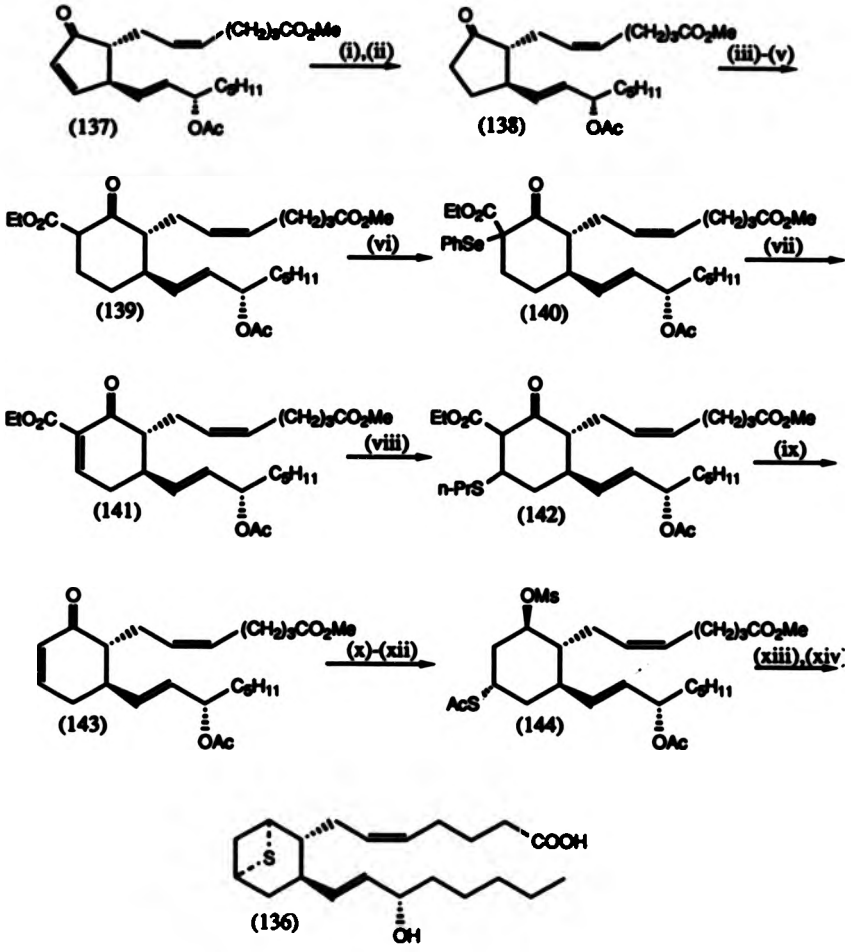
Scheme 21

(i) $NaBH_4$, MeOH then MsCl, then NaCN in HMPA (61%). (ii) KOH aq. (iii) $KI-I_2-KHCO_3$ then DBU, C_6H_6 (60%). (iv) $NaBH_4$, then DHP/H^+. (v) NaOH, H_2O, 100° (70%). (vi) $KI-I_2-KHCO_3$ then $n-Bu_3SnH$ (80%). (vii) Bu^i_2AlH then $Ph_3P=CH(CH_2)_3CO_2Na$ then CH_2N_2 (94%). (viii) PCC then $NaBH_4$, (70%). (ix) MsCl then NaSAc, DMSO, then H_2O/H^+ (30%). (x) $Ph_3SiCl-Et_3N$ then DHP-pTsOH then KF, HMPA (68%). (xi) Collins oxidation then $(MeO)_2P(O)-CH_2COC_5H_{11}$ (71%). (xii) $NaBH_4$, MeOH, separation. (xiii) AcCl, pyr then pyridinium tosylate then MsCl. (xiv) MeONa, 55°, 30 min (94%). (xv) KOH/H_2O.

Scheme 21 Reagents

The aldehyde (125) was prepared by Diels Alder reaction of methyl trans-4-oxobutenoate and butadiene. This reaction formed the basic cyclohexane skeleton and established the correct stereochemistry for the sidegroups. Formation of the thietane bridge was then the remaining problem. Homologation of the aldehyde, then two successive iodolactonisation steps were used to introduce the hydroxyl groups in the required 1,3 relationship. Conversion of C-3 hydroxyl to a thioacetate group and the C-1 hydroxyl to a mesylate gave (135) which was readily cyclised to form the bicyclic thietane. Deprotection then gave the thia-thromboxane A_2 analogue (136).

A chiral synthesis of the sulphur analogue (136) is presented in Scheme 22.[101] As with preparation of the oxygen analogue (124), ring expansion of a prostaglandin was used to obtain a pyran framework with the appropriate sidechains. In this case the prostaglandin derivative (137) was ring enlarged to yield the keto ester (139), which was subsequently transformed to the enone (143). Michael addition of thioacetate, reduction and mesylation of the C-3 hydroxyl provided the cyclisation precursor (144) which ultimately yielded the chiral product (136).

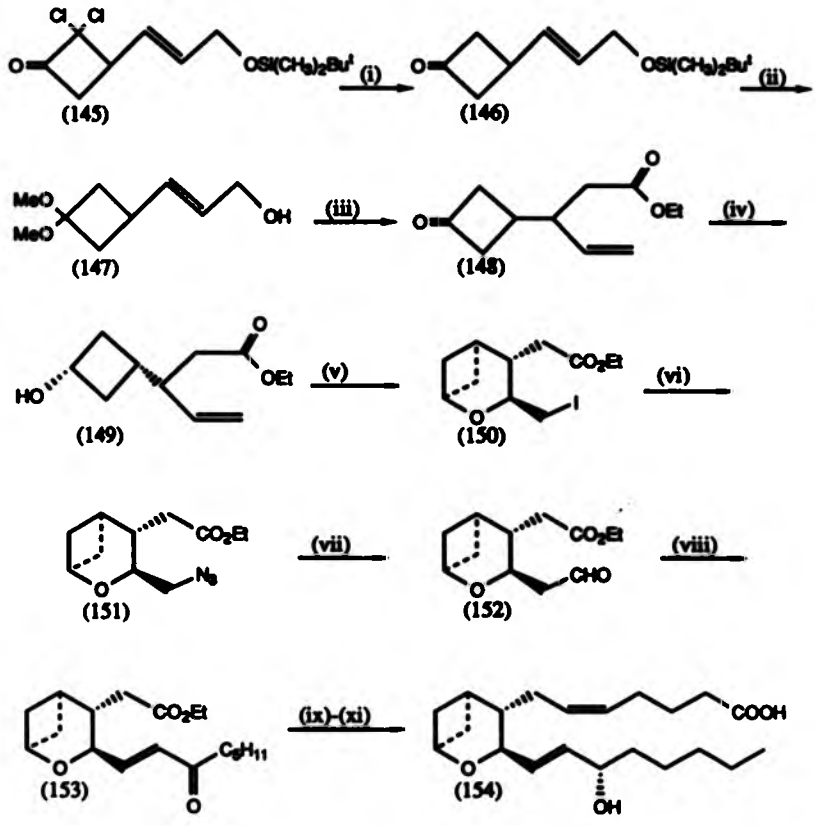


Scheme 22

(i) $NaBH_4$. (ii) PCC. (iii) $N_2CHCOOEt$, $BF_3O(Et)_2$.
(iv) $NaBH_4$, separation. (v) Collins oxidation. (vi) NaH, PhSeCl.
(vii) H_2O_2. (viii) nPrSH, Pr^i_2NH, DMF. (ix) NaCl, DMSO, 150°. (x) CH_3COSH, -78°. (xi) $Zn(BH_4)_2$. (xii) MsCl, py.
(xiii) NaOMe. (xiv) OH^-, then H_3O^+.

Scheme 22 Reagents

The first of the series with a 9,11 bridging methylene group (Type 73) is outline in Scheme 23.[102] 2,4-Pentadiene-1-ol was protected as a silyl ether and reacted with dichloro ketene to yield the cyclobutanone (145).



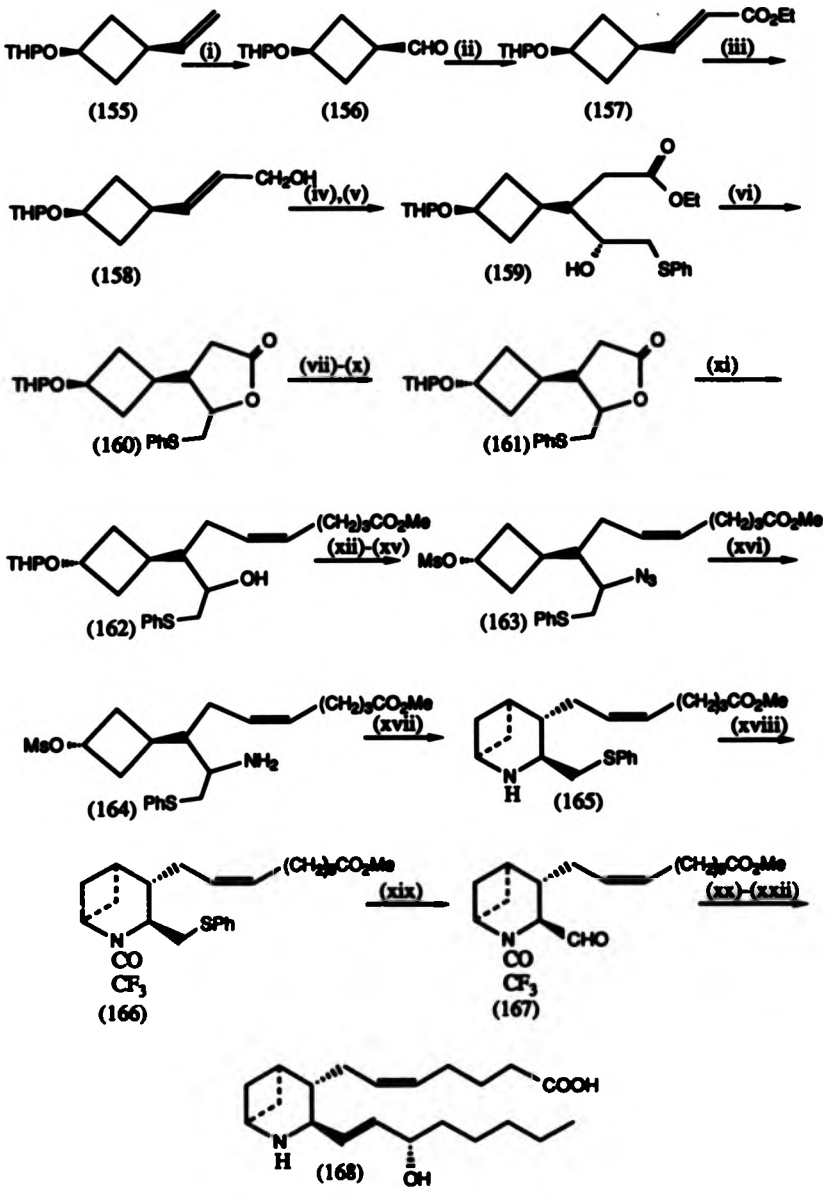
Scheme 23

(i) Zn/Cu/AcOH. (ii) H^+, MeOH. (iii) $(EtO)_3CCH_3$, propionic acid, 142°. (iv) $NaBH_4$, -60°. (v) $Hg(OAc)_2$, C_6H_6 then I_2. (vi) NaN_3, DMF, 100°. (vii) $MeOSO_2F$, 0°, CH_2Cl_2. (viii) $(MeO)_2P(O)CH_2COC_5H_{11}$. (ix) $ZnBH_4$,DME, 23°. (x) Bu^i_2AlH. (xi) $Ph_3P{=}CH(CH_2)_3CO_2Na$.

Scheme 23 Reagents

This was converted in a number of steps to the cis alcohol (149), which was cyclised to yield the bicyclic ester (150). This had exclusively the trans stereochemistry, the cis being unfavourable due to steric restrictions. This key step had therefore provided both the bicyclic nucleus and the correct relative stereochemistry for the sidechains. The synthesis was completed by displacement of iodide with azide and then conversion of this to the aldehyde (152), from which standard methodology followed.

A similar strategy was used to prepare the nitrogen analogue (168) (Scheme 24).[103] The cyclobutane derivative (155) was used as a building block to construct the cyclisation precursor (164). Reaction with sodium hydride in dimethyl formamide then provided the bicyclic amine (165) which was protected as the trifluoroacetyl amide (166). Once again exclusively trans stereochemistry resulted from cyclisation due to unfavourable steric requirements for the cis isomer. Oxidation of the sulphide and Pummerer reaction yielded the aldehyde (167) which permitted attachment of the lower sidechain.

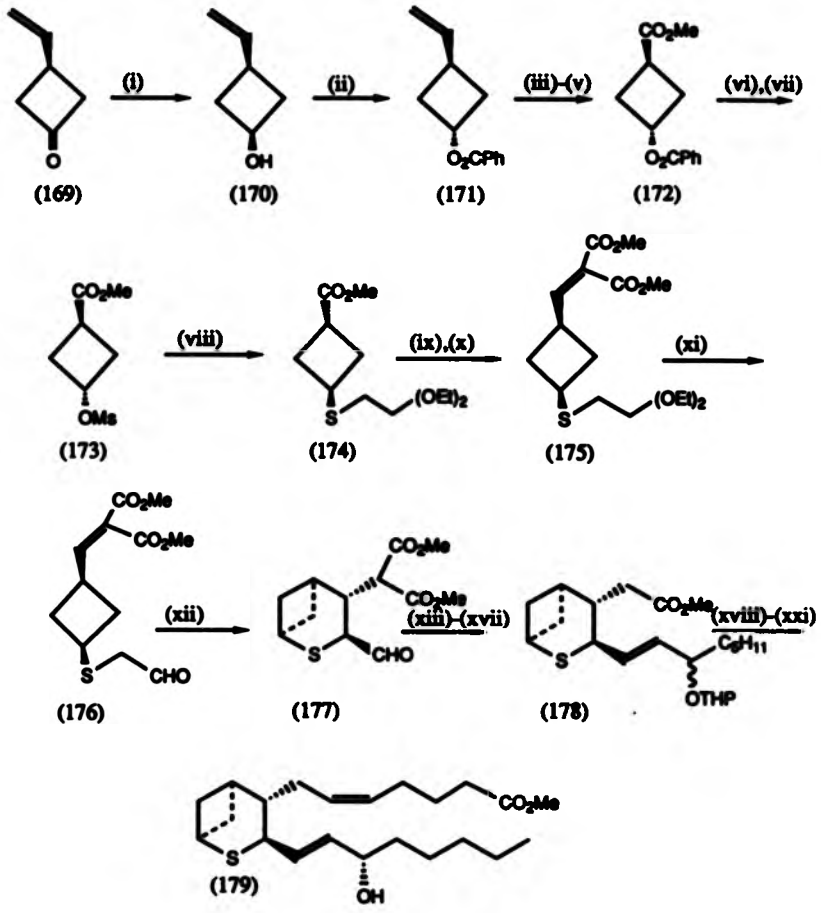

THPO
(i)
CHO
(ii)
CO2Et
(iii)
(155)
(156)
(157)
CH2OH
(iv),(v)
OEt
(vi)
HO
SPh
(158)
(159)
(vii)-(x)
(xi)
PhS
(160)
(161)
(CH2)3CO2Me
(xii)-(xv)
OH
(162)
MsO
N3
(xvi)
(163)
NH2
(xvii)
(164)
(xviii)
(165)
(xix)
CO
CF3
(166)
CHO
(xx)-(xxii)
(167)
COOH
(168)

Scheme 24

(i) OsO_4-$NaIO_4$. (ii) $Ph_3P{=}CHCO_2Et$ (80% from (155)). (iii) Bu^i_2AlH, -70°, toluene (73%). (iv) $(EtO)_3CCH_3$ (7 eq), pivalic acid, 160°, 1h (92%). (v) m-CPBA then PhSH, Et_3N, MeOH. (vi) H^+. (vii) H_2O, H^+. (viii) EtOCO-N=N-COOEt, Ph_3P, HCO_2H. (ix) K_2CO_3, MeOH. (x) DHP, H^+. (xi) Bu^i_2AlH then $Ph_3P{=}CH(CH_2)_3COONa$ then CH_2N_2, (63%). (xii) MsCl, Et_3N. (xiii) NaN_3, HMPA, 40°, 50h. (xiv) H_2O, H^+. (xv) MsCl, Et_3N (23% xii→xv). (xvi) $CrCl_2$. (xvii) NaH, DMF, 40°, 34h. (xviii) $(CF_3CO)_2O$ (30%, xvii→xviii). (xix) $NaIO_4$ then $(CF_3CO)_2O$ then $NaHCO_3/H_2O$. (xx) $(MeO)_2P(O)CHCOC_5H_{11}$. (xxi) $NaBH_4$, EtOH then chromatography (1:1 epimeric mixture). (xxii) LiOH, MeOH, 40°, 2h, H_3O^+

Scheme 24 Reagents

Finally, Scheme 25[104] outlines the synthesis of the regioisomeric sulphur analogue (179). In this case the vinyl ketone (169) was converted to the diester (176). This then underwent an intramolecular Michael addition to yield the bicyclic diester with trans stereochemistry. Attachment of the lower prostanoid chain and thermal decarboxylation followed to give (178), which was subsequently converted to the methyl ester (179).



Scheme 25

(i) $NaBH_4$, MeOH, (90%). (ii) EtOCO.N=N-COOEt, Ph_3P, PhCOOH, THF, 0° (87%). (iii) $NaIO_4$-OsO_4. (iv) Jones oxidation.
(v) CH_2N_2 (70%). (vi) K_2CO_3, MeOH. (vii) MsCl, Et_3N, (87% iv-v). (viii) $HSCH_2CH(OEt)_2$, DMSO, 50°, (68%).
(ix) Bu^i_2AlH. (x) Dimethyl malonate/pyrrolidine acetate cat, (87%). (xi) H^+ H_2O. (xii) Pyrrolidine acetate, C_6H_6, 25° (36%). (xiii) $Bu_3P{=}CHCOC_5H_{11}$. (xiv) $NaBH_4$. (xv) DHP/H^+.
(xvi) NaOH, H_2O. (xvii) quinoline, 160°, 30 mins (72%), CH_2N_2.
(xviii) Bu^i_2AlH. (xix) $Ph_3P{=}CH(CH_2)_3COONa$. (xx) CH_2N_2.
(xxi) H_2O, H^+, then separation.

Scheme 25 Reagents

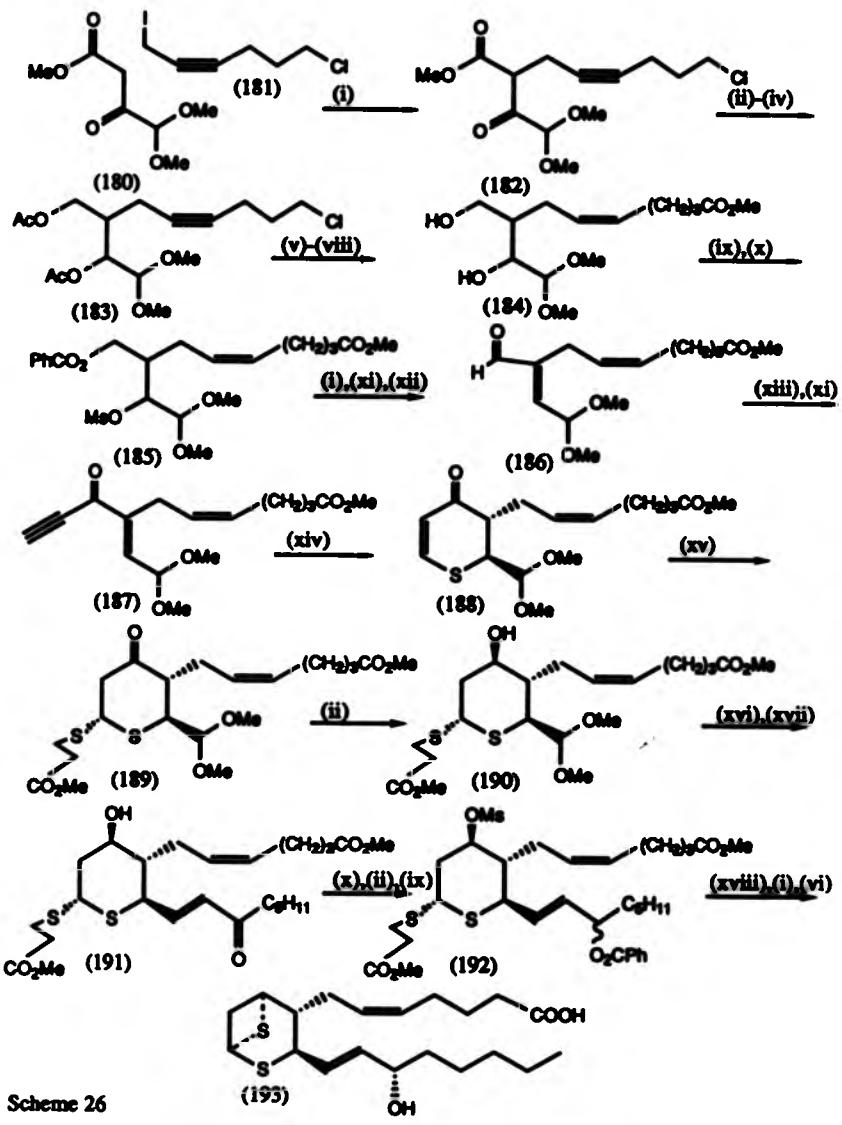
Heteroatom analogues

This series consists mainly of compounds where sulphur replaces either or both of the oxygen atoms in the bicyclic framework. However, for completeness the recent synthesis of thromboxane A_2 itself is also included.

The strategy adopted for these syntheses was primarily to construct a six-membered heterocyclic ring with the appropriate functionality in position. Formation of the bicyclic nucleus was then completed by closure of either an oxetane or thietane bridge.

The synthesis of dithia thromboxane A_2 (193) is outlined in Scheme 26.[105] Methyl 4,4-dimethoxy-acetoacetate (180) was reacted with 6-chloro-1-iodo-2-hexyne (181) to give (182). Subsequent conversion[0] ultimately yielded the acetylenic ketone (187) which provided the basic carbon skeleton. This was then cyclised by exposure to hydrogen sulphide, to yield predominantly the trans dihydro thiopyranone (188). Conjugate addition of methyl 3-mercaptopropanoate introduced the necessary sulphur nucleophile for thietane formation. Selective reduction of the keto group provided a potential leaving group at C-3. Attachment of the lower prostanoid chain followed and mesylation of the C-3 hydroxyl gave (192).

Cyclisation to the 2,6-dithia bicyclo (3,1,1) heptane skeleton was achieved using potassium t-butoxide in hexamethylphosphoramide (HMPA). Finally, separation of the C-15 epimers and deprotection provided dithia-thromboxane A_2 (193).

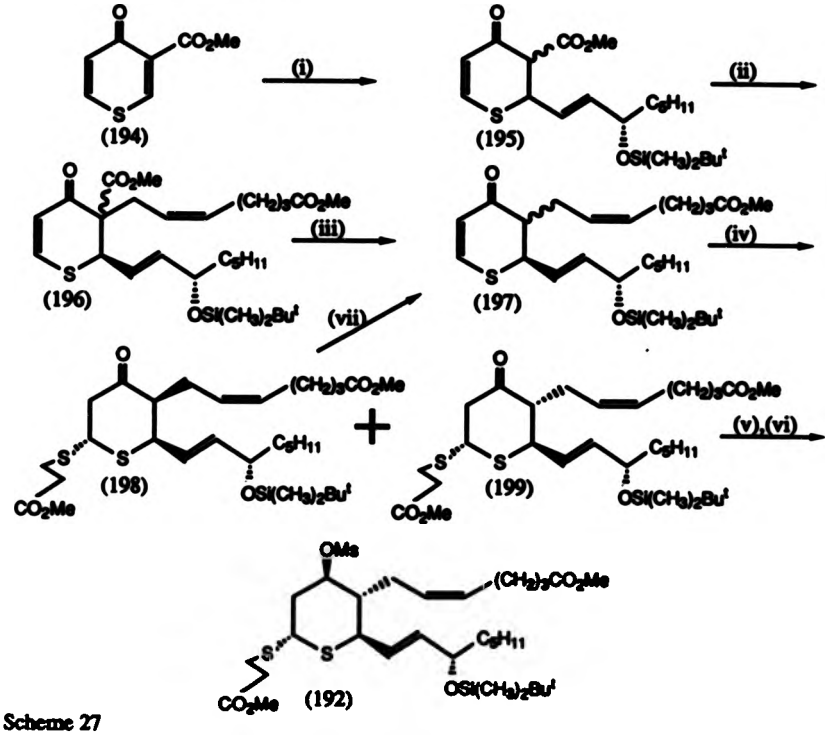


Scheme 26

(i) NaOMe. (ii) $NaBH_4$. (iii) Bu^i_2AlH. (iv) CH_3COCl, py.
(v) NaCN. (vi) NaOH aq. (vii) CH_2N_2. (viii) Lindlar cat.
(ix) PhCOCl, py. (x) MsCl, Et_3N. (xi) Collins Oxidation.
(xii) DBU. (xiii) LiC CH, HMPA, TMEDA. (xi) (xiv) H_2S, NaOAc.
(xv) $HS(CH_2)_2CO_2Me$, $(Pr^i)_2NH$, DMF. (xvi) H_3O^+.
(xvii) $Bu_3P{=}CHCOC_5H_{11}$. (x) MsCl, py. (xviii) ^tBuOK, HMPA, separation.

Scheme 26 Reagents

Scheme 27[106] illustrates another synthesis of dithiathromboxane A_2 (193) which employed the intermediate mesylate (192). Thiopyranone (194) underwent conjugate addition, anionic displacement of halogen and decarboxylation to yield the dihydrothiopyranone (197) as a cis, trans mixture. Michael addition of methyl 3-mercaptopropanoate furnished the adducts (198) and (199) which were chromatographically separable. The trans isomer (199) was subsequently converted to mesylate (192) and the cis (198) could be recycled.

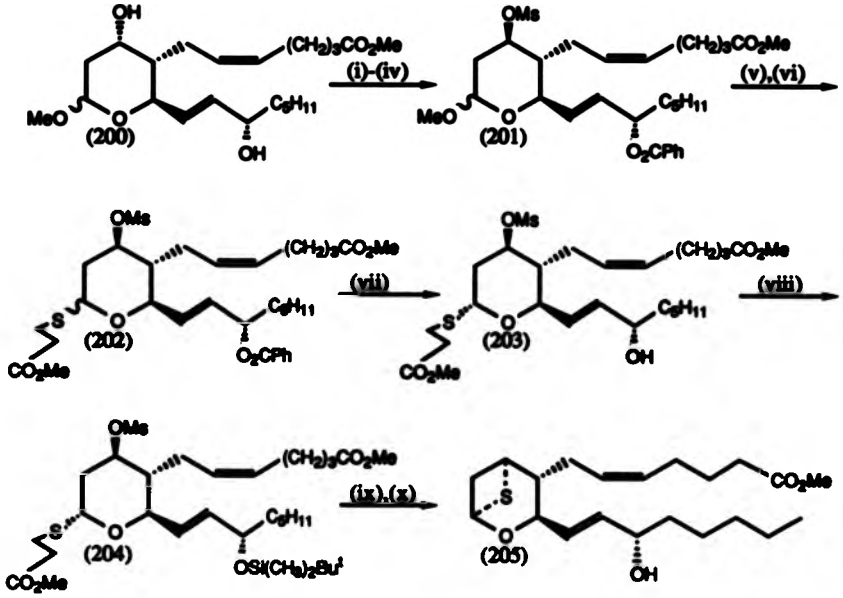


Scheme 27

(i) $(-CH{=}CHCH(OSiMe_2Bu^t)C_5H_{11})_2CuLi$. (ii) NaH, $ICH_2CH{=}CH(CH_2)_3CO_2Me$. (iii) $MgCl_2$, $DMF-H_2O$. (iv) $HSCH_2CH_2CO_2Me$, Pr^i_2NEt. (v) $NaBH_4$. (vi) MsCl, py. (vii) NaOMe.

Scheme 27 Reagents

This strategy for constructing the bicyclic system was also used to synthesise 9α, 11α-thiathromboxane A_2 (205), (Scheme 28).[107] The thromboxane B_2 derivative (200) was used as a starting material, thereby providing a pyran framework with the sidechains in position. Inversion of stereochemistry at C-3 and mesylation created the appropriate leaving group. Hydrolysis of the methyl acetal and reaction with methyl-3-mercapto propanoate introduced the sulphur atom, separation of the appropriate anomer and reaction with base gave the methyl ester (205).

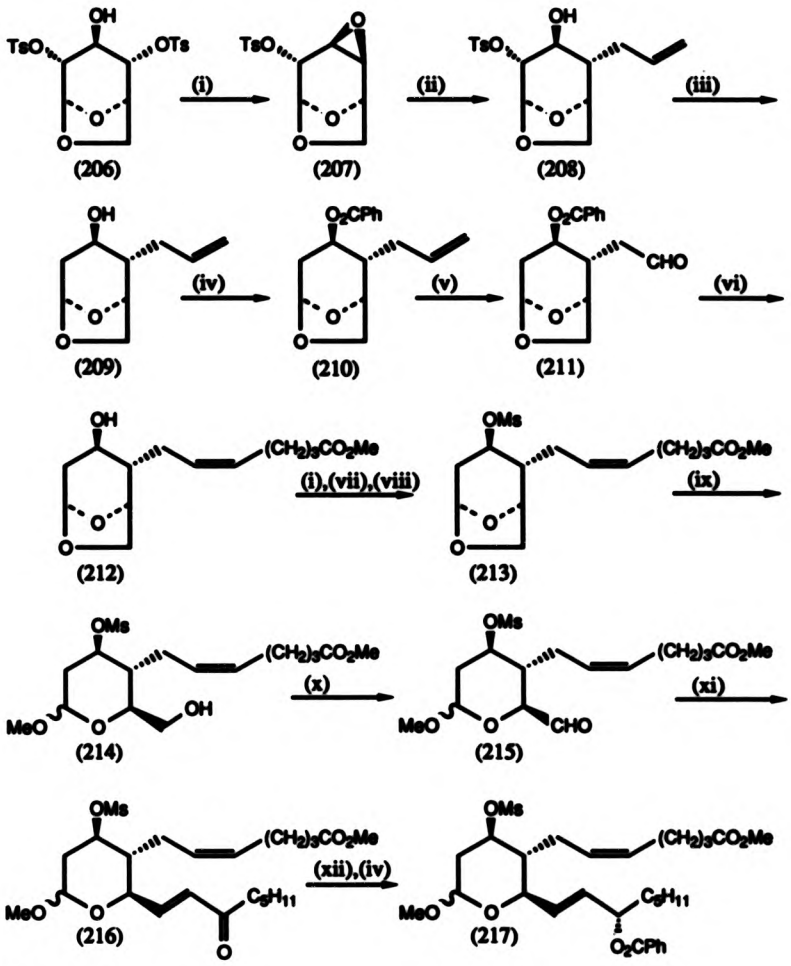


Scheme 28

(i) PhCOCl, py. (ii) $EtO_2CN{=}NCO_2Et$, Ph_3P, HCOOH. (iii) $NaHCO_3$, MeOH. (iv) MsCl, Et_3N. (v) H_3O^+. (vi) $SH(CH_2)_2CO_2Me$, $BF_3O(Et)_2$. (vii) NaOMe, separation. (viii) $^tBu(CH_3)_2SiCl$, py. (ix) NaN $(TMS)_2$, HMPA. (x) Bu_4NF.

