Synthesis of model compounds for the stereogenic centres of the C(1)–C(7) unit of the HO-FC_1 and iso-FC_1 fumonisins

by

CHANTAL HEFTI

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Supervisor : Prof. R. Vleggaar

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DECLARATION

I, Chantal Hefti declare that the thesis/dissertation, which I hereby submit for the degree MSc Chemistry at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature :..... **Date :**.....

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" To a synthetic chemist, the complex molecules of nature are as beautiful as any of her creations. The perception of that beauty depends on the understanding of chemical structures and their transformations, and, as with a treasured work of art, deepens as the subject is studied, perhaps even to a level approaching romance. It is no wonder that the synthetic chemist of today is filled with joy by the discovery of a new naturally occurring structure and the appearance of yet another challenge to synthesise[1]." *E.J. Corey*

[1] Corey, E.J. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 455.

SUMMARY

HO-FC_1 and iso-FC_1 are members of the fumonisin family of mycotoxins produced by *Fusarium verticillioides*, a fungus growing on maize, which is a staple food in many countries. Consumption of these mycotoxins over time can result in oesophageal cancer and neural tube birth defects in humans, while consumption of contaminated feed causes equine leukoencephalomalacia and porcine pulmonary edema.

There are three known classes of fumonisins, namely the A-, B- and C-classes. The structures of these mycotoxins have been published previously and while the configuration of the stereogenic centres for the A- and B-classes have been determined, the configuration for the C-class remains unknown. Based on this information, it is generally accepted that the configuration of the stereogenic centres on the left hand side of the fumonisins is invariant. The substitution pattern of the right hand side of the C-class exhibits differences and the absolute configuration of the stereogenic centres of this part of the HO-FC_1 and iso-FC_1 fumonisins is unknown.

This study focuses on the development and optimisation of the stereoselective synthesis of the C(1)–C(7) fragment 1-amino-7-[(*t*-butyldimethylsilanyl)oxy]-2,3-heptanediol and 1-amino-7-[(*t*-butyl-diphenylsilanyl)oxy]-2,3,4-heptanetriol of the iso-FC_1 and HO-FC_1 fumonisin backbones, respectively. By means of retrosynthetic analysis a synthetic pathway was designed which gives access to a number of stereoisomers for the C(1)–C(7) model compounds of both iso-FC_1 and HO-FC_1, with only minor modifications of selected steps and starting material. The synthetic procedure makes use of stereoselective reactions, carbon-carbon bond forming reactions, as well as protective group strategies.

The ^{13}C NMR spectroscopy data obtained for the model compounds was then compared to the data reported in the literature for iso-FC_1 and HO-FC_1, in order to determine the absolute configuration of the stereogenic centres.

LIST OF SELECTED ABREVIATIONS

Bn	-	Benzyl
Bz	-	Benzoyl
Cbz	-	Benzyloxycarbonyl
COSY	-	Correlation spectroscopy
DCM	-	Dichloromethane
DEAD	-	Diethyl azodicarboxylate
DEPT	-	Distortionless enhancement by polarisation transfer
DIAD	-	Diisopropyl azodicarboxylate
DIPT	-	Diisopropyl tartrate
DMAP	-	4-Dimethylaminopyridine
DMF	-	*N,N*-Dimethylformamide
DMP	-	Dess-Martin periodinane
DMSO	-	Dimethyl sulfoxide
d.r.	-	Diastereomeric ratio
e.e.	-	Enantiomeric excess
ELEM	-	Equine leukoencephalomalacia
HETCOR	-	Heteronuclear correlation spectroscopy
HPLC	-	High performance liquid chromatography
HSQC	-	Heteronuclear single-quantum correlation spectroscopy
IR	-	Infrared
MCPBA	-	*m*-Chloroperoxybenzoic acid
MHz	-	Megahertz
MS	-	Mass spectrometry
α-MTPAA	-	α-Methoxy-α-trifluoromethylphenylacetic acid
α-MTPACl	-	α-Methoxy-α-trifluoromethylphenylacetyl chloride
MTPPI	-	Methyltriphenylphosphonium iodide

N-Boc	-	*N-tert*-butyloxycarbonyl
NMR	-	Nuclear magnetic resonance
NOE	-	Nuclear Overhauser effects
Ph	-	Phenyl
ppm	-	Parts per million
PPO	-	Porcine pulmonary oedema
PPTS	-	Pyridinium *p*-toluenesulfonate
R_f	-	Retention factor
Salen		*N,N*-Bis(3,5-di-*t*-butylsalicylidene)-1,2-cyclohexanediamino
SPE	-	Solid-phase extraction
TBAI	-	Tetrabutylammonium iodide
TBDPS	-	*t*-Butyldiphenylsilanyl
TBDPSCl	-	*t*-Butyldiphenylsilanyl chloride
TBHP	-	*t*-Butylhydroperoxide
TBS	-	*t*-Butyldimethylsilanyl
TBSCl	-	*t*-Butyldimethylsilanyl chloride
TCA	-	Tricarballylic acid
THF	-	Tetrahydrofuran
TLC	-	Thin layer chromatography
p-TsOH	-	*p*-Toluenesulfonic acid
UV	-	Ultraviolet

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1. INTRODUCTION

1.1. General

Fungi occur throughout the world and while many are useful to humans as antibiotics, food and so forth, just as many are harmful as they are major pathogens of plants, animals and humans. Fungi have the ability to produce secondary metabolites called mycotoxins, which cause various diseases by means of ingestion, inhalation or skin contact.[1] The word mycotoxin was derived from the Greek "*mykes*" which means fungus and the Latin "*toxicum*" which means poison.[2] Many different definitions of what a mycotoxin is can be found in literature; however, the following is accepted as a useful working definition: mycotoxins are natural products that are produced by filamentous fungi that have the ability to induce a toxic response in higher vertebrates and other animals when introduced in low concentrations by natural means, while disease caused by mycotoxins is known as mycotoxicosis.[3]

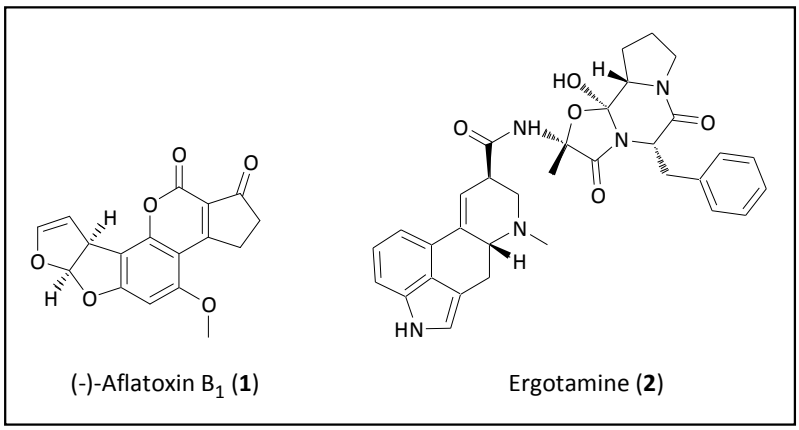

Figure 1: Structures of aflatoxin B_1 and ergotamine.

As far as recorded history goes there have been disease epidemics which have been caused by or are suspected of having been caused by exposure to mycotoxins.[1] However, the term mycotoxin only came into use in 1962 after more than 100 000 turkey poults died in the outbreak of "Turkey X Disease" near London.[4] It was eventually found that the disease was caused by the consumption of peanut meal that was contaminated with secondary

[1] Meggs, J.W. *Toxicol. Ind. Health* **2009**, *25*, 571.
[2] Goldblatt, L.A. *Clin. Toxicol*. **1972**, *5*, 453.
[3] Bennett, J.W. *Mycopathologia* **1987**, *100*, 3.
[4] Bennett, J.W.; Klich, M. *Clin. Microbiol. Rev.* **2003**, *16*, 497.

metabolites from *Aspergillus flavus*, called aflatoxins.[4] The aflatoxins have since been demonstrated to be acutely toxic and carcinogenic to many animal species,[5] including humans.[6] Aflatoxin B_1 (**1**) shown in **Figure 1** has been the focus of much research and is one of the most potent carcinogens known.[5,7] Since the discovery of the aflatoxins, research into mycotoxins has gained interest and resulted in the discovery of numerous new mycotoxins. Although this outbreak led to the first formal use of the word "mycotoxin", this is not the first reference to a mycotoxin that can be found in literature. It is believed that the earliest recorded reference can be found in an Assyrian tablet which is dated 600 BC, that describes a "noxious pustule in the ear of grain".[4,8] It is thought that this is a reference to the sclerotia of the fungus *Claviceps spp*, commonly known as ergot; however, this is not a certainty. The first unquestionable reference to ergot was published by Adam Lonitzer in 1582 in his *Kreuterbuch.*[9]

It has since been found that ergot produces a number of toxic alkaloids, such as ergotamine (**2**), which when consumed results in ergotism which was also known as "St. Anthony's Fire" in past times.[1] Ergotism takes on two main forms - gangrenous and convulsive. The gangrenous form affects the blood circulatory system leading to mortification of the limbs, while the convulsive form affects the central nervous system leading to spasms, convulsions and other nervous symptoms.[9,10] As a result of attempts to synthesise ergot alkaloid derivatives in the 20th century, the semisynthetic hallucinogenic lysergic acid diethylamide (LSD) was discovered.[4]

Other major mycotoxins which have received attention include citrinin (**3**), ochratoxin, zearalenone (**5**) and the trichothecenes, the structures of which are shown in **Figure 2**. Citrinin (**3**) is less well studied and has been isolated from several species of *Penicillium* and *Aspergillus*.[5] It has been associated with yellow rice disease in plants[11] and behaves as a

[5] Ciegler, A.; Bennett, J.W. *BioScience* **1980**, *30*, 512.
[6] Peers, F.G.; Linsell, C.A. *Br. J. Cancer* **1973**, *27*, 473.
[7] Hsu, I.C.; Metcalf, R.A.; Sun, T.; Welsh, J.A.; Wang, N.J.; Harris, C.C. *Nature* **1991**, *350*, 377.
[8] Hofmann, A. Ergot - A rich source of pharmacologically active substances. In *Plants in the development of modern medicine*. Swain, T (ed.). Harvard University Press, Cambridge, p. 236.
[9] Ainsworth, G.C. Introduction to the history of mycology. Cambridge University Press, Cambridge, **1976**, p. 186.
[10] Bennett, J.W.; Bentley, R. *Perspect. Biol. Med.* **1999**, *42*, 333.
[11] Saito, M.; Enomoto, M; Tatsuno, T. Yellowed rice toxins: luteroskyrin and related compounds, chlorine-containing compounds and citrinin. In Ciegler, A.; Kadis, S. *Microbial toxins. Vol. VI: Fungal toxins.* Ajl, S.J. (ed.). Academic Press, New York, **1971**, pp. 299-380.

nephrotoxin in animals.[12] It has also been found to act additively with ochratoxin A (**4**) to suppress RNA synthesis in mice kidneys.[13] Ochratoxin is also a metabolite of species of *Aspergillus* and has the potential to be as important as aflatoxins4 as it has been found to be a potent nephrotoxin as well as being a liver toxin, immunosuppressant, teratogen and carcinogen.[14,15]

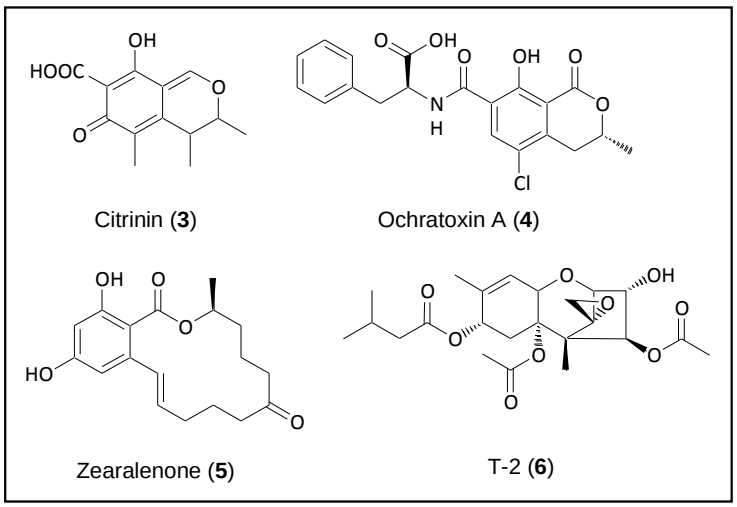

Figure 2: Structures of citrinin, ochratoxin A, zearalenone and the trichothecene T-2 toxins.

Although they are not as toxic as the previously mentioned mycotoxins, zearalenone (**5**) or T-2 toxins produced by species of *Fusarium* have the ability to bind to the estrogen receptors in mammalian cells and results in hyperestrogenic syndrome in swine, which is of agricultural importance.[5,16] The class of toxins known as the trichothecenes are produced by species of *Fusarium, Myrothecium, Phomopsis, Stachybotrys, Trichoderma, Trichothecium* to mention a few and comprises a group of more than 60 sesquiterpenoid metabolites[4] with T-2 toxin (**6**) the most well known. They contain a 12,13-epoxytrichothene skeleton, an olefinic bond and various side-chain substituents.[4] They are inhibitors of eukaryotic protein synthesis resulting in gastrointestinal, dermatological and neurological symptoms in humans and animals.[17] It has been proposed that trichothecenes are responsible for alimentary toxic aleukia in humans[4]

[12] Carlton, W.W.; Tuite, J. Metabolites of *P. viridicatum* toxicology in Rodricks, J.V.; Hesseltine, C.W. *Mycotoxins in human and animal health.* Mehlman, M.A. (ed.). Pathotox Publications Inc., Park Forest South, Ill., **1977**, pp. 525-555.
[13] Sansing, G.A.; Lillehoj, E.B.; Detroy, R.W.; Miller, M.A. *Toxicon*. **1976**, *14*, 213.
[14] Beardall, J.M.; Miller, J.D. Disease in humans with mycotoxins as possible causes. In Miller, J.D. *Mycotoxins in grains. Compounds other than aflatoxin.* Trenholm, H.L. (ed.). Eagan Press, St Paul, Minn, **1994**, pp. 487-539.
[15] Kuiper-Goodman, T.; Scott, P.M. *Biomed. Environ. Sci.* **1989**, *2*, 179.
[16] Kuiper-Goodman, T.; Scott, P.M.; Watanabe, H. *Regul. Toxicol. Pharmacol.* **1987**, *7*, 253.
[17] Trenholm, H.I.; Friend, D.; Hamilton, R.M.G.; Prelusky, D.B.; Foster, B.D. Lethal toxicity and nonspecific effects. In Trichothecene mycotoxicosis pathophysiologic effects, vol. 1. Beasley, V.L. (ed.). CRC Press, Boca Raton, Fla. **1989**, pp. 107-142.

and that the presence of *Stachybotrys* in damp buildings and materials with a high cellulose content has been associated with pulmonary bleeding in infants.[18] This has led to numerous law suits against various parties involved in the housing industry as a result of what is termed "sick building syndrome"; however, the relationship between cause and effect has been difficult to establish.[19]

However, it is not the *Fusarium* genus' ability to produce zearalenone (**5**) and trichothecenes that drew attention to this group of species, but their ability to produce the fumonisin mycotoxins. Fumonisins were first isolated from cultures of *Fusarium verticillioides* (formerly *Fusarium moniliforme*) strain MRC 826 in 1988 in South Africa.[20] It was subsequently shown to cause equine leukoencephalomalacia (ELEM)[20] and to be hepatocarcinogenic in rats.[21] At that stage it was known that *Fusarium verticillioides* was the fungus responsible for the disease pathologies but the specific causative agent was unknown. As a result, efforts were made to isolate and characterise the mutagen, leading to the isolation of fusarin C as a potential candidate. However, fusarin C was found not to be carcinogenic to rats.[22] The search continued and after further separation, assays and characterisation, the fumonisin class of mycotoxins, which reproduced the disease pathologies, were isolated from cultures of *F. verticillioides* in 1988.[23]

Details regarding the fumonisin mycotoxins are discussed in the following sections.

1.2. The Fumonisins

Fusarium verticillioides is one of the most commonly found fungi associated with the contamination of maize (*Zea mays*) throughout the world.[24] Maize is an important staple food for humans and a source of feed for livestock. Not only have *Fusarium spp.* been detected on maize, but they have also been found on rice, sorghum, wheat bran, soybean meal and in poultry feed.[24] The long term consumption of maize contaminated with *F. verticillioides* has been shown to result in various disease pathologies such as ELEM[20] and porcine pulmonary

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[19] Fung, F.; Clark, R.; Williams, S. *J. Toxicol. Clin. Toxicol.* **1998**, *36*, 79.
[20] Marasas, W.F.O.; Kellerman, T.S.; Gelderblom, W.C.A.; Coetzer J.A.; Thiel, P.G.; van der Lugt, J.J. *Onderstepoort J. Vet. Res.* **1988**, *55*, 197.
[21] Marasas, W.F.O.; Kriek, N.P.J.; Fincham, J.E.; van Rensburg, S.J. *Int. J. Cancer* **1984**, *34*, 383.
[22] Gelderblom, W.C.A.; Thiel, P.G.; Jaskiewicz, K.; Marasas, W.F.O. *Carcinogenesis* **1986**, *7*, 1899.
[23] Gelderblom, W.C.A.; Jaskiewicz, K.; Marasas, W.F.O.; Thiel, P.G.; Horak, R.M.; Vleggaar, R.; Kriek, N.P. *Appl. Environ. Microbiol.* **1988**, *54*, 1806.
[24] Stockmann-Juvala, H.; Savolainen, K. *Hum. Exp. Toxicol*. **2008**, *27*, 799.

oedema (PPO)[25] as well as being hepatotoxic to rats.[26] It has also been associated with a high rate of oesophageal cancer in humans in the Transkei area of South Africa[27] and regions of China.[28] Although a statistically strong correlation exists between the occurrence of *F. verticillioides* in maize and oesophageal cancer rates, the ability of the fungus to cause oesophageal cancer has not been proven experimentally.[27] Due to the implications of *F. verticillioides* to society in terms of maize and livestock farmers, as well as governmental and food regulatory bodies, it was of paramount importance that the fungus was studied further and that attempts were made to isolate and characterise the mycotoxin responsible for the various disease pathologies.

1.2.1. Discovery and Isolation

During 1970 a field outbreak of ELEM occurred in South Africa which was characterised by liquefactive necrosis in the white matter of equine brains. *F. verticillioides* was the principal fungus isolated from the mouldy maize responsible for the outbreak.[29] The ability of the fungus to cause ELEM was confirmed and described in detail in 1972.[29] Then in 1981 it was found that the fungus had the ability to cause PPO and was hepatotoxic and cardiotoxic in rats,[30] followed by the discovery that the fungus was hepatocarcinogenic to rats in 1984.[21] By this stage the mycotoxin responsible had still not been identified. Finally in 1988 a previously unidentified class of compounds was isolated after extensive extraction of cultures of *F. verticillioides* with aqueous methanol. The extract showed activity in rat liver histopathological and cancer initiation/promotion bioassays.[31]

This was the first isolation and purification of fumonisin B_1 (FB_1) (**7**) and B_2 (FB_2) (**8**), but dealt primarily with the identification of the cancer-promoting compounds produced by *F. verticillioides*. The techniques used by Gelderblom *et al.*[23] resulted in the purification of FB_1 and FB_2 with purity in excess of 90% by HPLC analysis. Due to little being known about the biological effects of the fumonisins it was necessary to develop more cost-effective and

[25] Harrison, L.R.; Colvin, B.M.; Greene, J.T.; Newman, L.E.; Cole, J.R. Jr. *J. Vet. Diagn. Invest.* **1990**, *2*, 217.
[26] Voss, K.A.; Norred, W.P.; Plattner, R.A.; Bacon, C.W. *Food Chem. Toxic.* **1989**, *27*, 89.
[27] Marasas, W.F.O.; Jaskiewicz, K.; Venter, F.S.; Van Schalkwyk, D.J. *S. Afr. Med. J.* **1988**, *74*, 110.
[28] Yang, C.S. *Cancer Res*. **1980**, *40*, 2633.
[29] Kellerman, T.S.; Marasas, W.F.O.; Pienaar, J.G.; Naude, T.W. *Onderstepoort J. Vet.Res.* **1972**, *39*, 205.
[30] Kriek, N.P.J.; Kellerman, T.S.; Marasas, W.F.O. *Onderstepoort J. Vet. Res.* **1981**, *48*, 129.
[31] Bezuidenhout, S.C.; Gelderblom, W.C.A.; Gorst-Allman, C.P.; Horak, R.M.; Marasas, W.F.O.; Spiteller, G.; Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1988**, 743.

efficient methods for the purification of large quantities for evaluation.[32] The extraction and purification steps reported previously[23] were used, but with minor modifications in order to isolate FB_1 (**7**) (see **Figure 3**) as well as other structurally related compounds.[32]

The prepared culture material was extracted with EtOAc in order to remove all the lipid-soluble material followed by extraction with methanol-water (3:1), after which the combined extracts were concentrated under vacuum. The dry residue was then partitioned between methanol-water (1:3) and chloroform to remove lipid-soluble material to prevent interference during the Amberlite XAD-2 column purification. An Amberlite XAD-2 column was equilibrated with methanol-water (1:3) and the aqueous phase was applied to the column. Gradient elution using various ratios of methanol-water and finally methanol was used in order to obtain the fumonisin mycotoxins. Separation of the individual fumonisins was accomplished using silica gel chromatography by means of different mobile phases. Two columns were required, with the first column being used to separate pigmented matter from the fumonisins and the second column separated the various fumonisins. This was followed by a final purification step of a reversed phase C_{18} column for each fumonisin. Use of this method resulted in the isolation of fumonisins A_1 (**9**), A_2 (**10**), B_1 (**7**), B_2 (**8**), B_3 (**11**) and B_4.[32] This was followed by the isolation of fumonisin C_1 (**12**) by Branham and Plattner[33] in 1993 and fumonisin C_4 by Plattner[34] in 1995.

Fungal strain KSU 819 was found to accumulate no FB_1 and FB_2, but instead accumulated high levels of FB_3 (**11**) and FB_4 as well as FA_3 and FC_4, which provided a convenient source of these mycotoxins for study. The use of solid-phase extraction (SPE) columns for the rapid isolation and purification of FB_3 (**11**) and FB_4 has been reported by Poling and Plattner.[35,36] The culture material was first extracted using CH_3CN-H_2O (1:1) and the extract stirred with IRA-68 (a weak anion exchange resin) followed by desorption of the fumonisins with 5% acetic acid in the same solvent. The mixture was diluted with water and the desorbed fumonisins were separated using a tC_{18} SPE cartridge. The various fractions obtained were subsequently purified using an SPE cartridge consisting of a propylamine NH_2-bonded phase with 5% acetic acid in methanol and increasing ratios of CH_3CN-H_2O to obtain FB_3 (**11**) and FB_4 with 90-95% purity.

[32] Cawood, M.E.; Gelderblom, W.C.A.; Vleggaar, R.; Behrend, Y.; Thiel, P.G.; Marasas, W.F.O. *J. Agric. Food Chem.* **1991**, *39*, 1958.
[33] Branham, B.E.; Plattner, R.D. *J. Nat. Prod.* **1993**, *56*, 1630.
[34] Plattner, R.D. *Nat. Toxins* **1995**, *3*, 294.
[35] Poling, S.M.; Plattner, R.D. *J. Agric. Food Chem.* **1996**, *44*, 2792.
[36] Poling, S.M.; Plattner, R.D. *J. Agric. Food Chem.* **1999**, *47*, 2344.

With time the isolation and purification procedure has been further refined and numerous minor fungal metabolites have since been isolated and characterised from cultures of *F. verticillioides* including the C-series of fumonisins, which lack the C-1 methyl group, such as fumonisin C_1 (**12**) (see **Figure 3**),[33,37] isofumonisin C_1,[38] hydroxyfumonisin C_1,[37] fumonisin C_3[37] and fumonisin C_4.[34,37] The isolation of the *N*-acetyl derivatives of the three fumonisin C_1 mycotoxins has also been reported by Seo *et al.*[38]

Other fumonisins isolated include FAK_1, which differs from FB_1 (**7**) in that the tricarballylic acid (TCA) functionality at C-15 is replaced by a ketone and the amino group is acetylated.[39] The P-series of fumonisins containing a 3-hydroxypyridinium moiety at the C-2 position instead of the amine group and designated FP_1, FP_2 and FP_3, were isolated in 1996[40] and are analogous to FB_1 (**7**), FB_2 (**8**) and FB_3 (**11**). Some more additions to the B-series of fumonisins include FB_5 and FBK_1,[41] iso-FB_1/FB_2/FB_3,[42,43] FBK_4,[43] *epi*-FB_3/FB_4,[44] FB_6[45] as well as the partially hydrolysed and fully hydrolysed fumonisins of the A- and B-series.[36,46]

As can be seen the list of known fumonisins has grown substantially since their discovery in 1988. However, work continues in this field as for many of these compounds the absolute structure is still to be elucidated.

1.2.2. Structure Elucidation

The structures of FA_1 (**9**), FA_2 (**10**), FB_1 (**7**) and FB_2 (**8**) were elucidated in 1988 by Bezuidenhout *et al.*[31] The structure of the FA_1 tetramethyl ester was elucidated by means of liquid secondary ion mass spectrometry, NMR spectroscopy and chemical derivatisation techniques. The data obtained suggested a molecular formula of $C_{40}H_{69}NO_{16}$ for the compound, while the presence of three hydroxy groups was indicated upon derivatisation with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide and acetylation. Hydrolysis of the FA_1 tetramethyl ester under

[37] Seo, J.-A.; Kim, J.-C.; Lee, Y.-W. *J. Nat. Prod.* **1996**, *59*, 1003.
[38] Seo, J.-A.; Kim, J.-C.; Lee, Y.-W. *J. Nat. Prod.* **1999**, *62*, 355.
[39] Musser, S.M.; Eppley, R.M.; Mazzola, E.P.; Hadden, C.E.; Shockcor, J.P.; Crouch, R.C.; Martin, G.E. *J. Nat. Prod.* **1995**, *58*, 1392.
[40] Musser, S.M.; Gay, M.L.; Mazzola, E.P.; Plattner, R.D. *J. Nat. Prod.* **1996**, *59*, 970.
[41] Musser, S.M.; Plattner, R.D. *J. Agric. Food Chem.* **1997**, *45*, 1169.
[42] MacKenzie, S.E.; Savard, M.E.; Blackwell, B.A.; Miller, J.D.; ApSimon, J.W. *J. Nat. Prod.* **1998**, *61*, 367.
[43] Bartók, T.; Szécsi, Á.;Szekeres, A.; Mesterházy, Á.; Bartók, M. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2447.
[44] Gelderblom, W.C.A.; Sewram, V.; Shephard, G.S.; Snijman, P.W.; Tenza, K.; Van der Westhuizen, L.; Vleggaar, R. *J. Agric. Food Chem.* **2007**, *55*, 4388.
[45] Månsson, M.; Klejnstrup, M.L.; Phipps, R.K.; Nielson, K.F.; Frisvad, J.C.; Gotfredsen, C.H.; Larsen, T.O. *J. Agric. Food Chem.* **2010**, *58*, 949.
[46] Sydenham, E.W.; Thiel, P.G.; Shephard, G.S.; Koch, K.R.; Hutton, T. *J. Agric. Food Chem.* **1995**, *43*, 2400.

basic conditions followed by methylation of the obtained salt gave trimethyl propane-1,2,3-tricarboxylate as the product. This was indicative of the presence of two ester linkages involving the C-14 and C-15 oxygen atoms with the terminal carboxy groups of the propane-1,2,3-tricarboxylate moiety as confirmed by NMR spectroscopic data. The location of the methyl groups was determined using ^{1}H-^{13}C NMR correlation experiments by means of long-range couplings. The final key was the fragmentation pattern in the electron impact mass spectrum which showed the linkage between the various fragments and led to the deduction of **9** in **Figure 3** as the structure for FA_1.

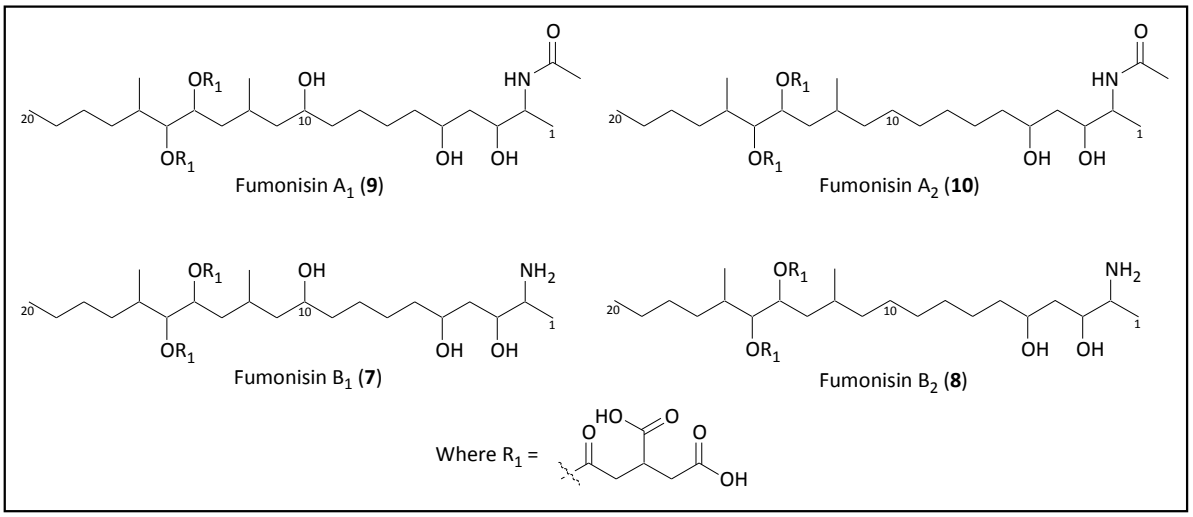


Figure 3: The 2D structures of the first fumonisins isolated by Bezuidenhout *et al.*[31]

The structure of FA_2 tetramethyl ester was determined by comparison of the electron impact mass spectral fragmentation patterns with those of FA_1, and found to have a molecular formula of $C_{40}H_{69}NO_{15}$. The experiments demonstrated that it is the C-10 hydroxy group that is absent, resulting in **10** as the structure for FA_2. A molecular formula of $C_{34}H_{59}NO_{15}$ was obtained for FB_1 (**7**) while that obtained for FB_2 (**8**) was $C_{34}H_{59}NO_{14}$. Comparison of the ^{13}C NMR spectral data indicated that a similar pattern was followed to that of FA_1 (**9**) and FA_2 (**10**), in that it was again the C-10 hydroxy group of FB_1 that was absent in FB_2. The absence of the *N*-acetyl group in the B-class has also been demonstrated by the absence of ^{1}H and ^{13}C resonances in the NMR spectra. This led to the deduction of **7** and **8** as the structures for FB_1 and FB_2, respectively.[31]

The 2D structure of FB_3 was similarly determined in 1992 by Plattner *et al.*[47] by comparison of ^{13}C NMR spectral data obtained with that of FB_2. They found that the hydroxy group on C-5

[47] Plattner, R.D.; Weisleder, D.; Shackelford, D.D.; Petersen, R.; Powell, R.G. *Mycopathologia* **1992**, *117*, 23.

was lacking and was instead located on C-10 to give **11** as the structure for FB_3. This was followed by the 2D structure **12** for FC_1 in 1993 by Branham and Plattner.[33]

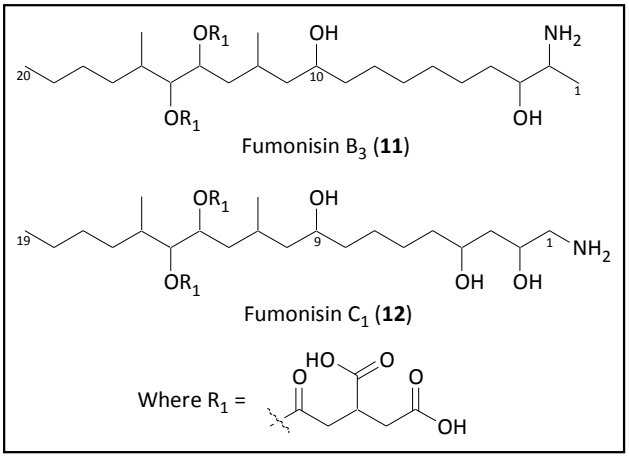


Figure 4: 2D structure of FB_3 obtained by Plattner *et al.*[47] and FC_1 obtained by Branham and Plattner [33]

Although it was known that the propane-1,2,3-tricarboxylate moieties were linked to the C-14 and C-15 oxygen atoms, the mode of linkage had not yet been ascertained. The esterification could involve either the terminal or the central carboxyl group of the tricarballylic acid fragment. The mode of linkage was investigated by Boer and it was determined that the linkage to the fumonisin backbone was via one of the terminal carboxyl groups of the tricarballylic acid fragment.[48]

The strategy used by Boer involved the selective reduction of the esters to alcohols in the presence of the carboxyl groups in order to obtain the hyroxydicarboxylate species, which could spontaneously undergo lactone formation. If the central carboxyl group of the tricarballylic acid was involved in ester formation, then reduction would result in the formation of an achiral compound, which could undergo lactonisation to form 2-oxo-tetrahydrofuran-4-acetic acid. However, if the terminal carboxyl group was involved then reduction would result in a chiral compound which could undergo lactonisation to form either the five-membered lactone, 2-oxo-tetrahydrofuran-3-acetic acid or the six-membered lactone, 2-oxo-tetrahydrofuran-4-carboxylic acid. It was expected that formation of the five-membered ring would be thermodynamically favoured over the six-membered ring.

The reduction was accomplished using sodium borohydride in *t*-BuOH-MeOH, and lactone

[48] Boer, A. Stereochemical studies on the fumonisins, metabolites of *Fusarium moniliforme*, M.Sc. Dissertation, University of Pretoria, Pretoria, May 1992.

formation did indeed occur as confirmed by IR and MS data. The NMR spectral data was then carefully examined, particularly the connectivity patterns in the HETCOR and COSY experiments. The magnitudes of the geminal and vicinal coupling constants obtained were compared to those of known compounds and this led to the conclusion that the five-membered lactone, 2-oxo-tetrahydrofuran-3-acetic acid, was the product, which proved unambiguously that the tricarballylic acid fragment was bound via a terminal carboxyl group. As a result of the terminal carboxyl group involvement in ester formation, a stereogenic centre with unknown absolute configuration at the time, is present within the tricarballylic acid moiety.

Although this meant that the 2-dimensional structures of the fumonisins had been established, the absolute configuration of the numerous stereogenic centres was yet to be elucidated. In addition to determining the linkage of the tricarballylic acid fragment, Boer also performed an investigation into the stereochemistry of the fumonisins and proposed **13** as the absolute structure for FB_1 shown in **Figure 5**.[48] This conclusion was based on the systematic determination of the absolute configuration of each of the stereogenic centres via a strategy based on the formation of conformationally rigid derivatives.

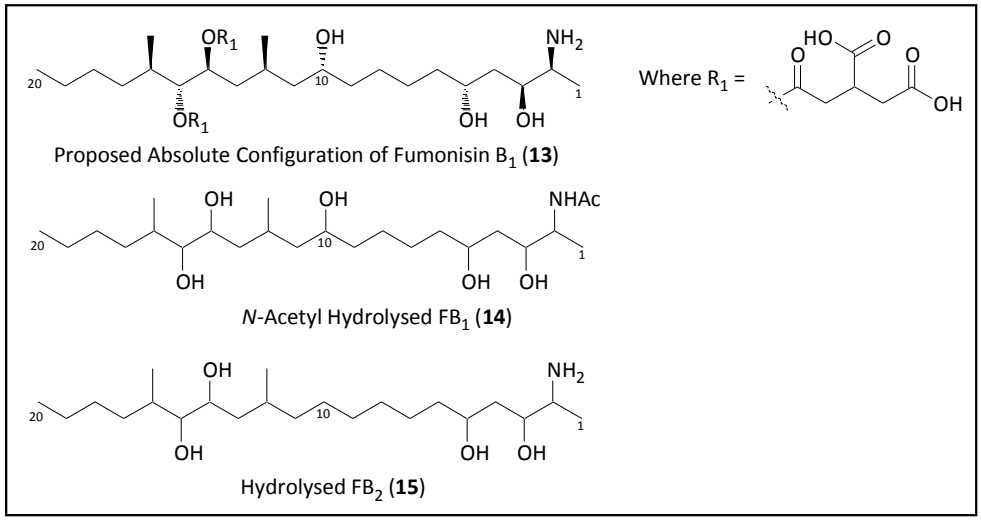


Figure 5: 3D Structure of fumonisin B_1 as proposed by Boer[48] and 2D structures of hydrolysed FB_1 and FB_2.

The oxazolidinone derivative of hydrolysed fumonisin B_2 (**15**) was prepared in order to determine the relative configuration of the C-2 and C-3 stereogenic centres. These were assigned using NMR spectral data, particularly proton-proton nuclear Overhauser effect (NOE) experiments. These experiments indicated a strong NOE between the protons of C-1 and C-3,

but not for the C-4 protons. This indicated a *trans* relationship between C-1 and C-4 in the oxazolidinone ring, and proved that the 2-amino and 3-hydroxy groups had the *syn* configuration.

In order to establish the relative configuration of the C-3 and C-5 stereogenic centres, the formylal derivative of fumonisin A_1 was prepared as this would possibly exist in a chair conformation which could be more easily analysed. Inspection of NOE experimental data indicated a strong NOE between the axial formylal proton and the protons of C-2 and C-5, whereas no NOE was observed for the equatorial formylal proton. This implied that the relative configuration between the C-3 and C-5 hydroxy groups was *anti*.

Rychnovsky[49] has demonstrated that the acetonides of *anti* and *syn* 1,3-diols can be distinguished by the ^{13}C NMR chemical shifts of the acetonide methyl groups. The ^{13}C NMR spectra of *syn* 1,3-diol acetonides exhibit the signal for the axial methyl group at δ_C 19.6 and the equatorial methyl group at δ_C 30.0. In contrast the *anti* 1,3-diol acetonides exhibit two methyl resonances at δ_C 24.7. The chemical shift of the acetal carbon is also indicative of the stereochemistry. The acetonide derivative of fumonisin B_1 was prepared and the ^{13}C NMR data analysed with signals being observed for the methyl groups at δ_C 24.57 and 26.12, which confirmed the proposed *anti* relationship obtained for the C-3 and C-5 hydroxy groups.

The absolute configuration of the stereogenic centre at C-5 was confirmed using the method of Horeau.[50] The methodology made use of the partial kinetic resolution of excess racemic α-phenylbutyric anhydride resulting in diastereomeric products which are produced at different rates. This means that preferential reaction will occur between the hydroxy group and one of the α-phenylbutyric anhydride enantiomers due to the difference in energy between the transition states. The excess anhydride is then hydrolysed, the formed acid recovered and its specific rotation determined. Horeau has shown that the sign of the specific rotation of the recovered acid can be correlated with the absolute configuration of the alcohol.

In order to determine the absolute configuration of the stereogenic centre at C-5, hydrolysed FB_2 (**15**) was taken and the hydroxy groups were protected. The oxazolidinone was prepared between the amino group on C-2 and the hydroxy group on C-3, and the 14,15-diol was protected as the 2,2-dimethyl-1,3-dioxolane. This left the hydroxy group on C-5 as the only

[49] Rychnovsky, S.D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511.
[50] Horeau, A. *Tetrahedron Lett.* **1961**, 506.

free hydroxy to react with excess racemic α-phenylbutyric anhydride to give two diastereomers in a ratio of 1:2. After hydrolysis and recovery of the unreacted α-phenylbutyric acid, the recovered acid had a specific rotation of +1.0, which corresponded to the *S* configuration for the acid and thus the *R* configuration for C-5 in the hydrolysed FB_2 (**15**). In conjunction with the previous results this led to the conclusion that the absolute configuration of the C-2–C-5 fragment was 2*S*,3*S*,5*R*.

To determine the absolute configuration of C-10 **14** was protected as the 3,5:14,15-diacetonide derivative and subjected to Horeau's method. A mixture of two diastereomers was obtained in a ratio of 5:4. Hydrolysis of the excess anhydride gave α-phenylbutyric acid with a specific rotation of +2.3, which corresponds to the *S* configuration. This meant that the configuration at C-10 must also be *R*.

The determination of the absolute configuration of the C-16 stereogenic centre made use of the oxidative cleavage of the 14,15-diol moiety by Kiliani's reagent (CrO_3/H_2SO_4), to yield 2-methylhexanoic acid, which was converted to the amide derivative using the chiral auxiliary (*S*)-α-methyl-*p*-nitrobenzylamine. The HPLC retention time for the amide derivative was correlated with those of the prepared derivatives of the enantiomers of 2-methylhexanoic acid, which led to the conclusion that the absolute configuration of C-16 was *R*.

The relative configuration of the C-14 and C-15 stereogenic centres was inferred from the magnitude of the proton-proton coupling constants for the 14,15-acetonide derivative. The coupling constant of 5.3 Hz indicated a *cis* relationship for the C-14 and C-15 hydroxy groups based on literature values.

The relative configuration for the C-15 and C-16 stereogenic centres was established once again by the magnitude of the proton-proton coupling constant (9.7 Hz) for the 14,15-acetonide derivative. On the basis of the Karplus equation [$J(\phi) = A \cos^2 \phi + B \cos \phi + C$] the two protons could have either an anti- or synperiplanar arrangement. Examination of the NOE data showed a strong NOE for the protons of C-14 and C-15 which indicated a *cis* arrangement for these groups. At the same time no NOE was observed for the C-16 protons, which was suggestive of an antiperiplanar conformation for the C-15 and C-16 groups. This led to the conclusion that the absolute configuration around these stereogenic centres must be 14*S*,15*R*,16*R*.

The absolute configuration of the C-12 stereogenic centre was based on the assumption that the C-12 and C-16 stereogenic centres were of the same enzymatic origin and thus the absolute configuration of C-12 must be *S*. The overall conclusion resulted in **13** as the proposed three-dimensional structure of FB_1.

It had been observed that both the fumonisins and AAL-toxins had the ability to inhibit sphingolipid biosynthesis and exhibited structural similarities in terms of the TCA moiety and the amino alcohol backbone as well (shown in **Figure 6**).[51] The absolute configuration of the stereogenic centres of the AAL-toxin T_A (**16**) had been derived by Boyle *et al.*[51] and it was suggested that the resemblance between the two toxins could be used to derive the absolute configuration of the stereogenic centres in the fumonisins. Boyle *et al.*[51] thus proposed **17** as the structure for FB_2 based on comparison of NMR data obtained for the AAL-toxin T_A and naturally occurring FB_2.

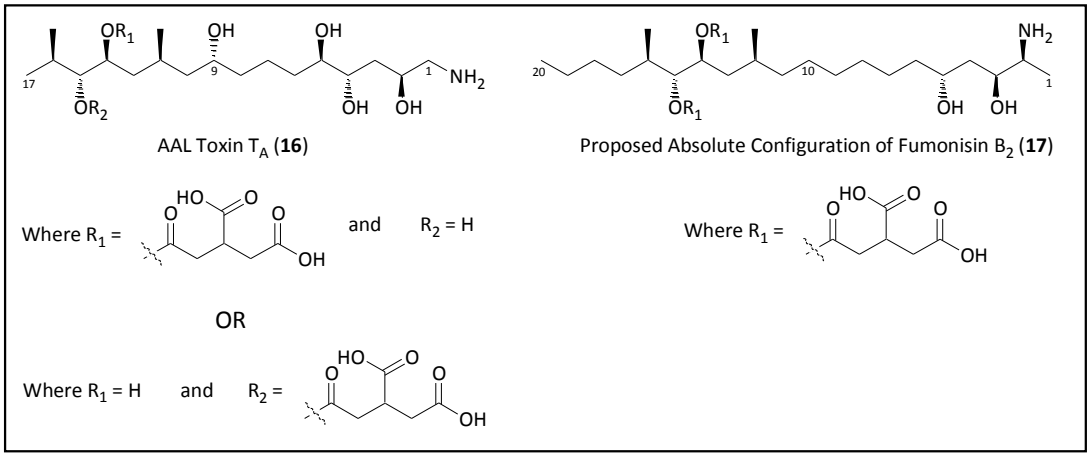


Figure 6: Similarity between the AAL toxins and fumonisins showing the proposed absolute configuration of FB_2.[51]

Harmange *et al.*[52] then proceeded to use a stepwise approach in order to elucidate the absolute configuration of FB_2 to confirm their proposed structure **17**. Their approach (shown briefly in **Figure 7**) involved the independent determination of the relative stereochemistry of the left and right hand halves followed by differentiation between the two possible diastereomers (**18** and **19**) using chiral shift reagents and finally determination of the absolute configuration of the amino alcohol by comparing (**18** and **21**), also using chiral shift reagents. The ^{1}H NMR spectroscopic data for the C-12–C-16 fragment of the *N*-acetylfumonisin B_1 methyl ester and those of AAL-toxin T_A (**16**) correlated well and confirmed that the

[51] Boyle, C.D.; Harmange, J.-C.; Kishi, Y. *J. Am. Chem. Soc.* **1994**, *116*, 4995.
[52] Harmange, J.-C.; Boyle, C.D.; Kishi, Y. *Tetrahedron Lett.* **1994**, *35*, 6819.

stereochemistry of the left hand half of the fumonisins corresponded to that of the AAL-toxins. It was also suggested that the absolute configuration of the C-3 and C-5 stereogenic centres in FB_2 would correspond to the C-2 and C-4 stereogenic centres of AAL-toxin T_A. Comparison of the ^{13}C NMR data of the C-1–C-4 fragment of the *N*-acetylfumonisin B_1 methyl ester with acetates derived from 2-aminotetradeca-5,7-dien-3-ols indicated that the relative stereochemistry of the C-2 and C-3 stereogenic centres was *syn*.

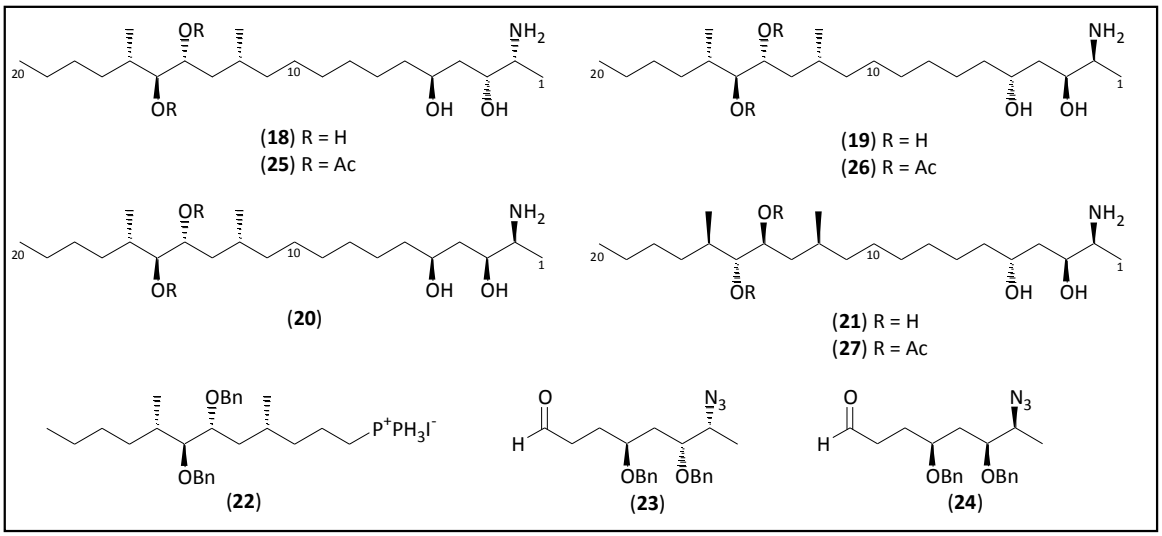


Figure 7: Structures used by Harmange *et al*.[52] to derive the absolute configuration of FB_2.

Based on the above assumptions, the backbone of FB_2 could have one of three possible configurations *viz*. **18, 19** and **20** in **Figure 7** or the enantiomers thereof. Proof for the suggested structure **17** came from the synthesis of the phosphonium salt (**22**) corresponding to the left hand half of the structure, which was synthesised from (*S*)-(–)-2-methyl-1-hexanol and (*R*)-(–)-citronellyl bromide. To obtain the right hand half, the diastereomeric azido aldehydes (**23** and **24**) bearing the C-2 and C-3 *syn* configurations were synthesised from L-glutamic acid. Wittig olefination followed by hydrogenation was then used to obtain the amino alcohol hydrochloride salts of **18** and **20,** from **23** and **24,** respectively and **19** from *ent*-**23**. ^1H NMR spectra of the hydrochloride salts were then compared to the amino alcohol hydrochloride salt of **21**, which was derived from natural FB_2 in order to determine which compounds represented the relative stereochemistry of the FB_2 backbone, resulting in the elimination of **20**.

The corresponding pentaacetates (**25** and **26**) were then synthesised in order to further differentiate between **18** and **19**. The ^1H NMR spectra were again indistinguishable from the peracetate of FB_2 (**27**), but in the presence of the chiral shift reagent $Eu(fod)_3$ differences were

observed. This established **18** as being representative of the relative stereochemistry of the FB_2 backbone.

The absolute stereochemistry was established using the same procedure as was used for the AAL-toxin T_A.[51] In the presence of (+)-Eu(hfc)$_3$ **25** and **27** behaved as two chemically different substances, while the signs of the specific rotation for **25** (+27) and **27** (–29) were opposite. This led to **21** being identified as having the same absolute configuration as that of natural FB_2, which agreed with the proposed structure (**17**). The structure obtained for FB_2 (**17**) compared well to that of FB_1 (**13**) obtained by Boer,[48] considering that FB_2 has one hydroxy group less than FB_1.

The findings reported for FB_2 were followed closely by the determination of the relative and absolute configuration of FB_1 using a different approach. The strategy used by Hoye *et al.*[53] involved chemical derivatisation and degradation studies of hydrolysed FB_1 (**28**) along with gas chromatography, NMR spectroscopy and molecular modelling in a similar manner to the approach used by Boer.[48]

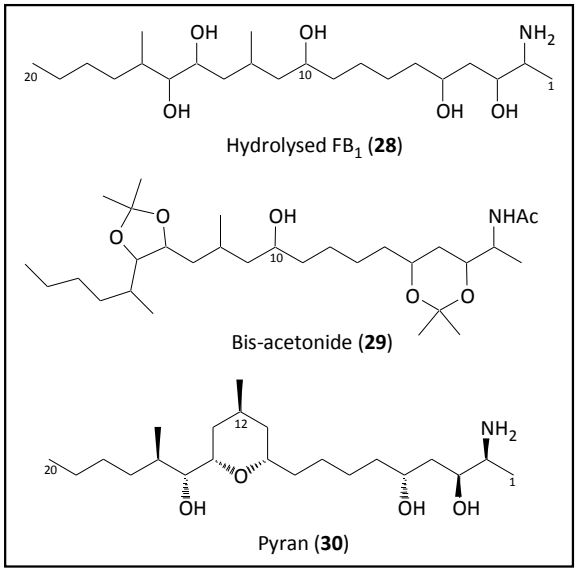


Figure 8: Structures used by Hoye *et al*.[53]

Hoye *et al.*[53] started with the determination of the absolute configuration at C-10 by subjecting **28** to peracetylation followed by penta-ester cleavage to give the *N*-acetyl derivative of **28**. Bis-acetonide (**29**) formation was used in order to obtain the dioxane

[53] Hoye, T.R.; Jiménez, J.I.; Shier, W.T. *J. Am. Chem. Soc.* **1994**, *116*, 9409.

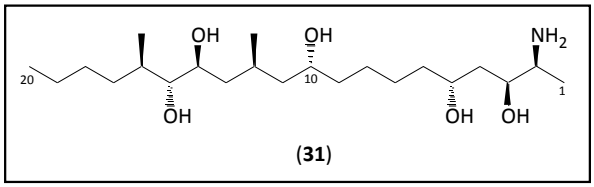
derivative involving the C-3 and C-5 hydroxy groups as well as the dioxolane derivative involving the C-14 and C-15 hydroxy groups, leaving the hydroxy group at C-10 free. Analysis of the C-10 Mosher esters established the 10*R* absolute configuration on the basis of the Δδ (where $\Delta\delta = \delta_S - \delta_R$) values obtained for the various protons in the ^{1}H NMR spectra for the diastereomeric esters: positive for the C-9 and C-5 protons and negative for the C-11, C-12, C-21 and C-22 protons.[53]

The peracetate derivative of **28** was used to determine the C-3 and C-5 relative configuration. The relationship was assigned as *anti* based on the chemical shifts of the diastereotopic protons H-4a (δ_H 1.75) and H-4b (δ_H 1.72) in the ^{1}H NMR spectra. Further validation of the *anti* relationship was obtained by means of Rychnovsky analysis[49] applied to the bis-acetonide (**29**). It was found that the acetal carbon of the dioxane ring resonated at δ_C 100.4 in the ^{13}C NMR spectrum, which was indicative of an *anti* relationship for the C-3 and C-5 stereogenic centres.[49,53]

The C-14 and C-15 relative configuration was assigned as *anti* based on the 5.0 Hz proton coupling constant for the C-14 and C-15 protons of the bis-acetonide (**29**). Monte Carlo conformational analysis via computational chemistry was then applied to the *cis*- and *trans*-disubstituted condensed model compounds followed by Boltzmann distribution to obtain a weighted average of the coupling constants. This result too was indicative of a 1,2-*anti* relationship for C-14 and C-15. The relative configuration between the C-2 and C-3 stereogenic centres was then assigned as *syn* using a similar strategy. These findings correlate well with those of Harmange *et al.*[52] published previously.

Comparison of the *in situ* GC analysis of the products formed in the $NaIO_4$ cleavage reaction of **28** with racemic and enantiomerically enriched 2-methylhexanal identified 2*R*-methylhexanal in the reaction mixture and confirmed the 16*R* absolute configuration. A different derivatisation procedure was applied to determine the absolute configuration of the C-2 stereogenic centre bearing the amino group. The amino group of **28** was selectively protected with CbzCl, followed by bis-acetonide formation and then hydrogenolysis to remove the Cbz. The Mosher amide esters were synthesised and the ^{1}H NMR Δδ values analysed using a similar procedure to that used to obtain the configuration of C-10. This resulted in the assignment of 2*S* absolute configuration.

All that remained was to determine the absolute configurations of the C-12, C-14 and C-15 stereogenic centres. This was done by taking the bis-acetonide (**28**) and transforming it into the C-10 mesylate followed by removal of the acetonides and formation of the pyran (**30**) with inversion of configuration at C-10. The three remaining free hydroxy groups were converted to the tris-(*S*) and tris-(*R*) Mosher esters. Analysis of the ^{1}H coupling constants indicated that the substituents on C-10 and C-14 had a *cis* orientation and that the protons on C-10 and C-14 were axially orientated, while the proton on C-12 was orientated equatorially. This meant that the absolute configuration of both C-12 and C-14 were *S*, while the absolute configuration of C-15 was arbitrarily assigned as *R* based on analysis of the tris-MTPA esters. These absolute configurations were confirmed by conformational analysis and calculated coupling constants for the possible diastereomers of the model pyrans, resulting in structure **31** (shown in **Figure 9**) as the backbone for hydrolysed FB_1.



(**31**)

Figure 9: The absolute configuration of the FB_1 backbone as determined by Hoye *et al.*[53]

Further confirmation of the relative configuration of the C-1–C-5 fragment of the fumonisins came from two separate studies using different strategies, but published simultaneously. ApSimon *et al.*[54] converted FB_1 tetramethyl ester to the 2,3-carbamate derivative using triphosgene followed by analysis of the proton coupling constants in the ^{1}H NMR spectrum. This indicated an *anti* relationship for the C-2 amino and C-3 hydroxy groups in FB_1. In a separate series of reactions the free C-2 amino group of FB_1 tetramethyl ester was converted into the *N*-*p*-bromobenzoyl derivative followed by reaction using phosgene to form the 3,5-carbonate. The proton coupling constants of the carbonate derivative were compared to those of model compounds and it was found that the C-3 and C-5 hydroxy groups were *anti* with respect to each other.[54]

In a parallel study Poch *et al.*[55] made use of a strategy similar to that applied by Boer,[48] in that hydrolysed FB_1 (**28**) was converted into the corresponding 2,3-oxazoline without inversion of configuration. The NMR spectroscopic data obtained for the 2,3-oxazoline was compared to

[54] ApSimon, J.W.; Blackwell, B.A.; Edwards, O.E.; Fruchier, A. *Tetrahedron Lett*. **1994**, *35*, 7703.
[55] Poch, G.K.; Powell, R.G.; Plattner, R.D.; Weisleder, D. *Tetrahedron Lett.* **1994**, *35*, 7707.

NMR spectroscopic data obtained for model compounds and to various literature values, resulting in the C-2, C-3 relative configuration being assigned as *anti*. This was in agreement with the results obtained by ApSimon *et al.*[54] and Boer.[48]

The absolute configuration of the stereogenic centres for the C-1–C-5 fragment was also studied by Hartl and Humpf.[56] using the circular dichroism (CD) exciton chirality method. The method studies the through-space interactions between two or more chromophores and results in bisignate CD curves. The absolute sense of the twist between the coupled chromophore transition dipole moments, which is dependent on the spatial arrangements of the functional groups nonempirically defines the signs of the CD curve. The amplitude of the resulting CD curves is then approximated by means of summation of the interacting basis pairs due to the additivity relation exhibited.[56]

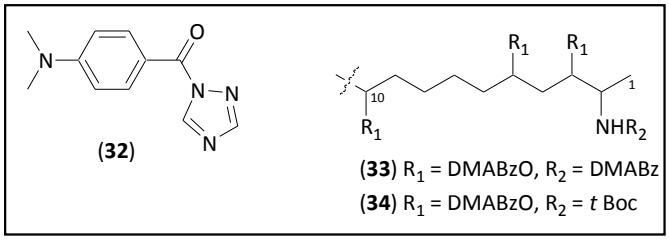


Figure 10: Structures used by Hartl and Humpf.[56]

The strategy used by Hartl and Humpf[56] to study FB_1 and FB_3 involved methylation of the carboxyl groups of the TCA side-chains in order to prevent interference during formation of the chromophore derivatives. The formed tetramethyl ester was subjected to derivatisation with *p*-dimethylaminobenzoyltriazole (**32**) to obtain the *p*-dimethylaminobenzoate chromophore derivative (**33**). In addition the C-2 *N*-Boc tetramethyl ester was also treated with *p*-dimethylaminobenzoyltriazole to give **34**. The *p*-dimethylaminobenzoate derivatives (**33** and **34**) obtained were purified and used in UV and CD studies. The negative twist sense between the chromophore substituents of C-3 and C-5 allowed the assignment of the absolute configuration of C-3 and C-5 as *S* and *R*, respectively. The positive twist sense for the C-2 and C-3 chromophore substituents indicated a *syn* relationship and thus the *S* absolute configuration for each of C-2 and C-3 in both FB_1 and FB_3.[56]

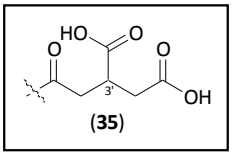
The relative and absolute configuration of the C-1–C-5 stereogenic centres was thus unambiguously established and all that remained was to establish the relative and absolute

[56] Hartl, M.; Humpf, H.-U. *Tetrahedron: Asymmetry* **1998**, *9*, 1549.

configuration of the C-10–C-16 fragment of the fumonisins as well as the absolute configuration of the TCA stereogenic centre. This became the focus of three separate studies using different strategies.

