Studies Towards a Total Synthesis of Tagetitoxin

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Abstract

Tagetitoxin was first isolated over thirty five years ago and a total synthesis has not been achieved to date. A vast amount of research has been carried out on the biological activity of tagetitoxin with hundreds of literature reports. However, very few papers have been published regarding the synthesis and within this thesis we will explore a number of synthetic pathways some towards tagetitoxin.

The first chapter reviews previous developments regarding the total synthesis of tagetitoxin. It also covers the discovery and biological work that has being carried out in identifying the structure and the biological activity of tagetitoxin.

The second part reports my studies towards a total synthesis of tagetitoxin. It details all the routes explored showing the key intermediates and novel compounds we obtained.

The third and final part contains the experimental data for all compounds discussed in part two of this thesis.

Abbreviations

Å-Ångström

Ac-Acetyl aq. -aqueous (solution)

Ar-aromatic (proton)

[α]D-specific optical rotation at the sodium D line

cat.-catalytic

cm^{-1}-wave number

°C-degrees Celsius

c-concentration

δ-chemical shift

DAM-Diacetone mannose

DBDMH-1,3-dibromo-5,5dimethylhydantoin

DCM-dichloromethane

DIPA-diisopropylamine

DMAP-4-(dimethylamino)pyridine

DMF-*N*,*N*-dimethylformamide

DM-Dess-Martin Periodinane

DMSO-dimethylsulfoxide

DMDO-dimethyldioxirane

eq-equivalent

FAB-fast atom bombardment

h-hour(s)

Hz-Hertz

IR-Infrared

Kdo-3-deoxy-D-*manno*-2-octulosonic acid

$LiAlH_4$-lithium aluminium hydride

LDA-lithium diisopropylamine

M-molarity

m-CPBA-*meta*-chloroperbenzoic acid

min-minute(s)

mp-melting point

m/z-mass to charge ratio

Ms-mesyl

MS-mass spectrometry

NBS-*N*-bromo succinimide

NMR-nuclear magnetic resonance

NTP-nucleotidyl triphosphate

PCC-pyridinium chlorochromate

PDC-pyridinium dichromate

ppm-parts per million

*p*TSA-*para*-toluenesulfonyl acid

pyr-pyridine

RNA-ribonucleic acid

r.t.-room temperature

sat.-saturated

SM-starting material(s)

TBAF-tetrabutylammonium fluoride

TBDMS-*tert*-butyldimethylsilyl

TBDPS-*tert*-butyldiphenylsilyl

TEA-triethylamine

Tgt-tagetitoxin

THF tetrahydrofuran

TIP- triisopropylsilyl

TLC-thin layer chromatography

Ts-tosyl

TMS-trimethylsilyl

Tr-trityl

All other abbreviations are used according to the IUPAC nomenclature or SI units.

Acknowledgments

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Finally, Kyriaki Gratsia made all of my PhD so much more bearable by being there for me outside of the lab. UEA will always be our special place and I thank you for all your help and support you have given me over the years to pick me up in both the good and bad times.

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Introduction:

Background:

Tagetitoxin is a relatively new toxin which was first isolated and purified in 1981, and since then has attracted a lot of attention in chemical research.[1] There has been much debate surrounding the structure of tagetitoxin, and to this date a crystal structure has not been obtained of tagetitoxin in its pure form. There has been a good deal of literature published in relation to its biological activity, but there are little papers in relation to the synthesis and biosyntheisis of tagetitoxin. Efforts are still ongoing to demonstrate the first total synthesis of tagetitoxin, and this has led our group to carry out its investigations towards the total synthesis.

Isolation

The isolation and purification of a chemical component from *Pseudomonas syringae pv. Tagetis* which was named tagetitoxin, was first published in 1981 by Mitchell and Durbin.[1] They began by obtaining a culture of *Pseudomonas syringae pv. Tagetis,* which had been obtained from Fahy.[2]

After extracting and carrying out purification of the toxin containing fractions and analyzing these toxin fractions, Mitchell and Durbin had observed that tagetitoxin was known to contain a sulfur atom, an amino group and a phosphate moiety.[1]

Mitchell and Durbin were the first group to have isolated and purified tagetitoxin successfully, and now more work was needed to characterize the structure of tagetitoxin.[2]

Structure

In 1983 Mitchell and Hart published a paper with the first proposed structure of tagetitoxin (**Figure 1**).[3] They believed the structure to contain an eight membered ring. They were unable to get an X-ray crystal structure of tagetitoxin due to the isolation of their sample of the toxin as a non-crystalline glassy residue. An IR spectrum of tagetitoxin only provided limited information indicating the presence of hydroxyl, carbonyl and phosphate group. Field desorption mass spectrometry determined that tagetitoxin gave *M/Z* of 435 for M^+ which indicated that tagetitoxin has a molecular weight of 435 corresponding to a molecular formula of $C_{11}H_{18}O_{13}SNP$. In addition, the hetroatomic components of tagetitoxin were identified as nitrogen in an amine from treatment with Ninhydrin which produed a purple colour (a key characteristic of nitrogen in an amine), and phosphorus in a phosphate ester from treatment

with a molybdate reagent producing a characteristic blue colour. In addition to these tests Mitchell carried out ^{31}P NMR and ^{35}S NMR spectroscopy. The sulfur was deduced to be present as either a thiol or a thioether and not in a sulfate ester as treatment of tagetitoxin with a strong acid did not liberate sulfate but instead a mixture of unknown products. However, treatment of tagetitoxin with sodium nitroprusside gave a positive colour, indicating the presence of a thiol. In the ^{31}P NMR spectrum it showed a doublet (J=11.5Hz) at δ 1.0 downfield from the phosphate, which was consistent with the presence of a phosphate ester or a secondary alcohol. The ^{13}C NMR spectrum had signals from 11 carbons in total, three present as carbonyl groups and five monosubstituted with oxygen. The functionalities contained in these five carbon substituents were deduced from ^{1}H and ^{13}C NMR chemical shifts to be one acetyl, one phosphate, and either three hydroxyl functions or one hydroxyl and one ether function.

Using both the NMR and mass spectral data, Mitchell and Hart suggested the molecular formula of tagetitoxin to be $C_{11}H_{18}O_{13}SNP$, and, furthermore, supported the presence of three hydroxyl functional groups rather than the alternative combination of one hydroxyl and one ether function. In accordance with the molecular formula and functional groups outlined, a fully saturated acyclic compound would have 20 hydrogens. Therefore, tagetitoxin has one unit of hydrogen unsaturation, and because the ^{13}C NMR showed there were no carbon-carbon double bonds present, tagetitoxin must be a single ring structure. These arguments are based on the sulfur of tagetitoxin having an oxidation state of -1, such as a thiol or thioether, and thus carrying no oxygenation. These observations supported the hypothesis that sulfur was not in a sulfate ester. Further tests were conducted on tagetitoxin by reacting it with sodium nitroprusside which showed no colour change, but when the toxin was pretreated with dilute hydrochloric acid a positive colour change was observed, indicating a thiol. A double labeling experiment was performed by growing *P.s. Tagetis* in the presence of $^{32}PO_4^{3-}$ and $^{35}SO_4^{2-}$, yielding a radiochemicallypure tagetitoxin which demonstrated a phosphorus-sulfur ratio of 1:1, showing the presence of one sulfur atom present in the tagetitoxin molecule. The ^{1}H NMR spectrum of tagetitoxin displayed a single methyl group (methyl in the acetyl) and well resolved multiplets and, therefore, gave useful structural information about the carbon skeleton of tagetitoxin. From both the NMR and mass spectrometry data and functional group knowledge from the group this led Mitchell and Hart to propose the first structure of tagetitoxin shown below (**Figure 1**).[3]

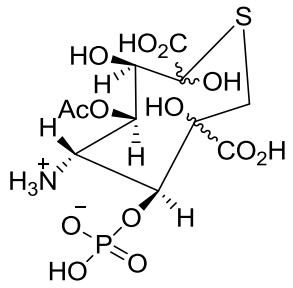

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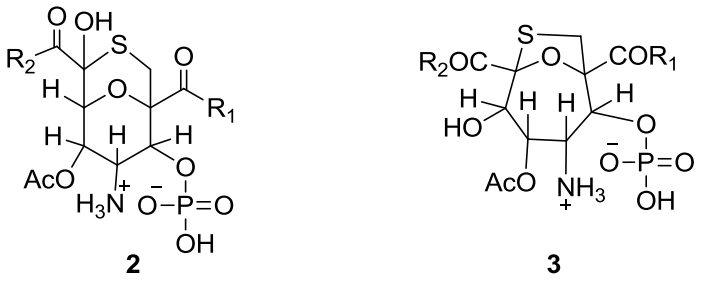
Figure 1: First Proposed structure of tagetitoxin.[3]

Mitchell *et al*. revised the structure of tagetitoxin in 1989 based on new mass spectrometry and NMR analysis they had carried out.[4] Higher resolution FAB Mass spectrometry gave a $(M+H)^+$ = 417.0316, indicating that tagetitoxin has a molecular formula of $C_{11}H_{17}N_2O_{11}PS$, not $C_{11}H_{18}O_{13}SNP$ which had been reported six years earlier by Mitchell. The functional group containing the additional nitrogen was deduced from NMR data to be an amide, since the ^{13}C NMR clearly indicated the presence of only one C-N bond, and since there was no phosphoramide nitrogen as evidenced by the ^{31}P chemical shift. The oxygen containing functional groups of tagetitoxin come from one acetyl, one phosphate, one carboxylic acid, one carboxamide and two oxygens, either as hydroxyl or ether groups. A fully saturated non-cyclic compound with these substituents would contain 21 hydrogens, and since the mass spectrum of tagetitoxin indicated the presence of only 17 hydrogens coupled with the lack of any carbon-carbon double bonds in the ^{13}C NMR data, tagetitoxin must contain two rings. Using the data obtained, in conjunction with chemical shifts, allowed Mitchell *et al*. to suggest only two possible bicyclic ring structures **2** and **3,** shown below (**Figure 2**).



a=R_1=OH, R_2=NH_2 or **b**=R_1=NH_2, R_2=OH

Figure 2: Revised Structure of Tagetitoxin by Mitchell *et* Hart.[4]

Both these bicyclic ring structures and substitution patterns were supported by nOe experiments. The coupling constants between the protons of the four-carbon series C5-C6 (*J*=3.6 Hz), C6-C7 (*J*=12.4 Hz), C7-C8 (*J*=6.0 Hz) allowed the dihedral angles to be deduced as follows for C5-C6 approximately 50°, C-6-C7 approximately 180° and finally C7-C8 aprroximately 35°. Using these angles, they are best accommodated by structure **2,** where the protons on C-6 and C-7 are in a true diaxial interrelationship. In contrast, the 5-membered ring structure **3** places a constraint on the 7-membered ring that does not allow a true diaxial relationship between C-6 and C-7 protons, whereas a diaxial interrelationship is fully supported by the structure proposed in structure **2**. On this basis, Mitchell *et al*. favoured structure **2**, although the data they obtained does not entirely eliminate the other proposed structure **3**.[4]

In 2005, Gronwald and colleagues reported that the structure of tagetitoxin which was proposed by Mitchell and his co-workers was incorrect due to an incorrect mass spectrum analysis.[5] Electrospray ionization (ESI) mass spectrometry gave ($M+H^+$) *m/z* = 679.5216 which indicated that the molecular weight of the tagetitoxin is 678, considerably greater than that previously reported of 416 by Mitchell *et al*. The NMR spectra reported by Mitchell *et al*. did however resemble the same NMR spectra obtained by Gronwald. Gronwald assumed that the additional mass units were accounted for due to the presence of oxygen, nitrogen, sulfur and exchangeable protons, which are not detected by 1D NMR. Their results indicated that the molecule contains a phosphate ester but does not contain a primary amine, and tagetitoxin cannot absorb UV or visible light. Having shown that the proposed structures by Mitchell *et al*. were incorrect, Gronwald however, failed to propose a molecular formula, let alone a structure of tagetitoxin and therefore structure **2** seems still the most plausible structure.[5]

Crystal Structure

In 2005 Vassylyev and co-workers published the first X-ray structure of tagetitoxin, which was obtained as a ttRNA-tagetitoxin complex, with tagetitoxin bound to the active sites of the RNA polymerase (**Figure 3**).[6] This structure confirmed that the structure proposed by Mitchell and his co-workers was the most probable and removed the uncertainty about the position of the carboxamide and the carboxylic acid functional groups within tagetitoxin This bicyclic structure is the target described within this thesis.

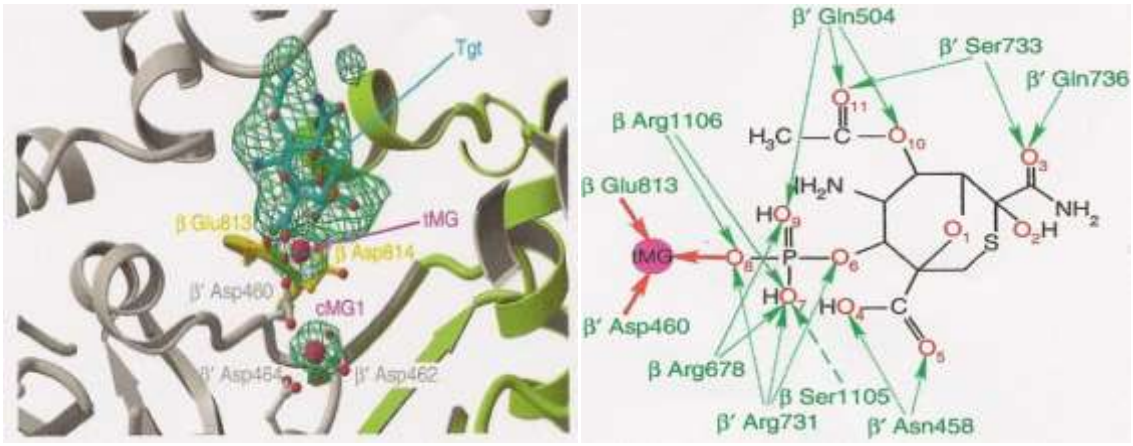


Figure 3: Left: Structure of Tagetitoxin-RNAP complex; Right: tagetitoxin binding site.[6]

Source of **Figure 3**: Vassylyev, D. G.; Svetlov, V.; Vassylyeva, M. N.; Perederina, A.; Igarashi, N.; Matsugaki, N.; Wakatsuki, S.; Artsimovitch, I. *Nat. Struct. Mol. Biol.* **2005**, *12* (12), 1086–1093.

Biological Activity

Tagetitoxin is a bacterial non-host specific phytotoxin, produced by *Pseudomonas syringae* pv *tagetis,* which causes apical chlorosis and leaf spot in host species of the Asteraceae family (e.g. zinnia (*Zinnia elgans* Jacq.) and sunflower (*Helianthus annuus*)].[6] The toxin which is produced *in planta*, is translocated to apical regions, where it inhibits chloroplast RNA polymerase III, which in turn blocks chloroplast biogensis. Tagetitoxin was shown also to inhibit RNAPs of bacteria, insects and vertebrates at micromolar levels of tagetitoxin *in vitro*, whereas in eukaryotic cells, RNA polymerase III was inhibited while RNA polymerases I and II were resistant.[6]

In 1990 Durbin and Matthews published a paper on the inhibition of eukaryotic transcription by RNA polymerase III caused by tagetitoxin.[7] Their work focused on the inhibition of RNA polymerase from chloroplasts and *Escherichia coli*. In isolated chloroplasts, tagetitoxin quickly and specifically reduced the incorporation of [^{3}H] uridine into RNA. When added to

transcriptionally active chloroplast protein extracts, the toxin directly inhibited incorporation of [^{32}P] UTP into RNA. In addition, tagetitoxin inhibited *in vitro* RNA synthesis directed by the RNA polymerase from *Escherichia Coli*. *In vitro* transcription reactions directed by chloroplast RNA polymerase or *E. coli* RNA polymerase are inhibited at tagetitoxin concentrations less than 1 µM. Nuclear RNA polymerase II purified from wheat grain was only affected at tagetitoxin concentrations greater than 100 µM during *in vitro* transcription. Tagetitoxin concentrations as high as 1 mM did not affect *in vitro* transcription reactions directed by RNA polymerase from bacteriophage T7 or SP6.

In 1992 Steinberg and Burgess published another paper which focused on testing the potential inhibition of eukaryotic RNAPs.[8] The toxin was shown to inhibit selectively RNAP III *in vitro* and in *xenopus oocytes*, showing effects on RNAP III from a wide range of organisms. They suggested that tagetitoxin acted directly against the RNAP III and not by binding transcription factors. This mode of action showed that in RNAP III there was a conserved structure to which the toxin could bind. Response differences could also be explained by the fact that the enzyme conformations could be different according to the template or protein cofactors in the transcription complexes. Mathews and Durbin carried out further work that indicated that tagetitoxin inhibition resulted in the small molecule reversibly binding to the discrete site of RNAP-template transcription complexes, creating an enhanced pausing in the transcription process, and this was template-dependent.[9]

Mechanism action of tagetitoxin

Vassylyev and his co-workers in 2005, having obtained the crystal structure of the tagetitoxin-RNAP complex of the bacterium *T. thermophilis* argued, against the already published simple mechanism of tagetitoxin, that tagetitoxin action would be in direct competition with the NTP substrate.[6,10,11] Vassylyev and his co-workers postulated that the mechanism by which tagetitoxin worked was by stabilizing some inactive intermediate during the substrate loading in the active site.

Structural analysis carried out by the authors suggests that an intermediate may be formed at either the pre-insertion or insertion step. The authors favored a concerted two-step model, in which the intermediate is preformed in the pre-insertion site and then is finally stabilized in the insertion complex.

The authors postulated that while binding to the pre-insertion site in the presence of tagetitoxin, the substrate phosphate that coordinates the cMG2 ion would, however, switch interactions to a well fixed tMG. This would in turn give a subsequent loss of cMG2. This proposal by the group would suggest that the substrate loading in the insertion was probably achieved by a simple rotation without repositioning of the phosphates in the NTP, and the interactions of NTP with tMG would not be disturbed during the isomerization. The more compact conformation of the active site in the insertion stage would result in a tighter binding of the tMG-bound substrate to prevent the dissociation of the substrate and the catalytic reaction, thereby irreversibly locking RNAP in a nonproductive state (**Figure 4**).[6]

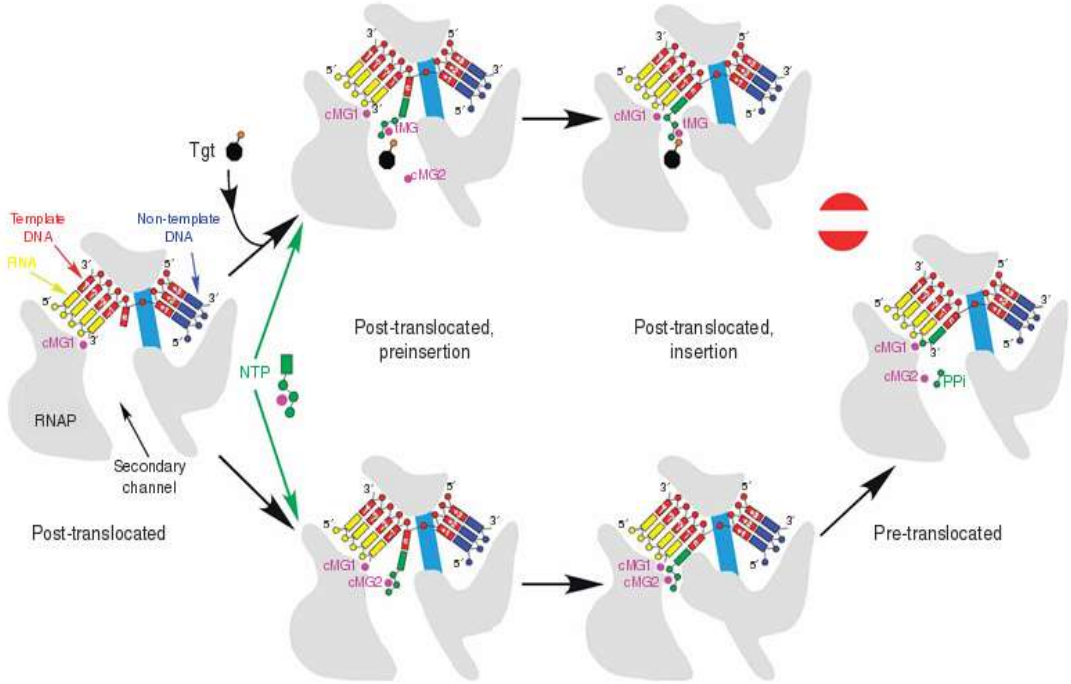


Figure 4: Proposed Mechanism of Tagetitoxin.[6]

Tagetitoxin inhibited all catalytic activities of RNAP and apparently competed with ppGpp for the effect on (and probably binding to) RNAP. The binding of tagetitoxin to RNA polymerase is exclusively by polar interactions, 9 of the 11 tagetitoxin oxygen atoms form 18 hydrogen bonds with the adjacent protein chains (**Figure 3**).

A basic and acidic set of side chains that constitute the tagetitoxin-binding site formed an extensive network not only with the inhibitor but also formed with each other. This concerted

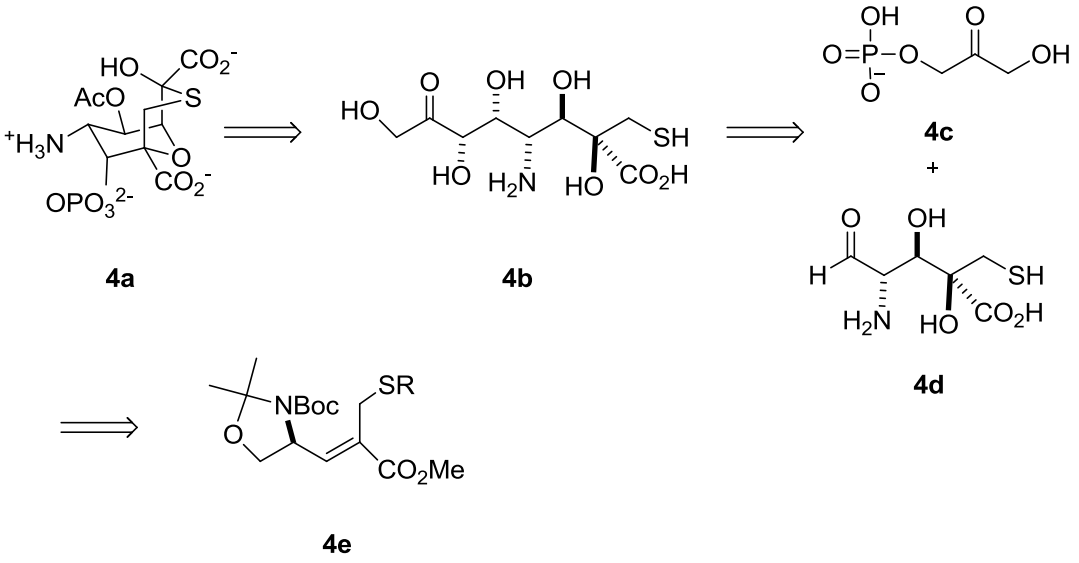
mode of recognition could be essential for the binding of tagetitoxin, which could be highly sensitive to subtle altercations either in conformation or position even for a single residue.

Tagetitoxin specifically interacts with three highly conserved basic residues (β Arg678, β Arg1106 and β' Arg731). The basic sidechain β' Asn458 is probably involved in substrate recognition, however, the tagetitoxin-binding and nucleotide triphosphate-binding sites do not overlap directly, suggesting that the competition with the substrate is not a major component of the tagetitoxin action.[12]

Vassylyev and his co-workers also postulated that an important structural feature of the RNAP-tagetitoxin complex was a well-fixed Mg^{2+} ion (tMG) that mediates RNAP interactions with tagetitoxin. It was shown that this Mg^{2+} ion was coordinated by the phosphate in tagetitoxin and by two other active site residues, β' Asp460 and β Glu813. The authors also compared this with the apo-holoenzyme, the side chain of the β' Asp460 which was better fixed in the complex by the bridging the two Mg^{2+} ions (cMG1 and tMG); this may favour coordination and strengthen binding of the catalytic cMG1. Tagetitoxin was shown to increase RNAP affinity for the major catalytic Mg^{2+} ion, cMG1.

Previous studies towards the synthesis of tagetitoxin

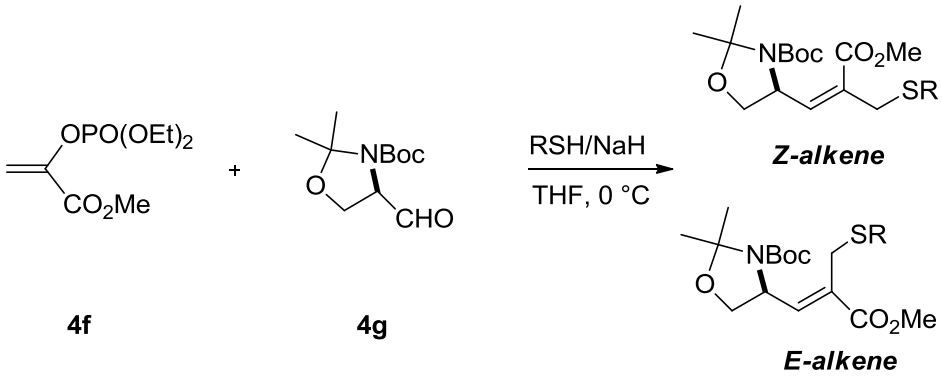
Sammakia *et al.*



Scheme 1: Retrosynthetic analysis of tagetitoxin.

In 1996 Sammakia *et al*. published a paper on the synthesis of tagetitoxin.[13] From the retrosynthetic analysis carried out, Sammakia, envisaged that an enzymatic coupling of dihyroxyacetone phosphate **4c** with aldehyde **4d** would form the fully functionalized tagetitoxin precursor **4b**. The next step would involve the cyclization of **4b** to give the proposed structure of tagetitoxin. Sammakia *et al*. proposed that aldehyde **4d** could be prepared from an oxazolidine olefin **4e** by a dihydroxylation followed by hydrolysis of the oxazolidine complete with an oxidation of the primary alcohol to give the desired aldehyde precursor **4d** (**Scheme 1**).

Sammakia therefore examined the preparation of alkenes **5a-e** and studied the effect of the protecting group on the ratio of dihydroxylation to sulfur oxidation (**Scheme 2 and table 1**). These alkenes were readily available in a one-pot synthesis with the generation of the phosphonate *in situ* followed by a condensation with the oxazolidine aldehyde. Using the above methodology Sammakia could vary the thiolate anion to produce a series of oxazolidine alkenes bearing protected thiols **5-*Z*** and **5-*E*** of different steric and electronic properties giving a mixture of products (**Table 1**). From the results it is clear that α, β-unsaturated esters were much more unreactive than the other systems explored by the group.

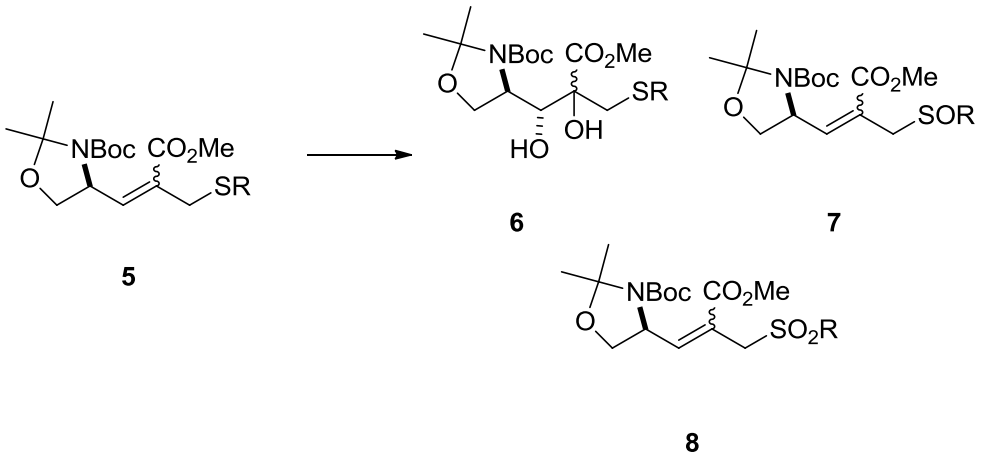


Scheme 2: Preparation of oxazolidine alkenes with varying sulfur protecting groups

R	Ethyl 5a	*i*-Propyl *5b*	*t*-butyl *Sc*	Phenyl 5d	Benzyl 5e
Z*:*E	60:40	70:30	100:0	20:80	30:70

Table 1: Ratio of *E: Z* products of olefin **5**

The group then attempted the dihydroxlation of **5**. The use of stoichiometric amounts of OsO_4 only produced sulfur oxidized products **7** and **8**, and therefore led the group to explore other ferricyanide oxidants. The use of AD-mix-β gave the poorest results and the group postulated that the bulky osmium-ligand complex was sterically demanding and reacted slower with the electron deficient and sterically hindered alkene substrates prepared (**Scheme 3 and table 2**). Changing the oxidant to $K_3Fe(CN)_6$ gave little of the desired product when used with substrates **5a**, **5b** and **5e,** with over-oxidation being observed and the major product being the sulfoxide instead of the desired sulfone product. Dihydroxylation of the phenyl sulfide **5d** followed the expected trend and gave less of the sulfur oxidized products, which were isolated with less bulky substrates. The t-butyl thiol substrate **5c**, was the only substrate to give the desired dihydroxylation product using osmium with $K_2Fe(CN)_6$ as the co-oxidant in good yield. This reaction gave isolated yields in the 50-63% range, and the osmylation gave a diastereoisomeric ratio of 25:1, the major product being the desired substrate for the synthesis of tagetitoxin. Sammakia reasoned that the major isomer was consistent with the osmium approaching the less hindered side opposite the BOC group in the minimum energy conformation.



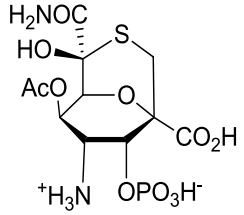
Scheme 3: Dihydroxylation of of olefin **5**

R	Oxidant	Recovered Starting Material	6	7	8
5-a	AD-mix-β	54	-	46	-
	$K_3Fe(CN)_6$	30	-	70	-
5-b	AD-mix-β	56	6	28	-
	$K_3Fe(CN)_6$	39	15	44	-
5-c	AD-mix-β	86	14	-	-
	$K_3Fe(CN)_6$	32	55	11	-
5-d	AD-mix-β	99	-	<1	-
	$K_3Fe(CN)_6$	34	27	-	39
5e	AD-mix-β	88	-	10	-
	$K_3Fe(CN)_6$	22	6	72	-

Table 2: Results of dihydroxlations of olefin **5** using varying oxidants

In conclusion, Sammakia developed a methodology to synthesize the required aldehyde **4d** as the major intermediate in their methodology in the total synthesis of tagetitoxin, however no more work on this approach has since been reported, and the quest continues to achieve a total synthesis of tagetitoxin.

Dent *et al.*



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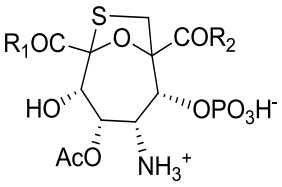

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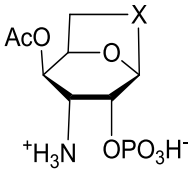
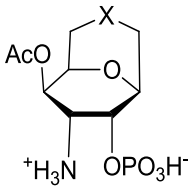


11

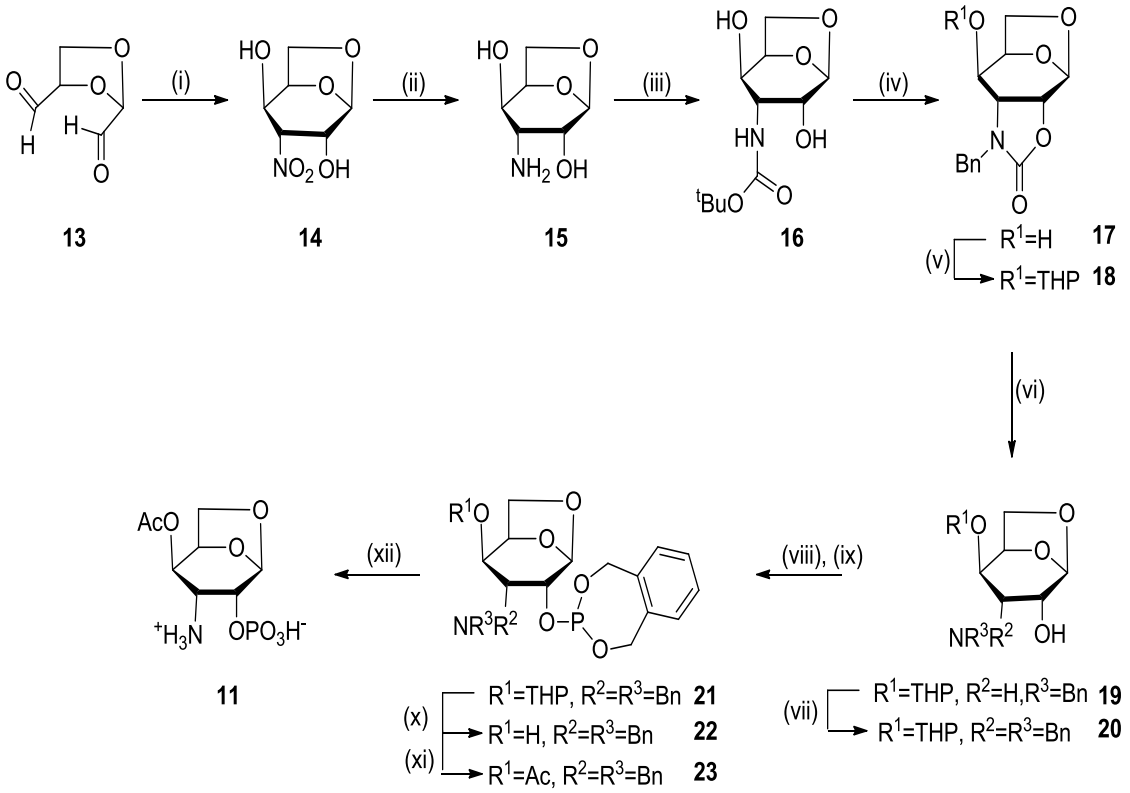
X=O, S



12

In 1999 Dent explored making different analogues of tagetitoxin due to its unique biological activity.[14] They based their studies on structure **9**; the authors hypothesized that the acetate, amine and phosphate groups are important for the activity while the C-S-C link was present to impose the required geometry. Dent believed that structure **9** matched tagetitoxin the best and studies began on analogues derived from D-sugars because they are readily available. These staring materials are structurally related to **9,** and have shown biological activity. In the paper, Dent described several approaches to these analogues based on structures **11** and **12**.[14]

The overall aim of the authors was to provide an insight into the structure-activity relationships of tagetitoxin, and also incidentally the construction of carbohydrate-based vicinal *cis*-amino phosphates. While compounds of **11** where synthesised, making bicyclic compounds like compund **12** where X=S,O had unexpected problems.



Scheme 4: i) $MeNO_2$, NaOMe, MeOH; **ii)** H_2, Pd/C, 2M HCl, 50 p.s.i.; **iii)** $(Boc)_2O$, Na_2CO_3, H_2O, THF; **iv)** $(Bu_3Sn)_2O$, Bu_4NBr, BnBr, toluene; **v)** dihydropyran, TsOH; **vi)** NaOH, EtOH; **vii)** Bu_2SnO, Bu_4NBr, BnBr, toluene; **viii)** *O*-xylylene-*N,N*-diethylphosphoramidite, 1H-tetrazole; **ix)** *m*-CPBA; **x)** 2M HCl; xi) Ac_2O, Pyridine **xii)** H_2, Pd/C, 50 p.s.i., EtOH, AcOH.

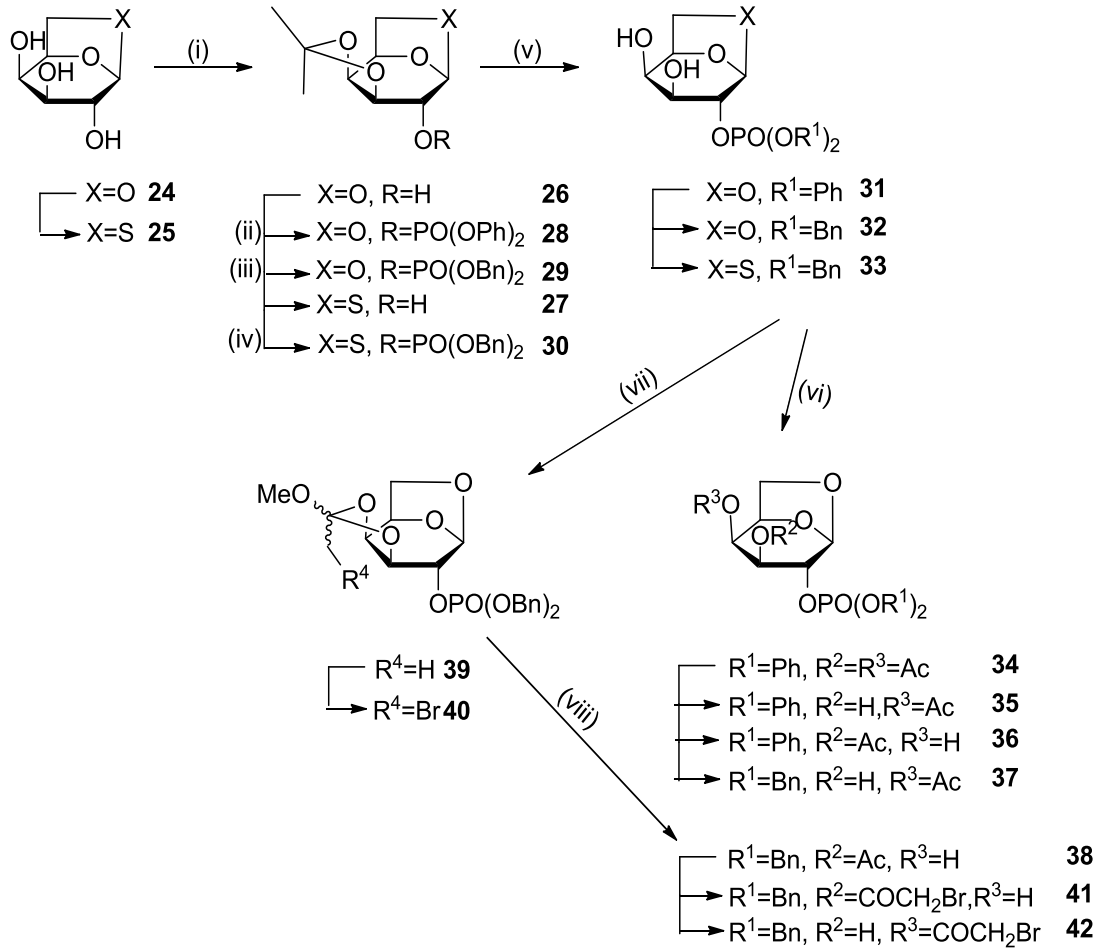
Dent began with a cyclization of the dialdehyde **13** with nitromethane and sodium methoxide to give the 1,6-anhydro-3-deoxy-3-nitro-D-gulose adduct **14**. The dialdehyde **13** was easily obtained by the periodate oxidation of levoglucosan. The nitro **14** product was subsequently reduced by a catalytic hydrogention to give the amine **15**. The configuration obtained was the D-gulo-configuration, which was supported by the large ^1H nmr coupling constant of $J_{3,4}$=9.9 Hz, and was also confirmed from the X-ray analysis of the hydrochloride salt of compound **15**. Dent decided it was desirable then to exploit the syn-disposition of the C-2 and C-3 subsituents in the anhydride **15** which permitted selective esterifcation on the C-2 and C-4 hydroxyl groups.

Amine **15** was then *N*-Boc protected to form compound **16**, which was then treated with bis(tributyltin) oxide, followed by tetrabutylammonium bromide and benzyl bromide in toluene under reflux giving the desired *N*-benzyl-protected cyclic carbamate **17**. Protection of the free hydroxyl on C-4 with THP under acidic conditions leading to the protected compound **18**, followed by alkaline cleavage of the carbamate group led to compound **19**, and selective *N*-benzylation with dibuytltin oxide followed by tetrabuytlammonium bromide and benzyl bromide to give the tertiary amine **20**. Phosphitylation with *o*-xylylene *N,N*-diethylphosphoramidite and 1*H*-tetrazole, followed by peracid oxidation, gave compound **21**. Removal of the THP protecting group on O-4 in acidic conditions gave the alcohol **22**, which was then acetylated with acetic anhydride in pyridine to afford **20**. Hydrogenolysis of compound **23** afforded the tagetitoxin analogue **11** (X=O) as a salt in quantitative yield (**Scheme 4**).

Dent subjected **11** to biological testing, and from the results the analogue was not active against the following pre- or post-emergent agriculturally important weeds: *Avena Fatua* (wild oat), *Setaria viridis* (green foxtail), *Amaranthus retroflexus* (redroot pigweed) or *Chenopodium album* (fat hen).

Dent also carried out a second approach based on compound **11** on the theory that it was necessary to functionalize O-2 and O-4 of 1,6-anhydro-D-galactose **24** and develop a good leaving group at C-3 to introduce the amino function with a configurational inversion. Phosphate **26,** which was prepared from **24**→**26**→**29**→**31** as shown in **scheme 5**, was treated with acetic anhydride in pyridine to give the diacetate **34** in 18% yield, and an inseparable mixture of the monoacetates **35** and **36** in 64% yield. The mixture contained over 90% of the 4-ester **35**, which was derived by selective reaction of the more accessible equatorial hydroxyl group. With selective acetylation established from compound **31**, the group decided to carry out selective acetylation on other closely related anhydrides **32** and **33** (**Scheme 5**). Work on these closely related anhydrides was unsuccessful due the sensitivity of compound **30** to acid and the failure to yield the diol **33**. This sensitivity they presumed was due to the participation of the sulfur atom in reactions of the carbocations generated under these conditions. Acetylation of **32** with acetic acid and pyridine, or followed by the reaction with dibutyltin oxide or bis(tributyltin) oxide, gave inseparable 1:1 mixtures of the monoacetates **37** and **38** contrasting with the selectivity seen in the model study of **31**. Treatment with bulkier pivaloyl chloride and dibuytltin oxide also gave a mixture of unsuitable monoesters. Dent postulated

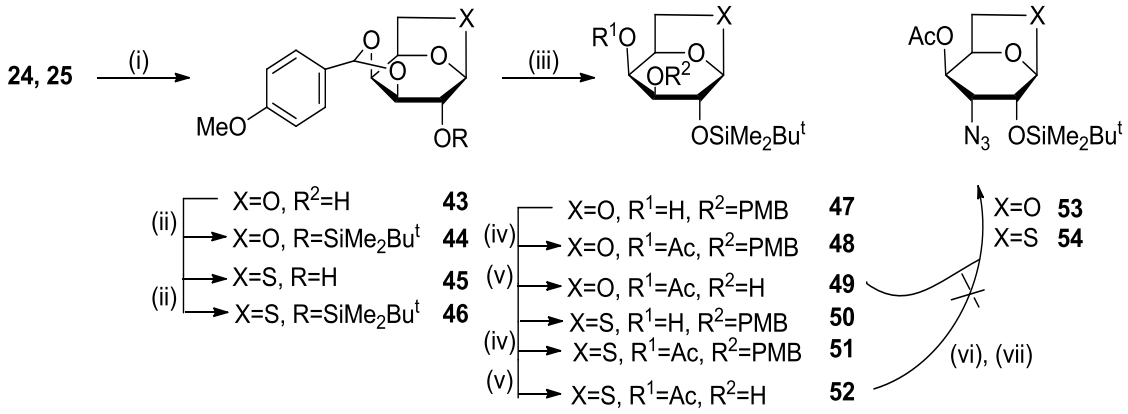
that these reactions might be victims of subsequent ester migration which would reduce the initial selectively. A selective acetylation was achieved when diol **32** was first converted into the cyclic orthoacetate **39** and then subjected to a mild hydrolysis. While this gave the desired acetate **38** with the new ester group axial at the O-3 position all attempts to turn this to an advantage were unsuccessful. The brominated orthoesters **40,** which were expected to give an O-3 ester that could be removed in the presence of an acetate at C-4, did not hydrolyse selectively but produced an inseparable mixture of bromoacetates **41** and **42**.



Scheme 5 i) $Me_2C(OMe)_2$, Me_2CO, TsOH; **ii)** $ClPO(OPh)_2$, pyridine; **iii)** BuLi, $[(BnO)_2PO]_2O$; **iv)** NaH, $[(BnO)_2PO]_2O$; **v)** 2M HCl **vi)** Ac_2O and (pyr or Bu_2SnO or $(Bu_3Sn)_2O$); **vii)** for **39**: $MeC(OEt)_3$, TsOH; for **40**: $BrCH_2C(OEt)_3$, TsOH; **viii)** AcOH, H_2O.

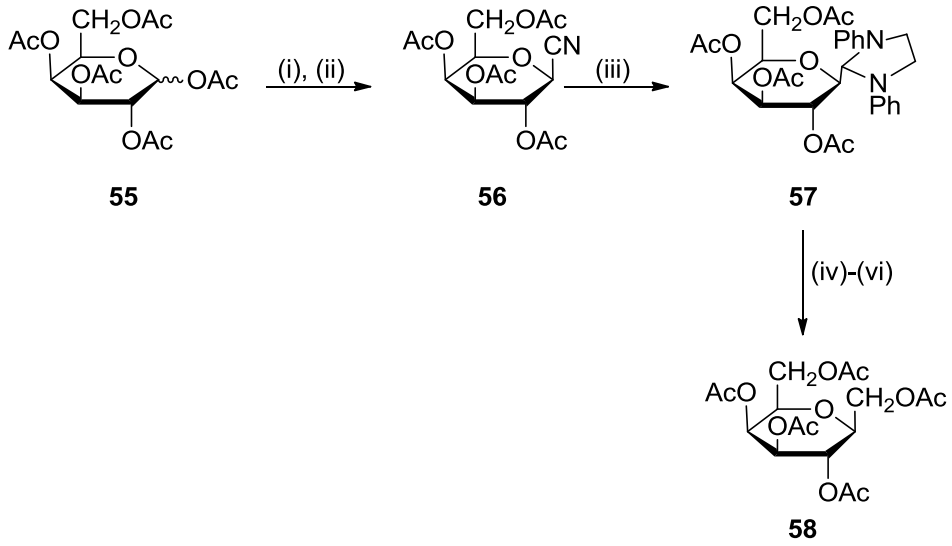
Dent worked on an alternative method of selectively functionalizing anhydrides **24** and **25,** based on the regioselective reductive cleavage of the *O*-benzyl and *O*-allyl derivatives of the *endo*-isomer of methoxybenzylidene acetal **43,** which was previously reported in the

literature.[15] Reduction of the 2-*O*-silyl derivatives **44** and **46**, derived from **43** and **45**, with $LiAlH_4$ and $AlCl_3$ gave the PMB protected O-3 ethers **47** and **50**. Acetylation gave esters **48** and **51**, followed by the removal of the PMB ethers to give the hydroxyl acetates **49** and **52,** followed by sulfonylation to give the derived 3-triflates, mesylates and tosylates, **49a**, **49b**, **49c** and **52a**, **52b**, **52c**. Attempts by Dent to obtain the desired azides **53** and **54,** from **49a**, **49b**, **49c** and **52a**, **52b**, **52c** all failed with sodium azide in DMF, HMPA or DMSO (**Scheme 6**). Dent presumed that this failure may have been due to the incoming nucleophile being impeded by the bulky substituent on O-2, and the desired product was not obtained, with just decomposition being observed.



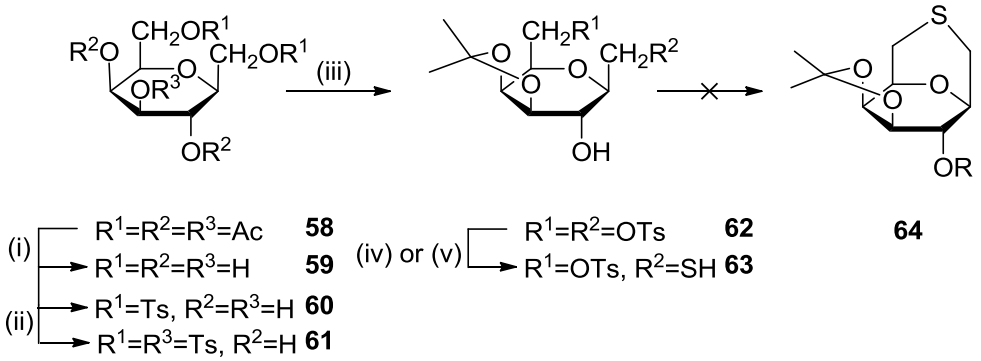
Scheme 6: i) $MeOC_6H_4CH(OMe)_2$, TsOH; ii) Bu^tMe_2SiCl, imidazole, DMF; iii) $LiAlH_4$, $AlCl_3$, THF; iv) Ac_2O pyridine; v) DDQ, DCM, H_2O; vi) Tf_2O, MsCl or TsCl, pyridine; vii) DMF, HMPA, or DMSO, NaN_3.

Having developed a route towards the synthesis of tagetitoxin analogue **11** (X=O), Dent then focused on the more closely related compound **9** (X=O,S), and envisaged making **12** from D-galactopyranose by a chain extension, by one carbon at C-1, followed by a ring closure *via* a linkage of the new atom and C-7 through sulfur. D-galactose pentacetate **55** was therefore brominated with HBr, and acetic acid to give the α-glycosyl bromide, which was then treated with mercury (II) cyanide in nitromethane to give the β-nitrile **56**. Reductive hydrolysis with Raney nickel, produced the unstable aldehyde, which was trapped with 1,2-dianilinoethane to give the imidazolidine **57**. Regeneration of the aldehyde followed by a reduction, and then acetylation, gave the known pentacetate **58** (**Scheme 7**).



Scheme 7: i) HBr, HOAc; **ii)** $Hg(CN)_2$, $MeNO_2$; **iii)** Raney Ni, NaH_2PO_2, $PhHN(CH_2)_2NHPh$; **iv)** TsOH, Me_2CO, DCM; **v)** $NaBH_4$ **vi)** Ac_2O, Pyridine

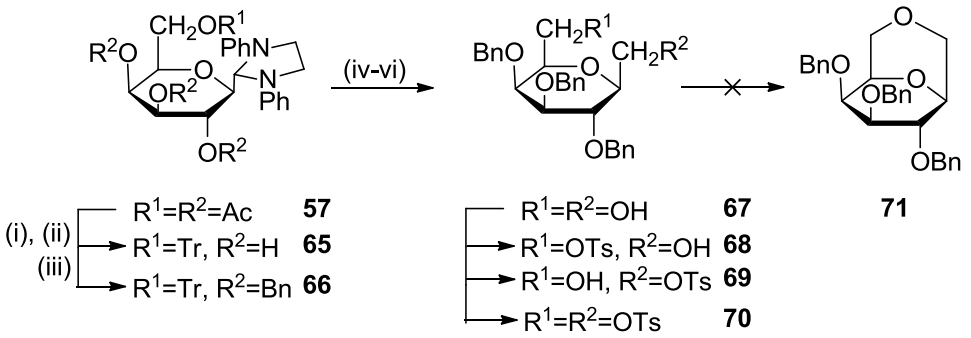
Deacetylation of **58** with sodium methoxide in methanol gave the pentaol **59,** followed by a selective tosylation at the primary centre, gave the ditosylate **60** in 33 % yield. Conversion of the ditosylate **60** into the acetonide was achieved easily to give **62**. Attempts to displace both tosylates with a divalent sulfur nucelophile failed to give the desired product **64**, and the only product isolated was **63** (**Scheme 8**).



Scheme 8: i) MeONa, MeOH; **ii)** TsCl, Pyridine; **iii)** $Me_2C(OMe)_2$, TsOH; **(iv)** Na_2S, DMF; **v)** Li_2S, DMF.

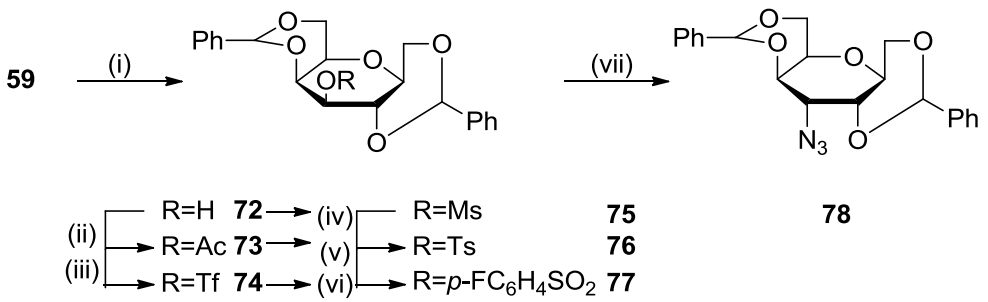
Other attempts were carried out by Dent involing the removal of the steric constraints from the isopropylidene protecting groups, which was carried out by making a modified substrate. Thus deacetylation of **57,** followed by tritylation of the primary alcohol gave **65**, followed by

perbenzylation to give **66**. Aldehyde unmasking followed by a reduction and detritylation gave the diol **67**. Ditosylate formation was achieved similar to the previous substrate to give **70**. Treatment of the ditosylate with sodium hydride gave a complex mixture, with no trace of the desired cyclic compound **71** being isolated (**Scheme 9**).



Scheme 9: i) MeONa, MeOH; **ii)** TrCl, Et_3N; **iii)** NaH, BnBr; iv) TsOH, Me_2CO, DCM; **(v)** $NaBH_4$; **vi)** TsOH, MeOH; **vii)** TsCl, pyridine.

Dent also explored the possibility of having an α-nitrogen bonded substituent at C-4 position, prior to ring closure, therefore eliminating any steric hindrance associated with at β-substituent at this position. Dent developed a suitable substrate with a good leaving group at C-4. Pentaol **59** was treated with benzaldehyde dimethyl acetal to give the diacetal **72,** which was then converted to make the already known acetate **73**. Several sulfonate esters were prepared from the diacetal **73**, triflate **74**, mesylate **75**, tosylate **76**, and the *p*-fluorobenzenesulfonate **77**. Dent carried out a range of nucleophilic displacements with sodium azide or tetrabutylammonium nitrite in DMF or DMSO. There was no reaction or decomposition was observed except with the tosylate **76** which provided a little of the azide product **78** in 10% yield. Dent could not improve on this result to gain a higher yielding reaction (**Scheme 10**).



Scheme 10: i) $PhCH(OMe)_2$, TsOH; **ii)** Ac_2O, pyridine; **iii)** Tf_2O, pyridine; **iv)** MsCl, pyridine; **v)** TsCl, DMAP, pyridine; **vi)** *p*-$FC_6H_4SO_2Cl$, DMAP, pyridine **vii)** NaN_3, DMSO.

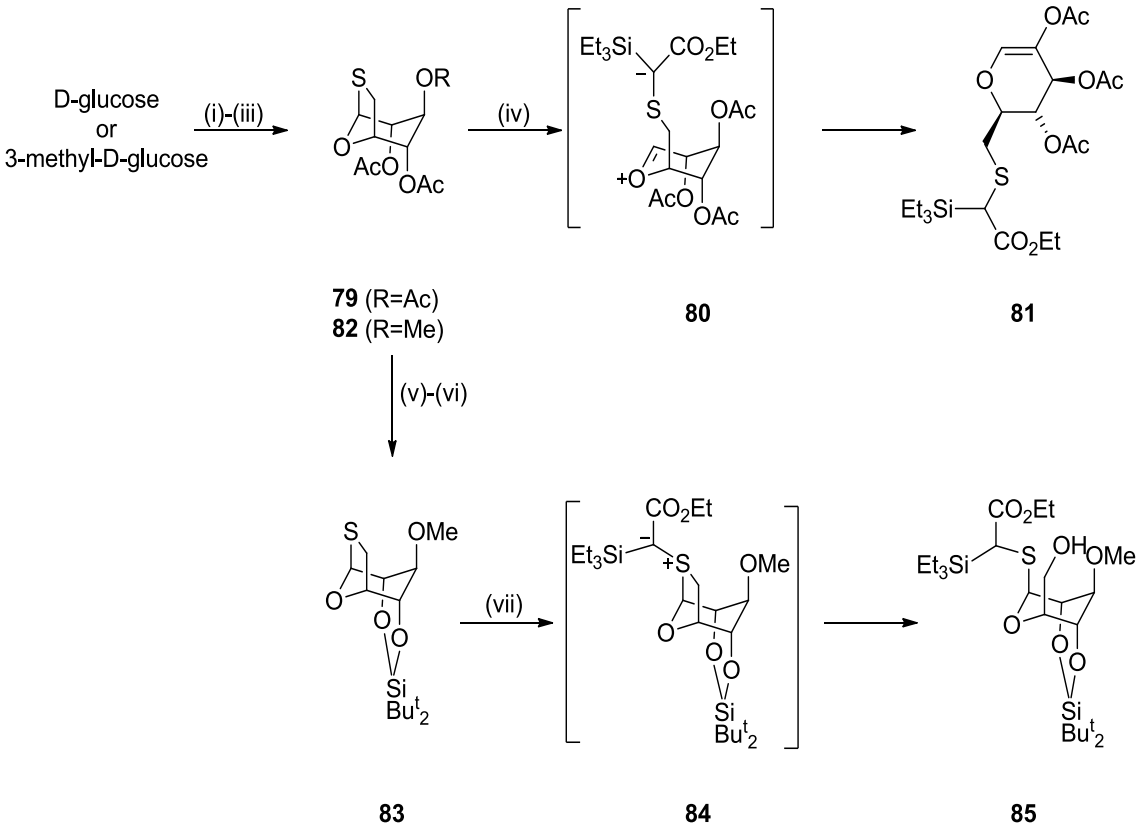
Dent in conclusion only made the tagetitoxin analogue **11** (**Scheme 1**), but all other routes towards the more closely related structure **12** were unsuccessful as well as all biological testing carried out on analogue **11,** and Dent finished his studies towards the total synthesis of tagetitoxin having obtained very little success with the synthesis.

Porter *et al.*

In 2006 Porter and Plet published a paper on the synthesis of the bicyclic core of tagetitoxin.[16] Porter's strategy focused on a carbene-mediated ring expansion of 1,3-oxathiolanes, on which his group had previously published.[17,18] Porter started his studies from D-glucose, which was converted into the bicyclic monothioacetal **79** through a displacement of an anomeric bromide, followed by a 6-tosylate displacement with potassium *O*-ethylxanthate. Ring expansion was then attempted using ethyl diazo(triethylsilyl)acetate, in the presence of catalytic rhodium(II) acetate, which the group had developed previously, but this did not lead to the anticipated bridged bicycle, but had led to the undesired glycal **81**.[17,18] Porter postulated that this product may have arisen through sulfur ylid formation and hetrocyclic C-S bond cleavage to give the zwitterion intermediate **80**, rather than the desired C-C bond formation. Porter presumed that this intermediate **80** underwent ring-flip to the more stable conformer followed by a proton transfer to afford the observed undesired product **81**.

In order to prevent this from happening, Porter designed a conformationally constrained substrate, where the zwitterionic intermediate would be incapable of ring flipping. Porter started from the readily available 3-methyl-D-glucose starting material, which was converted into bicyclic **82** using the same conditions as the previous substrate (**Scheme 1**). The acetate

groups were deprotected on bicycle **82**, and then treated with a di-*tert*-butylsilylene protecting group, to bridge the hydroxyl groups and give the tricycle **83**. Treatment of the tricycle **83** with ethyl diazo(triethylsilyl)acetate in the presence of rhodium(II) heptaflourobutyrate yielded the primary alcohol **85** as the only isolable product. Porter presumed that in this case, the sulfur ylid **84** intermediate formed as required, however rather than undergoing the required C-S bond heterolysis and ring expansion as expected; it was trapped by adventitious water to give the bicyclic alcohol **85** (**Scheme 11**).



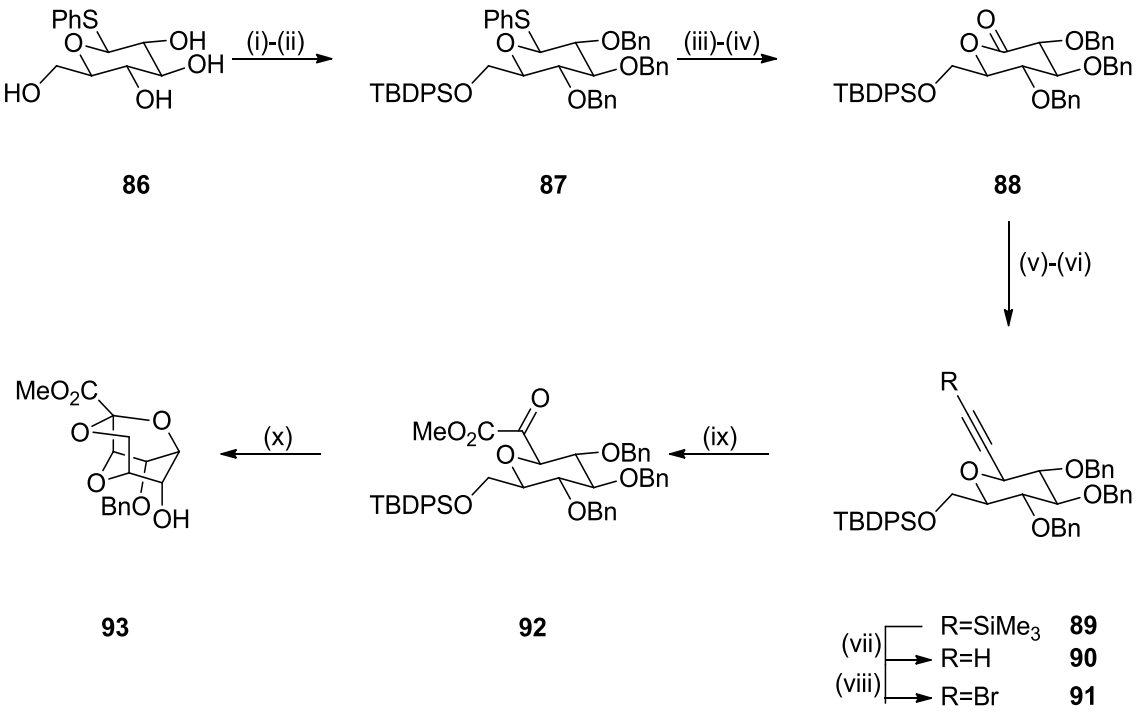
Scheme 11: i) TsCl, pyridine, Ac_2O; **ii)** HBr, AcOH; **iii)** KSCSOEt, DMF, 50 °C (46% over 3 steps) or KSCSOEt, acetone, reflux (47% over 3 steps)**; iv**) $Et_3SiC(N_2)CO_2Et$, $Rh_2(OAc)_4$, benzene, reflux 34%; **v)** NH_3, MeOH, H_2O, 50%; **vi)** $^tBu_2SiCl_2$, Et_3N, DCM, 86%; **vii)** $Et_3SiC(N_2)CO_2Et$, $Rh_2(O_2CC_3F_7)_4$, benzene, reflux, 21%.

Porter changed his strategy, since the initial approach had failed to achieve the tagetitoxin skeleton. The new strategy involved the synthesis of a 1,4-oxathiane ring, which would be

formed by the cyclization of a thiol onto an electron deficient ketone, rather than carrying out a carbene-mediated ring expansion, which had failed the earlier approaches.