Scheme 28 Reagents

The preparation of a key synthetic intermediate similar to (201) is shown in Scheme 29.[108] This started with the allylic alcohol (209) previously described by Kelly and Roberts.[109] This key compound provided the necessary pyran framework with trans stereochemistry. The synthesis was then completed by attaching the prostanoid sidechains and conversion of the C-3 alcohol to a mesylate.

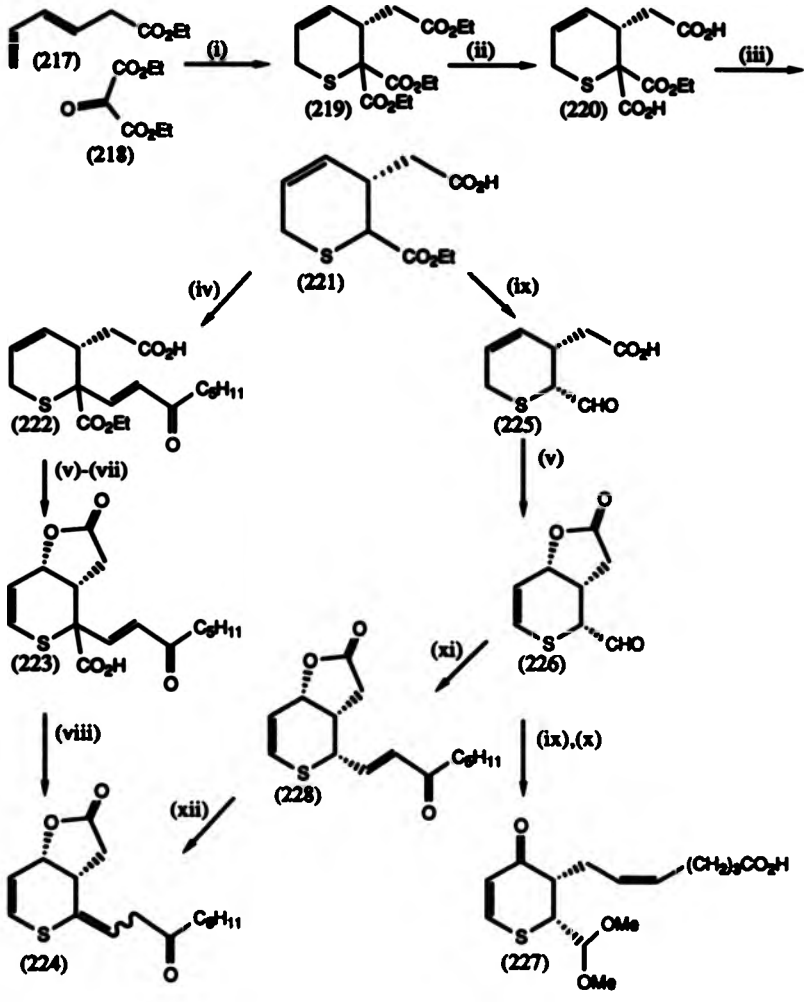


Scheme 29

(i) MeONa . (ii) BrMg CH_2CHCH_2\CuI. (iii) Et_3BHLi .
(iv) PhCOCl, py. (v) O_3,$(CH_3)_2S$. (vi) $Ph_3P{=}CH(CH_2)_3COONa$.
(vii) CH_2N_2. (viii) CH_3SO_2Cl/Py. (ix) MeOH, IRA 120
(H^+). (x) Collins Oxidation. (xi) $(CH_3O)_2POCH_2CO(C_5H_{11})$.
(xii) $NaBH_4$, separation.

Scheme 29 Reagents

More recently, further synthetic approaches to the synthesis of these sulphur analogues have been published by Sutherland *et al.*[110,111]. Scheme 30 outlines two different strategies adopted, which were based on the functionalised dihydrothiopyran (221).



Scheme 30

(i) P_4S_{10}, reflux 20h. (ii) EtOH, NaOH. (iii) , 130°.
(iv) $LiPr^i_2N$, $ClCH{=}CHCOC_5H_{11}$. (v) N-Chloro succinimide.
(vi) $NaBH_4$. (vii) LiOH. (viii) Jones oxidation. (ix) Bu^i_2AlH, -78°. (x) $Ph_3PCH(CH_2)_3CO_2Na$.
(xi) $(CH_3O)_2P(O)-CH_2COC_5H_{11}$. (xii) $KOBu^t$-THF.

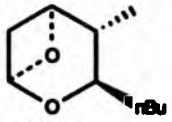
Scheme 30 Reagents

Attachment of the lower prostanoid chain and lactonisation yielded (223). However, decarboxylation resulted in isomerisation to the β,γ-unsaturated ketones (224). It proved to be impossible to reconvert these to the α,β-enone required.

Reduction of the dihydrothiopyran (221) yielded the cis aldehyde (225) which was subsequently lactonised to provide (226). Protection of the aldehyde, attachment of the top side chain and subsequent oxidation gave the cis ketone (227). This compound proved to be extremely difficult to epimerise to the necessary trans stereochemistry. Moreover, attachment of the lower side chain to the aldehyde (226) provided the lactone (228), which upon treatment with base immediately resulted in isomerisation to the - unsaturated ketone (224).

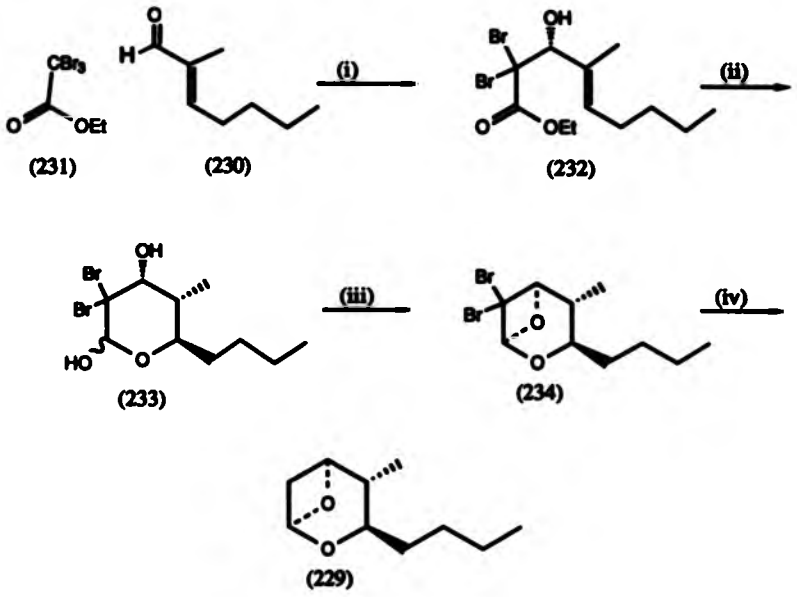
Due to the attendant difficulties of isomerisation described above and the undesirable biological activity of these analogues, reported by Hamanaka[105,106] these synthetic approaches were discontinued.

Finally, the introduction is concluded by a description of the superlative synthesis of thromboxane A_2 (15) itself from thromboxane B_2 (14).[112] However, prior to this fine synthetic achievement Still et al tackled the tricky problem of securing the labile 2,6-dioxabicyclo(3,1,1) heptane framework. This was carried out by model studies in the preparation of the oxetane (229), Scheme 31.[113]



(229)

Reformatsky reaction of aldehyde (230) with ethyl tribromoacetate (231) yielded the dibromo ester (232). This was cyclised to the dibromo hemiacetal (233) by hydroboration and work up with hydrogen peroxide. The key cyclisation to the bicyclic oxetane was accomplished by a modified Mitsunobo reaction. Finally, radical debromination provided the unsubstituted oxetane (229).

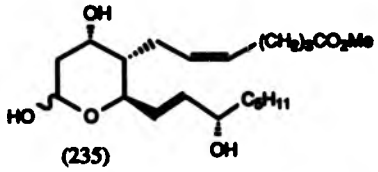


Scheme 31

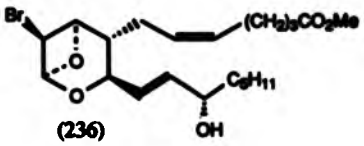
(i) Zn
(ii) $ThBH_2$, H_2O_2. (iii) $EtO_2CN{=}NCO_2Et$, $(MeO)_3P$.
(iv) Bu_3SnH.

Scheme 31 Reagents

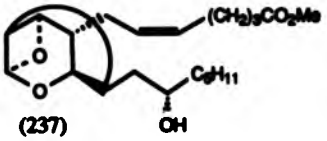
Application of this synthetic strategy to thromboxane B_2 methyl ester (235) resulted in the bromo oxetane (236). However, radical debromination of the oxetane led to an unwanted cyclisation reaction to yield (237).



(235)



(236)

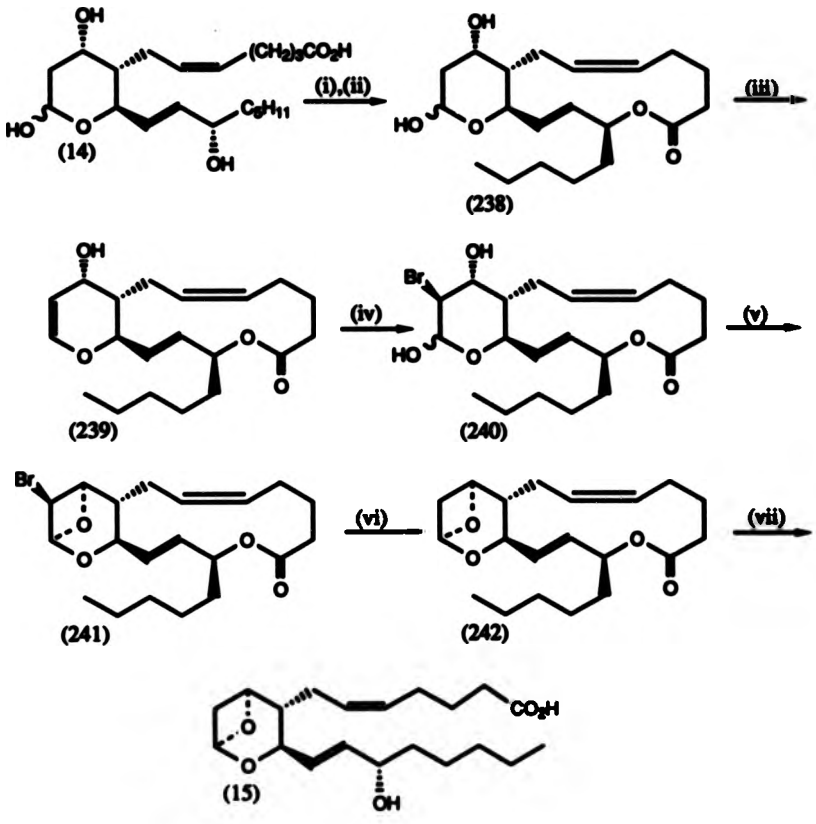


(237)

In order to circumvent this problem the prostanoid sidechains were conformationally restricted by formation of the 1,15-macrolactone (238) (Scheme 32). This was dehydrated to the glycal (239) and immediately converted to the bromohydrin (240). The key cyclisation to the dioxabicyclo (3.1.1) system was achieved using the Mitsunobo methodology previously developed. This gave low (20%) but reproducible yields of the oxetane (241). Radical debromination gave the macrolactone (242) and saponification with sodium hydroxide or potassium t-butoxide gave a compound with biological activity identical to natural thromboxane A_2.

Still comments that although this synthesis corroborated the original structural proposition made by Samuelson, it was not absolutely

conclusive. It was noted that the results were also compatible with *in vivo* conversion of (15), perhaps by addition of a biological nucleophile at C-11 to a derivative which is the endogeneous TXA_2.



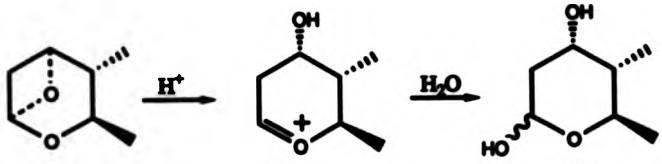
Scheme 32

(i) PyrSCOCl, Et_3N, Et_2O/CH_2Cl_2 0°C. (ii) $PhCH_3$, 110°C, 13 hrs. (iii) 2-chloro-1-methylpyridinium iodide, Et_3N. (iv) NBS, Et_2O, H_2O. (v) $(MeO)_3P$, DEAD. (vi) Polymer bound tin hydride. (vii) NaOH, MeOH or THF, $KOBu^t$.

Scheme 32 Reagents

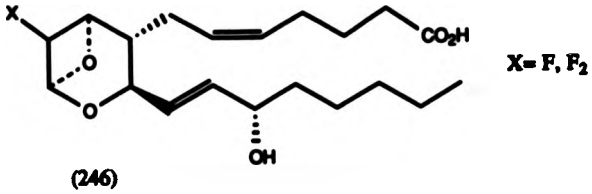
Where it has been determined, the biological activity of these structural analogues appears to be very potent. Most compounds possess arterial contractile activity and others also have platelet aggregatory activity. Unfortunately, for cardiovascular therapy these agonistic properties are undesirable.

The inherent instability of TXA_2 results from a hydrolytically labile functionality constrained within a bicyclic nucleus. That is, the relief of steric strain must facilitate the normal mechanism of acetal hydrolysis, in this case forming the oxonium ion (244), Scheme 33.



Scheme 33

The previous structural analogues of TXA_2 have sought to incorporate a more stable bicyclic unit by replacement of either one or both of the oxygen atoms. However, it is arguable that a 10-fluoro TXA_2 analogue (246) should also be stable. This would be a result of the electronegativity of fluorine destabilising the intermediate oxonium ion (244).



(246)

Furthermore, it is conceivable that a combination of the isosteric, electronegative, and lipophilic properties thus introduced would provide not only a stable structural analogue, but a compound altered enough so as to possess receptor antagonist properties.

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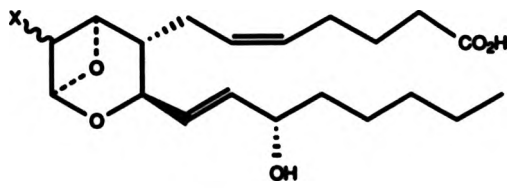
CHAPTER TWO

DISCUSSION

2.1 Introduction

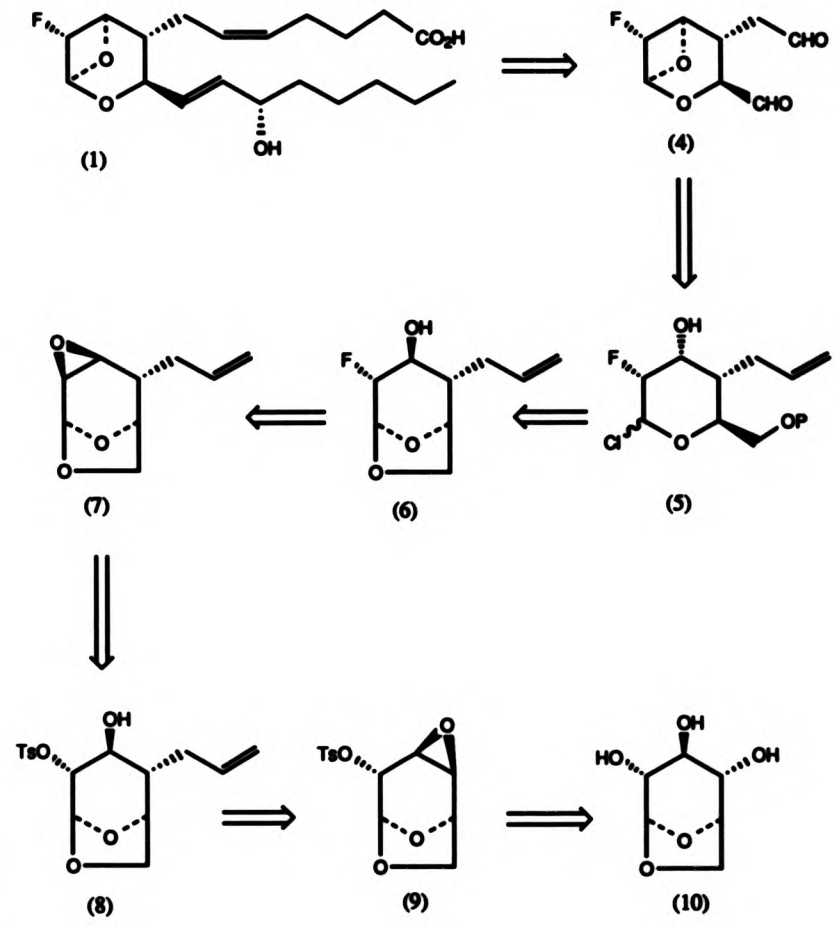
For the reasons outlined in section 1.3 (page 40) the aim of this project was to achieve a chiral synthesis of a 10-fluoro substituted thromboxane A_2 (1)-(3).



(1) X=α fluoro
(2) X=β fluoro
(3) X= difluoro

Analysis of this synthetic challenge identified four major problems. The first of these was the choice of an appropriate chiral starting material which would lead to the correct absolute stereochemistry of the TXA_2 derivative. The second consideration was the attachment of the two prostanoid sidechains with the required trans stereochemistry. The third point was the introduction of the fluorine substituent with, in the case of (1) and (2), the correct relative stereochemistry. Finally and potentially the most difficult hurdle was the attainment of the sensitive oxetane ring in terms of its stereochemistry and the timing of its introduction. Having identified these individual problems a synthetic strategy had to be devised in which all of them were taken into account, since they each impinged to a greater or lesser extent on one another.

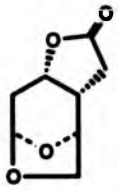
Taking the 10-αderivative (1) as the synthetic target, the retro-synthetic plan which was initially conceived is outlined in Scheme 1.


F
O
O
CO2H
OH
(1)
F
O
O
CHO
CHO
(4)
O
O
(7)
OH
F
O
O
(6)
OH
F
Cl
O
OP
(5)
OH
TsO
O
O
(8)
TsO
O
O
O
(9)
OH
HO
OH
O
O
(10)

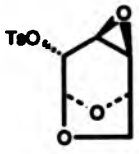
Scheme 1

The synthesis is based on the use of the 1,6-anhydro sugar levoglucosan (10). Its use as a chiral building block has previously been demonstrated not only in the synthesis of thromboxanes[1,2] but also in the preparation of ionophores,[3] avermectins[4] and macrolide antibiotics[5] to name a few.

Levoglucosan (10) can be readily converted to the epoxytosylate (9) by procedures outlined by Cerny et al.[6] Conversion of this to the allyl tosylate (8) was described by Kelly and Roberts[1] in an elegant synthesis of the known thromboxane B_2 intermediate (11). The stereospecific introduction of the allyl group by cleavage of the epoxide (9) solved the stereochemical requirements for attaching the prostanoid sidechains. The utility of this reaction was also demonstrated by its use in the chiral synthesis of thiathromboxane A_2.[2]



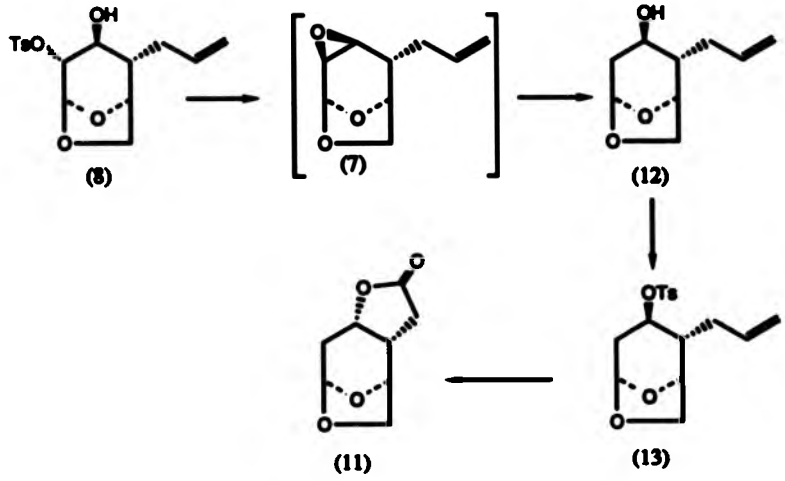
(11)



(9)

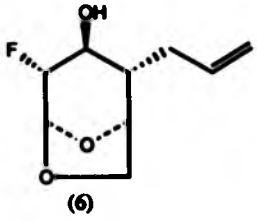
Kelly and Roberts[1] proposed that the allyl epoxide (7) was an intermediate in the lithium triethylborohydride reduction of the allyl tosylate (8) to the alcohol (12). This alcohol was then converted to the bicyclic lactone (11) by tosylation and oxidative lactonisation, Scheme 2.

It was anticipated that preparation and isolation of the epoxide (7) would allow introduction of fluorine by epoxide cleavage. Due to the rigidity of the pyran ring in 1,6-anhydro sugars and the known



Scheme 2

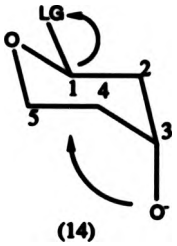
stereochemical requirements of epoxide cleavage[7] the trans diaxial 2-fluorohydrin (6) was the expected product.



This then would solve the problem of regio and stereospecific introduction of fluorine.

The problem of constructing the dioxa bicyclo[3.1.1]heptane unit then remained. From an inspection of models and previous syntheses of thromboxane A_2 analogues[2,8-12] the following relationships between

internal nucleophile and leaving group are required for oxetane formation. Either an equatorial leaving group at C-1 and an axial nucleophile at C-3 (14), or an equatorial leaving group at C-3 and an axial nucleophile at C-1 (15) would yield oxetanes of the correct stereochemistry.



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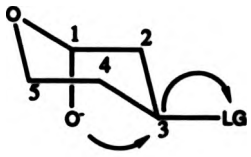
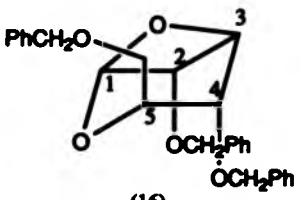
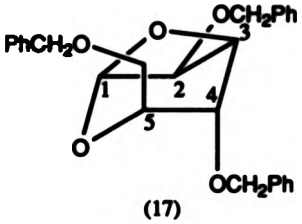


(15)

During the original planning of the synthesis the only known 2,6-dioxabicyclo (3.1.1) heptanes were the gluco and manno pyranose derivatives (16) and (17) that had been prepared by Scheurch.[13,14]

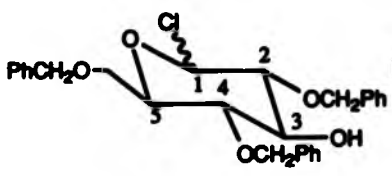


(16)

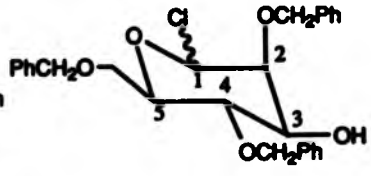


(17)

These had been prepared by reaction of the corresponding glycosyl chlorides (18) and (19) with base. Here a conformational change results in the halide ion being displaced by an axially orientated alkoxide at C-3 to form the oxetane.



(18)

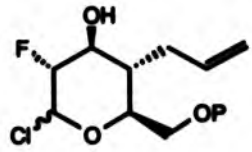


(19)

It was proposed by Scheurch that the unusual stability of the strained system was due to the electronegativity of the oxygen substituent at C-2 in the carbohydrate molecule. This therefore added further credence to the argument for stabilising the bicyclic oxetane with a fluorine substituent.

The above examples have an oxetane bridge with a β-configuration relative to the pyran ring instead of the α-configuration present in thromboxane A_2. However, using this cyclisation as a precedent it was hoped that the glycosyl chloride (20) would be available by cleavage of the 1,6-anhydro bridge with titanium tetrachloride. The required 1,3 relationship between the nucleophile and leaving group could then be created by inverting the stereochemistry of the hydroxyl group at C-3 to give (5). This should be possible by use of an adapted Mitsunobo reaction.[16]

Cyclisation and standard thromboxane/prostaglandin protocol should then allow completion of the synthesis.



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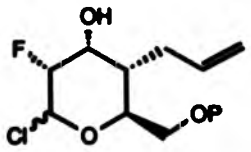
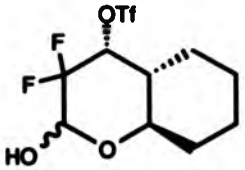
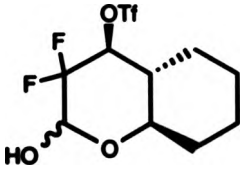


(5)

However, just prior to the start of this project a further synthesis of a bicyclic(3.1.1)oxetane was published. Fried et al[17] synthesised the anomeric triflates (21) and (22).

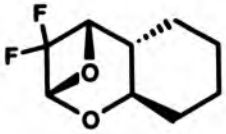


(21)

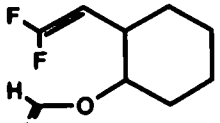


(22)

The 3-α anomer with an axial triflate (21) cyclised under basic conditions to give the bicyclic oxetane (23). However, the 3-β anomer (22) under the same conditions fragmented to yield the difluoro olefin formate (24).

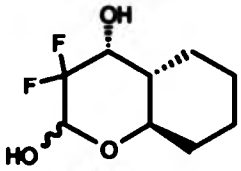


(23)



(24)

As with compounds (16) and (17) the compound (23) also had the 'incorrect' or β-configuration of the oxetane bridge. In order to produce a model compound with the correct stereochemistry relative to thromboxane A_2, the hemiacetal with an axial hydroxyl at C-3 (25) was converted to the C-1 mesylate (26) and cyclised to yield the bicyclic oxetane (27).



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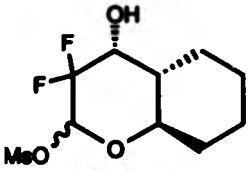

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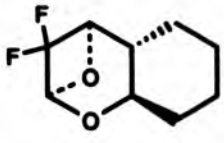
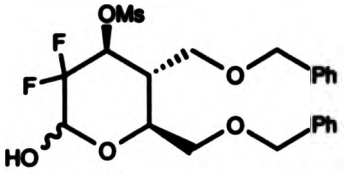
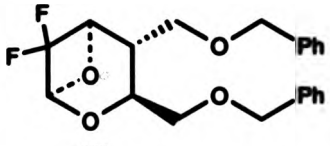


(27)

Fried argued that the rigid ring structure of triflate (22) had allowed the fragmentation process to occur more easily. To prove this hypothesis he prepared the 3β-mesylate (28), which cyclised smoothly to yield the bicyclic(3.1.1)oxetane (29).



(28)



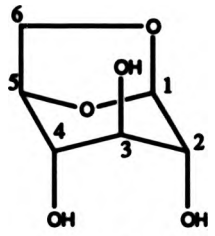
(29)

Kinetic studies on the hydrolysis of (29) revealed a 10^8 fold decrease in rate relative to natural thromboxane A_2, similarly a 10^2 fold decrease in rate relative to normal unstrained acetals was noted. This confirmed that fluorine would indeed stabilise the dioxa(3.1.1) heptane structure.

This therefore provided further precedent for the preparation of strained bicyclic oxetanes. Moreover, it would allow more flexibility to be built into the synthetic strategy.

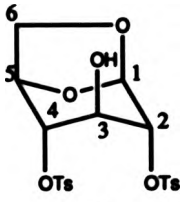
2.2 Synthesis of a functionalised bicyclic (3.1.1) oxetane, a precursor to 10-α-fluoro thromboxane A_2.

The compound levoglucosan (10) is a conformationally rigid molecule and is locked in a $^1C_4$ conformation with all the hydroxyl groups axially orientated. Extensive investigations by Cerny et al[18] have revealed that the trans diaxial arrangement of the hydroxyl groups coupled with the steric and electrostatic effects of the 1,6-anhydro bridge impart a large degree of selectivity in the reactions of levoglucosan (10).

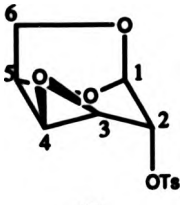


(10)

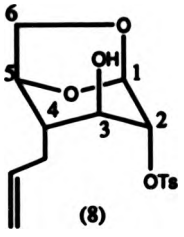
This was immediately obvious from the selectivity observed in the preparation of the functionalised derivatives (30) and (9); this also serves to emphasise the utility of levoglucosan for the synthesis of other chiral compounds.



(30



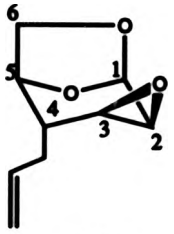
(9)



(8)

Using procedures outlined by Carlson,[19] the hydroxyl groups were initially differentiated by a selective diesterification to produce the ditosylate (30). This reaction is thought to occur because of unfavourable steric interactions between the 3-axial hydroxyl group and the 1,6-anhydro bridge. Reaction of the ditosylate (30) with base then produced the 3,4-epoxytosylate (9) almost exclusively. This regioselective internal displacement is considered to be due to an unfavourable electrostatic interaction between the alkoxide at C-3 and the oxygen of the anhydro bridge, hindering movement towards C-2.

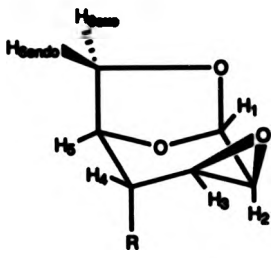
The 3,4-epoxytosylate was then cleaved in a regio- and stereoselective reaction to yield the allyl tosylate (8), reported previously by Kelly and Roberts.[1] As anticipated, reaction of the allyl tosylate (8) with base allowed further internal displacement yielding the allyl epoxide (7) as a colourless oil in 80% yield.



(7)

The expected stereochemistry was confirmed by high resolution ^{1}H nmr spectroscopy with extensive decoupling experiments. The data obtained are presented in Table 1, together with literature data[20] on other selected 1,6-2,3-dianhydro mannopyranose derivatives.

Table 1



R = $CH_2CH{=}CH_2$ (7)

R = OH (31)

R = OAc (32)

R = H (33)

^1H Chemical Shifts (ppm)

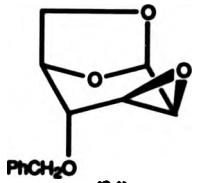
	H-1	H-2	H-3	H-4	H-5
(7)	5.64	3.34	2.9	2.01	4.23
(31)	5.69	3.45	3.14	3.91	4.42
(32)	5.72	3.47	3.13	4.96	4.41
(33)	5,68	3.36	3.13	1.98 (4_b 2.22)	4.43

^1H Coupling Constants (Hz)

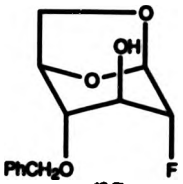
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
(7)	3.2	3.9	-	-
(31)	3.1	3.8	0.8	1.2
(32)	3.2	3.8	0.7	1.1
(33)	3.1	3.9	0.8	1.0

A comparison shows that the coupling constants $J_{1,2}$ and $J_{2,3}$ are almost identical to the known derivatives (31) (32) and (33). Similarly the ^1H chemical shifts compare favourably with the 4-deoxy derivative (33).