Blackwell *et al.*[57] continued their work on the fumonisins by taking the 3,5-carbonate previously prepared,[54] in which the C-10 hydroxy group had been replaced by chlorine via a S_N2 reaction. Base hydrolysis was used to remove the C-14,C-15 side chains and the carbonate ring, leaving the *N*-*p*-bromobenzoyl group in place. At the same time the C-14 hydroxy oxygen displaced the C-10 chorine atom with inversion of configuration to obtain the *N*-*p*-bromobenzoyl triol 10,14-cyclic ether. The relative configuration of the stereogenic centres was determined by analysis of NOE data. For the *N*-*p*-bromobenzoyl triol 10,14-cyclic ether NOEs were observed between H-10, H-12 and H-15, but not between H-10 and H-14 which indicated that the H-10 and H-12 protons were *cis* whereas the H-10 and H-14 protons were *trans*. The NOE data for the C-12–C-16 fragment of FB_1 was independently examined with positive NOE's observed between H-14 and H-16 and H-12, between H-15 and H-22 as well as between one of the C-13 protons and the C-21 methyl group. This meant that the groups on C-14 and C-15 were *syn* to each other while the methyl groups on C-12 and C-16 were *anti* to the hydroxy group on C-14.[57] These findings agreed with those of Boer[48] and Harmange *et al*.[52]

This left the absolute configuration of the stereogenic centre in the TCA side chains as the only unknown structural feature. It had been suggested that the TCA moieties serve as a detoxification mechanism in the producing fungi, as the hydrolysed fumonisins exhibit more activity than the intact toxins.[58] Shier *et al.*[58] determined the absolute configuration of the C-3' stereogenic centre of the TCA fragment (**35**) for both FB_1 and AAL toxin T_A using chiral gas chromatography methodology.



(**35**)

Figure 11: 2D structure of the TCA fragment showing the C-3' stereogenic centre.

The approach used by Shier *et al.*[58] depended on the conversion of the TCA side chains into derivatives resolvable on a chiral gas chromatography column. The *N*-acetyl derivative of FB_1

[57] Blackwell, B.A.; Edwards, O.E.; ApSimon, J.W.; Fruchier, A. *Tetrahedron Lett*. **1995**, *36*, 1973.
[58] Shier, W.T.; Abbas, H.K.; Badria, F.A. *Tetrahedron Lett.* **1995**, *36*, 1571.

was first prepared followed by selective reduction of the free carboxyl groups using diborane in order to differentiate the esterified carboxyl group from the free carboxyl groups after removal from the FB_1 backbone. Diborane was used for the reduction of the carboxyl groups in order to obtain a product which was converted to the *O*-tosylate followed by reduction with $LiAlH_4$ to obtain the TCA side-chains released as an alcohol, which were then oxidised to the corresponding carboxylic acids. The carboxylic acids were methylated using diazomethane to obtain the methyl esters, which were examined using gas chromatography on a Chiraldex GT-A column with comparison of the elution times to those of prepared standards. Shier *et al.*[58] determined the absolute configuration of the C-3' stereogenic centre of the TCA (**35**) fragment as *S* in both FB_1 and AAL toxin T_A.[58]

The absolute configuration of the C-3' stereogenic centre of the TCA (**35**) fragment of FB_1 was also determined by Boyle and Kishi.[59] However, they established the absolute configuration as *R*, a result opposite to that obtained by Shier *et al*.[58] The three-stage strategy employed by Boyle and Kishi.[59] required both enantiomers of the TCA dimethyl ester monocarboxylic acid. The key step for the first stage was the asymmetric Michael addition of a chiral allylphosphonamide to *t*-butyl sorbate using a modified Hanessian procedure followed by ozonolysis of the Michael adduct and reduction to the diol. The stereochemistry of the product obtained was verified by comparison with a compound of known absolute configuration. The diol was converted to the TCA dimethyl ester monocarboxylic acid in four steps and the optical purity determined.[59]

In the second stage natural FB_2 was esterified using diazomethane, followed by protection of the amino group using CbzCl and protection of the diol with TBSCl to obtain the bis-TCA tetramethyl ester with preservation of the stereochemistry in the TCA moiety. The TCA esters were selectively hydrolysed from the FB_2 backbone to obtain the diol. The third stage consisted of the re-esterification of the diol prepared in the second stage with the TCA dimethyl ester monocarboxylic acids prepared in the first stage, followed by comparison of their ^1H NMR spectra. This established the 3'*R* absolute configuration for the stereogenic centre of the TCA moiety (**35**). Boyle and Kishi.[60] then proceeded to demonstrate that the absolute configuration of the C-3' stereogenic centre of the TCA moiety (**35**) was *R* in both FB_1 and AAL toxin T_A, using the same methodology applied previously.[59] The results obtained were thus opposite to those obtained by Shier *et al*.[58]

[59] Boyle, C.D.; Kishi, Y. *Tetrahedron Lett.* **1995**, *36*, 4579.
[60] Boyle, C.D.; Kishi, Y. *Tetrahedron Lett.* **1995**, *36*, 5695.

In 1999 the absolute configuration of the C-3' stereogenic centre of the TCA portion was confirmed as *R* by Edwards *et al.*[61] in agreement with the results of Boyle and Kishi.[59,60] The approach used by Edwards *et al.*[61] closely resembled that of Shier *et al.*[58] It required the conversion of FB_1 into the *N*-acetyl triacetate bis-anhydride (**36**) using acetic anhydride followed by partial hydrolysis to the di-acid (**37**) and reduction using excess BH_3/THF to obtain the *N*-acetyl triacetyl tetraol (**38**). This was followed by complete hydrolysis under basic conditions with acidic work-up to obtain the hydroxy γ-lactone, which was subjected to benzoylation to give the benzoyloxy-lactone (**39**). An optically active standard of known absolute configuration was then prepared. Comparison of $[\alpha]_D^{25}$, MS, IR and NMR spectroscopic data confirmed that the benzoyloxy γ-lactone derived from natural FB_1 was the same as that of the prepared standard, leading to the conclusion that the absolute configuration of the C-3' stereogenic centre of the TCA portion was *R*.[61]

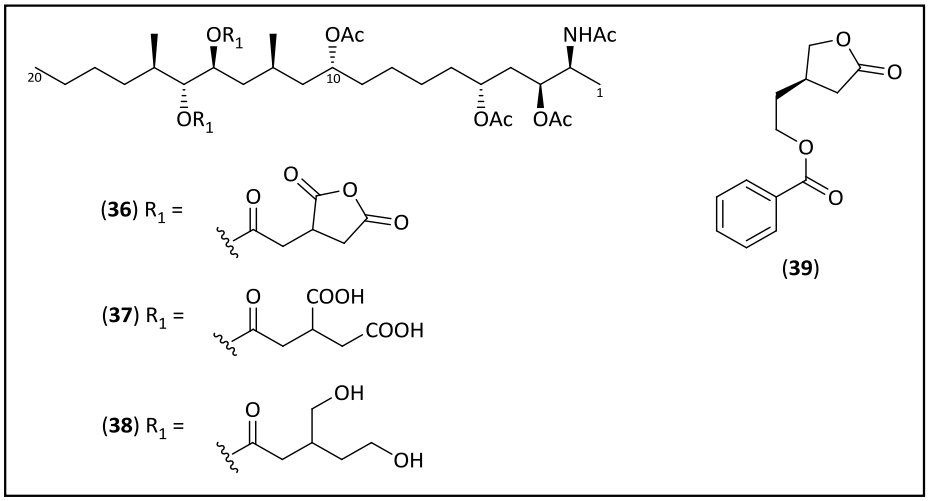


Figure 12: Structures of the compounds used by Edwards *et al.*[61] in the determination of the absolute configuration at C-3'.

These studies completed the three-dimensional structure elucidation of the most ubiquitous fumonisins. Since then numerous other analogues of FB_1 and FB_2 produced by *Fusarium spp*, *Aspergillus niger*[62] and other fungi have been identified. To date the complete set of fumonisins comprises a large number of different compounds classified into four different series, namely A, B, C and P, which are discussed below. Of the numerous different compounds only fumonisins A_1, A_2, B_1, B_2, B_3, B_4, C_1 and C_4 have been completely characterised.

[61] Edwards, O.E.; Blackwell, B.A.; Driega, A.B.; Bensimon, C.; ApSimon, J.W. *Tetrahedron Lett.* **1999**, *40*, 4515.
[62] Nielsen, K.F.; Mogensen, J.M; Johansen, M.; Larsen, T.O.; Frisvad, J.C. *Anal. Bioanal. Chem.* **2008**, *395*, 1225.

The A-series of fumonisins are characterised by a C_{20} backbone and the presence of an *N*-acetyl group on C-2. Various degrees and sites of hydroxyation are found within the series as can be seen in **Figure 13** and **Table 1**. Variability is also found at the sites of TCA esterification as some members of this class occur as monoesters of TCA, while yet other members completely lack the TCA portions. Different degrees of oxidation exist within the series, with the C-15 hydroxy group, which is normally esterified to TCA, being present as a ketone.

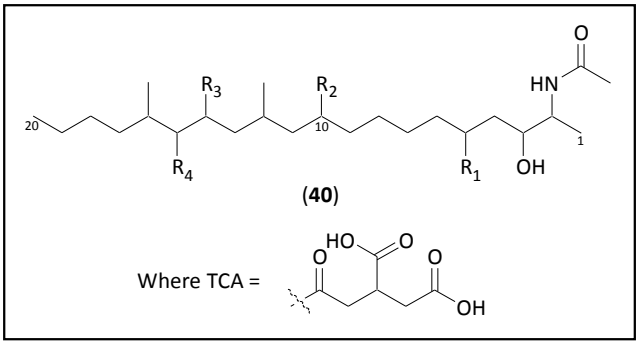


Figure 13: General structure of the A-series of the fumonisins.

Table 1: Substitution pattern of the A-series of the fumonisins.

Analogue	R_1	R_2	R_3	R_4
FA_1	OH	OH	TCA	TCA
FA_2	OH	H	TCA	TCA
FA_3	H	OH	TCA	TCA
FA_4	H	H	TCA	TCA
Iso-FA_1	H	H	TCA	TCA
FAK_1	OH	OH	TCA	=O
HFA_3	H	OH	OH	OH
$PHFA_{3a}$	H	OH	OH	TCA
$PHFA_{3b}$	H	OH	TCA	OH

The B-series of fumonisins (**Figure 14** and **Table 2**) are also characterised by a C_{20} backbone, but have a free amino group on C-2. Similar to the A-series, various degrees and sites of hydroxyation are found within the series, while variability is also observed at the sites of TCA esterification (monoesters and no TCA esterification). Different degrees of oxidation are also observed, specifically at the C-15 hydroxy group which can be oxidised to the ketone. Stereoisomers which are C-3 epimers of FB_3 and FB_4 have also been reported.[44]

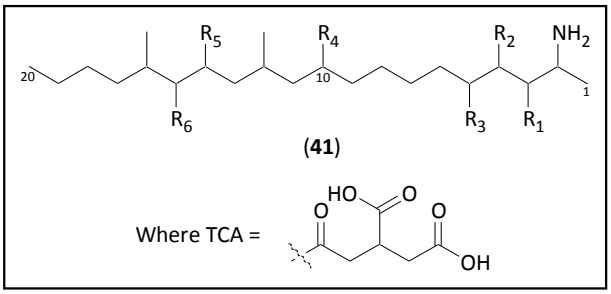


Figure 14: General structure of the B-series of fumonisins.

Table 2: Substitution pattern of the B-series of fumonisins.

Analogue	R_1	R_2	R_3	R_4	R_5	R_6
FB_1	OH	H	OH	OH	TCA	TCA
FB_2	OH	H	OH	H	TCA	TCA
FB_3	OH	H	H	OH	TCA	TCA
FB_4	OH	H	H	H	TCA	TCA
FB_5	Determined to have a hexahydroxy backbone					
FB_6	OH	OH	OH	H	TCA	TCA
Iso-FB_1	OH	OH	H	OH	TCA	TCA
HFB_1	OH	H	OH	OH	OH	OH
$PHFB_{1a}$	OH	H	OH	OH	OH	TCA
$PHFB_{1b}$	OH	H	OH	OH	TCA	OH
FBK_{1a}	OH	H	OH	OH	TCA	=O
FBK_{1b}	OH	H	OH	OH	=O	TCA
FBK_1 2TCA	=O	H	OH	OH	TCA	TCA
$PHFB_{2a}$	OH	H	H	H	OH	TCA
$PHFB_{2b}$	OH	H	H	H	TCA	OH
epiFB_3	OH	H	H	OH	TCA	TCA
epiFB_4	OH	H	H	H	TCA	TCA
FBK_4 2TCA	=O	H	OH	H	TCA	TCA

The fumonisins of the C-series with a C_{19} backbone (**Figure 15** and **Table 3**) lack the terminal methyl group (C-1) present in the A- and B-series, thereby placing the amino group terminal on C-1. Various degrees and sites of hydroxyation are also exhibited in this series as well as variability in esterification to TCA. To date, no variation in degree of oxidation has been observed for this series, however some *N*-acetyl derivatives have been reported.[38]

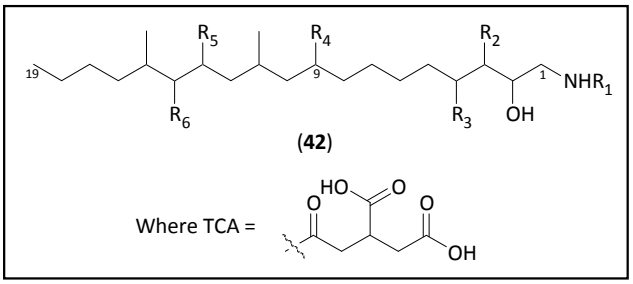


Figure 15: General structure of the C-series of fumonisins.

Table 3: Substitution pattern of the C-series of fumonisins.

Analogue	R_1	R_2	R_3	R_4	R_5	R_6
FC_1	H	H	OH	OH	TCA	TCA
FC_2	H	H	OH	H	TCA	TCA
FC_3	H	H	H	OH	TCA	TCA
FC_4	H	H	H	H	TCA	TCA
Iso-FC_1	H	OH	H	OH	TCA	TCA
HO-FC_1	H	OH	OH	OH	TCA	TCA
N-acetyl-FC_1	$COCH_3$	H	OH	OH	TCA	TCA
N-acetyl-Iso-FC_1	$COCH_3$	OH	H	OH	TCA	TCA
N-acetyl-HO-FC_1	$COCH_3$	OH	OH	OH	TCA	TCA
$PHFC_4$	H	H	H	H	OH ⟷	TCA

Fumonisins of the P-series (**Figure 16** and **Table 4**) consist of a C_{20} backbone and resemble the corresponding B-series fumonisins, but the amino group at C-2 has been replaced by a *N*-linked 3-hydroxypyridinium moiety. Only a small number of members have been identified in this series with various degrees and sites of hydroxyation. To date, no P-series fumonisins have been found that exhibit variability in TCA esterification or degree of oxidation.

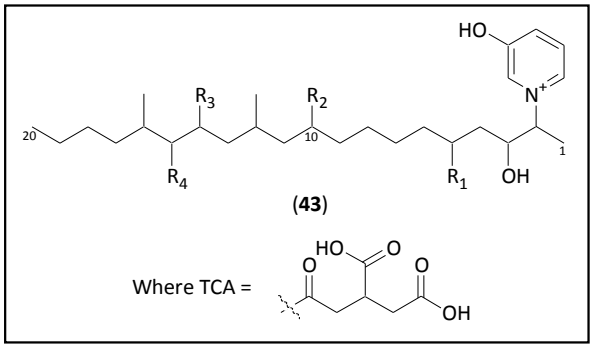


Figure 16: General structure of the P-series of fumonisins.

Table 4: Substitution pattern of the P-series of fumonisins

Analogue	R_1	R_2	R_3	R_4
FP_1	OH	OH	TCA	TCA
FP_2	OH	H	TCA	TCA
FP_3	H	OH	TCA	TCA

The above summarises all the fumonisins for which the 2-dimensional structures are currently known, while 3-dimensional structures have been determined for only a select few. However, it has been recently demonstrated that many more fumonisins possibly exist than listed above. Bartók *et al.*[43] successfully isolated thirty seven fumonisin and fumonisin-like compounds from a crude extract of a rice culture of *F. verticillioides* using reversed-phase high-performance liquid chromatography/electrospray ionisation ion trap mass spectrometry (RP-HPLC/ESI-IT-MS). The list of compounds detected included many of the previously known fumonisin isomers and many more novel compounds. The identification and tentative assignment of structures for the novel compounds was based on retention times on a C_{18} HPLC column, masses of the protonated molecules obtained in MS spectra as well as the characteristic product ions which included the backbones and neutral mass losses of the $[M+H]^+$ ion recorded in MS spectra. The results of this study indicated that TCA was not always bound at C-14 and C-15 and that TCA was not the only carboxylic acid that could be esterified to C-14 and C-15 of the fumonisin backbone. Other carboxylic acids detected included *cis*-aconitic acid, oxalylsuccinic acid and oxalylfumaric acid. In order to confirm the tentative structures assigned to the novel compounds further techniques such as NMR spectroscopy and X-ray crystallography will be required.[43]

As continuation of their work Bartók *et al.*[63] then proceeded to isolate twenty eight isomers of FB_1 from a crude extract of a rice culture of *F. verticillioides* using reversed-phase high-performance liquid chromatography/electrospray ionisation time-of-flight mass spectrometry (RP-HPLC/ESI-TOFMS) and RP-HPLC/ESI-IT-MS. Among the isomers isolated was FB_1 and iso-FB_1 and while no indication was given as to the structures of the various isomers it was proposed that TCA could also esterify other hydroxy groups other than those at C-14 and C-15, as this would explain the large range of retention times observed.[63]

[63] Bartók, T.; Tölgyesi, L.; Szekeres, A.; Varga, M.; Bartha, R.; Szécsi, Á.; Bartók, M.; Mesterházy, Á. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 35.

The number of fumonisin and fumonisin-like compounds isolated continues to increase but FB_1, FB_2 and FB_3 remain the focus of most research due to their predominant presence in rice and maize cultures. It has been found that on average FB_1 comprises 70-80%, FB_2 15-25% and FB_3 3-8% of total fumonisins.[64] Interest in the other fumonisins has grown of late as the possibility exists that although they are present in small amounts they too could be acutely toxic.

1.2.3. Synthetic Studies

The next challenge after the structure elucidation work was the synthesis of the C_{20} backbone of fumonisin B_1 (**7**) and B_2 (**8**). The fumonisins proved to be interesting targets and a notable synthetic challenge due to the presence of eight and seven stereogenic centres in FB_1 and FB_2, respectively, along with the sites of esterification and the single stereogenic centre of the TCA fragments which increased the complexity of the mycotoxin. Due to the biological implications of the fumonisins the development of a synthetic route which was both straightforward and easily modified to allow for the synthesis of all the fumonisin isomers/analogues was of paramount importance. The successful synthesis of the fumonisins would result in confirmation of the absolute stereochemistry assigned through comparison of physical and spectroscopic data as well as allow for a better understanding of the structural basis for the biological effects of the mycotoxin through investigations into structure-activity relationships, which could then lead to the design of analogues having modified properties.

The first paper published was on the enantioselective total synthesis of fumonisin B_2 (**44**) by Shi *et al*.[65] FB_2 (**44**) represented a simpler molecule to synthesise than FB_1 due to the absence of the C-10 hydroxy group. It was also recognised that FB_2 (**44**) could be divided into two distinct halves based on the concentration of stereogenic centres on the backbone: the right half consisting of C-1–C-9 and the left half consisting of C-10–C-20. It was hypothesised that each of the two different structural motifs are involved in different biological processes.

A convergent approach to the synthesis was used which divided the molecule into three easily coupled fragments: the left half (**45**), the right half (**46**) and the TCA moiety (**47**) shown in **Figure 17**. The synthesis was designed in such a way as to allow access to FB_2 and other analogues in order to explore their hypothesis.

[64] Rheeder, J.P.; Marasas, W.F.O.; Vismer, H.F. *Appl. Environ. Microbiol.* **2002**, *68*, 2101.
[65] Shi, Y.; Peng, L.F.; Kishi, Y. *J. Org. Chem.* **1997**, *62*, 5666.

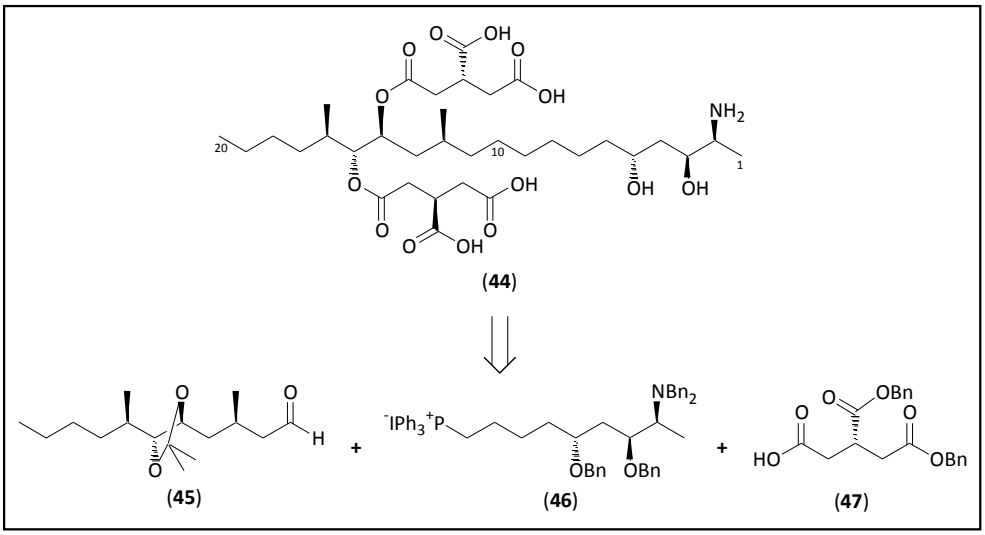
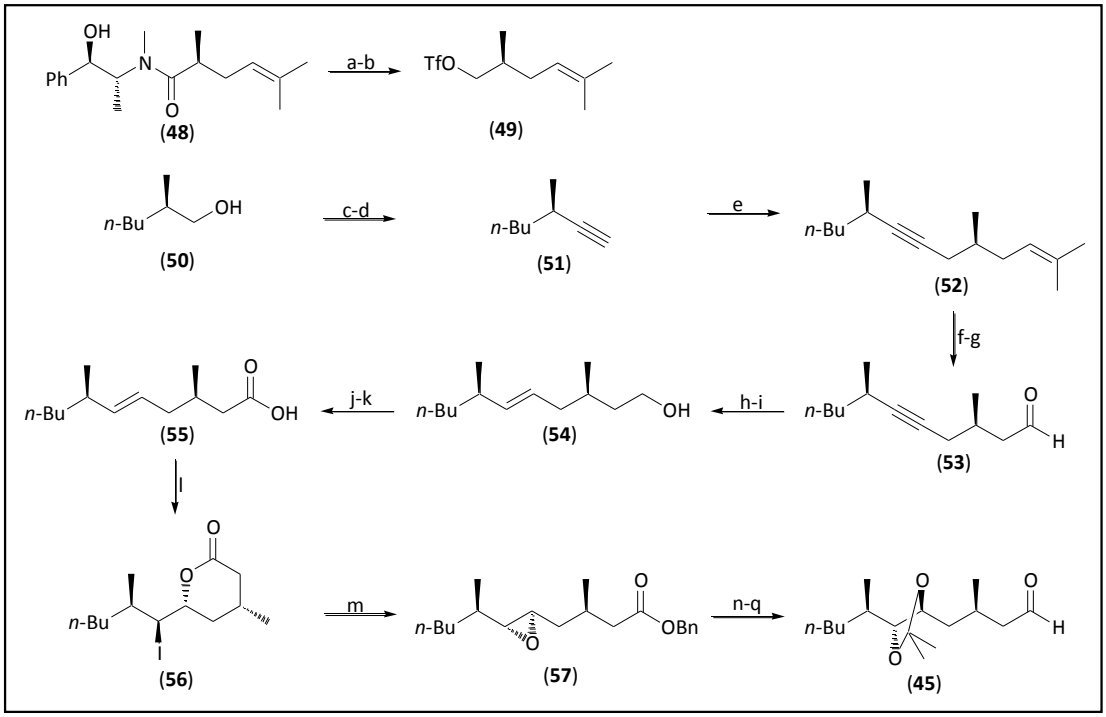


Figure 17: Retrosynthesis of FB_2 showing target fragments for convergent synthesis approach

Synthesis of the C-10–C-20 fragment (shown in **Scheme 1**) began with the asymmetric alkylation of the *N*-propionyl derivative of (*R*,*R*)-(–)-pseudoephedrine using 1-bromo-3-methylbut-2-ene to obtain **48**, which was subsequently reduced using pyrrolidine/BH_3/*n*-BuLi to obtain the alcohol which was converted to the triflate (**49**). The chiral alkyne (**51**) was prepared via the Swern oxidation of (*R*)-2-methylhexan-1-ol (**50**) followed by a Corey-Fuchs procedure to introduce the alkyne functionality. The prepared chiral alkyne (**51**) was lithiated and coupled with the triflate (**49**) to obtain the enyne (**52**) in 70% yield. **52** was subjected to site selective osmylation to obtain a mixture of diols which was subsequently cleaved using lead(IV) acetate to obtain aldehyde (**53**) as the product.

$NaBH_4$ reduction of the aldehyde (**53**) to the primary alcohol was followed by the stereoselective reduction of the alkyne using sodium in liquid ammonia to obtain the *trans*-alkene (**54**) in 92% yield. Swern oxidation followed by $NaClO_2$ oxidation converted the primary alcohol group in **54** to the corresponding acid (**55**) in 92% yield over two steps. The C-14 and C-15 vicinal hydroxy groups could then be introduced stereoselectively in three steps. Iodolactonisation of **55** under equilibrium conditions resulted in iodolactone (**56**) in 84% yield with a diastereomeric ratio of greater than 20:1. This was followed by ring opening of the lactone (**56**) using sodium benzyloxide to obtain the epoxide benzyl ester (**57**), which was subjected to hydrogenolysis to deprotect the benzyl ester with simultaneous epoxide ring opening to obtain a product that spontaneously underwent lactone formation to obtain a lactone alcohol with the required stereochemistry at C-14 and C-15. The lactone alcohol was reduced to the corresponding triol using $LiAlH_4$ followed by protection of the two vicinal

hydroxy groups as the acetonide and Swern oxidation of the primary alcohol to obtain the target aldehyde (**45**).[65]



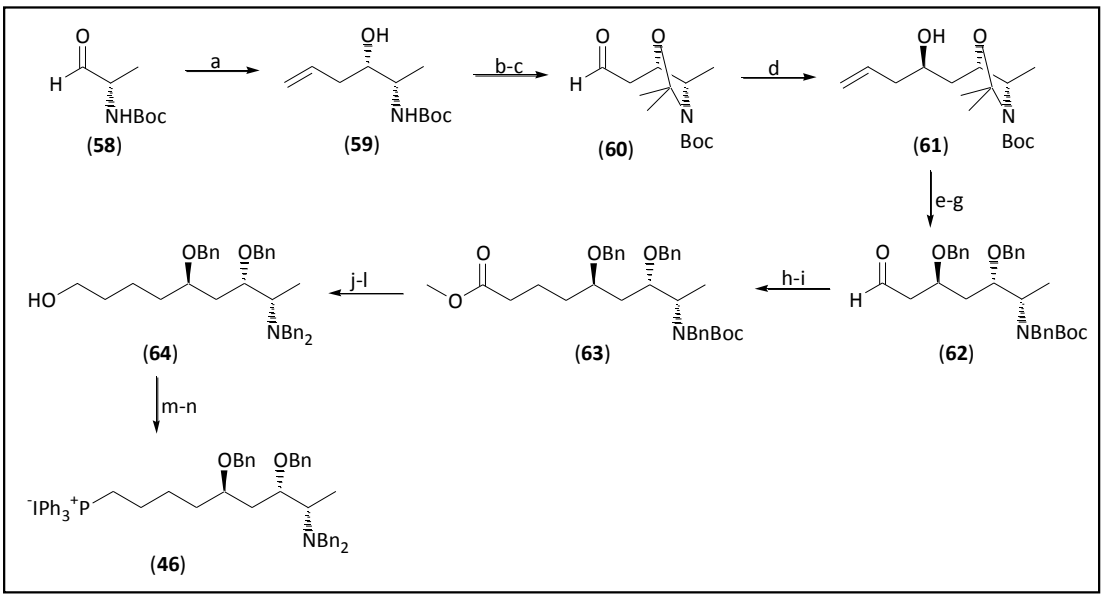
Scheme 1: Synthesis of the C-10–C-20 unit of FB_2 by Shi *et al*.[65]

Reagents : (a) Pyrrolidine, BH_3, *n*-BuLi; (b) Tf_2O; (c) $(COCl)_2$, DMSO, Et_3N; (d) CBr_4, PPh_3, *n*-BuLi, H_2O; (e) *n*-BuLi, **49**; (f) $K_2OsO_4{\cdot}2H_2O$; (g) $Pb(OAc)_4$; (h) $NaBH_4$; (i) Na/liq NH_3; (j) $(COCl)_2$, DMSO, Et_3N; (k) $NaClO_2$; (l) I_2, MeCN; (m) BnONa; (n) H_2, Pd/C, *p*-TsOH; (o) $LiAlH_4$; (p) Acetone, *p*-TsOH; (q) $(COCl)_2$, DMSO, Et_3N.

The synthesis of the right fragment (**46**) (shown in **Scheme 2**) began with the preparation of the protected α-amino aldehyde (**58**) from L-alanine,[66] with an optical purity of greater than 96% e.e. This was followed by the stereoselective allylation of the α-amino aldehyde (**58**) using Brown's chiral (–)-*B*-allyldiisopinocampheylborane[67] to obtain the *syn*-amino alcohol (**59**) in 75% yield and with a diastereoselectivity of 94%. The *syn*-amino alcohol (**59**) was then protected as the acetonide followed by ozonolysis of the alkene with a reductive work-up using dimethyl sulfide in order to obtain the aldehyde (**60**). Once again, stereoselective allylation of the aldehyde (**60**) was done, but using instead (+)-*B*-allyldiisopinocampheylborane in order to obtain the *anti*-alcohol (**61**) in 65% yield and with a diastereoselectivity of *ca.* 10:1. Deprotection of the acetonide was done using TsOH to obtain the amino alcohol followed by tribenzylation using NaH and benzyl bromide to give the protected alkene, which was

[66] Fehrentz, J.A.; Castro, B. *Synthesis* **1983**, *8*, 676.
[67] Brown, H.C.; Bhat, K.S.; Randad, R.S. *J. Org. Chem.* **1989**, *54*, 1570.

subjected to ozonolysis with reductive work-up to give the aldehyde (**62**). This was followed by a two-carbon chain extension using Horner-Wadsworth-Emmons olefination methodology to obtain the α,β-unsaturated esters in 91% yield with a *trans:cis* ratio of *ca.* 5:1 by NMR analysis. The resultant α,β-unsaturated esters were subjected to selective hydrogenolysis over Lindlar catalyst to obtain the ester (**63**) in an overall yield of 70%.



Scheme 2: Synthesis of the C-1–C-9 unit of FB_2 by Shi *et al.*[65]

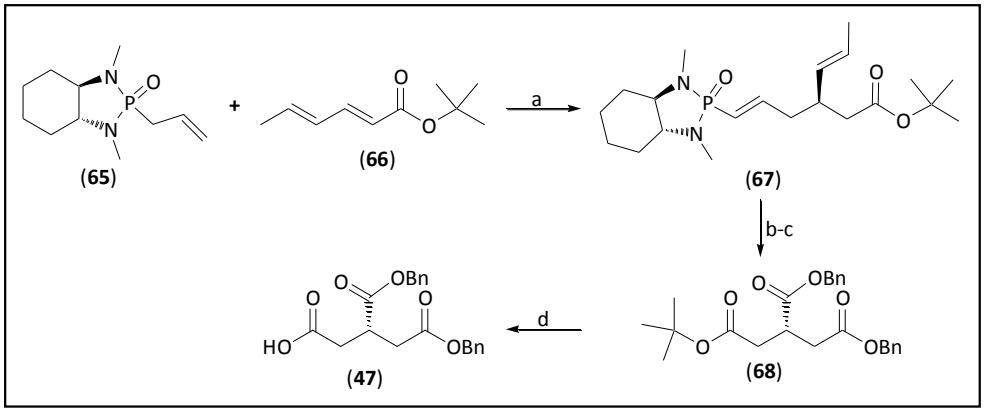
> *Reagents :* (a) (–)-Ipc_2B-allyl; (b) Acetone, *p*-TsOH; (c) O_3, Me_2S; (d) (+)-Ipc_2B-allyl; (e) *p*-TsOH, MeOH; (f) NaH, BnBr, TBAI; (g) O_3, Me_2S; (h) $(MeO)_2POCH_2COOMe$, NaH; (i) H_2, Lindlar cat.; (j) TFA, CH_2Cl_2; (k) BnBr, K_2CO_3; (l) DIBALH; (m) I_2, PPh_3, imidazole; (n) PPh_3, MeCN, reflux.

The Boc protecting group was then removed using trifluoroacetic acid followed by protection of the amino group with a second benzyl group using NaH and benzyl bromide. This was followed by DIBALH reduction of the methyl ester to generate the required alcohol (**64**) in 95% yield. The alcohol (**64**) was converted to the iodide which was treated with triphenylphosphine in acetonitrile to give the required phosphonium salt (**46**) in a yield of 75% over the two steps.[65]

Synthesis of the tricarballylic acid fragment (shown in **Scheme 3**) began with the asymmetric Michael addition of the allylphosphonamide (**65**) to the ester (**66**) to generate the Michael adduct (**67**) using the method of Hanessian.[68] Alkene (**67**) was subjected to ozonolysis and then Jones oxidation to generate the diacid, which was subsequently benzylated using benzyl

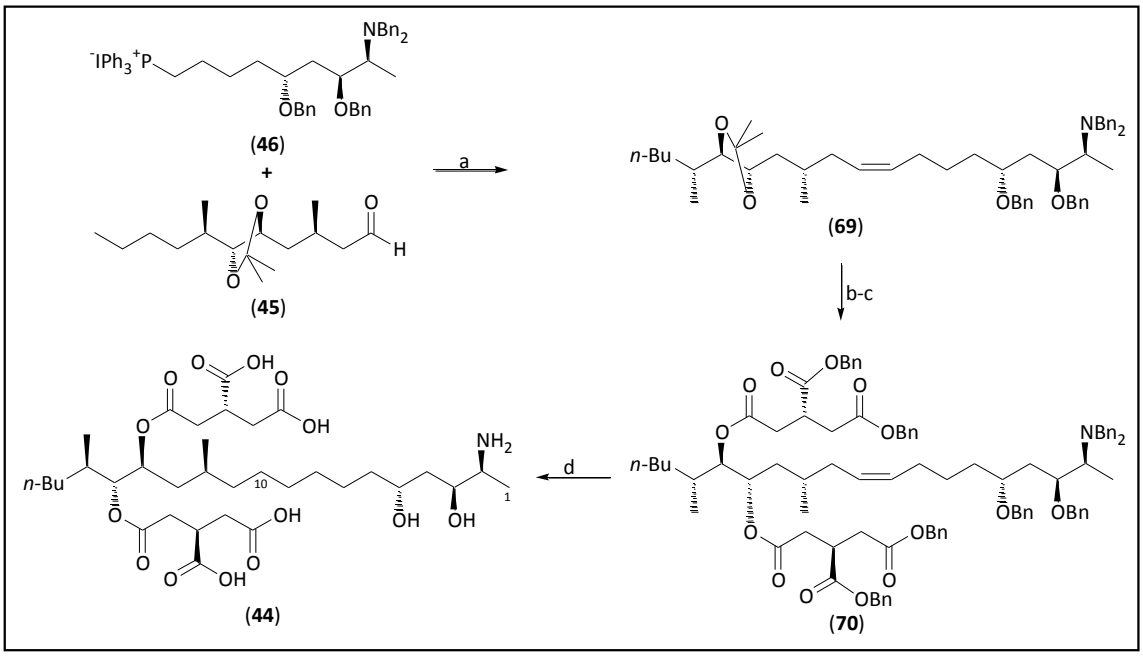
[68] Hanessian, S.; Gomtsyan, A.; Payne, A.; Herve, Y.; Beaudoin, S. *J. Org. Chem.* **1993**, *58*, 5032.

alcohol and EDCI to give the tri-ester (**68**). Reaction with trifluoroacetic acid resulted in the selective cleavage of the *t*-butyl ester to generate the target fragment (**47**).[65]



Scheme 3: Synthesis of the tricarballylic acid moiety (**47**).

Reagents: (a) *n*-BuLi; (b) i) O_3; ii) CrO_3, H_2SO_4; (c) BnOH, EDCI, DMAP; (d) TFA.



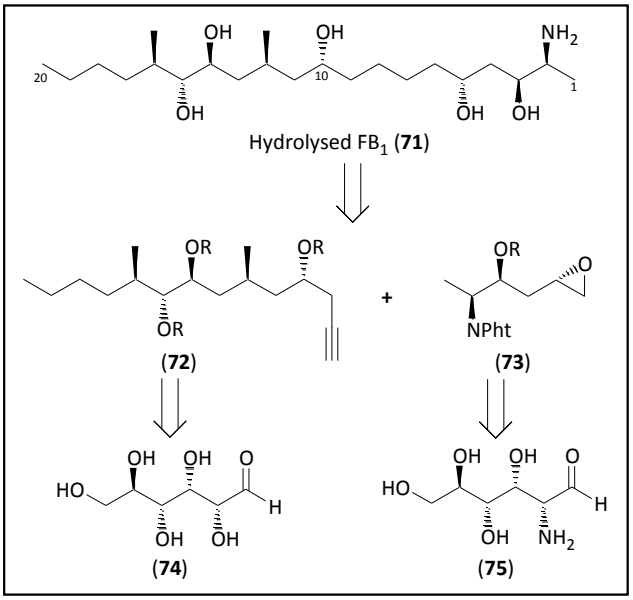
Scheme 4: Coupling of synthesised fragments to obtain FB_2.

Reagents : (a) *n*-BuLi; (b) TFA; (c) **47**, EDCI, DMAP; (d) H_2, Pearlman's cat., HCl.

Coupling of the three fragments (shown in **Scheme 4**) began with a Wittig reaction, which was achieved via the lithiation of **46** using *n*-BuLi to generate the ylide and reaction with aldehyde (**45**) to generate the *cis*-alkene (**69**) in 80% yield. Deprotection of the acetonide using trifluoroacetic acid released the 14,15-diol, freeing the hydroxy groups for esterification to **47** in the presence of EDCI and DMAP to obtain the protected fumonisin B_2 (**70**) in 90% yield. Hydrogenation using Pearlman's catalyst in the presence of HCl resulted in the reduction of

the alkene as well as the removal of all the benzyl protecting groups to obtain the completed fumonisin B_2 (**44**) in 60% yield identical in all respects to the naturally occurring FB_2.[65]

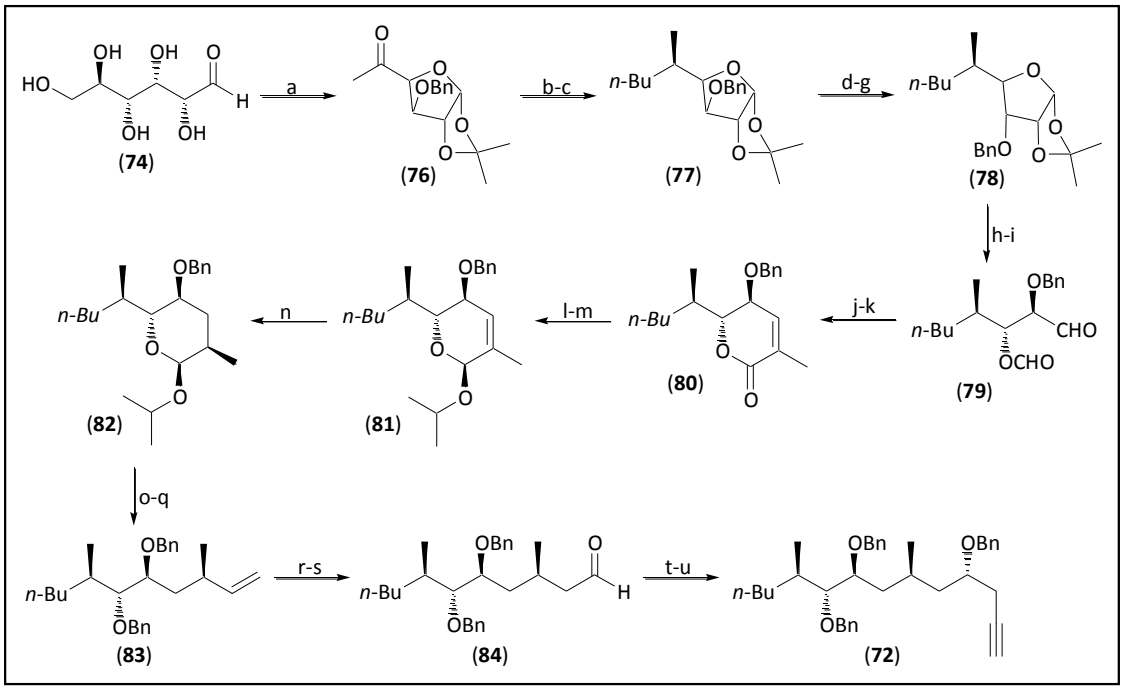
This work completed the synthesis of FB_2, however, the total synthesis of the most prevalent fumonisin B_1 was still to be achieved. The first published attempt of the synthesis of FB_1 was by Gurjar *et al*.[69] who synthesised a hexaacetate derivative of hydrolysed FB_1 (**71**) and not the complete FB_1. Their approach (shown in **Scheme 5**) also involved dividing the fumonisin backbone into two fragments with disconnection between C-6 and C-7 to obtain the left hand C-7–C-20 unit and the right hand unit, C-1–C-6 which could be synthesised from the carbohydrates D-glucose and D-glucosamine, respectively, in contrast to Shi *et al*.[65] who disconnected the backbone between C-9 and C-10.



Scheme 5: Retrosynthetic planning of Gurjar *et al*.[69]

The synthesis of the left hand unit (shown in **Scheme 6**) began with the conversion of D-glucose (**74**) into the 5-ulose derivative (**76**) via a previously published method.[70] This was followed by Wittig olefination of the triphenylphosphonium bromide obtained from *n*-butyl bromide with the ketone (**76**) to obtain an alkene, which was catalytically reduced over Pd-C to obtain a mixture of diastereomers that was separated by chromatography to give **77** in 50% yield (stereochemistry confirmed by chemical correlation experiments).[69]

[69] Gurjar, M.K.; Rajendran, V.; Rao, B.V. *Tetrahedron Lett.* **1998**, *39*, 3803.
[70] Araki, Y.; Arai, Y.; Endo, T.; Ishido, Y. *Chem. Lett.* **1989**, 1.

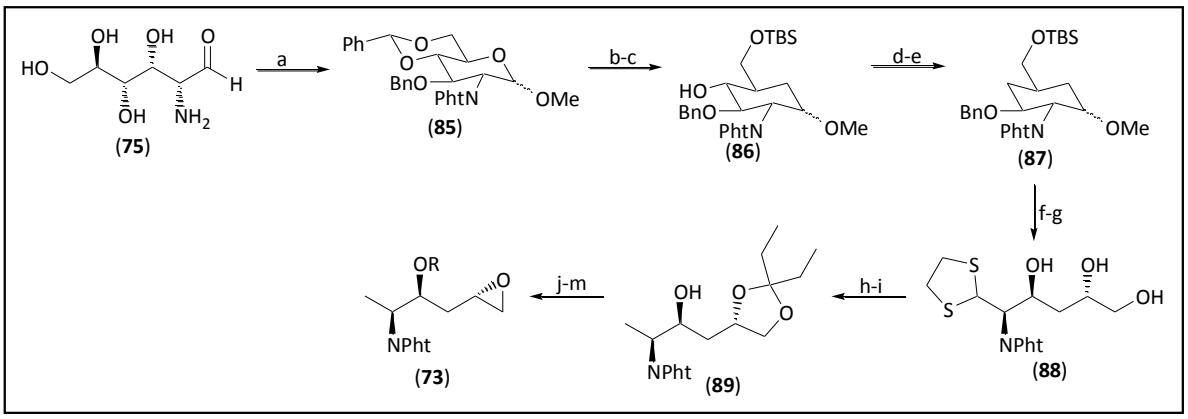


Scheme 6: Synthesis of the C-7–C-20 unit by Gurjar *et al*.[69]

Reagents : (a) ref. 70; (b) $CH_3(CH_2)_2CH_2P^+Ph_3Br^-$, *n*-BuLi; (c) Pd-C, H_2; (d) Ca/liq NH_3; (e) IBX, DMSO; (f) $NaBH_4$; (g) NaH, BnBr; (h) 70% aq. AcOH, H_2SO_4; (i) $NaIO_4$; (j) NaH, $(MeO)_2P(O)CH(CH_3)COOEt$,; (k) K_2CO_3; (l) DIBALH; (m) ^{i}PrOH, CSA; (n) Rh-Al_2O_3, H_2; (o) 70% aq. AcOH, H_2SO_4; (p) $(CH_3PPh_3)^+I^-$, *n*-BuLi; (q) BnBr, NaH; (r) i. 9-BBN, ii. NaOH, H_2O_2; (s) $(COCl)_2$, DMSO, Et_3N; (t) propargyl bromide, Zn, NH_4Cl; (u) NaH, BnBr.

The stereochemistry of the benzyl protected alcohol was inverted in four steps starting with reduction using calcium in liquid ammonia followed by oxidation of the hydroxy group to the ketone using IBX, subsequent reduction to the alcohol using $NaBH_4$ and benzylation using NaH and BnBr to obtain **78**. The protecting acetonide was then removed using aqueous acetic acid to obtain the vicinal diol, followed by oxidative cleavage of the free hydroxy groups using $NaIO_4$ to obtain the aldehyde (**79**). The aldehyde (**79**) was subjected to a Horner-Wadsworth-Emmons reaction with the required phosphonate ester followed by formation of the lactone (**80**) in the presence of K_2CO_3. For the purpose of greater stereoselective control later in the synthesis it was necessary to reduce the lactone to the corresponding lactol using DIBALH followed by reaction with isopropanol in the presence of CSA to obtain the isopropyl glycoside derivative (**81**) as the product. The absolute configuration at the anomeric carbon centre was confirmed as *S* based on the analysis of NMR spectra. Upon hydrogenation of **81** in the presence of Rh-Al_2O_3, **82** was obtained as the only product as determined from the proton coupling constant of 3.0 Hz between the anomeric proton and the C-2 proton indicating the additional effort had been worthwhile. The *O*-glycosidic bond was then hydrolysed using aqueous acetic acid followed by a one-carbon Wittig reaction and benzylation of the free

hydroxy group to generate the alkene (**83**) which underwent hydroboration-oxidation to obtain an alcohol that was converted to the aldehyde (**84**) by Swern oxidation. Reaction of **84** with propargyl bromide in the presence of zinc dust gave a diastereomeric mixture of alcohols which was separated by chromatography. Identification of the required diastereomer with the *S* configuration for the newly-formed hydroxy group was done via a modified Mosher ester method. The free hydroxy group was benzylated to give the target fragment (**72**).[69]



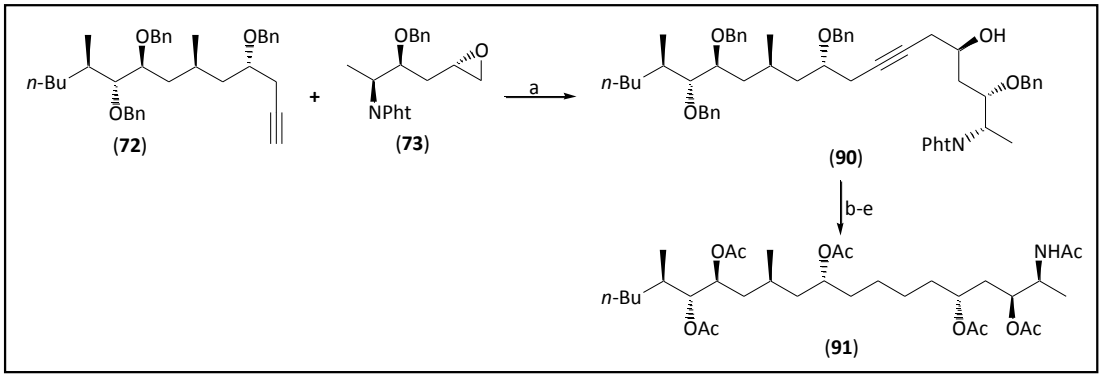
Scheme 7: Synthesis of the C-1–C-6 fragment by Gurjar *et al*.[69]

Reagents : (a) ref. 71; (b) 60% aq. AcOH; (c) TBSCl, Imidazole; (d) NaH, CS_2, MeI; (e) Bu_3SnH, AIBN; (f) *p*-TsOH; (g) $BF_3{\cdot}Et_2O$, $HS(CH_2)_2SH$; (h) Ra-Ni; (i) 3-pentanone, CSA; (j) NaH, BnBr; (k) *p*-TsOH; (l) TsCl, pyridine; (m) NaH.

Synthesis of the C-1–C-6 unit (shown in **Scheme 7**) began with the conversion of the D-glucosamine hydrochloride into the *N*-phthalimido methyl glycoside (**85**) via a previously published method.[71] This was followed by cleavage of the benzylidene protecting group using aqueous acetic acid to obtain the diol and the selective protection of the primary hydroxy group using TBSCl and imidazole to obtain **86** as the product. A Barton-McCombie radical deoxygenation procedure was performed to deoxygenate the C-4 free secondary hydroxy group resulting in the 4-deoxy derivative (**87**), which was subjected to glycosidic bond cleavage by *p*-TsOH. This was followed by treatment with 1,2-ethanedithiol and $BF_3{\cdot}OEt_2$ to give the dithiolane (**88**), which was subsequently reductively desulfurised over Raney-Ni to obtain the triol. 3-Pentanone-CSA was then used to regioselectively protect the C-1 and C-2 hydroxy groups to give the dioxolane (**89**), followed by benzylation of the C-4 hydroxy group using NaH and BnBr. Cleavage of the acetal using *p*-TsOH was followed by selective tosylation of the primary hydroxy group and then deprotonation of the secondary hydroxy group using NaH,

[71] Shigehiro, H. *Carbohydr. Res.* **1971**, *16*, 229.

leading to a stereospecific nucleophilic substitution reaction generating the target epoxide (**73**) in 52% yield.[69]



Scheme 8: Coupling of the C-7–C-20 unit with the C-1–C-6 unit.

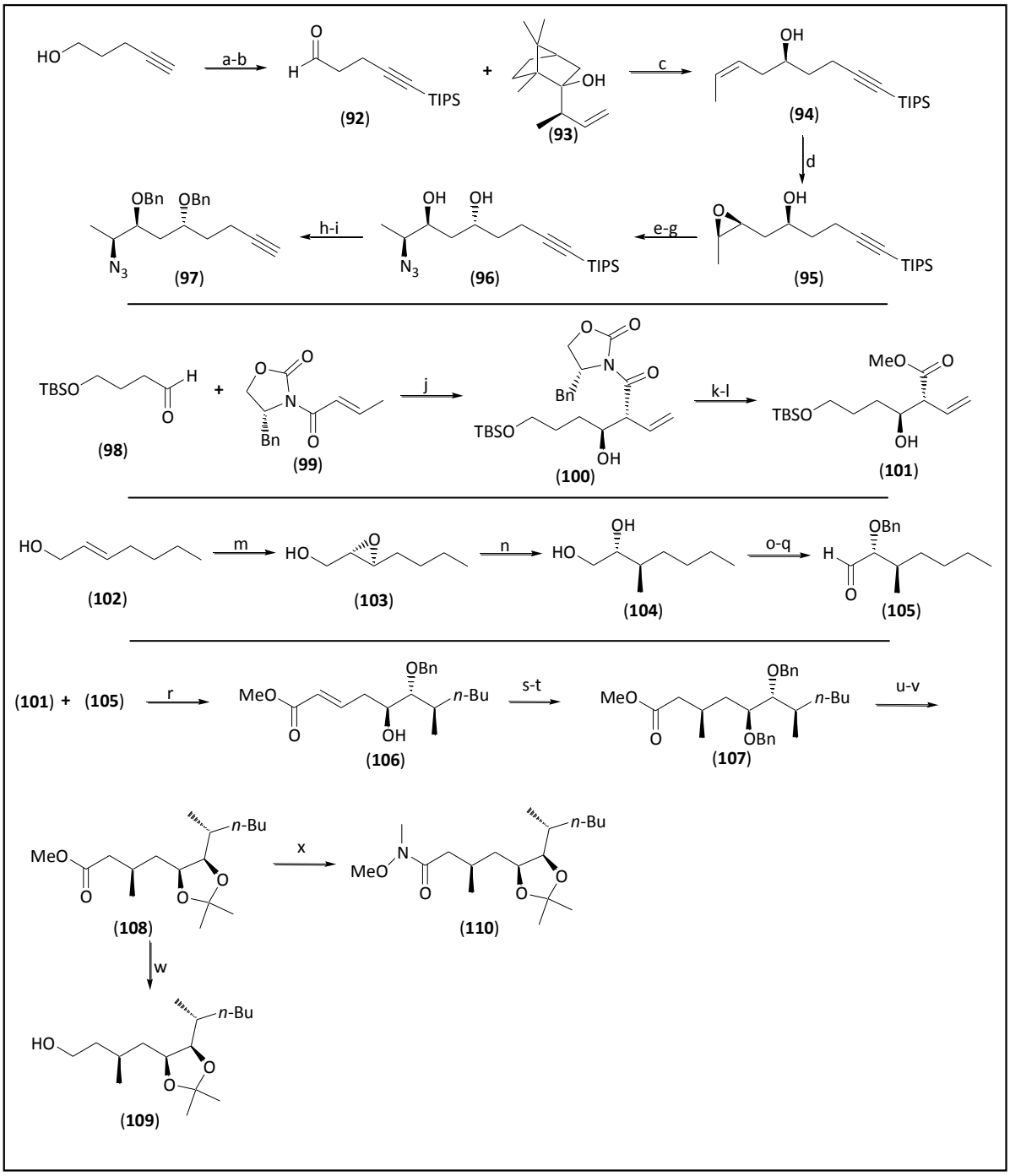
Reagents : (a) *n*-BuLi, $BF_3{\cdot}Et_2O$; (b) $MeNH_2$-MeOH, reflux; (c) Ac_2O, Et_3N; (d) Pd(OH), H_2; (e) Ac_2O, Et_3N.

The coupling of the alkyne (**72**) and the epoxide (**73**) (shown in **Scheme 8**) was done via the deprotonation of the alkyne using *n*-BuLi in the presence of $BF_3{\cdot}Et_2O$ with nucleophilic substitution of the epoxide to obtain **90** in 73% yield. This was followed by the removal of the phthalimido group using methylamine under reflux, acetylation of the free amino and hydroxy groups, followed hydrogenolysis over $Pd(OH)_2$ to remove the benzyl protecting groups. Treatment with acetic anhydride gave the *N*-acetyl hexaacetate FB_1 derivative (**91**) in 95% yield which was fully characterised by NMR spectroscopy and mass spectrometry.[69]

Gurjar *et al.*[69] successfully utilised carbohydrates as chiral starting materials, although why they chose to synthesise the hexaacetate derivative instead of hydrolysed FB_1 or the complete FB_1 esterified to TCA is not clear, since this could easily be achieved with a few modifications in the synthesis. The first total synthesis of FB_1 was published in 2009 by Pereira *et al.*,[72] also using a convergent approach based on the division of the fumonisin backbone into two main fragments (left C-10–C-20 and right C-1–C-9) by disconnection of the C-9–C-10 bond. The synthesised fragments were coupled and esterified to TCA to complete the synthesis of FB_1.

Synthesis of the C-1–C-9 fragment (**97**) (shown in **Scheme 9**) began with the conversion of 4-pentyn-1-ol to the TIPS protected alkyne using TIPSCl and ethylmagnesium chloride followed by oxidation of the alcohol to the aldehyde (**92**) using Dess-Martin periodinane (DMP) and water.

[72] Pereira, C.L.; Chen, Y.-H.; McDonald, F.E. *J. Am. Chem. Soc.* **2009**, *131*, 6066.



Scheme 9: Synthesis of the C-1–C-9 and C-10–C-20 fragments by Pereira *et al.*[72]

Reagents : (a) TIPSCl, EtMgCl; (b) DMP; (c) CSA; (d) $VO(acac)_2$, TBHP; (e) Ph_3P, DIAD, HOAc; (f) K_2CO_3, MeOH; (g) $Ti(O^iPr)_2(N_3)_2$; (h) TBAF; (i) NaH, BnBr, TBAI; (j) Bu_2BOTf, Et_3N; (k) LiOH, H_2O_2; (l) $TMSCHN_2$; (m) $Ti(O^iPr)_4$, L-(+)-DIPT, TBHP; (n) Me_3Al, NaF; (o) $PhCH(OMe)_2$, CSA; (p) DIBAL-H; (q) IBX, DMSO; (r) TMSOTf; (s) 2-Benzyloxy-1-methylpyridiniumtriflate, MgO; (t) (*R*)-Tol-BINAP, MeMgBr, CuI; (u) BCl_3; (v) $Me_2C(OMe)_2$, *p*-TsOH; (w) $LiAlH_4$; (x) $Me(MeO)NH_2Cl$, *i*-PrMgCl.