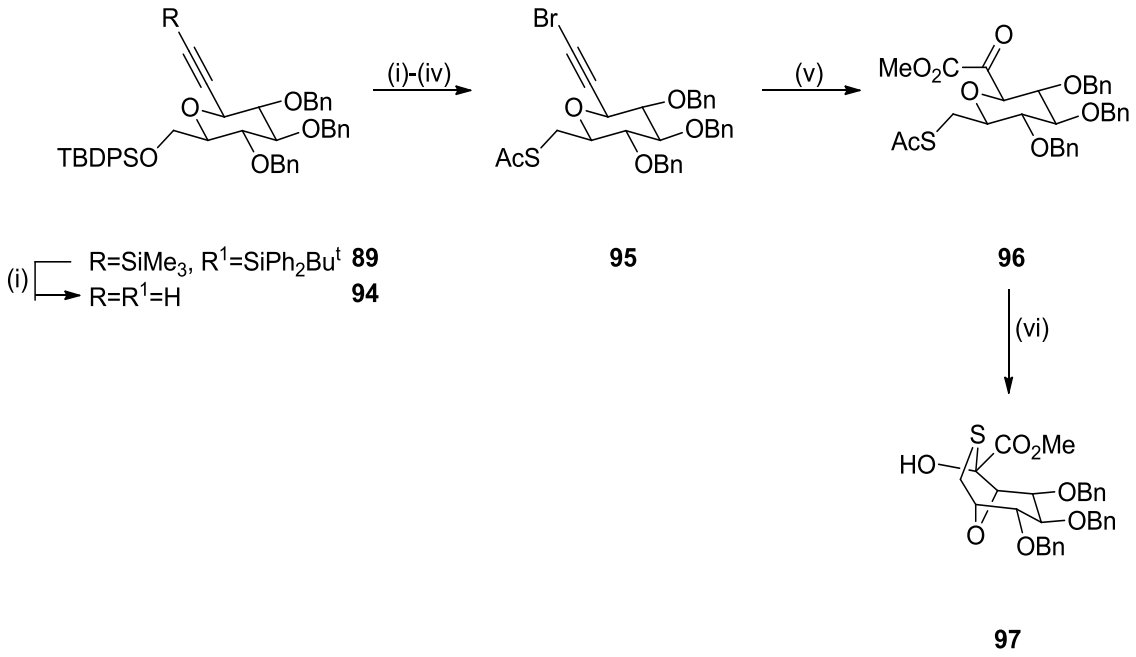
Porter began with phenyl 1-thio-β-D-glucopyranoside **86**; selective primary alcohol protection was achieved with TBDPS, followed by a benzyl protection of the other hydroxyl groups to give the fully protected analogue **87** in very good yield. NBS-promoted hydrolysis of the thioglycoside linkage was carried out, followed by oxidation of the lactol using Dess-Martin periodinane to give δ-lactone **88** in a reasonable yield. Treatment of **88** with cerium mediated addition of trimethylsilylacetylene, followed by deoxygenation and desilylation gave the terminal alkyne **90**. Bromination was then carried out with NBS to give **91**, followed by oxidation of **91** with $KMNO_4$ to give the desired α-ketoester **92** in high yield (**Scheme 12**).

Deprotection of the primary alcohol of **92** was needed in order to replace the primary alcohol with a good leaving group (eg MsCl), and carry out a displacement with potassium thioacetate, to introduce the sulfur atom at C-6 followed by a deprotection of the thioacetate and an in situ cyclization to give the bicyclic core. When Porter carried out this deprotection of the primary alcohol with TBAF, he ended up with an elimination of the 2-benzyloxy group, to form an enol ether, which is not what was expected. He then decided to carry out the deprotection using HF-pyridine on the silyl ether, and the sole product observed was the tricyclic acetal **93**, which he had not been expecting, and in which not only the silyl ether was cleaved but also the 3-benzyl and 4-benzyl ethers. Formation of an acetal between the ketone and the 3-OH and 6-OH groups had occurred (**Scheme 12**).



Scheme 12: i) TBDPSCl, imidazole, DMF, 99%; **ii)** BnBr, NaH, DMF, 87%; **iii)** NBS, acetone, 95%; **iv)** Dess-martin periodinane, pyridine, DCM, 69%; **v)**TMS=CH, n-BuLi, $CeCl_3.7H_2O$, THF, -78 °C to r.t., 96%; **vi)** Et_3SiH, TMSOTf, DCM, 74%; **vii)** NaOH, MeOH, DCM, 100%; **viii)** NBS, $AgNO_3$, acetone, 98%; **ix)** $KMNO_4$, $NaHCO_3$, $MgSO_4$, aq. MeOH, 84%; **x)** HF.pyridine, THF, -78 °C to r.t., 77%.

Porter decided since this method had failed to introduce the necessary sulfur atom on the C-6 position by carrying out an alternation of order of the the steps allowed completion of the synthesis of the tagetitoxin skeleton; thus double desilylation of substrate **89** was achieved with TBAF, to give the deprotected compound **94** (**Scheme 13**). Primary alcohol activation as the mesylate was achieved followed by subsequent displacement with potassium thioacetate to yield the desired sulfur atom at C-6 position. Bromination of the alkyne was achieved; similarly to the previous work with NBS to give compound **95**, followed by oxidation with $KMNO_4$ to give the desired α-ketoester **96**. Deprotection of the *S*-acetyl was then achieved using hydrazine hydrate in methanol, giving the free thiolate, which underwent an *in situ* cyclization onto the carbonyl group of the α-ketoester to yield the desired bicyclic hemithioacetal compound **97** of tagetitoxin (**Scheme 13**).



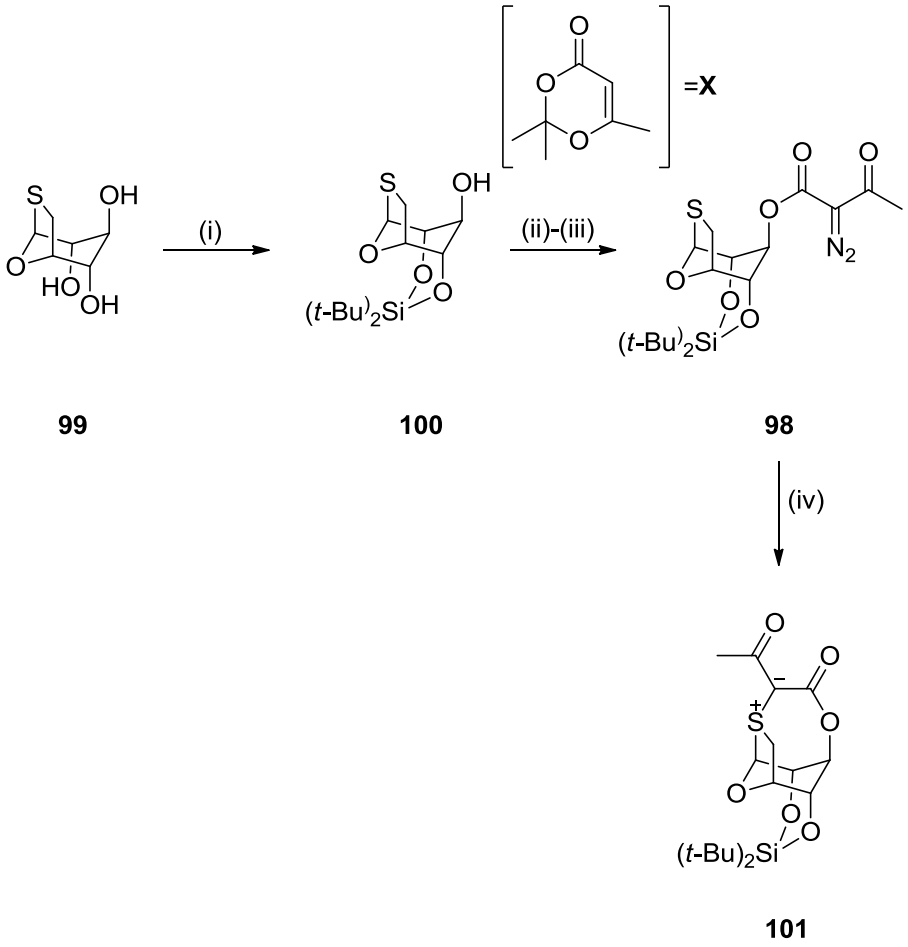
Scheme 13: i) TBAF, THF, 99%; **ii)** MsCl, Et_3N, DMAP, DCM, 95%; **iii)** KSAc, DMF, 99%; **iv)** NBS, $AgNO_3$, acetone, 99%; **v)** $KMnO_4$, $NaHCO_3$, $MgSO_4$, aq. MeOH, 71%; **vi)** $N_2H_4 \times H_2O$, MeOH, 88%.

In conclusion, Porter had achieved the synthesis of the bicyclic core of tagetitoxin through an *in situ* cyclization of the thiolate onto the carbonyl of the α-ketoester. This paper showed the importance of selecting the correct protecting groups as shown, and how to overcome obstacles in the synthesis of the core structure. Porter has been successful in achieving a skeleton closely related to tagetitoxin; however he is still missing a key carbonyl moiety on the anomeric carbon.

Porter *et al.* 2008

In 2008 Porter, published another paper on the synthesis of the bicyclic structure of tagetitoxin using a photo-Stevens rearrangement.[19] Porter had published a paper in 2006, on the metallocarbenoid-mediated ring expansion of 1,3-oxathiolanes, but had been unsuccessful in obtaining the desired compounds, and they postulated that this failure was likely due to the conformational flexibility of a monocyclic intermediate **80,** and that carrying out the ylide formation in an intramolecular fashion, this should give a more constrained intermediate, where the desired C-C bond formation would be favoured.[16,20]

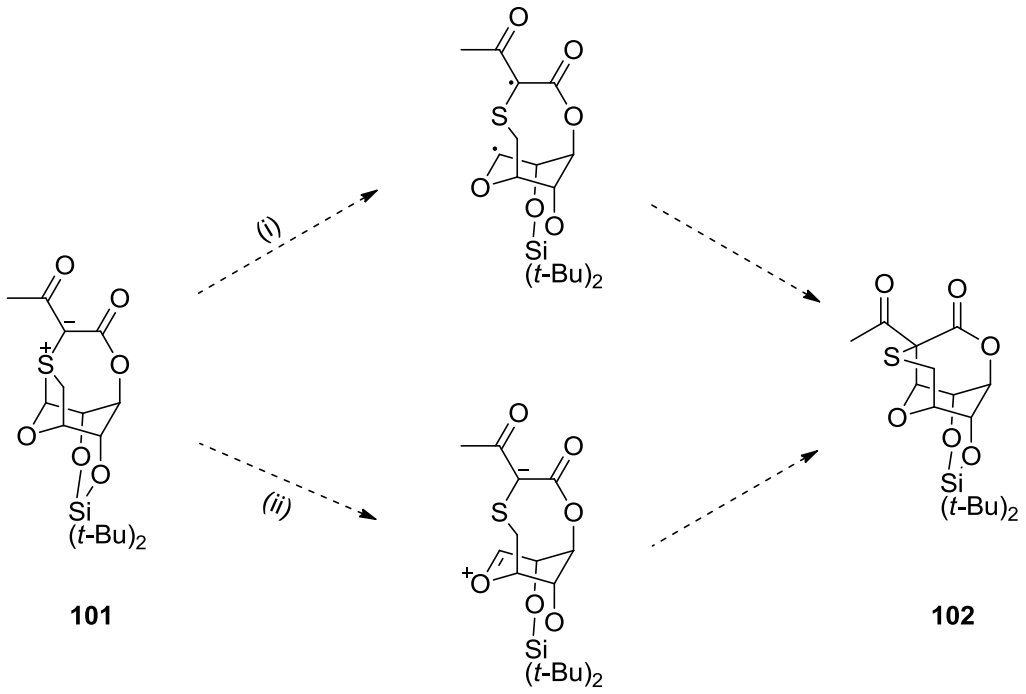
Glucose-derived C(3)-diazo esters such as **98** were chosen for test reactions. D-glucose was converted into the 1,6-thioanhydroglucose **99** in four steps from a literature preparation.[20] The C-2 and C-4 hydroxyl groups were selectively protected with a di-*tert*-butylsilylene bridge to give **100**. Acetoacetylation and diazo transfer using the Clemmens method proceeded smoothly to give the model substrate **98**. Treatment of **98** with 1 mol % of rhodium (II) acetate dimer gave the tetracyclic ylide **101**. Porter *was* fortunate to grow a crystal of **101**, and to obtain an X-ray crystal structure to prove the stereochemistry (**Scheme 14**).[21]



Scheme 14: i) *tert*-Bu_2SiCl_2, $AgNO_3$, Et_3N, DMF, 65%; **ii) X**, xylene, reflux, 88%; **iii)** *p*-$AcNHC_6H_4SO_2N_3$, Et_3N, MeCN, 100%; **iv)** $Rh_2(OAc)_4$ (1 mol %), benzene, reflux, 88%.

Porter suggested two mechanisms for the thermal rearrangement of ylide **101,** to give the desired tetracycle **102** (**Scheme 15**). The two mechanisms that Porter proposed were pathway A, the homolysis pathway, which is normally the more favoured mechanism for the Stevens

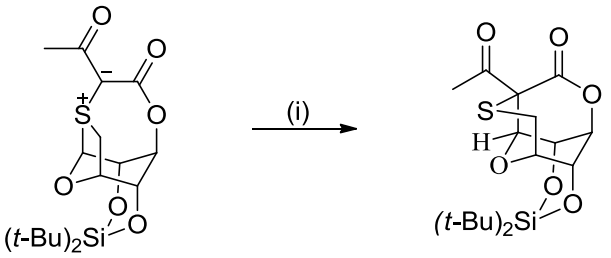
rearrangement as reported in previous examples, and pathway B, the heterolytic mechanism, which he believed was the most favoured in this case.[22–24] To understand the mechanism more closely, Porter tried to promote heterolysis by addition of protic (TFA, TfOH) or Lewis ($Cu(acac)_2$) acids to ylide **101**, with the expectation that this would increase the polarization of the C-S bond, but all these attempts were unsuccessful.



Scheme 15: Mechanistic Pathways for Thermal Rearrangement **i)** Pathway A Homolysis; **ii)** Pathway B Heterolytic

After the failure of the attempted induced rearrangement of the ylide thermally, Porter changed his strategy, to a photochemical Stevens rearrangement, which had been reported previously in the literature, but limited studies had been carried out on this topic (**Scheme 16**).[25–32]

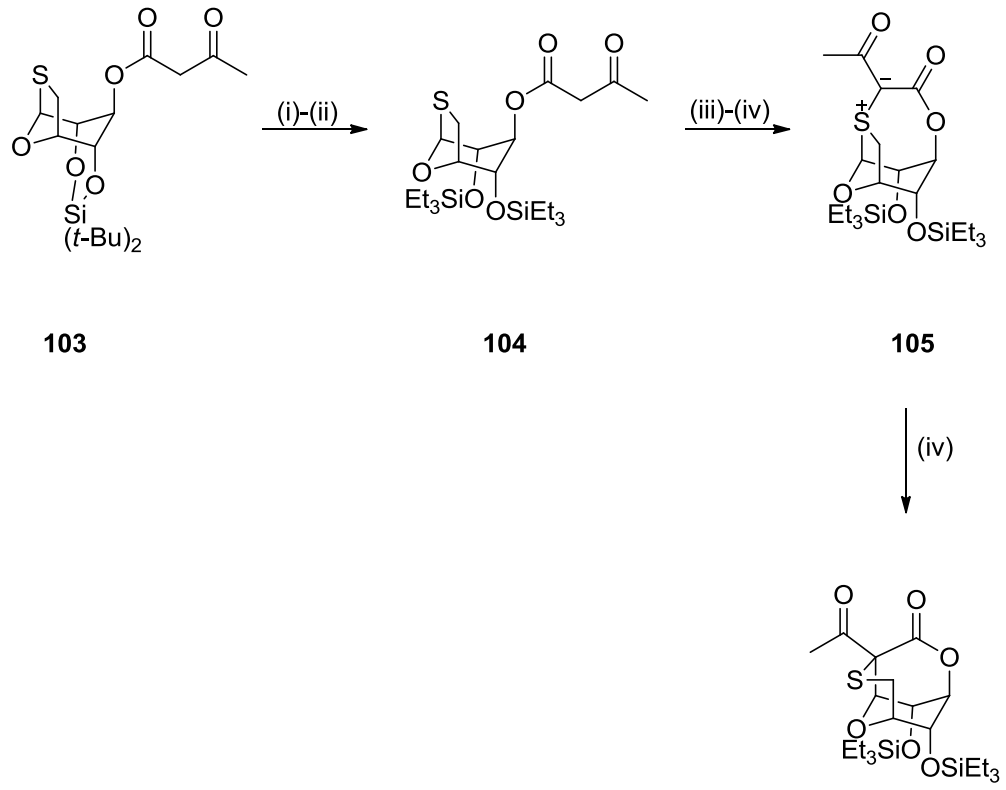
Ylide **101** was subjected to photolysis in acetonitrile, to give the desired rearranged tetracycle **102** within two hours.



101 **102**

Scheme 16: Photo-Stevens Reaction **i)** hv (> 290nm), MeCN, 69%.

Having achieved the target molecule by the Photo-Stevens rearrangement, Porter decided to investigate, which structural elements were essential for ylide formation and for this reaction. Acetoacetate **103** was deportected with TBAF, and the resulting diol was reprotected with TESCl to give **104** in good yield (**Scheme 17**). Diazo-transfer with *p*-carboxy-benzenesulfonyl chloride, and rhodium catalysed diazodecomposition gave **105**. Photolysis furnished the tricyclic ring-expanded product **106** with exactly the same results as **102**.

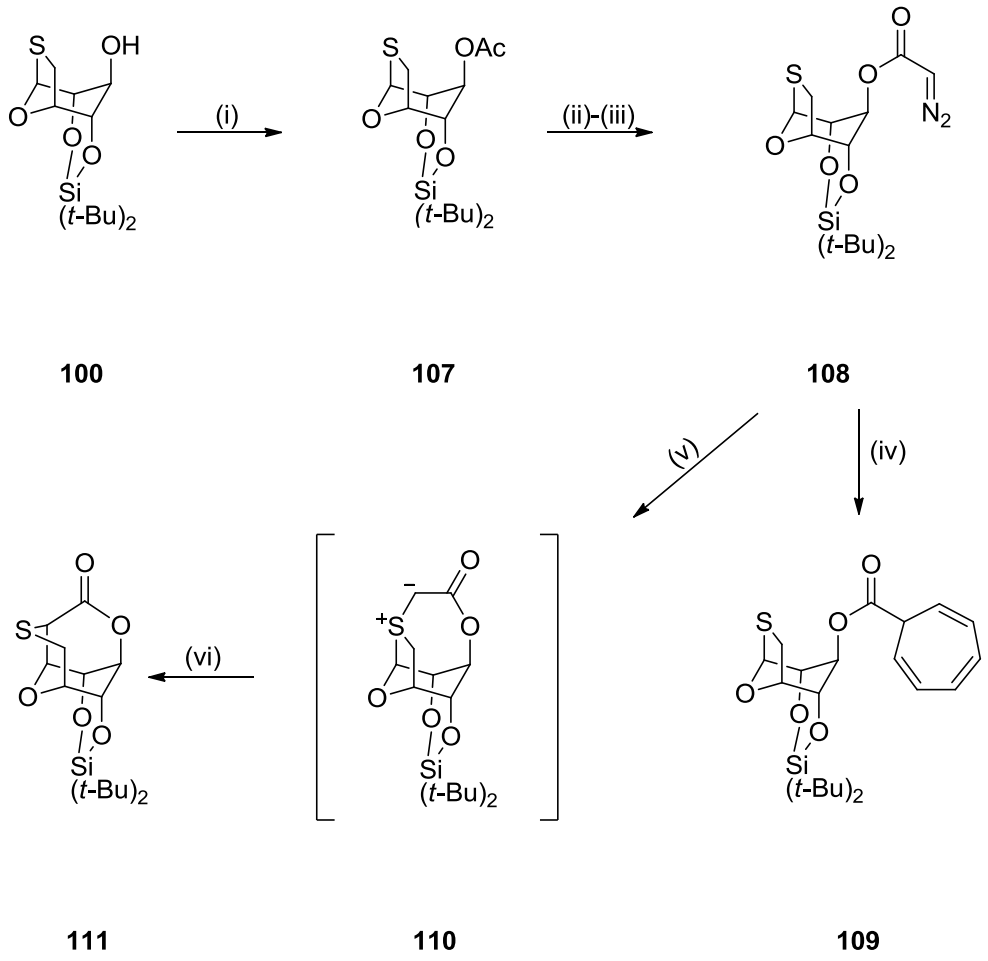


103 **104** **105**

106

Scheme 17: i) TBAF, THF, 76%; **ii)** Et_3SiCl, DMAP, Et_3N, DCM, 72%; **iii)** *p*-$HOOCC_6H_4SO_2N_3$, Et_3N, MeCN, 75%; **iv)** $Rh_2(OAc)_4$ 1 mol %, benzene, reflux, 53%; **v)** hv, MeCN, 65%

The authors also synthesized diazoacetate **108** from alcohol **100** from a previous reported synthesis (**Scheme 18**); acetylation of alcohol **100** gave ester **107**, which was converted into the diazoacete by Danheiser's detrifluoroacetylating diazo transfer method.[33] Rhodium (II) acetate treatment of **108** in benzene led to the unexpected cycloheptatriene **109** as the major product.[34] A simple change of the solvent to DCM, with the same reaction conditions, yielded the anticipated ylide intermediate **110**. Purification of this ylide intermediate was not possible due to the instability of the product, and therefore the crude reaction mixture was subjected to photolysis to afford the desired tetracycle **111** in a good yield over the two steps.



Scheme 18: i) Ac_2O, DMAP, Et_3N, DCM, 84%; **ii)** LiHMDS, THF. -78 °C, $CF_3CO_2CH_2CF_3$; **iii)** *p*-$HOOCC_6H_4SO_2N_3$, Et_3N,MeCN, 65%; **iv)** $Rh_2(OAc)_4$ 1 mol %, benzene, reflux, 39%; **v)** $Rh_2(OAc)_4$ 1 mol %, DCM, reflux; **vi)** hv, MeCN, 65%.

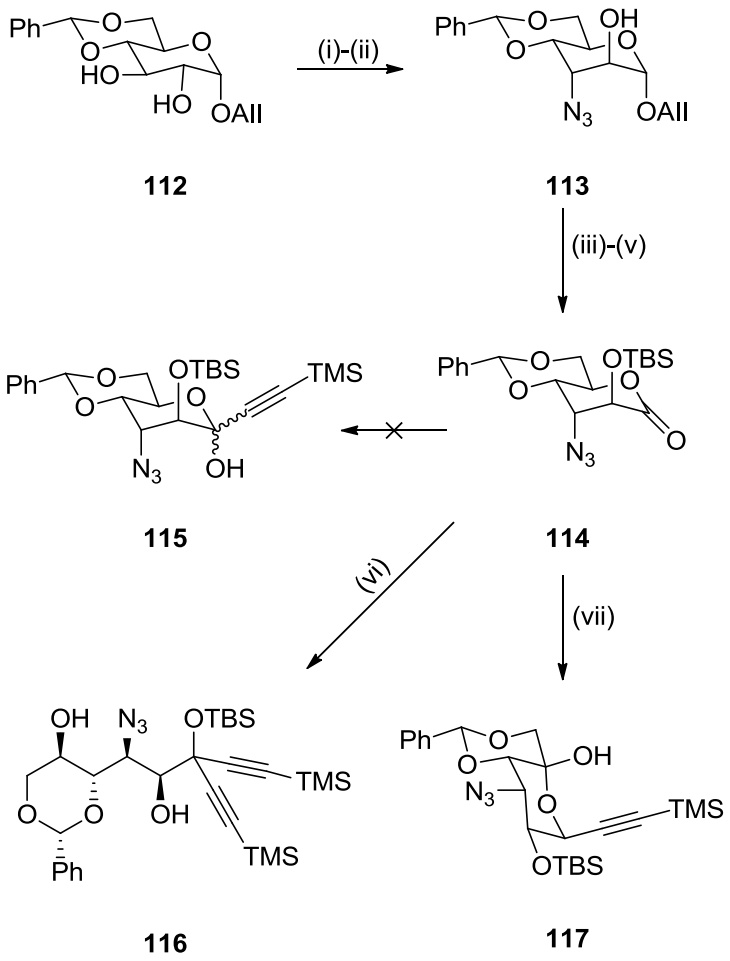
In conclusion Porter has synthesized the bicyclic core of tagetitoxin in two different ways: the first from an *in situ* cyclization of the free thiolate onto the carbonyl group of the electrophilic α-ketone to give the hemithioacetal, and the second from the photo-Stevens rearrangement. Similarly to the first publication on the previous studies, the bicyclic core synthesized by Porter in this case lack the key carbonyl moiety on the anomeric carbon, and the quest for the total synthesis of tagetitoxin continues.[16]

Porter *et al.* 2009

In 2009 Porter published a paper in Synlett on his continued studies towards the synthesis of tagetitoxin.[35] In this paper he describes efforts in applying the thiol cyclization route towards

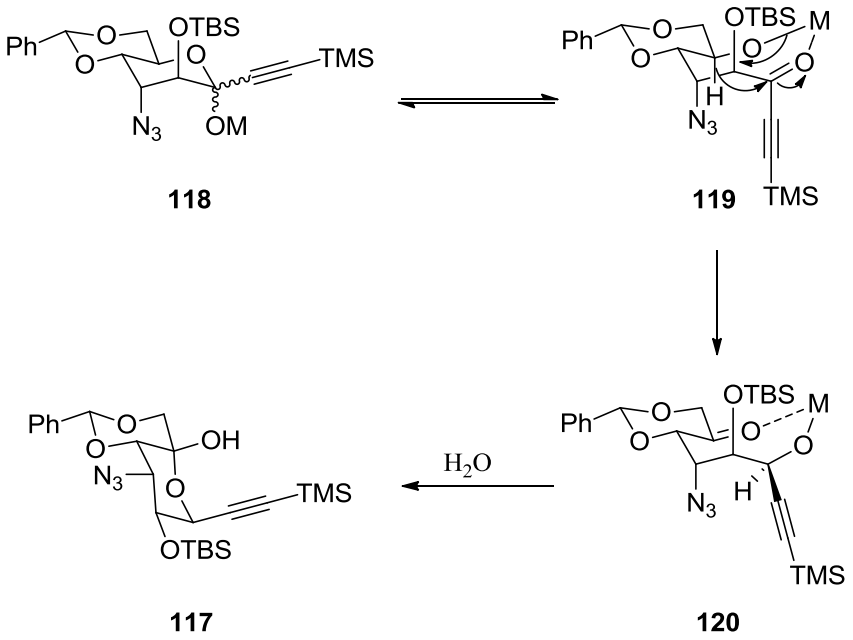
the synthesis of tagetitoxin.[16,19] The α-allyl glucsoide **112** was prepared using a literature procedure.[36] This was followed by a tosylation of the 2-hydroxyl group which was achieved with good selectivity and this was then accompanied by a ring closure to yield the desired β-epoxide, followed directly by treatment with sodium azide under acidic conditions to afford the *altro*-configured azidosugar **113** (**Scheme 19**).[37–40] Protection of the free hydroxyl was then achieved with TBSCl, followed by the deprotection of the allyl protecting group. The resulting lactol was then oxidized to give the desired lactone **114**. All attempts by the group to synthesize **115** through the addition of TMS-acetylide to the carbonyl group of **114** were unsuccessful. When the group used known conditions for a similar substrate reported in the literature, they were only able to isolate the undesirable compound **116**.[16] Porter postulated that the initial addition to give the lithium salt of **115** was followed by the ring opening to form a ketone, and a second organometallic addition must have taken place in order to produce **116**. Migration of the silyl group of the tertiary alkoxide, which was relatively unhindered due to the presence of two sp-hybridized substituent's, must have then occurred to give the undesired product **116.**

Porter then investigated the use of ytterbium triflate in place of cerium chloride as the additive in the lithium acetylide addition, as this had been used on other substrates.[41] To his surprise, by changing reagent he was able to achieve the desired bicycle **117,** which was obtained in around 48% yield.



Scheme 19: i) NaH, DMF, r.t. then Ts-imidazole, 50 °C **ii)** NaN_3, NH_4Cl, H_2O, 2-methoxyethanol, reflux, 92%; **iii)** TBSCl, imidazole, DMF, 80 °C, 98%; iv) Bu_3SnH, $ZnCl_2$, $Pd(PPh_3)_4$, THF, r.t., 90%; **v)** DMP, pyridine, DCM, r.t., 88%; **vi)** TMS-Acetylene, BuLi, $CeCl_3$, $Yb(OTf)_3$, THF, -78 °C to r.t., 32%; **vii)** TMS-Acetylene, BuLi, $Yb(OTf)_3$,THF, -78 °C to r.t., 48%.

Porter proposed a mechanism for the formation of **117** (**Scheme 20**), suggesting that it was obtained following the addition of the acetylide to give **118** and ring opening to afford a ketone **119**, by a transannular hydride shift to yield a secondary alkoxide **120**. Upon work-up, cyclization to the *cis*-fused bicyclic lactol **117** took place. Despite the earlier successes, attempts to furnish the key intermediate**117** were unsuccessful and only the starting lactone was recovered.



Scheme 20: Proposed mechanism for the formation of **117**

Having faced such difficulties in the synthesis of the key intermediate the group decided to change methodology, and instead introduce the alkyne moiety to a glucose derivative prior to inversion of the C-2 and C-3 sterogenic centres. 1,6-Anhydroglucose **121** was prepared according to previously literature procedures and this was converted to its 2,4-di-*O*-trietylsilyl derivative (**Scheme 21**).[42] Porter then used Vasella's method to steroselectively introduce a β-configured alkynyl substituent at C-1.[43] Using this procedure, lithium trimethylsilyl acetylide was reacted with aluminium trichloride prior to addition to the substrate. Having used these optimized conditions, an 81% yield of the diol **122** was obtained.

Removal of the triethylsilyl ethers of **122** was achieved, and the introduction of a *p*-methoxybenzylidene acetal gave the bicycle **123,** followed by treatment with numerous sulfonylation conditions (TsCl, Ts-imidazole, Ts_2O, or MsCl as reagent; NaH or pyridine as base). Porter found that no selectivity was obtained for one alcohol over another, and an inseparable mixture of 1:1 sulfonates was obtained.

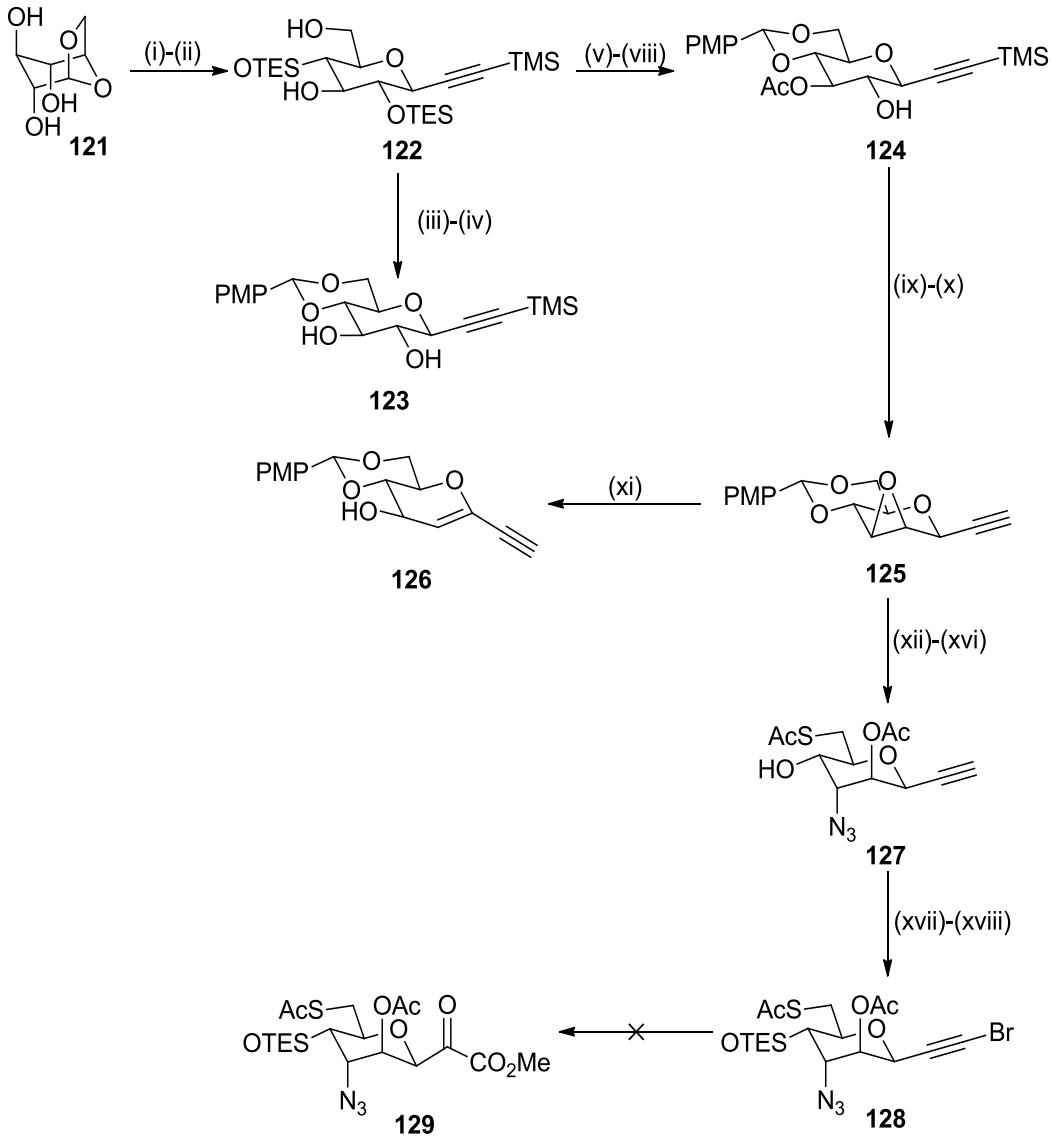
Porter, having been faced with this problem, came up with a solution by using different protecting groups on the O-2 and O-3 hydroxyl groups of **122**. Selective primary alcohol protection was carried out with a silyl ether, followed by acetylation of the secondary alcohol. This reaction required numerous attempts as it proved to be much more difficult than

anticipated. The acetylation only occurred in the presence of acetic anhydride, triethylamine and in the presence of 4-(1-pyrrolidino)pyridine.[44–46] Acid hydrolysis then cleaved the three silyl ethers and induced acetal formation to give the desired monoacetate **124**. This was followed by sulfonylation of the free hydroxyl group of **124,** using tosyl chloride in pyridine under reflux to give the desired unstable sulfonate intermediate, which was immediately subjected to treatment with sodium methoxide in methanol-dichloromethane to remove the acetate and TMS protecting group and initiate the cyclization to give the epoxide **125**.

The next problem Porter encountered was in the diaxial ring opening of the epoxide using acidic conditions, as this was unsuccessful due to the hydrolysis of the acetal protecting group in such conditions, whilst heating with sodium azide in DMF gave an elimination product **126**. However with the use of Yamamoto's conditions, Porter was able to achieve the ring opening of the epoxide, in which the epoxide was treated with a combination of ytterbium triisopropoxide and TMS-azide.[47] Acetylation of the 2-O hydroxyl group, followed by hydrolysis of the *p*-methoxybenzylidene acetal, was achieved. This was followed by a selective tosylation of the primary alcohol; followed by a tosylate displacement with potassium thioacetate, which gave the desired thioester **127**.

The secondary alcohol of **127** was then protected with TESCl, and the alkyne was brominated with a mixture of NBS / silver nitrate affording **128**.[48,49] However, all attempts to achieve the oxidation to the α-ketoester **129** were unsuccessful, only complete decomposition being observed.[50]

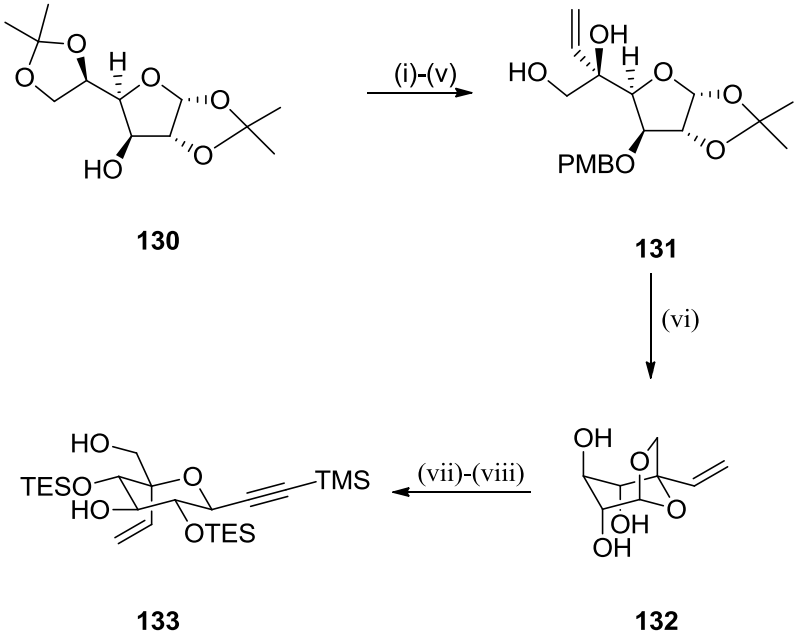
In order for Porter to complete the synthesis, he needed to form the α-ketoester followed by the introduction of a phosphate group, and the reduction of the azide, followed by the cleavage of the thioester to the thiol, which would undergo an *in situ* cyclization to give the desired natural product. However despite numerous attempts the group were unable to obtain the desired α-ketoester **129** and the completion of the synthesis of tagetitoxin could not be achieved (**Scheme 21**).



Scheme 21: i) TESCl, pyridine, 0 °C, 79%; **ii)** TMS-Acetylene, BuLi, $AlCl_3$, 2,4,6-collidine, toluene-THF, Sonnication, -15 °C to 50 °C, then add substrate, 130 °C, 81%; **iii)** AcOH, MeOH, H_2O, r.t., 92%. **iv)** 4-$MeOC_6H_4CH(OMe)_2$, TsOH, 4Å MS, MeCN, reflux, 95%; **v)** TESCl, pyridine, 0 °C, 92%; **vi)** Ac_2O, 4-(1-pyrrolidino)pyridine, Et_3N, r.t., 70%; **vii)** AcOH, THF, H_2O, 45 °C, 86%; **viii)** 4-$MeOC_6H_4CH(OMe)_2$, TsOH, 4Å MS, MeCN, 80 °C, 80%; **ix)** TsCl, pyridine, 120 °C, 72%. **x)** NaOMe, MeOH, DCM, r.t., 64%; **xi)** NaN_3, DMF, 90 °C, 49%; **xii)** $TMSN_3$, $Yb(OTf)_3$, LiO*i*-Pr, THF, 60 °C, 79%; **xiii)** Ac_2O, DMAP, pyridine , r.t., 99%; **xiv)** AcOH, THF, H_2O, 45 °C, 81%; **xv)** TsCl, pyridine, r.t., 67%; **xvi)** KSAc, DMF, r.t., 76%; **xvii)** TESCL, pyridine, 0-40 °C, 82%; **xviii)** NBS, $AgNO_3$, acetone, r.t., 73%.

Since his initial synthetic approach to tagetitoxin had not included the important carboxylic acid moiety on C-5, Porter decided to try to install this extra carbon substituent. Porter used a previously reported method in the literature from Rao in which a Grignard reagent was added steroselectively to a 5-keto glucose derivative (**Scheme 22**).[51]

The free hydroxyl group of diacetone-D-glucose **130** was protected with a PMB group, followed by a selective hydrolysis of the 5,6-acetonide, which was achieved using aqueous acetic acid. Silylation of the 6-hydroxyl group was achieved with TBSCl. Oxidation of the remaining secondary alcohol with a Swern oxidation proceeded smoothly to give the keto product. Steroselective Grignard addition was achieved using the Rao method, which gave the tertiary alcohol **131** (**Scheme 22**).[51] Porter then heated **131** for an extended period of 72 hours in a mixture of aqueous acetic acid and TFA, in the presence of thioanisole, and was able to achieve a global deprotection and a cyclization to give the desired 1,6-anhydro-5-*C*-vinylglucose **132**. Extensive Fischer esterification also took place under these conditions, and the crude material was treated subsequently with sodium methoxide to methanolyse the mixture of acetates to give the desired triol **132** in 70% yield. (Scheme 22)



Scheme 22: i) NaH, PMBCl, THF, r.t., 86%; ii) 60% aq ACOH, r.t., 80%; iii) TBSCl, imidazole, DMF, r.t., 81%; iv) $(COCl)_2$, DMSO, Et_3N, DCM, -78 °C, 79%; v) vinylmagnesium bromide, THF, r.t., 76%; vi) 80% aq AcOH, TFA, PhSMe, reflux then NaOMe, MeOH, r.t., 73% vii) TESCl, pyridine, r.t., 68%; viii) TMS-Acetylene, BuLi, $AlCl_3$, 2,4,6-collidine, toluene-THF, sonicate, -15 °C to 50 °C, then added substrate, 130 °C, 70%.

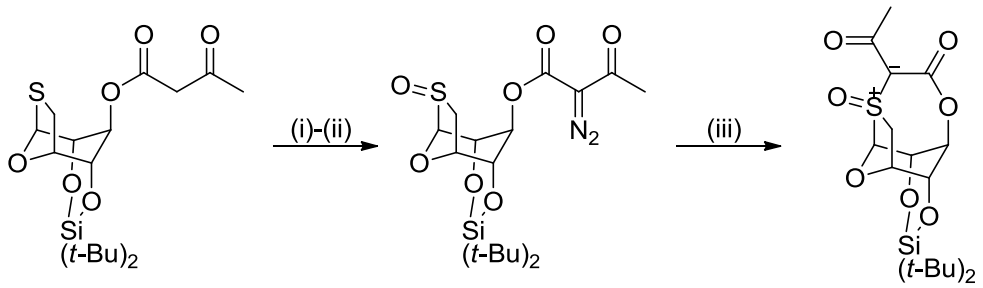
Selective desilylation was then carried out on triol **132**, which underwent aluminum-mediated alkynylation to give the *β*-*C*-glycoside **133** (**Scheme 22**).[43] However, carrying on from this work,

Porter was never able to achieve the desired natural product tagetitoxin using this methodology.

In conclusion Porter has developed a new methodology, but like previous reported papers he has been so far unsuccessful in achieving the total synthesis of tagetitoxin.

Porter *et al.* 2012

In 2012 Porter published another paper on the inter and intramolecular reactions of 1-deoxy-1-thio-1,6-anhydrosugars with α-diazoesters, as a continuation of his previous papers.[16,19,35,52] Following on from the previous work on synthesizing the tricylic ring-expanded product **106**, Porter carried out an oxidation of tricycle **103** with *m*-CPBA, which was followed by diazotransfer, and the sulfoxide product **134** was isolated as a single diastereoisomer (**Scheme 23**). Porter assumed that the sulfoxide stereochemistry was to be as depicted, since diazodecomposition with rhodium acetate led to the tetracyclic sulfoxonium ylide **135**. Compound **135** was subjected to Irradiation with *UV* light, and only decomposition was observed rather than the rearrangement that he had been hoping to observe based on previous studies.

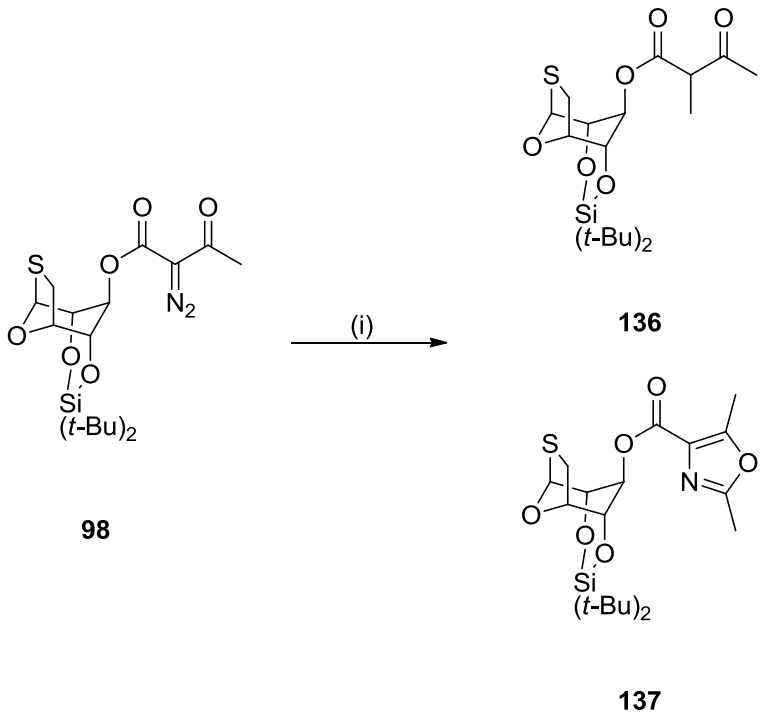


103 **134** **135**

Scheme 23: i) *m*-CPBA, $NaHCO_3$, DCM, 80%; **ii)** *p*-$HOOCC_6H_4SO_2N_3$, Et_3N, MeCN, 88%;**iii)** $Rh_2(OAc)_4$, benzene, reflux, 63%.

Porter then decided to see if he would be able to form the sulfur ylide **101,** by a photochemical generation of a free carbene from the diazo compound **98** (**Scheme 24**).[53] He had hoped that if this methodology worked, he could carry out the ylide formation, followed by the 1,3-rearrangement in a single photochemical operation and this would improve the synthesis of the bicyclic core. However, this methodology did not work as had been anticipated, due to the photolysis of the diazo compound **98** forming an inseparable mixture of compounds which

could not be characterized, but was tentatively assigned as a methylmalonate monoester **136** and an oxazole **137**. Porter had postulated that **136** had been formed through a Wolff rearrangement of the carbene intermediate, followed by hydrolysis of the resulting acylketene, and that **137** had been formed through the reaction of the carbene intermediate with solvent, acetonitrile in this case (**Scheme 24**). To prove this, Porter carried out the photolysis again in different solvent, using both dichloromethane and chloroform, and the only major product observed from the reaction was the malonate **136** with none of the desired compound tetracycle **102**.



98 **136** **137**

Scheme 24: Photolysis of diazocompound **98 i)** *hv* (>290nm), MeCN.

Porter had one further synthetic goal to achieve, involving the reactivity of substrates in which the diazo compound was tethered through equatorial oxygen functionality on the C-2, rather than axial on the C-3. Porter decided to introduce nitrogen functionality at the C-3 position, since this was present within the natural product, and to synthesize the decarboxy analogue of tagetitoxin. He started the synthesis from the readily available 4,6-O-benzylidene-α-D-glucopyranoside **138** (**Scheme 25**), which was converted into the 2,3-β-epoxy derivative, which was then ring opened with sodium azide under acidic conditions to give **139** following

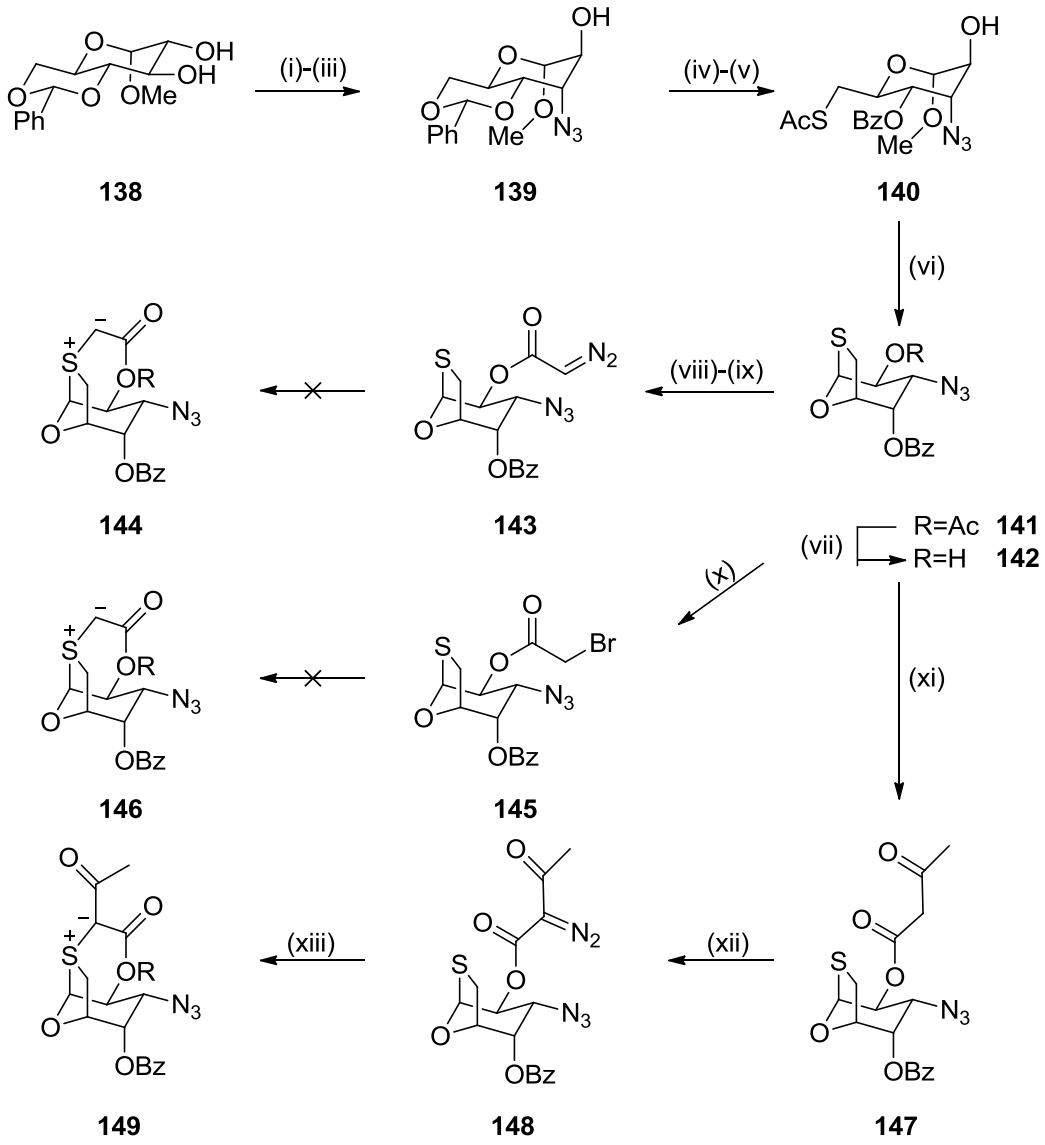
literature procedures.[54,55] A Hanessian-Hullar oxidative ring opening of **139** using NBS and Barium carbonate, led to the 6-bromo-4-benzoyl derivative from which the bromide was displaced with potassium thioacetate to give the thioester **140**.[56–5843] Treatment of the thioester **140** with acidic acetylating conditions for seven days not only acetylated the free hydroxyl group but caused cleavage of the thioacetate and an *in situ* cyclization to the desired bicyclic structure **141**. Conversion of the acetate **141** to the diazoacetate was the next step; however deprotonation of the acetate with LDA followed by triflouroacetylation gave none of the desired trifloroacetate, but instead gave a mixture of the acetoacetate **147** and alcohol **142**. Unfortunately, after many different attempts this pathway could not be suppressed by variations of base or temperature. Porter postulated that following initial deprotonation of **141**, either an intermolecular Clasien condensation takes place, or elimination occurs to give **142** and ketene, which further reacted with an ester enolate species to give **147**.

Having obtained this undesired reaction, the authors decided to selectively cleave the acetate of **141** with guanidine to give the alcohol **142**, which was then treated with a 2-triflouroacetyl derivative of Meldrum's acid, to give the trifluoroacetoacetate as previously reported.[59,60] Detrifluroacetylating diazo transfer then gave the desired diazoacetate **143** using known conditions.[33]

Treatment of **143** to give the desired ylide **144,** with the optimized conditions from the group, proved more difficult than with the C-3 diazo compound previously obtained by the group. Variation of catalysts for the diazo decomposition failed to yield the desired ylide **144**.

Porter then attempted to generate the desired ylide **144** using a sequence of intramolecular alkylation followed by deprotonation. Bromroacetate **145** was prepared by the reaction of the alcohol **142** with bromoacetylbromide in the presence of DMAP, but was unsuccessful in their attempts to cyclize **145** with or without silver salts to give the desired cyclic compound **146**.

Since Porter had earlier in previous reported synthesis observed the stability of acetyl substituted ylides **101**, **105** and **135,** he decided to prepare diazoacetoacetate **148** from the alcohol **142,** which was acetoacetylated to give **147**. Diazotransfer of **147** gave the desired diazoactoacetate **148**.[16,19,35] The final step was diazodecomposition of **148**; however the reaction worked in a very low yield, to give an impure mixture containing the desired ylide **149,** but did not produce the yields that Porter was expecting (**Scheme 25**).



Scheme 25: i) NaOMe, Ts-imidazole, DCM, reflux **ii)** NaOMe, MeOH, Reflux, 81% (2 steps); **iii)** NaN_3, NH_4Cl, $MeOCH_2CH_2OH$, H_2O, reflux, 83%; **iv)** NBS, $BaCO_3$, $CHCl_3$, reflux, 79%; **v)** KSAc, DMF, 95%; **vi)** Ac_2O, AcOH, H_2SO_4, 60%; **vii)** guanidine, EtOH/DCM, 83%; **viii)** 2,2-dimethyl-5-trifluoroacetyl-1,3-dioxan-4,6-dione, toluene, reflux **ix)** 4-$HO_2CC_6H_4SO_2N_3$, K_2CO_3, MeCN, 73% (2 steps); **x)** $BrCH_2COBr$, DMAP, DCM, 57%; **xi)** 2,2,6-trimethyl-4H-1,3-dioxin-4-one, xylene, reflux, 97%; **xii)** 4-$HO_2CC_6H_4SO_2N_3$, K_2CO_3, MeCN, 85%; **xiii)** $Rh_2(OAc)_4$, $CHCl_3$, reflux, 18%.

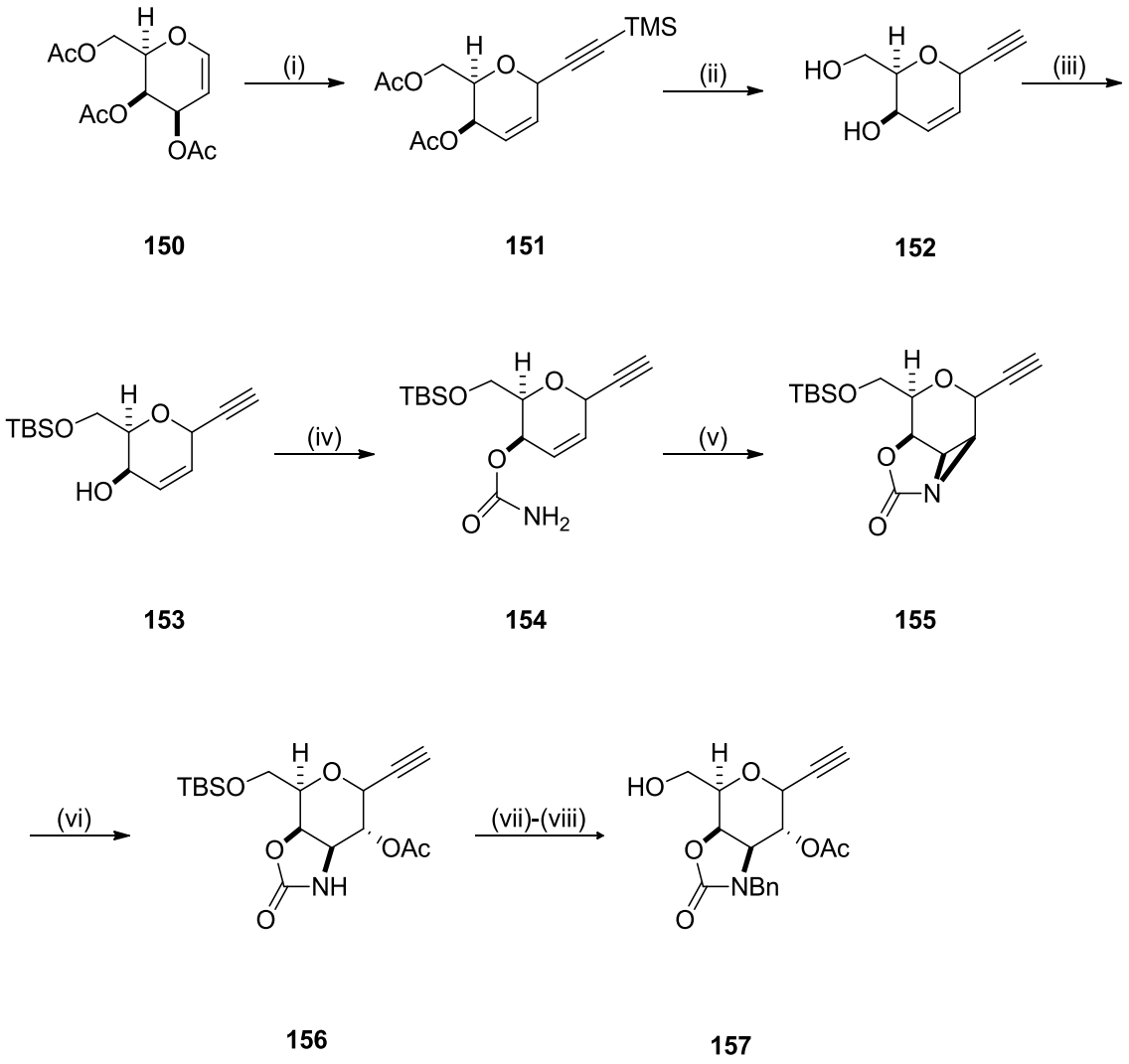
In conclusion Porter had hoped to be able to apply his methodology of a one carbon ring expansion of monocyclic 1,3-oxathiolanes using diazoesters to thioanydrosugars, however the reactions with these thioanydrosugars did not give the desired ylides but other ylide-derived products.[17,18] He also noted that tethering the diazo compound through an equatorial linkage at C-2 of the sugar was much less successful than the previous studies carried out, and he

postulated that these undesired reactions may be caused with an increase in strain in the ylide product. Porter also carried out DFT calculations on the photo-Stevens rearrangement, which strongly suggested that the process involved an initial homolysis of the carbon-sulfur bond followed by radical recombination to form a new carbon-carbon bond.[52]

Nishikawa *et al.*

Nishikawa *et al*. have just published a paper on the synthesis of the core structure of tagetitoxin in 2013.[61] Nishikawa began his sterocontrolled synthesis of the core structure of tagetitoxin with the readily available tri-O-acetyl-D-galactal **150** by a Ferrier type α-selective glycosylation, with an acetylene derivative using previously reported work from the group.[62–64] Compound **150** was treated with bis(trimethylsilyl)acetylene in the presence of the Lewis acid $SnCl_4$ to give the desired compound **151** in a poor yield of 35%. The use of different Lewis acids and solvent systems, did not improve on the previous yield. However, when Nishikawa changed from the silyl acetylene to tin acetylene, in the presence of TMSOTf he achieved the desired product **151** in a high yield with high stereoselectivity (α/β > 20:1).[65,66] Cleavage of the two acetates and the TMS group was achieved with sodium methoxide in methanol, to give the diol **152** in 95% in two steps from **150**. Selective protection of the primary alcohol was achieved with TBSCl, to give **153,** and the remaining alcohols of **153** were carbamolyated to give **154** in two steps, firstly trichloroacetyl carbamoylation with and then a hydrolysis to give the desired compound **154** in good yield. Compound **154** was subjected to standard aziridination conditions of $Rh(OAc)_2$ and $PhI(OAc)_2$ in the presence of MgO, and the desired aziridine **157** was obtained, but in a very low yield.[67] Simple adjustment of the conditions reported in the literature, by adding MS-4Å in DCM to $Rh_2(OAc)_4$ and $PhIO$, improved the yield of the desired aziridine **155** to 70%.[68]

Regioselective opening of aziridine **155** at the C-6 position was achieved using acetic acid, and heating gave the desired acetate **156**. Benzylation of the carbamate **156** using standard conditions proceeded smoothly, followed by deprotection of the primary alcohol TBS group with TBAF to give alcohol **157** (**Scheme 26**).



150 **151** **152**

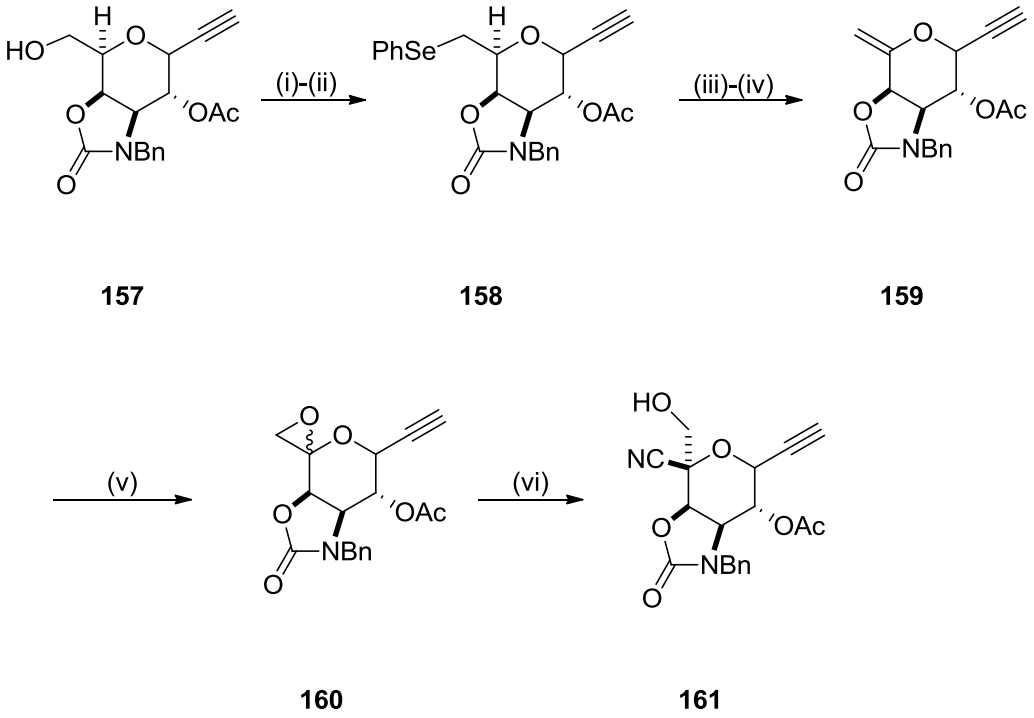
153 **154** **155**

156 **157**

Scheme 26: i) tributylstannyl(trimethylsilyl)ethyne, TMSOTf, DCM, 0 °C; **ii)** NaOMe, MeOH, 0 °C ; then Dowex®, 95% in 2 steps; **iii)** TBSCl, Et_3N, DMAP, DCM, r.t., 85%; **iv)** trichloroacetyl isocyanate, DCM, 0 °C ; then K_2CO_3, MeOH, H_2O, 0 °C , 73%; **v)** $Rh_2(OAc)_4$, PhIO, MS-4Å, DCM, r.t., 70%; **vi)** AcOH, 80 °C, 73%; **vii)** NaH, BnBr, TBAI, DMF, 0 °C, 90%; **viii)** TBAF, AcOH, THF, r.t., 78%.

The next part of the synthesis was to carry out a cyanation at the C-1 position from the preparation of a *spiro*-epoxyacetal. Attempts to form the *spiro*-epoxyacetal from the epoxidation of an *exo*-glycal, which Nishikawa had hoped to obtain by the elimination of a halide or sulfonate on the primary alcohol in **157**, had failed. Nishikawa postulated that the failure of the reaction was due to the steric hindrance around the C-1 position. He then decided to carry out an intramolecular elimination of a selenoxide. The selenide **158** was prepared from **157** in two steps by the triflation of the primary alcohol in **157** followed by a

nucleophilic substitution with lithium phenylselenide.[69] Oxidation of **158** with *m*-CPBA followed by heating of the selenoxide in the presence of a base gave the desired exo-glycal **159** in good yield. The next step was the formation of the desired *spiro*-epoxyacetal **160**, which was achieved by treatment of **159** with *m*-CPBA in the presence of a base to in a very good yield. Compound **160** proved to be surprisingly stable and purification on neutral silica gel gave a 3:2 diastereisomeric mixture. Treatment of the epoxide **160** with TMSCN in the presence of many different Lewis acids did not yield the desired cyano group on the C-1 position. After many trial runs a solution was found that had been reported previously, with the use of TMSCN in the presence of I_2 in a mixture of toluene and hexane at 0 °C.[70,71] These optimised conditions gave the desired nitrile **161** as a single diastereoisomer in 44% yield, after acid hydrolysis of the TMS-ether. The group obtained a crystal structure of **161** and so proved the configuration of the asymmetric centre at the C-1 position (**Scheme 27**).