Having obtained the epoxide (7), the next stage required its scission with fluoride to produce the desired 2-fluorohydrin (6). A survey of the literature indicated that a number of fluorocarbohydrates had previously been synthesised by this type of reaction using either potassium fluoride in molten acetamide or potassium hydrogenfluoride in ethylene glycol,[21] the latter reaction had also been applied to several 1,6-anhydro pyranosides. Moreover, potassium hydrogen fluoride/ethylene glycol and bubbled carbon dioxide had been used to produce the 1,6-anhydro-2-fluoro-2-deoxy glucopyranoside (35),[22] from the 1,6-2,3-dianhydro mannopyranoside (34), a particularly encouraging analogy.



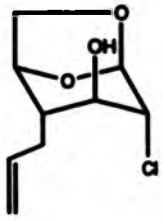
(34)



(35)

However, a report of epoxide cleavage with tetra n-butyl ammonium fluoride in acetonitrile[23] led to the belief that milder reaction conditions might be possible. Similarly, since both caesium fluoride and potassium fluoride/crown ether complexes had been used to displace sulphonate[24,25] ester groups it was thought that they might also be used for epoxide cleavage.

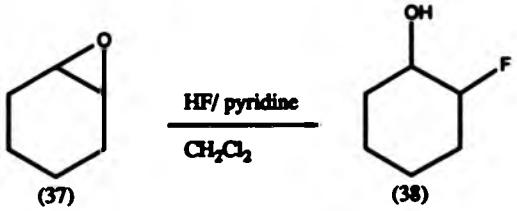
Reaction of the epoxide (7) with any of these reagents resulted in no change even after a prolonged reaction time. Use of the Lewis acid aluminium chloride to assist epoxide opening, resulted in a clean reaction giving a more polar product. Spectral analysis showed changes in the ^{1}H chemical shift of both H-2 and H-3 and evidence of a hydroxyl function therefore indicating that the epoxide had been opened. However, ^{13}C nmr showed no ^{13}C-^{19}F coupling proving that fluorine substitution had not occurred. Consequently, the chlorohydrin structure (36) was tentatively assigned. This could not easily be confirmed because there was no molecular ion in the mass spectrum.



(36)

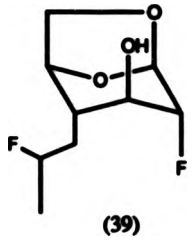
Using aluminium trifluoride as a Lewis acid unfortunately had no effect, and boron trifluoride etherate produced a plethora of compounds.

Epoxide cleavage has also been achieved by hydrogen fluoride[26] and more recently with hydrogen fluoride/pyridine complex.[27] Olah et al demonstrated that a series of aliphatic epoxides, for instance (37), could be cleaved to generate the appropriate fluorohydrins, eg (38) in respectable yields using this reagent.



(37) (38)

The hydrogen fluoride/pyridine complex is also a potent hydrofluorinating agent[28] and reaction with epoxide (7) is thought to have resulted in fluorination of the allylic sidechain as well as epoxide cleavage to yield the fluorohydrin (39).

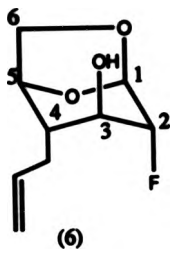


(39)

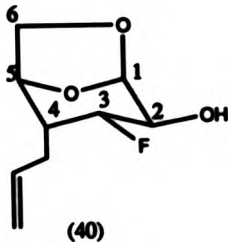
Once again this structural assignment is rather tentative. ^{1}H nmr indicated changes in the chemical shift of H-2 and H-3 relative to the epoxide (7). More significantly however, it displayed two highfield doublets (δ2.25, δ2.5) separated by 24Hz which is indicative of a methyl group with an α-fluoro substituent.

Having exhausted likely alternatives, the reaction with potassium hydrogen fluoride was the next logical step. Preliminary experiments based on Cerny's procedures[22] gave small yields of the regioisomeric fluorohydrins (6) and (40). Further experimentation showed that the bubbled carbon dioxide was unnecessary, but that rigorously anhydrous conditions were required for optimum yields. Ultimately reaction of epoxide (7) in refluxing ethylene glycol with a 1:1 w/w potassium hydrogen fluoride/potassium fluoride mixture for two hours produced the 2-fluorohydrin (6) as the major isomer (45%) and the 3-fluorohydrin (40) as the minor isomer (13%). Traces of starting material were always

present, along with more polar materials, which were assumed to be ethylene glycol adducts also noted by Cerny[22].



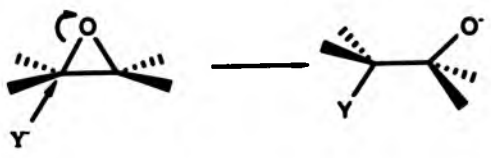
(6)



(40)

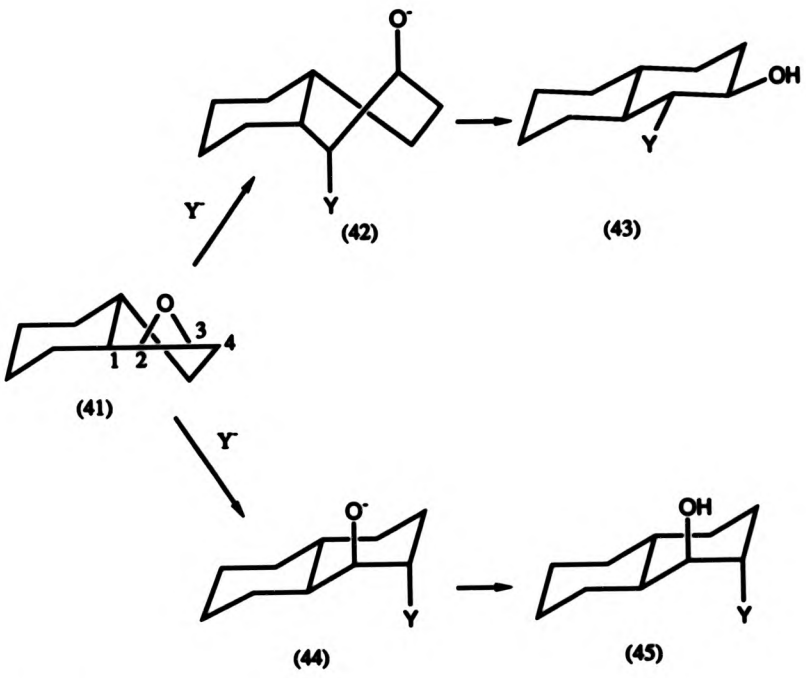
It was first proposed by Mills[29] that the Fürst-Plattner rule for the cleavage of steroid epoxides[7] might be applicable to carbohydrates. This rule predicts that epoxides open to give predominantly diaxial isomers. These experimental observations are now well established and mechanistically rationalised by stereoelectronic theory.[30]

The necessity for a colinear alignment between a nucleophile and a leaving group in an S_N2 type reaction means that opening of an epoxide by a nucleophile produces a product of defined stereochemistry, Scheme 3.



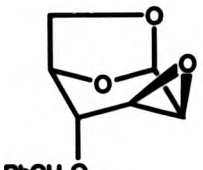
Scheme 3

In the case of a conformationally rigid epoxide eg (41), Scheme 4, this requirement means that reaction at C-2 proceeds through the twist boat intermediate (42) whereas reaction at C-3 gives the chair intermediate (44). These then give the diequatorial and diaxial products respectively. Formation of the transition state leading to (44) will be a lower energy process than that leading to (42), consequently the diaxial product (45) is preferentially formed under kinetically controlled conditions.

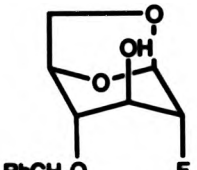


Scheme 4

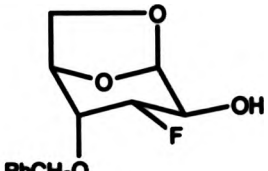
For the reasons outlined above, epoxide scission of most dianhydro hexopyranosides gives almost exclusively diaxial products. Cerny noted a 15:1 ratio of fluorohydrins (35) and (46) from the reaction of epoxide (34).



(34)



(35)

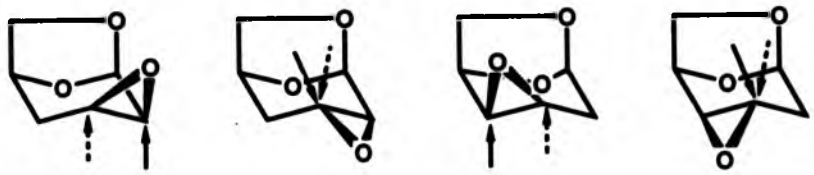


(46)

However, although allyl epoxide (7) gave a preponderance of the 2-fluorohydrin (6) the quantity of 3-fluorohydrin (46) was not insignificant. In this instance a 4:1 ratio of fluorohydrins was noted.

Non-selective product distributions have also been noted in the nucleophilic epoxide opening of the deoxy dianhydro sugars (47) and (49).[31] The lack of substitution at C-2 or C-4 in these compounds results in an unsymmetrical charge distribution within the molecule. This then allows polar effects to compete with the steric ones and sometimes results in epoxide cleavage at the most electron deficient carbon atom. In the series of deoxy sugars presented in Scheme 5, using hydroxide, hydride and iodide as nucleophiles, Cerny[32] has shown that when polar and steric effects are synergistic, eg cases (48) and (50), pure products are obtained. However, when they are antagonistic, eg cases (47) and (49) a mixture of regioisomers often results.

The increased proportion of 3-fluorohydrin (40) in this instance must be a consequence of a similar unsymmetrical charge distribution within the allyl epoxide (7).



(47) (48) (49) (50)

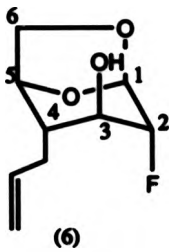
Polar direction

Steric direction

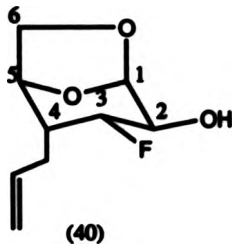
Scheme 5

Initially the stereochemical course of this reaction had been assumed on the basis of the previously discussed mechanistic considerations. However, analysis of ^{13}C spectra, Table 2, quickly established both the presence of fluorine and the constitution of the two isomers. The broad band decoupled spectra consisted of more lines than could be accounted for on the basis of carbon atoms alone. This was obviously due to coupling between carbon and fluorine producing a series of doublets in the spectrum and therefore provided evidance for the successful introduction of fluorine. Subsequent assignment of chemical shifts and the calculation of coupling constants provided the evidence for the position of substitution. This was obvious from the spread of the coupling constants. Fluorine attached to C-3 was coupled to all the carbon atoms in the pyran ring, C-1 to C-5, whereas fluorine attached to C-2 was coupled only to C-1, C-2 and C-3.

Table 2



(6)



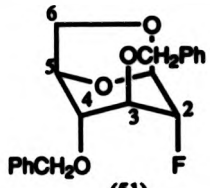
(40)

^{13}C Chemical Shifts, ppm (^{13}C-^{19}F coupling constants, Hz)

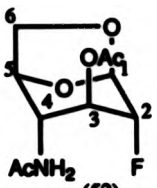
	C-1	C-2	C-3	C-4	C-5	C-6
(6)	99.5	89.5	69.8	43.2	74.6	68.2
	(27)	(183)	(26)			
(40)	102	71.8	92.8	43.4	64.5	67.9
	(10.8)	(176)	(185.8)	(16.3)	(6.8)	

The stereochemistry of these isomers was then determined from the high field ^{1}H nmr data presented in Table 3. Also presented in Table 3 are the pertinent nmr parameters of the 1,6-anhydro-2-fluoro glucopyranosides (51) and (52) and the 1,6-anhydro-3-fluoro altropyranose (53).[22,32-33] Cerny had made stereochemical assignments on the basis of the coupling constants $J_{2,3}$ and $J_{3,4}$ ie 9Hz and 4.5Hz for compound (53). These values are typical of a diaxial and an axial-equatorial orientation respectively, and compare favourably with those observed for the 3-fluorohydrin (40). For the 2-fluoro derivatives the coupling constants are either small 1.5hz (52) or unresolved (51). This was taken as being indicative of an equatorial configuration for H-2, H-3 and H-4. Analysis of the ^{1}H-^{19}F coupling constants corroborates this evidence, the data for compounds (6) and (40) comparing favourably with the relevant literature data in Table 3.

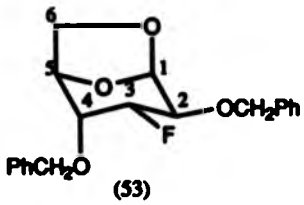
Table 3



(51)



(52)



(53)

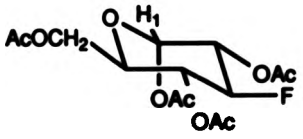
^1_H Coupling Constants, Hz.

	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
(6)	1.5	-	-	-
(51)	-	-	1.4	1.4
(52)	2.5	1.5	1.5	2.5
(40)	1.8	8.1	6.45	-
(53)	1.5	9	4.5	-

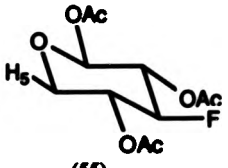
$^1_{H-}{}^{19}_F$ Coupling Constants Hz.

	$J_{H-1,F}$	$J_{H-2,F}$	$J_{H-3,F}$	$J_{H-4,F}$	$J_{H-5,F}$
(6)	1.5	46	17	-	-
(51)	4.9	45	-	-	-
(52)	1.0	43	15	-	-
(40)	6.9	14.6	52.2	-	5.8
(53)	6.5	13.0	48.8	-	-

They were also in agreement with literature data for other fluoro hexopyranose derivatives.[34] The vicinal coupling constants $J_{H-3,F-2}$ = 17Hz in compound (6) and $J_{H-2,F-3}$ = 14.6Hz in compound (40) are typical of an axial-equatorial orientation of a hydrogen and fluorine atom. The long range ^4J, $J_{H-1,F-3}$ and $J_{H-5,F-3}$ coupling constants for compound (40) are also indicative of a 1,3 diequatorial relationship between fluorine and the H-1 and H-5 hydrogen atoms.



(54)



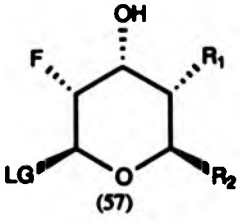
(55)

The 3-fluoro glucopyranose (54) displayed a 4.0Hz coupling between fluorine and H-1, similarly the 3-fluoro xylopyranose (55) showed a 4.2 Hz coupling between fluorine and H-5.[35] The ^4J coupling constants obtained for compound (40) are somewhat higher, namely 6.9 Hz and 5.8 Hz, and must be due to the conformational rigidity of the 1,6-anhydropyranose framework.

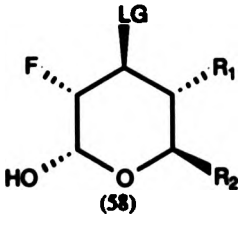
At a later date the preparation of a new fluorinating reagent, tetraphenyl-phosphonium hydrogen difluoride (56) was reported.[36] It was anticipated that this might have provided a milder and more efficient method of epoxide opening. However, reaction with the epoxide (7) in acetonitrile at room temperature gave no reaction. Unfortunately, a more extensive investigation into the potential of this reagent was never carried out.

Ph_4PHF_2 (56)

Having successfully introduced the fluorine in the correct position and having established its stereochemistry, the remaining major synthetic challenge was the formation of the oxetane bridge. In order to keep as many options as possible open for this step, it was decided to invert the stereochemistry at C-3. This would then allow the option of placing leaving groups at C-1 as in (57). Similarly further manipulation of the 2-fluorohydrin (6) would allow placement of leaving groups at C-3 as in (58).

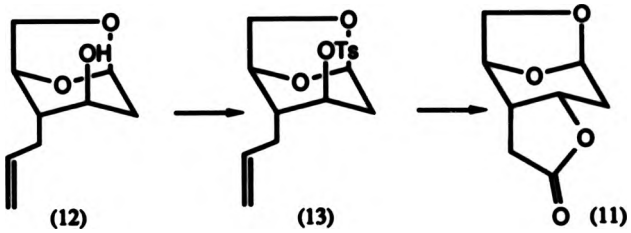


(57)



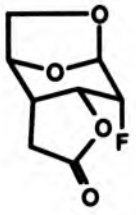
(58)

Instead of using the proposed Mitsunobo methodology for stereochemical inversion of the C-3 hydroxyl group, it was decided to utilise some previously established chemistry. In the synthesis of the thromboxane B_2 intermediate (11) from levoglucosan, Kelly and Roberts[1] achieved the inversion at C-3 in the following manner. The alcohol (12) was tosylated, then oxidative cleavage of the allyl sidechain with ruthenium tetroxide generated <u>in situ</u> yielded a carboxyl function which then lactonised under the reaction conditions to give the crystalline γ-lactone (11) in 90% yield.



(12) (13) (11)

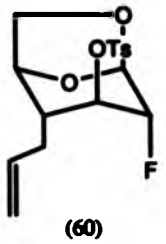
Since these reactions were relatively simple, it was hoped that the 2-fluorolactone (59) would be available by the same pathway.



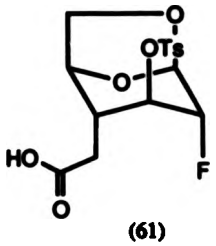
(59)

Tosylation of the C-3 axial alcohol (12) had required a prolonged reaction time (48 hours) in order to overcome the steric hinderance resulting from interaction with the 1,6-anhydro bridge. The fluorohydrin (6) however required not only a prolonged reaction time but an elevated temperature and use of a nucleophilic catalyst (Dimethylamino pyridine) to yield the tosylate (60). Oxidation of this tosylate (60) with ruthenium tetroxide then gave one major compound. The nmr spectrum

showed that this had lost the allyl function but still maintained the tosyl group, since the infra-red spectrum showed characteristic absorptions associated with an acid function, the structure (61) was assigned.

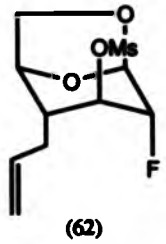


(60)

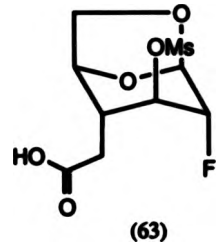


(61)

Similarly, preparation of the mesylate (62), followed by oxidation, yielded the corresponding acid (63).



(62)



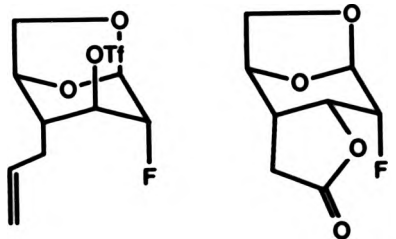
(63)

It is apparent from the above experimental details that there is a significant difference in reactivity between the alcohol (12), tosylate (13) and their fluorinated counterparts (6) and (60). Naturally, this must be due to the electronic effect of the fluorine atom. It would appear that the electron withdrawing capacity of fluorine is reducing the nucleophilicity of the C-3 hydroxyl group and consequently making the formation of the tosylate (60) even slower. Moreover, it is also slowing or preventing the subsequent displacement of leaving groups from C-3.

Similar changes in reactivity for an S_N2 displacement were noted

by McBee *et al*[37] who had studied the kinetics of iodide displacement of bromide in several perfluorinated alkyl bromides. A marked reduction in rate between these compounds and the unsubstituted alkyl bromides was observed. Furthermore, kinetic studies on benzyl tosylates[38] have indicated that for S_N2 reactions there is a substantial build up of positive charge on the methylene group in the transition state. In the light of this evidence it is not surprising that the fluorinated tosylate reacted sluggishly or not at all in the above reactions. This is because a fluorine atom will destabilise the transition state leading to lactone formation.

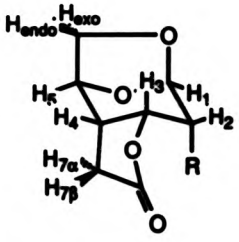
Since cyclisation of the mesylate (62) had also been unsuccessful it was reasoned that use of the more powerful, triflate leaving group might promote the reaction. It was therefore a pleasure to see that preparation of the triflate (64) followed by oxidation led to a smooth reaction yielding the highly crystalline 2-fluorolactone (59), in 65% yield. It was later shown that reaction of the acid (61) with potassium t-butoxide over a prolonged time period also led to formation of the lactone (59).

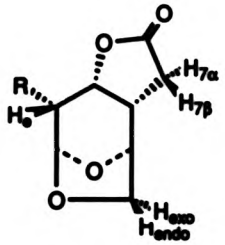


(64) (59)

Table 4 presents a comparison of the nmr parameters for both the tricyclic lactone (11) and the 2-fluoro analogue (59). Outside the

Table 4





^{1}H Chemical Shifts (ppm)

	H-1	H-2a	H-2e	H-3	H-4	H-5	H-6exo	H-6endo	H-7β	H-7α
R = H	5.52	1.59	2.37	4.83	2.62	4.53	3.82	3.85	2.51	2.76
R = F	5.57	-	4.58	4.69	2.68	4.61	3.84		2.46	2.81

^{1}H-^{1}H, ^{1}H-^{19}F Coupling Constants (Hz)

	1,2a	1,2e	2,2	2a,3	2e,3	3,4	4,5	7,7	6,6exo,endo	4,7β	4,7α	5,6	5,6	1,4	2e,5	2a,7β	2a,7α
R = H	1.74	2.05	14.0	8.55	7.9	7.8	1.7	16.0	7.5	7.77	11.91	4.78	1.22	0.5	0.87	-	-
R = F	0	2.43	49.8	20.5	4.9	7.43		16.2	-	7.97	12.5	-	-	-	-	2.67	3.02

^{13}C Chemical Shifts (^{13}C-^{19}F coupling constants Hz)

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
R = H	99.4	36.3	72.6	39.2	72.5	68.3	29.9	175.6
R = F	98.3	83.0	71.2	39.2	71.7	68.1	29.7	175.8
JC,F	(24.1)	(187.4)	(16.2)	(1.5)				

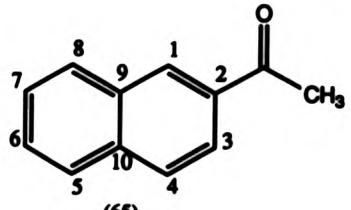
Interchangeable

sphere of influence of the fluorine atom, both the ^{13}C and the ^{1}H chemical shifts and the proton-proton coupling constants are similar for the two compounds. The similarity of $J_{3,4}$, $J_{4,7\alpha}$, $J_{4,7\beta}$ and $J_{1,2e}$ implies that the conformation of both lactones is the same. This therefore lends further credence to the idea that fluorine is isosteric with a hydrogen atom.

Due to the similar axial-axial and axial-equatorial values of the $J_{2,3}$ coupling constants Kelly[39] proposed that the pyranose ring in the tricyclic ketone (59) is slightly flattened from an ideal chair conformation. An X-ray crystal structure[40] of the 2-fluoro analogue (59) is shown in Figure 1. Calculations show that the pyranose ring adopts a chair conformation in which C-3 is flattened by 0.36Å.[40] These structural data therefore confirm both Kelly's proposal and the stereochemical assignments previously determined from nmr data.

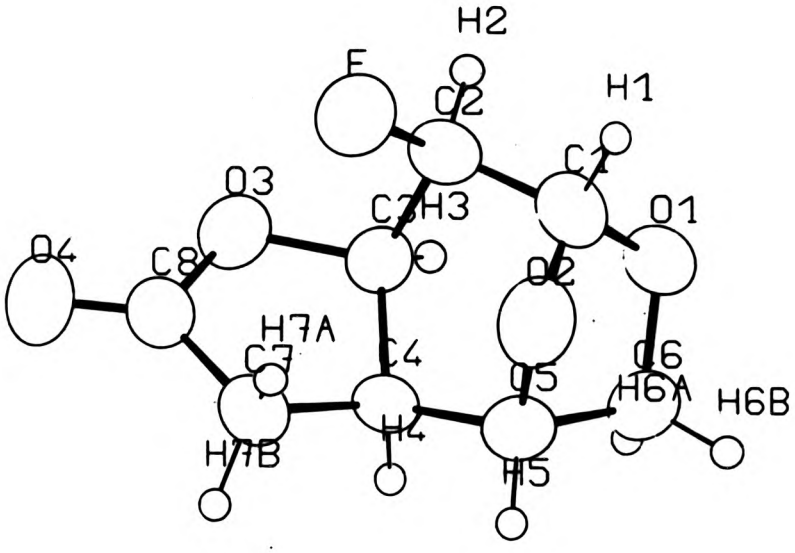
Also of interest are the long range coupling constants to the two H-7 protons in the lactone ring. Initially these were assumed to be due to coupling with fluorine. This was later confirmed using a technique known as 'hetero nuclear shift correlation spectroscopy, with broad band homonuclear decoupling' - a two dimensional nmr experiment.[41] A complex pulse sequence ultimately results in a proton decoupled ^{13}C spectrum on one axis and a proton decoupled ^{1}H spectrum along the other.

Figure 2 shows the two dimensional grid obtained for compound (65).



(65)

Figure 1



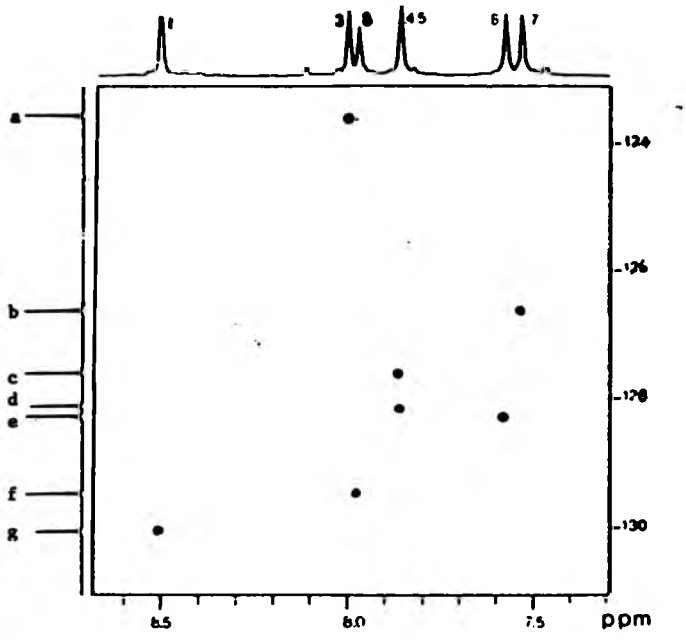


Figure 2

By taking slices across this two dimensional grid, it is possible to plot a proton spectrum which corresponds to each carbon resonance, Figure 3.

In the case of the fluorolactone (59) fluorine causes splitting of the carbon resonances and Figure 4 shows the ^{13}C spectrum of C-4 and C-7, each resonance being a doublet.

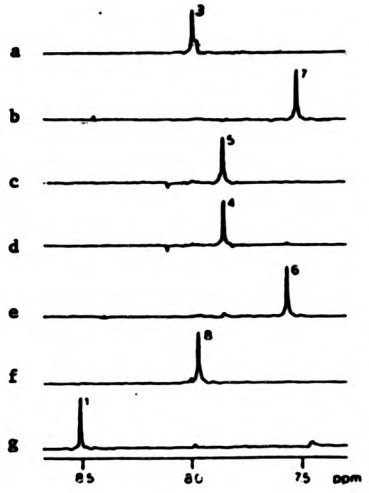


Figure 3

Figure 4

Figure 5 shows the proton spectrum corresponding to each of these carbon resonances. The differences in chemical shift between pairs of proton spectra, for example 1 and 2 or 3 and 4, correspond to the long range fluorine proton coupling constants. This experiment not only confirmed the $^{5}J_{H,F}$ coupling between the H-7 protons and fluorine but it also showed that there is a $^{4}J_{F,H-4}$ coupling $\sim$0.4Hz which had not been seen on the normal proton spectrum. This therefore indicates the sensitivity of this type of experiment.

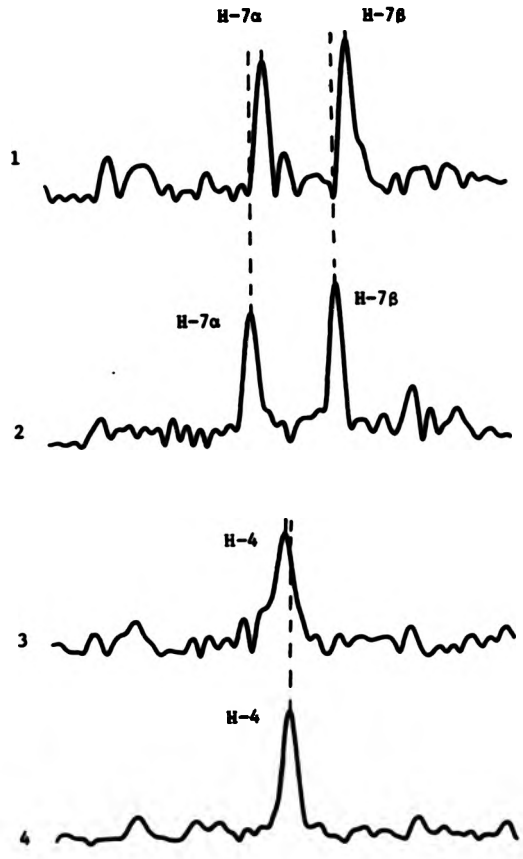
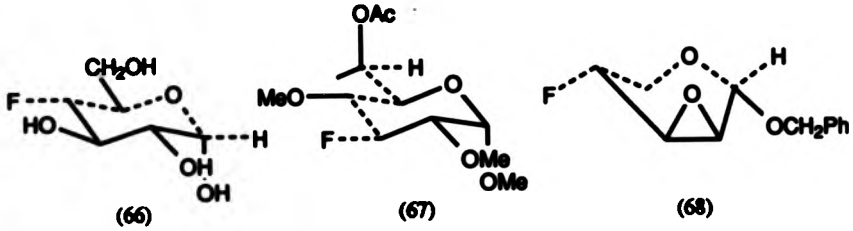


Figure 5

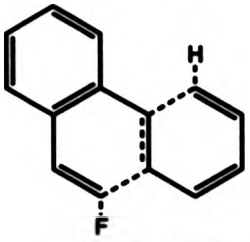
Within the carbohydrate literature long range $^{4}J_{H,F}$ coupling constants are well documented and it is now well established that they have a maximum value when the hydrogen and fluorine substituents are at the termini of a W-coplanar conformation.[35,42] This, however, is not the case with $^{5}J_{H,F}$ coupling constants, of which only a few examples have been cited. Among these are the compounds (66) (67) and (68)[35,42,43].



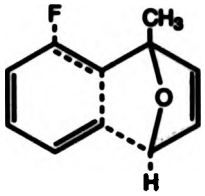
(66) (67) (68)

For these compounds it has been observed that the coupled nuclei (dotted lines) have a trans coplanar relationship to the bond which is the midpoint of the pathway between them.[35,42] A study of molecular models clearly shows that a similar geometric arrangement does not occur in the case of the 2-fluorolactone (59).

A wider search of the literature, however, has revealed that long range $^{5}J_{H,F}$ and even $^{6}J_{H,F}$ coupling constants are in fact well documented. From the examples cited, there appear to be two different situations where these couplings are possible. The first of these is similar to the previous pyranoside examples where coupling occurs when a zig zag pathway exists between the two nuclei,[44] as found in compounds (69) and (70) for example.

