A [2,3]-Wittig rearrangement approach towards the stereoselective synthesis of the C(10)–C(20) backbone of the fumonisins

by

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DECLARATION

I, Cara Slabbert, declare that the dissertation, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not previously been submitted by me for the degree at this or any other tertiary institution.

Signature: …….. Date: ……

C. Slabbert

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SUMMARY

Fusarium verticillioides (= Fusarium moniliforme) a common fungal contaminant of maize throughout the world has been associated with diseases in both man and animals. The structure of the fumonisins, a family of structurally related mycotoxins isolated from cultures associated with the high incidence of human oesophageal cancer in the Transkei region in South Africa and with equine leucoencephalomalacia, a neurological disorder in horses and donkeys, has been established. The main mycotoxin, fumonisin B_1 consists of the diester formed by the C(14) and C(15) hydroxyl groups of (2*S*,3*S*,5*R*,10*R*,12*S*,14*S*,15*R*,16*R*)-2-amino-12,16-dimethyleicosane-3,10,14,15-pentaol with the *Si* carboxy group of propane-1,2,3-tricarboxylic acid.

A comparison of the structures of the 28 known fumonisins reveals that they share a common structural motif for the C(11)–C(20) unit, and probably also the same stereochemistry for the 4 stereogenic centres present in this unit. Disconnection of the C(9)–C(10) bond in a retrosynthetic analysis of the fumonisins C_{20} backbone (C_{19} in the fumonisin C series) identifies (3*S*,5*S*,6*R*,7*R*)-3,7-dimethylundecane-1,5,6-triol as a common building block for the synthesis of any of the fumonisins.

In the dissertation the retrosynthetic analysis of this 3,7-dimethylundecane-1,5,6-triol building block identifies (3*S*,4*R*,5*R*)-5-methylnonane-1,3,4-triol as a viable target which in turn could be derived from a simple starting material *trans*-4-hexen-3-one. Key reactions identified to realise the required transformations leading to the identified target included kinetic enzymatic resolution of the racemic alcohol obtained from *trans*-4-hexen-3-one, and a pivotal role for both the [2,3]-Wittig rearrangement and the use of Sharpless asymmetric epoxidation methodology as these reactions generated the requisite stereogenic centres present in (3*S*,4*R*,5*R*)-5-methylnonane-3,4-diol. In this manner a synthetic route from *trans*-4-hexen-3-one to (2*R*,3*R*,4*R*,5*R*,6*E*)-4-(benzyloxy)-2,3-epoxy-5-methylnon-6-en-1-ol using appropriate functional group transformations and protective group strategies, with complete stereochemical control, were developed in this work. Alternative strategies to overcome problems encountered during the synthesis are presented for future work. The conversion of the 4-(benzyloxy)-2,3-epoxy-5-methylnon-6-en-1-ol intermediate to the protected 5-methylnonane-1,3,4-triol target could not be carried out due to time constraints and material shortages.

ABBREVIATIONS

AIBN	2,2′-Azobisisobutyronitrile
aq	aquous
Ar	Aryl
9-BBN	9-Borabicyclo[3.3.1]nonane
BINAL-H	2,2′-Dihydroxy-1,1′-binaphthyl lithium aluminium hydride
BINOL	1,1′-Bi-2,2′-naphthol
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
Bu	*n*-Butyl
Cbz	Benzyloxycarbonyl
CSA	Camphorsulfonic acid
d	Days
DCM	Dichloromethane
de	Diastereomeric excess
DET	Diethyl tartrate
DEAD	Diethyl azodicarboxylate
DIBALH	Diisobutylaluminium hydride
DIPT	Diisopropyl tartrate
DMAP	Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DMT	Dimethyl tartrate
dr	Diastereomeric ratio
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
ee	Enantiomeric excess
Et	Ethyl
IBX	2-Iodoxybenzoic acid
Ipc	Isopinocamphenyl
LAH	Lithium aluminium hydride
LDA	Lithium diisopropylamide
LHMDS	Lithium bis(trimethylsilyl)amide
MCPBA	m-Chloroperbenzoic acid
Me	Methyl
Ms	Mesyl (methanesulfonyl)
MS	Mass spectrometry
MTPA	α-Methoxy-α-trifluoromethylphenylacetyl
NBS	*N*-Bromosuccinimide

NMO	*N*-Methylmorpholine-*N*-oxide
Ph	Phenyl
PPL	Porcine pancreatic lipase
PPTS	Pyridinium *p*-toluenesulfonate
Pr	Propyl
Py	Pyridine
Red-Al	*bis*-(2-Methoxyethoxy)aluminum hydride
TBDPS	*t*-Butyldiphenylsilyl
TBDPS	*t*-Butyldiphenylsilyl
TBHP	*t*-Butyl hydroperoxide
TBS	*t*-Butyldimethylsilyl
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
THP	2-Tetrahydropyranyl
Ts	*p*-Toluenesulfonyl

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CHAPTER 1 INTRODUCTION

1.1 BACKGROUND

1.1.1 Environmental Health Criteria Monographs

The Environmental Health Criteria (EHC) Programme of the World Health Organization (WHO) was initiated in 1973[1] and the first EHC series was launched in 1976.

The objectives of publishing an EHC monograph are to evaluate information on the relationship between human health and exposure to environmental pollutants and to provide guidelines for national authorities in setting of exposure limits, to identify new or possible pollutants, to recognize breaches in knowledge regarding the health effects of pollutants and to promote management of toxicological and epidemiological methods in order to have internationally comparable results.[1] The data used originates from original studies, both published and unpublished.

The International Programme on Chemical Safety (IPCS) organizes meetings of scientists to compile a list of chemicals for subsequent evaluation. The chemicals are selected and evaluated according to an internationally agreed list of priorities.

The EHC monographs are widely established, used and recognized throughout the world. Each EHC follows a standard outline: A full summary of the document followed by information on identity, sources of exposure, environmental transport, distribution and transformation, environmental levels and human exposure, kinetics and metabolism in laboratory animals and humans, effects on laboratory animals and in *vitro* test systems, effects on humans, effects on other organisms in the laboratory and field, an overall evaluation and conclusions for the protection of human health and the environment at the end of each document, needs for further research and details of previous evaluations by international bodies.[1]

A WHO Task Group on Environmental Health Criteria for fumonisin B_1 met at the World Health Organization, Geneva, Switzerland from 10-14 May 1999. The Task Group reviewed

[1] WHO. Fumonisin B1 (Environmental Health Criteria 219). International Programme on Chemical Safety. World Health Organization, Geneva, **2000.**

and revised the draft monograph and made an evaluation of the risks for human health and the environment from exposure to fumonisin B_1.[1] Information regarding the abovementioned is therefore not covered; the reader is referred to reference 1 for the full EHC monograph. A review of the toxic effects and mechanisms of action of fumonisin B_1 has also been compiled recently by Stockmann-Juvala and Savolainen.[2]

1.1.2 The Fumonisins

The fungus *Fusarium moniliforme* Sheldon is now referred to as *Fusarium verticillioides* (Sacc.) Nirenberg. This decision was taken at the 8th International *Fusarium* Workshop in the United Kingdom in August 1998.[1]

F. verticillioides is one of the most widespread fungi and linked to the contamination of maize and products thereof throughout the world.[1] This fungal strain was isolated from a batch of mouldy maize in 1970.[1,3] In 1988 the fumonisins, a family of mycotoxins, were isolated and chemically characterized from cultures of *F. verticillioides* strain MRC 826.[3,4]

The fumonisins bear structural similarities to the sphingoid bases (**Figure 1**) and the biological activity of the fumonisins lies in their interference with sphingolipid biosynthesis. The sphingolipids are synthesized via a *de novo* pathway; the main steps include the formation of sphinganine (**1**) from the condensation of serine and palmitoyl-CoA. The enzyme ceramide synthase acylates both sphinganine (**1**) and sphingosine (**2**) to form dihydroxyceramide (**3**) and ceramide (**4**), respectively. Fumonisin B_1 (**5**) acts as an inhibitor of this enzyme (**Figure 2**).

Sphingolipids are present in all eukaryotic cells. They serve as structural molecules and as regulators in various cell functions, including signal transmission and cell recognition. All the sphingolipids that have been identified to date, contain a sphingoid base (long-chain base) backbone, its biosynthesis is therefore prone to inhibition by the fumonisins.

The effect of ceramide synthase inhibition snowballs. Free sphinganine (**1**) and sphingosine (**2**), reported to be cytotoxic, accumulate in the blood and urine of exposed animals. These compounds have been reported to be inhibitors of protein kinase C (PKC). PKC enzymes have a multitude of functions, including receptor desensitization, membrane modulation,

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2 Stockmann-Juvala, H.; Savolainen, K. *Human Exp. Toxicol.* **2008**, *27*, 799.
3 Gelderblom, W.C.A.; Jaskiewicz, K.; Marasas, W.F.O.; Thiel, P.G.; Horak, R.M.; Vleggaar, R.; Kriek, N.P.J. *Appl. Environ. Microbiol.* **1988**, *54*, 1806.
4 Bezuidenhout, S.C.; Gelderblom, W.C.S.; Gorst-Allman, C.P.; Horak, R.M.; Marasas, W.F.O.; Spiteller, G.; Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1988**, 743.

regulation of transcription, mediators in immune responses as well as regulators in cell growth.

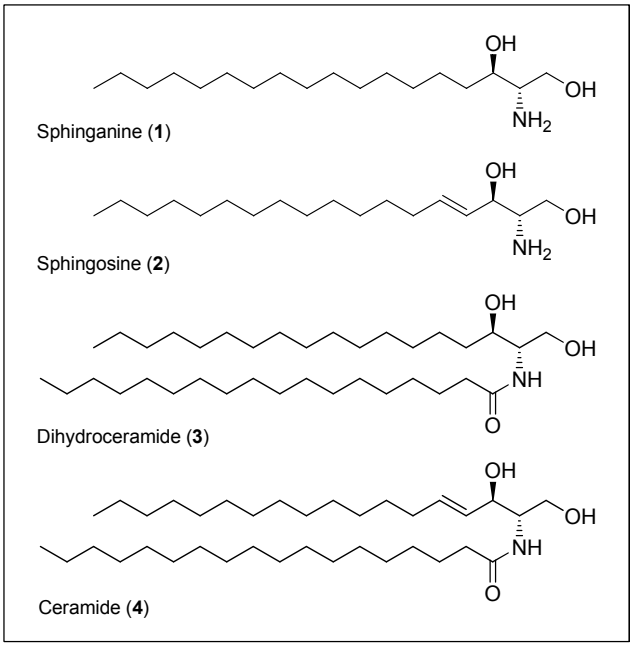


Figure 1: Structure of the sphingoid bases.[2]

Ceramide synthase inhibition and the accumulation of free sphinganine and sphingosine in the blood and urine result in detrimental biological conditions in the exposed animal. EHC monograph 219 states that, "FB_1 is carcinogenic in mice and rats and induces fatal diseases in pigs and horses at levels of exposure that humans encounter." In humans oesophageal cancer and neural tube defects have been linked to ingestion of food produce contaminated with FB_1.[5]

1.2 THE FUMONISINS

Twenty-eight different fumonisins have been identified and 15 of the more important fumonisins, divided into four main categories, A, B, C and P,[1,6] are listed here. Fumonisin B_1 (**5**) is by far the most abundant. Samples containing FB_1 (**5**) are usually co-contaminated with FB_2 (**6**) and FB_3 (**7**), which are structurally very similar to FB_1 (**5**), but the levels of the last two compounds are usually quite low.[3,4] The fumonisins are polar diesters with a C_{20} polyhydroxylated backbone (C_{19} in the fumonisin C series). The differences between the metabolites lie in the hydroxylation patterns and are indicated in **Figures 3-5**.[7]

[5] Rheeder, J.P.; Marasas, W.F.O.; Thiel, P.G.; Sydenham, E.W.; Shephard, G.S.; Van Schalkwyk, D.J. *Phytopathol.* **1992**, *82,* 353.
[6] Dutton, M.F. *Pharmacol. Ther.* **1996,** *70*, 137.
[7] Rheeder, J.P., Marasas, W.F.O., Vismer, H.F. *Appl. Environ. Microbiol.* **2002**, *68*, 2101.

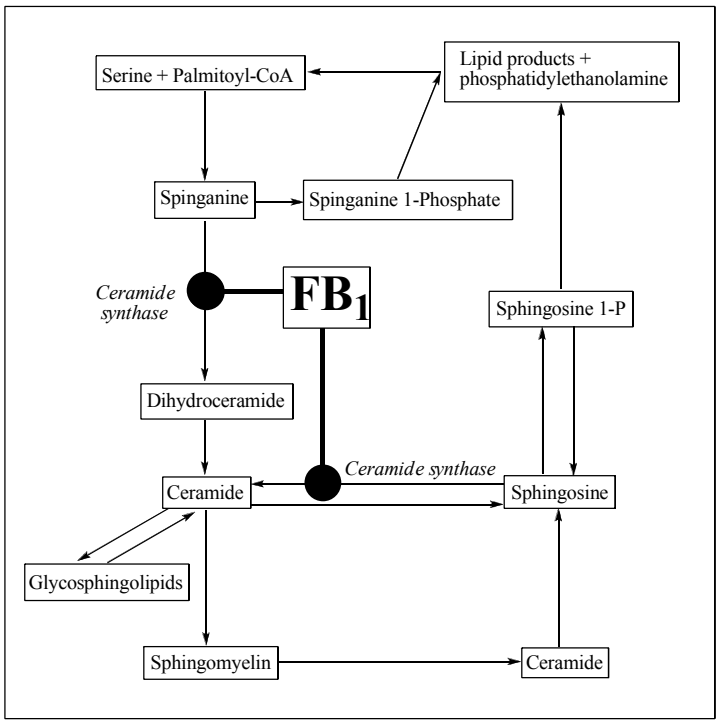
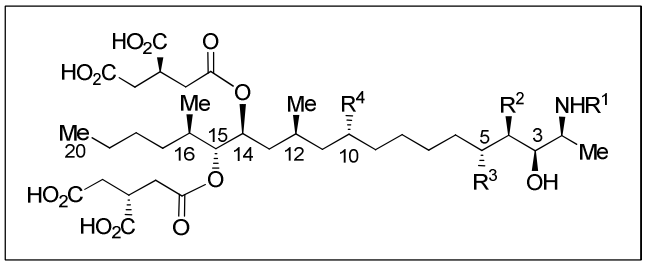
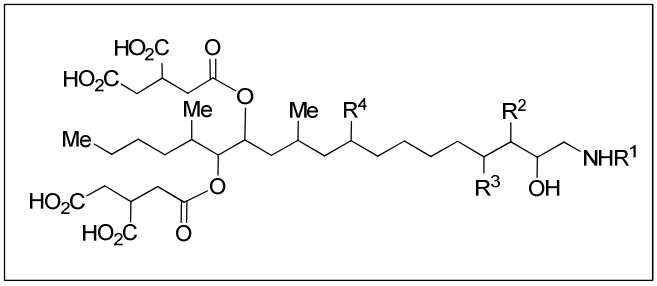


Figure 2: Schematic pathway of *de novo* sphingolipid synthesis and turnover in mammalian cells.[2]



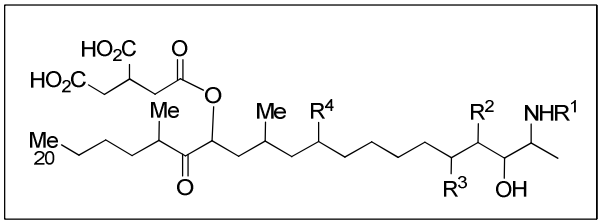
Mycotoxin	R^1	R^2	R^3	R^4
Fumonisin A (FA) series				
Fumonisin A_1 (FA_1) (**8**)	$COCH_3$	H	OH	OH
Fumonisin A_2 (FA_2) (**9**)	$COCH_3$	H	OH	H
Fumonisin B (FB) series	**R^1**	**R^2**	**R^3**	**R^4**
Fumonisin B_1 (FB_1) (**5**)	H	H	OH	OH
Fumonisin B_2 (FB_2) (**6**)	H	H	OH	H
Fumonisin B_3 (FB_3) (**7**)	H	H	H	OH
Fumonisin B_4 (FB_4) (**10**)	H	H	H	H
Iso-Fumonisin B_1 (iso-FB_1) (**11**)	H	OH	H	OH

Figure 3: The fumonisin A and B series.



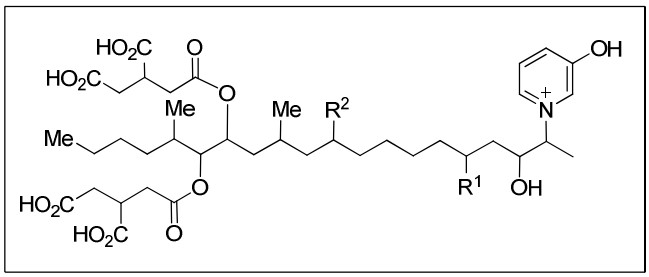
Fumonisin C (FC) series	R^1	R^2	R^3	R^4
Fumonisin C_1 (FC_1) (**12**)	H	H	OH	OH
Fumonisin C_3 (FC_3) (**13**)	H	H	H	OH
Fumonisin C_4 (FC_4) (**14**)	H	H	H	H
Hydroxylated Fumonisin C_1 (**15**)	H	OH	OH	OH

Figure 4: The fumonisin C series.



	R^1	R^2	R^3	R^4
Fumonisin AK_1 (FAK_1) (**16**)	$COCH_3$	H	OH	OH

Figure 5: The fumonisin AK_1 series.



Fumonisin P (FP) series	R^1	R^2
Fumonisin P_1 (FP_1) (**17**)	OH	OH
Fumonisin P_2 (FP_2) (**18**)	OH	H
Fumonisin P_3 (FP_3) (**19**)	H	OH

Figure 6: The fumonisin FP series.

1.2.2 Absolute Configuration

Fumonisin B_1 does not crystallize and X-ray analysis is therefore not possible. A significant amount of research has been done in order to determine the stereochemistry of these compounds. The absolute configuration at each stereogenic centre of the FB_2 (**6**) backbone and the AAL toxins has been determined by Kishi and co-workers.[8,9] The approach involved the independent synthesis and NMR analysis and comparison of all the diastereomers relating to the individual halves of FB_2 (**6**). The approach adopted by Hoye *et al.*[10] made use of a series of derivatisation and degradation studies in conjunction with NMR spectroscopy and chiral gas chromatography to assign the relative and absolute configuration of the stereogenic centers in the FB_1 (**7**) backbone. The results were consistent with that arrived at by Oikawa *et al.*[11] for the C(10)-C(17) fragment of the AAL toxins.

The relative configuration of the C(1)-C(5) fragment of FB_1 (**5**) were independently arrived at by the research groups of ApSimon[12,13] who based their research on the NMR analysis of 2,3-cyclic carbamates while Poch *et al.*[14] focused on oxazoline derivatives. The relative configuration of the C(10)-C(16) fragment of FB_1 (**5**) was also arrived at by ApSimon *et al.*[13] who based their research on the NMR analysis of a 10,14-cyclic ether derivative.

Earlier stereochemical studies on the eight stereogenic centres of the FB_1 (**5**) backbone were conducted by Boer.[15] The strategy was based on the formation of conformational rigid 1,3-oxazolidinone, 1,3-dioxane and 1,3-dioxolane derivatives involving the 2-amino and 3-hydroxy groups and determining the relative stereochemistry using a method developed by Rychnovsky[16] by ^{13}C NMR analysis.

NMR analysis, Mosher ester analysis, chiral and achiral shift reagents, degradation, derivatisation, circular dichroism (CD), exciton chirality method and chiral chromatography are thus all methods employed in elucidating the absolute configuration at each of the 8 stereogenic centers contained in mentioned fumonisins. [8,10,13,14,17]

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[8] Harmange, J.-C., Boyle, C.B., Kishi, Y. *Tetrahedron Lett*., **1994**, *35*, 6819.
[9] Boyle, C.D.; Harmange, J.-C.; Kishi, Y. *J. Am. Chem. Soc.* **1994**, *116*, 4995.
[10] Hoye, T.R.; Jimenez, J.I.; Shier, W.T. *J. Am. Chem. Soc.* **1994**, *116*, 9409.
[11] Oikawa, H.; Matsuda, I.; Ichihara, A.; Kohmoto, K. *Tetrahedron Lett.* **1994**, *35*, 1223.
[12] ApSimon, J.W.; Blackwell, B.A.; Edwards, O.E.; Fruchier, A.; Miller, J.D.; Savard, M.; Young, J.C. *Pure Appl. Chem.,* **1994**,*66*, 2315.
[13] ApSimon, J.W.; Blackwell, B.A.; Edwards, O.E.; Fruchier, A. *Tetrahedron Lett.***1994**, *35*, 7703.
[14] Poch, G.K.; Powell, R.G.; Plattner, R.D., Weisleder, D. *Tetrahedron Lett.,* **1994**, *35*, 7707.
[15] Boer, A. Stereochemical Studies on the Fumonisins, Metabolites of *Fusarium moniliforme*, M.Sc. Dissertation, University of Pretoria, 1992.
[16] Rychnovsky, S.D.; Skalitzky, D.J. *Tetrahedron Lett.*, **1990**, *31*, 945.
[17] Hartl, M.; Humpf, H.-U. *Tetrahedron Asymmetry* **1998**, *9*, 1549.

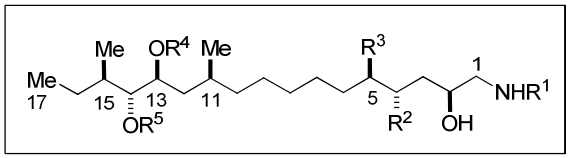
The absolute stereochemistry of the tricarballylic acid (TCA) side-chain has been assigned as *R* by independent research groups.[18,19,20]

1.3 RELATED COMPOUNDS

1.3.1 AAL-toxins

The AAL-toxins (TA_1, TA_2 and TB_1) (**Figure 7**) were isolated in 1981 from *A. alternata* f. sp. *lycopersici*. These phytotoxins cause stem canker in susceptible tomato cultivars[21,22,23] and also act as inhibitors of sphingolipid biosynthesis.[24] The fumonisins and the AAL-toxins, structurally related compounds, are believed to follow similar biosynthetic pathways.

The AAL-toxins are C_{17} hydroxylated alkylamines with one propane-1,2,3-tricarboxylic acid side-chain per molecule. The stereochemistry of the AAL-toxin TA backbone and the TCA-side-chain have been determined to be the same as that of FB_1.[9,11]



AAL Toxin	R^1	R^2	R^3	R^4	R^5
TA_1 (**20**)	H	OH	OH	TCA	H
TA_2 (**21**)	H	OH	OH	H	TCA
TB_1 (**22**)	H	OH	H	TCA	H
TB_2 (**23**)	H	OH	H	H	TCA
TC_1 (**24**)	H	H	H	TCA	H
TC_2 (**25**)	H	H	H	H	TCA
TD_1 (**26**)	Ac	OH	H	TCA	H
TD_2 (**27**)	Ac	OH	H	H	TCA
TE_1 (**28**)	Ac	H	H	TCA	H
TE_2 (**29**)	Ac	H	H	H	TCA

Figure 7: The AAL-toxins.

[18] Boyle, C.D.; Kishi, Y. *Tetrahedron Lett.* **1995**, *36*, 5695.
[19] Edwards, O.E.; Blackwell, B.A.; Driega, A.B.; Bensimon, C.; ApSimon, J.W. *Tetrahedron Lett.* **1999**, *40*, 4515.
[20] Hartl, M.; Humpf, H.-U. *J. Org. Chem.* **2001**, *66*, 3678.
[21] Bottini, A.T.; Bowen, J.R.; Gilchrist, D.G. *Tetrahedron Lett.* **1981**, *22*, 2723.
[22] Bottini, A.T.; Gilchrist, D.G. *Tetrahedron Lett.* **1981**, *22*, 2719.
[23] Abbas, H.K.; Tanaka, T.; Duke, S.O. *J. Phytopathol.* **1995**, *143*, 329.
[24] Merrill, A.H., Jr.; Wang, E.; Gilchrist, D.G.; Riley, R.T. *Adv. Lipid Res.* **1993**, *26*, 215.

A number of marine natural products[25,26,27,28,29] and the sphingofungins[30,31,32] have also been found to be structurally related in that the terminal 2-amino-3-hydroxy motif contained in the C_{20} backbone of the fumonisins, is present in either the *syn* or the *anti* relative configuration.

1.3.2 Biosynthesis of the Fumonisins

The structural similarity between the sphingoid bases and the fumonisins pointed researchers in the direction of a common biosynthetic pathway.[33] Sphingolipid biosynthesis has been investigated in both yeast[34] and mammals.[35] Sphingosine is produced by the condensation of palmitoyl CoA and serine (**Figure 2**).[36]

Labeling experiments have shown that the 18 carbon atoms represented by C(3)–C(20) of the C_{20} backbone of the fumonisins are derived from acetate. The C(1) and C(2) atoms and the C(2) amino group are derived from (2*S*)-alanine. The C_{18}-polyketide ester chain then condenses with (2*S*)-alanine to form the C_{20}-backbone. The methyl groups at C(12) and C(16) are derived from (2*S*)-methionine and the hydroxyls at C(5), C(10), C(14) and C(15) from molecular oxygen.[37,38,39] The tricarballylic acid units are formed by condensation between α-ketoglutarate from the citric acid cycle,[37] and another acetylCoA unit prior to esterification to the backbone.

ApSimon[36] incorporated specifically enriched ^{13}C-labelled acetates into fungal cultures as a preliminary step in preparing radiolabelled compounds. The studies showed that ^{13}C-labelled acetate is incorporated into the fumonisins in such a way as to suggest a polyketide

[25] Gulavita, N.K.; Scheuer, P.J. *J. Org. Chem.* **1989**, *54*, 366.
[26] Jiménez, C.; Crews, P. *J. Nat. Prod.* **1990**, *53,* 978.
[27] Gelderblom, W.C.A.; Sewram, V.; Shephard, S.D.; Snyman, P.W.; Tenza, K.; van der Westhuizen, L.; Vleggaar, R. *J. Agric. Food Chem.* **2007**, *55*, 4388.
[28] Sata, N.U.; Fusetani, N. *Tetrahedron Lett.* **2000**, *41*, 489.
[29] Garrido, L.; Zubía, E.; Ortega, M.J.; Naranjo, S.; Salvá, J. *Tetrahedron* **2001**, *57*, 4579.
[30] VanMiddlesworth, F.; Giacobbe, R.A.; Lopez, M.; Garrity, G.; Bland, J.A.; Bartizal, K.; Fromtling, R.A.; Polishook, J.; Zweerink, M.; Edison, A.M.; Rozdilsky, W.; Wilson, K.E.; Monaghan, R.L. *J. Antibiot.* **1992**, *45*, 861.
[31] VanMiddlesworth, F.; Dufresne, C.; Wincott, F.F.; Mosley, R.P.; Wilson, K.F. *Tetrahedron Lett.* **1992**, 33, 297.
[32] Horn, W.S.; Smith, T.L.; Bills, G.F.; Raghoobar, S.L.; Helms, G.L.; Kurts, M.B.; Marrinan, J.A.; Frommer, B.R.; Thornton, R.A.; Mandara, S.M. *J. Antibiot.* **1992**, *45*, 1692
[33] Plattner, R.D.; Shackelford, D.D. *Mycopathol.* **1992**, *117*, 17.
[34] Snell, E.E.; Dimari, S.J.; Brady, R.N. *Chem. Phys. Lipids* **1970**, *5*, 116.
[35] Kanfer, J.N. *Chem. Phys. Lipids* **1970**, *5*, 159.
[36] ApSimon, J.W. *Environmental Health Perspectives* **2001,** 109, 245.
[37] Blackwell, B.A.; Edwards, O.E.; Fruchier, A.; ApSimon, J.W.; Miller, J.D. *Adv. Exp. Med. Biol.* **1996**, *392*, 75.
[38] Branham, B.E.; Plattner, R.D. *Mycopathol.* **1993**, *124*, 99.
[39] Caldas, E.D.; Sadilkova, K.; Ward, B.L.; Jones, A.D.; Winter, C.K.; Gilchrist, D.G. *J. Agric. Food Chem.* **1998**, *46*, 4734.

biosynthetic pathway. The labeling pattern observed after incorporation of ^{13}C-labelled acetate (**Figure 8**) was consistent with the head-to-tail pattern expected for the condensation of acetyl CoA units in both fatty acid and polyketide biosynthesis. The polyketide pathway is favoured due to the fact that considerable enrichment occurs after initial lipid synthesis in cultures that are not carbon compromised.

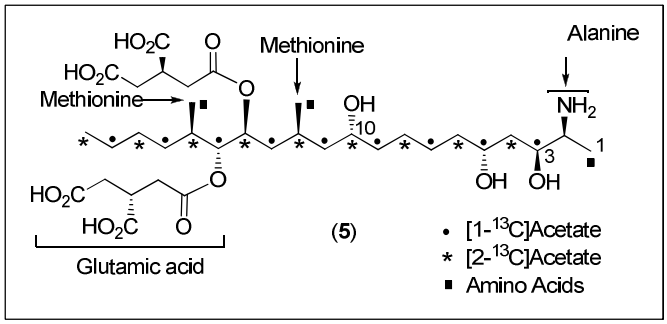


Figure 8: Incorporation of ^{13}C labeled precursors in fumonisin B_1 (**5**).[36]

Later biosynthetic research cloned and characterized the gene cluster (*FUM1-FUM3, FUM6-FUM8, FUM10, FUM11* and *FUM13-FUM19)* responsible for the biosynthesis of the fumonisins in *F. verticillioides.*[40,41,42] Researchers were able to construct a biosynthetic pathway from the data obtained by studying these genes and their associated enzymes using genetic and biochemical approaches.[43,44]

The biosynthesis starts with the Fum1p polyketide synthase enzyme catalyzing the formation of a dimethylated and saturated C_{18} polyketide chain. Subsequent carbon-carbon bond formation between (2*S*)-alanine and the C_{18}-polyketide chain, which is still bound to the Fum1p polyketide synthase enzyme, is facilitated by the pyridoxalphosphate-dependent aminoacyl transferase enzyme Fum8p. The formation of the carbon-carbon bond occurs with concomitant loss of CO_2 to form a 3-keto intermediate with retention of configuration at C(2). Ketoreductase Fum13p stereospecifically reduces the carbonyl group of the 3-keto intermediate at the *ReS* face to yield the hydroxyl group at C(3) with the *S* configuration. This reduction results in the *syn*-2,3 amino alcohol motif.[45,46,47] The *anti-2,3* amino alcohol motif present in sphinganine is formed in a similar fashion by reduction of 3-ketosphinganine.[45]

[40] Proctor, R.H.; Desjardins, A.E.; Plattner, R.D.; Hoh, T.M. *Fungal Genet. Biol.* **1999,** *27*, 100.
[41] Proctor, R.H.; Brown, D.W.; Plattner, R.D.; Desjardins, A.E. *Fungal Genet. Biol.* **2003,** *38*, 237.
[42] Seo, J.A.; Proctor, R.H.; Plattner, R.D. *Fungal Genet. Biol.* **2001,** *34*, 155.
[43] Bojja, R.S.; Cerny, R.L.; Proctor, R.H.; Du, L. *J. Agric. Food Chem.* **2004**, *52*, 2855.
[44] Butchko, R.A.; Plattner, R.D.; Proctor, R.H. *J. Agric. Food Chem.* **2006,** *54,* 9398.
[45] Butchko, R.A.; Plattner, R.D.; Proctor, R.H. *J. Agric. Food Chem.* **2003,** *51,* 3000.
[46] Ding, Y.; Bojja, R.S.; Du, L. *J. Agric. Food Chem.* **2004,** *52,* 1931.
[47] Yi, H.; Bojja, R.S.; Fu, J.; Du, L. *J. Agric. Food Chem.* **2005,** *53,* 5456.

Reduction of the 3-keto group has been proved to be one of the early steps in the biosynthesis of the fumonisins, while the incorporation of the C(5) hydroxyl group by a 2-ketoglutarate-dependent dioxygenase Fum3p has been proven to be the last step.[44,45,47]

Enzymatic pathways, which differ early in the biosynthetic pathway, are thought to be responsible for the different FB analogues, since *F. verticillioides* fails to convert added FB_2 or FB_3 into FB_1.[48]

1.4 FUMONISIN B_1 (5)

Fumonisin B_1 (FB_1) (**5**), $C_{34}H_{59}NO_{15}$, is the diester of propane-1,2,3-tricarboxylic acid and 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane. The compound is a hygroscopic white powder, which must be stored in a desiccator to prevent the formation of a hard, glassy material which is very slow to dissolve. It is soluble in water and polar organic solvents such as acetonitrile and methanol. It is unstable in methanol due to the possible formation of fumonisin methyl esters, but stable in acetonitrile-water (1:1), light and food processing temperatures.[33]

1.5 PREVIOUS SYNTHETIC APPROACHES

Shi *et al.*[49] were the first to report the enantioselective total synthesis of FB_2 (**6**) in 1997. The synthetic route was designed by disconnecting FB_2 (**6**) (see **Figure 3**) in a retrosynthetic analysis into three building blocks: the C_{11} C(10)-C(20) unit (**30**), the C_9 C(1)-C(9) unit (**31**), and the tricarballylic acid (TCA) moiety (**32**) (**Figure 9**). These units were synthesized separately and linked in a convergent synthesis to give FB_2.