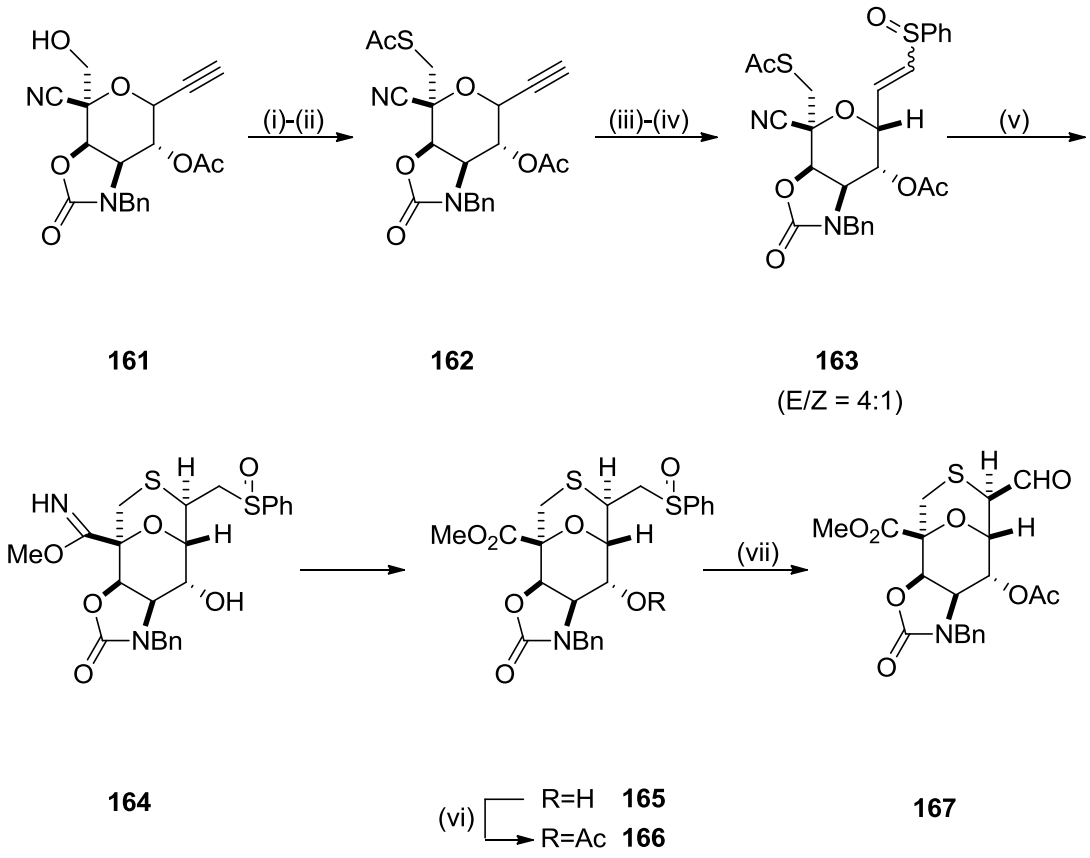


157 **158** **159**

160 **161**

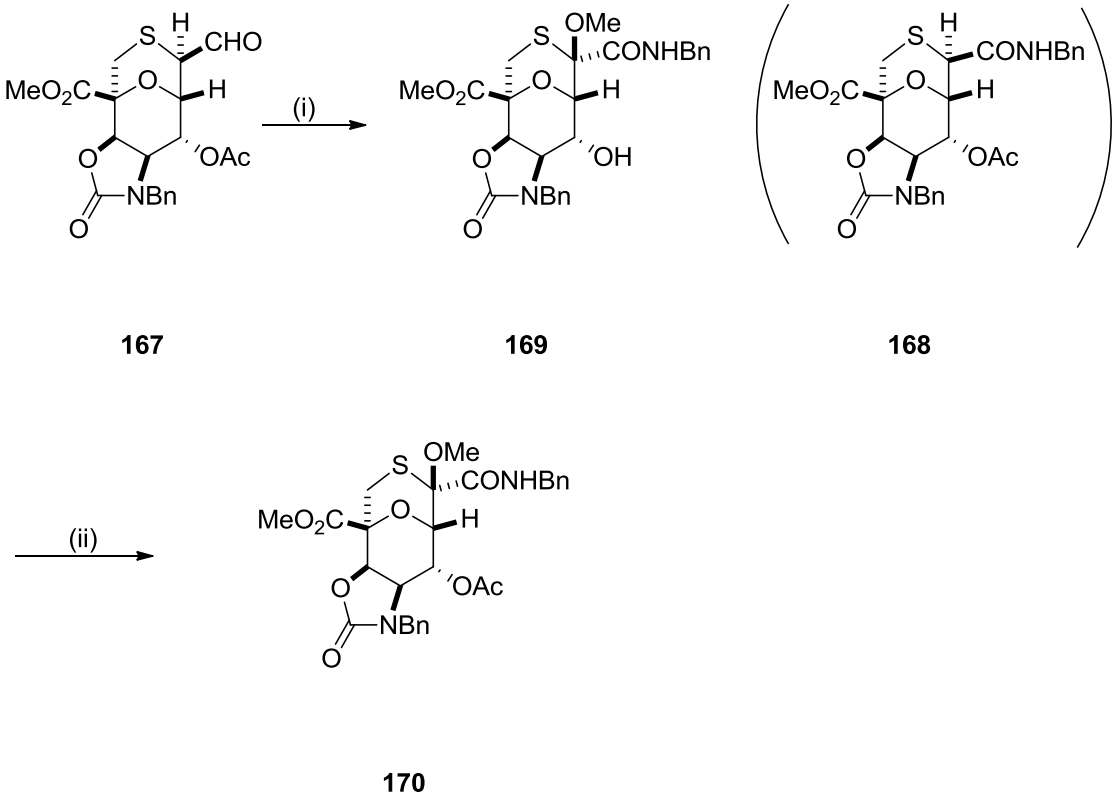
Scheme 27: i) Tf_2O, 2-6-lutidine, CH_3CN, -20 °C, 93%; **ii)** $(PhSe)_2$, *n*-BuLi, THF, -20 °C, 86%; **iii)** *m*-CPBA, DCM, -40 °C; **iv)** $NaHCO_3$, THF, 65 °C, 88% in 2 steps; **v)** *m*-CPBA, $NaHCO_3$, DCM, r.t., 98%; **vi)** TMSCN, I_2, toluene, hexane, 0 °C, then 1 M HCl aq. r.t., 44%.

Nishikawa having successfully synthesized the pyranose core structure of tagetitoxin, started to carry out the synthesis of the cyclic sulfide (**Scheme 28**). Triflation of the hydroxyl group of **161** followed by a displacement reaction with potassium thioacetate introduced the thioacetate group to give compound **162**. Treatment of **162** with LiSMe, to remove the acetyl group, gave an undesired product which was unexpected forming a 7-memebered sulfide product.[72] It was postulated that the generated thiolate underwent the undesired *7-endo-dig* cyclization. The group decided to prevent this undesired cyclization from happening by utilizing an intramolecular conjugate addition of a thiolate to a vinyl sulfoxide from **162**. Vinyl sulfoxide **163** was synthesized by a radical addition of thiophenol to the acetylene, followed directly by an oxidation with *m*-CPBA to give a 4:1 *E:Z* mixture of **163**. Treatment of the sulfoxide **163** with lithium hydride allowed the desired intramolecular conjugate addition of the thiolate. The acetate had been removed and the nirtile group was transformed into the imidate, giving the desired product **164**, which was then hydrolysed with HCl to give the desired methyl ester **165** in good yield. Acetylation of **165** gave **166** in 71% yield for the two steps from **163**. Pummerer rearrangement transformed the phenyl sulfoxide of **166** into the desired aldehyde, but unfortunately this was not achieved with standard conditions for a Pummerer rearrangement.[73–76] It was observed that when **166** was treated with triflouroacetic anhydride, only starting material was observed, but interestingly the diasteromeric ratio of sulfoxide, had changed, implying that activation of the sulfoxide had in fact occurred. Further work on conditions to achieve the desired aldehyde was carried out, finding that the combination of TMSOTf and TEA gave the desired rearrangement to give the desired aldehyde **166** in 63% yield (**Scheme 28**).[77]



Scheme 28: i) Tf_2O, 2-6-lutidine, CH_3CN, -20 °C; **ii)** AcSH, NaH, DMF, r.t., 89% in 2 steps; **iii)** PhSH, AIBN, *t*-BuOH, 100 °C; **iv)** m-CPBA, DCM, -40 °C, 62% in 2 steps; **v)** LiH, MeOH, 0 °C to r.t., then 1 M HCl aq., 0 °C **vi)** Ac_2O, pyridine, DCM, r.t., 77% in 2 steps; **vii)** TMSOTf, Et_3N, DCM, 0 °C, 63%.

The final transformations required were amide formation from the aldehyde **167** and hydroxylation at the C-4 position. Aldehyde **167** underwent oxidative amidation conditions using benzylamine, having been previously treated with iodine in methanol using previously reported conditions, did not obtain the expected product **168,** but instead the thioacetal **169** was obtained as a single diastereoisomer, confirmed by extensive NMR analysis using HMBC correlations, and isolated as the acetate **170.**[78] This reaction thus provided a fully functionalized core structure of tagetitoxin in 30% yield (**Scheme 29**).



167 **169** **168**

170

Scheme 29: i) $BnNH_2$, I_2, MeOH, r.t.; **ii)** Ac_2O, pyridine, r.t., 30% in 2 steps.

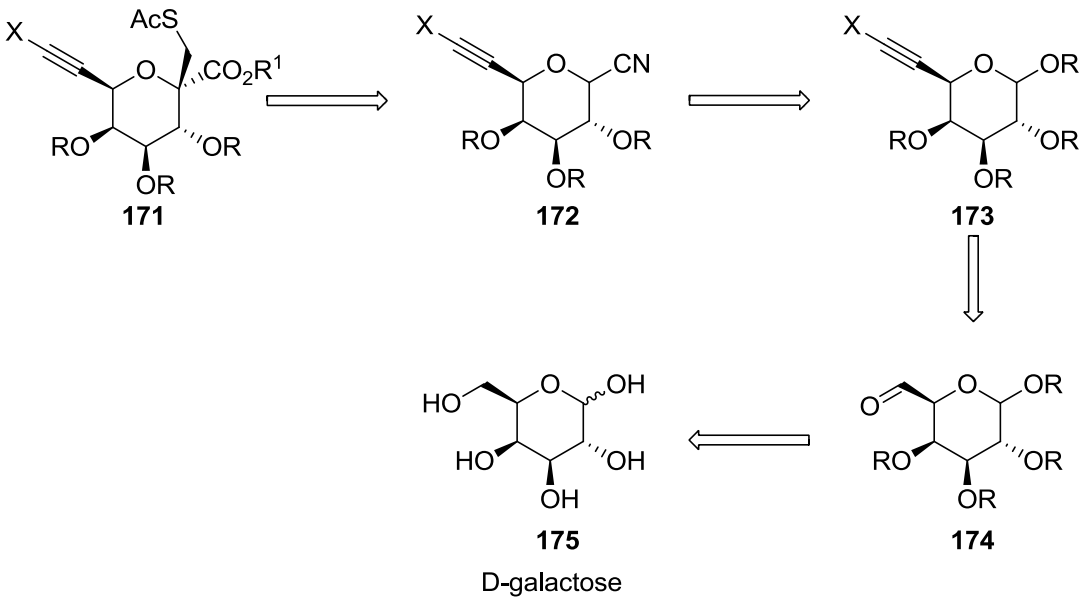
In conclusion, Nishikawa has achieved the synthesis of the tagetitoxin core using several very interesting methodologies such as *C*-glycosylation of the galcatal **150** with tin acetylene in the presence of TMSOTf, stereoselective introduction of a cyano group through the opening of a *spiro*-epoxyacetal **160** with TMSCN, and the construction of the 6-membered sulfide through an intramolecular conjugate addition of thiol to vinyl sulfoxide. To date, this is the first synthesis of a functionalized core structure of tagetitoxin.

Previous Work within the Page Group

The Page group has previously attempted a number of synthetic routes to prepare the bicyclic core of tagetitoxin. First of all, Claud-Eric Roy's work had used different sugars as starting materials in order to generate the target bicyclic structure of tagetitoxin.[79]

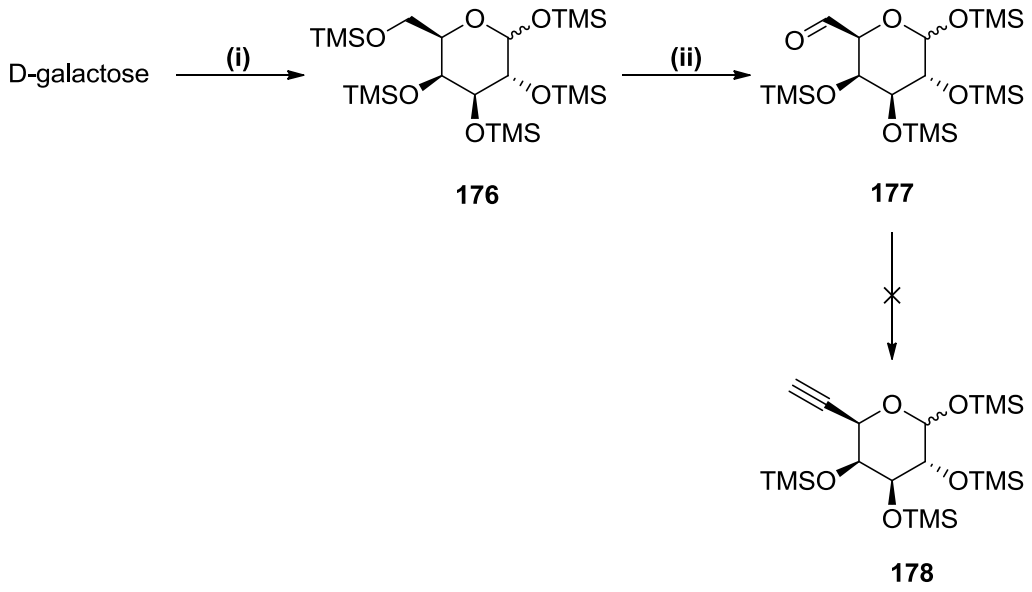
D-galactose Route

The first route by the group focused on the functionilization of a pyranose ring where O-2, O-3 and O-4 configurations would be preserved until their conversion into the desired moieties (**Scheme 30**).



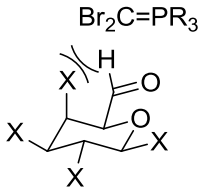
Scheme 30: Proposed Retrosynthesis of tagetitoxin from D-galactose

Starting from the readily available D-galactose and following a short sequence reported in the literature, the aldehyde intermediate **174** was synthesized.[80] The alkyne **173** would be prepared from the aldehyde **174** using a known methodology previously reported in the literature. The cyano moiety would be inserted by a glycosidation reaction.[81,82] The next step would be the introduction of the thioacetate functionality, which would be derived from the condensation with formaldehyde on the anomeric carbon following a previously reported literature procedure, after a displacement of the protected hydroxyl with a thioacetate anion.[83] The final step of the synthesis would be the conversion of the cyano group into an ester, using well documented transformation procedures (**Scheme 30**).[84]



Scheme 31: i) TMSCl, pyridine, 3 h **ii)** $(COCl)_2$, DMSO, DCM, -78 °C then Et_3N, -50 °C.

The penta-silylation of D-galactose proceeded smoothly in quantitative yield, using pyridine and TMSCl to give **176**. The next step involved the oxidation of the alcohol of **176**, via a Swern oxidation, which led to the desired aldehyde **177** been obtained in very good yields of between 80-98%. The next step was for conversion of the aldehyde into the desired alkyne using a Corey-Fuchs procedure previously reported in the literature, but unfortunately this reaction did not yield the desired alkyne (**Scheme 31**).[85] An explanation was postulated for the failure of this reaction, due to the hindrance generated by the –OTMS group on the C-4 position (**Scheme 32**).



179

X=OTMS

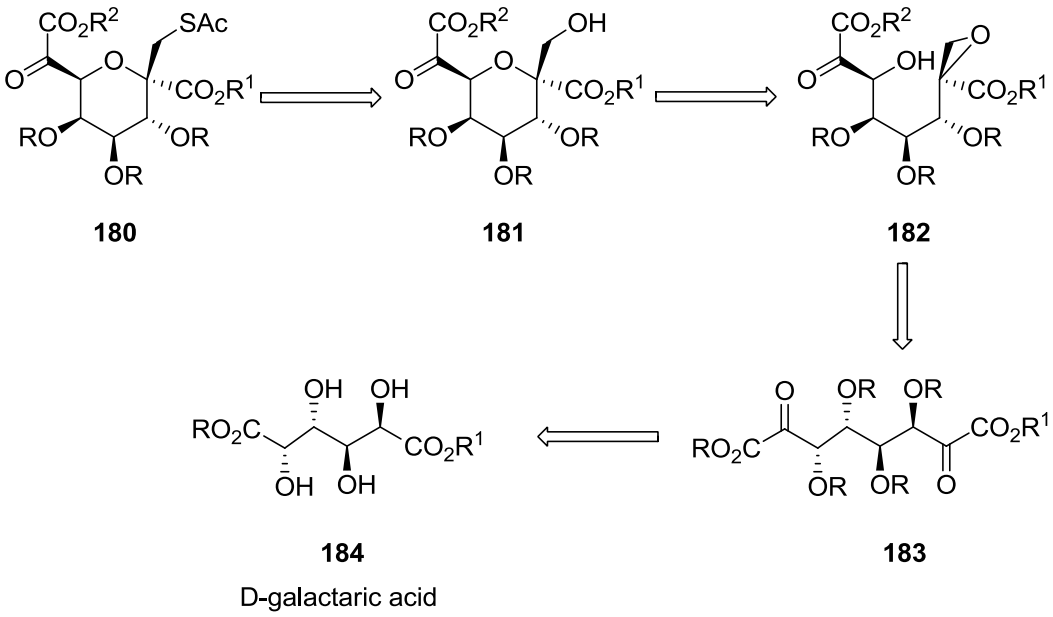
Scheme 32: Steric hindrance generated by the OTMS on the C-4 position.

Several other methods were carried out by the group to overcome this problem including using other known alkyne synthesis methodologies such as the Seyferth-Gilbert reaction, using the

Ohira-Bestmann protocol, however all attempts proved to be unsuccessful.[86–89] The group attempted other reactions to obtain the desired alkyne **178**, but with the problems encountered, the group decided to change their focus and develop a new strategy from a different starting material.

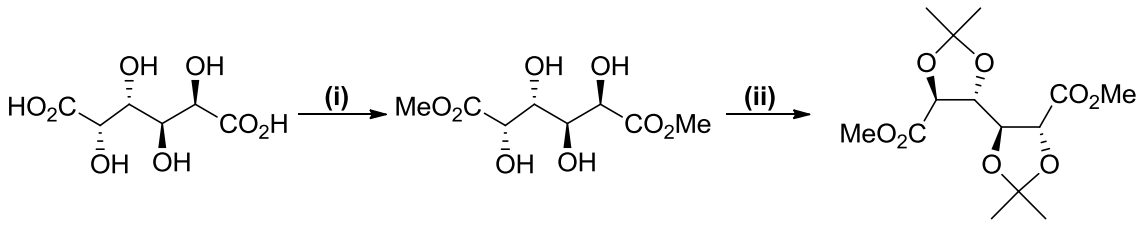
D-galactaric acid

The next strategy carried out by the group involved starting from a non-cyclic readily available starting material D-galactaric acid **184** (**Scheme 33**).



Scheme 33: Proposed retrosynthesis of tagetitoxin from D-galactaric acid

In this strategy, the formation of the pyranose ring would proceed from an ether synthesis through a ring closure by attack of a nucleophilic oxygen onto an epoxide to yield an advanced synthetic intermediate **181**. The targeted precursor **180** would be derived from a displacement of an activated hydroxyl group by a thioacetate. Intermediate **181,** which is fully functionalized, could be derived from a terminal epoxide **182**. This intermediate could be formed from a di-ketoester **183** through a previously reported Tebbe olefination-epoxidation sequence, or a Corey-Chaykovsky reaction on one of the two ketone carbonyls, as reported in the literature.[90–92] This intermediate **183** could be synthesized from the readily available starting material D-galactaric acid **184** in a few simple steps (**Scheme 33**).

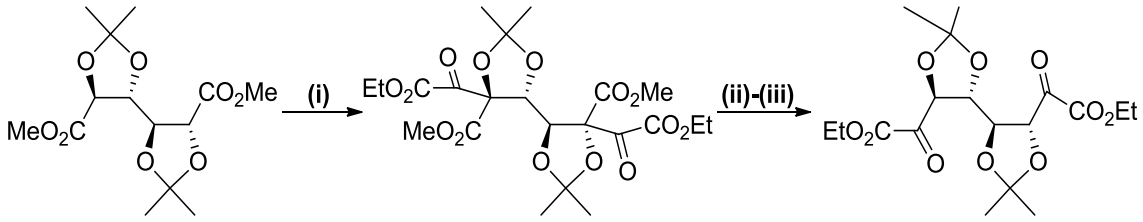


185 **186** **187**

Scheme 34: i) H_2SO_4, CH_3OH, 90-9%; ii) C_3H_6O, $FeCl_3$, Reflux, 2.5 h 17-25%.

The first part of the synthesis was to obtain the di-ketoester **187,** which was prepared following a literature procedure reported by Hirsch.[93] The initial step was the synthesis of the bis-methyl-ester **186** using a mixture of methanol and conc sulfuric acid which was obtained in 90-99% yield. The next step was the formation of the *bis*-ketal **187** using acetone and a Lewis acid, but the product was obtained in very low yields (17-25%) despite numerous attempts and changing of the acid catalyst for Brønsted acids (H_2SO_4, *p*TSA, HCl), however this generally tended to reduce yields or lead to decomposition. Spectral and single crystal X-ray data for bis-ketal **187** product were analysed and they were found to match the previously reported structure (**Scheme 34**).[79,93]

The second part of this strategy was to obtain the diketoester intermediates, with the simplest route appearing to be the use of enolate chemistry, with the formation of the enolates of the ester functions on **187**, allowing the enolate anion to attack a derivative of oxalic acid, diethyl oxalate. After addition to diethyl oxalate, the intermediate **188** was decarboxylated under acidic conditions and subsequent esterification to give the desired diketoester **189** (**Scheme 35**).[94]



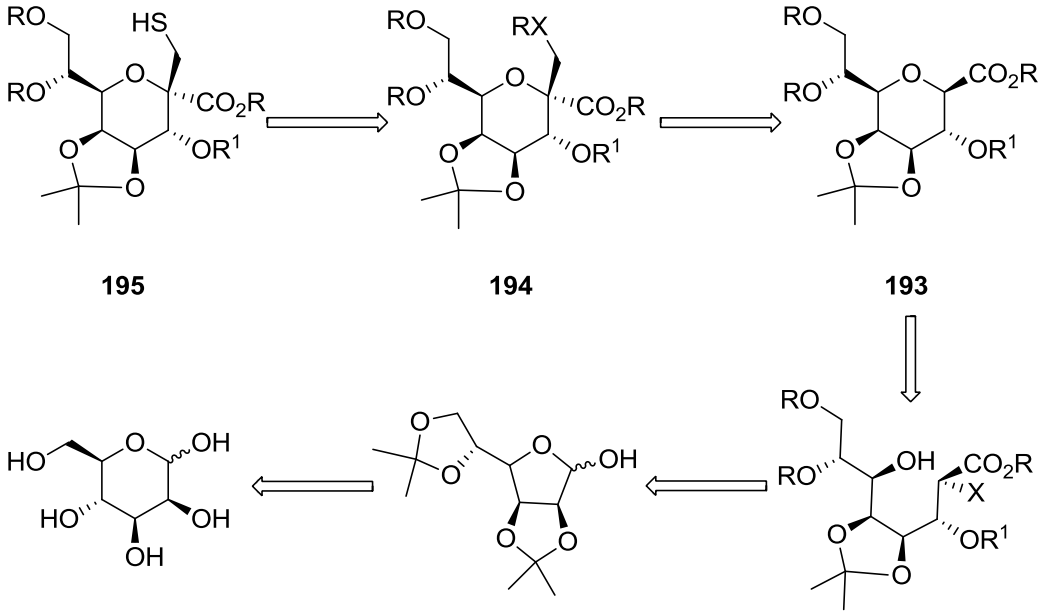
187 **188** **189**

Scheme 35: i) Na metal, EtOH, r.t., $(CO_2Et)_2$; **ii)** $HCl_{conc.}$, H_2O, reflux, 6 h; **iii)** *p*-TSA, EtOH, toluene, reflux, 6 h, 64% (over 2 steps).

However when the group carried out this reaction using Kagan's conditions, using sodium ethoxide as a base, unfortunately the desired di-ketoester **189** was not obtained and only starting material was recovered.[94] The group also carried out the reaction with the more basic sodium hydride or the commonly used LDA however the desired product was not obtained. The group carried out further attempts to alkylate the intermediate **187**, in order to assess the reactivity and the accessibility of the carbon to be deprotonated. The group used ethyl iodide as a reactant, in order to ascertain the feasibility of the addition onto a small alkyl group, using both the same bases, as in the previous reaction with LDA and sodium hydride. Unfortunately all these reactions failed to yield the product and only starting material was obtained again.

The group decided to abandon this route due to the low yields for the bis-ketal they obtained and also the lack of success obtaining the desired bis-ketoester **189** and decided to focus on other potentially more successful strategies.

D-mannose route

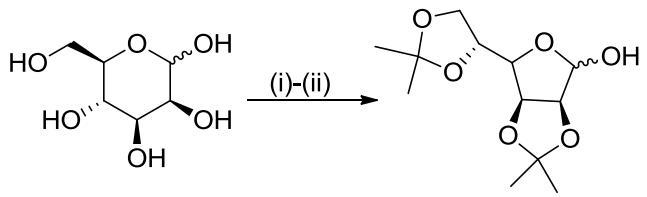


D-mannose

Scheme 36: Proposed Retrosynthesis of tagetitoxin from D-mannose

The next strategy (**Scheme 36**) carried out by the group involved the development of the ester intermediate **191** which could be prepared from **192**. Intermediate **192** could be prepared from an open carbohydrate **193** by a cyclization process. Alcohol **193** could be easily prepared from diacetone mannose **194**. Diacetone mannose **194** was easily obtained following a literature procedure, from the readily available cheap starting material D-mannose **195**.[95] This strategy provides many advantages, especially the fewer steps from diacetone mannose. The insertion of a hydroxymethyl group has been reported in the literature previously on a similar intermediate to that of **192**, using enolate chemistry.[83]

The first step in the synthesis was the protection of D-mannose to form diacetone mannose **190**, using a classic reaction in carbohydrate chemistry developed by Schmidt in 1963 (**Scheme 36**).[95]

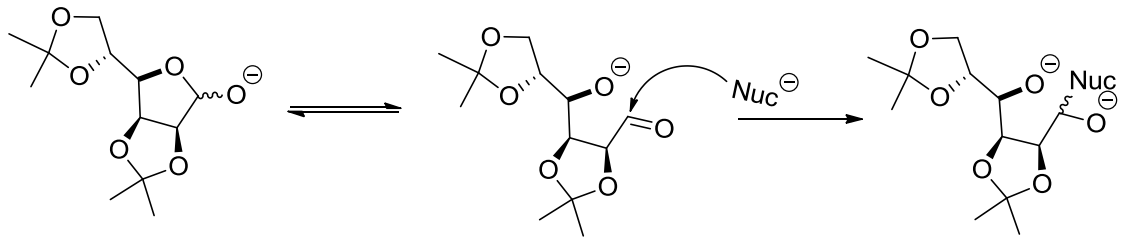


190 **191**

D-mannose

Scheme 37: i) H_2SO_4, acetone, r.t., 3 h; **ii)** Na_2CO_3, charcoal, acetone, reflux, 1 h.

The next step in the synthesis was the addition of a masked α-ketoester onto an aldehyde moiety of diacetone mannose **191**. Diacetone mannose exists as an equilibrium between the lactol form which exists as a mixture of α and β anomers and the open hydroxyl aldehyde form (**Scheme 38)**, and therefore a nucelophilic species was chosen to add to the aldehyde function. Numerous methods were used by the group to condense ethyl diazoacetate under various conditions from the literature, however all attempts proved to be unsuccessful and the group had to move on to another method to produce the desired ketoester **196** intermediate.[96]



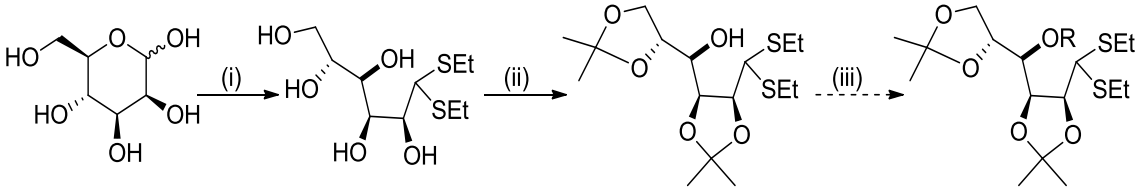
191 **196**

Scheme 38: Proposed reactivity of lactol-hydroxy aldehyde equilibrium.

With the lack of success from this route, another pathway was investigated by the group. Whilst still using diacetone mannose the group again focused on the aldehyde moiety and the hydroxyl group in the open form would be protected before any reaction would be carried out on the aldehyde. Initially the group tried to carry out the protection of the open form hydroxyl group directly from diacetone mannose. Despite several procedures to protect the 4-OH

hydroxyl of diacetone mannose using a variety of silyl ethers, the group obtained either a protection of the 1-OH hydroxyl or starting material, and no desired compound was observed.

A new route again was envisaged by the group to synthesize the targeted aldehyde. It involved the protection of the aldehyde function, masked in the lactol form, as a thioacetal, thus, several protections of the various hydroxyls and hydrolysis of the sulfur would give the desired intermediate (**Scheme 39**).[97,98]



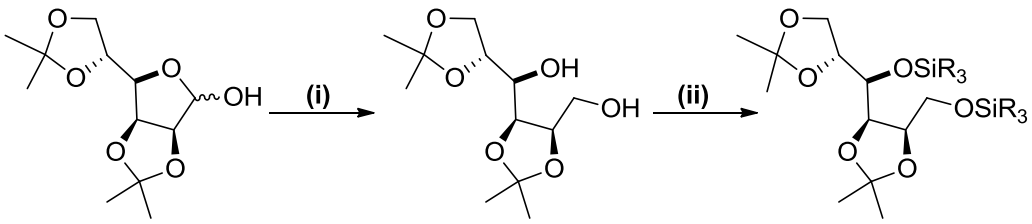
190 **197** **198** **199**

D-mannose

Scheme 39: i) EtSH, HCl, 89%; **ii)** acetone, H_2SO_4; **iii)** protection, cleavage.

The initial thioacetal formation was performed in a good yield of 89% using ethanethiol as solvent and HCl as the acid catalyst to give the desired product **197**. The second step involved the selective 2,3-5,6-diisopropylidene formation to produce compound **198**. The use of acetone with sulfuric acid under similar conditions as for the formation of diacetone mannose **191** led to an inseparable mixture of multiple acetonides or degradation of the starting material.

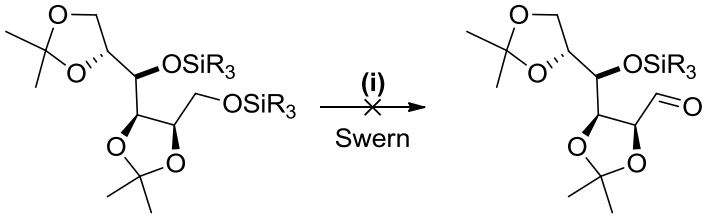
Having had little success with the thioacetal route the group decided to try a sequential silylation protection method on diacetone mannose. The synthesis began with the reduction of diacetone mannose 191 using lithium hydride, which was previously reported in the literature to give the diol **200** in very high yields.[99–101] Several silylation procedures were carried out using TMSCl and TESCl, leading to the diprotected species of both silyl ethers **201** in good yields (**Scheme 40**).



191 **200** **201**

Scheme 40: i) $LiAlH_4$, Et_2O, 4-5 h, 0 °C to r.t.; **ii)** base, R_3SiCl, solvent.

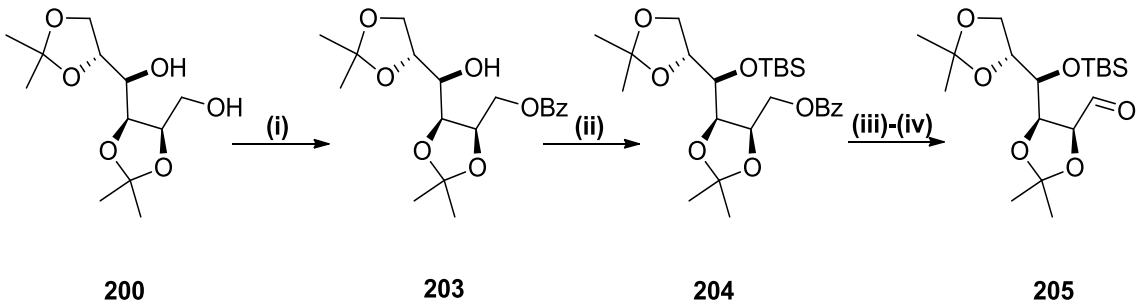
The next step of the synthesis was the selective oxidation of the primary silyl ether. Following previously reported literature procedures for the precedent on silyl ether oxidation.[100,101] Swern oxidation reactions were preformed on the desilylated species, however no desired aldehdye **198** was obtained.



201 **202**

Scheme 41: i) $(COCl)_2$, DMSO, DCM, TEA, -70 °C.

Several attempts were made in order to form the desired aldehyde intermediate **202**, however none of the reactions yielded the desired intermediate, and the group decided to abandon this pathway (**Scheme 41**).



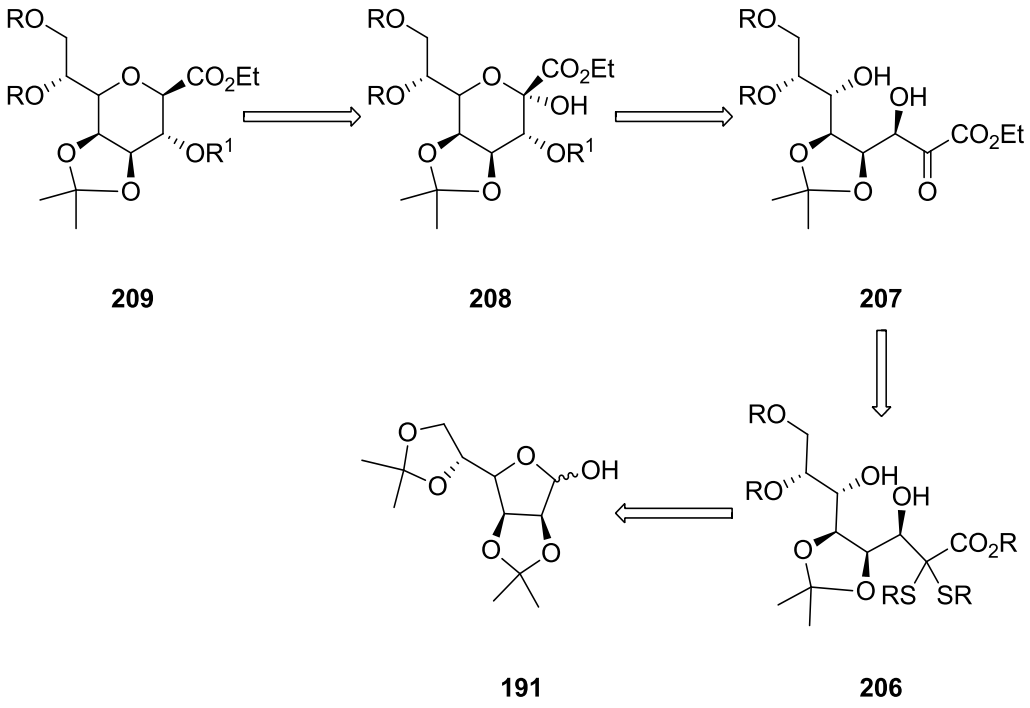
Scheme 42: i) BzCl, pyridine, 0 °C to r.t.; **ii)** TBSOTf, DIPEA, Et_2O, 0 °C; **iii)** 1N NaOH/MeOH, MeOH, r.t. overnight; **iv)** oxidation conditions.

Carrying on from the failure of the strategy above the group decided to revise the synthesis for the desired silylated aldehyde and complete a five step synthesis to obtain the desired aldehyde following a previously reported literature by Hashimoto (**Scheme 42**).[99] It firstly consisted of a protection of the primary alcohol of diol **200** with a benzoyl. The secondary alcohol of the diol was then protected with TBSCl to obtain the TBS protected compound 204, however no desired reaction was obtained so they decided to change the conditions an use TBSOTf in TBME and were able to obtain the desired TBS intermediate **204** in 37% yield. The next step was the saponification of the ester to give the hydroxyl function which proceeded in quantitative yield, With the free hydroxyl the next step was to oxidize to give the desired aldehyde **205,** but they were unable to produce the desired aldehyde **205** using Parikh-Doering, IBX-mediated, Swern or Moffat oxidation conditions.[102–105]

The group decided to abandon this route since they could not obtain the desired aldehyde and moved on to alternative routes.

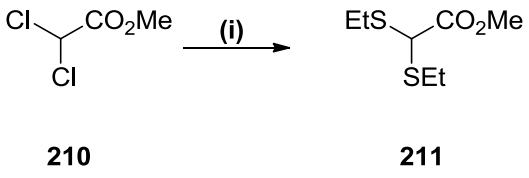
Dithioacetal Route

The final strategy investigated by Claude-Eric Roy within the group was a nucleophilic addition of a synthetic equivalent of an α-ketoester onto diacetone mannose (**Scheme 43**).



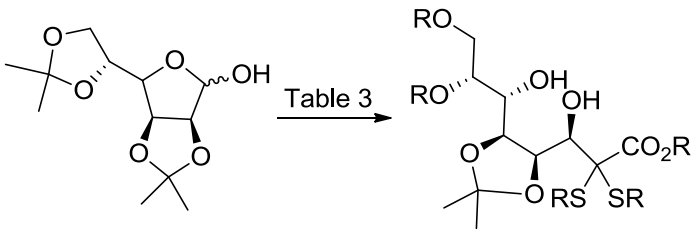
Scheme 43: Retrosynthesis of of Pyranoside ester **209**.

This route was largely inspired from a reported synthesis by Schmidt of KDO (3-deoxy-D-manno-2-octulusonic acid), which at the time was highly topical, involving the use of a dithiomercaptal and dithiane species to afford the required intermediate **209.**[106] The first step in the synthesis was the making of the diethymercaptal species **211** following a literature procedure, formed from the reaction of methyl dichloroacetate and ethane thiol (**Scheme 44**).[107]



Scheme 44: i) MeONa, then EtSH, 82%.

Having synthesized this intermediate, the next step along with the other two mercaptals which were commercially available was the nucleophilic addition onto diacetone mannose following a literature procedure.[106]



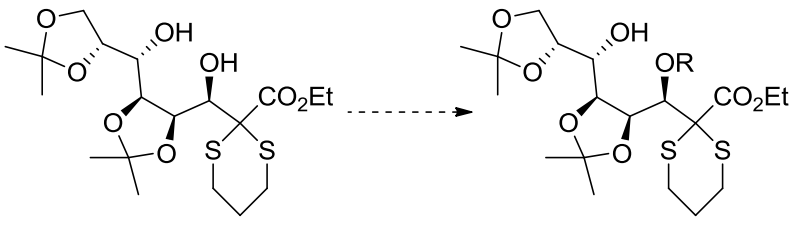
191 **206**

Scheme 45: Addition onto diacetone mannose **206**.

Entry	Thioacetal	Conditions	Yield
1	Diethyl mercaptal	DIPA, BuLi then mercaptal, then $MgBr_2$, then **191**.	SM
2	Ethyl dithiane carboxylate	DIPA, BuLi then dithiolane, then $MgBr_2$, then **191**.	SM
3	Ethyl dithiane carboxylate	DIPA, BuLi then dithiane, then $MgBr_2$, then **191**.	SM
4	Ethyl dithiane carboxylate	DIPA, BuLi then dithiane, then $MgBr_2$, then **191**.	65% **212**

Table 3: Reagents and conditions for the nucleophilic addition reaction.

Despite many attempts, the reported nucelophilic attack using entry 1 and 2 were unsuccessful and only starting material was observed. The addition of the dithiane ester (entry 4) led to the desired product **212** in good yield and as a single diasterisomer. It was also shown that the same reaction without the intermediate formation of the organomagnesium bromide derivative of the dithiane species prevented the addition and only starting material was observed (**Scheme 45 and Table 3**).

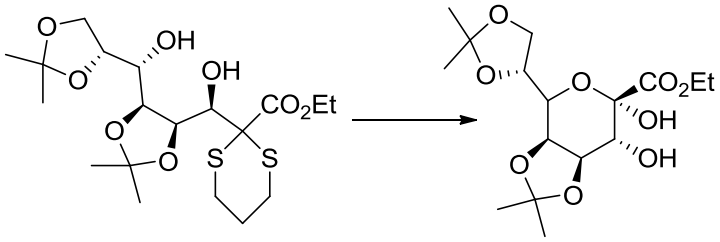


212 **213**

Scheme 46: Protection of the C-3 hydroxyl of intermediate **212**.

Further work was carried out by the group on the selective protection of the C-3 hydroxyl group on intermediate **212** and dethioketalization to trigger cyclization to form the desired six membered intermediate **213** (**Scheme 46**). Several protecting groups were used such as TBS, benzyl and benzoyl but only starting material, decomposition or diacetone mannose was observed and the group were unable to selectively protect at this position (**Scheme 46**).

Having obtained such poor results with the selective protection of the C-3 hydroxyl the group decided to alter the sequence of the steps so that the cyclization step would be performed first. Thus the dethioketalization was attempted using conditions reported in the literature (**Scheme 47**).[106]



212 **214**

Scheme 47: Dethioketalization/Cyclization of compound **212**.
i) NBS, Acetone/water

Initial attempts using the previous reported conditions from the literature did not reproduce the same results, and gave a dramatically low yield of 18% for intermediate **212** compared to the 76% yield reported in the literature (**Scheme 47**).[106] When a scale up was performed on this

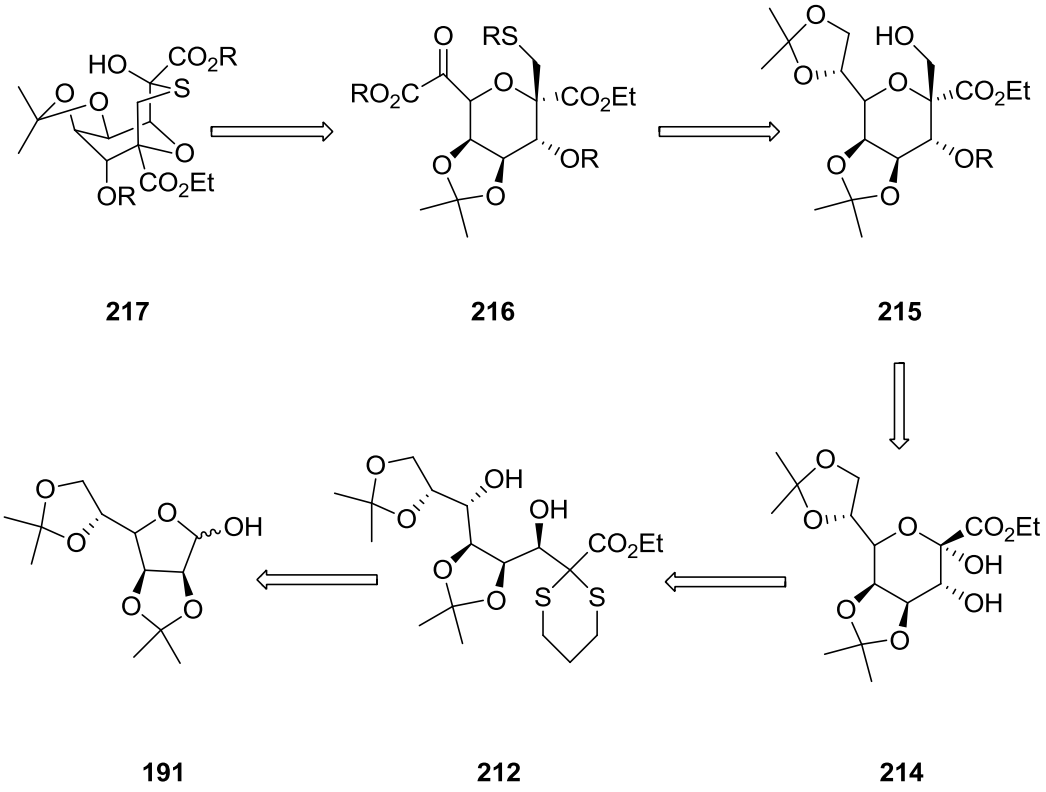
reaction even poorer results were obtained, and even controlling the purity of the reagents and solvents (recrystalization of NBS, HPLC grade acetone and distilled water) led to no change in the poor yields obtained. The group carried out many deprotection procedures of dithiane using reagents such as NIS, I_2 and different halogen sources, but this led to no greater increase in yield.

Only a small amount of the desired 6-membered pyranose **214** was obtained from the described procedures above, and purification of this led to decomposition or loss of the desired pyranose.

This initial work carried out by the Page group on the synthesis of tagetitoxin led to a number of interesting intermediates, and some routes which would not be investigated further due to the problems encountered.

Pearce

Following on from the work carried out within the group by Claude-Eric Roy, Chris Pearce carried on using the dithioacetal route as this had given the most promising results towards a synthesis of the bicyclic core of tagetitoxin, and optimizing some of these results could provide the shortest and most efficient route towards the synthesis of the bicyclic core. Pearce continued the work on the addition of a dithioacetal-protected ketoester onto diacetone mannose **191** (**Scheme 48**).[108]



Scheme 48: Retrosynthesis via the dithioacetal route.

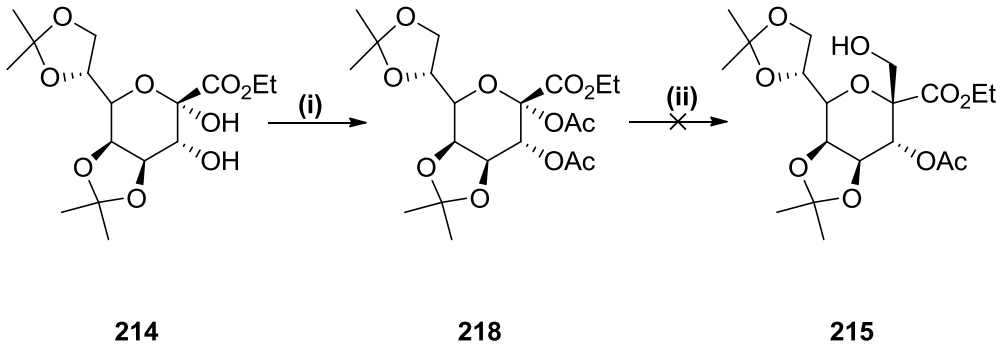
The first step in the synthesis was the conversion of D-mannose into diacetone mannose **191** using the same procedure reported above.[72] The next step in the synthesis was the nucleophilic addition of the dithiane protected ketoester onto diacetone mannose to give compound **212**. The group used the same procedure carried out previously and optimized the reaction conditions to obtain **212** in very high yield of 98%.[77] The next step in the synthesis was the removal of dithiane group in **212**, and the cyclization to form the six membered pyranose ring **214**. The group carried out the deprotection and cyclization using previously reported conditions by Schmidt, using NBS in acetone and water, however despite numerous attempts the group was never able to obtain the reported yields and the highest they obtained of pyranose **214** was 40%.[106] Pearce obtained an X-ray crystal structure for compound **214** which confirmed its stereochemistry (**Scheme 48**).[108]

Having obtained such poor results for the deprotection of the dithiane and the cyclization to give **214**, Pearce carried out numerous literature dithiane deprotection reactions, including mercury reagents, halogen donors and several oxidants.[109–114] Unfortunately, none of these

procedures gave higher yields than the use of NBS, and sometimes the only isolated product was diacetone mannose.

Despite the very low yields obtained for **214** the group decided to continue with this route. The group carried out investigations on the removal of the anomeric hydroxyl group of **214** and came across a report of a samarium diiodide-promoted coupling of anomeric acetate with carbonyl compounds.[115] The group believed they could apply this methodology to compound **214** to remove the anomeric hydroxyl group and introduce the methylene alcohol group in one step (**Scheme 49**).

The group used a modified version of the samarium diiodide procedure for the conversion of the diacetate **218** to the alcohol **215** using formaldehyde as the carbon source, hoping that this would give the desired alcohol. Diacetate **218** was easily prepared using standard acetate conditions from pyranose **214** (**Scheme 49**) in very high yield. The next step was the samarium diiodide-mediated coupling reaction. However after numerous attempts the group was unable to obtain the desired alcohol **215**.[116]

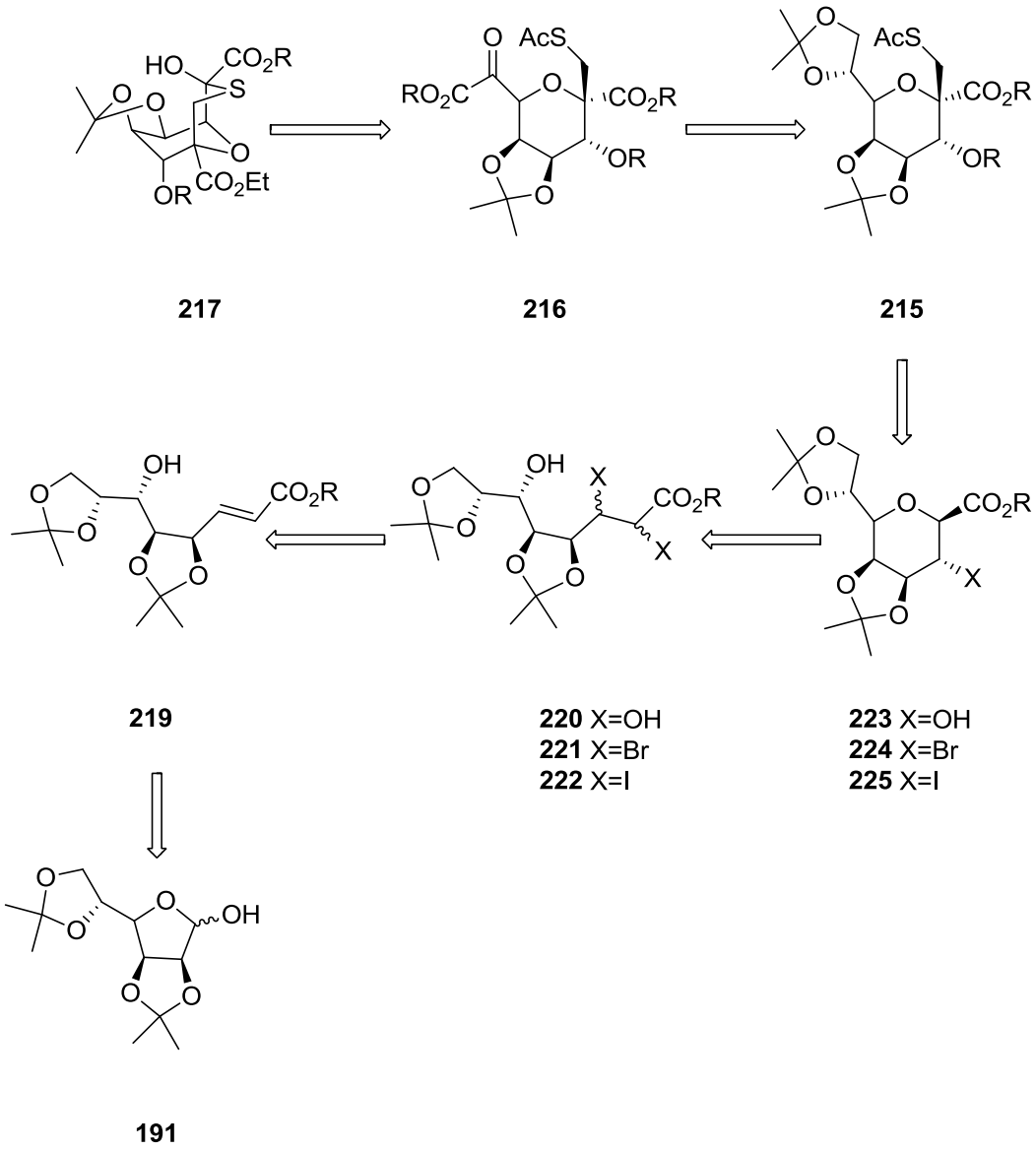


214 **218** **215**

Scheme 49: i) Ac_2O, Et_3N, DMAP, DCM, 88%; **ii)** SmI_2, CH_2O, THF.

Unfortunately, having tried numerous attempts and procedures the group were forced to abandon this route.

Wittig Route

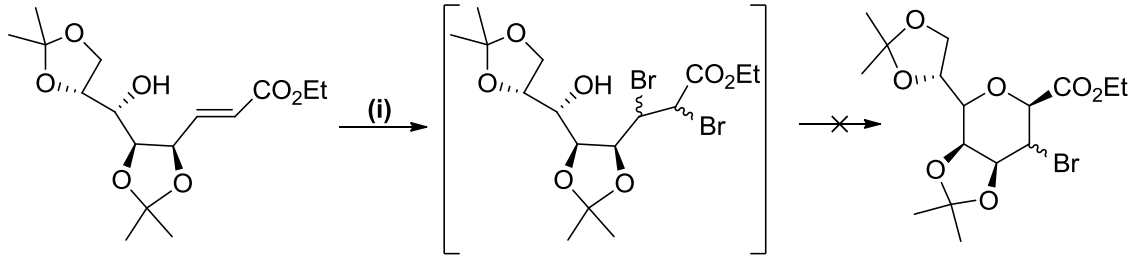


Scheme 50: Retrosynthesis from diacetone mannose using Wittig route.

Pearce then decided to move on and envisaged a new route involving a Wittig reaction to introduce the ester moiety (**Scheme 50**). The group decided to start again from diacetone mannose **191**, carry out a Wittig reaction on the aldehyde function of the open furanose to produce the alkene **219**, after a simple protection of the secondary alcohol. The alkene then would be subjected to either a bromination, iodination or a dihydroxylation to give the desired product **220-222**. The group then postulated that removal of the protecting group on the secondary hydroxyl group and a displacement of either the halogen or a manipulated hydroxyl

group alpha to the ester function would lead to the cyclized pyranose **223-225**. The next step would be the introduction of the thiol function, which the group postulated could be introduced using enolate chemistry to obtain the desired primary alcohol on the anomeric position, and then a displacement with a thioacetate anion to give the desired intermediate **215**. The final steps would involve the selective removal of the acetal and then oxidation and a protection to give the compound **216**. The final step of the postulated synthesis was the removal of the acetate on the thioacetate to give the free thiol, which would undergo an *in situ* cyclization to give the desired bicyclic structure **217** (**Scheme 50**).

The group began with the first step of the synthesis of the target bicyclic structure **217** by carrying out a Wittig reaction on diacetone mannose **191** using literature conditions, where diacetone mannose was heated at reflux with the corresponding ester ylid to produce a range of alkenes.[117] Under these conditions the *E*-alkene was the major product and easily separated from the *Z*-alkene. The next step was the protection of the hydroxyl moiety, which was carried out easily using standard acetate protection conditions, which gave the product in good yield, and also a TMS protection which gave very low yields. The group carried out a dihydroxylation on the TMS-protected alkene, which was then followed by a selective tosylation of the hydroxyl α to the ester moiety, but the tosylation gave very low yields due to the mild conditions. The next step was the removal of the TMS protecting group and a cyclization to give the 6-memebered pyranose intermediate, however only decomposition was observed, and this led the group to explore other methods to reach this intermediate.

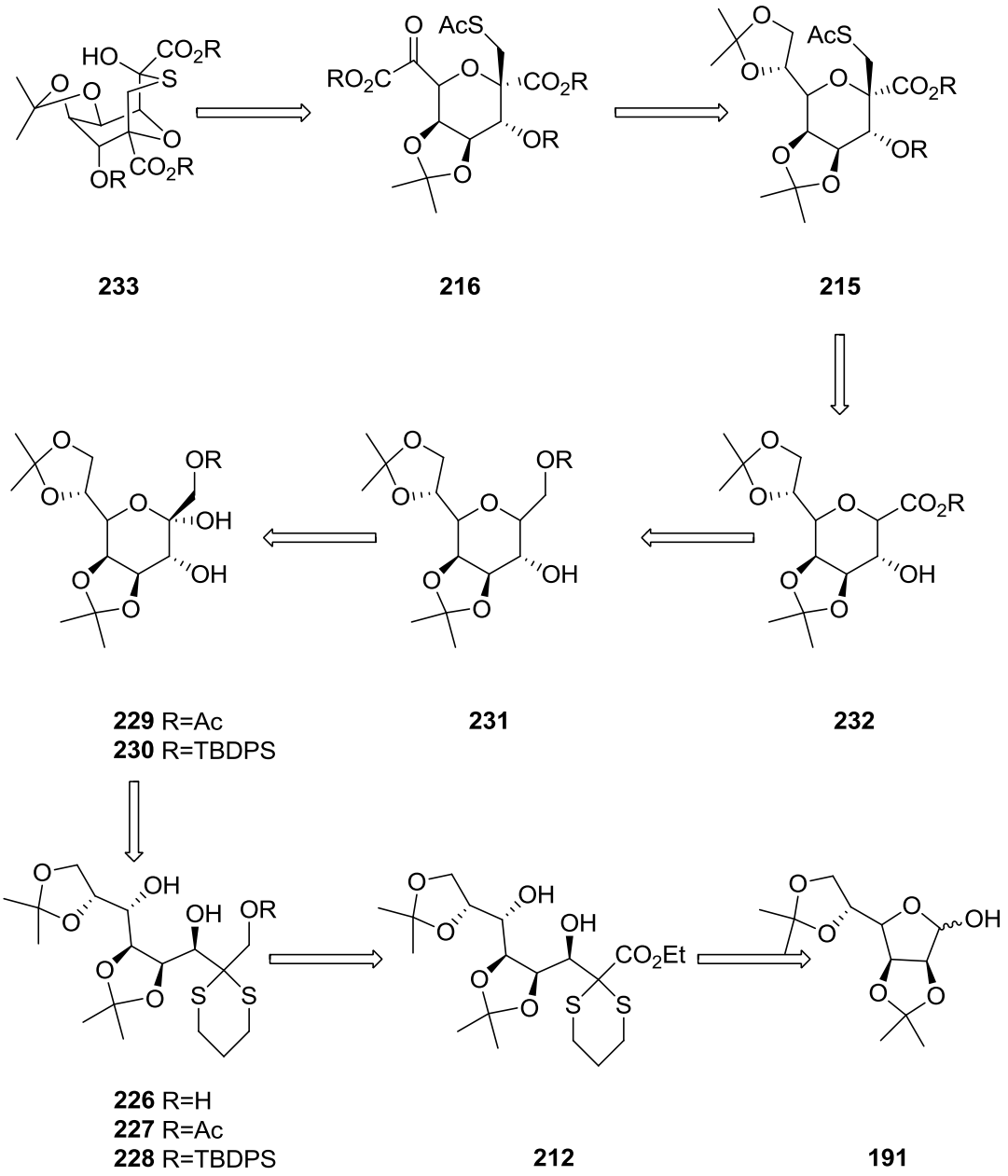


219 **221** **224**

Scheme 51: i) Br_2, DCM, 0 °C.

The group then decided to functionalize the alkene. The group postulated that a direct bromination of the alkene **219** could lead to a cyclization to form the desired intermediate **224** in one step (**Scheme 51**). However following bromination conditions the group was unable to obtain the desired cyclized product, and decomposition of the starting material was observed. Other methods to brominate the alkene and reach the desired cyclized product **224** were unsuccessful and forced the group to abandon this route and look for an alternative method (**Scheme 51**).

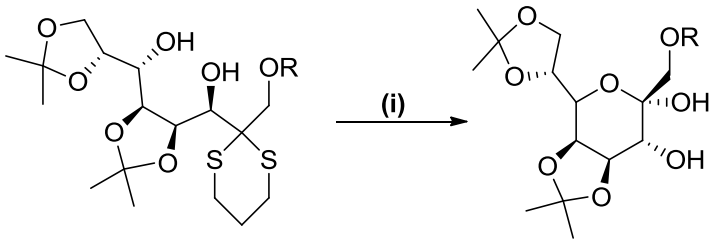
Reduction Route



Scheme 52: Retrosynthesis via the reduction route.

Following on from the lack of success in achieving the desired bicyclic structure, Pearce, decided to go back to the dethioketalization, strategy but this time to remove the ester moiety completely (**Scheme 52**).[108] The first step was the nucleophilic attack of ethyl ester dithiane on diacetone mannose **191**, which had been carried previously and optimized by the group. The next step was the reduction of the ethyl ester **212** using lithium aluminium hydride in THF to give the triol **226**, in which the primary alcohol was selectively acetate protected to give **227**

(R=Ac) in very high yields without using DMAP as this had produced the triacetate product. The group had also protected the primary group with a tertiarybutyldiphenylsilyl ether to give **228** (R=TBDPS), however the reaction was very low yielding (18%). The group believed that the removal of the ester moiety would give a reduction in the likelihood of the reversion back to diacetone mannose **191**; following reaction with NBS, without the ester moiety present the dithiane anion, generated during the production of diacetone mannose when reacting **227** and **228** with NBS, would be far less stable.



227 R=Ac
228 R=TBDPS

229 R=Ac
230 R=TBDPS

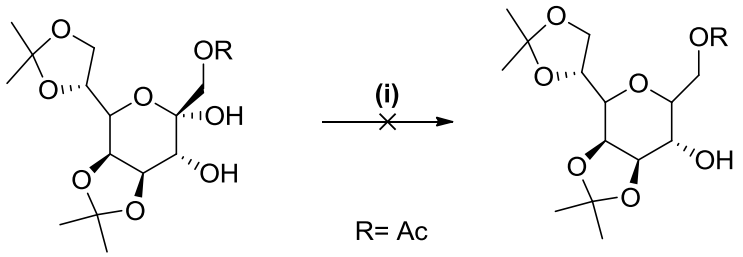
Scheme 53: **i)** DBDMH, 95% acetone, 0 °C, 3 min (66-100% R=Ac) (67% R=TBDPS.

When compounds **227** and **228** were subjected to the dethioketalization reaction using NBS no desired product was obtained. When compounds **227** and **228** were treated with 1,3-dibromo-5,5-dimethylhydantoin (DBDMH), however, the group obtained the desired protected pyranoses **229** and **230** in good yields (**Scheme 53**).

The next step in this route was the removal of the anomeric hydroxyl on **229** and **230**. Following the removal of the hydroxyl group to give **229** and **230**, the ester moiety would need to be restored giving compound **232**. The group had then postulated that having obtained the ester moiety to give compound **232**; this would enable the enolate addition of formaldehyde and a subsequent displacement of the primary alcohol with a thioacetate anion to give compound **215**. The group then postulated that the rest of the synthesis would be the same as the previous dithiane route, with the generation of the keto-ester **216**, followed by the deprotection and *in situ* cyclization to give the desired bicyclic structure **233** (**Scheme 52**).