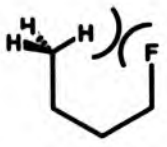


(69, $^5J_{HF}$ = 2 Hz)



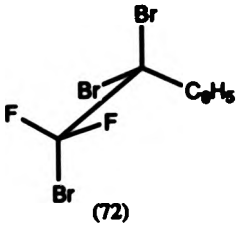
(70, $^5J_{HF}$ = 2 Hz)

The second situation is where the two coupled nuclei are very close together, as depicted (71) and here a 'through space' or direct coupling mechanism is thought to occur.



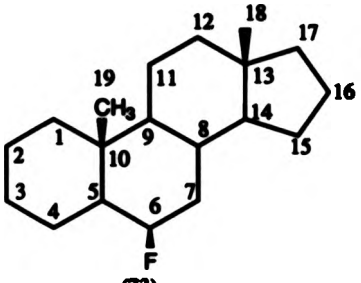
(71)

Long range coupling of this type was first recorded[45] in compound (72) where a $^5J_{H,F} \sim 1.6$Hz was observed between the gauche fluorine and the protons at positions 2 and 6 in the phenyl ring.

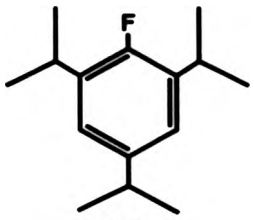


(72)

Similar long range couplings were then noted in 6-β-fluorosteroids[46] eg (73) and have since been observed in a large number of others,[47] with coupling constants ranging from 1.0Hz to 5.3Hz.



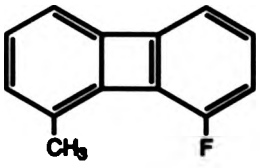
(73)



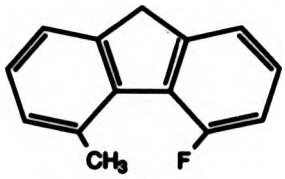
(74)

Studies on a series of alkylfluorobenzenes[48] eg (74) showed that the magnitude of the $^{5}J_{H,F}$ coupling constant was dependent on the internuclear distance. Correlation of this evidence with the data previously obtained for a series of 6-β-fluorosteroids[49] indicated that once the distance between the methyl carbon and the fluorine atom was greater than 3A the coupling constant became very small (~1.0Hz). It was concluded that when the carbon and fluorine were separated by this distance or when the hydrogen and fluorine were separated beyond ~2.5A (the sum of their Van der Waals radii) a through-space mechanism would be no longer possible.

Since these early studies further examples of long range $^{5}J_{H,F}$ and $^{6}H_{H,F}$ coupling have provided more evidence for the 'through space' mecnanism. A study on the compounds (75) and (76) for instance[50] showed that (76) has a large $^{6}J_{H,F}$ (8.3Hz), and an internuclear hydrogen-fluorine distance of 1.44A. Compound (75) however shows no significant coupling between the methyl group and fluorine and here the internuclear distance is 2.84A.

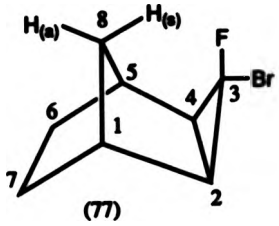


(75)



(76)

The tricyclic octane[51] (77) is of particular interest since a $^5J_{H,F}$ coupling is observed for both the syn and the anti C-8 protons ($^5J_{H,F}$ = 3.6Hz and 3.0Hz respectively).

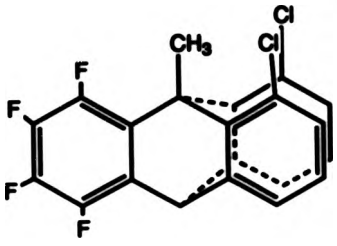


(77)

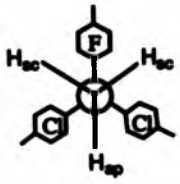
The internuclear distance between the syn proton and the fluorine atom is about 1.6A and a through space mechanism is considered likely. Naturally, this cannot be the case for the anti proton since it is directed away from the fluorine atom. It is thought that the spin information may be relayed through the agency of the syn proton or by overlap of the fluorine with the small rear lobe of the anti carbon hydrogen bond. Despite there being two possible mechanisms the paper[51] concluded that "the satisfaction of the space criterion for a

proximal proton will ensure that the geminal, aprotic proton couples as well. Consequently, for the cases of $^{5}J_{H,F}$ coupling involving methyl groups it has to be considered probable that all three methyl protons are simultaneously coupled."

Coupling to the individual protons within a methyl group in this manner has recently been demonstrated using the triptycene derivative[52] (78). At low temperature the internal rotation of the methyl group is frozen, and rotamer (79) displays a $^{5}J_{H,F}$ coupling of 8.7Hz to the ap proton and of 6.1 Hz to both the sc protons.



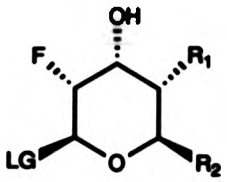
(78)



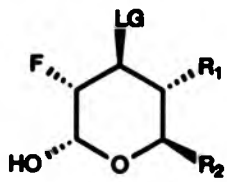
(79)

From a study of these examples it would appear that the long range coupling observed in the 2-fluorolactone (59) is somewhat anomalous. Firstly, the geometric arrangement between the fluorine atom and both the C-7 hydrogen atoms is not favourable for a through bond coupling mechanism. Secondly, the internuclear distance[39] of 2.75A between the fluorine atom and H-7 or the distance of 4.0A between the fluorine atom and C-7 are unfavourable for the direct or 'through space' coupling mechanism.

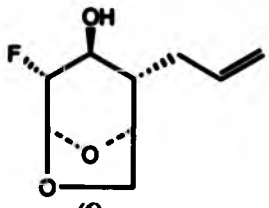
In order to prepare either of the cyclisation precursors (57) or (58) previously discussed, a means of cleaving the 1,6-anhydro bridge had to be found.



(57)



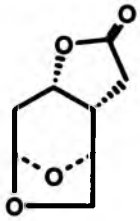
(58)



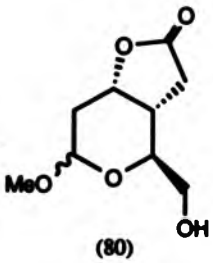
(6)

Concurrent with the preparation of the 2-fluorolactone (59), ways of hydrolysing the acetal bridge in the 2-fluorohydrin (6) were investigated. The electronic influence of fluorine in altering chemical reactivity has already been seen. This effect was however much more apparent in the stabilisation of the 1,6-anhydro bridge. Naturally, this was to be expected because ultimately the role of fluorine was to stabilise an acetal bridge in the thromboxane A_2 analogue (1).

Kelly and Roberts[1] described a procedure using an acidic resin in methanol that gave the ring-opened alcohol (80) from the tricyclic lactone (11)

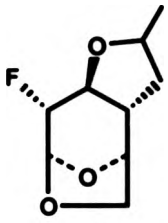


(11)



(80)

Under these conditions, the 2-fluorohydrin (6) was totally inert. Similarly, reaction with numerous mineral and organic acids led to incomplete reactions after prolonged reaction times. In certain cases the production of what was thought to be the cyclic ether compound (81) occurred.



(81)

Once again this structural assignment is tentative and is primarily based on the nmr spectrum which confirmed the loss of the allyl group and displayed a high field (δ1.35) three proton doublet (J~6Hz), indicating the presence of a methyl group.

Reaction with titanium tetrachloride and bromide[53] as initially proposed produced several products along with unreacted starting material as did the use of the palladium-based acetal exchange catalyst (82).[54]

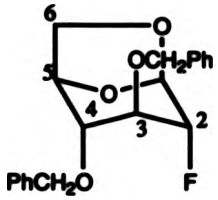
$$PdCl_2(CH_3CN)_2$$

(82)

This had been used to remove an acetal protecting group which had also proved to be inert to several methods of acid hydrolysis.

Once the 2-fluorolactone (59) had been prepared it proved to be totally inert in aqueous acids as well. Even under the conditions reputed to have cleaved the 1,6-anhydro-2-fluoro glucopyranoside (51)

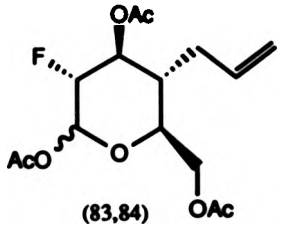
namely 50% (v/v) aqueous methane sulphonic acid at 120°C for 30 minutes.[33]



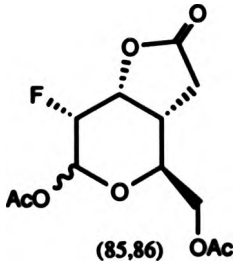
(51)

This hydrolytic stability had also been noted by Cerny during the preparation of 2-fluoro glucose from 1,6-anhydro-2-fluoro-glucopyranoside.[22] A small yield of the hydrolysis products was obtained eventually using p-toluene sulphonic acid in a sealed tube. In a later paper[55] however, an acetolysis procedure was described; this cleaved the 1,6-anhydro bridge to produce the peracetylated pyranosides as an anomeric mixture. These could then be readily converted to the hemiacetals by simple transesterification using methoxide.

Treatment of the 2-fluorohydrin (6) with acetic anhydride and perchloric acid, gave a reasonable yield (60%) of the anomeric triacetates (83) and (84).



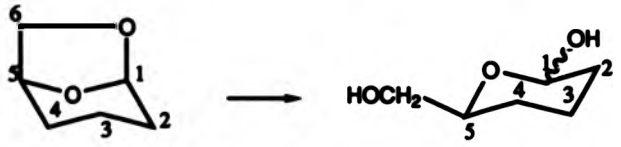
(83,84)



(85,86)

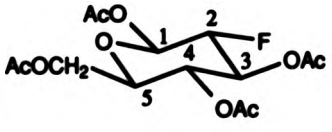
Moreover, reaction of the 2-fluorolactone (59) under the same conditions gave a cleaner reaction leading to the anomeric diacetates (85) and (86). In this case it proved to be possible to separate and purify the individual anomers. The nmr parameters for both anomers are presented in Table 5.

Normally after cleavage of the 1,6-anhydro bridge the pyranose ring undergoes a conformational change from $^{1}C_{4}$ (a) to $^{4}C_{1}$ (b).

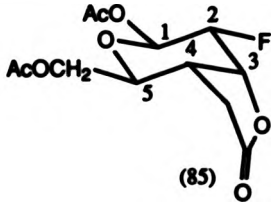


(a) $^{1}C_{4}$ (b) $^{4}C_{1}$

Analysis of the coupling constants and comparison with literature values[56] show that the best correlation of data is obtained if a $^{4}C_{1}$ conformation (b) is assumed for compound (85), it has therefore been assigned the β-configuration at the anomeric centre. The coupling constants $J_{1,2}$ and $J_{H\text{-}1,F}$ for the tetraacetate (87) are 8.1 Hz and 3.2Hz respectively,[56] the somewhat lower value of $J_{1,2}$ =5.6Hz for acetate (85) was attributed to a flattening out of the pyranose ring, resulting in a dihedral angle of less than 180° between the coupled nuclei.

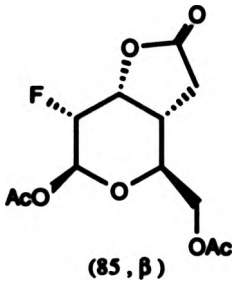


(87)

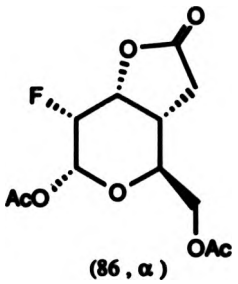


(85)

Table 5



(85, β)



(86, α)

^{1}H Chemical Shifts (ppm)

	H-1	H-2	H-3	H-4	H-5	H-6	H-6'	H-7	H-7'
(85)	6.08	4.67	4.88	2.77	3.88	4.15		2.68	2.41
(86)	6.12	5.05	4.67	2.88	4.13			2.62	2.37

^{1}H Coupling Constants (Hz)

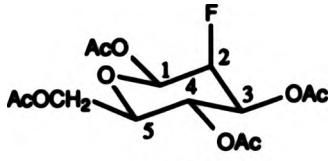
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,7}$	$J_{4,7'}$	$J_{7,7'}$
(85)	5.6	3.6	6.14	8.2	3.4	17.4
(86)	3.776	2.6	8.7	9.8	8.3	17.7

^{1}H-^{19}F Coupling Constants (Hz)

	$J_{H-1,F}$	$J_{H-2,F}$	$J_{H-3,F}$	$J_{H-7,F}$	$J_{H-7'F}$
(85)	-	47.1	15.7	0.7	3.4
(86)	17.45	50.2	28.3	1.7	2.9

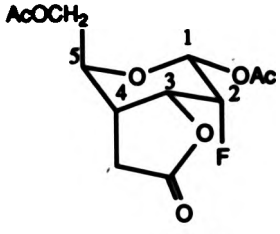
The data for (86) are anomalous with respect to normal pyranosides which have the α-configuration at C-1 and a $^4C_1$ conformation (b). The very large $J_{H-1,F}$ (17.45 Hz) and $J_{H-3,F}$ (28.3 Hz) coupling constants can only be accounted for by a <u>trans</u> diaxial arrangements between fluorine and both H-1 and H-3.

This geometric arrangement is often found in 2-fluoro mannopyranoside derivatives for instance (88)[56]

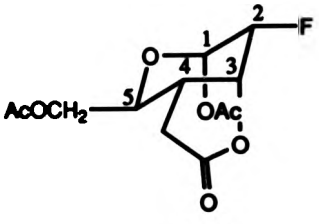


(88)

Here $J_{H-1,F}$ and $J_{H-3,F}$ are 18.9 Hz and 25.6 Hz respectively. It was therefore concluded that compound (86) is the α-anomer but adopting a $^1C_4$ conformation (a).



(86,a)

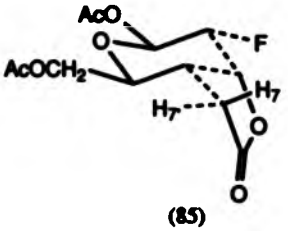


(86,b)

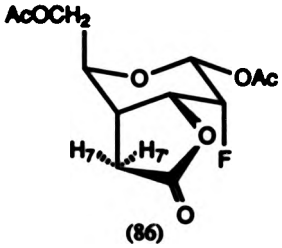
A conformational change to $^4C_1$ (b) would result in a 1,3 diaxial orientation of the substituents at C-1 and C-3. Presumably this is sufficiently destabilising to overcome any stabilisation that may result from the anomeric effect and an equatorial disposition of the C-6 acetate.

Given that one of these anomers has undergone a conformational change and that the other has not, it is interesting to note that both display long-range $^5J_{H,F}$ coupling constants. The mystery concerning the possible mechanisms for these interactions is now even more intriguing.

Compound (85) in a $^4C_1$ conformation has an equatorial fluorine atom and a geometric relationship similar to the pyranoside examples seen previously (66) - (68).



(85)

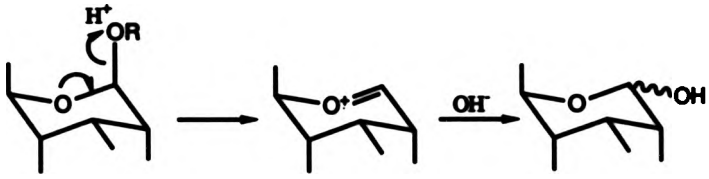


(86)

In this case a through bond mechanism might explain the larger $^5J_{H\text{-}7',F}$ (3.4 Hz) coupling accompanied by a much smaller coupling (0.7 Hz) to H-7. The coupling constants observed for the α-anomer (86) are 1.7 Hz and 2.9 Hz, since this compound has maintained the $^1C_4$ conformation the coupling mechanism must be the same as that for the 2-fluorolactone (59). The difference in magnitude between these coupling constants and those of the lactone must however reflect a slight

change in the bond angles between the coupled nuclei or a change in the internuclear distance.

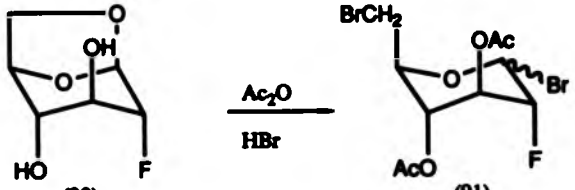
The cleavage of the 1,6-anhydro bridge under these conditions poses some intriguing mechanistic problems. The unusual hydrolytic stability of both fluoro-acetals[17] and 1,6-anhydro-2-fluoro sugars[55] has been accounted for on the basis of destabilisation of the intermediate oxonium ion (89) by the inductive effect of fluorine.



(89)

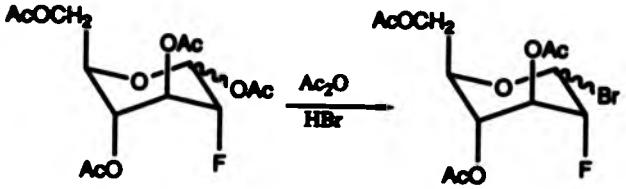
Acetolysis of these compounds however gives good yields of the peracetylated ring-opened pyranoses.

Cerny et al[55] observed that treatment of the 1,6-anhydro-2-fluoro glucopyranose (90) with acetic anhydride, perchloric acid and hydrogen bromide yielded the 6-bromo glycosyl bromide (91)



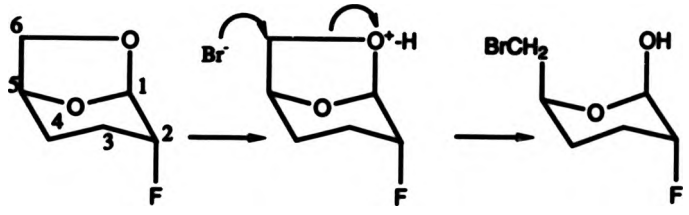
(90) (91)

whereas treatment of the C-6 acetate (92) under the same conditions yielded only the glycosyl bromide (93).



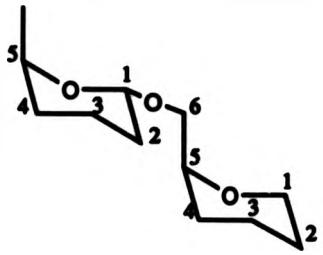
(92) (93)

It was therefore concluded that under the acetolysis conditions the C-6 oxygen bond was broken by nucleophilic attack of bromide at C-6, Scheme 6.

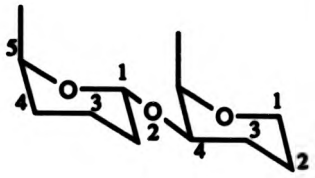


Scheme 6

This proposal therefore provides a mechanism for cleavage of the 1,6-anhydro bridge without the intermediacy of an oxonium ion. The acetolysis of polysaccharides and oligosaccharides[57] is known to cleave selectively 1→6 glycosyl linkages (94) rather than the 1→4 linkages (95).



(94)

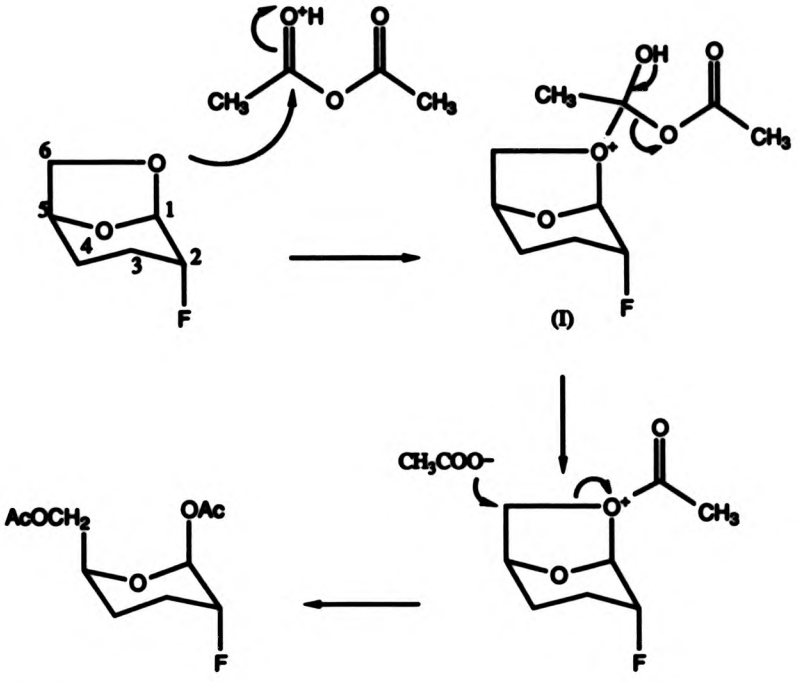


(95)

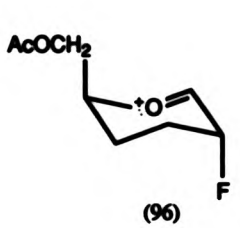
This selectivity is believed to occur because carbon oxygen bond cleavage occurs by nucleophilic attack at C-6. A similar attack at the secondary C-4 site is unlikely. Previous studies[58] on the mechanistic aspects of acetolysis concluded that the reaction is initiated by the acetylium cation. It is therefore likely that acetolysis of the 1,6-anhydro sugars proceeds through a similar mechanism as outlined in Scheme 7.

Attack of the C-6 oxygen on the protonated acetic anhydride would give the tetrahedral intermediate (I). Collapse of this would then generate acetate, allowing nucleophilic attack at C-6 and breaking of the C-6 oxygen bond, hence yielding the acetylated product.

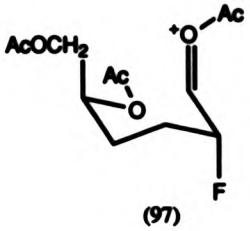
Unfortunately this mechanism only explains the production of the β-anomer, whereas it is known that both anomers are produced. Similarly, when either of the pure anomers (85) or (86) were subjected to the same reaction conditions anomeric mixtures were obtained. In order to account for any anomerisation the reaction must proceed through either of the oxonium ions (96 and (97).



Scheme 7



(96)

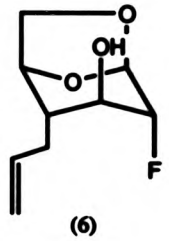


(97)

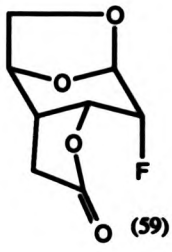
Compound (96) can be generated by loss of acetic anhydride or acetic acid from C-1 and (97) can be generated by breaking the C-1, O-5 bond, again catalysis by either a proton or an acetylium cation being possible. Naturally both of these will be destabilised due to the fluorine atom. However, (97) will be additionally destabilised since a positive charge build up on the oxygen will be unfavourable because of the electron withdrawing carbonyl function.

Given that the reaction must proceed through an unfavourable cationic intermediate, the following mechanism can be argued. Initially cleavage of the C-6 oxygen bond occurs to generate primarily the β-anomer, as outlined in Scheme 7. This is then followed by a subsequent and presumably slower anomerisation reaction via the oxonium ion (96).

Having now found a means of breaking the anhydro bridge, the major synthetic problem remaining was to close the oxetane bridge hence constructing the bicyclic (3.1.1) acetal unit. The stereochemical prerequisites for this ring closure have been discussed earlier and the possibility for either method is available through either the fluorohydrin (6) or the fluorolactone (59).



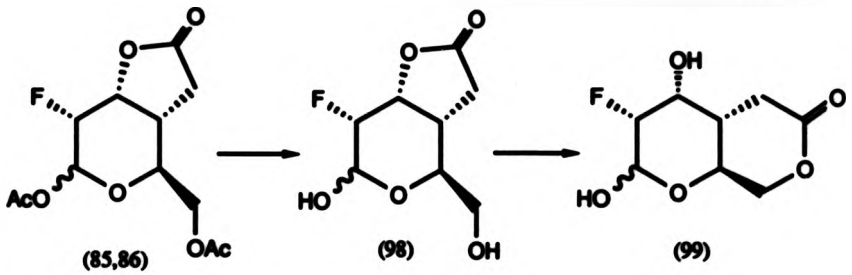
(6)



(59)

In order to try both these methods the following synthetic manipulations

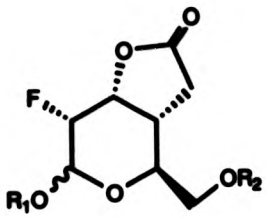
were attempted. Having already prepared the anomeric diacetates (85, 86) it was assumed that these could be deprotected to yield the diols (98). It was then hoped that these might be isomerised to the δ-lactones (99) using an acid catalyst, Scheme 8.

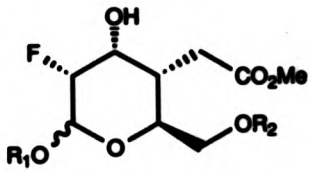


Scheme 8

The δ-lactones (99) would provide ideal intermediates to attempt cyclisation via a C-1 leaving group and also be ideal for addition of the prostanoid sidechains via the corresponding lactol. Treatment of the acetates (85, 86) with methoxide in methanol yielded a mixture of at least three compounds which proved to be inseparable by column chromatography. Given that the lactone ring might be unstable to the transesterification conditions the number of possible compounds resulting from this reaction is quite large (98), (100)-(104).

It was decided, however, that the isomerisation to the δ-lactones (99) should still be possible even with this mixture of compounds. The unresolved mixture was therefore treated with p-toluene sulphonic acid in tetrahydrofuran. After several days stirring at room temperature the mixture had resolved into two closely running spots. The reaction was worked up and the product acetylated to yield compounds chromatographically and spectroscopically identical to the starting acetates (85, 86).





(98) $R_1 = R_2 = H$

(100) $R_1 = Ac$ $R_2 = H$

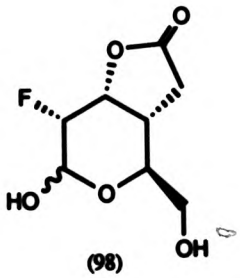
(101) $R_1 = H$ $R_2 = Ac$

(102) $R_1 = R_2 = H$

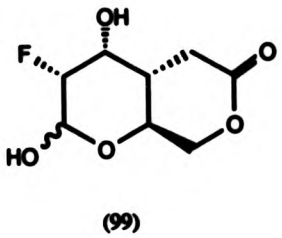
(103) $R_1 = Ac$ $R_2 = H$

(104) $R_1 = H$ $R_2 = Ac$

It was concluded that acid-catalysed equilibration had yielded the diols (98), therefore indicating that easy preparation of the δ-lactones (99) was unlikely.

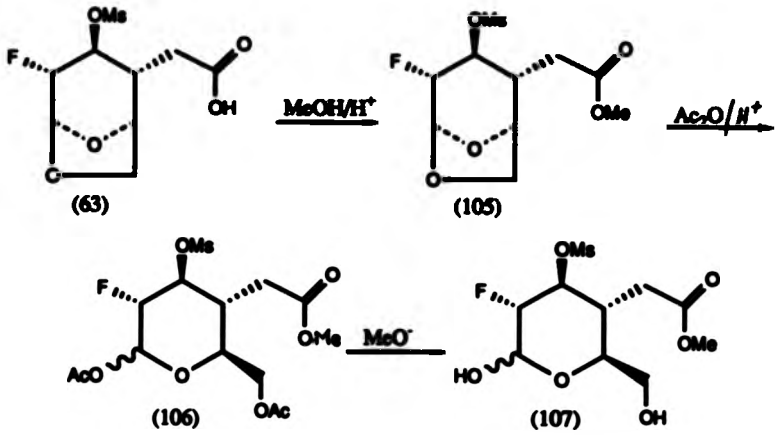


(98)



(99)

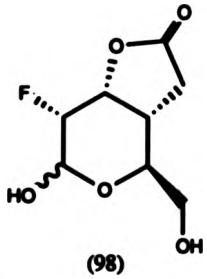
During the preparation of the 2-fluorolactone (59) the mesylate (63) had been isolated and characterised. This had a C-3 leaving group already in place, so it was thought that acetolysis of the methyl ester (105) and subsequent deacetylation would yield the diols (107), Scheme 9.



Scheme 9

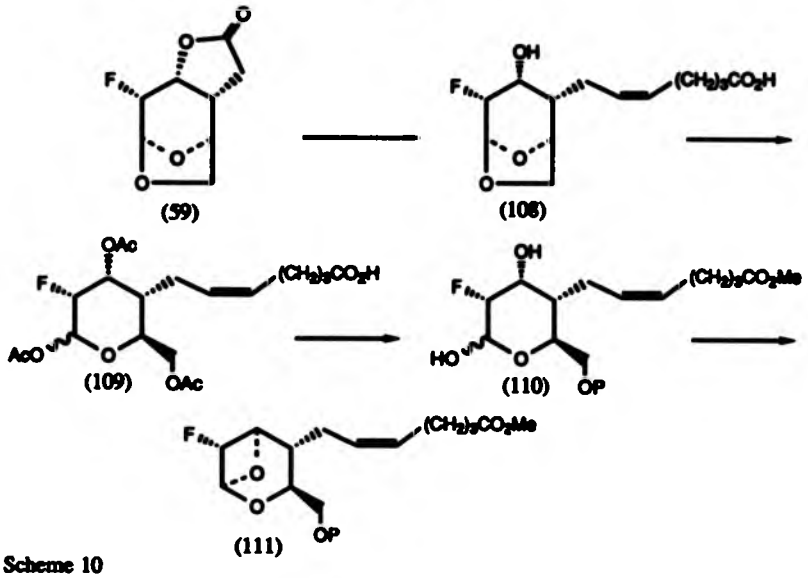
Availability of these diols (107) would allow an attempt at cyclisation using the Fried analogy,[17] ie with a C-3 leaving group. This route was also appealing since it appeared from a study of molecular models that protection of the primary hydroxyl group at C-6 would be unnecessary since the corresponding alkoxide could not displace the C-3 mesylate.

Preparation of the methyl ester (105) proved to be straightforward as did the acetolysis, to yield the acetates (106). However, deprotection with potassium carbonate in methanol did not give the desired diols (107). Analysis by nmr spectroscopy showed that both the mesylate group and the methyl ester function were missing. Since there was no evidence of the elimination products it was concluded that the γ-lactone (98) was the likely product, although this was never confirmed.



(98)

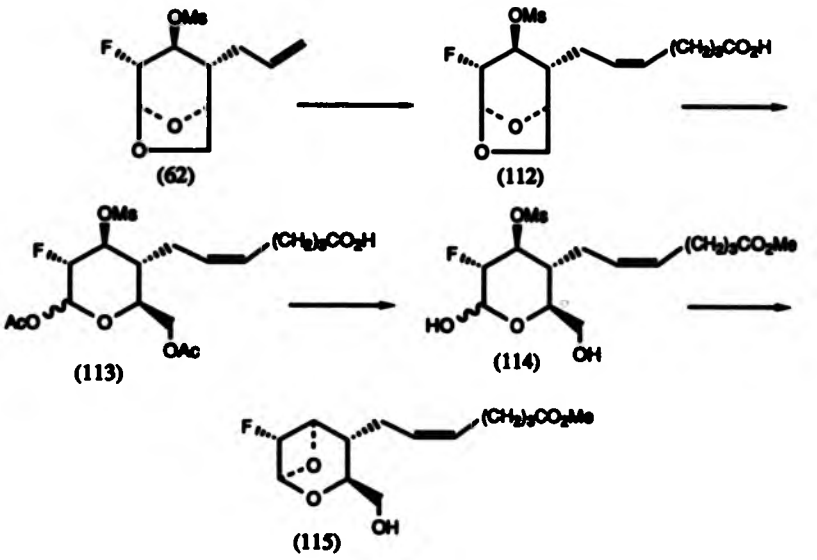
Since both of these routes had been unproductive, the following synthetic schemes were envisaged. It was hoped that the problems previously encountered may be circumvented in a constructive manner by attachment of the top prostanoid side chain.