The alkynyl aldehyde (**92**) was reacted with the camphor derived reagent (**93**) in a stereospecific allylic transfer catalysed by CSA to obtain the homoallylic alcohol (**94**) in 70%

yield with stereochemical control at C-5 and selectivity for the *cis* alkene (ratio of > 95:5 *cis:trans*), while the stereochemistry at C-5 was confirmed by Mosher ester analysis. Epoxidation of the *cis* alkene directed by $VO(acac)_2$ gave the epoxide (**95**) in 73% yield and with 91% enantiomeric excess followed by Mitsunobu reaction to invert the stereochemistry of the hydroxy group at C-5 and introduction of the azide at C-2 using the chelating reagent $Ti(O^iPr)_2(N_3)_2$ to obtain the azidodiol (**96**). The stereochemistry of the diol was confirmed by formation of the acetonide and subsequent NMR analysis. TBAF deprotection of the terminal alkyne followed by benzyl protection of the two free hydroxy groups gave the right hand target molecule (**97**).[72]

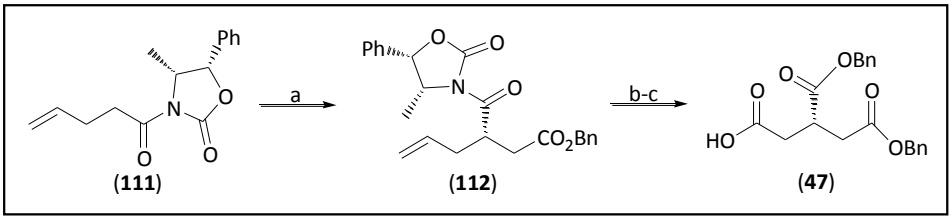
The synthesis of the C-10–C-20 unit (**110**) (also shown in **Scheme 9**) posed a greater challenge than that of the C-1–C-9 unit (**97**) and was done in three stages. Stage one consisted of the monoprotection of 1,4-butanediol with TBSCl followed by Swern oxidation to obtain aldehyde (**98**), which was reacted with the chiral imide (**99**) (prepared according to published methodology)[73] to obtain the hydroxy imide (**100**) in 96% yield. **100** was treated with LiOH and H_2O_2 to obtain the acid which on treatment with $TMSCHN_2$ gave the homoallylic alcohol (**101**), the target compound for the first stage.[72]

The second stage involved the Sharpless epoxidation of the allylic alcohol (**102**) to the epoxy alcohol (**103**), which subsequently underwent ring opening at C-3 using trimethylaluminium to give the diol (**104**). The diol was protected as the benzylidene acetal, followed by DIBALH reduction to give the *O*-benzyl protected secondary alcohol and a primary hydroxy group which was oxidised using IBX to give the aldehyde (**105**), the target compound for the second stage.[72]

A stereospecific allylic transfer between **101** and **105** in the presence of TMSOTf was the key step in the synthesis of the C-10–C-20 unit and resulted in the hydroxy alkenyl ester (**106**). The formation of this product (**106**) proceeded via the formation of a seven-membered ring acetal intermediate which then underwent an oxonia-Cope rearrangement resulting in complete *trans* alkene selectivity and 95% e.e. for the hydroxy group as determined by Mosher ester analysis. Benzylation of the free hydroxy group to give the dibenzyl ether followed by treatment with CuI, (*R*)-Tol-BINAP and MeMgBr resulted in the ester (**107**) in 69% yield. Subsequent debenzylation using BCl_3 resulted in a mixture of the diol and corresponding

[73] Evans, D.A.; Bartoli, J.; Shih, T.L. *J. Am. Chem. Soc.* **1981**, *103*, 2127.

lactone, which was treated with 2,2-dimethoxypropane and *p*-TsOH to obtain the acetonide (**108**), which would allow for the selective deprotection of the C-14 and C-15 hydroxy groups at a later stage. While reduction of the acetonide (**108**) with $LiAlH_4$ gave the alcohol (**109**) which correlated spectroscopically with an intermediate made by Shi *et al.*,[65] the target molecule was the Weinreb amide (**110**), obtained from the reaction of the acetonide (**108**) with $Me(MeO)NH_2Cl$ and *i*-PrMgCl in 83% yield.[72]



(**111**) (**112**) (**47**)

Scheme 10: Synthesis of the tricarballylic acid dibenzyl ester by Pereira *et al.*[72]

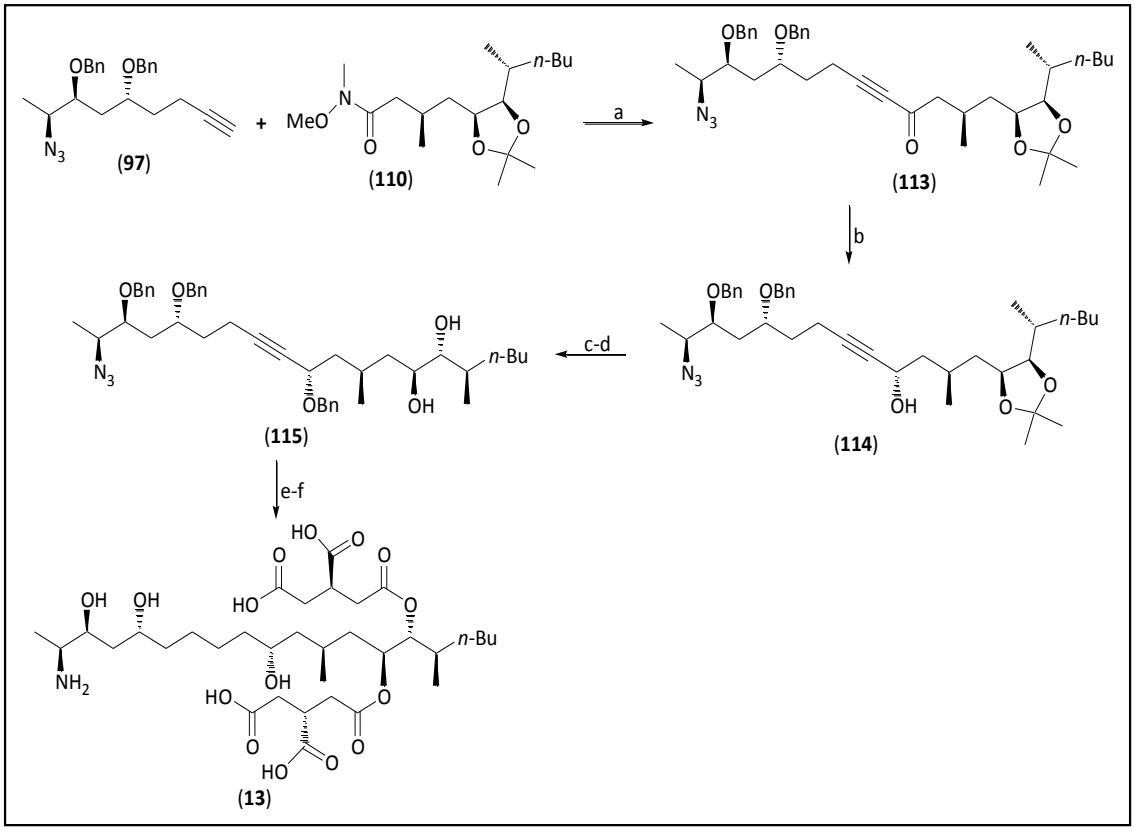
Reagents: (a) benzyl bromoacetate, LiHMDS; (b) *n*-BuLi, BnOH; (c) $NaIO_4$, $RuCl_3{\cdot}H_2O$.

Pereira *et al*.[72] chose to synthesise the same TCA fragment (**47**) as Shi *et al*.[65] but developed a shorter pathway which was based on stereoselective enolate alkylation and alkene oxidation to the carboxylic acid.[72] The synthesis (shown in **Scheme 10**) began with the preparation of the chiral imide (**111**) by a previously published method[74] followed by reaction with LiHMDS and benzyl bromoacetate to obtain the imide (**112**), which was reacted with *n*-BuLi and benzyl alcohol to give the dibenzyl ether. Oxidation of the alkene functionality using sodium periodate and $RuCl_3$ gave the target acid (**47**) in 91% yield. The enantiomeric purity was confirmed as 93% e.e. by synthesis of the menthyl ester from (–)-menthol.[72]

Coupling of all the fragments (shown in **Scheme 11**) to obtain the complete FB_1 began with the lithiation of the alkyne (**97**) and reaction with the Weinreb amide (**110**) to give the alkynyl ketone (**113**) in 65% yield. Enantioselective reduction of the ketone functionality using (*R*)-B-butyldiphenyloxazaborolidine and catecholborane gave the propargylic alcohol (**114**) in 71% yield and a 9:1 d.r. by ^1H NMR analysis. The free hydroxy group was protected as the benzyl ether using NaH and BnBr, followed by the acid catalysed removal of the acetonide group using Amberlite-120 H^+ to obtain the 14,15-diol (**115**). Esterification of the C-14 and C-15 hydroxy groups with the tricarballylic acid dibenzyl ester (**47**) in the presence of EDCI and DMAP followed by total hydrogenation over Pearlman's catalyst gave FB_1 (**13**) in 45% yield,

[74] Li, Y.; Hale, K.J. *Org. Lett*. **2007**, *9*, 1267.

which upon analysis of the spectroscopic data was found to be identical to a commercial FB_1 sample.[72]

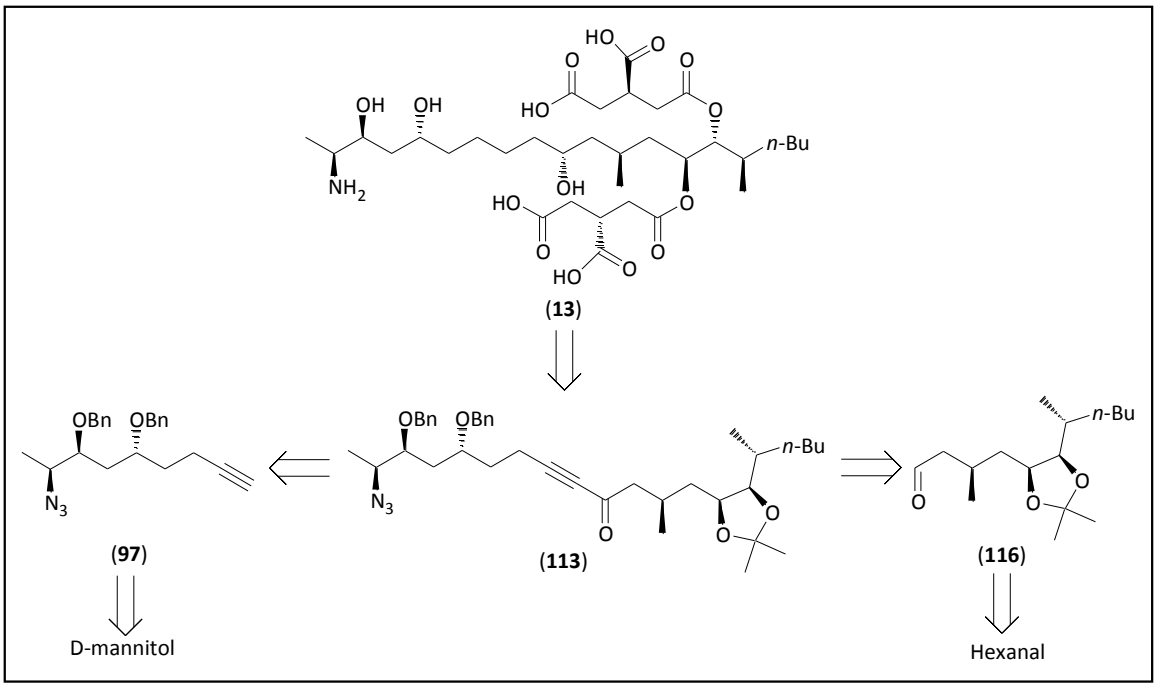


Scheme 11: Coupling of units and synthesis of FB_1.[72]

Reagents: (a) *n*-BuLi; (b) (*R*)-B-butyldiphenyloxazaborolidine, catecholborane; (c) NaH, BnBr, TBAI; (d) Amberlite-120 H^+, MeOH; (e) **47**, EDCI, DMAP; (f) H_2, Pearlman's cat.

Pereira *et al.*[72] also synthesised the hydrolysed FB_1 and hexaacetyl FB_1 derivatives via minor modifications to the synthetic pathway. **107** was reacted with $Me(MeO)NH_2Cl$ and *i*-PrMgCl to obtain the corresponding Weinreb amide, which was reacted with lithiated alkyne (**97**) to give the corresponding alkynyl ketone. This was subjected to enantioselective reduction of the ketone functionality using (*R*)-B-butyldiphenyloxazaborolidine and catecholborane to obtain the propargylic alcohol followed by global hydrogenation of the molecule to obtain hydrolysed FB_1 (**71**). The hexaacetate derivative (**91**) was prepared from **71** by reaction with acetic anhydride, DMAP and Et_3N. The spectroscopic characteristics of the prepared hydrolysed FB_1 and hexaacetate derivatives were found to be identical with the prepared standard and published data,[69] respectively.[72]

More recently Chandrasekhar and Sreelakshmi[75] have also published a paper on the total synthesis of FB_1. Their approach also involved disconnecting the fumonisin backbone into two fragments consisting of the C-1–C-9 and C-10–C-20 units, similar to Pereira *et al.*[72] The principal target of their synthesis was the alkynyl ketone (**113**) reported by Pereira *et al.*,[72] which was subsequently analysed retrosynthetically (shown in **Scheme 12**) resulting in D-mannitol and hexanal being identified as suitable starting materials for the C-1–C-9 and C-10–C-20, respectively.[75]

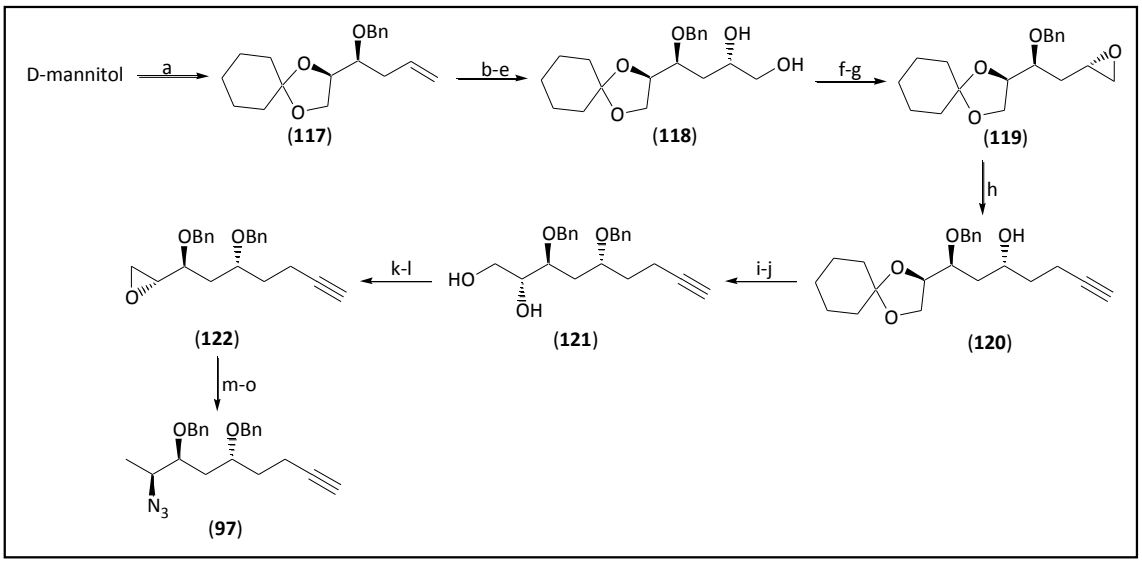


Scheme 12: Retrosynthetic analysis of Chandrasekhar and Sreelakshmi.[75]

The synthesis of the C-1–C-9 fragment (**97**) (shown in **Scheme 13**) began with the preparation of the homoallyl triol (**117**) from D-mannitol in four steps using previously published methodology.[76] Hydroboration of the alkene functionality using BH_3·DMS gave the primary alcohol followed by oxidation to the aldehyde, which could be used in an organocatalytic MacMillan α-hydroxyation reaction to insert the hydroxy group required at C-5. The aldehyde was subjected to 40 mol % D-proline and PhNO, and prompt reduction using $NaBH_4$ to obtain an unstable anilinoxy compound, followed by subsequent treatment with $CuSO_4 \cdot 5H_2O$ in MeOH to give the diol (**118**) in 64% yield and 92% d.r. Selective tosylation of the primary hydroxy group using TsCl and dibutyltin oxide gave the tosylate which was treated with K_2CO_3 to give the epoxide (**119**). Reaction of the epoxide (**119**) with propargyl magnesium bromide

[75] Chandrasekhar, S.; Sreelakshmi, L. *Tetrahedron Lett.* **2012**, *53*, 3233.
[76] Chattopadhyay, A.; Mamdapur, V.R. *J. Org. Chem.* **1995**, *60*, 585.

resulted in the acetylenic alcohol (**120**), which was subsequently benzylated using NaH and BnBr to form the benzyl ether followed by cleavage of the cyclohexylketal using *p*-TsOH to give the diol (**121**). The primary hydroxy group was again selectively tosylated followed by reaction with K_2CO_3 to obtain the epoxide (**122**), which was subjected to regioselective reductive ring opening at C-1 using $LiAlH_4$ to give the secondary alcohol. The alcohol was mesylated using MsCl and subsequently replaced with azide using NaN_3 via an S_N2 reaction to obtain the target azide (**97**) in 83% yield over the two steps.[75]



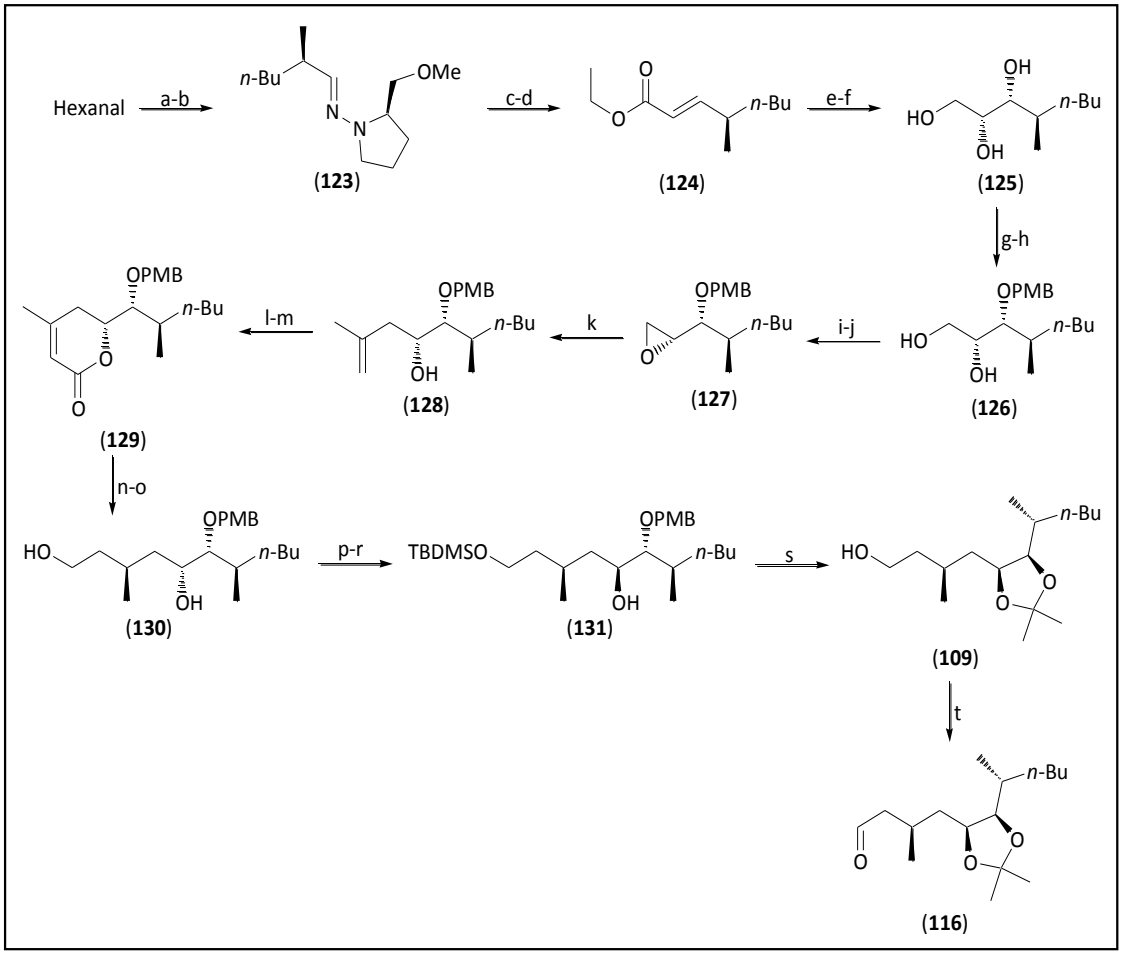
Scheme 13: Synthesis of the C-1–C-9 fragment by Chandrasekhar and Sreelakshmi.[75]

Reagents: (a) 4 steps, ref 76; (b) BH_3·DMS; (c) IBX, DMSO; (d) D-proline, PhNO, DMSO then $NaBH_4$; (e) $CuSO_4{\cdot}5H_2O$; (f) TsCl, Et_3N, Bu_2SnO; (g) K_2CO_3; (h) propargyl magnesium bromide; (i) NaH, BnBr; (j) *p*-TsOH; (k) TsCl, Et_3N; (l) K_2CO_3; (m) $LiAlH_4$; (n) MsCl, Et_3N; (o) NaN_3.

Synthesis of the C-10–C-20 unit (**116**) (shown in **Scheme 14**) began with the conversion of hexanal into the Ender's imine (**123**) via reaction with RAMP followed by diastereoselective alkylation using LDA and methyl iodide.[77] The imine was oxidised using ozone resulting in the aldehyde, followed by reaction with ethoxycarbonylmethylene triphenylphosphorane to obtain the α,β-unsaturated ester (**124**). Reduction of the ester (**124**) with DIBALH gave the allylic alcohol, which was subjected to Sharpless asymmetric dihydroxyation using AD-mix-β to give the triol (**125**) in 90% yield and greater than 90% diastereomeric excess. This was followed by various protective group manipulations starting with the transformation of the triol (**125**)

[77] Waalboer, D.C.J.; van Kalkeren, H.A.; Schaapman, M.C.; van Delft, F.L.; Rutjes, F.P.J.T. *J. Org. Chem.* **2009**, *74*, 8878.

into the *p*-methoxybenzylidene acetal and reductive ring opening using DIBALH to give the diol (**126**).



Scheme 14: Synthesis of the C-10–C-20 unit (**116**) by Chandrasekhar and Sreelakshmi.[75]

Reagents: (a) RAMP; (b) LDA, MeI; (c) O_3; (d) PPh_3CHCO_2Et; (e) DIBALH; (f) AD-mix-β, $CH_3SO_2NH_2$; (g) $PMPCH(OMe)_2$; (h) DBALH; (i) TsCl, Et_3N; (j) K_2CO_3; (k) 2-propenyl magnesium bromide; (l) acryloyl chloride, DIPEA; (m) Grubbs II; (n) 10% Pd-C, H_2; (o) $LiBH_4$; (p) TBDMSCl, imidazole; (q) DMP; (r) LiI, $LiAlH_4$; (s) 10% Pd-C, H_2, *p*-TsOH then *p*-TsOH, acetone; (t) DMP.

Tosylation of the primary hydroxy group followed by treatment with K_2CO_3 furnished the epoxide (**127**), which was subjected to regioselective ring opening using 2-propenyl magnesium bromide in a Grignard reaction to obtain the alkene (**128**). Treatment of the hydroxy group with acryloyl chloride and DIPEA generated the diene, which was a precursor for the ring closing metathesis reaction catalysed by 5 mol % Grubbs II catalyst to give the lactone (**129**) in 85% yield. Hydrogenation of the α,β-unsaturated ester using 10% Pd-C generated the C-12 methyl group with the required stereochemistry followed by reduction of the lactone to the diol (**130**) using $LiBH_4$ and subsequent protection of the primary hydroxy

group as a silyl ether using TBDMSCl and imidazole. The stereochemistry of the remaining free hydroxy group was then inverted via oxidation to the ketone with DMP followed by $LiAlH_4$ reduction in the presence of LiI to furnish the alcohol (**131**) in 86% yield and 90% diastereomeric excess. Deprotection of the silyl and PMB protecting groups was accomplished via hydrogenation using 10% Pd-C and *p*-TsOH to obtain the vicinal diol, which was then protected as the acetonide (**109**) using *p*-TsOH and acetone, while freeing the primary hydroxy group. It should be noted that **109** was also prepared by Pereira *et al*.[72] in their synthesis of FB_1 and that comparison of the spectroscopic characteristics indicated that the compounds were indeed identical. Oxidation of **109** using DMP generated the target aldehyde (**116**) in 82% yield.[75]

In order to couple the two fragments the acetylene (**97**) was lithiated using *n*-BuLi and reacted with the aldehyde (**116**) to furnish the complete backbone with a hydroxy group at C-10 as a diastereomeric mixture (60:40) and 75% yield. For stereochemical reasons the hydroxy group at C-10 was oxidised using DMP to obtain the ketone (**113**), identical with the ketone produced by Pereira *et al*.[72] based on comparison of spectroscopic data. From this point Chandrasekhar and Sreelakshmi[75] continued the synthesis using the same methodology as Pereira *et al*.[72] to generate the complete FB_1 mycotoxin.

The synthesis and confirmation of the absolute stereochemistry of the two most important fumonisins (FB_1 and FB_2) have been successfully accomplished. However, there is still much work to be done in this field as the structures of many more fumonisins are still to be elucidated as well as synthesised in order to fully understand this class of mycotoxins and their biological effects.

1.2.4. Biological Aspects

Since the recognition of *Fusarium spp* as a serious agricultural problem, a vast amount of research has been done on its toxicology. The synthesis and characterisation of the fumonisins was an important step in understanding the complex mechanisms of action as well as the toxic effects of the mycotoxin in plants, animals and humans alike. Although a lot of work has been done on the fumonisins, it still remains a relatively ignored health issue, especially in low-income countries, where the risk of staple food contamination is highest. While the link between fumonisins and human disease has not been conclusively established, the data

linking fumonisins to animal disease is extensive. Fumonisins have been shown to cause ELEM[20] and PPO[11] as well as being hepatotoxic and responsible for liver tumours in rats,[21] to mention the main disease pathologies. The various biological implications will be discussed further in the following sections.

1.2.4.1. Occurrence and Distribution

Fumonisins are a common contaminant of corn throughout the world[78] with reports of contamination coming from Argentina,[79] Austria,[79] Botswana,[79] Brazil,[79,80] Bulgaria,[79] Canada,[79] China,[79,81] France,[79] Germany,[79] Great Britain,[79] Hungary,[24,79] India,[79] Iran,[79,82] Italy,[79] Japan,[79] Kenya,[79] Nepal,[79] New Zealand,[79] Peru,[79] Poland,[79] South Africa,[27,79] Switzerland,[79] United States[24,79] and Venezuela.[79] The levels of contamination vary widely across the world but it is estimated that the average daily intake of fumonisins ranges between 12 and 140 µg per person per day with some people being exposed to levels as high as 2500 µg per day depending on geographical factors.[83]

Fumonisins have also been detected in other sources of food including sorghum, rice, soybean meal, wheat bran and poultry feed,[24] as well as in numerous foodstuffs available commercially such as flours, cereals and breads. Weidenbörner[84] has written an extensive review article on fumonisins in food summarising the levels of fumonisins detected in various foodstuffs from numerous countries. There have also been reports of *Fusarium spp* that have been detected in air samples and samples taken from damp, mouldy walls where moisture damage is evident meaning that people are possibly being exposed unknowingly to fumonisins.[85] It has been found that *F. verticillioides* is endemic to maize,[86] occurring in the leaves, stems, roots and kernels of maize (*Zea mays*),[87] and that it grows well at temperatures above 26 °C.[88]

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[78] Marasas, W.F.O.; Nelson, P.E.; Toussoun, T.A. Toxigenic *Fusarium* species: Identity and Mycotoxicology. University Park, PA, Pennsylvania State University Press, **1984**.
[79] Marasas, W.F.O. in Fumonisins in Food. Jackson, L.S.; de Vries, J.W.; Bullermann, L.B. (eds), Plenum Press, New York, **1996**.
[80] Sydenham, E.W.; Marasas, W.F.O.; Shephard, G.S.; Thiel, P.G.; Hirooka, E.Y. *J. Agric. Food Chem.* **1992**, *40*, 994.
[81] Li, F.-Q.; Yoshizawa, T.; Kawamura, O.; Luo, X.-Y.; Li, Y.-W. *J. Agric. Food Chem.* **2001**, *49*, 4122.
[82] Shephard, G.S.; Marasas, W.F.O.; Leggott, N.L.; Yazdanpanah, H.; Rahimian, H.; Safavi, N. *J. Agric. Food Chem.* **2000**, *48*, 1860.
[83] WHO. Safety evaluation of certain mycotoxins in food (WHO food additives series 47). International programme on chemical safety. Geneva, World Health Organisation; **2001**, pp. 103-279.
[84] Weidenbörner, M. *Eur. Food Res. Technol.* **2001**, *212*, 262.
[85] Hunter, C.A.; Grant, C.; Flannigan, B.; Bravery, A.F. *Int. Biodeterior.* **1988**, *24*, 81.
[86] Miller, J.D. *Environ. Health Perspect.* **2001**, *109*, 321.
[87] Foley, D.C. *Phytopathology* **1962**, *68*, 1331.
[88] Reid, L.M.; Nicol, R.W.; Quellet, T.; , M.; Miller, J.D.; Young, J.C.; Stewart, D.W.; Schaafsma, A.W. *Phytopathology* **1999**, *89*, 1028.

An increase in the production of fumonisins has been associated with hot, dry weather in conjunction with insect damage. Thus the main factors determining fumonisin contamination include climate, location and the susceptibility of the plants to insect damage, crop stress/drought stress, fungal invasion as well as the maize genotype.[86,89]

When *Fusarium spp* are cultured on maize, 56-84% of the total fumonisins produced can be attributed to the fumonisin B analogues (with FB_1 contributing about 70%, FB_2 15- 25% and FB_3 3-8%),[33,64] while the fumonisin C analogues are found at levels which are 5-20% those of the B series.[90] The P-series have also been detected at levels up to 30% of FB_1 in some *Fusarium spp*.[40] When cultured on rice the levels of fumonisins produced tend to be lower when compared to maize cultures.[85] The other fumonisin analogues are generally present at levels of about 1% of FB_1 and are frequently difficult to detect except by the most sensitive instruments.

1.2.4.2. Biochemistry and Mechanisms of Action

Examination of the fumonisin backbone indicates that it closely resembles the sphingoid base backbone of a special class of lipids known as the sphingolipids. Sphingolipids are found in all eukaryotic cells, particularly in the plasma membrane and other associated membranes. They consist of a long chain sphingoid base backbone linked to other biological molecules, resulting in the various functional sphingolipids. In animals the sphingoid base is sphingosine (**132**) and in plants, phytosphingosine (**133**). The fumonisins closely resemble these molecules structurally as can be seen in **Figure 18**.[91] The sphingosine backbone consists of a long chain alkylamine with a *trans* double bond between C-4 and C-5 and hydroxy groups on C-1 and C-3, while phytosphingosine lacks the double bond and instead has an additional hydroxy group at C-4.

Sphingolipids play an important role in the maintenance of membrane structures as they serve as a site for the binding of extracellular matrix proteins, as well as regulate growth factor receptors.[92] They are involved in the signal transduction pathways functioning as precursors for second messengers that control cell growth, cell differentiation, cell functions as well as

[89] Wild, C.P.; Gong, Y.Y. *Carcinogenesis* **2010**, *31*, 71.
[90] Sewram, V.; Mshicileli, N.; Shephard, G.S.; Vismer, H.F.; Rheeder, J.P.; Lee, Y.-W.; Leslie, J.F.; Marasas, W.F.O. *J. Agric. Food Chem.* **2005**, *53*, 4861.
[91] Shier, W.T.; Shier, A.C. *J. Toxicol. - Toxin Rev.* **2000**, *19*, 189.
[92] Iwabuchi, K.; Handa, K.; Hakomori, S.I. *J. Biol. Chem.* **1998**, *273*, 33766.

apoptosis (programmed cell death).[93] More specific amongst the many different sphingolipids, sphingosine and sphingosine-1-phosphate have been shown to play a regulatory role in cell differentiation,[94] growth,[95] calcium movement,[96] motility[97] and the activity of many enzymes including the important intracellular regulatory enzyme protein kinase C.[98]

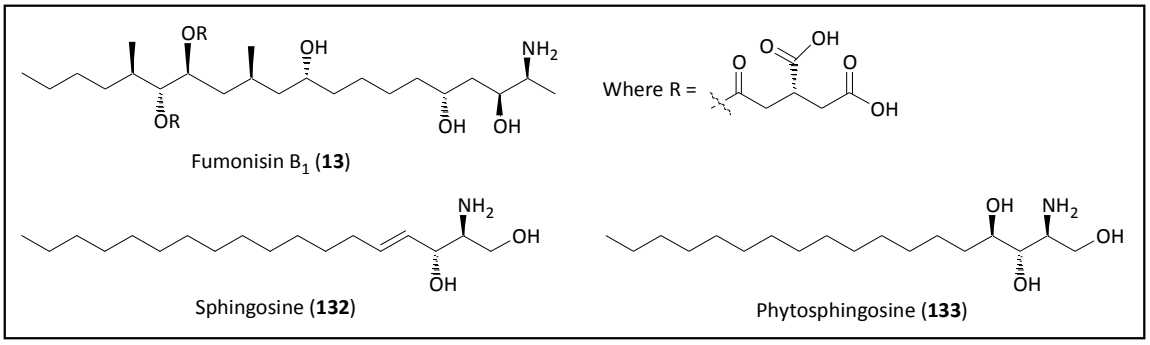

Figure 18: Resemblance between fumonisin B_1, sphingosine and phytosphingosine.[91]

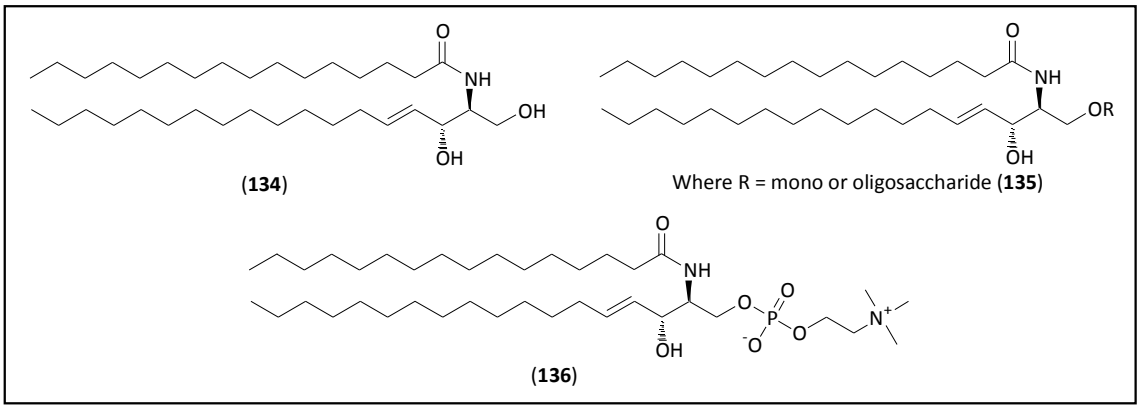

Figure 19: Structures of ceramide (**134**), glycosphingolipids (**135**) and sphingomyelin (**136**).[91]

Other sphingolipids, such as the *N*-acyl sphingosine, ceramide (**134**), where the 2-amino group has been acylated with the long-chain fatty acid stearic acid, are second messengers in cytokine signal transduction,[93] and play a regulatory role in cell growth[99] and apoptosis.[100]

[93] Merrill Jr, A.H.; Schmelz, E.-M.; Dillehay, D.L.; Spiegel,S.; Shayman, J.A.; Schroeder, J.J.; Riley, R.T.; Voss, K.A.; Wang, E. *Toxicol. Appl. Pharmacol.* **1997**, *142*, 208.
[94] Merrill Jr, A.H.; Sereni, A.M.; Stevens, V.L.; Hannun, Y.A.; Bell, R.M.; Kinkade Jr, J.M. *J. Biol. Chem.* **1986**, *261*, 12610.
[95] Olivera, A.; Spiegel, S. *Nature* **1993**, *365*, 557.
[96] Ghosh, T.K.; Bian, J.; Gill, D.L. *Science* **1990**, *248*, 1653.
[97] Igarashi, Y.; Sadahira, Y.; Yamamura, S.; Hakomori, S. Inhibition of mouse B16 melanoma cell motility by sphingosine-1-phosphate eicosanoids and other bioactive lipids. in *Cancer, Inflammation, and Radiation Injury.* Honn, K.V. (ed.), **1997**, Plenum Press, New York, p. 693.
[98] Igarashi, Y.; Kitamura, K.; Toyokuni, T.; Dean, B.; Fenderson, B.; Ogawa, T.; Hakomori, S. *J. Biol. Chem.* **1990**, *265*, 5385.
[99] Olivera, A.; Buckley, N.A.; Spiegel, S. *J. Biol. Chem*. **1992**, *267*, 26121.
[100] Obeid, L.M.; Lenardic, C.M.; Karolak, L.A.; Hannun, Y.A. *Science* **1993**, *259*, 1769.

Glycosphingolipids (**135**) such as cerebrosides and gangliosides, which are glycosylated derivatives of ceramide, have been shown to be involved in processes such as cell adhesion,[101] transmembrane signalling[102] and transport.[103] An important class of structural sphingolipids are the sphingomyelins (**136**) (a type of phospholipid), which play a major role in the formation of cell membranes. They consist of a phosphorylcholine which is esterified to the C-1 hydroxy group of ceramide yielding a moiety which is both hydrophilic and hydrophobic in nature.[91] Proof for the importance of sphingomyelins to membrane stability comes from the treatment of cells with sphingomyelinase enzymes (found in some bacteria, spider and snake venoms) which catalyse the hydrolysis of the phosphodiester bond, resulting in membrane disruption and cytolysis.[104]

The biosynthesis, distribution and breakdown of sphingolipids has been extensively studied due to the importance of these molecules and their influence in many disease pathologies. The *de novo* biosynthesis of sphingolipids (shown in **Scheme 15**) begins with the serine palmitoyltransferase enzyme catalysed condensation of palmitoyl-CoA (**137**) with serine (**138**) to form 3-ketosphinganine (**139**) with the loss of carbon dioxide from the carboxylic acid of serine. This is followed by reduction of **139** with a NADPH-dependent reductase to give sphinganine (**140**) (also known as dihydrosphinganine). Ceramide synthase (also known as sphingosine *N*-acyl transferase) then catalyses the acylation of the 2-amino group with a fatty acid from a coenzyme A derivative to obtain dihydroceramide (**141**). A stereoselective desaturase enzyme then introduces the *trans* 4,5 double bond to give ceramide (**134**). It should be noted that sphingosine is not part of the *de novo* biosynthesis pathway as the direct desaturation of sphinganine to sphingosine does not occur. Sphingosine is a degradation product of ceramide, catalysed by the enzyme ceramidase, and it is a substrate for the enzyme ceramide synthase. Thus it can again be acylated by ceramide synthase to obtain a ceramide, resulting in constant recycling of the sphingolipids and a reduction in the need for the *de novo* biosynthesis. The importance of this process has not been established. From ceramide the biosynthetic pathway branches out extensively to obtain the numerous other possible complex sphingolipids by means of a variety of enzymes.[91,104]

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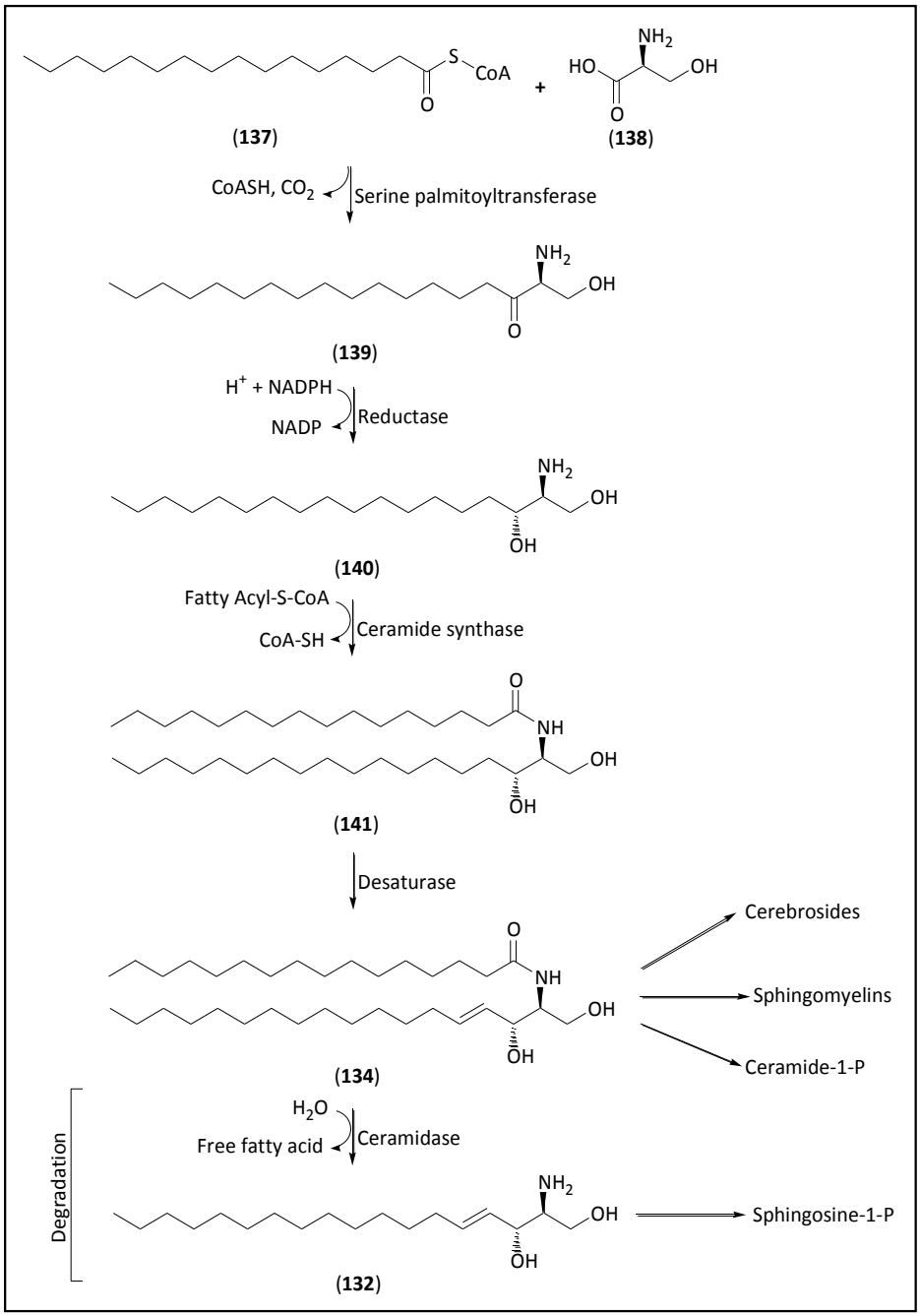
[101] Shayman, J.A.; Deshmukh, G.D.; Mahdiyoun, S.; Thomas, T.P.; Wu, D.; Barcelon, F.S.; Radin, N.S. *J. Biol. Chem.* **1991**, *266*, 22968.
[102] Hakamori, S.I. *Perspect. Cancer Res.* **1996**, *56*, 5309.
[103] Sandvig, K.; Dubinina, E.; Garred, O.; Prydz, K.; Kozlov, Y.V.; Hansen, S.H.; Van Deurs, B. *Biochem. Soc. Trans.* **1992**, *20*, 724.
[104] Harvey, A.L. Cytolytic Toxins in *Handbook of Toxinology*. Shier, W.T., Mebs, D. (eds.), Marcel Dekker Inc., New York, **1990**, p. 1.



Scheme 15: The *de novo* biosynthesis of sphingolipids.[91]

It is due to the structural resemblance between sphinganine (**140**) and the backbone of the fumonisins that it was first hypothesised by Wang *et al*.[105] that the target of the fumonisins was sphingolipid biosynthesis. This hypothesis they proved in 1991 using rat liver hepatocytes as a model. The influence of fumonisin B_1 and B_2 on the *de novo* biosynthesis of sphingolipids was studied by monitoring the amount of [^{14}C]-serine incorporated into sphingolipids.[105] Their approach involved incubating cells in the presence of [^{14}C]-serine either in the presence or

[105] Wang, E.; Norred, W.P.; Bacon, C.W.; Riley, R.T.; Merrill Jr, A.H. *J. Biol. Chem.* **1991**, *266*, 14486.

absence of fumonisins followed by extraction and acid hydrolysis of the lipids to obtain the free long- chain bases. TLC was used to separate the sphinganine and sphingosine followed by determination of the radiolabel profile which indicated that the formation of [^{14}C]-sphingosine was much lower in hepatocytes exposed to fumonisins than in the control group. The inhibitory action of fumonisins was found to occur relatively quickly and be persistent, while no effect was observed on the incorporation of [^{14}C]-serine in other biosynthetic pathways requiring serine, which indicated that the radiolabeled serine had no influence on the functioning of the hepatocytes.[105]

The site of action of the fumonisins on sphingolipid metabolism was investigated further by studying the activities of the various enzymes in the presence and absence of fumonisins. Serine palmitoyltransferase showed no inhibition, while for the second step there was no accumulation of the 3-ketosphinganine. It was found that inhibition occurred at the step where [^{14}C]-sphinganine was converted to dihydroceramide as the amount of free [^{14}C]-sphinganine increased 110 fold in the presence of fumonisin B_1, with a corresponding decrease in free sphingosine over the same time period. The inhibition of ceramide synthase was then directly demonstrated using *in vitro* assays on rat liver microsomes in which the IC_{50} for inhibition was found to be 0.1 µM for FB_1.[105] Further confirmation of these results came from additional studies conducted by Yoo *et al.*[106] on renal cell lines and by Merrill *et al*.[107] on mouse cerebellar neurons in culture who found that fumonisin inhibition was of the competitive type with an IC_{50} of 0.075 µM for cerebellar microsomes. Merrill *et al*.[107] also investigated the effect of inhibition of ceramide synthase on the formation of some of the more complex sphingolipids.

Mechanistically speaking it was known that fumonisins inhibit ceramide synthase, but the question that remained was how this was done. Merrill *et al*.[107] proposed that FB_1 possibly had two modes of interaction with ceramide synthase which could explain its potency. The first being the interaction with the sphinganine binding site via the "sphinganine-like" domain of FB_1, and secondly interaction of the negatively charged tricarballylic acid groups with the fatty acyl-CoA binding site due to resemblance of the TCA to the polyanionic phosphate groups of CoA.[107] The TCA interaction was deemed to be the least important as FB_1 lacks the ability to inhibit other CoA-dependent enzymes, while removal of the TCA groups has been shown to

[106] Yoo, H.; Norred, W.P.; Wang, E.; Merrill Jr, A.H.; Riley, R.T. *Toxicol. Appl. Pharmacol.* **1992**, *114*, 9.
[107] Merrill Jr, A.H.; van Echten, G.; Wang, E.; Sandhof, K. *J. Biol. Chem.* **1993**, *268*, 27299.

reduce the amount of inhibition with a 10-fold increase in the IC_{50} value.[108] Further evidence that supports this model of binding and inhibition of ceramide synthase comes from data that indicates the potency of FB_1 inhibition is sensitive to the concentrations of sphingoid bases and fatty acyl-CoA,[107] while removal of the TCA groups (as in hydrolysed FB_1) results in a compound that is not only an inhibitor but also a substrate for acylation by ceramide synthase.[109] This could possibly be an explanation why aminopentol-like compounds often remain toxic to animals even though they are not such potent inhibitors of ceramide synthase.[110]

The mechanism responsible for the carcinogenicity of fumonisins is still unknown, but it has been determined that FB_1 is not genotoxic,[111] although it has been found to act as a tumour promoter and is thus classified as a mitogen.[112] In terms of structure-activity relationship studies, three conclusions were reached regarding fumonisins : firstly, it was determined that the free amino group is necessary for binding as the A-series of fumonisins is inactive; secondly, a long alkyl chain is necessary and thirdly, a hydroxy group is possibly required on the carbon adjacent to the one bearing the free amino group.[91] However, it has been proven that a C-1 hydroxy group is not necessary for enzyme activity.[109]

The various observed disease pathologies are thought to be related to the disruption of sphingolipid biosynthesis by the fumonisins. Due to the vast number of biological pathways that are possibly influenced by sphingolipids, the extent to which the sphingolipids are affected and play a role in disease are still unknown.

1.2.4.3. Toxic Effects and Biodistribution

The toxic effects, biodistribution and target organs of fumonisins have been extensively studied in numerous species to determine the impact of fumonisins on society. Fumonisins have the largest agricultural impact due to the regulation of levels in maize as well as the toxicoses of humans, animals and plants. Studies indicate that the main target organs of fumonisins are the kidneys and liver, but species, strain and sex dependent differences

[108] Merrill Jr, A.H.; Wang, E.; Gilchrist, D.G.; Riley, R.A. *Adv. Lipid Res.* **1993**, *26*, 215.
[109] Humpf, H.-U.; Schmelz, E.-M.; Meredith, F.I.; Vesper, H.; Vales, T.R.; Wang, E.; Menaldino, D.S.; Liotta, D.C.; Merrill Jr, A.H. *J. Biol. Chem.* **1998**, *273*, 19060.
[110] Desai, K.; Sullards, M.C.; Allegood, J.; , E.; Schmelz, E.M.; Hartl, M.; Humpf, H.-U.; Liotta, D.C.; Peng, Q.; Merrill Jr, A.H. *Biochim. Biophys. Acta*. **2002**, *1585*, 188.
[111] Norred, W.P.; Plattner, R.D.; Vesonder, R.F.; Bacon, C.W.; Voss, K.A. *Food Chem. Toxicol.* **1992**, *30*, 233.
[112] Riley, R.T.; Voss, K.A.; Yoo, H.-S.; Gelderblom, W.C.A.; Merrill Jr, A.H. *J. Food Protect.* **1994**, *57*, 638.

occur.[113] Another concern is whether fumonisin residues could contaminate milk, eggs and meat, which could potentially increase exposure of the consumer to these mycotoxins.