The group began the first part of the synthesis with the removal of the anomeric hydroxyl group from compound **229** using a previously reported procedure from the literature, to give

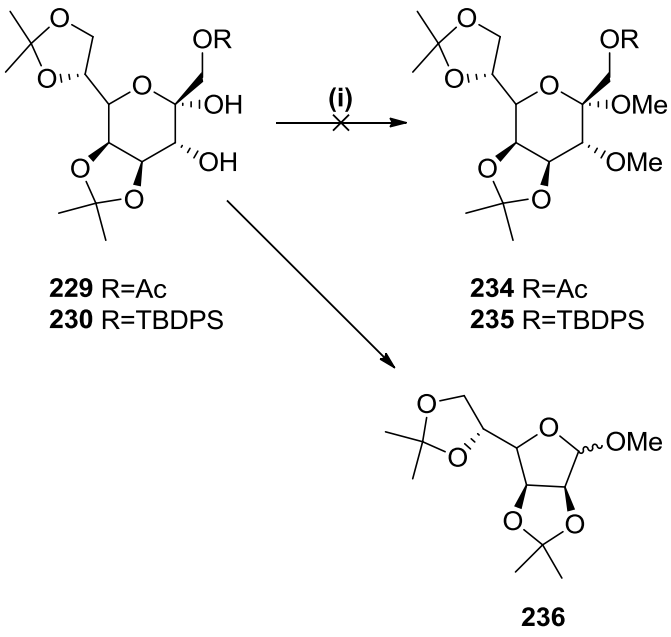
231 (**Scheme 54**), unfortunately this procedure was unsuccessful, and despite numerous attempts led only to complete decomposition of the starting material.[118]



229 **231**

Scheme 54: **i)** TMSOTf, Et_3SiH, MeCN.

The group decided that despite these disappointing results with the removal of the anomeric hydroxyl on **229**, instead to firstly convert the free hydroxyl groups on compounds **229** and **230** (R=Ac or TBDPS) to a methoxy group in the hope it would aid the removal of the group on the anomeric position. However the group were unsuccessful in carrying out the conversion with methyl iodide in the presence of sodium hydride and the group observed complete decomposition to the starting material. When the reaction was carried out on the acetate protected compound **229** no desired product **234** was observed. When they applied the same conditions to the TBDPS protected compound **230,** they also observed decomposition of the starting material and none of the desired product **235** but they also identified one of the decomposition compounds as the methoxy diacetone mannose compound 236 (**Scheme 55**).



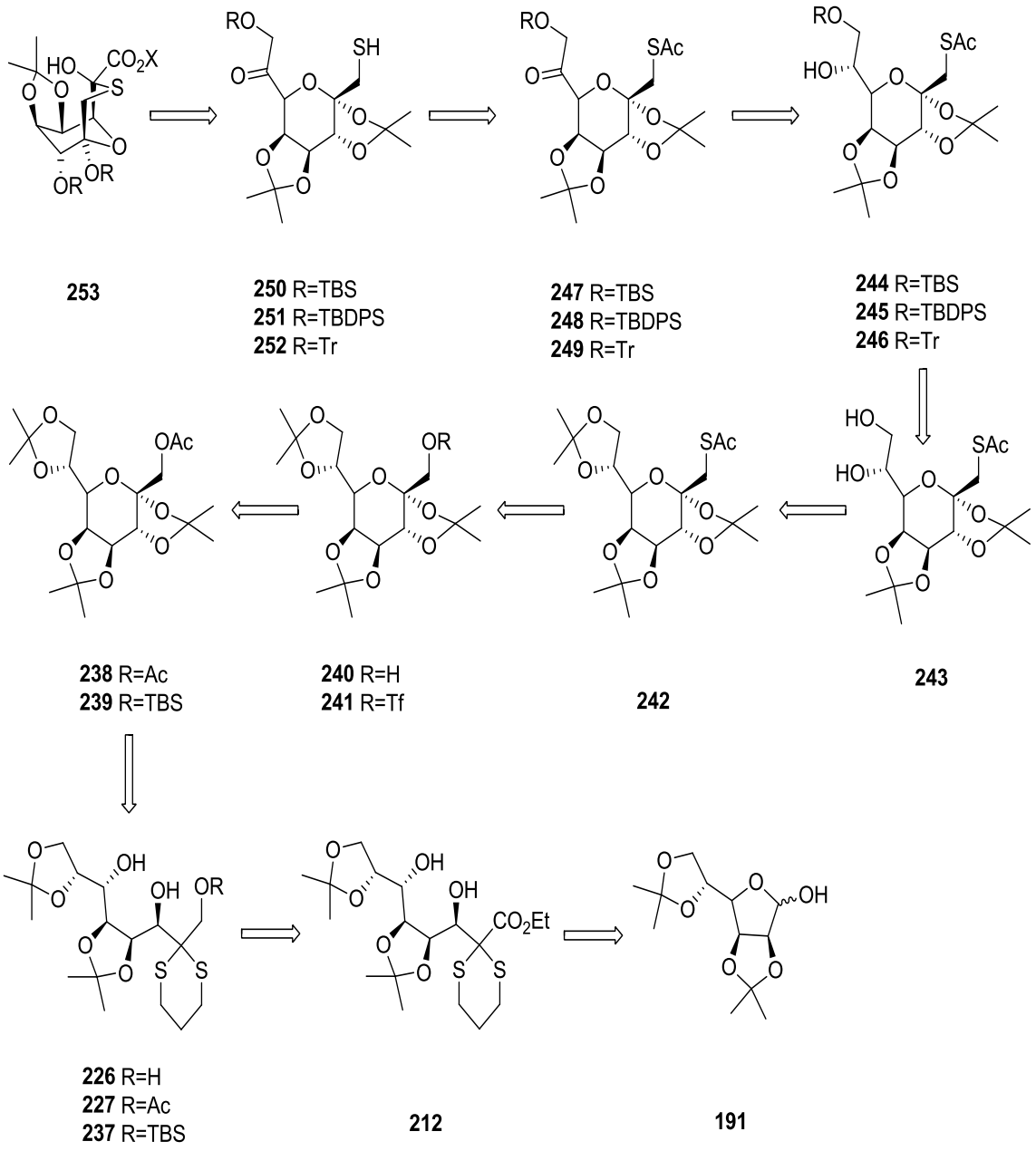
Scheme 55: **i)** NaH, MeI, DMF.

Following further disappointing results the group decided to abandon this route and carried out other routes towards obtaining a synthesis of the bicyclic structure of tagetitoxin, however all these routes did not lead to any success, and with time constraints no further work was carried out on these strategies by Pearce.[108]

The previous work carried out within the Page group had led to a number of interesting intermediates and some of these were investigated and are described within this thesis.

Project

Following on from previous work within the group, my aim was to continue on from previous intermediates especially, the dethioketalization strategy, but involving the reduction of the ester moiety completely to the alcohol, as this had produced some of the most successful intermediates in our aim to complete a total synthesis of the bicyclic core of tagetitoxin.[79,108]

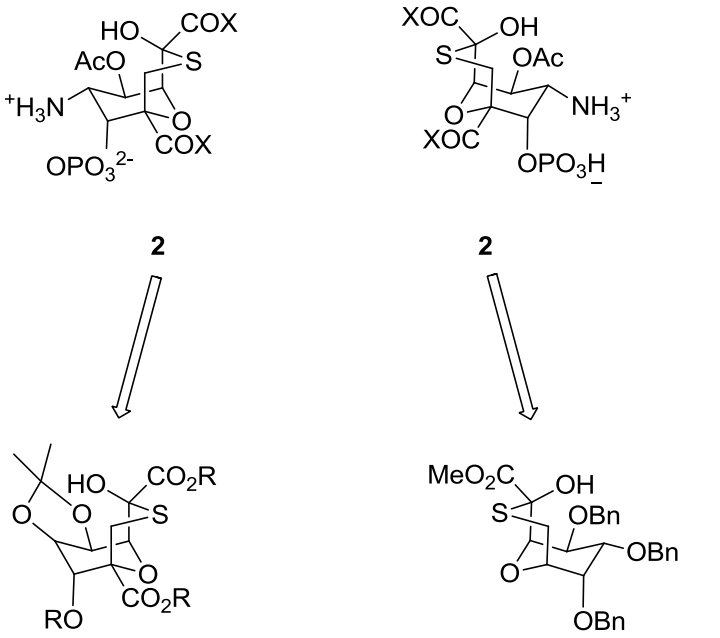


Scheme 56: Retrosynthesis for taget bicylic compound **253.**

Our initial goal was to produce the bicyclic structure of tagetitoxin, but without the acid functional group on the anomeric position. We would develop later a suitable synthesis to complete this later once this methodology had been carried out (**Scheme 56**). Starting from diacetone mannose **191,** made from readily available D-mannose, the next step was the nucleophilic addition of the dithiane protected ketoester to give compound **212**. Reduction of the ethyl ester to the alcohol could be achieved using lithium aluminium hydride in THF to give

the triol **226**, followed by a selective protection of the primary alcohol to produce acetate **227** or tert-butyl silyl **237**. The next step was to carry out the dethioketalization and the *in situ* cyclization to give pyranose **238** (R=Ac) or **239** (R=TBS). Deprotection of the acetate to the free primary alcohol could be carried out using conventional acetate deprotection methods to give pyranose **240** (X=OH). Triflation of the primary alcohol could be carried out easily using previously reported conditions to give **241**, followed subsequently by a thioacetate anion displacement of the triflate using sodium thioacetate to give **242**.[119] The next step involved the selective acetal deprotection using a procedure previously reported in the literature to give the diol **243**.[120] Selective primary alcohol protection using a silyl ether TBS **244**, TBDPS **245** or a trityl **246** protecting group and an oxidation of the secondary alcohol to produce ketone **247, 248 and 249**. The final step involved the deprotection of the thioacetate to the thiols **250, 251** and **252**, and *in situ* cyclization to produce the bicyclic structure **253** (**Scheme 56**). From this structure, theoretically all functional groups could be introduced using known carbohydrate chemistry, and the readjustment of the synthesis to introduce the carboxylic acid moiety on the anomeric to give the natural product.

It is also noteworthy that the synthetic pathways within the group are designed in order for the synthesis to start from the D-series and to obtain a pyranose core **229**, as opposed to the strategy developed by Porter previously to afford **249** (**Scheme 56**). Our aim was to synthesize the bicyclic core structure **248**, and with a simple modification of this methodology to obtain the bicyclic structure **229**.

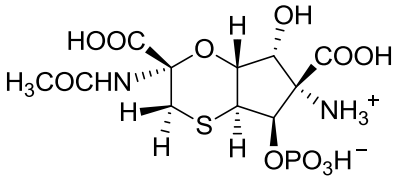


233
Absolute configuration
intended by Page Group

254
Absolute configuration
intended by Porter group

Scheme 57: Targeted absolute configuration

Since the production of this thesis a new paper has been published by Aliev, Karu, Mitchell and Porter in October 2016.[121] Aliev *et al.* report new NMR and MS data for tagetitoxin which contradicts previous published structures of tagetitoxin and reported a new proposed structure of tagetitoxin **255** (**Figure 5**).[3–6] A sample was obtained which had been previously isolated and purified by Mitchell and this was analysed.[1,3,4] Most of the spectral features in the ^{1}H NMR were the same as those observed by Gronwald *et al.* two peaks were not observed at 1.75 and 2.53 ppm.[5] In the ^{13}C NMR no peak was observed at 181.45 ppm. Based on the NMR analysis obtained Aliev *et al.* postulated that the material studied by Gronwald *et al.* was not as pure as that which was extracted by Mitchell *et al*.[3–5] The mass spectrum data obtained also during this work contained no species with a molecular weight of 678 which Gronwald *et al.* had reported as the revised molecular weight for tagetitoxin.[5]



255

Figure 5: Latest proposed structure of tagetitoxin.[121]

Micthell in 1989 revised the structure of tagetitoxin in which his revised structure had been proposed based on the analysis of 1H and 13C NMR spectra in particular the ^{1}H NOES and the COLOC spectrum for ^{1}H-^{13}C long range correlations.[4] In this paper Aliev *et al.* based on HMBC spectra as well as values of long range $^{n}J_{CH}$ couplings revealed several correlations which they used to rule out the previously reported published structures of tagetitoxin structures **1** and **2**.[121] In particular the authors discussed the following points in which they were in disagreement with the previously reported structures and helped them rule out structures **1** and **2**:

1. A cross-peak was observed for the C11-H8 pair which is in disagreement with structure **2a** with six bonds between C11 and H8.
2. A strong cross peak C10-H2' observed in disagreement with structure **2b** with four bonds between C10 and H2'. They noted that $^{4}J_{CH}$ correlations were observed in the

HMBC spectra, but a value for the J_{C10H2} coupling which was derived from the HMBC-JC spectrum had a value equal to 5.0 Hz in which could not be attributed to a $^{4}J_{CH}$ coupling.

3. Cross peaks observed for C7-H2 (J_{CH}=5 Hz) and C7-H2 (J_{CH}=3 Hz), was in total disagreement to the structures **1**, **2a** and **2b**, with four bonds separation between C7 and H2.
4. Lastly for structure 2a the dihedral angle between C4 and C6 is approximately 180° and they only observed a weak HMBC cross peak in the HMBC. The HMBC-JC value of J_{C4H6} was 1.4 Hz.[121]

In particular the authors discussed the following points in which they were in disagreement with the previously reported structures:

1. A Cross-peak was observed for C11-H8 pair which was due to the $^{3}J_{CH}$ coupling in **255.**
2. A Cross-peak was observed for C10-H2 which was due to the $^{3}J_{CH}$ coupling in **255.**
3. Cross-peaks were observed for C7-H2 (J_{CH}=5Hz) and C7-H2 (J_{CH} =5Hz) which was due to the $^{3}J_{CH}$ coupling in **255**
4. They assumed a chair conformation of the six membered ring, where the dihedral angle between C4 and H6 would be approximately 60°, which is in agreement with the coupling they observed for $^{3}J_{C4H6}$=1.4 Hz which is based on the Karplus type relationships for $^{3}J_{CH}$ couplings.[121]

Aliev *et al.* also analysed all the $^{3}J_{CH}$ couplings in which they believed them to be in good agreement with their new proposed structure **255**.[121] They also analysed the few J_{HH} couplings in tagetitoxin. They observed a large value for $^{3}J_{H6H7}$=12.2 Hz which they suggest is in favour for the *trans* fusion of the two cycles with both protons occupying axial orientations.

Further data was collected to support their claim for the new predicted structure of tagetitoxin which can be found in the latest paper attached in Appendix A of the thesis. However, a total synthesis has still to be completed for tagetitoxin to prove which structure is in fact correct.

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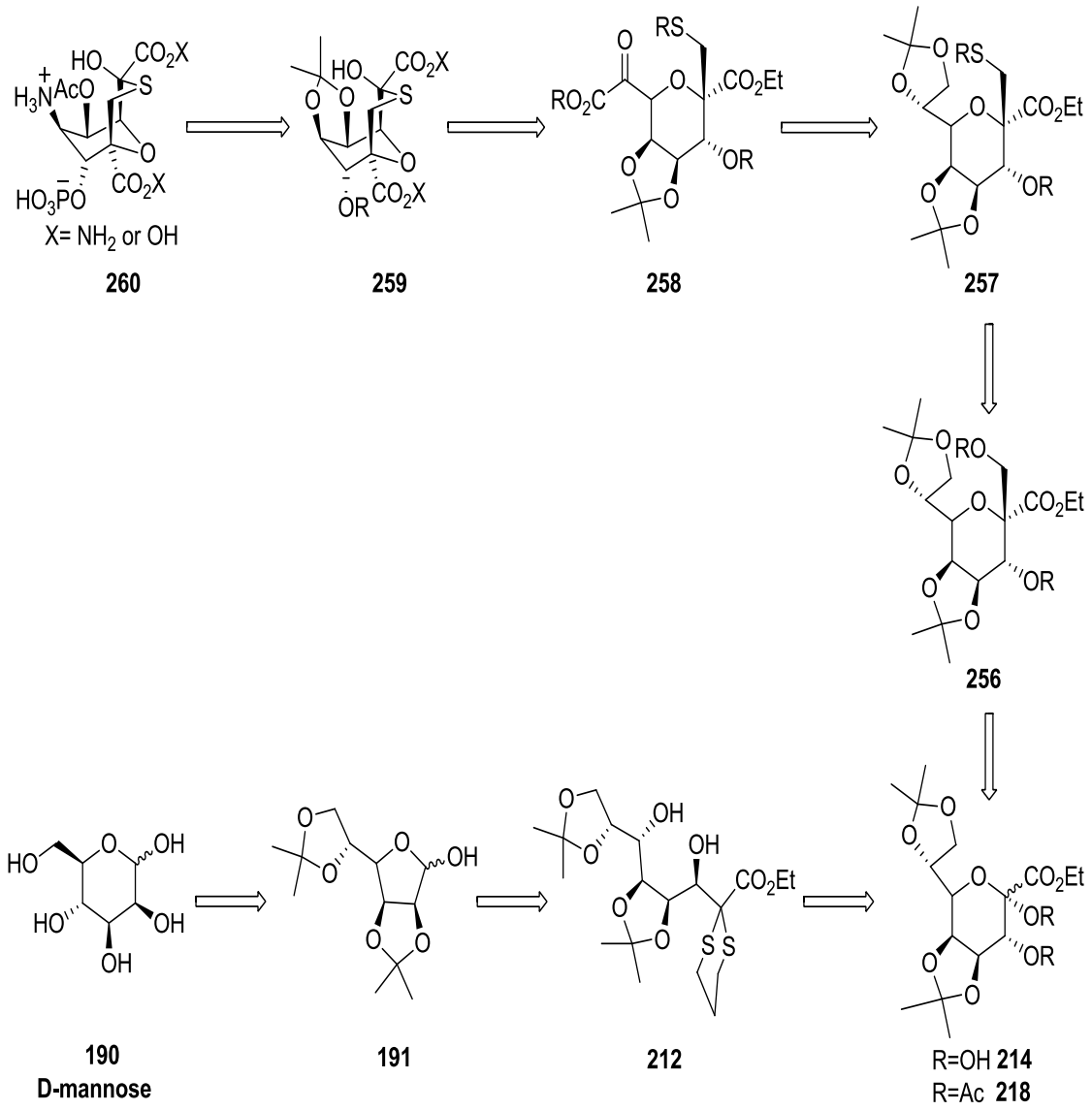
Results and Discussion:

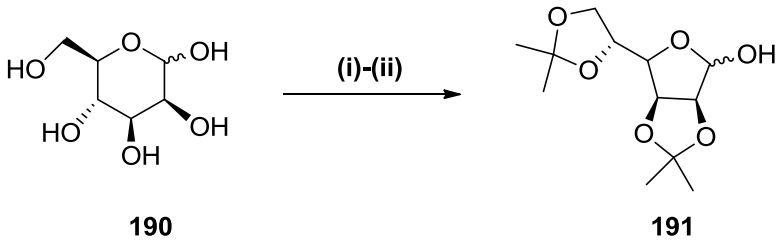
Route 1 Dithiane Route:

Several retrosynthetic pathways were envisaged. A continuation of the work already carried out within the Page group on the dithiane route is where this research began. The first two steps had previously been worked on within the group and this project began with the optimization of these steps and to continue further to complete the synthetic route towards the bicyclic structure of tagetitoxin **260** (**Scheme 58**).



Scheme 58: Retrosynthesis for the targeted bicylic structure **260**.

The first step in the synthesis involved the conversion of D-mannose into diacetone mannose DAM **191** using a literature procedure in 70-90% yield after crystallization to give the product as a mixture of anomers which was used in the next step (**Scheme 37**).[1]

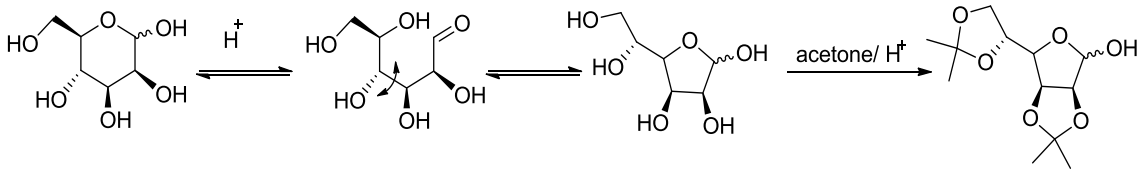


190 **191**

D-mannose

Scheme 37: i) H_2SO_4, acetone, r.t., 3 h; **ii)** Na_2CO_3, charcoal, acetone, reflux, 1 h, 92% yield

The reaction occurs through a displacement of the lactol equilibrium from the 6-membered ring to the 5-membered ring (**Scheme 59**).

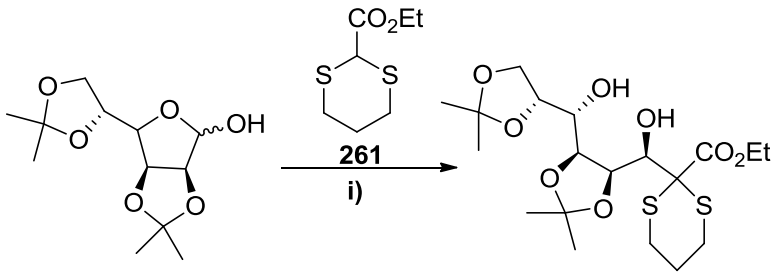


190
D-mannose

191

Scheme 59: Proposed mechanism for the formation of diacetone mannose **191** from D-mannose

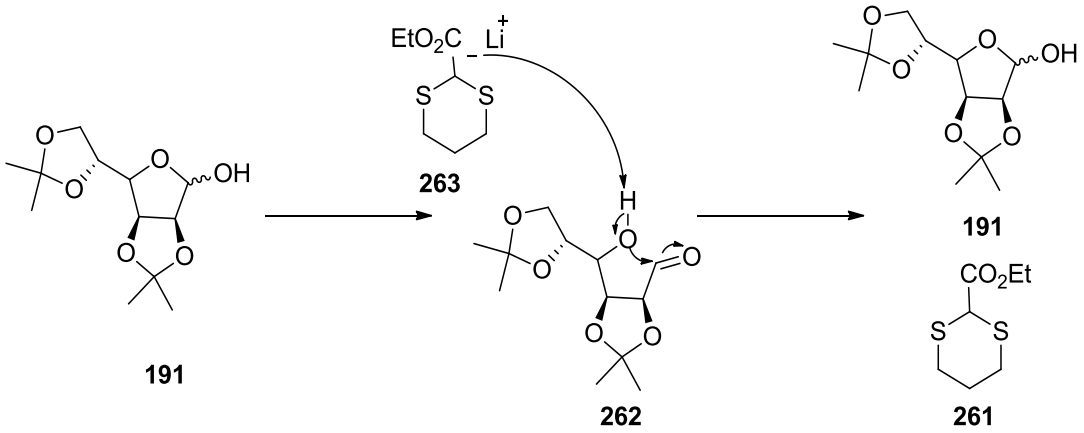
The next step involved the nucleophilic addition of a masked α-ketoester to diacetone mannose **191** using Schmidt's previously reported procedure (**Scheme 60**).[2]



191 **212**

Scheme 60: Nucleophilic addition of ethyl-1,3-dithiane-2-carboxylate to diacetone mannose **191**.**i)** LDA, $MgBr_2$, dithiane **261**, 99% yield.

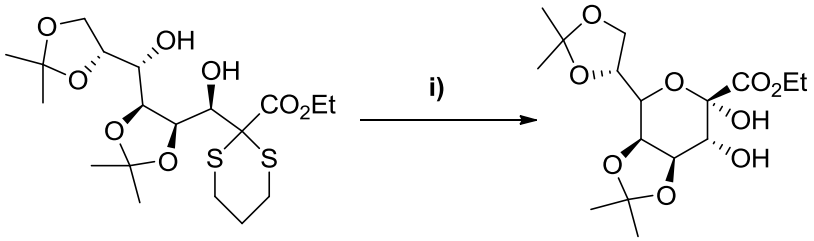
The addition of ethyl 1,3-dithiane-2-carboxylate **261** to diacetone mannose **191** proceeded smoothly in very high yields of up to 98% to give **212** after optimization. We found working with less than 10 g of diacetone mannose reduced the yield and more than three equivalents of dithiane were needed to achieve a high yield (**Scheme 60**).



Scheme 61: Proposed mechanism for the failed dithiane addition onto **191** in the absence of $MgBr_2$.

Attempts to add ethyl 1,3-dithiane-2-carboxylate **261** onto diacetone mannose **191** in the absence of $MgBr_2$ led only to starting material **191** being obtained because lithiated dithiane **263** works as a base, which leads to the ring closing of the hydroxy-aldehyde **262** (**Scheme 61**). The addition of ethyl 1,3-dithiane-2-carboxylate **261** onto diacetone mannose in the presence of $MgBr_2$ led to the formation of an organomagnesium bromide intermediate which led to nucleophilic attack to give compound **212** (**Scheme 60**).

The next step involved the removal of the dithiane moiety on **212** and the *in situ* cyclization to form the six membered pyranose **214** (**Scheme 47**).[2]

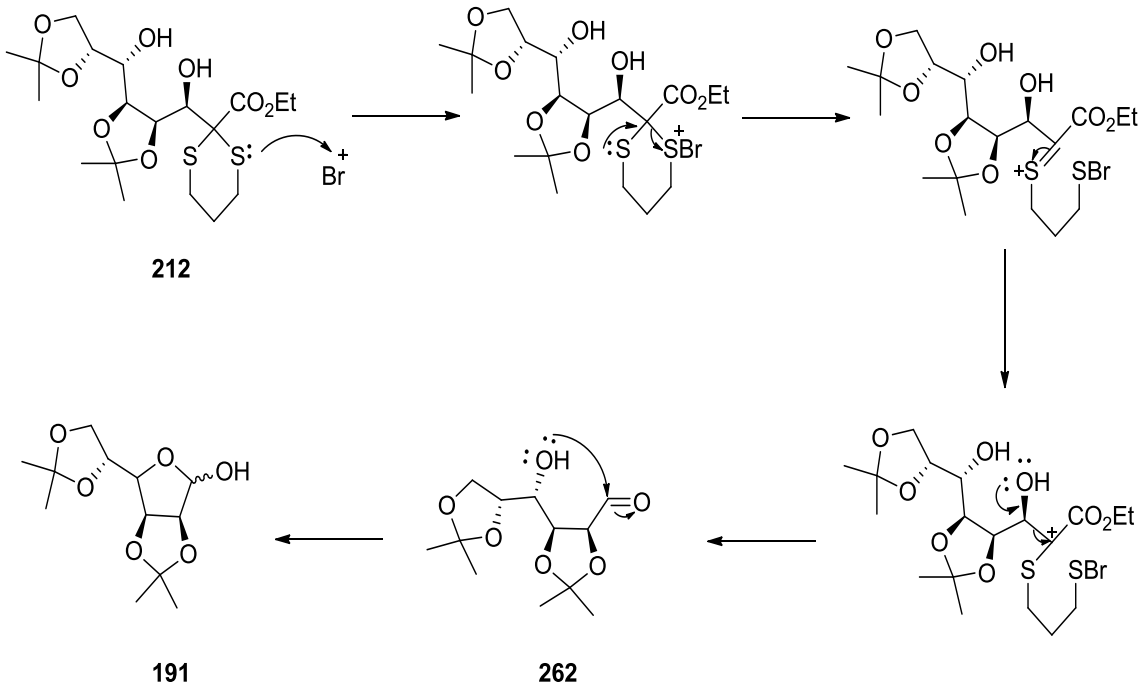


212 **214**

Scheme 47: Dethioketalization of **212** to give pyraonse **214**.
i) NBS, Acetone/water, 40% yield.

Our initial work involved the use of Schmidt's conditions for the hydrolysis of the dithiane with the use of NBS in 95% acetone. This reaction proved to be highly troublesome and failed to reproduce the yields reported by Schmidt.[2] The highest yielding method that we were able to devise involved the reaction being completed in 12 tubes in a carousel, with 0.1g of **212** in each tube using acetone and NBS at 0 °C, with a yield of between 30-40% being obtained. We decided to investigate what was happening in this reaction, and we isolated and characterized the byproduct in high yields which proved to be the starting material DAM **191**.

We postulated a reaction mechanism for its formation, shown below, and since the reaction is in equilibrium we see that the five membered ring is more favoured than the six membered ring under these conditions. We postulated that this may be due to mechanism of the NBS mediated cleavage of the ditihiane group: an intramolecular reaction is taking place which led to the removal of the entire ethyl 1,3 dithiane-2-caboxylate group. Removing this group led to the formation of the hydroxy aldehyde **262** which in turn led back to DAM **191** (**Scheme 62**).

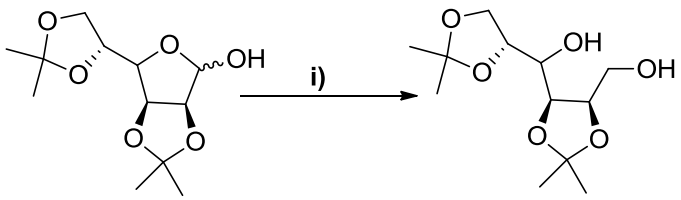


Scheme 62: Proposed mechanism for the intermolecular reaction during NBS mediated cleavage.

Having had limited success we decided to carry out a variety of deprotections of dithiane on substrate **212**, using known reactions from the literature in collaboration with Pearce using NIS, NCS, DBDMH, mercury chloride and mercury oxide but no improvement was obtained.[3]

We postulated that the yield of the reaction could be improved by the protection of the free hydroxyl groups of **200** before the dethioketalization with NBS, as we believed that the protection of the free hydroxyl group on the α-position of the dithiane would prevent the intramolecular reaction during the NBS-mediated cleavage of the dithiane and improve the yield of the reaction.

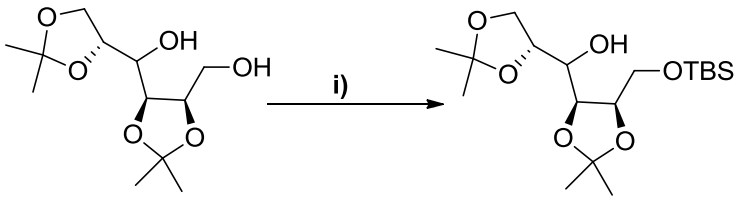
This synthetic pathway started with the reduction of the diacetone mannose **191** with $LiAlH_4$ in THF to provide 2,3-5,6-di-*O*-isopropyliden-D-mannitol **200** according to the literature in a high yield of up to 98% yield (**Scheme 63**).[4]



191 **200**

Scheme 63 : Reduction of DAM to give diol **200**
i) $LiAlH_4$, THF, 0 °C to r.t., 83% yield.

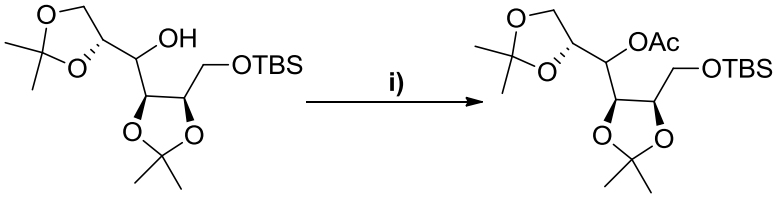
The next step carried out was the selective protection of the primary hydroxyl group of **200**, with TBSCl to give the silyl derivative **264** in a high yield of 99% (**Scheme 64**).[4]



200 **264**

Scheme 64: Selective protection of primary alcohol to give silyl product **264**
i) TBSCl, Imidazole, DMF, 96% yield.

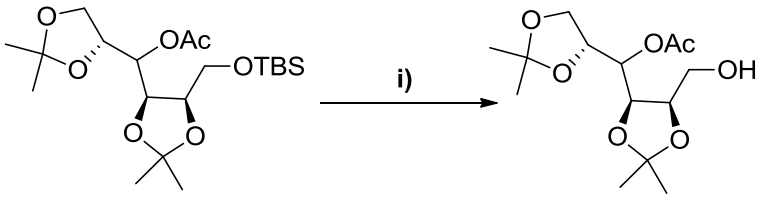
After protecting the primary hydroxyl group successfully with TBSCl, the secondary hydroxyl group of **264** was protected with an acetate group using acetic anhydride, DMAP and TEA to give compound **265** in a high yield of over 95% (**Scheme 65**).



264 **265**

Scheme 65: Protection of the free hydroxyl group of **264** with an acetate to give **265**
i) Ac_2O, DMAP, Et_3N, DCM, 90%.

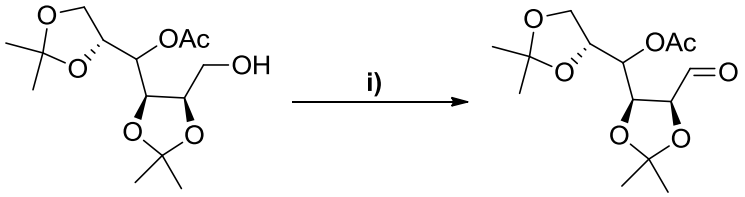
The next step was the selective deprotection of silyl group on the primary hydroxyl of **265**. The selective deprotection of the TBS group of compound **265** was carried out using TBAF which gave the corresponding alcohol **266** in a very good yield of over 90% (**Scheme 66**).



265 **266**

Scheme 66 : Deprotection of the TBS group on the primary hydroxyl group of **265** to give **266** **i)** TBAF, THF.0 °C, 78%.

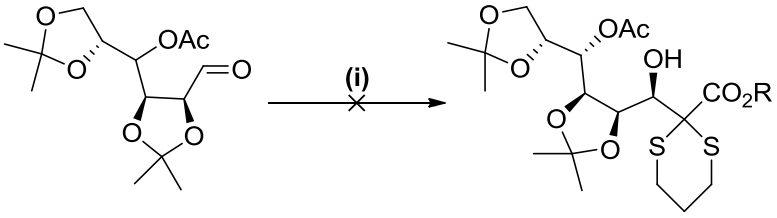
After the selective deprotection of the primary hydroxyl, the next step we wanted to carry out was to oxidize this primary hydroxyl **266** to the aldehyde **267** (**Scheme 67**).[6]



266 **267**

Scheme 67: Swern oxidaion of compound **266** to give aldehyde **267**.
i) DMSO, oxayl chloride, DCM, -78 °C for 2 h, Et_3N, -78 °C to r.t., 91%.

We completed this by carrying out a Swern oxidation on the corresponding alcohol **266** to provide the aldehyde **267** in a high yield of up to 91% (**Scheme 67**). The obtained aldehyde **267** was unstable because it could easily be converted back to the starting material diacetone mannose **191**. For that reason the aldehyde **267** was used directly for the next step without further purification.



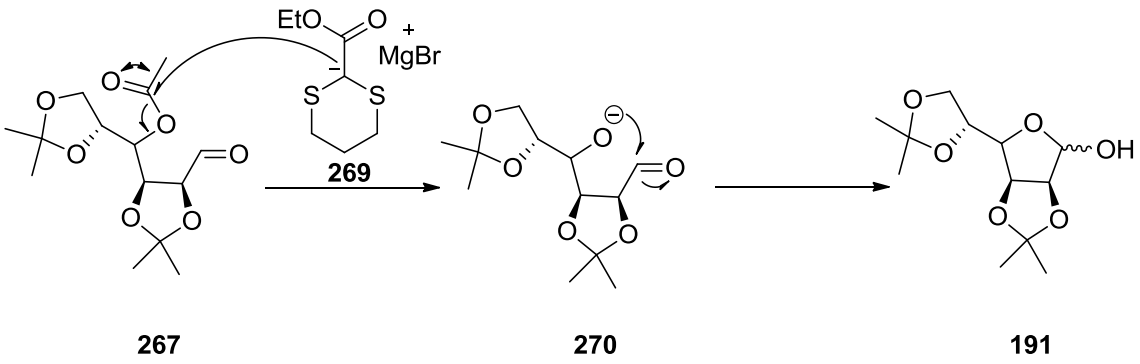
267 **268**

R=Me or Et

Scheme 68: Attempted synthesis of **268**
i) LDA, $MgBr_2$, ethyl/methyl 1,3-dithiane-2-carboxylate, THF.

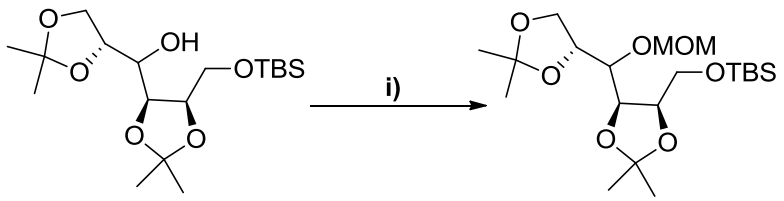
The addition of a masked α-ketoester onto the aldehyde **267** was attempted. This next step involved the addition of ethyl 1,3-dithiane-2-carboxylate or methyl 1,3-dithiane-2-carboxylate onto the aldehyde **267** to provide compound **268** (**Scheme 68**), using Schmidt's conditions.[2] However after reaction work up and purification, analysis of the NMR spectrum showed that only diacetone mannose **191** was obtained.

The only rationale for the formation of DAM **191** was that the organomagnesium bromide intermediate **269** attacked the carbonyl group of the acetate instead of the carbonyl group of the aldehyde, which led to the formation an anionic oxygen **270**. The anionic oxygen attacked the aldehyde which led to the formation of diacetone mannose **191** (**Scheme 69**).



Scheme 69: Postulated mechanism for the failed synthesis of **268**

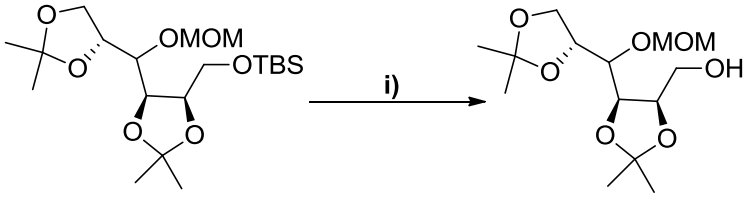
In order to overcome this problem we decided to change the protecting group from an acetate to another protecting group to prevent this side reaction from occurring. The free hydroxyl group of compound **264** was protected with MOMCl to provide compound **271** (**Scheme 70**) as described in the literature.[4]



264 **271**

Scheme 70: Protection of the free hydroxyl group of **264** to give **271**
i) 264, MOMCl, $^{i}Pr_2NEt$, DCM, 0 °C, 63%.

After protecting the free hydroxyl group of compound **264** successfully, the silyl group of compound **271** was deprotected using TBAF in THF to provide alcohol **272** (**Scheme 71**).[4]



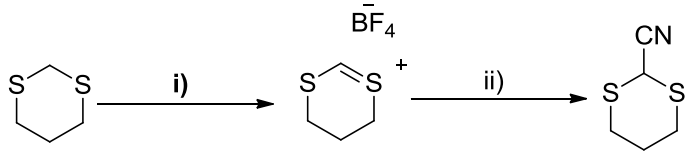
271 **272**

Scheme 71: Selective deprotection of silyl group on **271** to give **272**
i) 271, TBAF, THF, 0 °C, 79%.

We again attempted the oxidation of the alcohol to the aldehyde and the nucleophilic addition of the dithiane as described in the previous step, however we again only isolated diacetone mannose **191** and we therefore decided to look at alternative ways to reach the bicyclic core **260**.

Having had limited success with improving the yield of the dethioketalization of compound **212** we decided to try an alternative strategy in order to improve the reaction yield. This time we decided to incorporate a nitrile functionality on the dithiane instead of the ester. We could eventually carry out the hydrolysis of the nitrile to give the carboxylic acid.

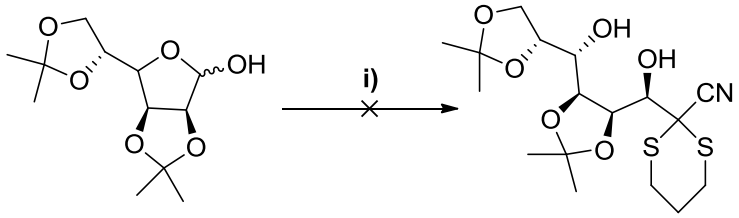
We first had to synthesize the 2-cyano-1,3-dithiane substrate **275**, which we were able to obtain using previous reported literature by the Page group on dithiane work.[7,8] 1,3-dithiane **273** was treated with triphenylcarbenium tetrafluoroborate in DCM to give the tetrafluoroborate salt **274** after work up. The next step to form the desired nitrile-derived dithiane, was the addition of TMSCN to **274** in DCM at -20 °C to give nitrile **275** in up to 60% yield for the two steps (**Scheme 72**).



273 **274** **275**

Scheme 72: Synthesis of 2-cyano-1,3-dithiane
i) $PhC^{+}BF_4^{-}$, DCM, reflux, 45 min **ii) 274**, TMSCN, DCM, -20 °C, 1 h, 58%.

The next task was the nucleophilic attack of the anion of the nitrile-dithiane onto DAM **191**. We used the same conditions as Schmidt, however we were unable to obtain the desired product **276** (**Scheme 73**) with only starting material being observed.[2] Having being unsuccessful in obtaining the desired product **276**, we believed that the lithiation of the cyano dithiane was not being achieved. Work in conjunction with Pearce was attempted to see if we were able to displace the proton on the C-2 position with a deuterium atom and by comparison of NMR to show the displacement was carried out however this proved to be unsuccessful and this route was dropped.[3]



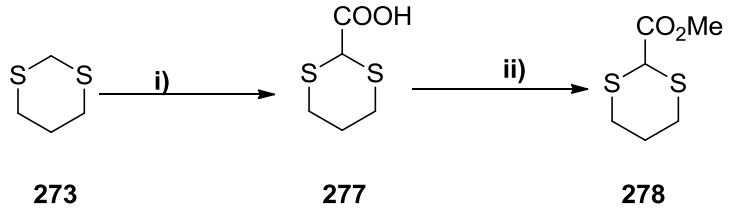
191 **276**

Scheme 73: Nucleophilic addition of **275** onto diacetone mannose **191**.
i) LDA, $MgBr_2$, **191**, THF, -20 °C to r.t.

We also obtained the methyl ester-derived dithiane to see if an alternative ester on the C-2 position of the dithiane would have an influence on the dethioketalization reaction and help improve the yield. From Schmidt's reported work, higher yields were obtained using a methyl ester rather than an ethyl ester when carrying out the dethioketalization reaction.[2]

We synthesized the methyl ester derived dithiane **278** from 1,3-dithiane **273**, following a literature procedure, with the lithiation using *n*-BuLi, followed by the addition of solid carbon dioxide and an acidic work up to give the carboxylic acid derivatized dithiane **277**. HCl gas was

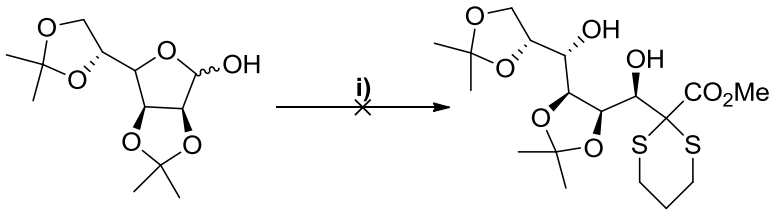
then bubbled through a solution of the carboxylic acid derivatized dithiane **277** in MeOH to give the desired methyl ester derivatized dithiane **278** (**Scheme 74**).[9]



273 **277** **278**

Scheme 74: Synthesis of methyl ester dithiane **278.**
i) 273, *n*-BuLi, CO_2(s), THF, -78 °C to r.t. 68%. **ii) 277**, HCl(g), MeOH, 10 min, 85%.

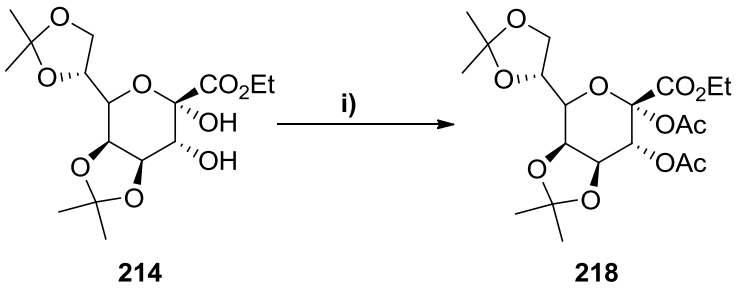
Having obtained the 2-methyl ester dithiane **278,** we used the same conditions as Schmidt for the coupling of the 2-methyl ester dithiane derivative **278** with diacetone mannose **191** however we were unable to obtain the desired compound 279 after numerous attempts using the same conditions as above (**Scheme 75**).[2]



191 **279**

Scheme 75: Nucleophilic addition of 2-methyl ester dithiane to diacetone mannose **191**.**i)** LDA, $MgBr_2$, dithiane **278,** THF, -20 °C to r.t.

Despite the poor yield obtained in the synthesis of ethyl ester **214** (30-40% after optimization) and the unsuccessful synthesis of methyl ester 279, we decided to carry on to the next step: the selective protection of the secondary hydroxyl at the C-3 position, followed by the activation and removal of the tertiary hydroxyl at the C-2 position, to see if our methodology would work.

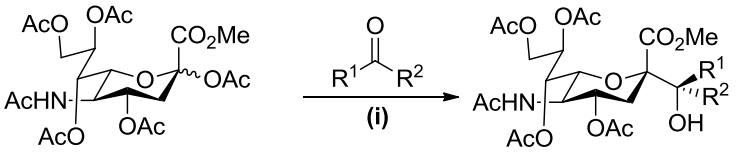


214 **218**

Scheme 76: Acetate protection of pyranose **214**
i) Ac_2O, DMAP, Et_3N, DCM, 84%.

Converting the free hydroxyl groups to the corresponding acetate groups was highly successful, often taking place in 99% yield with Ac_2O, Et_3N and DMAP (**Scheme 76**). The diacetylated compound **218** was also a crystalline material, enabling an X-ray crystal structure determination to be made, proving the stereochemistry. This was carried out previously within the group.[3]

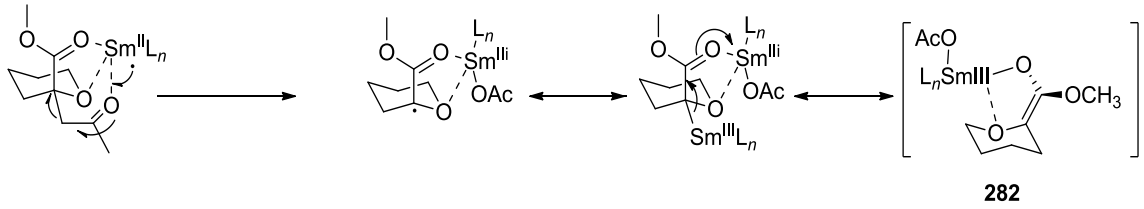
We were then tasked with the removal of the anomeric hydroxyl group on pyranose **218.** A paper in the literature described a samarium diiodide-promoted coupling of anomeric acetates with carbonyl compounds.[11] Malapelle described how to convert the anomeric acetate of their substrate *N*-acetylneuraminic acid derivative to the corresponding alcohols using samarium diiodie and a series of different aldehydes and ketones (**Scheme 77**).[10]



280 **281**

Scheme 77: Samarium diiodie coupling between acetate and carbonyl compounds
i) SmI_2, 3 equivalents, THF, r.t [10]

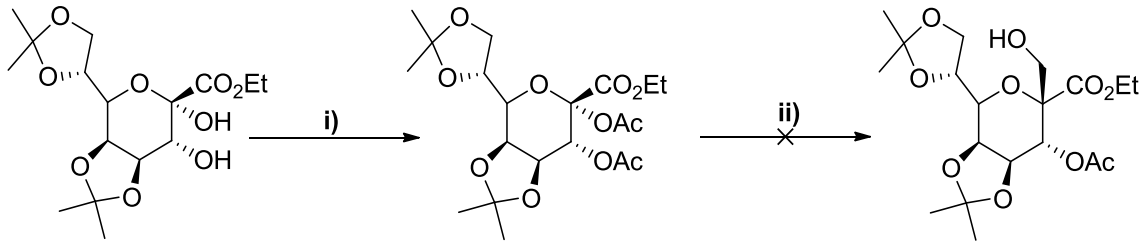
Malapelle also proposed a mechanism for the formation of the samarium enolate **282** that reacted with the carbonyl substrates performed by the group (**Scheme 78**).



282

Scheme 78: Proposed mechanism for the formation of the samarium enolate **282.**[10]

We decided to apply this methodology to compound **218** to see if we could remove the anomeric hydroxyl group and introduce a methylene alcohol group, all in one step. Modifying the conversion of the diacetate **218** to the alcohol could be done using samarium diioide, and formaldehyde could be used as the carbonyl source.

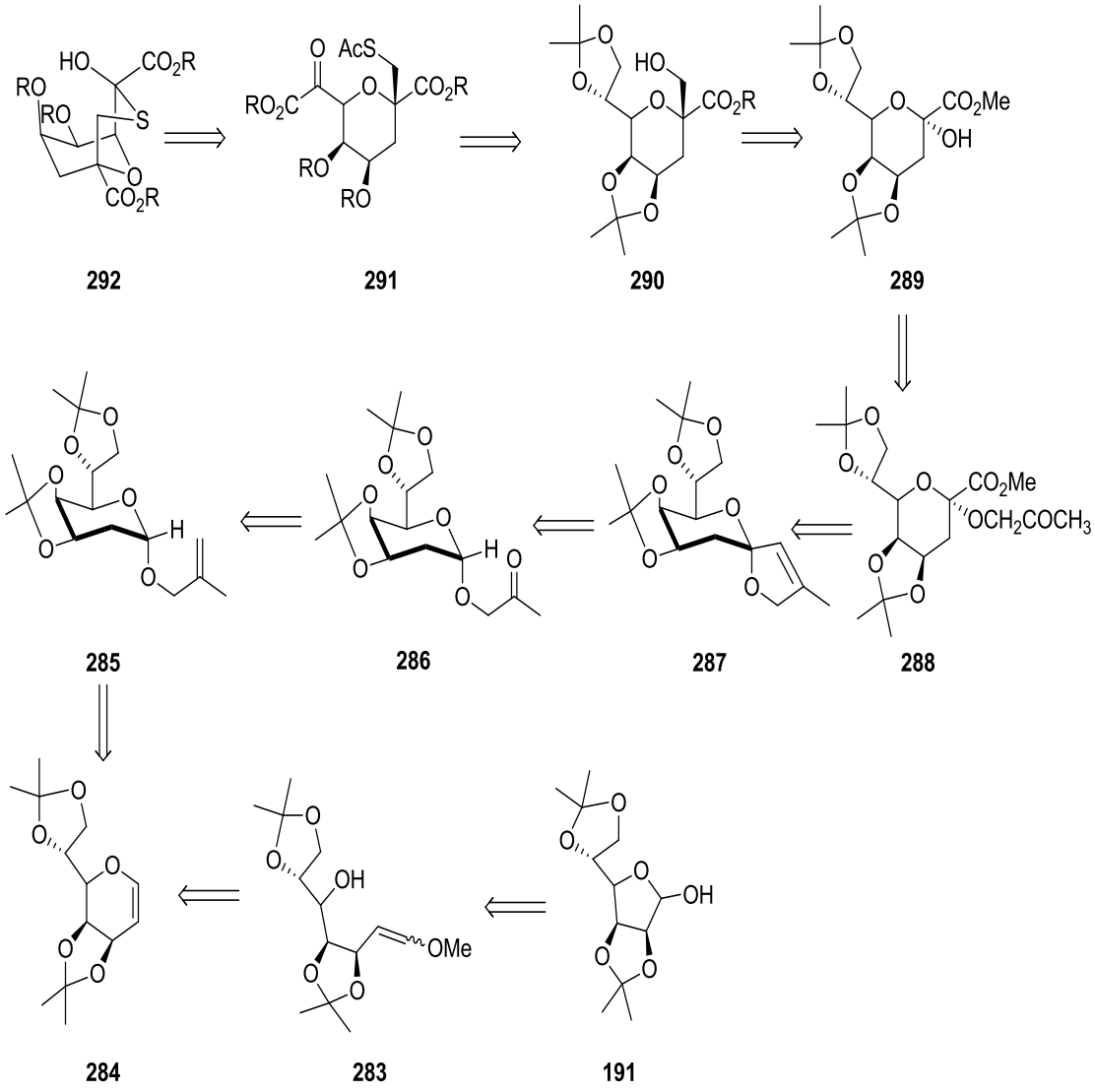


214 **218** **215**

Scheme 49: Our attempted synthesis of **215 i)** Ac_2O, Et_3N, DMAP, DCM **ii)** SmI_2, CH_2O, THF

However after numerous attempts we were unable to achieve the removal of the anomeric hydroxyl moiety or introduce the methylene alcohol group to give **215** using samarium diioide in the presence of paraformaldehyde (**Scheme 49**). We initially thought the issue might have been with the commercially available samarium iodide solution in THF we were using since we noticed it degraded rapidly and tried to make our own using literature procedures, however we were unable to obtain any Samarium Iodide solution which was noted by the absence of a dark blue solution. Since working with samarium iodide was extremely difficult we decided to move onto other routes to try and synthesize the desired bicyclic core **260**.

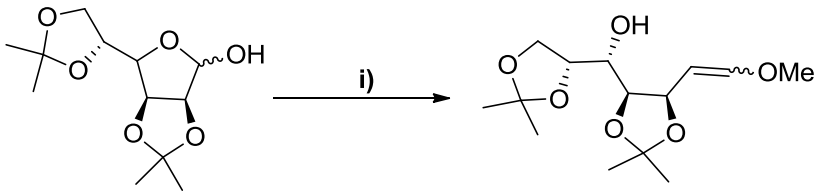
Route 2 KDO Route:



Scheme 79: Retrosynthesis for KDO route starting with DAM **191.**

Following on from the poor yields obtained in route 1 the next route attempted in our strategy (**Scheme 79**) involved again D-mannose and its conversion into diacetone mannose **191**.[1] Alkene **283** was targeted, a product of the Wittig condensation between (methoxymethyl)triphenylphosphonium chloride and diacetone mannose. We synthesized the required Wittig salt on a 300 g scale using literature procedures by reacting triphenylphosphine with MOMCl in toluene under reflux for 24 hours.[12]

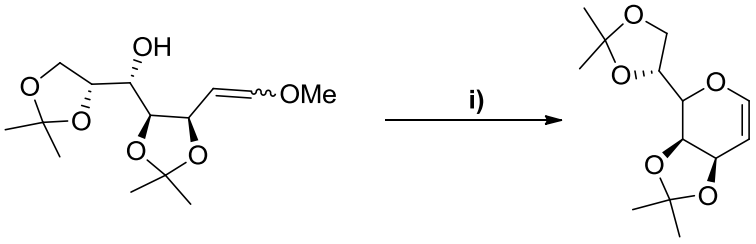
Alkene **283**, was prepared using the Wittig reaction with the Wittig salt derived from (methoxymethyl)triphenylphosphonium chloride, potassium *tert*-butoxide and diacetone mannose **191** to give alkene **283** in over 93% yield as a mixture of 70:30 *E/Z* isomers.[13]



191 **283**

Scheme 80: Wittig product **283**
i) 191, $Ph_3PCH_2(OMe)Cl$, *t*-BuOK, THF, reflux, 4 h, 96%

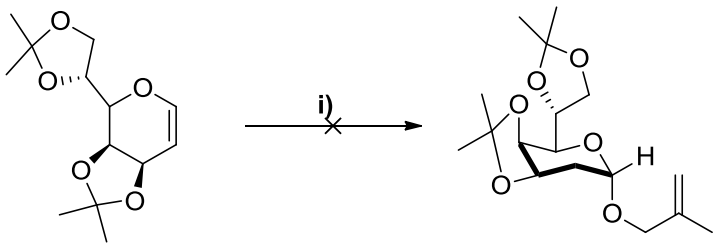
Pyrolysis of **283**, using a bulb-to-bulb distillation apparatus under reduced pressure in the presence of a catalytic mercury acetate led to the six membered alkene ring **284** through an intramolecular vinyl esterification reaction in up to 67% yield (**Scheme 81**).[14] After optimization, a yield of over 70% was obtained when the reaction was carried out on a one gram scale.



283 **284**

Scheme 81: Formation of the six membered pyranose **284**.
i) 283, $Hg(OAc)_2$, DCM, 110 °C, 20 mmHg, 2 h, 67%.

After forming the six membered alkene ring **284**, the next step in the synthesis was the acid catalysed addition of the methallyl alcohol in the presence of TPHB, to give the α-glycoside **285** with complete diastereoselectivity, however we were unable to obtain the desired product **285** despite replicating the literature conditions numerous times (**Scheme 82**).[15] .

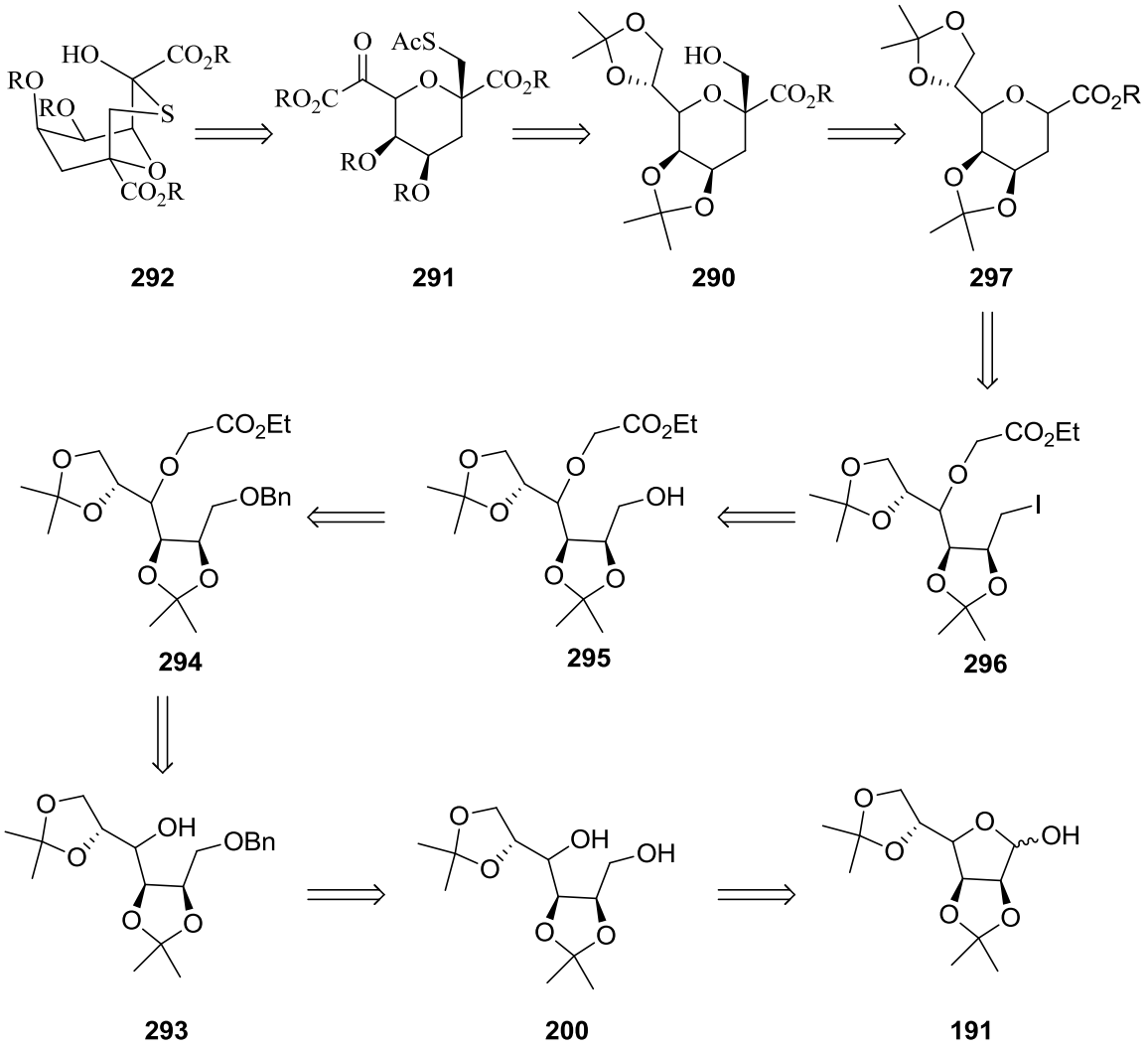


284 **285**

Scheme 82: Attempted formation of glycoside **285**
i) 284, methallyl alcohol, TPHB (3 mol%), DCM, r.t., 15 min, 81%

After being unable to obtain **285** we decided to abandon this route to obtain KDO and move on to alternative routes to obtain the desired bicyclic compound **292**.

Route 3 Alternative route towards KDO



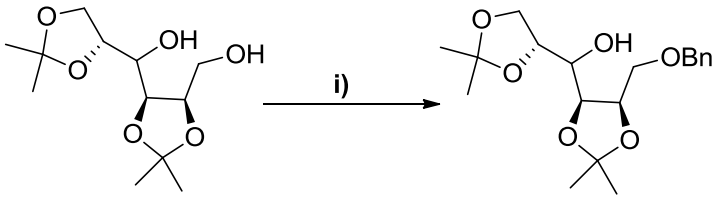
Scheme 83: Retrosyntheic analysis from **191** using alternative KDO method

While trying to improve the synthesis and form the bicyclic structure **292** we came across a paper in the literature on an alternative route towards the synthesis of KDO by Ohrui and co-workers.[5] They had proposed the construction of the tetrahydropyran ring of 2-deoxy-β-KDO by an intramolecular C-C bond formation.[5] They had selected a 2,3-O-isopropylidene-4-*O*-alkoxycarbonylmethyl-D-mannitol derivative having a leaving group on the C-1 position as the key intermediate. They had tried placing a tosyl group on this position; however the authors describe the problems encountered with the tosyl, with the major product being starting material. After a slight modification with Iodine and triphenylphosphine in DCM they were able to obtain the 1-iodide product in up to 77% yield with only a slight amount of the starting material recovered. The last part in obtaining the desired KDO involved the intramolecular C-C bond formation using lithium diisopropylamide (LDA) in THF at -75 °C to give the 2-deoxy-β-Kdo derivative stereospecifically in 84% Yield.[5] We decided to apply this methodology to our substrate in the hope of achieving **292**.

The first step was the reduction of diacetone mannose **191** to the diol **200** using lithium aluminium hydride in THF in 98% yield as seen above (**Scheme 63**).

We then introduced a base stable protecting group on the O-1 position using a benzyl group in order to introduce an ethoxycarbonylmethyl group on the O-4 position. The authors had tried a TBDMS group previously; however extensive *O*-desilylation was observed when this substrate was treated with sodium hydride and ethyl bromoacetate.[5]

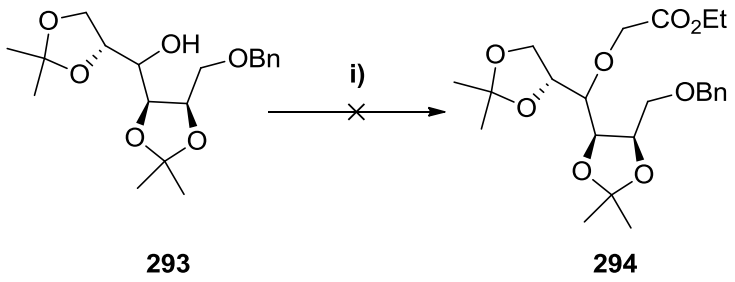
In order to achieve the benzylation of **200**, we used benzyl bromide in the presence of sodium hydride in THF to give the benzyl protected **293** in a high yield of 98% (**Scheme 84**).[5]



200 **293**

Scheme 84: Benzylation to give **293**
i) 200, NaH, DMF, $BnBr_2$, 0 °C to r.t., 90%.