Scheme 10

Scheme 10 shows the intended synthetic sequence for manipulation of the 2-fluorolactone (59). Dibal reduction and Wittig reaction should yield the hydroxy acid (108). Acetolysis should then provide the triacetates (109) which could be deprotected to the corresponding triol. Esterification of the acid function and selective protection of the primary alcohol should then give (110), a suitable precursor for cyclisation via a C-1 leaving group.

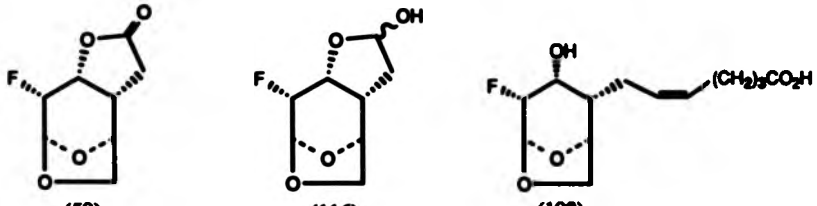
Scheme 11 shows the intended sequence for conversion of the mesylate (62). Oxidative cleavage of the allyl group and Wittig reaction should yield the acid (112).



Scheme 11

Esterification and acetolysis would give the diacetates (113). In this case transesterification should produce the diol (114) because internal displacement of the mesylate by the carboxyl group is unlikely. As mentioned previously, reaction with base should then furnish the bicyclic (3.1.1) acetal without the necessity of protecting the primary C-6 hydroxyl function. Unfortunately, pressure of time and materials never allowed this synthetic scheme to be explored any further.

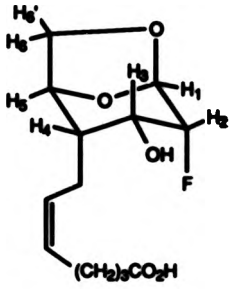
Using previously established prostaglandin methodology[59] the 2-fluorolactone (59) was reduced to the lactol (116) and Wittig reaction with the appropriate phosphorane yielded the hydroxy acid (108) in 70% yield.



(59) (116) (108)

Table 6 shows the nmr data obtained from the high field spectrum. Of interest here are the $J_{2,3}$ and $J_{3,4}$ values 3.66 Hz and 6.1 Hz respectively, typifying the axial-equatorial orientation of the hydrogen atoms. However, more diagnostic is the $J_{H-3,F}$ value, 29 Hz, which is clearly indicative of a trans diaxial orientation of fluorine and the hydrogen atom.[56] This therefore fixed the stereochemistry of the 3-hydroxyl group as equatorial.

Table 6



(108)

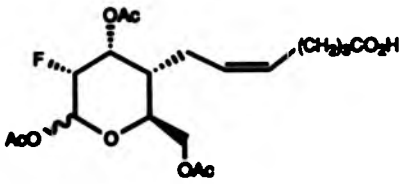
^{1}H Chemical Shifts (ppm)

H-1	H-2	H-3	H-4	H-5	H-6	H-6'
5.4	4.48	4.08	-	4.5	4.78	

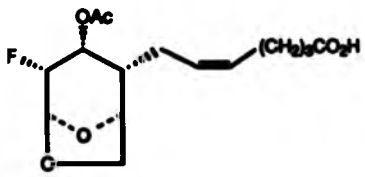
^{1}H-^{1}H, ^{1}H-^{19}F Coupling Constants (Hz)

$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{H-2,F}$	$J_{H-3,F}$
2.44	3.66	6.1	50.7	29.3

Acetolysis of the hydroxy acid (108), however, did not give the expected triacetates.

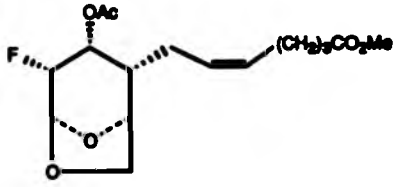


(109)



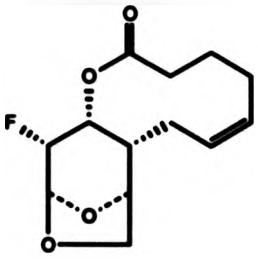
(117)

The major product, along with a plethora of minor components, was the mono acetate (117). This was confirmed by spectroscopic means and an independent synthesis using acetic anhydride and pyridine. Similarly, acetolysis of the monoacetate (117) led to recovery of starting material along with numerous decomposition products. Initial protection of the acid as the methyl ester (118) was equally unproductive. Acetolysis in this case produced a whole string of compounds, none of which have been characterised.

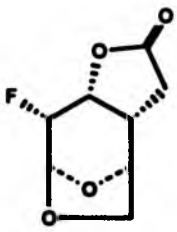


(118)

In order to overcome these difficulties it was reasoned that the macrolactone (119) might give a cleaner reaction on acetolysis. It was believed that this compound would bear a closer parallel with the γ-lactone (59), in terms of functionality.



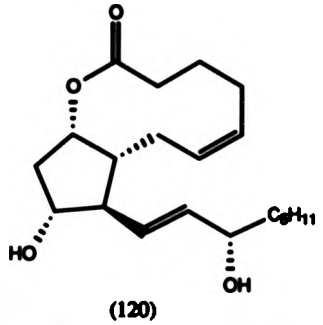
(119)



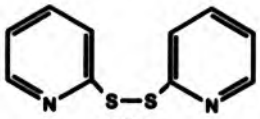
(59)

A similar ten-membered ring lactone (120) was prepared by Corey et al[60] by prior preparation of the thiopyridyl ester, followed by thermal lactonisation.

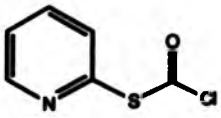
Using the same procedure[61] the crystalline macrolactone (119) was isolated in about 10% yield. Use of a more efficient preparation[62] of the thiopyridyl ester allowed this to be improved to ~30%. The original preparation used the dimeric bipyridyl bisulphide (121) to form the thiopyridyl esters. The latter process used thiopyridyl chloroformate (122) which has the advantage of yielding volatile by products, namely carbon dioxide and hydrogenchloride.



(120)



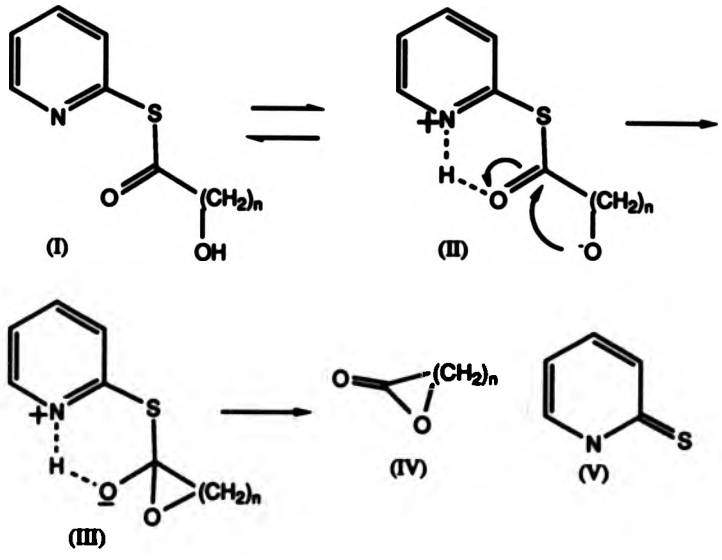
(121)



(122)

Further experimental work using either silver salt catalysis[63] or changing addition rates and concentrations provided no significant improvements. During these experiments small quantities of the dimeric species were often isolated, here structural assignment was based on the compound having a molecular ion of m/e 512 and a very similar nmr spectrum. Other products were usually observed on tlc and some starting material was always recovered. This however never accounted for the large mass imbalance between isolated products and starting material. It is therefore likely that polymerisation or decomposition are not insignificant side reactions.

Retrospective rationalisation has led to the belief that once again, the electronegativity of the fluorine substituent might be responsible. The normal mechanism for the lactonisation of 2-pyridyl thioesters is outlined in Scheme 12.[61]



Scheme 12

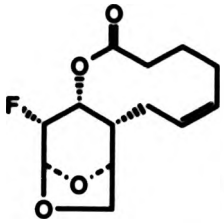
It was reasoned that with pyridyl thio esters that the internal proton transfer (I)⟶(II) would be more favourable than for simple esters. The dipolar intermediate (II) was then believed to undergo a facile, electrostatically driven cyclisation to (III) which would then yield lactone (IV) by elimination of the 2-pyridthione (V).

As already seen in the tosylation reaction of alcohol (12), the presence of a α-fluoro substituent will reduce the nucleophilicity of the C-3 alkoxide residue. In the case of an intermediate of type (ii) this will reduce the efficiency of the intramolecular cyclisation with respect to intermolecular side reactions. This suggests that even higher dilution conditions might favour the intramolecular process.

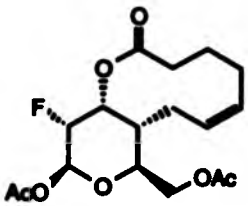
Although the yields of this step were disappointing it was gratifying to find that acetolysis gave a relatively clean reaction to yield the acetates (123) and (124). Once more, it was possible to separate and characterise the individual acetates. The appropriate nmr details are presented in Table 7 along with those for the macrolactone (119).

As with the hydroxy acid (108) the ^1H-^1H coupling constants $J_{1,2}$, $J_{2,3}$ and $J_{3,4}$ for the macrolactone are indicative of an axial-equatorial orientation. Once again the conformation of the pyran ring was confirmed by the $J_{H-3,F}$ coupling constant, (~27 Hz) this being typical of a <u>trans</u> diaxial arrangement of the fluorine and hydrogen atoms.[56] Naturally, these values all change for the acetates (123) and (124). Since the $J_{H-3,F}$ value now appears to be too small to measure, it implies that both anomers have undergone a conformational change from $^1C_4$ to $^4C_1$.

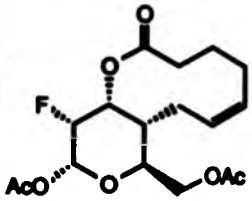
Table 7



(119)



(123)



(124)

^1_H Chemical Shifts (ppm)

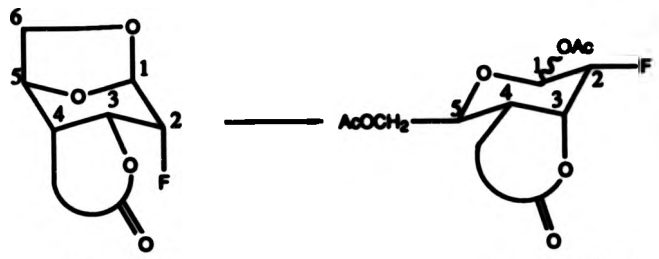
	H-1	H-2	H-3	H-4	H-5	H-6	H-6'
119	5.57	4.51	5.12	2.2	4.51	3.78	3.89
123	5.94	4.47	5.38	(2.5-1.5)	3.98	4.31	4.15
124	6.32	4.7	5.28	(2.5-1.5)	4.1	4.29	4.18

^1_H Coupling constants (Hz)

	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
119	1.3	4.1	6.7	-	5.3	0.9	7.7
123	9.0	3.0	-	-	1.5	4.5	12.0
124	4.0	4.0	-	-	1.5	4.5	12.0

^1_H-$^{19}_F$ Coupling constants (Hz)

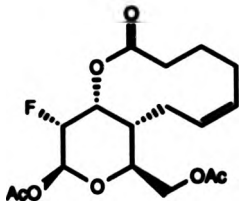
	$J_{H-1,F}$	$J_{H-2,F}$	$J_{H-3,F}$
119	-	50.4	27.0
123	2.0	46.5	-
124	0	43.5	-



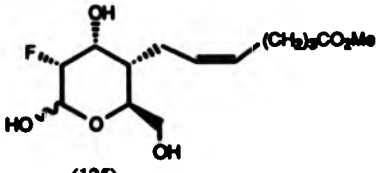
(119, $^1C_4$) (123,124, $^4C_1$)

This was corroborated by the $J_{2,3}$ values for both anomers being similar and typical of axial-equatorial orientation. Moreover, the $J_{1,2}$ values of 9Hz and 4Hz were typical of a axial and an axial-equatorial orientation respectively, hence allowing assignment of the anomeric configuration.

Having finally achieved the cleavage of the anhydro bridge, all that remained to be done was some appropriate protection before cyclisation to the oxetane. It was hoped that the diacetates (123, 124) could be transesterified to the anomeric methyl ester (125).

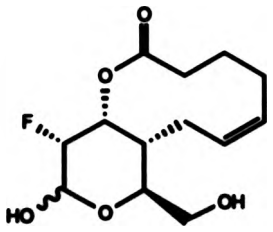


(123,124)



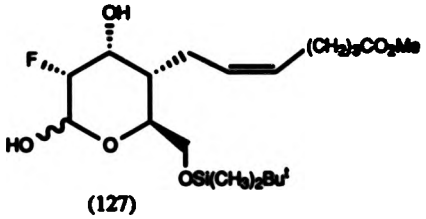
(125)

Reaction with potassium carbonate in methanol however led to a smooth conversion of the diacetate to the anomeric diol (126).



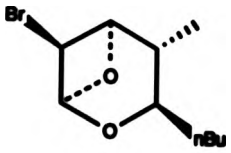
(126)

Several attempts to transesterify this diol were attempted, but under both acidic and basic conditions the lactone remained intact. Ultimately the diol (126) was hydrolysed with potassium hydroxide in aqueous methanol and work-up with diazomethane gave the methyl ester (125) as a anomeric mixture in moderate yield. Finally, selective protection of the primary hydroxyl group gave the silyl ether (127), a suitable precursor for cyclisation.

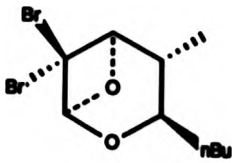


(127)

During the course of this synthetic endeavour, the synthesis of the dioxabicyclo(3.1.1) heptanes (128) and (129) had been published by Still *et al.*[64]

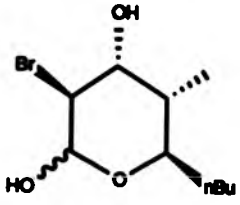


(128)

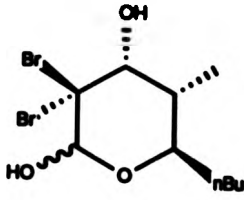


(129)

Both these bicyclic oxetanes had been prepared by cyclisation of the corresponding hemiacetals (130) and (131), using a modified Mitsunobo reaction.

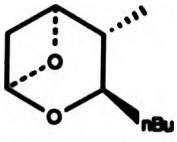


(130)



(131)

Still et al. were then able to remove the bromine atoms using tributyltin hydride to yield the unsubstituted oxetane (132).

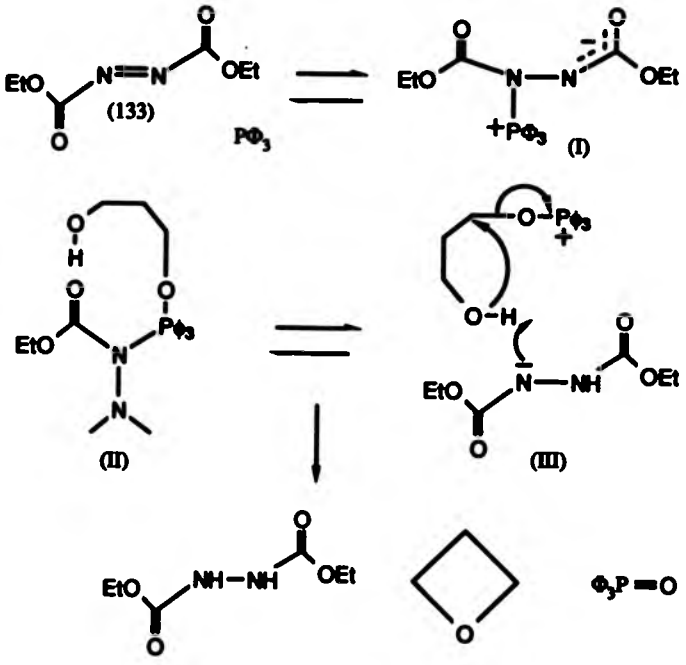


(132)

The methodology developed on these model systems was later used to synthesise thromboxane A_2 [65] (p. 66).

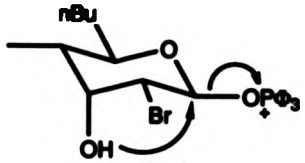
Use of the Mitsunobo reaction for the synthesis of oxetanes and other oxygen heterocycles was first reported by Carlock and Mack.[66] It was demonstrated that treatment of various diols with diethylazodicarboxylate (133) and triphenyl phosphine led to their efficient conversion to the corresponding cyclic ethers. That is, (1,2), (1,3) and (1,4) diols gave epoxides, oxetanes and tetrahydrofurans respectively. The mechanism postulated by Carlock and Mack[66] for these cyclisation reactions is outlined in Scheme 13.

The reaction is thought to proceed first through the betaine (I) which then interacts with the diol to form either the adduct (II) or the salt (III). Collapse of these intermediates then generates the cyclic ether, triphenylphosphine oxide and dicarbethoxyhydrazine.



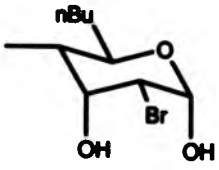
Scheme 13

Analysis of the cyclisation of the hemiacetals (130, 131) in the light of this mechanism implies that the salt (134) (β-anomer, $^4C_1$ conformation) is the only viable intermediate likely to produce an α-oxetane bridge.

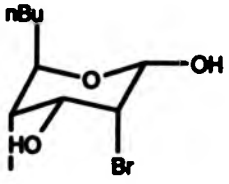


(134)

Reaction of the α-anomer in either the $^4C_1$ (135) or the $^1C_4$ (136) conformation is extremely unfavourable on stereoelectronic grounds. That is because internal nucleophiles at either C-1 or C-3 can never attain the correct orientation for displacement of a leaving group.

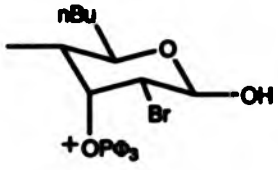


(135, $^4C_1$)

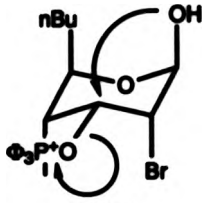


(136, $^1C_4$)

Similarly, having a C-1 hydroxyl as an internal nucleophile in the anomer (137) can be discounted on the same grounds.



(137)



(138)

This does, however, have the potential to form a β-oxetane bridge by reaction through the less stable $^{1}C_{4}$ conformer (138) with most substituents axially orientated. Finally, an intramolecular process as in the type II adducts proposed by Carlock and Mack was also discounted. It is thought that the steric requirements for oxetane formation are too restricting to incorporate a ten-membered cyclic transition state.

Cyclisation via an intermediate such as (134) is also in agreement with the previously discussed stereochemical requirements for α-oxetane bridge formation. In this instance the Mitsunobo reagents create an equatorial leaving group at C-1 and an axial internal nucleophile at C-3.

The anology between the bromo hemiacetals (130) and (131) and the protected fluoro hemi acetal (127) was obvious and naturally this cyclisation reaction was attempted. As anticipated on the basis of the results obtained by Still,[65] reaction of (127) with diethylazodicarboxylate/trimethylphosphite adduct gave a less polar spot, well resolved from the starting material and other products. Separation by flash chromatography gave a compound homogeneous on tlc and displaying the nmr spectrum in Figure 6 and the parameters presented in Table 8.

Analysis of nmr data from previously synthesised dioxa bicyclo

(3.1.1) heptanes and their analogues, Table 9, showed the following characteristics. Firstly, all these compounds have a large $J_{1,3}$ coupling constant of approximately 4-5 Hz. Secondly, compounds that have a β-hydrogen substituent at C-2 (132), (140)-(142), show a zero coupling with both H-1 and H-3.

Examination of the nmr spectrum, Figure 6, clearly shows H-3 as a doublet of doublets centred at δ4.54. The larger coupling constant 13 Hz was assumed to be $J_{H-3,F}$ and the smaller 4.8 Hz was presumed to be the expected long range $J_{1,3}$ coupling constant. Unfortunately, this could not be conclusively proved by either further inspection or a simple decoupling experiment since the H-1 resonance was buried amongst the alkene resonances δ5.3-5.5. However, this problem was overcome using a decoupling difference experiment. Here irradiation of a specified proton is carried out in the normal manner, then the original spectrum is subtracted from the decoupled spectrum to provide a difference spectrum. The net result of this manipulation provides a spectrum which shows negative peaks for the irradiated proton and both positive and negative peaks for the decoupled proton. For the decoupled proton, the negative peaks correspond to the spectrum before irradiation and the positive peaks to the spectrum after irradiation.

Table 8

^{1}H Chemical Shift (ppm)

H-1	H-2	H-3	H-4	H-5	H-6	H-6'	H-7	H-8	H-9	H-10	H-11	H-12
5.5	4.7	4.54	(2.2-2.4)	3.83	3.78	3.63	(2.2-2.4)	5.35	5.5	2.08	1.68	2.3

^{1}H Coupling Constants (Hz)

$J_{1,2}$	$J_{1,3}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,6'}$	$J_{4,7}$	$J_{9,10}$	$J_{10,11}$	$J_{11,12}$
0.0	4.8	0.0	-	-	2.9	11.1	-	7.5	7.4	7.4

^{1}H-^{19}F Coupling Constants (Hz)

$J_{H-1,F}$	$J_{H-2,F}$	$J_{H-3,F}$
4.4	59.3	13.5

Figure 6

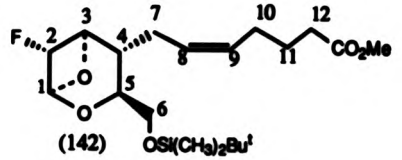
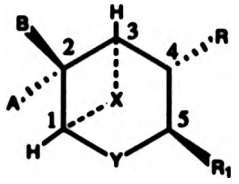


Table 9



Compound[Ref]	X	Y	A	B	$J_{1,A}$	$J_{1,B}$	$J_{3,B}$	$J_{3,A}$	$J_{1,3}$	$J_{A,B}$
27[17]	O	O	F	F	0.0	0.0	0.0	8.5	4.6	185.1
29[17]	O	O	F	F	0.0	0.0	0.0	7.9	4.9	176.2
139[64]	O	O	S	S	-	-	-	-	4.3	0.0
129[64]	O	O	Br	Br	-	-	-	-	4.3	0.0
132[64]	O	O	H	H	4.0	0.0	0.6	6.6	4.0	8.9
128[64]	O	O	H	Br	3.3	-	-	5.9	3.9	0.0
140[67]	S	S	H	H	5.7	0.0	0.0	6.0	3.8	10.1
141[68]	S	O	H	H	3.0	0.0	0.0	5.0	5.0	9.5
142	O	O	F	H	4.84	0.0	0.0	13.55	4.84	59.3

The results of this experiment are shown in Figures 7 and 8. Figure 7 shows the spectrum obtained by irradiation of the H-3 resonance. At δ4.55 the doublet of doublets are seen as negative peaks. However, at δ5.45 a triplet is seen below the line and a doublet above. Since spectral subtraction has removed any interference from the alkene protons H-1 is clearly seen as a triplet (negative) which collapses to a doublet (positive) when H-3 is irradiated. This shows that H-1 is coupled to H-3 and indicates that H-1 is also coupled to fluorine with a coupling constant of similar magnitude. The converse experiment is shown in

Figure 8 in which protons at δ5.45, that is in the approximate region of H-1, were irradiated.

Irradiation at δ4.55

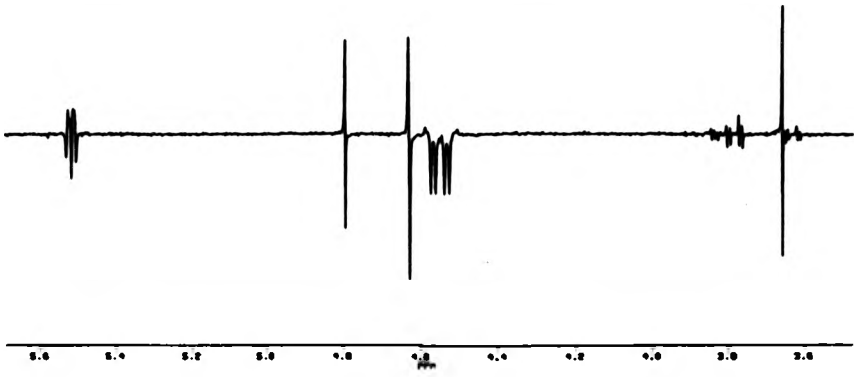


Figure 7

Irradiation at δ5.45

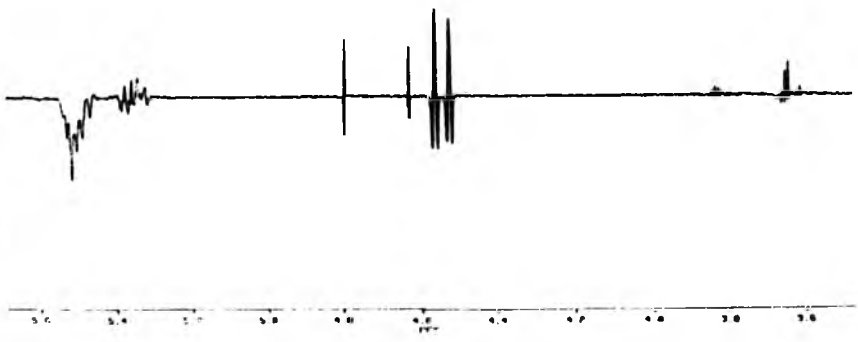
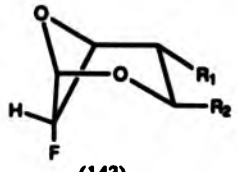


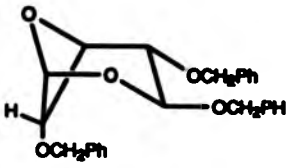
Figure 8

At δ4.55 the H-3 doublet of doublets (negative) collapses to a doublet (positive) on irradiation. This confirmed that H-1 and H-3 are coupled as outlined above.

Further analysis of the spectrum, Figure 6, shows H-2 as a doublet centred at δ4.7, once again this large coupling ~59 Hz is assumed to be $J_{H-2,F}$. This shows that there is a zero coupling to both H-1 and H-3, therefore providing the evidence for formation of an α-oxetane bridge. Had the β-oxetane (143) been formed the dihedral angle between H-1 and H-2 would have been close to 0^{o} and a larger coupling constant would have been seen.



(143)

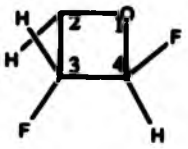


(16)

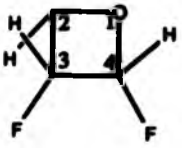
The 1,3-anhydro glucopyranoside (16) synthesised by Schuerch[13] has a $J_{1,2}$ value of ~4 Hz.

Finally, the magnitude of the $^{1}H-^{19}F$ coupling constants were confirmed from an ^{19}F nmr spectrum. Given that the (H-3, F-2) and (H-1, F-2) dihedral angles are identical the $J_{H-1,F}$ (4.4 Hz) and $J_{H-3,F}$ (13.5 Hz) coupling constants would appear anomalous. This discrepancy can only result from the different electronegativities of the substituents on the oxetane ring, the $J_{H-1,F}$ being lower because of two oxygen substituents on C-1. A similar variation in coupling constants

has also been observed in the unsymmetrically substituted fluoro-oxetanes[69] (144) and (145).



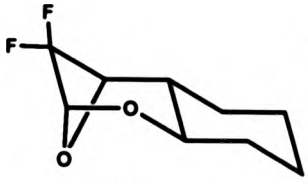
(144)



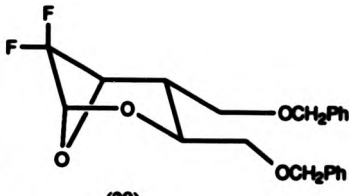
(145)

Oxetane (144) displayed $J_{H-2,F-3}$ = 13 Hz and $J_{H-2,F-3}$ = 7 Hz whereas oxetane (145) had $J_{H-2,F-3}$ = 12 Hz and $J_{H-4,F-3}$ = 3.5 Hz. In these cases the lower $J_{H-4,F-3}$ values must be a result of the fluorine substituent on C-4.

It was also reasoned that these substituent effects resulted in no fluorine proton coupling being observed in the gem difluoro oxetanes (27) and (29).[17]



(27)

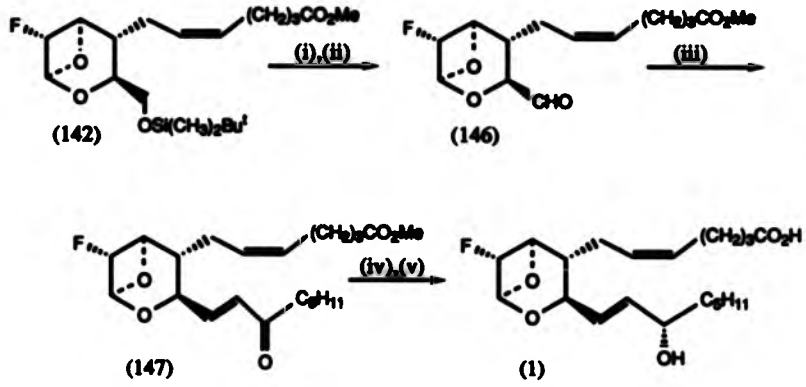


(29)

Zero coupling between the H-1, H-3 protons and the β-fluoro substituent would be observed due to a 90° dihedral angle, $^{1}H-^{19}F$ vicinal

coupling having a similar angular dependence as $^{1}H-^{1}H$ vicinal coupling.[70] It was then argued that any coupling between these protons and the α-fluoro substituent would be reduced to negligible values due to the electronegativity of the β-fluoro substituent and the oxygen substituents.

Having prepared and satisfactorily characterised the 2,6-dioxa bicyclo (3.1.1) heptane (142) all the major synthetic hurdles had been overcome. All that remained to complete the synthesis of 10-fluoro-TXA_2 (1) was attachment of the bottom prostanoid side chain which should follow the usual prostaglandin methodology, Scheme 14.



Scheme 14

(i) Bu_4NF (ii) [O] (iii) $(MeO)_2POCH_2COC_5H_{11}$
(iv) $NaBH_4$, column chromatography (v) H_3O^+

Scheme 14

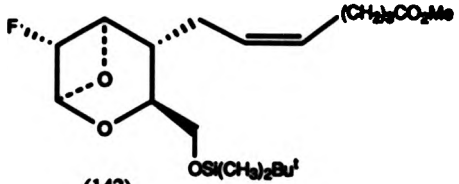
Completion of the synthesis in this manner will naturally depend on the chemical stability of the oxetane bridge. As yet, nothing is really known about this, excepting that due to a time lapse between running the ^{1}H and ^{19}F spectra the compound was stored at low temperature in solution for several days without any hydrolysis occurring. The hydrolytic stability of the difluoro oxetanes[17] (27) and (29) are also very encouraging in this respect.

It is regretted that insufficient time and materials were available to either complete the total synthesis or to progress beyond this point.