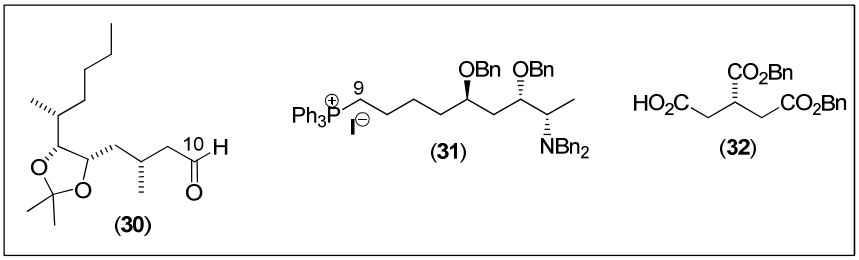


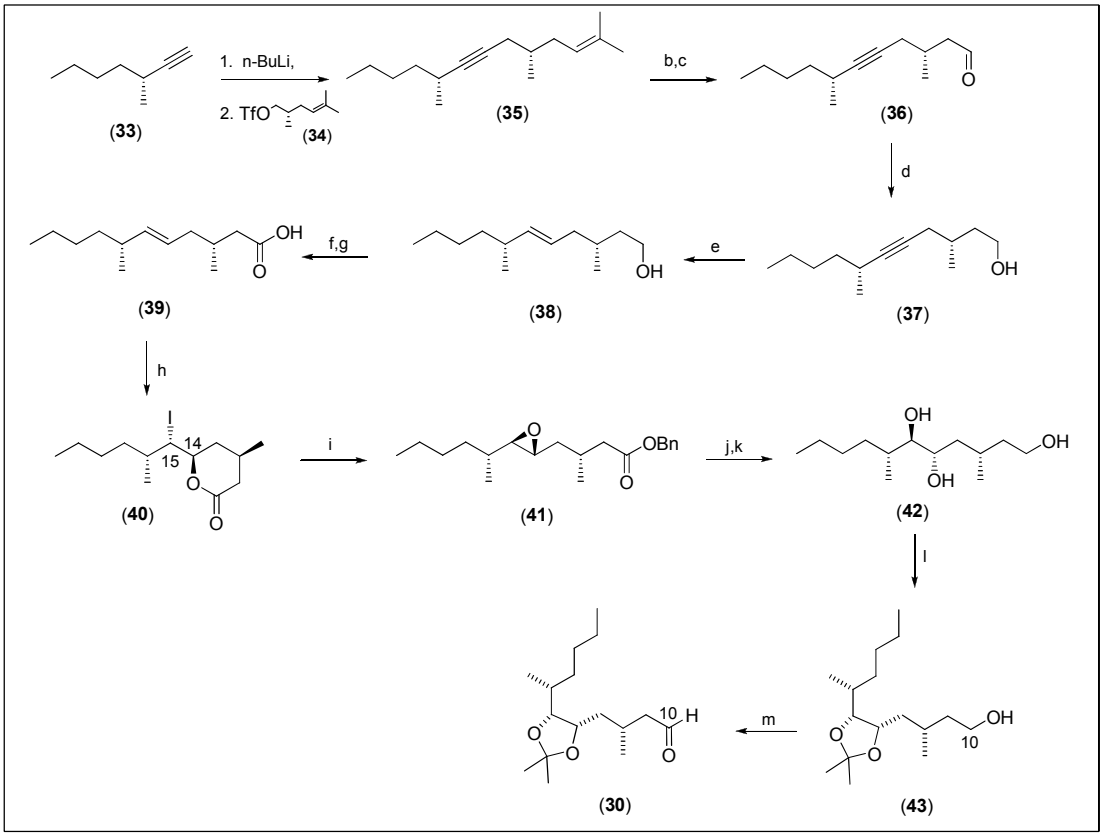
Figure 9: Building blocks for the synthesis of FB_2.[49]

The backbone was formed by linking the chiral aldehyde (**30**) and the ylide derived from the triphenylphosphonium iodide salt (**31**) in a Wittig reaction. Esterification of the TCA segments (**32**) was subsequently done at C(14) and C(15) of the backbone. The benzyl group was chosen as common protecting group of the C(3) and C(5) hydroxyl, the C(2) amino

[48] Powell, R.G.; Plattner, R.D. in *Alkaloids: Chemical and Biological Perspectives,* Pelletier, S.W. (Ed.); Pergamon Press, Oxford, **1995**, pp 247-278.
[49] Shi Y.; Peng F.P.; Kishi Y. *J. Org. Chem.* **1997**, *62*, 5666.

functionality and the TCA carboxylic acid groups in order to allow for a one-step deprotection protocol at the end of the synthesis.

The synthesis of the aldehyde (**30**), the C(10)-C(20) unit, was done by coupling of the chiral terminal alkyne (**33**) and the *O*-triflate (**34**) to give the alkyne (**35**) in 70% yield. Both the alkyne (**33**) and the triflate (**34**) have the methyl bearing stereocenters of the required configuration in place. The alkyne (**33**) was synthesized by Myers[50] from (2*R*)-2-methyl-1-hexanol making use of a Swern oxidation[51] followed by a Corey-Fuchs protocol.[52] The triflate (**34**) was synthesized from 1-bromo-3-methyl-2-butene with (*R,R*)-(−)-pseudoephedrine amide as chiral auxiliary using a asymmetric alkylation method also developed by Myers.[50]



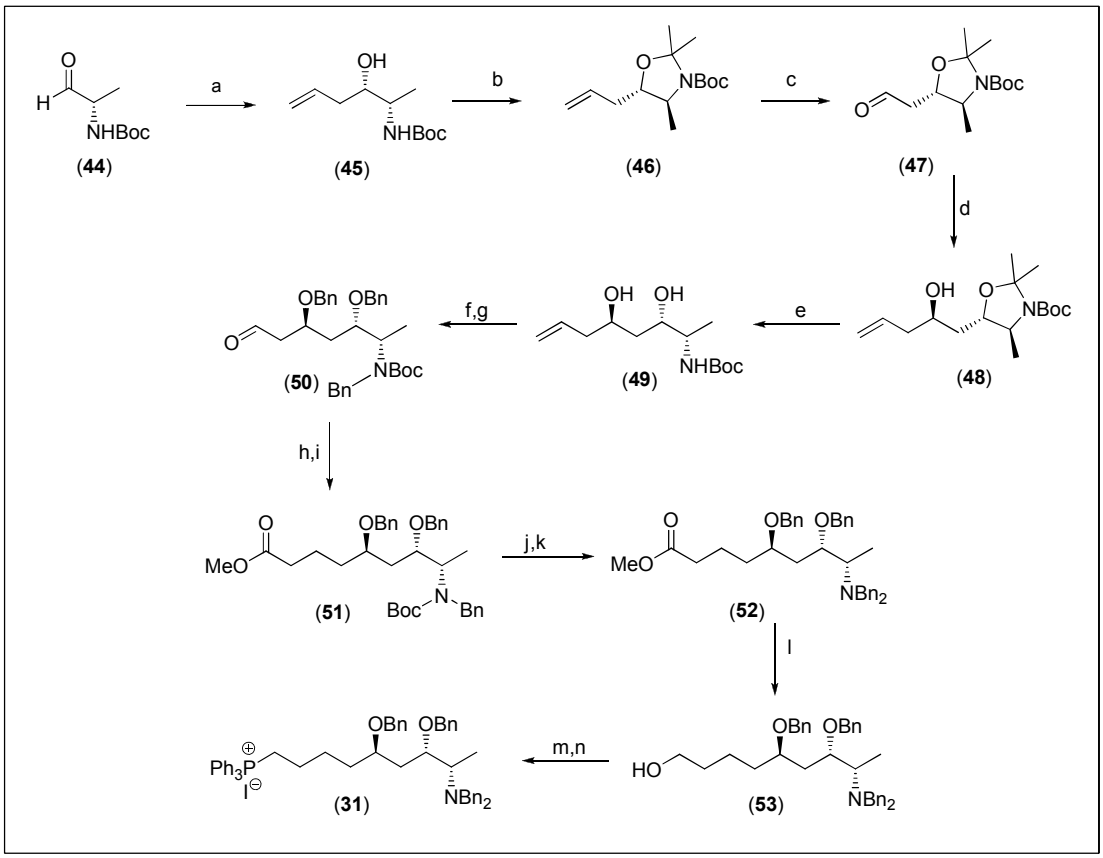
Scheme 1: Synthesis of the C(10)-C(20) unit (**30**) by Shi *et al.*[49]

Reagents: a. 1. *n*BuLi, 2. Triflate (**34**) (70%); b. $K_2OsO_4.2H_2O$ (cat.); c. $Pb(OAc)_4$; d. $NaBH_4$; e. Na/liq. NH_3; f. $(COCl)_2$, DMSO, Et_3N; g. $NaClO_2$ (77%, 6 steps); h. I_2, CH_3CN (84%); i. BnONa; j. H_2, Pd-C, *p*-TsOH (cat); k. LAH; l. *p*-TsOH (cat.), acetone; m. $(COCl)_2$, DMSO, Et_3N (79%, 5 steps).

Alkyne (**35**) was converted to the *trans*-alkene acid (**39**) in 6 steps with an overall yield of 77% (**Scheme 1**). A brief overview of the steps is as follows: Dihydroxylation of the double bond in **35** using site-selective osmylation gave a 1,2-diol which was cleaved by lead

[50] Myers, A.G.; Yang, B.H.; Chen, H.; Gleason, J.L. *J. Am. Chem. Soc.* **1994**, *116*, 9361.
[51] Mancuso, A.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.
[52] Corey, E.J.; Fuchs, P.L. *Tetrahedron Lett.* **1972**, *13*, 3769.

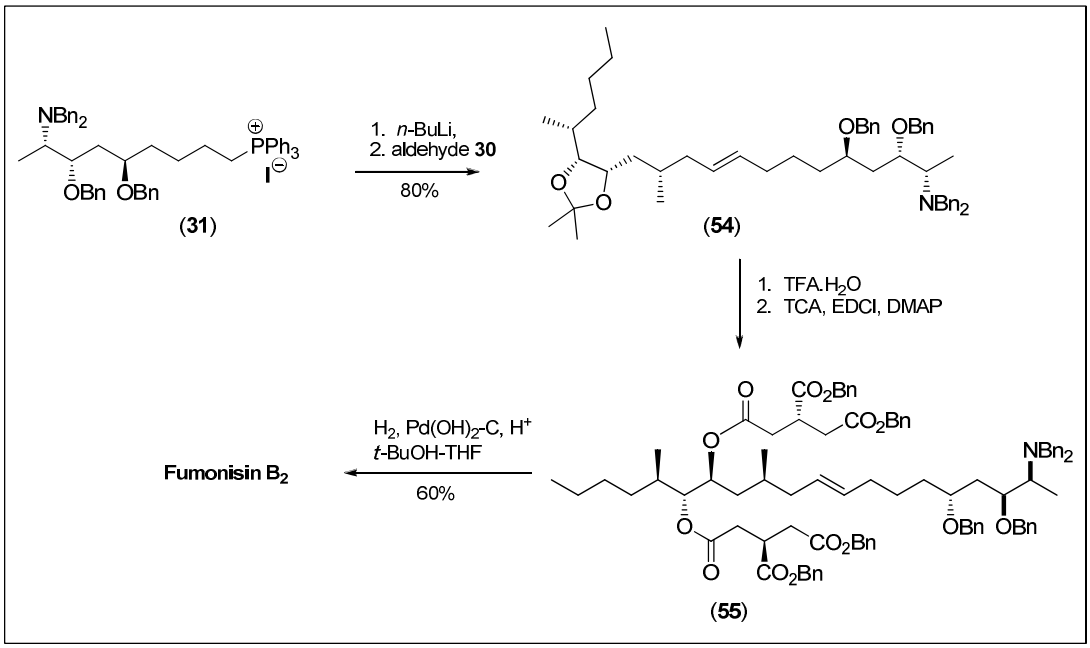
tetraacetate ($Pb(OAc)_4$) to give the aldehyde (**36**), which was subsequently reduced to the alcohol (**37**) using $NaBH_4$. The alkyne functionality was reduced to the *trans* alkene (**38**) with Na in liquid NH_3. The primary alcohol was then converted to the acid by a two-step sequence in which the primary alcohol was oxidized to the aldehyde using Swern oxidation, followed by oxidation to the acid (**39**) using sodium chlorite ($NaClO_2$). Treatment of the unsaturated carboxylic acid with iodine resulted in an iodolactonization reaction which produced the iodolactone (**40**) with the *anti* configuration for the C(14) and C(15) centres with a diastereomeric ratio >20:1 in 84% yield. The reaction of the iodolactone (**40**) with NaOBn resulted in the opening of the lactone ring to afford a benzyl ester and an alkoxide which in an intramolecular S_N2 reaction then formed an epoxide by displacement of iodide to give (**41**). Hydrogenolysis of the benzyl ester with Pd-C as catalyst uncovered an acid which under acid catalysis caused the opening of the epoxide ring and the formation once again of a lactone ring. Reduction of this lactone to the triol (**42**) left the C(14) and C(15) hydroxyl groups to be protected as an acetonide and the terminal alcohol to be oxidized to the required aldehyde (**30**) by Swern oxidation with the last 5 steps in an overall yield of 79%.[49]



Scheme 2: Synthesis of the C(1)-C(9) unit (**31**) by Shi *et al.*[49]

Reagents: a. (–)-Ipc_2B-allyl (75%); b. *p*-TsOH (cat.), acetone; c. O_3, Me_2S, d. (–)-Ipc_2B-allyl (65%, 3 steps); e. *p*-TsOH(cat.), MeOH; f. NaH, BnBr; g. O_3, Me_2S; h. $(MeO)_2P(O)CH_2COOMe$, NaH; i. H_2, Lindlar catalyst (70%, 5 steps); j. TFA, DCM; k. BnBr, K_2CO_3; l. DIBALH; m. I_2, PPh_3, imidazole; n. PPh_3, CH_3CN, reflux (75%, 5 steps).

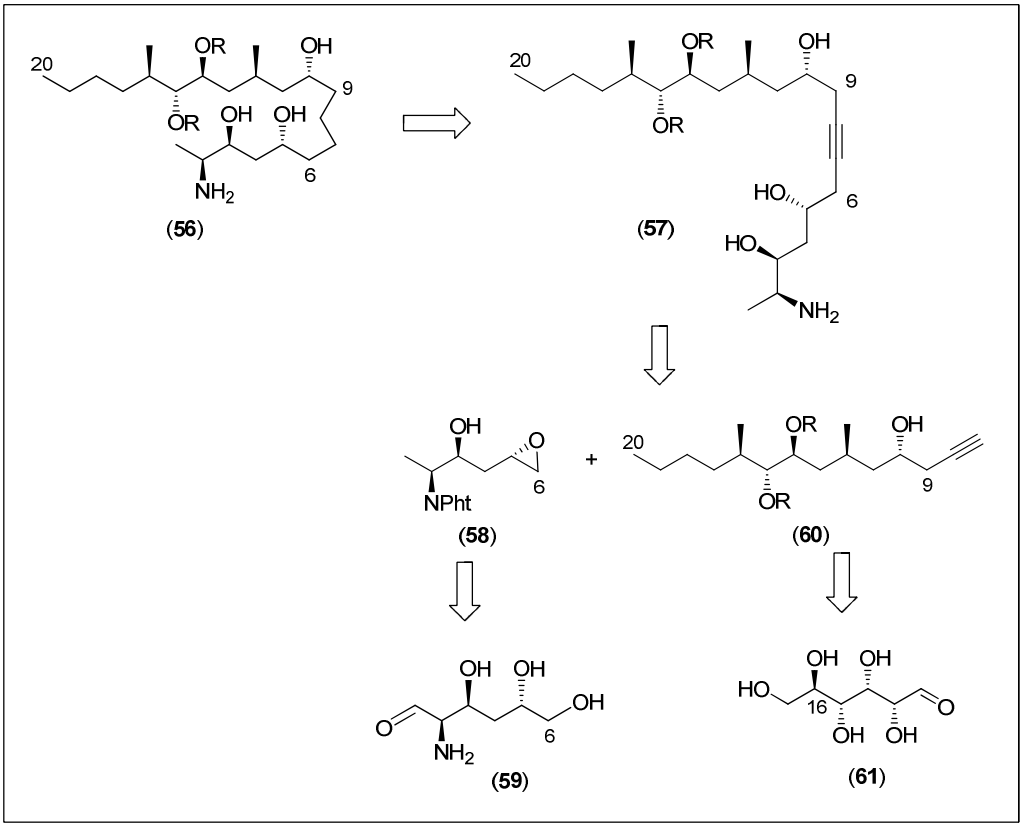
Synthesis of the triphenylphosphonium iodide segment (**31**) is shown in **Scheme 2**. The C_3 2*S* amino aldehyde chain (**44**), derived from (2*S*)-alanine, was extended with three carbon atoms utilizing (−)-*B*-allyldiisopinocampheylborane as chiral ligand in a Brown allylation reaction to give the *syn* amino alcohol (**45**) (94% de) in 75% yield which was protected as the acetonide (**46**). An aldehyde (**47**) was then formed by reductive ozonolysis of the terminal alkene group with dimethyl sulfide work-up. A second Brown allylation reaction extended the carbon chain to give the *anti* alcohol (82% de) (**48**). These 3 reactions proceeded with an overall yield of 65%. Acid-catalysed (*p*-TsOH) removal of the acetonide protecting group gave a diol which was then converted to the (*O*,*O*,*N*)-benzyl derivative (**49**). Reductive ozonolysis of the terminal double bond in **49** yielded the aldehyde (**50**). Horner-Wadsworth-Emmons olefination of this aldehyde followed by hydrogenation resulted in a two-carbon chain extension to give the methyl ester (**51**) in a 70% yield from **48**. The Boc protecting group was removed by treatment with TFA, followed by benzyl protection of the amine to give **52**. DIBALH reduction of the methyl ester then gave the primary alcohol (**53**) which was transformed into an alkyl iodide using Ph_3P-I_2 reagent. Treatment of this iodide with PPh_3 gave the C(1)-C(9) phosphonium salt (**31**) in a 75% yield from **51**.



Scheme 3: Linkage of the left- (**30**), right-hand (**31**) and TCA (**32**) units.[49]

The left (**30**) and right side (**31**) units were coupled under Wittig reaction conditions (**Scheme 3**) to give the alkene (**54**) in 80% yield. Acid-catalysed deprotection of the acetonide provided a diol, which was acylated with the TCA segments (**32**) (**Figure 10**) at C(14) and C(15) in a 90% yield from **54**. Catalytic hydrogenation of the C(9)-C(10) double bond and hydrogenolysis of the benzyl protecting groups of (**55**) gave synthetic FB_2 (**8**) in an overall yield of 1.7% over 44 steps.

Synthetic studies by Gurjar *et al.*[53] in 1998 worked towards the synthesis of a hexaacetate derivative of the FB_1 backbone. Retrosynthetic analysis of the backbone resulted in two target compounds whose synthesis were embarked on using D-glucose (**61**) and D-glucosamine (**59**) as ultimate starting materials (**Scheme 4**).

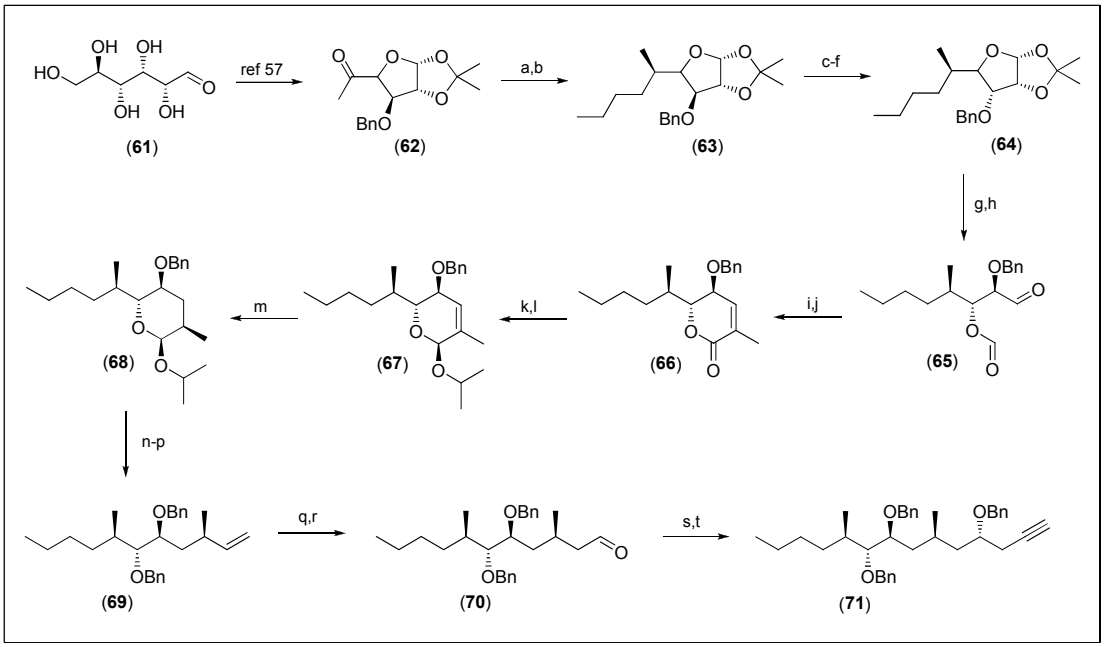


Scheme 4: Retrosynthetic analysis of FB_1 (**5**) by Gurjar *et al.*[53]

The alkyne (**60**) was synthesized from D-glucose (**61**) in 25 steps (**Scheme 5**). D-Glucose (**61**) was transformed into the 5-ulose derivative (**62**) by a stereoselective photochemical cyclization sequence described by Araki *et al.*[54] A 4-carbon chain extension was accomplished using the appropriate C_4 phosphonium ylid ($C_4H_9P^+Ph_3Br^-$). Subsequent hydrogenation yielded a diastereomeric mixture of **63** which was separated chromatographically to yield 50% of the wanted isomer. The next four steps were dedicated to an oxidation-reduction sequence in order to invert the stereocentre at C(3) to give **64**. Acetal hydrolysis followed by $NaIO_4$ oxidation yielded aldehyde (**65**). Aldehyde (**65**) was then converted to the lactone (**66**) by firstly subjecting it to Horner-Wadsworth-Emmons conditions with the appropriate phosphonate, ethyl 2-(dimethoxyphosphino)propanoate ($(MeO)_2P(O)CH(CH_3)COOEt$), where after lactone (**66**) was formed using K_2CO_3-MeOH to promote cyclization.

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

The next step included converting the lactone (**66**) to a lactol and protecting the lactol as a isopropanol glycoside with the anomeric carbon in the *S* configuration by treating the lactol with isopropanol and CSA to give **67**. Having the anomeric carbon in this configuration set the molecule up for stereoselective hydrogenation to exclusively yield **68**. Acid hydrolysis of the *O*-glycosidic bond contained in **68** followed by a one carbon Wittig olefination with the appropriate C_1 phosponium ylid ($CH_3P^+Ph_3I^-$) gave **69** after the C(15) hydroxyl was protected as the benzyl ether. Alkene (**69**) was then subjected to a hydroboration-oxidation procedure to yield a primary alcohol which was subsequently converted to aldehyde (**70**) *via* Swern oxidation. The addition of the propargylic group, contained in 3-bromoprop-1-yne, to the terminal aldehyde of **70** yielded a diastereomeric mixture of alcohols that was separated by chromatography where after it was converted to the *O*-benzyl derivative to yield **71**.



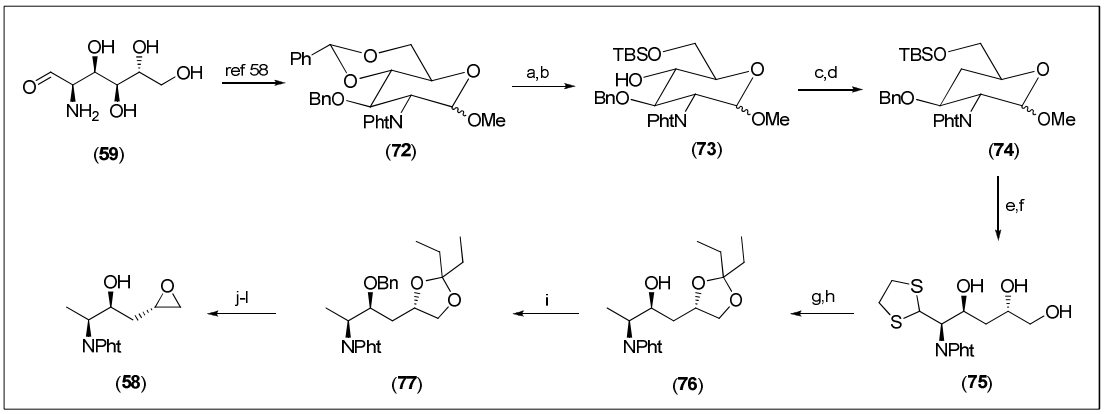
Scheme 5: Synthesis of alkyne (**71**) from D-glucose.[53]

Reagents: a. $C_4H_9P^+Ph_3Br^-$, *n*-BuLi (86%); b. Pd-C, H_2 (50%); c. Ca, NH_3(l); d. IBX, DMSO; e. $NaBH_4$, MeOH; f. BnBr, NaH (72%, 4 steps); g. CH_3COOH, H_2SO_4 (70%); h. $NaIO_4$, MeOH-H_2O (4:1) (77%); i. $(MeO)_2P(O)CH(CH_3)COOEt$, NaH; j. K_2CO_3, MeOH (88%); k. DIBALH, PhMe; l. ^{i}Pr-OH, CSA (98%); m. Rh-Al_2O_3, H_2; n. CH_3COOH, H_2SO_4(70%); o. $CH_3P^+Ph_3I^-$, *n*-BuLi (78%); p. BnBr, NaH; q. 9-BBN, THF, NaOH, H_2O_2; r. $(COCl)_2$, DMSO (82%, 3 steps); s. C_3H_3Br, Zn dust, NH_4Cl (70%); t. NaH, BnBr (85%).

The epoxide (**58**) was synthesized from D-glucosamine (**59**) in 16 steps (**Scheme 6**). D-Glucosamine hydrochloride was converted into *N*-phtalimido methyl glycoside (**72**).[55] The benzylidene group was subsequently cleaved and the selective protection of O(6) as the TBS-ether yielded **73**. Removal of the hydroxyl group at C(4) by Barton radical deoxygenation yielded **74**. Ring opening was effected by treating **74** with 1,2-ethanedithiol and $BF_3.OEt_2$ to

[55] Shigehiro, H. *Carbohydr. Res,* **1971**, *6*, 229.

give the triol (**75**). Reductive desulfurisation with Raney-nickel and subsequent protection of the 1,2-diol gave **76**. The free hydroxyl group at C(4) was then protected as a benzyl ether giving **77**. Acetal hydrolysis, followed by tosylation provided a compound which was neatly set up to form the epoxide (**58**) after treatment of the hydroxyl group with base.



Scheme 6: Synthesis of the epoxide unit (**58**).[53]

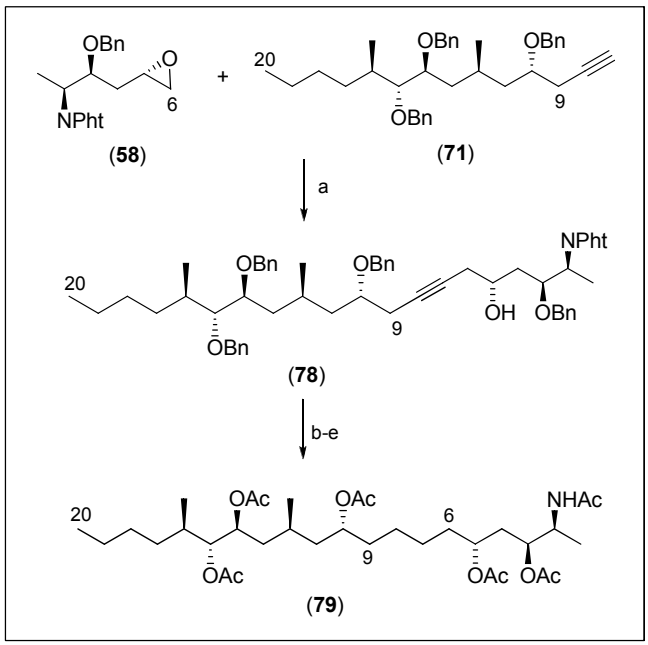
Reagents: a. 60% CH_3COOH; b. TBS-Cℓ, imidazole (81%); c. NaH, CS_2, MeI; d. Bu_3SnH, AIBN(cat), PhMe (80%); e. MeOH, *p*-TsOH; f. $BF_3.Et_2O$, $HSCH_2CH_2SH$ (73%); g. Ra-Ni (85%); h. $(CH_3CH_2)_2CO$, CSA (92%); i. NaH, BnBr; j. MeOH, *p*-TsOH; k. TsCl, Py; l. NaH (52%).

The C-C coupling of the alkyne (**71**) and epoxide (**58**) segments was accomplished using *n*-BuLi-$BF_3.OEt_2$ in order to abstract the acetylenic proton and so mediate nucleophilic attack on the C(6) carbon of the epoxide to give compound (**78**) (**Scheme 7**). Removal of the phthalimido group was followed by acetylation at both the amino group and the C(5) carbon. Hydrogenolysis using $Pd(OH)_2$-C as catalyst afforded, after acetylation, the hexaacetate FB_1-derivative (**79**).

The use of carbohydrates as chiral starting materials installed most of the stereocenters of the FB_1 backbone, although the stereocentre at C(14) had to be inverted. This 45 step synthesis by Gurjar *et al.*[53] yielded the hexaacetate derivative (**79**) of the FB_1-backbone in an overall yield of 0.9%.

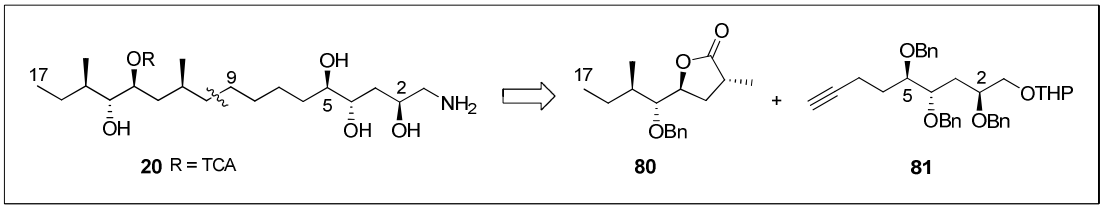
The C(9)-C(10) bond of the fumonisin related AAL-toxin TA_1 was identified in the retrosynthetic analysis by Oikawa *et al.*[56] in 1999 as a strategic bond (**Scheme 8**). Disconnection of this bond yielded two synthetic target molecules (**80**) and (**81**).

[56] Oikawa, H.; Yamawaki, D.; Kagawa, T.; Ichihara, A. *Tetrahedron Lett.* **1999**, *40*, 6621.



Scheme 7: Synthesis of the C_{20} backbone hexaacetate derivative (**79**) of FB_1.[53]

Reagents: a. *n*-BuLi, $BF_3.Et_2O$ (73%); b. $MeNH_2$-MeOH; c. Ac_2O, Et_3N (85%); d. $Pd(OH)_2$, H_2, MeOH; e. Ac_2O, Et_3N (95%).



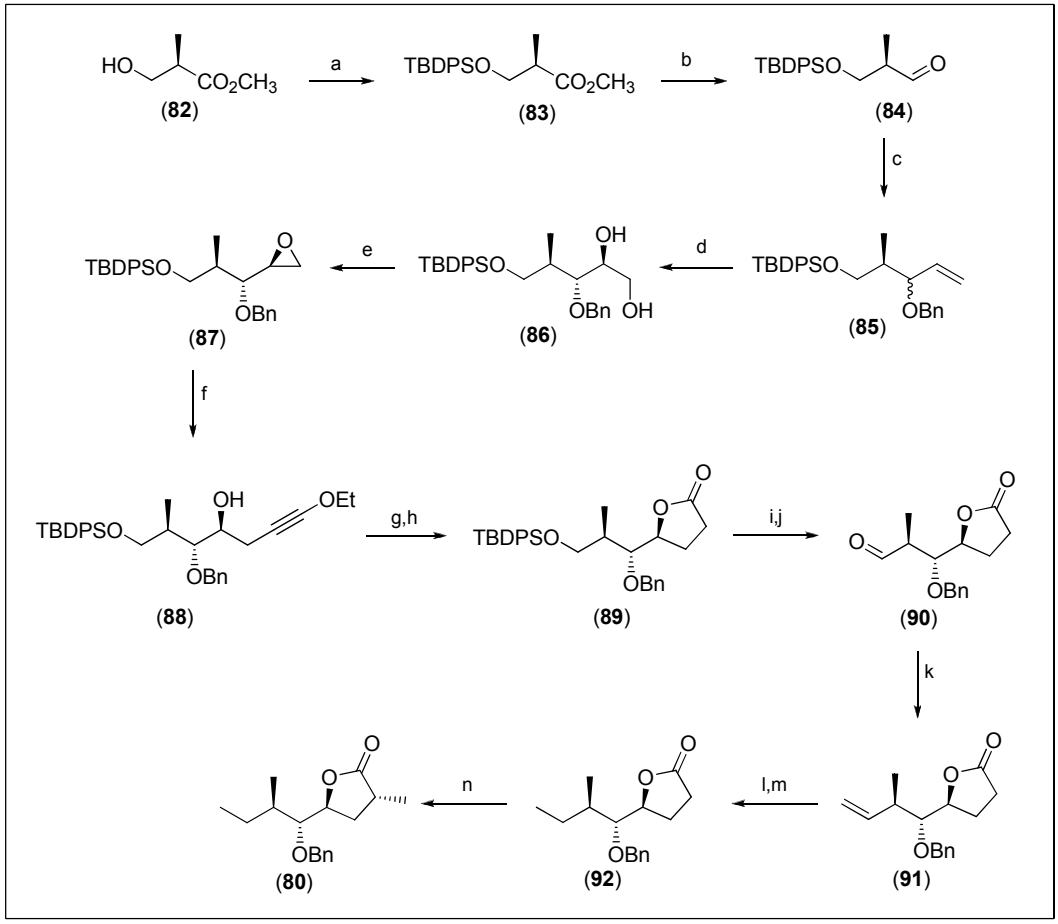
Scheme 8: Synthetic approach towards AAL-toxin TA_1 (**20**) by Oikawa *et al.*[56]

The lactone (**80**) was synthesized from (2*R*)-methyl-3-hydroxy-2-methylpropionate (**82**) in 14 steps (**Scheme 9**). The methyl ester (**82**) was protected as the silyl ether (**83**) where after the ester group was reduced to the aldehyde (**84**) using DIBALH. A Grignard reaction was then used to install the C(14) hydroxyl functionality and yield the diastereomeric allylic alcohol, which was then protected as the benzyl ether (**85**). After separation of the wanted benzyl protected diastereomer by column chromatography, treatment with OsO_4 and NMO stereoselectively delivered diol (**86**). This compound (**86**) was then transformed to the epoxide (**87**). Treatment of the epoxide with lithium acetylide in the presence of $BF_3.Et_2O$ gave alkyne (**88**) which was converted to the lactone (**89**) using a set of hydrolytic conditions. The lactone moiety served as protection of the C(13) hydroxy group as well as to allow for the stereoselective methylation to the bottom face of the ring later in the synthesis.