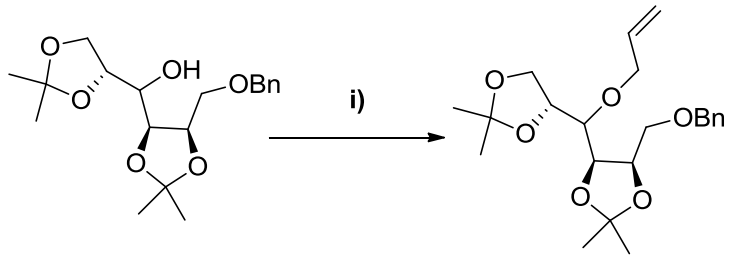
We hoped treatment of **293** with ethyl bromoacetate in the presence of sodium hydride in THF would give **294** in a good yield like Ohrui and co-workers achieved. However, after numerous attempts using their conditions and varying the amount of sodium hydride or ethyl bromoacetate we were unable to synthesise the desired compound and the route had to be abandoned (**Scheme 85**).



293 **294**

Scheme 85: Treatment of **293** with ethyl bromoaceate
i) 293, NaH, THF reflux 1h, $CH_2BrCO_2C_2H_5$, 0 °C, 6 h at r.t.

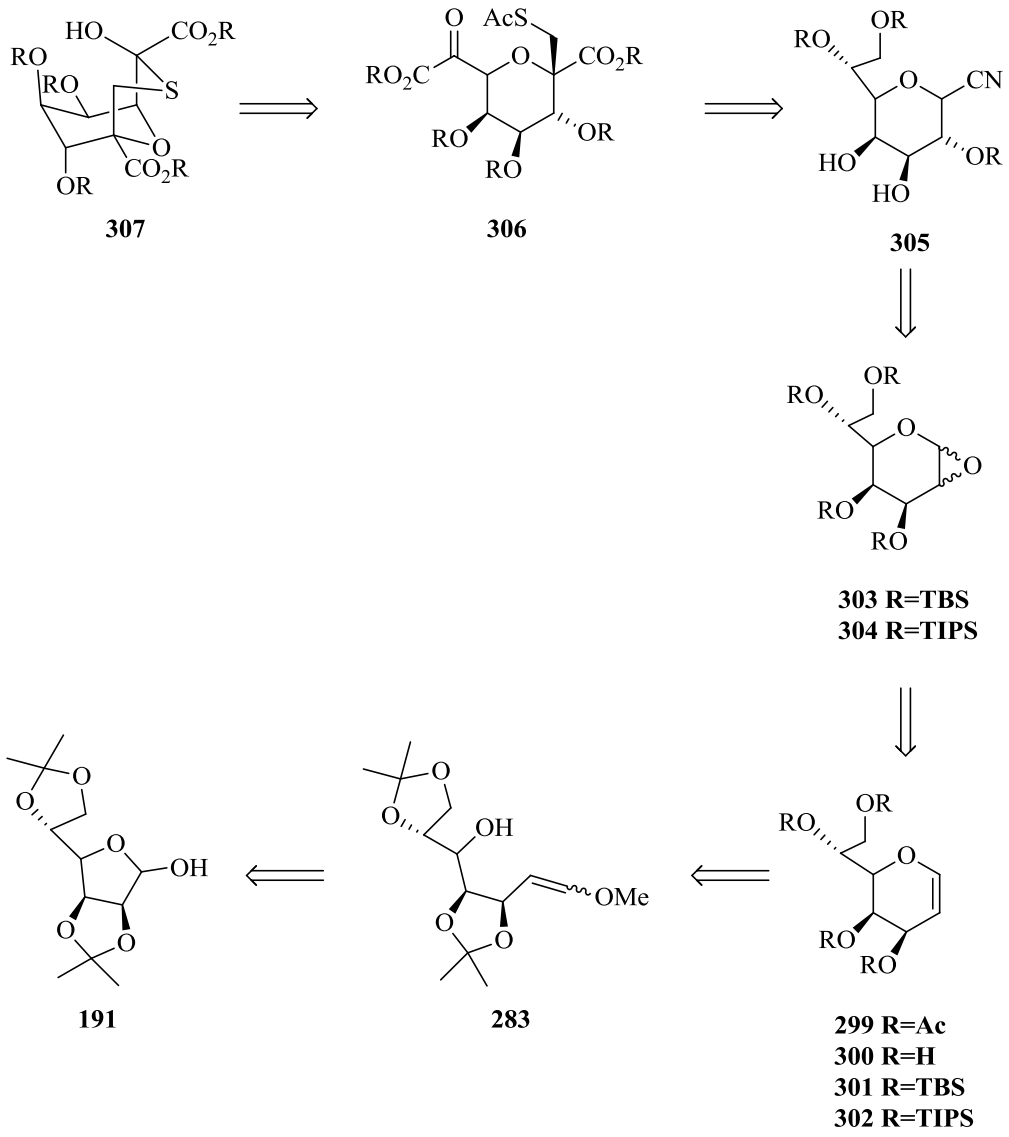
We also were able to introduce allyl group on the O-4 position of **293** using sodium hydride and allyl bromide in DMF and were able to obtain the desired substrate **298** in 96% yield (**Scheme 86**). Having obtained **298** we had hoped to carry out modifications to the allyl group, however we decided to continue with other routes and this route could be further explored in the future.



293 **298**

Scheme 86: Treatment of **293** with allylbromide.
i) 293, NaH, DMF, allylbromide, 0 °C, 1.5 h, 96%.

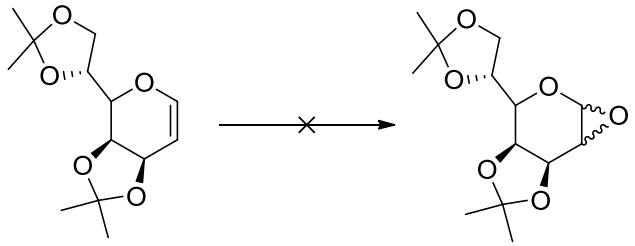
Route 4 Epoxidation Route



Scheme 87: Retrosynthesis for the targeted bicylic structure **307**

As described previously (**Scheme 80-81**) the key intermediate **284** was prepared on a 50g scale in up to 70% yield.

We then wanted to carry out the epoxidation of the alkene, which was tried on the protected alkene **284** but could not be achieved either with *m*-CPBA or other oxidants with only starting material being observed (**Scheme 88**). After numerous attempts and conditions and varying the equivalents of the oxidant, we were unable to form the epoxide **308** from the alkene **284** (**Table 4**).

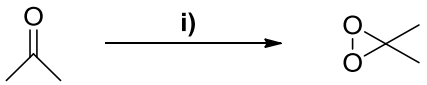


Scheme 88: Attempted synthesis of **308**

Reagents	Product
***m*-CPBA (1 equiv.), DCM, 0 °C**	SM
***m*-CPBA (1.5 equiv.), DCM, 0 °C**	SM
***m*-CPBA (2 equiv.), DCM, 0 °C**	SM
DMDO (0.03 M) (1.2 equiv.), DCM, 0 °C	SM
DMDO (0.03 M) (2.4 equiv.), DCM, 0 °C	SM

Table 4: Expoxidation conditions attempted on alkene **284**

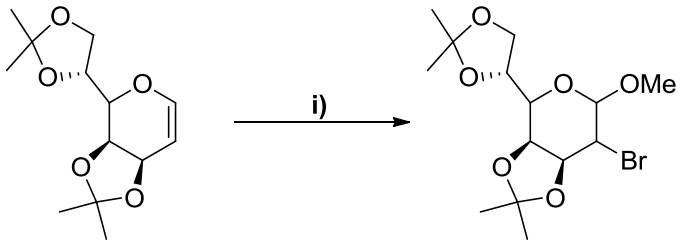
We then decided to make some freshly prepared DMDO **310** which we were able to make from acetone, $NaHCO_3$, and oxone **(Scheme 89)**.[16] However, again even after numerous attempts, with different amounts of DMDO, we were again unable to synthesize the epoxide **308** from the alkene **284** (**Table 4**).



309 **310**

Scheme 89: Sytheisis of DMDO **310**
i) 309, oxone, $NaHCO_3$, H_2O, 0 °C to 30-40 °C, 2 h, 5%.

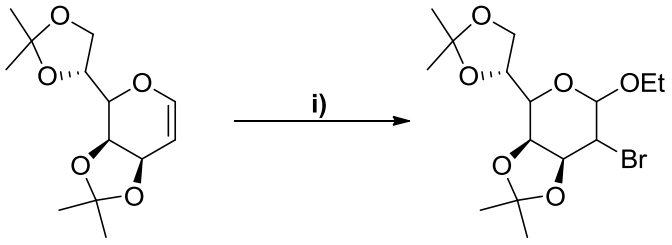
After the unsuccessful epoxidation of the alkene **284** we decided to remove the isopropylidene groups and use other protecting groups as we believed that the isopropylidene groups could be causing some steric hindrance and prevent the epoxidation from happening. In order to be able to deprotect the isopropylidene groups and not affect the alkene we first had to carry out a bromination with NBS and MeOH of the alkene **284** to give the methoxy bromo derivative **311** in up to 96% yield (**Scheme 90**).[17] We noticed that anhydrous methanol was needed in order for the reaction to take place.



284 **311**

Scheme 90: Synthesis of methoxy-bromo derivative **311**
i) 284,NBS, MeOH, -50 °C to r.t. over 12 h, 94%.

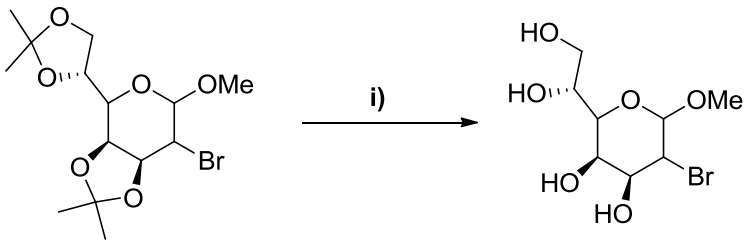
We also synthesised the ethoxy-bromo derivative **312,** using ethanol instead of methanol and we obtained the ethoxy-bromo derivative **312** in 91% yield (**Scheme 91**).



284 **312**

Scheme 91: Synthesis of ethoxy-bromo derivative **312**
i) 284,NBS, EtOH, -50 °C to r.t. over 12 h, 91%.

Having obtained substrate **311** we were then tasked with the deprotection of the isopropylidene groups on pyranose **311**. We came across a paper in the literature where both isopropylidene groups had been removed using TFA in DCM on a different compound and decided to try these conditions on our substrate **311**. We were able to obtain the deprotected pyranose compound **313** in quantitative yields (**Scheme 92**).[2]

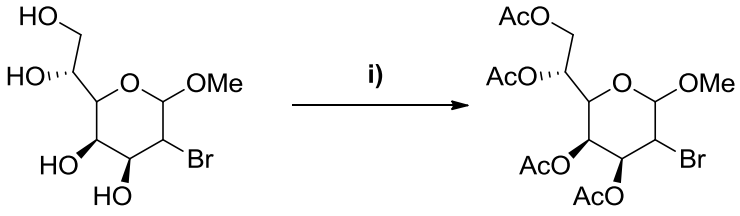


311 **313**

Scheme 92: Deprotection of the isopropylidene groups to form **313**
i) 80% TFA, **311**, DCM, 90%.

Having successfully deprotected both isopropylidene groups and having obtained a pure sample of compound **313,** we then wanted to look at introducing different protecting groups on the hydroxyl groups and to reform the alkene to carry out the epoxidation of the alkene or to introduce a cyano functionality on the anomeric carbon which we could hydrolyse to the carboxylic acid.

We decided to look at acetate protecting groups on the deprotected compound **313** to see the effect on forming the epoxide after the alkene was reformed. We took substrate **313** and carried out standard acetate protection conditions using 4 equivalents of acetic anhydride with DMAP in anhydrous DCM to give tetra-acetate pyranose **314** in over 90-94% yield (**Scheme 93**).

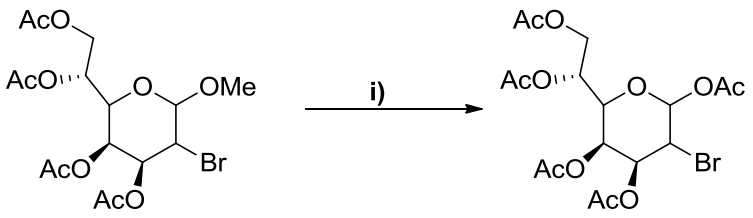


313 **314**

Scheme 93: Acetate protection of the free hydroxyls to give **314**
i) Ac_2O, DMAP, TEA, **313**, DCM 0 °C to r.t. 2 h, 77%.

We were then tasked with reformation of the alkene to carry out the epoxidation. Consulting the literature, we came across a paper where the author had been able to carry out the reformation of the alkene on a substrate similar to **314**, except with an acetate on the anomeric position and a bromine at the C-2 position. They used zinc dust in the presence of sodium acetate acetic acid water mixture and due to the affinity of zinc to bromine they reformed the alkene. In order to carry out the reaction using our substrate we had a problem since we had a methoxy group on the anomeric position. We decided to carry out the reaction on the methoxy substrate **314**, however we were unable to reform the alkene with only starting material observed.[17]

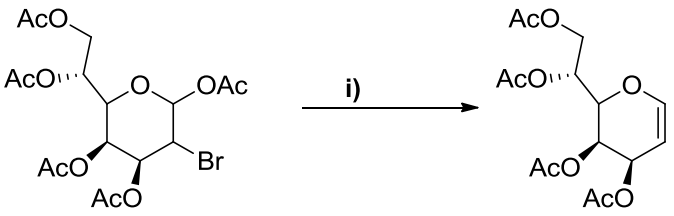
In order to reform the alkene, we first needed to convert the methoxy group on the anomeric position to acetate and form 3**15**. This was obtained using a trace amount of sulfuric acid in acetic anhydride. After dilution with chloroform and washing with an ice water mixture, base and water, we dried the organic layer and after chromatography we were able to obtain the desired penta acetate **315** in quantitative yield (**Scheme 94**).[18]



314 **315**

Scheme 94: Conversion of the anomeric methoxy to an acetate **315**
i) 314, Ac_2O, 2% H_2SO_4 in Ac_2O, r.t. 2 h, 85%

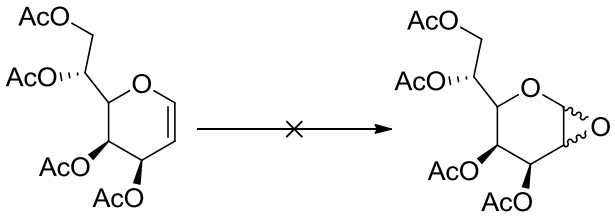
After the formation of the acetate on the anomeric carbon **315** we then attempted to reform the alkene. The usage of zinc dust in the presence of sodium acetate and acetic acid led to the formation of the tetra-acetate alkene **299** (**Scheme 95**).



315 **299**

Scheme 95: Formation of alkene **299**
i) Zn (dust), AcONa, AcOH, H_2O, $CuSO_4$(Sat), **315**, Ac_2O, 82%.

Having formed the tetra-acetate alkene **299** we then wanted to see if we would be able to afford the epoxide **316**, which we were hoping to ring open with TMSCN in the presence of a Lewis acid to introduce a cyano group on the anomeric group, which we hoped to hydrolyse to the carboxylic acid (**Scheme 96**).



299 **316**

Scheme 96: Attempted epoxidiation of **316**

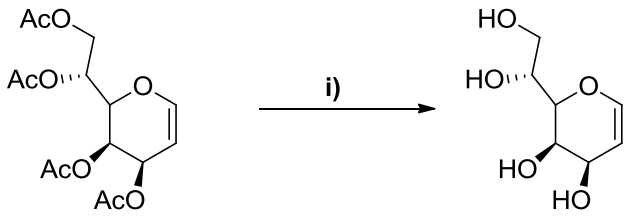
The first reaction we tried involved the epoxidation of the tetra acetate alkene **299** using *m*-CPBA. Again, after many attempts we were unable to form the desired expoxide **316**. We again tried DMDO on this substrate but unfortunately after numerous attempts we were unable to achieve the desired epoxide **316** (**Table 5**).

Reagents	Product
***m*-CPBA (1 equiv.), DCM, 0 °C**	SM
***m*-CPBA (1.5 equiv.,) DCM, 0 °C**	SM
DMDO (0.03 M) (1.2 equiv.) , DCM, 0 °C	SM
DMDO (0.03 M) (2.4 equiv.) , DCM, 0 °C	SM

Table 5: Epoxidation conditions attempted on alkene **299**

We then decided to try silyl groups to see the effect they might have on forming the epoxide. We started this time with the tetra acetate alkene **299.** A simple deprotection was carried out

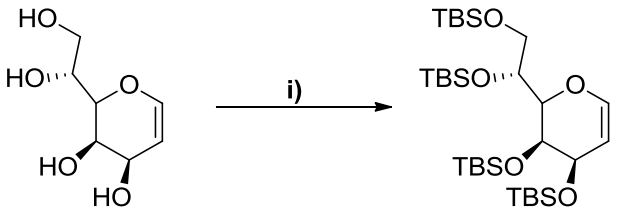
using an excess of sodium methoxide in anhydrous methanol to achieve the free hydroxyl groups on compound **300** and after flash chromatography we obtained the product **300** in 95% yield (**Scheme 97**).



299 **300**

Scheme 97: Deprotection of the acetates to form **300**
i) 299, DCM, NaOMe, MeOH, 95%.

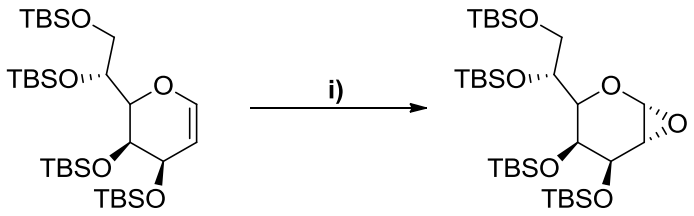
The next step involved the protection of the free hydroxyl groups of alkene **300** using over 4 equivalents of TBSCl and imidazole in DCM to achieve the tetra *tert*-butyl silyl alkene **301** in up to 60% yield (**Scheme 98**).[19] We again decided to carry out the epoxidation of alkene **301,** firstly with *m*-CPBA, but again we were unsuccessful.



300 **301**

Scheme 98: *tert*-butyldimethylsilyl protection to form **301**
i) 300, TBSCl, DMF, Imidazole, 60%

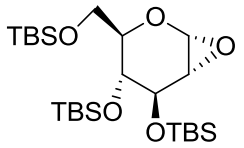
After the disappointment of being unable to get the epoxidation reaction to work with *m*-CPBA, we came across some papers in the literature where Danishefsky had carried out epoxidation of glucals with similar protecting groups with DMDO, and had been very successful.[20–22] We carried out the epoxidation of the alkene **301** with DMDO and to our pleasure we obtained the desired epoxide **303** in quantitative yield after around 2 hours at 0 °C whilst monitoring the reaction completion by tlc (**Scheme 99**).



301 **303**

Scheme 99: Epoxidation of alkene **301** to form epoxide **303**
i) 301, DMDO**,** DCM, 0 °C, quantitative.

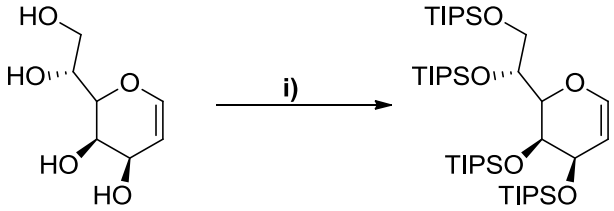
We were able to complete the assignment of the stereochemistry using NMR analysis and comparing it to previous work in the literature on similar susbtrates.[21] Based on the ^{1}H NMR spectrum assignment we obtained a doublet for the proton in the H-1 position with a coupling constant of 2.2 Hz at 4.90 ppm and a triplet for the proton on the H-2 position with a coupling of 2.1 Hz at 2.79 ppm. Comparing this to Danishefsky α-epoxide substrate **317** in which he obtained a doublet for the proton on the H-1 position with a coupling constant of 2.20 Hz at 4.90 ppm, and he also obtained a doublet for the H-2 position with a coupling constant of 2.45 Hz at 2.92 ppm (**Figure 6**).[21] Danishefsky had run his ^{1}H NMR on a 250 MHz NMR compared to ours which was run at 500 MHz. Danishefsky had also predicted his compound to be the α-epoxide. Within the paper Danishefsky stated that while the NMR spectra of the crude product did not rigorously define the stereochemical sense of epoxidation, this point was established by a sequence of methanolysis followed by acetylation to prove it with the α-epoxide subjected to methanolysis and acetylation to give the β-product. When their β-epoxide was subjected to methanolysis and acetylation the α-product was obtained. When the coupling constants we obtained were compared to the literature values, we could show that the α-epoxide **303** is the only product obtained (**Scheme 99**). For the β-epoxide on our substrate, we should observe larger coupling constants for the H-1 and H-2 protons of between 7-10 Hz, which we did not observe. Danishefsky also observed similar coupling constants for his β-epoxide derivatives of 7-10 Hz compared to his α-epoxides with coupling constants of 2-3 Hz.[21]



317

Figure 6: Epoxide substrate synthesized by Danishefsky et al.[22]

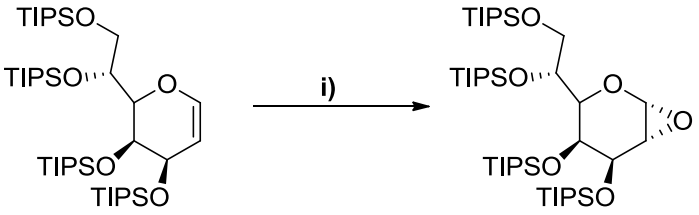
Having being successful we also introduced the TIPS protecting groups on the free hydroxyl groups of alkene **300** using over 4 equivalents of TIPSCl in the presence of imidazole to obtain the *tetra*-TIPS alkene product **302** in around 50% yield (**Scheme 100**).[19]



300 **302**

Scheme 100: TIPS protection to form alkene **302**
i) 300, TIPSCl, Imidazole, DMF 51%

Having obtained another substrate, we carried out the epoxidation of the alkene **302** with DMDO and to our pleasure we obtained the desired epoxide **304** in quantitative yield after 2 hours at 0 °C whilst monitoring the reaction completion by tlc (**Scheme 101**). Simple evaporation under reduced pressure removed the acetone byproduct and after drying under high vacuum we were able to obtain pure **304** without further flash chromatography.



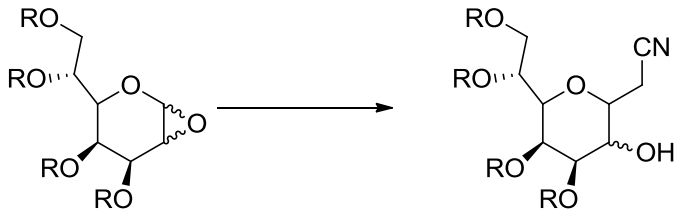
302 **304**

Scheme 101: Epoxidation of TIPS alkene **302** to form **304**
i) 302, DMDO**,** DCM, 0 °C, quantitative.

We were able to complete the assignment of the stereochemistry using NMR analysis and comparing it to previous work in the literature on similar substrates and the work carried out above on **303**.[21] Based on the ^{1}H NMR spectrum assignment we obtained a doublet for the proton in the H-1 position with a coupling constant of 2.4 Hz at 4.91 ppm and a doublet for the proton on the H-2 position with a coupling of 2.4 Hz at 2.86 ppm. Comparing this again to Danishefsky α-epoxide substrate in which he obtained a doublet for the proton on the H-1 position with a coupling constant of 2.20 Hz at 4.90 ppm, and he also obtained a doublet for the H-2 position with a coupling constant of 2.45 Hz at 2.92 ppm we can show that we have obtained the α-epoxide as a single product **304** (**Scheme 101**).

Having obtained both epoxides **303** and **304** from DMDO we then wanted to introduce a cyano group on the anomeric position and then hydrolyse this cyano group to an acid or ester functionality.

We began trying to ring open the epoxides **303** and **304** using TMSCN in the presence of a Lewis acid, however after numerous attempts shown (**Table 6**) below and trying many different conditions and Lewis acid combinations or using TMSCN on its own we were unable to obtain the desired compound **318** and only decomposition was observed (**Scheme 102**).[23–25]



303 R=TBS or
304 R=TIPS

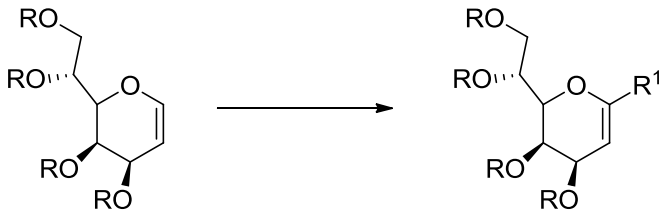
318

Scheme 102: Ring opening of an epoxide with conditions listed in table 2 below

Reagents	Product
TMSCN, Al(O^{i}Pr)$_3$, Hexane	Decomposition
Me$_2$PhSiCH$_2$MgCl, CuI, THF	Decomposition
TMSCN	Decomposition
Vinylmagnesium Bromide, DCM -60 °C,	Decomposition

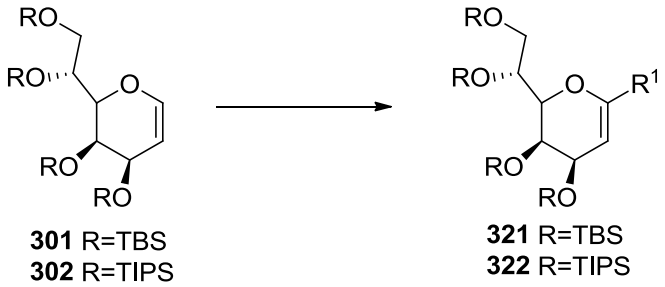
Table 6: Attempted ring opening of epoxides conditions on **303** and **304**

Having been unable to ring open epoxides **303** and **304** using conditions described above we decided to change strategy and introduce a functional group on alkenes **301** and **302**. In order to check if this strategy could be carried out deprotonation was attempted on the anomeric position using *t*-BuLi. We quenched the reaction with D_2O and we were able to see the disappearance of the anomeric proton in the NMR spectrum. We were able to obtain the deuterated compounds **319** and **320** (**Scheme 103**)**.**



Scheme 103: Deprotonation of the anomeric position of alkene **301** and **302** and workup with D_2O

Having discovered that it was possible to deprotonate at the anomeric position, we examined a range of reactions using a range of bases from *t*-BuLi to Schlosser's base, a mixture of *n*-BuLi and *t*-BuOK, to carry out the deprotonation on alkenes **301** and **302** (**Scheme 104**). Quenching the reactions with reagents such as ethyl or methyl chloroformate should allow us to introduce ester moieties.



Scheme 104: Attempted deprotonation of the anomeric position of alkene **301** and **302** and workup with reagents listed below

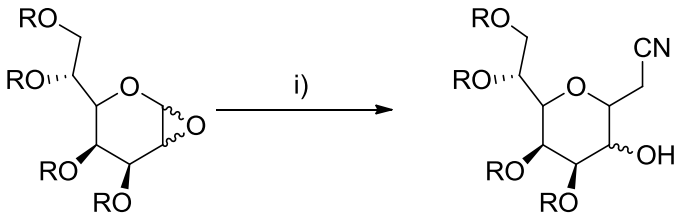
Unfortunately, we were unable to introduce an ester moiety, with only starting material observed. We also tried another procedure from the literature with the use of

paraformaldehye with an excess of *t*-BuLi, but again we were unable to obtain the desired functionalization of the anomeric position of the alkene. After several attempts listed (**Table 7**) below we were unable to introduce a functional group on the anomeric position even after showing that the deprotonation can occur using D_2O.[26–30]

Reagents	Product
***t*-BuLi, Ethyl chloroformate, THF**	SM
***t*-BuLi, Methyl chloroformate, THF**	SM
***t*-BuLi, Methyl chloroformate, t-BuOK, THF**	SM[31]
***t*-BuLi, Paraformaldehyde, THF**	SM [27]
***t*-BuLi, CO_2, THF**	SM [28]
***n*-BuLi, t-BuOk, Bu_3SnCl, THF**	SM [29]
***t*-BuLi, THF, Bu_3SnCl**	SM [30]

Table 7: Conditions carried out for the attempted synthesis of **321** and **322**.

Since writing my thesis a paper has been published by Nishikawa in which they have successfully ring opened an epoxide by using TMSCN in the presence of Iodine in toluene to introduce a cyano functional group on the anomeric group. This methodology could be tried within our group on substrates **303** and **304** in the future (**Scheme 105**).[31]

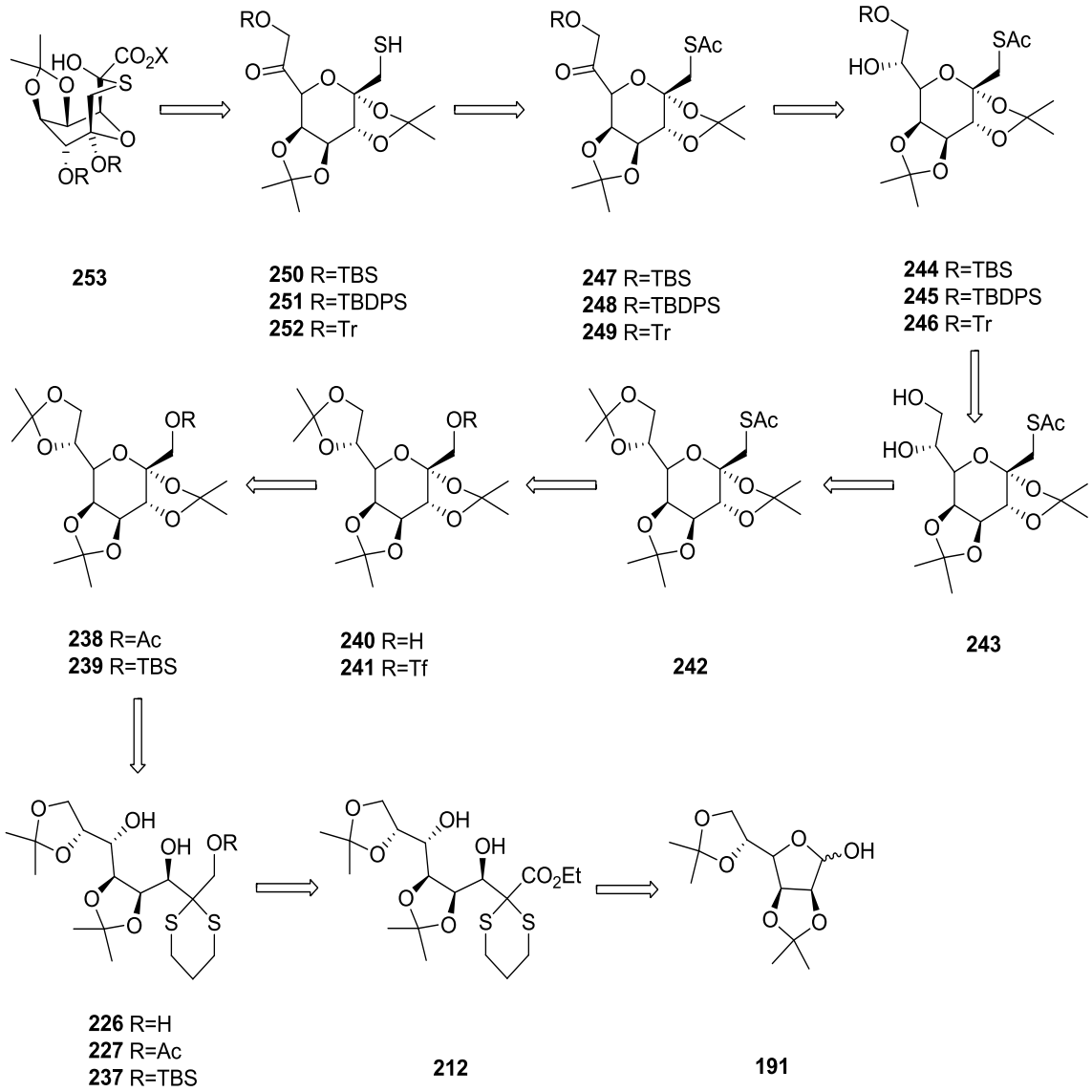


303 R=TBS
304 R=TIPS

318

Scheme 105: Future work to be crried out on the ring opening of epoxides **303** or **304**
i) TMSCN, I_2, toluene, hexane, 0 °C, then 1 M HCl aq. r.t.

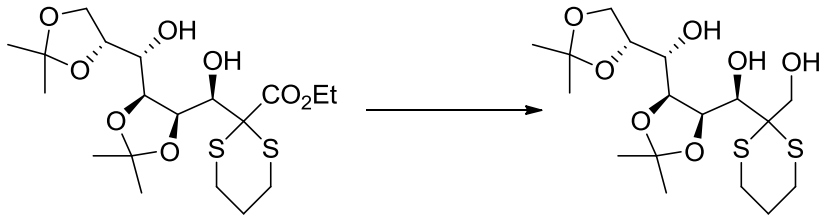
Route 5 Reduction route:



Scheme 56: Retrosynthesis for taget bicylic compound **253.**

The next route attempted in our strategy (**Scheme 56**) involved steps described previously (**Schemes 37 and 47**), with the key intermediate **212** being prepared from D-mannose on a 50g scale in 98% yield.[2]

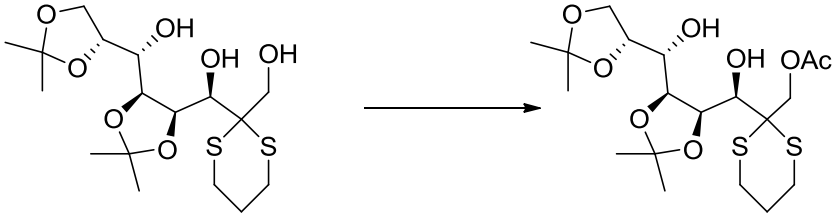
However, this time, instead of carrying out the deprotection and the *in situ* cyclization, we reduced the ethyl ester of the dithiane **212** to the alcohol **226** using $LiAlH_4$ to give **226** in yields above 98% (**Scheme 106**). The next step was to carry out the deprotection of the dithiane on **226** using NBS and acetone and the *in situ* cyclization; however the desired product was not obtained, with just starting material **226** being observed.



212 **226**

Scheme 106: Reduction of the ethyl ester **212** to alcohol **226**
i) 212, $LiAlH_4$, THF, 0 °C, 98-99%.

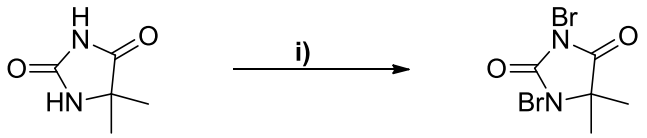
Having had no success in obtaining the desired compound, we decided to selectively protect the primary alcohol of **226** with an acetate to give **227** and then carry out the deprotection and the cyclization again using the same conditions (**Scheme 107**). Again only diacetone mannose **191** was obtained.



226 **227**

Scheme 107: Acetate protection of primary alcohol **226** to form **227.**
i) 226, Ac_2O, TEA, DCM, 0 °C, 91%.

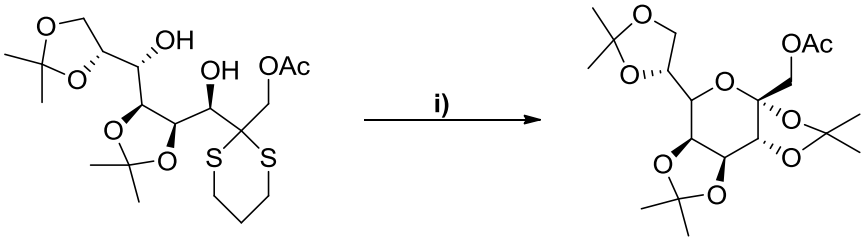
We decided then to look at DBDMH **324** to carry out the deprotection of the dithiane and *in situ* cyclization. We synthesized it freshly within the lab, prior to use, using previously reported conditions, starting with 5,5-dimethylhydantoin **323,** which was dissolved in a 5% NaOH solution and added to neat bromine and stirred until a colourless precipitate was formed, which was filtered and dried (**Scheme 108**).[45]



323 **324**

Scheme 108: Synthesis of DBDMH **324**
i) 323, NaOH, Br_2, H_2O, 89%.

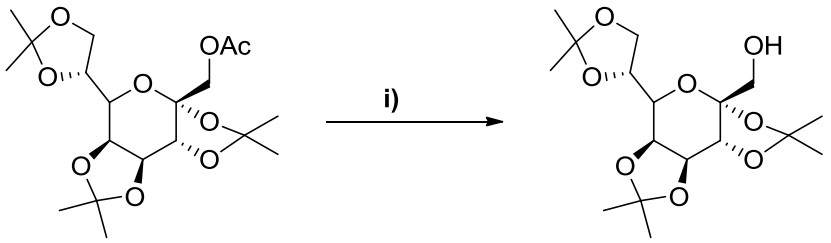
Instead of carrying out the usual NBS deprotection reaction and *in situ* cyclization we used DBDMH **324** on the acetate protected compound **227**. We found using DBDMH instead of NBS gave to our surprise a very high yield of the desired triacetonide pyranose **238,** and after the optimization of the conditions, we were able to improve the yield to 70-90% (**Scheme 109**).



227 **238**

Scheme 109: Dethioketalization of protected **227** to form **238**
i) 227, DBDMH, Acetone 67%

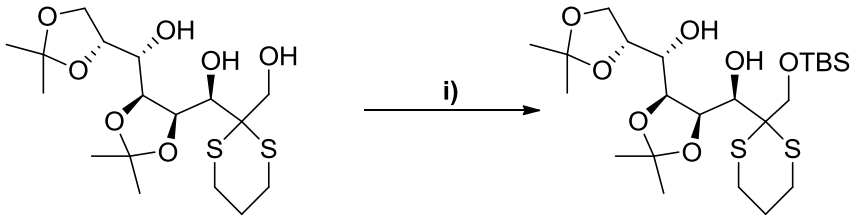
We finally obtained the reaction in a good yield. The next step was the deprotection of the acetate on pyranose **238** to give the primary alcohol **240**. Using sodium methoxide, freshly prepared prior to usage, we were able to obtain the desired compound **240** in a quantitative yield (**Scheme 110**).



238 **240**

Scheme 110: Deprotection of acetate **238** to form primary alcohol **240**
i) 238, NaOMe, MeOH, 95%.

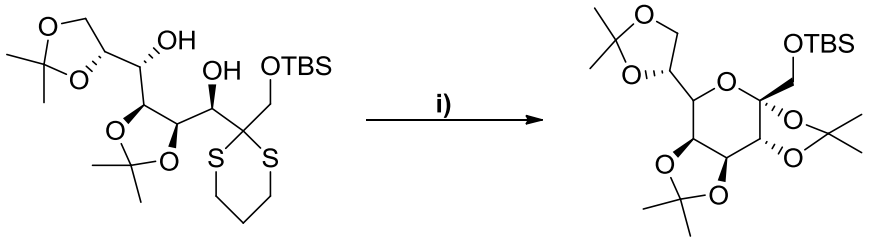
We decided also to protect the primary alcohol with a silyl group using TBSCl to see if this could increase the yield further on the deprotection and cyclization step compared to the acetate protected substrate **227**. We were able to obtain the TBS protected substrate **237** in up to 76% yield (**Scheme 111**) compared with the acetate protected substrate **227** obtained in over 90% yield.



226 **237**

Scheme 111: Acetate protection of primary alcohol **226** to form **237.**
i) 237, Imidazole, TBSCl, DMF, r.t., 76%.

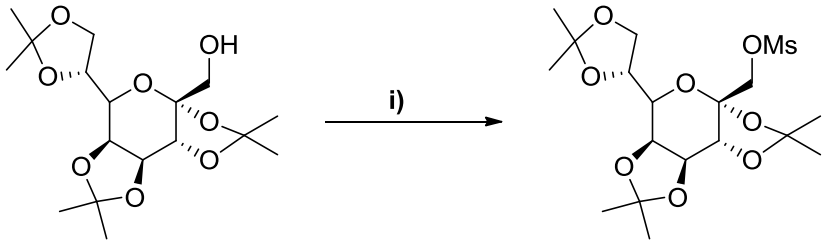
Having obtained the TBS protected substrate **237**, we carried out the deprotection and *in situ* cyclization using DBDMH and we were able to obtain the desired triacetonide pyranose **239** in up to 70% yield (**Scheme 112**). Since the yield of the protection step with TBS to give **237** was lower than the acetate **227** we decided to continue using the acetate substrate **227** for the remainder of this route.



237 **239**

Scheme 112: Dethioketalization of protected **237** to form **239**
i) 237, DBDMH, Acetone, 70%.

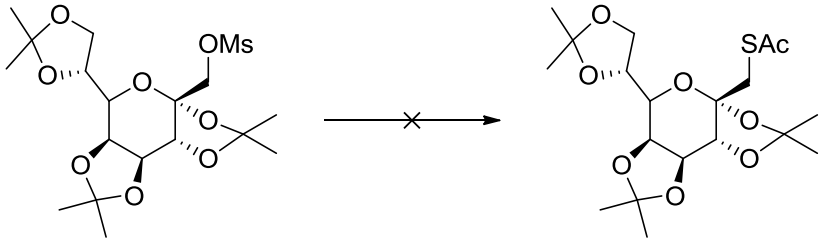
The next step was the conversion of the primary hydroxyl group into a leaving group on substrate **240** in order to carry out a displacement with a thiol. The first reaction we tried involved the introduction of a mesylate group. Using standard conditions, methanesulfonylchloride in pyridine was added to the primary alcohol **240** and stirred for one hour.[32] After work up and purification by column chromatography mesylate **325** was obtained in 89% yield (**Scheme 113**).



240 **325**

Scheme 113: Mesylation of primary alcohol **240** to form **325**
i) 240, MsCl, pyridine. 82%

Having obtained such success forming the mesylated compound **325,** we then moved onto the displacement of the mesylate with potassium thioacetate to introduce the thiol functionality at this position. We found that this reaction proved very difficult and only starting material was recovered. We tried many different conditions with some shown in (**Table 8**) in order to obtain the thioaceteate product **242**, but we were unable to obtain it. We postulated that the mesylate might be sterically hindered due to the presence of the tri-acetonides on compound **325**, and this might explain why we were unsuccessful in obtaining our desired compound **242** (**Scheme 114**).[33,34]



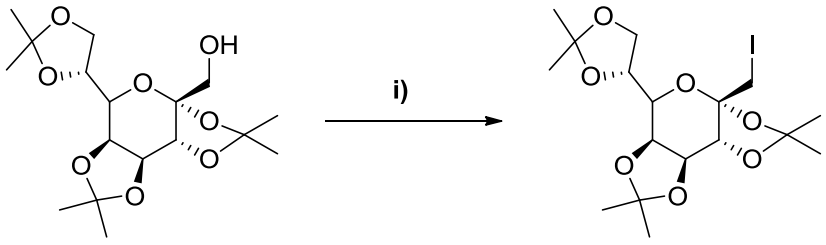
325 **242**

Scheme 114: Displacment of the mesylate **325** group to for the thioacetate **242**

Reagents	Products
KSAc (2 equiv.), Butanone, Reflux	SM
KSAc (4 equiv.), Butanone, Reflux	SM
KSAc (2 equiv.), DMF, Reflux	SM
KSAc (4 equiv.), DMF, Reflux	SM

Table 8: Conditions tried in order to displace the mesylate to form thioacetate **242**.

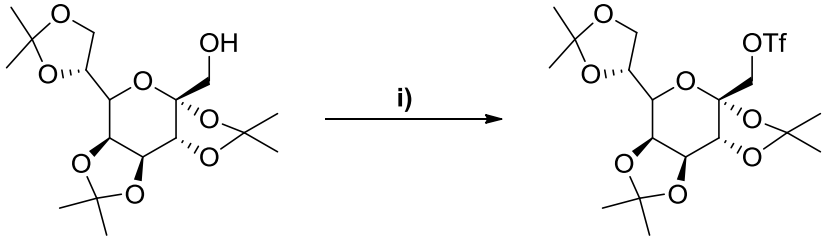
Having been unsuccessful in obtaining pyranose **242** we looked at other leaving groups. The next step we tried involved using iodine in the presence of triphenylphosphine and imidazole in toluene to introduce the iodide on substrate **240**. We obtained pyranose **326** in a very good yield (**Scheme 115**). We then carried out the displacement reaction using potassium thioacetate in DMF, but again to our surprise we were unable to obtain pyranose **242**.[11]



240 **326**

Scheme 115: Iodination of **240** to form **326**
i) 240, PPh3, Imidaozle, Toluene, I_2, 60 °C, 70%.

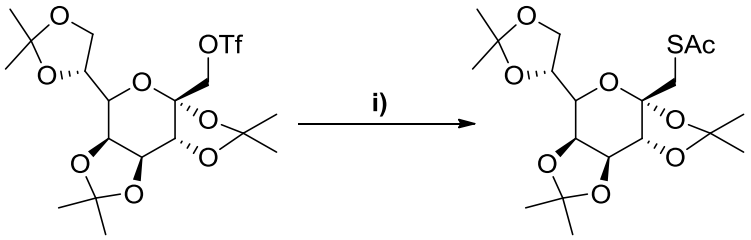
We had experienced limited success so we then decided to try to introduce a triflate on the primary alcohol of **240** as triflates are known to be very good leaving groups. Using triflic anhydride in DCM at -78 °C and stirring for 1 hour with an aqueous work up we were able to obtain the triflated pyranose **241** in very high yields of up to 90-96% without purification (**Scheme 116**).[35]



240 **241**

Scheme 116: Triflation of **240** to form **241**
i) 240, Tf_2O, pyridine, DCM, 0 °C, 45 min, 96%.

We were successful in obtaining the triflated pyranose **241** and the next step was to attempt the displacement with potassium thioaceteate in DMF. Monitoring the reaction closely by tlc we were able to see total consumption of our starting material **241** and a new product being formed. After a work up and purification we were able to obtain the thioacetate pyranose **242**. After optimization of the reaction, using 4 equivalents of potassium thioacetate in dry DMF, we were able to obtain pyranose **242** in yields greater than 90% (**Scheme 117**).

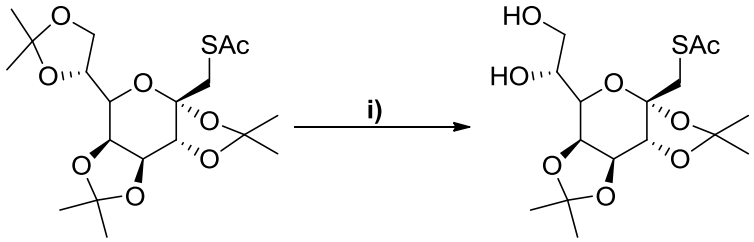


241 **242**

Scheme 117: Displacement of **241** to form thioacetate **242**
i) 241, KSAc, DMF, 80 °C for 1 h, 93%.

The next step involved the selective deprotection of one of the acetonides so we could oxidize the secondary alcohol to the ketone and then carry out the *in situ* cyclization to prove our methodology could be achieved to form the bicyclic structure. We came across a paper in the

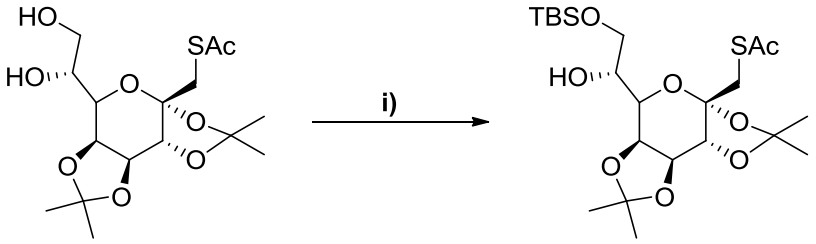
literature where they had carried out a selective deprotection of an acetonide on a different substrate using 80% aqueous acetic acid.[36] We decided to carry this out on our triacetonide substrate pyranose **242**, and after monitoring by tlc we saw that the reaction had reached completion after around 2 hours. After aqueous work up and purification using flash chromatography we were able to obtain our selectively deprotected acetonide **243** in 80-94% yield (**Scheme 118**). We noticed that if the reaction was left for more than 3 hours, the deprotection of the other acetonides occurred.



242 **243**

Scheme 118: Selective deprotection of acetonide **242** to form **243**
i) 242, 80% AcOH, 50°C for 3 h, 89%.

The next step involved the selective protection of the primary alcohol over the secondary on **243**. We decided to use TBSCl as the protecting group and using standard TBSCl protection conditions in the presence of imidazole in DMF, using only one equivalent of TBSCl in order to avoid the protection of both alcohol moieties. We were able to obtain the selectively protected pyranose **244** in very good yields of up to 70% (**Scheme 119**).[37,38]

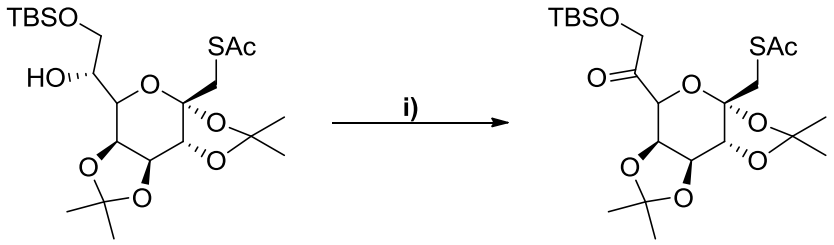


243 **244**

Scheme 119: Selective protection of primary alcohol **243** with TBSCl to form **244**
i) 243, imidazole, TBSCl, DCM. 72%.

After obtaining the selectively protected primary alcohol **244** we then wanted to oxidize the secondary alcohol to the ketone, and after deprotecting the thioacetete to attempt an *in situ*

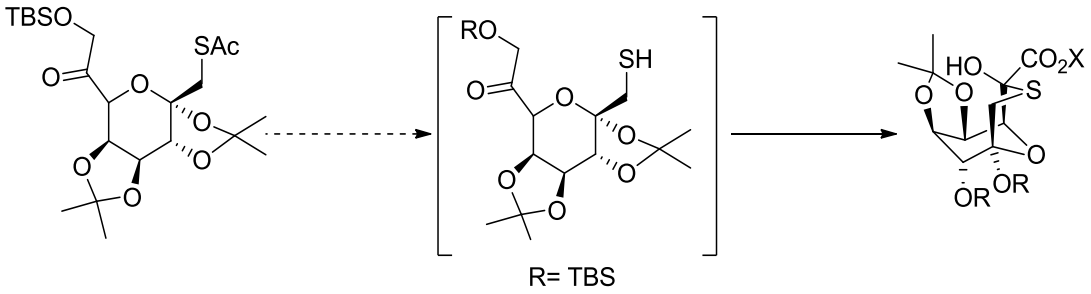
cyclization to obtain the bicyclized product **253**. We initially carried out a Dess-Martin oxidation to obtain the ketone **247**, but we were unable to repeat this reaction using the literature conditions.[39] We then carried out a Swern and we were able to obtain the desired ketone pyranose **247** in quantitative yields (**Scheme 120**).[40]



244 **247**

Scheme 120: Oxidation of **244** to form ketone **247**
i) DMSO, Oxalyl chloride, DCM, -78 °C for 30 min, **244**, TEA, 100%

The next step we were tasked with was the deprotection of the thioacetate of **247** to give the thiol intermediate **250** and the *in situ* cyclization to form the bicyclic product **253** (**Scheme 121**). We tried the thioacetate deprotection on the TBS silyl protected ketone **247** with multiple methods used for different substrates from the literature shown below (**Table 9**), from the most common deprotection conditions such as sodium methoxide in methanol, right through to other methods for thioacetate deprotections with only decomposition being observed (**Table 9**). This was very disappointing as we were very close to obtaining the desired bicyclic structure **253** to prove our methodology. We thought that perhaps there was some steric hindrance between the bulky TBS silyl group and the acetonide groups. We thought about the deprotection of the acetonides before carrying out the reaction, or in earlier steps, to see if this steric hindrance was preventing the cyclization from happening. We also studied the reaction by monitoring it by NMR spectroscopy, but were unable to see what was happening and we could only observe decomposition.



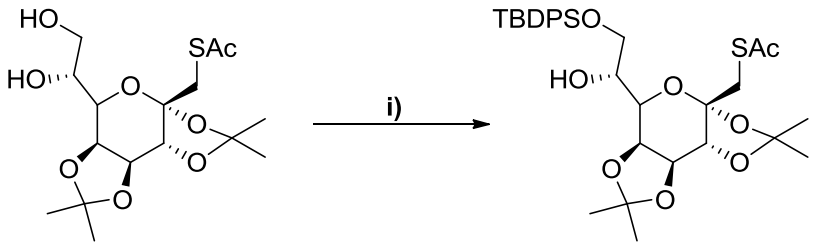
247 **250** **253**

Scheme 121: Proposed deprotection of thioacetate **247** to thiol **250** and the in-situ cyclisation to form bicyclic stucture **253**

Reagents	Product
NaOMe, MeOH	Decomposition
TBAF (1.0 M), THF	Decomposition
Hydrazine Acetate, THF	Decomposition
Hydrazine mono-hydrate, AcOH, DMF	Decomposition
2-methoxy ethanol, H_2O, $NaHCO_3$	Decomposition
K_2CO_3, MeOH	Decomposition
KOH, MeOH, H_20	Decomposition
LiOH, THF, H_2O	Decomposition

Table 9: Thioacetate deprotection conditions carried out on thioacetate **247 and 248**.[33,35–38,41–43]

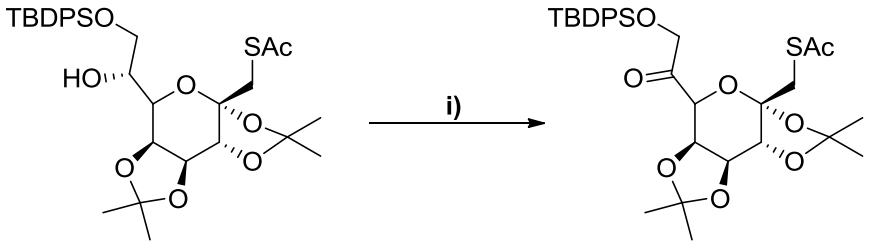
As we were unable to obtain bicyclic compound **253** or the deprotected thiol **250,** we decided to look at other silyl protecting groups with primary alcohol selectivity and decided to use TBDPS instead of TBS. Using standard TBDPS protection conditions in the presence of imidazole in DMF using only one equivalent of TBDPSCl in order to avoid the protection of both alcohol moieties, we were able to obtain the selectively protected pyranose **245** in a yield of up to 74% (**Scheme 122**). [38]



243 **245**

Scheme 122: Selective protection of primary alcohol **243** with TBDPSCl to form **245**
i) TBDPSCl, Imidazole, **243**, DMF, 50 °C for 48h, 74%

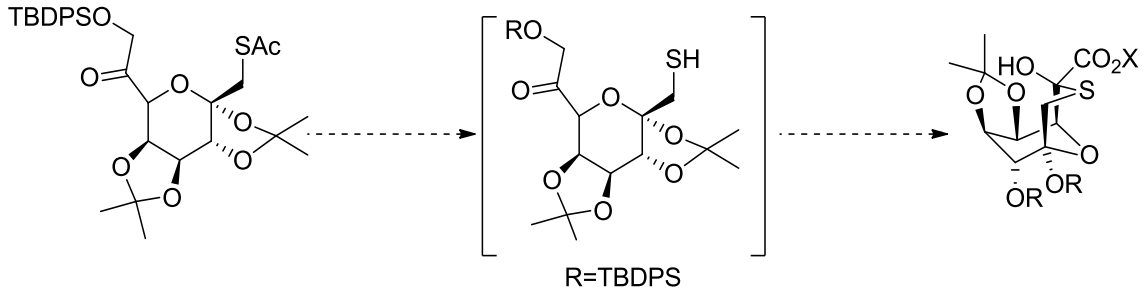
After achieving the selective protection of the primary alcohol to form **245** we carried out a Swern oxidation in order to obtain the ketone pyranose **248** in a quantitative yield (**Scheme 123**).



245 **248**

Scheme 123: Oxidation of **245** to form ketone **248**
i) DMSO, Oxalyl chloride, DCM, -78 °C for 30 min, **245**, TEA, 100%

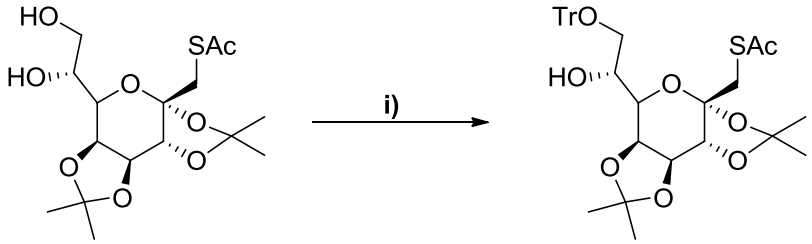
We then attempted the thioacetate deprotection on the TBDPS silyl protected ketone **248** with multiple methods as used with the TBS substrate (**Table 9**). Unfortunately, only decomposition was observed and intermediate **251** or bicyclic structure **253** were not obtained (**Scheme 124**).



248 **251** **253**

Scheme 124: Proposed deprotection of thioacetate **248** to thiol **251** and the in-situ cyclisation to form bicyclic stucture **253**

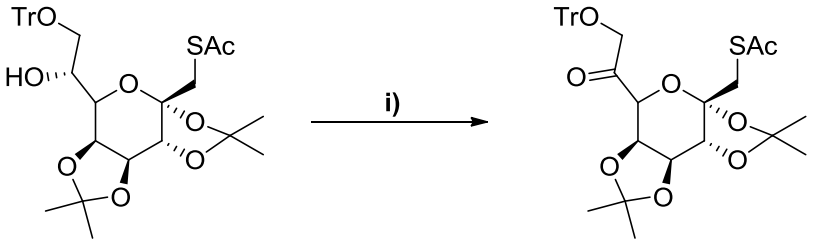
Further to this we decided to look at other protecting groups besides the silyl groups to see if these would influence the reaction. We decided to selectively protect the primary alcohol of pyranose **243** with a trityl group.[3444] We obtained the trityl protected compound **246** in very high yield after purification on deactivated silica gel to obtain the product **246** in yields of up to 90% (**Scheme 125**).



243 **246**

Scheme 125: Selective protection of primary alcohol **243** with trityl chloride to form **246**
i) 243, DCM, TEA, TrCl, 0 °C, 89%.

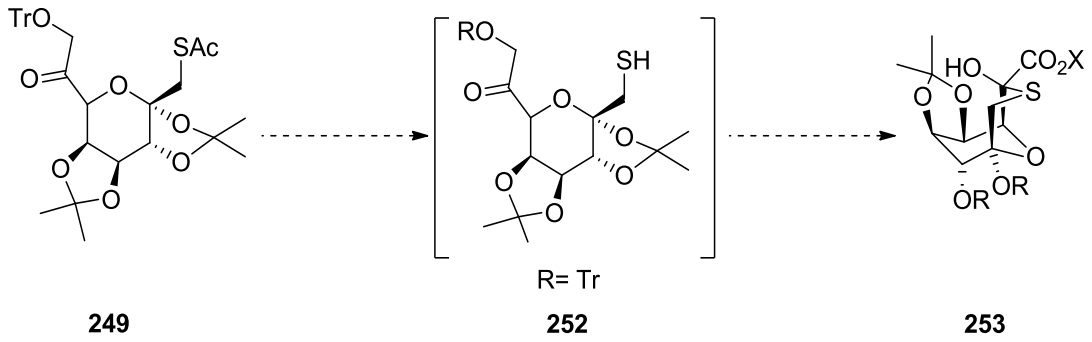
We then oxidized the secondary alcohol of **246** to the ketone **249** using the Swern oxidation conditions reported above, and obtained the ketone **249** in a yield of up to 51% (**Scheme 126**).



246 **249**

Scheme 126: Oxidation of **246** to form ketone **249.**
i) DMSO, Oxalyl chloride, DCM, -78 °C for 30 min, **246**, TEA, 51%

After achieving the desired ketone **249**, the next step was the deprotection of the thioacetate to give the thiol intermediate **252** and the *in situ* cyclization to form the bicyclic compound **253** (**Scheme 127**). We first tried similar conditions as used with the previous substrate but to our disappointment only complete decomposition was observed. After numerous attempts we then decided to look at other ways to cleave the thioacetate to the thiol. However, from all conditions carried out listed (**Table 10**) below, only complete decomposition was observed. Further work was carried out using bases such as LiOH and NaOH which showed promising results, but further work needs to be carried out in order to achieve the bicyclic compound **253**.



249 **252** **253**

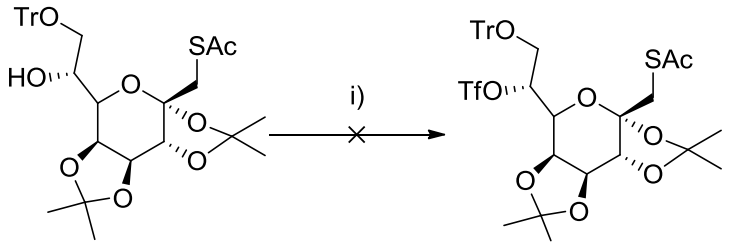
Scheme 127: Proposed deprotection of thioacetate **249** to thiol **252** and the in-situ cyclisation to form bicyclic stucture **253**

Reagents	Product
Hydrazine acetate, DMF	Decomposition
Hydrazine Hydrate, Acetic Acid, DMF	Decomposition
2-methoxy ethanol, H_2O, $NaHCO_3$	Decomposition
K_2CO_3, MeOH	Decomposition
KOH, MeOH, H_20	Decomposition
LiOH, THF, H_2O	Decomposition

Table 10: Thioacetate deprotection conditions carried out on thioacetate **249.** [33,35–38,41–43]

We also decided to try and introduce a triflate on the secondary alcohol instead of oxidizing to the ketone on substrate **246**. We believed by having a good leaving group in this position and trying to carry out the deprotection of the thioacetate to the thiol should aid an *in situ* cyclization to form a bicyclic structure. We began with the triflation of **246** using triflation

conditions we used previously and after two or three attempts we were unable to obtain the desired compound **327** with only decomposition observed (**Scheme 128**).



246 **327**

Scheme 128: Attempted triflation of the secondary alcohol of **i) 246,** Tf_2O, pyridine, 0 °C, DCM.

Conclusion and future work:

By the end of our studies towards the synthesis of tagetitoxin, a range of routes had been explored (**Routes 1-5**), producing the key intermediates shown below (**Scheme 129**).

Within our first route the use of dithiane followed by a dethioketalization produced intermediate **214** in low yield and the acetate protected derivative **218**. We had hoped to complete the removal of the anomeric hydroxyl moiety or the introduction of a methylene group to give **215**, however we were unable to introduce this after numerous attempts. We also tried to improve the yield of the key intermediate **214** using different deprotection conditions of the ditihiane or by synthesizing other dithiane derivatives, for example, protecting the free hydroxyl group on the α-position of intermediate **268** prior to the NBS mediated cleavage of the dithiane. However no improvement could be made on the initial reaction discussed in route one.

Route two (**Scheme 79**) involved a Wittig reaction to produce alkene **283** which, following pyrolysis after an intramolecular esterification, led to the key intermediate **284.** Intermediate **284** provided a lot of interesting work following Waldrop's route, however we were unable to replicate the literature route to KDO after obtaining intermediate **284**.[15] This intermediate led us to complete further work on alkene intermediate **284** discussed in route four.

In route three we tried an alternative route towards KDO (**Scheme 83**).[5] In this route we started again from starting material **191**. We then carried out a reduction of diacetone mannose to give **200** and protected the O-1 hydroxyl with a benzyl group to give intermediate **293**. Intermediate **293** was treated with ethyl bromoacetate in the presence of sodium hydride in THF to introduce an ethoxycarbonylmethyl group on the O-4 position to give **294** in the same way as Ohrui and co-workers achieved. However, after numerous attempts using their conditions and varying the amount of sodium hydride or ethyl bromoacetate we were unable to synthesize the desired compound **294**.This route would have provided a quick route towards achieving a KDO substrate which could be modified so further work could be completed on this in the future.