2.3 Concluding Remarks

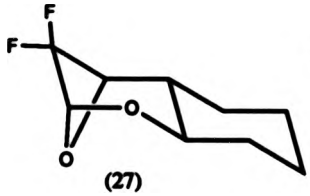
The aim of this project was to achieve a total synthesis of a fluorinated thromboxane A_2 analogue, in the belief that this would be a more stable compound than TXA_2 itself, and that it might have an interesting biological profile. Furthermore, it was hoped that such a compound might have antagonistic properties with respect to natural TXA_2 and thus be therapeutically useful.

Although completion of this ambitious project has not been realised in full, a major part of it has been fulfilled in the form of the synthesis of the 10-α-fluoro TXA_2 precursor (142).

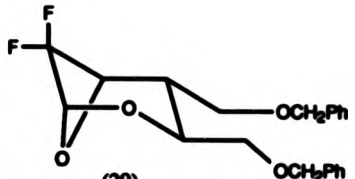


(142)

This has been particularly rewarding since it proves that the original idea concerning the stabilisation of the sensitive bicyclic (3.1.1) acetal by the electron withdrawing properties of fluorine, was fundamentally correct. This was also borne out by the elegant studies of Fried et al[17] in their synthesis of the hydrolytically stable difluoro-acetals (27) and (29).



(27)



(29)

The synthetic progress towards the oxetane (142) has also continually emphasised the influence of fluorine substitution on chemical reactivity (p.17). Indeed it is now hoped that a greater awareness and understanding of these properties should allow some optimisation of the synthetic sequence.

The first area requiring improvement however is the fluorination step itself. It is felt that further experimental work using (56), the reagent developed by Clark and Brown,[35] might allow some improvement.

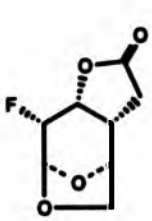
$$Ph_4PHF_2$$
(56)

Use of the non-nucleophilic solvents dimethyl formamide or dimethyl sulphoxide will prevent competitive epoxide cleavage from the solvent and allow more forcing conditions than tried previously. Another fluorinating reagent recently reported[71] is tetrabutylammonium bifluoride (148). This could be regarded as an organic equivalent to potassium hydrogen fluoride and consequently may also be a useful reagent.

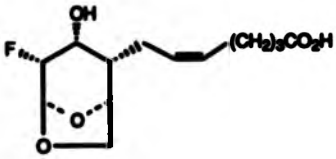
$$Bu_4NHF_2$$
(148)

The influence of the fluorine atom first became apparent during the preparation of the tricyclic lactone (59), that is when the use of a triflate leaving group was required to complete lactonisation. However, this influence was more significant in increasing the stability of the 1,6-anhydro bridge towards hydrolysis. In order to overcome this problem a strongly acidic acetolysis procedure was required.

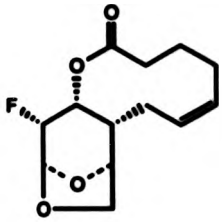
Unfortunately, under these conditions the hydroxy acid (108) decomposes or yields only the C-3 acetate.



(59)



(108)



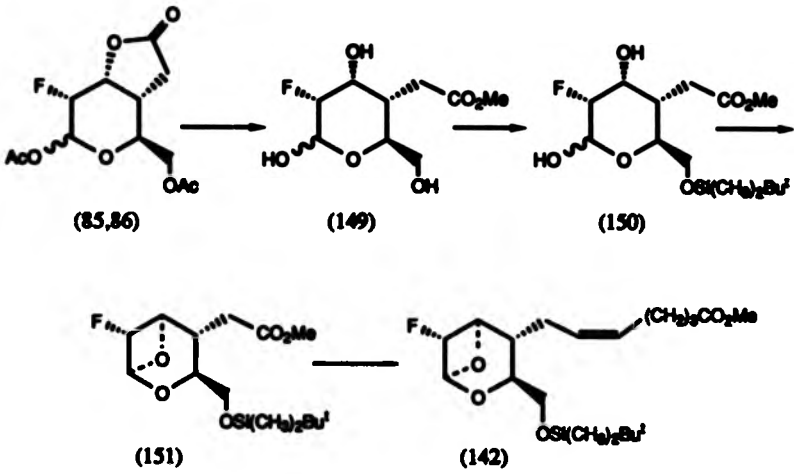
(119)

Acetolysis of the hydroxy acid was however facilitated by preparation of the macrolactone (119) but this could only be achieved in poor yields (~30%). It is possible that an alternative procedure for lactonisation may improve this step, for instance use of a different thio ester and catalysed lactonisation with silver or mercury salts.[72] However, it is arguable that a change in the sequence of synthetic steps may be more appropriate in this case.

It was demonstrated that acetolysis of the tricyclic lactone (59) proceeded smoothly to yield the acetates (85, 86). Therefore introduction of the top prostanoid sidechain after cleavage of the 1,6-anhydro bridge and formation of the oxetane bridge may be a better alternative, Scheme 15.

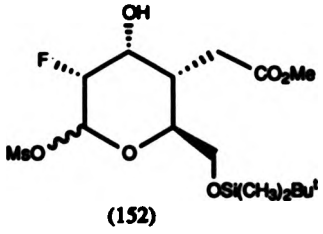
Hydrolysis of the acetates (85, 86) and work up with diazomethane should yield the methyl ester (149). Protection of the primary alcohol followed by cyclisation would provide the oxetane (151) and finally Dibal reduction, Wittig reaction and esterification would give the TXA_2 precursor (142). This sequence will only be viable however if a more efficient cyclisation procedure can be found and if the oxetane bridge is

stable to several chemical conversions.

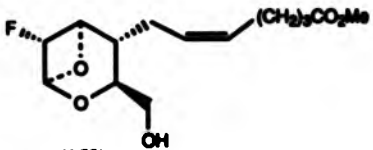


Scheme 15

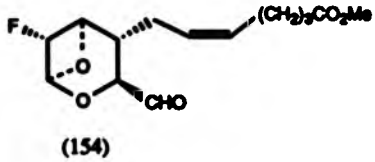
Use of the Mitsunobo reagents for this reaction has been thoroughly investigated by Still et al[60] so any optimisation of this reaction is unlikely. A better yield might be possible however by preparation of the C-1 mesylate (152) and reaction with base according to Fried et al[17].



The oxetane (142) is unlikely to have any intrinsic biological activity itself. However, it provides easy access to the alcohol (153) and aldehyde (154) which would be valuable intermediates for the preparation of a series of thromboxane A_2 analogues.



(153)



(154)

It is known that the bottom sidechain is not generally required for the biological activity of many prostanoid derived pharmaceuticals.[73] The aldehyde (154) and alcohol (153) would therefore provide suitable precursors for attaching a variety of substituents for the investigation of biological activity.

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CHAPTER THREE

EXPERIMENTAL

Experimental

i) Spectroscopy

^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded in the solvents stated and using tetramethylsilane (TMS) as an internal standard. All chemical shifts are quoted in ppm downfield from TMS. Routine spectra were recorded on a Perkin-Elmer R32 (90 MHz) or a Bruker WP80 (80 MHz) spectrometer. High field spectra were recorded either at Edinburgh University (360 MHz spectra) or at ICI Pharmaceuticals, Alderley Park (400 MHz spectra).

^{19}F spectra were also recorded at Edinburgh University using deuterochloroform as solvent and trifluoroacetic acid as an external reference.

Infra-red spectra were recorded on a Shimadzu IR-435 instrument either in solution (10% in CH_2Cl_2) or as thin films. Mass spectra and high resolution mass determinations were obtained by Mr D F Dance or Mr T Moodie using a Jeol JMS-D 100 mass spectrometer.

ii) Chromatography

Analytical tlc plates (Merck Kieselgel 60 F_{254} Art 5735) were eluted in the solvent systems stated, then examined under ultra violet light. These plates were then stained with iodine vapour and/or ceric ammonium sulphate followed by heating to 150°C.

Chromatographic separations were achieved using columns with Kieselgel 60 (Merck 9385 or Fluka 60738) and the procedures of Still et al (J.Org.Chem., 43, 2923, 1978) were normally utilised. Where elution rates are not quoted columns were pressurised using a hand pump and the elution rates unknown.

Prior to loading onto columns all samples were bound onto silica. This was achieved by dissolving the compounds in an appropriate solvent (usually CH_2Cl_2 or MeOH), adding small quantities of silica (100-200 mg per 100 mg of sample), mixing then removing the solvent *in vacuo*. This process was repeated until a dry free flowing powder was obtained. The sample was then applied to the column by pouring the powder into the head of solvent above the column. This was followed by tapping gently to remove air bubbles and level out the sample. The columns were then eluted in the normal manner.

iii) General

All solvents were routinely distilled from appropriate drying agents and commercially available reagents were used as obtained.

For reactions requiring anhydrous conditions, the solvents were either redistilled immediately before use or dried by storage over activated molecular sieve 3A. Liquid reagents were purified by distillation and dried by storage over molecular sieve, whereas solid reagents were predried by storage at reduced pressure over P_2O_5 or by azeotropic distillation with dry toluene. Reactions were performed in flame dried flasks fitted with Suba-seal rubber septa under an atmosphere of dry nitrogen. Transfer of solutions was accomplished using syringe technique.

Finally, all melting points are uncorrected and were determined on a Köffler hot stage apparatus. Micro analyses were determined by Mrs G Berry at Stirling University.

Preparation of levoglucosan (10)

a) Pyrolysis of starch[1]

Oven-dried maize starch (~50 gms) was placed in a wide-necked round-bottom flask (500 ml). A conical flask in an ice bath was attached to this via a wide bore glass tube and the system evacuated under water pump pressure.

The starch was then pyrolysed using a luminous yellow bunsen flame for 30 mins. The collected brown distillate was dissolved in acetone, transferred to a round bottom flask and concentrated. The resulting syrup was azeotroped with more acetone (2x50 mls), dissolved in a minimum quantity of warm acetone and placed in a freezer overnight.

If no crystallisation had occurred, the syrup was bound onto silica gel (60-120 mesh), the resulting free flowing powder was then poured onto a column of silica (18x5 cm) with a small head of solvent above it. Once the sample was loaded the column was eluted with 10% MeOH/90% EtOAc. The later fractions yielded clean crystalline levoglucosan (~1.5 gms) mp 170°C, (lit[1] mp 172°) tlc mobility in 10% MeOH/90% EtOAc was identical to an authentic sample Rf ≈0.1.

b) Hydrolysis of phenyl β-D-glucopyranoside[2]

Phenyl β-D-glucopyranoside (10 gms) was dissolved in 1.3M potassium hydroxide solution (500 mls) and refluxed overnight, tlc monitoring (90% EtOAc/10% MeOH) indicating a complete reaction.

The reaction mixture was cooled and the pH adjusted to 3.5 with 1.5M sulphuric acid solution (78 mls) then it was evaporated to dryness azeotroping with ethanol if necessary. The crystalline residue was extracted with several portions of hot ethanol and filtered to leave the unwanted sodium sulphate.

The combined filtrate was then concentrated and recrystallised from ethanol (20 mls) to yield levoglucosan (4.5 gms, 65%).

c) From glucose[3,4]

i) Penta-O-acetyl-β-D-glucopyranose[3]

A suspension of anhydrous sodium acetate (50 gms) in acetic anhydride (700 mls) in a 2-litre round-bottom flask was heated over a flame to its boiling point. A portion of α-D-glucose (3 gms) from a larger supply (100 gms) was added and without shaking, the flask was heated carefully at the point nearest the sugar lying on the bottom. Initiation of the reaction was indicated by continued boiling after removal of the flame; the flask was then placed on a cork ring and the remainder of the α-D-glucose was added in small portions to maintain the boiling temperature of the mixture. Occasionally the flask was shaken to prevent an accumulation of solid sugar on the bottom. If the reaction stops it should be started again by heating before much more sugar is added to the flask. After the addition of all the sugar and after the reaction had subsided, the solution was brought to a full boil. It was then cooled and poured with stirring onto cracked ice (2 litres). After standing for 3 hrs with occasional stirring the crystalline material was filtered with suction and washed with cooled water. Recrystallisation from ethanol followed by filtration once the solution had cooled to room temperature, yielded β-D-glucopyranose penta acetate, mp 128°-131°, (lit[3] mp 132°C).

ii) Phenyl tetra-o-acetyl-β-D-glucopyranoside[4]

To a solution of p-toluene sulphonic acid monohydrate (0.75 gms) in warm phenol (50 gms) was added penta-o-acetyl-β-D-glucopyranose (58.5 gms). The flask was fitted with a capillary tube and arranged for distillation. The mixture was heated strongly on a steam bath under reduced pressure (20mm Hg) for 30 minutes after the liquid began to distill, then under 10-12mm Hg pressure for 15 minutes. At this point distillation was interrupted and a solution of sodium hydroxide

(0.25 gms) in warm phenol (15 mls) was added. The distillation was continued at 10-12 mm Hg until distillation had nearly ceased. The pressure was then reduced to about 1 mm Hg and distillation was continued for as long as phenol came over. The thick residue was stirred with hot water (100 mls) and the mixture was allowed to cool. The water was decanted, the residue dissolved in hot ethanol (50 mls) and the solution was allowed to stand at room temperature for 15-20 hours. The resulting crystals of phenyl tetra-o-acetyl-β-D-glucopyranoside were filtered and washed with 70% ethanol (~15 mls), then air dried. Yield 33.2 gms (52%)# mp 118-122°, (lit[4] mp 120-122°C).

1,6-anhydro-β-D-glucopyranose[4] (levoglucosan), (10)

To a solution of sodium hydroxide (42.9 gms) in water (333 mls) contained in a 500 ml round-bottom flask was added phenyl tetra-o-acetyl-β-D-glucopyranoside (55.1 gms, 0.13 moles). The mixture was then refluxed gently for 20 hours.

After refluxing, the solution was cooled to room temperature and neutralised by the addition of conc sulphuric acid (45.4 gms) diluted with an equal weight of ice. The solution was then concentrated to dryness under reduced pressure and the residue was extracted with boiling ethanol (250 mls) and filtered. The undissolved salts were washed with two portions (50 mls) of hot ethanol and the combined ethanol extracts were concentrated to dryness. The resulting crude syrup containing levoglucosan was then used in the preparation of 1,6:3,4-dianhydro-2-O-p-toluenesulphonyl β-D-galactopyranose (9).

This was the best yield obtained and was from commercially available Penta-O-acetyl-β-D-glucopyranose (supplied by Fluka). Using the penta acetate prepared as above the yields of phenyl tetra acetate were typically 25-30%. This presumably indicates a substantial contamination from penta-o-acetyl-α-D-glucopyranose.

1,6:3,4-Dianhydro-2-O-p-toluenesulphonyl-β-D-galactopyranose[5] (9)

Levoglucosan (10 gms) was dissolved in pyridine (50 mls) and cooled to 0°C. A solution of p-toluene sulphonyl chloride (24.4 gms) in chloroform (100 mls) and pyridine (70 mls) was then added dropwise. The resulting solution was warmed to room temperature and stirred for 2 days.

Water (10 mls) was added and the resulting solution stirred for 2 hrs. This was then washed with water (10 mls), 5% sulphuric acid solution (3x100 mls) and finally with water (100 mls) again. The organic solution was then dried ($MgSO_4$), filtered and concentrated to leave crude 1,6-anhydro-2,4-di-o-p-toluenesulphonyl-β-D-glucopyranose as a syrup.

This was dissolved in either dichloromethane or chloroform (250 mls) and to this a 5% sodium methoxide solution (100 mls) was added dropwise. The resulting solution was stirred at room temperature overnight during which time a white precipitate appeared. The reaction was quenched with water (50 mls) so the precipitate dissolved and then the phases were separated, the aqueous phase being washed with several portions of dichloromethane (3x10 mls). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated to leave the crude crystalline material. Recrystallisation from 4:1 methanol/chloroform gave needle-shaped crystals of the epoxide (9) (9.7 gms, 55%) mp 151-152°C lit[5](147°-148°C).

1,6-Anhydro-2-o-p-toluenesulphonyl-4-deoxy-4-(2-propenyl)-β-D-glucopyranose[6] (8)

Dry hexane-washed magnesium turnings (20 gms) were placed in a flame-dried flask under nitrogen and suspended in dry tetrahydrofuran (250 mls). A few crystals of iodine and ethylene dibromide (0.2 ml) were added and the suspension stirred mechanically until the iodine

colouration had gone. It was then cooled to -15°C (carbon tetrachloride/dry ice bath) and a solution of allyl bromide (7.6 gms) in dry tetrahydrofuran (20-30 mls) was added dropwise over 1.5-2 hours, and then the mixture was stirred for a further 0.5 hours.

The resulting metallic grey coloured solution prepared above was added dropwise to a mechanically stirred suspension of the epoxide (9) (5g) and copper(I) iodide (1g) in dry tetrahydrofuran (50 mls) at 0°C under nitrogen. During the addition the suspension went yellow then eventually black. The resulting mixture was then stirred overnight at 0°C.

2M Hydrocaloric acid (12 ml) was added dropwise to the cold stirred solution, so the solution was acidic. The resultant clear supernatant layer was then decanted from the black granular precipitate. The precipitate was washed with small portions of dichloromethane and the combined organic fractions concentrated. The sludgy residue was extracted by washing with several portions (5x20 mls) of dichloromethane. The combined organic fractions were then washed with brine (2x20 mls) dried ($MgSO_4$) and concentrated to an oil.

The crude oil was normally processed through the next stage without further purification. However, it was easily purified by column chromatography. Column chromatography using silica gel (300g, K9385) and eluting with chloroform/ethyl acetate (19:1) increasing to (88:12) yielded pure 1,6-anhydro-2-O-p-toluenesulphonyl-4-deoxy-4-(2-propenyl)-β-D-glucopyranose (8) (3.7g, 64%) mp 65-68°C (lit[6] mp 65-67°).

1,6:2,3-Dianhydro-4-deoxy-4-(2-propenyl)-β-D-manno-pyranose (7)

The crude oily residue from the previous preparation was dissolved in dichloromethane (100 mls) and 5% sodium methoxide solution (50 mls) was added dropwise to the stirred solution at room temperature. After

addition the resulting solution was stirred overnight.

The mixture was then quenched with water (20 mls) to dissolve the white precipitate. The phases were separated and the aqueous phase washed with dichloromethane (2x10 mls). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The crude oil was bound onto silica, placed on a column (10x3 cm) and eluted at 35 mls/min with 10% ethyl acetate/90% hexane to yield 1,6-2,3-dianhydro-4-deoxy-4-(2-propenyl)-β-D-mannopyranose (7) (1.6 gms, 54% from epoxide (9)) as a slightly yellow oil. This was suitable for further synthetic work. However, analytically pure material was obtained by Kugelrohr distillation.

^{1}H NMR (360 MHz, $CDCl_3$)

δ5.76-5.88 (1H, m, -CH=C, J=7, 10.4, 17 Hz), 5.65 (1H, dd, H-1, J=3.2, 0.5 Hz), 5.11-5.17 (2H, m, C=CH_2 J=10.4, 17 Hz), 4.22-4.24 (1H, m, H-5, J=1.2, 0.5 Hz), 3.68-3.73 (2H, m, H-6_{exo}, 6_{endo}), 3.33-3.35 (1H, m, H-2, J=3.9, 3.2, 0.7 Hz), 2.93-2.94 (1H, m, H-3, J=3.9, 1.2 Hz), 2.29-2.34 (2H, m, -CH_2-C, J=7.5, 7 Hz), 1.98-2.03 (1H, bt, H-4, J=7.5 Hz);

^{13}C NMR ($CDCl_3$)

δ135.4 (C-8), 117.8 (C-9), 98.2 (C-1), 71.2 (C-5), 68.6 (C-6), 50.4 (C-3)*, 53.9 (C-2)*, 39.2 (C-4), 35.2 (C-7).

*Interchangeable.

IR (neat) 3070, 1640 cm^{-1}

MS, m/e found 123

Anal. calcd for $C_9O_3H_{12}$ (168) C, 64.3% H, 7.1%, found C, 63.0% H, 7.4%

TLC (50% EtOAc/hexane) Rf = 0.46.

Fluorination of 1,6:2,3-dianhydro-4-deoxy-4-(2-propenyl)-β-D-manno pyranose (7)

a) Reaction with potassium hydrogen fluoride[7]

The epoxide (100 mg, 0.5 mmol) was dissolved in dry ethylene glycol (2 mls) and potassium hydrogen fluoride (250 mg, 3.2 mmol, 6 eq) was rinsed in with more ethylene glycol (2 mls). The mixture was heated to reflux temperature and the resulting solution refluxed for 2 hours, tlc (25% EtOAc/hex) indicated that most of the starting material had been consumed.

The reaction mixture was cooled and poured into a 5% potassium bicarbonate solution (10 mls). The aqueous solution was then extracted with dichloromethane (3x10 mls). The organic extracts were dried ($MgSO_4$), filtered, and bound to silica for column chromatography. The column (13x1 cm) was eluted with 20% ethyl acetate/80% hexane and yielded 10 mgs (~10%) of suspected fluorohydrin along with numerous more polar compounds.

b) Reaction with potassium fluoride/18.crown.6 complex

Potassium fluoride (200 mg, 3.4 mmols) and 18.crown.6 (910 mg, 3.4 mmols) were dissolved in methanol (10 mls). After a few minutes stirring the solution was evaporated to dryness, azeotroped with benzene (2x2 mls) and then placed at 40°C under reduced pressure (0.5mm Hg) for 1 hour.

Acetonitrile (10 mls) was added to the complex (6.9 eq) above and the mixture brought to reflux temperature. The epoxide (100 mgs, 0.5 mmol, 1 eq) in acetonitrile (5 mls) was added and the solution refluxed, and monitored by tlc (50% EtOAc/50% hexane). Refluxing for five days produced no change.

c) Reaction with tetrabutylammonium fluoride[8]

Tetrabutylammonium fluoride (1 gm, 3.15 mmols, 6.3 eq) was weighed and placed into a round bottom flask under reduced pressure (0.5 mm Hg) for about forty hours.

The flask was then flushed with nitrogen and dry acetonitrile (5 mls) added. The epoxide (100 mg, 0.5 mmol, 1 eq) dissolved in acetonitrile (2 mls) was also added and the mixture warmed to reflux temperature. The resulting solution was then refluxed and monitored by tlc (50% EtOAc/50% hexane) over several days, in which time no significant change was observed.

d) Reaction with caesium fluoride and aluminium trichloride

Caesium fluoride (500 mg, 6.6 eq) was placed in a flask under reduced pressure for several hours. The flask was then flushed with nitrogen and dry tetrahydrofuran (5 mls) was added. To this was added first the epoxide (100 mg) in tetrahydrofuran (2 ml) then aluminium trichloride (200 mg, 2 eq) also in tetrahydrofuran (5 mls). The resulting suspension was stirred at room temperature under nitrogen and monitored by tlc (50% EtOAc/50% hexane).

After six days, 5% ammonium chloride solution was added until the precipitate had disappeared, then the aqueous phase was extracted with chloroform (3x10 mls). The organic extracts were dried ($MgSO_4$), bound to silica and chromatographed. The column (10x1 cm) was eluted with 20% ethylacetate/80% hexane and gave a small quantity of starting material followed by the major product. Spectral data clearly indicated that the epoxide had been opened, but it was not consistent with a fluorinated compound (no ^{13}C-^{19}F coupling). It was therefore tentatively assigned as the chlorohydrin (36).

An identical experiment using potassium fluoride/18.crown.6 complex

(6 eq) and aluminium trichloride also produced the same compound.

^{1}H NMR (90 MHz, $CDCl_3$)

δ6.1-5.55 (2H, m, H-1, -CH=C), 5.3-5.1 (2H, m, C=CH_2), 4.5 (1H, bd, H-5, $J_{5,6\ exo}$ = 5 Hz), 4.15 (1H, d, H-6_{endo}, $J_{endo,\ exo}$ = 7 Hz), 4.0-3.75 (3H, m, H-2, H-3, H-6_{exo}). 2.95 (1H, d, -OH), 2.5 (2H, bt, -CH_2-), 1.8 (1H, bt, H-4).

^{13}C NMR ($CDCl_3$)

δ135.6 (C-8), 117.8 (C-9), 102.1 (C-1), 74.7 (C-2)*, 72.6 (C-5)*, 68.6 (C-6), 58.8 (C-3)*, 44.2 (C-4), 36.3 (C-7),

*Interchangeable

IR (neat) 3450 broad, 3070, 1640 cm^{-1}

MS, m/e found: 169.

a) <u>Reaction with hydrogenfluoride-pyridine complex</u>[9]

A small polypropylene vial with a magnetic stirrer was fitted with a septum and cooled to 0°C. The hydrogenfluoride-pyridine complex (100 µl) in chloroform (1 ml) was syringed into the vial then the epoxide (100 mgs) in chloroform (1 ml) was added dropwise over fifteen minutes. Monitoring by tlc (50% EtOAc/50% hexane) showed no change after 2 hours, so the solution was allowed to warm to room temperature and stirred overnight. Since there was still little change, a large excess of complex (1 ml) was added.

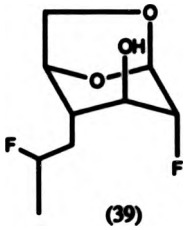
Five hours stirring resulted in consumption of the starting material, so the reaction was worked up by addition of saturated sodium bicarbonate solution (5 mls) and extraction with chloroform (5x5 mls). The organic extracts were dried ($MgSO_4$), filtered, and bound to silica for chromatography.

The column (12x1 cm) was eluted with 20% ethyl acetate/80% hexane and gave one major compound, which was tentatively assigned the structure (39).

^{1}H NMR (90 MHz, $CDCl_3$)

δ5.4 (1H, bd, H-1), 4.3-5.2 (3H, m), 3.6-3.9 (3H, m), 1.6-2.7 (4H,m) 1.35 (3H, q, $-CH_3$, J = 33.7, 6.7 Hz).

MS m/e found: 149.



(39)

f) Reaction with aluminium trifluoride and caesium fluoride

Aluminium trifluoride (200 mg, 2 eq) was weighed out under nitrogen (Glove bag).

Caesium fluoride (1.1 gm, 6 eq) was added and the mixture placed under a positive pressure of nitrogen. The epoxide (200 mg, 1 eq, 1 mmol), dissolved in dry tetrahydrofuran (10 mls) was added and the resulting suspension stirred at room temperature and monitored by tlc. (50% EtOAc/50% hexane).

Several days stirring produced no change.

g) Reaction with tetraphenylphosphonium hydrogen difluoride

Ph_4PHF_2 (50 mg) was dried at 70°C under reduced pressure for 20 hours. The flask was flushed with nitrogen and the epoxide (10 mg) in freshly distilled acetonitrile (2 mls) was added. The resulting solution was stirred at room temperature and monitored by tlc (50% EtOAc/50% Hex).

Twenty-four hours stirring, followed by several days refluxing, produced no change.

2-Deoxy-2-fluoro-4-deoxy-4-(2-propenyl)-β-D-glucopyranose[7,11-12] (6)

A 1:1 w/w mixture of potassium hydrogen fluoride (10 gm) and potassium fluoride (10 gm) dried over P_2O_5 at reduced pressure was suspended in dry ethylene glycol (100 mls) under nitrogen and brought to reflux, thus causing the salts to go into solution.

The epoxide (5 gm), suspended in dry ethylene glycol (5 mls), was then added dropwise through a septum to the refluxing solution. The resulting brown solution was refluxed for 2.5-3 hours, then cooled to room temperature and quenched slowly with 5% potassium bicarbonate solution (100 mls). This was then thoroughly extracted with dichloromethane (5x20 ml) and the combined organic extracts dried ($MgSO_4$), filtered, concentrated, and bound onto silica. The material was then split into two fractions and each chromatographed in the following manner. The columns (10x3.5 cm) were eluted at 35 mls/min with 15% ethyl acetate/85% hexane to yield, in total 2-deoxy-2-fluoro-4-deoxy-4-(2-propenyl)-β-D-glucopyranose (2.69 gm, 48%) as an oil. This was usually well separated from its regio isomer 1,6-anhydro-3-deoxy 3-fluoro-4-deoxy-4-(2-propenyl)-β-D-altro pyranose (0.75 gm, 13%) a crystalline material mpt 101-102°C. Analytically pure samples were obtained by Kugelrohr distillation for the 2-fluorohydrin (6) and recrystallisation from ethanol for the 3-fluorohydrin (40).

2-fluorohydrin (6)

^{1}H NMR (360 MHz, $CDCl_3$)

δ5.72-5.84 (1H, m, -CH=C, J=7, 10.3, 16.9 Hz), 5.55 (1H, t, H-1, $J_{1,2}$=1.5, $J_{1,F}$=1.5 Hz), 5.10-5.16 (2H, m, C=CH_2, J=10.3, 16.9 Hz), 4.43 (1H, bd, H-5, $J_{5,6\ exo}$ = 4.9, $J_{5,6\ endo}$ = 0.7 Hz), 4.24 (1H, dd, H-2, $J_{1,2}$=1.5, $J_{2,F}$=46 Hz), 4.05 (1H, dd, H-6_{endo}, $J_{5,6\ endo}$=0.7, $J_{exo,endo}$ = 7.1 Hz), 3.71-3.78 (2H, m, H-6_{exo}, H-3), 2.35-2.4 (2H, m,

$-CH_2$, J=7, 7.7 Hz), 1.67-1.72 (1H, bt, H-4 J=7.7 Hz).

^{13}C NMR ($CDCl_3$)

δ135.6 (C-8), 117.9 (C-9), 99.5 (C-1, J_{CF}=27 Hz), 89.5 (C-2, J_{CF} = 183 Hz), 74.6 (C-5), 69.8 (C-3, J_{CF} = 26 Hz), 68.2 (C-6), 43.2 (C-4), 35.5 (C-7).

IR (neat) 3450 broad, 3070, 1640 cm^{-1}.

MS, m/e found, 143.

Anal. calc. for $C_9O_3FH_{13}$ (188) C, 57.4%, H, 6.9%; found C, 57.8%, H, 7.07%.

TLC (50% ethylacetate/50% hexane) Rf =0.3

3-fluorohydrin (40).

^1H NMR (360 MHz, $CDCl_3$)

δ5.69-5.81 (1H, m, $-CH=C$, J = 8, 10, 19 Hz), 5.33-5.35 (1H, bd, H-1, J_{HF} = 6.9 Hz), 5.08-5.17 (2H, m, $C=CH_2$, J = 10, 19 Hz), 4.67 (1H, dt, H-3, $J_{H,F}$ = 52.2, $J_{2,3}$ = 8.1, $J_{3,4}$ = 6.4 Hz), 4.53 (1H, bt, H-5, $J_{5,F}$=5.8, $J_{5,6\ exo}$ = 5.3, $J_{5,6\ endo}$ 0.8 Hz), 3.77-3.81 (1H, m, $H\text{-}6_{exo}$, $J_{5,6\ exo}$ = 5.3, $J_{exo,endo}$ = 7.3 Hz), 3.68-3.75 (2H, m, H-2, $H\text{-}6_{endo}$, $J_{2,3}$ = 8.1, $J_{2,F}$ = 14.6, $J_{5,6\ endo}$ = 0.8, $J_{exo,\ endo}$ = 7.3 Hz), 2.43-2.48 (1H, m, H-7), 2.11-2.23 (2H, m, H-4, H-7').