Removal of the silyl protecting group gave a primary alcohol intermediate which was converted to the aldehyde (**90**) by Swern oxidation and transformed to the olefin (**91**) using a

C_1 Wittig reaction. Hydrogenation of the alkene also resulted in removal of the benzyl groups which after re-benzylation produced **92** which was then stereoselectively methylated to give unit (**80**).

The alkyne (**81**) was prepared from the 4-pentyn-1-ol protected as the silyl ether in 7 steps, but is not be discussed here as its structure is quite far removed from the right hand side unit of the fumonisins.

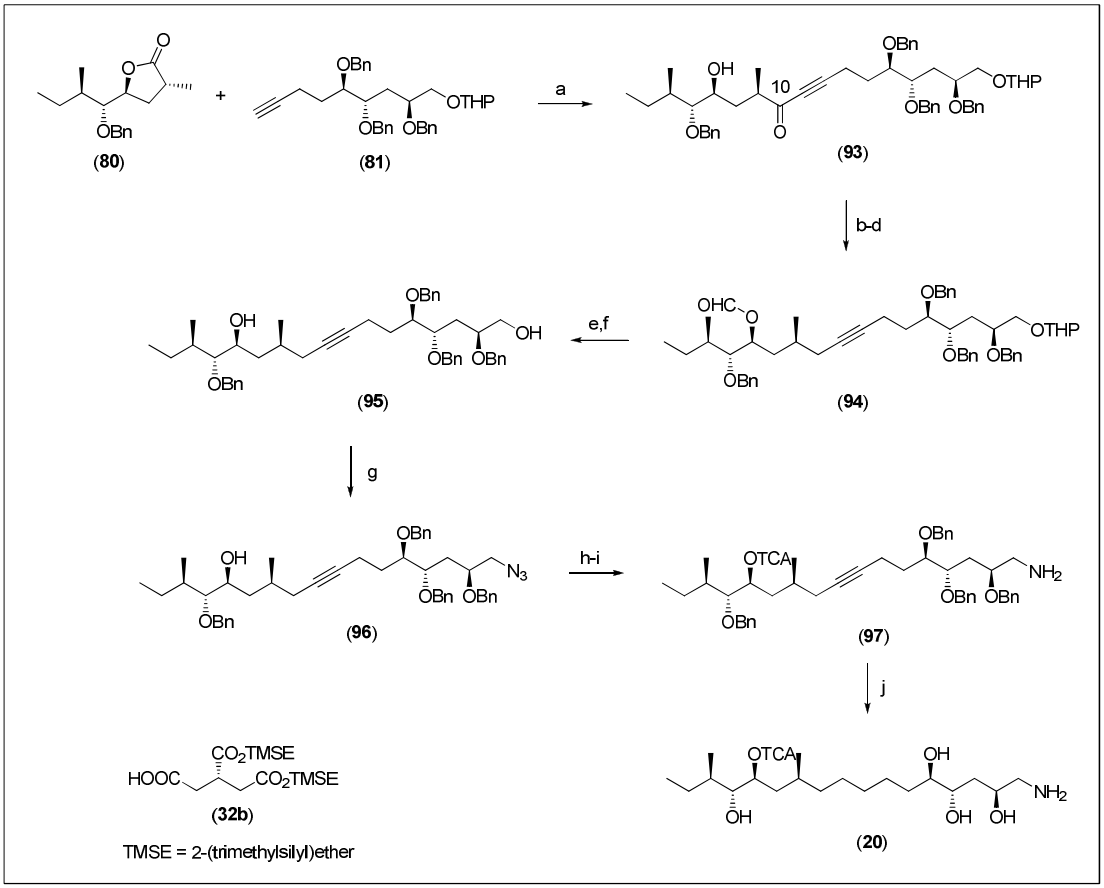


Scheme 9: Synthesis of target molecule **80.**[56]

Reagents: a. TBDPS-Cℓ, Imidazole, DMF (100%); b. DIBALH; c. (i) $CH_2CHMgBr$ (77%); (ii) NaH, BnBr, n-Bu_4NI (91%, chromatographic separation); d. OsO_4, NMO (91%); e. (i) $MeC(OMe)_3$, PPTS (cat); (ii) AcBr; (iii) K_2CO_3, MeOH (77%); f. ethyl ethynyl ether, *n*-BuLi, $BF_3.Et_2O$; g. $HgCl_2$, EtOH; h. K_2CO_3, MeOH, 3M HCl (59%, 3 steps); i.TBAF (80%); j. Swern oxidation; k. Ph_3PCH_3Br, *n*-BuLi (19%, 2 steps); l. Pd-C, H_2; m. $CCl_3C(=NH)OBn$, TfOH (57%, 2 steps); n. LiHMDS, CH_3I (68%).

Linkage of the lactone (**80**) with the acetylene (**81**) (**Scheme 10**) was accomplished by treating the lactone (**80**) with the anion obtained by removal of the acetylenic proton of **81** to yield **93**. The carbonyl functionality at C(10) in **93** was reduced to a methylene group following a three-step procedure, which included Luche reduction, formylation of the formed alcohol and palladium-catalyzed deoxygenation to produce **94**. Deprotection of the acyl and

terminal THP protected hydroxyl groups afforded a diol (**95**) which could be converted regioselectively to the azide (**96**) under Mitsunobu conditions. Acylation of C(13) was done with the tricarballyic acid moiety (**32b**), which after deprotection with TBAF provided the diacid (**97**). Hydrogenolysis of the benzyl groups and reduction of the azide and alkyne functionalities gave the final AAL-toxin TA_1 (**20**).



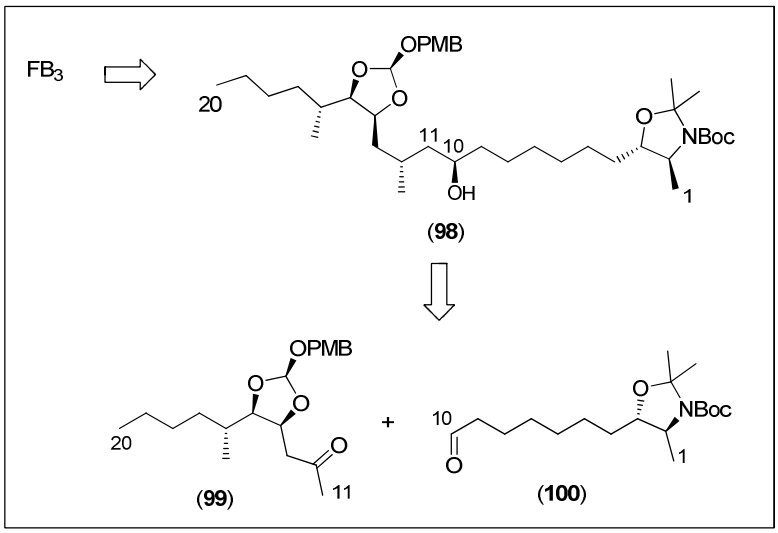
Scheme 10: Linkage of **80** and **81** leading towards AAL-toxin TA_1 (**20**).[56]

Reagents: a. *n*-BuLi (75%); b. $NaBH_4$, $CeCl_3$, MeOH (85%); c. Ac_2O, HCO_2H, Py (97%); d. $Pd(OAc)_2$, *n*-Bu_3P (84%); e. LAH; f. PPTS (89%, 2 steps); g. HN_3, Ph_3P, DEAD (69%); h. 2,4-$NO_2C_6H_4COCl$, **32b**, DMAP (71%); i. TBAF; j. Pd-C, H_2, *t*-BuOH-THF-1M HCl (76%).

Several of the AAL-toxin analogues were subsequently synthesized and proved useful for further SAR investigations.

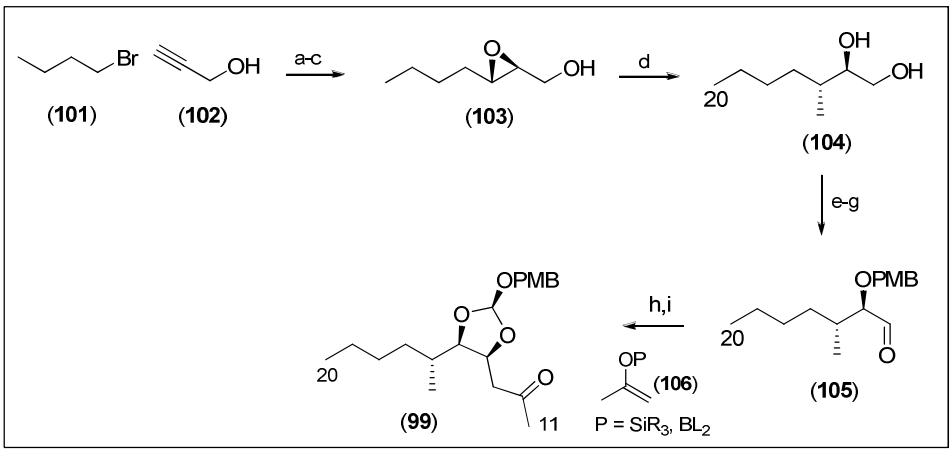
A synthetic approach by Issa[57] utilizes a convergent strategy aimed to improve the protocol of Shi *et al.*[49] Using retrosynthetic analysis, FB_3 (**7**) (**Scheme 11**) was divided into three units. Disconnection of the C(10)-C(11) bond provided a left (**99**) and a right side (**100**) unit as synthetic intermediates. The third unit is the TCA derivative.

[57] Issa, F. Studies towards the total synthesis of the fumonisin B natural products, Ph.D. Thesis, University of Sydney, 2003.



Scheme 11: Retrosynthetic analysis of FB_3 (**7**) by Issa.[57]

The methyl ketone segment (**99**) was realized in 9 steps from hept-2-yn-1-ol using propargyl alcohol (**101**) and *n*-butyl bromide (**102**) as starting materials (**Scheme 12**). The propargyl alcohol was deprotonated and subsequently alkylated at the triple bond by the addition of *n*-butyl bromide (**102**). Reduction of the formed alkyne was afforded using LAH, which yielded a *trans* allylic alcohol intermediate. Sharpless-Katsuki asymmetric epoxidation of the allylic alcohol using (*R*,*R*)-(+)-diisopropyl tartrate yielded **103**. Treatment of the (*S*,*S*)-epoxide (**103**) afforded the 14,15-diol (**104**).



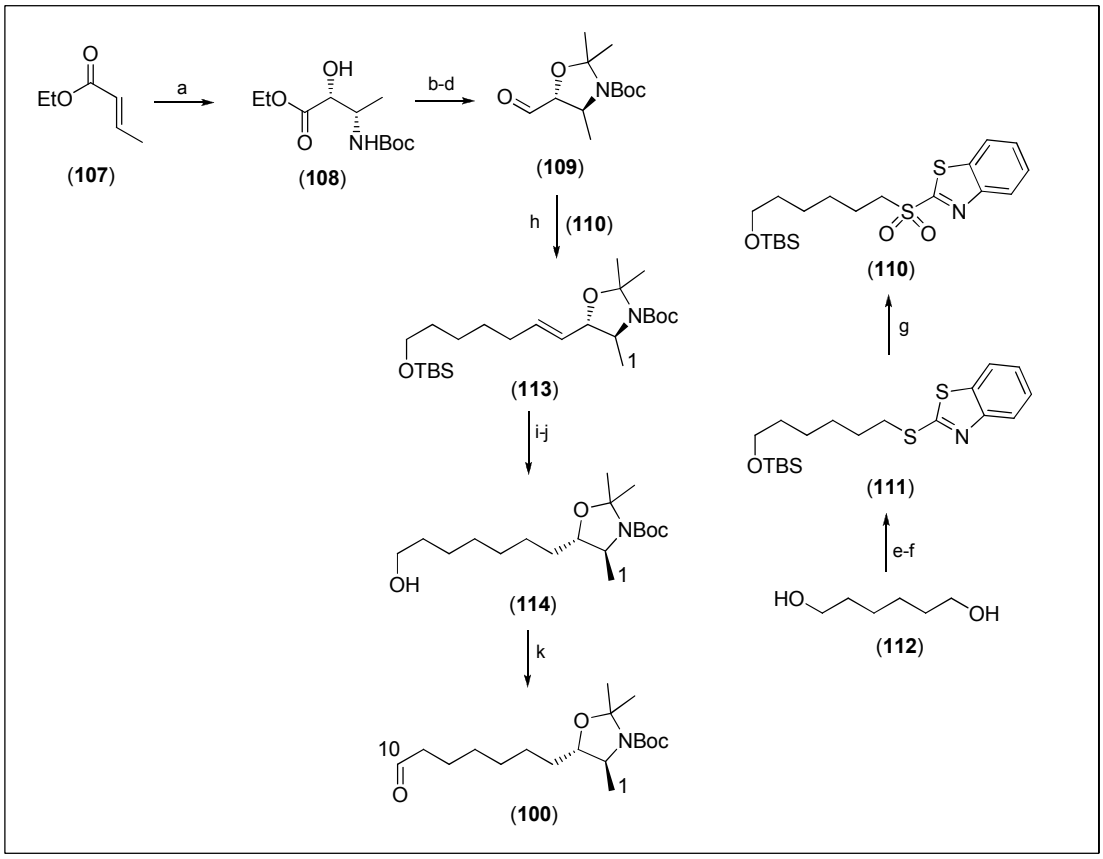
Scheme 12: Synthesis of the C(11)-C(20) unit of FB_3 (**7**) by Issa.[57]

Reagents: a. Li/NH_3, $FeCl_3$ (74%); b. LAH (80%); c. $Ti(O^iPr)_4$, L-(+)-DIPT, TBHP (86%); d. Me_3Al (71%); e. PMBDMA, TsOH (cat) (92%); f. DIBALH (83%); g. $(COCl)_2$, $(CH_3)_2SO$, Et_3N (91%); h. **106**, *R*-Proline (40 mol%), DMSO, acetone (33%); i. DDQ, DCM (88%).

The diol (**104**) was then converted to the *p*-methoxybenzylidene acetal. Regioselective reductive cleavage of the acetal with DIBALH afforded the PMB ether of the secondary hydroxyl group. The primary hydroxyl group was then converted to the aldehyde (**105**) under

Swern reaction conditions. An aldol reaction between **106** and aldehyde (**105**) yielded a product which when subjected to oxidative anhydrous conditions yielded the PMB-acetal (**99**).[57]

Synthesis of the aldehyde (**100**) was realized in 9 steps from 1,6-hexanediol (**112**) and ethyl crotonate (**107**) as starting materials (**Scheme 13**). The ethyl crotonate (**107**) was subjected to a carbamate-based Sharpless aminohydroxylation in order to install the 2,3-*syn* amino alcohol of **108**. The amino and the hydroxyl groups were then protected as the *N*,*O*-acetonide using 2,2-dimethoxypropane and the ester group was transformed by a reduction-oxidation sequence to form aldehyde (**109**).



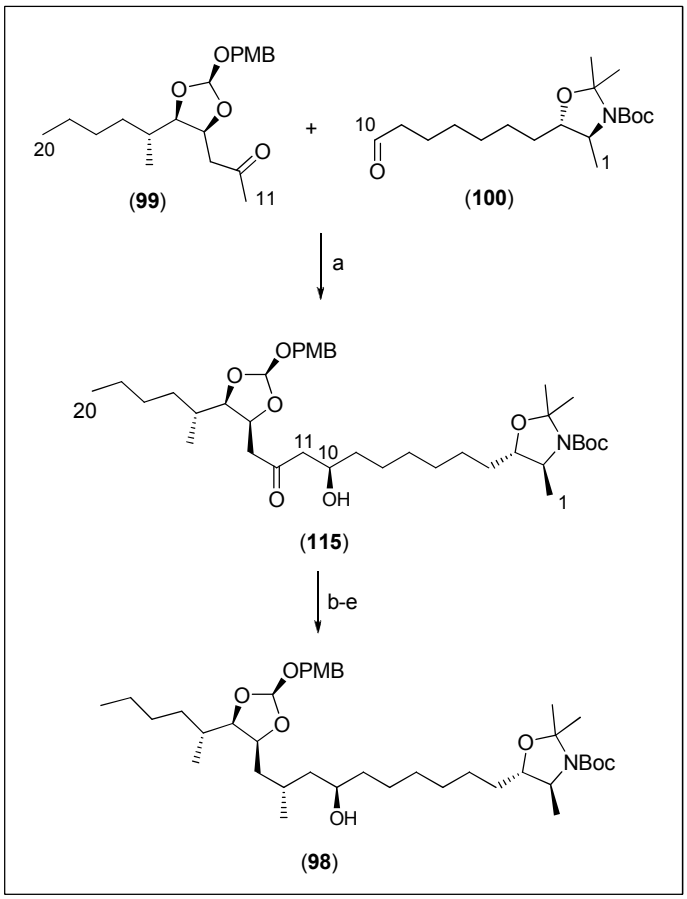
Scheme 13: Synthesis of the right side unit (**100**) of FB_3.[57]

Reagents: a. *t*-Butyl carbamate, NaOH, ^{t}BuOCl, $(DHQ)_2PHAL$, $K_2OsO_4.2H_2O$ (21%); b. 2,2-DMP, *p*-TsOH (74%); c. LAH (93%); d. Swern oxidation; e. DMF, imidazole, TBSCl (66%); f. BTSH, PPh_3, DIAD (90%); g. $(NH_4)_6Mo_7O_{24}.4H_2O$ (94%); h. LDA, THF (22%); i. Pt-C, H_2, MeOH (100%); j. TBAF; k. Swern oxidation (92%).

The sulfone intermediate (**110**) was introduced at this stage. It was synthesized from 1,6-hexanediol (**112**). The diol was converted to a mono-OTBS protected product which was subsequently transformed to a thioether (**111**). The thioether (**111**) was oxidized to the

corresponding sulfone (**110**). The two segments (**109**) and (**110**) were combined to yield an *E/Z* mixture of alkene (**113**). Hydrogenation followed by deprotection using TBAF yielded **114**. Oxidation to aldehyde (**100**) was accomplished under Swern reaction conditions to yield the synthetic target of this leg of the synthesis.[57]

Synthesis of the sulfone unit (**110**) could also be circumvented using instead Wittig methodology. In that methodology the 1,6-hexanediol (**112**) was converted to the phosphonium bromide salt. Reaction between the phosphonium bromide salt and aldehyde (**109**) produced the Wittig olefination product, the alkene (**113**), which after subsequent hydrogenation and Swern oxidation yielded **100**.[57]



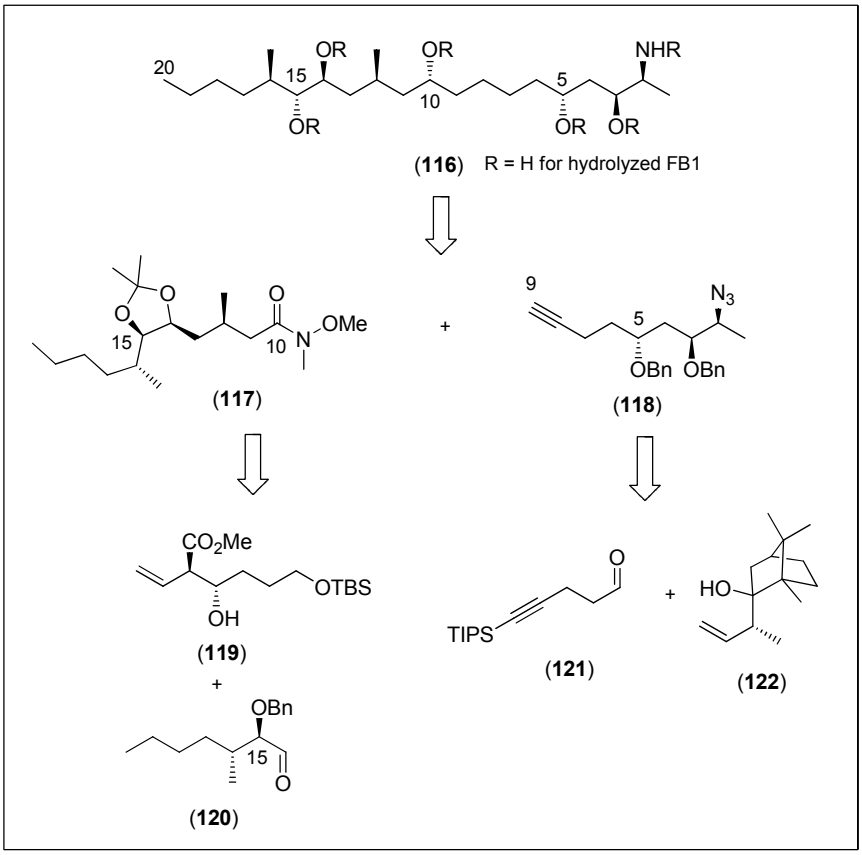
Scheme 14: Linking of the left (**99**) and right side (**100**) units of FB_3 (**7**).[57]

Reagents: a. *c*-Hexyl$_2$BCl, Et_2O, Et_3N (65%); b. TESCl, pyridine, DMAP (90%); c. Cp_2TiMe_2, THF (74%); d. TBAF, THF (71%); e. Pt-C, H_2 (84%).

The two segments (**99** and **100**) were linked using boron-mediated aldol coupling to stereoselectively generate the C(10) hydroxyl functionality to provide the aldol product (**115**) (**Scheme 14**). Protection of the hydroxyl groups in the aldol adduct (**115**) as silyl ethers yielded an intermediate, which after Petasis olefination, yielded the homoallylic alcohol

which was neatly set up for stereoselective construction of the 1,3-methyl-hydroxyl motif *via* directed hydrogenation. Hydrogenation, however yielded a 50:50 mixture of the 10,12-*syn* and 10,12-*anti* products (**98**) which were separated by HPLC. The final product could be obtained by global deprotection and installation of the TCA side chains, although this was not reported.[57]

Pereira *et al.*[58] was the first to accomplish the total synthesis of FB_1 in 2009. A convergent approached was used with final linkage between C(9)-C(10) (**Scheme 15**). Retrosynthetic analyses identified the disconnection of the C(9)-C(10) bond to give the alkyne (**118**) and amide (**117**) as synthetic intermediates (**Scheme 15**). The Weinreb amide (**117**) was formed from the methyl ester in order to be able to couple the two segments using the amide and the acetylide functionalities.

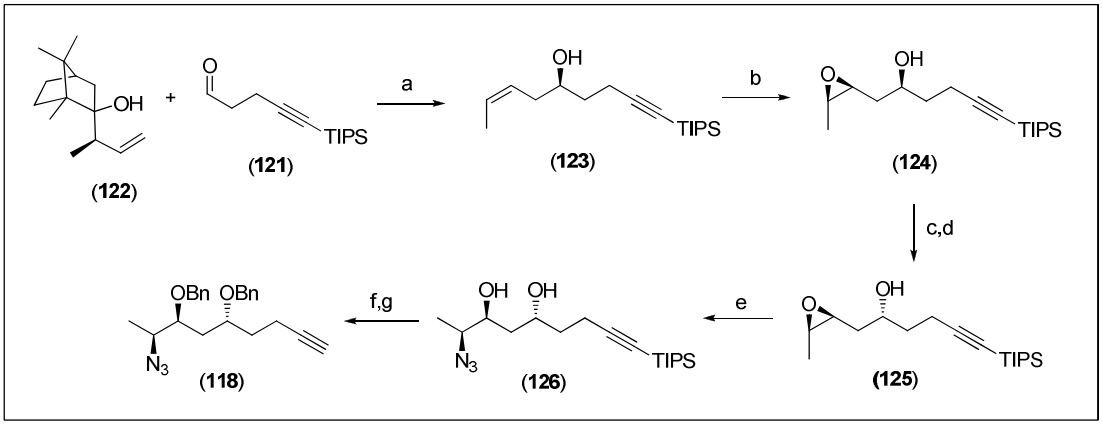


Scheme 15: Total synthesis of FB_1 (**5**) by Pereira *et al.*[58]

The alkyne (**118**) was synthesized from the camphor-derived reagent (**122**) and the alkenyl aldehyde (**121**) (**Scheme 16**). This first reaction yielded only the *cis* allylic alcohol (**123**) with complete stereochemical control at the C(5) hydroxyl functionality to yield the product in 90% ee. Epoxidation of the *cis* double bond gave the epoxide (**124**). Mitsunobu inversion of

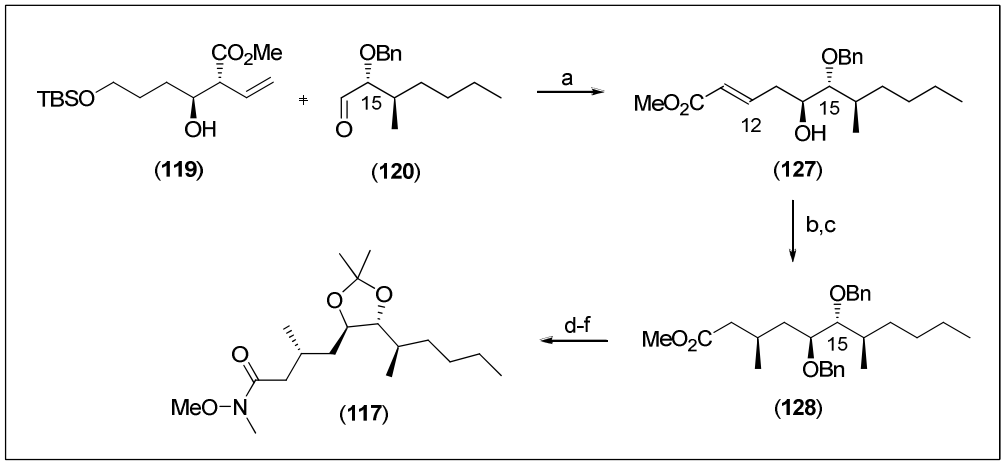
[58] Pereira L.C.; Chen Y; McDonald, F.E. *J. Am. Chem. Soc*. **2009**, *131*, 6066.

the C(5) alcohol yielded compound (**124**) with the correct stereochemistry. The epoxide contained in the molecule, allowed the introduction of the azide functionality at C(2), with concomitant opening of the epoxide, giving the azido diol (**126**). Deprotection of the terminal alkyne was followed by protection of the two alcohol functionalities as benzyl ethers to yield the complete segment (**118**) in 7 steps.[58]



Scheme 16: Synthesis of the C(1)-C(9) unit by Pereira *et al.*[58]

Reagents: a. CSA (cat., (70%); b. $VO(acac)_2$ (cat.), *t*-BOOH (73%); c. Ph_3P, DIAD, HOAc (87%); d. K_2CO_3, MeOH (85%); e. $Ti(O^iPr)_2(N_3)_2$ (64%); f. Bu_4NF (84%); g. NaH, BnBr (85%).

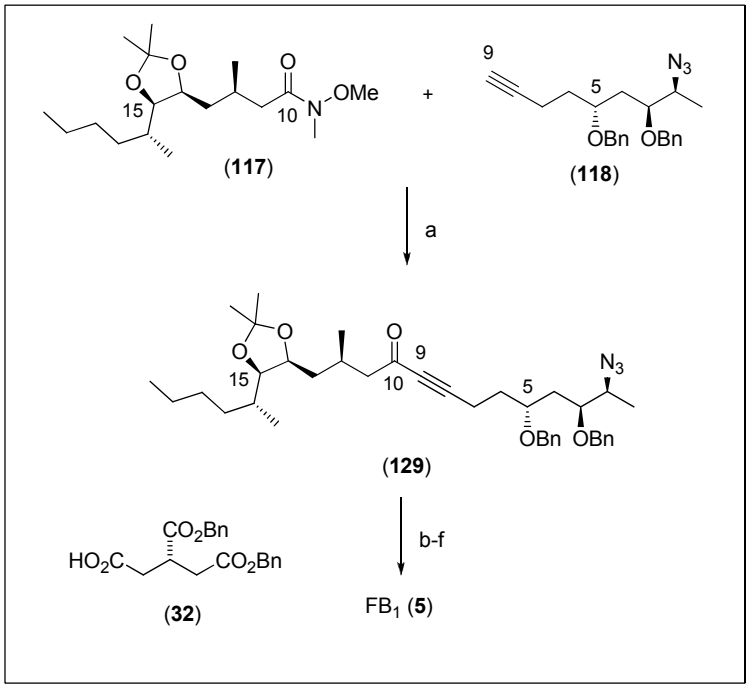


Scheme 17: Synthesis of the C(10)-C(20) unit by Pereira *et al.*[58]

Reagents: a. TMSOTf (61%); b. 2-Benzyloxy-*N*-methylpyridinum triflate, MgO (66%); c. MeMgBr, CuI (cat), (*R*)-Tol-BINAP, MTBE (69%); d. BCl_3 (88%); e. $Me_2C(OMe)_2$, TsOH (cat.) (80%); f. Me(MeO)NH.HCl, *i*-PrMgCl (83%).

The C(10)-C(20) segment combined the aldol product (**119**) with the chiral non-racemic aldehyde (**120**) using a stereospecific allylic transfer reaction as key step (**Scheme 17**). This provided the intermediate (**127**) with the stereocentre at C(14) in place and with the *trans* double bond neatly set up for catalytic asymmetric conjugate addition of the methyl group needed at C(12). The addition reaction yielded **128.** The benzyl protecting groups were

removed at this stage and replaced with the acetonide protecting group. At this point the Weinreb amide was installed to yield the amide segment (**117**) in 7 steps.[58]



Scheme 18: Formation of the C_{20} backbone of FB_1 (**5**).[58]

Reagents: a. *n*-BuLi (76%); b. (*R*)-CBS, catecholborane (75%); c. NaH, BnBr, THF (86%); d. Amberlite-120 H^+ (80%); e. EDCl, DMAP, (**32**) (71%); f. $Pd(OH)_2$-C, H_2 (94%).

After coupling of **117** and **118** the formed alkynyl ketone (**129**) was reduced enantio-selectively to install the correct stereochemistry at C(10) using (*R*)-CBS (**Scheme 18**). Benzyl ether formation at C(10), followed by acid-catalyzed removal of the acetonide protecting group yielded a 14,15-diol. The C(14) and C(15) hydroxyls were then esterified with **32**. Global hydrogenation of the esters, the benzylic ethers, the alkyne and the azide provided FB_1 (**5**).[58]

1.6 PRESENT APPROACH TOWARDS THE C(10)-C(20) SEGMENT OF FB_1

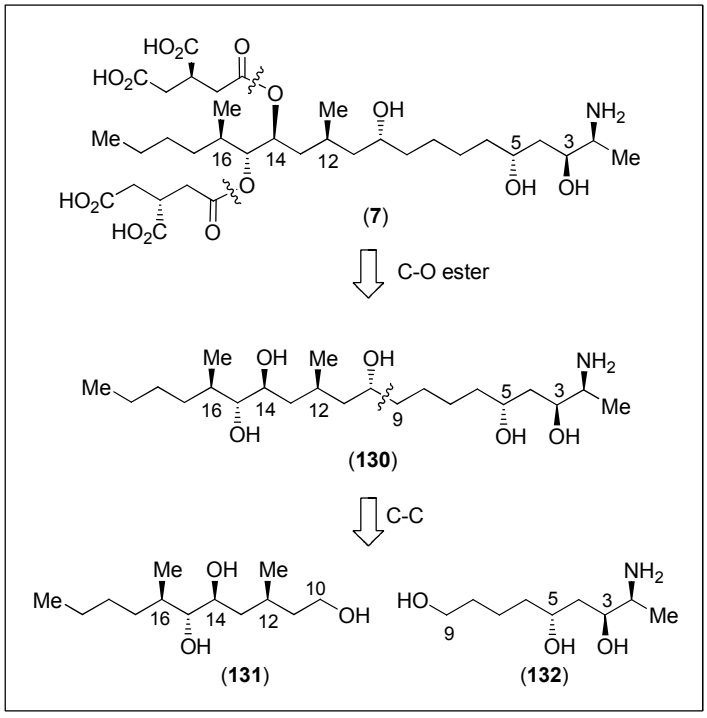
Upon examination of the previous synthetic approaches, it can be concluded that a shorter, simpler approach towards the synthetic targets is needed. The aim of this project is then also the development of a shorter and more efficient synthetic route towards the synthesis of the C(10)-C(20) segment of FB_1 (**5**). Key reactions identified to realize transformation of the starting material leading to the identified target include kinetic enzymatic resolution, a [2,3]-Wittig rearrangement and the use of Sharpless asymmetric epoxidation methodology and is discussed in detail in the following chapters.

RETROSYNTHETIC ANALYSIS *CHAPTER* 2

"Retrosynthetic (or *antithetic)* analysis is a problem-solving technique for transforming the structure of a *synthetic target* (TGT) molecule to a sequence of progressively simpler structures along a pathway which ultimately leads to simple or commercially available starting materials for a chemical synthesis." E.J. Corey.[1]

More about the beautiful art that is retrosynthesis will not be covered in this text, as it can simply not be better worded than in the article of E.J. Corey.[1]

2.1 RETROSYNTHETIC ANALYSIS OF THE FUMONISIN B_1 BACKBONE



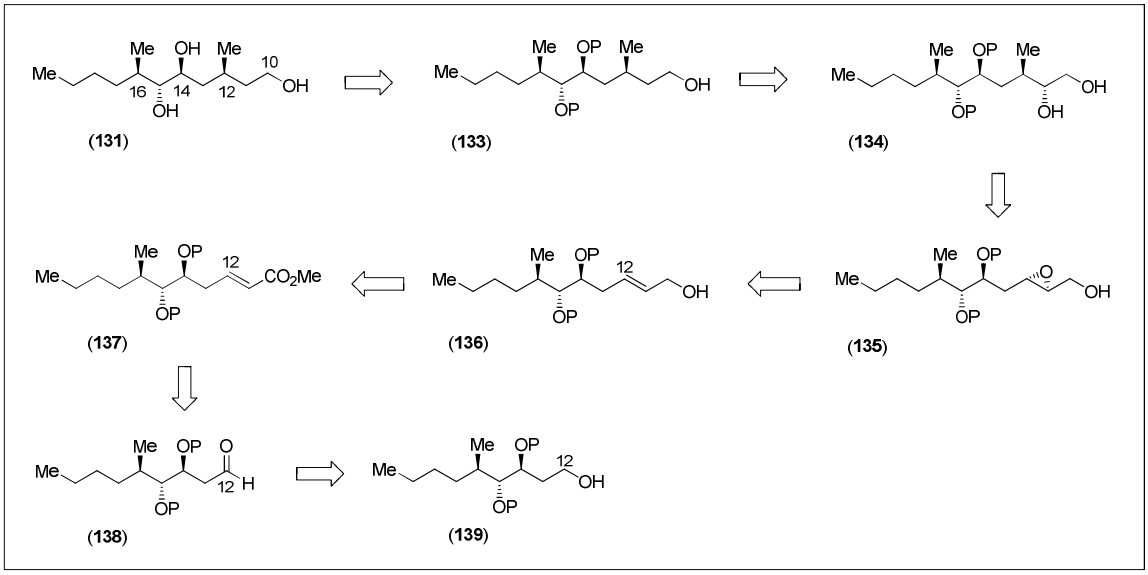
Scheme 19: Retrosynthetic analysis of the C_{20} backbone of fumonisin B_1 (**5**).