In route four (**Scheme 87**) we started with intermediate **284** previously prepared in route two. Epoxidation of the alkene moiety followed by epoxide ring opening to introduce functional groups on the anomeric position was envisaged. However, this provided difficult as no epoxide

was formed using substrate **284.** In order to change the isopropylidene protecting groups and to allow the formation of epoxide derivatives, we first had to carry out a bromination of the alkene moiety on **284**. This produced the methoxy-bromo derivative **311**, the isopropylidene groups of **311** were removed and acetate protecting groups were introduced to form intermediate **314**. In order to reform the alkene we first had to convert the methoxy group on the anomeric position of **314** to an acetate to produce **315**. Having obtained **315** we could then reform the alkene using zinc dust in the presence of sodium acetate and acetic acid to form tetra-acetate alkene **299**. We also tried to form the epoxide from alkene **299** using *m*-CPBA or DMDO; however, we were unsuccessful. We decided to look at alternative protecting group and deprotected the acetate groups on alkene **299** using sodium methoxide in methanol to give **300**. We were then able to protect the hydroxyl groups with TBS and TIPS groups to produce alkenes **301** and **302**. We then completed the epoxidation of both alkenes using DMDO to give solely the α-epoxides **303** and **304**. Numerous attempts were carried out to successfully ring open epoxides **303** and **304** which proved unsuccessful but more work should be carried out especially since this would provide a fast way to introduce functional groups.

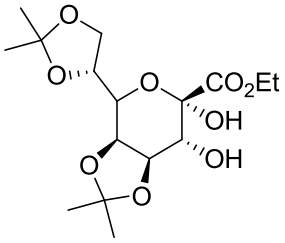
Route five (**Scheme 56**) provided the closest route we had in obtaining ~~a~~ bicyclic intermediate **253**. Using the same chemistry as in route one with the reduction of the ethyl ester to alcohol which was then acetate protected and the use of DBDMH to carry out the dethioketalization we were able to increase significantly the yield. Acetate deprotection followed by the introduction of a good leaving group produced triflate **241**. The triflate group was displaced using potassium thioacetate to form the intermediate **242**. The selective deprotection of an isopropylidene group on **242** to produce **243** was achieved in high yield. Selective protection of the primary alcohol with silyl and trityl groups followed by the oxidation of the secondary alcohol produced key intermediates **247**, **248** and **249**. The next step involved the deprotection of the thioacetate to produce the thiol which we had hoped would allow an *in situ* cyclization to form the bicyclic structure **253**. However after numerous attempts to deprotect the thioacetate on substrates **247**-**249** to the thiol **250**-**252** we were unable to obtain the desired product. Further work should be carried out to try and achieve this with the use of other protecting groups which might favor the cyclization.

A number of key intermediates and novel compounds were synthesized in our aim towards a total synthesis of tagetitoxin which are shown (**Scheme 129** and **130**, respectively). Route four

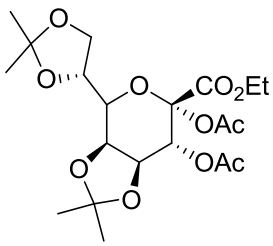
and five produced the best results. Further work should explore the ring opening of epoxides **303** and **304** using a set of recently reported conditions (**Scheme 105**).

Further work should also be carried out on route three as this could lead to a fast route in obtaining a KDO derivative which could be modified to form a bicyclic derivative **292** and prove our methodology.

Route five produced the best results with higher yields and less steps and the thioacetate deprotections reaction on thioacetate intermediates **247-249** to the corresponding thiols **250-252** and *in situ* cyclization to achieve bicyclic structure **253** should be further explored to show that the methodology works. Further modifications in the synthesis would be required to introduce functional groups to achieve a total synthesis of tagetitoxin.



214



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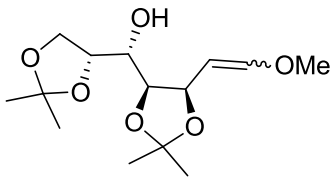
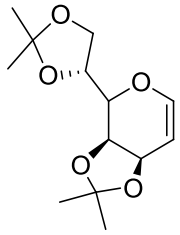
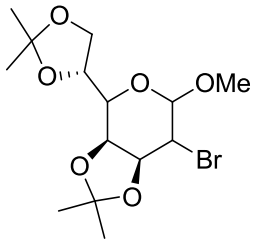


283



284



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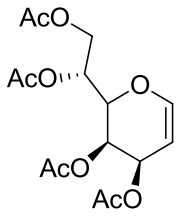

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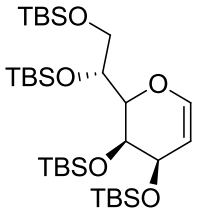
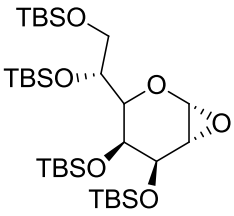
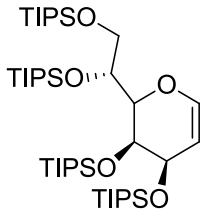


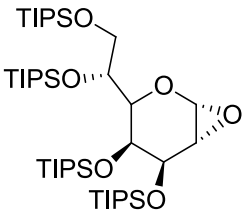
301



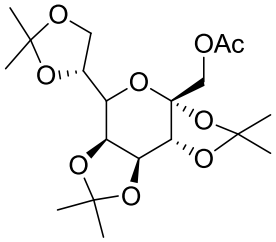
303



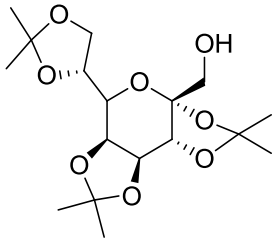
302



304



238



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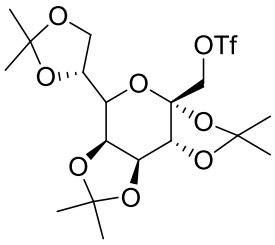
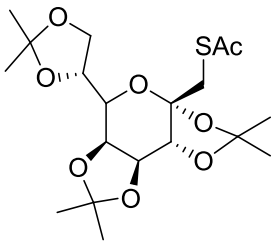


241



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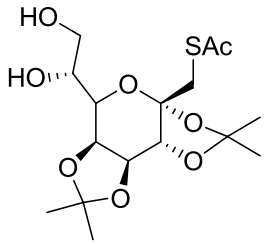

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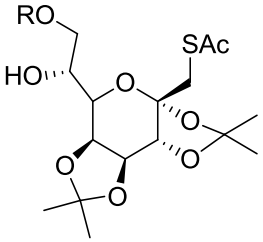
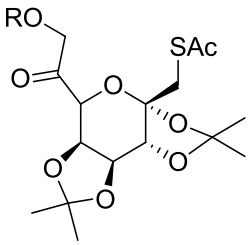
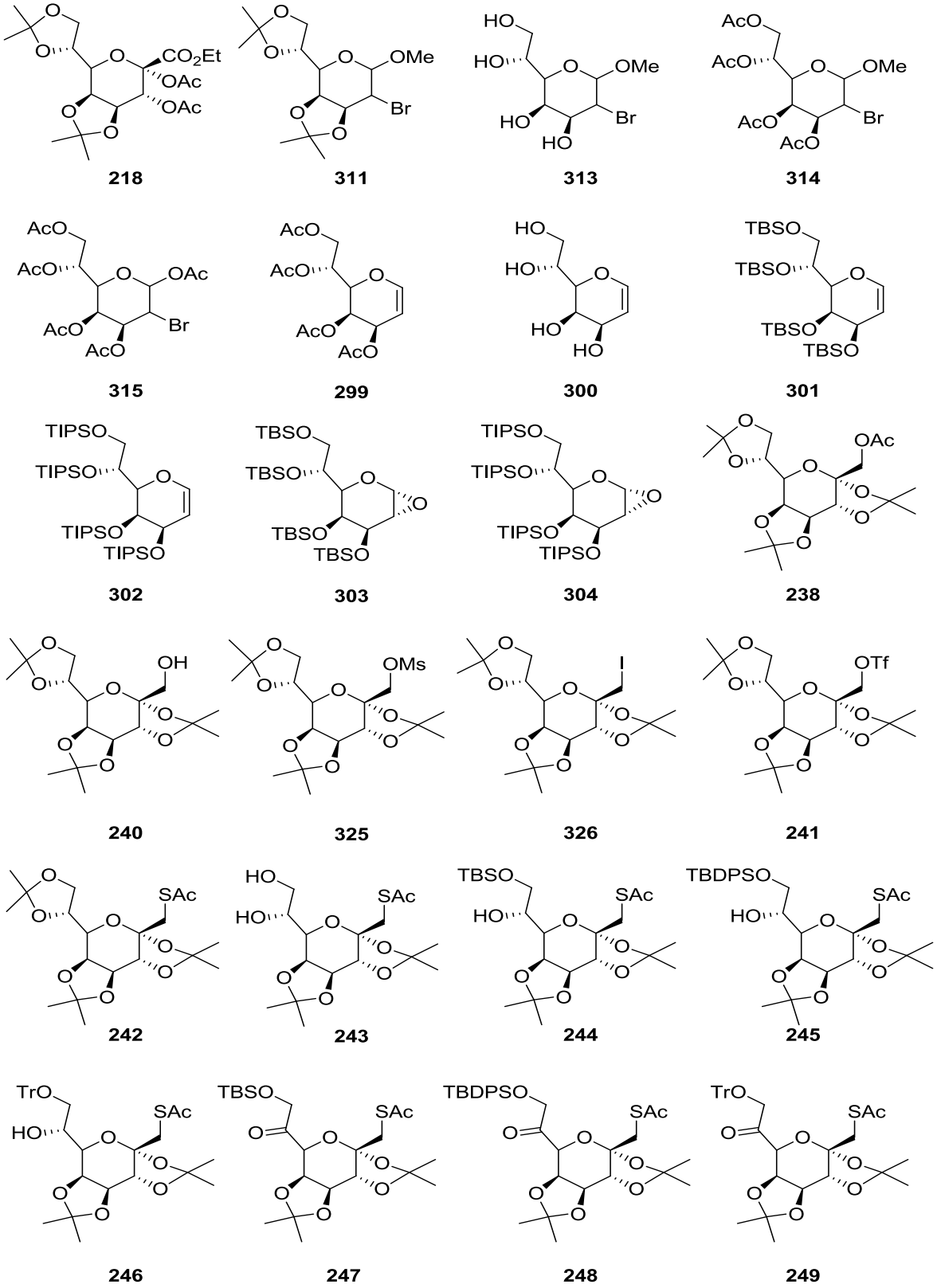


244 R=TBS
245 R=TBDPS
246 R=Tr



247 R=TBS
248 R=TBDPS
249 R=Tr

Scheme 129: Key intermediates synthesised during the project



Scheme 130: Novel intermediates obtained throughout the project

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Experimental:

Experimental Procedures

Air sensitive reactions were run using flame-dried glassware and under an argon atmosphere. Extractions were performed using the reported organic solvent and, if not indicated, were equivolumetric. Where petroleum ether fractions 40/60 has been used, it is referred to in the term “petrol”.

Chromatography on silica gel was performed using a standard purification procedure using Fluka Kieselgel 60, 0.023-0.063 mm particle size with the reported solvent systems. Thin layer chromatography was performed using Merck aluminium-backed plates coated with Kieselgel 60 F254 silica coating. The plates were visualized by U.V. irradiation at a wavelength of 254 nm, or by dipping the plate in an ethanolic solution of phosphomolybdic acid, or potassium permanganate solution.

Fourier transformation Infrared spectroscopy was recorded using a Perkin Elmer Model spectrophotometer in the range of 4000-500 cm^{-1}. Samples were dissolved in the reported solvent and applied onto a sodium chloride plate as thin films.

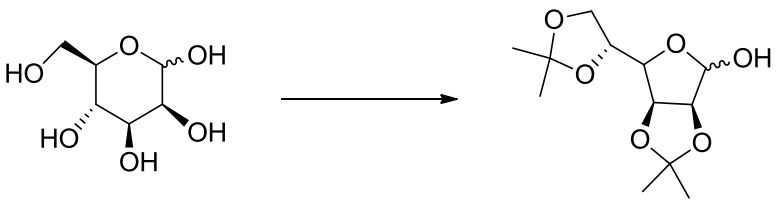
Nuclear magnetic resonance spectroscopy was acquired using a Bruker 400 and 500 MHz Spectrometer instrument for ^{1}H NMR analyses and for ^{13}C NMR analyses. The spectra were calibrated where possible to the signals of tetramethylsilane, at d=0.00 ppm, or else using the residual peak of $CHCl_3$ present in $CDCl_3$, at δ=7.26 ppm. Chemical shifts (δ) are reported in ppm. When possible, coupling constants (J) are shown denoting the multiplicity as: singlet (s), doublet (d), triplet (t), quarter (q), multiplet (m) or any combination of those. The size of the coupling constant is given in Hertz. Carbons are shown as follows: CH_3, CH_2, CH and Cq (quaternary carbons).

Optical rotations values were measured with a Bellingham and Stanley ADP-440 polarimeter, operating at λ = 589 nm, corresponding to the sodium line (D), at the temperatures indicated. The solvent used for these measurements were of spectrophotometric grade and the solutions for these measurements were prepared in volumetric flasks for maximum accuracy.

High resolution mass spectroscopy was carried out by the EPSRC national mass spectrometry service at the University of Wales, Swansea, utilizing electrospray (ES), nanoelectrospray (NESP) and MALDI-TOF ionization techniques.

Melting points were obtained using an Electrothermal-IA 9100 melting point instrument

2,3:5,6-di-O-isopropylidene-α-D-mannofuranose (191) [1]



d-mannose

190

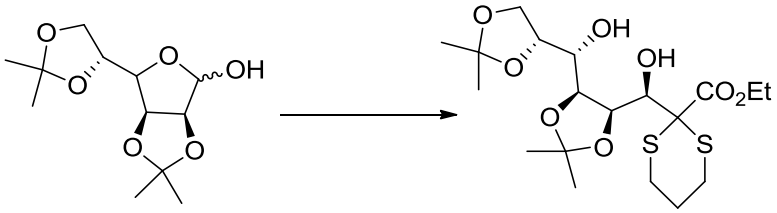
191

α-D-Mannose **190** (20 g) was dissolved in acetone (600 ml). Concentrated sulfuric acid (14 ml) was added and the mixture was left to stir for 2 h until all the sugar had dissolved. After 2 h. the mixture was neutralized with anhydrous sodium carbonate and was filtered off. The filtrate was then heated under reflux for 1 h with activated charcoal and anhydrous sodium carbonate (2-3 g). After 1 h the mixture was filtered over celite and the filtrate was evaporated to dryness to give a colourless solid. The crude product was subjected to crystallisation by dissolving in diethyl ether and the product was precipitated with petroleum ether. This was carried out 2 times with the precipitate filtered of using a Buchner funnel. 20 g was obtained from the first recrystallization and a further 6-7 g was obtained from the mother liquor. White crystalline solid obtained **191**. Yield of 92%.

mp 122-123 °C (Lit. 122-123 °C); IR γ_{max} (film)/cm^{-1}: 3428, 2979, 2948, 2899, 2948, 1457, 1372, 1350 1318, 1237, 1202, 1087; ^{1}H NMR (400 Hz, $CDCl_3$): δ 1.33 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.46 (3H, s, CH_3 isopropylidene), 1.47 (3H, s, CH_3 isopropylidene), 3.49 (1H, s, OH), 4.03 (1H, dd, *J* = 8.7, 4.8 Hz, H-6), 4.09 (1H, dd, *J* = 8.6, 6.2 Hz, H-6), 4.19 (1H, dd, *J* = 7.1, 3.6 Hz, H-5), 4.43 – 4.38 (1H, m, H-4), 4.63 (1H, d, *J* = 5.9 Hz, H-2), 4.82 (1H, dd, *J* = 5.9, 3.7 Hz, H-3), 5.38 (1H, s, H-1); ^{13}C NMR (100 MHz, in $CDCl_3$): δ 24.42 (1C, CH_3 isopropylidene), 25.12 (1C, CH_3 isopropylidene), 25.81 (1C, CH_3 isopropylidene), 26.76 (1C, CH_3 isopropylidene), 66.48 (1C, CH_2, C-6), 72.28 (1C, C-5), 79.62 (1C, C-4) 80.08 (1C, C-3), 85.49 (1C. C-2), 101.16 (1C, C-1), 109.10 (1C, Cq isopropylidene), 112.60 (1C, Cq isopropylidene).

Data is in agreement with the literature reference.

Ethyl-2-deoxy-4,5:7,8-di-O-isopropylidene-D-glycero-D-galacto-0ctulosonate-1,3-propane-dithio-acetal (212) [2]



191 **212**

A solution of diisopropylamine (17.80 mL, 126 mmol) in anhydrous THF (200 mL) was treated with *n*-BuLi (50.26 mL, 2.5 M in hexane, 126 mmol) at −20 °C. After stirring for 15 min ethyl 1,3-dithiane-2-carboxylate (18.1 mL, 115 mmol) was slowly added. The dark red solution was kept for 2 h at −20 °C and then added to a cooled suspension of $MgBr_2$ (−20 °C) in anhydrous THF (250 mL) made from magnesium (4.2 g, 173 mmol) and 1,2-dibromoethane (13.25 mL, 153 mmol). Then **191** (10 g, 38.4 mmol) was added without any solvent. The reaction mixture was warmed to room temperature over a period of 3 h, stirred for 3 h at 50 °C, then poured into ice-cold, satd. Aqueous NH_4Cl (500 mL) and extracted with EtOAc (900 mL). The combined extracts were washed with water, dried ($MgSO_4$) and evaporated; the residue was purified by flash chromatography (toluene:EtOAc, 5:1)to afford excess ethyl 1,3-dithiane-2-carboxylate and **212** (17.24 g, 99%) as a light brownish syrup; TLC (toluene:EtOAc, 5:1): *R*f 0.54.

IR γmax (film)/cm^{-1}: 3454, 2983, 2934, 1727, 1370, 1213, 1158, 1063 and 850; ^{1}H NMR (400 Hz, $CDCl_3$): δ 1.38 (3H, t, J=7.2 Hz, CH_3 ester), 1.45 (9H, s, 3 x CH_3 isopropylidene), 1.54 (3H, s, CH_3 isopropylidene), 1.94 (1H, m, CH_2 dithiane), 2.11 (1H, m, CH_2 dithiane), 2.82 (2H, m, CH_2 dithiane), 3.06 (1H, dd, *J*= 11.3 Hz, 3.0 Hz, CH_2 dithiane), 3.26 (1H, dd, *J*= 11.5 Hz, 2.8 Hz, CH_2 dithiane), 3.67 (1H, d, *J*= 7.3 Hz, H-5), 3.77 (1H, s, OH), 3.95 (1H, s, OH), 4.10 - 4.06 (1H, m, H-2), 4.19 - 4.12 (2H, m, H-6, H-6), 4.36 - 4.27 (3H, m, CH_2 ester), 4.46 (1H, dd, *J*= 7.6, 1.0 Hz, H-4), 4.61 (1H, d, *J*=7.6 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.08 (1C, CH_3 ester), 24.16 (1C, CH_2, dithiane), 25.13 (1C, CH_3, isopropylidene), 25.34 (1C, CH_3, isopropylidene), 26.09 (1C, CH_3 isopropylidene), 26.92 (1C, CH_3 isopropylidene), 27.24 (1C, CH_2 dithiane), 27.55 (1C, CH_2 dithiane), 58.67 (1C, Cq dithiane), 62.74 (1C, CH_2 ester), 67.59 (1C, CH_2, C-6), 70.63 (1C, C-4), 72.50 (1C, C-5), 74.17 (1C, C-1), 75.72 (1C, C-2), 77.22 (1C, C-3), 109.19 (1C, Cq isopropylidene), 109.35 (1C, Cq isopropylidene), 169.92 (1C, carbonyl of ester).

Data is in agreement with the literature reference.

Ethyl 4,5:7,8-di-O-isopropylidene-α-D-glycero-D-galacto-2-octulopyranosanate (214) [2]

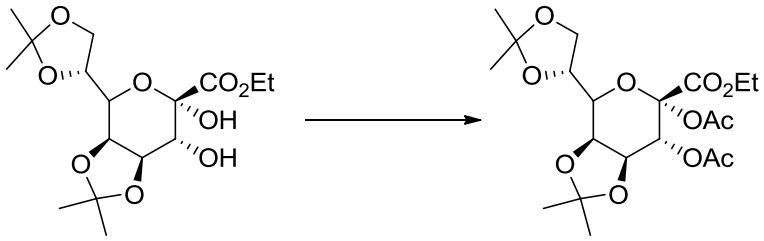
212 **214**

A solution of NBS (1.38 g, 7.73 mmol) in acetone (60 ml) was added slowly to a solution of **212** (1 g, 2.21 mmol) in acetone (60 ml) at 0 °C and the reaction mixture was stirred for 5 min at 0 °C. Saturated aqueous solutions of $Na_2S_2O_3$ and $NaHCO_3$ were added to the reaction mixture which was then extracted with EtOAc. The combined organic layers were washed with water, dried over $MgSO_4$ and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (Petrol/ EtOAc, 1:1) to give the pure compound **214** as a white solid (0.32 g, 40%).

mp 127-128 °C (Lit. 128 °C); IR γmax (film)/cm^{-1}: 3419, 2982, 2950, 2899, 1732, 1458, 1437, 1374, 1350, 1279, 1253, 1026; ^{1}H NMR (400 MHz in $CDCl_3$): δ 1.27 (3H, t, *J*=7.1Hz, CH_3 ester), 1.30 (3H, s, CH_3 isopropylidene), 1.33 (3H, s, CH_3 isopropylidene), 1.34 (3H, s, CH_3 isopropylidene), 1.49 (3H, s, CH_3 isopropylidene), 4.01 - 3.88 (3H, m, H-3, H-4, H-6), 4.13 - 4.06 (2H, m, CH_2 ester), 4.32 - 4.22 (4H, m, H-2, H-5, H-7, H-7); ^{13}C NMR (100 MHz, in $CDCl_3$): δ 13.98 (1C, CH_3, ester), 25.49 (1C, CH_3 isopropylidene), 26.37 (1C, CH_3 isopropylidene), 26.88 (1C, CH_3 isopropylidene), 28.21 (1C, CH_3 isopropylidene), 63.31 (1C, CH_2 ester), 66.78 (1C, CH_2, C-7), 69.86 (1C, C-5), 70.87 (1C, C-2), 73.01 (1C, C-3), 74.0 (1C, C-4), 77.26 (1C, C-6), 95.07 (1C, Cq), 109.39 (1C, Cq isopropylidene), 109.79 (1C, Cq isopropylidene), 169.16 (1C, Cq ester).

Data is in agreement with the literature reference.

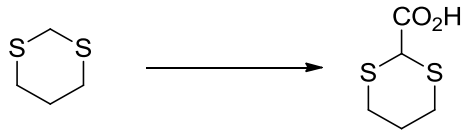
(3a*S*,4*R*,6*S*,7*R*,7a*S*)-4-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)(ethoxycarbonyl)-2,2-dimethyltetrahydro-3aH-[1,3]dioxolo[4,5-c]pyran-6,7-diyl diacetate (218)



Ac_2O (0.46 ml, 4.84 mmol), DMAP (92 mg, 0.75 mmol), and Et_3N (0.675 ml, 4.84 mmol) were added to a solution of **214** (0.5 g, 1.1 mmol) at 0 °C in anhydrous dichloromethane, and the reaction mixture was stirred for 3 h at room temperature. Saturated $NaHCO_3$ was added the mixture was extracted with dichloromethane. The organic layer was washed with water and brine then dried over $MgSO_4$. Purification by column chromatography (Petrol: EtOAc, 1:1) furnished the pure compound as a white solid **218** (0.414 g, 84.4%).

IR γmax (film)/cm^{-1}: 2988, 2933, 2853, 1740, 1691, 1456, 1380, 1370, 1325, 1282, 1258, 1211, 1178, 1117, 1096, 1042, 1004, 902; ^{1}H NMR (400 MHz in $CDCl_3$): δ 1.25 (3H, t, *J* = 7.1 Hz, CH_3, ester), 1.36 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 1.53 (3H, s, CH_3 isopropylidene), 2.11 (3H, s, CH_3 acetate), 2.15 (3H, s, CH_3 acetate), 3.70 (1H, dd, *J* = 8.2, 1.9 Hz, H-5), 3.97 (1H, dd, *J* = 9.1, 4.1 Hz, H-7), 4.10 (1H, dd, *J* = 9.1, 6.2 Hz, H-7), 4.18 (2H, qd, *J* = 7.1, 2.2 Hz, CH_2 ester), 4.39-4.36 (2H, m, H-4, H-3), 4.44 (1H, ddd, *J* = 8.2, 6.2, 4.1 Hz, H-6), 5.23 (1H, d, *J* = 6.9 Hz, H-2); ^{13}C NMR (100 MHz, CDCl3): δ 14.0 (1C, CH_3 ethyl ester), 20.9 (1C, CH_3 acetate), 21.0 (1C, CH_3 acetate) 25.4 (1C, CH_3, isopropylidene), 26.3 (1C, CH_3 isopropylidene), 27.1 (1C, CH_3 isopropylidene), 27.5 (1C, CH_3 isopropylidene), 62.8 (1C, CH_2, ester), 67.1 (1C, CH_2, C-7), 69.7 (1C, C-2), 71.7 (1C, C-5), 72.3 (1C, C-4), 73.6 (1C, C-3), 73.8 (1C, C-6), 95.9 (1C, Cq, C-1), 109.7 (1C, Cq isopropylidene), 110.7 (1C, Cq isopropylidene), 164.9 (1C, Cq acetate), 168.1 (1C, Cq acetate), 169.8 (1C, Cq ester). *m/z* $[M+NH_4]^+$: 464.2123 ; $[C_{20}H_{30}O_{11}+NH_4]^+$ requires 464.2126.

1,3-dithiane-2-carboxylic acid (277)[3]



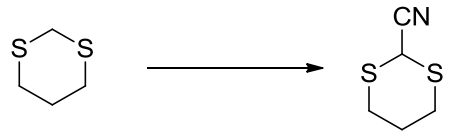
273 **277**

1,3-Dithiane **273** (5 g, 41.6 mmol) was dissolved in anhydrous THF (150 ml) and the mixture was cooled to -20 °C. *n*-BuLi (18.3 mL, 2.5 M in Hexane , 45.74 mmol) was added and the reaction mixture was allowed to stir at -20 °C for 1.5 h, then CO_2 (dry ice) was added quickly and the reaction mixture was allowed stir for 1 h at -20 °C. The reaction mixture was then allowed to reach room temperature and allowed to stir at r.t. for 2 h. After quenching the reaction mixture with NH_4Cl (sat), the mixture was evaporated to reduce the amount of solvent. The aqueous layer was acidified to p=3 with a 1 M HCl solution, extracted with ethyl acetate and dried over anhydrous $MgSO_4$, filtered and evaporated to dryness. The crude mixture was purified by column chromatography (Petrol: EtOAc, 4:1) to afford the product **277** as a white solid (4.687 g, 68%). mp 114-116 °C (Lit. 114-116 °C);

IR γmax (film)/cm^{-1}: 2971, 2942, 2927, 2908, 2826, 2681, 2570, 1692, 1422, 1212, 1301, 1242, 922 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD): δ 1.84–1.98 (2H, m, SCH_2), 2.48–2.55 (2H, m, SCH_2), 3.15–3.24 (2H, m, SCH_2), 4.14 (1H, s), 4.80 (1H, s); ^{13}C NMR (75 MHz, CD_3OD): δ 25.18 (1C, CH_2), 26.07 (2C, 2 x CH_2), 41.08 (1C, CH), 172.17 (1C, C=O).

Data is in agreement with the literature reference.

1,3-dithiane-2-carbonitrile (275)[4]



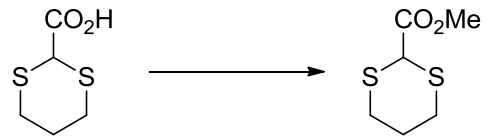
273 **275**

Triphenylcarbenium tetrafluoroborate (2.26 g, 6.8 mmol) was added to a solution of 1,3-dithiane **273** (1.5 g, 12.48 mmol) in anhydrous dichloromethane (150 ml). The mixture was heated under reflux for 1 h and then allowed to cool to room temperature. The solvents were removed under reduced pressure to yield an orange solid. Trituration with cold diethyl ether gave 1,3-dithienium tetrafluoroborate (2.33 g) as a yellow solid. TMSCN (1.41 mL, 1.12g, 11.31 mmol) was added to the 1,3-dithienium tetrafluoroborate (2.33 g, 11.31 mmol) in anhydrous dichloromethane under a nitrogen atmosphere at −20 °C. The reaction mixture was stirred at -20 °C for 1 h then quenched by the acidification of 1 M HCL (ml). The resulting mixture was washed with saturated aqueous NH_4Cl, the aqueous layer was extracted with dichloromethane twice (2x 25 ml). The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (Petrol: EtOAc, 9:1) to give the 2-cyano-1,3-dithane **275** as a pale brown solid (0.95 g, 58%). mp 86-87 °C (Lit. 87-88 °C)

IR γmax (film)/cm^{-1}: 2931, 2908, 2843, 2228, 1669, 1438, 1427, 1412, 1286, 1274, 1242, 1212, 942, 910, 766; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.95-2.08 (1H, m, CH_2), 2.16-2.26 (1H, m, CH_2), 2.76-2.85 (2H, m, SCH_2), 3.28-3.38 (2H, m, SCH_2), 4.42 (1H, s, SCHCN); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.0 (1C, CH_2 dithi), 26.9 (1C, SCH_2), 28.6 (1C, SCH), 116.0 (1C, CN).

Data is in agreement with the literature reference.

Methyl 1,3-dithiane-2-carboxylate (278)[4]



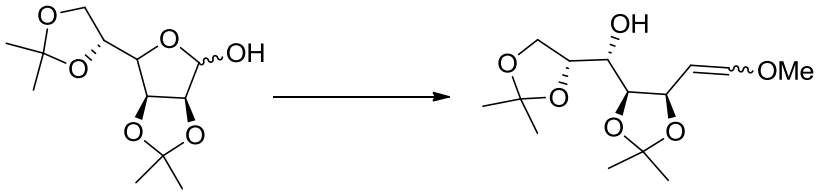
277 **278**

$HCL_{(gas)}$ was bubbled through a methanolic solution (40 mL) of the carboxylic acid **277** (4.687 g, 28.4 mmol)**.** The solvent was removed under reduced pressure and the crude product was distilled in a kugelrohr apparatus. The desired ester **278** (4.315 g, 85%) crystallized upon standing at room temperature. mp 26-28 °C (Lit. 28 °C).[4]

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.93-2.22 (2H, m, CH_2), 2.50-2.66 (2H, m, SCH_2), 3.31-3.46 (2H, m, SCH_2), 3.78 (3H, s, CH_3), 4.19 (1H, s, SCH); ^{13}C NMR δ (75 MHz, $CDCl_3$): 25.0, 26.1, 26.3, 40.0, 170.6.

Data is in agreement with the literature reference.

(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-(4*S*,5*R*)-5-(2-methoxyvinyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol (283)[5]



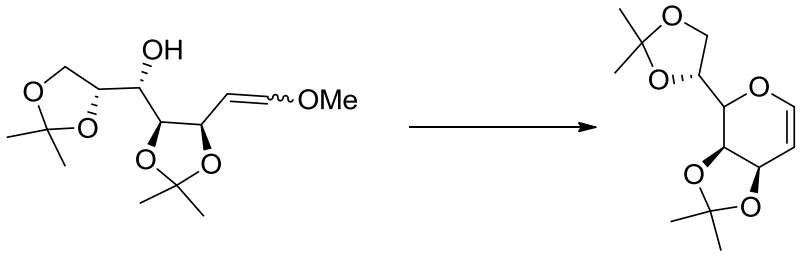
191 **283**

Methoxymethyltriphenylphosphonium chloride (9.26 g, 27 mmol) was added in portions over 5 min to a stirred solution of potassium *t*-butoxide (3.03 g, 27 mmol) in anhydrous THF (250 ml) at 0 °C. After stirring for 30 min at 0 °C a solution of DAM **191** (2.37 g, 9.1 mmol) in anhydrous THF (50 ml) was added dropwise. The red suspension was left to stir overnight at room temperature and the reaction was quenched by addition of aqueous brine solution (100 ml). After equilibration and separation of the layers, the aqueous layer was extracted with diethyl ether 3 times. The combined organic layers were dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography on silica gel (Petrol: EtOAc, 7: 3) to give a mixture of pure products of *cis* and *trans* isomers of the vinyl ether **283** as a colourless syrup (2.43 g, 93%).

IR γmax (film)/cm^{-1}: 3542, 2988, 2980, 2835, 1650 1455, 1411, 1372, 1321, 1259, 1121, 1070, 1042, 935, 889, 853; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35 (3H, s, CH_3 isopropylidene), 1.39 (3H, s, CH_3 isopropylidene), 1.40 (3H, s, CH_3 isopropylidene), 1.40 (3H, s, CH_3 isopropylidene), 3.61 (3H, s, CH_3 methoxy), 4.04 – 3.99 (3H, m, 3 x CH), 4.12 – 4.06 (2H, m, 2 x CH), 4.30 (1H, dd, *J* = 7.4, 1.1 Hz, H-6), 4.34 (1H, dd, *J* = 7.3, 1.4 Hz, H-6), 4.66 (1H, dd, *J* = 9.6, 7.4 Hz, CH), 4.74 (1H, dd, *J* = 8.7, 6.3 Hz, C=C, *cis*-alkene), 5.05 (1H, dd, *J* = 12.7, 9.6 Hz, CH=CH, *trans*-alkene), 5.27 (1H, ddd, *J* = 8.6, 7.4, 1.1 Hz), 6.13 (1H, dd, *J* = 6.3, 1.2 Hz, CH=CH, *cis*-alkene), 6.64 (d, *J* = 12.7 Hz, CH=CH, *trans*-alkene); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.30, 24.39, 25.29, 25.35, 26.71, 26.82, 26.84 (8 x CH3 one overlapping), 56.19, 60.10, 66.88, 67.07, 70.78, 70.85, 71.24, 75.98, 76.14, 76.17, 76.22, 76.72, 97.96, 101.97, 107.75, 107.92, 109.20, 109.31, 150.17, 153.03; *m/z* $[M]^+$:288.1567; $[C_{14}H_{24}O_6]^+$ requires 288.1573.

Data is in agreement with the literature reference.

(3a*R*,4*R*,7a*R*)-4-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-2,2-dimethyl-4,7a-dihydro-3aH-[1,3]dioxolo[4,5-c]pyran (284)[5]



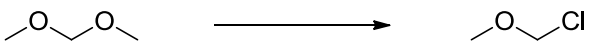
283 **284**

A solution of the hydroxyl-vinyl ether **283** (1.471 g, 4.46 7mmol) and mercury (II) acetate (0.285 g, 0.893 mmol) in anhydrous dichloromethane (30 ml) was evaporated to give a colourless viscous oil, which was heated in a Kugelrohr at 110 °C and 20 mm Hg for 2 h. The product was collected in the Kugelrohr flask and gave the pure cyclic compound **284** (0.755 g, 67%) without any further purification.

$[\alpha]^{25}_{D}$ -7.9 (*c* 0.0107 in $CHCl_3$); IR γmax (film)/cm^{-1}: 2986, 2934, 2888, 1648, 1456, 1371, 1236, 1138, 1099, 1061, 1028, 992, 969; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (6H, s, 2 x CH_3 isopropylidene), 1.45 (3H, s, CH_3 isopropylidene), 1.47 (3H, s, CH_3 isopropylidene), 3.80 (1H, d, *J* = 7.9, 0.9 Hz, H-5), 4.09 (1H, dd, *J* = 8.9, 5.0 Hz, H-7), 4.14 (1H, dd, *J* = 8.9, 6.1 Hz, H-7), 4.41-4.37 (1H, m, H-6), 4.45 (1H, dt, *J* = 6.2, 1.0 Hz, H-4), 4.68 (1H, dd, *J* = 6.2, 2.8 Hz, H-3), 4.81 (1H, ddd, *J* = 6.2, 2.8, 1.5 Hz, H-2), 6.37 (1H, d, *J* = 6.3, H-1); ^{13}C NMR (125 MHz, in $CDCl_3$): δ 25.31 (1C, CH_3 isopropylidene), 26.88 (1C, CH_3 isopropylidene), 27.07 (1C, CH_3 isopropylidene), 28.14 (1C, CH_3 isopropylidene), 66.70 (1C, CH_2, C-7), 68.46 (1C, C-5), 72.06 (1C, C-6), 74.18 (1C, C-4), 75.10 (1C, C-3), 103.07 (1C, Cq C-2), 109.44 (1C, Cq isopropylidene), 110.58 (1C, Cq isopropylidene), 144.52 (1C, Cq C-1); *m/z* $[M+NH_4]^+$: 274.1648; $[C_{13}H_{20}O_5+NH_4]^+$ requires 274.1649.

Data is in agreement with the literature reference.

Chloro(methoxy)methane (329)[6]



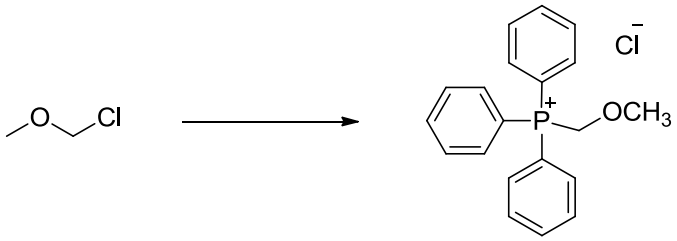
328 **329**

A three-neck 250 ml flask fitted with a thermometer, reflux condenser, and addition funnel was charged with dimethoxymethane **328** (44.25 ml, 0.5 mol), toluene (133 ml), and $Zn(OAc)_2$ (9.2 mg, 0.01%). Acetyl chloride (33.5 ml, 0.50 mol) was placed in the addition funnel, and was introduced into the reaction mixture at a constant rate over 5 min. $Zn(OAc)_2$ dissolved shortly after the addition of AcCl was started. During the next 15 min, the reaction mixture warmed slowly to 45 °C, and cooled to ambient temperature over 3 h, at which time analysis of an aliquot of the reaction by NMR indicated complete consumption of dimethoxymethane to **329**.

^{1}H NMR ($CDCl_3$): δ 2.03 (3H, s, MeOAc), 3.49 (3H, s, MOMCl), 3.64 (3H, s, MeOAc), 5.44 (2H, s, MOMCl).

Data is in agreement with the literature reference.

(Methoxymethyl)triphenylphosphonium chloride (330)[7]



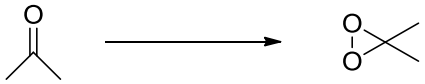
329 **330**

MOMCl **329** (2.1 M in toluene, 25.43 mL, 50.83 mmol) was added to a solution of triphenylphosphine (20 g, 76.25 mmol) in toluene. The solution was heated under reflux overnight and the white salt that precipitated out was filtered off, washed with toluene, and dried under reduced pressure to yield the product compound **330** (16.35 g, 94%).

mp 187-188 °C (Lit. 185-195 °C); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.69 (3H, s, methoxy), 5.89 (2H, d *J = 4.0* Hz, CH2-OMe), 7.87–7.61 (15H, m, CH aryl).

Data is in agreement with the literature reference.

3,3-dimethyldioxirane (310)[8]



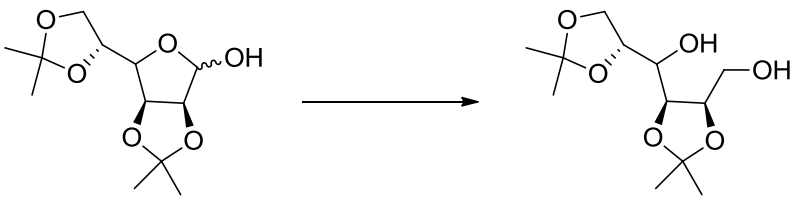
309 **310**

Sodium hydrogenocarbonate (29.0 g) was added in a mixture of water (127 mL) and acetone **309** (96 mL) through a solid addition funnel. The resulting white suspension was stirred vigorously, and the reaction was allowed to cool to 0 °C in an ice bath. Oxone (60.0 g) was added to the reaction mixture under vigorous stirring. The solid addition funnel was then removed and replaced with a thermometer adapter fitted with the thermometer in order to keep the temperature of the exothermic reaction mixture between 0 and 5 °C. After 15 min, a conelflexible tubing adapter was connected to a pump and a moderate vacuum (100 mmHg) was applied. The reaction mixture was allowed to rise to 30 °C by means of a warm water bath. The pale yellow effluent of dimethyldioxirane-acetone solution was collected in the previously cooled receiving flask at − 78 °C over 4 h. When the distillation was over, the pump was disconnected and a nitrogen flux was connected to the conelflexible tubing adapter. The dimethyldioxirane solution obtained was kept on molecular sieves (4 A) in a freezer. Iodometric titration allowed us to determine the concentration of dimethyldioxirane **310** in the solution (75 mL, 0.05-0.08 M, 5% yield);

IR γ_{max} (neat)/cm^{-1}: 2980 (O-C-O), 1210 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65 (6H, s, $6CH_3$); ^{13}C NMR (400 MHz, $CDCl_3$): δ 22.1 (2C, 2 x CH_3), 214.0 (1C, $C(CH_3)_2$).

Data is in agreement with the literature reference.

(*R*)-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-(4*S*,5*R*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl-methanol (200)[9]



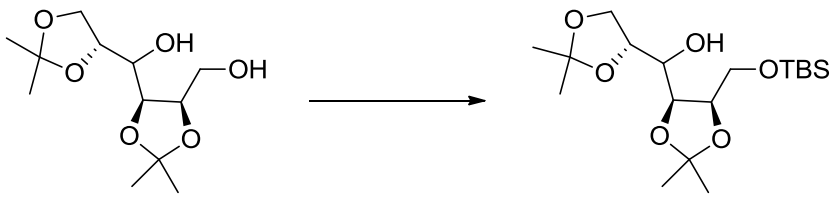
191 **200**

DAM **191** (10 g, 38.42 mmol) was dissolved in anhydrous THF (200 ml) at 0 °C and $LiAlH_4$ (1.6 g, 42.26 mmol) was slowly added portion-wise. The mixture was stirred at room temperature for 2 h. The excess $LiAlH_4$ was carefully destroyed by the addition of 1.5 g of Na_2SO_4, followed by the slow addition of few drops of water. The mixture was filtered through celite and extracted with diethyl ether. The combined filtrate was concentrated under reduced pressure, and the crude product was purified by column chromatography (Petrol:EtOAc, 1:2) on silica gel to give the pure diol **200** as a colourless syrup (8.314 g, 83%).

IR γ_{max} (film)/cm^{-1}: 3427, 2987, 1381, 1216, 1068, 850; ^{1}H NMR (400 MHz in $CDCl_3$): δ 1.39 (3H, s, CH_3 isopropylidene), 1.33 (3H, s, CH_3 isopropylidene), 1.43 (3H, s, CH_3 isopropylidene), 1.45 (3H, s, CH_3 isopropylidene), 2.79 (s, 1H, 4-OH), 3.52 (1H, dd, *J*=7.6, 1.5 Hz, H-4), 3.76 (1H, dd, *J*=12.2, 4.4Hz, CH_2, H-1), 3.83 (1H, dd, *J*=12.2, 4.4Hz, CH_2, H-1), 4.09-3.93 (3H, m, H-5, H-6), 4.24 (1H, dt, *J*=7.3, 4.4Hz, H-2), 4.32 (1H, dd, *J*=7.3, 1.5Hz, H-3). ^{13}C NMR (100 MHz, in $CDCl_3$): δ 24.79 (1C, CH_3 isopropylidene), 25.25 (1C, CH_3 isopropylidene), 26.77 (1C, CH_3 isopropylidene), 26.80 (1C, CH_3 isopropylidene), 60.98 (1C, CH_2, C-1), 67.33 (1C, CH_2, C-6), 70.26 (1C, C-4), 75.71 (1C, C-3), 76.08 (1C, C-5), 77.23 (1C, C-2), 108.38 (1C, Cq isopropylidene), 109.46 (1C, Cq isopropylidene).

Data is in agreement with the literature reference.

(*R*)-(4*S*,5*R*)-5-(tert-butyldimethylsilyl-oxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-methanol (264)[9]



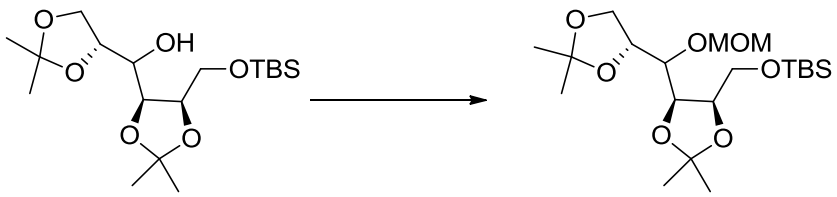
200 **264**

Diol **200** (8.314 g, 31.73 mmol) was dissolved in anhydrous DMF (35 ml) at 0 °C and TBSCl (5.74 g, 38.08 mmol) and imidazole (6.55 g, 96.142 mmol) were added. The mixture was stirred at room temperature for 4 h, then poured into water (50 mL) , extracted with Et_2O (50 mLx4). The combined organic layers were washed with brine, dried over $MgSO_4$, then filtered, concentrated under reduced pressure, and the residue was purified by column chromatography (Petrol:EtOAc, 4:1) on silica gel to give pure compound **264** as a colourless syrup (11.489 g, 96%).

IR γ_{max} (film)/cm^{-1}: 3394, 2985, 1370, 1241, 1208, 1059 and 845; ^{1}H NMR (400 MHz in $CDCl_3$): δ -0.0 (6H, s, $(Si(CH_3)_2)$), 0.80 (9H, s, $SiC(CH_3)_3$), 1.25 (3H, s, CH_3 isopropylidene), 1.28 (3H, s, CH_3 isopropylidene), 1.30 (3H, s, CH_3 isopropylidene), 1.39 (3H, s, CH_3 isopropylidene), 3.06 (1H, d, *J* = 6.0 Hz, OH), 3.56 (1H, dd, *J*= 7.6 Hz, 5.8 Hz, H-4), 3.72 (1H, dd, *J*=11.0 Hz, 3.8 HZ, H-1), 3.94-3.87 (2H, m, H-1, H-5), 4.04-3.96 (2H, m, H-6), 4.13 (1H, dt, *J*=6.7 Hz, 3.8 Hz, H-2), 4.28 (1H, dd, *J*= 7.1 Hz, 1.0 Hz, H-3). ^{13}C NMR (100 MHz, in $CDCl_3$): δ -5.51 (1C, $SiCH_3$), -5.48 (1C, $SiCH_3$), 18.26 (1C, Cq SiC), 24.82 (1C, CH_3 isopropylidene), 25.31 (1C, CH_3 isopropylidene), 25.81 (3C x $SiC(CH_3)_3$), 26.66 (1C, CH_3 isopropylidene), 26.87 (1C, CH_3 isopropylidene), 61.56 (1C, CH_2, C-1), 67.50 (1C, CH_2, C-6), 70.30 (1C, C-5), 75.72 (1C, C-4), 75.79 (1C, C-3), 77.35 (1C, C-2), 108.19 (1C, Cq isopropylidene), 109.23 (1C, Cq isopropylidene).

Data is in agreement with the literature reference.

Tert-butyl(4*R*,5*R*)-5-(*R*)-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-methoxymethoxy-methyl-2,2-dimethyl-1,3-dioxolan-4-yl-methoxy-dimethylsilane (271)[9]



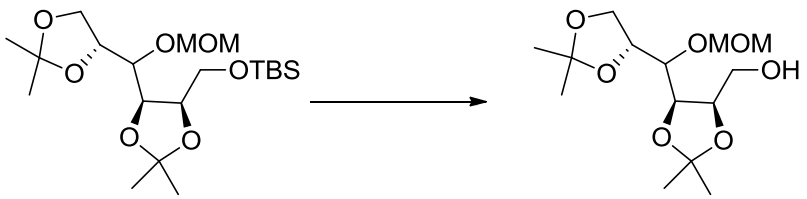
264 **271**

264 (11.5 g, 36.07 mmol) was dissolved in anhydrous dichloromethane (50 mL) at 0 °C and $^{i}Pr_2NEt$ (18.37 mL, 112.53 mmol) and MOMCl (8.42 mL, 104.6 mmol) were added. The mixture was stirred overnight at room temperature, and then diluted with Et_2O (100 mL), washed with water, saturated aqueous NH_4Cl, and brine, and dried over $MgSO_4$. The solution was concentrated under reduced pressure, and then the residue was purified by column chromatography (Petrol: EtOAc, 5:1) on silica gel to give pure compound **271** as a colourless syrup (9.49 g, 63%).

IR γ_{max} (film)/cm^{-1}: 2987, 2931, 2858, 1463, 1370, 1252, 1212, 1150, 1092, 1064, 1006, 970, 894; ^1H NMR (400 MHz, in $CDCl_3$): δ 0.1 (6H, s, $Si(CH_3)_2$), 0.9 (9H, s, $SiC(CH_3)_3$), 1.34 (3H, s, CH_3 isopropylidene), 1.35 (3H, s, CH_3 isopropylidene), 1.40 (3H, s, CH_3 isopropylidene), 1.45 (3H, s, CH_3 isopropylidene), 3.42 (3H, s, OCH_2OCH_3), 3.72 (1H, dd, *J*=10.5, 4.7 Hz, H-6), 3.85 (1H, dd, *J*=10.5, 7.0 Hz, H-6), 4.1 (4H, m, H-1, H-1, H-2, H-4), 4.12 (1H, m, H-3), 4.12 (1H, m, H-5), 4.83 (2H, s, OCH_2OCH_3).

Data is in agreement with the literature reference.

(4*R*,5*R*)-5-(*R*)-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-methoxymethoxy-methyl-2,2-dimethyl-1,3-dioxolan-4-yl-methanol (272)[9]



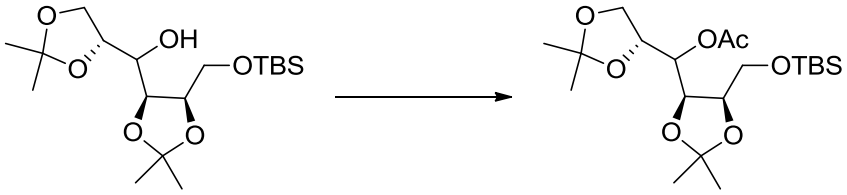
271 **272**

271 (9.451 g, 22.4 mmol) was dissolved in THF (50 mL) at 0 °C and TBAF (1 M solution in THF, 22.4 mL, 22.4 mmol) was added. After stirring for 2 h at room temperature, the mixture was diluted with Et_2O, washed with water and brine, dried over $MgSO_4$, and then concentrated under reduced pressure, and the residue was purified by column chromatography (Petrol:EtOAc, 1:1) on silica gel to give pure compound **272** as a colourless syrup (5.394 g, 79%).

IR γ_{max} (film)/cm^{-1}: 3407, 2985, 2931, 2858, 1462, 1371, 1250, 1212, 1156, 1063 and 939, 921, 834; ^1H NMR (400 Hz, $CDCl_3$): δ 1.34 (3H, s, CH_3 isopropylidene), 1.41 (3H, s, CH_3 isopropylidene), 1.48 (6H, s, CH_3 isopropylidene), 3.40 (3H, s, OCH_2OCH_3), 3.65 (1H, dd, *J*=11.1, 7.0 Hz, CH, H-6), 3.83 (1H, dd, *J*=11.1, 4.5 Hz, CH, H-6), 3.87 (1H, t, *J*=6.8 Hz, H-1), 3.94 (1H, t, *J*=7.7 Hz, CH, H-1), 4.08 (1H, dt, *J*=12.7, 6.3 Hz, CH, H-6), 4.21-4.011 (3H m, CH, H-5, H-3, H-2), 4.75 (1H, d, *J*=6.8 Hz, OCH_2OCH_3), 4.80 (1H, d, *J*=6.8 Hz, OCH_2OCH_3).

Data is in agreement with the literature reference.

(*R*)-(4*R*,5*R*)-5-(tert-butyldimethylsilyl-oxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-methyl acetate (265)

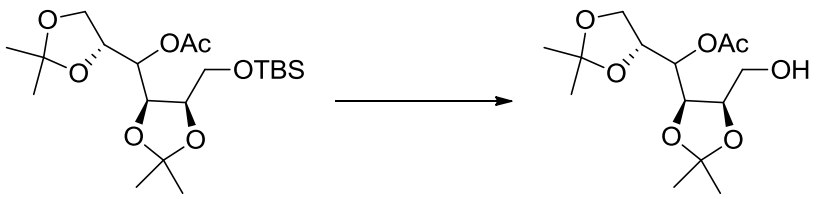


264 **265**

Acetic anhydride (4.5 ml, 47.74 mmol), DMAP (0.9 g, 7.4 mmol) and TEA (6.63 ml, 47.74 mmol) were added to a solution of **264** (8.15 g, 21.7 mmol) in anhydrous dichloromethane (40 ml) at 0 °C. The reaction mixture was then stirred for 2 h at room temperature. The reaction mixture was extracted with aqueous $NaHCO_3$ and dichloromethane and then washed with water, brine and dried over $MgSO_4$. The solvent was removed under reduced pressure to give the crude product which was then purified by flash chromatography (Petrol:EtOAc, 4:1) on silica gel to give the pure compound **265** as a colourless syrup (8.2 g, 90%).

IR γ_{max} (film)/cm^{-1}: 2954, 2934, 1749, 1371, 1250, 1216, 1069 and 837; ^{1}H NMR (400 MHz in $CDCl_3$): δ 0.01 (6H, s, $Si(CH_3)_2$), 0.82 (9H, s, $SiC(CH_3)_3$), 1.27 (3H, s, CH_3 isopropylidene), 1.29 (3H, s, CH_3 isopropylidene), 1.29 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 2.02 (3H, s, CH_3 acetate), 3.57 (1H, dd, *J*=10.5, 7.3 Hz, CH_2, H-1), 3.67 (1H, dd, *J*=10.5 Hz, CH_2, H-1), 3.85 (1H, dd, *J*=8.5, 6.8 Hz, H-6), 3.92 (1H, dd, *J*=8.5, 6.3Hz, H-6), 4.17 (2H, m, H-3, H-5), 4.25 (1H, dd, *J*=6.4, 2.4 Hz, H-2), 5.14 (1H, dd, *J*=5.9, 2.4 Hz, H-4) . ^{13}C NMR (100 MHz, in $CDCl_3$): δ -5.42 (1C, $SiCH_3$), -5.45 (1C, $SiCH_3$), 18.26 (1C, Cq Si), 21.25 (1C, CH_3, acetate), 25.50 (1C, CH_3 isopropylidene), 25.59 (1C, CH_3 isopropylidene), 25.86 (3C, 3 x $SiC(CH_3)_3$), 26.45 (1C, CH_3 isopropylidene), 26.59 (1C, CH3 isopropylidene), 61.46 (1C, CH2, C-1), 65.90 (1C, CH2, C-6), 70.20 (1C, CH, C-5), 75.39 (1C, CH, C-4), 75.70 (1C, CH, C-3), 77.24 (1C, CH, C-2), 109.03 (1C, Cq isopropylidene), 109.09 (1C, Cq isopropylidene), 169.83 (1C, Cq C=O); *m/z* $[M+H]^+$: 419.2449; $[C_{20}H_{38}O_7Si+H]^+$ requires 419.2460.

(*R*)-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-(4*R*,5*R*)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl-methyl acetate (266)

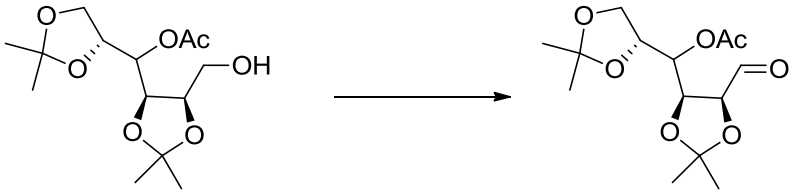


265 **266**

265 (4.681 g, 16.26 mmol) was dissolved in THF (50 mL) at 0 °C and TBAF (1 M solution in THF, 16.26 mL) was added. After stirring for 2 h at room temperature, the mixture was diluted with Et_2O, washed with water and brine, dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (Petrol:EtOAc, 1:1) on silica gel to give pure compound **266** as a colourless syrup (3.88 g, 78%).

IR γ_{max} (film)/cm^{-1}: 3504, 2987, 1741, 1372, 1215, 1158, 1041 and 851; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (3H, s, CH_3 isopropylidene), 1.40 (3H, s, CH_3 isopropylidene), 1,41 (3H, s, CH_3 isopropylidene), 1.52 (3H, s, CH_3 isopropylidene), 2.10 (3H, s, CH_3 acetate), 2.18 (1H, s, OH), 3.52 (1H, t, *J*=7.9 Hz, H-1), 4.04 - 3.98 (2H, m, CH_2, H-1, H-6), 4.10 (1H, td, *J*=8.2, 3.8 Hz, H-6), 4.31 (1H, m, H-3), 4.47 - 4.40 (3H, m, H-2, H-4, H-5): ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.20 (1C, CH_3 acetate), 20.93 (1C, CH_3 isopropylidene), 24.59 (1C, CH_3 isopropylidene), 25.24 (1C, CH_3 isopropylidene), 26.82 (1C, CH_3 isopropylidene), 63.9 (1C, CH_2, C-1), 67.17 (1C, C-6), 70.20 (1C, C-5), 75.02 (1C, C-4), 75.23 (1C, C-2), 76.15 (1C, C-3), 108.89 (1C, Cq isopropylidene), 109.5 (1C, Cq isopropylidene), 170.75 (1C, Cq C=O); *m/z* $[M+NH_4]^+$: 322.1855; $[C_{14}H_{24}O_7+NH_4]^+$ requires 322.1860.

(*R*)-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-(4*S*,5*S*)-5-formyl-2,2-dimethyl-1,3-dioxolan-4-yl-methyl acetate (267)[12]

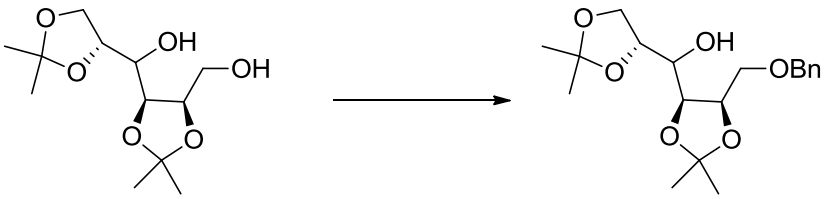


DMSO (1.93 mL, 27.13 mmol) was slowly added to a solution of oxalyl chloride (1.3 mL, 14.73 mmol) in anhydrous dichloromethane (30 ml) at -78 °C and the solution was stirred for 1 h at this temperature. A solution of 4-*O*-acetoxy-2,3:5,6-di-*O*-isopropylidene-D-mannitol **266** (2.36 g, 7.8 mmol) in anhydrous dichloromethane (15 ml) was added dropwise to the reaction mixture and the solution was stirred for further 1 h. TEA (5.17 mL, 37.2 mmol) was slowly added to the reaction mixture at the same temperature and then the solution was allowed to warm up to 0 °C and the mixture was stirred for a further 30 min at 0 °C. Then the reaction mixture was allowed to warm up to room temperature and was allowed to stir for 30 min at this temperature. Water, saturated aqueous solution of NH_4Cl and 2 M aqueous HCl were added to the reaction mixture which was then extracted with dichloromethane. The organic layer was washed further with water and brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give pure compound **267** (2.32 g, quantitative) without any further purification.

IR γ_{max} (film)/cm^{-1}: 2988, 1742, 1374, 1216, 1068 and 852 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.29 (6H, s, 2 x CH_3 isopropylidene), 1.30 (3H, s, CH_3 isopropylidene), 1.46 (3H, s, CH_3 isopropylidene), 1.97 (3H, s, CH_3 acetate) 3.97 (1H, dd, *J*=6.4 Hz, 8.8 Hz, CH_2, H-6), 4.09 (1H, dd, *J*=6.4, 8.8 Hz, CH_2, H-6), 4.18 (2H, m, 2 x H-5, H-3), 4.54 (1H, ddd, *J*=2.0, 8.0 Hz, H-2), 4.75 (1H, m, H-4) ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.63 (1C, CH_3 acetate), 24.48 (1C, CH_3 isopropylidene), 24.63 (1C, CH_3 isopropylidene), 25.84 (1C, CH_3 isopropylidene), 26.66 (1C, CH_3 isopropylidene), 61.8 (1C, CH), 65.62 (1C, CH_2, C-6), 70.19 (1C, CH), 75.37 (1C, CH), 78.88 (1C, CH, C-2), 110.06 (1C, Cq isopropylidene), 110.87 (1C, Cq isopropylidene), 170.38 (1C, C=O ester), 204.49 (1C, C=O aldehyde).

Data is in agreement with the literature reference.

(*R*)-(4*S*,5*R*)-5-benzyloxy-methyl-2,2-dimethyl-1,3-dioxolan-4-yl-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-methanol (293)[11]



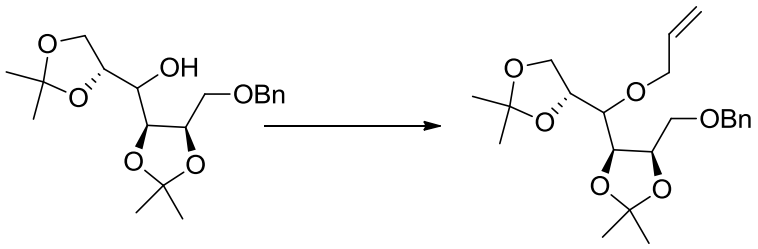
200 **293**

200 (3.315 g, 12.64 mmol) was dissolved in anhydrous DMF and the solution cooled to 0 °C. NaH (0.319 g, 13.272 mmol) was added and the mixture was stirred for 10 min at this temperature before being allowed to warm up to room temperature. Benzyl bromide (1.80 mL, 15.2 mmol) was added dropwise, and the mixture was stirred for 5 min, before water was added at 0 °C. The mixture was extracted with EtOAc and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by column chromatography (Toluene:EtOAc, 9:1) to give pure compound **293** as a colourless syrup (4.02 g, 90%).

IR γ_{max} (film)/ cm^{-1}: 3478, 3089, 3064, 3031, 2988, 2936, 2876, 1877, 1738, 1713, 630, 1587, 1496, 1454, 1308, 1249, 1042; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.34 (3H, s, CH_3 isopropylidene), 1.37 (3H, s, CH_3 isopropylidene), 1.39 (3H, s, CH_3 isopropylidene), 1.51 (3H, s, CH_3 isopropylidene), 2.80 (1H, d, *J* = 6.8 Hz, 4-OH), 3.50 (1H, d, *J* = 7.9 Hz, H-4), 3.75 (1H, dd, *J* = 10.3, 5.1 Hz, H-1), 3.81 (1H, dd, *J* = 10.3, 4.9 Hz, H-1), 4.01 – 3.96 (1H, m, H-5), 4.07 – 4.01 (1H, m, H-6), 4.15 – 4.07 (1H, m, H-6), 4.37 (1H, dd, *J* = 7.2, 1.0 Hz, H-3), 4.42 (1H, dt, *J* = 7.2, 5.0 Hz, H-2), 4.59 (2H, s, CH_2Ph), 7.32 – 7.28 (1H, m, CH aryl), 7.36 – 7.32 (4H, m, CH aryl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.75 (1C, CH_3 isopropylidene), 25.31 (1C, CH_3 isopropylidene), 26.65 (1C, CH_3 isopropylidene), 26.86 (1C, CH_3 isopropylidene), 67.30 (1C, CH_2 C-7), 68.52 (1C, CH carbo), 70.45 (1C, CH carbo), 73.82 (1C, CH_2Ph), 75.56 (1C, CH carbo), 75.81 (1C, CH carbo), 75.94 (1C, CH carbo), 108.52 (1C, Cq isopropylidene), 109.32 (1C, Cq isopropylidene), 127.95 (1C, CH aryl), 128.02 (2C, 2 x CH aryl), 128.51 (2C, 2 x CH aryl), 137.40 (1C, Cq aryl); *m/z* $[M+H]^+$: 353.1960; $[C_{19}H_{28}O_6+H]^+$ requires 353.1959.