Horses have been shown to be the most sensitive animals to fumonisin mycotoxins with the consumption of fumonisins resulting in equine leukoencephalomalacia (ELEM), characterised by the liquefactive necrosis of the white matter in the brain and which is fatal.[80] Other symptoms include apathy, paralysis, lack of co-ordination and aggressiveness.[80,113] The first report of ELEM induced in a horse was by Marasas *et al*.,[20] which was achieved by repeated intravenous administration of pure FB_1. The presence of fumonisins in feeds associated with field cases of ELEM was subsequently proven although the concentrations detected were not reported.[26] As more sensitive techniques became available it became possible to determine the concentrations of fumonisins present in implicated feeds with one study finding a range of 1-126 µg/g FB_1 over 98 samples.[114] Numerous other studies have been done since measuring the levels of fumonisins in feeds,[80] and the pattern that has been observed is that concentrations >10 ppm FB_1 are generally involved in ELEM cases.[115] Ross *et al*.[116] did a more in-depth study on the effect of feed contaminated with fumonisins on horses and found not only ELEM but also hepatic and histopathological lesions indicating that fumonisins are also hepatotoxic in horses,[116] while cardiotoxicity has been reported by Smith *et al*.[117]

Consumption of fumonisin contaminated feed by swine was first recorded in 1981 and was found to result in porcine pulmonary oedema (PPO) and a swollen hydrothorax.[24] In PPO the thoracic cavity becomes filled with a yellow liquid along with edema of the lungs, a condition which is fatal.[25] The first experimental reproduction of PPO was by Harrison *et al*.,[25,118] who conducted a feeding and intravenous injection study, which confirmed the ability of fumonisins to cause PPO. They also found pancreatic lesions and changes in liver condition in all pigs exposed to fumonisins.[25,118] Confirmation of these results came from a detailed study conducted by Haschek *et al*.[119] Another study by Ross *et al*.[114] who analysed the concentrations of fumonisins in feeds associated with field cases of PPO found the fumonisin

[113] Voss, K.A.; Smith, G.W.; Haschek, W.M. *Anim. Feed Sci. Technol.* **2007**, *137*, 299.
[114] Ross, P.F.; Rice, L.G.; Plattner, R.D.; Osweiler, G.D.; Wilson, T.M.; Owens, D.L.; Nelson, H.A.; Richard, J.L. *Mycopathologia* **1991**, *114*, 129.
[115] Ross, P.F.; Rice, L.G.; Osweiler, G.D.; Nelson, P.E.; Richard, J.L.; Wilson, T.M. *Mycopathologia* **1992**, *117*, 109.
[116] Ross, P.F.; Ledet, A.E.; Owens, D.L.; Rice, L.G.; Nelson, H.A.; Osweiler, G.D.; Wilson, T.M. *J. Vet. Diagn. Invest.* **1993**, *5*, 69.
[117] Smith, G.W.; Constable, P.D.; Foreman, J.H.; Eppley, R.M.; Waggoner, A.L.; Tumbleson, M.E.; Haschek, W.M. *Am. J. Vet. Res.* **2002**, *63*, 538.
[118] Colvin, B.M.; Harrison, L.R. *Mycopathologia* **1992**, *117*, 79.
[119] Haschek, W.M.; Motelin, G.; Ness, D.K.; Harlin, K.D.; Hall, W.F.; Vesonder, R.F.; Peterson, R.E.; Beasley, V.R. *Mycopathologia* **1992**, *117*, 83.

levels ranged from 1-330 µg/g over 83 samples. Exposure to fumonisins has also been found to result in some sows aborting within a few days of onset of clinical symptoms, while loss of appetite is a common occurrence.[25,115] The short-term ingestion of fumonisins has also been shown to result in decreased left-ventricular contractility of the heart along with a reduced mechanical efficiency in swine, a finding which Constable *et al*.[120] used to suggest that PPO was a result of sphingosine-mediated left-sided heart failure.[120] The distribution of fumonisins in the body of pigs was found to be as follows : liver >> kidney > large intestine > brain > lung, heart, adrenal gland, spleen.[121] It has been found that pigs develop lethal PPO within 4-7 days at fumonisin concentrations of ≥16 mg/kg body weight per day, which is more than what is required to cause ELEM.[121] Reports of immunosuppression with a reduction in phagocytosis of particulates and bacteria have also been published.[121] Research shows that orally ingested fumonisins are poorly absorbed from the intestinal tract and are mainly excreted in faeces and urine, however that which is absorbed tends to accumulate in the liver and kidneys.[113]

The majority of studies on the effects of fumonisins have been done on rats and mice. Initially in 1984[21] it was known that consumption of the fungus *F. verticillioides* was highly toxic resulting in ELEM and hepatocarcinogenicity in rats; however, the causative metabolite (fumonisins) was only identified in 1988. One of the first studies done was by Gelderblom *et al*.,[23] with the aim of proving that fumonisins were responsible for the hepatocarcinogenicity observed. Their approach involved male BD IX rats which were given an initiation treatment followed by incorporation of fumonisins into the diet, which resulted in weight loss, toxic hepatitis, necrosis and cancer promotion when compared to the control group, confirming the hepatocarcinogenicity of fumonisins in rats.[23] These results were confirmed by Voss *et al*.[26] who fed maize samples that were associated with field cases of ELEM to male Sprague-Dawley rats in a short-term feeding study, which also resulted in hepatotoxicity and renal toxicity. They also determined that serum chemical and liver histopathological examinations could be used to examine *F. verticillioides* culture material for the presence of hepatotoxins in feeding studies.[26,122]

Primary rat hepatocytes were used in a study by Norred *et al*.[123] to determine the effects of fumonisins (complete and hydrolysed forms) on sphingolipid biosynthesis by measuring the

[120] Constable, P.D.; Smith, G.W.; Rottinghaus, G.E.; Haschek, W.M. *Toxicol. Appl. Pharmacol.* **2000**, *162*, 151.
[121] Haschek, W.M.; Gumprecht, L.A.; Smith, G.; Tumbleson, M.E.; Constable, P.D. *Environ. Health Perspect.* **2001**, *109*, 251.
[122] Voss, K.A.; Plattner, R.D.; Bacon, C.W.; Norred, W.P. *Mycopathologia* **1990**, *112*, 81.
[123] Norred, W.P.; Wang, E.; Yoo, H.; Riley, R.T.; Merrill Jr, A.H. *Mycopathologia* **1992**, *117*, 73.

incorporation of radiolabelled serine into sphingosine. The results showed that the fumonisins were potent inhibitors of sphingolipid biosynthesis with an IC_{50} of 0.1 μM. They also determined the site of inhibition as the enzyme ceramide synthase, as no effect was observed on other biosynthetic pathways.[123] Gelderblom *et al*.[124] investigated the structure-activity relationships (SAR) of fumonisins using male Fischer rats in a feeding study followed by analysis of the primary hepatocytes, leading to the conclusion that the free amino group was necessary for cytotoxicity, a study that became the basis for future investigations into SAR. Further studies found that fumonisins are poorly absorbed by the intestines and that they are rapidly excreted, mostly in faeces and some in urine, although there is noticeable accumulation in the liver and kidneys.[125,126] Effects on pregnant rats and mice have also been examined and fumonisins have been found not to be teratogenic, but are embryotoxic at higher doses, resulting in skeletal anomalies.[127,128,129] Female rats appear to be more sensitive to the effects of fumonisins than male rats, while in mice the main target organ is also the liver with females also being more sensitive than males.[24] Additionally the exposure of mouse embryos to fumonisins resulted in neural tube defects as a result of inhibited folate uptake due to the effect of fumonisins on the sphingolipids regulating this pathway.[130,131]

The effects of fumonisins on other animals has also been investigated. Adult ruminants exhibit no toxic effects when exposed to fumonisins and absorption of fumonisins from the intestines has been shown to be minimal,[132] while intravenous doses of fumonisins are rapidly cleared from the plasma.[133] However, fumonisins have been found to be hepatotoxic and nephrotoxic to calves in high-dose short-term studies[134] as well as in low-dose long-term studies.[135] Feeding studies conducted on lamb and goat kids gave similar results to those obtained for calves,[136]

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[124] Gelderblom, W.C.A.; Cawood, M.E.; Snyman, S.D.; Vleggaar, R.; Marasas, W.F.O. *Food Chem. Toxic.* **1993**, *31*, 407.
[125] Norred, W.P.; Plattner, R.D.; Chamberlain, W.J. *Nat. Toxins* **1993**, *1*, 341.
[126] Shephard, G.S.; Thiel, P.G.; Sydenham, E.W.; Alberts, J.F. *Food Chem. Toxicol.* **1994**, *32*, 489.
[127] Collins, T.F.X.; Shackelford, M.E.; Sprando, R.L.; Black, T.N.; LaBorde, J.B.; Hansen, D.K.; Eppley, R.M.; Trucksess, M.W.; Howard, P.C.; Bryant, M.A. *Food Chem. Toxicol.* **1998**, *36*, 697.
[128] Collins, T.F.X.; Sprando, R.L.; Black, T.N.; Shackelford, M.E.; Laborde, J.B.; Hansen, D.K.; Eppley, R.M.; Trucksess, M.W.; Howard, P.C.; Bryant, M.A. *Food Chem. Toxicol.* **1998**, *36*, 673.
[129] Reddy, R.V.; Johnson, G.; Rottinghaus, G.E.; Casteel, S.W.; Reddy, C.S. *Mycopathologia* **1996**, *134,* 161.
[130] Sadler, T.W.; Merrill Jr, A.H.; Stevens, V.L.; Sullards, M.C.; Wang, E.; Wang, P. *Teratology* **2002**, *66*, 169.
[131] Gelineau-van Waes, J.; Starr, L.; Maddox, J.; Aleman, F.; Voss, K.A.; Wilberding, J.; Riley, R.T. *Birth Defects Res. (Part A). Clin. Mol. Teratol.* **2005**, *73*, 487.
[132] Smith, J.; Thakur, R. Occurrence and fate of fumonisins in beef in Fumonisins in Food, Jackson, L. (ed), **1996**, Plenum Press, New York. pp. 39.
[133] Prelusky, D.B.; Savard, M.E.; Trenholm, H.L. *Nat. Toxins.* **1995**, *3*, 389.
[134] Mathur, S.; Constable, P.D.; Eppley, R.M.; Waggoner, A.L.; Tumbleson, M.E.; Haschek, W.M. *Toxicol. Sci.* **2001**, *60*, 385.
[135] Osweiler, G.D.; Kehrli, M.E.; Stabel, J.R.; Thurston, J.R.; Ross, P.F.; Wilson, T.M. *J. Anim. Sci.* **1993**, *71*, 459.
[136] Edrington, T.S.; Kamps-Holtzapple, C.; Harvey, R.B.; Kubena, L.F.; Elissalde, M.H.; Rottinghaus, G.E. *J. Anim. Sci.* **1995**, *73*, 508.

indicating that fumonisins were potentially toxic only to young animals. In poultry fumonisins administered to laying hens were rapidly cleared from the plasma and excreted with only small amounts detectable in livers and kidneys,[137] while broiler chicks exposed to fumonisins in a feeding study exhibited hepatic necrosis along with other side effects,[138] which was confirmed by a second study.[139] The same toxic effects were found in turkey poults as identified for broiler chicks,[140] while studies on ducks resulted in the identification of hepatic injury following feeding studies.[141,142] Marasas *et al*.[143] determined that *F. verticillioides* culture material was highly toxic to ducklings and this was confirmed by several other studies.[144,145] Studies have also been conducted on fish, primarily on adult channel catfish, carp and trout with observed hematologic changes and reduced resistance to bacterial infection although there is disagreement about neurotoxicity.[146,147,148]

Possibly the most relevant studies to humans are those which have been conducted on primates as these studies give a good idea as to the potential effects of fumonisins on humans. Initial studies were conducted on baboons by Kriek *et al*.,[30] who found acute congestive heart failure as well as liver cirrhosis in some animals. A study was then conducted by Jaskiewicz *et al*.[149] in which vervet monkeys were fed diets contaminated with *F. verticillioides*, which found that the monkeys developed hepatitis indicating that fumonisins could potentially contribute to human liver disease.[149] These results were confirmed by a later study, also on vervet monkeys, done by Fincham *et al*.[150] which found hepatotoxic effects after chronic feeding. Males appeared to be more sensitive than females, with the threshold for chronic liver damage being in the range of 0.10-0.18 mg FB/kg bw/day.[149]

Thus far the effects of fumonisins on humans are inconclusive as causality between fumonisin consumption and the occurrence of human disease has not been proven conclusively,

[137] Vudathala, D.K.; Prelusky, D.B.; Ayroud, M.; Trenholm, H.L.; Miller, J.D. *Nat. Toxins.* **1994**, *2*, 81.
[138] Ledoux, D.R.; Brown, T.P.; Weibking, T.S.; Rottinghaus G.E. *J. Vet. Diagn. Invest.* **1992**, *4*, 330.
[139] Weibking, T.S.; Ledoux, D.R.; Bermudez, A.J.; Turk, J.R.; Rottinghaus, G.E. *Poultry Sci.* **1993**, *72*, 456.
[140] Weibking, T.S.; Ledoux, D.R.; Brown, T.P.; Rottinghaus G.E. *J. Vet. Diagn. Invest.* **1993**, *5*, 75.
[141] Tardieu, D.; Bailly, J.D.; Benard, G.; Tran, T.S.; Guerre, P. *Poultry Sci.* **2004**, *83*, 1287.
[142] Bailly, J.D.; Benard, G.; Jouglar, J.Y.; Durand, S.; Guerre, P. *Toxicology* **2001**, *163*, 11.
[143] Marasas, W.F.O.; Kriek, N.P.J.; Wiggins, V.M.; Steyn, P.S.; Towers, D.K.; Hastie, T.J. *Phytopathology* **1979**, *69*, 1181.
[144] Jeschke, N.; Nelson, P.E.; Marasas, W.F.O. *Poultry Sci.* **1987**, *66*, 1619.
[145] Thiel, P.G.; Shephard, G.S.; Sydenham, E.W.; Marasas, W.F.O.; Nelson, P.E.; Wilson, T.M. *J. Agric. Food Chem.* **1991**, *39*, 109.
[146] Lumlertdacha, S.; Lovell, R.T. *J. Aquat. Anim. Health.* **1995**, *7*, 1.
[147] Lumlertdacha, S.; Lovell, R.T.; Shelby, R.A.; Lenz, S.D.; Kemppainen, B.W. *Aquaculture* **1995**, *130*, 201.
[148] Kovačić, S.; Pepeljnjak, S.; Petrinec, Z.; Šegvić Klarić, M. *Arhiv za Higijenu Rada i Toksikologiju* **2009**, *60*, 419.
[149] Jaskiewicz, K.; Marasas, W.F.O.; Talgaard, J.J.F. *J. Comp. Path.* **1987**, *97*, 281.
[150] Fincham, J.E.; Marasas, W.F.O.; Taljaard, J.J.F.; Kriek, N.P.; Badenhorst, C.J.; Gelderblom, W.C.A.; Seier, J.V.; Smuts, C.M.; Faber, M.; Weight, M.J. *Atherosclerosis* **1992**, *94*, 13.

although fumonisins are suspected to be involved in oesophageal[27] and liver[151] cancers as well as neural tube defects[131] and cardiovascular problems[150] amongst populations where the staple food is maize. Epidemiologic studies have found a correlation between the consumption of maize contaminated with *F. verticillioides* and the occurrence of oesophageal cancer in the Transkei area of South Africa,[27,152,153] as well as in countries such as China,[28,81] Iran,[154,83] Italy,[83] Kenya,[83] Zimbabwe,[83] United States[83] and Brazil.[83] Similarly a correlation between the consumption of contaminated maize and the occurrence of primary liver cancer (PLC) in China was found although fumonisins could not be specifically linked to PLC as it co-occurred with a number of other fungal toxins.[151] A study by Tolleson *et al*.[155] using human keratinocytes, fibroblasts, oesophageal epithelial and hepatoma cells found that FB_1 had an anti-proliferative and apoptotic effect on these cell lines, meaning that FB_1 had the ability to inhibit growth of cells in culture. The tumour promoting activity of fumonisins was thus determined to be of the non-TPA-type as protein kinase C activity was not stimulated but decreased, however further experiments were required.[155] Another epidemiologic study amongst people where the staple food was maize was conducted in which the correlation between *F. verticillioides* consumption and the occurrence of neural tube defects (NTD's) was investigated. It was found that typically amongst the black population there was a lower rate of NTD's than among Hispanic and non-Hispanic whites. The rate of NTD's amongst the black population in the Transkei region of South Africa was 10 times higher than for the black population in Cape Town.[156,157] A similar trend, concerning rural vs. urban dwellers, has been reported in China[158] and South Texas.[159] Gelineau-van Waes *et al*.[131] used pregnant LM/Bc mice injected with different doses of fumonisins, followed by examination of the foetuses to evaluate the risk of NTD's when there is maternal exposure to fumonisins. The results obtained indicated a dose-dependent increase in the number of NTD's in the exposed offspring. It was hypothesised that fumonisin induced inhibition of sphingolipid biosynthesis results in sphingolipid depletion which results in inhibited vitamin and folate uptake by the glycosylphosphatidylinositol (GPI)-anchored folate

[151] Ueno, Y.; Iijima, K.; Wangi, S.D.; Sugiura, Y.; Sekijima, M.; Tanaka, T.; Chen, C.; Yu, S.-Z. *Food Chem. Toxicol.* **1997**, *35*, 1143.
[152] Sydenham, E.W.; Thiel, P.G.; Marasas, W.F.O.; Shephard, G.S.; Van Schalkwyk, D.J.; Koch, K.R. *J. Agric. Food Chem.* **1990**, *38*, 1900.
[153] Rheeder, J.P.; Marasas, W.F.O.; Thiel, P.G.; Sydenham, E.W.; Shephard, G.S.; van Schalkwyk, D.J. *Phytopathology* **1992**, *82*, 353.
[154] Shephard, G.S.; Marasas, W.F.O.; Leggott, N.L.; Yazdanpanah, H.; Rahimian, H.; Safavi, N. *J. Agric. Food Chem.* **2000**, *48*, 1860.
[155] Tolleson, W.H.; Melchior Jr, W.B.; Domon, O.E.; Muskhelishvili, L.; James, S.J.; Howard, P.C. *Carcinogenesis* **1996**, *17*, 239.
[156] Ncayiyana, D.J. *S. Afr. Med. J.* **1986**, *69*, 618.
[157] Cornell, J.; Nelson, M.M.; Beighton, P. *S. Afr. Med. J.* **1983**, *64*, 83.
[158] Moore, C.A.; Li, S.; Li, Z.; Hong, S.X.; Gu, H.Q.; Berry, R.J.; Mulinare, J.; Erickson, J.D. *Am. J. Med. Genet.* **1997**, *73*, 113.
[159] Hendricks, K. *Epidemiology* **1999**, *10*, 198.

receptor thus compromising biological processes dependent on folate such as the development and closing of the neural tube in the fetus.[131]

In plants *F. verticillioides* grows over the pericarp of kernels and is also located within the kernel away from the vascular bundles. The fungus results in stalk, root and ear rot of maize which results in huge economic losses agriculturally throughout the world, while fumonisin mycotoxins do not appear to have any adverse effects on the maize plants.[160] Fumonisins have been shown to be a virulence factor in jimsonweed[161] and duckweed[162] resulting in wilt and necrotic lesions on the stems and leaves. Fumonisins also disrupt sphingolipid biosynthesis in plants by inhibiting the enzyme ceramide synthase resulting in the accumulation of free sphinganine and free phytosphinganine.[162] A study done by Gutiérrez-Nájera *et al*.[163] showed that FB_1 was a potent inhibitor of H^+-ATPase in the plasma membrane of maize embryos via an uncompetitive inhibitory mechanism that resulted in an increase in membrane fluidity along with ceramide synthase inhibition.

Abbas *et al*.[164] then chose to do phytotoxicity and cytotoxicity studies using the C- and P-series of fumonisins which had not been done previously by any group. They found that the C-series of fumonisins were as phytotoxic to duckweed as FB_1, with FC_3 and FC_4 being only slightly less toxic at a concentration of 1 µM, while the P-series became phytotoxic to duckweed at concentrations of 50 µM. The cytotoxicity of the C and P series fumonisins was then examined using H4TG and MDCK cell lines, with the C-series showing cytotoxicity at concentrations similar to FB_1 (10-25 µM), with FC_3 and FC_4 again being slightly less cytotoxic. The P-series fumonisins were only cytotoxic to the H4TG cell line and were much less cytotoxic than FB_1. This study indicates that the C-series of fumonisins should be considered as important as the B-series in terms of toxicity and should not be ignored due to the small amounts detected.[164]

The primary mechanisms of fumonisin induced toxicity are thus oxidative stress, apoptosis, cytotoxic and immunotoxic effects which can potentially affect several different pathways. Oxidative stress is a result of the production of reactive oxygen species (ROS),[24] which were found to increase in human and rat glioblastoma cells and in mouse hypothalamic cells in

[160] Bacon, C.W.; Williamson, J.W. *Mycopathologia* **1992**, *117*, 65.
[161] Abbas, H.K.; Paul, R.N.; Boyette, C.D.; Duke, S.O. *Can. J. Bot.* **1992**, *70*, 1824.
[162] Abbas, H.K.; Tanaka, T.; Duke, S.O.; , J.K.; Wray, E.M.; Hodges, L.; Sessions, A.E.; Wang, E.; Merrill Jr, A.H.; Riley, R.T. *Plant Physiol.* **1994**, *106*, 1085.
[163] Gutiérrez-Nájera, N.; Muñoz-Clares, R.A.; Palacios-Bahena, S.; J.; Sanchez-Nieto, S.; Plasencia, J.; Gavilanes-Ruiz, M. *Planta* **2005**, *221*, 589.
[164] Abbas, H.K.; Shier, W.T.; Seo, J.A.; Lee, Y.W.; Musser, S.M. *Toxicon* **1998**, *36*, 2033.

studies done by Stockmann-Juvala *et al*.[165,166] A study done by Yin *et al*.[167] found that FB_1 was able to stimulate an increase in the oxidation rate and so promote the formation of ROS along with increasing the rate of chain reactions at the lipid peroxidation membranes. Conversely another study showed no change in the production of ROS[168] indicating that more studies are required in this field. While apoptosis has been demonstrated for a range of different cell types, it has also been proven that FB_1 induction of apoptosis is not due to inhibition of ceramide synthase alone,[169] but that FB_1 initiates apoptosis by binding to the TNF (tumour necrosis factor) receptors.[170] Immunological effects have been observed in several studies where the cytokine profile in different cell types and organs was monitored for changes resulting in weaker immune responses.[171,172,173] However, since the immune response consists of numerous chain reactions further studies are required to better understand the effect of fumonisins on the immune system.[24]

As a result of all the studies done, along with the wide range of toxicities displayed by fumonisins, the International Agency for Research on Cancer (IARC) in 2000 classified FB_1 as a group 2B compound, that is, as a possible human carcinogen [131] While in 2001, the Joint Food and Agricultural Organisation of the United Nations (FAO)/WHO Expert Committee on Food Additives (JECFA) put forward a level of 2 µg/kg bw for the maximum tolerable daily intake of fumonisins.[174] Most developed countries have regulations governing FB concentrations in foodstuffs in place but in less developed countries where maize is a staple food for a large part of the population the regulation of FB concentrations is either not enforced or is completely non-existent.[89]

1.2.4.4. Diagnosis, Treatment and Prevention of Toxicosis and Control of Fumonisins

The diagnosis of fumonisin toxicosis is currently based on a history of consumption of

[165] Stockmann-Juvala, H.; Mikkola, J.; Naarala, J.; Loikkanen, J.; Elovaara, E.; Savolainen, K. *Free Radic. Res.* **2004**, *38*, 933.
[166] Stockmann-Juvala, H.; Mikkola, J.; Naarala, J.; Loikkanen, J.; Elovaara, E.; Savolainen, K. *Toxicology* **2004**, *202*, 173.
[167] Yin, J.-J.; Smith, M.J.; Eppley, R.M.; Page, S.W.; Sphon, J.A. *Biochem. Biophys. Acta.* **1998**, *1371*, 134.
[168] Galvano, F.; Russo, A.; Cardile, V.; Galvano, G.; Vanella, A.; Renis, M. *Food Chem. Toxicol.* **2002**, *40*, 25.
[169] Seefelder, W.; Humpf, H.-U.; Schwerdt, R.; Freudinger, R.; Gekle, M. *Toxicol. Appl. Pharmacol.* **2003**, *192*, 146.
[170] Ciacci-Zanella, J.R.; Merrill Jr, A.H.; Wang, E.; Jones, C. *Food Chem. Toxicol.* **1998**, *36*, 791.
[171] Bhandari, N.; Sharma, R.P. *Toxicology* **2002**, *172*, 81.
[172] Stockmann-Juvala, H.; Alenius, H.; Savolainen, K. *Food Chem. Toxicol.* **2008**, *46*, 1444.
[173] Halloy, D.J.; Gustin, P.G.; Bouchet, S.; Oswald, I.P. *Toxicology* **2005**, *213*, 34.
[174] Bolger, M.C.; Coker, R.D.; DiNovi, M.; Gaylor, D.; Gelderblom, W.C.A; Olsen, M.; Paster, N.; Riley, R.T.; Shephard, G.S.; Speijers, G.J.A. Fumonisins. In : Safety evaluation of certain mycotoxins in food. Food and Agriculture Organisation of the United Nations, World Health Organisation. Geneva, Food Additives, Series 47. **2001**, *74*, 103.

contaminated maize along with the identification of characteristic clinical symptoms. Generally the detection of fumonisins in feed at levels greater than 10 ppm is considered potentially toxic.[113] However, the analysis of feed is often not indicative of toxicosis as the feed sample submitted for analysis is often from a different batch to that which caused disease.[113] Thus the levels of the ratio sphinganine:sphingosine are considered to be a much better biomarker for exposure to fumonisins as an increase in ratios in tissues and body fluids exhibits a dose and time dependent relationship.[175] However, all studies to date indicate that this is only applicable to animals and not to humans. In humans FB_1 does not appear to alter this ratio enough to be considered a useful biomarker for exposure.[176,177]

Since there is no therapy available for fumonisin toxicosis, the principle of prevention is better than cure applies, which is why strict guidance levels have been implemented for animal feeds and human consumption. The levels stipulated for feeds vary according to species sensitivity to fumonisins although the margins set are potentially too narrow.[113] The FDA has set a maximum level of 5 ppm total fumonisins for horse feed and 20 ppm total fumonisins for pig feed, while for most other animals the level is set at 30 ppm or more total fumonisins.[113] For humans the recommended maximum level of fumonisin intake is 2 µg/kg bw/day.[174]

The control of fumonisin levels in maize requires more attention to pre-harvest procedures as well as subsequent food preparation and processing. Controls at the pre-harvest level include any agricultural practices which can reduce crop stress such as pest control and improved irrigation.[89] Fumonisins are moderately heat stable compounds,[36] thus food processing procedures such as sorting can lead to reduced exposure, while the method of food preparation can also limit fumonisins in food. Certain processing methods can however result in the conversion of intact fumonisins into their hydrolysed forms resulting in increased human exposure due to the hydrolysed forms having a broader range of activities.[36]

The dry-milling of maize results in two main fractions – the fraction used for human consumable products having little or no detectable fumonisins and the bran fraction used mainly in animal feeds which contains the bulk of fumonisin contamination. For wet-milling a similar trend was observed where fumonisins were detected in the by-products destined for

[175] Shephard, G.S.; van der Westhuizen, L.; Sewram, V. *Food Addit. Contam.* **2007**, *24*, 1196.
[176] van der Westhuizen, L.; Brown, N.L.; Marasas, W.F.O.; Swanevelder, S.; Shephard, G.S. *Food Chem. Toxicol.* **1999**, *37*, 1153.
[177] Solfrizzo, M.; Chulze, S.N.; Mallmann, C.; Visconti, A.; De Girolamo, A.; Rojo, F.; Torres, A. *Food Addit. Contam.* **2004**, *21*, 1090.

animal feeds, but not in the products destined for human consumption.[178] The process of nixtamalisation used to produce masa and tortillas, where the maize is cooked and steeped overnight in an alkaline solution resulted in a substantial reduction of fumonisins detected in the rinsed nixtamal with most of the fumonisins being extracted into the alkaline solution. However, with time the fumonisins are converted into hydrolysed fumonisins within the alkaline solution which are also washed away during rinsing. As such it was found that relatively little hydrolysed fumonisins could be detected in the end products (masa and tortillas).[178] Cooking and baking was found to have very little to no effect on the levels of fumonisins while canning resulted in a small reduction in fumonisin levels and extrusion (where cornmeal is subjected to high pressures, temperatures and mechanical shearing) showed the greatest reduction in fumonisin levels.[178] However, no truly effective process for the complete detoxification of fumonisins has been developed at this stage.

Methods of biological control in the field (pre-harvest) were investigated using the biocontrol bacterium *B. subtilis* RRC101.[179] It was shown that the bacterium was randomly distributed intercellularly around the plant and had plant enhancing characteristics as well as the ability to protect the plant from pathogenic organisms while no signs of damage caused by the bacterium could be observed. Further studies demonstrated that *B. subtilis* RRC101 had the ability to inhibit the colonisation and growth of *F. verticillioides* on maize plants with a resultant decrease in the amount of fumonisins produced even under conditions of plant stress.[179] Studies using *Trichoderma spp* for post-harvest control were also done with positive signs of inhibition of *Fusarium spp* growth and inhibition of fumonisin production.[179] The genetic modification of maize is another avenue of research that is being explored for the reduction of fumonisin contamination. Although a substantial amount of research is still required in this field the potential for transgene mediated fungal resistance is slowly becoming a reality.[180]

1.2.5. Biosynthesis

The biosynthesis of fumonisins is a topic of high importance in terms of the pathways involved as well as the mechanisms that regulate production of fumonisins and the modes of action. Numerous biosynthetic studies have been carried out on fumonisins using isotopically labelled

[178] Saunders, D.S.; Meredith, F.I.; Voss, K.A. *Environ. Health Perspect.* **2001**, *109*, 333.
[179] Bacon, C.W.; Yates, I.E.; Hinton, D.M.; Meredith, F.I. *Environ. Health Perspect.* **2001**, *109*, 325.
[180] Duvick, J. *Environ. Health Perspect.* **2001**, *109*, 337.

compounds in solid and liquid media. Two possible routes of biosynthesis have been proposed – the polyketide biosynthetic pathway and the fatty acid biosynthetic pathway, both of which are known to make use of the C_2 building block, acetate. Studies done up till now have managed to elucidate the origins of most of the atoms constituting FB_1.

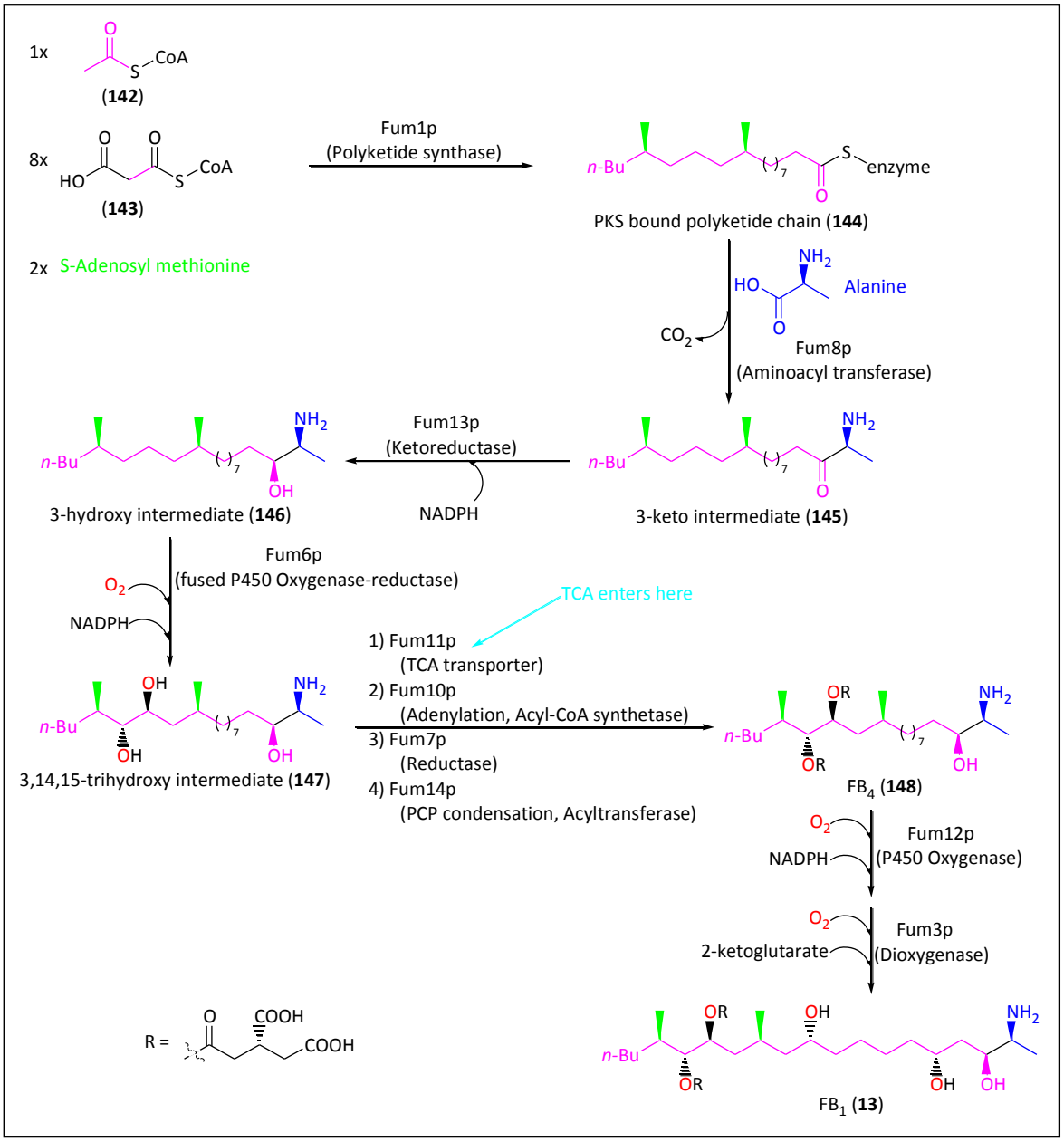
The first study done determined the source of the C-12 and C-16 methyl groups as being L-methionine in an experiment where *F. verticillioides* cultures were incubated in medium containing [*methyl-d*$_3$]-L-methionine, which resulted in incorporation of exactly 6 or 3 deuterium atoms, with the positions of incorporation confirmed by EI (electron ionisation) spectra and NMR spectra.[181] These results were confirmed by Alberts *et al*.[182] using [methyl-^{14}C]-L-methionine. Experiments conducted by Branham *et al*.[183] demonstrated that L-alanine was the source of C-1, C-2 and the amino group on C-2 of the fumonisin backbone using [^{2}H,^{13}C]-L-alanine.

The C-3–C-20 part of the backbone was found to be derived from acetate in an experiment where *F. verticillioides* cultures were incubated in mediums containing [1-^{13}C]-, [2-^{13}C]- and [1,2-^{13}C]-acetate, resulting in FB_1 with a ^{13}C enriched backbone.[184] Blackwell *et al*.[184] also came to the conclusion that the fumonisin backbone was of polyketide origin. Studies using labelled oxygen ($^{18}O_2$) indicated that molecular oxygen is the source of the C-5, C-10, C-14 and C-15 hydroxy oxygen atoms while studies using $H_2{}^{18}O$ demonstrated that water was the source of oxygens for the TCA side chains.[185] The source of the C-3 hydroxy group was shown to be an acetate derived carbonyl group.[184] The source of the TCA esters has not been determined conclusively but is thought to come from the citric acid (Krebs) cycle.[186]

Proof that fumonisins are biosynthesised via a polyketide synthesis pathway came from studies in which disruption of the *FUM1* (previously *FUM5*) gene resulted in a disruption of fumonisin production. The biosynthesis (shown in **Scheme16**) starts with the formation of the

[181] Plattner, R.D.; Shackelford, D.D. *Mycopathologia* **1992**, *117*, 17.
[182] Alberts, J.F.; Gelderblom, W.C.A.; Vleggaar, R.; Marasas, W.F.O.; Rheeder, J.P. *Appl. Environ. Microbiol.* **1993**, *59*, 2673.
[183] Branham, B.E.; Plattner, R.D. *Mycopathologia* **1993**, *124*, 99.
[184] Blackwell, B.A.; Miller, J.D.; Savard, M.E. *J. AOAC Int.* **1994**, *77*, 506.
[185] Caldas, E.D.; Sadilkova, D; Ward, B.L.; Jones, A.D.; Winter, C.K.; Gilchrist, D.G. *J. Agric. Food Chem.* **1998**, *46*, 4734.
[186] Zaleta-Rivera, K.; Xu, C.; Yu, F.; , R.A.E.; Proctor, R.H.; Hidalgo-Lara, M.E.; Raza, A.; Dussault, P.H.; Du, L. *Biochemistry* **2006**, *45*, 2561.

C_{18} polyketide chain catalysed by the highly reducing Fum1p polyketide synthase (coded for by the *FUM1* gene in the *FUM* cluster) to give the enzyme-bound polyketide chain (**144**).[187]



Scheme 16: The biosynthesis of fumonisin B_1 showing the origin of all the components.[188]

This is followed by carbon-carbon bond formation between L-alanine and **144** catalysed by the pyridoxal-phosphate dependent Fum8p aminoacyltransferase enzyme (coded by the *FUM8* gene) to obtain the 3-keto intermediate (**145**).[189] This enzyme is also responsible for the

[187] Proctor, R.H.; Desjardins, A.E.; Plattner, R.D.; Hohn, T.M. *Fungal Genet. Biol.* **1999**, *27*, 100.
[188] Du, L.; Zhu, X.; Gerber, R.; Huffman, J.; Lou, L.; Jorgenson, J.; Yu, F.; Zaleta-Rivera, K.; Wang, Q. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 455.
[189] Seo, J.-A.; Proctor, R.H.; Plattner, R.D. *Fungal Genet. Biol.* **2001**, *34*, 155.

release of the C_{18} polyketide chain and this is thought to occur by a nucleophilic attack on the carbonyl carbon of the polyketide acyl chain by a carbanion derived from the α-carbon of alanine via decarboxylation.[190,191] The *FUM13* gene produces the Fum13p ketoreductase which stereoselectively reduces the C-3 ketone at the *ReS* face to the alcohol to give the 3-hydroxy intermediate (**146**) having the *syn*-2,3 amino alcohol orientation.[44,192,193]

The hydroxyations and esterifications that follow have been less well studied, but it is thought that the C-14 and C-15 hydroxyations are catalysed by the Fum6p P450 monooxygenase enzyme (coded by the *FUM6* gene), while the C-10 hydroxy group is introduced by the Fum12p P450 monooxygenase (coded for by the *FUM12* gene). This has, however, not been confirmed.[186,190] The esterification of the TCA moieties to the C-14 and C-15 hydroxy groups is thought to catalysed by a nonribosomal peptide synthetase (NRPS) complex comprising Fum11p, Fum10p, Fum7p and Fum14p coded for by *FUM11*, *FUM10*, *FUM7* and *FUM14* genes, respectively.[185,186] Zaleta-Rivera *et al*.[186] proposed a mechanism for the esterification reaction involving 3 steps. The first step is the ATP-dependent Fum10p activation of a tricarboxylate substrate to obtain an acyl-AMP, which is then transferred to the PCP domain of Fum14p. The second step is the condensation between the acyl-S-PCP of Fum14p and HFB_3 or HFB_4 catalysed by the C-domain of Fum14p to give a didehydro intermediate. The double bond of the didehydro intermediate is then reduced by Fum7p to obtain FB_3 or FB_4 (**148**).[185] *FUM11* codes for Fum11p which is thought to function as a tricarboxylate transporter from the inner mitochondria to the cytoplasm. It has been shown that *FUM11* is not necessary for acquiring the TCA moiety but that it greatly enhances the amount of esterification.[194] The final step in the biosynthesis is the C-5 hydroxyation which is catalysed by the 2-ketoglutarate dependent Fum3p dioxygenase (coded by *FUM3* previously known as *FUM9*) resulting in FB_1 (**13**) as the product.[195,196]

The isotope studies have demonstrated that the B-series of fumonisins is biosynthesised via the addition of alanine to the polyketide chain, thus it is hypothesised that the C-series of fumonisins are formed by the addition of glycine. However, for a strain to simultaneously produce both series, the Fum8p aminoacyltransferase must either be able to use both amino

[190] Bojja, R.S.; Cerny, R.L.; Proctor, R.H.; Du, L. *J. Agric. Food Chem.* **2004**, *52*, 2855.
[191] Gerber, R.; Lou, L.; Du, L. *J. Am. Chem. Soc.* **2009**, *131*, 3148.
[192] Butchko, R.A.E.; Plattner, R.D.; Proctor, R.H. *J. Agric. Food Chem.* **2003**, *51*, 3000.
[193] Yi, H.; Bojja, R.S.; Fu, J.; Du, L. *J. Agric. Food Chem.* **2005**, *53*, 5456.
[194] Butchko, R.A.E.; Plattner, R.D.; Proctor, R.H. *J. Agric. Food Chem.* **2006**, *54*, 9398.
[195] Butchko, R.A.E.; Plattner, R.D.; Proctor, R.H. *Appl. Environ. Microbiol.* **2003**, *69*, 6935.
[196] Ding, Y.; Bojja, R.S.; Du, L. *Appl. Environ. Microbiol.* **2004**, *70*, 1931.

acids or there is another form of the enzyme present in the cell, meaning that there would be an additional gene sequence that has not been identified yet.[90]

An understanding of the biosynthetic pathway that results in fumonisin production is important if research efforts towards finding a method to detoxify contaminated food and feed and controlling fumonisin production are to be successful. Although elucidating the biosynthesis of fumonisins has come a long way there is still much that needs to be done before this pathway is fully understood.

1.3. Conclusion

As a result of the fumonisins biological importance and the large number of analogues in this class of toxins there is still a vast amount of research that can be done. Inspection of the structures for the fumonisins that have been elucidated highlights the C-11–C-20 unit which is conserved across all the classes (A, B, C and P), while closer inspection indicates that the absolute stereochemistry along this fragment is also conserved. The majority of variation occurs in the stereochemistry and functional group substitution of the C-1–C-10 fragment and even here in going from class to class, distinct patterns can be observed. The main fumonisins (FB_1 and FB_2) have been completely synthesised, but with some modifications it would be possible to gain access to many of the other analogues across all the classes by synthesising enough of the conserved C-11–C-20 unit and then combining it with the various C-1–C-10 units. The newly synthesised compounds could then be characterised completely followed by comparison of the physical and spectral properties to those of the natural product and in the process obtain confirmation of the absolute configuration of all the stereogenic centres. This would also make material available for structure-activity relationship studies, which would further improve our knowledge of the biological effects and mechanisms of toxicity with the aim of developing an efficient detoxification method.

Work on the synthesis of the conserved C-11–C-20 fragment has already been done previously in our laboratory[197]. An efficient synthesis for the different stereoisomers of the C-1–C-7 unit with a focus on the iso-FC_1 and HO-FC_1 fumonisins, for which there is very little information available, and thereby establishing the absolute configuration of the stereogenic centres in this unit, is the aim of this dissertation.

[197] Thompson, S. Synthetic studies toward the four invariant stereogenic centres of the left side of the backbone of the fumonisins and AAL toxins. M.Sc. Dissertation, University of Pretoria, Pretoria, September 2011.

2. RETROSYNTHESIS

2.1. Introduction

In the early days of organic chemistry the focus was predominantly on *carbogens* (carbon-containing molecules) and the transformations they could undergo. The reactions used were classified according to the types of substrates that underwent a chemical change and the types of reactions and methodology available was limited. As a result of this, any molecules which were deemed more complicated (*e.g.* cholesterol) were considered too difficult for synthesis at the time.[1] At this stage most of the syntheses that were done came from selecting a *starting material* through trial and error based on which material appeared suitable and most closely matched the *target* molecule. A set of reactions was then found which in the end could result in the formation of the target molecule.[1] The method, however, often met with limited success and the necessity to restart the synthesis using different starting materials.

Then in the fall of 1957 the concept of *retrosynthetic* or *antithetic analysis* was developed by E.J. Corey (amongst others) and this concept revolutionised the way that synthesis in organic chemistry was approached as it led to a different way of designing a synthesis.[2] By this method the *target* molecule (TGT) became the *starting point* and was subjected to a process of deconstruction which corresponded to the reverse of the synthetic reaction in order to convert the target structure into much simpler precursor structures, while avoiding any assumptions in terms of starting materials.[1] Each of the precursors thus generated were then subjected to the same process which was repeated until structures were obtained that corresponded to more simple materials. The repetition of this process produces a "tree" of intermediates in which the intermediate structures are representative of nodes and the various pathways correspond to synthetic routes.[2] These trees which are derived from the TGT are often complicated due to the extensive branching that can occur at each node and thus each synthesis ultimately needs to be evaluated individually, based on its merits.[2]

[1] Corey, E.J.; Cheng, X.-M. *The Logic of Chemical Synthesis*, John Wiley & Sons, New York, 1989.
[2] Corey, E.J. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 455.

In retrosynthetic analysis each step has a target structure which contains a key structural feature or *retron*, which will allow for further transformation. The most useful *retrons* are those which can reduce the complexity of the molecule by means of a number of major strategies as proposed by E.J. Corey.[1,2] These strategies include transform-based strategies: the application of a step which greatly simplifies the structure and synthesis as a whole; structure-goal strategies: based on the identification of a potential starting material; topological strategies: the identification of strategic bond disconnections; stereochemical strategies: based on simplification of stereochemistry which arises by transform or substrate control; and functional group-based strategies: the transformation of various functional groups in order to assist the synthetic procedure.[2] A combination of all these strategies is generally applied to a target and this approach to synthesis allowed more complicated molecules to be synthesised and also encouraged the development of new procedures.

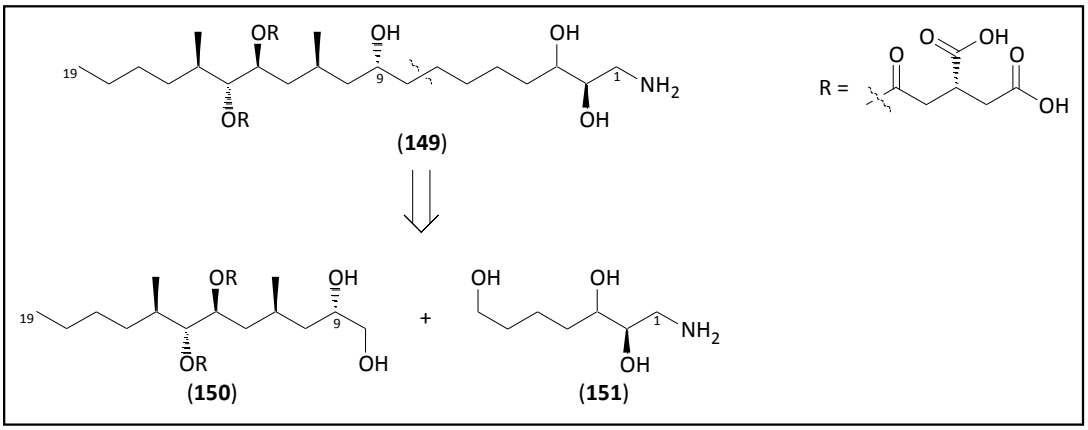
The aim of this dissertation was the identification and implementation of short, stereoselective syntheses of the stereoisomers of the right-hand side of the iso-FC_1 and HO-FC_1 fumonisins which contain two and three stereogenic centres, respectively. The synthesis should also be easily modified in order to give access to the various other classes of fumonisins should it be required. Eventually these right-hand units can be used to determine the absolute configuration of the stereogenic centres of the iso-FC_1 and HO-FC_1 fumonisins, which to date has not been established. Coupling of the right-hand units to the left-hand unit, followed by comparison of the spectroscopic and physical data of the synthetic compound to that of the natural fumonisins would thus allow for the assignment of the absolute configuration of the toxins of unknown stereochemistry. This would also provide a route for the total synthesis of the fumonisin toxins.

2.2. Retrosynthetic analysis of the fumonisins

2.2.1. Disconnection of the Fumonisin Backbone

Analysis of the fumonisin mycotoxin backbone results in the identification of two different units, a left-hand and right hand side. These units are defined by the concentration of stereogenic centres separated by a five-carbon alkyl chain (in the case of iso-FC_1 fumonisin) and the retrosynthetic disconnection of the two units can be done using any bond in this alkyl region. The question was thus which bond should be disconnected, and for strategic reasons the C-7–C-8 bond was chosen. The disconnection at this point allows for the use of various

coupling methodologies which can lead to the unfunctionalised C-9 or hydroxyated C-9 depending on the required target molecule. This disconnection between the C-7–C-8 bond of the fumonisin backbone is an example of a topological strategy and results in retrons **150** and **151** shown in **Scheme 17** using fumonisin iso-FC_1 (**149**) as an example.



Scheme 17: Retrosynthetic analysis of fumonisin iso-FC_1, showing the target (**151**) for the right-side.

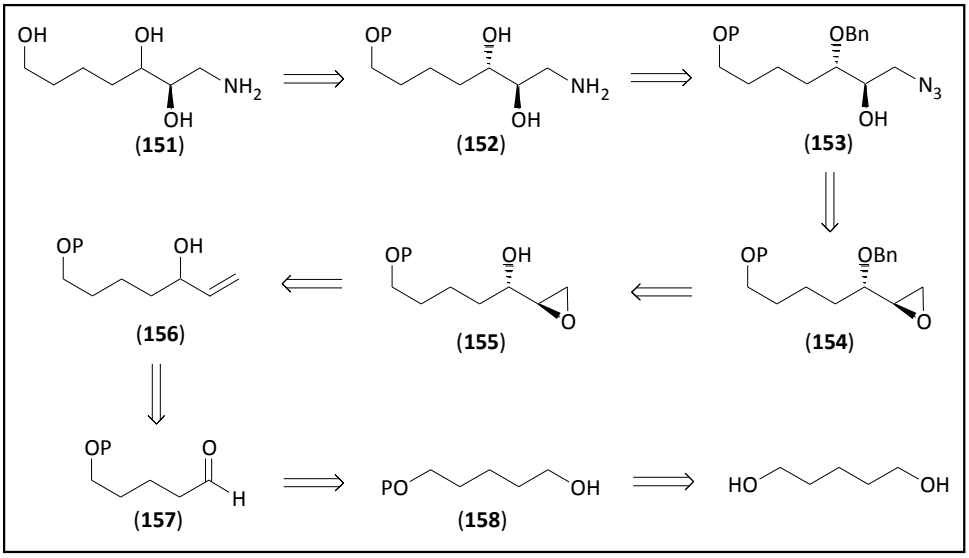
The structure of the left-hand C_{12} unit (**150**) has been found to be invariant among all classes of the fumonisins whereas the right-hand C_7 unit (**151**) exhibits various structural differences which are class dependent. The C_7 unit (**151**) representing the right-hand side of the fumonisin iso-FC_1 backbone was the target of interest for this project and will be analysed further in the next section.

2.2.2. Disconnection of the Right-hand Unit of Iso-FC_1 (151)

On examination of the right-hand unit (**151**) it becomes clear that protecting group strategies will be required in the execution of its synthesis. This is due to the presence of three hydroxy functional groups, one of which is required for the coupling of the left-hand and right-hand units. This terminal hydroxy group requires protection by a group which is stable under a wide range of reaction conditions due to its introduction early on in the synthesis, but which will be easy to remove when required to do so. For this reason the silanyl family of protecting groups was given consideration, as other protecting groups can be introduced and removed without affecting the silanyl protecting group. A wide range of silanyl protecting groups is available to choose from with differing ease of removal and stabilities. For the purpose of this analysis, the TBS protecting group was chosen as it is relatively stable under a range of conditions,

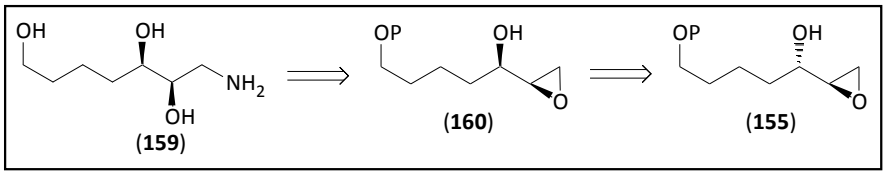
excluding extremely acidic or basic conditions and should not be too difficult to remove by TBAF when required.

The retrosynthetic analysis of the rest of the right-hand unit (**151**) involves a series of functional group transformations (see **Scheme 18**). The protection of the hydroxy group and transformation of the terminal amine (**152**) generates the azide (**153**), which can be obtained from the ring opening reaction of the epoxide (**154**) at C-1 with retention of stereochemistry at C-2. Epoxide (**154**) is obtained from the protection of the C-3 hydroxy group of epoxide (**155**) with a benzyl protecting group. The next step in the analysis is to determine the origin of the epoxide (**155**) in such a way as to have excellent stereochemical control. Chiral *anti*-epoxy alcohols such as (**155**) can be formed using Sharpless epoxidation via the asymmetric kinetic resolution of racemic allylic alcohols which leads to the identification of allylic alcohol (**156**) in the retrosynthetic analysis. The allylic alcohol (**156**) is obtained from the disconnection of the C-2–C-3 bond and involves a Grignard reaction on aldehyde (**157**) using vinyl magnesium bromide. The aldehyde (**157**) can be obtained from the Swern oxidation of the monoprotected 1,5-pentanediol (**158**), which in turn is available as 1,5-pentanediol.



Scheme 18: Retrosynthetic analysis of the C-1–C-7 unit of fumonisin iso-FC_1.

A stereoisomer of (**152**) above can be synthesised in which the configuration of the stereogenic centre at C-3 is inverted to obtain isomer (**159**). The retrosynthetic procedure is the same as that presented in **Scheme 18,** with the exception of the inversion of configuration at C-3 by means of a Mitsunobu reaction on epoxy alcohol (**155**) to obtain epoxy alcohol (**160**) in **Scheme 19**.

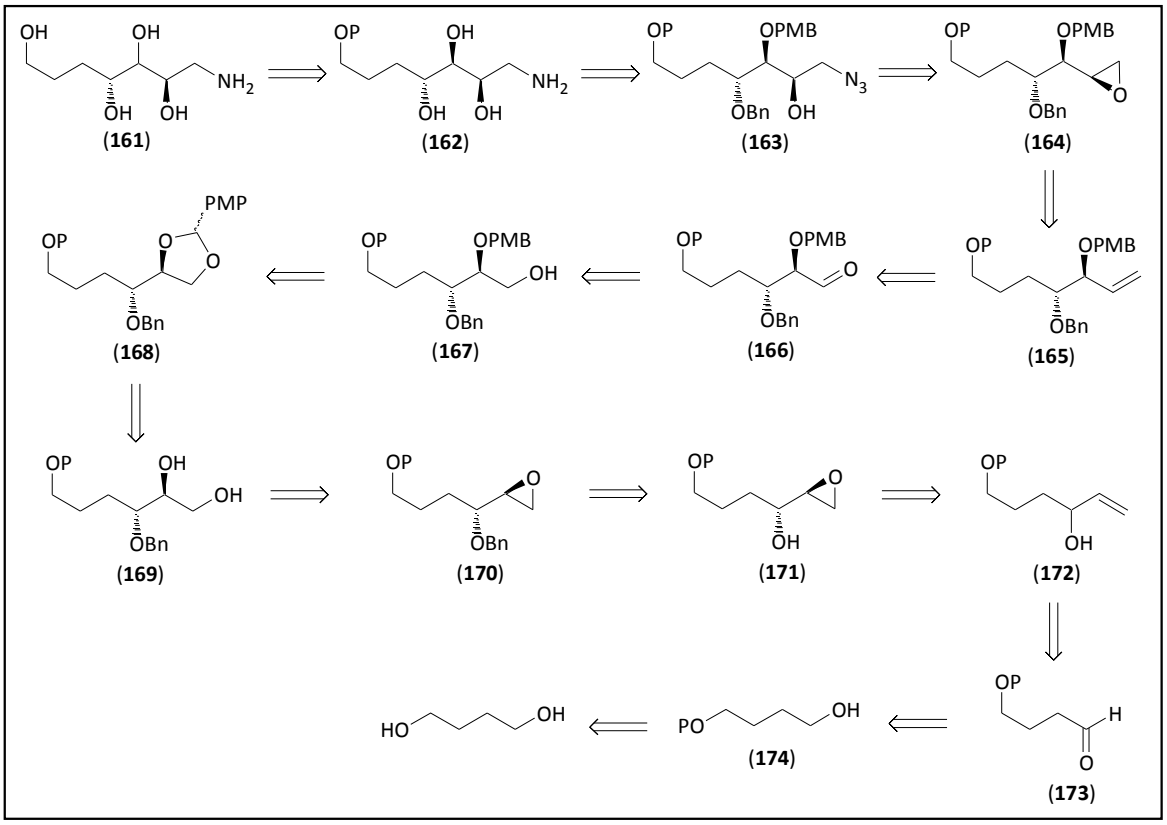


Scheme 19: Inversion of configuration at C-3.

2.2.3. Disconnection of the Right-hand Unit of HO-FC_1 (161)

The retrosynthetic analysis of the HO-FC_1 fumonisin (**161**) is similar to that of iso-FC_1 discussed in section 2.2.2. above, except that HO-FC_1 has an additional stereogenic centre at C-4 of the C_7 unit which complicates matters and indicates the necessity to introduce additional steps. Once again protective group strategies are required as mentioned previously. For this analysis the TBDPS protecting group was chosen as it is more robust than the TBS group and should be able to withstand the even greater range of reaction conditions that will be experienced due to the additional steps required, which includes the addition and removal of other protecting groups as will be discussed shortly.

The retrosynthetic analysis of the rest of the C_7 unit involves a series of functional group transformations (see **Scheme 20).** Protection of the C-3 hydroxy group as a PMB ether and the C-4 hydroxy group as a Bn ether along with transformation of the terminal amine (**162**) generates the protected azide (**163**) which is obtained from the ring opening reaction of epoxide (**164**) at C-1 with retention of stereochemistry at C-2. The origin of the epoxide (**164**) must then be deduced in order to maintain stereochemical control. The chiral *syn* epoxide (**164**) is formed using Jacobson's (*R*,*R*)-salen-Mn(II) catalyst for the epoxidation of an olefin in which the *cis* configuration of the olefin is favoured. This leads to the identification of the alkene (**165**) which is obtained from the disconnection of the C-1–C-2 bond and involves a Wittig reaction on aldehyde (**166**) using methyltriphenylphosphonium iodide. Aldehyde (**166**) can be obtained from the Swern oxidation of the primary hydroxy group in the C-2 PMB protected ether (**167**) after the PMP protecting group has been selectively opened at the C-1 side of (**168**). **168** is generated by the PMP protection of the 1,2-diol (**169**), which is obtained from the asymmetric ring opening of an epoxide by means of the selective (*S*,*S*)-salen-Co(II) catalyst.



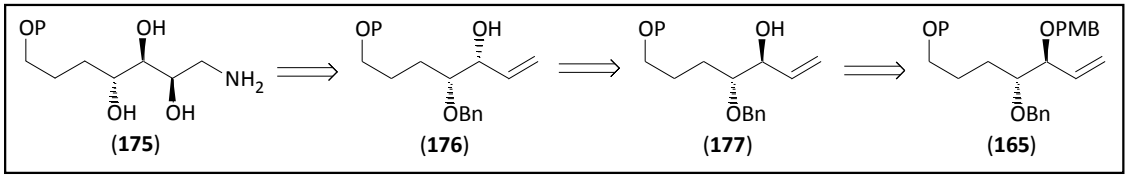
Scheme 20: Retrosynthetic analysis of the C-1–C-7 unit of fumonisin HO-FC_1.

This led to the identification of epoxide (**170**) which is generated from the epoxy alcohol (**171**) by means of protection of the hydroxy group as the benzyl ether and which also serves the purpose of protecting the C-3 hydroxy group during the Swern oxidation reaction to form (**166**) at a later stage.

The chiral *anti* epoxy alcohol (**171**) is formed using Sharpless epoxidation via the asymmetric kinetic resolution of a racemic allylic alcohol, which led to the identification of allylic alcohol (**172**) as substrate. The allylic alcohol (**172**) can be obtained from the disconnection of the C-2–C-3 bond and involves a Grignard reaction on aldehyde (**173**) using vinyl magnesium bromide. The aldehyde (**173**) is obtained from the Swern oxidation of the monoprotected 1,4-butanediol (**174**), which in turn is available as 1,4-butanediol.

Another stereoisomer of (**162**) can be synthesised in which the configuration of the stereogenic centre at C-3 is inverted to obtain isomer (**175**). The retrosynthetic procedure follows that presented in **Scheme 20** above. The inversion of configuration at C-3 is again achieved by means of a Mitsunobu reaction, but this time on the allylic alcohol (**177**) to obtain allylic alcohol (**176**) in **Scheme 21.** Allylic alcohol (**177**) is obtained by the selective

deprotection of the PMB group present in (**165**). The inversion of configuration reaction is another reason for the necessity of the benzyl protecting group for the C-4 hydroxy group in (**165**) in order to prevent the inversion of configuration occurring at C-4 rather than at C-3.



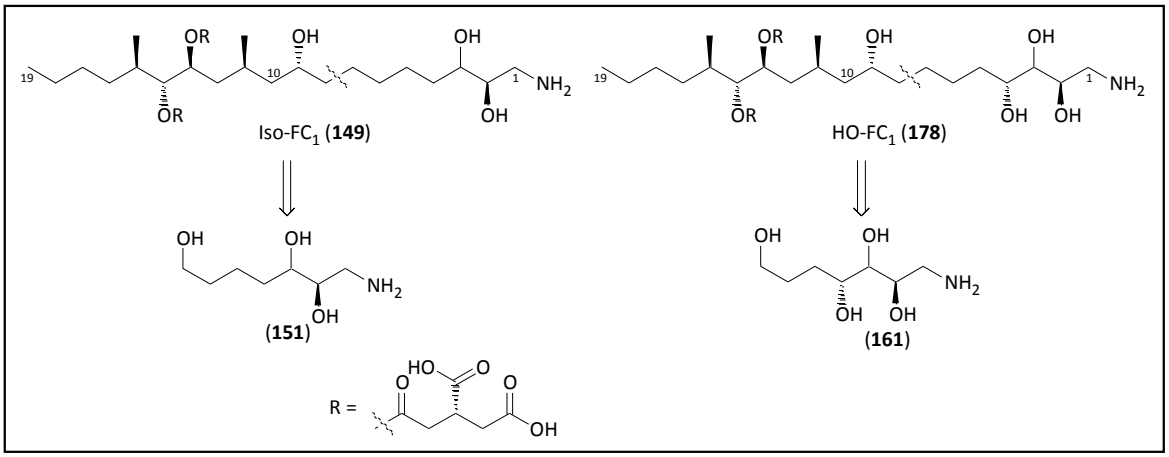
Scheme 21: Inversion of configuration at C-3.

The aim of this project was the the synthesis and optimisation of the synthetic routes for the right-hand C_7 units of the iso-FC_1 and HO-FC_1 fumonisin toxins. This would allow access to amounts of the right-hand units for coupling to the left-hand unit in the total synthesis and absolute structure determination of the fumonisin mycotoxins.

3. SYNTHETIC DISCUSSION

3.1. Introduction

The aim of this dissertation is the synthesis and optimisation of a synthetic route for the right-hand fragments of the iso-FC_1 and HO-FC_1 fumonisin toxins in order to determine the absolute configuration of the stereogenic centres in these toxins. The retrosynthetic analysis (Chapter 2) identified **151** and **161** as ideal targets for the iso-FC_1 and HO-FC_1 synthesis, respectively, as these targets can be readily synthesised from available diols using protecting group strategies and stereoselective reaction methodologies. A synthetic route based on the use of 1,n-alkanediols would allow for a wide range of fumonisin analogues to be synthesised having a range of functional groups and chain lengths between the left and right-hand fragments. The proposed synthetic routes and key associated reactions for the C-1–C-7 fragment of the iso-FC_1 and HO-FC_1 fumonisins are discussed in this chapter.



Scheme 22: Retrosynthetic analysis of iso-FC_1 (**149**) and HO-FC_1 (**178**) fumonisins showing the right-hand target fragments.

The proposed synthetic routes for the C-1–C-7 fragments of the iso-FC_1 and HO-FC_1 fumonisins are shown in **Scheme 23** and **Scheme 24**, respectively, while the notable theoretical aspects are discussed in Section 3.2 below.