Retrosynthetic analysis of fumonisin B_1 (**5**) points firstly to the disconnection of the C-O ester bond of the TCA-ester moieties to yield the C_{20} amino alcohol backbone (**130**) (**Scheme 19**). In the synthetic direction this can be achieved by esterification of the tricarballylic ester

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

moieties and the C(14) and C(15) hydroxyl groups of the C_{20} backbone. With a convergent synthesis and the stereochemical demands in the synthesis direction in mind, the next step is to identify a strategic bond to be disconnected. The disconnection of the C(9)–C(10) bond or the C(10)–C(11) bond of the C_{20} backbone (**130**) results in two compounds of approximately equal length. Disconnection of the C(9)–C(10) bond was preferred. Synthesis of this bond in the forward direction can easily be envisioned from the reaction between the aldehyde formed from (**131**) and an appropriate nucleophilic derivative of **132**. Retrosynthesis of the C_{11} compound (**131)** is easily envisioned as outlined the following section.

2.2 THE C_{10} –C_{20} FRAGMENT

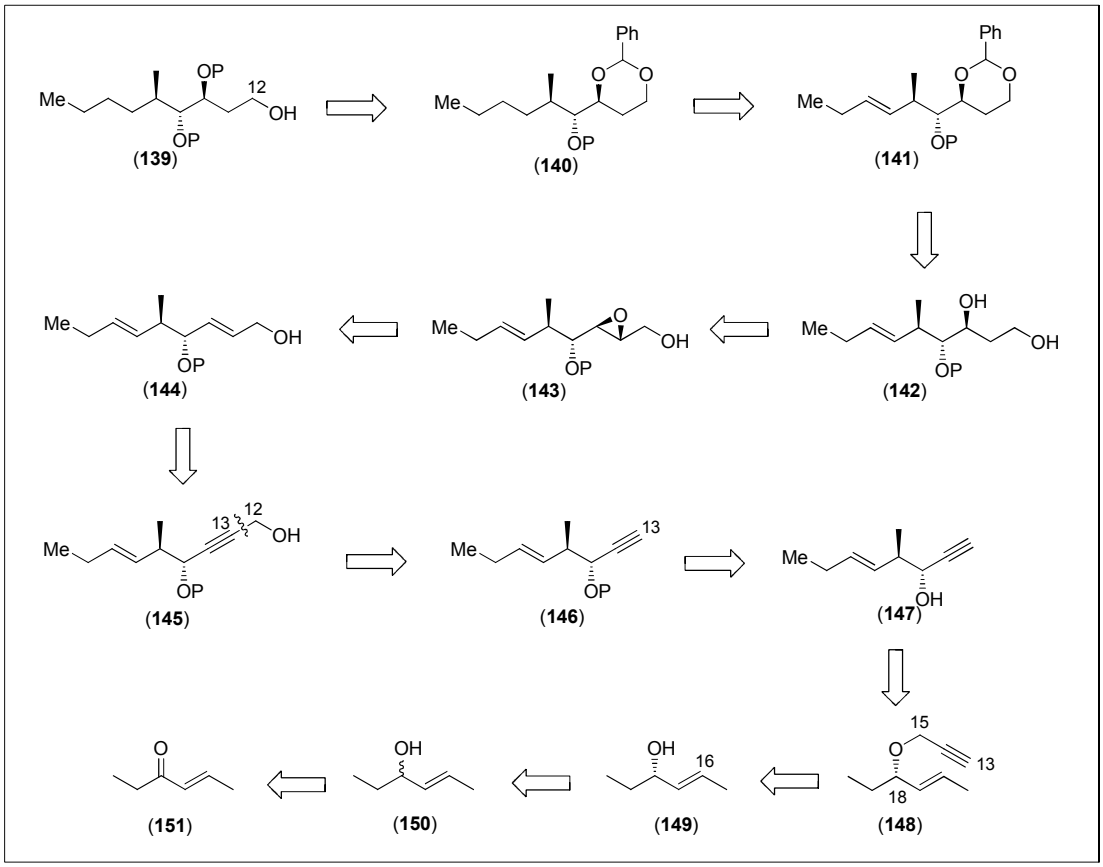


Scheme 20: Retrosynthetic analysis of the C(10) –C(20) moiety (**133**) of fumonisin B_1.

The disconnection of the C(9)–C(10) bond in **130** provided the C_{11} compound (**133**) which contains the four invariant stereocentres of all the fumonisins as well as three hydroxyl functionalities (**Scheme 20**). Protection of the C(14) and C(15) secondary hydroxyl groups in **131** leaves molecule **133** with a primary hydroxyl group and methyl substituent in the *R* configuration at C(12). The 1-hydroxy-3-methyl terminus present in **133** can be seen as to come from **134** by deoxygenation at C(11). This 1,2-dihydroxy-3-methyl motif with the required stereochemistry in place, can in turn be obtained from the regio- and stereoselective ring opening of the epoxide alcohol (**135**). In the synthesis direction this requires an epoxide with the (2*S,3S*) configuration. The methyl group at C(12) can be selectively introduced, to give the 1,2-dihydroxy-3-methyl motif with the desired stereochemistry by using Me_3Al as reagent in the ring opening reaction. The epoxide (**135**) is obtainable from allylic alcohol (**136**) using Sharpless methodology. The stereochemistry of the epoxide is established using

the diethyl (*S,S*)-tartrate ligand in the Sharpless asymmetric epoxidation reaction. The allylic alcohol (**136**) is the product of a DIBALH reduction reaction using an α,β-unsaturated ester (**137**) as substrate. This ester can be formed from aldehyde (**138**) using Wadsworth-Emmons methodology which will ultimately lead to the allylic alcohol (**136**) with the *E*-configuration of the double bond. The necessary aldehyde (**138**) can in turn be obtained by oxidation of the primary alcohol (**139**) using Swern oxidation.

2.3 THE C_{12} –C_{20} FRAGMENT



Scheme 21: Retrosynthetic analysis of the C(12)–C(20) fragment.

Retrosynthetic analysis of the C(12)–C(20) building block (**139**) envisions the formation of the 1,3-diol derivative as the product of a regioselective ring opening reaction by disconnecting the outer C-O bond of the 1,3-benzylidene derivative (**140**) (**Scheme 21**). This will produce the 1,3-diol derivative (**139**) with the primary alcohol unprotected and the secondary alcohol protected as a benzyl ether. At this stage in the analysis an isolated double bond is introduced in synthon (**140**) using a reduction transform to give synthon (**141**). Removal of the benzylidene protecting group leads to the 1,3-diol system of **142** which can be formed by the reductive ring opening of an epoxide (**143**). The secondary alcohol at C(14) has

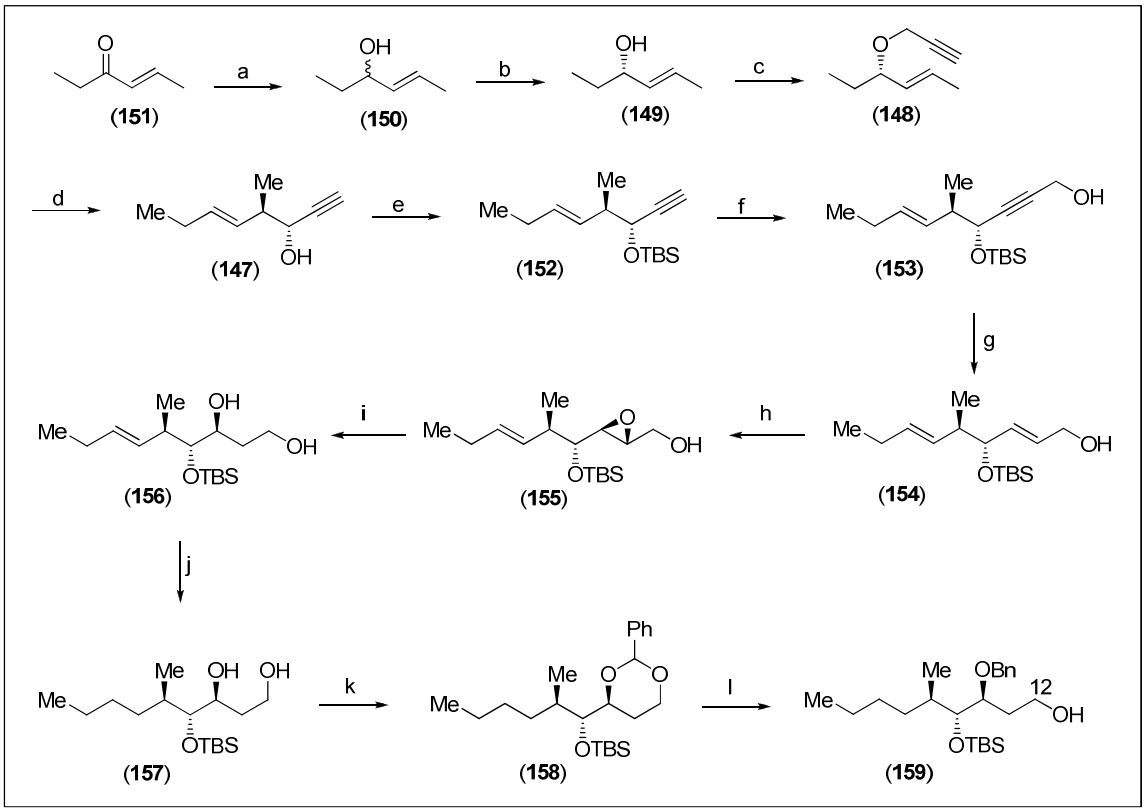
the *S* configuration, necessitating the (2*R*,3*S*) configuration for the epoxide. The epoxide (**143**) can be formed stereoselectively using Sharpless asymmetric epoxidation methodology with (*S,S*)-tartrate as a chiral ligand with the *E* allylic alcohol (**144**) as the substrate. This allylic alcohol (**144**) is the product of the stereoselective reduction of the alkyne (**145**). Terminal alkynes can be used as functionalities leading to C-C bond formation. Thus hydroxymethylation of the terminal alkyne (**146**) should therefore lead to alkyne (**145**). Disconnection of the C(12)–C(13) bond is thus a viable option and leads to the terminal alkyne (**145**) as synthon. Protection of the secondary alcohol is predicted to be vital at this stage, since hydroxymethylation requires a strong base. Alcohol (**147**) is the product of a [2,3]-Wittig sigmatropic rearrangement. Stereochemically, the 15*R,16R* configuration for the stereocentres at C(15) and C(16) is provided using the [2,3]-Wittig sigmatropic rearrangement methodology and a substrate with the appropriate configuration for both the stereogenic centre and the double bond. The electronic state of the substrate required for the rearrangement reaction can be seen to come from allyl ether (**148**). The allyl ether can be derived from the allylic alcohol (**149**) with the *S* configuration. The configuration of this alcohol can be obtained by kinetic enzymatic resolution of the racemic alcohol substrate (**150**), which is the 1,2-reduction product of the α,β-unsaturated ketone (**151**).

Retrosynthetic reasoning therefore predicts *trans*-4-hexen-3-one (**151**) as commercially available starting material in the synthetic sequence leading to **139** as ultimate target of this project. The synthesis of the identified target molecule from *trans*-4-hexen-3-one is discussed in detail in Chapters 3 and 4.

SYNTHETIC STUDIES CHAPTER 3

3.1 PROPOSED SYNTHETIC SEQUENCE

The retrosynthetic analysis of the C_{20} backbone of fumonisin B_1 (**5**) as discussed in Chapter 2 identified the C(12)-C(20) unit (**159**) as the synthetic target of this project. The synthesis of this C_9 intermediate using *trans*-4-hexen-3-one (**151**) as starting material is outlined in **Scheme 22**.



Scheme 22: Proposed reaction scheme for the synthesis of building block (**159**).

Reagents: a. $NaBH_4$, $CeCl_3$, MeOH; b. PPL, Et_2O; c. Propargyl bromide, 50% NaOH; d. *n*-BuLi; e. TBSCl; f. 1. *n*-BuLi, 2. H_2CO; g. LAH, THF; h. Sharpless; i. Red-Al; j. Pd-C, H_2; k. $PhCH(OCH_3)_2$, *p*-TsOH; l. DIBALH.

The synthesis starts with the 1,2-reduction of ketone (**151**) using Luche reagent.[1] This reaction provided the racemic secondary *E* allylic alcohol (**150**). Kinetic enzymatic resolution is envisioned to provide the required configuration for the C(18) stereogenic centre with an

[1] Gemal, A.L.; Luche J.L. *J. Am. Chem. Soc.* **1981**, *103,* 5454.

acceptable enantiomeric excess using porcine pancreatic lipase (PPL) as enantioselective acylating agent.[2] Formation of the propargylic ether (**148**), from the resolved secondary alcohol (**149**) is hoped to set the molecule up for a [2,3]-Wittig sigmatropic rearrangement reaction which will produce an intermediate product (**147**), with the correct stereochemistry at both the methyl bearing C(16) carbon and the hydroxyl bearing C(15) carbon.[3] The [2,3]-Wittig sigmatropic rearrangement reaction leaves the molecule with a terminal alkyne group as shown in **147**, which after protection of the secondary alcohol at C(15) can be hydroxymethylated[4] to give **153**. After selective reduction of the internal alkyne (**153**) using lithium aluminium hydride (LAH),[5] which produces the *E*-allylic alcohol (**154**), the molecule should be neatly set up for Sharpless asymmetric epoxidation[6] using an (*S,S*)-tartrate ester to obtain the epoxide (**155**) with the required stereochemistry in place. Selective opening of the expected epoxide is predicted to yield the 1,3-diol (**156**) with the C(14) stereogenic centre bearing the secondary alcohol in the *S* configuration. Hydrogenation of the double bond using Pd-C as catalyst is planned to be done at this stage to produce **157**. The secondary alcohol can then be selectively protected as the benzyl ether by firstly protecting the 1,3-diol as the benzylidene derivative (**158**). Regioselective opening of the benzylidene (**158**) with DIBALH produces the secondary hydroxy group protected as the benzyl ether (**159**) with retention of configuration.

Each step in the proposed synthetic route is discussed in the following sections using a format which firstly covers the theoretical aspects of the reaction, followed by a detailed experimental discussion.

The initial steps of the synthesis are given in **Scheme 23.** After formation of the [2,3]-Wittig rearrangement product (**147**), the synthetic approach diverges into two distinct routes. In **Route 1**, the protecting group strategy entailed protection of the secondary alcohol contained in **147** to eventually set the molecule up for Sharpless asymmetric epoxidation using the primary allylic alcohol as directing moiety (**Scheme 40**). Different protecting groups were tested. In **Route 2** (**Scheme 48**), the primary alcohol, installed after hydroxymethylation of **147**, was protected. The secondary alcohol was therefore left unprotected in order to be used as the directing allylic alcohol system in Sharpless asymmetric epoxidation. Again, different protecting groups were investigated.

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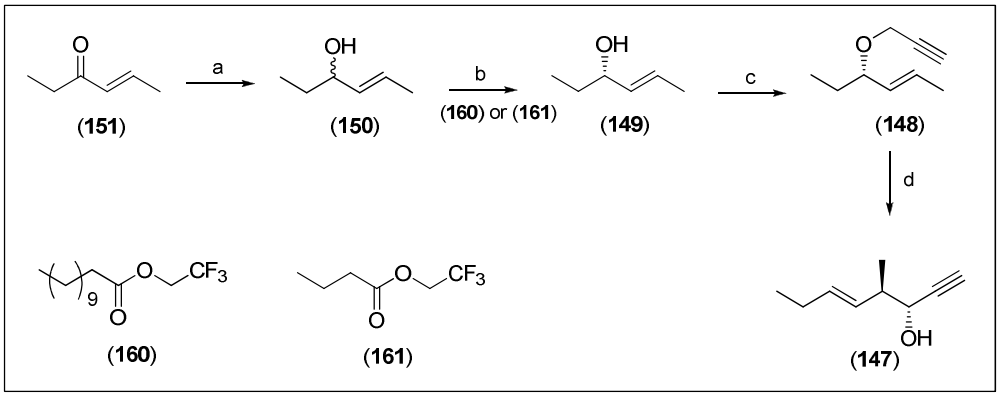
[2] Morgan, B.; Oehlshlager, A.C.; Stokes, T.M. **1991**, *47*, 1611.
[3] Nakai, T.; Mikami, K. *Chem. Rev.* **1986**, *86*, 855.
[4] Gilman, H.; Catlin, W.E. *Org. Synth. Coll*. **1941**, *1*, 188.
[5] Osby, J.O; Stephen, W.; Heinzman, S.W.; Ganem, B. *J. Am. Chem. Soc.* **1986,** *108,* 67.
[6] Gao, Y.; Hanson, R.M.; Klunder, J.M.; Ko, S.; Masamune, H.; Sharpless, K.B. *J. Am. Chem. Soc.* **1987,** *109*, 5165.



Scheme 23: Synthesis towards the [2,3]-Wittig rearrangement product (**147**).

3.2 1,2-SELECTIVE REDUCTION

The first step towards the synthesis of the target C(12)–C(20) unit from *trans*-4-hexen-3-one (**151**) was the regioselective 1,2-reduction of the α,β-unsaturated ketone moiety in (**151**) (**Scheme 23**).

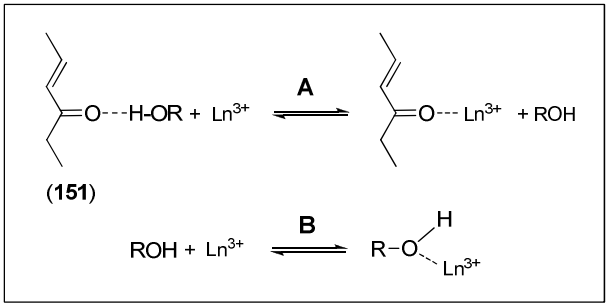
3.2.1 THEORETICAL ASPECTS

Sodium borohydride ($NaBH_4$) has been identified as a selective and mild reducing agent of carbonyl compounds and gives a mixture of 1,2- and/or 1,4-addition products. The presence of a lanthanide salt in the $NaBH_4$ reduction of α,β-enones has been shown to be regioselective and delivers the allylic alcohol as main product. This reaction has been studied in depth by Luche[1,7,8,9] and the reagent is thus referred to as the Luche reagent. The mechanism describing the regioselective 1,2-reduction of α,β-enones in an alcohol solution of lanthanide chlorides has been extensively studied using theoretical, stereochemical and kinetic approaches.[10]

Luche[1] studied the role of lanthanide ions on the selectivity and reaction rate in the 1,2-reduction of α,β-enones with $NaBH_4$ in alcohol solution and determined that the choice of the metallic ion, solvent effects and the overall reaction concentration were important.[1] The highest regioselectivity was found using cerium(III) chloride as metal salt. Substituting the lanthanide salt with a different metal salt resulted in a decrease in regioselectivity of the reaction. Lanthanides are well known for their high coordinative abilities with many functional groups. The role of the lanthanide ion was at first thought to modify the geometry or electron density of the enone by complexation. This would consequently affect the

[7] Luche, J.L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.
[8] Luche, J.L.; Rodriguez-Hahn, L.; Crabbe, M.P. *J. Chem. Soc., Chem. Commun.* **1978**, 601.
[9] Gemal, A.L.; Luche, J.L. *J. Org. Chem.* **1979**, *44*, 4187.
[10] Lefour, J.M.; Loupy, A. *Tetrahedron* **1978**, *34*, 2597.

reactivity of the system. Using a lanthanide ion as complexing agent in an aprotic solvent, renders the C(2) carbon of the conjugated system more prone to attack and favours 1,2- over 1,4-selectivity. It was also found that the use of a complexing agent in a protic solvent resulted in an increase in selectivity and reaction rate.[1] Varying protic solvents also influenced regioselectivity and reaction rate to a different extent. The highest regioselectivity and reaction rate was found using methanol as protic solvent in the reduction reaction.[1]



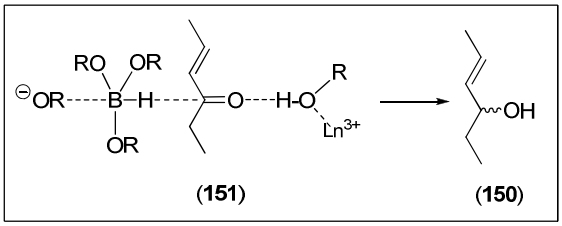
Scheme 24: Complexation equilibrium established in the 1,2-reduction reaction of (**151**).[1]

A further increase in regioselectivity was found on decreasing the overall reaction concentration. This fact, combined with the increase in regioselectivity and reaction rate observed on using a protic solvent prompted further investigation of the suggested complexation control. On decreasing the overall reaction concentration, the equilibrium shifts to the left of reaction **A** given in **Scheme 24**, nullifying complexation between the carbonyl oxygen of (**151**) and lanthanide ion. It is also known that lanthanide ions preferentially complex with alcohols rather than carbonyls. The second equilibrium reaction (**B**) given in **Scheme 24** should therefore shift to the right. The combination of these two effects nullifies the suggested complexation effect of the cation and reaction regioselectivity should therefore become the same to that without lanthanide as complexing ion. This was not the case observed though. Complexation of the cation to the alcohol increases the acidity of the medium and with cerium(III) being a hard Lewis acid, a positive contribution to both the 1,2 selectivity and the reaction rate is made. A push-pull type mechanism (**Scheme 25**) proposed by Wigfield,[11] which focuses on the electrophilic assistance of the solvent, can therefore be used to explain the results observed.

The actual reducing agent was found to be the alkoxyborohydride $NaBH_{4-n}(OR)_n$ and not BH_4^-.[1] Evidence confirming alkoxyborohydrides as actual reducing species, was provided by further investigation into the observed reaction rate and regioselectivity in different protic solvents and in comparing results with those observed in aprotic solvents with and without the

[11] Wigfield, D.C.; Gowland, F.W. *Tetrahedron Lett*. **1979**, *20*, 2209.

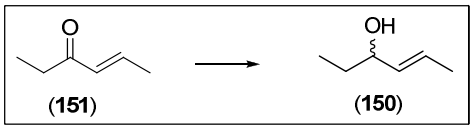
presence of the complexing agent. Interpretations resulting from experimental observations can also be confirmed by the hard acid-base theory (HSAB) in that the alkoxyborohydrides are harder acids than the BH_4^- species and thus favour attack at the hard site of the enone. A reasonable mechanistic interpretation is given in **Scheme 25**.



Scheme 25: Mechanistic interpretation of the push-pull type mechanism formulated by Gemal and Luche.[1]

The conclusions that can be drawn from the studies conducted by Gemal and Luche[1] on the role of lanthanide ions on the selectivity and reaction rate of the selective 1,2 reduction of α,β-enones by $NaBH_4$ in alcoholic solution is that methanol is the most suitable solvent providing the highest regioselectivity and reaction rate. The overall concentration in methanol has also been found to positively influence both the regioselectivity and reaction rate on decreasing the concentration of the reacting species.[1]

3.2.2 EXPERIMENTAL DISCUSSION



Scheme 26: 1,2-Selective reduction of (**151**).

Reagents: $NaBH_4$, $CeCl_3.7H_2O$, MeOH (100%).

The general procedure described by Gemal and Luche[1] was followed for the selective reduction of (*E*)-hex-4-en-3-one (**151**) to (*E*)-hex-4-en-3-ol (**150**). The product formed quantitatively. The polarity and volatility of the compound (bp 47 ºC, 15 Torr) necessitated the use of Et_2O as extracting solvent. Subsequent removal of the solvent employing rotary evaporation in a cold waterbath proved necessary due to volatility of the product. ^{1}H NMR spectroscopy confirmed the formation of the 1,2-reduction product: the protons of the C(4)-C(5) double bond appeared at δ_H 5.61 (ddq, $^3J_{4,5}$ 15.2, $^3J_{5,6}$ 6.5, $^4J_{3,5}$ 1.1, H-5) and 5.42 (ddq, 1H, $^3J_{4,5}$ 15.2, $^3J_{3,4}$ 7.0, $^4J_{4,6}$ 1.3, H-4). The coupling constant of 15.2 Hz between the H(4) and H(5) protons confirmed the *E* configuration of the double bond. The signal at δ_H 1.79 was assigned to the OH proton. In the ^{13}C NMR spectrum, the C(4) and C(5) shifts appeared at δ_C

134.03D and 126.80D, respectively. Further evidence for the presence of the –OH group was the broad band at 3344 cm^{-1} in the IR spectrum.

3.3 KINETIC ENZYMATIC RESOLUTION

The next challenge was the kinetic resolution of the racemic alcohol in order to obtain the *S*-enantiomer from the racemic mixture. A kinetic enzymatic resolution procedure was investigated using the 2,2,2-trifluoroethyl esters of both dodecanoic and butanoic acid. The enantioselectivity in all experiments was determined by converting both the resolved and racemic products into Mosher ester derivatives using (*R)*-(−)-α-methoxy-α-trifluoro-methylphenylacetic acid (*R*-MTPA) and determining the enantiomeric excess by analysis of the signals in the ^{19}F NMR spectrum.

3.3.1 THEORETICAL ASPECTS

The use of enzymes as catalysts in organic synthesis is widely accepted. One of the main problems encountered with enzyme catalysis is specificity. Enzymes, in nature, are designed to function in a specific biochemical pathway leading to substrate specificity. A wide range of enzymes are utilized in organic synthesis. One of the most common enzymes used in synthesis is probably baker's yeast which is predominantly good at reducing ketones yielding excellent enantioselectivities with ketones bearing β-esters as substrate.[12] The enzyme investigated in this work was a lipase (triglycerol acyl-hydrolase, E.C. 3.1.1.3). It has been identified as a valuable catalyst for the resolution of racemic secondary alcohols.

The Enzyme Commission's (E.C.) classification number of the enzyme (E.C. 3.1.1.3) is assigned as follows: the first digit refers to the main class of hydrolase enzymes. The second digit refers to the type of bond hydrolysed; in this case the 1 indicates that an ester is being hydrolysed. The third digit further describes the type of bond hydrolysed: the 1 refers to enzymes that are carboxylic ester hydrolases. The Commission also requires the source of the enzyme used: here the source is the porcine pancreas.[13]

The transesterification of one enantiomer of a racemic acyclic secondary alcohol in organic solvents by porcine pancreatic lipase (PPL) has been shown to proceed with high enantioselectivity.[14] Racemic secondary alcohols can thus be resolved by separating the acylated enantiomer from the unreacted enantiomer. PPL is an insoluble, inexpensive, stable

[12] Servi, S., *Synthesis* **1990**, 1.
[13] Palmer, T., Enzymes: Biochemistry, Biotechnology, Clinical chemistry, 2nd Ed., Horwood Publishing, Chichester, **2003**, p. 6.
[14] Morgan, B.; Oehlshlager, A.C.; Stokes, T.M. *Tetrahedron,* **1991**, *47*, 1611.

enzyme that has been shown to be effective on a wide range of alcohols. It has to be noted however, that pure PPL shows little to no enantioselectivity; the crude enzyme which also shows amylase and protease activity is also used as transesterification reagent.[14] Up to now, the active enzyme has not been isolated.[15] The catalytic process using crude PPL, involves initial acylation of the enzyme by the acylating agent, forming an acyl-enzyme complex. Enantioselective acylation of the secondary alcohol by the acyl-enzyme complex then takes place.

Enantioselective transesterification is influenced by a variety of factors *viz.* 1) dehydration of the enzyme, 2) chain length of the acylating agent, 3) chain length and branching of the racemic substrate, 4) overall reaction concentration and to a lesser extent the solvent and reaction time.[16]

Extensive research investigating all the above mentioned factors have been done by the research groups of Morgan[14], Nogawa[15] and Stokes.[16] Assumptions made during investigations, include process irreversibility, which implies independence of the enantiomeric ratio on degree of conversion and substrate and enzyme concentrations.[14]

1) Dehydration of the enzyme: This factor was found to increase the stability of the enzyme and resulted in an increase in enantioselectivity. Dehydration, unfortunately, also brings about a decrease in catalytic activity.[17] Investigation of this factor in previous studies done by Stokes[16] using (±)-sulcatol as racemic substrate, showed a three-fold increase in enantioselectivity on changing from off-the-shelf enzyme to the dehydrated equivalent. Dehydration is routinely achieved by drying the enzyme to a constant weight at room temperature by washing the enzyme with cold acetone (–20 ºC), followed by Et_2O and drying the enzyme under vacuum. The reactions were performed using Et_2O as solvent. Drying time was found to parallel to amount of enzyme used as exposure of the enzyme to air results in a 3-4% weight increase.

2) Chain length of the acylating agent: Activated esters were used in most studies as acylating agents. An activated ester such as 2,2,2-trifluoroethyl dodecanoate or butanoate increases the reaction rate of the acylation process. Using the latter ester results in the reaction proceeding somewhat slower, but has the advantage of it being volatile (113 ºC, 747 torr), making it easier to remove it from crude product mixture. Having the weakly

[15] Nogawa, M.; Shimojo, M.; Matsu; Moto, K.; Okudomi, M.; Nemoto, Y.; Ohta, H. *Tetrahedron*, **2006,** *62,* 7300.
[16] Stokes, T.M.; Oehlschlager, A.C. *Tetrahedron Lett.* **1987**, *28*, 2091.
[17] Zaks, A.; Klibanov, A.A. *Proc. Natl. Acad. Sci. USA* **1985**, *82,* 3192.

nucleophilic trifluoroethanol as leaving group, annuls competition of it with the substrate alcohol and therefore also speeds up the reaction.[18] Rapid transesterification also helps in achieving higher enantioselectivity. Butanoic anhydride has also been investigated as acylating agent, but selectivity proved poor compared to the activated esters.

Changing the chain length of the acylating ester showed a marked difference in enantioselectivity. Systematic change in chain length was investigated by Amici *et al.*[19] Chain lengths of C_4 to C_{10} (2,2,2-trifluoroethyl butanoate to 2,2,2-trifluoroethyl dodecanoate) showed that enantioselectivity increased with increase in chain length. Studies done by Stokes and Oehlschlager[16] showed as much as a four-fold increase in enantioselectivity by changing from 2,2,2-trifluoroethyl butanoate to 2,2,2-trifluoroethyl dodecanoate. This trend is ascribed to one of two factors: Either an effect of the chain length on the equilibrium constant[20] or steric effects in the active site of the enzyme.[19]

Another acylating agent investigated by Kamal and Fao[21] for use in lipase-catalysed resolution of secondary alcohols containing an amino group is trichloroacetic anhydride with dioxane as solvent.

3) Nature of the racemic substrate: Morgan *et al.*[14] investigated the effect of chain length, branching, position and nature of other functionalities contained by a range of 2-alkanol substrates on PPL-catalysed enantioselective transesterification reactions. 2,2,2-Trifluoroethyl dodecanoate and butanoate were used as acylating agents with the assumption that the reactions proceeded irreversibly. The degree of enantioselectivity increased rapidly with an increase in chain length of the 2-alkanol substrate from C_4 to C_6. Further elongation did not result in a marked increase in enantioselectivity. A sixteen-fold increase in enantioselectivity was found when the substrate contained a methyl group substituent at C-3 of the 2-alkanol substrate. Methyl substituents further down the linear alkyl chain failed to result in further increases in enantioselectivity. By comparing enantiomeric ratios obtain by using 2-alkanols bearing a cyclohexyl or phenyl group at C-2 with the results obtained with 2-pentanol as substrate, indicated that the flexibility of the substituent out competes absolute size as determinant of enantioselectivity. An adjacent benzyl group, conversely, was found to decrease selectivity. Alcohols that contained distant saturated and unsaturated esters showed lower enantioselectivities relative to straight chain analogues. Alkanols bearing terminal

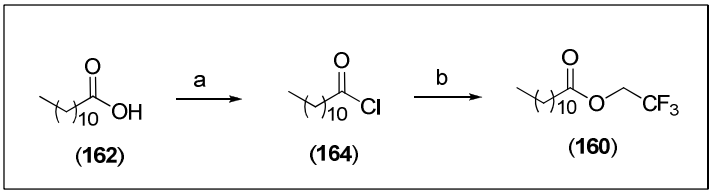
[18] Kirchner, G.; Scollar, M.P.; Klibanov, A.M. *J. Am. Chem. Soc.* **1985***, 107*, 7072.
[19] De Amici, M.; De Micheli, C.; Carrea, G.; Spezia, S. *J. Org. Chem.* **1989,** *54,* 2646.
[20] Chen, C.-S.; Wu, S.-H.; Girdaukas, G.; Sih, S.J. *J. Am. Chem. Soc.* **1987***, 109*, 7072.
[21] Kamal, A.; Fao, M.V. *Tetrahedron Asymmetry*, **1991**, *2*, 751.

carboxylic acids showed no enantioselectivity.

4) Overall reaction concentration: Substrate and acyl donor concentrations were also investigated and found to mainly affect the reaction rate. This is an important factor since the rate of acylation increases enantioselectivity. A linear increase in reaction rate was found by increasing substrate concentration from 0.2M to 1.0M with a constant acyl donor concentration of 1.25M and enzyme concentration of 0.33-0.40 g/mL.[16]

3.3.2 EXPERIMENTAL DISCUSSION

The method used in this study to synthesize the acylating agent, 2,2,2-trifluoroethyl dodecanoate (**160**), from dodecanoic acid (**162**) and 2,2,2-trifluoroethanol (**165**), required derivatisation of the carboxylic acid to the acyl chloride. 2,2,2-Trifluoroethanol was then added as nucleophile to the electrophilic acyl chloride in the presence of a base. The base serves to abstract the proton from the alcohol. A common base used in these reactions is pyridine (pK_{aH} 5.5). Dimethylaminopyridine (DMAP) is added as catalyst. Having a pK_{aH} of 9.7, it attacks the acyl chloride first to form an extremely electrophilic intermediate. The DMAP-substituted intermediate subsequently reacts with the alcohol to produce ester **160**.