Data is in agreement with the literature reference.

(4*R*,5*R*)-4-(*R*)-(allyloxy)-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-methyl-5-benzyloxy-methyl-2,2-dimethyl-1,3-dioxolane (298)

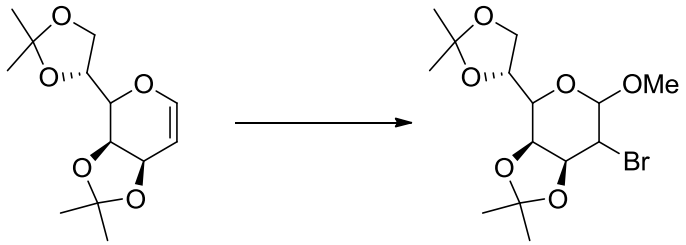


293 **298**

293 (1 g, 2.714 mmol) was dissolved in anhydrous DMF (20 mL) and NaH (0.103 g, 4.071 mmol) was added at 0 °C in small portions. The reaction mixture was stirred for a further 30 min at this temperature. Allyl bromide (0.274 mL, 3.17 mmol) was then added over a period of 5 min dropwise. After being stirred for 1 h, the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with diethyl ether. The organic extracts were washed with brine, then dried over $MgSO_4$ and evaporated under reduced pressure. The crude mixture was purified by column chromatography (Toluene:EtOAc, 9:1) to give pure compound **298** as a colourless syrup (1.021 g, 96%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32 (3H, s, CH_3 isopropylidene), 1.34 (3H, s, CH_3 isopropylidene), 1.37 (3H, s, CH_3 isopropylidene), 1.47 (3H, s, CH_3 isopropylidene), 3.70 – 3.61 (3H, m, H-6, H-6, H4), 4.07 – 3.93 (3H, m, H-1, H-1, H-3), 4.12 (1H, dd, *J* = 6.4, 4.4 Hz, H-3), 4.20 – 4.14 (1H, m, H-2), 4.30 (1H, ddt, *J* = 13.0, 5.3, 1.5 Hz, CH_2Ph), 4.37 (1H, dt, *J* = 12.0, 5.7 Hz, CH_2Ph), 4.53 (1H, d, *J* = 12.0 Hz, CH_2-C=C), 4.60 (1H, d, *J* = 12.1 Hz, CH2-C=C), 5.10 (1H, ddd, *J* = 10.5, 3.2, 1.5 Hz, CH=CH_2), 5.29 – 5.24 (1H, m, CH=CH_2), 5.92 – 5.79 (1H, m, CH=CH_2), 7.37 – 7.30 (5H, m, CH aryl); ^{13}C (125 MHz, $CDCl_3$): δ 25.18 (1C, CH_3 isopropylidene), 25.68 (1C, CH_3 isopropylidene), 26.29 (1C, CH_3 isopropylidene), 26.98 (1C, CH_3 isopropylidene), 65.88 (1C, CH_2 C-7), 69.07 (1C, CH_2 C-1), 72.75 (1C, O-CH_2), 73.58 (1C, CH_2Ph), 76.20 (1C, C-5), 76.98 (1C, C-2), 77.78 (1C, C-4), 78.10 (1C, C-3), 108.36 (1C, Cq isopropylidene), 108.89 (1C, Cq isopropylidene), 116.10 (1C, CH_2 alkene), 127.77 (1C, CH aryl), 127.94 (2C, 2 x CH aryl), 128.42 (2C, 2 x CH aryl), 134.89 (1C, CH=CH_2), 137.91 (1C, Cq aryl); *m/z* $[M+NH4]^+$: 410.2534; $[C_{22}H_{32}O_6+NH_4]^+$ requires 410.2537.

(3a*S*,4*R*,7a*S*)-7-bromo-4-((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)-6-methoxy-2,2-dimethyltetrahydro-3aH-[1,3]dioxolo[4,5-c]pyran (311)

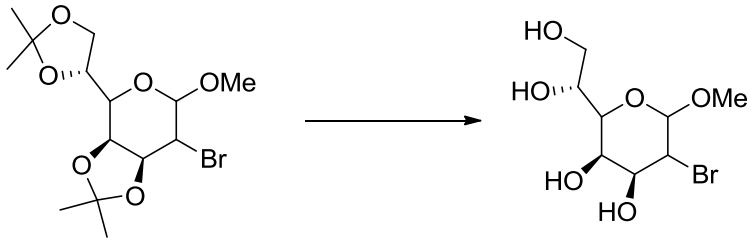


284 **311**

A solution of **284** (0.781 g, 3.05 mmol) in anhydrous MeOH (18 mL) was added dropwise to a stirred and cooled -50 °C solution of NBS (0.543 g, 3.05 mmol) in anhydrous MeOH. The cooling bath was left in place but not recharged and stirring was continued overnight. Most of the MeOH was removed under reduced pressure and the residue was diluted with diethyl ether. The mixture was washed with a saturated solution of $Na_2S_2O_3$, water, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by column chromatography (Petrol: EtOAc, 4:1) over silica gel to give pure compound **311** (1.053 g, 94%) as a colourless syrup and a single diastereoisomer.

IR γ_{max} (film)/ cm^{-1}: 2946, 2940, 2938, 1747, 1434, 1372, 1382, 1230, 1140, 1060, 985, 911; ^1H NMR (500 MHz, $CDCl_3$): δ 1.40 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 1.46 (3H, s, CH_3 isopropylidene), 1.56 (3H, s, CH_3 isopropylidene), 3.56 (3H, s, CH_3 methoxy), 3.75–3.67 (2H, m, CH_2, H-7), 4.03-4.06 (2H, m, H-5, H-6), 4.21 (1H, dd, *J* = 5.3, 2.1 Hz, H-4), 4.35 (1H, d, *J* = 8.9 Hz, H-3), 4.45–4.38 (2H, m, H-1, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.33 (1C, CH_3 isopropylidene), 26.22 (1C, CH_3 isopropylidene), 27.00 (1C, CH_3 isopropylidene), 28.17 (1C, CH_3 isopropylidene), 53.06 (1C, C-2), 57.04 (1C, CH_3 methoxy), 66.62 (1C, CH_2 C-7), 73.54 (1C, C-4), 73.60 (1C, C-6), 74.06 (1C, C-3), 80.68 (1C, C-5), 102.85 (1C, Cq C-1), 109.55 (1C, Cq isopropylidene), 110.67 (1C, Cq isopropylidene); *m/z* $[M+H]^+$: 367.0757; $[C_{14}H_{23}BrO_6+H]^+$ requires 367.0751.

2*R*,3*R*,4*S*-5-bromo-2-(*R*)-1,2-dihydroxyethyl-6-methoxytetrahydro-2H-pyran-3,4-diol (313)

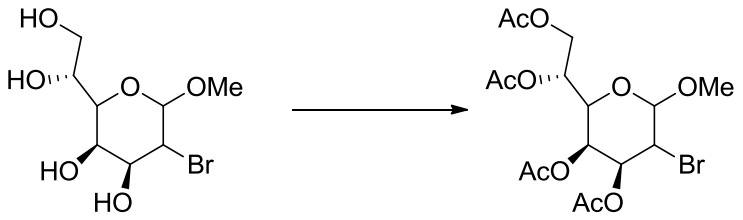


311 **313**

Aqueous 80% TFA (20.12 mL) was added to a solution of **311** (1.01 g, 2.76 mmol) in dichloromethane (40 mL). The mixture was stirred for 24 h at room temperature, then concentrated and co-evaporated with toluene several times. The reaction mixture was concentrated under reduced pressure and the crude product was purified by column chromatography (Petrol:EtOAc, 1:1) to give compound **313** (0.72 g, 90%) as a colourless syrup.

Used in the next step straight after chromatography.

(2*R*,3*S*,4*S*)-5-bromo-2-(*R*)-1,2-diacetoxyethyl-6-methoxytetrahydro-2H-pyran-3,4-diyl-diacetate (314)

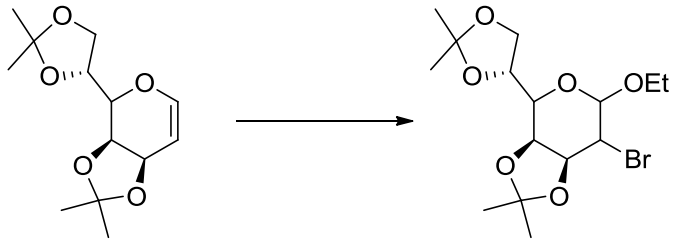


313 **314**

Acetic anhydride (0.193 ml, 2.05 mmol), DMAP (39 mg, 0.32 mmol) and TEA (0.284 ml, 2.05 mmol) were added to a solution of **313** (0.132 g, 0.466 mmol) in anhydrous dichloromethane (40 ml) at 0 °C. The reaction mixture was then stirred for 2 h at room temperature. The reaction mixture was extracted with aqueous $NaHCO_3$ and dichloromethane and then washed with water, brine and dried over $MgSO_4$. The solvent was evaporated to dryness to give the desired compound and then purified by flash chromatography (Petrol:EtOAc, 2:1) on silica gel to give pure compound as a colourless syrup **314** (0.1 g, 77%).

IR γ_{max} (film)/ cm^{-1}: 2946, 2940, 2938, 1747, 1434, 1372, 1382, 1230, 1140, 1060, 985, 911; ^{1}H NMR (400 MHz, $CDCl_3$): δ ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.94 (3H, s, CH_3 acetate), 1.99 (3H, s, CH_3 acetate), 2.03 (3H, s, CH_3 acetate), 2.03 (3H, s, CH_3 acetate), 3.52 (3H, s, CH_3 methoxy), 3.81 (1H, dd, *J* = 9.6, 0.7 Hz, H-4), 3.88 (1H, dd, *J* = 11.2, 8.6 Hz, H-6), 4.21 (1H, dd, *J* = 12.3, 3.9 Hz, H-7), 4.35 (1H, dd, *J* = 12.3, 2.3 Hz, H-7), 4.42 (1H, d, *J* = 8.6 Hz, H-6), 5.06 – 4.98 (1H, m, H-5), 5.13 – 5.06 (1H, m, H-2), 5.25 (1H, d, *J* = 2.6 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5 (1C, CH_3, acetate), 20.5 (1C, CH_3 acetate), 20.6 (1C, CH_3 acetate), 20.7 (1C, CH_3 acetate), 47.9 (1C, C-2), 57.5 (1C, CH_3 methoxy), 62.0 (1C, CH_2, C-7), 66.3 (1C, C-4), 67.4 (1C, C-6), 70.6 (1C, C-5), 73.0 (1C, C-3), 103.8 (1C, C-1), 169.5 (1C, Cq C=O), 169.7 (1C, Cq C=O), 170.2 (1C, Cq C=O), 170.5 (1C, Cq C=O); *m/z* $[M+NH_4]^+$: 472.0818; $[C_{16}H_{23}BrO_{10}+NH_4]^+$ requires 472.0813.

(3a*S*,4*R*,7a*S*)-7-bromo-4-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-6-ethoxy-2,2-dimethyltetrahydro-3aH-[1,3]dioxolo[4,5-c]pyran (312)

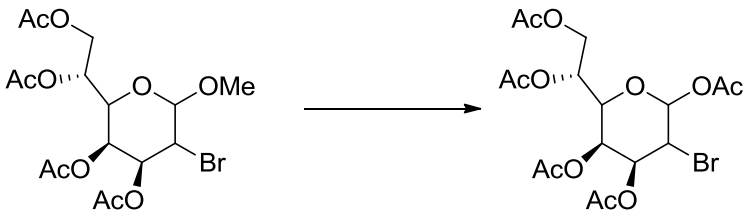


284 **312**

A solution of **284** (0.1 g, 0.39 mmol) in anhydrous EtOH (10 mL) was added dropwise to a stirred and cooled -50 °C solution of NBS (0.07 g, 0.39 mmol) in anhydrous EtOH. The cooling bath was left in place but not recharged and stirring was continued overnight. Most of the EtOH was removed under reduced pressure and the residue was diluted with diethyl ether. The mixture was washed with a saturated solution of $Na_2S_2O_3$, water, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by column chromatography Petrol: EtOAc, 4:1) on silica gel to give pure compound **312** as a colourless syrup (0.135 g, 91%) and as a single diastereoisomer.

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (3H, t, *J* = 7.1 Hz, CH_3 ethoxy), 1.40 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 1.46 (3H, s, CH_3 isopropylidene), 1.56 (3H, s, CH_3 isopropylidene), 3.68 – 3.61 (1H, m, H-5), 3.72 (2H, ddd, *J* = 8.3, 5.7, 3.7 Hz, CH_2, H-7), 3.94 (1H, dq, *J* = 9.6, 7.1 Hz, H-6), 4.11 (2H, qd, *J* = 8.9, 5.2 Hz, CH_2 ethoxy), 4.20 (1H, dd, *J* = 5.2, 2.1 Hz, H-4), 4.45 – 4.38 (3H, m, H-1, H-2, H-3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.96 (1C, CH_3 ethoxy), 25.33 (1C, CH_3 isopropylidene), 26.24 (1C, CH_3 isopropylidene), 27.03 (1C, CH_3 isopropylidene), 28.17 (1C, CH_3 isopropylidene), 53.41 (1C, C-2), 65.68 (1C, CH_2, C-7), 66.72 (1C, CH_2 ethoxy), 73.56 (2C, C-6, C-4), 74.03 (1C, C-3), 80.79 (1C, C-5), 101.76 (1C, Cq C-1), 109.55 (1C, Cq isopropylidene), 110.63 (1C, Cq isopropylidene); *m/z* $[M+NH_4]^+$: 398.1181; $[C_{15}H_{25}BrO_6+NH_4]^+$ requires 398.1173.

(4*S*,5*S*,6*R*)-3-bromo-6-(*R*)-1,2-diacetoxyethyl-tetrahydro-2H-pyran-2,4,5-triyl triacetate (315)

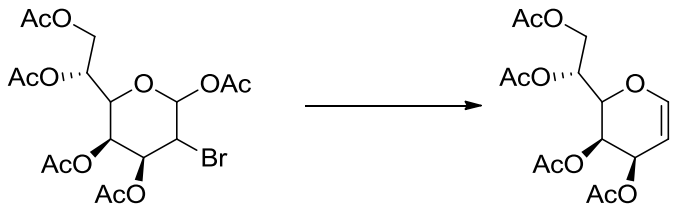


314 **315**

Tetra-acetate **314** (0.111 g, 0.244 mmol) was dissolved in acetic anhydride (0.5 ml) and 2 mL of 2% H_2SO_4 in acetic anhydride was added. The reaction mixture was maintained at room temperature for 2 h, then diluted with chloroform, and washed successively with an ice-water mixture (2 x 200 ml), saturated aqueous $NaHCO_3$, and water. The organic layer was dried over $MgSO_4$ and concentrated, and the crude product was purified by column chromatography (Petrol:EtOAc, 1:1) on silica gel to give compound as a colourless syrup **315** (0.1 g, 85%).

$[\alpha]^{25}_D$ -81.3 (*c* 0.00603 in $CHCl_3$); IR γ_{max} (film)/ cm^{-1}: 2979, 1838, 1751, 1440, 1373, 1227, 1133, 1070, 1043, 1010; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01 (3H, s, CH_3 acetate), 2.08 (3H, s, CH_3 acetate), 2.08 (3H, s, CH_3 acetate), 2.13 (3H, s, CH_3 acetate), 2.21 (3H, s, CH_3 acetate), 4.05 – 4.18 (2H, m, CH_2, H-7), 4.30 – 4.37 (2H, m, H-2, H-4), 4.44 (1H, dd, *J* = 12.3, 2.4 Hz, H-5), 5.11 (1H, ddd, *J* = 9.8, 4.2, 2.4 Hz, H-6), 5.40 (1H, dd, *J* = 11.5, 3.3 Hz, H-3), 5.46 (1H, dd, *J* = 3.4, 1.4 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4 (1C, CH_3 acetate), 20.5 (1C, CH_3 acetate), 20.5 (1C, CH_3 acetate), 20.6 (1C, CH_3 acetate), 20.7 (1C, CH_3 acetate), 44.2 (1C, C-2), 62.0 (1C, CH_2, C-7), 66.7 (1C, C-3), 67.2 (1C, C-6), 68.3 (1C, C-4), 69.5 (1C, C-5), 91.0 (1C, C-1), 168.3 (1C, Cq C=O, acetate), 169.6 (1C, Cq, C=O acetate), 169.7 (1C, Cq C=O acetate), 170.1 (1C, Cq C=O acetate), 170.2 (1C, Cq C=O acetate); *m/z* $[M+NH_4]^+$: 500.0759; $[C_{17}H_{23}BrO_{11}+NH_4]^+$ requires 500.0762.

(*R*)-1-(2*R*,3*R*,4*R*)-3,4-diacetoxy-3,4-dihydro-2H-pyran-2-yl-ethane-1,2-diyl-diacetate (299)

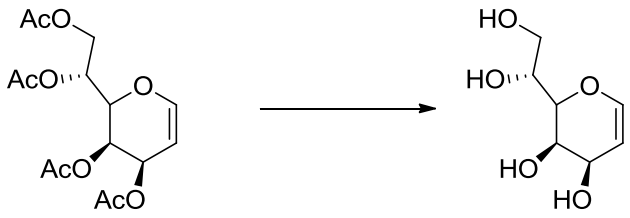


315 **299**

Zinc dust (1.78 g, 27.28 mmol) was suspended into a stirred solution of AcONa (2.1 g, 25.55 mmol) and AcOH (6 mL) in water (1.5 mL), and saturated aqueous $CuSO_4$ (1.2 mL) was then added. The blue colour disappeared. A solution of **315** (0.269 g, 0.56 mmol) in Ac_2O (2 mL) was then added at a fast dropwise rate, and stirring was continued for 3 h. The mixture was diluted with dichloromethane and filtered through a pad of celite. The combined organic extracts were washed with saturated aqueous $NaHCO_3$, brine and dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (Petrol:EtOAc, 1:3) over silica gel to give a pure compound **299** (0.162 g, 82%) as a colourless oil.

$[\alpha]^{25}_D$ 10.7 (*c* 0.0105 in $CHCl_3$); IR γ_{max}(film)/ cm^{-1}: 1749, 1654, 1436, 1372, 1226, 1151, 1128, 1063, 1040, 1013; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.03 (3H, s, CH_3 acetate), 2.06 (3H, s, CH_3 acetate), 2.10 (3H, s, CH_3 acetate), 2.12 (3H, s, CH_3 acetate), 4.17 (1H, dd, *J* = 12.3, 4.5 Hz, H-7), 4.25 (1H, d, *J* = 9.7 Hz, H-5), 4.55 (1H, dd, *J* = 12.3, 2.3 Hz, H-7), 4.71 (1H, dt, *J* = 6.3, 1.9 Hz, H-6), 5.19 (1H, ddd, *J* = 9.7, 4.5, 2.4 Hz, H-4), 5.47 (1H, dd, *J* = 3.3, 1.3 Hz, H-3), 5.63 (1H, ddd, *J* = 3.9, 3.0, 2.0 Hz, H-2), 6.47 (1H, dd, *J* = 6.3, 2.1 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.61 (1C, CH_3 acetate), 20.69 (1C, CH_3 acetate), 20.75 (1C, CH_3 acetate), 20.78 (1C, CH_3 Acetate), 61.53 (1C, CH_2, C-7), 62.14 (1C, C-3), 64.25 (1C, C-6), 67.48 (1C, C-4), 72.27 (1C, C-5), 99.34 (1C, C-2), 144.97 (1C, C-1), 169.68 (1C, Cq C=O acetate), 170.32 (1C, Cq C=O acetate), 170.43 (1C, Cq C=O acetate), 170.60 (1C, Cq C=O acetate); *m/z* $[M+NH_4]^+$: 362.1450; $[C_{15}H_{20}O_9+NH_4]^+$ requires 362.1446.

2*R*,3*R*,4*R*-2-*R*-1,2-dihydroxyethyl-3,4-dihydro-2H-pyran-3,4-diol (300)

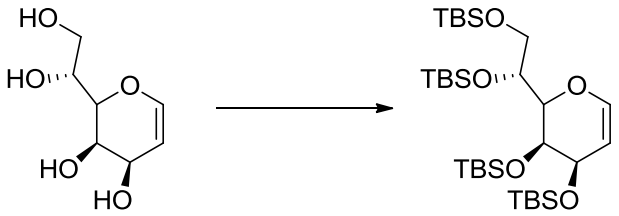


299 **300**

A solution of **299** (1 g, 2.91 mmol) in anhydrous methanol (12 mL) was treated with of sodium methoxide (2 M, 0.2 mL) in 3 mL of anhydrous methanol. The combined solution was stirred at room temperature overnight and then concentrated to dryness. The crude product was purified by column chromatography (Petrol: EtOAc, 1:2) to give pure compound **300** (0.487 g, 95%).

Used straight in the next step after purification.

(*R*)-5-(2*R*,3*S*,4*R*)-3,4-bis((tert-butyldimethylsilyl)oxy)-3,4-dihydro-2H-pyran-2-yl-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane (301)

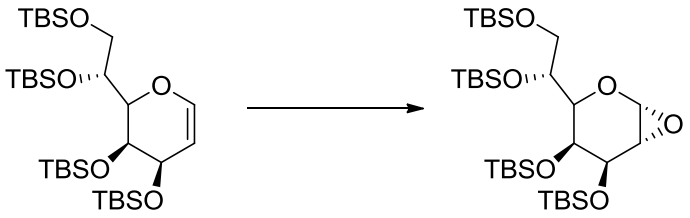


300 **301**

Compound **300** (0.901 g, 2.62 mmol) was taken up in 11 mL of anhydrous DMF and treated with imidazole (1.71 g, 25.14 mmol), followed by *tert*-butyldimethylchlorosilane (1.9 g, 12.6 mmol). The resulting solution was stirred at room temperature for 16 h, diluted with 200 mL of diethyl ether, washed with water (1 x 100 mL) and a copper (II) sulfate solution (2 x 100 mL). The aqueous layers were back extracted with diethyl ether, and then the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (Petrol:Ether, 98:2) to furnish compound **301** (0.92 g, 60%) as a clear, homogenous, colourless oil.

IR γ_{max} (film)/ cm^{-1}: 2943, 2892, 2887, 1651, 1463, 1452, 1384, 1241, 1148, 1112, 1099, 1001; $[\alpha]^{25}_{D}$ -16 (*c* 0.0115 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ -0.07 (3H, s, $SiCH_3$), -0.07 (3H, s, $SiCH_3$), -0.00 (6H, s, 2 x $SiCH_3$), 0.01 (6H, s, 2 x $SiCH_3$), 0.02 (6H, s, 2 x $SiCH_3$), 0.78 (12H, s, 4 x $SiC(CH_3)$), 0.78 (12H, s, 4 x $SiC(CH_3)$), 0.80 (12H, s, 4x $SiC(CH_3)$), 3.59 (2H, dd, *J* = 10.7, 4.3 Hz, CH_2 H-7), 3.67 (1H, dd, *J* = 10.6, 2.6 Hz, H-5), 3.91 – 3.87 (1H, m, H-6), 3.94 – 3.92 (1H, m, H-4), 4.39 – 4.35 (2H, m, H-2, H-3), 6.20 (1H, dd, *J* = 6.1, 1.3 Hz, H-1); ^{13}C (125 MHz, $CDCl_3$): δ -5.47, -5.35, -5.04, -4.85, -4.70, -4.29 (8C, 8 x $SiCH_3$), 18.20, 18.21, 18.48 (4C, 4 x Cq, SiC), 25.82, 25.90, 26.00 (12C, 12 x $SiC(CH_3)_3$), 63.54 (1C, CH carbo), 64.59 (1C, CH_2, C-7), 65.72 (1C, CH carbo), 71.29 (1C, CH carbo), 75.13 (1C, CH carbo), 102.70, (1C, C-1), 144.03 (1C, C-2); *m/z* $[M+NH_4]^+$: 650.4460; $[C_{31}H_{68}O_5Si_4+NH_4]^+$ requires 650.4482.

(*R*)-5-(1*S*,3*R*,4*S*,5*R*,6*R*)-4,5-bis((tert-butyldimethylsilyl)oxy)-2,7 dioxabicyclo[4.1.0]heptan-3-yl-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane (303)

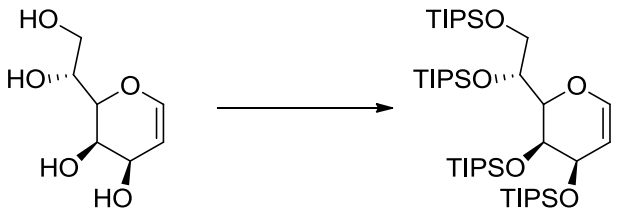


301 **303**

The glycal **301** (0.083 g, 0.131 mmol) was dissolved in 5 mL of dichloromethane, and the resulting solution was cooled to 0 °C. A solution of dimethyldioxirane in acetone (5.3 mL, 0.16 mmol, ca. 0.03 M) was added dropwise. The resulting mixture was stirred at 0 °C for 1 h or until TLC indicated complete consumption of the starting material. The solution was evaporated to dryness and the residue was dried under reduced pressure to afford the 1,2-anhydro sugar **303** in quantitative yield as a white solid (0.084 g).

$[\alpha]^{25}_{D}$ -24.0 (*c* 0.001 in $CHCl_3$); IR γ_{max} (film)/ cm^{-1}: 2952, 2928, 2856, 2885, 1472, 1462, 1438, 1389, 1360, 1329, 1249, 1148, 1112, 1096, 1062, 1027, 1005; ^{1}H NMR (500 MHz, $CDCl_3$): δ -0.00 (4H, s), 0.00 (4H, s), 0.05 (4H, s), 0.06 (4H, s), 0.11 (4H, s), 0.12 (4H, s) (24H, 4 x $Si(CH_3)_2$), 0.82 (12H, s, 4 x $SiC(CH_3)$), 0.83 (12H, s, 4 x $SiC(CH_3)$), 0.89 (12H,s, 4x $SiC(CH_3)$), 2.79 (1H, t, *J* = 2.1 Hz, H-2), 3.47 (1H, d, *J* = 7.4 Hz, H-6), 3.60 (1H, dd, *J* = 10.6, 4.9 Hz, H-7), 3.70 (1H, dd, *J* = 10.6, 3.8 Hz, H-7), 3.80 – 3.78 (1H, m, H-3), 3.89 (1H, d, *J* = 4.0 Hz, H-4), 3.94 – 3.90 (1H, m, H-5), 4.90 (1H, d, *J* = 2.2 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): δ -5.43, -5.41, -5.05, -4.89, -4.78, -4.38 (8C x $SiCH_3$), 18.16, 18.24, 18.43 (4C x Cq, SiC), 25.79, 25.85, 25.88, 25.97 (12C x $SiC(CH_3)_3$), 52.65 (1C, C-2), 62.32 (1C, C-5), 64.49 (1C, CH_2, C-7), 68.44 (1C, C-3), 68.85 (1C, C-4), 72.30 (1C, C-3), 77.40 (1C, C-1); *m/z* $[M-H]^-$: 647.4004; $[C_{31}H_{68}O_6Si_4-H]^-$ requires 647.4009.

(*R*)-5-2*R*,3*S*,4*R*-3,4-bis-triisopropylsilyl-oxy-3,4-dihydro-2H-pyran-2-yl-3,3,8,8-tetraisopropyl-2,9-dimethyl-4,7-dioxa-3,8-disiladecane (302)

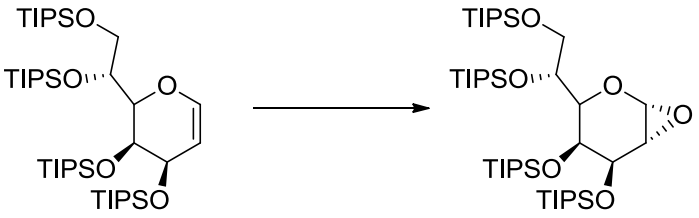


300 **302**

Compound **300** (0.6 g, 3.41 mmol) was dissolved in anhydrous DMF (17 mL) and treated with imidazole (1.86 g, 27.3 mmol), followed by Triisopropylsilyl chloride (3.36 mL, 15.69 mmol). The resulting solution was stirred at 60 °C for 16 h, then diluted with 200 mL of diethyl ether, washed with water (1 x 100 mL) and a copper (II) sulfate solution (2 x 100mL). The aqueous layers were back extracted with diethyl ether, and then the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (Petrol : Ether, 98:2) to furnish compound **302** (1.44 g, 51%) as a clear, homogenous, colourless oil.

IR γmax (film)/cm^{-1}: 2944, 2892, 2867, 2908, 1658, 1464, 1384, 1367, 1243, 1102, 1067, 1014, 997, 883; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.98-1.02 (84H, m, 4 x $Si(CH(CH_3)_2)_3$), 3.45 (1H, dd, *J* = 8.9, 2.2 Hz, H-6), 3.86 (1H, dd, *J* = 9.8, 3.4 Hz, H-7), 3.91 (1H, dd, *J* = 9.8, 3.8 Hz, H-7), 3.96 (1H, ddd, *J* = 8.3, 5.6, 3.8 Hz, H-4), 4.31 – 4.16 (1H, m, H-3), 4.98 – 4.80 (1H, m, H-5), 5.80 – 5.65 (1H, m, H-2), 6.17 – 6.00 (1H, m, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): δ 11.85, 12.30, 12.92 (12C, 4 x $Si(CH)_3$), 17.70, 17.97, 17.98, 18.12 (24C, 4 x $SiCH(CH_3)_2$), 63.81 (1C, C-4), 64.71 (1C, CH2, C-7), 65.87 (1C, C-6), 71.78 (1C, C-3), 75.08 (1C, C-5), 103.01 (1C, C-2), 143.93 (1C, C-1); *m/z* $[M+NH_4]^+$: 818.6354; $[C_{43}H_{92}O_5Si_4+NH_4]^+$ requires 818.6360.

(5*R*)-5-(3*R*,4*S*,5*R*)-4,5-bis-triisopropylsilyl-oxy-2,7-dioxabicyclo[4.1.0]heptan-3-yl-3,3,8,8-tetraisopropyl-2,9-dimethyl-4,7-dioxa-3,8-disiladecane (304)

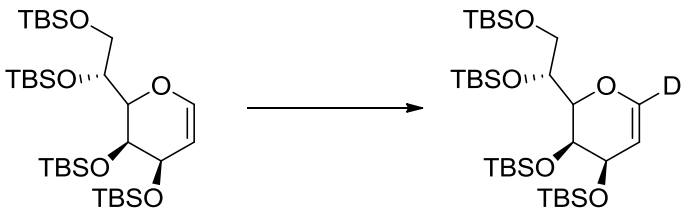


302 **304**

The glycal **302** (0.1 g, 0.125 mmol) was dissolved in 5 mL of dichloromethane, and the resulting solution was cooled to 0 °C. A solution of dimethyldioxirane in acetone (10.6 mL, 0.3 mmol, ca. 0.03 M) was added dropwise. The resulting mixture was stirred at 0 °C for 1 h or until TLC indicated complete consumption of the starting material. The solution was evaporated to dryness and the residue was dried under reduced pressure to afford the 1,2-anhydro sugar **304** (0.101 g) as a colourless oil in quantitative yield.

$[\alpha]^{25}_{D}$ -0.6 (*c* 0.077 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 - 0.98.(84H, m, 4X Si$(CH(CH_3)_2)_3$), 2.86 (1H, d, *J* = 2.4 Hz), 3.09 (1H, s, H-4), 3.59 (1H, d, *J* = 6.4 Hz, H-6), 3.77 (1H, dd, *J* = 10.5, 5.1 Hz, H-7), 3.83 (1H, dd, *J* = 10.5, 4.2 Hz, H-7), 3.94 (1H, s, H-5), 4.10 (1H, dd, *J* = 10.9, 4.8 Hz, H-3), 4.91 (1H, d, *J* = 2.4 Hz, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.01, 12.12, 12.77 (12C, 4X Si$(CH)_3$), 17.93, 17.97, 17.98, 18.12 (24C, 4X SiCH$(CH_3)_2$), 53.04 (1C, C-2), 62.84 (1C, C-4), 64.55 (7C, CH_2), 68.68 (1C, C-6), 69.36 (1C, C-5), 73.09 (1C, C-3), 77.64 (1C, C-1); *m/z* $[M+H]^+$: 817.6044; $[C_{43}H_{92}O_6Si_4+H]^+$ requires 817.6044.

1-$^{2}H_{1}$-(*R*)-5-(2*R*,3*S*,4*R*)-3,4-bis((tert-butyldimethylsilyl)oxy)-3,4-dihydro-2H-pyran-2-yl-2,2,3,3,8,8,9,9-octamethyl-4,7-dioxa-3,8-disiladecane (319)

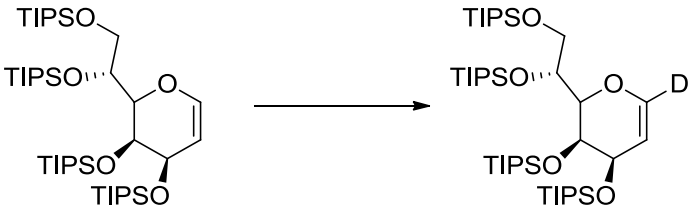


301 **319**

Glycal **301** (0.1 g, 0.125 mmol) was dissolved in anhydrous THF at -78 °C and *t*-BuLi (0.4 mL, 0.75 mmol) was added dropwise. The reaction was allowed to stir at -78 °C for 30 min, before allowing the solution to warm up to 0 °C where it was stirred for a further 30 min. After which the solution was cooled to -78 °C where an excess of D_2O was added (5 equiv). The reaction mixture was quenched with a saturated solution of NH_4Cl, and then the solution was filtered through a pad of Na_2SO_4. The solution was evaporated to dryness and the residue was dried under reduced pressure to yield compound **319** (0.078 g, quantitative) without further purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ -0.07 (s, 6H), -0.07 (s, 6H), -0.00 (s, 6H), 0.00 (s, 6H), 0.78 (s, 12H), 0.78 (s, 12H), 0.80 (s, 12H), 3.63 – 3.57 (m, 2H), 3.68 – 3.65 (m, 1H), 3.90 (dd, *J* = 6.2, 2.9 Hz, 2H), 4.41 – 4.34 (m, 2H).

1-$^{2}H_{1}$-(*R*)-5-2*R*,3*S*,4*R*-3,4-bis-triisopropylsilyl-oxy-3,4-dihydro-2H-pyran-2-yl-3,3,8,8-tetraisopropyl-2,9-dimethyl-4,7-dioxa-3,8-disiladecane (320)

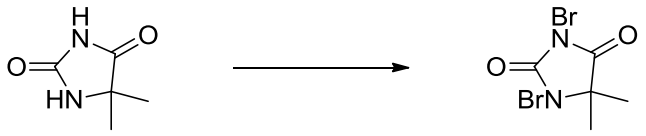


302 **320**

Glycal **302** (0.1 g, 0.125 mmol) was dissolved in anhydrous THF at -78 °C and *t*-BuLi in *n*-hexane (0.4 mL, 0.75 mmol, 1.9 M) was added dropwise. The reaction was allowed to stir at -78 °C for 30 min, before allowing the solution to warm up to 0 °C where it was stirred for a further 30 min. After which the solution was cooled to -78 °C where an excess of D_2O was added. The reaction mixture was quenched with a saturated solution of NH_4Cl, and then the solution was filtered through a pad of Na_2SO_4. The solution was evaporated to dryness and the residue was dried under reduced pressure to yield compound **320** (0.1 g, quantitative) without further purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 - 0.98 (84H, m, 4X $Si(CH(CH_3)_2)_3$). 3.45 (dd, *J* = 8.9, 2.2 Hz, 1H), 3.86 (dd, *J* = 9.8, 3.4 Hz, 1H), 3.91 (dd, *J* = 9.8, 3.8 Hz, 1H), 3.96 (ddd, *J* = 8.3, 5.6, 3.8 Hz, 1H), 4.31 – 4.16 (m, 1H), 4.88 – 4.80 (m, 1H), 5.78 – 5.67 (m, 1H).

1,3-dibromo-5,5-dimethylimidazolidine-2,4-dione (324)[10]



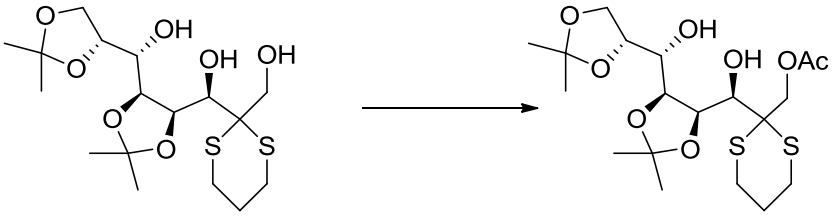
323 **324**

5,5-dimethylhydantoin **323** (15 g, 117 mmol) was dissolved in a 5% solution of NaOH at room temperature. Neat bromine (12 mL, 234 mmol) was added to the solution dropwise and left to stir for 1 h. After formation of a colourless precipitate, the solution was filtered and the solid was washed with an ice-water mixture until the filtrate ran clear. The solid was then dried in a vacuum oven at 50 °C for 2 h to give the pure compound **324** as a pale yellow solid (29.8 g, 89%).

mp 197-198 °C; IR γ_{max} (film)/ cm^{-1}: 1777, 1716, 1456, 1387, 1355, 1209, 1072, 854, 734; ^{1}H NMR δ ($CDCl_3$): 1.38 (s, 6H, 2 x CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.8 (2 x CH_3), 68.9 (Cq C-5), quaternary carbonyls not observed.

Data is in agreement with the literature reference.

2-(1*R*)-(4*R*,5*S*)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4yl-hydroxy-methyl-2,2-dimethyl-1,3-dioxolan-4-yl-hydroxy-methyl-1,3-dithian-2-yl-methyl acetate (227)

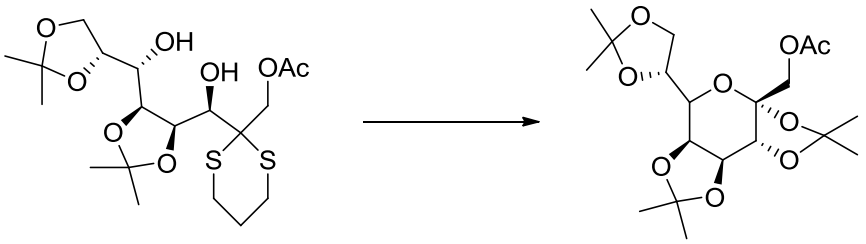


226 **227**

Acetic anhydride (0.24 mL, 2.53 mmol) followed by TEA (0.35 mL, 2.53 mmol) was added to a solution of **226** (0.925 g, 2.25 mmol) in anhydrous dichloromethane at 0 °C. The mixture was warmed to room temperature and left to stir overnight. A saturated solution of $NaHCO_3$ was added and the mixture was extracted with dichloromethane. The combined organic layers were washed further with water, brine, dried over $Na_2SO_4$and the solvent removed under reduced pressure. The crude product was purified by column chromatography (Petrol: EtOAc, 5:1) on silica gel to furnish the pure compound **227** as a colourless oil (0.930 g, 91%).

$[\alpha]^{25}_{D}$ -6.1 (*c* 0.0105 in $CHCl_3$); IR γ_{max} (film)/ cm^{-1}: 3456, 2985, 2941, 1742 1379, 1218, 1144, 1066, 913, 881; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35 (3H, s, CH_3 isopropylidene), 1.41 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 1.53 (3H, s, CH_3 isopropylidene), 2.11 (3H, s, CH_3 acetate), 2.80 – 2.62 (2H, m, SCH_2), 3.03 – 2.86 (2H, m, SCH_2), 3.60 (3H, t, *J* = 12.6 Hz, 4-H, SCH_2), 4.06 – 3.94 (2H, m, H-7), 4.11 (3H, qd, *J* = 6.8, 4.0 Hz, CH_2OAc, H-3), 4.48 – 4.38 (3H, m, H-5, 2-OH, H-6), 4.70 (1H, t, *J* = 7.1 Hz, H-2), 4.78 (1H, dd, *J* = 11.8, 7.7 Hz, 5-OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.98 (1C, CH_3 acetate), 24.32 (1C, CH_2 dithi), 25.08 (1C, CH_3 isopropylidene), 25.26 (1C, CH_3 isopropylidene), 25.74 (1C, CH_3 isopropylidene), 25.78 (1C, CH_3 isopropylidene), 26.05 (1C, CH_2 dithi), 26.89 (1C, CH_2 dithi), 56.59 (1C, C_q dithi), 63.75 (1C, CH_2, C-6), 67.32 (1C, C-4), 70.58 (1C, C-1), 72.14 (1C, CH_2OAc), 73.56 (1C, C-5), 75.77 (1C, C-3), 76.93 (1C, C-2), 108.97 (1C, Cq isopropylidene), 109.25 (1C, Cq isopropylidene), 170.19 (1C, Cq, C=O).

(3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-2,2,7,7-tetramethyltetrahydro-3aH-bis[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl acetate (238)

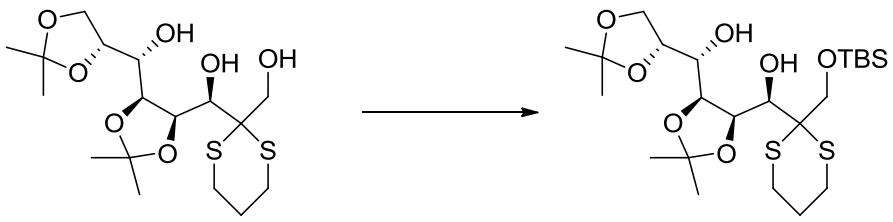


227 **238**

227 (1.302 g, 2.9 mmol) was dissolved in reagent grade acetone and DBDMH (2.33 g, 8.15 mmol) in acetone was added at 0 °C. The mixture was stirred at 0 °C for a further 30 min. A saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic extracts were washed further with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (Petrol: EtOAc, 4:1) on silica gel to furnish the pure compound **238** as a colourless oil (0.78 g, 67%).

$[\alpha]^{25}_D$ -8.7 (*c* 0.0117 in $CHCl_3$); IR γ_{max}(film)/ cm^{-1}: 2989, 2937, 1752, 1457 1381, 1259, 1224, 1167, 1158, 1119, 1072, 984, 899, 847; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31 (3H, s, CH_3 isopropylidene), 1.32 (3H, s, CH_3 isopropylidene), 1.35 (3H, s, CH_3 isopropylidene), 1.37 (3H, s, CH_3 isopropylidene), 1.40 (3H, s, CH_3 isopropylidene), 1.48 (3H, s, CH_3 isopropylidene), 2.04 (3H, s, CH_3 Acetate), 3.67 (1H, dd, *J* = 8.8, 1.8 Hz, H-7), 3.90 (1H, d, *J* = 11.7 Hz, H-2), 3.94 (1H, dd, *J* = 8.8, 3.8 Hz, H-7), 4.00 (1H, dd, *J* = 8.8, 6.0 Hz, H-3), 4.22 – 4.16 (1H, m, H-4), 4.30 (1H, d, *J* = 2.7 Hz, H-6), 4.34 (1H, dd, *J* = 7.9, 1.8 Hz, CH_2OAc), 4.39 (1H, d, *J* = 11.7 Hz, H-5), 4.62 (1H, dd, *J* = 7.9, 2.7 Hz, CH_2OAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.77 (1C, CH_3 Acetate), 24.18 (1C, CH_3 isopropylidene), 25.14 (1C, CH_3 isopropylidene), 25.21 (1C, CH_3 isopropylidene), 25.71 (1C, CH_3 isopropylidene), 26.48 (1C, CH_3 isopropylidene), 27.11 (1C, CH_3 isopropylidene), 64.53 (1C, CH_2OAc), 66.99 (1C, CH_2, C-7), 69.40 (1C, C-3), 70.55 (1C, C-4), 70.64 (1C, C-5), 70.73 (1C, C-2), 73.23 (1C, C-6), 102.12 (1C, C_q C-1), 108.90 (1C, Cq isopropylidene), 109.34 (1C, Cq isopropylidene), 109.43 (1C, Cq isopropylidene), 169.87 (1C, Cq C=O); *m/z* $[M+H]^+$: 403.1970; $[C_{19}H_{30}O_9+H]^+$ requires 403.1963.

(1*R*)-2-tert-butyldimethylsilyl-oxy-methyl-1,3-dithian-2-yl-(4*R*,5*S*)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-hydroxy-methyl-2,2-dimethyl-1,3-dioxolan-4-yl-methanol (237)

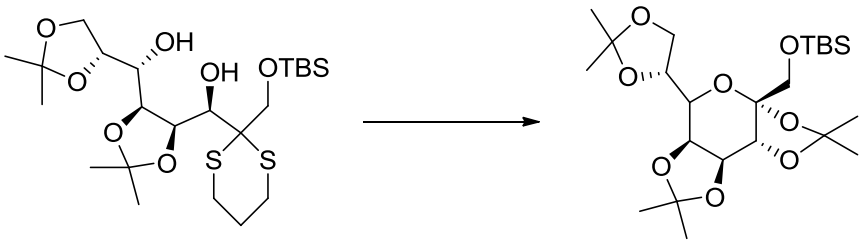


226 **237**

Imidazole (0.10 g, 1.47 mmol) and TBSCl (0.09 g, 0.6 mmol) were added to a solution of **226** (0.2 g, 0.49 mmol) in anhydrous DMF (5 mL). The resulting solution was stirred at room temperature for 16 h, and then diluted with (20 mL) of diethyl ether, washed with water (1 x 100 mL) and a copper (II) sulfate solution (2 x 100 mL). The aqueous layers were back extracted with diethyl ether, and then the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (Petrol:EtOAc, 4:1) to furnish pure compound **237** (0.195 g, 76%) as a clear, colourless oil.

Refer to provisional structure assignment: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.09 (3H, s, $SiCH_3$), 0.10 (3H, s, $SiCH_3$), 0.89 (9H, s, $SiC(CH_3)_3$), 1.34 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.52 (3H, s, CH_3 isopropylidene), 2.00 – 1.93 (2H, m, SCH_2), 2.83 – 2.72 (4H, m, 2 x SCH_2), 3.52 (1H, d, *J* = 8.0 Hz, H-5), 3.94 (1H, dd, *J* = 10.2, 1.1 Hz, H-3), 4.02 – 3.98 (1H, m, H-7), 4.16 – 4.09 (3H, m, H-2, H-4, H-7), 4.22 (1H, d, *J* = 10.2 Hz, H-1), 4.34 (1H, d, *J* = 7.4 Hz, CH_2-OTBS), 4.69 (1H, d, *J* = 7.4 Hz, CH_2-OTBS); ^{13}C NMR (125 MHz, $CDCl_3$): δ -5.72 (1C, $SiCH_3$), -5.65 (1C, $SiCH_3$), 18.19 (1C, Cq, SiC), 24.64 (1C, CH_2 dithi), 25.15 (1C, CH_2 dithi), 25.34 (1C, CH_3 isopropylidene), 25.65 (1C, CH_3 isopropylidene), 25.70 (3C, $SiC(CH_3)_3$), 25.95 (1C, CH_2 dithi), 26.22 (1C, CH_3 isopropylidene), 26.94 (1C, CH_3 isopropylidene), 56.45 (1C, Cq, dithi), 67.32 (1C, CH_2OSi), 67.80 (1C, CH_2, C-6), 71.42 (1C, C-4), 73.07 (1C, C-2), 73.93 (1C, C-1), 75.32 (1C, C-3), 77.38 (1C, C-5), 108.93 (1C, Cq isopropylidene), 109.16 (1C, Cq isopropylidene); *m/z* $[M-H]^-$: 523.2224; $[C_{23}H_{44}O_7S_2Si-H]^-$ requires 523.2225.

Tert-butyl-(3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methoxy-dimethylsilane (239)

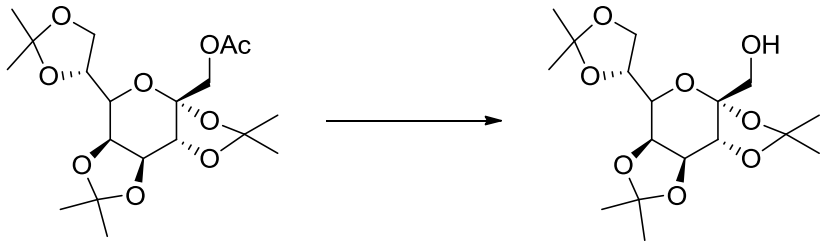


237 **239**

237 (0.174 g, 0.332 mmol) was dissolved in acetone reagent grade and a solution of DBDMH (0.27 g, 0.94 mmol) in acetone was added at 0 °C. The mixture was stirred at 0 °C for a further 30 min. A saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ was added and the mixture was extracted with EtOAc. The combined organic extracts were washed further with water, brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (Petrol: EtOAc, 4:1) on silica gel to furnish compound **239** as a colourless syrup (0.11 g, 70%).

Refer to provisional structure assignment: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.07 (3H, s, $SiCH_3$), 0.08 (3H, s, $SiCH_3$), 0.91 (9H, s, $SiC(CH_3)_3$), 1.36 (6H, s, 2 x CH_3 isopropylidene), 1.41 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 1.53 (3H, s, CH_3 isopropylidene), 3.62 (1H, d, *J* = 10.5 Hz, H-7), 3.74 – 3.70 (2H, m, H-7, H-4), 3.99 (1H, dd, *J* = 8.8, 3.8 Hz, CH_2OSi), 4.05 (1H, dd, *J* = 8.7, 6.0 Hz, CH_2OSi), 4.24 – 4.18 (1H, m, H-3), 4.39 (1H, dd, *J* = 7.9, 1.8 Hz, H-6), 4.43 (1H, d, *J* = 2.6 Hz, H-5), 4.65 (1H, dd, *J* = 7.9, 2.6 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ -5.63 (1C, $SiCH_3$), -5.34 (1C, $SiCH_3$), 18.48 (1C, Cq SiC), 24.17 (1C, CH_3 isopropylidene), 25.31 (1C, CH_3 isopropylidene), 25.72 (1C, CH_3 isopropylidene), 25.80 (1C, CH_3 isopropylidene), 25.96 (3C, $SiC(CH_3)_3$), 26.72 (1C, CH_3 isopropylidene), 27.20 (1C, CH_3 isopropylidene), 64.28 (1C, CH_2 7-C), 67.25 (1C, CH_2OSi), 69.31 (1C, C-3), 69.52 (1C, C-4), 70.90 (1C, C-5) , 70.99 (1C, C-2), 73.40 (1C, C-6), 103.90 (1C, Cq C-1), 108.65 (1C, Cq isopropylidene), 109.25 (1C, Cq isopropylidene), 109.47 (1C Cq isopropylidene); *m/z* $[M+NH_4]^+$: 492.2978; $[C_{23}H_{42}O_8Si+NH_4]^+$ requires 492.2987.

(3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methanol (240)

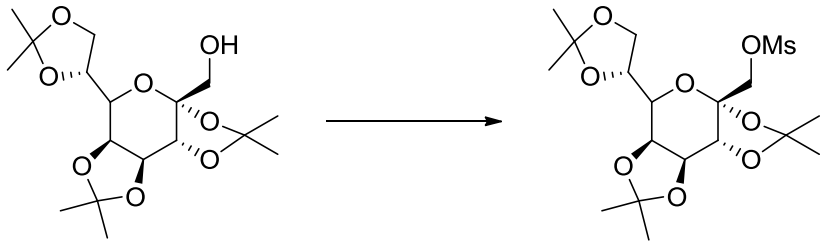


238 **240**

238 (0.75 g, 1.86 mmol) was dissolved in 10 mL of anhydrous methanol and several drops of methanolic 2 M sodium methoxide solution were added at room temperature. The mixture was stirred overnight at room temperature after which the mixture was concentrated. The crude mixture was purified by column chromatography (Petrol:EtOAc, 4:1) on silica gel to furnish pure compound **240** (0.64 g, 95%).

$[\alpha]^{25}_{D}$ -25.6 (*c* 0.0078 in $CHCl_3$); IR γ_{max} (film)/ cm^{-1}: 3501, 2989, 2941, 2857, 1372, 1252, 1211, 1072; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (3H, s, CH_3 isopropylidene), 1.37 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 1.44 (3H, s, CH_3 isopropylidene), 1.53 (3H, s, CH_3 isopropylidene), 2.13 (1H, s, OH), 3.69 –3.59 (2H, m, H-4, H-3), 3.72 (1H, dd, *J* = 8.9, 1.9 Hz, H-5), 3.98 (1H, dd, *J* = 8.8, 3.7 Hz, H-7), 4.06 (1H, dd, *J* = 8.8, 3.7 Hz, H-7), 4.27-4.23 (1H, m, H-6), 4.36 (1H, d, *J* = 2.7 Hz, H-2), 4.39 (1H, dd, *J* = 7.9, 1.9 Hz, CH_2OH), 4.66 (1H, dd, *J* = 7.9, 2.7 Hz, CH_2OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.18 (1C, CH_3 isopropylidene), 25.28 (1C, CH_3 isopropylidene), 25.39 (1C, CH_3 isopropylidene), 25.67 (1C, CH_3 isopropylidene), 26.52 (1C, CH_3 isopropylidene), 27.22 (1C, CH_3 isopropylidene), 65.24 (1C, CH_2-OH), 67.15 (1C, CH_2 C-7), 69.56 (1C, C-3), 70.62 (1C, C-4), 70.72 (1C, C-5), 71.35 (1C, C-2), 73.30 (1C, C-6), 103.72 (1C, C_q C-1), 108.88 (1C, C_q isopropylidene), 109.51 (1C, C_q isopropylidene), 109.53 (1C, C_q isopropylidene); *m/z* $[M+NH_4]^+$: 378.2122; $[C_{17}H_{28}O_8+NH_4]^+$ requires 378.2122.

(3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl methanesulfonate (325)

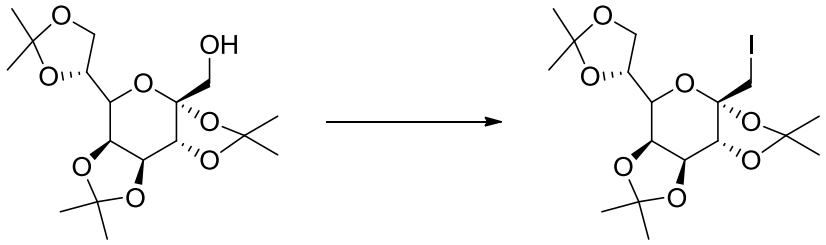


240 **325**

Alcohol **240** (0.07 g, 0.194 mmol) was dissolved in pyridine (5 mL), methanesulfonylchloride (0.025 mL, 0.305 mmol) was added and after 1 h dichloromethane was added. The organic layer was washed subsequently with a 1 M HCl (100 mL), saturated aqueous $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL) and dried over Na_2SO_4. The solvent was concentrated under reduced pressure and the crude product was purified by column chromatography (Petrol:EtOAc, 1:2) on silica gel to furnish the pure compound **325** as a colourless liquid (0.069 g, 82%).

IR γ_{max} (film)/ cm^{-1}: 3444 2989, 2938, 1634, 1457, 1371, 1256, 1209, 1177, 1117, 1072, 1047; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (3H, s, CH_3 isopropylidene), 1.31 (3H, s, CH_3 isopropylidene), 1.34 (3H, s, CH_3 isopropylidene), 1.35 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.47 (3H, s, CH_3 isopropylidene), 2.98 (3H, s, CH_3 mesylate), 3.67 (1H, dd, *J* = 8.7, 1.8 Hz, CH_2-OSO_2CH_3), 3.91 (1H, dd, *J* = 8.8, 3.7 Hz, CH_2-OSO_2CH_3), 3.98 (1H, dd, *J* = 8.8, 6.0 Hz, H-7), 4.12–4.18 (2H, m, H-4, H-7), 4.22 (1H, d, *J* = 10.8 Hz, H-3), 4.27 (1H, d, *J* = 2.7 Hz, H-6), 4.33 (1H, dd, *J* = 7.9, 1.8 Hz, H-5), 4.61 (1H, dd, *J* = 7.9, 2.7 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.2 (1C, CH_3 isopropylidene), 25.2 (1C, CH_3 isopropylidene), 25.3 (1C, CH_3 isopropylidene), 25.8 (1C, CH_3 isopropylidene), 26.6 (1C, CH_3 isopropylidene), 27.2 (1C, CH_3 isopropylidene), 37.6 (1C, CH_3 mesylate), 67.0 (1C, CH2, C-7), 69.2 (1C, C-4), 69.7 (1C, CH_2-OSO_2CH_3), 70.5 (2C, C-3, C-5), 70.6 (1C, C-2), 73.2 (1C, C-6), 101.3 (1C, C_q C-1), 109.6 (1C, C_q isopropylidene), 109.6 (1C, C_q isopropylidene), 109.7 (1C, C_q isopropylidene); *m/z* $[M+NH_4]^+$: 456.1894; $[C_{18}H_{30}O_{10}S+NH_4]^+$ requires 456.1898.

(3a*S*,5*R*,5a*S*,8a*S*,8bR)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-3a-iodomethyl-2,2,7,7 tetramethyltetrahydro-3aH-bis([1,3]dioxolo[4,5-b:4',5'-d]pyran (326)

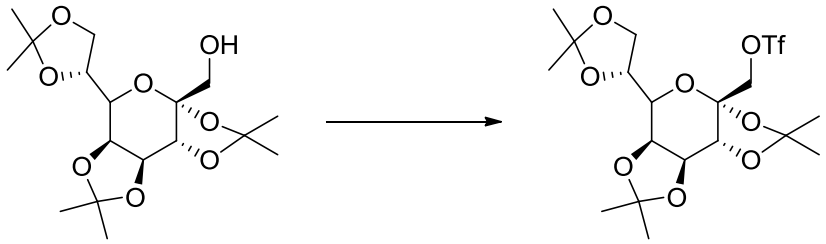


240 **326**

Alcohol **240** (0.11 g 0.305 mmol) was dissolved in anhydrous toluene (20 mL) at 60 °C under vigorous stirring and triphenylphosphine (0.21 g, 0.8 mmol) and Imidazole (0.08 g, 1.10 mmol) were added. After dissolution, Iodine (0.203 g, 0.8 mmol) was added and the heating was continued for 4 h. The solution was cooled and diluted with EtOAc (200 mL). The organic layer was washed with saturated solution of Na_2SO_3 (10 mL) then water (2 x 20 mL), 1 M HCl (20 mL), water and finally saturated $NaHCO_3$ (20 mL) and water (20 mL). After drying over magnesium sulfate, the solvent was removed under reduced pressure and the residue purified by column chromatography (Petrol:EtOAc 4:1) on silica gel to furnish pure compound **326** (0.1 g, 70%).

Refer to provisional structure assignment: ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (s, 3H, CH_3 isopropylidene), 1.37 (s, 3H, CH_3 isopropylidene), 1.42 (s, 3H, CH_3 isopropylidene), 1.43 (s, 3H, CH_3 isopropylidene), 1.44 (s, 3H, CH_3 isopropylidene), 1.53 (s, 3H, CH_3 isopropylidene), 3.32 (d, *J* = 10.7 Hz, 1H, CH_2-I), 3.51 (d, *J* = 10.7 Hz, 1H, CH_2-I), 3.73 (dd, *J* = 8.7, 1.9 Hz, 1H, H-7), 4.03 (dd, *J* = 8.8, 3.9 Hz, 1H, H-7), 4.09 (dd, *J* = 8.8, 6.0 Hz, 1H, H-4), 4.27 (ddd, *J* = 8.7, 6.0, 3.9 Hz, 1H, H-3), 4.33 (d, *J* = 2.8 Hz, 1H, H-6), 4.37 (dd, *J* = 7.9, 1.9 Hz, 1H, H-5), 4.64 (dd, *J* = 7.8, 2.8 Hz, 1H, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 10.3 (1C, CH_2I), 24.4 (1C, CH_3 isopropylidene), 25.4 (1C, CH_3 isopropylidene), 25.5 (1C, CH_3 isopropylidene), 25.9 (1C, CH_3 isopropylidene), 26.7 (1C, CH_3 isopropylidene), 27.2 (1C, CH_3 isopropylidene), 67.1 (1C, CH_2 C-7), 70.1 (1C, CH carbo), 70.7 (1C, CH carbo), 71.3 (1C, CH carbo), 72.0 (1C, CH carbo), 73.3 (1C, CH carbo), 100.8 (1C, Cq C-1), 108.8 (1C, Cq isopropylidene), 109.5 (1C, Cq isopropylidene), 109.7 (1C, C_q isopropylidene); *m/z* $[M+NH_4]^+$: 488.1128; $[C_{17}H_{27}IO_7+NH_4]^+$ requires 488.1140.

(3a*R*,5*R*,5a*S*,8a*S*,8b*R*)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl-trifluoromethanesulfonate (241)



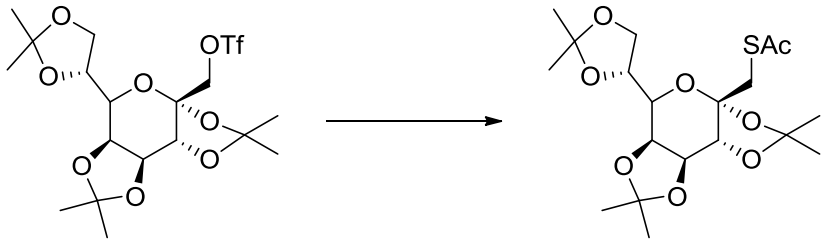
240 **241**

Alcohol **240** (0.1 g, 0.2774 mmol) and pyridine (0.023 mL, 0.2774 mmol) were dissolved in anhydrous dichloromethane. The solution was then added to a solution of Trifluoromethanesulfonic anhydride (0.05 mL, 0.2774 mmol) in anhydrous dichloromethane at 0 °C, dropwise over a 15 min period. The reaction mixture was stirred for a further 30 min period at 0 °C, reaction was then allowed to warm up to room temperature for a further 30 min. The reaction mixture was then transferred to a separating funnel and washed quickly with an ice-water mixture (2 x 20 mL). The combined organic layers was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography (Petrol:EtOAc, 1:2) on silica gel to furnish the pure compound **241** as a colourless oil (0.131 g, 96%).

$[\alpha]^{25}_D$ -6.0 (*c* 0.0168 in $CHCl_3$); IR γ_{max}(film)/ cm^{-1}: 2991, 2939, 2253, 1456, 1419, 1382, 1346, 1320, 1249, 1211, 1168, 1117, 1046; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.35 (3H, s, CH_3 isopropylidene), 1.36 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.41 (3H, s, CH_3 isopropylidene), 1.43 (3H, s, CH_3 isopropylidene), 1.54 (3H, s, CH_3 isopropylidene), 3.73 (1H, dd, *J* = 8.8, 1.9 Hz, CH_2-CF_3SO_3), 3.97 (1H, dd, *J* = 8.9, 3.6 Hz, CH_2-CF_3SO_3), 4.04 (1H, dd, *J* = 8.9, 6.0 Hz, H-7), 4.21 (1H, ddd, *J* = 8.8, 6.0, 3.6 Hz, H-7), 4.31 (1H, d, *J* = 2.8 Hz, H-4), 4.36 (1H, d, *J* = 10.5 Hz, H-3), 4.39 (1H, dd, *J* = 7.9, 1.9 Hz, H-6), 4.51 (1H, d, *J* = 10.5 Hz, H-5), 4.68 (1H, dd, *J* = 7.9, 2.8 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.1 (1C, CH_3 isopropylidene), 25.1 (1C, CH_3 isopropylidene), 25.2 (1C, CH_3 isopropylidene), 25.6 (1C, CH_3 isopropylidene), 26.6 (1C, CH_3 isopropylidene), 27.2 (1C, CH_3 isopropylidene), 67.0 (CH_2, CH_2-CF_3SO_3), 69.9 (1C, C-7), 70.3 (1C,

C-3), 70.4 (1C, C-4), 70.4 (1C, C-5), 73.1 (1C, C-2), 73.9 (1C, CH_2, C-6), 100.5 (1C, C-1), 109.6 (1C, Cq isopropylidene), 109.7 (1C, Cq isopropylidene), 109.7 (1C, Cq isopropylidene), 110.2 (1C, Cq CF_3); *m/z* $[M+H]^+$: 493.1343 ; $[C_{18}H_{27}F_3O_{10}S+H]^+$ requires 493.1350.