3.2. Theoretical Aspects

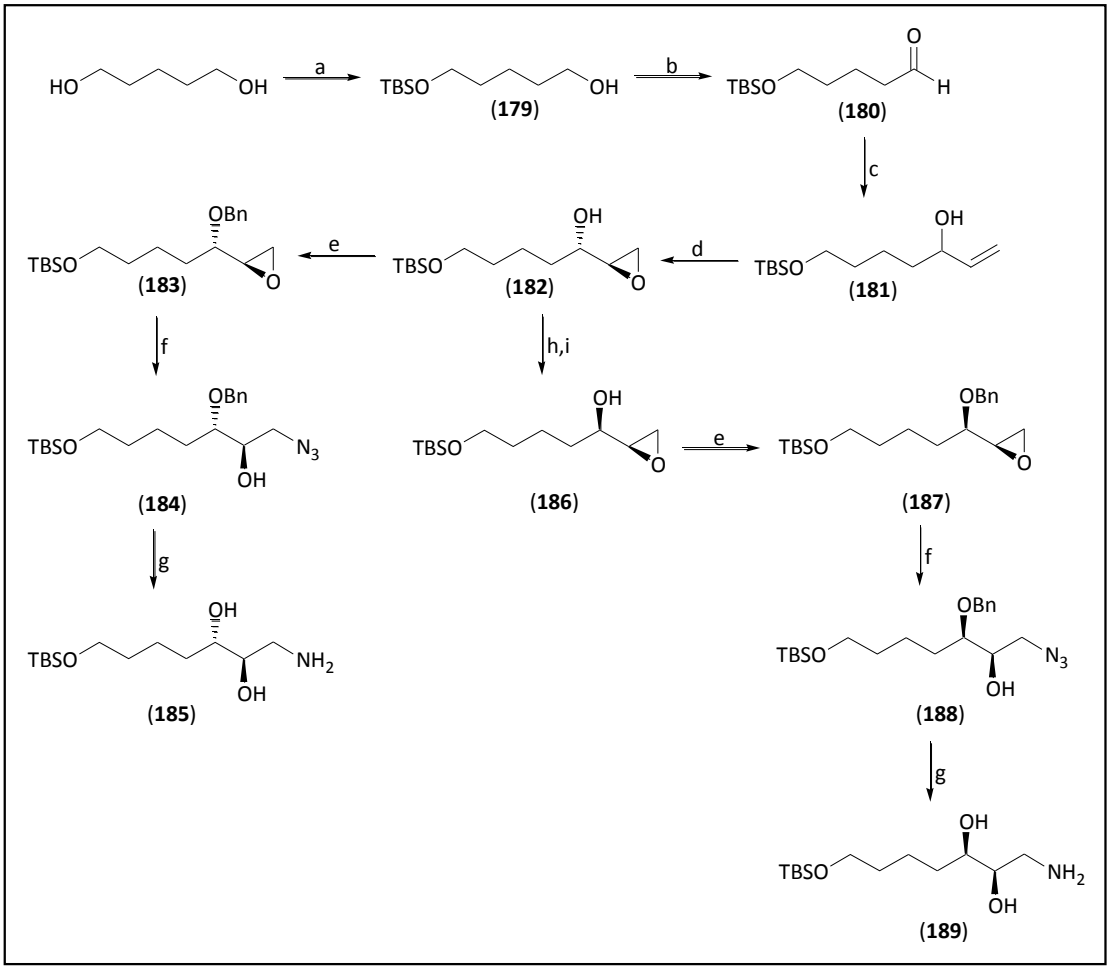
3.2.1. Protection Strategies

The choice of a protecting group strategy is just one of the deciding factors in the realisation of the synthesis and can potentially result in the success or failure of the synthesis.[1] The proposed synthetic routes require the long term protection of a primary hydroxy group throughout the synthesis as well as secondary hydroxy groups which are introduced later in the synthesis. Numerous protecting groups for a primary hydroxy group can be found in the literature including various alkyl ethers, aromatic and substituted aromatic ethers, silyl ethers, esters, ester derivatives, carbonates and carbamates. Esters and their derivatives are not ideal for long term protection due to their reactivity under acidic and basic conditions while alkyl ethers can be difficult to remove. Thus the silyl ethers are the most attractive option as they are stable under a wide range of reaction conditions excluding extremely acidic or basic conditions, and there is a wide range of silyl ethers available to choose from having varying degrees of stability. All the silyl ethers can be selectively cleaved using fluoride (due to the high affinity of silicon for fluorine) while leaving other protecting groups in place, which will be required upon coupling of the left fragment to the right fragment of the fumonisins in the future.

In literature the silyl ethers are some of the most commonly used protecting groups for hydroxy groups.[2] This is due to the ease with which they can be introduced and selectively removed, while their reactivity, electronic and steric effects can be modified by the substituents present on the silicon atom. The presence of electron-withdrawing substituents increases the susceptibility of the group towards base hydrolysis while increasing resistance to acid hydrolysis. Thus the *t*-butyldimethylsilyl (TBS) ether is more susceptible to acid than *t*-butyldiphenylsilyl (TBDPS) ether, while both are equally sensitive to base.[3] The selective cleavage of the silyl ethers by the fluoride ion has made their use widespread in organic synthesis, with cleavage most often being effected using TBAF in THF at room temperature.[4,5] The TBS group can also be selectively removed in the presence of the TBDPS group. The

[1] Schelhaas, M.; Waldmann, H. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2056.
[2] Van Look, G.; Simchen, G.; Heberle, J. *Silylating Agents*. **1995**, Fluka Chemie AG.
[3] Wuts, P.G.M.; Greene, T.W. *Greene's Protective Groups in Organic Synthesis.* **2007**, John Wiley & Sons; New Jersey.
[4] Hanessian, S.; Lavallee, P. *Can. J. Chem*. **1975**, *53*, 2975.
[5] Corey, E.J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

literature available for silyl ethers in terms of protection and deprotection reactions is extensive and has been reviewed by Nelson and Crouch,[6] and Crouch.[7]

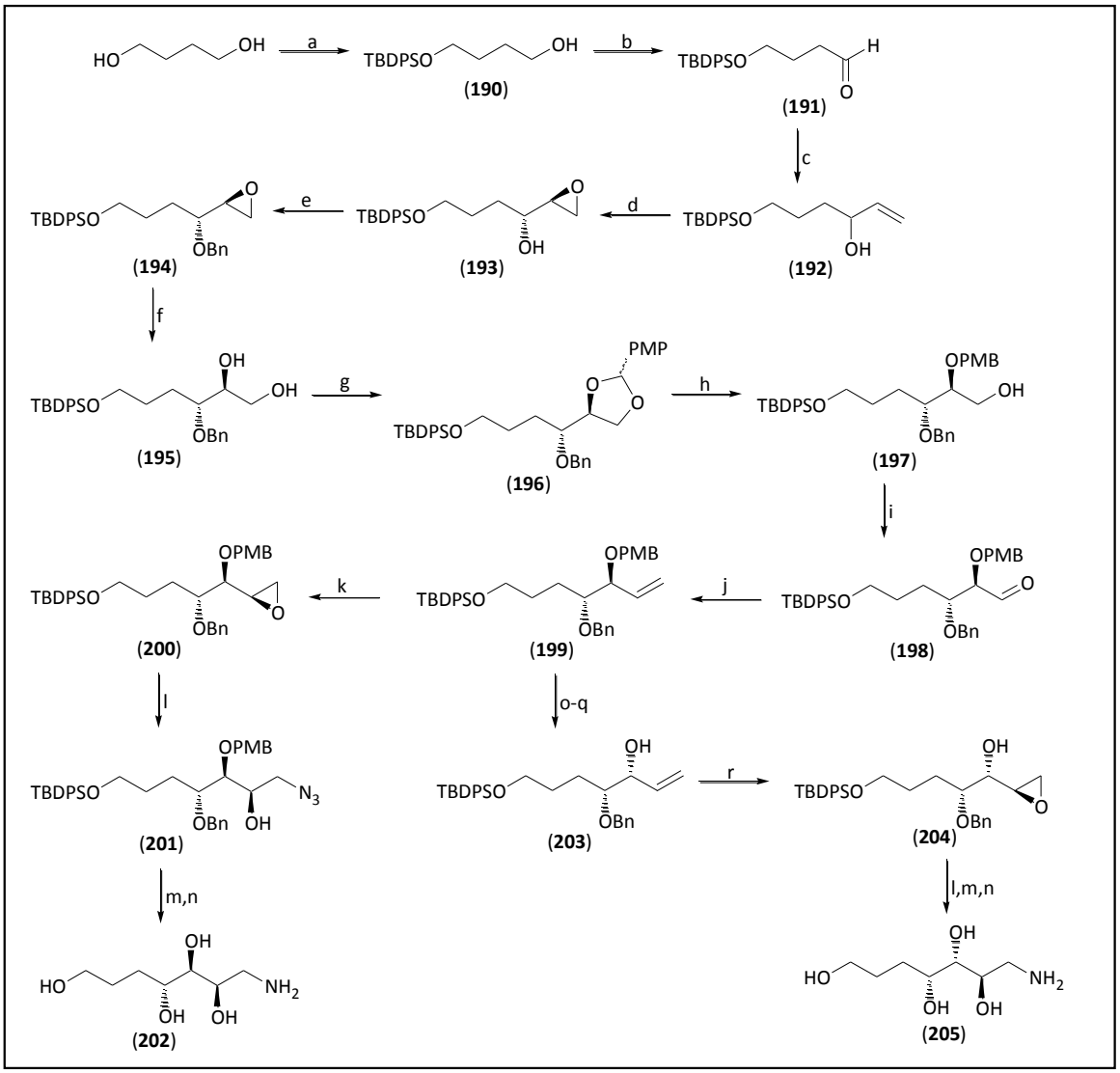


Scheme 23: Proposed synthesis for the C-1–C-7 fragment of iso-FC_1.

Reagents: (a) NaH, TBSCl; (b) $(COCl)_2$, DMSO, Et_3N; (c) vinyl magnesium bromide; (d) $Ti(O^iPr)_4$, (*R,R*)-(+)-DIPT, TBHP; (e) Dudley's reagent, MgO; (f) NaN_3, NH_4Cl; (g) H_2, Pd-C; (h) PPh_3, benzoic acid, DEAD; (i) K_2CO_3, MeOH.

Another commonly used protecting group is the *O*-benzyl ether which is also stable to a wide range of reaction conditions including acidic and basic conditions as well as being orthogonal to many other protecting groups including silyl ethers. *O*-Benzyl ethers are generally cleaved by hydrogenolysis over Pd-C, which means that it would be an ideal protecting group for the secondary hydroxy groups but not the primary hydroxy group due to the hydrogenation step required to obtain the terminal amine, as this would expose the primary hydroxy group prematurely.

[6] Nelson, T.D.; Crouch, R.D. *Synthesis* **1996**, *9*, 1031.
[7] Crouch, R.D. *Tetrahedron* **2004**, *60*, 5833.



Scheme 24: Proposed synthesis for the C-1–C-7 fragment of HO-FC_1.

Reagents: (a) NaH, TBDPSCl; (b) $(COCl)_2$, DMSO, Et_3N; (c) vinyl magnesium bromide; (d) $Ti(O^iPr)_4$, (*S,S*)-(–)-DIPT, TBHP; (e) Dudley's reagent, MgO; (f) Salen cat.; (g) $ArCH(OMe)_2$, TsOH; (h) DIBALH; (i) $(COCl)_2$, DMSO, Et_3N; (j) Ph_3PCH_3I, *n*-BuLi; (k) Salen cat.; (l) NaN_3, NH_4Cl; (m) TBAF; (n) H_2, Pd-C; (o) PPTS, MeOH; (p) Ph_3P, benzoic acid, DEAD; (q) K_2CO_3, MeOH; (r) $Ti(O^iPr)_4$, (*R,R*)-(+)-DIPT, TBHP.

The protection of a secondary hydroxy group is generally more challenging than that of a primary hydroxy group due to steric considerations, while if silyl ether protecting groups are present the possible migration of the silyl group also needs to be considered.3 In the case of 1,2-diols this becomes more complicated. One method is to selectively protect the primary hydroxy group in the presence of the secondary hydroxy group (procedures are available for this), and then to protect the secondary hydroxy group with an orthogonal group; however, this involves the addition of extra steps to the synthesis. An alternative is to form the *O*-*p*-methoxyphenyl or *O*-benzylidene acetals, which can then be selectively cleaved to release

either the primary or secondary hydroxy group, depending on the reagents and reaction conditions used. Thus the secondary hydroxy group can be protected while leaving the primary hydroxy group available for further reaction.

Using the above analysis the protecting group strategies are shown in **Scheme 23** and **Scheme 24** for the iso-FC_1 and HO-FC_1 fragments respectively. The silyl ethers were chosen for the protection of the primary hydroxy group at the beginning of the synthesis and the *O*-benzyl group for the secondary hydroxy group generated at a later stage. The synthesis of the C-1–C-7 fragment of HO-FC_1 fumonisin required a third protecting group for which the *O*-*p*-methoxyphenyl group was chosen which could be selectively opened to the C-2 *O*-PMB ether leaving the primary hydroxy group free. These protecting groups are able to withstand the wide range of reaction conditions required while also allowing for the selective deprotection of the various hydroxy groups when necessary.

3.2.2. Epoxidation Strategies

As intermediates the epoxide group is both useful and versatile and can be used in the formation of more complex compounds, as can be seen from their regular use in synthesis. The proposed synthetic routes (**Schemes 23** and **24**) make use of epoxidations as key transformations for the stereoselective introduction of a hydroxy group at C-2 in the iso-FC_1 fragment and at C-2 and C-3 in the HO-FC_1 fragment. Two different procedures were chosen, the first being the Sharpless asymmetric epoxidation for the formation of an *anti* epoxy-alcohol (required in both the iso-FC_1 and HO-FC_1 fragment synthesis) and the second being the Jacobsen epoxidation for the formation of the *syn* epoxy-alcohol (required in the HO-FC_1 fragment synthesis). These procedures were chosen for their ability to produce epoxides reliably and with high enantioselectivity.

3.2.2.1. Sharpless Asymmetric Kinetic Resolution

In 1980 the discovery of the Sharpless epoxidation resulted in a process which was found to be more selective than any of the previously known methods. The procedure was elegantly simple and the required reagents were commercially available at moderate cost. The reaction involved the stoichiometric use of quantities of $Ti(O^iPr)_4$, diethyl tartrate (DET) and *t*-butyl hydroperoxide (TBHP) and allowed the synthesis of highly enantiopure epoxy-alcohols from

allylic alcohols.[8] Since its discovery the reaction has undergone very little modification except for the introduction of a catalytic version of the reaction which includes the addition of 4Å molecular sieves and which requires only 5-10 mol % of the $Ti(O^iPr)_4$ and 6-13 mol % of the tartrate ester.[9] This reaction system has been found to accept a wide range of allylic alcohol substrates and is also ideal for the kinetic resolution of racemic allylic alcohols.

The efficient epoxidation of allylic alcohols has been shown to be effectively catalysed by the high-valent early transition metals such as titanium(IV) and vanadium(V) as a result of the strong associative reactions between the catalyst and its substrate.[10] The preparation of the active catalyst consists of an "aging" period in which the $Ti(O^iPr)_4$, tartrate ester and TBHP react. The initial structure of the active catalyst is believed to be a dimer consisting of two titanium atoms bridged by two tartrate ligands. Upon addition of the oxidising agent (TBHP) one of the isopropoxide ligands and one of the tartrate carbonyl groups are displaced. The oxidising complex then reacts with the allylic alcohol via coordination of the alcohol group with the titanium and displacement of an isopropoxide ligand, with the orientation of the alcohol group dictated by the stereochemistry of the tartrate used.[11] The presence of water in the reaction results in inhibition of the catalyst which explains the reduced yields and enantioselectivity observed for reactions in the absence of molecular sieves.

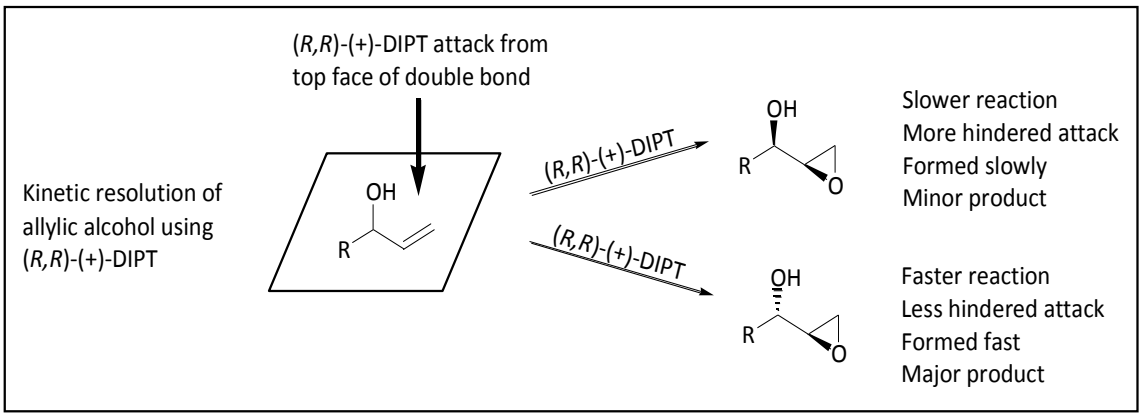


Figure 20: Enantioselectivity in the Sharpless asymmetric kinetic resolution epoxidation.

[8] Katsuki, T.; Sharpless, K.B. *J. Am. Chem. Soc.* **1980**, *102*, 5976.
[9] Hanson, R.M.; Sharpless, K.B. *J. Org. Chem.* **1986**, *51*, 1922.
[10] Adolfsson, H.; Balan, D. Metal-catalyzed Synthesis of Epoxides. In *Aziridines and Epoxides in Organic Synthesis*. Yudin, A.K. (ed.). Wiley-VCH, Weinheim, **2006**, pp. 185-192.
[11] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*. Oxford University Press, Oxford, **2008**, pp. 1239-1241.

The principle of kinetic resolution is based on the selective reaction of one enantiomer over another in the presence of a chiral catalyst or reagent in order to obtain a mixture of enantio-enriched starting material and product with subsequent isolation of the enantio-enriched product.[12] The major drawback of kinetic resolution is that the maximum theoretical yield possible is usually 50% unless the starting material can be racemised or converted back to the required enantiomer. As can be seen in **Figure 20** when the tartrate (*R,R*)-(+)-DIPT is used attack occurs from the *Re* face of the allylic alcohol with the less sterically hindered allylic alcohol having a faster rate of reaction than its more hindered enantiomer resulting in the formation of the less hindered epoxide as the major product with >95% enantioselectivity. (*S,S*)-(–)-DIPT will attack from the *Si* face of the allylic alcohol. As a result of this consistency in attack the enantiomer that will be more readily formed can be predicted.

3.2.2.2. Jacobsen's Asymmetric Epoxidation

In 1990 Jacobsen *et al*.[13] and Katsuki *et al*.[14] almost simultaneously discovered that chiral Mn-Salen complexes had the ability to catalyse the enantioselective epoxidation of olefins and since then their use has become widespread in the asymmetric synthesis of epoxides. The reaction involves 3-10 mol % of the Mn-Salen catalyst, NaOCl as the oxidant and an imidazole additive in order to synthesise highly enantiopure epoxides. In the Mn-Salen epoxidation there is no pre-coordination of the olefin to the catalyst which means that unfunctionalised olefins can be selectively and efficiently oxidised. In contrast the Sharpless asymmetric epoxidation requires an allylic hydroxy group for coordination. However, the substitution pattern of the olefin does have an influence on the enantioselectivity of the reaction.[15]

Mechanistically this reaction has been the focus of much discussion with the most widely accepted mechanism involving a two-step reaction. The initial step was determined to be the transfer of oxygen from the terminal oxidant to the Mn(III)-Salen catalyst to form a Mn(V)-oxo intermediate[16] followed by transfer of the oxygen from the transition metal centre to the olefin to form the epoxide, the details regarding the mode of transfer are yet to be elucidated.

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[12] Keith, J.M.; Larrow, J.F.; Jacobsen, E.N. *Adv. Synth. Catal.* **2001**, *343*, 5.
[13] Jacobsen, E.N.; Zhang, W.; Loebach, J.L.; Wilson, S.R. *J. Am. Chem. Soc.* **1990**, *112*, 2801.
[14] Katsuki, T.; Irie, R.; Noda, K.; Ito, Y.; Matsumoto, N. *Tetrahedron Lett*. **1990**, *31*, 7345.
[15] Adolfsson, H.; Balan, D. Metal-catalyzed Synthesis of Epoxides. In *Aziridines and Epoxides in Organic Synthesis*. Yudin, A.K. (ed.). Wiley-VCH, Weinheim, **2006**, pp. 204-208.
[16] Feichtinger, D.; Plattner, D.A. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1718.

The basis for the enantioselectivity of the reaction is yet to be confirmed. Various different routes for the olefin approach to the catalyst have been proposed; however, it is mainly thought to originate from the olefin approaching from the side of the aromatic ring (shown in **Figure 21**).[17] The stereogenic centres positioned near the diimine bridge induce folding of the ligand which creates a chiral pocket with the low-energy path into this chiral pocket being a side-on approach over one of the aromatic rings.[16] Typically the oxidant used for the Mn-Salen asymmetric epoxidations is NaOCl but experiments using other oxidants have been conducted for which the yields and enantioselectivities have not been as high. Numerous different variants of the Mn-Salen catalyst with different attached ligands have been prepared. It has also been determined that imidazole additives are important for the formation of the active Mn(V)-oxo intermediate while the presence of water has an inhibitory/deactivating effect on the catalyst.[17]

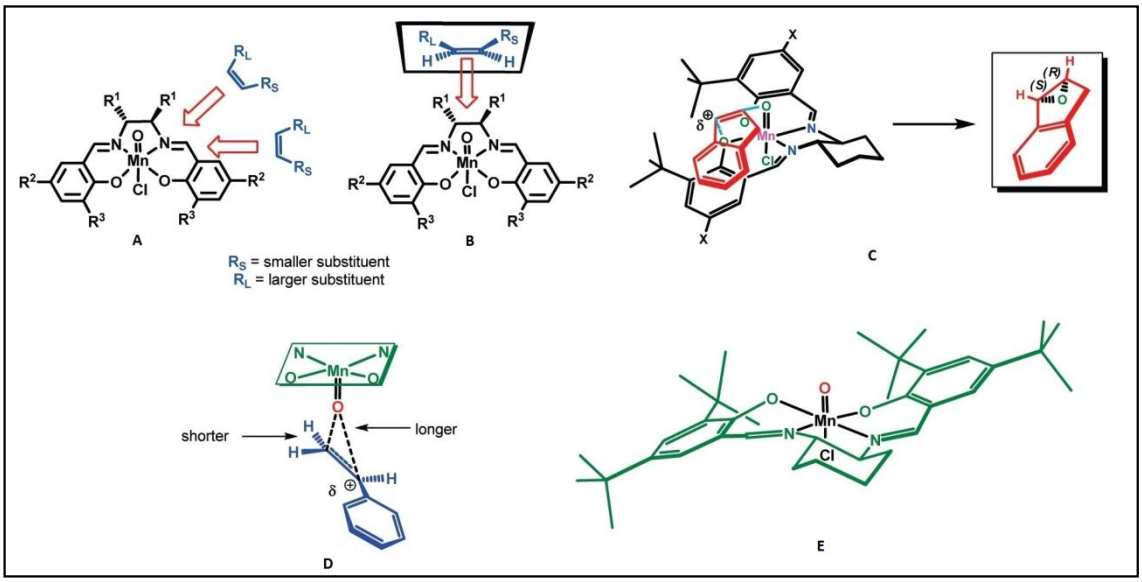


Figure 21: (A) and (B) Proposed approaches of the olefin to the Mn-Salen catalyst; (C) Most widely accepted approach route; (D) Transition state for epoxidation; (E) Folding of the ligand to form the chiral pocket.[17]

3.2.3. Inversion of Configuration - The Mitsunobu Reaction

In 1967 Mitsunobu[18] discovered a method for the dehydrative coupling of an alcohol to a pronucleophile in the presence of an azo oxidising agent (usually DEAD) and a phosphine reducing agent (usually Ph_3P) under mild to neutral reaction conditions. This reaction has become one of the most widely used methods for the preparation of esters, aryl esters, cyclic

[17] Kürti, L.; Blewett, M.M.; Corey, E.J. *Org. Lett.* **2009**, *11*, 4592.
[18] Mitsunobu, O.; Yamada, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380.

ethers, C-C, C-O and C-N bonds.[19] The attraction of the reaction is that the coupling of the alcohol and pronucleophile starting reagents occur with inversion of configuration at the stereogenic centre carrying the hydroxy functional group. The major disadvantage of the Mitsunobu reaction is that in addition to the alcohol and pronucleophile, two other reagents (DEAD and Ph_3P) are required, both of which produce by-products leading to difficulties in isolating the desired product. As a result research efforts have been directed towards finding alternative reagents to DEAD and Ph_3P that avoid the production of by-products which are difficult to remove.[19]

The mechanism of the reaction has been extensively studied due to the widespread use and importance of the Mitsunobu reaction. In the currently accepted mechanism (shown in **Figure 22**) the first step is the irreversible nucleophilic addition of the Ph_3P to DEAD to form a reactive intermediate which deprotonates the acid (H—Nuc) to form the reactive nucleophile. Due to the strong affinity of oxygen for phosphorus the alcohol attacks the positively charged phosphorus atom resulting in the displacement of the nitrogen anion and formation of the phosphorus–oxygen bond. The reactive nucleophile formed earlier then attacks the phosphorus derivative of the alcohol in an S_N2 reaction to obtain the product with inversion of configuration.

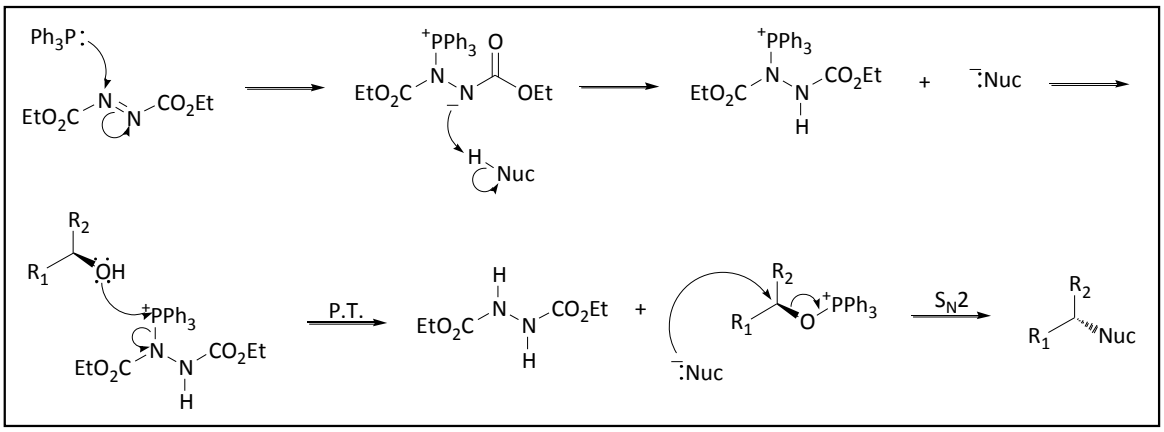


Figure 22: Mechanism for the Mitsunobu reaction.[20]

3.2.4. Ring opening of Epoxides

The ring-opening reactions of epoxides has been the focus of much research due to the

[19] But, T.Y.S.; Toy, P.H. *Chem. Asian J.* **2007**, *2*, 1340.
[20] Kürti, L.; Czakó, B. Strategic Applications of Named Reactions in Organic Synthesis. Academic Press Inc. **2005**, pp. 294.

versatility of epoxides in synthesis and because the epoxide ring can easily be opened under a wide range of reaction conditions. Additionally the ring-opening reactions of epoxides are normally stereospecific and regioselective, leading to products having a 1,2 functional group relationship of known stereochemistry.[21] The proposed synthetic routes (**Schemes 23** and **24**) make use of asymmetric ring-opening reactions of epoxides for the introduction of the terminal amine group in the iso-FC_1 and HO-FC_1 fragments and for the formation of the 1,2-diol in the HO-FC_1 fragment. Two different procedures were required, the first being the nucleophilic ring-opening using azide as a source of the amine (required in both the iso-FC_1 and HO-FC_1 fragment synthesis) and the second being the asymmetric ring-opening using the chiral Co(III)-Salen catalyst and water to obtain the 1,2-diol (required in the HO-FC_1 fragment synthesis). These procedures were chosen for their ability to open epoxide rings reliably and regioselectively.

3.2.4.1. Ring-opening Using Azide

Azides are widely used as nucleophiles as a result of their high nucleophilicity, low basicity and stability towards a wide range of reaction conditions. The azidolysis of epoxides is an attractive route to the formation of 1,2-amino alcohols with excellent stereocontrol and in good yields.[22] The most commonly used method for azidolysis makes use of NaN_3 as the nucleophile and NH_4Cl as the coordinating salt using an alcohol-water mixture as the solvent under reflux. The only disadvantage of this method is that it requires long reaction times.[23] The regioselective nucleophilic attack of azide on an epoxide takes place at the least substituted carbon by means of an S_N2 reaction resulting in inversion of configuration at the site of ring-opening. This means that for terminal epoxides the azide nucleophile will attack at the C-1 carbon resulting in a 1,2-azido alcohol as product. Numerous different procedures for the ring-opening of epoxides are available using different sources of azide, different coordinating salts, addition of catalysts and different solvents in attempts to reduce the length of the required reaction times. Azidolysis reactions for the kinetic resolution of racemic epoxides have also been developed.

3.2.4.2. Ring-opening Using Co(III)-Salen

[21] Behrens, C.H.; Ko, S.Y.; Sharpless, K.B.; Walker, F.J. *J. Org. Chem.* **1985**, *50*, 5687.
[22] Nielsen, L.P.C.; Jacobsen, E.N. Catalytic Asymmetric Epoxide Ring-opening Chemistry. In *Aziridines and Epoxides in Organic Synthesis*. Yudin, A.K. (ed.). Wiley-VCH, Weinheim, **2006**, pp. 229-243.
[23] Fringuelli, F.; Piermatti, O.; Pizzo, F.; Vaccaro, L. *J. Org. Chem.* **1999**, *64*, 6094.

The Co(III)-Salen catalysed asymmetric ring-opening of terminal epoxides in the presence of water has become an important method for the synthesis of highly enantiopure 1,2-diols, which are valuable building blocks in organic synthesis.[24] The reaction involves the use of Co(III)-Salen catalyst, an acid for activation of the catalyst, the nucleophile which is water and the epoxide substrate in order to synthesise enantiopure 1,2-diols. Since its discovery many different versions of the catalyst have been developed for use with a variety of nucleophiles. The catalysed hydrolysis occurs at room temperature and under relatively neutral conditions and accepts a wide range of substrates.[25]

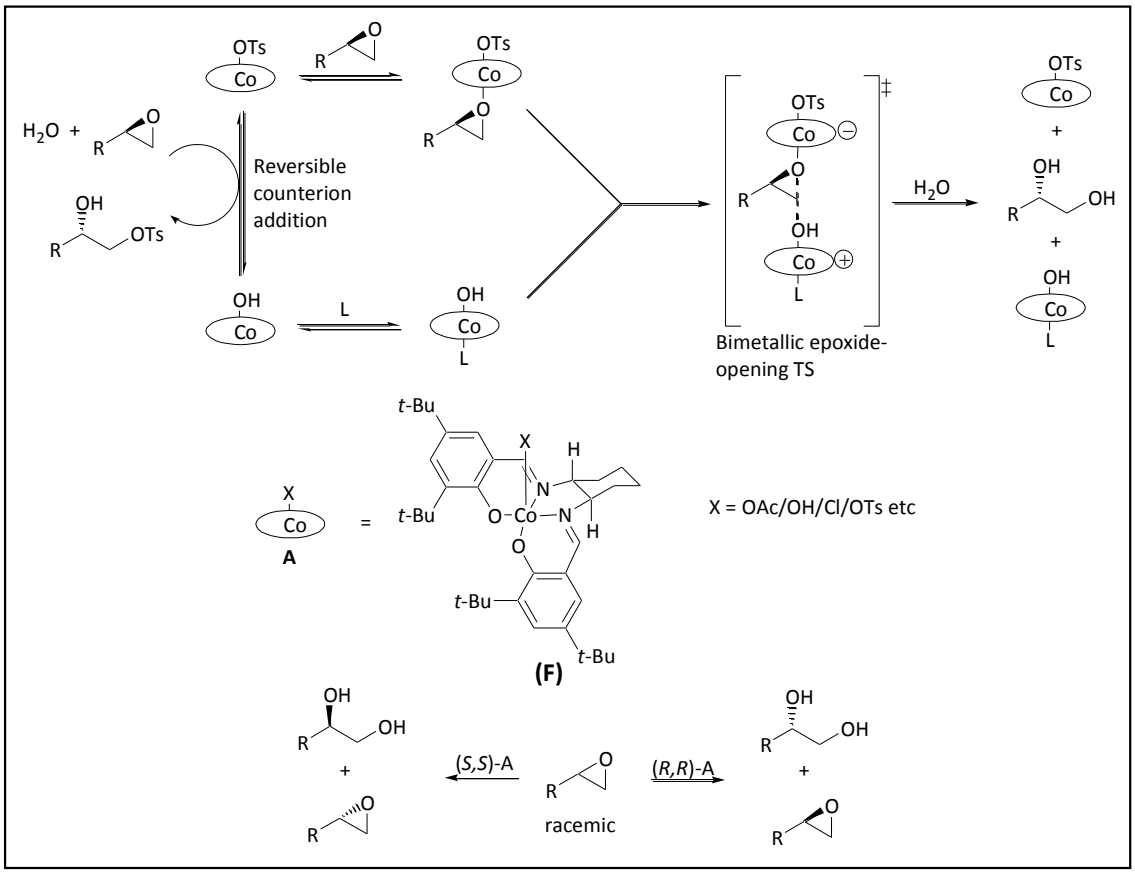


Figure 23: Mechanism of epoxide opening and enantioselectivity of reaction.[25]

The key feature of the reaction mechanism is the cooperative action of two different Co(III)-Salen units [(Salen)Co-X and (Salen)Co-OH] in the ring-opening of the epoxide with the rate of reaction determined by the ratio of nucleophilic (Salen)Co-OH catalyst to the Lewis acidic (Salen)Co-X catalyst. In the presence of water and the substrate the (Salen)Co-X is converted to the nucleophilic (Salen)Co-OH by a mechanism that involves reversible counterion addition

[24] Tokunaga, M.; Larrow, J.F.; Kakiuchi, F.; Jacobsen, E.N. *Science* **1997**, *277*, 936.
[25] Nielsen, L.P.C.; Zuend, S.J.; Ford, D.D.; Jacobsen, E.N. *J. Org. Chem.* **2012**, *77*, 2486.

to the epoxide. The two Co(III)-Salen units then combine to form the bimetallic epoxide opening transition state and react with more water to obtain the 1,2-diol. The enantioselectivity of the reaction is determined by the chirality of the catalyst used. The stereochemistry of the C-2 stereogenic centre of the epoxide can be conserved through correct choice of catalyst chirality.[25]

3.2.5. The Wittig Reaction

The Wittig reaction[26] was first published in 1953 and has become one of the most powerful methods to form carbon-carbon double bonds used in natural product synthesis[27] and industrial processes.[28] The reaction can be controlled regioselectively and stereoselectively as a result of double bond introduction in the place of a carbonyl functionality.[29] The reaction involves the synthesis of an ylide (formed from a phosphonium salt and base) and reaction with an aldehyde in order to stereoselectively synthesise alkenes. Since its discovery many different types of ylides and bases have been tested to prepare a variety of alkenes and in attempts to elucidate the mechanism of the reaction.

Although the reaction is used extensively the complete mechanism is still a topic of intense debate as several different mechanisms have been proposed since the reaction was first published.[30] The most widely accepted mechanism (shown in **Figure 24**) is based on a [2+2] cycloaddition of the ylide and carbonyl group to obtain a four-membered ring oxaphosphetane (OPA). The formed OPA then undergoes a facile pseudo-rotation which places the ring oxygen in an apical position in the OPA trigonal bipyramid. The alkene and phosphine oxide by-product are subsequently formed via an irreversible, stereospecific *syn*-cycloreversion of OPA.[30] The driving force for the reaction comes from the formation of the very strong oxygen-phosphorus bond in the phosphine oxide by-product that is formed.[31]

The stereoselectivity of the Wittig reaction is dependent on the nature of the substituent on the carbon atom of the ylide used. Ylides are strongly nucleophilic and attack the carbonyl carbon of aldehydes and ketones leading to the formation of the OPA intermediates. There are

[26] Wittig, G.; Geissler, G. *Liebigs Ann. Chem.* **1953**, *580*, 44.
[27] Nicolau, K.C.; Härter, M.; Gunzer, J.; Nadin, A. *Liebigs Ann./Recl.* **1997**, *7*, 1283.
[28] Pommer, H. *Angew. Chem.* **1977**, *89*, 437.
[29] Pascariu, A.; Mracec, M.; Berger, S. *Magn. Reson. Chem.* **2005**, *43*, 451.
[30] Byrne, P.A.; Gilheany, D.G. *J. Am. Chem. Soc.* **2012**, *134*, 9225.
[31] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*. Oxford University Press, Oxford, **2008**, pp. 814-818.

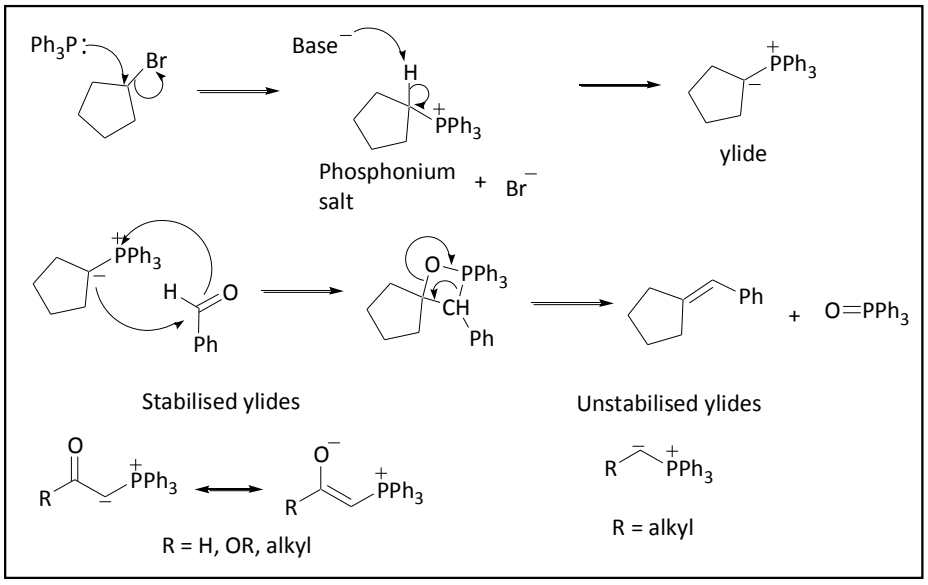


Figure 24: Mechanism and types of ylides for the Wittig reaction.[31]

two types of ylides that can be used in a Wittig reaction: stabilised and unstabilised.[31] Stabilised ylides have a conjugating or anion-stabilising substituent adjacent to the negative charge which means that the negative charge is not only stabilised by the phosphorus atom but by the adjacent functional group as well. Unstabilised ylides are those ylides which have no stabilising group adjacent to the negative charge. Wittig reactions using stabilised ylides lead to *E* selectivity while unstabilised ylides lead to *Z* selectivity for the double bond. Stabilised ylides can be isolated; however, they are usually prepared and used directly.[31]

3.3. Experimental Aspects

3.3.1. Synthesis of the C-1–C-7 Fragment of the Iso-FC_1 Fumonisin (151)

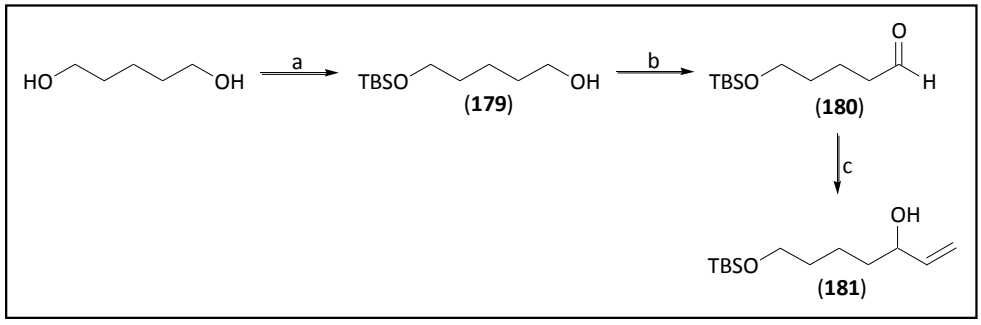
3.3.1.1. Via the Benzylation Protection Route

1,n-Alkanediols are important bifunctional synthons in many syntheses of which monoprotection is often the first step.[32] Many procedures are available for the selective protection of unsymmetrical diols; however, the selective monoprotection of a symmetric diol is more problematic. If stoichiometric equivalents of the protecting reagents are used, then a statistical mixture of 1:2:1 of the unprotected:monoprotected:diprotected products is expected with a maximum yield of 50% for the required monoprotected product.[32] Procedures have been developed which somewhat avoid this problem, which make use of a large excess

[32] McDougal, P.G.; Rico, J.G.; Oh, Y.-I.; Condon, B.D. *J. Org. Chem.* **1986**, *51*, 3388.

of the diol to protecting reagent,[33] use polymer supports[34] or continuous extraction.[35] The most effective procedure, however, appears to be that of McDougal *et al.*[32] in which the mono-sodium alkoxide salt is silylated.

The initial steps of the synthesis are shown in **Scheme 25**, and begin with the selective mono-protection of 1,5-pentanediol using TBSCl, following the procedure developed by McDougal *et al.*[32], to obtain the TBS ether (**179**) in 76% yield. In this procedure the diol is reacted with NaH in THF to form an insoluble alkoxide salt to which the silylating agent is added, with the selectivity of the reaction being due to only a small amount of the alkoxide salt being available in solution and able to react with the silylating agent. The TBS group was chosen as a suitable protecting group for the primary hydroxy group as it is stable under a wide range of reaction conditions (excluding very acidic or basic conditions) and other protecting groups can be selectively introduced and removed in its presence. The OH group of the TBS-ether (**179**) was characterised by a broad ^{1}H NMR signal at δ_H 2.27 (s) and a broad band at ν_{max} 3321 cm^{-1} in the IR spectrum while the ^{13}C NMR spectrum exhibited a signal at δ_C 62.58 (T) for C-1.



Scheme 25: Synthesis of the racemic allylic alcohol (**181**).

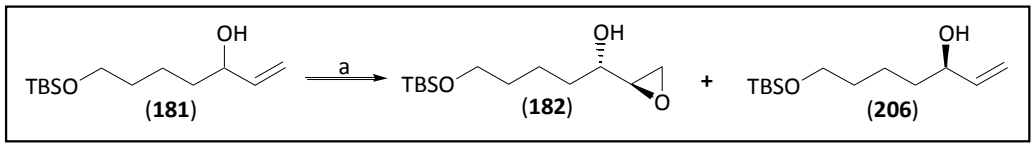
Reagents: (a) NaH, TBSCl (76%); (b) $(COCl)_2$, DMSO, Et_3N (106% crude); (c) Vinyl magnesium bromide (80%).

The extension of the backbone of the TBS-ether (**179**) by two carbons is based on Grignard methodology and requires the aldehyde (**180**), which is obtained by the Swern oxidation[36] of the primary hydroxy group. The procedure is well known and has been extensively studied and makes use of oxalyl chloride, dimethylsulfoxide and triethylamine at −78 °C to oxidise the hydroxy group to give the aldehyde (**180**), which was used directly in the Grignard reaction due to poor stability of the aldehyde.

[33] Sheehan, M.; Spangler, R.J.; Djerassi, C. *J. Org. Chem.* **1971**, *36*, 3526.
[34] Leznoff, C.C. *Acc. Chem. Res.* **1978**, *11*, 327.
[35] Pattison, F.L.M.; Stothers, J.B.; Woolford, R.G. *J. Am. Chem. Soc.* **1956**, *78*, 2255.
[36] Omura, S.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.

The Grignard reaction is one of the most commonly used methods for the extension of a carbon backbone, while generating a hydroxy group from a carbonyl. Vinyl magnesium bromide was reacted with **180** to generate the racemic allylic alcohol (**181**) in 80% yield, after intensive chromatographic purification due to a large number of impurities generated during the reaction. Work-up was done using saturated NH_4Cl instead of aqueous acid to conserve the acid labile TBS group. The OH group of the allylic alcohol (**181**) exhibited a broad band at ν_{max} 3360 cm^{-1} in the IR spectrum, while analysis of the ^{13}C NMR spectrum indicated the presence of the C=C double bond as the C-1 carbon appeared at δ_C 114.55 (T) and the C-2 carbon appeared at δ_C 141.20 (D), which is characteristic of a terminal double bond. The ^1H NMR spectrum exhibited a signal at δ_H 5.83 (ddd) for the protons of C-2 showing a *trans* coupling of 17.2 Hz with H-1a (δ_H 5.18 ddd) and a *cis* coupling of 10.5 Hz with H-1b (δ_H 5.06 ddd).

Resolution of the racemic allylic alcohol (**181**) was done using Sharpless asymmetric kinetic resolution, which would lead to a single stereoisomer of the epoxy-alcohol having the required stereochemistry at the two stereogenic centres. The absolute stereochemistry of the stereogenic centres is determined by the stereochemistry of the tartrate ester used, as discussed previously under theoretical aspects (**section 3.2.2.1.**).



Scheme 26: Sharpless asymmetric kinetic resolution of the allylic alcohol (**181**).

Reagents: (a) *(R,R)*-(+)-DIPT, $Ti(O^iPr)_4$, TBHP (49%).

$Ti(O^iPr)_4$ (0.5 equivalents) was reacted with (*R,R*)-(+)-DIPT (0.6 equivalents), followed by reaction with TBHP (0.6 equivalents) to allow for the formation of the reactive dimer species responsible for the stereoselective epoxidation. This dimer species was reacted with **181** to form the *anti* epoxy-alcohol (**182**) in 49% yield (maximum yield in the kinetic resolution reaction is 50%) and a diastereomeric ratio of 96:4. Analysis of the ^{13}C NMR spectrum showed the typical signals for a terminal epoxide group, with the signal for C-1 appearing at δ_C 43.42 (T) and that of C-2 appearing at δ_C 54.58 (D). The ^1H NMR spectrum exhibited a signal at δ_H 2.97 (ddd) for the protons of C-2 showing a *cis* coupling of 2.8 Hz with H-1a (δ_H 2.76 dd) and a *trans* coupling of 4.0 Hz with H-1b (δ_H 2.69 dd). In addition a geminal coupling of 5.1 Hz was observed between H-1a and H-1b. Long range coupling between the protons of C-1 and the

proton of C-3 was observed with a signal appearing at δ_H 2.76 (dd) with coupling constants of 2.9 Hz and 5.1 Hz. The *anti* arrangement between the protons of C-2 and C-3 resulted in a coupling constant of 3.1 Hz.

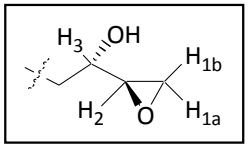


Figure 25: Numbering of the protons in the epoxy alcohol (**182**).

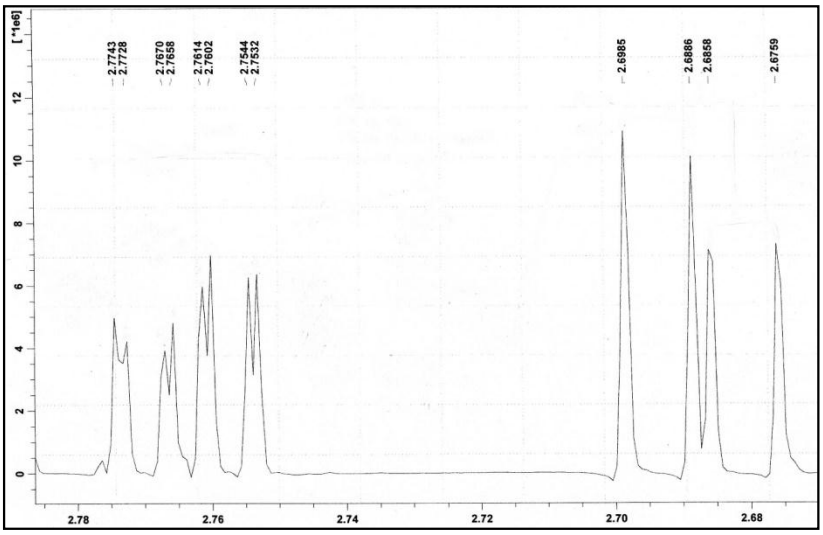


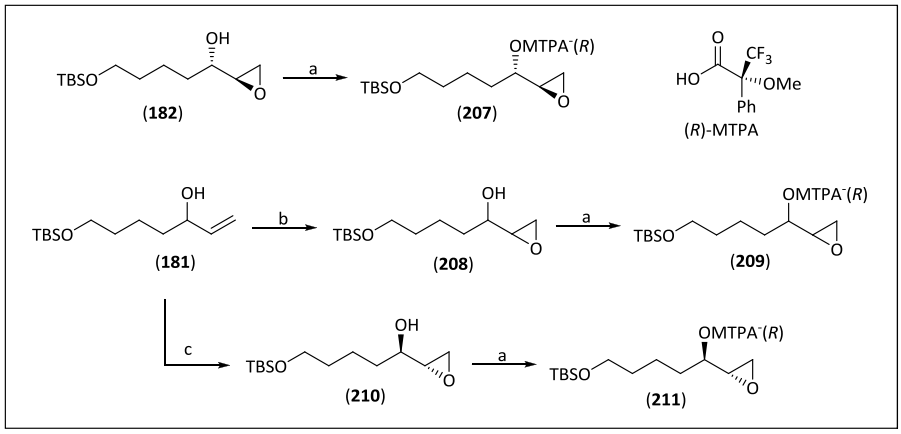
Figure 26: ^{1}H NMR spectrum of the C-1 proton signals of the epoxy-alcohol (**182**).

To establish the enantiomeric excess of the epoxides formed in the Sharpless reaction, the epoxides were converted into the corresponding α-methoxy-α-trifluoromethylphenylacetate (α-MTPA) derivatives. This leads to the formation of diastereomers that can be differentiated by NMR spectroscopy.[37] The derivative used was α-methoxy-α-trifluoromethylphenylacetic acid (α-MTPAA) which is converted to the acyl chloride and then esterified with the alcohol. This derivative was chosen due to the availability of both enantiomers, the relative ease with which the esters can be prepared and resolved and its stability towards racemisation.[38,39] An additional advantage is the presence of the α-CF_3 group allowing for ^{19}F NMR spectroscopy. The differences in the ^{19}F chemical shifts have been found to be greater than those of the corresponding proton signals while the ^{19}F NMR signals also appear in a relatively

[37] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*. Oxford University Press, Oxford, **2008**, pp. 1231.
[38] Dale, J.A.; Dull, D.L.; Mosher, H.S. *J. Org. Chem.* **1969**, *34*, 2543.
[39] Sullivan, G.R.; Dale, J.A.; Mosher, H.S. *J. Org. Chem.* **1973**, *38*, 2143.

unobstructed area of the spectrum. The e.e. of a reaction is thus determined by integrating and comparing the ratios of the signals obtained in the simpler ^{19}F NMR spectrum.[39]

The enantioselectivity of the reaction was determined by conversion of the (2*R*,3*S*)-epoxy-alcohol (**182**) to the (*R*)-α-methoxy-α-trifluoromethylphenylacetate [(*R*)-MTPA] derivative (shown in **Scheme 27**) using the protocol developed by Ward and Rhee.[40] followed by analysis of the ^{19}F NMR spectrum. The Mosher acid chloride was prepared by the addition of oxalyl chloride to a DMF-hexane solution of (*R*)-MTPAA, stirring for 1 hour and followed by filtration to remove the DMFCl contaminant that precipitates out in hexane. The concentrated acyl chloride solution was dissolved in DCM, after which Et_3N and DMAP were added, followed by a solution of the epoxy-alcohol in DCM and stirring for 3 hours till TLC analysis indicated completion of reaction. Work-up of the reaction gave the crude Mosher ester derivative (**207**) in 74% yield. Analysis of the ^{1}H NMR spectrum showed a signal at δ_H 3.70 (ddd) for the proton of C-3, whereas the protons of the methoxy group gave a signal at δ_H 3.83 (s). The ^{19}F NMR spectrum exhibited a single major signal at δ_F –71.57 (96%) and a minor signal at δ_F –71.42 (4%).



Scheme 27: Synthesis of the racemic epoxide (**208**), (2*S*,3*R*)-epoxy-alcohol (**210**) and the corresponding Mosher ester derivatives (**207**), (**209**), and (**211**).

Reagents: (a) (*R*)-(+)-α-MTPAA, $(COCl)_2$, Et_3N, DMAP (**207** 74%, **209** 86%, **211** 74%); (b) MCPBA (79%); (c) *(S,S)*-(–)-DIPT, $Ti(O^iPr)_4$, TBHP (30%).

In order to assist in the determination of the enantioselectivity of the asymmetric kinetic resolution reaction using the ^{19}F NMR data it was necessary to prepare the racemic epoxy-alcohol (shown in **Scheme 27**) containing all four of the possible stereoisomers. The allylic alcohol (**181**) was thus reacted with MCPBA to give racemic epoxy-alcohol (**208**) in 79% yield,

[40] Ward, D.E.; Rhee, C.K. *Tetrahedron Lett.* **1991**, *32*, 7165.

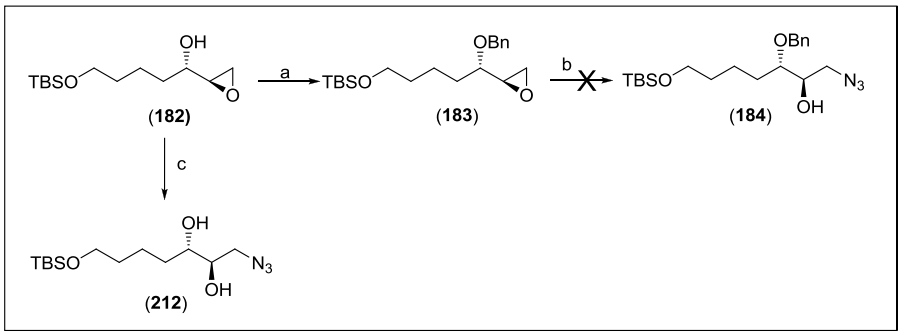
followed by conversion to the (*R*)-MTPA derivative (**209**). The ^{19}F NMR spectrum exhibited four major signals at δ_F −71.41, −71.48, −71.57 and −71.76 in the ratio 1.4:1.8:1.9:1. The (2*S*,3*R*)-epoxy-alcohol (**210**) was also prepared (shown in **Scheme 27**) under the same asymmetric kinetic resolution conditions as used for the preparation of the (2*R*,3*S*)-epoxy-alcohol, but using *(S,S)*-(–)-DIPT instead, followed by conversion to the (*R*)-MTPA derivative (**211**). Examination of the ^{19}F NMR spectrum revealed a major signal at δ_F −71.42 (95%) and a minor signal at δ_F −71.57 (5%). Using all the ^{19}F chemical shifts it was then possible to determine the enantiomeric excess for **182** as 96% and that of **210** as 95%.

In terms of protecting groups, the benzyl ethers are some of the most important and commonly used groups.3 They are stable to a wide range of reaction conditions and have minimal impact on the electronic properties of the oxygen atom to which they are attached. Typically the most popular protocols require harsh reaction conditions and are therefore limited to substrates which can tolerate strongly acidic or basic conditions. The Williamson ether synthesis[41] which is an S_N2-type of reaction between an alkali metal alkoxide and benzyl bromide is one such method which can be too harsh. Another is the reaction using benzyl trichloroacetimidate promoted by trifluoromethanesulfonic acid.[42] These methods are too harsh for protecting groups such as the silyl ethers. An alternative is to use Dudley's reagent which is a bench-stable pyridinium salt for the formation of the benzyl ether under reaction conditions that are mild and nearly neutral, following a mix-and-heat procedure.[43]

The protection of the secondary hydroxy group (shown in **Scheme 28**) with a benzyl group in epoxy-alcohol (**182**) was thus achieved using Dudley's reagent (Bn-OPT) in the presence of benzotrifluoride and vacuum dried MgO, following the procedure of Poon and Dudley.[43] This reaction was done under neutral conditions allowing for the conservation of the TBS protecting group as well as the epoxide, which can be difficult to work with under more acidic or basic conditions. The benzyl group can also be cleaved by hydrogenation without affecting the TBS group, which led to its choice as a suitable protective group for the C-3 hydroxy group resulting in the benzyl ether (**183**) as product in 41% yield. Analysis of the NMR spectroscopic data indicated the characteristic signals in the ^{1}H NMR spectrum for the aromatic protons in the δ_H 7.34-7.29 region and the diastereotopic protons of the O**CH_2**Ph group formed an AB system and appeared as a set of two doublets at δ_H 4.65 and δ_H 4.49, respectively, with a

[41] Fuhrmann, E.; Talbiersky, J. *Org. Process Res. Dev.* **2005**, *9*, 206.
[42] Iversen, T.; Bundle, D.R. *J. Chem. Soc., Chem. Commun.* **1981**, *23*, 1240.
[43] Poon, K.W.C.; Dudley, G.B. *J. Org. Chem.* **2006**, *71*, 3923.

coupling constant of 11.6 Hz. In the ^{13}C NMR spectrum the benzylic carbon atom appeared at δ_C 72.29 (T) and the C-3 signal at δ_C 78.04 (D).



Scheme 28: The regioselective ring opening of the epoxide at C-1.

Reagents : (a) Bn-OPT, MgO, benzotrifluoride (41%); (b) NaN_3, NH_4Cl, 60% EtOH (0%); (c) NaN_3, NH_4Cl, 8:1 EtOH/water (77%).

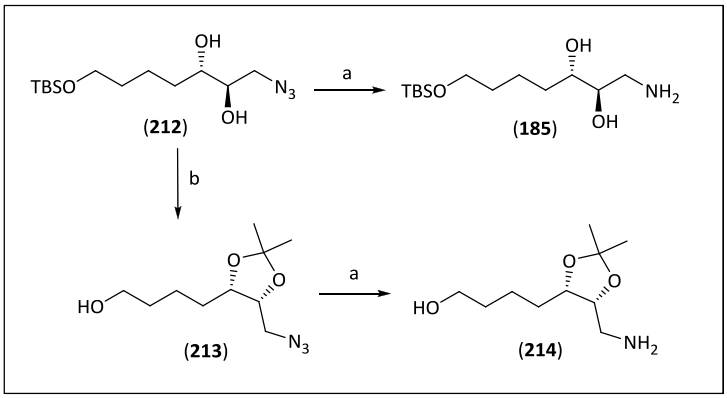
Reaction of the epoxide (**183**) with sodium azide and NH_4Cl in 60% ethanol followed by reflux at 60 °C for 3 hours failed to result in the regioselective ring opening of the epoxide at C-1 to form the azido-alcohol (**184**). The reaction conditions caused the loss of the benzyl and TBS protecting groups.

3.3.1.2. Via the Omission of Benzylation Protection Step

It was then decided to attempt the regioselective ring opening reaction on epoxide (**182**) (shown in **Scheme 28**) which lacks the benzyl protecting group. Reaction of **182** with sodium azide and NH_4Cl in 8:1 EtOH/water following the procedure of Behrens *et al.*[44] resulted in the ring opening of the epoxide at C-1 by the azide to form the azidodiol (**212**) in 77% yield, which indicated that the presence of the benzyl protecting group was somehow interfering with the reaction. This result led to a change in the synthetic methodology as it was determined that the benzyl group was not necessary in order to complete the synthesis. The azidodiol (**212**) was characterised by a broad band for the hydroxy groups at ν_{max} 3389 cm^{-1} and a sharp band for the azide at ν_{max} 2092 cm^{-1} in the IR spectrum. The ^{13}C NMR spectrum showed a characteristic signal at δ_C 52.97 (T) for the C-1 azide-bearing carbon atom, as well as signals at δ_C 73.17 (D) and 72.77 (D) representing the hydroxy-bearing C-2 and C-3 carbon atoms. The protons of the C-1 methylene group appeared as the AB-part of an ABX spin system at δ_H 3.42 and 3.39 with J_{AB} 12.6, J_{AX} 7.4 and J_{BX} 3.2 Hz in the ^1H NMR spectrum. The protons of the two hydroxy groups were characterised by broad signals at δ_H 2.71 (s) and 2.48 (s).