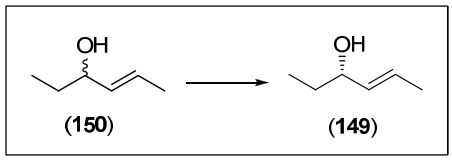
(162) (164) (160)

Scheme 27: Synthesis of 2,2,2-trifluoroethyl dodecanoate **160**.

Reagents: a. $SOCl_2$; b. 2,2,2-trifluoroethanol (**165**), pyridine, DMAP. (99%)

^{13}C NMR spectroscopy confirmed formation of the acyl chloride with the acyl carbon shift being at δ_C 173.74S in comparison to the dodecanoic acid with the carbonyl carbon shift at δ_C 180.53S. The acyl carbon shift lies upfield relative to the acid carbon shift due to increased deshielding afforded by the neighbouring chlorine atom.

The acyl chloride (**164**) was used without further purification in the next reaction to form the final ester product (**160**) in 99% yield. Formation of ester (**160**) was confirmed by the ^{13}C NMR data with the carbonyl carbon signal at δ_C 172.11 and the CF_3 signal at δ_C 123.03 as a quartet with a coupling constant of J = 279 Hz due to one-bond coupling with the fluorine atoms and the methylene signal as a quartet at δ_C 60.08 with coupling constant of J = 38 Hz due to two-bond fluorine coupling.



Scheme 28: Kinetic enzymatic resolution of **150.**

Reagents: PPL, 2,2,2-trifluoroethyl dodecanoate, Et_2O (46%)

The general reaction procedure devised by Stokes and Oehlschlager[16] was followed. A solution of the acylating agent (**160**), in anhydrous Et_2O, was firstly added to the dried enzyme to allow formation of the enzyme-acyl complex prior to the addition of the racemic substrate (**150**).

The enantioselectivity in all experiments was determined by converting both the resolved and racemic products into diastereomeric Mosher ester derivatives using (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (*R*-MTPA). Determination of the enantiomeric excess was carried out by analysis of the signals in the ^{19}F NMR spectrum. The presence of the trifluoromethyl group permits the use of ^{19}F NMR spectroscopy for the quantitative analysis of the enantiomeric composition of the parent alcohol, chiral or racemic.[22]

The first obvious factor that was found to influence the enantioselectivity was dehydration of the enzyme. Initially, dehydration of the enzyme increased the enantiomeric excess (e.e.) from 76% using the enzyme directly, to 92% by using the dried enzyme with in each case 2,2,2-trifluoroethyl butanoate as acylating agent. An increase in drying time proved necessary to maintain the % e.e. when using a larger amounts of enzyme in scaled-up reactions.

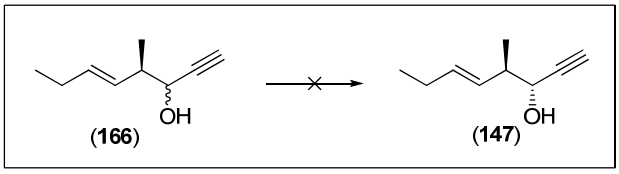
The second factor investigated was the chain length of the acylating agent. Initially, commercially available 2,2,2-trifluoroethyl butanoate (**161**) was used. The best e.e. obtained was 92%. The use of 2,2,2-trifluoroethyl dodecanoate (**160**) was investigated next. This decision was prompted by the fact that it would be more cost effective to synthesize the acylating agent ourselves and in the hope that an increase in enantioselectivity and reaction rate on increasing the chain length could be obtained.[14,16]

Optimized conditions for the enzymatic kinetic resolution of the racemic alcohol were obtained using PPL, washed with acetone and dried under reduced pressure, 2,2,2-trifluoroethyl dodecanoate (**160**) as acylating agent in anhydrous Et_2O and a reaction time of 96 hours. A 99.8% ee was obtained with a yield of 46% working on a 20.0 mmol scale (2.00

[22] Dale, J.A.; Dull, D.L.; Mosher, H.S. *J. Org. Chem.* **1969**, *34*, 2543.

g) of the alcohol substrate. The signals of the *R*- and *S*-enantiomers appeared at δ_F 70.07 and 70.16, respectively in the ^{19}F NMR spectrum.

Scale-up of the reaction turned out to be rather wasteful and brought about a decrease in e.e. and alternative methods were investigated. To circumvent difficulties caused by the volatility of the secondary alcohol substrate **150**, PPL-catalysed resolution was tried on a racemic version of the [2,3]-Wittig rearrangement product (**166**) synthesized in a later stage of the synthetic sequence (**Scheme 29**).



Scheme 29: Attempted enzymatic resolution of **166**.

Reagents: PPL, 2,2,2-trifluoroethyl dodecanoate, Et_2O.

The reaction proceeded with disappointingly low enantioselectivity, resulting in an enantiomeric excess of only 41%. PPL has been shown to successfully resolve a range of 2-alkanols bearing a methyl substituent adjacent to the secondary alcohol. Although a methyl substituent is present in the substrate, the reason for PPL failing to resolve this substrate could be that it is not a 2-alkanol and the lack of flexibility of the alkyne functionality present adjacent to the alcohol group on the other side.

3.3.3 ALTERNATIVE APPROACHES

The enantioselective reduction of the carbonyl group in the starting material, *trans*-4-hexen-3-one (**151**) was also investigated and is discussed in the following section.

3.3.3.1 ENANTIOSELECTIVE REDUCTION BY BINAL-H REAGENT

The enantioselective reduction of the prochiral carbonyl substrate (**151**) with the chiral 2,2′-dihydroxy-1,1′-binaphtyl modified aluminium hydride reagent (BINAL-H) was also investigated. This reagent was developed and its use reported by Noyori *et al.*[23] in 1984. Only a limited number of methods are available for controlling the stereochemistry of simple acyclic systems, and investigations towards these transformations are therefore imperative.[24] The aim of the Noyori groups' study was to develop a new enantioselective reducing reagent

[23] Noyori, R.; Tomino. I.; Tanimoto, Y.; Nishizawa, M. *J. Am. Chem. Soc.* **1984**, *106*, 6709.
[24] Noyori, R.; Tomino, I.; Yamada, M.; Nishizawa, M. *J. Am. Chem. Soc.* **1984**, *106*, 6717.

reactive towards a broad variety of prochiral carbonyl substrates. Modified LAH species generally do not result in a high level of stereoselectivity. This problem has been construed as being due to the fact that these reactive species typically form more than one reactive species placed in different chemical and chiral surroundings.[23] The approach by Noyori is based on the consideration that a minimum number of reactive hydride species should increase enantioselectivity.

One problem they were confronted with, was that the reaction of LAH with 2 equivalents of a chiral alcohol (R*OH) yields a compound represented as $LiAlH_2(OR^*)_2$. This compound tends to disproportionate to produce a mixture of $LiAl(OR^*)_4$, $LiAlH_2(OR^*)_{4-n}$ (n = 1-3) and achiral LAH as reactive species. Another problem was the number of conformational possibilities in which the chiral ligands could be present. The challenge was therefore to decrease the likelihood of disproportionation of the aluminum hydrides and at the same time fix the conformation of the ligand used.[23] Ligands commonly used are bifunctional molecules such as diols, di-amines and amino alcohols from naturally occurring chiral compounds. In most cases, H_a and H_b in $LiAlH_2(OR^*)_2$ derived from these compounds are diastereotopic with different behaviour in the enantioselective reaction; therefore the ideal would be for the H_a and H_b protons to be homotopic in order to halve the type of hydrogens attached to aluminum. A modified reagent suited for this is 2,2′-dihydroxy-1,1′-binaphthyl (**A**) and (**B**) which is an axially dissymmetric bifunctional molecule (**Figure 10**).[23]

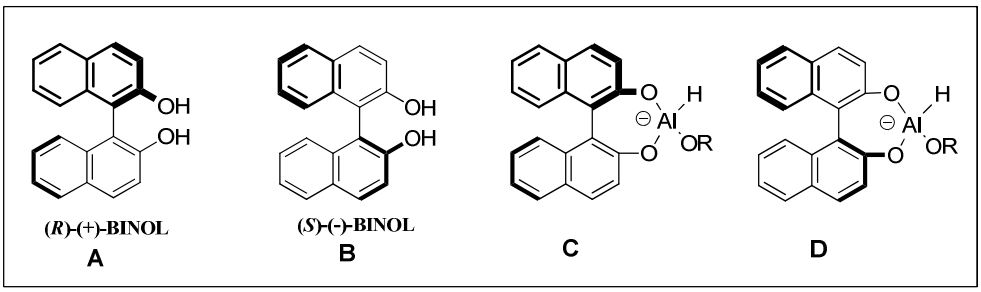


Figure 10: Structure of 2,2′-dihydroxy-1,1′-binaphthyl reagents.

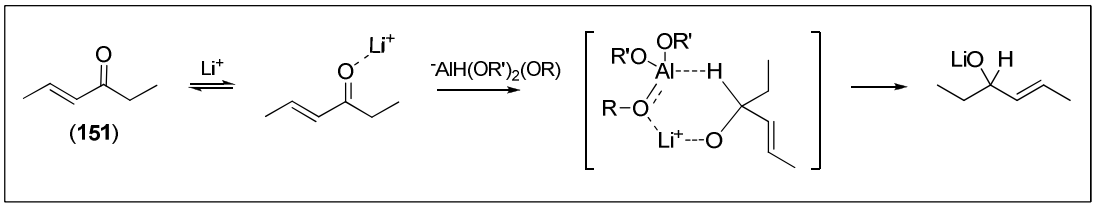
The BINOL ligands (**A**) and (**B**) have limited mobility and can attain a stable chelate structure by accommodating an aluminum atom by suitable rotation about the C(1)-C(1′) axis and the C-O bonds. The seven-membered ring system that results is conformationally locked due to the presence of the sp^2-hybridized carbon atoms of the 1,1′-binaphthyl system.

Investigations by Nyori and co-workers[23,24] into the concept described, using prochiral acetophenone as substrate, produced disappointingly low levels of asymmetric induction (2% e.e.).[23] This was thought to be the result of the hydrogens being homotopic in the produced

reagent. Further modification using a simple alcohol was examined: Replacement of either hydrogen by an alkoxide (RO^-) moiety would result in a single aluminum hydride reagent. A significant increase in optical yield was found on testing the binaphthol-modified aluminum hydride reagent (**Figure 10**: **C** and **D**) (BINAL-H), again using acetophenone as substrate.

Reaction temperature, molar equivalents of reducing agent and the nature of the alkoxide group all seemed to influence the degree of enantiofacial differentiation. The best results, with acetophenone as substrate, were obtained with RO^- being $CH_3CH_2O^-$ at −100 to −78 ºC using 2-3 equivalents of the reducing agent. Investigation of the temperature effect showed that the % e.e. of the acetophenone reduction reaction increased linearly by lowering the reaction temperature. It was also found that reductions by (*R*)-BINAL-H produced the *R*-carbinol product and reaction with (*S*)-BINAL-H produced the *S*-carbinol product.[23]

The extent of enantioselectivity varies with the R-group: Investigation found that the % e.e. increases gradually going from acetophenone to carbonyl substrates carrying long primary alkyl group side-chains. A sudden decrease in enantioselectivity with an increase in side chain bulkiness was also observed. To determine the effect and contribution of these two influencing factors, a series of carbonyl substrates were tested. Analogues varied from carbonyl substrates bearing primary alkyl side chains to the isopropyl equivalent. It was found that electronic properties were the main determinant of the substrates' reactivity towards the reducing agent.[23]



Scheme 30: Mechanistic interpretation of the reduction of **151**.

In order to explain the empirical rule for the orientation observed, the following mechanistic hypothesis was formulated: The reduction of the carbonyl substrate with $LAH(OR)_3$-type reagents was taken to proceed as indicated in **Scheme 30**. Complex formation of Li^+, which is a Lewis acid, with the carbonyl oxygen atom, activates the carbonyl carbon. Hydride transfer then occurs from aluminum to the carbonyl carbon *via* the Zimmerman-Traxler transition state.[23] The oxygen from the alkoxide group, acts as bridging atom due to higher basicity compared to the oxygens attached to aluminum.

The two diastereotopic binaphthoxy oxygens in (*S*)-BINAL-H introduce the possibility of different transition states. Transition state **A** (**Figure 11**) is the favoured transition state. Modeling the unsaturated prochiral ketone substrate, UnCOR', into transition state **A**, delivers two possible diasteromeric transition states, **B** and **C**. Observed results back the empirical rule for the orientation observed and favours transition state **B.** Stability is controlled by the 1,3-diaxial interactions, which are sterically repulsive, but either repulsive or attractive when considering electronic effects. Transition state **C,** is postulated to be destabilized by the n–π electronic interactions whereas these interactions are absent in **B**. Transition state **B** is thus generally favoured over transition state **C**. The electronic factors therefore outweigh the increasing 1,3-diaxial steric repulsion brought about by an increase in the size of the R-group.[23]

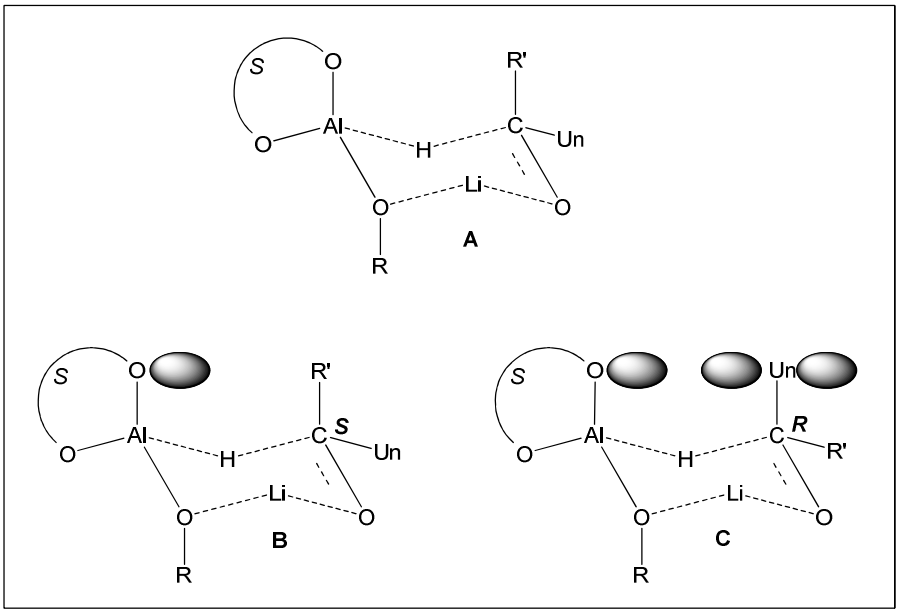
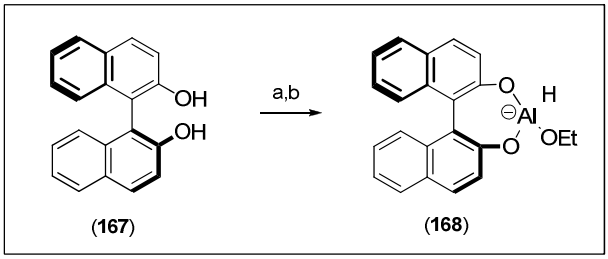


Figure 11: Modeled effects of steric and electronic factors affecting enantioselectivity.[23]

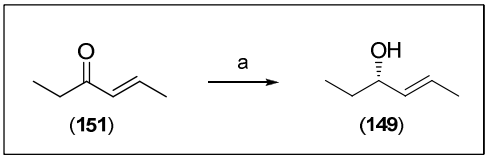
3.3.3.2 EXPERIMENTAL DISCUSSION



Scheme 31: Formation of the BINAL-H reagent **168.**

Reagents: a. 2M LAH, THF; b. EtOH.

The binaphthol-modified reagent (**168**) was prepared following the procedure by Noyori *et al.*[24] The reaction was performed following a prescribed procedure. Formation of a homogenous mixture of the reducing agent is required to ensure a high degree of enantioselectivity. Since the optical yield increases with a decrease in reaction temperature, the reducing agent was cooled to −100 ºC upon addition, where after the reaction proceeded at −78 ºC.



Scheme 32: Reduction of **151** using BINAL-H reagent.

Reagents: a. (*S*)-BINAL-H (**168**)

The reaction was quenched with methanol, since the use of strong acids or bases may cause racemization of BINAP, which can be recovered by recrystallization and re-used. The recovery of the secondary alcohol product (**149**) proved difficult due to its volatility (47 ºC, 15 Torr) and evaporation of the THF solvent resulted in considerable losses. The product was therefore left in THF and the Mosher ester derivative prepared directly in order to determine the % e.e. of the product. The reaction was not successful in that a disappointingly low level of asymmetric induction (20% e.e.) was obtained and this route was consequently abandoned.

3.3.3 ALTERNATIVE APPROACHES (CONT.)

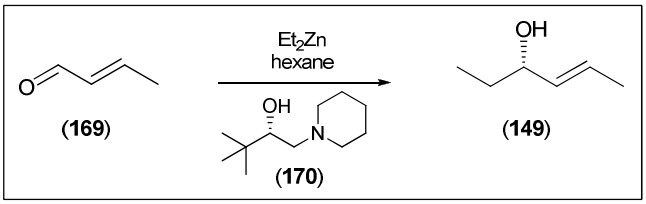
Other methods to enantioselectively reduce the prochiral ketone (**151**) also exist.

The first of these is the CBS reagent, a chiral borohydride heterocycle developed by Corey *et al.*[25,26] Proline provides the chiral amino alcohol derivative and produces the active reducing agent as a heterocycle complexed with borane. Only catalytic amounts are needed. A possible limitation of the reagent is that the best results were obtained using ketone substrates with two sterically well-differentiated substituents. This is not the case with *trans* 4-hexen-3-one.

Liang *et al.*[27] too experienced problems in the scale-up of the kinetic enzymatic resolution of the racemic *trans*-4-hexen-3-ol (**150**) discussed earlier. They developed a protocol for larger scale reactions but with a trade-off in a lower enantioselectivity: crotonaldehyde (**169**) and

[25] Corey, E.J.; Bakshi, R.K.; Shibata, S *J.Am. Chem. Soc*. **1987**, *109*, 5551.
[26] Corey, E.J.; Bakshi, R.K.; Shibata, S *J.Am. Chem. Soc*. **1987**, *109*, 7925.
[27] Liang, J.; Hoard, D.W.; Khau, V.V.; Martinelli, M.J.; Moher, E.D.; Moore, R.E.; Tius, M.A. *J. Org. Chem.* **1999,** *64,* 1459.

diethylzinc in the presence of 1 mol % (*S*)-1-piperidino-3,3-dimethyl-2-butanol (**170**) gave *trans-(S)*-4-hexen-3-ol (**149**) (**Scheme 33**) in 64% yield with a 83% e.e.



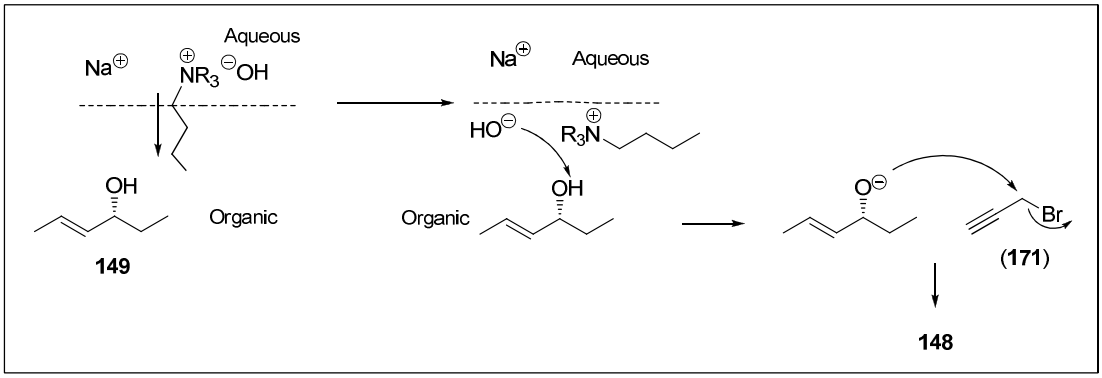
Scheme 33: Synthesis of **149** from crotonaldehyde (**169**).[27] (64%. 83% e.e).

3.4 ETHER SYNTHESIS: PHASE TRANSFER CATALYSIS

The next step in the reaction sequence involved the formation of an ether bond from the homochiral *trans-(3S)*-4-hexen-3-ol (**149**) and propargyl bromide.

3.4.1 THEORETICAL ASPECTS

Formation of the ether bond between **149** and propargyl bromide was accomplished using phase transfer catalysis. A phase transfer catalyst facilitates the migration of reactants in a biphasic system from one phase to another. Phase transfer catalysts for anions are usually quaternary ammonium salts, whereas crown ethers are used for cations.[28] Phase transfer catalysis has also cemented its place in green chemistry by allowing the use of water and reducing the use of organic solvents.[29]



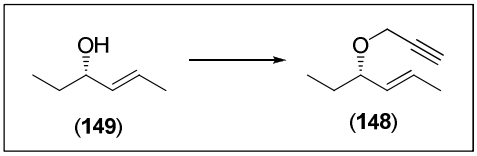
Scheme 34: Mechanistic interpretation of ether formation using phase transfer catalysis.

The phase transfer catalyst used in this reaction was a quaternary ammonium salt, tetrabutylammonium hydrogensulfate following the procedure by Liang *et al.*[27] The catalyst ferries sufficient hydroxide ions from the aqueous phase to the organic phase to deprotonate

[28] Stark, C.M. *J. Am. Chem. Soc.* **1971**, *93,* 195.
[29] Metzger, J.O. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2975.

the secondary alcohol, promoting an S_N2 reaction between the formed alkoxide and propargyl bromide, while preventing a S_N2 reaction between the hydroxide ions and the propargyl bromide.

3.4.2 EXPERIMENTAL DISCUSSION



Scheme 35: Propargyl ether synthesis under phase transfer conditions.

Reagents: Propargyl bromide, *n*-Bu_4NHSO_4, 50% NaOH.

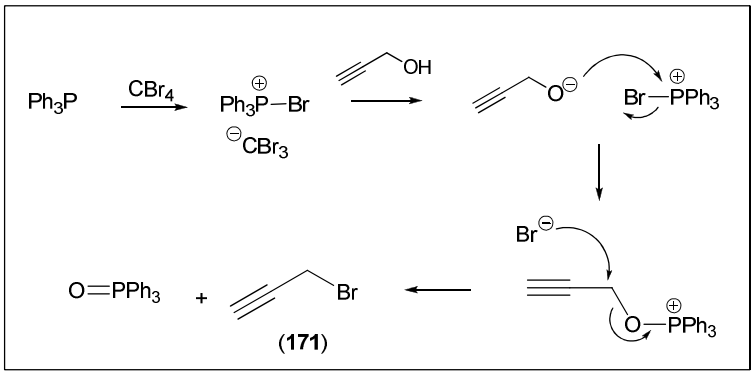
The synthesis of propargyl bromide (**171**) was attempted from propargyl alcohol which was available in our laboratories.

3.4.2.1 SYNTHESIS OF PROPARGYL BROMIDE (171)

Methods tried to synthesize propargyl bromide from the propargylic alcohol included:

a. From CBr_4 and PPh_3.

A mechanistic interpretation of the Appel reaction[30] is given in **Scheme 36**. This reaction functions as a mild method for halide formation using alcohols. Formation of the (tribromomethyl)phosphonium bromide salt sets the reaction up for nucleophilic displacement of the bromide by the alcohol. The reaction then proceeds by an S_N2 process to form the desired alkyl halide, while the formation of triphenylphosphine oxide, which is a stable solid, drives the reaction to completion.



Scheme 36: Mechanistic explanation of the Appel reaction.

[30] Appel, R. *Angew. Chem. Int. Ed. Engl.* **1975,** *14*, 801.

b. From MsCl and LiBr.

Methanesulfonyl chloride, in the presence of a base forms methanesulfonate esters with alcohols. Bromination is then accomplished by the addition of lithium bromide with the bromide ion acting as the nucleophile in a nucleophilic substitution reaction with mesylate as leaving group.

c. From NBS and PPh_3.

The reaction mechanism is similar to that of the mechanistic explanation given for the Appel reaction given earlier using CBr_4 and PPh_3.

None of the three methods proved successful: This was thought to be due to the volatility of the ether product which rendered it difficult to remove the solvent used in the reaction mixtures. This left a solution with unknown molarity, making it impossible to calculate the correct molar amounts needed in the reaction forming the ether. Costs of reagents needed to synthesize the propargyl bromide (**171**) from propargyl alcohol proved wasteful. The commercially available propargyl bromide was purchased since it was inexpensive in comparison.

The reaction itself posed no difficulties. It was found, however, that an increase in reaction time resulted in better results. The procedure followed[27] recommended a reaction time of 24 h, but it was found that a period of 60 h was needed to get optimum results. Volatility of the product (**148**) prevented measurement of its optical rotation and calculation of an accurate yield due to difficulty in removing toluene from the sample. The toluene stems from the propargyl bromide reagent which comes as an 80% solution in toluene. The product (**148**) was also found to be unstable and decomposed on storage.

Formation of the propargyl ether (**148**) was confirmed by its ^{1}H NMR data. The acetylenic proton appeared as a triplet at δ_H 2.32 with $^4J_{HH}$ 2.3 Hz due to long-rang coupling with the C(1′) methylene protons. The methylene protons are diastereotopic and appeared as an AB spin system at δ_H 4.11 and 3.96, respectively each as a double doublet with a geminal coupling constant of 15.8 Hz and a long range coupling constant of 2.3 Hz due to coupling with the acetylenic proton. In the ^{13}C NMR spectrum, ether formation was also confirmed by the shift in the methylene signal from δ_C 21.85 in propargyl bromide to δ_C 54.66 in the ether product (**148**). The carbons of the triple bond appeared at δ_C 73.36D and 80.46S, respectively. It was of interest to note that the latter signal assigned to the C(2′) quaternary carbon atom

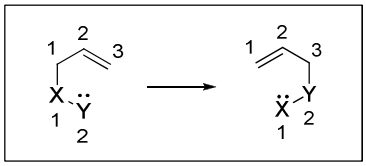
analysed as a doublet in the DEPT-135 spectrum due to the two-bond ($^{13}C,^{1}H$) coupling of 49 Hz with the acetylenic proton.

The propargylic ether (**148**) could now be used to effect a two-carbon chain extension and the creation of an additional stereogenic centre under the control of the existing centre using a [2,3]-Wittig rearrangement reaction to give compound (**147**).

3.5 [2,3]-WITTIG REARRANGEMENT

3.5.1 THEORETICAL ASPECTS

The generalized [2,3]-sigmatropic reaction is shown in **Scheme 37**. The reaction can be defined as a thermal isomerization that proceeds through a six-electron, five-membered cyclic transition state.



Scheme 37: The [2,3]-Wittig rearrangement.[31]

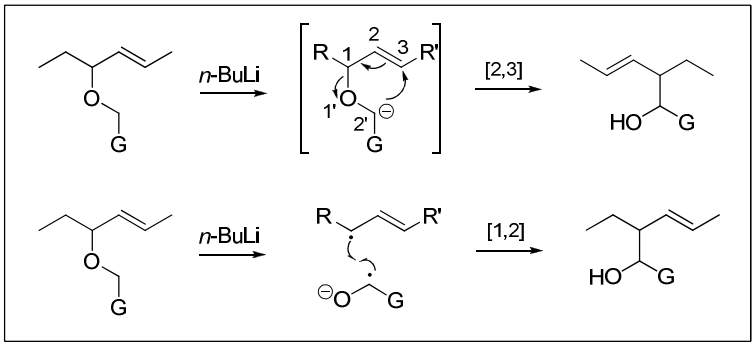
A vast number of variants exist regarding both the atom pair X and Y and the electronic state of Y which can be present as an anion, an atom with lone pair electrons or as an ylide (neutral dipolar molecule with the negatively charged carbon). The [2,3]-rearrangement involving oxycarbanions as migrating end, with X = oxygen and Y = carbanion, is now formally termed the [2,3]-Wittig sigmatropic rearrangement, stemming from the classic Wittig 1,2-alkyl shift of oxycarbanions.[31]

The [2,3]-Wittig rearrangement reaction includes a number of extremely valuable features which include the regiospecific C-C bond formation with allylic transposition of the oxygen functionality, generation of specific geometries of the olefin functionality, stereoselective creation of vicinal stereogenic centers and the transfer of chirality.[31]

The [1,2]-Wittig rearrangement proceeds through a radical-pair dissociation-recombination mechanism. The initially formed carbanion undergoes homolytic cleavage of the α-carbon-oxygen bond leads to the formation of a radical pair which then recombines to a rearranged alkoxide. Aqueous workup yields the alcohol.[32] The general [1,2]-shift is shown in **Scheme 38** as opposed to the [2,3]-shift which is a concerted and thermally allowed complying with

[31] Nakai, T.; Mikami, K. *Chem. Rev.* **1986**, *86*, 885.
[32] Strunk, S.; Schlosser, M. *Eur. J. Org. Chem.* **2006**, *19*, 4393.

the Woodward-Hoffmann rule[33] or Fukui's frontier orbital theory[34]. The driving force for the rearrangements is the transfer of the negative charge from carbon to the more electronegative oxygen atom. The [2,3]-shift product prevails at low temperatures over the [1,2]-shift product and on using THF as only solvent.



Scheme 38: Comparison of the [2,3] shift with the competing [1,2] shift.[31]

The [2,3] shift can therefore be described as an S_Ni' reaction, proceeding suprafacially with respect to both ends (**Scheme 3.24**).

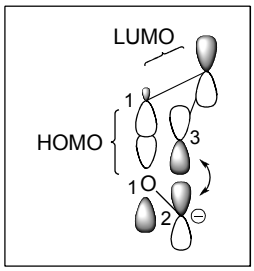
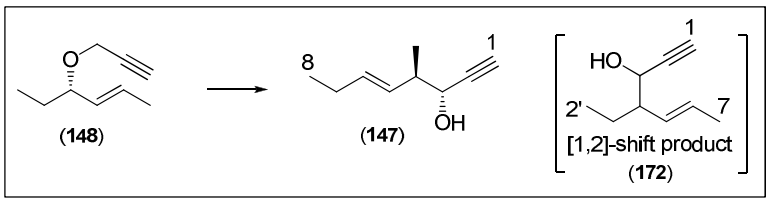


Figure 12: Orbital depiction of the [2,3] shift.[31]

Theoretically, the smaller the energy gap between the LUMO of the allylic site ($LUMO_{allyl}$) and the HOMO of the carbanion site ($HOMO_{anion}$), the more likely the rearrangement is to occur. This implies that the less stable the carbanion formed, the faster the rearrangement. A semi-quantitative study done on the thio-[2,3]-Wittig variants by Nakai and Mikami[31] showed that reaction rate decreases on changing the group G (**Scheme 38**) in the following order: G = Ph > CO_2Li > CN > CO_2Et > CO_2Me and R = Ph > H > Me for G = CO_2Et and CN. Therefore, it can be said that substituents which lower the $HOMO_{anion}$ and raise the $LUMO_{allyl}$ lower the reaction rate.

3.5.2 EXPERIMENTAL DISCUSSION

[33] Woodward, R.B.; Hoffmann, R. *The Conservation of Orbital Symmetry,* Academic Press, New York, **1970**.
[34] Fukui, K. *Theory of Orientation and Stereoselection,* Springer-Verlag, Berlin, **1971**.



Scheme 39: Formation of **147.**

Reagents: n-BuLi, THF (93%).

The solvent of an *n*-BuLi solution (1.6M in hexane) was removed *in vacuo* and the propargylic ether (**148**) in THF was added to the solid *n*-BuLi at –90 ºC in order to minimize the occurrence of the competing [1,2]-shift. Three equivalents of *n*-BuLi were used to compensate for the presence of the acidic acetylenic proton and the fact that *n*-BuLi is known to react with the solvent, THF. The acetylenic alcohol (**147**) compound was formed in 93% yield. The ^{1}H NMR spectrum of **147** showed the C(3) proton as a double doublet at δ_H 4.12 due to coupling with H(4) (*J* 6.2 Hz) and long-range coupling with H(1) (*J* 2.1 Hz) whereas the corresponding proton H(4) in the starting material **148**, appeared as a doublet of triplets as a result of coupling with the C(3) olefinic proton and the C(5) methylene protons.

The [1,2]-shift product (**172**) (**Scheme 39**) did not form. This was confirmed by analysis of the ^{1}H NMR and the COSY spectrum. The formation of **172** would result in the protons of one of the two methyl groups, H(7), to appear as a doublet of doublets due to coupling with H(6) and H(5). This was not the case and the protons of the two methyl groups appeared as a doublet (*J* = 7.0 Hz) (4-CH_3) at δ_H 1.10 and a triplet (*J* = 6.8 Hz) (H(8)) at δ_H 0.97, respectively. Thus it was concluded that the [1,2]-shift product did not form under the reaction conditions of the experiment.