***S*-(3a*S*,5*R*,5a*S*,8a*S*,8b*R*)-5-(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl-ethanethioate (242)**

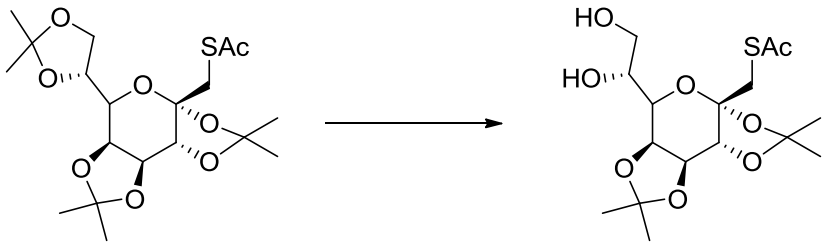


241 **242**

A mixture of the triflate **241** (0.097 g, 0.203 mmol) and potassium thioacetate (0.0463 g, 0.405 mmol) in anhydrous DMF (5 mL) was heated at 80 °C for 1 h (colour change from yellow to brown observed) or until TLC (Petrol:EtOAc 4:1) showed complete consumption of the starting material. The DMF was evaporated under reduced pressure. After diethyl ether was added to the reaction mixture and the mixture was washed with water, brine and the combined organic layers were then dried over Na_2SO_4. The solvents were removed under reduced pressure and the crude product was purified by column chromatography (Petrol:EtOAc, 4:1) on silica gel to furnish pure compound as a colourless liquid **242** (0.079 g, 93%).

$[\alpha]^{25}_{D}$ -24.4 (*c* 0.0087 in $CHCl_3$); IR γ_{max} (film)/ cm^{-1}: 2994, 2937, 1697, 1371, 1210, 1100, 1070 996, 911; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.29 (6H, s, 2 x CH_3 isopropylidene), 1.33 (3H, s, CH_3 isopropylidene), 1.34 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 1.43 (3H, s, CH_3 isopropylidene), 2.28 (3H, s, CH_3 thioacetate), 3.26 (1H, d, *J* = 13.8 Hz, CH_2SAC), 3.32 (1H, d, *J* = 13.8 Hz, CH_2SAC), 3.61 (1H, dd, *J* = 8.5, 1.8 Hz, H-7), 3.91 (1H, dd, *J* = 8.8, 4.0 Hz, H-7), 3.99 (1H, dd, *J* = 8.8, 6.1 Hz, H-3), 4.14 (1H, d, *J* = 2.6 Hz, H-4), 4.17 (1H, ddd, *J* = 8.5, 6.1, 4.1 Hz, H-6), 4.29 (1H, dd, *J* = 7.9, 1.8 Hz, H-5), 4.55 (1H, dd, *J* = 7.9, 2.6 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.30 (1C, CH_3 isopropylidene), 25.14 (1C, CH_3 isopropylidene), 25.36 (1C, CH_3 isopropylidene), 25.72 (1C, CH_3 isopropylidene), 26.48 (1C, CH_3 isopropylidene), 27.14 (1C, CH_3 isopropylidene), 30.39 (1C, CH_3 thioacetate), 37.17 (1C, CH_2, CH_2SAc), 67.00 (1C, CH_2, C-7), 69.70 (1C, C-3), 70.47 (1C, C-4), 71.03 (1C, C-5), 73.07 (1C, C-2), 73.39 (1C, C-6), 102.96 (1C, Cq C-1), 108.73 (1C, Cq isopropylidene), 109.37 (1C, Cq isopropylidene), 109.56 (1C, Cq isopropylidene), 194.61 (1C, Cq C=O); m/z $[M+H]^+$: 419.1731; $[C_{19}H_{30}O_8S+H]^+$ requires 419.1734.

_S_-(3a_S_,5_R_,5a_S_,8a_S_,8b_R_)-5-(_R_)-1,2-dihydroxyethyl-2,2,7,7 tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl-ethanethioate (243)

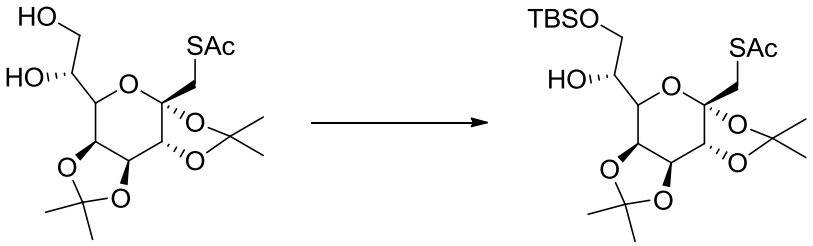


242 **243**

The triacetonide (0.130 g, 0.311 mmol) **242** was dissolved in 80% aqueous acetic acid (2.2 mL) and the mixture was stirred at 50 °C for 3 h or until TLC (EtOAc, $KMNO_4$ stain) showed complete consumption of the starting material and a single product was observed. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (EtOAc, 100%) to furnish the pure compound as a white solid **243** (0.105 g, 89%).

IR γ_{max} (film)/ cm^{-1}: 3442, 2994, 2935, 1697, 1374, 1256, 1210, 1166, 1070, 997, 887, 627; ^1H NMR (500 MHz, $CDCl_3$): δ 1.35 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.48 (3H, s, CH_3 isopropylidene), 1.49 (3H, s, CH_3 isopropylidene), 2.34 (3H, s, CH_3 thioacetate), 3.32 (1H, d, *J* = 13.7 Hz, CH_2SAC), 3.37 (1H, d, *J* = 13.7 Hz, CH_2SAC), 3.72 – 3.81 (3H, m, H-6, H-7, H-7), 3.85 – 3.89 (1H, m, H-5), 4.20 (1H, d, *J* = 2.6 Hz, H-4), 4.42 (1H, dd, *J* = 7.9, 1.9 Hz, H-3), 4.63 (1H, dd, *J* = 7.9, 2.6 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.4 (1C, CH_3 isopropylidene), 25.0 (1C, CH_3 isopropylidene)., 25.8 (1C, CH_3 isopropylidene), 26.3 (1C, CH_3 isopropylidene), 30.4 (1C, CH_3 thioacetate), 37.4 (1C, CH_2, CH_2SAc), 64.1 (1C, CH_2, C-7), 69.0 (1C, C-3), 69.9 (1C, C-4), 70.6 (1C, C-6), 71.2 (1C, C-5), 73.0 (1C, C-2), 102.9 (1C, Cq C-1), 108.9 (1C, Cq isopropylidene), 109.6 (1C, Cq isopropylidene), 194.6 (1C, Cq C=O); *m/z* $[M+H]^+$: 379.1419; $[C_{16}H_{26}O_8S+H]^+$ requires 379.1421.

_S_-(3a_S_,5_R_,5a_S_,8a_S_,8b_R_)-5-(_R_)-2-((tert-butyldimethylsilyl)oxy)-1-hydroxyethyl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl-ethanethioate (244)

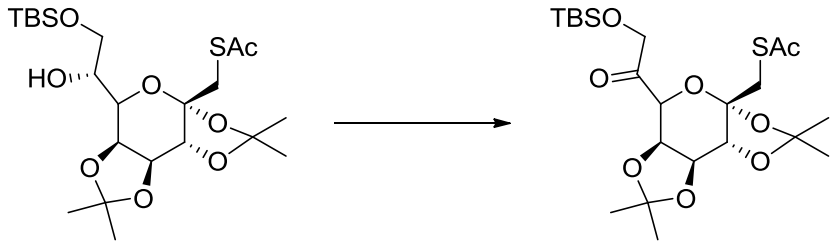


243 **244**

Imidazole (0.043 g, 0.633 mmol) and TBSCl (0.04 g, 0.253 mmol) were added to a solution of **243** (0.08 g, 0.211 mmol) in anhydrous DMF (5 mL). The resulting solution was stirred at room temperature for 16 h, and then diluted with (20 mL) of diethyl ether, washed with water (1 x 100 mL) and a copper (II) sulfate solution (2 x 100 mL). The aqueous layers were back extracted with diethyl ether, and then the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (Petrol:EtOAc, 4:1) to furnish pure compound **244** (0.075 g, 72%) as a clear, colourless oil.

IR γ_{max} (film)/ cm^{-1}: 3529, 2966, 2934, 2933, 2957, 1697, 1462, 1382, 1253, 1210, 1072; ^{1}H NMR (500 MHz, $CDCl_3$): δ -0.00 (3H, s, $SiCH_3$), 0.01 (3H, s, $SiCH_3$), 0.82 (9H, s, $SiC(CH_3)_3$), 1.29 (3H, s, CH_3 isopropylidene), 1.31 (3H, s, CH_3 isopropylidene), 1.40 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 2.27 (3H, s, CH_3 thioacetate), 2.59 (1H, s, OH), 3.27 (1H, d, *J* = 4.0 Hz, CH_2SAC), 3.30 (1H, d, *J* = 13.7 Hz, CH_2SAC), 3.59 – 3.54 (1H, m, H-6), 3.63 (1H, dd, *J* = 8.5, 1.8 Hz, H-4), 3.78 – 3.73 (2H, m, H-7, H-7), 4.10 (1H, dd, *J* = 9.3, 2.5 Hz, H-3), 4.38 (1H, dd, *J* = 7.9, 1.9 Hz, H-3), 4.54 (1H, dd, *J* = 7.9, 2.5 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ -5.4 (1C, $SiCH_3$), -5.3 (1C, $SiCH_3$), 18.3 (1C, Cq, SiC), 24.3 (1C, CH_3 isopropylidene), 25.0 (1C, CH_3 isopropylidene), 25.8 (1C, CH_3 isopropylidene), 26.0 (3C, $SiC(CH_3)_3$), 26.6 (1C, CH_3 isopropylidene), 30.3 (1C, CH_3 SAc), 37.5 (1C, CH_2OSAc), 64.0 (1C, CH_2 C-7), 68.3 (1C, C-3), 69.5 (1C, C-4), 70.6 (1C, C-6), 71.1 (1C, C-5), 73.0 (1C, C-2), 102.8 (1C, Cq C-1), 108.6 (1C, Cq isopropylidene), 109.4 (1C, Cq isopropylidene), 194.8 (1C, Cq C=O); *m/z* $[M+H]^+$: 493.2280; $[C_{22}H_{40}O_8SSi+H]^+$ requires 493.2286.

_S_-(3a_S_,5_S_,5a_R_,8a_S_,8b_R_)-5-2-tert-butyldimethylsilyloxyacetyl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl-ethanethioate (247)



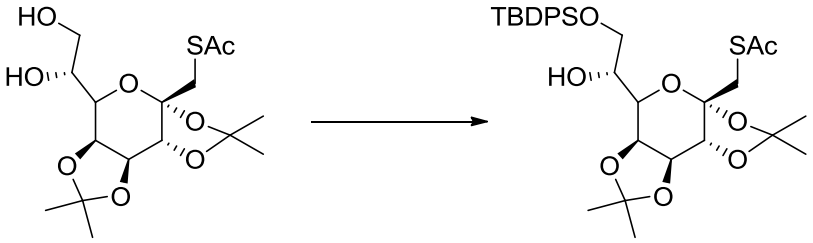
244 **247**

DMSO (0.02 mL, 0.28 mmol) was slowly added to a solution of oxalyl chloride (0.011 mL, 0.122 mmol) in anhydrous dichloromethane at -78 °C and the solution was stirred for a further 30 min at this temperature. A solution of **244** (0.04 g, 0.081mmol) in anhydrous dichloromethane was added dropwise to the reaction mixture and the solution was stirred for 1 h. TEA (0.05 mL, 0.39 mmol) was slowly added to the reaction mixture at the same temperature and the solution was then warmed up to 0 °C and stirred at this temperature for a further 30 min. After the mixture was allowed to warm up to room temperature, water, a saturated solution of NH_4Cl and a 2 M HCl were added the mixture was extracted with dichloromethane. The combined organic layers were further washed with water, brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the product **247** as a colourless syrup (0.039 g, quantitative).

IR γ_{max} (film)/ cm^{-1}: 2989, 2931, 2856, 2856, 1742, 1699, 1462, 1384, 1324, 1256, 1210, 1128, 1071; ^1H (500 MHz, $CDCl_3$): δ -0.00 (3H, s, $SiCH_3$), 0.01 (3H, s, $SiCH_3$), 0.82 (9H, s, $SiC(CH_3)_3$), 1.29 (3H, s, CH_3 isopropylidene), 1.31 (3H, s, CH_3 isopropylidene), 1.40 (3H, s, CH_3 isopropylidene), 1.42 (3H, s, CH_3 isopropylidene), 2.27 (3H, s, CH_3 thioacetate), 3.32 – 3.24 (2H, m, CH_2SAc), 3.59 – 3.55 (1H, m, H-3), 3.63 (1H, dd, *J* = 8.5, 1.9 Hz, H-4), 3.76 (1H, d, *J* = 1.9 Hz, H-2), 4.11 (1H, d, *J* = 2.5 Hz, H-5), 4.38 (1H, dd, *J* = 7.9, 1.9 Hz, H-7), 4.55 – 4.52 (1H, m, H-7); ^{13}C NMR (125 MHz, $CDCl_3$): δ -5.48 (1C, $SiCH_3$), -5.26 (1C, $SiCH_3$), 18.52 (1C, Cq, SiC) 24.86 (1C, CH_3 isopropylidene), 25.59 (1C, CH_3 isopropylidene), 25.89 (3C, $SiC(CH_3)_3$), 25.95 (1C, CH_3 isopropylidene), 26.35 (1C, CH_3 isopropylidene), 30.40 (1C, CH_3, SAc), 37.45 (1C, CH_2OSAc), 64.01 (1C, CH_2 C-7), 70.94 (1C, C-4), 71.91 (1C, C-3), 73.01 (1C, C-5), 74.65 (1C, C-2), 102.80 (1C, Cq C-1), 109.05 (1C, Cq

isopropylidene), 109.81 (1C, Cq isopropylidene), 194.23 (1C, Cq C=O, SAc), 205.65 (1C Cq, C=O); *m/z* $[M+NH_4]^+$: 508.2383; $[C_{22}H_{38}O_8SSi+NH_4]^+$ requires 508.2395.

_S_-(3a_S_,5_R_,5a_S_,8a_S_,8b_R_)-5-(_R_)-2-tert-butyldiphenylsilyl-oxy-1-hydroxyethyl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl-ethanethioate (245)

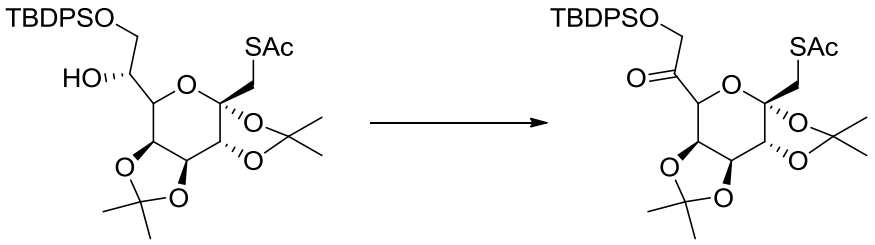


243 **245**

TBDPSCl (0.25 mL, 0.941 mmol) and Imidazole (0.13 g, 1.882 mmol) were added to a solution of **243** (0.356 g, 0.941 mmol) in anhydrous DMF (25 mL). The reaction was heated at 50 °C and left to stir for 48 h. After 48 h water (100 mL) was added, and the mixture was extracted with diethyl ether three times. The combined organic layer was washed successively with water, brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (Petrol:EtOAc, 4:1) to yield the pure compound **245** (0.43 g, 74%) as a colourless oil.

IR γ_{max} (film)/ cm^{-1}: 3551, 3072, 3049, 2989, 2934, 2895, 2858, 1695, 1472, 1462, 1428, 1382, 1373, 1256, 1210, 1186, 1132, 1105, 1073, 906; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (9H, s, $Si(CH_3)_3$), 1.35 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.41 (3H, s, CH_3 isopropylidene), 1.47 (3H, s, CH_3 isopropylidene), 1.96 (1H, s, OH), 2.30 (3H, s, CH_3 thioacetate), 3.26 (2H, s, CH_2SAC), 3.80 – 3.72 (3H, m, H-4, H-2, H-3), 3.85 (1H, ddd, *J* = 8.7, 5.1, 3.9 Hz, H-6), 4.12 (1H, d, *J* = 2.5 Hz, H-5), 4.38 (1H, dd, *J* = 7.9, 1.9 Hz, H-7), 4.54 (1H, dd, *J* = 7.9, 2.5 Hz, H-7), 7.37 – 7.29 (6H, m, CH aryl), 7.70 (4H, m, CH aryl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.35 (1C, Cq, SiC), 24.43 (1C, CH_3 isopropylidene), 25.09 (1C, CH_3 isopropylidene), 25.87 (1C, CH_3 isopropylidene), 26.63 (1C, CH_3 isopropylidene), 27.02 (3C, $SiC(CH_3)_3$), 30.45 (1C, CH_3 thioacetate), 37.66 (1C, CH_2SAc), 64.66 (1C, CH_2, C-7), 68.20 (1C, C-3), 69.91 (1C, C-4), 70.79 (1C, C-6), 71.28 (1C, C-5), 73.09 (1C, C-2), 102.95 (1C, Cq, C-1), 108.63 (1C, Cq, isopropylidene), 109.42 (1C, Cq, isopropylidene), 127.84 (2C, 2CH aryl), 127.90 (2C, 2CH aryl), 129.78 (1C, CH aryl), 129.91 (1C, CH aryl), 133.02 (1C, CH aryl), 133.20 (1C, CH aryl), 135.77 (2C, 2CH aryl), 135.79 (2C, 2CH aryl), 194.93 (1C, Cq C=O thioacetate); *m/z* $[M+NH_4]^+$: 634.2857; $[C_{32}H_{44}O_8SSi+NH_4]^+$ requires 634.2864.

_S_-(3a_S_,5_S_,5a_R_,8a_S_,8b_R_)-5-2-tert-butyldiphenylsilyl-oxy-acetyl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl) ethanethioate (248)



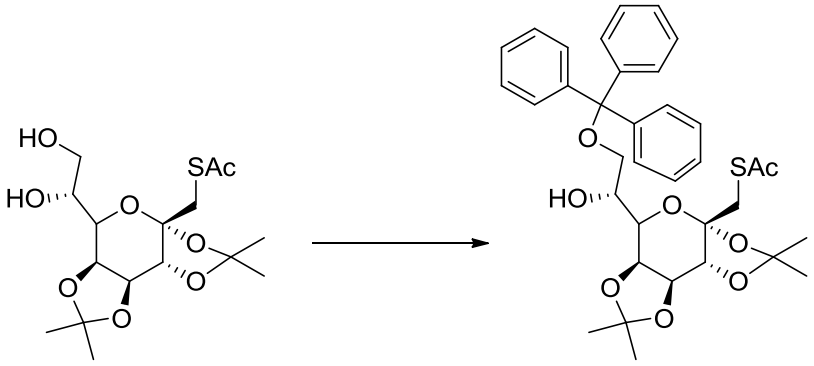
245 **248**

DMSO (0.114 mL, 1.6 mmol) was slowly added to a solution of oxalyl chloride (0.061 mL, 0.713 mmol) in anhydrous dichloromethane at -78 °C and the solution was stirred for a further 30 min at this temperature. A solution of **245** (0.293 g, 0.48 mmol) in anhydrous dichloromethane was added dropwise to the reaction mixture and the solution was stirred for 1 h. TEA (0.318 mL, 2.28 mmol) was slowly added to the reaction mixture at the same temperature and the solution was then warmed up to 0 °C and stirred at this temperature for a further 30 min. After the mixture was allowed to warm up to room temperature, water, a saturated solution of NH_4Cl and a 2 M HCl were added the mixture was extracted with dichloromethane. The combined organic layers were further washed with water, brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the product **248** as a colourless syrup. (0.294 g, quantitative)

IR γ_{max} (film)/ cm^{-1}: 3072, 3050, 2990, 2934, 2896, 2858, 1962, 1892, 1742, 1704, 1695, 1589, 1473, 1462, 1428, 1384, 1373, 1324, 1307, 1256, 1210, 1163, 1113; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 (9H, s, $Si(CH_3)_3$), 1.18 (3H, s, CH_3 isopropylidene), 1.18 (3H, s, CH_3 isopropylidene), 1.30 (3H, s, CH_3 isopropylidene), 1.37 (3H, s, CH_3 isopropylidene), 2.15 (3H, s, CH_3 thioacetate), 3.16 (1H, d, J = 13.8 Hz, CH_2SAc), 3.33 (1H, d, J = 13.8 Hz, CH_2SAc), 4.11 (1H, d, J = 2.2 Hz, H-3), 4.29 (1H, d, J = 1.8 Hz, H-4), 4.42 (1H, m, H-7), 4.54 – 4.49 (2H, m, H-2, H-5), 4.60 (1H, m, H-7), 7.37 – 7.27 (6H, m, CH aryl), 7.63 – 7.58 (4H, m, CH aryl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.36 (1C, Cq, SiC), 24.07 (1C, CH_3 isopropylidene), 24.85 (1C, CH_3 isopropylidene), 25.36 (1C, CH_3 isopropylidene), 26.33 (1C, CH_3 isopropylidene), 26.77 (3C, $SiC(CH_3)_3$), 30.22 (1C, CH_3 thioacetate), 37.31 (1C, CH_2SAc), 69.03 (1C, CH_2, C-7), 70.92 (1C, C-4), 71.86 (1C, C-3), 72.93 (1C, C-5), 74.66 (1C, C-2), 102.76 (1C, Cq, C-1), 109.00 (1C, Cq, isopropylidene), 109.83 (1C, Cq,

isopropylidene), 127.70 (2C, 2CH aryl), 127.75 (2C, 2CH aryl), 129.68 (1C, CH aryl), 129.72 (1C, CH aryl), 133.02 (1C, CH aryl), 133.26 (1C, CH aryl), 135.57 (2C, 2CH aryl), 135.64 (2C, 2CH aryl), 194.18 (1C, Cq C=O, SAc), 204.68 (1C, Cq C=O); *m/z* $[M+NH4]^+$: 632.2702; $[C_{32}H_{42}O_8SSi+NH_4]^+$ requires 632.2708.

S-(3aS,5R,5aS,8aS,8bR)-5-(R)-1-hydroxy-2-trityloxy-ethyl-2,2,7,7-tetramethyltetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl-ethanethioate (246)



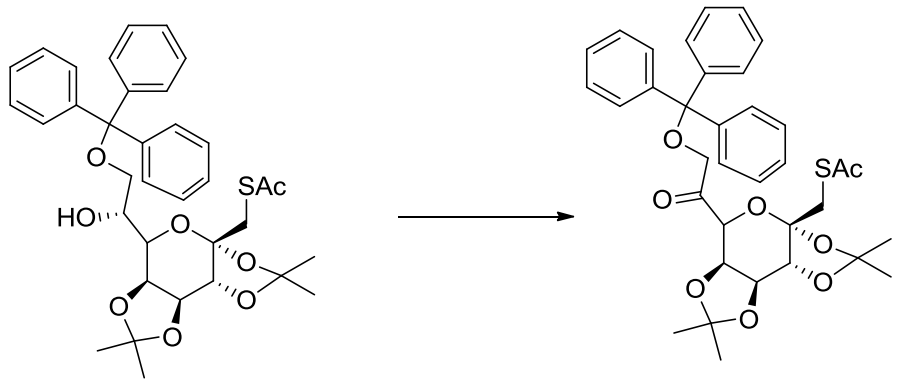
243 **246**

Diol **243** (0.733 g, 1.94 mmol) and TEA (0.54 mL, 3.88 mmol) were dissolved in anhydrous dichloromethane. Trityl chloride (0.6 g, 2.134 mmol) was added dropwise in a dichloromethane solution at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred a further 8 h or until TLC showed complete consumption of the starting material. Water was added to the reaction mixture and the mixture was extracted with dichloromethane (50 mL x 2). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (Petrol:EtOAc, 4:1) on silica gel to give the pure compound **246** as a white solid (1.066 g, 89%). Prior to chromatography a few drops of TEA 2% of eluent was added to neutralise the silica gel.

IR γ_{max} (film)/ cm^{-1}: 3434, 3059, 2988, 2945, 1693, 1494, 1451, 1382, 1321, 1309, 1258, 1172, 1140, 995, 908; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32 (3H, s, CH_3 isopropylidene), 1.38 (3H, s, CH_3 isopropylidene), 1.40 (3H, s, CH_3 isopropylidene), 1.45 (3H, s, CH_3 isopropylidene), 2.33 (3H, s, CH_3 thioacetate), 2.66 (1H, s, OH), 3.29 (1H, d, *J* = 6.8 Hz, CH_2SAc), 3.33 (2H, d, *J* = 3.0 Hz, H-2, H-4), 3.43 (1H, d, *J* = 6.8 Hz, CH_2SAc), 3.93 (2H, s, H-7, H-7), 4.19 (1H, d, *J* = 2.3 Hz, H-5), 4.37 (1H, d, *J* = 7.9 Hz, H-3), 4.59 (1H, dd, *J* = 7.9, 2.3 Hz, H-6), 7.29-7.23 (10H, m, CH aryl), 7.45 (5H, m, CH aryl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.3 (1C, CH_3 isopropylidene), 25.0 (1C, CH_3 isopropylidene), 25.8 (1C, CH_3 isopropylidene), 26.4 (1C, CH_3 isopropylidene), 30.4 (1C, CH_3 thioacetate), 37.5 (1C, CH_2SAc), 63.7 (1C, CH_2, C-7), 68.0 (1C, C-6), 69.5 (1C, C-3), 70.8 (1C, C-4), 71.2 (1C, C-2), 72.9 (1C, C-5), 86.7 (1C, Cq aryl), 103.0 (1C, Cq C-1), 108.6 (1C, Cq isopropylidene), 109.3 (1C, Cq isopropylidene), 127.0 (3C, 3CH, aryl), 127.9 (6C, 6CH, aryl),

128.8 (6C, 6CH, aryl), 143.9 (3C, 3Cq, aryl), 195.0 (1C, Cq, C=O, SAc); *m/z* $[M+Na]^+$: 643.2327; $[C_{35}H_{40}O_8S+Na]^+$ requires 643.2336.

_S_-(3a_S_,5_S_,5a_R_,8a_S_,8b_R_)-2,2,7,7-tetramethyl-5-2-trityloxy-acetyl-tetrahydro-3aH-bis-[1,3]dioxolo-[4,5-b:4',5'-d]pyran-3a-yl-methyl-ethanethioate (249)



246 **249**

DMSO (0.077 mL, 1.08 mmol) was slowly added to a solution of Oxalyl chloride (0.0412 mL, 0.48 mmol) in anhydrous dichloromethane at -78 °C and the solution was stirred for a further 30 min at this temperature. A solution of **246** (0.2 g, 0.322 mmol) in anhydrous dichloromethane was added dropwise to the reaction mixture and the solution was stirred for 1 h. TEA (0.216 mL, 1.55 mmol) was slowly added to the reaction mixture at the same temperature and the solution was then warmed up to 0 °C and stirred at this temperature for a further 30 min. After the mixture was allowed to warm up to room temperature, water, a saturated solution of NH_4Cl and a 2 M HCl were added the mixture was extracted with dichloromethane. The combined organic layers were further washed with water, brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the product was purified by column chromatography (Petrol:EtOAc, 4:1) on silica gel to give pure compound **249** (0.102 g, 51%) Prior to column chromatography 2% TEA was added to the eluent to neutralise the silica gel.

IR γ_{max} (film)/ cm^{-1}: 3087, 3059, 3024, 2989, 2936, 2945, 1738, 1697, 1491, 1449, 1442, 1384, 1373 1323, 1162, 1099, 1070, 1033, 954, 906; ^1H NMR (500 MHz, $CDCl_3$): δ 1.12 (3H, s, CH_3 isopropylidene), 1.19 (3H, s, CH_3 isopropylidene), 1.32 (3H, s, CH_3 isopropylidene), 1.41 (3H, s, CH_3 isopropylidene), 2.26 (3H, s, CH_3 thioacetate), 4.02 – 4.11 (3H, m, CH_2SAC, H-3), 4.12 – 4.15 (1H, m, H-4), 4.26 – 4.29 (1H, m, H-7), 4.48 – 4.52 (1H, m, H-7), 4.52 – 4.56 (2H, m, H-2, H-5), 7.16 – 7.29 (10H, m, CH aryl), 7.40 – 7.45 (5H, m, CH aryl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.1 (1C, CH_3 isopropylidene), 24.8 (1C, CH_3 isopropylidene), 25.4 (1C, CH_3 isopropylidene), 26.3(1C,

CH_3 isopropylidene), 30.3 (1C, CH_3 thioacetate), 37.4 (1C, CH_2OSAc), 69.2 (1C, CH_2 C-7), 70.9 (1C, C-4), 72.0 (1C, C-3), 72.9 (1C, C-5), 74.6 (1C, C-2), 86.9 (1C, Cq aryl), 102.7 (1C, Cq, C-1), 109.1 (1C, Cq isopropylidene), 109.8 (1C, Cq isopropylidene)127.1 (3C, 3CH aryl), 128.0 (6C, 6CH aryl), 128.7 (6C, 6CH aryl), 143.5 (3C, 3Cq aryl), 194.3 (1C, C=O ketone), 203.4 (1C, C=O, thioacetate); *m/z* $[M+NH_4]^+$: 636.2624; $[C_{35}H_{38}O_8S+NH_4]^+$ requires 636.2626.

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Appendix A

Organic & Biomolecular Chemistry

PAPER

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The structure of tagetitoxin†

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Based on detailed analysis of newly acquired NMR data, we show that the previously revised structure of tagetitoxin is incorrect. A new structure of tagetitoxin is proposed which is consistent with the NMR and MS data.

Introduction

Tagetitoxin is a toxin isolated from the plant pathogenic bacterium *Pseudomonas syringae* pv. *tagetis*.[1] It is known to cause chlorosis in young plant leaves, which has been attributed to inhibition of RNA polymerase in chloroplasts.[2] Tagetitoxin also inhibits bacterial RNA polymerase,[2] and is the only natural product known to inhibit eukaryotic RNA polymerase III in a specific manner.[3] Recently, Yuzenkova *et al.* have shown that tagetitoxin neither affects the chemistry of RNA synthesis nor competes with the nucleoside triphosphate in the active centre.[4] Instead, tagetitoxin increases the stability of the pre-translocated state of the elongation complex, thus slowing down addition of the following nucleotide.[4]

The first published structure of tagetitoxin by Mitchell and Hart in 1983 consisted of an eight-membered heterocycle with a sulfur atom (structure **1** in Fig. 1, molecular weight 435, $C_{11}H_{18}NO_{13}PS$).[5]

It was found that heteroatomic components comprised of oxygen, nitrogen in an amine, phosphorus in a phosphate ester and sulfur. Investigations of the structure of tagetitoxin continued based on new MS and NMR data, after attempts to obtain crystals for X-ray analysis failed.[5] In 1989, a revised bicyclic structure of tagetitoxin based on the 9-oxa-3-thiabicyclo[3.3.1]nonane ring system was proposed by Mitchell *et al.* (Fig. 1).[6] FAB mass spectrometry showed $(M + H)^+ = 417.0361$ ($C_{11}H_{18}N_2O_{13}PS$ requires 417.0369) indicating that tagetitoxin has a molecular formula $C_{11}H_{17}N_2O_{13}PS$. Structure **2** was favoured, although the spectroscopic data did not rule out the closely related structure **3** (Fig. 1).[6]

In 2005, a crystal structure of the RNA polymerase from *Thermus thermophilus* with tagetitoxin bound to the active site was published by Vassylyev *et al.*[7] Although the difference electron density map revealed an electron density attributed to tagetitoxin in this crystal structure, the structure of tagetitoxin was not investigated and a stereoisomer of structure **2** was used by Vassylyev *et al.* without further verification.[7] In the same year, Gronwald *et al.* published their purification protocol and partial characterization of tagetitoxin.[8] According to their analysis, the revised structure of Mitchell *et al.*[6] is incorrect. Based on electrospray ionization mass spectrometry in 50% methanol : H_2O, Gronwald *et al.* reported that the molecular weight of tagetitoxin is 678, although the NMR spectrum of tagetitoxin published by them indicated that tagetitoxin contained additional peaks at 2.53 ppm and 1.75 ppm not observed previously by Mitchell *et al.*[5,6] Despite the ambiguity of the structure of tagetitoxin, several reports have been published to date, detailing synthetic approaches to tagetitoxin and its analogues with the basic bicyclic ring structure **2**,[9] though none of these has successfully delivered the full structure **2**.

Here, we report the results of our analysis of NMR and MS data for tagetitoxin and show that neither of the published structures of tagetitoxin is correct. A new structure of tagetitoxin is reported which is in agreement with NMR and MS data.

Results and discussion

The sample studied was that originally isolated and purified by Mitchell.[1,5] In order to illustrate the purity of the compound studied, the proton NMR spectrum of tagetitoxin in D_2O is shown in Fig. 2. Note that additional peaks of smaller intensity appeared in ^1H NMR spectrum of tagetitoxin kept in D_2O solution over 4–6 weeks (see Fig. S2 in ESI†), suggesting that tagetitoxin gradually decomposes in aqueous solutions. Although most of the spectral features in Fig. 1 resemble those observed in the ^1H NMR spectrum of tagetitoxin by Gronwald *et al.*,[8] no peaks are observed at 1.75 and 2.53 ppm. Similarly, no ^{13}C peak was observed at 181.45 ppm. These observations suggest the material studied by Gronwald *et al.*[8] was less pure

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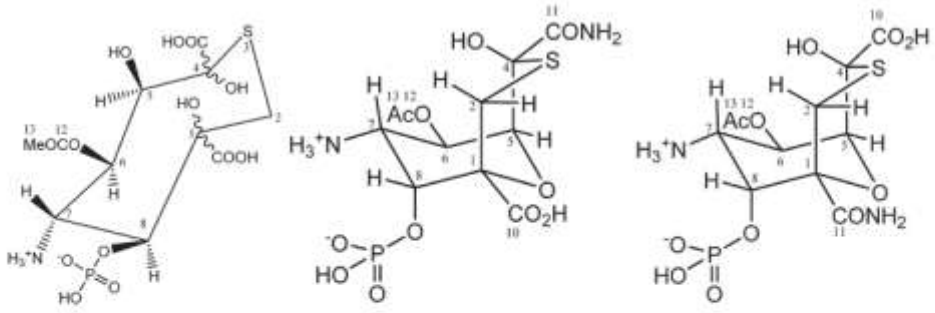

1 2 3

Fig. 1 Previously proposed structures of tagetitoxin.

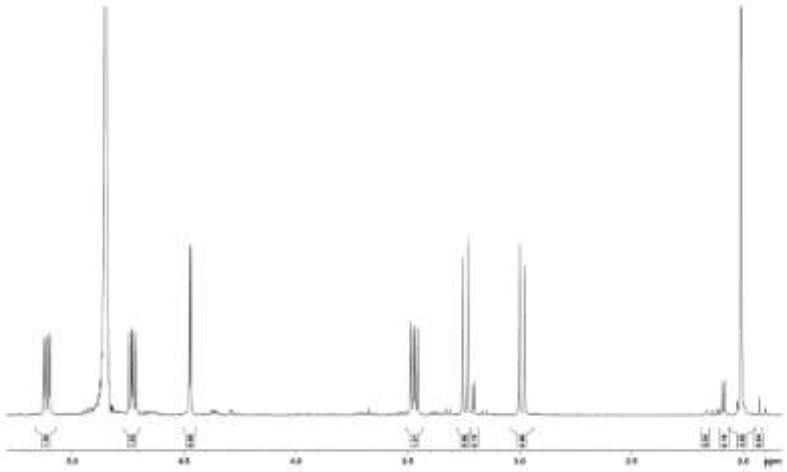

Fig. 2 ^{1}H NMR spectrum of tagetitoxin in D_2O at 293 K (600 MHz). Integral intensities are shown between the chemical shift axis and the spectrum. The most significant impurity peaks are observed as doublets at 3.21 ppm with 4.0 Hz splitting (integral intensity 0.15) and at 2.09 ppm with 5.0 Hz splitting (integral intensity 0.18).

compared to that extracted by Mitchell *et al.*[3,6] From the analysis of the MS data obtained in this work (see ESI† for full details), no species with the molecular weight of 678 were found, which is reported by Gronwald *et al.*[8] as a revised molecular weight of tagetitoxin.

In 1989, the revised structure 2 (Fig. 1) was deduced based on the analysis of ^{1}H and ^{13}C NMR spectra, ^{1}H NOEs and the COLOC spectrum for ^{1}H–^{13}C long-range correlations.[6] The latter is expected to provide information similar to that from the HMBC spectrum, although it is significantly less sensitive than HMBC, which is usually used for identification of 2 or 3 bond correlations between ^{1}H and ^{13}C nuclei. The HMBC spectrum shown in Fig. 3, as well as the values of long-range $^nJ_{CH}$ couplings (Table 1), revealed several correlations which allowed us to rule out structures 1–3 shown in Fig. 1. In particular, some of the disagreements are as follows:

(1) A cross-peak is observed for the C11–H8 pair which is in disagreement with structure 2 with six bonds between C11 and H8.

(2) A strong cross-peak C10–H2′ is in disagreement with structure 3 with four bonds between C10 and H2′. In principle, $^4J_{CH}$ correlations can be observed in HMBC spectra, however,

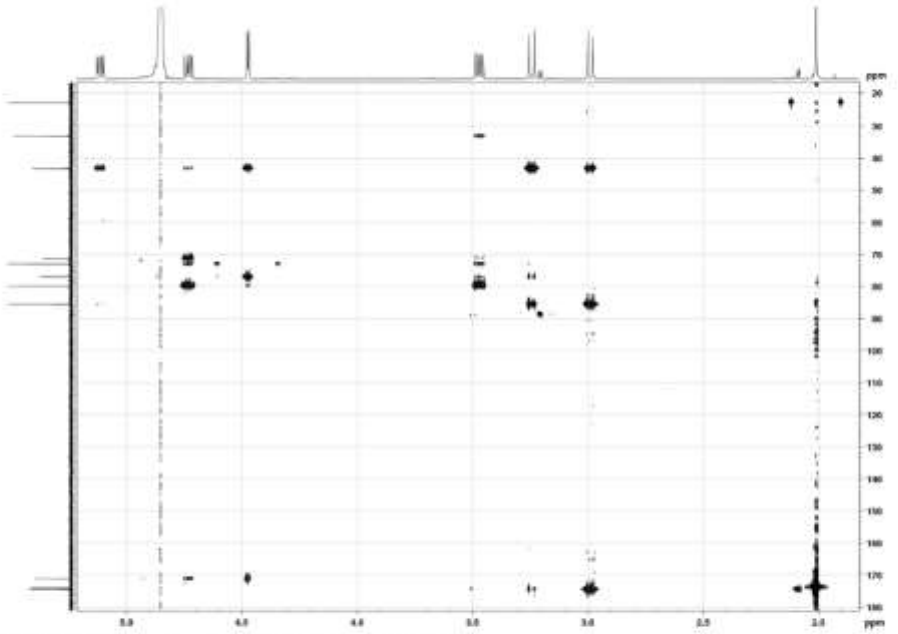

Fig. 3 ^{1}H–^{13}C HMBC NMR spectrum of tagetitoxin in D_2O at 293 K (600 MHz).

the value of $J_{C10H2'}$ coupling derived from the HMBC-JC spectrum is 5.0 Hz, which cannot be attributed to a $^{n}J_{CH}$ coupling.

(3) Cross-peaks are observed for C7–H2 (J_{CH} = 5 Hz) and C7–H2′ (J_{CH} = 3 Hz), which are in disagreement with all three structures shown in Fig. 1, with four bond separation between C7 and H2.

(4) Dihedral angle between C4 and H6 is ~180° in structure 2, while only a weak HMBC cross-peak is observed in the HMBC spectrum. From HMBC-JC, the value of J_{C4H6} is 1.4 Hz.

Furthermore, the 1D NOESY spectrum with selective excitation of methyl protons at 2.01 ppm showed a negative exchange enhancement at 2.16 ppm with the integral intensity ratio 68 : 1 for singlets at 2.01 and 2.16 ppm in the ^{1}H NMR spectrum (Fig. S3 in ESI†). Such a slow exchange at room temperature between two sites with unequal populations is characteristic for an amide group NHCOMe, but not for OCOMe shown in structures 1–3 (Fig. 1). ^{1}H NMR spectrum recorded in H_2O : D_2O (9 : 1) showed a singlet at 8.47 ppm (Fig. S13 in ESI†), which is in agreement with the presence of the NHCOMe group. In addition, the ^{1}H–^{15}N HMBC spectrum in D_2O (Fig. S20 in ESI†) showed a correlation for the methyl protons with the ^{15}N signal at 140.5 ppm, in agreement with the expected ^{15}N chemical shift for a secondary amide in the range 110–160 ppm (relative to liquid NH_3).

Based on mainly ^{1}H–^{13}C HMBC correlations and the values of long-range J_{CH} couplings a new structure was derived shown in Fig. 4. The above noticed disagreements (1)–(4) for structures 1–3 were verified for structure 4:

(1) The cross-peak observed for C11–H8 pair is due to $^{3}J_{CH}$ coupling in 4;

(2) The cross-peak C10–H2′ is due to $^{3}J_{CH}$ coupling in 4.

(3) Cross-peaks observed for C7–H2 (J_{CH} = 5 Hz) and C7–H2′ (J_{CH} = 3 Hz) are due to $^{3}J_{CH}$ coupling in 4.

(4) Assuming a chair conformation of the six membered ring, the dihedral angle between C4 and H6 is ~60° in structure 4, in agreement with the measured value of $^{3}J_{C4H6}$ = 1.4 Hz based on the Karplus-type relationship for $^{3}J_{CH}$ couplings.

In a similar fashion, we have analysed the measured values of all the vicinal $^{3}J_{CH}$ couplings, which show good agreement with structure 4. There are relatively few J_{HH} couplings in tagetitoxin. Nevertheless, the large value of the $^{3}J_{H6H7}$ = 12.2 Hz is in favour of the *trans* fusion of two cycles with both protons occupying axial orientations. Furthermore, from the measured signal enhancements in 1D NOESY spectra (Table 2), the NOE is relatively small for the H6–H7 pair (0.5%) compared to, for example, H5–H6 (1.4–1.5%) or H7–H8 (1.2–1.3%). Combined with the values of vicinal couplings ($^{3}J_{H5H6}$ = 4.1 Hz and $^{3}J_{H7H8}$ = 7.8 Hz), these NOEs are in favour of the *trans* configuration

Table 1 Experimental values of long-range ^{1}H–^{13}C coupling constants ($^nJ_{CH}$, Hz) of tagetitoxin in D_2O. The signs of coupling constants measured using HSQC–HECADE are included in brackets. The calculated values (at the DFT B3LYP/6-311+G(2d,p) IEFPCM(H_2O) level of theory) for individual conformers, as well as averaged values, ($^nJ_{CH}$), over two conformers, 4-chair (75%) and 4-twisted-chair (25%), are shown

	Exper. $^nJ_{CH}$/Hz	4-Chair Calc. $^nJ_{CH}$/Hz	4-Tw.-chair Calc. $^nJ_{CH}$/Hz	4 Calc. ($^nJ_{CH}$)/Hz
C1–H7	1.3	1.20	2.05	1.41
C1–H8	2.4	−2.07	−2.46	−2.17
C1–H5	~0	−0.62	−0.88	−0.69
C1–H6	~0	0.16	−0.01	0.12
C2–H7	1.5	2.82	0.02	2.10
C4–H2	2.3	−2.32	−1.63	−2.15
C4–H2′	4.1	−4.02	−2.20	−3.56
C4–H13	0.8	0.53	0.48	0.51
C4–H6	1.4	0.79	−0.10	0.56
C5–H7	(+)1.1	1.28	1.02	1.22
C5–H8	(+)0.9	0.72	0.41	0.64
C5–H6	(+)0.2	0.17	0.95	0.37
C6–H5	(−)0.7	−0.14	−0.01	−0.11
C6–H7	(−)5.6	−5.44	−5.65	−5.49
C6–H8	(+)8.0	7.26	6.32	7.02
C7–H2	5.0	6.98	−0.10	5.17
C7–H2′	3.0	2.44	5.99	3.35
C7–H5	(+)6.1	5.53	5.31	5.47
C7–H6	(−)2.7	−2.36	−1.60	−2.17
C7–H8	(−)1.1	−2.12	−1.96	−2.08
C8–H2	1.4	1.39	−0.17	0.99
C8–H5	(+)6.2	6.35	6.40	6.36
C8–H6	(+)0.3	0.50	0.22	0.43
C8–H7	(−)0.4	−1.12	−0.44	−0.95
C10–H2	1.2	1.35	1.64	1.42
C10–H2′	5.0	7.03	1.54	5.63
C11–H8	1.5	1.86	2.89	2.12
C11–H5	2.7	2.12	1.95	2.07
C12–H13	6.0	−5.51	−5.52	−5.51
C12–NH	3.7	4.57	5.14	4.72
rms$_J$/Hz	—	0.72	1.50	0.52

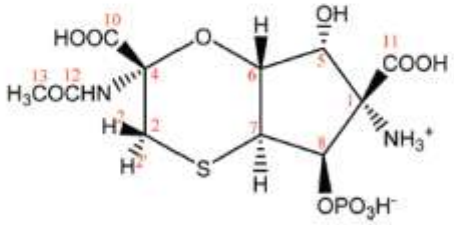

Fig. 4 The proposed revised structure of tagetitoxin based mainly on the analysis of ^{1}H–^{13}C HMBC correlations and the values of long-range J_{CH} couplings. The atom numbering used corresponds to that in 2 (Fig. 1).

of protons H6 and H7, the *cis* configuration of protons H5 and H6 and the *cis* configuration of protons H7 and H8. A very small enhancement (0.1%) observed for the H6–H8 pair is in agreement with their *trans* configuration in the five-membered ring.

Protons of the methylene group in tagetitoxin are labelled as 2 and 2′ (Fig. 4). The methylene proton with the *cis* configuration relative to proton H6 is denoted as H2 (the high-frequency CH_2 signal in the ^{1}H NMR spectrum), while the other methylene proton with the *trans* configuration relative to H6 is denoted as H2′ (the low-frequency CH_2 signal in the ^{1}H NMR spectrum). Thus, in a chair conformation of the six-membered ring with the axial orientation of H6, such a definition of H2 and H2′ corresponds to the equatorial orientation of H2 and the axial orientation of H2′. Relatively strong NOE (0.6%) was observed for proton pair H2–H6 (Table 2), which led to a consideration of a twisted chair conformation for the six-membered ring. Both chair and twisted-chair conformations were included into our computational analysis and the final lowest energy conformations derived from DFT M06-2X/def2-TZVP geometry optimisations are shown in Fig. 5. The free energy of the twisted-chair conformation relative to that of the chair conformation is +1.22 kcal mol^{-1}. On the assumption of a two-site fast exchange (in the NMR timescale) between chair and twisted-chair conformations, the predicted populations by DFT M06-2X/def2-TZVP calculations are 89% and 11% for chair and twisted-chair conformations, respectively. A more reliable estimate of the conformational populations was achieved using experimental values of 30 long-range J_{CH} couplings and predicted values of corresponding coupling constants in chair and twisted-chair conformations at the DFT B3LYP/6-311+G (2d,p) level of theory (Table 1; regarding the performance of B3LYP calculations for predictions of J couplings, see ref. 10). The populations of conformers derived from this analysis were 75% and 25% for chair and twisted-chair conformations, respectively.

No HMBC correlations were observed for C1–H5 and C1–H6 pairs separated by two and three bonds, respectively, in structure 4. DFT calculations confirmed that the expected values of the corresponding $^{2,3}J_{CH}$ couplings are indeed small, *e.g.*, −0.62 Hz and 0.16 Hz in the 4-chair conformation shown in Fig. 5 (−0.88 Hz and −0.01 Hz in the 4-twisted-chair conformation).

In order to determine orientations of substituents in position 4, we have used weak NOEs observed for the amide NH proton with H7 in $H_2O + D_2O$ (9 : 1) solution, as well as the fact that the NOE for the NH–H2′ pair is significantly stronger than that for the NH–H2 pair (~4.4 times based on the volume integration of the corresponding cross-peaks; the volume integration ratio for the cross-peaks of the amide proton NH with H7, H2, H2′, Me was 1.0 : 2.3 : 10.1 : 9.2; Fig. S13 in ESI†). Furthermore, the *cis* orientation of the NHCOMe group relative to proton H7 was confirmed by the analysis of vicinal J_{CH} couplings of the adjacent carboxylic carbon based on the Karplus-type relationship: $^3J_{C10H2} = 1.2$ Hz and $^3J_{C10H2'} = 5.0$ Hz. These agree well with the DFT predicted values of 1.4 and 5.6 Hz on the assumption of the equilibrium between 4-chair (75%) and 4-twisted-chair (25%) conformations (Fig. 5 and Table 1).

The determination of orientations of substituents in position 1 required consideration of both alternatives in DFT calculations and the analysis of $^3J_{CH}$ couplings of the carboxylic

Table 2 NOE Enhancements (in %) from 1D NOESY experiments. No NOEs were observed on selective excitation of methyl protons. The measured distances (in Å) in DFT-optimised geometries of 4-chair/4-twisted-chair conformations are shown in brackets

	"Touched" protons					
	H2'	H2	H7	H5	H8	H6
H2'	—	5.4 (1.77/1.77)	0.8 (2.37/3.92)			0.1 (3.93/3.73)
H2	5.6 (1.77/1.77)	—				0.6 (4.12/2.30)
H7	1.1 (2.37/3.92)		—	0.1(3.72/3.74)	1.2 (2.26/2.21)	0.5 (3.04/3.03)
H5			0.1 (3.72/3.74)	—		1.5 (2.44/2.45)
H8			1.3 (2.26/2.21)		—	0.1 (3.76/3.80)
H6		0.7 (4.12/2.30)	0.5 (3.04/3.03)	1.4 (2.44/2.45)	0.1 (3.76/3.80)	—

4-chair 4-twisted-chair

Fig. 5 Geometries of 4-chair and 4-twisted-chair conformations derived from DFT M06-2X/def2-TZVP calculations. One of the carboxylic protons of structure 4 (Fig. 4) is delocalized between COO^- and OPO_3H^- groups in both conformations.

carbon C11 with protons H5 and H8. Structures of 5-chair and 5-twisted-chair, in which the orientations of N^+H_3 and COOH are interchanged at C1 compared to 4, are shown in Fig. S1 (ESI†). The free energy of the 5-twisted-chair conformation relative to that of the 5-chair conformation is +0.81 kcal mol^{-1}. On the assumption of a two-site exchange between chair and twisted-chair forms, the predicted populations by DFT M06-2X/def2-TZVP calculations are 81% and 19% for 5-chair and 5-twisted-chair conformations, respectively. From the analysis of experimental values of 30 long-range J_{CH} couplings and predicted values of corresponding coupling constants in 5-chair and 5-twisted-chair conformations at the DFT B3LYP/6-311+G (2d,p) level of theory (Table S3†), the populations of conformers were 78% and 22% for 5-chair and 5-twisted-chair conformations, respectively. However, the rms deviation between experimental and calculated couplings is 1.24 Hz for the conformational equilibrium 5-chair/5-twisted-chair, compared to 0.52 Hz for the conformational equilibrium 4-chair/4-twisted-chair. The predicted values for $^3J_{C1\,1H8}$ and $^3J_{C1\,1H5}$ were 3.7 and 0.3 Hz for the 5-chair/5-twisted-chair equilibrium, which are in disagreement with the experimental values of 1.5 and 2.7 Hz. In the case of the 4-chair/4-twisted-chair equilibrium, the predicted values for $^3J_{C1\,1H8}$ and $^3J_{C1\,1H5}$ were 2.1 and 2.1 Hz. Thus, the *cis* configuration of the phosphate and carboxylic groups at C8 and C1, respectively, can be deduced based on the analysis of experimental and calculated J_{CH} couplings.

In addition to the measured values, we have also determined the sign of some of the J_{CH} couplings (Table 1). It is well known that ^{13}C–^1H couplings over one and three bonds are positive, while those over two or four bonds ($^2J_{CH}$ or $^4J_{CH}$) are either positive or negative. Thus, if we know that the sign of $^nJ_{CH}$ is negative, then the number of bonds between C and H cannot be three and using the absolute value of the coupling constant we could deduce whether it corresponds to $^2J_{CH}$ or $^4J_{CH}$. We have used the HSQC-HECADE spectrum for sign determinations.[11] Note that the sign of only some of the $^nJ_{CH}$ couplings are available from this spectrum (*e.g.*, $^nJ_{CH}$ correlations of quaternary carbons are not detectable).[11] Nevertheless, all the measured negative values (for spin pairs C6–H5, C6–H7, C7–H6 and C8–H7) can be attributed to geminal $^2J_{CH}$ couplings, while $^3J_{CH}$ couplings have a positive sign (for spin pairs C5–H7, C5–H8, C6–H8, C7–H5, C8–H5 and C8–H6). Thus, these results additionally support the sequence in which the corresponding C and H atoms are arranged. The signs of these couplings predicted by the DFT calculations were in agreement with the HSQC-HECADE measurements (Table 1).

The EASY-ROESY method was also used, which is known to provide accurate integration of cross-peaks for quantitative estimates.[12] We have analysed the observed rotational Overhauser effects (ROEs) using a simplified version of the growth rates method in order to estimate internuclear ^1H–^1H distances.[13] The satisfactory performance of the simplified

growth rates method has been demonstrated previously for cyclic organic compounds.[14] The standard deviations for distance measurements were typically 10% of the corresponding mean values.[14] Volume integrals of ROE cross-peaks for 6 proton pairs were measured for tagetitoxin (Table 3 and Fig. S14 in ESI†). Using r = 1.77 Å as the reference value for the geminal H2–H2′ pair, internuclear distances for other proton pairs were calculated using the r^{-6} dependence of ROEs.[13] In Table 3, we compare experimental values with those from interatomic distances (r_i^{calc}, Å) derived from M062X/def2-TZVP-optimised geometries. The individual chair and twisted-chair conformers showed the rms deviations (rms_d, Å) of 0.62 Å and 0.71 Å, respectively, for five pairs of protons (excluding the reference geminal pair). Significantly improved agreement is observed with rms_d = 0.11 Å on considering a two-site fast exchange between 4-chair (75%) and 4-twisted-chair (25%), with the populations determined from the analysis of $^n J_{CH}$ couplings above.

The populations of chair (75%) and twisted chair (25%) conformers obtained from the analysis of $^3J_{CH}$ couplings also agree well with the combined analysis of experimental and calculated ^{1}H and ^{13}C chemical shifts. The methodology used here has been verified previously for cyclic organic compounds with known structures.[14,15] In particular, optimised geometries of 4-chair and 4-twisted-chair were used in GIAO B3LYP/6-311+G(2d,p) chemical shielding calculations. The conformationally averaged values of the isotropic shieldings $\langle\sigma^{calc}(i)\rangle$ were calculated. The averaged values of the isotropic shieldings were then converted into conformationally averaged values of chemical shifts, $\langle\delta^{calc}(i)\rangle$, using both the slope and the intercept of the σ^{calc} *vs.* δ^{exp} plot, as described previously.[14] From the results obtained (Table 4), the rms_δ values for 4 were 0.08 ppm (^{1}H) and 2.2 ppm (^{13}C). For comparison, the rms_δ values for a closely related structure 5 (78% chair and 22% twisted chair) were 0.21 ppm (^{1}H) and 2.8 ppm (^{13}C), showing high sensitivity of both ^{1}H and ^{13}C chemical shifts to the change in the orientation of substituents. Overall, the relatively small values of rms deviations for chemical shifts (rms_δ ^{1}H 0.08 ppm and ^{13}C 2.2 ppm), together with the ROE analysis of interproton distances (rms_d 0.11 Å), further support the validity of structure 4 for tagetitoxin. The NMR-derived structure 4 of tagetitoxin was also consistent with the accurate mass measurements and gas-phase fragmentation patterns, full details of which are included in ESI.†

Table 3 Internuclear distances (in Å) in **4** obtained from NMR ROE measurements in D_2O and DFT M06-2X/def2-TZVP calculations in H_2O with the IEFPCM solvation model. The rms deviations (rms_d, in Å) from the experimental NMR values are shown

Proton pair	NMR $\langle r^{exp}\rangle^a$ (Å)	4-Chair r^{calc} (Å)	4-Twisted-chair r^{calc} (Å)	4 $\langle r^{calc}\rangle^b$ (Å)
2–2′	1.77	1.77	1.77	1.77
7–8	2.21 ± 0.02	2.26	2.21	2.24
5–6	2.31 ± 0.01	2.44	2.45	2.44
6–7	2.85 ± 0.02	3.04	3.03	3.03
2–6	2.76 ± 0.03	4.12	2.30	2.85
2′–7	2.43 ± 0.01	2.37	3.92	2.47
rms_d	—	0.62	0.71	0.11

[a] Uncertainties in experimental values were estimated using volume integrations of cross-peaks above and below the diagonal. [b] In calculations of averaged values of $\langle r^{calc}\rangle$, the calculated ROEs were weighted using populations of conformers 4-chair (75%) and 4-twisted-chair (25%).

Table 4 Experimental and calculated ^{1}H and ^{13}C chemical shifts in **4** (δ, ppm). Optimised geometries from M062X/def2-TZVP IEFPCM(H$_2$O) were used in GIAO B3LYP/6-311+G(2d,p) IEFPCM(H$_2$O) chemical shift calculations. The rms deviation (rms_δ, in ppm) and the largest residual deviation (Δ^{max}, in ppm)between the calculated and experimental values are shown

Proton	δ_H^{exp} (ppm)	$\langle\delta_H^{calc}\rangle^a$ (ppm)	Carbon	δ_C^{exp} (ppm)	$\langle\delta_C^{calc}\rangle^a$ (ppm)
2	3.25	3.23	1	71.2	73.1
2′	2.98	2.87	2	33.1	36.2
5	4.46	4.39	4	85.6	82.8
6	5.13	5.15	5	72.9	72.8
7	3.48	3.65	6	79.8	80.4
8	4.73	4.72	7	43.2	44.8
13	2.01	2.02	8	77.0	78.4
			10	174.4	174.4
			11	171.2	170.1
			12	173.8	174.3
			13	22.9	17.7
rms_δ	—	0.08	rms_δ	—	2.2
Δ^{max}	—	0.17	Δ^{max}	—	−5.2

[a] Calculated chemical shifts [$\delta^{calc}(i) = (\sigma^{calc}(i) - b)/a$] were determined using the slope [$a(^1H) = -1.14$ and $a(^{13}C) = -0.98$] and the intercept [$b(^1H) = 32.31$ ppm and $b(^{13}C) = 177.66$ ppm] derived from the least squares fittings [$\sigma^{calc}(i) = a\delta^{exp}(i) + b$].

Experimental

NMR spectroscopy

Purified tagetitoxin was received in non-crystallised solid form from Robin Mitchell.[1,5,6] ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer equipped with a 5 mm cryoprobe (^{1}H 600.13 MHz and ^{13}C 150.90 MHz). These spectra showed no change from the data of Mitchell *et al.*[6] ^{15}N and ^{31}P NMR spectra were recorded on a Bruker Avance III 400 MHz NMR spectrometer equipped with a 5 mm ^{1}H–^{13}C–^{15}N–^{31}P probe (^{1}H 400.13 MHz, ^{15}N 40.55 MHz and ^{31}P 161.98 MHz). Data acquisition and processing were performed using standard TopSpin software (versions 2.1 and 3.2). ^{1}H and ^{13}C chemical shifts were calibrated indirectly, using dioxane shifts in D_2O (^{1}H 3.75 ppm, ^{13}C 67.19 ppm). ^{15}N and ^{31}P NMR chemical shifts were calibrated using $^{15}N_2$-urea dissolved in DMSO-d_6 (77.6 ppm relative to liquid NH_3) and 85% H_3PO_4 (0 ppm). Unless otherwise specified, NMR measurements were carried out at 293 K. Temperature calibration was carried out using a sample of 99.8% deuterated MeOD in a 5 mm NMR tube. In addition to standard 1D and 2D spectra, additional techniques were employed for measur-

ing long-range J_{CH} couplings, including HMBC-JC[16] and HSQC-HECADE.[12]

One- and two-dimensional NOE measurements were undertaken for establishing spatial proximities of protons.[13] Standard pulse sequences and those with the elimination of strong interference caused by zero-quantum coherence were employed.[17] 2D EASY-ROESY spectra were also acquired. The main advantage of this experiment is that artifacts due to *J*-couplings are minimised. It has also been shown to yield reliable intramolecular distances without a sample-specific setup.[12]

Calculations

Initial structures for quantum-mechanical calculations were built and optimized using PCMODEL (version 8.5).[18] The MMX force field was used for energy evaluations.[18,19] Relaxed grid search (RGS) analysis[18] was carried out for each conformer considered using PCMODEL. RGS is a systematic method, which involves creation of a large number of starting configurations and mapping out the shape of the potential energy surface. In this method the rotatable bonds of interest are first identified. The calculation starts by evaluating the energy when all the rotatable bonds are set to 180°. The bonds are then rotated sequentially and all the structures are minimized and sorted based on their total energy, with any duplicate configurations removed. Since the total number of energy evaluations can be very large (usually several hundreds or thousands depending on the number of rotatable bonds), the energies of conformers were calculated using molecular mechanics method and the MMX force field.

In some cases, the RGS derived structures were further optimized *via* semi-empirical PM6[20] calculations using Gaussian 09. The reaction field method IEFPCM[21] was used to account for water solvent effects in PM6 calculations.

All quantum mechanical calculations were carried out using Gaussian 09.[22] For geometry optimizations using density functional theory (DFT), the M06-2X[23] functional with def2-TZVP basis set was used.[24] The performance of M06-2X functional has been compared extensively to other DFT methods and MP2.[23,25] Its superior performance has been illustrated in a comprehensive review article by Zhao and Truhlar,[25] in which they have included comparisons of M06-2X with SCS-MP2 and B2PLYP-D. The choice of the def2-TZVP basis set is dictated primarily by the presence of sulfur and phosphorus atoms in tagetitoxin. At the DFT level the def2-TZVP basis set has been shown to produce results that are not too far from the DFT basis set limit.[24] For optimization of structure parameters, the def2-TZVP errors in bond lengths are typically smaller than 1 pm and that in bond angles are smaller than 1°.[24] The ultrafine numerical integration grid (with 99 radial shells and 590 angular points per shell) was used in our M06-2X/def2-TZVP geometry optimisations, combined with the "verytight" convergence condition (requesting the root-mean-square forces to be smaller than 1×10^{-6} Hartree $Bohr^{-1}$). Additional frequency calculations were also undertaken in order to verify that the optimized geometries correspond to true minima. The reaction field method IEFPCM[21] was used to account for water solvent effects. NMR chemical shieldings and *J* couplings were computed at the B3LYP/6-311+G(2d,p) level using the GIAO method.[26] Water solvent effects were used in all the quantum mechanical calculations using the reaction field method IEFPCM.[21]

Conformationally averaged interatomic distances from the QM calculations were determined in a way similar to that used in NMR measurements: (i) internuclear distances (r_i) for pairs of hydrogen atoms were measured in each conformer *i*; (ii) a quantity equal to r_i^{-6} was calculated as a measure of the expected NOE in each conformer, η_i; (iii) the sum of $p_i r_i^{-6}$ was calculated, where values of populations p_i were derived from the analysis of experimental long range J_{CH} couplings using their QM-predicted boundary values in each conformer *i*; (iv) using $r = 1.77$ Å as the reference H2–H2′ distance for geminal protons, internuclear distances for other proton pairs were calculated using the $\eta \sim r^{-6}$ relationship.[13]

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