^{13}C NMR ($CDCl_3$)

δ 135.6 (C-8), 117.8 (C-9), 102 (C-1, J_{CF} = 10.8 Hz), 92.8 (C-3, J_{CF} = 185.81 Hz), 74.5 (C-5, J_{CF} = 6.8 Hz), 71.8 (C-2, J_{CF} = 176 Hz), 67.9 (C-6), 43.4 (C-4, J_{CF} = 16.3 Hz), 29.4 (C-7, J_{CF} = 6.7 Hz).

MS, m/e found, 147.

Anal. calc for $C_9O_3FH_{13}$ (188) C, 57.4%, H, 6.9%, found C, 56.6%, H 6.9%.

mp 101-102°C.

TLC (50% ethyl acetate/50% hexane) Rf = 0.26.

Reactions of 2-deoxy-2-fluoro-4-deoxy-4-(2-propenyl)-β-D-glucopyranose (6)

a) With titanium tetrachloride[13]

The fluorohydrin (7 mgs) was dissolved in trifluoroacetic acid (1 ml) and brought to reflux. Titanium tetrachloride (100 μl) was then added and refluxing continued for another three hours. The solution was cooled, methanol (2-3 mls) added and the mixture stirred then concentrated. TLC analysis with (50% EtOAc/hexane) and 90% EtOAc/hexane) indicated residual starting material along with several other products.

b) With titanium tetrabromide[13]

The fluorohydrin (9 mg) and titanium tetrabromide (85 mg, added under N_2; the flask was then sealed and reweighed) were placed together in a flask under a positive pressure of nitrogen. Trifluoroacetic acid (1 ml) was added and the mixture was refluxed for three hours.

The solution was cooled, methanol (2-3 mls) was added and the mixture stirred and then concentrated. Tlc analysis using both (50% EtOAc/hexane) and (75% EtOAc/hexane) once again showed a mixture of several products.

c) With palladium(II) chloride/acetonitrile complex[14]

Palladium(II) chloride (11 mgs) was added to acetonitrile (2 mls) and the mixture refluxed to form a yellow solution. It was then cooled and shielded from the light.

The fluorohydrin (50 mg) was dissolved in acetonitrile (1 ml) under nitrogen. To this an aliquot (1 ml) of the above solution and water (0.5 mls) were added and the resulting solution stirred at room temperature and monitored by tlc (50% EtOAc/hexane).

After two weeks stirring, substantial amounts of starting material were still present, along with numerous other products.

d) With palladium(II) chloride/acetonitrile complex in acetone[14]

The remaining solution of complex from the previous experiment was concentrated to leave a yellow crystalline solid.

This was dissolved in acetone (2 mls) and added to the fluorohydrin (57 mg) dissolved in acetone (1 ml) and water (0.5 ml). The resulting solution was stirred at room temperature and monitored by tlc (50% EtOAc/50% hexane).

As with the previous experiment, after two weeks stirring, residual starting material was still present along with a plethora of other products.

e) With Amberlyst 15 in methanol[15]

Activated amberlyst 15 (50 mg) was placed in a flame dried flask under nitrogen. The fluorohydrin (50 mg) in dry methanol (2 mls) was added and the mixture refluxed and monitored by tlc (50% EtOAc/50% hexane). Forty eight hours refluxing showed no change.

f) Reaction with aqueous hydrochloric acid

The fluorohydrin (10 mg) was dissolved in a 1:1 mixture of acetonitrile and acid (1 ml). (Acid strength was varied to provide solutions of 0.05M, 0.5M, 1M and 5M.) The solutions were refluxed and monitored by tlc (50% EtOAc/hexane).

For the three dilute acid solutions, some reaction had occurred after 2-3 days refluxing. However, further prolonged reaction times never drove the reaction to completion.

Reaction with the 5M acid solution produced numerous products.

g) Reaction with organic acid[16]

i) The fluorohydrin (12 mg) was dissolved in trifluoroacetic acid (1 ml), water (200 µl) was added, and the solution heated at 90°C for twenty four hours. The mixture was then cooled, concentrated to dryness

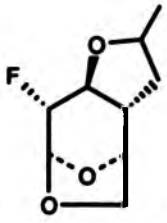
and dissolved in chloroform (1 ml).

Analysis by tlc (75% EtOAc/hexane) showed loss of starting material with one major new spot. However, the polarity of this material was inconsistent with a hydrolysis product.

ii) The fluorohydrin (8 mg) was dissolved in 1:1 v/v mixture of methane sulphonic acid and water (1 ml) and then heated at 130°C for thirty minutes. The solution was cooled and saturated sodium bicarbonate (3 mls) added. The aqueous solution was extracted with chloroform (3x1 ml), and the combined organic phases then dried, filtered and concentrated to 1 ml for tlc analysis (50% EtOAc/hexane or 90% EtOAc/10% MeOH). This also showed a new compound with a polarity similar to the one prepared in the previous experiment.

iii) The fluorohydrin (90 mgs) was dissolved in 1:1 v/v methanesulphonic acid/H_2O (5 mls) and refluxed for 2 hours. The solution was cooled and 5% aq potassium bicarbonate (20 mls) was added. The solution was extracted with chloroform (4x10 mls), dried ($MgSO_4$) filtered and then bound to silica for chromatography.

The column (12x1 cm) was eluted with 5% ethyl acetate/hexane increasing to 10% ethyl acetate/hexane and gave the new product (30 mg) contaminated with a small quantity of starting material (tlc). This compound was tentatively assigned structure (81) based on limited spectral data.



(81)

^{1}H NMR (90M Hz, $CDCl_3$)

δ5.45 (1H, bs, H-1), 3.9-4.7 (6H, m, H-2, H-3, H-5, $H\text{-}6_{exo,endo}$-CH=C),

1.5-2.8 (3H, m, H-4, $-CH_2-$), 1.35 (3H, d, $-CH_3$, J = 6.7 Hz).

MS; m/e found, 188

TLC (50% Ethylacetate/50% hexane) Rf =0.29.

Acetolysis of 1,6-anhydro-2-deoxy-2-fluoro-4-deoxy-4-(2-propenyl)-β-D-glucopyranose (6)[17]

The fluorohydrin (6), (250 mg) was dried by azeotroping with benzene (3x5 mls) then dissolved in freshly distilled acetic anhydride (2.5 mls). To this was added a mixture of dry acetic anhydride (2.5 mls) and perchloric acid (10 µl). The resulting solution turned dark brown in colour and was stirred at room temperature overnight.

Saturated sodium bicarbonate solution (10 mls) was added and the solution stirred for 0.5 hour, then extracted with chloroform (3x10 mls). The combined organic extracts were dried ($MgSO_4$), filtered, concentrated and azeotroped with toluene to remove any residual acetic anhydride. Analysis by tlc (50% Ethyl acetate/hexane) showed one major compound with several minor constituents. The residue was then bound to silica and chromatographed. The column (8x1.5 cm) was eluted with 10% ethylacetate/90% hexane to give the major constituent (300 mg, 60%) which was spectroscopically consistent with the anomeric triacetate structure (83, 84).

^1H NMR (90 MHz, $CDCl_3$).

δ6.35 (1H, d, H-1, J=4 Hz), 3.5-6.1 (8H, m, H-2, H-3, H-5, $H\text{-}6_{exo,endo}$, $-CH{=}C$, $C{=}CH_2$), 1.3-3.0 (12H, m, H-4, $-CH_2$, 3xOAc).

^{13}C NMR ($CDCl_3$)

δ169.9, 169.4, 168.3 (3xC=O), 132.3 (C-8), 117.6 (C-9), 88.5 (C-1, J_{CF} = 21.7 Hz), 87.4 (C-2, J_{CF} = 192.6 Hz), 70.3 (C-5), 68.8 (C-3, J_{CF} = 17.6 Hz), 62.4, (C-6), 39.3 (C-4, J_{CF} = 4.1 Hz), 30.2 (C-7), 20.2 ($-CH_3$).

IR (neat) 1740, 1640 cm^{-1}

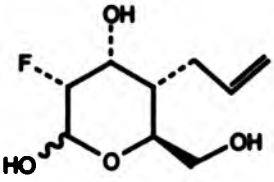
MS, m/e found 273, $m^+ - CH_3CO_2$

TLC (50% EtOAc/50% hexane) Rf ≈ 0.36.

Deprotection of the triacetates (83, 84).[17]

The triacetates (300 mg) were dissolved in methanol (15 mls) and 5% sodium methoxide solution (5 drops) added. The resulting solution was stirred at room temperature under nitrogen. After five hours tlc (50% EtOAc/hex) showed no starting material was left. The solution was neutralised with Amberlite IR 120 H^+ resin and filtered through a short silica column (4xl cm). The filtrate was then concentrated to yield a syrup.

^1H NMR (90MHz, CD_3OD), showed only a series of poorly resolved multiplets. However, since the acetate groups were not present the compound was tentatively assigned the triol structure below



1,6-Anhydro-2-deoxy-2-fluoro-2-o-p-toluenesulphonyl-4-deoxy-4-(2-propenyl)-β-D-glucopyranose (60)[18]

The fluorohydrin (100 mg) was dissolved in pyridine (0.5 mls) under nitrogen. To this a solution of p-toluene sulphonyl chloride (410 mg, 4 eq) and dimethylaminopyridine (260 mg,4 eq) in chloroform/pyridine (1.5 ml/1 ml) was added dropwise. The resulting solution was then stirred at room temperature and monitored by tlc. After five days the reaction was incomplete, so the mixture was warmed to 60°C and stirred for a further 3 days. It was then quenched with water (5 mls), stirred for 2 hours and then extracted with chloroform (3x5 mls). The combined organic extracts were then dried ($MgSO_4$), filtered, bound to silica and chromatographed. The column (10xl cm) was eluted with 10% ethyl

acetate/90% hexane at 4 mls min^{-1}. This gave the required tosylate (60) as an oil (100 mg, 55%).

^1H NMR (90 MHz, $CDCl_3$).

δ7.3-7.8 (4H, q, aromatic H), 5.4-5.85 (2H, m, H-8, H-1), 4.95-5.2 (2H, m, $C=CH_2$), 4.45-4.55 (2.5H, m, H-5, H-3, 0.5 H-2), 4.05 (1H, d, $H\text{-}6_{endo}$, $J_{exo,endo}$ = 7 Hz), 3.95 (0.5H, m, 0.5 H-2), 3.7-3.85 (1H, m, $H\text{-}6_{exo}$, $J_{5,6\ exo}$ = 5, $J_{exo,endo}$ = 7 Hz), 2.3-2.5 (5H, m, $-CH_2$, $-CH_3$), 1.75 - 1.95 (1H, bt, H-4).

^{13}C NMR ($CDCl_3$)

δ134.5 (C-8), 129.0 (C-9), 131.5, 128.5 (aromatic C), 99.0 (C-1, J_{CF} = 40.3 Hz), 85.5 (C-2 J_{CF}=181.4), 74.0 (C-5), 68.0 (C-6), 42.0 (C-4), 35.5 (C-7), 22.5 ($-CH_3$).

IR (neat); 3060, 1640, 1660 cm^{-1}.

MS; m/e found 342.

HRMS; calc for $C_{16}H_{19}O_5SF$ 342.0938 measured 342.0950.

TLC (50% ethylacetate/50% hexane) Rf = 0.4.

<u>1,6-Anhydro-2-deoxy-2-fluoro-3-O-methanesulphonyl-4-deoxy-4-(2-propenyl)-β-D-glucopyranose</u> (62)

The fluorohydrin (100 mg) was dissolved in a 1:1 mixture of chloroform and pyridine (2 mls) under nitrogen and cooled to 0°C. Methanesulphonyl chloride (100 µl) was added dropwise, the resulting solution was allowed to warm to room temperature and stirred overnight. The appearance of a white precipitate was noted.

Water (5 mls) was added and the mixture stirred for 1.5 hrs, then extracted with chloroform (3x5 mls), dried ($MgSO_4$), filtered, concentrated and azeotroped with toluene.

Kugelrohr distillation 120°C, 1mm Hg gave the pure mesylate (62) (100 mg, 70%) as an oil.

^{1}H NMR (90 MHz, $CDCl_3$).

5.5-6.1 (2H, m, H-1, -CH=C), 5.1-5.3 (2H, m, C=CH_2), 4.4-4.8 (2.5H, m, H-3, H-5 0.5 H-2), 4.15 (0.5 H, bs, 0.5 H-2), 4.05 (1H, d, H-6_{endo}, $J_{exo,endo}$ = 7 Hz), 3.7-3.88 (1H, m, H-6_{exo}), 3.05 (3H, s, $-CH_3$), 2.35-2.55 (2H, bt, $-CH_2-$), 1.85-2.05 (1H, bt, H-4).

IR (neat) 3060, 1640 cm^{-1}.

MS; m/e found 266.

HRMS; calc for $C_{10}H_{15}O_5SF$ 266.0625 found 266.0640.

TLC (50% EtOAc/hexane) Rf = 0.3 (identical to starting material).

<u>1,6-Anhydro-2-deoxy-2-fluoro-3-O-trifluoromethanesulphonyl-4-deoxy-4-(2-propenyl)-β-D-glucopyranose</u> (64)

The fluorohydrin (1 gm, 5 mM) was dissolved in dichloromethane (20 mls) under nitrogen and cooled to 0°C. Pyridine (1.2 mls, 3 eq) was added, then triflic anhydride (1.34 mls, 1.5 eq) was added dropwise to the stirred solution. The resultant mixture was stirred for 3.5 hrs, tlc monitoring (50% EtOAc/hexane) indicating a complete reaction.

Water (5 mls) was added and the mixture stirred for 15 minutes. The phases were separated and the aqueous phase washed with dichloromethane (2x5 mls). The combined organic extracts were then concentrated, azeotroped with toluene and bound to silica for chromatography. The column (10x3 cm) was eluted with 50% ethyl acetate/50% hexane at 35 mls/min and yielded the triflate (64) (1.5 gms, 85%) as a colourless oil.

^{1}H NMR (90 MHz, $CDCl_3$).

δ5.05-6.05 (2H, m, H-1, -CH=C), 5.1-5.3 (2H, m, C=CH_2), 4.85-5.0 (1H, bd, H-3 $J_{3,F}$ = 14 Hz), 4.18-4.68 (1H, d, H-2, $J_{2,F}$ = 45 Hz), 4.5 (1H, d, H-5, $J_{5,6exo}$ = 5 Hz), 4.05 (1H, d, H-6_{endo}, $J_{exo,endo}$ = 7.5 Hz), 3.75-3.9 (1H, m, H-6_{exo}), 2.5 (2H, bt, $-CH_2-$), 2.0 (1H, bt, H-4

$J_{4,-CH_2}$ = 8 Hz).

^{13}C NMR ($CDCl_3$)

δ133.7 (C-8), 126.4, 110.5 (CF_3 J_{CF} = 320 Hz), 119.7 (C-9), 98.2 (C-1, J_{CF} = 27.2 Hz), 85.3 (C-2, J_{CF} = 184.4 Hz), 82.4 (C-3, J_{CF} = 32.6 Hz), 73.6 (C-5), 67.5 (C-6), 41.7 (C-4), 34.7 (C-7).

IR (neat) 3060, 1640 cm^{-1}.

MS; m/e found, 320.

HRMS calc. for $C_{10}H_{12}O_5F_4S$ 320.0340, found 320.0360.

TLC (50% EtOAc/50% hexane) Rf = 0.4.

Oxidation of 1,6-anhydro-2-deoxy-2-fluoro-3-O-p-toluenesulphonyl-4-deoxy-4-(2-propenyl)-β-D-glucopyranose (60)[18,19]

The tosylate (130 mg 0.36 mM) and sodium metaperiodate (320 mg 4.1 eq) were suspended in a mixture of carbontetrachloride (2 mls), acetonitrile (2 mls) and water (1 ml). Ruthenium trichloride (5 mg) was rinsed with more water (2 mls) and the resulting dark brown, biphasic mixture was stirred vigorously overnight.

Water (3 mls) was added and the aqueous phase extracted with dichloromethane (4x5 mls). The organic extracts were dried ($MgSO_4$), filtered and bound to silica for chromatography. The column (10x1 cm) was eluted with 50% ethyl acetate/50% hexane at 4 mls/min and gave the corresponding carboxylic acid (61), (120 mg, 80%) as a crystalline compound.

^1H NMR (90 MHz, $CDCl_3$).

δ9.5 (1H, bs, COOH), 7.3-7.85 (4H, q, aromatic H), 5.45 (1H, s, H-1), 4.45-4.6 (2.5H, m, H-3, H-5, 0.5 H-2), 4.15 (1H, d, $H\text{-}6_{endo}$, $J_{exo,endo}$ = 7 Hz), 4.0 (0.5H, s, 0.5 H-2), 3.7-3.85 (1H, m, H-6 exo), 2.7-2.85 (2H, m, $-CH_2$), 2.2-2.5 (4H, m, H-4, $-CH_3$).

^{13}C NMR ($CDCl_3$)

145.2 (C=O), 97.8 (C-1, J_{CF} = 27.2 Hz), 84.8 (C-2, J_{CF} = 183.2 Hz), 76.2 (C-3, J_{CF} 32.5 Hz), 73.7 (C-5), 67.2 (C-6), 37.8 (C-4, C-7), 21.6 ($-CH_3$).

TLC (50% EtOAc/50% hexane) Rf = 0.0-0.2.

Oxidation of 1,6-anhydro-2-deoxy-2-fluoro-3-O-methanesulphonyl-4-deoxy-4-(2-propenyl)-β-D-glucopyranose (62)[19]

The mesylate (140 mg, 0.52 mM) and sodium metaperiodate (460 mg, 4 eq) were suspended in a mixture of carbon tetrachloride (2 mls) acetonitrile (2 mls) and water (1 ml). Ruthenium trichloride (5 mg) was rinsed in with water (2 mls) and the resulting biphasic mixture stirred vigorously. Monitoring by tlc (50% EtOAc/hexane) showed that the reaction was complete after three hours. Water (2 mls) was added, the phases were separated and the aqueous phase extracted with dichloromethane (4x5 mls). The combined organic extracts were dried ($MgSO_4$) filtered and bound to silica for chromatography. The column (6x1 cm) was eluted with ethyl acetate and gave the carboxylic acid (63) (100 mgs, 66%) as a crystalline compound.

^1H NMR (90 MHz, $CDCl_3$)

δ7.3 (1H, bs, COOH), 5.5 (1H, s, H-1), 4.55-4.75 (2.5H, m, H-3, H-5, 0.5 H-2), 4.25 (0.5H, s, 0.5 H-2), 4.15 (1H, d, $H\text{-}6_{endo}$, $J_{exo,endo}$ = 7 Hz), 3.7-3.85 (1H, m, $H\text{-}6_{exo}$), 3.75 (3H, s, $-CH_3$), 2.8 (2H, d, $-CH_2-$ J_{4,CH_2} = 7 Hz), 2.35-2.5 (1H, m, H-4).

TLC (50% EtOAc/50% hexane) Rf = 00-0.13.

Oxidation of 1,6-anhydro-2-deoxy-2-fluoro-3-O-trifluoromethanesulphonyl-4-deoxy-4-(2-propenyl)-β-D- glucopyranose (64)[19]

The triflate (1.5 gms, 4.5 mM) and sodium metaperiodate (4.5 gms, 4 eq) was suspended in a mixture of carbon tetrachloride (12 mls), acetonitrile (12 mls) and water (10 mls). Ruthenium trichloride (10 mgs) was rinsed in with water (10 mls) and the resulting dark brown biphasic mixture stirred vigorously for five hours.

Water was added to dissolve precipitated sodium iodate and then an equal volume of dichloromethane, and isopropanol (3 mls) were added and the mixture stirred for 0.5 hour. The biphasic mixture was then filtered through a celite pad into a separating funnel. The phases were separated and the aqueous phase extracted with dichloromethane (3x20 mls). The combined organic extracts were dried ($MgSO_4$), filtered, and bound to silica for chromatography.

The column (10x3 cm) was eluted with 50% ethyl acetate/50% hexane at 35 mls/min. and gave the 2-fluorolactone (59) (650 mg, 65%) as a white crystalline material.

^1H NMR (360 MHz, $CDCl_3$).

δ5.57 (1H, d, H-1, $J_{1,2}$ = 2.43 Hz), 4.63-4.73 (1.5H, m, H-3, 0.5 H-2, $J_{2,3}$ = 4.9, $J_{3,4}$ = 7.4, $J_{3,F}$ = 20.5 Hz), 4.6-4.61 (1H, bs, H-5), 4.49-4.51 (0.5H, m, 0.5 H-2, $J_{2,3}$ = 4.9, $J_{1,2}$ = 2.43, $J_{2,F}$ = 49.8 Hz), 3.82-3.86 (2H, m, H-$6_{exo,endo}$), 2.76-2.84 (1H, m, H-7 , $J_{7\alpha\beta}$ = 16.2, $J_{4,7\alpha}$ = 12.5, $J_{F,7\alpha}$ = 3.02 Hz), 2.63-2.72 (1H, m, H-4), 2.42-2.49 (1H, m, H-7 , $J_{7\alpha\beta}$ = 16.2, $J_{4,7\beta}$ = 7.9, $J_{F,7\beta}$ = 2.67 Hz).

^{13}C NMR ($CDCl_3$).

δ175.9 (C=O), 98.3 (C-1, J_{CF} = 24.1 Hz), 83.0 (C-2, J_{CF} = 187.4 Hz), 71.7 (C-5), 71.2 (C-3, J_{CF} = 16.2 Hz), 66.1 (C-6), 39.2 (C-4, J_{CF} = 1.5 Hz), 29.7 (C-7).

IR ($CHCl_3$), 1780 cm^{-1}.

MS; m/e found 188.

HRMS calc. for $C_8O_4H_9F$ 188.0485 found 188.0485.

Anal. calc. for $C_8O_4H_9F$ (188), C, 51.1%, H, 4.9%, found C, 51.1%, H, 4.8%.

Mp 124-127°C.

TLC (50% EtOAc/50% hexane) Rf = 0.15.

Reactions of 1,6-anhydro-2-deoxy-2-fluoro-4-deoxy-4-(carboxymethylene)-β-D-allo hexopyranose-γ-lactone (59)

a) With hydrochloric acid.

The fluorolactone (10 mg) was refluxed in 1M HCl solution (1 ml) for three days. A work up of cooling, evaporating to dryness, and then dissolving in 1:1 CH_3CN/H_2O allowed tlc analysis (90% EtOAc/10% MeOH). This showed no significant change in the starting material.

b) With organic acids.[16]

i) The fluorolactone (10 mg) was dissolved in 50% v/v aqueous methanesulphonic acid (1 ml) and heated at 115-120°C for thirty minutes. The solution was neutralised by the addition of Amberlite IR 45 resin. This was then filtered off and the residual solution evaporated to dryness. Analysis by tlc (50% EtOAc/hexane) showed no change in the starting material.

ii) The fluorolactone (10 mg) was dissolved in 1M aqueous trifluoroacetic acid solution (1 ml), and refluxed overnight. The solution was cooled and evaporated to leave an oily residue, tlc analysis (90% EtOAc/10% Methanol) showed no change in the starting material.

iii) The fluorolactone (10 mg) was dissolved in 1M aqueous triflic acid (1 ml) and refluxed overnight. The solution was cooled and neutralised with saturated sodium bicarbonate solution. This was then evaporated to dryness and dissolved in a 2:1 H_2O/CH_3CN mixture (1 ml). Tlc (90% EtOAc/10% MeOH)) showed no change in the starting material.

Acetolysis of 1,6-anhydro-2-deoxy-2-fluoro-4-deoxy-4-(carboxy - methylene)-β-D-allo hexopyranose-γ-lactone (59)[17]

The fluorolactone (200 mg) was dissolved in acetic anhydride (5 mls) under nitrogen. Perchloric acid (10 µl) was added and the resulting solution stirred overnight at room temperature. A colour change to a yellowish solution was noted and tlc (75% ethyl acetate/hex) showed a complete reaction.

Water (5 mls) was added and the mixture stirred for 0.5 hours. The aqueous solution was extracted with chloroform (3x5 mls), then combined organic fractions were dried ($MgSO_4$), filtered and concentrated. Residual acetic anhydride was removed on an oil pump or by azeotropic distillation with toluene, the remaining gum was then bound to silica and chromatographed.

The column (9x1.5 cm) was eluted with 20% ethyl acetate/hexane increasing to 30% ethyl acetate/hexane and gave first a small quantity of an as yet unidentified compound followed by the anomeric acetates (240 mg, 75%).

The column chromatography above caused a partial separation of the anomeric mixture. It was noted that the later fractions were rich in the more polar anomer and that these crystallised readily. Two successive recrystallisations of these residues from ethanol followed by washing with ether provided a pure sample of the more polar β-anomer (85) Mpt 116-118°C.

The mother liquors from the recrystallisations were then rechromatographed using a column (6x1.5 cm) eluted with 20% ethyl acetate/hexane to provide a pure sample of the less polar α-anomer (86) as a syrup.

β-anomer (85)

^{1}H NMR (360 MHz $CDCl_3$)

δ6.1 (1H, t, H-1, $J_{1,2}$ = 5.6, $J_{1,F}$ = 5.6 Hz), 4.85-4.92 (1H, m, H-3, $J_{2,3}$ = 3.56, $J_{3,4}$ = 6.14, $J_{3,F}$ = 15.7 Hz), 4.59-4.75 (1H, m, H-2, $J_{1,2}$ = 5.6, $J_{2,3}$ = 3.56, $J_{2,F}$ = 47.1 Hz), 4.11-4.18 (2H, m, H-6,6'), 3.85-3.9 (1H, m, H-5), 2.73-2.81 (1H, m, H-4), 2.65-2.7 (1H, m, H-7 , $J_{7,7'}$ = 17.4, $J_{4,7}$ = 8.2, $J_{F,7}$ = 0.7 Hz), 2.37-2.44 (1H, m, H-7', $J_{7,7'}$ = 17.4, $J_{4,7'}$ = 3.4, $J_{F,7'}$ = 3.4 Hz), 2.13 (3H, s, $-CH_3$), 2.07 (3H, s, $-CH_3$).

^{13}C NMR ($CDCl_3$)

δ173.7, 170.4, 168.4 (3xC=O), 89.7 (C-1, J_{CF} = 27.9 Hz), 85.8 (C-2 J_{CF} = 189.3 Hz), 75.3 (C-3, J_{CF} = 15.3 Hz), 73.5 (C-5), 63.9 (C-6), 36.3 (C-4), 32.3 (C-7), 20.54, 20.67 ($-CH_3$).

IR ($CHCl_3$) 1780, 1740 cm^{-1}.

Anal. Calc. for $C_{12}H_{15}O_7F$ C, 49.6%, H, 5.2%, found C, 49.5%, H, 5.29%

Mp 116-118°C.

α-anomer (86)

^{1}H NMR (360 MHz, $CDCl_3$).

δ6.09-6.15 (1H, q, H-1, $J_{1,2}$ = 3.8, $J_{1,F}$ = 17.4 Hz), 4.97-5.12 (1H, m, H-2, $J_{1,2}$ = 3.8, $J_{2,3}$ = 2.6, $J_{2,F}$ = 50.2 Hz), 4.61-4.72 (1H, m, H-3, $J_{2,3}$ = 2.6, $J_{3,4}$ = 8.7, $J_{3,F}$ = 28.3 Hz), 4.09-4.21 (3H, m, H-5, H-6,6'), 2.83-2.92 (1H, m, H-4, $J_{3,4}$ = 8.7, $J_{4,7}$ = 9.8, $J_{4,7}$ = 8.3 Hz), 2.58-2.66 (1H, m, H-7 , $J_{7,7'}$ = 17.75, $J_{4,7}$ = 9.8, $J_{F,7}$ = 1.7 Hz), 2.33-2.41 (1H, m, H-7 , $J_{7,7'}$ = 17.75, $J_{4,7'}$ = 8.3, $J_{F,7'}$ = 2.96 Hz), 2.15 (3H, s, $-CH_3$), 2.08, (3H, s, $-CH_3$).

^{13}C NMR ($CDCl_3$).

δ174.4, 170.3, 169.1 (3xC=O), 88.7 (C-1, J_{CF} = 16.0 Hz), 85.5 (C-2, J_{CF} = 191.5 Hz), 74.2 (C-3, J_{CF} = 16.4 Hz), 72.7 (C-5), 64.3 (C-6),

35.0 (C-4), 31.7 (C-7), 20.7, 20.5 (2x-CH_3).

IR ($CHCl_3$) 1780, 1740 cm^{-1}.

Deprotection of 1,6-di-O-acetyl-2-deoxy-2-fluoro-4-deoxy-4-(carboxymethylene)-α- and -β-D-allopyranose-γ-lactones(85) (86)[17]

The diacetates (240 mg) were dissolved in methanol (5 mls) and a small quantity of a 5% sodium methoxide solution was added (5 drops). The resulting yellowish solution was stirred overnight at room temperature under nitrogen, tlc (50% EtOAc/hexane) indicating that the reaction was complete.

The solution was neutralised with Amberlite IR 120 resin, filtered and then concentrated.

Further tlc (90% EtOAc/10% MeOH) showed that a mixture of at least three products was present, these proved to be inseparable by chromatography. A column (9x1 cm) eluted with 79% ethyl acetate/1% methanol/20% hexane gave only poorly resolved fractions. Since it was likely that these compounds were a mixture of the deacetylated and monoacetylated lactones or possibly methyl esters, the fractions were recombined and an acid catalysed equilibration was attempted.

Acid catalysed equilibration of transesterification products

The combined material from the previous experiment was dissolved in dry tetrahydrofuran (10 mls) and a few crystals of p-toluene sulphonic acid were added. The resulting solution was refluxed and monitored by tlc (90% ethylacetate/10% methanol).

After five days only one major product was present (this comprised two closely running spots on tlc); the solution was cooled and neutralised with Amberlite IR 45 (OH) resin. It was then filtered, concentrated and chromatographed.

Column (6x1 cm) eluting with 60% ethylacetate/39% hexane/1% methanol

gave the major component.

Analysis by tlc and nmr identified this as one of the transesterification products observed previously and it was tentatively assigned the anomeric diol structure (98). This was confirmed by acetylation. The compound above was dissolved in dry pyridine (3 mls) and acetic anhydride (1 ml) was added dropwise to the cooled solution. This was stirred for 2-3 hours, then water (5 mls) was added and stirring continued for a further 15 minutes. The mixture was then extracted with chloroform (3x3 mls). The organic phases were combined then azeotroped with several portions of toluene to remove residual acetic anhydride, pyridine and water. The isolated compound was spectroscopically identical to the anomeric diacetates (85) (86).

1,6-Anhydro-2-deoxy-2-fluoro-3-O-methanesulphonyl-4-deoxy-4-(methyl ethanoate)-β-D-glucopyranose (105).

The acid (63) (100 mg) was dissolved in dry methanol (10 mls) and conc sulphuric acid (20 l) was added. A small quantity of molecular sieve 3A was added and the mixture stirred overnight.

The solution was filtered and the methanol removed in vacuo. The residue was treated with sodium bicarbonate solution (5 mls) and then extracted with dichloromethane (3x3 mls). The organic fractions were dried ($MgSO_4$) filtered and bound to silica for chromatography. The column (13.5x2 cm) was eluted with 50% ethylacetate/50% hexane at 16 mls min^{-1} to give the methyl ester (105), as a colourless oil (50 mg, 47%).