[44] Behrens, C.H.; Ko, S.Y.; Sharpless, K.B.; Walker, F.J. *J. Org. Chem.* **1985**, *50*, 5687.

The next step in the synthesis was the reduction of the azido group to an amine (shown in **Scheme 29**) by means of hydrogenation of the azidodiol (**212**) over Pd-C at 45 psi overnight to obtain the target amino-diol (**185**) in 77% yield. The NMR spectra were obtained in both $CDCl_3$ and MeOH-d_4 for the purposes of comparison to literature values obtained by Seo *et al*.[45] as described at a later stage (see section **3.3.1.3**). The IR spectrum exhibited a characteristic broad band for the hydroxy groups at ν_{max} 3347 cm^{-1}, while the N–C stretch band for the amine was observed at ν_{max} 1593 cm^{-1}. Analysis of the ^{13}C NMR spectrum in $CDCl_3$ showed a characteristic signal at δ_C 42.54 (T) for the C-1 amino-bearing carbon atom, while the hydroxy-bearing C-3 and C-2 signals appeared at δ_C 73.96 (D) and 73.56 (D), respectively. The ^1H NMR spectrum exhibited characteristic broad signals at δ_H 3.85 (s), 1.91 (s) and 1.87 (s) representing the protons of the amino group and two hydroxy groups, respectively. The ^{13}C NMR data obtained in MeOH-d_4 can be found in Table 5 (section **3.3.1.3**).



Scheme 29: Reactions of the azidodiol (**212**) to obtain the target amines.

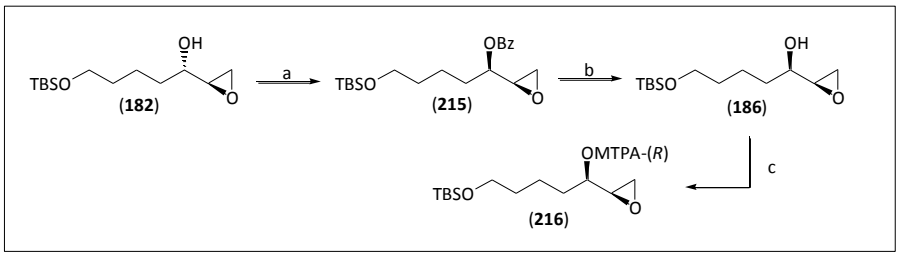
Reagents: (a) H_2, Pd-C, (**185** 77%, **214** 92%); (b) 2,2-Dimethoxypropane, *p*-TsOH, (71%).

As a result of the availability of the azidodiol (**212**), it was decided to protect the 2,3-diol as the *O*-isopropylidene (**213**) (shown in **Scheme 29**) as this would be an ideal protection strategy for the hydroxy groups at a later stage, should the C-1–C-7 fragment be coupled to the C-8–C-19 fragment. Azidodiol (**212**) was reacted with 2,2-dimethoxypropane, catalysed by *p*-TsOH in acetone, to obtain the azido-*O*-isopropylidene (**213**) in 71% yield. The reaction conditions did result, however, in the hydrolysis of the TBS protecting group. This result was deemed not to be a problem, as the envisaged coupling reaction would require deprotection of the TBS group in any case. If required the TBS group could easily be re-inserted. The IR spectrum was found to exhibit a broad band at ν_{max} 3381 cm^{-1} for the primary hydroxy group and an intense band

[45] Seo, J.-A.; Kim, J.-C., Lee, Y.-W. *J. Nat. Prod.* **1999**, *62*, 355.

at ν_{max} 2091 cm^{-1} for the azide. Analysis of the ^{13}C NMR spectrum indicated characteristic peaks at δ_C 108.51 (S) for the acetal carbon atom and at δ_C 28.18 (Q) and 25.49 (Q) for the two methyl groups. The signal for C-1 appeared at δ_C 51.19 (T), while signals were observed at δ_C 76.88 (D) and 76.52 (D) for C-2 and C-3, respectively. The characteristic signals indicative of the TBS group at δ_C 25.92 (Q), 18.32 (S) and –5.33 (Q) in **212** were absent in the ^{13}C NMR spectrum of **213** while in the ^{1}H NMR spectrum a broad signal was observed at δ_H 1.86 representing the proton of the hydroxy group on C-7.

The azide group of the azido-*O*-isopropylidene (**213**) was then reduced to the amine (shown in **Scheme 29**), using hydrogenation over Pd-C at 45 psi overnight to obtain the amino-*O*-isopropylidene (**214**) in 92% yield. A broad band at ν_{max} 3763 cm^{-1} was observed for the primary hydroxy group and a broad band of ν_{max} 2989 cm^{-1} was observed for the C–N stretching of the amine in the IR spectrum. In the ^{1}H NMR spectrum a characteristic broad signal at δ_H 3.50 (s) was observed for the amine protons, while the ^{13}C NMR spectrum exhibited signals at δ_C 108.11 (S) for the acetal carbon and δ_C 28.42 (Q) and 25.70 (Q) for the methyl groups. The signal for C-1 appeared at δ_C 41.49 (T) while signals were observed at δ_C 77.80 (D) and 77.05 (D) for C-2 and C-3. This concluded the synthesis of one of the target stereoisomers of the C-1–C-7 fragment of the iso-FC_1 fumonisin.



Scheme 30: Mitsunobu reaction to invert stereochemistry at C-3 and Mosher ester derivatisation.

Reagents: (a) PPh_3, benzoic acid, DEAD, (111%, crude); (b) K_2CO_3, MeOH, (61%); (c) (*R*)-(+)-α-MTPAA, $(COCl)_2$, Et_3N, DMAP, (75%).

The Mitsunobu reaction is a widely used and versatile reaction due to its stereospecificity, mild reaction conditions and scope when it comes to the formation of C-O, C-S, C-N or C-C bonds using primary and secondary alcohols as substrates, with chiral secondary alcohols experiencing inversion of configuration. In order to obtain the second stereoisomer of the C-1–C-7 fragment of the iso-FC_1 fumonisin it was necessary to do a Mitsunobu reaction on epoxy-alcohol (**182**), in order to invert the stereochemistry at C-3 to obtain epoxy-alcohol

(**186**). The Mitsunobu reaction consists of two parts, with the first part being the synthesis of the benzoate ester (**215**) via an S_N2 reaction with inversion of configuration, followed by conversion to the epoxy-alcohol (**186**) as shown in **Scheme 30**. The benzoate ester (**215**) was formed by reaction of epoxy-alcohol (**182**) with triphenylphosphine, followed by a solution of benzoic acid in benzene and then DEAD with stirring overnight. The benzoate ester (**215**) was found to be unstable and substantially reduced yields were obtained when the crude product was purified by column chromatography using silica gel. Thus the crude product (contaminated by triphenylphosphine oxide) was taken directly into the next reaction, in which **215** was reacted with K_2CO_3 in MeOH which resulted in transesterification of the benzoate ester under the basic conditions and the formation of the epoxy-alcohol (**186**) in 61% chemical yield and a diastereomeric ratio of 97:3.

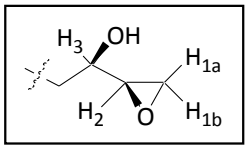


Figure 27: Numbering of the protons in the epoxy alcohol (**186**).

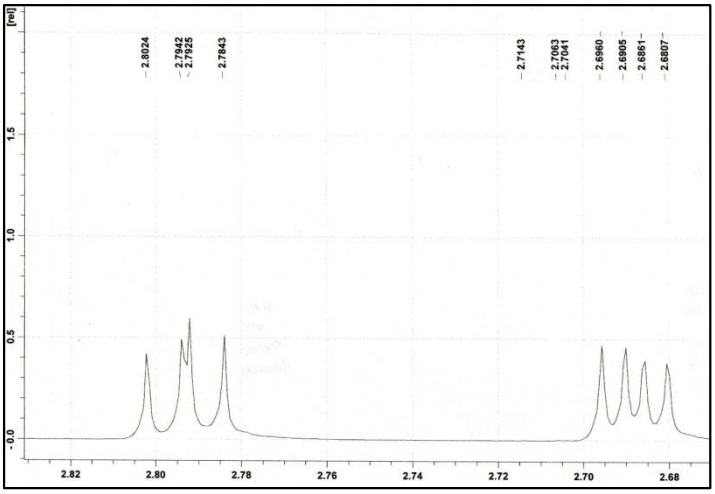
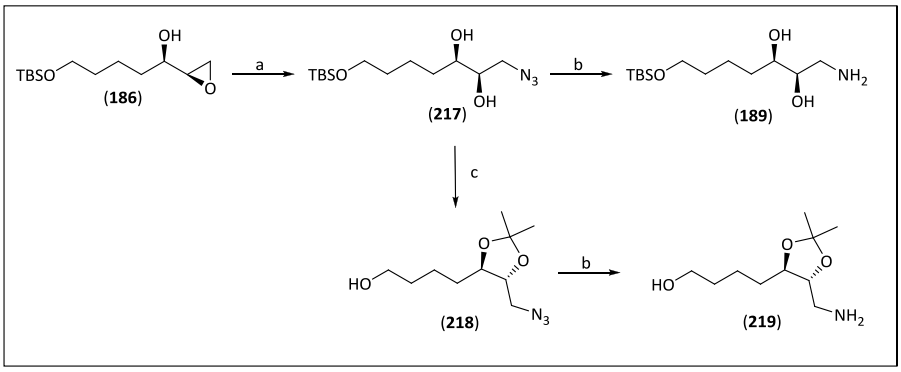


Figure 28: ^{1}H NMR spectrum of the C-1 proton signals of the epoxy-alcohol (**186**).

Analysis of the ^{13}C NMR spectrum showed the typical signals for a terminal epoxide group with the C-1 signal appearing at δ_C 45.14 (T) and C-2 at δ_C 55.33 (D). In the ^{1}H NMR spectrum the C-2 protons appeared at δ_H 2.95 (ddd) and showed a *trans* coupling of 2.5 Hz with H-1a (δ_H 2.79 dd) and a *cis* coupling of 1.6 Hz with H-1b (δ_H 2.69 dd). A geminal coupling of 3.0 Hz was observed between H-1a and H-1b. The *syn* arrangement between the protons of C-2 and C-3 resulted in a coupling constant of 1.7 Hz.

The enantioselectivity of the reaction was determined by conversion of the (2*R*,3*R*)-epoxy alcohol (**186**) to the (*R*)-MTPA derivative (shown in **Scheme 30**) using the protocol developed by Malkov *et al*.[46], followed by analysis of the ^{19}F NMR spectrum. The experimental procedure was changed as the procedure of Ward *et al*.[40] results in a large impurity signal in the ^{19}F NMR spectrum that could potentially interfere with the Mosher ester signals, which is not the case using the method of Malkov *et al*.[46] The procedure differs in that the acyl chloride is allowed to form over 2 hours, while in the second stage the Et_3N is not added and the reaction is left to stir overnight. Work-up of the reaction gave the crude Mosher ester derivative (**216**) in 75% yield. Analysis of the ^{1}H NMR spectrum showed a signal at δ_H 4.80 (ddd) for the C-3 proton, whereas the protons of the methoxy group gave a signal at δ_H 2.96 (s). The ^{19}F NMR spectrum exhibited a single major signal at δ_F –71.48 (97%) and a minor signal at δ_F –71.76 (3%), which was used to determine the enantiomeric excess of **186** as 97%.



Scheme 31: Synthesis of the target amine (**189**) and corresponding *O*-isopropylidene (**219**).

Reagents: (a) NaN_3, NH_4Cl, 8:1 EtOH/H_2O, (53%); (b) H_2, Pd-C, (**189** 33%, **219** 51 %); (c) 2,2-dimethoxypropane, *p*-TsOH, (40%).

The subsequent transformation of the epoxide (**186**) into the target amine (**189**) (shown in **Scheme 31**) was accomplished in two steps similar to those described for **185** earlier, namely 1) regioselective ring opening of the epoxide with azide to obtain **217**, and 2) reduction of the azide by hydrogenation to obtain **189**. The corresponding azido-*O*-isopropylidene (**218**) and amino-*O*-isopropylidene (**219**) were also prepared following the same protocol as described earlier, while the details regarding structural analysis are given below.

The ^{13}C NMR spectrum of the azidodiol (**217**) showed a characteristic signal at δ_C 54.14 (T) for the C-1 azide-bearing carbon atom as well as signals at δ_C 72.91 (D) and 71.82 (D) representing the hydroxy-bearing C-2 and C-3 carbon atoms. The protons of the two hydroxy groups were

[46] Malkov, A.V.; Gordon, M.R.; Stončius, S; Hussain, J.; Kočovsky, P. *Org. Lett.* **2009**, *11*, 5390.

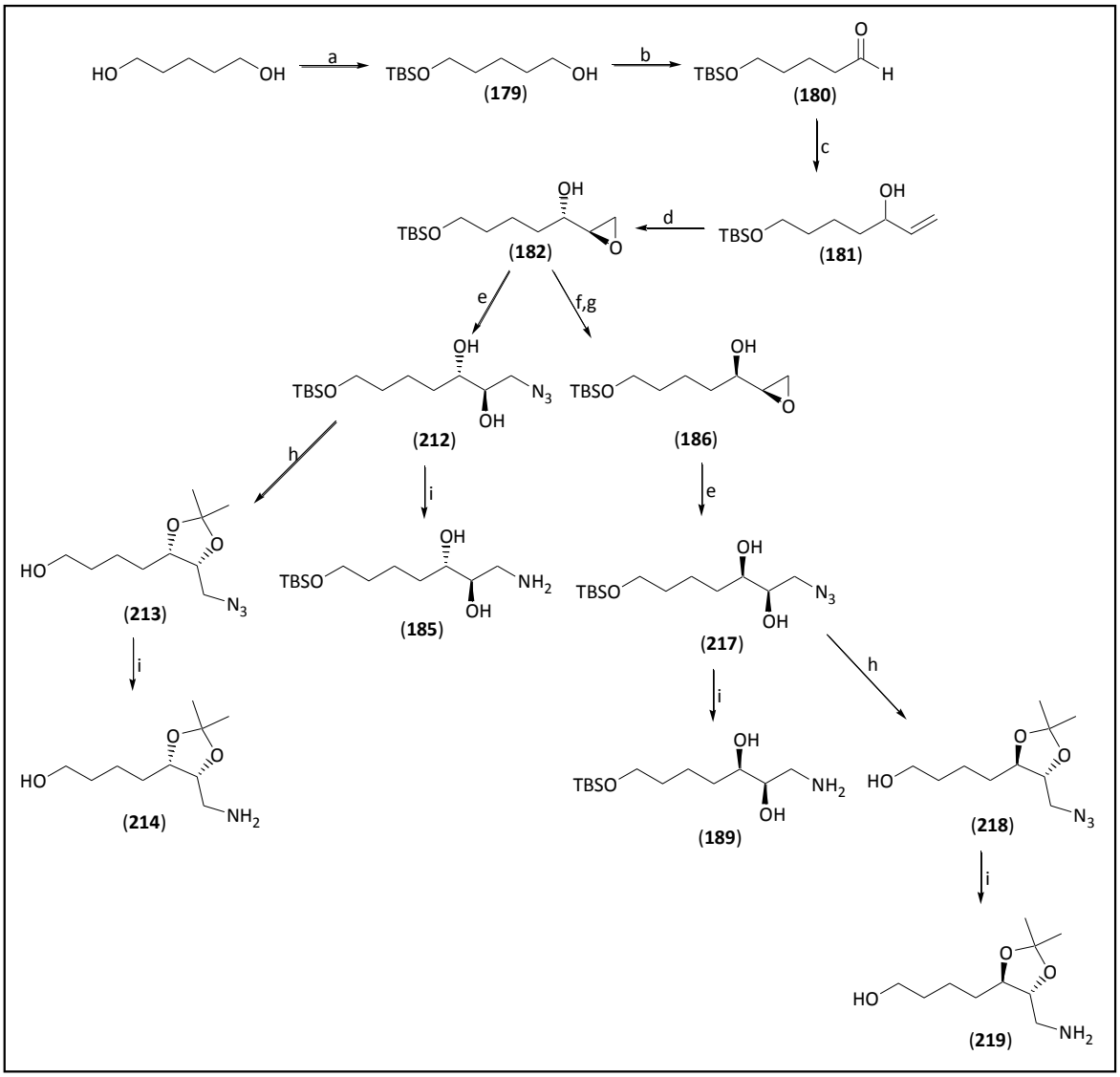
characterised by broad signals at δ_H 2.98 (s) and 2.64 (s) in the ^{1}H NMR spectrum. The NMR spectra of the target amine (**189**) were once again obtained in both deuterated chloroform and deuterated methanol. Analysis of the ^{13}C NMR spectrum in $CDCl_3$ showed a characteristic signal at δ_C 48.69 (T) for the C-1 amino-bearing carbon atom while the hydroxy-bearing C-3 and C-2 signals appeared at δ_C 73.27 (D) and 71.47 (D). The ^{1}H NMR spectrum exhibited characteristic broad signals at δ_H 4.17 (s) and 1.90 (s, 2H) representing the protons of the amino group and two hydroxy groups, respectively. The protons of C-1 were observed at δ_H 3.19 and 2.95 and formed the AB part of an ABX spin system with coupling constants of J_{AB} 12.2, J_{AX} 7.1 and J_{BX} 5.3 Hz. The ^{13}C NMR data obtained in deuterated methanol can be found in Table 5 (section **3.3.1.3**). The yields observed for amine (**189**) were found to be consistently poor due to solubility problems.

The ^{13}C NMR spectrum of azido-*O*-isopropylidene (**218**) indicated characteristic peaks at δ_C 109.18 (S) for the acetal carbon atom and at δ_C 27.32 (Q) and 26.76 (Q) for the two methyl groups. The characteristic signals indicative of the TBS group in the diol (**217**) at δ_C 26.42 (Q), 19.17 (S) and –5.16 (Q) were absent in the ^{13}C NMR spectrum, while in the ^{1}H NMR spectrum a broad signal was observed at δ_H 1.67 (s) representing the proton of the hydroxy group on C-7. The protons of C-1 appeared at δ_H 3.49 (dd) and 3.24 (dd), while those of C-2 and C-3 were observed at δ_H 3.76 (ddd) and 3.85 (m), respectively. In the ^{1}H NMR spectrum of the amino-*O*-isopropylidene (**219)** a characteristic broad signal at δ_H 2.93 (s) was observed for the amine protons, while the protons of C-1 appeared at δ_H 3.34 (dd) and 3.10 (dd) and those of C-2 and C-3 appeared at δ_H 4.02 (ddd) and 3.90 (ddd), respectively. The ^{13}C NMR spectrum exhibited signals at δ_C 109.53 (S) for the acetal carbon and δ_C 27.30 (Q) and 27.05 (Q) for the methyl groups. This concluded the synthesis of the second target stereoisomer of the C-1–C-7 fragment of the iso-FC_1 fumonisin.

3.3.1.3. Conclusion

A short and elegant synthetic route was developed and optimised to synthesise the required stereoisomers of the C-1–C-7 fragment of the iso-FC_1 fumonisin using readily available 1,5-pentanediol. Both the target isomers (**185** and **189**) as well as their corresponding amino-*O*-isopropylidenes (**214** and **219**) were successfully synthesised and completely characterised. The final synthetic pathway is shown in **Scheme 32**.

^{13}C NMR spectroscopic data was obtained for the two amines (**185** and **189**) in CD_3OD and



Scheme 32: The final synthetic route for the C-1–C-7 stereoisomers of iso-FC_1.

Reagents: (a) NaH, TBSCl; (b) $(COCl)_2$, DMSO, Et_3N; (c) Vinyl magnesium bromide; (d) *(R,R)*-(+)-DIPT, $Ti(O^iPr)_4$, TBHP; (e) NaN_3, NH_4Cl, 8:1 EtOH/water; (f) PPh_3, benzoic acid, DEAD; (g) K_2CO_3, MeOH; (h) 2,2-dimethoxypropane, *p*-TsOH; (i) H_2, Pd-C.

$CDCl_3$ as well as in CD_3OD/TCA where the ratio of amine:TCA was 1:1. TCA was added to the NMR sample in an attempt to mimic the conditions under which Seo *et al*.[45] obtained their data. The natural sample of Seo *et al*. [45] contained the TCA portions esterified to the C-14 and C-15 hydroxy groups, which induces folding of the fumonisin backbone as a result of protonation of the amino group by the carboxyl groups, and influences the chemical shifts of C-1, C-2 and C-3. The absolute configuration of the stereogenic centres for the C-1–C-7 fragment of the iso-FC_1 fumonisin could now possibly be determined by comparison of the ^{13}C NMR data obtained for the two amines (**185** and **189**) with the literature values obtained by Seo *et al*.[45] as shown in **Table 5**.

Table 5: Comparison of ^{13}C NMR data obtained (ppm)

	Iso-FC_1 lit.[45] in CD_3OD	**185** in CD_3OD	**185** in $CDCl_3$	**185** in CD_3OD and TCA	**189** in CD_3OD	**189** in $CDCl_3$	**189** in CD_3OD and TCA
C-1	43.7	43.53	42.54	43.46	44.26	48.69	43.69
C-2	71.8	73.88	73.56	73.84	73.28	71.47	71.83
C-3	73.6	74.86	73.96	74.82	73.79	73.27	74.53
δ(C-3–C-2)	1.8	0.98	0.40	0.98	0.51	1.8	2.7

In the presence of TCA the ^{13}C NMR signals of the *anti*-**185** are not significantly shifted resulting in a difference between the C-3 and C-2 signals which is smaller than that observed in the data of Seo *et al.*, while the signals of the *syn*-**189** are significantly shifted resulting in a much larger difference between the C-3 and C-2 signals, similar to that what is observed in the data of Seo *et al.* The difference in signals for **189** is thus a closer match than that of **185**. Based on this comparison of the data, the absolute configuration of the stereogenic centres for the C-1–C-7 fragment of the iso-FC_1 fumonisin is most probably that of **189**. Thus when combined with the C-8–C-19 fragment, for which the absolute stereochemistry has been confirmed, results in **220** as the proposed absolute structure for the iso-FC_1 fumonisin (shown in **Figure 29**).

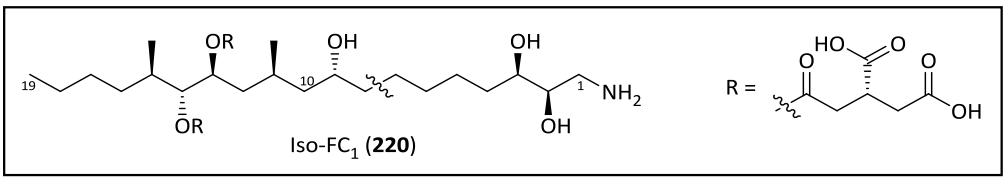


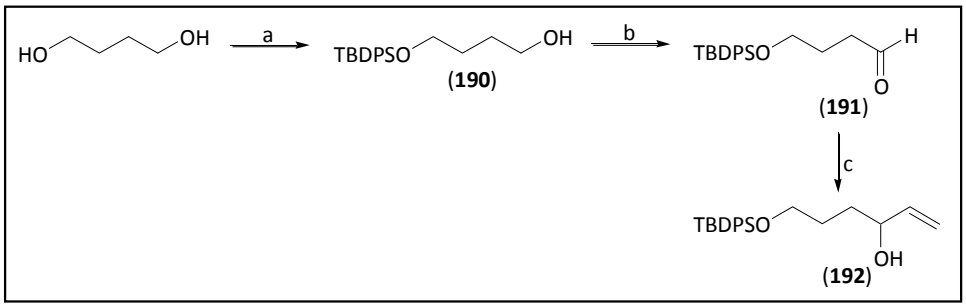
Figure 29: Proposed absolute stereochemistry for iso-FC_1.

3.3.2. Synthesis of the C-1–C-7 Fragment of the HO-FC_1 Fumonisin (161)

3.3.2.1. Via the Benzylidene Protection Route

The initial steps of the synthesis closely resemble those used for iso-FC_1 and are shown in **Scheme 33**. The synthesis began with the selective mono-protection of 1,4-butanediol using TBDPSCl, following the procedure developed by McDougal *et al.*,[32] to obtain the TBDPS-ether (**190**) in 91% yield. The TBDPS group was chosen as a suitable protecting group as it is more robust than the TBS group and should be able to withstand the even greater range of reaction conditions that would be experienced due to the additional steps required in the synthesis, which includes the selective addition and removal of other protecting groups in the presence of the TBDPS group. The OH group of the TBDPS-ether (**190**) was characterised by a broad

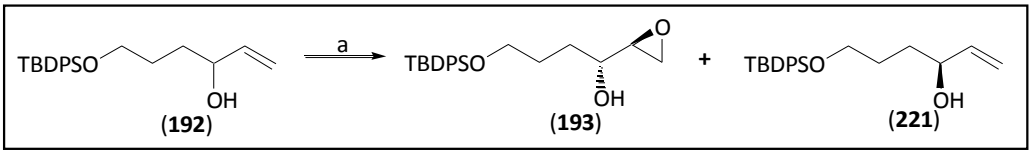
band at ν_{max} 3335 cm^{-1} in the IR spectrum and a broad ^1H NMR signal at δ_H 2.15 (s), while the aromatic protons of the TBDPS group exhibited a typical signal at δ_H 7.68-7.36 (m). The ^{13}C NMR spectrum exhibited a signal at δ_C 62.75 (T) for the C-1 hydroxy-bearing carbon atom.



Scheme 33: Synthesis of the racemic allylic alcohol (**192**).

Reagents: (a) NaH, TBDPSCl, (91%); (b) $(COCl)_2$, DMSO, Et_3N, (97%); (c) Vinyl magnesium bromide, (80%).

The extension of the TBDPS-ether (**190**) backbone by two carbons was also based on Grignard methodology and required the aldehyde (**191**), which was obtained by the Swern oxidation[36] of the primary hydroxy group. The aldehyde (**191**) was used directly in the Grignard reaction due to poor stability of the aldehyde. A Grignard reaction was done in which vinyl magnesium bromide was reacted with **191** to generate the racemic allylic alcohol (**192**) in 80% yield. Work-up was done using saturated NH_4Cl instead of aqueous acid to conserve the acid labile TBDPS group, after which the allylic alcohol required intensive chromatographic purification due to a large number of impurities generated during the reaction. The OH group of the allylic alcohol (**192**) exhibited a broad band at ν_{max} 3367 cm^{-1} in the IR spectrum, while analysis of the ^{13}C NMR spectrum indicated the presence of the C=C double bond by the signals at δ_C 114.49 (T, C-1) and δ_C 141.14 (D, C-2) typical of a terminal double bond. The ^1H NMR spectrum exhibited a signal at δ_H 5.86 (ddd) for the protons of C-2 showing a *trans* coupling of 17.2 Hz with H-1a (δ_H 5.22 ddd) and a *cis* coupling of 10.5 Hz with H-1b (δ_H 5.10 ddd).



Scheme 34: Sharpless asymmetric kinetic resolution of the allylic alcohol (**192**).

Reagents: (a) *(S,S)*-(–)-DIPT, $Ti(O^iPr)_4$, TBHP, (42%).

Resolution of the racemic allylic alcohol (**192**) was done using the Sharpless asymmetric kinetic resolution (shown in **Scheme 34**). For this epoxidation, 0.5 equivalents $Ti(O^iPr)_4$ was reacted

with 0.6 equivalents (*S,S*)-(–)-DIPT and 0.6 equivalents TBHP, followed by reaction with **192** to form the *anti* epoxy-alcohol (**193**) in 42% yield (maximum yield in the kinetic resolution reaction is 50%) and a diastereomeric ratio of 99:1. Analysis of the ^{13}C NMR spectrum showed the typical signals for a terminal epoxide group with the signal for C-1 appearing at δ_C 43.81 (T) and that of C-2 appearing at δ_C 54.46 (D). The ^{1}H NMR spectrum exhibited a signal at δ_H 2.97 (ddd) for the protons of C-2 showing a *cis* coupling of 2.8 Hz with H-1a (δ_H 2.76 dd) and a *trans* coupling of 3.9 Hz with H-1b (δ_H 2.72 dd). A geminal coupling of 5.2 Hz was observed between H-1a and H-1b. Long range coupling between the protons of C-1 and C-3 was observed with a signal appearing at δ_H 2.77 (dd) with coupling constants of 2.8 Hz and 5.3 Hz. The *anti* arrangement between the protons of C-2 and C-3 resulted in a coupling constant of 2.8 Hz. The ^{1}H NMR spectrum had the same appearance for the signals of the protons in the epoxide portion as that shown in **Figure 26**. The 3*S*-alkene (**221**) was also isolated and subsequently subjected to a Mitsunobu reaction to invert the stereochemistry of the hydroxy group at C-3. This was done via the formation of the benzoate ester, followed by transesterification to obtain the 3*R*-alkene, which then underwent Sharpless asymmetric kinetic resolution to obtain more **193** and increased the amount of material available for the synthesis.

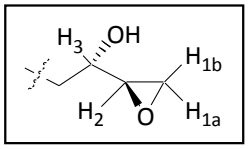
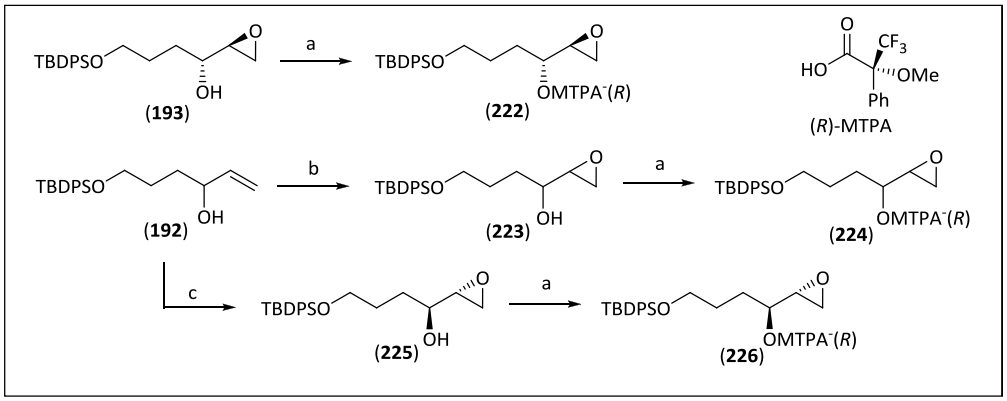


Figure 30: Numbering of the protons in the epoxy-alcohol (**193**).

The enantioselectivity of the reaction was determined by conversion of the (2*S*,3*R*)-epoxy-alcohol (**193**) to the (*R*)-α-methoxy-α-trifluoromethylphenylacetate [(*R*)-MTPA] derivative (**222**) (shown in **Scheme 35**) using the protocol developed by Malkov *et al*.[46] The crude Mosher ester derivative (**222**) was obtained in 86% yield and an enantiomeric excess of 99%. Analysis of the ^{1}H NMR spectrum showed a signal at δ_H 5.09 (ddd) for the C-3 proton, while the protons of the methoxy group gave a signal at δ_H 3.62 (s). The ^{19}F spectrum exhibited a single major signal at δ_F –71.24 (99%) and a minor signal at δ_F –71.48 (1%).

Once again in order to assist the determination of the enantioselectivity of the asymmetric kinetic resolution reaction using the ^{19}F NMR data, it was necessary to synthesize the racemic epoxy-alcohol (shown in **Scheme 35**) containing all four of the possible diastereomers. The allylic alcohol (**192**) was reacted with MCPBA to give racemic epoxy-alcohol (**223**) in 96% yield, followed by conversion to the (*R*)-MTPA derivative (**224**). The ^{19}F spectrum exhibited four



Scheme 35: Synthesis of the racemic epoxide (**223**), (2*R*,3*S*)-epoxy-alcohol (**225**) and the corresponding Mosher ester derivatives (**222**, **224**, **226**).

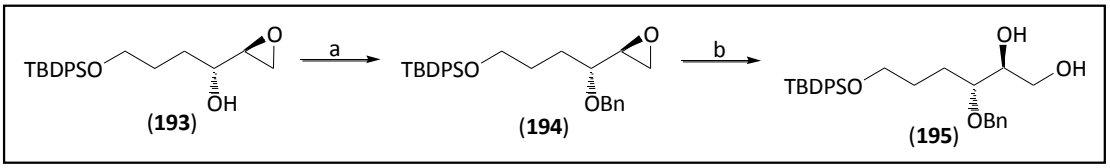
Reagents: (a) (*R*)-(+)-α-MTPAA, $(COCl)_2$, Et_3N, DMAP, (**222** 86%; **224** 86%; **226** 88%); (b) MCPBA (96%); (c) *(R,R)*-(+)-DIPT, $Ti(O^iPr)_4$, TBHP, (44%).

major signals at δ_F −71.34, −71.39, −71.47 and −71.64 in a ratio of 1:1.2:1.3:1.3. The (2*R*,3*S*)-epoxy-alcohol (**225**) was also prepared (shown in **Scheme 35**) under the same asymmetric kinetic resolution conditions as used for the preparation of the (2*S*,3*R*)-epoxy-alcohol, but using *(R,R)*-(+)-DIPT instead, followed by conversion to the (*R*)-MTPA derivative (**226**). Examination of the ^{19}F NMR spectrum revealed a major signal at δ_F −71.35 (98%) and a minor signal at δ_F −71.60 (2%). Using the ^{19}F chemical shifts it was then possible to determine the enantiomeric excess for **193** as being 99% and that of **225** as being 98%.

The protection of the secondary alcohol (shown in **Scheme 36**) with a benzyl group in epoxy-alcohol (**193**) was attempted using Dudley's reagent (Bn-OPT) in the presence of benzotrifluoride and vacuum dried MgO following the procedure of Poon *et al.*,[43] but failed to produce the required benzyl ether (**194**). The protection of the hydroxy group with a benzyl group was achieved using an alternative procedure reported by Jin and Weinreb.[47] The benzylation of the C-3 hydroxy group occurs via the formation of an alkoxide which could potentially result in the ring-opening of the epoxide giving a complex mixture of benzyl ethers. This problem can, however, be avoided by careful sequential addition of the reagents. In this procedure the epoxy-alcohol (**193**) was deprotonated using NaH suspended in THF followed by reaction with benzyl bromide and tetrabutylammonium iodide (TBAI) and stirring overnight to obtain the benzyl ether (**194**) in 92% yield. The benzyl group can once again be selectively cleaved in the presence of the TBDPS group, which makes it a suitable protecting group for the C-3 hydroxy group. Analysis of the NMR data indicated the characteristic signals in the ^1H NMR

[47] Jin, J.; Weinreb, S.M. *J. Am. Chem. Soc.* **1997**, *119*, 2050.

spectrum for the aromatic protons in the 7.73-7.26 (m) ppm region while the diastereotopic protons of the OC**H$_2$**Ph group appeared as an AB spin system with a coupling constant of 11.6 Hz at δ_H 4.65 (d) and δ_H 4.49 (d). The C-3 signal appeared at δ_C 78.04 (D) in the ^{13}C NMR spectrum.



Scheme 36: The regioselective ring opening of the epoxide at C-1.

Reagents: (a) NaH, BnBr, TBAI, (92%); (b) (*S,S*)-**F**, *p*-TsOH, H_2O, (75%).

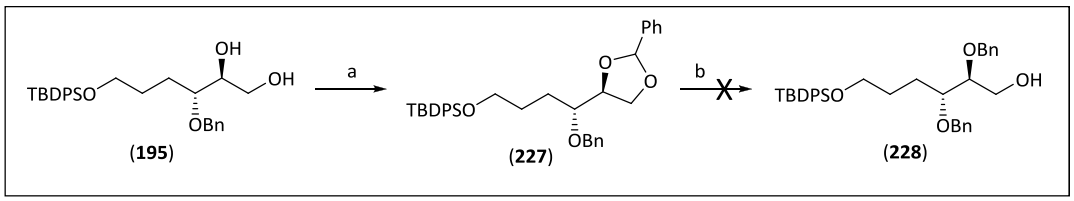
The next key step in the synthesis was the regioselective ring opening of the epoxide to obtain the diol (shown in **Scheme 36**) with retention of the 2*S* stereochemistry. Reaction of the epoxide (**194**) with water and (*S,S*)-Co-Salen complex (**F**) (**Figure 23**) activated by AcOH using the procedure of Schaus *et al*.[48], followed by stirring overnight at room temperature failed to result in the regioselective ring opening of the epoxide at C-1 to form the 1,2-diol (**195**). No reaction occurred and the starting epoxide (**194**) was recovered, indicating that the AcOH failed to activate the (*S,S*)-Co-Salen complex (**F**) sufficiently. The reaction was repeated again but under reflux at 65 °C overnight but with no success – no reaction occurred. It was then decided to first activate the (*S,S*)-Co-Salen complex (**F**) with *p*-TsOH instead of AcOH using a procedure optimised in the group.[49] In this procedure the (*S,S*)-Co-Salen complex (**F**) was added to a solution of the *p*-TsOH in DCM and stirred open to the air for two hours resulting in the formation of a dark green residue upon concentration. A solution of the epoxide (**194**) in THF was then added to the activated catalyst followed by water and heating at 35-40 °C over 48 hours, which after work-up gave the 1,2-diol (**195**) in 75% yield. The diol was characterised by a broad band at ν_{max} 3386 cm^{-1} for the hydroxy groups while the ^{1}H NMR exhibited two broad signals for the hydroxy groups at δ_H 2.89 (s) and δ_H 2.66 (s) which disappeared on addition of D_2O to the sample. The signals at δ_C 63.27 (T) and 80.64 (D) in the ^{13}C NMR spectrum were assigned to C-1 and C-2, respectively.

Various protection strategies were then examined for the selective protection of the C-2 hydroxy group in the presence of the primary C-1 hydroxy group. The latter group is required

[48] Schaus, S.E.; Brandes, B.D.; Larrow, J.F.; Tokunaga, M.; Hansen, K.B.; Gould, A.E.; Furrow, M.E.; Jacobsen, E.N. *J. Am. Chem. Soc.* **2002**, *124*, 1307.
[49] Bischofberger, K. Personal communication.

for oxidation to the aldehyde in order to execute the Wittig reaction at a later stage. The protecting group used on the C-2 hydroxy group needs to be easily and selectively removed in the presence of TBDPS and Bn groups. Some of the protecting groups that were considered were the *O*-benzylidene and *O*-*p*-methoxybenzylidene groups which would protect both the C-1 and C-2 hydroxy groups simultaneously, but which can be regioselectively opened to reveal the C-1 hydroxy group. An alternative strategy would be to selectively protect the C-1 hydroxy group with the TBS protecting group, followed by protection of the C-2 hydroxy group with the *O*-PMB or *O*-benzyl protecting groups. The TBS group could then be selectively hydrolysed in the presence of the existing protecting groups: TBDPS at C-6, Bn at C-3 and the chosen protecting group installed on the hydroxy group at C-2, leaving the primary hydroxy group available for oxidation to the aldehyde.

The benzylidene protection route was initially investigated (shown in **Scheme 37**). To this end, α,α-dimethoxytoluene was freshly prepared by the reaction of benzaldehyde and trimethyl-orthoformate catalysed by *p*-TsOH. The benzylidene (**227**) was formed by reaction of the 1,2-diol (**195**) with the α,α-dimethoxytoluene in the presence of *p*-TsOH, to obtain a mixture of diastereomers in 76% yield. The ^{13}C NMR spectrum exhibited signals at δ_C 104.07 (S) and δ_C 103.79 (S) for the acetal carbon of the benzylidene group for the two diastereomers, while all expected ^{13}C NMR signals for the TBDPS [δ_C 26.89 (Q) and 19.20 (S)] and Bn [δ_C 72.56 (T), 72.33 (T) and δ_H 4.63 (d), 4.57 (d)] protecting groups, were still present.



Scheme 37: The benzylidene protection route.

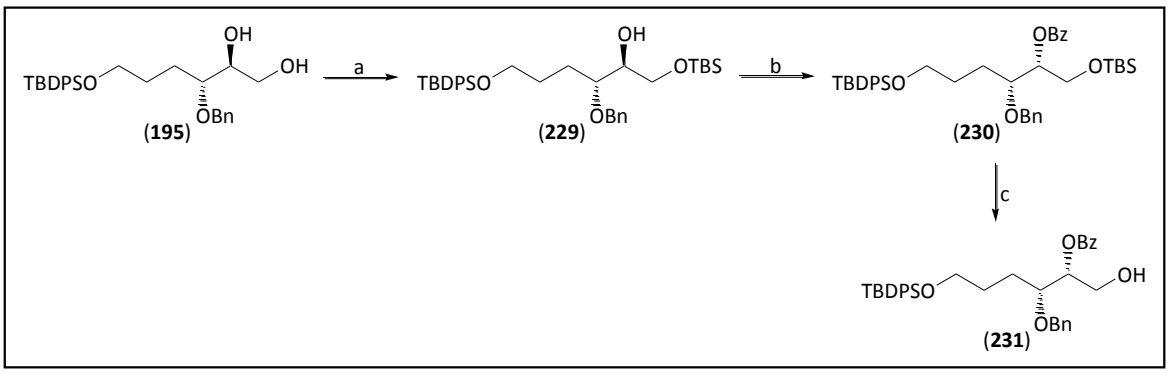
Reagents: (a) α,α-Dimethoxytoluene, *p*-TsOH, (76%); (b) $BH_3{\cdot}Me_2NH$, $BH_3{\cdot}Et_2O$.

Reaction of a solution of benzylidene (**227**) and $BH_3{\cdot}Me_2NH$ with $BH_3{\cdot}Et_2O$ at –40 °C over two hours failed to open the benzylidene selectively to release the primary hydroxy group and form the dibenzyl ether (**228**). Loss of the protecting groups occurred and the compound of interest could not be isolated. An additional problem that would be encountered at a later stage using the dibenzyl ether (**228**) is that the C-2 benzyl group cannot be selectively removed in the presence of the C-3 benzyl group in order to invert the stereochemistry at C-2 using the Mitsunobu reaction. At this point it was decided not to pursue this route.

3.3.2.2. Via the Benzoyl Protection Route

At this point an alternative, but longer route, in which the C-1 hydroxy group was protected with a TBS group followed by protection of the C-2 hydroxy group as the benzoate ester (shown in **Scheme 38**) was investigated. The TBS group was chosen for protection of the C-1 hydroxy group as it is easily introduced and can also be selectively removed in the presence of the TBDPS and Bn groups.

A cooled solution of the 1,2-diol (**195**), imidazole and DMAP was reacted with a solution of TBSCl at 0 °C for two hours to give the TBS ether (**229**) in 91% yield. The TBS ether (**229**) was characterised by a broad band at ν_{max} 3462 cm^{-1} in the IR spectrum and a signal at δ_H 2.43 (s) in the ^1H NMR spectrum for the hydroxy group on C-2. Analysis of the ^{13}C NMR spectrum showed the characteristic signals for a TBS group at δ_C 25.88 (Q) and δ_C –5.38 (Q) for the *t*-butyl protons and the methyl protons attached to silicon, respectively, while the signal for C-1 appeared at δ_C 63.96 (T) and that of C-2 appeared at δ_C 78.99 (D).



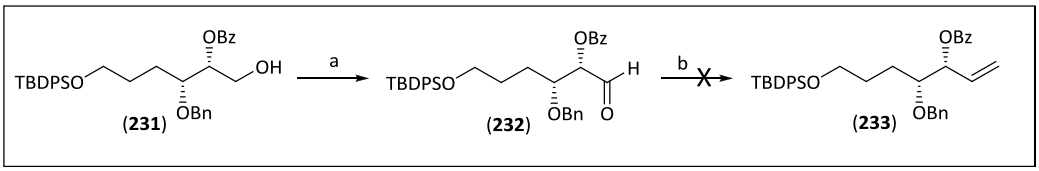
Scheme 38: The benzoyl protection route.

Reagents: (a) Imidazole, DMAP, TBSCl, (91%); (b) PPh_3, benzoic acid, DIAD, (99%); (c) *p*-TsOH, dry MeOH, (105% crude).

The benzoate ester (**230**) was prepared (**Scheme 38**) by reaction of a cooled solution of the TBS ether (**229**) and triphenylphosphine with benzoic acid and DEAD followed by stirring overnight. The benzoate ester (**230**) was obtained in a crude yield of 99%. Purification by column chromatography using silica gel resulted in substantially reduced yields. The crude benzoate ester (**230**), therefore, was taken directly into the next reaction which was the hydrolysis of the TBS protecting group to release the C-1 hydroxy group. The ^{13}C NMR spectrum of the benzoate ester (**230**) was characterised by a signal at δ_C 166.50 (S) for the benzoate ester carbonyl carbon. All the expected NMR signals for the TBS [δ_C 26.19 (Q), 18.56

(S), –5.00 (Q) and δ_H 0.08 (s), 0.05 (s)]; the TBDPS [δ_C 27.23 (Q), 19.57 (S) and δ_H 1.03 (s)] and the Bn [δ_C 73.04 (T) and δ_H 4.69 (d), 4.62 (d)] protecting groups were still present.

The procedure of Corey and Roberts[50] for the deprotection of the TBS group (**Scheme 38**) was used in which the benzoate ester (**230**) was reacted with PPTS in EtOH and heated at reflux overnight at 55-60 °C. Deprotection of the TBS group to give the alcohol (**231**) occurred, but unfortunately the reaction did not even approach 50% completion. Longer reaction times improved yields only slightly. An alternative procedure in which **230** was reacted with 5% w/w *p*-TsOH in dry MeOH resulted in the deprotection reaction going to completion to obtain the alcohol (**231**) in a crude yield of 105%. Purification of the crude product by column chromatography using silica gel again resulted in substantially reduced yields. An important consideration for this procedure is that the *p*-TsOH has the ability to hydrolyse both the TBS and TBDPS protecting groups, with preferential hydrolysis of the TBS group. Thus it was necessary to carefully monitor the reaction by TLC analysis on a continuous basis, as long reaction times would also lead to hydrolysis of the TBDPS group. The alcohol (**231**) was characterised by a broad band at ν_{max} 3466 cm^{-1} in the IR spectrum, while the ^{1}H NMR spectrum exhibited a broad signal at δ_H 2.19 (s) for the C-1 hydroxy group and the signal for C-1 appeared at δ_C 62.10 (T) in the ^{13}C NMR spectrum. The signals for the various protecting groups were still present with the TBDPS group signals observed at δ_C 26.79 (Q) and 19.12 (S), the benzyl group was confirmed by the presence of the signal at δ_C 72.27 (T) and the Bz group was confirmed by the signal observed at δ_C 166.49 for the benzoate ester carbonyl group.



Scheme 39: One-carbon extension of the carbon backbone.

Reagents: (a) $(COCl)_2$, DMSO, Et_3N, (59%); (b) MTPPI, *n*-BuLi.

The crude alcohol (**231**) obtained was used directly in the Swern reaction[36] to oxidise the C-1 hydroxy group to the aldehyde (**232**) in a crude yield of 59% (shown in **Scheme 39**). Analysis of the ^{1}H NMR spectrum indicated that the aldehyde had indeed formed due to the characteristic signal observed at δ_H 9.68 (d). The possibility of epimerisation of the C-2 stereogenic centre as

[50] Corey, E.J.; Roberts, B.E. *J. Am. Chem. Soc.* **1997**, *119*, 12425.

well as decomposition of the aldehyde, precluded purification and led to its direct use in the Wittig reaction.

The Wittig ylide required for the one-carbon extension of the carbon backbone of the aldehyde (**232**) was prepared by deprotonation of methyltriphenylphosphonium iodide (MTPPI) using a strong base. The MTPPI compound was formed as a white solid in 96% yield by reaction of methyl iodide with a solution of triphenylphosphine in toluene. In the Wittig reaction the methyl group of MTPPI was deprotonated with *n*-BuLi and reacted with crude **232** (shown in **Scheme 39**), followed by column chromatography on silica gel. A product was isolated which lacked the typical signals in the NMR spectrum of a terminal double bond. In order to establish the conditions for a successful Wittig reaction it was decided to repeat the reaction using fresh *n*-BuLi. The same result was obtained - no formation of the target alkene.

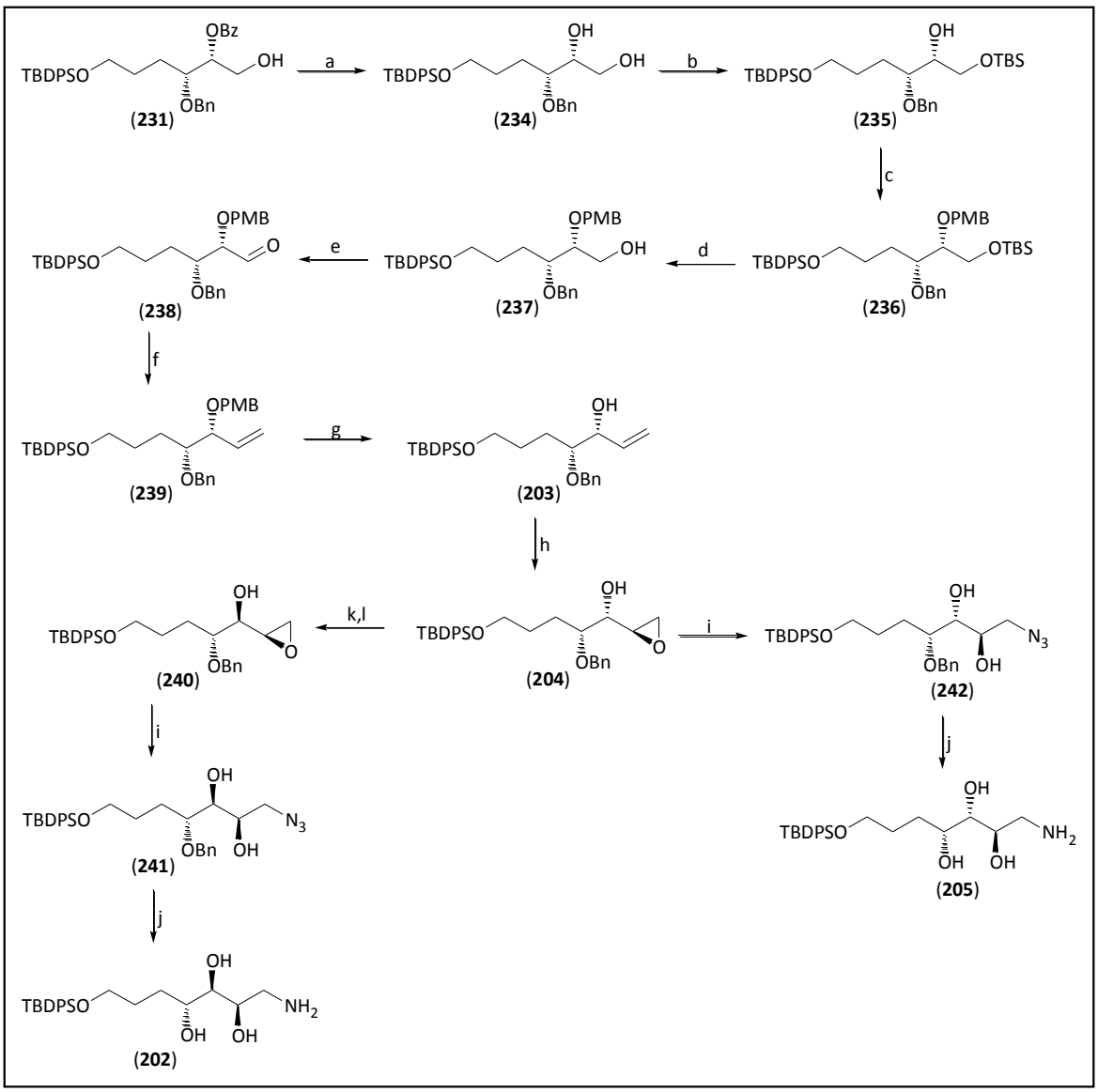
The experimental procedure used for the Wittig reaction was then questioned. A test reaction was done using the prepared MTPPI reagent, hexanal as the aldehyde and n-BuLi as base. This resulted in the formation of the required 1-heptene which was confirmed by the presence of ^{1}H NMR signals in the range 5.69 - 5.53 (m) and 5.19 - 5.08 (m), which are indicative of a terminal double bond.

It was concluded that the presence of the C-2 *O*-benzoate group was potentially a problem, especially with purification using silica gel column chromatography as extensive losses occurred. The decision was made to replace the benzoyl group with a PMB protecting group, starting from the alcohol (**195**).

3.3.2.3. Via the PMB Protection Route

The decision to replace the benzoate protecting group with the PMB group necessitated a re-examination of the synthetic route. Transesterification of the benzoate ester using the second step of the Mitsunobu reaction to obtain the 1,2-diol (**234**) was identified as the most suitable synthetic route. Protection of the primary hydroxy group with TBSCl to obtain **235**, followed by protection of the secondary hydroxy group with PMBCl to obtain **236** and consequent deprotection of the primary hydroxy group would result in the alcohol (**237**). The aldehyde (**238**) required for the Wittig reaction can be prepared by oxidation of **237,** followed by a Wittig reaction and deprotection of the secondary hydroxy group, which would result in the allylic alcohol (**203**). Sharpless epoxidation methodology would allow for the synthesis of the

more easily obtained *anti* epoxy-alcohol (**204**), followed by a Mitsunobu reaction to invert the stereochemistry at C-3 to obtain the *syn* epoxy-alcohol (**240**). Completion of the synthesis would then once again involve ring opening of the epoxide with azide, followed by hydrogenation. The new proposed synthetic route is shown in **Scheme 40**.

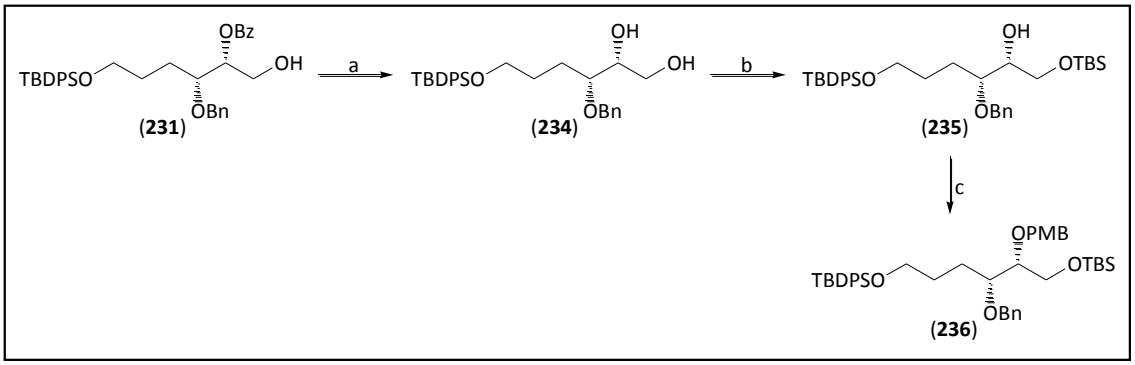


Scheme 40: New proposed synthetic route from **231** to the target HO-FC_1 stereoisomers.

Reagents : (a) K_2CO_3, MeOH; (b) Imidazole, DMAP, TBSCl; (c) NaH, PMBCl; (d) *p*-TsOH, dry MeOH; (e) $(COCl)_2$, DMSO, Et_3N; (f) MTPPI, *n*-BuLi; (g) PPTS, MeOH; (h) $Ti(O^iPr)_4$, (*R*)-(+)-DIPT, TBHP; (i) NaN_3, NH_4Cl; (j) H_2, Pd-C; (k) PPh_3, benzoic acid, DEAD; (l) K_2CO_3, MeOH.

The benzoyl-alcohol (**231**) was reacted with K_2CO_3 in dry MeOH, using the procedure for the second stage of the Mitsunobu reaction which resulted in the formation of the 1,2-diol (**234**) in a yield of 56% (**Scheme 41**). The diol was characterised by a broad band at ν_{max} 3401 cm^{-1}

for the hydroxy groups, while the ^{1}H NMR spectrum exhibited a broad signal (2H) for the hydroxy groups at δ_H 2.25 (s) which disappeared in the presence of D_2O. The signals at δ_C 63.71 (T) and δ_C 79.34 (D) in the ^{13}C NMR spectrum were assigned to C-1 and C-2, the carbons bearing the primary and secondary hydroxy group, respectively.



Scheme 41: Replacement of the Bz group with PMB.

Reagents : (a) K_2CO_3, MeOH, (56%); (b) Imidazole, DMAP, TBSCl, (66%); (c) NaH, PMBCl, (0%).

The C-1 hydroxy group of the 1,2-diol (**234**) was once again protected with a TBS group using TBSCl in the presence of imidazole and DMAP to obtain the TBS-ether (**235**) (shown in **Scheme 41**). The expected **235** formed in 66% yield and was characterised by a broad band at ν_{max} 3460 cm^{-1} in the IR spectrum and a signal at δ_H 2.09 (s) in the ^{1}H NMR spectrum for the hydroxy group on C-2. Analysis of the ^{13}C NMR spectrum showed the characteristic signals for a TBS group at δ_C 25.88 (Q) and δ_C –5.38 (Q) for the *t*-butyl methyl protons and the methyl protons attached to silicon respectively while the signals for C-1, C-2 and C-3 appeared at δ_C 63.93 (T), δ_C 78.56 (D) and δ_C 73.05 (D), respectively. The assignment of the signals was assisted by correlation with specific proton resonances using 2D (^{13}C-^{1}H) heteronuclear single-quantum coherence (HSQC) experiments while the multiplicities were deduced from proton-decoupled DEPT-135 spectra.

The protection of the C-2 hydroxy group with PMBCl (shown in **Scheme 41**) followed the procedure of Kobayashi *et al.*[51], in which **235** was reacted with NaH and PMBCl in DMF overnight at room temperature. This resulted in no reaction as the starting TBS-ether (**235**) and PMBCl were the only products. The reaction was repeated with the addition of an equivalent of KI to assist the reaction, which resulted in the isolation of a complicated mixture of products, none of which was the required PMB ether (**236**) as shown by NMR spectroscopic

[51] Kobayashi, Y.; Kumar, G.B.; Kurachi, T.; Acharya, H.P.; Yamazaki, T.; Kitazume, T. *J. Org. Chem.* **2001**, *66*, 2011.

analysis. The procedure was repeated once more, but without the addition of KI and instead of reaction overnight at room temperature it was heated at reflux at 65-70 °C, which resulted in a slightly less complex mixture of products, but again the required PMB-ether (**236**) was not obtained.