3.6 ROUTE 1

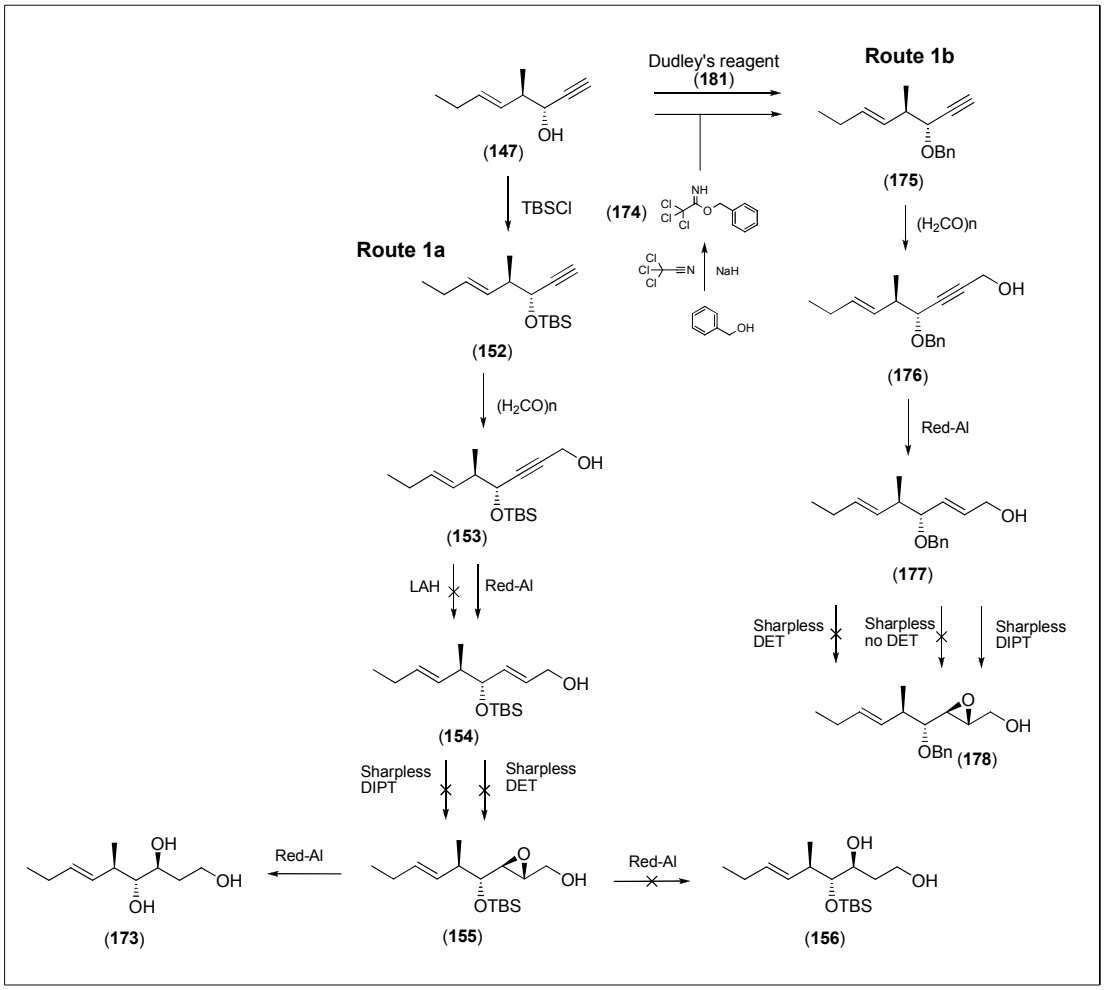
At this stage of the synthetic route the matter of a protecting group strategy for the secondary hydroxyl group had to be investigated. Protecting groups are used in functional group transformations: multifunctional compounds will require protecting groups in order to facilitate free manipulation of a specific functional group without affecting other functional groups contained in the same molecule. The choice of protecting group will be influenced by the type of functional group that needs protection, the reaction conditions in subsequent reactions and the ease of removal.

In this dissertation two different approaches towards the target molecule were investigated. The first approach employed a strategy in which the secondary hydroxyl group of the [2,3]-

Wittig rearrangement product (**147**) was first protected followed by the a chain extension of the acetylene functional group in a hydroxymethylation reaction. The different protecting groups used caused problems of their own which are also discussed in this section. The second approach protected the primary hydroxyl group formed after hydroxymethylation of the [2,3]-Wittig rearrangement product.

3.6.1 PROTECTION OF THE SECONDARY HYDROXY GROUP

The different protecting group methodologies, used in the protection of the secondary hydroxy group, are shown in **Scheme 40** which also indicates problems encountered later in the synthetic sequence which prompted the use of the different protecting groups employed.

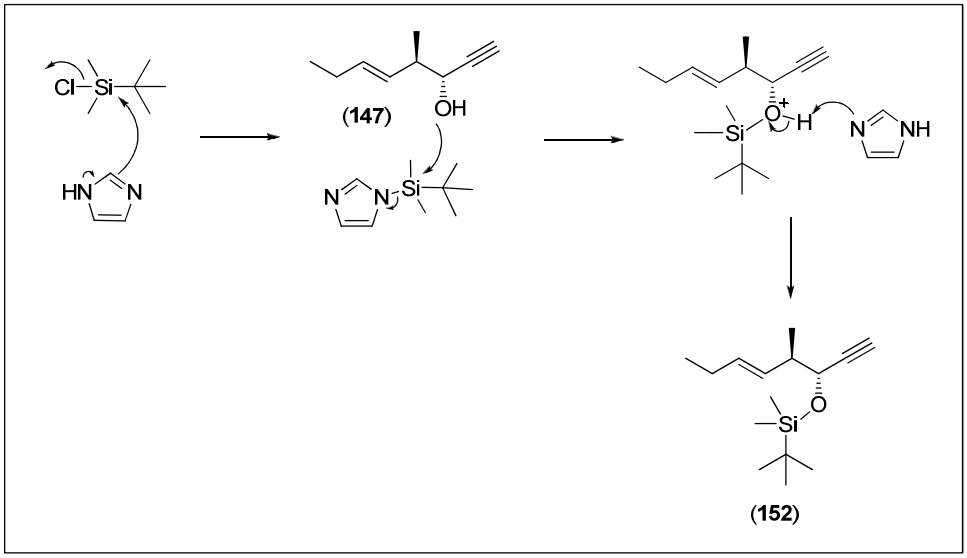


Scheme 40: Schematic presentation of **Route 1.**

3.6.1.1 ROUTE 1A: *t*-BUTYLDIMETHYLSILYL PROTECTION

A silyl ether was the first choice in protection of the secondary hydroxy group contained in the [2,3]-Wittig rearrangement product (**147**). Silyl ethers are a popular choice as far as

protecting groups go. Their chemical properties infer ease of formation, selective removal and stablility towards a wide range of reaction conditions. Stability of silyl ethers towards nucleophilic attack and hydrolysis are dictated by the steric bulk of the groups attached to the central silicon atom. The steric bulk of the silyl ether thus predicts its stability towards different reaction conditions. Silicon's affinity for fluorine is used to selectively deprotect hydroxy groups protected as silyl ethers in the presence of other protecting groups present in the molecule. The fluoride ion contained in tetra-*n*-butylammonium fluoride (TBAF) readily cleaves silyl ethers in aprotic medium.



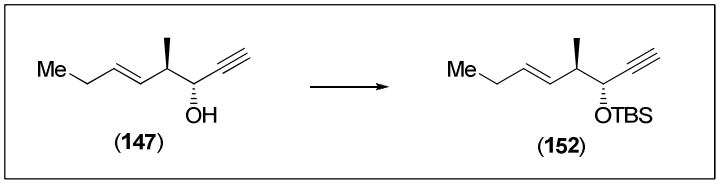
Scheme 41: Mechanistic interpretation of the silylation of **147** using imidazole as base.

t-Butyldimethylsilyl has become one of the most popular protecting groups of oxygenated functionalities since its introduction by Corey and Venkateswarlu.[35] Studies proved TBS ethers to be stable towards metal hydrides *e.g*. LAH, aprotic bases and Grignard reagents at temperatures below 0 ºC, but unstable towards acid hydrolysis (acetic acid-water 2:1) and is prone to migrate in substrates containing more than one hydroxyl functionality. Conventional silylation techniques using excess TBSCl and dry pyridine in THF gave unsatisfactory results due to a sluggish reaction rate and poor yields.[35] Reaction rates were improved by using imidazole as catalyst and acid scavenger and dimethylformamide (DMF) as solvent under mild conditions. Dichloromethane can also be used as solvent, depending on the polarity of the substrate. The conjugate acid of the intermediate formed, *N*-dimethyl-*tert*-butylsilylimidazole, is an extremely reactive silylating agent.[35] Chaudhary and Hernandez[36] found that the reaction rate can be increased further still by using 4-dimethylaminopyridine

[35] Corey, E.J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
[36] Chaudhary, S.K.; Hernandez, O. *Tetrahedron Lett*. **1979**, *20*, 99.

(DMAP) as group transfer agent in the presence of triethylamine, which serves as acid scavenger and to regenerate the catalyst. Both DMAP and imidazole also scavenge any acid produced during the reaction.[35,36] A mechanistic explanation of TBS protection is given in **Scheme 41.**

3.6.1.2 EXPERIMENTAL DISCUSSION



Scheme 42: TBS protection of **147.**

Reagents: TBS-Cl, imidazole, DCM (67%)

Treatment of the acetylenic alcohol (**147**) with TBSCl and imidazole in anhydrous DCM gave the *O*-TBS ether (**152**) in 67% yield. The characteristic signals for the TBS group appeared at δ_C –4.87Q ($SiMe_2$), 18.23S [-**C**$(CH_3)_3$] and 25.75Q [-C(**C**$H_3)_3$] in the ^{13}C NMR spectrum.

3.6.1.3 ROUTE 1B: *O*-BENZYL PROTECTION

The benzyl group is another common protecting group used in organic synthesis. The advantages of benzyl ethers include their stability toward a wide range of reaction conditions, minimal electronic impact imparted on the oxygen atom it is linked to, effectively a small protecting group due to free rotation around the benzyl carbon and the flat sp^2 hybridized ring, versatility in methods of cleavage which includes hydrogenolysis as well as a set of mild reaction conditions.[37,38,39,40]

A number of methods for the generation of benzyl ethers are available. The classical method is the Williamson ether synthesis, which is an S_N2 reaction between an alkali metal alkoxide and benzyl chloride or bromide. Reaction conditions are thus basic as the alkoxide is formed on treatment with a strong alkali metal base, such as NaH. Benzyl bromide can also be used in the presence of silver oxide in DMF for substrates containing acidic protons other than the hydroxy proton.[41] Benzylation under acidic conditions can be accomplished using benzyl trichloroacetimidate catalysed by trifluoromethanesulfonic acid. Nucleophilic cleavage of

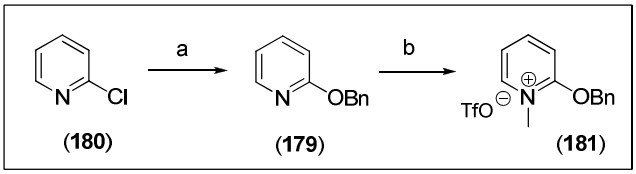
[37] Jobron, L.; Hindsgaul, O. *J. Am. Chem. Soc.* **1999**, *121*, 5835.
[38] Plante, O.; Buchwald, S.L.; Seeberger, P.H. *J. Am. Chem.Soc.* **2000**, *122*, 7148.
[39] Lam, H.; House, S.E.; Dudley, G.B. *Tetrahedron Lett.* **2005**, *46*, 3283.
[40] Dudley. G.B.; Poon, W.C. *J. Org. Chem.* **2006**, *27*, 3923.
[41] Kuhn, R.; Louw, I.; Trishmann, H. *Chem. Ber.* **1975**, *90,* 203.

oxiranes[42] and regioselective benzylidene cleavage[43] also results in the formation of O-benzyl ethers. The latest method developed by Dudley and Poon in 2006, employs 2-benzyloxy-1-methylpyridinium triflate for the benzylation of alcohols under neutral conditions.[40]

3.6.1.4 BENZYLATION OF ALCOHOLS UNDER NEUTRAL CONDITIONS USING DUDLEY'S REAGENT

The investigation by Dudley and Poon was prompted by the observation that 2-alkoxypyridinium bromides decompose to form bromoalkanes and pyridones and that 2-alkoxypyridinium sulfonates do not decompose spontaneously to the alkyl sulfonates.[40] Therefore, if an imidate substitute can be irreversibly activated by alkylation in the same manner that protonation activates benzyl trichloroacetimidate (**Scheme 45**), benzylation can be affected without the need for added base or acid i.e. under neutral conditions. 2-Benzyloxypyridine (**179**), was used as the imidate substitute in the hope that decomposition of the 2-alkoxypyridinium sulfonates would produce the alkyl ether and pyridone.[40]

Dudley's reagent (Bn-OPT) was synthesized from benzyl alcohol and coupled with 2-chloropyridine (**180**) to afford 2-benzyloxypyridine (**179**) (**Scheme 43**). Irreversible covalent activation was effected using methyl triflate as alkylating agent. The white crystalline 2-benzyloxy-1-methyl-pyridinium triflate, (**181**), (Bn-OPT) is stable and can be stored for months without loss in reactivity.[40]



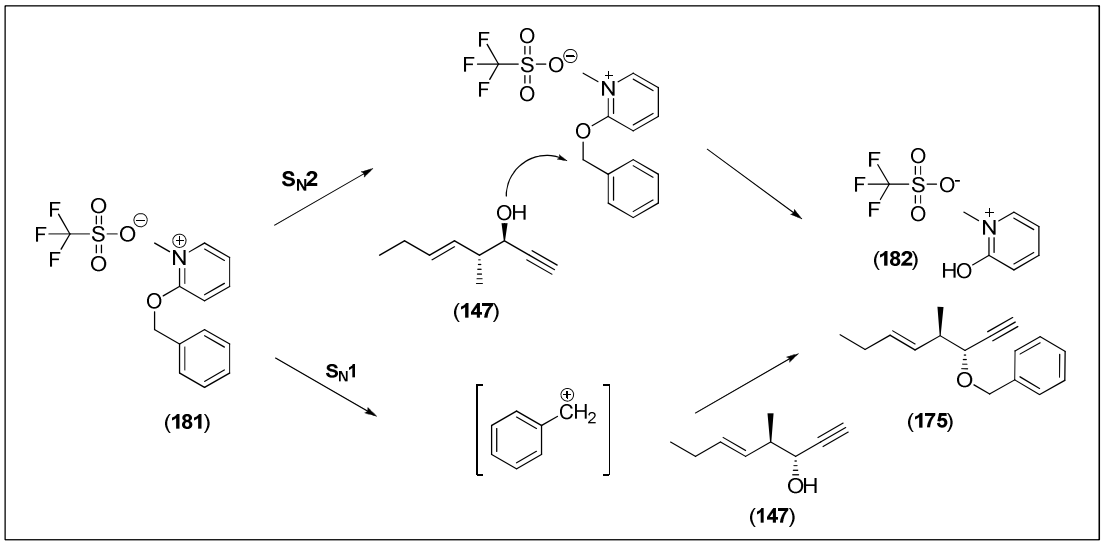
Scheme 43: Synthesis of Dudley's reagent.

Reagents: a. KOH, BnOH, 18-crown-6, toluene (96%); b. MeOTf, toluene (91%).

The *N*-methyl hydroxypyridinium triflate (**182**) formed in the benzylation reaction is mildly acidic and magnesium oxide (MgO) was used as acid scavenger. The benzylation reaction also produced two byproducts *viz*. *N*-methylpyridone (the conjugate base of *N*-methyl hydroxypyridinium) and dibenzyl ether (Bn_2O). Aqueous extraction rids the product mixture of the soluble pyridone, while the dibenzyl ether had to be separated out. Dudley and Poon[40] had trouble separating the benzyl ether from many of the target products formed during their investigation. Bn-OPT has limited solubility and subsequent solvent screening pointed to

[42] Posner, G.H.; Rogers, D.Z.; *J. Am. Chem. Soc.* **1977,** *99*, 8208.
[43] Curtis, N.R.; Holmes, A.B.; Looney, M.G. *Tetrahedron Lett.* **1992**, *33*, 671.

2,2,2-trifluorotoluene as solvent. Added advantages of Bn-OPT included low-cost, moderate volatility (bp 100-103 ºC) and it is seen as environmentally friendly. The success of this protocol is also determined by tolerance of sensitive functionalities contained in a multifunctional compound. The benzylation of a primary alcohol in the presence of a primary silyl ether group occurred without loss of the silyl protecting group.[40]



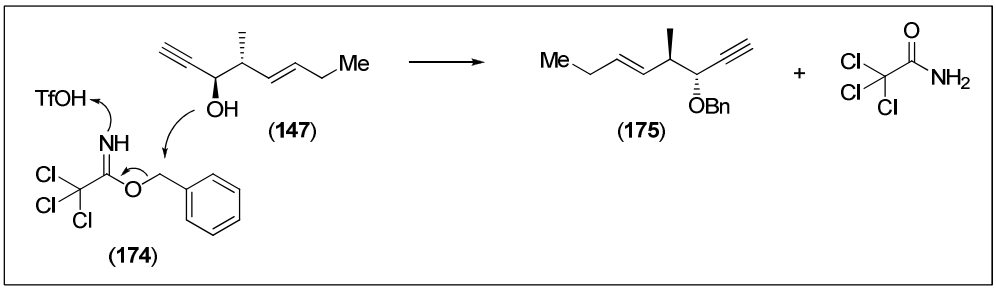
Scheme 44: Mechanistic interpretation of *O*-benzyl protection using Dudley's reagent.

The reaction mechanism is thought to lie somewhere between an S_N1 and S_N2 type reaction (**Scheme 44**). The S_N1-type reaction mechanism was favoured after finding 1-benzyl-2-methylbenzene (*ortho* product) and 1-benzyl-4-methylbenzene (*para* product) as byproducts in a reaction performed using toluene as solvent. These products were assumed to come from a Friedel-Crafts alkylation of toluene, indicating the formation of a benzyl cation, which in turn, favours an S_N1 pathway. The lack of reactivity when using the methoxypyridium triflate salt also points away from an S_N2-type mechanism.[40]

3.6.1.5 BENZYLATION OF ALCOHOLS UNDER ACIDIC CONDITIONS USING TRICHLOROACETIMIDATE

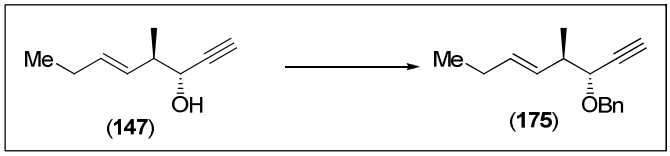
Benzyl trichloroacetimidate (**174**), is a convenient reagent for the benzylation of hydroxy groups under acidic conditions.[44] Imides, esters and acetal protecting groups are compatible with the reaction conditions employed. Formation of the benzylating agent is afforded by base catalyzed addition of benzyl alcohol to trichloroacetonitrile. The benzylation reaction mechanism of the alcohol substrate (**147**) is illustrated in **Scheme 45**.

[44] Wessel, H-P.; Iversen, T., Bundle, D.R. *J. Chem. Soc., Perkin Trans.1* **1985**, 2217.



Scheme 45: Mechanistic interpretation of *O*-benzylation under acidic conditions.

3.6.1.6 EXPERIMENTAL DISCUSSION



Scheme 46 Benzylation of **147** under acidic conditions.

Reagents: Benzyl trichloroacetimidate, TfOH, C_6H_{12}-DCM (7:1) (73%).

Protection of the secondary alcohol (**147**) as the benzyl ether, was initially attempted using Dudley's reagent.[40] The yield obtained using this approach was disappointingly low. Dibenzyl ether (Bn_2O) formed as byproduct which was difficult to separate from the generated product (**175**). This route was therefore abandoned.

Benzylation of alcohols under acidic conditions using trichloroacetimidate (**174**) was investigated next. Benzyl-2,2,2-trichloroacetimidate was prepared from benzyl alcohol and trichloroacetonitrile in anhydrous Et_2O using NaH as base in 81% yield. The ^{1}H NMR spectrum showed the characteristic NH signal at δ_H 8.45. The reagent was used without further purification and could be stored at 4 ºC under an atmosphere of argon for later use.

A 7:1 mixture of anhydrous cyclohexane and DCM was used as solvent. A minimum amount of DCM should be used and can be adjusted depending on the polarity of the substrate. Two equivalents of benzyl-2,2,2-trichloroacetimidate were added per hydroxyl group in the substrate (**147**). The mechanism of the reaction is explained in **Scheme 45**. The *O*-benzyl ether (**175**) was obtained in 73% yield. The aromatic proton signals were present as a multiplet at δ_H 7.33-7.36 and the benzylic protons as an AB-spin system, with $^2J_{ab}$ 12.1 Hz at δ_H 4.81 and 4.50. The corresponding benzylic carbon signal appeared at δ_C 70.45T in the ^{13}C NMR spectrum.

The benzylation under basic conditions was not considered due to the acidity of the acetylenic proton (H-1).

Different protecting group strategies are discussed in **Section 3.8**.

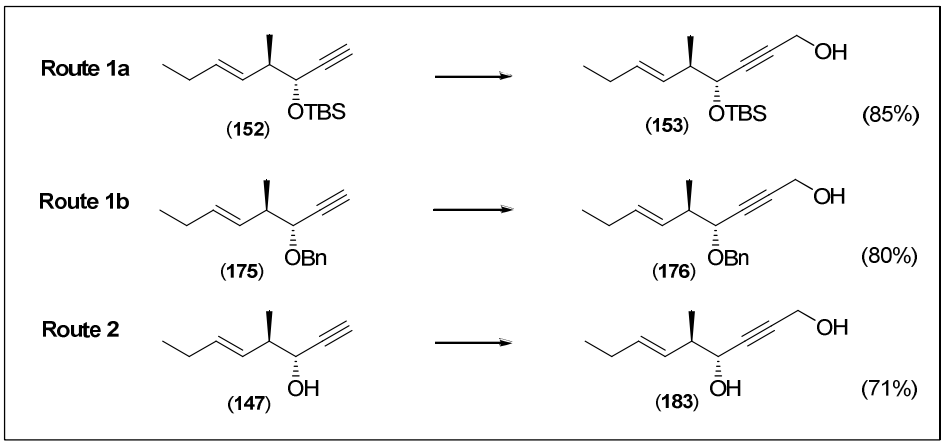
3.7 HYDROXYMETHYLATION

3.7.1 THEORETICAL ASPECTS

Formaldehyde, a gas with bp −21 ºC, is a handy reagent for the synthesis of primary alcohols and is available as the solid paraformaldehyde, a polymer with n=8. Gaseous formaldehyde can be obtained from paraformaldehyde by depolymerization or 'cracking' through heating. Repolymerization and consequential deposition of 'cracked' formaldehyde causes uncertainty regarding the stoichiometry of the reaction and paraformaldehyde is therefore used in considerable excess.[45]

The hydroxymethylation reaction is a nucleophilic acyl addition reaction to the carbonyl carbon to form an alkynyl alcohol. The carbon nucleophile in this case is an acetylide, which is formed by removal of the acidic hydrogen (pK_a ~25) by a suitable base such as one of the following bases: sodium hydride, potassium amide, sodium amide, lithium dialkyl amides, organolithium reagents and Grignard reagents.[46]

3.7.2 EXPERIMENTAL DISCUSSION



Scheme 47: Hydroxymethylation reactions performed.

Reagents: *n*-BuLi, $(H_2CO)_n$, THF.

[45] Gilman, H.; Catlin, W.E. *Org. Synth.* **1941,** *Coll. Vol. 1,* 188.
[46] Kříz, J.; Beneš, M.J.; Peška, J. *Tetrahedron Lett.* **1965**, *6*, 2881.

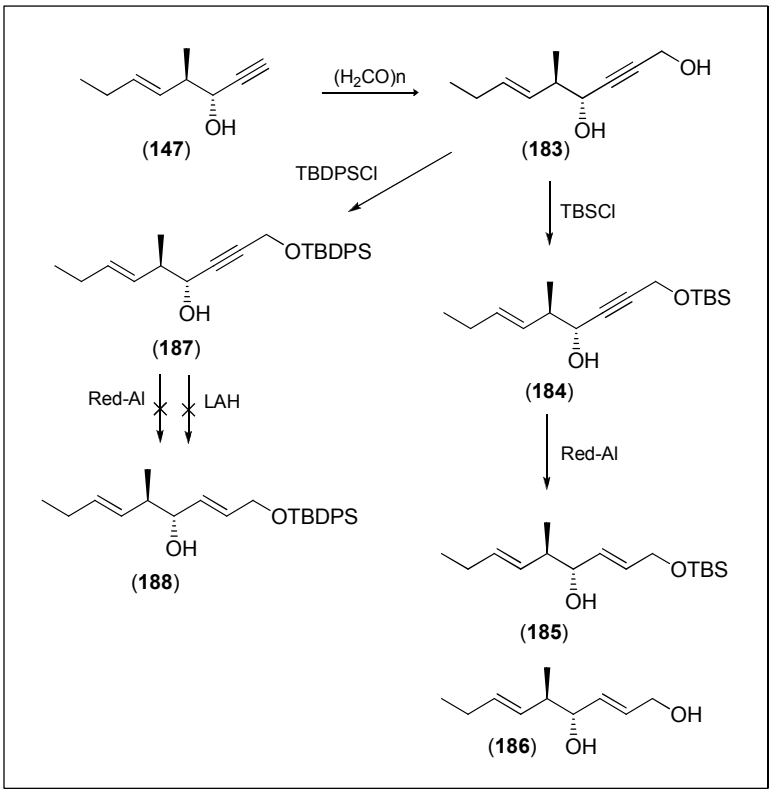
A procedure by Gilman and Catlin,[47] in which dried paraformaldehyde was cracked in a separate flask by heating to 180-200 ºC, was attempted at first. The flask containing the formaldehyde was connected with a tube to a second flask containing the acetylide solution. Gaseous formaldehyde was bubbled through the acetylide solution *via* a gas inlet using argon as carrier gas. This method proved technically difficult as the gas inlet had to be unclogged frequently as the formaldehyde repolymerized and deposited on every possible surface.

Another procedure involved the direct addition of the dried and finely ground paraformaldehyde to the solution containing the acetylide at 0 ºC. The acetylide anion was formed first by abstracting the acetylenic proton using *n*-BuLi as base at −78 ºC. Hydroxymethylation of the *O*-TBS protected substrate (**152**) required one equivalent of base to form the anion, while the *O*-Bn protected (**175**) and the unprotected substrate **147** required two equivalents due to the presence of the benzylic protons and the hydroxy proton, respectively. Formation of the anions could be seen by an observable colour change in the different reaction mixtures. A change in colour from yellow to orange indicated the abstraction of the most acidic proton to form an anion in all the substrates (**152**, **147** and **175**). A further colour change, from orange to wine red, was observed for substrates **147** and **175**, when the second acidic proton was abstracted forming the di-anion. At this stage the solution was allowed to warm up to 0 ºC at which time the dried, powdered paraformaldehyde was added in one shot. Formation of the hydroxymethylated products could be seen by reversal of the mentioned colour changes in 85%, 80% and 71% yields for **153**, **176** and **183,** respectively.

Formation of the hydroxymethylated product (**153**) was confirmed by the absence of an acetylenic proton signal present in the ^{1}H NMR spectrum of **152** at δ_H 2.34 and the appearance of the methylene protons and –OH proton signals at δ_H 4.27 and 1.59, respectively. The ^{13}C NMR spectrum confirmed the formation of the quaternary *sp* carbon (C-2) by the appearance of a signal at δ_C 86.39S. The signal at δ_C 51.38T was assigned to the C(1) methylene carbon. The corresponding signals for the hydroxymethyl group in the ^{1}H and ^{13}C NMR spectra appeared at δ_H 4.33 (H-1), and δ_C 83.58S (C-2) and δ_C 51.91T (C-1), respectively, for **176** and δ_H 4.28 (H-1), and δ_C 85.19S (C-2) and at δ_C 50.87T (C-1), respectively, for **183.**

3.8 ROUTE 2

[47] Gilman, H.; Caltlin, W.E. *Org. Synth.* **1941**, *Coll. Vol. 1,* 188*;* **1926**, *6,* 22.



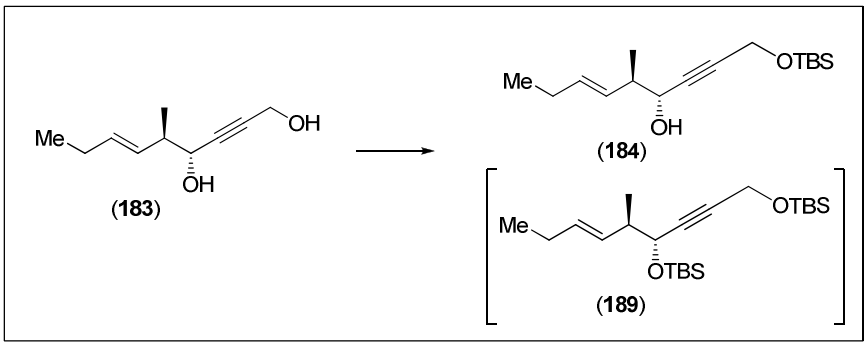
Scheme 48: Schematic outline of **Route 2.**

3.8.1 PROTECTION OF PRIMARY ALCOHOL USING *t*-BUTYLDIMETHYLSILYL ETHER

The second approach towards the target molecule as stated in **Section 3.6** explored protection of the primary hydroxyl group present after hydroxymethylation of the [2,3]-Wittig rearrangement product to give **183** (**Scheme 47**). This approach would eventually set the Sharpless asymmetric epoxidation up to be directed by a secondary allylic alcohol. The first choice in protecting group was once again the TBS group. Silylation of primary alcohols in the presence of secondary alcohols has been reported.[36] Reactions carried out in the presence of DMAP and Et_3N using a diol containing a primary and secondary hydroxyl group as substrate, demonstrated preferential formation of the monosilylated product, while the same reaction carried out using the imidazole procedure only showed a slight preference for the monosilylated product.

3.8.2 EXPERIMENTAL DISCUSSION

The procedure described by Chaudhary[36] delivered disappointing results. The reaction was performed at 0 ºC with one equivalent of TBS-Cl and monitoring the progress of the reaction by TLC. The monosilylated product (**184**) did form first as predicted, but the diprotected product (**189**) formed before the starting material was consumed. A *ca*. 2:1 mixture of the



Scheme 49: Protection of **183** as the *O*-TBS ether.

Reagents: TBS-Cl, Et_3N, DMAP (67%)

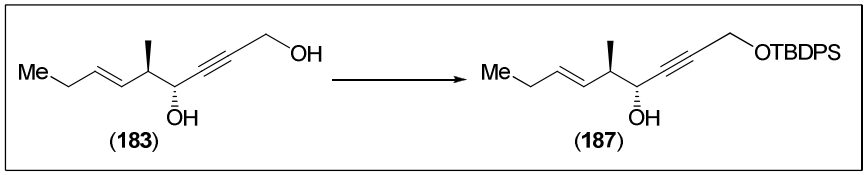
mono- and di-silyl ethers formed. The monosilyl ether (**184**) was isolated and used in subsequent steps. To circumvent formation of the disilyl ether, the reaction was repeated but at a temperature of −15 ºC for 16 hours. Performing the reaction at this temperature prevented formation of the unwanted product. Formation of the monosilylated product was confirmed by comparing the ^{1}H NMR spectra of the unprotected (**183**), and monosilylated product (**184**). The signal of the methylene protons shifted slightly downfield from δ_H 4.28 (**183**) to δ_H 4.34 and of course, the presence of a single set of the characteristic peaks of the –TBS group. This protecting group strategy was abandoned due to complications encountered at a later stage in the synthesis (**Section 3.9.3**). It was decided to use the more sterically demanding and stable *t*-butyldiphenylsilyl (TBDPS) protecting group instead, which should bring about a shorter reaction time and a significant reduction in the likelihood of forming the bis-protected product.

3.8.3 PROTECTION OF PRIMARY ALCOHOL AS *t*-BUTYLDIPHENYLSILYL ETHER

Hanessian and Lavallee[48] first reported the use of the *t*-butyldiphenylsilyl group (TBDPS) as selective protecting group of primary alcohols in 1975. TBDPS outweighs TBS regarding stability towards a wide range of reagents, reaction conditions and selectivity in introduction and removal. Due to the sheer bulk of TBDPS, the reagent effectively discriminates between primary and secondary alcohols, introducing a means of selectively protecting primary alcohols. Formation of TBDPS ethers is accomplished by either treating the primary alcohol with TBDPS-Cl in DMF or DCM with imidazole or Et_3N as base and a catalytic amount of DMAP. An alternative method employs TBDPSOTf with 2,6-lutidine as base.[49] Cleavage of TBDPS ethers can also be achieved by using TBAF, NaF, HF.pyridine or HF.Et_3N as fluoride ion source in aprotic medium.

[48] Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *53*, 2975.
[49] Grieco. P.A.; Henry, K.J.; Nunes, J.J.; Matt, J.E. *J. Chem. Soc., Chem. Commun.* **1992**, 368.

3.8.4 EXPERIMENTAL DISCUSSION



Scheme 50: Protection of the primary hydroxy group in **183** as the TBDPS ether (**187**).

Reagents: TBDPS-Cl, Et_3N, DMAP, DCM (69%).

Protection of the primary hydroxyl group in the diol (**183**) as the TBDPS ether was attempted next. The reaction proved to be extremely selective, protecting exclusively the primary hydroxyl group. The reaction was complete in 2 hours and delivered the mono-protected TBDPS-ether (**187**) as only product in 69% yield. The presence of the TBDPS group was evident from the ^{13}C and ^{1}H spectra. The signals for the *t*-butyl group appeared at δ_C 18.99S and 26.66Q in the ^{13}C NMR spectrum and at δ_H 1.07 in the ^{1}H NMR spectrum.

3.9 STEREOSELECTIVE REDUCTION OF PROPARGYL ALCOHOL

The next step in the synthetic sequence, required the stereoselective reduction of the propargylic alcohols **153**, **176**, **184** and **187** to deliver the respective *E*-allylic alcohols. The allylic systems can then serve as templates for the Sharpless asymmetric epoxidation reaction in the synthetic route to deliver an epoxide with the desired stereochemistry in place. The *E*-allylic alcohol is required, since it has been shown by Katsuki *et al.* [50] that the *Z* diastereomer is a poorer substrate which results in a diminished yield, slower reaction rate and lower selectivity in most instances.

In this section, different means to accomplish stereoselective reduction of alkynes to both the *E*- and the *Z*-alkenes are briefly discussed.