^1H NMR (220 MHz, $CDCl_3$)

δ5.52 (1H, bs, H-1), 4.56-4.7 (1H, m, H-3, $J_{3,F}$ = 15 Hz), 4.46 (1H, bd, H-5), 4.33-4.56 (1H, m, H-2, $J_{2,F}$ = 45 Hz), 4.6 (1H, d, H-6_{endo}), 3.75-3.85 (1H, m, H-6_{exo}). 3.72 (3H, s, $-OCH_3$), 3.12 (3H, s, SO_2-CH_3), 2.75-2.8 (2H, m, $-CH_2$), 2.3-2.43 (1H, bt, H-4).

IR (neat) 1710 cm^{-1}.

1,6-Di-O-Acetyl-2-deoxy-2-fluoro-3-O-methanesulphonyl-4-deoxy-4-(methyl ethanoate)-α and β-D-glucopyranoses (106).

The ester (105) (50 mg) was dissolved in acetic anhydride (2 mls) under nitrogen and perchloric acid (10 1) was added. The resulting brown solution was stirred overnight.

Sodium bicarbonate solution (2 mls) was added and the mixture was stirred for a further 2 hours then extracted with dichloromethane (3x2 mls). Azeotropic distillation with toluene removed any residual acetic anhydride and water to yield anomeric acetates (106) (60 mgs, 94%) as a colourless oil.

^{1}H NMR (220 MHz, $CDCl_3$).

δ6.4 (d, H-1), 5.75-5.81 (m, H-1β), 5.1-5.3 (1H, m, H-3), 4.5-4.83 (1H, m, H-2, $J_{2,F}$ = 48 Hz), 4.08-4.38 (3H, m, H-5, H-6,6'), 3.68 (3H, s, -OMe), 3.08 (3H, s, SO_2-Me), 2.55-2.7 (2H, m, $-CH_2$), 2.25-2.45 (1H, m, H-4), 2.2 (S, , -OAc), 2.15 (s, 3, -OAc), 2.05 (3H, s, C-6, -OAc).

IR (neat) 1730 cm^{-1}.

Attempted deprotection of diacetate (106)

The diacetate (106) (60 mg) was dissolved in methanol (5 mls) under nitrogen. 5% sodium methoxide solution (5 drops) was added and the resulting yellow solution stirred overnight.

The solution was acidified with Amberlite IR 120 H^+ resin, filtered, concentrated and chromatographed. The column (13x1 cm) was eluted with 60% ethyl acetate/40% hexane at 4 mls min^{-1} to give one major compound (10 mgs).

^{1}H NMR (220 MHz CD_3OD) gave a scrappy spectrum of poorly resolved multiplets. However, no mesylate or methyl groups were present. Since only trace amounts of olefinic material were present, the lactone structure (98) was tentatively assigned.

Reduction of 1,6-anhydro-2-deoxy-2-fluoro-4-deoxy-4-carboxymethylene-β-D-allo hexopyranose-γ-lactone (59)[20]

The lactone (59) (1 gm) was dissolved in a mixture of toluene (25 mls) and dimethoxymethane (6.25 mls) under nitrogen, warming with an air blower was usually required to get the lactone into solution. The solution was then cooled to -70°C (dry ice/acetone bath) and the diisobutyl aluminium hydride solution (7.5 mls, 1M in toluene) added dropwise. The resulting solution was stirred at -70°C for 2 hours, tlc (50% EtOAc/hexane) showing a complete reaction. Methanol (10 mls) was added and the solution warmed to room temperature, then brine (10 mls) and ethyl acetate (10 mls) were added and the mixture stirred for 15 minutes. The mixture was then filtered through a sinter directly into a separating funnel and the white precipitate was washed copiously with ethyl acetate. The phases were separated and the aqueous phase was washed with further portions of ethyl acetate. The combined organic extracts were dried ($MgSO_4$), filtered and bound onto silica for column chromatography.

The column (10x3 cm) was eluted with 60% ethyl acetate/hexane at 35 mls min^{-1} to give the expected lactol (116) (750 mg, 75%) as a white crystalline material.

^1H NMR (220 MHz $CDCl_3$).

δ5.5-5.7 (2H, m, H-1, -CH-), 4.15-4.7 (3H, m, H-5, H-3, H-2), 3.8 (2H, m, $H\text{-}6_{exo,\ endo}$), 3.1 (1H, bs, -OH), 2.5-2.75 (1H, m, H-4), 2.2-2.5 (1H, m, $H\text{-}7_{\beta}$), 1.85-2.0 (1H, m, $H\text{-}7_{\alpha}$).

IR ($CHCl_3$) 3550, 3450, 3000 cm^{-1}.

MS m/e found 190.

HRMS calc. for $C_8H_{11}O_4F$ 190.0641, found 190.0627

Mp 78-81°C.

TLC (75% EtOAc/25% Hex) Rf = 0.08-0.38.

1,6-Anhydro-2-deoxy-2-fluoro-4-deoxy-4-(6-carboxy-hexen-2-Z-yl) allo hexapyranose (108)[21,22]

a) Generation of dimsyl sodium

Sodium hydride (1 gm, 50% dispersion in oil) was placed under nitrogen and washed with dry hexane (3x10 mls). Residual hexane was removed by evacuation of the flask to leave the sodium hydride as a dry free flowing powder. Nitrogen was reintroduced into the flask and the hydride was suspended in dry dimethyl sulphoxide (20 mls). The suspension was warmed to 60°-70°C and stirred until evolution of hydrogen had ceased, usually 1.5-2 hours was enough, the residual sodium hydride was allowed to settle and the greenish yellow supernatent used directly for generation of the phosphonium ylide.

b) Generation of phosphonium ylide

(4-Carboxy butyl) triphenyl phosphonium bromide (2.35 gms 2 eq) was dissolved in dry dimethyl sulphoxide (20 mls) under N_2. The dimsyl solution above was then titrated into the reaction vessel, a permanent yellow colour marked the first end point, then slightly less than one more equivalent was added to generate a bright orange coloured solution (~10 mls in total).

The lactol (116), (500 mg) which had been dissolved in dimethyl sulphoxide (10 mls) and left standing at room temperature for one hour was then added to the ylide solution. On addition of the lactol the colour intensity faded a little and the resulting solution was stirred for a further 2.5 hours.

Water (50 mls) was added and the solution acidified with HCl (2M), more water (50 mls) was then added and the resulting solution extracted with dichloromethane (5x30 mls). The organic extracts were dried ($MgSO_4$) filtered and concentrated, residual dimethylsulphoxide was

removed by oil pump vacuum. The residue was then bound to silica and chromatographed using a column of dimensions (10x3.5 cm) and eluting with 40% EtOAc/59% hexane/0.5% CHOOH at 35 mls min^{-1}.

Formic acid was removed from the combined acid containing fractions by azeotroping with toluene. If any trace impurities remained, tlc (45% EtOAc/53% hexane/2% CHOOH) usually uv active, they were removed by dissolving the residue in 10% potassium hydroxide solution (20 mls) and washing with ether (50 mls). The aqueous phase was then acidified and extracted with dichloromethane (3x10 mls). The organic extracts were then dried ($MgSO_4$), filtered and concentrated to yield the desired hydroxy acid (108) (600 mg, 70%).

^1H NMR (400 MHz, $CDCl_3$).

δ5.45-5.6 (2H, m, HC=CH), 5.35-5.45 (1H, m, H-1), 4.45-4.55 (1.5H, m, H-5, 0.5 H-2), 4.38 (0.5H, m, 0.5 H-2, $J_{1,2}$ = 2.44, $J_{2,3}$ = 3.66, $J_{2,F}$ = 50.66 Hz), 4.05-4.15 (1H, m, H-3, $J_{2,3}$ = 3.66, $J_{3,4}$ = 6.1, $J_{3,F}$ = 29.3 Hz), 3.7-3.8 (2H, m, $H\text{-}6_{exo,endo}$), 1.7-2.5 (9H, m).

^{13}C NMR ($CDCl_3$).

δ178.3 (C=O), 131.3 (C-8)*, 128.2 (C-9)*, 98.6 (C-1, J_{CF} = 24.4 Hz), 87.7 (C-2, J_{CF} = 183.1 Hz), 73.5 (C-5), 66.7 (C-6), 63.9 (C-3, J_{CF} = 18.3 Hz), 43.9 (C-4), 33.2 (C-7) 26.3 (C-10), 22.8 (C-11), 24.5 (C-12).

*Interchangeable.

IR ($CHCl_3$) 3500-2600, 1710 cm^{-1}.

MS, m/e found 274.

HRMS calc. for $C_{13}O_5H_{19}F$ 274.1217 found 274.1228.

TLC (45% EtOAc/53% hexane/2% CHOOH) Rf = 0.24.

Acetolysis of 1,6-Anhydro-2-deoxy-2-fluoro-4-deoxy-4-(6-carboxy-hexen-2-2-yl) allo hexopyranose (108)[17]

The hydroxy acid (200 mg) was dissolved in dry acetic anhydride (5 mls) under nitrogen and perchloric acid (10 µl) was added. The resulting solution was stirred at room temperature and monitored by tlc (45% ethyl acetate 55% hexane 2% Formic acid and 75% ethyl acetate/hexane with trace of acid).

After four days stirring tlc showed the major product to be 3-acetyl compound (117) with numerous minor components.

1,6-Anhydro-2-deoxy-2-fluoro-3-O-acetyl-4-deoxy-4-(6-carboxy-hexen-2-2-yl)-allo hexopyranose (117)

The acid (108) (130 mg) was dissolved in pyridine (2 mls) under nitrogen. Acetic anhydride (0.5 mls) was added and the solution stirred overnight.

Water (2 mls) was added and the mixture stirred for five minutes. It was then extracted with dichloromethane (3x2 mls). Residual water and pyridine were removed from the organic extracts by azeotroping with toluene. The remaining oil was bound to silica and chromatographed. A column (10x1 cm) was eluted with 50% ethyl acetate/50% hexane at 4 mls min^{-1} to give the monoacetate (117) in a quantitative yield.

^1H NMR (90 MHz, $CDCl_3$).

δ5.15-5.6 (3H, m, H-8, H-9, H-1), 4.2-5.05 (3H, m, H-2, H-3, H-5), 3.85 (2H, m, $H\text{-}6_{exo,endo}$), 1.6-2.0 (9H, m), 2.15 (3H, s, $-CH_3$).

Acetolysis of 3-acetyl hydroxyacid (117)[17]

The acetate (100 mg) was dissolved in dry acetic anhydride (3 mls) under nitrogen and perchloric acid (10 µl) added. The resulting solution was stirred overnight and monitored by tlc (75% EtOAc/Hex + trace formic acid). This showed a large amount of unreacted starting material along with numerous minor components.

1,6-Anhydro-2-deoxy-2-fluoro-3-O-acetyl-4-deoxy-4-(methyl-6-carboxy-hexan-2-Zyl) allo hexopyranose

The hydroxy acid (108) (80 mg) was dissolved in dry methanol (5 mls) under N_2 and perchloric acid (50 µl) was added. The resulting solution was stirred overnight at room temperature.

The methanol was removed in vacuo then saturated sodium bicarbonate solution (1 ml) and water (2 mls) were added. The aqueous phase was extracted with dichloromethane and the organic fractions dried by azeotropic distillation with toluene.

The residue was dissolved in dichloromethane (5 mls), pyridine (1 ml), acetic anhydride (0.5 mls) were then added and the resulting solution stirred for two hours. Water was added, the phases separated and the aqueous phase extracted with more dichloromethane. The combined organic extracts were then azeotropically distilled with toluene to remove pyridine and water. Finally the residue was bound to silica for chromatography.

A column (10x1 cm) was eluted with 25% ethylacetate/hexane at 4 mls min^{-1} and gave the acetylated methyl ester (118).

i) Methyl ester.

^1H NMR (90 MHz, $CDCl_3$).

δ5.35-5.65 (3H, m, H-1, H-8, H-9), 4.0-4.8 (3H, m, H-2, H-3, H-5), 3.75-4.0 (2H, m, $H\text{-}6_{exo,endo}$), 3.68 (3H, s, -OMe), 1.6-2.6 (9H, m).

ii) Acetyl methyl ether (118).

^1H NMR (90 MHz, $CDCl_3$).

δ5.3-5.7 (3H, m, H-9, H-8, H-1), 4.0-4.8 (3H, m, H-5, H-3, H-2), 3.75-4.0 (2H, m, $H\text{-}6_{exo,endo}$), 3.68 (3H, s, $-OCH_3$), 1.6-2.6 (9H, m), 2.05 (3H, s, $-CH_3$).

Acetolysis of 3-acetyl methyl ester (118)[17]

The methyl ester was dissolved in dry acetic anhydride (3 mls) under nitrogen, perchloric acid (20 µl) was added and the resulting solution stirred overnight. Sodium bicarbonate solution (1 ml) and water (2 mls) were added and the solution stirred for 0.5 hours.

Extraction with dichloromethane and azeotropic removal of water and acetic anhyride with toluene gave the crude oil. Tlc analysis (45% ethyl acetate 2% CHOOH, 53% hexane) showed a plethora of compounds.

1,6-anhydro-2-deoxy-2-fluoro-4-deoxy-4-(6-carboxy-hexen-2-Z-yl) allo hexopyranose-macrolactone (119)[25]

a) Preparation of 2-pyridyl chloro formate solution[24]

A 10-15% phosgene solution in dichloro methane (5 mls, 5 mmols) was diluted with toluene (5 mls) and cooled to 0°C under N_2. A solution of triethylamine (1.08 mmol 0.15 ml) and 2-pyridine thiol (1.0 mmol, 110 mg) in methylene chloride (5 mls) was added dropwise over five minutes. The resultant solution was stirred for 10 minutes, then any excess phosgene and methylene chloride were removed *in vacuo*. Hexane (10 mls) was added and the precipitated triethylamine hydrochloride was removed by filtration. The flask was rinsed with an additional volume of toluene (5 mls) and hexane (10 mls) and the solution was filtered. The combined filtrates were concentrated *in vacuo* to yield the reagent as a colourless oil. The reagent was dissolved in methylene chloride (5 mls) and either used directly or stored in a freezer protected from moisture (N_2, parafilm seal).

b) Preparation of 2-pyridinethiol ester[24]

The hydroxy acid (108) (250 mg, 0.9 mmol) was dissolved in methylene chloride (5 mls) under nitrogen. Triethylamine (150 µl) was added and the solution cooled to 0°C. Finally, the 2-pyridyl chloroformate solution (5 mls, 1 mmol) was added dropwise and the resulting solution stirred for 0.5 hrs.

The solution was diluted with dichloromethane (25 mls) and washed with *cold* 10% $NaHCO_3$ (10 mls) *cold* 5% HCl (10 mls) and finally saturated NaCl solution (10 mls). After drying ($MgSO_4$), the residue was azeotroped with toluene (3x10 mls) and placed under reduced pressure (1 mmHg) for several hours prior to use.

c) Lactonisation of thio-pyridyl ester[25]

The thio-pyridyl ester was dissolved in freshly distilled dry xylene (40 mls) and added dropwise over several hours (2-3) to refluxing freshly distilled dry xylene (300 mls). Once the addition was complete the resulting yellow solution was refluxed overnight. It was then cooled, concentrated and bound to silica for chromatography. The column (13x1.5 cm) was eluted with 30% ethylacetate/70% hexane at 8-9 mls min^{-1}. This gave the macrolactone (119) (70-80 mg, ~30%) and smaller quantities (~40 mg) of a compound tentatively identified as the dimeric species.

Experiments changing both refluxing times and addition rates had little effect on these yields.

^{1}H NMR (400 MHz, $CDCl_3$).

δ5.57 (1H, d, H-1, $J_{1,2}$ = 1.3 Hz), 5.31-5.45 (2H, m, H-8, H-9), 5.07-5.18 (1H, m, H-3, $J_{2,3}$ = 4.1, $J_{3,4}$ = 6.7, $J_{3,F}$ = 27.0 Hz), 4.51 (1H, d, $J_{5,6\ exo}$ = 5.3 Hz), 4.43-4.60 (1H, m, H-2, $J_{2,3}$ = 4.1, $J_{1,2}$ = 1.3, $J_{2,F}$ = 50.4 Hz), 3.89 (1H, d, $H\text{-}6_{endo}$, $J_{exo,endo}$ = 7.7 Hz), 3.77-3.82 (1H, m, $H\text{-}6_{exo}$, $J_{5,6\ exo}$ = 0.9, $J_{exo,endo}$ = 7.7 Hz), 3.06-3.14 (1H, m, H-7, $J_{4,7}$ = 8.6, $J_{7,8}$ = 10.8, $J_{7,7'}$ = 14.2 Hz), 2.8-2.9 (1H, m, H-10'), 2.39-2.5 (2H, m, H-12,12'), 2.19 (1H, bt, H-4), 1.93-2.06 (3H, m, H-7', H-10' H-11), 1.6-1.7 (1H, m, H-11').

^{13}C NMR ($CDCl_3$).

δ171.54 (C=O), 129.7, 129.3 (C-8, C-9) , 99.1 (C-1, J_{CF} = 25 Hz), 85.8 (C-2, J_{CF} = 189 Hz), 77.8 (C-5), 67.4 (C-6), 66.2 (C-3, J_{CF} = 15.1), 39.8 (C-4), 32.5 (C-7), 26.6, 23.3, 20.9 (C-10, C-11, C-12).

IR ($CHCl_3$) 1715 cm^{-1}.

MS m/e found 256.

HRMS Calc. for $C_{13}O_4H_{17}F$ 256.1112. Found 256.1116.

Mp 181° sub, 205-209°C.

TLC (45% EtOAc/53% hexane/2% CHOOH) Rf = 0.42, or (50% EtOAc/50% hexane) Rf = 0.53.

Dimeric compound.

^{1}H NMR (90 MHz, $CDCl_3$).

δ5.2-5.65 (3.5H, m, H-9, H-8, 0.5 H-3, H-1), 5.0 (0.5H, m, 0.5 H-3), 4.2-4.8 (1H, m, H-2, $J_{2,F}$ = 48 Hz), 3.8 (2H, m, H-$6_{exo,endo}$), 1.55-2.6 (9H, m).

MS m/e found 512.

HRMS Calc. for $C_{26}O_8H_{34}F_2$ 512.2223. Found 512.2243.

TLC (45% EtOAc/53% hexane/2% CHOOH) Rf = 0.34.

1,6-Di-O-acetyl-2-deoxy-2-fluoro-4-deoxy-4-(6-carboxy-hexen-2-2-yl)-α and β- allo hexopyranose-macrolactones(123, 124)[17]

The macrolactone (330 mg) was dissolved in dry acetic anhydride (10 mls) under a nitrogen atmosphere. To this 70% perchloric acid (20 μl) was added and the solution stirred for 2 hours. Since no reaction had occurred (usually indicated by substantial darkening of solution and confirmed by tlc, 50% EtOAc/Hex) a further 30 μl of acid was added. This caused immediate darkening and the resulting solution was stirred for a further 4-5 hours until the reaction was complete (tlc).

5% Potassium bicarbonate solution (5 mls), water (5 mls) and dichloromethane (10 mls) were added and the mixture stirred for 0.5 hours. The phases were separated and the aqueous phase extracted with dichloromethane (2x5 mls). The combined organic extracts were then concentrated and azeotroped with toluene to remove water and excess acetic anhydride. The residue was bound to silica and chromatographed. The column (15x1.5 cm) was eluted with 30% EtOAc/70% hexane at 8-9 mls min^{-1} to yield the diacetate (300 mg, 65%) as a 4:1 anomeric mixture which was crystallised by trituration with ethanol.

Anomeric separation and purification

The column chromatography above caused a partial separation of the anomeric mixture. The very late fractions were pure in the more polar minor α-anomer, these were kept separate and recrystallised from ethanol. Similarly, recrystallisation of a small sample from the bulk material which was rich in the major isomer gave a pure sample of the β-anomer (123).

^1H NMR (400 MHz $CDCl_3$)

δ5.94 (1H, q, H-1, $J_{1,2}$ = 9, $J_{1,F}$ = 2 Hz), 5.43-5.51 (1H, m, CH=C), 5.24-5.4 (2H, m, H-3, CH=C), 4.4-4.56 (1H, m, H-2, $J_{1,2}$ = 9, $J_{2,3}$ =

3, $J_{2,F}$ = 46.5 Hz), 4.28-4.35 (1H, m, H-6, $J_{6,6'}$ = 12, $J_{5,6}$ = 1.5 Hz), 4.14-4.2 (1H, m, H-6', $J_{6,6'}$ = 12, $J_{5,6'}$ = 4.5 Hz), 3.95-4.03 (1H, m, H-5), 1.55-2.5 (9H, m), 2.12 (3H, s, $-CH_3$), 2.17 (3H, s, $-CH_3$).

^{13}C NMR ($CDCl_3$).

δ173.3, 170.7, 169.2 (3xC=O), 133.4, 124.1 (C-8, C-9), 89.9 (C-1, J_{CF} = 25.6 Hz), 86.6 (C-2, J_{CF} = 193.7 Hz), 75.0 (C-5), 68.8 (C-3), 63.2 (C-6), 37.3 (C-4)*, 35.0 (C-7)*, 26.6, 25.4, 24.6 (C-10, C-11, C-12), 20.9, 20.8 (2xCH_3).

TLC (50% EtOAc/50% hexane) Rf = 0.48

α-anomer (124).

^1H NMR (400 MHz, $CDCl_3$).

δ6.32 (1H, d, H-1, $J_{1,2}$ = 4 Hz), 5.44-5.53 (1H, m, CH=C), 5.23-5.34 (2H, m, H-3, CH=C), 4.64-4.78 (1H, m, H-2, $J_{1,2}$ = 4, $J_{2,3}$ = 4, $J_{2,F}$ = 43.5 Hz), 4.25-4.33 (1H, m, H-6, $J_{5,6}$ = 1.5, $J_{6,6'}$ = 12 Hz), 4.16-4.22 (1H, m, H-6', $J_{5,6'}$ = 4.5, $J_{6,6'}$ = 12 Hz), 4.08-4.14 (1H, m, H-5), 1.6-2.5 (9H, m), 2.12 (3H, s, $-CH_3$), 2.15 (3H, s, $-CH_3$).

^{13}C NMR ($CDCl_3$).

δ173.4, 170.7, 169.2 (3xC=O), 139.6, 123.9 (C-8, C-9), 88.0 (C-1, J_{CF} = 25.6 Hz), 83.9 (C-2, J_{CF} = 195.9), 68.8 (C-5), 66.3 (C-3), 63.2 (C-6), 35.9 (C-4)*, 35.1 (C-7)*, 26.5, 25.4, 24.8 (C-10, C-11, C-12), 20.9, 20.7 (2xCH_3). *Interchangeable.

TLC (50% EtOAc/50% hexane) Rf = 0.46.

Mixture

IR ($CHCl_3$) 3010, 1740 cm^{-1}.

MS m/e found 298, m^+ -60.

Mp 111-121° then 136-142°C.

2-deoxy-2-fluoro-4-deoxy-4-(6-carboxy-hexen-2-2-yl)- α and β -allo hexopyranose-3-O-macrolactones(126)

The diacetate (123, 124) (300 mg) was dissolved in methanol (15 ml) and solid potassium bicarbonate (350 mg) was added. The resulting suspension was stirred at room temperature for one hour, tlc (50% EtOAc/50% hexane) showing a complete reaction. The reaction mixture was bound onto silica and chromatographed. The column (15x1.5 cm) was eluted with 10% MeOH/40% EtOAc/50% hexane at 8-9 mls min^{-1} to yield the diol (126) (180 mg, 75%) as a white crystalline material.

^1H NMR (90 MHz, CD_3OD)

δ5.1-5.5 (3H, m, H-1, H-8, H-9), 4.4-4.95 (1.5 H, m, H-3, 0.5 H-2), 4.0 (0.5 H, m, 0.5 H-2), 3.5-3.9 (3H, m, H-5, H-6,6'), 1.6-2.5 (9H, m).

^{13}C NMR (CD_3OD).

δ175.5 (C=O), 126.5, 133.5 (C-8, C-9), 94.3 (C-1, J_{CF} = 26.9 Hz), 89.5 (C-2, J_{CF} = 139.2), 78.2 (C-5), 71.8 (C-3, J_{CF} = 17.1), 62.9 (C-6), 38.3 (C-4), 35.9 (C-7), 25.8, 26.6, 27.4, (C-10, C-11, C-12).

Further confirmation was obtained by acetylation and spectroscopic comparison with the diacetates(123, 124).

Attempted transesterification of macrolactone diols(126)

a) Under acidic conditions

The diol (11 mgs) was dissolved in methanol (2 mls) under nitrogen. Perchloric acid (2 μl) was added and the solution stirred and monitored by tlc (70% ethyl acetate/25% hexane/5% methanol).

Several hours showed no change. Similarly, addition of more acid (50 μl) prolonged stirring and refluxing of the reaction mixture caused no change.

Use of p-toluene sulphonic acid under similar reaction conditions also caused no change.

b) Under basic conditions

The diol (11 mgs) and potassium ^{t}butoxide (23 mg, 5 eq) were dissolved in dry methanol (2 mls). The resulting solution was stirred overnight at room temperature under nitrogen. Monitoring by tlc (70% ethyl acetate/25% hexane/5% methanol) showed no change, so the solution was refluxed for 3-4 hours.

The reaction mixture was then cooled, acidified with 2M HCl solution, then concentrated and azeotroped with toluene. The residue was then acetylated with pyridine and acetic anhydride. Subsequent isolation and tlc analysis of the acetates showed the products to be a mixture of the acetylated macrolactone (123, 124) and a new product, assumed to be the acetylated trihydroxy acid (109).

c) Aqueous basic hydrolysis

The diol (9 mg) and potassium hydroxide (22 mg, 12 eq) were dissolved in methanol (2 mls) and water (100 µl) was added.

Stirring at room temperature for 3-4 hours caused no change so the solution was refluxed overnight. The solution was then acidified with 2M HCl solution, concentrated and azeotroped with toluene.

Acetylation of the residue and tlc analysis of the resulting acetates indicated that the acetylated trihydroxy acid (109) had been isolated.

2-Deoxy-2-fluoro-4-deoxy-4-(methyl-6-carboxy-hexan-2-2-yl)- α and β =allo hexopyranoses(125)

The diol (90 mg) was dissolved in methanol (10 mls) and to this solid potassium hydroxide (150 mg) and water (0.5 ml) were added. The resulting solution was refluxed for 3 hours, then cooled, neutralised (2M, HCl) concentrated and azeotropically dried with toluene.

The residue was then reacted with ethereal diazomethane[26] solution (3-4 mls). Excess diazomethane was removed under a stream of nitrogen and the residue extracted with dichloromethane. The organic extracts were bound to silica and chromatographed. The column (13x1.5 cm) was eluted with 48% EtOAc/2% MeOH/50% hexane at 8-9 mls min^{-1} and yielded the methyl ester (125) (42 mg, 41%).

^1H NMR ($CDCl_3$).

δ5.1-5.6 (3H, m, H-1, H-8, H-9), 3.4-4.8 (5H, m, H-2, H-3, H-5, H-6,6'), 3.68 (3H, s, $-OCH_3$), 1.6-2.5 (9H, m).

TLC (50% EtOAc/50% hexane) of anomeric mixture Rf = 0.0-0.13, or (75% EtOAc/20% hexane/5% MeOH) Rf = 0.31 and 0.21.

6-O-^{t}Butyldimethylsilyl-2-deoxy-2-fluoro-4-deoxy-4-(methyl-6-carboxy-hexen-2-Z-yl)-α and β-allo hexopyranoses(129)[27]

The trihydroxy methyl ester (125) (85 mg), 0.27 mM) was dissolved in dichloromethane (1.5 mls) with a small quantity of dimethylaminopyridine (5 mg) under a nitrogen atmosphere.

Concurrently, tertiary butyldimethylsilyl chloride (101 mg, 0.67 mM) and triethylamine (112 µl, 1.2 eq) were dissolved in dichloromethane (1 ml) under a nitrogen atmosphere and stirred for fifteen minutes. An aliquot from the solution of silylating agent, (450 µl, 1 eq) was then added to the substrate solution above.

The resulting solution was stirred overnight and worked up despite the reaction being incomplete by tlc (50% EtOAc/50% hexane). The solution was washed with water (1 ml) then saturated ammonium chloride solution (1 ml) and dried ($MgSO_4$).

Finally, the residue was bound to silica and chromatographed. The column (10x1 cm) was eluted with 50% ethylacetate/50% hexane at 4 mls min^{-1} and gave the silyl ether (127) (24 mg, 20%) as an oil.

^{1}H NMR (90 MHz, $CDCl_3$).

δ5.5-5.2 (m), 3.7-4.3 (m), 1.6-2.5 (m), 3.66 (3H, s, OCH_3), 0.9 (9H, s, $C(CH_3)_3$), 0.1 (6H, s, $2xCH_3$).

TLC (50% EtOAc/50% hexane) of anomeric mixture. Rf = 0.62 and 0.53.

1,3-Anhydro-6-O-^{t}butyldimethylsilyl-2-deoxy-2-fluoro-4-deoxy-4-(methyl-6-carboxy-hexen-2-Z-yl)-α-allo hexapyranose (142)[25]

The silyl ether (30 mg, 0.07 mM) was dissolved in dry dichloromethane (1 ml), under nitrogen. Concurrently, diethylazodicarboxylate (50 µl, 0.41 mmol) and trimethyl phosphite (50 µl, 0.31 mmol) were dissolved in dry dichloromethane (1 ml) under nitrogen and stirred for 10-15 minutes. An aliquot (300 µl) was then added to the silyl ether solution above at 0°C, the resulting solution was then stirred at room temperature for forty minutes. Tlc (20% EtOAc/hexane) clearly showed a well separated less polar substance.

The solution was concentrated and placed onto a pre-prepared column (in this instance the crude reaction mixture was not dry loaded).

The column (10x1 cm) was eluted with 20% ethyl acetate/2%triethylamine/78% hexane at 4 mls min^{-1} and yielded the oxetane (142) (3.5 mg, 12%).

^{1}H NMR (360 Mz, $CDCl_3$).

δ5.33-5.54 (3H, m, H-1, H-8, H-9), 4.62-4.79 (1H, d, H-2, $J_{2,F}$ = 59.3 Hz), 4.51-4.56 (1H, q, H-3, $J_{1,3}$ = 4.8, $J_{3,F}$ = 13.5 Hz), 3.82-3.85 (1H, m, H-5), 3.76-3.80 (1H, m, H-6, $J_{5,6}$ = 2.9, $J_{6,6'}$ = 11.1 Hz), 3.66 (3H, s, $-OCH_3$), 3.61-3.65 (1H, m, H-6', $J_{6,6'}$ = 11.1 Hz), 2.2-2.4 (3H, m, H-4, H-7,7'), 2.3 (2H, t, H-12,12', $J_{11,12}$ = 7.4 Hz), 2.04-2.09 (2H, m, H-10,10' $J_{10,11}$ = 7.4, $J_{9,10}$ = 7.5 Hz), 1.64-1.72 (2H, m, H-11,11', $J_{10,11}$ = 7.4, $J_{11,12}$ = 7.4 Hz), 0.89 (9H, s, $C(CH_3)_3$), 0.064-0.068 (6H, d, Si $(CH_3)_2$).

^{19}F NMR ($CDCl_3$).

(-201.75) to (-200.72) 8 lines, $J_{F,H-2}$ = 59.3, $J_{F,H-3}$ = 13.5, $J_{F,H-1}$ = 4.3 Hz.

TLC (20% EtOAc.80% hexane) Rf = 0.35.

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