3.3.2.4. Conclusion

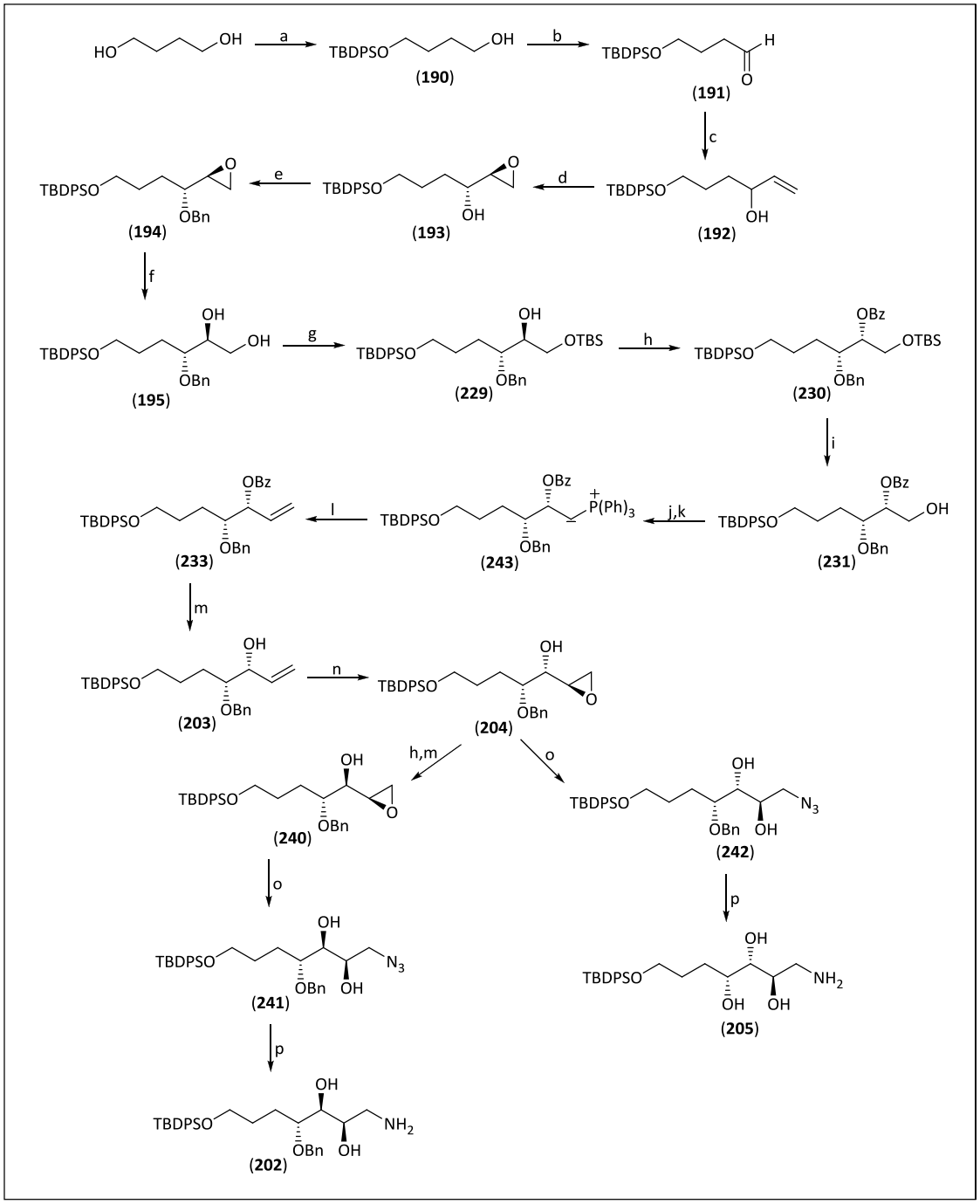
Due to time constraints, other protecting strategies that would lead to the free C-1 hydroxy group needed for oxidation to the aldehyde required for the Wittig reaction could not be investigated, and could potentially encounter the same difficulties as already experienced.

Thus an entirely different synthetic pathway would need to be devised with a re-evaluation of the protection strategies used as well as the reactions used for extension of the carbon backbone. The synthesis of the C-1–C-7 fragment of the HO-FC_1 fumonisin could not be completed and the target stereoisomers (**202** & **205**) could not be obtained, therefore no conclusions can be drawn regarding the absolute structure of the HO-FC_1 fumonisin as was done for the iso-FC_1 fumonisin.

3.4. Future Work

Although a short, elegant synthetic route for the synthesis of the C-1–C-7 fragment of the iso-FC_1 fumonisin was successfully devised and optimised using Sharpless asymmetric kinetic resolution, regioselective opening of an epoxide and various protection strategies, the synthesis of the C-1–C-7 fragment of the HO-FC_1 fumonisin using similar methodology could not be achieved. Future work on the C-1–C-7 fragment of the HO-FC_1 fumonisin requires an alternative synthetic route to the target stereoisomers, followed by implementation and optimisation of the synthesis. The absolute configuration of the C-1–C-7 unit of HO-FC_1 fumonisin can only be derived once this synthesis is completed.

The problematic area in the previous attempts to synthesise the C-1–C-7 stereoisomers of the HO-FC_1 fumonisin was identified to be at the Wittig reaction. An alternative synthesis is given in **Scheme 42** and it can be seen that the first ten steps and the last five steps have not been altered as from experience it is known that these reactions give the required targets in good yields. A Wittig reaction can still be used for the insertion of the single carbon atom required; however, the source of the ylide and aldehyde need to change.



Scheme 42: Alternative synthesis for the C-1–C-7 fragment of HO-FC_1.

Reagents : (a) NaH, TBDPSCl; (b) $(COCl)_2$, DMSO, Et_3N; (c) vinyl magnesium bromide; (d) $Ti(O^iPr)_4$, (*S,S*)-(–)-DIPT, TBHP; (e) NaH, BnBr, TBAI; (f) (*S,S*)-Co-Salen complex **F**, *p*-TsOH, H_2O; (g) Imidazole, DMAP, TBSCl; (h) PPh_3, benzoic acid, DIAD; (i) *p*-TsOH, dry MeOH; (j) CBr_4, PPh_3; (k) PPh_3; (l) *n*-BuLi, formaldehyde; (m) K_2CO_3, MeOH; (n) $Ti(O^iPr)_4$, (*R,R*)-(+)-DIPT, TBHP; (o) NaN_3, NH_4Cl; (p) H_2, Pd-C.

The aldehyde (**232**) (see **Scheme 39**) was identified as potentially being one of the major problems in the previous synthesis due to stability issues and possible decomposition before reaction with MTPPI could occur. This can be circumvented by converting alcohol (**231**) into

the corresponding bromide using CBr_4 and PPh_3, followed by reaction with PPh_3 to form the ylide (**243**) which is required for the Wittig reaction with formaldehyde to obtain the target alkene (**233**). Once the alkene (**233**) has been obtained the synthesis can continue following known methodology.

4. EXPERIMENTAL

4.1. General Procedures

Air- and moisture-sensitive reactions were performed under an argon atmosphere in glassware dried overnight in an oven at 110 °C. Room temperature (rt) refers to temperatures in the range 18-25 °C. Concentrations and evaporations were performed under reduced pressure. All reagents used were of synthetic grade and were used without further purification unless stated otherwise. When required, solvents and reagents were dried prior to use using standard methods.[1]

All reactions were monitored by means of thin-layer chromatography using Merck silica gel $60F_{254}$ sheets. UV light (254 and 266 nm) as well as phosphomolybdic acid and anisaldehyde-sulfuric acid were used to visualise the TLC plates. R_f values given are for the solvent systems used in column chromatography. Column chromatography was performed on Fluka silica gel 60 (0.063-0.2 mm, 70-230 mesh). Eluent ratios are given as v/v and 2 mL Et_3N was added to every 1000 mL eluent prepared. Yields refer to pure products obtained after column chromatography unless stated otherwise.

Mass spectra were recorded at the University of Pretoria on a Waters SYNAPT G2 Q-TOF Ultima spectrometer using the electrospray ionisation (ESI) technique and detection of positive ions with m/z > 99. FTIR spectroscopy was performed using a Perkin-Elmer RXI FT-IR spectrometer fitted with a PIKE technologies ATR accessory making use of the reflectance technique. Optical rotations were obtained using a Perkin-Elmer 341 polarimeter and a 10.0 cm cell at the wavelength of the sodium D-line (λ = 589 nm). Optical rotation values are given in units of 10^{-1}deg.cm^2.g^{-1} while concentrations are given in g/100 mL. All measurements were done in chloroform unless stated otherwise.

Nuclear magnetic resonance (NMR) spectra were obtained for $CDCl_3$ solutions (unless specified otherwise) using Bruker spectrometers: AVANCE-III-300 operating at 300.13 MHz for ^{1}H, 75.47

[1] Perrins, D.D.; Armarego, W.L.F. *Purification of laboratory chemicals*. 3rd Ed., Pergamon Press, Oxford, **1992**.

MHz for ^{13}C and 282.37 MHz for ^{19}F; AVANCE-III-400 operating at 400.21 MHz for ^{1}H and 100.64 MHz for ^{13}C and AVANCE-500-DRX operating at 500.13 MHz for ^{1}H and 125.73 MHz for ^{13}C. Chemical shifts are reported as δ (ppm) values downfield from Me_4Si using $CHCl_3/CDCl_3$ as an internal standard (δ_H = 7.24 and δ_C = 77.00, respectively). Proton coupling constants (*J*) are given in Hz. The spectral coupling patterns are designated as follows: s/S - singlet; d/D - doublet; t/T - triplet; q/Q - quartet; m - multiplet; br - broad signal. Assignments of signals marked with an * are interchangeable.

The assignments of the signals in the ^{1}H NMR spectra are based on first-order analysis of the spin systems and when required were confirmed by two-dimensional (2D) (^{1}H-^{1}H) homonuclear chemical shift correlation (COSY) experiments. The ^{13}C shifts were obtained from proton-decoupled ^{13}C NMR spectra. The multiplicities of the ^{13}C signals were deduced from proton-decoupled DEPT-135 spectra. The signals of the proton-bearing carbon atoms were correlated with specific proton resonances using 2D (^{13}C-^{1}H) heteronuclear single-quantum coherence (HSQC) experiments by utilising the one-bond spin-spin couplings. Standard Bruker pulse programs were used in the experiments.

4.2. Reagent Preparation

4.2.1. Visualisation Reagents

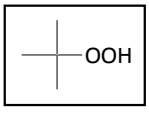
Phosphomolybdic acid TLC dip

A solution was prepared by dissolving phosphomolybdic acid (12 g) in EtOH (250 mL). Compounds visualised using this dip result in blue or green spots against a green background upon heating with a heat gun.

Anisaldehyde - sulfuric acid TLC dip

A solution was prepared by dissolving anisaldehyde (15 g) in EtOH (250 mL) followed by slow addition of concentrated H_2SO_4 (2.5 mL). Compounds visualised in this dip result in pink or blue spots against a pink background upon heating with a heat gun.

4.2.2. Freshly Prepared Reagents



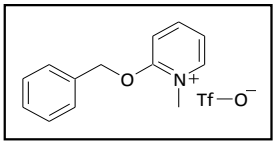
Anhydrous *tert*-butyl hydroperoxide in toluene

Aqueous *t*-butyl hydroperoxide (70 %, 350 mL) and toluene (700 mL) were swirled together in a separatory funnel followed by separation of the phases. The organic phase was equipped with a Dean-Starke trap and refluxed at 115 °C under argon for 5 h to allow for the azeotropic removal of water. The solution was cooled to rt and stored in a brown glass bottle over 4Å molecular sieves. The anhydrous TBHP solution in toluene was standardised by means of ^{1}H NMR spectroscopy. The molarity of the solution was determined as 4.2 M using the formula

$$\mathrm{M} = \frac{x}{0.1x+0.32y}$$

where *x* and *y* are the integrated values in millimetres (mm) of the *tert*-butyl and toluene methyl group proton signals, respectively.

δ_H 2.39 (s, C**H$_3$**); 1.29 (s, *t*-butyl).



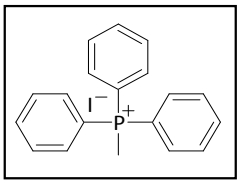
Dudley's reagent[2]

Benzyl alcohol (12.0 mL, 116 mmol) and 2-chloropyridine (18.1 mL, 191 mmol) were dissolved in toluene (230 mL) followed by addition of freshly ground KOH (21.5 g, 383 mmol) and 18-crown-6 (1.54 g, 5.8 mmol). The reaction mixture was attached to a Dean-Starke trap, heated at reflux and the water azeotropically removed followed by cooling to rt. The reaction mixture was diluted with EtOAc (100 mL) and washed with water (2×100 mL) and brine (1×100 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$, filtered and concentrated. The red oil obtained was purified by column chromatography using 3:1 hexane/EtOAc as eluent to obtain a yellow oil (10.8 g, 85%); R_f = 0.48.

Methyl trifluoromethanesulfonate (10.0 g, 60.9 mmol) was added rapidly to a solution of the oil prepared above (10.8 g, 58.0 mmol) in toluene (58.0 mL) cooled to 0 °C. The mixture was allowed to warm to rt over 3 h resulting in a white precipitate. The mixture was vacuum filtered and the solid washed with cold toluene (3×15 mL), followed by drying under high vacuum to obtain Dudley's reagent as a white powder (15.3 g, 75%).

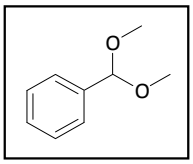
δ_C: 159.41 (S, C-2); 147.98 (D, C-6); 143.68 (D, C-3); 132.46, 129.48, 128.95, 128.47 (Ph ring carbons); 120.58 (Sq, J_{CF} 320 Hz, **C**F$_3$); 118.92 (D, C-4); 112.00 (D, C-5); 74.33 (T, O**C**H$_2$Ph); 41.83 (Q, N**C**H$_3$).

[2] Poon, K.W.C.; Dudley, G.B. *J. Org. Chem.* **2006**, *71*, 3923.



Methyltriphenylphosphonium iodide (MTPPI)

Methyl iodide (3.2 mL, 51.4 mmol) was added dropwise to a solution of triphenylphosphine (10.0 g, 38.1 mmol) in toluene (50.0 mL) followed by stirring at rt for 18 h. The reaction was cooled to 0 °C and vacuum filtered to collect the solids followed by washing with cold toluene (2×30 mL) and drying under reduced pressure at 50 °C to obtain methyltriphenylphosphonium iodide as an off-white powder (14.8 g, 96%), which was used without further purification.



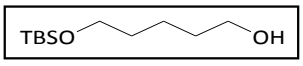
α,α-Dimethoxytoluene

p-TsOH (0.53 g, 2.8 mmol) and trimethyl orthoformate (60.0 mL, 548 mmol) were added to a solution of benzaldehyde (51.0 mL, 505 mmol) in anhydrous MeOH (170 mL) followed by refluxing at 75-80 °C for 4 h. The reaction was allowed to cool to rt and diluted with EtOAc (200 mL) followed by washing with 1:1 KOH/brine (2×100 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated to obtain a light yellow oil (69.4 g, 90%) which was used without further purification.

4.3. Procedures

4.3.1. Synthesis of the C-1–C-7 Fragment of Iso-FC_1 Fumonisin

4.3.1.1. Via the Benzylation Protection Route



5-[(*t*-Butyldimethylsilanyl)oxy]-1-pentanol (179)[3]

NaH (60% dispersion, 3.80 g, 95.0 mmol) was washed with hexane (2×20 mL) and suspended in THF (150 mL). 1,5-Pentanediol (10.0 mL, 95.0 mmol) was added at rt and stirred for 60 min to obtain a white precipitate. TBSCl (14.4 g, 95.0 mmol) dissolved in THF (30 mL) was added dropwise to the reaction mixture at rt and stirred for 50 min. The reaction mixture was diluted

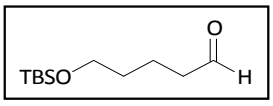
[3] McDougal, P.G.; Rico, J.G.; Oh, Y-I.; Condon, B.D. *J. Org. Chem.* **1986**, *51*, 3388.

with Et_2O (150 mL) and water (80 mL) followed by washing with brine (2×80 mL) and saturated aq. $KHCO_3$ (1×50 mL). The Et_2O fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 2:1 hexane/EtOAc as eluent to obtain the *O*-TBS ether (**179**) (15.7 g, 76%) as a light yellow oil; R_f = 0.38; ν_{max} 3321 cm^{-1}.

δ_H: 3.56 (t, 4H, *J* 6.5, H-1, H-5); 2.27 (br s, 1H, O**H**); 1.56-1.46 (m, 4H, H-2, H-4); 1.38-1.30 (m, 2H, H-3); 0.84 (s, 9H, SiC(C**H₃**)₃); −0.01 (s, 6H, Si(C**H₃**)₂).

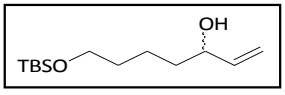
δ_C: 63.09 (T, C-5); 62.58 (T, C-1); 32.40 (T, C-4); 32.34 (T, C-2); 25.88 (Q, SiC(**C**H_3)$_3$); 21.94 (T, C-3); 18.28 (S, Si**C**$(CH_3)_3$); −5.37 (Q, Si(**C**H_3)$_2$).

HRMS (ESI): *m/z* 201.1672 $[(M+H)-H_2O]^{+\bullet}$ Calculated for $C_{11}H_{25}OSi$: 201.1675.
m/z 219.1776 [M+H]; Calculated for $C_{11}H_{27}O_2Si$: 219.1780.



5-[(*t*-Butyldimethylsilanyl)oxy]-pentanal (180)

DMSO (4.2 mL, 59.1 mmol) was added dropwise to a solution of $(COCl)_2$ (2.6 mL, 29.8 mmol) in DCM (180 mL) at −78 °C and stirred for 30 min. The alcohol (**179**) (4.01 g, 18.3 mmol) in DCM (95 mL) was added dropwise at −78 °C and stirred for 1 h. Et_3N (14.8 mL, 106.2 mmol) was added at −78 °C and stirred for 1 h followed by warming to rt. TLC analysis was done using 4:1 hexane/EtOAc as eluent (R_f = 0.49) to determine reaction progress. Water (100 mL) was added to the reaction mixture and the phases separated. The aqueous phase was washed with Et_2O (3×80 mL), the organic fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The murky oil obtained was dissolved in a minimum of Et_2O and the white crystals that formed were filtered off, followed by concentration to obtain the aldehyde (**180**) (4.21 g, 19.5 mmol, 106%). The crude material was used immediately without further purification or characterisation for the next reaction.



(3*RS*)-7-[(*t*-Butyldimethylsilanyl)oxy]-hept-1-en-3-ol (181)[4]

The aldehyde (**180**) (4.59 g, 21.2 mmol) dissolved in THF (45 mL) was added dropwise to vinyl magnesium bromide (42.5 mL, 42.5 mmol, 1 M in THF) at rt followed by stirring for 90 min.

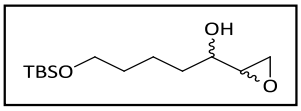
[4] Gowrisankar, P.; Pujari, S.A.; Kaliappan, K.P. *Chem. Eur. J.* **2010**, *16*, 5858.

The reaction mixture was cooled to 0 °C, quenched with saturated NH_4Cl and diluted with EtOAc (70 mL). The EtOAc fraction was washed with water (2×70 mL) and brine (1×40 mL). The organic fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 5:1 hexane/EtOAc as eluent to obtain the allylic alcohol (**181**) (4.17 g, 80%) as a yellow oil; R_f = 0.38; ν_{max} 3360, 1093 cm^{-1}.

δ_H: 5.83 (ddd, 1H, *J* 6.3, 10.4, 17.2, H-2); 5.18 (ddd, 1H, *J* 1.4, 1.4, 17.2, H-1a); 5.06 (ddd, 1H, *J* 1.4, 1.3, 10.5, H-1b); 4.09-4.04 (m, 1H, H-3); 3.58 (t, 2H, *J* 6.4, H-7); 1.78 (br s, 1H, O**H**); 1.57-1.46 (m, 4H, H-4, H-6); 1.45-1.34 (m, 2H, H-5); 0.86 (s, 9H, SiC(C**H₃**)$_3$); 0.01 (s, 6H, Si(C**H₃**)$_2$).

δ_C: 141.20 (D, C-2); 114.55 (T, C-1); 73.13 (D, C-3); 63.06 (T, C-7); 36.67 (T, C-4)*; 32.58 (T, C-6)*; 25.93 (Q, SiC(**C**H_3)$_3$); 21.60 (T, C-5); 18.32 (S, Si**C**$(CH_3)_3$); –5.31 (Q, Si(**C**H_3)$_2$).

HRMS (ESI): *m/z* 227.1827 $[(M+H)-H_2O]^{+\bullet}$; Calculated for $C_{13}H_{27}O_1Si$: 227.1831.



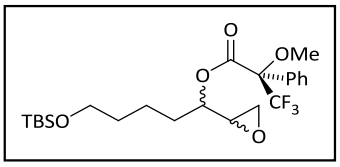
(2*RS*,3*RS*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptanol (208)

MCPBA (0.50 g, 2.9 mmol) dissolved in DCM (10 mL) was dried using anhydrous $MgSO_4$ followed by filtration. The MCPBA was added to a solution of **181** (0.33 g, 1.4 mmol) in DCM (10 mL) and stirred for 2 days at rt. The DCM fraction was washed with saturated aq. $KHCO_3$ (3×20 mL), dried ($MgSO_4$) and concentrated. The oil obtained was purified by column chromatography using 2:3 hexane/Et_2O as eluent to give the epoxy alcohol (**208**) as a light yellow oil (0.28 g, 79%); R_f = 0.33; *dr* 1.3:1; ν_{max} 3426, 1736 cm^{-1}.

δ_H: 3.77 (ddd, 1H, *J* 3.6, 3.6, 7.3, H-3); 3.59 (t, 2H, *J* 6.2, H-7); 3.58 (t, 2H, *J* 6.2, H-7); 3.38 (ddd, 1H, *J* 5.6, 5.6, 7.1, H-3); 2.97 (ddd, 1H, *J* 3.1, 3.1, 3.9, H-2); 2.93 (ddd, 1H, *J* 2.8, 4.1, 5.2, H-2); 2.78 (dd, 1H, *J* 4.1, 4.9, H-1a); 2.76 (dd, 1H, *J* 2.3, 2.8, H-1b); 2.69 (dd, 1H, *J* 4.0, 5.1, H-1a); 2.67 (dd, 1H, *J* 2.8, 4.9, H-1b); 2.31 (br s, 1H, O**H**); 1.63-1.36 (m, 6H, H-4, H-5, H-6); 0.85 (s, 9H, SiC(C**H₃**)$_3$); 0.00 (s, 6H, Si(C**H₃**)$_2$).

δ_C: 71.69, 68.39 (D, C-3); 62.96, 62.89 (T, C-7); 55.40, 54.49 (D, C-2); 45.11, 43.42 (T, C-1); 33.98, 33.12 (T, C-4)*; 32.63, 32.59 (T, C-6)*; 25.89 (Q, SiC(**C**H_3)$_3$); 21.61, 21.59 (T, C-5); 18.28 (S, Si**C**$(CH_3)_3$); –5.35 (Q, Si(**C**H_3)$_2$).

HRMS (ESI): *m/z* 283.1725 $[M+Na]^{+\bullet}$; Calculated for $C_{13}H_{28}O_3SiNa$: 283.1705.



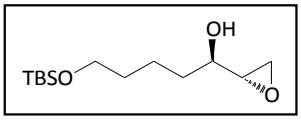
(2*RS*,3*RS*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptanyl (*R*)-α-methoxy-α-trifluoro-methyl-phenylacetate (209)

DMF (470 µL, 6.1 mmol) was added to a solution of (*R*)-(+)-α-MTPACA (0.25 g, 1.1 mmol) in hexane (7 mL) followed by dropwise addition of $(COCl)_2$ (94 µL, 1.1 mmol) and stirring at rt for 1 h. The hexane was decanted and the solvent removed to obtain the acyl chloride.

Et_3N (755 µL, 5.4 mmol) and DMAP (1 crystal) were added to a solution of the above acyl chloride in DCM (6.7 mL). **208** (0.27 g, 1.1 mmol) dissolved in DCM (6.7 mL) was added to the solution followed by stirring for 3 h at rt. The mixture was diluted with DCM (13 mL) followed by washing with 0.1 M HCl (10 mL) and saturated $NaHCO_3$ (20 mL). The DCM fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated to obtain the Mosher ester (**209**) as a light yellow oil (0.43 g, 86%); R_f = 0.54 (2:1 hexane/EtOAc); *dr* 1.4:1.8:1.9:1.

δ_C: 170.55 (S, **C**OO); 135.75, 129.52, 128.29, 128.27, 128.23, 128.07, 127.63, 127.59, 127.39, 127.21 (aromatic carbons); 124.79, 123.21, 123.19, 123.16 (Sq, J_{CF} 288 Hz, **C**F_3); 77.80, 74.68, 74.16, 68.55 (D, C-3); 62.93, 62.86, 62.59, 62.52 (T, C-7); 55.40, 55.26, 54.73, 54.41 (D, C-2); 52.41, 52.36, 51.71, 51.65 (Q, O**C**H_3); 45.33, 44.69, 44.50, 43.48 (T, C-1); 32.62, 32.56, 32.29, 32.24 (T, C-4)*; 31.46, 31.11, 30.85, 30.76 (T, C-6)*; 25.84, 25.80 (Q, SiC(**C**$H_3)_3$); 21.54, 21.35, 21.15, 20.96 (T, C-5); 18.23, 18.19 (S, Si**C**$(CH_3)_3$); –5.40, –5.46 (Q, Si(**C**$H_3)_2$).

δ_F: –71.42; –71.49; –71.57; –71.76.



(2*S*,3*R*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptanol (210)

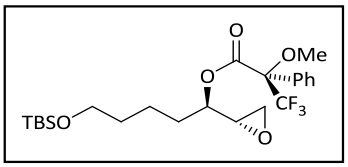
Powdered 4Å molecular sieves (0.41 g) were suspended in DCM (49 mL) and cooled to –20 °C. (*S,S*)-DIPT (0.9 mL, 4.4 mmol) was added followed by dropwise addition of $Ti(O^iPr)_4$ (1.1 mL, 3.7 mmol) and stirred for 30 min. TBHP (4.92 M, 0.9 mL, 4.4 mmol) was added dropwise and stirred for 30 min. The allylic alcohol (**181**) (1.80 g, 7.4 mmol) dissolved in DCM (26 mL) was added dropwise and stirred for 1 h at –20 °C followed by reaction for 18 h in the freezer at –10 °C. The reaction mixture was warmed to 0 °C, vacuum filtered to remove the molecular sieves, water added (50 mL) and stirred for 45 min while warming to rt. The mixture was

vacuum filtered to break the emulsion. The phases were separated and the aqueous phase extracted with DCM (3×20 mL). The DCM fractions were pooled, cooled to 0 °C and 15% NaOH in brine (30 mL) was added and stirred for 1 h. The reaction mixture was diluted with water (60 mL), the phases were separated and the aqueous phase extracted with DCM (3×20 mL). The DCM layers were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 2:1 hexane/EtOAc as eluent gave the epoxy alcohol (**210**) as a light yellow oil (0.57 g, 59%); R_f = 0.24; $[\alpha]_D^{20}$ –5.7 (*c* 1.0); *dr* = 95:5; ν_{max} 3443, 1730 cm^{-1}.

δ_H: 3.80 (ddd, 1H, *J* 3.4, 3.4, 9.6, H-3); 3.60 (t, 2H, *J* 6.2, H-7); 2.98 (ddd, 1H, *J* 3.0, 3.0, 4.0, H-2); 2.78 (dd, 1 H, $J_{1,3}$ 2.8, 5.0, H-1); 2.78 (dd, 1H, *J* 2.8, 5.1, H-1a); 2.70 (dd, 1H, *J* 4.0, 5.1, H-1b); 1.94 (br s, 1H, O**H**); 1.63-1.41 (m, 6H, H-4, H-5, H-6); 0.86 (s, 9H, SiC(C**H**$_3$)$_3$); 0.02 (s, 6H, Si(C**H**$_3$)$_2$).

δ_C: 68.37 (D, C-3); 62.99 (T, C-7); 54.47 (D, C-2); 43.40 (T, C-1); 33.13 (T, C-6)*; 32.66 (T, C-4)*; 25.93 (Q, SiC(**C**H_3)$_3$); 21.63 (T, C-5); 18.32 (S, Si**C**$(CH_3)_3$); –5.31 (Q, $Si(CH_3)_2$).

HRMS (ESI) : *m/z* 283.1719 $[M+Na]^{+\bullet}$; Calculated for $C_{13}H_{28}O_3SiNa$: 283.1705.



(2*S*,3*R*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptanyl (*R*)-α-methoxy-α-trifluoro-methyl-phenylacetate (211)

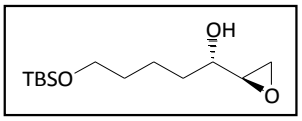
DMF (100 µL, 1.3 mmol) and $(COCl)_2$ (1.3 mL, 14.9 mmol) were added to a solution of *R*-(+)-MTPAA (0.34 g, 1.5 mmol) in hexane (66 mL) followed by stirring at rt for 2 h. The mixture was filtered and the solvent removed under reduced pressure to obtain the acyl chloride as a yellow oil.

DMAP (0.36 g, 2.9 mmol) and **210** (0.15 g, 0.6 mmol) dissolved in DCM (29 mL) were added to the yellow oil obtained above followed by stirring at rt overnight. The reaction was quenched by addition of saturated NH_4Cl (20 mL) and diluted with DCM (10 mL) followed by separation of the phases. The organic phase was washed with saturated aq. $NaHCO_3$ (2×10 mL) and brine (1×10 mL). The organic phase was dried using anhydrous $MgSO_4$ and concentrated to obtain the Mosher ester (**211**) as a dark yellow oil (0.21 g, 74%); R_f = 0.54 (2:1 hexane/EtOAc); *ee* = 95%.

δ_H: 7.59 - 7.24 (m, 5H, aromatic protons); 5.07 (ddd, 1H, *J* 4.9, 4.9, 7.3, H-3); 3.50 (t, 2H, *J* 6.6, H-7); 3.13 (s, 3H, OC**H$_3$**); 3.03 (ddd, 1H, *J* 2.7, 4.2, 4.2, H-2); 2.72 (dd, 1H, *J* 3.9, 5.1, H-1a); 2.69 (dd, 1H, *J* 2.7, 5.1, H-1b); 1.78-1.64 (m, 2H, H-4); 1.54-1.34 (m, 2H, H-6); 1.40-1.09 (m, 2H, H-5); 0.85 (s, 9H, SiC(C**H$_3$**)$_3$); 0.00 (s, 6H, Si(C**H$_3$**)$_2$).

δ_C: 169.24 (S, **C**OO); 132.16, 129.58, 128.32, 127.26 (aromatic carbons); 123.23 (Sq, J_{CF} 288 Hz, **C**F$_3$); 74.23 (D, C-3); 62.65 (T, C-7); 51.77 (D, C-2); 44.57 (T, C-1); 40.01 (Q, O**C**H$_3$); 32.28 (T, C-6)*; 30.91 (T, C-4)*; 25.86 (Q, SiC(**C**H$_3$)$_3$); 21.01 (T, C-5); 18.25 (S, Si**C**(CH$_3$)$_3$); –5.41 (Q, Si(**C**H$_3$)$_2$).

δ_F: –71.42 (major).



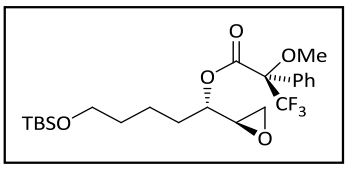
(2*R*,3*S*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptanol (182)

Powdered 4Å molecular sieves (1.63 g) were suspended in DCM (85 mL) and cooled to –20 °C. (*R,R*)-DIPT (1.6 mL, 7.7 mmol) dissolved in DCM (20 mL) was added followed by dropwise addition of Ti(O^{i}Pr)$_4$ (1.9 mL, 6.4 mmol) and stirred for 30 min. TBHP (4.93 M, 1.6 mL, 7.9 mmol,) was added dropwise and stirred for 30 min. The allylic alcohol (**181**) (3.14 g, 12.9 mmol) dissolved in DCM (45mL) was added dropwise and stirred for 1 h at –20 °C followed by reaction for 18 h in the freezer at –10 °C. The reaction mixture was warmed to 0 °C, vacuum filtered to remove the molecular sieves, water added (150 mL) and stirred for 45 min while warming to rt. The mixture was vacuum filtered to break the emulsion. The phases were separated and the aqueous phase extracted with DCM (3×50 mL). The DCM fractions were pooled, cooled to 0 °C and 15 % NaOH in brine (100 mL) was added and stirred for 1 h. The reaction mixture was diluted with water (100 mL), the phases were separated and the aqueous phase extracted with DCM (3×70 mL). The DCM layers were pooled, dried using anhydrous MgSO$_4$ and concentrated. The oil obtained was purified by column chromatography using 2:1 hexane/EtOAc as eluent to obtain the epoxy alcohol (**182**) as a light yellow oil (1.63 g, 97%); R_f = 0.24; $[\alpha]_D^{20}$ +10.2 (*c* 1.3); *dr* = 96:4; ν_{max} 3435, 1737 cm^{-1}.

δ_H: 3.78 (ddd, 1H, *J* 3.6, 3.6, 7.1, H-3); 3.59 (t, 2H, *J* 6.2, H-7); 2.97 (ddd, 1H, *J* 3.1, 3.1, 4.0, H-2); 2.76 (dd, 1H, $J_{1,3}$ 2.9, 5.1, H-1); 2.76 (dd, 1H, *J* 2.8, 5.0, H-1a); 2.69 (dd, 1H, *J* 4.0, 5.1, H-1b); 2.07 (br s, 1H, O**H**); 1.61-1.39 (m, 6H, H-4, H-5, H-6); 0.85 (s, 9H, SiC(C**H$_3$**)$_3$); 0.01 (s, 6H, Si(C**H$_3$**)$_2$).

δ_C: 68.39 (D, C-3); 62.97 (T, C-7); 54.48 (D, C-2); 43.42 (T, C-1); 33.13 (T, C-4)*; 32.64 (T, C-6)*; 25.91 (Q, SiC(**C**H_3)$_3$); 21.60 (T, C-5); 18.30 (S, Si**C**(CH$_3$)$_3$); –5.34 (Q, Si(**C**H_3)$_2$).

HRMS (ESI): *m/z* 243.1773 $[(M+H)-H_2O]^{+\bullet}$; Calculated for $C_{13}H_{27}O_2Si$: 243.1780.
m/z 261.1881 $[M+H]^{+\bullet}$; Calculated for $C_{13}H_{29}O_3Si$: 261.1886.



(2*R*,3*S*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptanyl (*R*)-α-methoxy-α-trifluoro-methyl-phenylacetate (207)

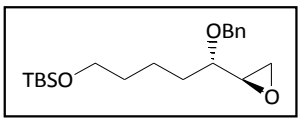
DMF (304 µL, 3.9 mmol) was added to a solution of (*R*)-(+)-α-MTPAA (0.16 g, 0.7 mmol) in hexane (4.7 mL) followed by dropwise addition of $(COCl)_2$ (61 µL, 0.7 mmol) and stirring at rt for 1 h. The hexane was decanted and the solvent removed to obtain the acyl chloride.

Et_3N (806 µL, 5.8 mmol) and DMAP (1 crystal) were added to a solution of the above acyl chloride in DCM (7.2 mL). The epoxy alcohol (**182**) (0.29 g, 1.1 mmol) in DCM (7.2 mL) was added to the solution followed by stirring for 3 h at rt. The mixture was diluted with DCM (14 mL) followed by washing with 0.1 M HCl (10 mL) and saturated $NaHCO_3$ (20 mL). The DCM fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated to obtain the Mosher ester (**207**) as a light yellow oil (0.39 g, 74%); R_f = 0.54 (2:1 hexane/EtOAc); *ee* = 96 %.

δ_H: 7.66-7.21 (m, 5H, aromatic protons); 3.83 (s, 3H, OC**H$_3$**); 3.70 (ddd, 1H, *J* 3.7, 3.7, 10.5, H-3); 3.57 (t, 2H, *J* 6.3, H-7); 2.94-2.88 (m, 1H, H-2); 2.73-2.64 (m, 2H, H-1); 1.57-1.37 (m, 2H, H-4, H-5, H-6); 0.83 (s, 9H, SiC(C**H$_3$**)$_3$); –0.01 (s, 6H, Si(C**H$_3$**)$_2$).

δ_C: 170.47 (S, **C**O); 135.72, 127.60, 127.56, 127.24 (aromatic carbons); 124.75 (Sq, J_{CF} 288 Hz, **C**F_3); 68.53 (D, C-3); 62.91 (T, C-7); 54.44 (D, C-2); 52.91 (Q, O**C**H_3); 43.49 (T, C-1); 33.18 (T, C-4)*; 32.59 (T, C-6)*; 25.82 (Q, SiC(**C**H_3)$_3$); 21.51 (T, C-5); 18.21 (S, Si**C**(CH$_3$)$_3$); –5.43 (Q, Si(**C**H_3)$_2$).

δ_F: –71.57 (major).



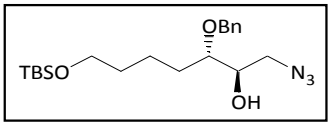
(2*R*,3*S*)-3-Benzyloxy-1,2-epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-heptane (183)

Vacuum dried MgO (0.09 g, 2.2 mmol) was added to Dudley's reagent (0.73 g, 2.1 mmol) dissolved in benzotrifluoride (2.3 mL). The epoxy alcohol (**182**) (0.27 g, 1.0 mmol) was added followed by refluxing at 80-85 °C overnight. The reaction mixture was cooled to rt, filtered through celite and concentrated. The oil obtained was purified by column chromatography using 5:1 hexane/EtOAc as eluent to obtain the *O*-benzyl ether (**183**) as a light yellow oil (0.15 g, 41%); R_f = 0.43; $[\alpha]_D^{20}$ −1.2 (*c* 1.1); ν_{max} 1716, 722, 686 cm^{-1}.

δ_H: 7.32-7.31 (m, 5H, aromatic protons); 4.65 (d, 1H, *J* 11.6, OC**H**$_2$Ph); 4.49 (d, 1H, *J* 11.6, OC**H**$_2$Ph); 3.59 (t, 2H, *J* 6.2, H-7); 3.25 (ddd, 1H, *J* 5.2, 5.2, 6.9, H-3); 2.92 (ddd, 1H, *J* 2.7, 3.9, 5.4, H-2); 2.77 (dd, 1H, *J* 3.9, 5.3, H-1a); 2.71 (dd, 1H, *J* 2.7, 5.3, H-1b); 1.68-1.39(m, 6H, H-4, H-5, H-6); 0.88 (s, 9H, SiC(C**H**$_3$)$_3$); 0.04 (s, 6H, Si(C**H**$_3$)$_2$).

δ_C: 138.51, 128.32, 127.65, 127.59 (aromatic carbons); 78.04 (D, C-3); 72.29 (T, O**C**H$_2$Ph); 63.02 (T, C-7); 53.49 (D, C-2); 45.57 (T, C-1); 32.78 (T, C-6)*; 32.64 (T, C-4)*; 25.94(Q, SiC(**C**H$_3$)$_3$); 21.55 (T, C-5); 18.32 (S, Si**C**(CH$_3$)$_3$); −5.30 (Q, Si(**C**H$_3$)$_2$).

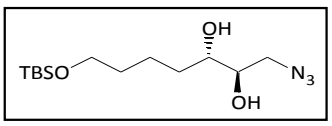
HRMS (ESI): *m/z* 373.2179 $[M+Na]^{+\bullet}$; Calculated for $C_{20}H_{34}O_3SiNa$: 373.2175.



(2*R*,3*S*)-1-Azido-3-benzyloxy-7-[(*t*-butyldimethylsilanyl)oxy]-2,3-heptanediol (184)

NaN_3 (0.55 g, 8.4 mmol) and NH_4Cl (0.19 g, 3.6 mmol) were added to a solution of **183** (0.59 g, 1.7 mmol) in 60 % EtOH (10 mL) followed by refluxing at 60 °C for 4 h. The reaction mixture was cooled to rt and concentrated followed by suspension of the crude oil in Et_2O (15 mL) and washing with water (3×10 mL). The Et_2O fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 15:1 hexane/EtOAc (R_f = 0.33) as eluent to obtain a light yellow oil which was not the required **184**.

4.3.1.2. Via the Omission of Benzylation Protection Step



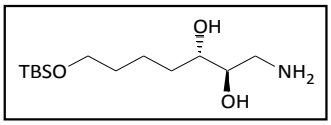
(2*R*,3*S*)-1-Azido-7-[(*t*-butyldimethylsilanyl)oxy]-2,3-heptanediol (212)

NaN_3 (0.29 g, 4.5 mmol) and NH_4Cl (0.20 g, 3.7 mmol) were added to a solution of **182** (0.23 g, 0.9 mmol) in 8:1 EtOH/water (12 mL) followed by refluxing at 80 °C overnight. The reaction mixture was cooled to rt and concentrated followed by suspension of the crude oil in EtOAc (30 mL) and washing with water (3×30 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 3:2 hexane/EtOAc as eluent to obtain the azide (**212**) as a light yellow oil (0.21 g, 77%); R_f = 0.40; $[\alpha]_D^{20}$ +0.8 (*c* 0.8); ν_{max} 3389; 2092 cm^{-1}.

δ_H: 3.69-3.63 (m, 2H, H-2, H-3); 3.60 (t, 2H, *J* 5.4, H-7); 3.42 and 3.39 (AB part of ABX system, 1H each, J_{AB} 12.6, J_{AX} 7.4, J_{BX} 3.2, H-1); 2.71 (br s, 1H, O**H**); 2.48 (br s, 1H, O**H**); 1.57-1.36 (m, 6H, H-4, H-5, H-6); 0.86 (s, 9H, SiC(C**H**$_3$)$_3$); 0.02 (s, 6H, Si(C**H**$_3$)$_2$).

δ_C: 73.17 (D, C-2)†; 72.77 (D, C-3)†; 62.99 (T, C-7); 52.97 (T, C-1); 32.32 (T, C-6)*; 32.05 (T, C-4)*; 25.92 (Q, SiC(**C**H$_3$)$_3$); 22.11 (T, C-5); 18.32 (S, Si**C**(CH$_3$)$_3$); –5.33 (Q, Si(**C**H$_3$)$_2$).
*,† may be interchanged

HRMS (ESI): *m/z* 304.2064 $[M+H]^{+\bullet}$; Calculated for $C_{13}H_{30}N_3O_3Si$: 304.2065.
m/z 326.1880 $[M+Na]^{+\bullet}$; Calculated for $C_{13}H_{29}N_3O_3SiNa$: 326.1876.



(2*R*,3*S*)-1-Amino-7-[(*t*-butyldimethylsilanyl)oxy]-2,3-heptanediol (185)

Pd-C (0.17 g, 10 %) was added to a solution of the azidodiol (**212**) (1.60 g, 5.3 mmol) in EtOH (96 mL) and the mixture was shaken under a H_2 atmosphere (45 psi) in a Parr hydrogenator overnight followed by filtration through celite. The filtrate was concentrated and the oil obtained was purified by column chromatography using 9:1 EtOH/Et_3N as eluent to obtain the aminoalcohol (**185**) as a light yellow oil (1.12 g, 77 %); R_f = 0.18; $[\alpha]_D^{20}$ –1.6 (*c* 1.1); ν_{max} 3347; 3292, 1593 cm^{-1}.

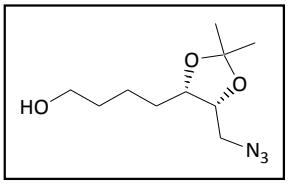
δ_H(CD_3OD): 3.65 (t, 2H, *J* 8.3, H-7); 3.48 - 3.18 (m, 4H, H-1, H-2, H-3); 1.75 - 1.28 (m, 6H, H-4, H-5, H-6); 0.89 (s, 9H, SiC(C**H**$_3$)$_3$); 0.05 (s, 6H, Si(C**H**$_3$)$_2$).

δ_C(CD_3OD): 74.86 (D, C-3); 73.88 (D, C-2); 64.33 (T, C-7); 43.53 (T, C-1); 33.99 (T, C-6)*; 33.80 (T, C-4)*; 26.42 (Q, SiC(**C**H$_3$)$_3$); 23.23 (T, C-5); 19.16 (S, Si**C**(CH$_3$)$_3$); –5.17 (Q, Si(CH$_3$)$_2$).

δ_H ($CDCl_3$): 3.85 (br s, 2H, N**H**$_2$); 3.54 (t, 2H, *J* 6.1, H-7); 3.49 (ddd, 1H, *J* 9.4, 4.2, 4.2, H-3); 3.38 (ddd, 1H, *J* 7.6, 4.0, 4.0, H-2); 2.76 and 2.66 (AB part of ABX system, 1H each, J_{AB} 13.3, J_{AX} 7.7, J_{BX} 3.3, H-1); 1.91 (br s, 1H, O**H**); 1.87 (br s, 1H, O**H**); 1.51 - 1.23 (m, 6H, H-4, H-5, H-6); 0.81 (s, 9H, SiC(C**H**$_3$)$_3$); –0.03 (s, 6H, Si(C**H**$_3$)$_2$).

δ_C ($CDCl_3$): 73.96 (D, C-3); 73.56 (D, C-2); 63.08 (T, C-7); 42.54 (T, C-1); 32.67 (T, C-6)*; 32.66 (T, C-4)*; 25.90 (Q, SiC(**C**H$_3$)$_3$); 22.24 (T, C-5); 18.28 (S, Si**C**(CH$_3$)$_3$); –5.35 (Q, Si(CH$_3$)$_2$).

HRMS (ESI): *m/z* 260.2051 $[(M+H)-H_2O]^{+\bullet}$; Calculated for $C_{13}H_{30}O_2NSi$: 260.2046.
m/z 278.2182 $[M+H]^{+\bullet}$; Calculated for $C_{13}H_{32}O_3NSi$: 278.2151.
m/z 300.1978 $[M+Na]^{+\bullet}$; Calculated for $C_{13}H_{31}O_3NSiNa$: 300.1971.



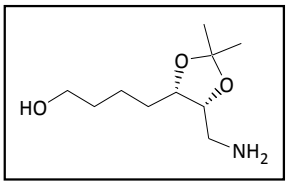
(2*R*,3*S*)-1-Azido-2,3-*O*-isopropylidene-heptane-2,3,7-triol (213)

2,2-Dimethoxypropane (90 µL, 0.7 mmol) and *p*-TsOH (6 mg, 0.03 mmol) were added to a solution of **212** (0.21 g, 0.7 mmol) in acetone (2.0 mL). The mixture was stirred for 1 h at rt followed by addition of Et_3N (0.6 mL) and evaporation of the solvent at rt due to volatility. The oil obtained was purified by column chromatography using 1:1 hexane/EtOAc as eluent to obtain a light yellow oil of the acetonide (**213**) (0.11 g, 71%); R_f = 0.30; $[\alpha]_D^{20}$ +36.0 (*c* 0.7); ν_{max} 3381; 2985; 2091 cm^{-1}.

δ_H: 4.16 (ddd, 1H, *J* 4.5, 5.9, 7.5, H-2); 4.13 (ddd, 1H, *J* 5.8, 4.2, 12.7, H-3); 3.64 (t, 2H, *J* 6.2, H-7); 3.33 (dd, 1H, *J* 7.5, 12.6, H-1a); 3.17 (dd, 1H, *J* 4.3, 12.7, H-1b); 1.86 (br s, 1H, O**H**); 1.63-1.38 (m, 6H, H-4, H-5, H-6); 1.46 (s, 3H, C**H**$_3$); 1.33 (s, 3H, C**H**$_3$).

δ_C: 108.51 (S, O_2**C**(CH$_3$)$_2$); 76.88 (D, C-2)†; 76.52 (D, C-3)†; 62.54 (T, C-7); 51.19 (T, C-1); 32.42 (T, C-6); 28.69 (T, C-4); 28.18, 25.49 (Q, O_2C(**C**H$_3$)$_2$); 22.90 (T, C-5).
† may be interchanged

HRMS (ESI): *m/z* 252.1334 $[M+Na]^{+\bullet}$; Calculated for $C_{10}H_{19}N_3O_3Na$: 252.1324.



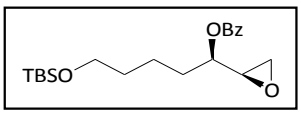
(2*R*,3*S*)-1-Amino-2,3-*O*-isopropylidene-heptane-2,3,7-triol (214)

Pd-C (0.04 g, 10 %) was added to a solution of the azide (**213**) (0.37 g, 1.6 mmol) in EtOH (6.0 mL) and the mixture was shaken overnight under a H_2 atmosphere (45 psi) in a Parr hydrogenator followed by filtration through celite. The filtrate was concentrated and the oil obtained was purified by column chromatography using 9:1 EtOH/Et_3N as eluent to obtain the protected amine (**214**) as a light yellow oil (0.30 g, 92%); R_f = 0.35; $[\alpha]_D^{20}$ +33.2 (*c* 1.1); ν_{max} 3763; 2989 cm^{-1}.

δ_H: 4.09 (ddd, 1H, *J* 4.3, 5.8, 8.8, H-2); 3.98 (ddd, 1H, *J* 3.6, 5.7, 9.2, H-3); 3.59 (t, 2H, *J* 6.1, H-7); 3.50 (br s, 2H, N**H$_2$**); 2.71 (dd, 1H, *J* 9.1, 12.8, H-1a); 2.63 (dd, 1H, *J* 3.5, 12.8, H-1b); 1.91 (br s, 1H, O**H**); 1.60-1.35 (m, 6H, H-4, H-5, H-6); 1.41, 1.31 (s, 3H each, C(C**H$_3$**)**$_2$**).

δ_C: 108.11 (S, O_2**C**$(CH_3)_2$); 77.80 (D, C-2)†; 77.05 (D, C-3)†; 61.96 (T, C-7); 41.49 (T, C-1); 32.29 (T, C-6); 28.53 (T, C-4); 28.42, 25.70 (Q, O_2C(**C**$H_3)_2$); 22.60 (T, C-5).
† may be interchanged

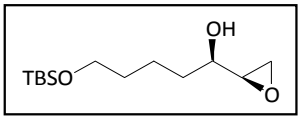
HRMS (ESI): *m/z* 204.1607 $[M+H]^{+\bullet}$; Calculated for $C_{10}H_{22}NO_3$: 204.1600.
m/z 226.1422 $[M+Na]^{+\bullet}$; Calculated for $C_{10}H_{21}NO_3Na$: 226.1419.



(2*R*,3*R*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptyl benzoate (215)

Triphenylphosphine (0.94 g, 3.6 mmol) was added to a solution of the epoxyalcohol (**182**) (0.80 g, 3.1 mmol) in THF (25.0 mL) and cooled to 0 °C. A solution of benzoic acid (0.44 g, 3.6 mmol) in benzene (10 mL) was added slowly to the reaction mixture and allowed to stir for 5 min at 0 °C followed by the addition of DEAD (0.56 mL, 3.5 mmol). The mixture was allowed to stir overnight at rt. The solvent was evaporated and the crude product suspended in hexane (30 mL) and water (20 mL) followed by vacuum filtration. The filtrate was taken and the phases separated, the organic phase was washed with water (2×20 mL) and brine (20 mL). The organic fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated to give the benzoate

ester (**215**) as a yellow oil (1.24 g, 111%, R_f = 0.78 (1:1 hexane/EtOAc)). The crude product was used directly in the next reaction without further purification or characterisation.



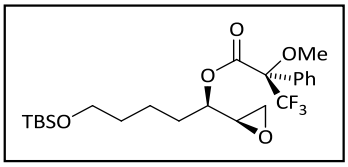
(2*R*,3*R*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptanol (186)

Anhydrous K_2CO_3 (1.41 g, 10.2 mmol) was added to a solution of the crude benzoate ester (**215**) (1.11 g, 3.1 mmol) in dry MeOH (70 mL) followed by stirring at rt for 2 h. The solvent was removed and the crude oil obtained suspended in water (50 mL) and extracted with Et_2O (3×30 mL). The Et_2O fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 1:1 hexane/EtOAc as eluent to obtain the epoxy alcohol (**186**) as a light yellow oil (0.48 g, 61 %); R_f = 0.43; $[\alpha]_D^{20}$ –1.6 (*c* 1.0); *dr* = 97:3; ν_{max} 3415; 1384 cm^{-1}.

δ_H: 3.60 (t, 2H, *J* 3.8, H-7); 3.41 (ddd, 1H, *J* 3.4, 3.4, 6.7, H-3); 2.95 (ddd, 1H, *J* 1.7, 2.5, 3.1, H-2); 2.79 (dd, 1H, *J* 2.5, 3.0, H-1a); 2.69 (dd, 1H, *J* 1.6, 3.0, H-1b); 1.96 (br s, 1H, O**H**); 1.63-1.39 (m, 6H, H-4, H-5, H-6); 0.86 (s, 9H, SiC(C**H₃**)₃); 0.02 (s, 6H, Si(C**H₃**)₂).

δ_C: 71.66 (D, C-3); 62.94 (T, C-7); 55.33 (D, C-2); 45.14 (T, C-1); 34.10 (T, C-4)*; 32.63 (T, C-6)*; 25.94 (Q, SiC(**C**H₃)₃); 21.66 (T, C-5); 18.33 (S, Si**C**(CH₃)₃); –5.31 (Q, Si(**C**H₃)₂).

HRMS (ESI): *m/z* 283.1718 $[M+Na]^{+\bullet}$; Calculated for $C_{13}H_{28}O_3SiNa$: 283.1705.



(2*R*,3*R*)-1,2-Epoxy-7-[(*t*-butyldimethylsilanyl)oxy]-3-heptanyl (*R*)-α-methoxy-α-trifluoro-methyl-phenylacetate (216)

DMF (70 µL, 0.9 mmol) and $(COCl)_2$ (850 µL, 9.7 mmol) were added to a solution of *R*-(+)-MTPAA (0.26 g, 1.1 mmol) in hexane (7 mL) followed by stirring at rt for 2 h. The mixture was filtered and the solvent removed to give the acyl chloride as a yellow oil.

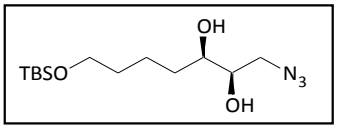
DMAP (0.24 g, 2.0 mmol) and the epoxy alcohol (**186**) (0.10 g, 0.4 mmol) dissolved in DCM (19 mL) were added to the acyl chloride obtained above followed by stirring at rt overnight. The

reaction was quenched by addition of saturated NH_4Cl (20 mL) and diluted with DCM (10 mL) followed by separation of the phases. The organic phase was washed with saturated aq. $NaHCO_3$ (2×20 mL) and brine (20 mL). The organic phase was dried using anhydrous $MgSO_4$ and concentrated to obtain the Mosher ester (**216**) (0.14 g, 75%); R_f = 0.54 (2:1 hexane/EtOAc); *ee* = 97%.

δ_H: 7.57-7.35 (m, 5H, aromatic protons); 4.80 (ddd, 1H, *J* 6.4, 7.2, 7.2, H-3); 3.53 (t, 2H, *J* 6.3, H-7); 3.08 (ddd, 1H, *J* 2.6, 4.1, 7.4, H-2); 2.96 (s, 3H, OC**H**$_3$); 2.85 (dd, 1H, *J* 4.1, 4.8, H-1a); 2.67 (dd, 1H, *J* 2.6, 4.9, H-1b); 1.74-1.65 (m, 2H, H-4); 1.52-1.41 (m, 2H, H-6); 1.3-1.25 (m, 2H, H-5); 0.86 (s, 9H, SiC(C**H**$_3$)$_3$); 0.01 (s, 6H, Si(C**H**$_3$)$_2$).

δ_C: 165.99 (S, ester **C**O); 132.23, 129.58, 128.35, 127.28 (aromatic carbons); 123.27 (Sq, J_{CF} 288 Hz, **C**F$_3$); 77.83 (D, C-3); 62.59 (T, C-7); 52.48 (D, C-2); 45.38 (T, C-1); 38.95 (Q, O**C**H$_3$); 32.31 (T, C-6)*; 30.83 (T, C-4)*; 25.87 (Q, SiC(**C**H$_3$)$_3$); 21.21 (T, C-5); 18.26 (S, Si**C**(CH$_3$)$_3$); –5.39 (Q, Si(**C**H$_3$)$_2$).

δ_F: –71.48 (major).



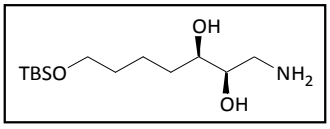
(2*R*,3*R*)-1-Azido-7-[(*t*-butyldimethylsilanyl)oxy]-2,3-heptanediol (217)

NaN_3 (1.02 g, 15.7 mmol) and NH_4Cl (0.69 g, 12.9 mmol) were added to a solution of the epoxy alcohol (**186**) (0.81 g, 3.1 mmol) in EtOH/water (8:1, 45 mL) followed by refluxing overnight at 80 °C. The reaction mixture was cooled to rt and concentrated followed by suspension of the crude oil in EtOAc (40 mL) and washing with water (3×20 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 3:2 hexane/EtOAc as eluent to obtain the azide (**217**) as a light yellow oil (0.50 g, 53%); R_f = 0.38; $[\alpha]_D^{20}$ +9.0 (*c* 0.9); ν_{max} 3384; 2093 cm^{-1}.

δ_H: 3.60 (t, 2H, *J* 6.2, H-7); 3.61-3.52 (m, 2H, H-2, H-3); 3.39 and 3.37 (AB part of ABX system, 1H each, J_{AB} 12.6, J_{AX} 7.2, J_{BX} 3.6, H-1); 2.98 (br s, 1H, O**H**); 2.64 (br s, 1H, O**H**); 1.57-1.36 (m, 6H, H-4, H-5, H-6); 0.86 (s, 9H, SiC(C**H**$_3$)$_3$); 0.02 (s, 6H, Si(C**H**$_3$)$_2$).

δ_C: 72.91 (D, C-2); 71.82 (D, C-3); 63.00 (T, C-7); 54.14 (T, C-1); 33.15 (T, C-6)*; 32.33 (T, C-4)*; 25.91 (Q, SiC(**C**H$_3$)$_3$); 21.83 (T, C-5); 18.31 (S, Si**C**(CH$_3$)$_3$); –5.34 (Q, Si(**C**H$_3$)$_2$).

HRMS (ESI): *m/z* 326.1889 $[M+Na]^{+\bullet}$; Calculated for $C_{13}H_{29}N_3O_3SiNa$: 326.1876.