3.9.1 THEORETICAL ASPECTS

3.9.1.1 LINDLAR CATALYST

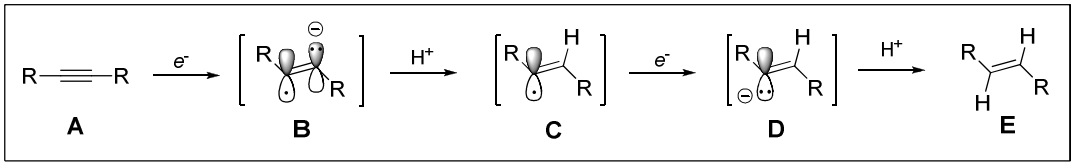
Addition of hydrogen atoms to alkynes can produce either *E*- or *Z*-alkenes. The *Z* specific reduction can be accomplished using H_2 and Lindlar catalyst (Pd-$CaCO_3$, $Pb(OAc)_2$)) to control chemoselectivity in reducing alkynes to alkenes. The two hydrogen atoms add in a *syn* fashion producing a *Z*-alkene. The *E* selective reduction can be accomplished using sodium in

[50] Katsuki, T.; Lee, A.W.M.; Ma, P.; Martin, V.S.; Masamune, S.; Sharpless, K.B.; Tuddenham, D.; Walker, F.J. *J. Org. Chem,* **1982**, *47*, 1373.

liquid ammonia, LAH or Red-Al (**Section 3.9.1.3** and **3.9.1.4**). LAH and Red-Al require the substrate to have a hydroxy or ether functionality in close proximity of the alkyne as complexation to the oxygen atom delivers the reducing species to the triple bond.

3.9.1.2 METAL REDUCTION

One of the main uses of metal reduction is the conversion of alkynes to *E* alkenes (**Scheme 51**). The metal here refers to sodium metal in liquid ammonia ($Na/NH_3(\ell)$). A one-electron transfer to alkyne **A** initiates the reaction by forming a radical anion **B**. Repulsion between the lone electron in one p orbital and the carbanion electrons in the other p orbital forces them to be as far away from each other as possible thus giving rise to the observed *trans* geometry. Protonation produces the vinyl radical **C,** which after a second one-electron transfer again forms a carbanion **D**, which on protonation generates the final *trans* hydrogenated allylic alcohol **E**. The percentage *cis* alkene increases on using protic solvents such as ethanol.



Scheme 51: Mechanistic explanation of the metal reduction of alkynes.

3.9.1.2.1 LITHIUM ALUMINUM HYDRIDE

Studies on the reduction of cinnamic acid and cinnamaldehyde by Nystrom and Brown[51] and Hochstein and Brown[52] using LAH, made the researchers consider the possibility of partial or complete reduction of acetylenic compounds, using the same reducing agent. The reduction of propargylic alcohols to *E*-allylic alcohols using LAH as reducing agent has since become an established synthetic method.

Stereoselective reduction of propargylic alcohol systems using LAH has been shown to proceed by a site-specific hydride transfer mechanism. Studies by Corey *et al.*[53], however, showed that this was not the case for certain substrates such as 2-alkyn-1-ols. The reaction with phenyl substituted substrates on the other hand, proceeded by site-specific hydride attack which was ascribed to the directive influence of the phenyl substituent attached to the C(3) carbon. The stereoselective reduction is therefore also structure sensitive.

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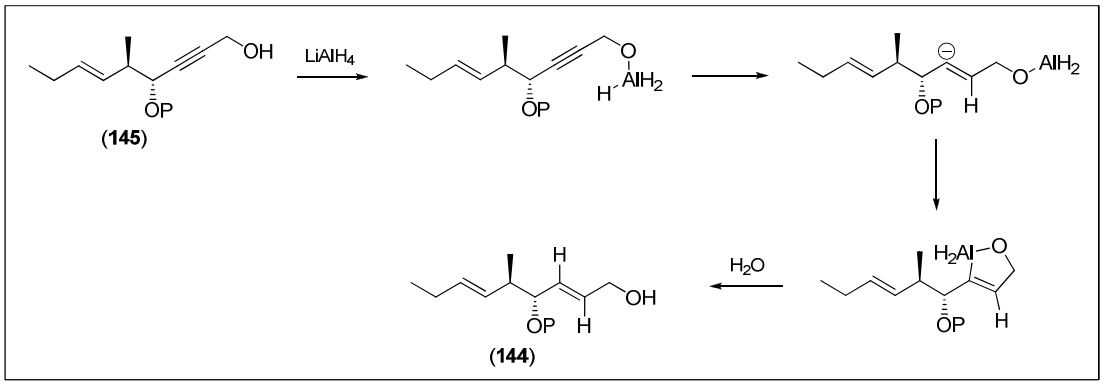
[51] Nystrom, R.F.; Brown, W.G. *J. Am. Chem. Soc.* **1947**, *69*, 1197.
[52] Hochstein, F.A.; Brown, W.G. *J. Amer. Chem. Soc.* **1948**, *70*, 3483.
[53] Corey, E.J.; Katzenellenbogen, J.A.; Posner, G.H. *J. Am. Chem. Soc.* **1967**, *89,* 4245.

The hydroxyl group adjacent to the triple bond facilitates reduction by binding to the aluminium atom contained in the reducing agent, contributing to the substrate specificity mentioned above. Hydride transfer occurs from the aluminum to the C(2) carbon. Subsequent hydrolysis then delivers the *trans* allylic alcohol (**Scheme 52**).



Scheme 52: Mechanistic explanation of alkyne reduction using LAH.

Studies of solvent effects on the stereoselectivity indicated that there exists a reciprocal relationship between the Lewis basicity of the solvent and the proportion of the *cis* reduction product obtained in substrates with a secondary carbon at C(2): Djerassi and Grant[54] reported a ratio of 3:1 in favour of the *cis* isomer on using isopropyl ether as solvent. *Cis* reduction indicates that intramolecular stabilization of the carbanion by the bound aluminum does not occur.[54] The degree of solvation of the Lewis acid in the reaction medium plays an integral role. Lewis acids, in this case Li^+, act as counterions and so stabilize the carbanion formed. Weaker Lewis base solvents, *e.g*. Et_2O, solvate the counterions to a lesser extent. The counter ions are thus available in a higher concentration to stabilize the carbanion, resulting in the formation of the *trans* product. The opposite is true for strong Lewis base solvents, *e.g*. THF, where the counterions are not as readily available for stabilization of the anionic sites. The molecule therefore adopts a configuration with the greatest separation of these sites, and the result being formation of the *cis* product. The ability of the solvent to solvate Lewis acids is therefore inversely related to the proportion of *cis* product formed. It was also found that the addition of a crown ether to a strong Lewis base solvent, increases the percentage *trans* reduction, strengthening the proposed mechanism. Lowering the temperature of the reaction was also found to favour formation of the *trans* product.[54]

3.9.1.2.2 SODIUM *bis*-METHOXYETHOXYALUMINUM DIHYDRIDE (Red-AL®)

[54] Grant, B.; Djerassi, C. *J. Org. Chem.* **1974**, *39,* 968.

The name Red-Al is a trademark of Sigma-Aldrich. The reducing power and reactivity profile of Red-Al (**Figure 13**) is comparable to that of LAH. Properties that give Red-Al an advantage over the use of LAH is its thermal and atmospheric stability and its solubility in Et_2O and aromatic hydrocarbon solvents.

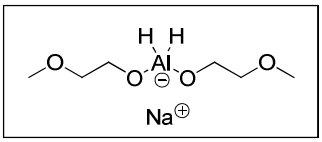
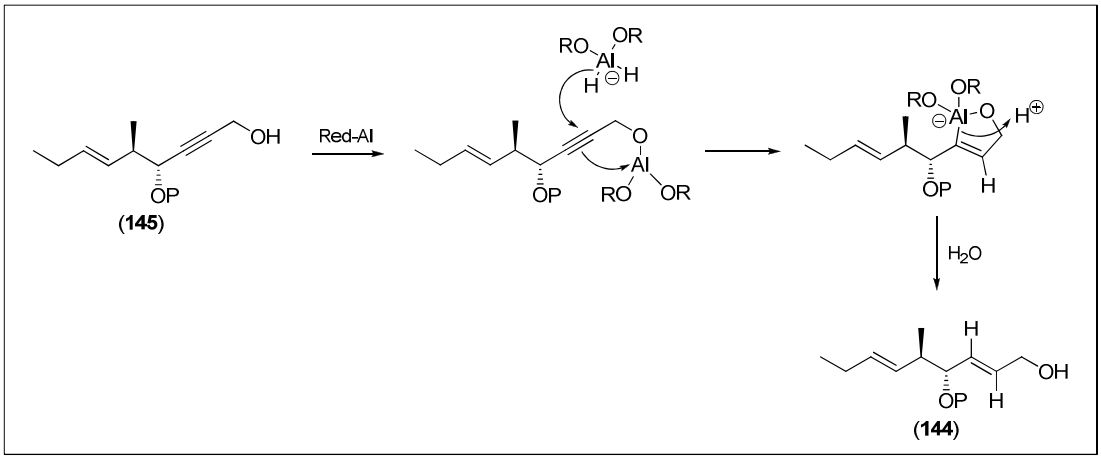


Figure 13: Structure of Red-Al.

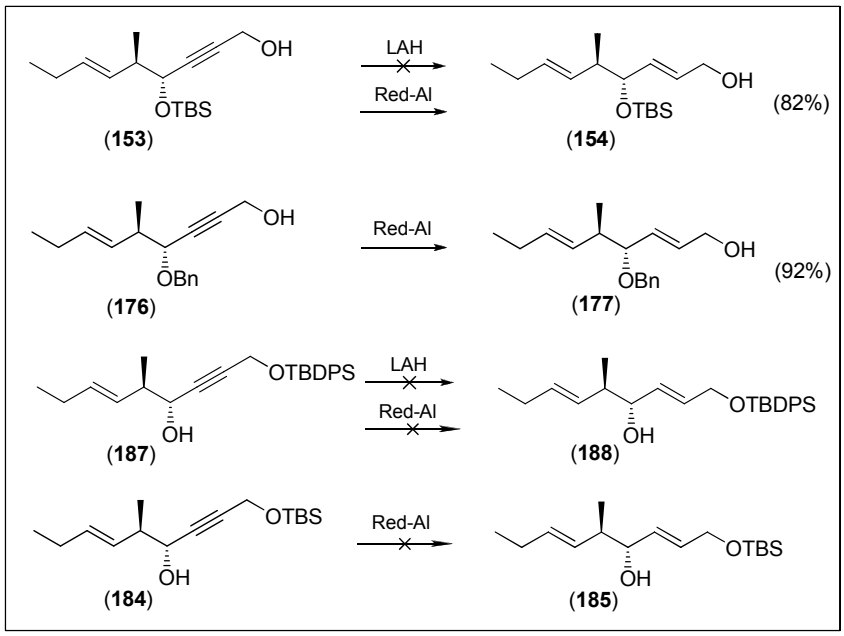
The mechanistic explanation of *trans* hydroalumination is very similar to that of LAH (**Scheme 53**). The hydroxyl group adjacent to the triple bond again facilitates reduction by binding to the aluminium atom contained in the reducing agent. External nucleophilic attack from another Red-Al molecule provides the first hydride ion at C(2) during the hydroalumination process. Subsequent hydrolysis then results in the formation of the *trans* allylic alcohol.



Scheme 53: Mechanistic explanation of alkyne reduction using Red-Al

3.9.3 EXPERIMENTAL DISCUSSION

Reduction of the triple bond of substrate (**153**) was first attempted using LAH as reducing agent. This proved to be a futile exercise as removal of the TBS protecting group proceeded at the same rate as reduction of the triple bond to produce an unwanted diol (**186**) (see **Scheme 48**). The reduction of the propargylic alcohol (**153**) with Red-Al using Et_2O as solvent to promote formation of the *trans* product as described earlier proceeded cleanly to give the *E* allylic alcohol (**154**) in 82% yield. Similarly the reduction of the allylic alcohol (**176**) gave once again only the *E* allylic alcohol (**177**) in 92% yield.



Scheme 54: Attempted alkyne reduction of substrates **153**, **176**, **180** and **182**.

The ^{13}C NMR spectrum of the *E* allylic alcohol (**154**) showed the signals for the newly-formed olefinic carbon atoms, C(2) and C(3), at δ_C 129.48D and 133.18D, respectively. The ^{13}C NMR spectrum also lacked the signals present at δ_C 82.97S (C-3) and 86.39S (C-2) assigned to the acetylene carbon atoms in the starting material (**153**). In the ^{1}H NMR spectrum of **154** the signals of the newly-formed olefinic protons appeared at δ_H 5.72 (H(2)) and 5.62 (H(3)) with a value of 15.4 Hz for one of the coupling constants which established the *E* configuration for this double bond. Analysis of the ^{1}H and ^{13}C NMR spectra of the reduction product (**177**) showed the signals for the newly-formed olefinic carbon atoms, C(2) and C(3), at δ_C 133.77D and 130.27D and the corresponding protons at δ_H 5.78 (H-2) and 5.60 (H-3) with a 15.6 Hz coupling constant.

Red-Al reduction did result in reduction of the alkyne functionality in the monosilylated substrate **184**, but also resulted in the deprotection of the *O*-TBS group to give a diol (**190**). This result led to a change in the protecting group strategy and the use of the TBDPS protecting group (see **Section 3.8.2**).

Reduction of the alkyne in the *O*-TBDPS protected substrate (**187**)**,** using both LAH and Red-Al failed. In the Red-Al reduction experiments, only starting material was recovered. This was ascribed to the sheer bulk of the protecting group which prevented complexation of the secondary alcohol to the aluminium. Modeling studies were carried out to test the plausibility of this proposal. Geometry optimization of **187** was done on Gaussian 03 using the Hartree

Fock method and 6-31G as the basis set. **Table 1** describes the colour codes used for the respective atoms in **Figure 13** and **Figure 14.**

Table 1: Colour coding of atoms in **Figure 13** and **Figure 14**

Light blue	Silicon
White	Hydrogen
Gray	Carbon
Red	Oxygen

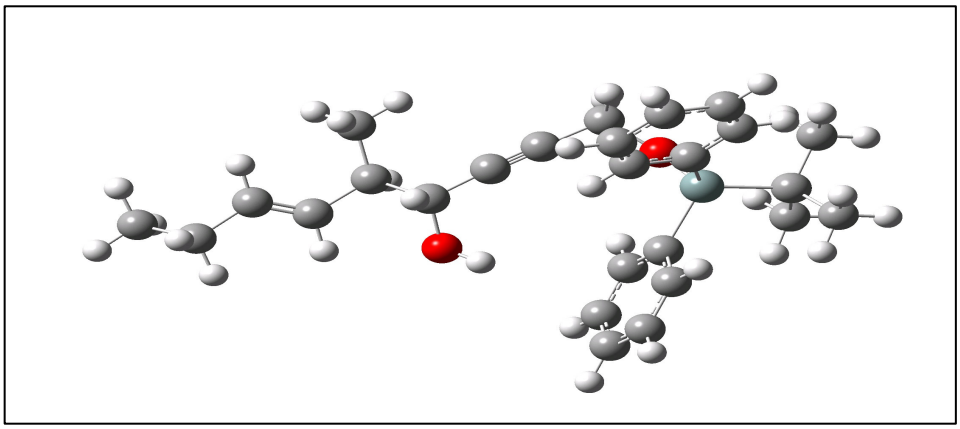


Figure 13: A ball representation of the geometry optimised **187** in GaussView.

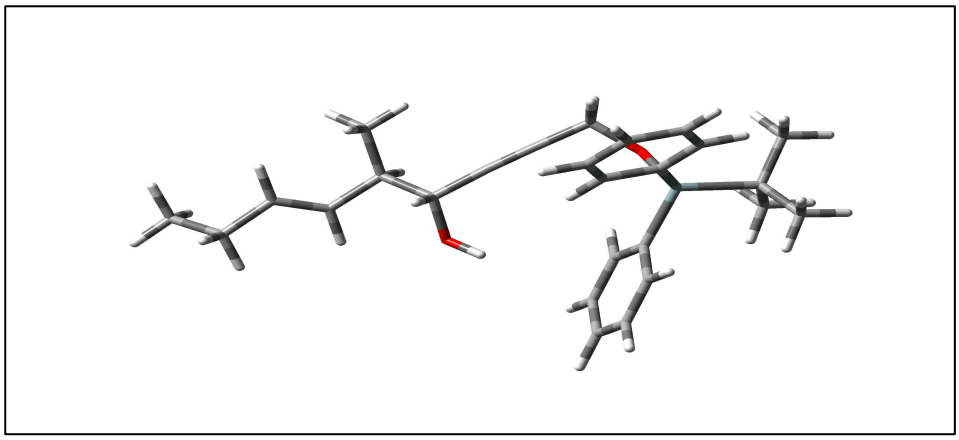


Figure 14: A tube representation of the geometry optimised **187** in GaussView.

The modeling studies revealed that the phenyl groups contained in TBDPS bend downwards shielding the secondary hydroxyl group at C(4), when the geometry of the molecule is optimized. This most probably prevents aluminium from complexing with the C(4) hydroxyl group thereby preventing the subsequent reduction.

The attempted LAH reduction of **187** resulted in the deprotection of the *O*-TBDPS ether to form **183**. The reaction progress was monitored by TLC and ^{1}H NMR. TLC confirmed that the *O*-TBDPS ether was being deprotected, while the ^{1}H NMR spectra indicated the absence of any sign of alkene formation as no additional signals were present in the 5.00 - 6.00 ppm region of the ^{1}H NMR spectrum.

The *E*-allylic alcohol motif present in both **154** and **177** meet the structural requirements for the introduction of an epoxide functional group with the requisite stereochemistry using the Sharpless asymmetric epoxidation methodology.

Short descriptions of the most common approaches to form epoxides are given in the following section. Sharpless asymmetric epoxidation was used in this work and is discussed in more detail in **Section 3.10.4**.

3.10 EPOXIDATION REACTIONS

Epoxidation reactions are indispensable tools in organic synthesis: Two neighbouring stereogenic centers are formed in one step and stereoselective opening of the epoxide ring provides the chemist with a 1,2 functionality.

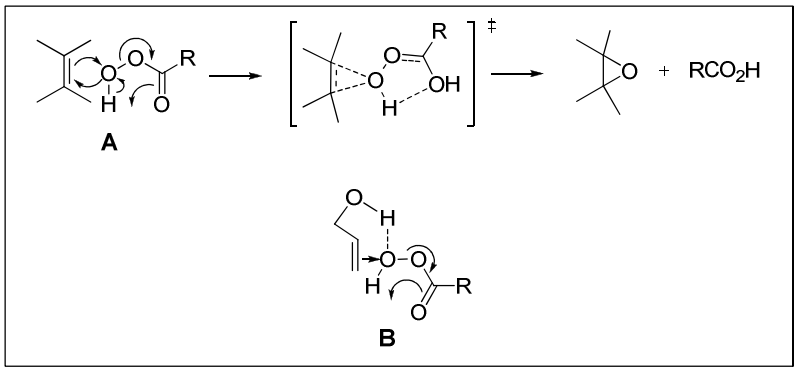
Numerous synthetic methods for the preparation of epoxides exist. Epoxides can be formed by either nucleophilic or electrophilic peroxide reagents,[55] the Darzens reaction[56] and by condensation of carbonyl compounds with ylides of certain main group elements.[57-58]

3.10.1 PERACID EPOXIDATION

Prileschajew discovered the epoxidation of alkenes using peracids in 1909.[59] In these reactions the peracid fulfills the role of the electrophile and the alkene that of the nucleophile. Although this seems illogic, it has been backed experimentally: Increasing electron density of the alkene or decreasing electron density of the peracid increases the reaction rate. Placement of an electron withdrawing group *e.g.* a methyl ether, in the allylic position of the olefin containing substrate, decreases the reaction rate, whereas a hydroxyl group in the same position increases the reaction rate, although still slower than the simple olefin.[60] A

[55] Berti, G. *Top. Stereochem.* **1973**, *7*, 93.
[56] Gladiali, S.; Soccolini, F. *Synth. Commun.* **1982**, *12*, 355.
[57] Still, W.C.; Novack, V.J. *J. Am. Chem. Soc.* **1981**, *103*, 1283.
[58] Corey, E.J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.
[59] Prileschajew, M. *Chem. Ber.* **1906**, *42*, 4811.
[60] Henbest, H.B.; Wilson, R.A.L. *J. Chem. Soc.* **1957**, 1958.

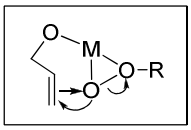
mechanistic explanation of the epoxidation of both an olefin (**A**) and an allylic alcohol (**B**) are given in **Scheme 55**.



Scheme 55: Peracid epoxidation of allylic alcohol and olefin systems.

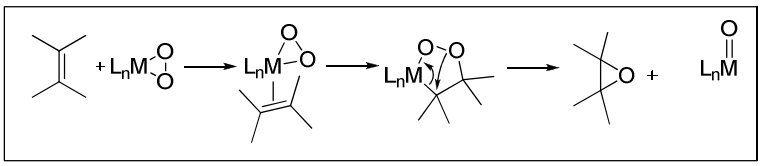
3.10.2 TRANSITION METAL HYDROPEROXIDE EPOXIDATION

This epoxidation reaction proceeds by nucleophilic attack by the olefin on the hydroperoxide. The hydroperoxide, in turn, is activated by covalent bonding to the metal centre *via* the oxygen atom furthest from the alkyl group.[61] Both the allylic alcohol and the peroxide are therefore bound to the d^0 transition metal centre during epoxidation. A mechanistic interpretation by Sharpless and Chong[61] is given in **Scheme 56**.



Scheme 56: Mechanistic interpretation of the transition metal hydroperoxide epoxidation.

3.10.3 PEROXYMETALLATION



Scheme 57: The peroxymetallation epoxidation reaction.

The mechanistic interpretations of these epoxidation reactions stems from the ability of the alkene to act as ligand for the transition metal. The transition metals mostly used are rhodium

[61] Chong, A.O.; Sharpless, K.B. *J. Org. Chem.* **1977,** *42*, 1587.

and platinum. Coordinated alkenes inserted into a metal-peroxo bond result in a five-membered metallocycle which decomposes to deliver an epoxide and carbonyl compound (**Scheme 57**).

3.10.4 SHARPLESS ASYMMETRIC EPOXIDATION

Asymmetric metal-catalyzed epoxidation reactions were already attempted in 1977 by Yamada *et al.*[62] In 1980 Katsuki and Sharpless succeeded in developing an extremely effective method for the asymmetric epoxidation (AE) of primary allylic alcohols.[63] In 1981 Martin *et al.*[64] expanded on this by using the same system for the kinetic resolution (KR) of racemic allylic alcohols.

Epoxidation reactions performed earlier[62,65,66,67], also employed transition metals as catalysts of the type $ML_n(OR)_m$. Depending on the metal used, L represents one to two oxo ligands and $(OR)_m$, two to four alkoxide ligands. An intermediate complex forms and the epoxidation reaction proceeds after covalent bonding between the metal, a hydroperoxide and the allylic alcohol containing substrate.

Titanium(IV), vanadium(V), molybdenum(VI) and tungsten(VI) are commonly used as epoxidation catalysts and are typified by being Lewis acidic, have low redox potentials, exist in d^0 oxidation state, are prone to alkoxide ligand substitution and polymerize as polymeric hydrates on water exposure. What differentiates titanium(IV) from the other metal catalysts is its ability to form four covalent bonds to alkoxide ligands, whereas vanadium(V), molybdenum(VI) and tungsten(VI) bind to one, three and two alkoxide ligands, respectively. The significance of this is that the titanium(IV) can at the same time coordinate with the alkyl hydroperoxide, the allylic alcohol and a bidentate ligand, such as dialkyl tartrates and tartramides in a non-polar organic solvent enabling the successful introduction of asymmetry.[62]

The titanium metal-catalyst exists as a dimer containing two dialkyl tartrates or tartramides as covalently bound ligands. During epoxidation the allylic alcohol substrate and the hydroperoxide can bind to either of the two metal centers. Titanium tetraisopropoxide is the

[62] Yamada, S.; Mashiko, T.; Terashima, S. *J. Am Chem. Soc.* **1977**, *99*, 1988.
[63] Katsuki, T.; Sharpless, K.B. *J. Am. Chem. Soc.* **1980**, *102*, 5974.
[64] Martin, V.S.; Woodard, S.S.; Katsuke, T., Yamada, Y.; Ikeda, M., Sharpless, K.B. *J. Am. Chem. Soc.* **1981**, *103*, 6237.
[65] Michaelson, R.C.; Palermo, R.E.; Sharpless, K.B. *J. Am. Chem. Soc.* **1977**, *99*, 1990.
[66] Kagan, H.B.; Mimoun, H.; Mark, C.; Shurig, V. *Ang. Chem. Int. Ed.* **1979**, *18*, 485.
[67] Tani, K.; Hanafusa, M.; Otsuka, S. *Tetrahedron Lett.* **1970**, *20*, 3017.

most commonly used reagent, but different alkoxides can be used. It has been found that substrates prone to nucleophilic attack e.g. epoxy alcohols, perform better on using a bulkier alkoxide. Ring opening of the formed epoxy alcohols needs to be prevented since the formed diol acts as an inhibitor. Other transition metals of group IV and V have been screened as possible asymmetric epoxidation (AE) catalysts but proved to have a negative effect on either the reaction rate or have the reversed asymmetric inductive effect than that observed for titanium.[62]

Up to date, dialkyl esters of tartaric acid and tartramides have performed best as ligands used in metal catalyzed AE reactions. The most popular tartrate ligands include dimethyl tartrate (DMT), diethyl tartrate (DET) and diisopropyl tartrate (DIPT). Catalyst properties can be modified by either increasing or decreasing the steric bulk of the ester. Bulkier ligands such as DIPT are preferred: It is thought that the formed product is thus protected from ring opening and so prevents inhibition of the catalyst, which in turn, has a positive influence on the reaction rate and percentage completion.

The titanium alkoxide epoxidation catalyst functions best with allylic alcohols as substrates. Increased olefin electron density has proven to increase reaction rates. On the other hand the more reactive the allylic alcohol substrate, the more readily it decomposes.[68] Alkyl substituents at C-2 assist in stabilizing the more reactive epoxides against decomposition. The steric environment surrounding the substrate also exhibits a profound effect on reaction rate. The *Z* allylic alcohols, secondary allylic alcohols and homoallyllic alcohols all show a marked decrease in reaction rate when compared to the corresponding *E* allylic analogues.[50]

Molecular weight measurements in DCM utilizing differential vapour phase osmometry and the isopiestic Signer method, electron impact (EI) mass spectrometry, IR spectroscopy and NMR all point to the catalyst existing as a dimer in solution.[69] Researchers are not excluding the possibility that a minor, catalytically active structure, may be present though. Two main hypothetical structures of the dimer are given in **Figure 15. X** represents a six-coordinate structure and **Y** a five-coordinate structure. Most data collected points to the six-coordinate structure with the bridging oxygens provided by the two tartrate ligands. A fluxional equilibrium, confirmed by NMR spectra at room temperature, proved the two halves of the molecule to be identical. It has been proposed that the fluxional equilibrium goes through the

[68] Finn, M.G.; Sharpless, D.B. *Asymmetric Synthesis"* (J. Morrison, Ed.), Academic Press. Orlando, **1985**, 247.
[69] Clark, E.P. *Ind. Eng. Chem. Anal. Ed.* **1941**, *13,* 820.

ten-membered ring structure **Y** as the transition state. The reaction proceeds on one of the two metal centers which have a local C_2 symmetry.

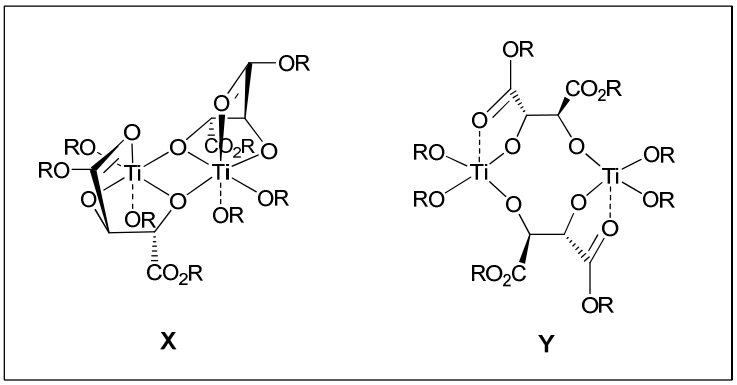


Figure 15: Proposed structures for the titanium catalyst complex in Sharpless asymmetric epoxidation.

On forming the catalyst complex (**Figure 16**), the two non-reactive alkoxide ligands are both replaced by the hydroperoxide which links to the metal centre in a bidentate manner. The oxygen distal to the peroxide alkyl group occupies the equatorial position in order to comply with steric demands imposed by the hydroperoxide. It is proposed that the peroxide oxygen furthest from the alkyl group is the oxygen transferred to the alkene.[61] The allylic hydroxyl group occupies the axial position of the catalyst complex. The alkene group approaches the oxygen atom along the axis of the oxygen-oxygen bond to be broken, with the midpoint of the double bond perpendicular to the oxygen atom furthest from the hydroperoxide alkyl side chain.

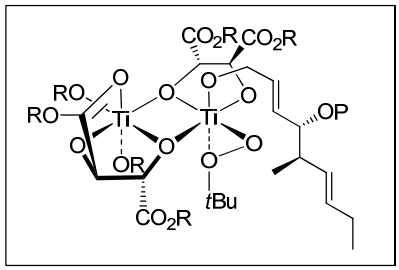


Figure 16: Octahedral titanium coordination geometry of the catalyst complex with either **154** or **177** as allylic alcohol.

Only one of the enantiofaces of the substrate double bond can be presented to the peroxide oxygen-oxygen bond due to steric constraints between the alkene substrate and the tartrate ester (**Figure 16**). Meridonial arrangement of the complex in **Figure 16** gives rise to four conformations shown in **Figure 17.**

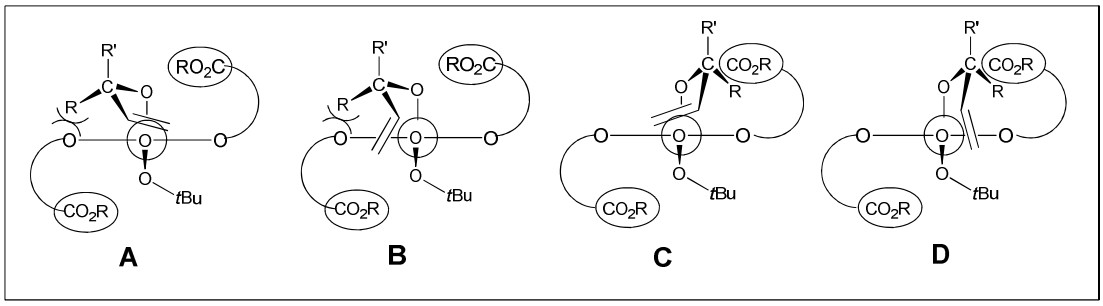
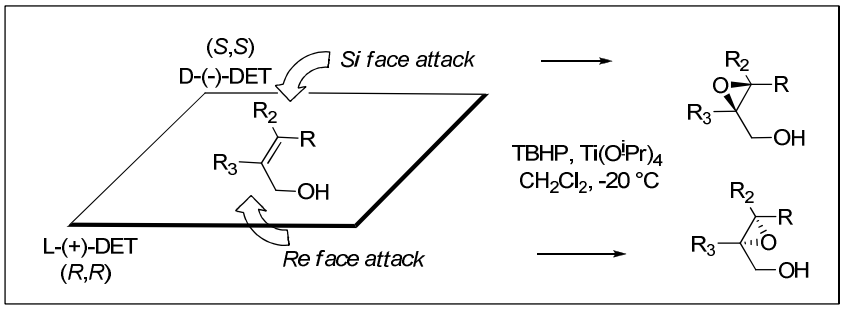


Figure 17 Possible conformers of the meridonial arrangement of the catalyst complex.

A plausible explanation for the specificity in enantiofacial presentation can be due to the substrate's performance as ligand: The two enantiomeric allylic alcohols show different affinities for the metal centre. This explanation is valid when considering steric interactions but does not provide an explanation for the fact that **A** and **B** are preferred over **C** and **D**. Stereoelectronic factors are thought to be the determining factor in **A** being preferred over **B**. Four different explanations have been brought forward: 1) The geometry adopted by **A** in preparation of the S_N2 attack makes it easier for the alkene to reach the peroxide. 2) **A** forms a spiro transition state versus the planar transition state adopted by **B**. The spiro transition state aligns the lone pair of electrons of the transferred oxygen with the π* orbital of the alkene. This transition state leads to the enantiofacial preference observed.[70] 3) In the planar geometry adopted by **B**, the titanium atom is far removed from the lone pair electrons contained by the epoxy oxygen. The structure therefore needs to adopt an unfavourable twisted conformation to accommodate binding of the epoxy alkoxide moiety to titanium. 4) The different allylic alcohol conformations result in the two systems having different dihedral angles (**A** ~ 30º and **B** ~120º). This dihedral angle (C=C-C-O) influences reactivity of the alkene towards the coordinated hydroperoxide. Maximum reactivity is observed when the dihedral angle is ~ 90º.



Scheme 58: The enantiofacial selection rule for AE.

Regardless of which of the above-mentioned explanations provides the true reason for **A** being the favoured transition state it provides a consistent model for explaining and predicting

[70] Sharpless, K.B.; Woodard, S.S.; Finn, M.G. *Pure Appl. Chem.* **1983**, *55,* 1823.

results obtained in AE reactions. It therefore enables the chemist to predict the stereochemical outcome of the reaction. To date no exception has been found regarding the stereofacial selection rule (**Scheme 58**).

3.10.5 EXPERIMENTAL CONSIDERATIONS

REACTION CONDITIONS

It has been shown experimentally that the ratio of titanium to ligand used contributes to the degree of enantioselectivity observed. Optimum results have been obtained with a titanium to ligand ratio of 1:1.2. An excess tartrate negatively affects the reaction rate in that it occupies the catalytic sites on the titanium. The solvent most commonly used is DCM. Coordinating solvents should be avoided, due to an impeding effect on the reaction rate.

TBHP

Commercial grade TBHP is available as a 70% aqueous solution. The water content is much too high for the reagent to be useful as water decreases the reaction rate and reduces enantioselectivity. The water is removed by partitioning between toluene and water and removal of the water from the toluene phase by azeotropic distillation. The hydroperoxide content of the resulting solution is determined by ^{1}H NMR spectroscopy or iodometric titration. The TBHP solution can be stored in a dark glass bottle for extended periods of time without a loss in reactivity. For AE, 1.5-2.0 equivalents of TBHP have proven to lead to optimum results.

TITANIUM REMOVAL

Methods for the removal of the tartrate ligands differ with water-solubility of the formed epoxide. Water-insoluble epoxides can be isolated by using a 10% tartaric or citric acid solution. This extracts the titanium from the organic into the aqueous phase as the acid complex. Iron(II) sulfate is also commonly added to the acid solution and destroys the excess TBHP.