(2*R*,3*R*)-1-Amino-7-[(*t*-butyldimethylsilanyl)oxy]-2,3-heptanediol (189)

Pd-C (0.04 g, 10 %) was added to a solution of the azide (**217**) (0.30 g, 1.0 mmol) in EtOH (6.0 mL) and the mixture was shaken overnight under a H_2 atmosphere (45 psi) in a Parr hydrogenator followed by filtration through celite. The filtrate was concentrated and the oil obtained was purified by column chromatography using 9:1 EtOH/Et_3N as eluent to obtain the aminoalcohol (**189**) as a light yellow oil (0.09 g, 33%); R_f = 0.16; $[\alpha]_D^{20}$ +7.6 (*c* 1.1); ν_{max} 3459; 3084 cm^{-1}.

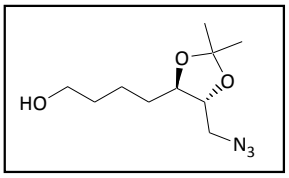
δ_H (CD_3OD): 3.65 (t, 2H, *J* 6.0, H-7); 3.56 (ddd, 1H, *J* 3.9, 3.9, 8.0, H-2); 3.48 (ddd, 1H, *J* 3.8, 3.8, 7.8, H-3); 2.90 and 2.84 (AB part of ABX system, 1H each, J_{AB} 12.8, J_{AX} 7.9, J_{BX} 3.8, H-1); 1.62-1.30 (m, 6H, H-4, H-5, H-6); 0.90 (s, 9H, $SiC(C\mathbf{H}_3)_3$); 0.06 (s, 6H, $Si(C\mathbf{H}_3)_2$).

δ_C (CD_3OD): 73.79 (D, C-3); 73.28 (D, C-2); 64.29 (T, C-7); 44.26 (T, C-1); 33.92 (T, C-6)*; 33.81 (T, C-4)*; 26.42 (Q, $SiC(\mathbf{C}H_3)_3$); 23.32 (T, C-5); 19.17 (S, $Si\mathbf{C}(CH_3)_3$); –5.16 (Q, $Si(\mathbf{C}H_3)_2$).

δ_H ($CDCl_3$): 4.17 (br s, 2H, $N\mathbf{H_2}$); 3.59 (t, 2H, *J* 6.3, H-7); 3.60 (ddd, 1H, *J* 2.4, 6.3, 6.3, H-3); 3.51 (ddd, 1H, *J* 1.3, 6.0, 7.4, H-2); 3.19 and 2.95 (AB part of ABX system, 1H each, J_{AB} 12.2, J_{AX} 7.1, J_{BX} 5.3, H-1); 1.58-1.33 (m, 6H, H-4, H-5, H-6); 0.87 (s, 9H, $SiC(C\mathbf{H}_3)_3$); 0.02 (s, 6H, $Si(C\mathbf{H}_3)_2$).

δ_C ($CDCl_3$): 73.27 (D, C-3); 71.47 (D, C-2); 63.14 (T, C-7); 48.69 (T, C-1); 33.47 (T, C-6)*; 32.81 (T, C-4)*; 25.99 (Q, $SiC(\mathbf{C}H_3)_3$); 22.07 (T, C-5); 18.34 (S, $Si\mathbf{C}(CH_3)_3$); –5.26 (Q, $Si(\mathbf{C}H_3)_2$).

HRMS (ESI): *m/z* 278.2123 $[M+H]^{+\bullet}$; Calculated for $C_{13}H_{32}NO_3Si$: 278.2151.



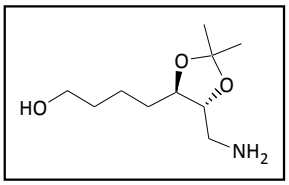
(2*R*,3*R*)-1-Azido-2,3-*O*-isopropylidene-heptane-2,3,7-triol (218)

2,2-Dimethoxypropane (200 µl, 1.6 mmol) and *p*-TsOH (0.01 g, 0.05 mmol) were added to a solution of **217** (0.45 g, 1.5 mmol) in acetone (4.0 mL). The mixture was stirred for 1 h at rt followed by addition of Et_3N (0.3 mL) and evaporation of the solvent at rt due to volatility. The oil obtained was purified by column chromatography using 1:1 hexane/EtOAc as eluent to give the protected azide (**218**) as a light yellow oil (0.13 g, 40%); R_f = 0.26; $[\alpha]_D^{20}$ +60.4 (*c* 0.8), ν_{max} 3402; 2098 cm^{-1}.

δ_H: 3.85 (m, 1H, H-3); 3.76 (ddd, 1H, *J* 3.7, 5.0, 8.6, H-2); 3.63 (t, 2H, *J* 6.2, H-7); 3.49 (dd, 1H, *J* 3.7, 13.1, H-1a); 3.24 (dd, 1H, *J* 5.0, 13.1, H-1b); 1.67(s, br, 1H,O**H**); 1.63-1.38 (m, 6H, H-4, H-5, H-6); 1.40 (s, 3H, C**H**$_3$); 1.39 (s, 3H, C**H**$_3$).

δ_C: 109.18 (S, O_2**C**$(CH_3)_2$); 79.71 (D, C-2); 77.96 (D, C-3); 62.58 (T, C-7); 51.79 (T, C-1); 32.48 (T, C-4 and T, C-6); 27.32, 26.76 (Q, O_2C(**C**$H_3)_2$); 22.25 (T, C-5).

HRMS (ESI): *m/z* 252.1332 $[M+Na]^{+\bullet}$; Calculated for $C_{10}H_{19}N_3O_3Na$: 252.1324.



(2*R*,3*R*)-1-Amino-2,3-*O*-isopropylidene-heptane-2,3,7-triol (219)

Pd-C (0.02 g, 10 %) was added to a solution of the azide (**218**) (0.13 g, 0.6 mmol) in EtOH (6.0 mL) and the mixture was shaken overnight under a H_2 atmosphere (45 psi) in a Parr hydrogenator followed by filtration through celite. The filtrate was concentrated and the oil obtained was purified by column chromatography using 9:1 EtOH/Et_3N as eluent gave the amine (**219**) as a light yellow oil (0.06 g, 51%); R_f = 0.41; $[\alpha]_D^{20}$ +11.6 (*c* 1.2); ν_{max} 3375, 2988 cm^{-1}.

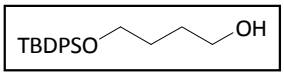
δ_H: 4.02 (ddd, 1H, *J* 3.2, 7.4, 7.9, H-2); 3.90 (ddd, 1H, *J* 5.6, 6.6, 8.0, H-3); 3.62 (t, 2H, *J* 5.7, H-7); 3.34 (dd, 1H, *J* 3.2, 13.3, H-1a); 3.10 (dd, 1H, *J* 7.1, 13.2, H-1b); 2.93 (br s, 2H, N**H$_2$**); 1.94 (br s, 1H, O**H**); 1.71-1.47 (m, 6H, H-4, H-5, H-6); 1.38 (s, 6H, C(C**H$_3$**)$_2$).

δ_C: 109.53 (S, O_2**C**$(CH_3)_2$); 78.21 (D, C-3); 76.95 (D, C-2); 61.72 (T, C-7); 41.89 (T, C-1); 32.06 (T, C-6); 31.92 (T, C-4); 27.30, 27.05 (Q, O_2C(**C**$H_3)_2$); 21.68 (T, C-5).

HRMS (ESI): *m/z* 204.1607 $[M+H]^{+\bullet}$; Calculated for $C_{10}H_{22}NO_3$: 204.1600.
m/z 226.1421 $[M+Na]^{+\bullet}$; Calculated for $C_{10}H_{21}NO_3Na$: 226.1419.

4.3.2. Synthesis of the C-1–C-7 Fragment of the HO-FC$_1$ Fumonisin

4.3.2.1. Via the Benzylidene Protection Route



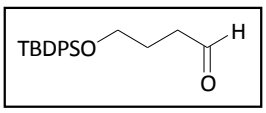
4-[(*t*-Butyldiphenylsilanyl)oxy]-1-butanol (190)[3]

NaH (60% dispersion, 4.54 g, 113 mmol) was washed with hexane (2x20 mL) and suspended in THF (250 mL). 1,4-Butanediol (10.0 mL, 113 mmol) was added at rt and stirred for 60 min to obtain a white precipitate. TBDPSCl (29.4 mL, 113 mmol) dissolved in THF (60 mL) was added dropwise to the reaction mixture at rt and stirred for 50 min. The reaction mixture was diluted with Et_2O (250 mL) and water (110 mL) followed by washing with brine (2×100 mL) and aq. $KHCO_3$ (1×80 mL). The Et_2O fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using gradient elution starting with hexane followed by 5:1 hexane/EtOAc as eluent to obtain the *O*-silyl ether (**190**) as a light yellow oil (33.9 g, 91%); R_f = 0.38 (2:1 hexane/EtOAc) ; ν_{max} 3335 cm^{-1}.

δ_H: 7.68-7.36 (m, 10H, aromatic protons); 3.69 (t, 2H, *J* 5.8, H-4); 3.65 (t, 2H, *J* 6.1, H-1); 2.15 (br s, 1H, O**H**); 1.71-1.61 (m, 4H, H-2, H-3); 1.05 (s, 9H, SiC(C**H$_3$**)$_3$).

δ_C: 135.53, 133.62, 129.61, 127.63 (aromatic carbons); 63.98 (T, C-4); 62.75 (T, C-1); 29.76 (T, C-3); 29.22 (T, C-2); 26.79 (Q, SiC(**C**H$_3$)$_3$); 19.13 (S, Si**C**(CH$_3$)$_3$).

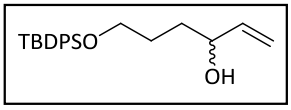
HRMS (ESI): *m/z* 351.1761 $[M+Na]^{+\bullet}$; Calculated for $C_{20}H_{28}O_2SiNa$: 351.1756.



4-[(*t*-Butyldiphenylsilanyl)oxy]-butanal (191)

DMSO (3.5 mL, 49.3 mmol) was added dropwise to a solution of $(COCl)_2$ (2.1 mL, 24.1 mmol) in DCM (150 mL) at −78 °C and stirred for 30 min. *O*-Silyl ether (**190**) (5.00 g, 15.2 mmol) dissolved in DCM (80 mL) was added dropwise at −78 °C and stirred for 1 h. Et_3N (12.3 mL, 88.2 mmol) was added at −78 °C and stirred for 1 h followed by warming to rt. TLC analysis was done using 4:1 hexane/EtOAc as eluent (R_f = 0.44) to determine reaction progress. Water (120 mL) was added to the reaction mixture and the phases separated. The aqueous phase was washed with Et_2O (3×90 mL), the organic fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The murky oil obtained was dissolved in a minimum of Et_2O and the

white crystals formed were filtered off, followed by concentration to give the aldehyde (**191**) as a dark yellow oil (4.80 g, 97%). The crude material was used immediately without further purification or characterisation for the next reaction.



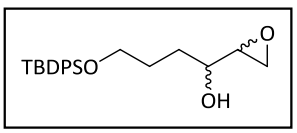
(3*RS*)-6-[(*t*-Butyldiphenylsilanyl)oxy]-hex-1-en-3-ol (192)[5]

A solution of the aldehyde (**191**) (9.50 g, 29.1 mmol) dissolved in THF (64 mL) was added dropwise to vinyl magnesium bromide (1 M in THF, 60.9 mL, 60.9 mmol,) at rt followed by stirring for 90 min. The reaction mixture was cooled to 0 °C, quenched with saturated NH_4Cl and diluted with EtOAc (130 mL). The EtOAc fraction was washed with water (2×90 mL) and brine (1×60 mL). The organic fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 5:1 hexane/EtOAc as eluent to give the racemic allylic alcohol (**192**) as a yellow oil (8.20 g, 80%); R_f = 0.27; ν_{max} 3367, 919 cm^{-1}.

δ_H: 7.68-7.36 (m, 10H, aromatic protons); 5.86 (ddd, 1H, *J* 6.1, 10.5, 17.2, H-2); 5.22 (ddd, 1H, *J* 1.4, 1.4, 17.2, H-1a); 5.10 (ddd, 1H, *J* 1.4, 1.4, 10.5, H-1b); 4.15-4.11 (m, 1H, H-3); 3.69 (t, 2H, *J* 5.8, H-6); 2.11 (br s, 1H, O**H**); 1.70-1.60 (m, 4H, H-4, H-5); 1.05 (s, 9H, SiC(C**H**$_3$)$_3$).

δ_C: 141.14 (D, C-2); 135.54, 133.66, 129.60, 127.62 (aromatic carbons); 114.49 (T, C-1); 72.77 (D, C-3); 63.98 (T, C-6); 33.87 (T, C-4); 28.36 (T, C-5); 26.81 (Q, SiC(**C**H$_3$)$_3$); 19.15 (S, Si**C**(CH$_3$)$_3$).

HRMS (ESI) : *m/z* 337.1993 $[(M+H)-H_2O]^{+\bullet}$; Calculated for $C_{22}H_{29}OSi$: 337.1988.
m/z 377.1922 $[M+Na]^{+\bullet}$; Calculated for $C_{22}H_{30}O_2SiNa$: 377.1913.



(2*RS*,3*RS*)-1,2-Epoxy-6-[(*t*-butyldiphenylsilanyl)oxy]-3-hexanol (223)

A solution of MCPBA (0.19 g, 1.1 mmol) in DCM (6 mL) was dried using anhydrous $MgSO_4$ followed by filtration. The MCPBA was added to a solution of the allylic alcohol (**192**) (0.19 g, 0.5 mmol) in DCM (7 mL) and stirred for 5 days at rt. The DCM fraction was washed with

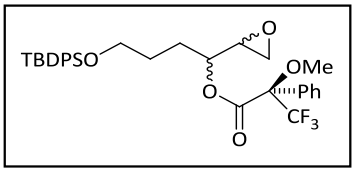
[5] Jana, N.; Mahapatra, T.; Nanda, S. *Tetrahedron: Asymmetry* **2009**, *20*, 2622.

saturated aq. $KHCO_3$ (3×10 mL), dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 2:1 hexane/EtOAc as eluent to obtain the epoxy alcohol (**223**) as a light yellow oil (0.19 g, 96%); R_f = 0.22; *dr* 1.3:1 ; ν_{max} 3414, 1732 cm^{-1}.

δ_H: 7.68-7.34 (m, 10H, aromatic protons); 3.73-3.69 (m, 1H, H-3); 3.70 (t, 2H, *J* 5.9, H-6); 3.70 (t, 2H, *J* 5.8, H-6); 3.48-3.43 (m, 1H, H-3); 2.98 (ddd, 1H, *J* 2.80, 3.9, 3.9, H-2); 2.96 (ddd, 1H, *J* 2.8, 4.0, 5.0, H-2); 2.78 (dd, 1H, *J* 4.1, 5.0, H-1a); 2.78 (dd, 1H, *J* 5.1, 0.3, H-1a); 2.73 (dd, 1H, *J* 3.9, 5.1, H-1b); 2.68 (dd, 1H, *J* 2.8, 5.0, H-1b); 2.46; (br s, 1H, O**H**); 2.46 (br s, 1H, O**H**); 2.38 (br s, 1H, O**H**); 2.36 (br s, 1H, O**H**); 1.82-1.62 (m, 4H, H-4, H-5); 1.05 (s, 9H, SiC(C**H₃**)$_3$); 1.04 (s, 9H, SiC(C**H₃**)$_3$).

δ_C: 135.51, 133.64, 133.57, 129.61, 129.60, 127.62 (aromatic carbons); 71.29, 68.86 (D, C-3); 63.89, 63.74 (T, C-6); 55.29, 54.47 (D, C-2); 44.91, 43.89 (T, C-1); 31.01, 30.39 (T, C-4); 28.34, 28.30 (T, C-5); 26.79 (Q, SiC(**C**H$_3$)$_3$); 19.13 (S, Si**C**(CH$_3$)$_3$).

HRMS (ESI): *m/z* 393.1869 $[M+Na]^{+\bullet}$; Calculated for $C_{22}H_{30}O_3SiNa$: 393.1862.



(2*RS*,3*RS*)-1,2-Epoxy-6-[(*t*-butyldiphenylsilanyl)oxy]-3-hexanyl (*R*)-α-methoxy-α-trifluoromethyl-phenylacetate (224)

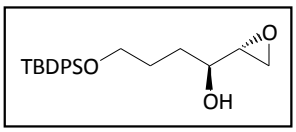
DMF (795 µL, 10.3 mmol) and $(COCl)_2$ (795 µL, 9.1 mmol) were added to a solution of *R*-(+)-MTPAA (0.22 g, 0.9 mmol) in hexane (6 mL) followed by stirring at rt for 2 h. The mixture was filtered and the solvent removed to obtain the acyl chloride as a yellow oil.

DMAP (0.23 g, 1.8 mmol) and **223** (0.14 g, 0.4 mmol) dissolved in DCM (25 mL) were added to the acyl chloride obtained above followed by stirring at rt overnight. The reaction was quenched by addition of saturated NH_4Cl (25 mL) and diluted with DCM (20 mL) followed by separation of the phases. The organic phase was washed with saturated aq. $NaHCO_3$ (2×20 mL) and brine (20 mL). The organic phase was dried using anhydrous $MgSO_4$ and concentrated to give the Mosher ester (**224**) as a dark yellow oil (0.18 g, 86%); R_f = 0.54 (2:1 hexane/EtOAc); *dr* = 1:1.2:1.3:1.3.

δ_C: 165.89, 165.83 (S, **C**O); 135.39, 133.57, 133.53, 132.09, 129.57, 129.54, 128.28, 127.58, 127.37, 127.19 (aromatic carbons); 123.19 (Sq, J_{CF} 288 Hz, **C**F$_3$); 77.62, 77.10, 74.53, 74.02 (D, C-3); 62.99, 62.96, 62.90, 62.88 (T, C-6); 52.41, 52.34,

51.71, 51.62 (D, C-2); 45.28, 44.71, 44.61, 44.42 (T, C-1); 38.84 (Q, O**C**H_3); 27.94, 27.86, 27.80, 27.64 (T, C-4)*; 27.62, 27.55, 27.51, 27.45 (T, C-5)*; 26.72 (Q, SiC(**C**H_3)$_3$); 19.08 (S, Si**C**$(CH_3)_3$).

δ_F: –71.34; –71.40; –71.47; –71.64.



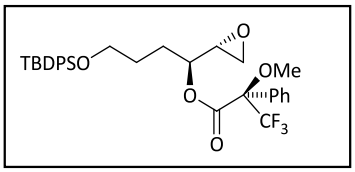
(2*R*,3*S*)-1,2-Epoxy-6-[(*t*-butyldiphenylsilanyl)oxy]-3-hexanol (225)

Powdered 4Å molecular sieves (0.41 g) were suspended in DCM (10 mL) and cooled to –20 °C. (*R,R*)-DIPT (180 µl, 0.9 mmol) dissolved in DCM (3.0 mL) was added followed by dropwise addition of Ti(O^{i}Pr)$_4$ (200 µL, 0.7 mmol) and stirred for 30 min. TBHP (170 µl, 0.8 mmol, 4.92 M) was added dropwise and stirred for 30 min. A solution of the allylic alcohol (**192**) (0.50 g, 1.4 mmol) in DCM (7 mL) was added dropwise and stirred for 1 h at –20 °C followed by reaction for 18 h in the freezer at –10 °C. The reaction mixture was warmed to 0 °C, vacuum filtered to remove the molecular sieves, water added (20 mL) and stirred for 45 min while warming to rt. The mixture was vacuum filtered to break the emulsion. The phases were separated and the aqueous phase extracted with DCM (3×10 mL). The DCM fractions were pooled, cooled to 0 °C and 15 % NaOH in brine (15 mL) was added and stirred for 1 h. The reaction mixture was diluted with water (15 mL), the phases were separated and the aqueous phase extracted with DCM (3×20 mL). The DCM layers were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 2:1 hexane/EtOAc as eluent to give the epoxy alcohol (**225**) as a light yellow oil (0.23 g, 88%); R_f= 0.23; $[\alpha]_D^{20}$ –8.4 (*c* 1.4); *dr* = 98:2; ν_{max} 3429, 1736 cm^{-1}.

δ_H: 7.68-7.35 (m, 10H, aromatic protons); 3.77-3.70 (m, 1H, H-3); 3.70 (t, 2H, *J* 5.7, H-6); 2.98 (ddd, 1H, *J* 2.8, 3.8, 3.8, H-2); 2.79 (dd, 1H, $J_{1,3}$ 2.8, 0.5, H-1); 2.77 (dd, 1H, *J* 2.9, 0.5, H-1a); 2.73 (dd, 1H, *J* 3.9, 5.1, H-1b); 2.52 (br s, 1H, O**H**); 1.84-1.55 (m, 4H, H-4, H-5); 1.05 (s, 9H, SiC(C**H$_3$**)$_3$).

δ_C: 135.50, 133.50, 129.62, 127.62 (aromatic carbons); 68.84 (D, C-3); 63.87 (T, C-6); 54.47 (D, C-2); 43.92 (T, C-1); 30.40 (T, C-4); 28.29 (T, C-5); 26.76 (Q, SiC(**C**H_3)$_3$); 19.11 (S, Si**C**$(CH_3)_3$).

HRMS (ESI) : *m/z* 393.1863 $[M+Na]^{+\bullet}$; Calculated for $C_{22}H_{30}O_3SiNa$: 393.1862.



(2*R*,3*S*)-1,2-Epoxy-6-[(t-butyldiphenylsilanyl)oxy]-3-hexanyl (R)-α-methoxy-α-trifluoro-methyl-phenylacetate (226)

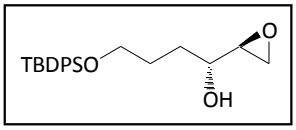
DMF (78 µL, 1.0 mmol) and $(COCl)_2$ (880 µL, 10.1 mmol) were added to a solution of *R*-(+)-MTPAA (0.25 g, 1.1 mmol) in hexane (7 mL) followed by stirring at rt for 2 h. The mixture was filtered and the solvent removed to obtain to give the acyl chloride as a yellow oil.

DMAP (0.25 g, 2.1 mmol) and epoxy alcohol (**225**) (0.14 g, 0.4 mmol) dissolved in DCM (26 mL) were added to the acyl chloride obtained above followed by stirring at rt overnight. The reaction was quenched by addition of saturated NH_4Cl (25 mL) and diluted with DCM (20 mL) followed by separation of the phases. The organic phase was washed with saturated aq. $NaHCO_3$ (2×20 mL) and brine (20 mL). The organic phase was dried using anhydrous $MgSO_4$ and concentrated to give the Mosher ester (**226**) as a dark yellow oil (0.14 g, 62%); R_f = 0.54 (2:1 hexane/EtOAc); *ee* = 98 %.

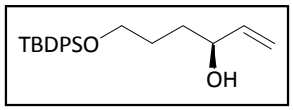
δ_H: 7.64-7.32 (m, 15H, aromatic protons); 5.09 (ddd, 1H, *J* 4.6, 4.6, 7.9, H-3); 3.61 (t, 2H, *J* 6.1, H-6); 3.02 (ddd, 1H, *J* 2.6, 3.9, 4.4, H-2); 2.96 (s, 3H, OC**H₃**); 2.71 (dd, 1H, *J* 3.9, 5.1, H-1a); 2.68 (dd, 1H, *J* 2.6, 5.2, H-1b); 1.90-1.73 (m, 4H, H-4, H-5); 1.04 (s, 9H, SiC(C**H₃**)$_3$).

δ_C: 168.77 (S, **C**O); 135.47, 133.81, 132.44, 129.56, 129.44, 128.45, 127.60, 127.32 (aromatic carbons); 124.78 (Sq, J_{CF} 288 Hz, **C**F_3); 74.35 (D, C-3); 63.08 (T, C-6); 55.26 (Q, O**C**H_3); 51.75 (D, C-2); 44.52 (T, C-1); 27.69 (T, C-5)*; 27.56 (T, C-4)*; 26.82 (Q, SiC(**C**H_3)$_3$); 19.14 (S, Si**C**$(CH_3)_3$).

δ_F: −71.35.



and



(2*S*,3*R*)-1,2-Epoxy-6-[(*t*-butyldiphenylsilanyl)oxy]-3-hexanol (193) and *(3S)*-1,2-epoxy-6-[(*t*-butyldiphenylsilanyl)oxy]-3-hexanol (221)

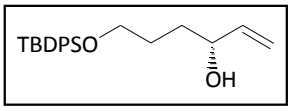
Powdered 4Å molecular sieves (1.63 g) were suspended in DCM (76 mL) and cooled to −20 °C.

(*S,S*)-DIPT (1.4 mL, 6.6 mmol) dissolved in DCM (18 mL) was added followed by dropwise addition of $Ti(O^iPr)_4$ (1.7 mL, 5.7 mmol) and stirred for 30 min. TBHP (1.4 mL, 6.8 mmol, 4.92 M) was added dropwise and stirred for 30 min. Allylic alcohol (**192**) (4.00 g, 11.3 mmol) in DCM (57 mL) was added dropwise and stirred for 1 h at –20 °C followed by reaction for 18 h in the freezer at –10 °C. The reaction mixture was warmed to 0 °C, vacuum filtered to remove the molecular sieves, water added (100 mL) and stirred for 45 min while warming to rt. The mixture was vacuum filtered to break the emulsion. The phases were separated and the aqueous phase extracted with DCM (3×50 mL). The DCM fractions were pooled, cooled to 0 °C and 15 % NaOH in brine (100 mL) was added and stirred for 1 h. The reaction mixture was diluted with water (60 mL), the phases were separated and the aqueous phase extracted with DCM (3×50 mL). The DCM layers were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 2:1 hexane/EtOAc as eluent to obtain a light yellow oil of epoxy alcohol (**193**) (1.75 g, 84%); R_f = 0.30; $[\alpha]_D^{20}$ –9.3 (*c* 1.1); *dr* = 99:1; ν_{max} 3426, 1729 cm^{-1}.

δ_H: 7.68-7.35 (m, 10H, aromatic protons); 3.77-3.73 (m, 1H, H-3); 3.72 (t, 2H, *J* 5.9, H-6); 2.97 (ddd, 1H, *J* 2.8, 3.8, 3.8, H-2); 2.77 (dd, 1H, $J_{1,3}$ 2.8, 5.3, H-1); 2.76 (dd, 1H, *J* 2.8, 5.2, H-1a); 2.72 (dd, 1H, *J* 3.9, 5.1, H-1b); 2.27 (br s, 1H, O**H**); 1.81-1.68 (m, 4H, H-4, H-5); 1.06 (s, 9H, SiC(C**H₃**)₃).

δ_C: 135.56, 133.79, 129.61, 127.64 (aromatic carbons); 68.93 (D, C-3); 63.95 (T, C-6); 54.46 (D, C-2); 43.81 (T, C-1); 30.39 (T, C-4); 28.33 (T, C-5); 26.88 (Q, SiC(**C**H_3)₃); 19.18 (S, Si**C**(CH₃)₃).

HRMS (ESI): *m/z* 393.1864 $[M+Na]^{+\bullet}$; Calculated for $C_{22}H_{30}O_3SiNa$: 393.1862.



(3*R*)-6-[(*t*-Butyldiphenylsilanyl)oxy]-hex-1-en-3-ol (237)

Triphenylphosphine (20.1 g, 76.6 mmol) was added to a solution of allylic alcohol (**221**) (23.8 g, 67.1 mmol) in THF (550 mL) and cooled to 0 °C. A solution of benzoic acid (9.35 g, 76.6 mmol) in a minimum of benzene was slowly added to the reaction mixture and allowed to stir for 5 min at 0 °C followed by the addition of DIAD (15.1 mL, 76.7 mmol). The mixture was allowed to stir overnight at rt. The solvent was evaporated and the crude product suspended in hexane (200 mL) and water (200 mL) followed by vacuum filtration. The filtrate was taken and the phases separated, followed by washing of the organic phase with water (2×100 mL) and brine

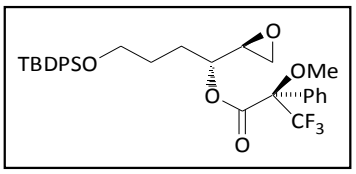
(1×100 mL). The organic fraction was dried using anhydrous $MgSO_4$ and concentrated to obtain the crude benzoate ester (34.4 g, 112%); R_f = 0.52 (2:1 hexane/EtOAc).

Anhydrous K_2CO_3 (30.7 g, 222 mmol) was added to a solution of the crude benzoate (34.4 g, 75.0 mmol) in dry MeOH (800 mL) followed by stirring at rt overnight. The solvent was removed and the crude oil obtained suspended in water (400 mL) and EtOAc (200 mL). The phases were separated and the aqueous phase extracted with EtOAc (2×150 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 6:1 hexane/EtOAc as eluent to obtain the enantiomeric allylic alcohol (**237**) as a light yellow oil (17.8 g, 75%); R_f = 0.19; $[\alpha]_D^{20}$ –1.4 (*c* 1.0); ν_{max} 3388, 912 cm^{-1}.

δ_H: 7.69-7.36 (m, 10H, aromatic protons); 5.87 (ddd, 1H, *J* 6.0, 10.5, 17.2, H-2); 5.23 (ddd, 1H, *J* 1.5, 1.5, 17.2, H-1a); 5.10 (ddd, 1H, *J* 1.3, 1.5, 10.5, H-1b); 4.16-4.09 (m, 1H, H-3); 3.70 (t, 2H, *J* 5.8, H-6); 2.17 (br s, 1H, O**H**); 1.72-1.60 (m, 4H, H-4, H-5); 1.06 (s, 9H, SiC(C**H₃**)₃).

δ_C: 141.13 (D, C-2); 135.53, 133.64, 129.58, 127.61 (aromatic carbons); 114.47 (T, C-1); 72.74 (D, C-3); 63.96 (T, C-6); 33.85 (T, C-4); 28.35 (T, C-5); 26.80 (Q, SiC(**C**H_3)₃); 19.13 (S, Si**C**$(CH_3)_3$).

HRMS (ESI): *m/z* 377.1912 $[M+Na]^{+\bullet}$; Calculated for $C_{22}H_{30}O_2SiNa$: 377.1913.



(2*S*,3*R*)-1,2-Epoxy-6-[(t-butyldiphenylsilanyl)oxy]-3-hexanyl (*R*)-α-methoxy-α-trifluoro-methyl-phenylacetate (222)

DMF (74 µL, 1.0 mmol) and $(COCl)_2$ (835 µl, 9.6 mmol) were added to a solution of *R*-(+)-MTPAA (0.29 g, 1.2 mmol) in hexane (8 mL) followed by stirring at rt for 2 h. The mixture was filtered and the solvent removed to obtain the acid chloride as a yellow oil.

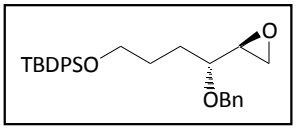
DMAP (0.24 g, 2.0 mmol) and epoxy alcohol (**193**) (0.14 g, 0.4 mmol) dissolved in DCM (27 mL) were added to the acid chloride obtained above followed by stirring at rt overnight. The reaction was quenched by addition of saturated NH_4Cl (25 mL) and diluted with DCM (20 mL) followed by separation of the phases. The organic phase was washed with saturated aq.

$NaHCO_3$ (2×20 mL) and brine (20 mL). The organic phase was dried using anhydrous $MgSO_4$ and concentrated to to give the Mosher ester (**222**) (0.19 g, 86%); R_f = 0.54 (2:1 hexane/EtOAc); *ee* = 99%.

δ_H: 7.76-7.39 (m, 15H, aromatic protons); 5.09 (ddd, 1H, *J* 5.9, 3.5, 3.5, H-3); 3.70 (t, 2H, *J* 6.0, H-6); 3.62 (s, 3H, OC**H₃**); 3.03 (ddd, 1H, *J* 2.0, 2.9, 3.4, H-2); 2.72 (dd, 1H, *J* 2.9, 3.9, H-1a); 2.69 (dd, 1H, *J* 2.0, 3.9, H-1b); 2.01-1.78 (m, 2H, H-5); 1.71-1.53 (m, 2H, H-4); 1.14 (s, 9H, SiC(C**H₃**)$_3$).

δ_C: 165.78 (S, **C**O); 135.32, 133.48, 132.05, 129.49, 128.20, 127.52, 127.09 (aromatic carbons); 123.20 (Sq, J_{CF} 288 Hz, **C**F_3); 73.81 (D, C-3); 62.81 (T, C-6); 55.19 (Q, O**C**H_3); 51.59 (D, C-2); 44.21 (T, C-1); 27.43 (T, C-5)*; 27.37 (T, C-4)*; 26.63 (Q, SiC(**C**H_3)$_3$); 18.98 (S, Si**C**$(CH_3)_3$).

δ_F : –71.48.



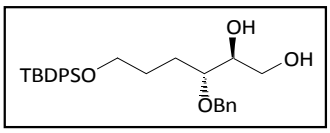
(2*S*,3*R*)-3-Benzyloxy-1,2-epoxy-6-[(*t*-butyldiphenylsilanyl)oxy]-hexane (194)

NaH (60% dispersion, 0.04 g, 1.0 mmol) was washed with hexane (2x3 mL) and suspended in THF (2.0 mL). A solution of epoxy alcohol (**193**) (0.10 g, 0.30 mmol) dissolved in THF (1 mL) was added to the NaH followed by stirring for 30 min. BnBr (50 µL, 0.4 mmol) and TBAI (0.02 g, 0.04 mmol) were added followed by stirring overnight at rt. MeOH (3 mL) was added and allowed to stir for 30 min followed by dilution with water (4 mL) and brine (2 mL). The mixture was extracted with EtOAc (3×10 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 15:1 hexane/EtOAc as eluent to provide the *O*-benzyl ether (**194**) as a yellow oil (0.12 g, 92%); R_f = 0.33 (5:1 hexane/EtOAc); $[\alpha]_D^{20}$ +5.9 (*c* 0.9); ν_{max} 1730 cm^{-1}.

δ_H: 7.73-7.26 (m, 15H, aromatic protons); 4.67 (d, 1H, *J* 11.6, OC**H₂**Ph); 4.50 (d, 1H, *J* 11.6, OC**H₂**Ph); 3.72 (t, 2H, *J* 5.1, H-6); 3.32 (ddd, 1H, *J* 5.1, 5.1, 5.1, H-3); 2.95 (ddd, 1H, *J* 2.6, 2.6, 2.6, H-2); 2.80 (dd, 1H, *J* 3.9, 5.3, H-1a); 2.74 (dd, 1H, *J* 2.6, 5.4, H-1b); 1.89-1.78 (m, 2H, H-5); 1.77-1.66 (m, 2H, H-4); 1.10 (s, 9H, SiC(C**H₃**)$_3$).

δ_C: 138.43, 135.51, 133.87, 129.49, 128.29, 127.60, 127.55 (aromatic carbons); 77.67 (D, C-3); 72.13 (T, O**C**H_2Ph); 63.63 (T, C-6); 53.45 (D, C-2); 45.43 (T, C-1); 28.98 (T, C-5)*; 28.08 (T, C-4)*; 26.81 (Q, SiC(**C**H_3)$_3$); 19.15 (S, Si**C**$(CH_3)_3$).

HRMS (ESI): *m/z* 483.2333 $[M+Na]^{+\bullet}$; Calculated for $C_{29}H_{36}O_3SiNa$: 483.2331.



(2*S*,3*R*)-3-Benzyloxy-6-[(*t*-butyldiphenylsilanyl)oxy]-hexan-1,2-diol (195)

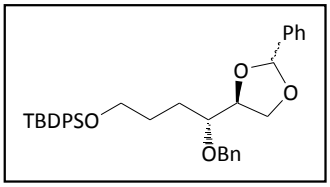
(*S,S*)-Salen-Co complex (**F**) (2.25 g, 3.7 mmol) was added to a solution of *p*-TsOH (0.64 g, 3.3 mmol) in DCM (47 mL) and stirred open to air for 2 h. The mixture was concentrated and co-evaporated with THF (45 mL).

The *O*-benzyl ether (**194**) (8.52 g, 18.5 mmol) dissolved in THF (50 mL) was added to the catalyst prepared above, stirred for 5 min followed by the addition of water (3.0 mL, 166.6 mmol) and stirring overnight at 35-40 °C. The mixture was concentrated and the oil obtained was purified by column chromatography using 1:3 hexane/EtOAc followed by EtOAc as eluent to elute the 1,2-diol (**195**). The catalyst fractions were pooled and concentrated followed by suspension in MeOH and vacuum filtration to retrieve the catalyst. The 1,2-diol (**195**) was obtained as a yellow oil (6.60 g, 75%); R_f = 0.48 (1:3 hexane/EtOAc); $[\alpha]_D^{20}$ –4.3 (*c* 1.0); ν_{max} 3386 cm^{-1}.

δ_H: 7.75-7.29 (m, 15H, aromatic protons); 4.58 (d, 1H, *J* 11.4, OC**H**$_2$Ph); 4.52 (d, 1H, *J* 11.4, OC**H**$_2$Ph); 3.76-3.71 (m, 3H, H-1, H-3); 3.75 (t, 2H, *J* 5.9, H-6); 3.54 (ddd, 1H, *J* 4.5, 4.5, 6.4, H-2); 2.89 (br s, 1H, O**H**); 2.66 (br s, 1H, O**H**); 1.78-1.55 (m, 4H, H-4, H-5); 1.06 (s, 9H, SiC(C**H**$_3$)$_3$).

δ_C: 138.04, 135.46, 133.72, 129.52, 128.38, 127.74, 127.56 (aromatic carbons); 80.64 (D, C-2); 72.35 (D, C-3); 72.29 (T, OCH_2Ph), 63.64 (T, C-6); 63.27 (T, C-1); 28.00 (T, C-5); 26.78 (Q, SiC(CH_3)$_3$); 26.30 (T, C-4); 19.11 (S, Si**C**(CH$_3$)$_3$).

HRMS (ESI): *m/z* 501.2437 $[M+Na]^{+\bullet}$; Calculated for $C_{29}H_{38}O_4SiNa$: 501.2437.



(2*S*,3*R*)-1,2-*O*-Benzylidene 3-benzyloxy-6-[(*t*-butyldiphenylsilanyl)oxy]-hexane-1,2-diol (227)

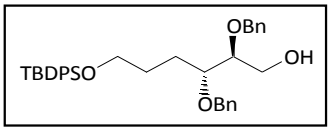
α,α-Dimethoxytoluene (35.0 mL, 239 mmol) and *p*-TsOH (0.50 g, 2.6 mmol) were added to a solution of the 1,2 diol (**195**) (6.15 g, 12.8 mmol) in DCM (27 mL) followed by stirring overnight

at rt. The reaction was neutralised with Et_3N (7 mL) and diluted with EtOAc (50 mL) followed by washing with water (2×50 mL) and brine (1×40 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 2:1 hexane/EtOAc as eluent to yield the benzylidene derivative (**227**) as a light yellow oil (5.54 g, 76%); R_f = 0.57; $[\alpha]_D^{20}$ –2.2 (*c* 1.0); ν_{max} 2334, 906 cm^{-1}.

δ_H: 7.68-7.28 (m, 20H, aromatic protons); 5.91 and 5.78 (s, 1H each, $O_2C\mathbf{H}Ph$); 4.63 (d, 1H, *J* 11.4, $OC\mathbf{H_2}Ph$); 4.57 (d, 1H, *J* 11.4, $OC\mathbf{H_2}Ph$); 4.22-4.18 (m, 1H, H-3); 3.70 (t, 2H, *J* 7.0, H-6); 3.68-3.60 (m, 1H, H-2); 3.64 (dd, 1H, *J* 7.0, 9.6, H-1a); 3.55 (dd, 1H, *J* 7.0, 9.6, H-1b); 1.84-1.61 (m, 4H, H-4, H-5); 1.07 (s, 9H, $SiC(C\mathbf{H_3})_3$).

δ_C: 138.18-126.36 (aromatic carbons); 104.07, 103.79 (S, $O_2\mathbf{C}HPh$); 78.90 (D, C-2); 78.44, 78.12 (D, C-3); 72.56, 72.33 (T, $O\mathbf{C}H_2Ph$); 63.84, 63.79 (T, C-6); 61.19 (T, C-1); 28.05, 27.78 (T, C-5)*; 27.40, 27.32 (T, C-4)*; 26.89 (Q, $SiC(\mathbf{C}H_3)_3$); 19.20 (S, $Si\mathbf{C}(CH_3)_3$).

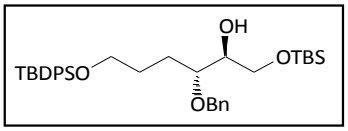
HRMS (ESI): *m/z* 589.2749 $[M+Na]^{+\bullet}$; Calculated for $C_{36}H_{42}O_4SiNa$: 589.2750.



(2*S*,3*R*)-2,3-Dibenzyloxy-6-[(*t*-butyldiphenylsilanyl)oxy]-1-hexanol (228)

$BH_3{\cdot}Me_2NH$ (1.53 g, 26.0 mmol) and $BF_3{\cdot}Et_2O$ (4.5 mL, 49.0 mmol) were added to a solution of the benzylidene derivative (**227**) (5.54 g, 9.8 mmol) in DCM (112 mL) cooled to –40 °C followed by stirring for 2 h at 0 °C. The reaction was quenched with satd. $NaHCO_3$ solution (75 mL) and extracted with chloroform (3×50 mL). The chloroform fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated to obtain a yellow oil which was not the required (**228**).

4.3.2.2. Via the Benzoyl Protection Route



(2*S*,3*R*)-3-Benzyloxy-1-[(*t*-butyldimethylsilanyl)oxy]-6-[(*t*-butyldiphenylsilanyl)oxy]-hexan-2-ol (229)

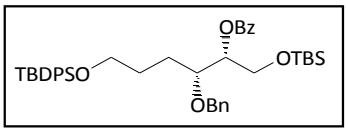
Imidazole (0.84 g, 12.3 mmol) and DMAP (0.03 g, 0.3 mmol) were added to a solution of the 1,2-diol (**195**) (4.44 g, 9.3 mmol) dissolved in DCM (35 mL) followed by cooling to 0 °C. A solution of TBSCl (1.44 g, 9.6 mmol) dissolved in DCM (10 mL) was added dropwise to the

reaction mixture followed by stirring at 0 °C for 2 h. The mixture was concentrated and the residue obtained partitioned between EtOAc (45 mL) and water (3×20 mL). The organic phase was dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 4:1 hexane/EtOAc as eluent to give the *O*-TBS derivative (**229**) as a light yellow oil **229** (4.97 g, 91%); R_f = 0.48; $[\alpha]_D^{20}$ –4.0 (*c* 1.0); ν_{max} 3462, 1248 cm^{-1}.

δ_H: 7.70-7.27 (m, 15H, aromatic protons); 4.56 (s, 2H, OC**H**$_2$Ph); 3.78-3.65 (m, 3H, H-1, H-3); 3.71 (t, 2H, *J* 5.3, H-6); 3.53-3.47 (m, 1H, H-2); 2.43 (br s, 1H, O**H**); 1.83-1.60 (m, 4H, H-4, H-5); 1.07 (s, 9H, C-6 SiC(C**H**$_3$)$_3$); 0.92 (s, 9H, C-1 SiC(C**H**$_3$)$_3$); 0.09 (s, 6H, C-1 Si(C**H**$_3$)$_2$).

δ_C: 138.55, 135.55, 134.01, 129.49, 128.32, 127.78, 127.56 (aromatic carbons); 78.99 (D, C-2); 72.77 (D, C-3); 72.06 (T, O**C**H$_2$Ph); 63.96 (T, C-1)†; 63.85 (T, C-6)†; 28.02 (T, C-5); 26.86 (Q, C-6 SiC(**C**H$_3$)$_3$); 26.21 (T, C-4); 25.88 (Q, C-1 SiC(**C**H$_3$)$_3$); 19.20 (S, C-6 Si**C**(CH$_3$)$_3$); 18.25 (S, C-1 Si**C**(CH$_3$)$_3$); –5.38 (Q, C-1 Si(CH$_3$)$_2$).
† may be interchanged

HRMS (ESI): *m/z* 593.3483 $[M+H]^{+\bullet}$; Calculated for $C_{35}H_{53}O_4Si_2$: 593.3482.
m/z 615.3298 $[M+Na]^{+\bullet}$; Calculated for $C_{35}H_{52}O_4Si_2Na$: 615.3302.



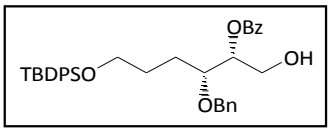
(2*R*,3*R*)-3-Benzyloxy-1-[(*t*-butyldimethylsilanyl)oxy]-6-[(*t*-butyldiphenylsilanyl)oxy]-2-hexanyl benzoate (230)

Triphenylphosphine (7.18 g, 27.4 mmol) was added to a solution of the *O*-TBS derivative (**229**) (13.37 g, 22.6 mmol) in THF (420 mL) and cooled to 0 °C. A solution of benzoic acid (3.34 g, 27.4 mmol) in a minimum volume of benzene was added slowly to the reaction mixture and allowed to stir for 5 min at 0 °C followed by the addition of DIAD (5.3 mL, 26.9 mmol). The mixture was allowed to stir overnight at rt. The solvent was evaporated and the crude product suspended in hexane (200 mL) and water (200 mL) followed by vacuum filtration. The filtrate was taken and the phases separated, followed by washing of the organic phase with water (2×100 mL) and brine (1×100 mL). The organic fraction was dried using anhydrous $MgSO_4$ and concentrated to give the benzoate ester (**230**) (15.60 g, 99%); R_f = 0.49 (6:1 hexane/EtOAc); $[\alpha]_D^{20}$ +4.6 (*c* 1.0); ν_{max} 1711, 1260 cm^{-1}. The crude material obtained was used as is for the next reaction.

δ_H: 8.11-8.08; 7.68-7.25 (m, 20H, aromatic protons); 5.34 (ddd, 1H, *J* 4.6, 4.6, 6.1, H-3); 4.69 (d, 1H, *J* 11.6, OC**H$_2$**Ph); 4.62 (d, 1H, *J* 11.6, OC**H$_2$**Ph); 3.97 (dd, 1H, *J* 4.5, 11.0, H-1a); 3.90 (dd, 1H, *J* 6.1, 11.0, H-1b); 3.81 (ddd, 1H, *J* 4.7, 4.7, 6.9, H-2); 3.66 (t, 2H, *J* 5.7, H-6); 1.93-1.50 (m, 4H, H-4, H-5); 1.03 (s, 9H, C-6 SiC(C**H$_3$**)$_3$); 0.88 (s, 9H, C-1 SiC(C**H$_3$**)$_3$); 0.05 and 0.04 (s, each 3H, C-1 Si(C**H$_3$**)$_2$).

δ_C: 166.50 (S, Ph**C**OO); 138.88, 135.94, 134.31, 133.27, 130.73, 130.15, 129.92, 128.70, 128.68, 128.26, 127.99 (aromatic carbons); 77.78 (D, C-2); 76.26 (D, C-3); 73.04 (T, O**C**H$_2$Ph); 64.08 (T, C-6); 61.85 (T, C-1); 29.09 (T, C-5); 27.23 (Q, C-6 SiC(**C**H$_3$)$_3$); 27.19 (T, C-4); 26.19 (Q, C-1 SiC(**C**H$_3$)$_3$); 19.57 (S, C-6 Si**C**(CH$_3$)$_3$); 18.56 (S, C-1 Si**C**(CH$_3$)$_3$); –5.00 (Q, C-1 Si(CH$_3$)$_2$).

HRMS (ESI): *m/z* 679.3629 $[(M+H)-H_2O]^{+\bullet}$; Calculated for $C_{42}H_{55}O_4Si_2$: 679.3639.
m/z 719.3554 $[M+Na]^{+\bullet}$; Calculated for $C_{42}H_{56}O_5Si_2Na$: 719.3564.



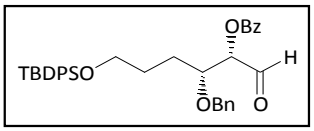
(2*R*,3*R*)-3-Benzyloxy-6-[(*t*-butyldiphenylsilanyl)oxy]-1-hydroxy-2-hexyl benzoate (231)

p-TsOH (0.77 g, 4.0 mmol) was added to a solution of the benzoate ester (**230**) (15.29 g, 21.9 mmol) in dry MeOH (250 mL) followed by stirring at rt for 35 min using TLC analysis to follow reaction progress. Water (100 mL) was added followed by dilution with EtOAc (300 mL). The phases were separated and the EtOAc fraction extracted with brine (100 mL) and water (2×100 mL). The EtOAc fraction was dried using anhydrous $MgSO_4$ and concentrated to give the primary alcohol (**231**) as an yellow oil (13.4 g, 105%); R_f = 0.14 (4:1 hexane/EtOAc); $[\alpha]_D^{20}$ –2.7 (*c* 1.2); ν_{max} 3466, 1712 cm^{-1}. The crude material obtained was used as is for the next reaction.

δ_H: 8.09-8.06; 7.66-7.29 (m, 20H, aromatic protons); 5.33 (ddd, 1H, *J* 4.3, 4.3, 6.1, H-3); 4.64 (s, 2H, OC**H$_2$**Ph); 3.98 (dd, 1H, *J* 4.1, 11.9, H-1a); 3.88 (dd, 1H, *J* 6.2, 11.9, H-1b); 3.79 (ddd, 1H, *J* 4.6, 4.6, 7.3, H-2); 3.66 (t, 2H, *J* 5.8, H-6); 2.19 (br s, 1H, O**H**); 1.89-1.55 (m, 4H, H-4, H-5); 1.03 (s, 9H, SiC(C**H$_3$**)$_3$).

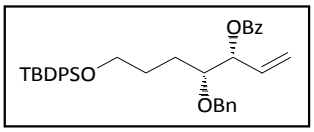
δ_C: 166.49 (S, Ph**C**OO); 137.95, 135.49, 133.79, 133.16, 129.77, 129.53, 128.39, 127.94, 127.78, 127.58 (aromatic carbons); 78.06 (D, C-2); 75.35 (D, C-3); 72.27 (T, O**C**H$_2$Ph); 63.50 (T, C-6); 62.10 (T, C-1); 28.56 (T, C-5); 26.79 (Q, SiC(**C**H$_3$)$_3$); 26.30 (T,C-4); 19.12 (S, Si**C**(CH$_3$)$_3$).

HRMS (ESI): *m/z* 565.2771 $[(M+H)-H_2O]^{+\bullet}$; Calculated for $C_{36}H_{41}O_4Si$: 565.2774.
m/z 605.2700 $[M+Na]^{+\bullet}$; Calculated for $C_{36}H_{42}O_5SiNa$: 605.2699.



(2*R*,3*R*)-3-Benzyloxy-6-[(*t*-butyldiphenylsilanyl)oxy]hexanal 2-*O*-benzoate (232)

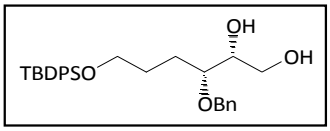
DMSO (136 µL, 1.9 mmol) was added dropwise to a solution of $(COCl)_2$ (84 µL, 1.0 mmol) in DCM (6.0 mL) at −78 °C and stirred for 30 min. The primary alcohol (**231**) (0.35 g, 0.6 mmol) dissolved in DCM (5.6 mL) was added dropwise at −78 °C and stirred for 1 h. Et_3N (485 µL, 3.5 mmol) was added at −78 °C and stirred for 1 h followed by warming to rt. TLC analysis was done using 4:1 hexane/EtOAc as eluent (R_f = 0.31) to determine reaction progress. Water (15 mL) was added to the reaction mixture and the phases separated. The aqueous phase was washed with EtOAc (3×7 mL), the organic fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The murky oil obtained was dissolved in a minimum of Et_2O and the white solid formed was filtered off, followed by concentration to give the aldehyde (232) as a yellow oil (0.21 g, 59%). The crude material was used immediately without further purification or characterisation for the next reaction.



(2*R*,3*R*)-3-Benzyloxy-6-[(*t*-butyldiphenylsilanyl)oxy]-hex-1-en-2-yl benzoate (233)

n-BuLi (1.0 mL, 1.6 mmol, 1.6 M in hexane) was added dropwise to a solution of methyl-triphenylphosphonium iodide (0.61 g, 1.5 mmol) in THF (16 mL) cooled to 0 °C followed by warming to rt over 45 min. A solution of **231** (0.21 g, 0.4 mmol) in THF (13 mL) was added dropwise to the reaction followed by stirring overnight at rt. The reaction was quenched by dropwise addition of satd. NH_4Cl solution (5 mL), concentrated, followed by suspension in EtOAc (10 mL) and ammonium chloride (15 mL) and removal of the solids by vacuum filtration. The phases were separated and the aqueous phase was washed with EtOAc (3×10 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography after dry loading using 4:1 hexane/EtOAc (R_f = 0.53) as eluent to obtain a light yellow oil which was not the required (**233**).

4.3.2.3. Via the PMB Protection Route



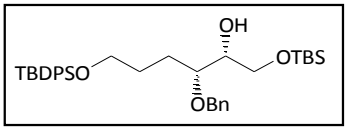
(2*R*,3*R*)-3-Benzyloxy-6-[(*t*-butyldiphenylsilanyl)oxy]-hexan-1,2-diol (234)

Anhydrous K_2CO_3 (10.1 g, 72.9 mmol) was added to a solution of the primary alcohol (**231**) (12.7 g, 21.8 mmol) in dry MeOH (500 mL) followed by stirring at rt for 2 h. The solvent was removed and the crude oil obtained suspended in water (500 mL), brine (100 mL) and extracted with EtOAc (3×250 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using gradient elution starting with 6:1 hexane/EtOAc, then 1:1 hexane/EtOAc as eluent to obtain the 1,2-diol (**234**) as a light yellow oil (5.80 g, 56%); R_f = 0.22 (1:1 hexane/EtOAc); $[\alpha]_D^{20}$ –13.0 (*c* 1.0); ν_{max} 3401 cm^{-1}.

δ_H: 7.66-7.63, 7.43-7.26 (m, 15H, aromatic protons); 4.62 (d, 1H, *J* 11.3, OC**H**$_2$Ph); 4.43 (d, 1H, *J* 11.3, OC**H**$_2$Ph); 3.72-3.57 (m, 5H, H-1, H-3, H-6); 3.48 (ddd, 1H, *J* 5.4, 5.4, 5.4, H-2); 2.25 (br s, 2H, 2x O**H**); 1.72-1.59 (m, 4H, H-4, H-5); 1.04 (s, 9H, SiC(C**H**$_3$)$_3$).

δ_C: 137.93, 135.53, 133.82, 129.58, 128.51, 127.90, 127.62 (aromatic carbons); 79.34 (D, C-2); 72.70 (D, C-3); 71.95 (T, OC**H**$_2$Ph); 63.89 (T, C-6); 63.71 (T, C-1); 27.88 (T, C-5); 26.84 (Q, SiC(C**H**$_3$)$_3$); 26.19 (T, C-4); 19.17 (S, Si**C**(CH$_3$)$_3$).

HRMS (ESI): *m/z* 501.2436 $[M+Na]^{+\bullet}$; Calculated for $C_{29}H_{38}O_4SiNa$: 501.2437.



(2*R*,3*R*)-3-Benzyloxy-1-[(*t*-butyldimethylsilanyl)oxy]-6-[(*t*-butyldiphenylsilanyl)oxy]-hexan-2-ol (235)

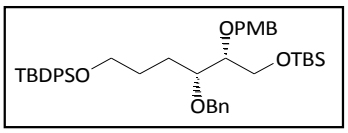
Imidazole (1.09 g, 15.9 mmol) and DMAP (0.05 g, 0.4 mmol) were added to a solution of the 1,2-diol (**234**) (5.80 g, 12.1 mmol) in DCM (46 mL) followed by cooling to 0 °C. A solution of TBSCl (1.90 g, 12.6 mmol) in DCM (13 mL) was added dropwise to the reaction mixture followed by stirring at 0 °C for 2 h. The mixture was concentrated and the residue obtained suspended in EtOAc (100 mL) followed by washing with water (2×80 mL) and brine (50 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil

obtained was purified by column chromatography using 4:1 hexane/EtOAc as eluent to obtain the O-TBS ether (**235**) **as a** light yellow oil (4.77 g, 66%); R_f = 0.43; $[\alpha]_D^{20}$ –5.1 (*c* 1.0); ν_{max} 3460, 1253 cm^{-1}.

δ_H: 7.68-7.65, 7.43-7.25 (m, 15H, aromatic protons); 4.60 (d, 1H, *J* 11.4, OC**H**$_2$Ph); 4.54 (d, 1H, *J* 11.4, OC**H**$_2$Ph); 3.75-3.61 (m, 5H, H-1, H-3, H-6); 3.55-3.52 (m, 1H, H-2); 2.09 (br s, 1H, O**H**); 1.80-1.63 (m, 4H, H-4, H-5); 1.05 (s, 9H, C-6 SiC(C**H**$_3$)$_3$); 0.89 (s, 9H, C-1 SiC(C**H**$_3$)$_3$); 0.06 (s, 6H, C-1 Si(C**H**$_3$)$_2$).

δ_C: 138.46, 135.54, 133.94, 129.52, 128.35, 127.86, 127.58 (aromatic carbons); 78.56 (D, C-2); 73.05 (D, C-3); 72.39 (T, O**C**H$_2$Ph); 63.93 (T, C-1); 63.82 (T, C-6); 28.51 (T, C-5); 26.85 (Q, C-6 SiC(**C**H$_3$)$_3$); 26.54 (T, C-4); 25.88 (Q, C-1 SiC(**C**H$_3$)$_3$); 19.19 (S, C-6 Si**C**(CH$_3$)$_3$); 18.23 (S, C-1 Si**C**(CH$_3$)$_3$); –5.38 (Q, C-1 Si(CH$_3$)$_2$).

HRMS (ESI): *m/z* 593.3486 $[M+H]^{+\bullet}$; Calculated for $C_{35}H_{53}O_4Si_2$: 593.3482.
m/z 615.3303 $[M+Na]^{+\bullet}$; Calculated for $C_{35}H_{52}O_4Si_2Na$: 615.3302.



(2*R*,3*R*)-3-Benzyloxy-1-[(*t*-butyldimethylsilanyl)oxy]-6-[(*t*-butyldiphenylsilanyl)oxy]-2-[4-methoxybenzyloxy]-hexane (236)

NaH (60% dispersion, 0.02 g, 0.6 mmol) was washed with hexane (2x2 mL) and suspended in DMF (1.5 mL). A solution of the hexan-2-ol (**235**) (0.20 g, 0.3 mmol) in DMF (1.0 mL) was added to the NaH suspension and stirred for 30 min at rt. PMBCl (58 µL, 0.4 mmol) was added to the reaction mixture followed by reflux at 60-65 °C overnight. The reaction was quenched with brine (5 mL) and washed with EtOAc (3×5 mL). The EtOAc fractions were pooled, dried using anhydrous $MgSO_4$ and concentrated. The oil obtained was purified by column chromatography using 6:1 hexane/EtOAc (R_f = 0.39) as eluent to obtain a light yellow oil which was not the required (**236**).