For water-soluble and acid sensitive epoxides, a saturated solution of sodium sulfate can be used to precipitate the titanium, which can then be removed by filtration.

For the removal of titanium from more sensitive epoxides (epoxides prone to ring opening either by nucleophilic or intramolecular attack and also the more water-soluble species) a

non-aqueous method has been developed. Here the reaction mixture is subjected to an Et_2O or Et_2O-acetone solution of citric acid. This results in precipitation of the titanium acid complex.

Another non-aqueous method developed for the isolation of epoxides with a low molecular weight is to reduce the TBHP with Me_2S. The reaction mixture is then treated with triethanolamine which again results in precipitation of the titanium species.

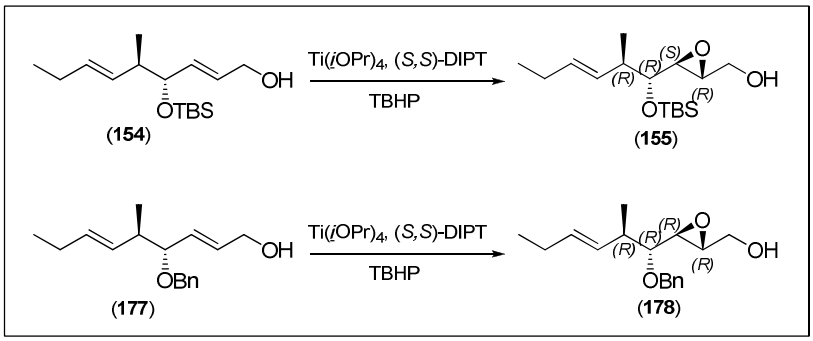
REMOVAL OF TARTRATE ESTERS

The tartrate esters can be removed in three ways depending on reaction scale and volatility of the formed epoxide. If the epoxide is more volatile than the ester ligands, separation can be effected by distillation. For small scale reactions the ligand can simply be removed by column chromatography. Reactions on larger scale require the removal of the ligand by washing the reaction mixture with a brine solution of NaOH. A brine solution is required to minimize the epoxides contact with the NaOH to prevent the Payne rearrangement reaction.[71] This work-up is also important with the more water-soluble epoxides.

TBHP REMOVAL

Usually the TBHP is simply removed by chromatography in small scale reactions, but can also be removed by azeotropic distillation with toluene using rotary evaporation, or by reductive means in larger scale reactions where distillation may be dangerous. Reagents employed include Me_2S, $P(OMe)_3$, aq. Na_2SO_4, aq. $NaHSO_4$ and $NaBH_4$. [72,73,74]

3.10.6 EXPERIMENTAL DISCUSSION



Scheme 3.59: Sharpless asymetric epoxidation of **155** and **178.**

Reagents: $Ti(O^iPr)_4$, *S,S*-DIPT, TBHP, DCM.

[71] Payne, G.B. *J. Org. Chem.* **1962**, *27*, 3819.
[72] Sharpless, K.B.; Verhoeven, T.R. *Aldrichimica Acta,* **1979**, *12*, 63.
[73] Bessodes, M.; Abushanab, E.; Antonakis, K. *Tetrahedron Lett.* **1984**, *25*, 5899.
[74] Pickenhagen, W.; Brönner-Schindler, H. *Helv. Chim. Acta,* **1984**, *67,* 947.

The allylic alcohol (**154**) synthesised in **Route 1a** was now subjected to the Sharpless AE reaction using *S,S*-DET in order to ensure attack on the *Si*-face of the allylic alcohol to produce the (2*R*,3*S*) epoxide (**155**). An important experimental consideration is aging of the catalyst complex. In every AE epoxidation reaction, twenty minutes was allowed for the complex to form at −15 ºC prior to addition of the oxidizing agent. Stereofacial selection proved disappointingly low with a (2*R*,3*S*):(2*S*,3*R*) ratio of 3:1. At first it was thought that the TBS protecting group was too bulky and prevented formation of the catalyst complex, but modeling studies showed no interference of the protecting group with formation of the catalyst complex.

Keeping the idea of protecting group bulkiness in mind, the protecting group was changed to a benzyl ether (**177**) (**Route 1b**), which is much smaller and more maneuverable than the TBS group. Again asymmetric epoxidation (AE) using (*S,S*)-DET did not take place with satisfactory stereofacial selection. In a control experiment epoxidation was done using the Sharpless methodology but without the (*S,S*)-DET, to investigate the possibility of mismatching between the directing influence of the substrate and that of the chiral catalyst. However the results showed that epoxidation takes place with a preference towards the required (2*R*,3*R*) epoxide with a (2*R*,3*R*): (2*S*,3*S*) ratio 4.7:1, which shuns this possibility.

This result prompted the change from (*S*,*S*)-DET to (*S,S*)-DIPT in the AE reaction and using the *O*-TBS protected allylic alcohol (**154**) as substrate. A slight improvement in selectivity was observed with a diastereomeric ratio of (2*R*,3*S*):(2*S*,3*R*) of 4.7:1. The Sharpless AE with reaction of the *O*-Bn protected allylic alcohol (**177**) as substrate finally resulted in the reaction proceeding with complete stereofacial selection yielding the(2*R*,3*S*)-epoxide (**178**) as a single diastereomer.

Formation of the epoxide was confirmed by examination of the ^{13}C and ^{1}H NMR spectra with the H-2 signal present at δ_H 3.11 as a double triplet and the H-3 signal as a double doublet at δ_H 2.96. The corresponding carbon signals were present at δ_C 56.56D and 55.94D, respectively.

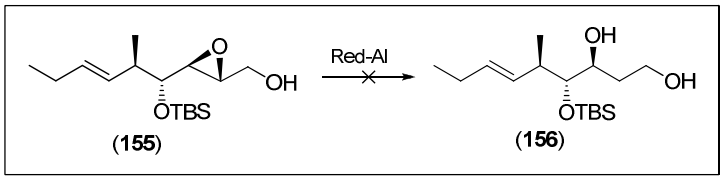
The formed epoxides **155** and **178** can now be opened to deliver the next product in the reaction sequence. Too little of **178** was left and the reaction was done on the epoxy alcohol (**155**) only.

3.11 SELECTIVE REDUCTION OF 2,3-EPOXY ALCOHOL

Red-Al is used as the reducing agent in the selective reduction of the 2,3-epoxide. Some theoretical background on the reagent has already been given in an earlier section and will not be repeated again.

Red-Al regioselectively reduces aliphatic 2,3-epoxy alcohols at the less hindered carbon to produce a 1,3-diol product.[75] The type of solvent used influences the product distribution which is given as a ratio of the 1,3- and 1,2-diol. This effect is more pronounced in epoxides having similar steric hindrance at the epoxide carbons.

3.11.1 EXPERIMENTAL DISCUSSION



Scheme 60: Selective reduction of **155.**

A standard procedure by Gao and Sharpless[75] was followed in attempting the 1,3-reduction reaction of substrate **155**. The reaction produced disappointing results in that reaction conditions resulted in deprotection of the secondary alcohol to form the triol (**173**) as product (**Scheme 40**). Further investigation was not conducted due to time constraints and insufficient amount of material left.

3.12 CONCLUSION AND FUTURE WORK

An efficient synthetic route towards the synthesis of the C(12)-C(20) segment of FB_1 (**5**) was developed. The [2,3]-Wittig rearrangement approach provides efficient methodologies up to the benzyl protected epoxide (**178**) stage towards the target molecule (**159**) of this project. Kinetic enzymatic resolution, the [2,3]-Wittig rearrangement, and Sharpless asymmetric epoxidation reactions provided efficient means of installing the required stereocentres with satisfactory optical purity.

Problems associated with the use of different protecting groups following **Route 1** were solved and future work will focus on using the *O*-Bn as protecting group. The obstacles encountered in **Route 2** (**Scheme 48**) regarding the inability to reduce the primary *O*-TBDPS

[75] Gao,Y.; Sharpless, K.B. *J. Org. Chem.* **1988**, *53*, 4081.

protected alkyne (**187**) to the *trans*-alkene (**188**) need further investigation. Using sodium metal in liquid ammonia to afford the reduction reaction must definitely be looked into.

The Achilles' heel of the [2,3]-Wittig rearrangement approach towards the identified target molecule (**159**) lies in the kinetic enzymatic resolution step. The optimized reaction procedure on a 20 mmole scale produces (3*S*,4*E*)-hex-4-en-3-ol (**149**) in 99.8% e.e. but with a maximum yield of 1.00 g. The kinetic enzymatic resolution step is step 2 in a proposed 12 step synthesis and scale-up of the reaction comes at a cost in optical purity. It is therefore obvious why an alternative route is required.

Alternative methods, in addition to, or to completely circumvent the enzymatic kinetic resolution step, should be investigated further: The possibility of converting the acylated *R* enantiomer with subsequent conversion to the *S* enantiomer exists and can be executed in two ways: 1. Mitsunobu methodology to invert the stereocentre to the *S* enantiomer as an ester and subsequent hydrolysis or 2. Firstly hydrolyzing the *R*-ester followed by tosylation of the formed alcohol. The tosylated product can then be subjected to a carboxylate anion nucleophile in an S_N2 reaction to again form a hydrolysable ester to yield the wanted enantiomer.

Methods to enantioselectively reduce the prochiral ketone **151** also exist: 1. The CBS reagent developed by Corey, Bakshi and Sibita, which is a chiral borane heterocycle. The use of proline provides the amino alcohol derivative and is responsible for producing the active reducing agent. 2. A method developed by Liang *et al.*[27] utilizing crotonaldehyde and diethylzinc, in the presence of 1 mol % (*S*)-1-piperidino-3,3-dimethyl-2-butanol to deliver **149** from crotonaldehyde (**169**).

CHAPTER 4 EXPERIMENTAL

4.1 GENERAL

All air sensitive reactions were carried out under an atmosphere of argon in oven-dried (120 ºC) glassware. Solvents and reagents used for air-sensitive reactions were dried according to standard methods.[1] Reactions done at rt refer to 20-25 ºC. Solvents used for chromatography or extraction were only distilled.

Thin layer chromatography (TLC) analysis was done using aluminium sheets coated with silica gel (60 F_{254}) from Merck. TLC plates were examined under UV light (254 and 366 nm) and/or after colouring and subsequent heating with cerium(IV) sulfate–ammonium heptamolybdate reagent or cerium(IV) sulfate–sulfuric acid reagent.. Preparative column chromatography was carried out using Merck silica gel (70-230 mesh).

Optical rotations were measured using a Perkin Elmer 341 polarimeter with the sodium D line (589 nm) at ambient temperature. Rotations were recorded in chloroform in 1.00 dm length cells. Specific rotations $[\alpha]_D$, are expressed in units of $dm^{-1}g^{-1}cm^3$. Mass spectra were recorded at the University of Stellenbosch on a Waters API Q-TOF Ultima spectrometer using the electrospray ionisation (ESI) technique and detection of positive ions with m/z > 99. FTIR spectra were recorded using a Perkin Elmer RX 1 spectrophotometer fitted with a PIKE Technologies ATR accessory using the reflectance technique. Samples were prepared as chloroform ($CHCl_3$) solutions.

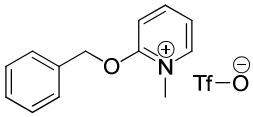
^1H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE III-400 (9.4T) or AVANCE 500-DXR (11.7T) spectrometers for solutions in $CDCl_3$ (unless indicated otherwise). Data are given as chemical shift values (δ) in parts per million (ppm) downfield from tetramethylsilane (Me_4Si) using $CDCl_3$ as internal standard with δ_H 7.24 and δ_C 77.00. Coupling constants are given as *J*-values in Hz. Spectral coupling patterns are designated as follows: S/s: singlet; D/d: doublet; T/t: triplet; Q/q: quartet; m: multiplet; br: broad signal. ^{19}F NMR spectra were recorded on a Bruker AMX-300 (7.0 T) or

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

AVANCE III-400 (9.4T) using $CFCl_3$ as external standard. Peaks at highfield shift values were assigned negative values.

Analyses and peak assignments in ^1H NMR spectra were done by first order analysis of the spin systems present. Two-dimensional (^1H,^1H) homonuclear and (^{13}C,^1H) heteronuclear chemical shift correlation experiments were performed to confirm first order analysis. Multiplicities of the different ^{13}C chemical shifts were determined from proton-decoupled CH, CH_2 and CH_3 subspectra using the DEPT pulse sequence.

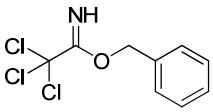
4.2. PREPARED REAGENTS



4.2.1 2-(Benzyloxy)-1-methylpyridinium trifluoromethanesulfonate (Dudley's Reagent) (181)

A mixture of benzyl alcohol (2.00 g, 18.5 mmol), 2-chloropyridine (**180**) (3.46 g, 30.5 mmol), powdered KOH (3.42 g, 61.0 mmol), toluene (37.0 mL), and 18-crown-6 (24.4 mg, 0.93 mmol) was heated at reflux for 1 h with azeotropic removal of water by means of a Dean-Stark trap. The reaction mixture was allowed to cool to rt and partitioned between EtOAc (20.0 mL) and water (10.0 mL). The organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The product was purified on silica gel with hexane-EtOAc (100:1) as eluent to provide 2-benzyloxypyridine (3.28 g, 96% yield) as a yellow liquid.

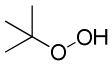
To a cold (0 ºC) solution of 2-benzyloxypyridine (**179**) (100 mg, 0.54 mmol) in toluene (0.54 mL), methyl trifluoromethanesulfonate (64.0 μL, 0.57 mmol) was added. The mixture was allowed to warm to rt. This resulted in the formation of a white crystalline precipitate. After 40 min, the volatiles were removed in *vacuo*, yielding Dudley's reagent (**181**) (0.172 g, 91% yield) as a white microcrystalline solid.



4.2.2 Benzyl trichloroacetimidate (174)

Sodium hydride (0.869 g, 21.0 mmol) washed with pentane, was suspended in anhydrous Et_2O (20 mL). A solution of benzyl alcohol (21.7 mL, 210 mmol) in Et_2O (30 mL) was added dropwise with stirring under an atmosphere of argon. After 20 min, the solids dissolved and

the solution was cooled to 0 ºC using a salt-ice bath. Trichloroacetonitrile (20.1 mL, 200 mmol) was added dropwise over 15 min, after which the reaction mixture was allowed to warm to 20 ºC over a period of 60 min. The reaction mixture was concentrated and pentane (20 mL) containing anhydrous MeOH (0.8 mL) was added. Vigorous shaking followed this addition and resulted in the formation of solids which were removed by filtration. The filtrate was washed with pentane (3×20 mL) and concentrated, after which the resultant imidate (**174**) (41.9 g, 81%) was obtained as a clear yellow liquid and used without further manipulation. The liquid was stored under argon at 4 ºC.



4.2.3 *t*-Butyl hydroperoxide (TBHP)

A mixture of 70% aq *t*-butyl hydroperoxide (500 mL) and toluene (1 L) was swirled in a separatory funnel. The aqueous phase was removed, and the organic phase dried by azeotropic water removal using a Dean-Stark trap. The solution was refluxed until water collection ceased. The TBHP solution was stored in a dark bottle at rt. The concentration of TBHP in the solution was determined by ^{1}H NMR using the following equation:

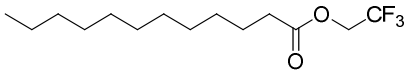
Molarity = X/(0.1X + 0.32Y)

X = integral value for the *t*-butyl methyl resonance

Y = integral value for the toluene methyl resonance

δ_H: 2.81 (s, CH_3)

1.50 (*t*-butyl)



4.2.4 2,2,2-Trifluoroethyl dodecanoate (160)

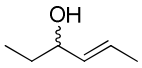
Dodecanoic acid (**162**) (100 g, 499 mmol) was gradually added to thionyl chloride (255 mL, 3.49 mol). The mixture was heated to reflux under Ar for 1 h when the evolution of SO_2 (g) and HCℓ (g) ceased. The excess thionyl chloride was removed under reduced pressure to give the acid chloride which was used directly in the next step.

The acyl chloride (**164**) (50.0 g, 229 mmol) was added to a cold (0°C) solution of 2,2,2-trifluoroethanol (25.0 mL, 343 mmol) and DMAP (2.79 g, 22.9 mmol) in anhydrous DCM (229 mL) and pyridine (20.3 mL, 251 mmol). The milky, light yellow solution was stirred for 16 h at rt under argon and then quenched by addition of H_2O (115 mL). The layers were separated and the aqueous layer extracted with DCM (3×150 mL). The combined organic

layers were washed with satd $NaHCO_3$ solution (300 mL), dil HCl (300 mL), and brine (300 mL), dried ($MgSO_4$), and evaporated to yield the crude product as a bright orange liquid. The crude product was purified by column chromatography with hexane-EtOAc (95:5) as eluent to yield the ester (**160**) (63.9 g, 99 %) as a light yellow liquid. R_f = 0.43 (hexane- Et_2O 95:5).

The method followed in each synthetic route is identical up to the formation of the [2,3]-Wittig rearrangement product. Thereafter the method diverges into three different routes, the beginning of which is marked clearly in each case.

4.3 PROCEDURES

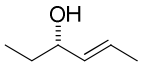


4.3.1 (3*RS*,4*E*)-Hex-4-en-3-ol (150)

(4*E*)-4-Hexen-3-one (**151**) (15.0 mL, 131 mmol) was added to a solution of cerium(III) chloride (64.6 g, 262 mmol) in MeOH (500 mL). The reaction was cooled to −78 °C (dry ice-acetone cold bath), after which $NaBH_4$ (9.92 g, 262 mmol) was added in a single batch. The milky white reaction mixture was left to warm to rt and quenched by addition of a satd. NH_4Cl solution (250 mL). The layers were separated and the aqueous layer extracted with Et_2O-pentane (1:1, 3×250 mL). The combined organic layers were washed with brine (400 mL), dried ($MgSO_4$), and evaporated under reduced pressure and a bath temperature of <30 ºC to yield the crude product as a colourless liquid. The crude product was purified by column chromatography with Et_2O-pentane (1:1) as eluent to quantitatively yield **150** (13.1 g, 100%) as a volatile, colourless liquid. R_f = 0.39 (hexane-EtOAc 1:1); ν_{max} 3344 (broad), 2961, 2931, 2920, 2876, 2856 cm^{-1}.

δ_H: 5.61 (ddq, 1H, $^3J_{4,5}$ 15.2, $^3J_{5,6}$ 6.5, $^4J_{3,5}$ 1.1, H-5)
5.42 (ddq, 1H, $^3J_{4,5}$ 15.2, $^3J_{3,4}$ 7.0, $^4J_{4,6}$ 1.3, H-4)
3.91 (ddt, 1H, $^3J_{3,4}$ 7.0, $^3J_{2,3}$ 6.5, $^4J_{3,5}$ 1.1, H-3)
1.66 (br, 1H, OH)
1.66 (dd, $^3J_{5,6}$ 6.5, $^4J_{4,6}$ 1.3, H-6)
1.49 (m, 2H, H-2)
0.86 (t, 3H, $^3J_{1,2}$ 7.5, H-1)

δ_C: 134.03D (C-4), 126.80D (C-5), 74.41D (C-3), 30.05T (C-2), 17.59Q (C-6), 9.68Q (C-1).



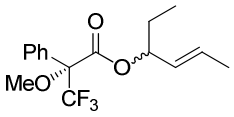
4.3.2 *(3S,4E)*-Hex-4-en-3-ol (149)

a. 2,2,2-Trifluoroethyl dodecanoate (10.48 g, 40.0 mmol) was added to a light brown suspension of porcine pancreatic lipase (PPL) (8.0 g) (washed with acetone and vacuum dried) in Et_2O (165 mL). After 10 min the racemic alcohol (**150**) (2.00 g, 20.0 mmol) was added and the suspension stirred for 96 h under an atmosphere of argon. The reaction mixture was filtered through celite to remove the enzyme, after which the filtrate was concentrated by rotary evaporation (waterbath <30 ºC). The product was purified by column chromatography with pentane-Et_2O (95:5 → 1:1) as eluent to give **149** (0.92 g, 46 % yield) as a colourless liquid. $[\alpha]_D$ −9.0 (*c* 8.2, $CHCl_3$), 99.8% e.e.

δ_H: 5.61 (ddq, 1H, $^3J_{4,5}$ 15.2, $^3J_{5,6}$ 6.5, $^4J_{3,5}$ 1.1, H-5)
5.42 (ddq, 1H, $^3J_{4,5}$ 15.2, $^3J_{3,4}$ 7.0, $^4J_{4,6}$ 1.3, H-4)
3.91 (ddt, 1H, $^3J_{3,4}$ 7.0, $^3J_{2,3}$ 6.5, $^4J_{3,5}$ 1.1, H-3)
1.66(br, 1H, OH)
1.66 (dd, $^3J_{5,6}$ 6.5, $^4J_{4,6}$ 1.3, H-6)
1.49 (m, 2H, H-2)
0.86 (t, 3H, $^3J_{1,2}$ 7.5, H-1)

δ_C: 134.03D (C-4), 126.80D (C-5), 74.41D (C-3), 30.05T (C-2), 17.59Q (C-6), 9.68Q (C-1).

b. The same reaction procedure was followed on scale-up of the reaction using 82.5 mmol (8.26 g) of racemic alcohol (**150)** with 2,2,2-trifluoroethyl dodecanoate (46.6 g, 165 mmol) and porcine pancreatic lipase (PPL) (33.0 g) in Et_2O (165 mL). The alcohol (**149**) (3.84 g, 46.5%) was obtained as a colourless liquid. $[\alpha]_D$ −9.0 (*c* 8.2, $CHCl_3$), 96% e.e.



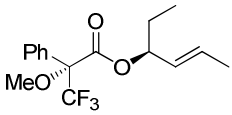
4.3.3 (3*RS*,4*E*)-Hex-4-en-yl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate

Oxalyl chloride (0.22 mL, 0.50 mmol) and DMF (220 µL, 2.84 mmol) were added to a solution of *R*-(+)-MTPA (117 mg, 0.50 mmol) in hexane (3 mL). After 1 h the hexane solution was decanted to leave DMF-Cl behind as oily droplets. The hexane solution was evaporated and the residual oil, the acid chloride (114 mg, 90%) was used directly in the next reaction.

A solution of the alcohol (**150**) (50 mg, 0.50 mmol) in DCM (2.5 mL) was added to a solution of the acid chloride, DMAP (6.1 mg, 0.05 mmol) and Et_3N (0.35 mL, 2.52 mmol) in DCM

(2.5 mL). The reaction stirred for 3 h at rt. The solution was diluted with DCM (5 mL) and washed with 0.1M HCl (2.5 mL) and satd. $NaHCO_3$ (2.5 mL) solution, dried ($MgSO_4$), filtered and evaporated to yield the Mosher ester (134 mg, 85%), a yellow oil, as a 1:1 mixture of diastereomers.

δ_F: −70.07, −70.16

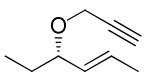


4.3.4 [(3*S*,4*E*)-Hex-4-en-3-yl] (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate

Oxalyl chloride (0.22 mL, 0.50 mmol) and DMF (220 µL, 2.84 mmol) were added to a solution of *R*-(+)-MTPA (117 mg, 0.50 mmol) in hexane (3 mL). After 1 h the hexane solution was decanted to leave DMF-Cl behind as oily droplets. The hexane solution was evaporated and the residual oil, the acid chloride (105 mg, 83%) was used directly in the next reaction.

A solution of **149** (50 mg, 0.50 mmol) in DCM (2.5 mL) was added to a solution of the acid chloride (**187**), DMAP (6.1 mg, 0.05 mmol) and Et_3N (0.35 mL, 2.52 mmol) in DCM (2.5 mL). The reaction stirred for 3 h at rt. The solution was diluted with DCM (5 mL) and washed with 0.1M HCl (2.5 mL) and satd. $NaHCO_3$ (2.5 mL) solution, dried ($MgSO_4$), filtered and evaporated to yield the Mosher ester (126 mg, 80%), a yellow oil, as a 1:1 mixture of diastereomers.

δ_F: −70.16 (*S*-enantiomer)



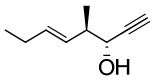
4.3.5 (2*E*,4*S*)-4-[(Prop-2-ynyl)oxy]hex-2-ene (148)

To a vigorously stirred solution of *(3S,4E)*-hex-4-en-3-ol (**149**) (4.10 g, 40.9 mmol) and *n*-tetrabutylammonium hydrogensulfate (1.39 g, 4.09 mmol), sodium hydroxide (50%) (20.0 g, 500 mmol) was added. The reaction was cooled to 0 °C prior to the careful dropwise addition of propargyl bromide (13.7 mL, 123 mmol). The two-phase solution was stirred for 48 h. The solution was neutralized with 6M HCl at 0 °C, after which the layers were separated. The aqueous layer was extracted with pentane (3×100 mL), and the combined organic layers washed with brine (150 mL), dried ($MgSO_4$) and evaporated to yield the propargyl ether (**148**) as a volatile light yellow liquid. The crude product was used directly in next step to avoid losses due to volatility. R_f = 0.63 (pentane-Et_2O 1:1); ν_{max} 2100 (weak) cm^{-1}.

δ_H: 5.64 (dq, 1H, $^3J_{2,3}$ 15.2, $^3J_{1,2}$ 6.5, H-2)
5.43 (ddq, 1H, $^3J_{2,3}$ 15.2, $^3J_{3,4}$ 8.6, $^4J_{1,3}$ 1.6, H-3)
4.11 (dd, 1H, $^2J_{1'a,b}$ 15.8, $^4J_{1'-3'}$ 2.3, H-1'a)
3.96 (dd, 1H, $^2J_{1'a,b}$ 15.8, $^4J_{1',3'}$ 2.3, H-1'b)
3.69 (dt, 1H, $^3J_{3,4}$ 8.6, $^3J_{4,5}$ 6.5, H-4)
2.32 (t, 1H, $^4J_{1',3'}$ 2.3, acetylene H)
1.67 (dd, 3H, $^3J_{1,2}$ 6.5, $^4J_{1,3}$ 1.6, H-1)
1.58 (m, 1H, H-5a)
1.45 (m, 1H, H-5b)
0.85 (t, 3H, $^3J_{5,6}$ 7.5, H-6)

δ_C: 130.75D (C-3), 129.88D (C-2), 81.06D (C-4), 80.46S (C-2'), 73.36D (C-3'), 54.66D (C-1'), 28.21T (C-5), 17.57Q (C-1), 9.70Q (C-6).

ESI-MS: *m/z* 138 $[M+H]^+$. Exact mass: Calculated for $C_9H_{14}O$, 138.1045; Found, 138.1024.



4.3.6 (3*R*,4*R*,5*E*)-4-Methyloct-5-en-1-yn-3-ol (147)

An aliquot of *n*-BuLi (1.6M in hexanes, 18.5 mL, 29.6 mmol) was added to a Schlenk tube. The hexane was evaporated in *vacuo* and the residue cooled to −90 °C (hexane-$N_2(\ell)$ slush bath). A solution of the propargyl ether (**148**) (1.46 g, 10.6 mmol) in THF (29 mL) was then slowly added and the reaction allowed to warm to rt overnight. The reaction vessel was cooled to −78 ºC (acetone-dry ice) before quenching with H_2O (15 mL). The THF was evaporated under reduced pressure. The aqueous residue was extracted with EtOAc (3×50 mL) and the combined organic layers were washed with brine (50 mL), dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography using hexane-EtOAc (95:5) as eluent to yield the acetylenic alcohol (**147**) (1.36 g, 93%) as light yellow oil. R_f = 0.46 (hexane-EtOAc 1:1); $[\alpha]_D$ +31.9 (*c* 1.0, $CHCl_3$); ν_{max} 3292 (broad), 2108 (weak) cm^{-1}.

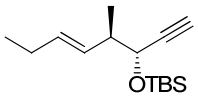
δ_H: 5.62 (ddt, 1H, $^3J_{5,6}$ 15.3, $^3J_{6,7}$ 6.5, $^4J_{4,6}$ 1.3, H-6)
5.34 (ddt, 1H, $^3J_{5,6}$ 15.3, $^3J_{4,5}$ 7.8, $^4J_{5,7}$ 1.5, H-5)
4.12 (dd, 1H, $^3J_{3,4}$ 6.2, $^4J_{1,3}$ 2.1, H-3)
2.44 (d, 1H, $^4J_{1,3}$ 2.1, H-1)
2.39 (m, 1H, H-4)
2.03 (m, 2H, H-7)
1.61 (OH)
1.10 (d, 3H, $^3J_{3,methyl}$ 7.0, 4-methyl)

0.97 (t, 3H, $^{3}J_{7,8}$ 6.8, H-8)

δ_C: 135.33D (C-6), 129.20D(C-5), 83.42S (C-2), 73.70D (C-1), 66.27D (C-3), 43.40D (C-4), 25.65T (C-7), 15.81Q (-CH_3), 13.75Q (C-8).

ESI-MS: *m/z* 138 $[M+H]^+$. Exact mass: Calculated for $C_9H_{14}O$, 138.1045; Found, 138.1040.

4.4 ROUTE 1A



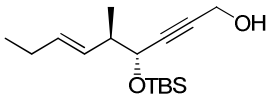
4.4.1 (3*R*,4*R*,5*E*)-3-[(*t*-Butyldimethylsilyl)oxy]-4-methyloct-5-en-1-yne (152)

Imidazole (1.48 g, 21.7 mmol) was added to a solution of the alkyne (**147**) (2.00 g, 14.5 mmol) in anhydrous DCM (145 mL). The solution was cooled to 0°C in an ice-bath and TBS-Cl (3.93 g, 26.0 mmol) was slowly added. The reaction was stirred at rt for 48 h. The reaction was quenched by the addition of H_2O (75 mL). The aqueous phase was extracted with DCM (3×100 mL) and the combined organic layers washed with brine (100 mL), dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography (hexane-EtOAc 95:5) to give the *O*-TBS ether (**152**) (1.84 g, 67%) colourless oil. R_f = 0.66 (hexane-EtOAc 1:1); $[\alpha]_D$ +38.0 (*c* 1.0, $CHCl_3$); ν_{max} 3060, 2960 (medium) cm^{-1}.

δ_H: 5.51 (ddt, 1H, $^{3}J_{5,6}$ 15.2, $^{3}J_{6,7}$ 6.2, $^{4}J_{4,6}$ 1.0, H-6)
5.34 (ddt, 1H, $^{3}J_{5,6}$ 15.2, $^{3}J_{4,5}$ 7.7, $^{4}J_{5,7}$ 1.5, H-5)
4.25 (dd, 1H, $^{3}J_{3,4}$ 6.0, $^{4}J_{1,3}$ 2.3, H-3)
2.34 (d, 1H, $^{4}J_{1,3}$ 2.3, H-1)
2.32 (m, 1H, H-4)
2.03 (m, 2H, H-7)
1.06 (d, 3H, $^{3}J_{4,methyl}$ 6.8, methyl)
0.96 (t, 3H, $^{3}J_{7,8}$ 7.5, H-8)
0.89 (s, 9H, *t*-butyl)
0.09 (s, 6H, $SiMe_2$)

δ_C: 133.02D (C-6), 130.36D (C-5), 84.25S (C-2), 72.81D (C-1), 67.28D (C-3), 43.75D (C-4), 25.75Q (*t*-butyl CH_3), 25.66T (C-7), 18.23S (*t*-butyl C), 15.45Q (CH_3), 13.74Q (C-8), –4.87Q ($SiMe_2$).

ESI-MS: *m/z* 252 $[M+H]^+$. Exact mass: Calculated for $C_{15}H_{28}O_2Si$, 252.1909; Found, 252.1892.

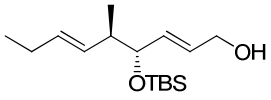


4.4.2 (4*R*,5*R*,6*E*)-4-[(*t*-Butyldimethylsilyl)oxy]-5-methylnon-6-en-2-yn-1-ol (153)

n-BuLi (1.6M in hexanes, 1.24 mL, 1.98 mmol) was added dropwise to a stirred solution of the *O*-TBS ether (**152**) (3.80 g, 1.98 mmol) in THF (5 mL) at −78 °C. After 30 min the reaction was allowed to warm to 0°C and dried paraformaldehyde (89.0 mg, 2.97 mmol) was added. The reaction was stirred at 30-35 °C for 16 h and then quenched at −78°C with satd NH_4Cl soln. (2.5 mL). The reaction mixture was diluted with EtOAc (50 mL) and the aqueous layer extracted with EtOAc (3×50 mL). The combined organic layers were dried ($MgSO_4$) and the solvent evaporated. The crude product was purified by column chromatography using hexane-EtOAc (9.5:1→1:1) as eluent to yield the acetylenic alcohol (**153**) (3.61 g, 85%) as colourless oil R_f = 0.65 (hexane-EtOAc 1:1); $[\alpha]_D$ +17.2 (*c* 1.0, $CHCl_3$); ν_{max} 3180-3579 (broad), 2856-2960 (strong) cm^{-1}.

δ_H: 5.46 (dtd, 1H, $^3J_{6,7}$ 15.4, $^3J_{7,8}$ 6.3, $^4J_{7,5}$ 1.0, H-7)
5.43 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{5,6}$ 7.8, $^4J_{6,8}$ 1.5, H-6)
4.27 (br s, 2H, H-1)
4.18 (dt, 1H, $^3J_{4,5}$ 6.0, $^3J_{1,4}$ 1.8, H-4)
2.30 (m, 1H, $^3J_{5,6}$ 7.8, $^3J_{4,5}$ 6.0, $^3J_{5,methyl}$ 6.8, $^4J_{7,5}$ 1.0, H-5)
2.00 (m, 2H, H-8)
1.59 (br s, OH)
1.04 (d, 3H, $^3J_{5,methyl}$ 6.8, methyl)
0.95 (t, 3H, $^3J_{8,9}$ 7.3, H-9)
0.88 (s, 9H, *t*-butyl)
0.10 (s, 6H, $SiMe_2$)

δ_C: 133.00D (C-7), 130.44D (C-6), 86.39S (C-2), 82.97S (C-3), 67.44D (C-4), 51.25T (C-1), 43.84D (C-5), 25.78Q (*t*-butyl CH_3), 25.66T (C-8), 18.25S (*t*-butyl C), 15.61Q (CH_3), 13.78Q (C-9), −4.78Q ($SiMe_2$).



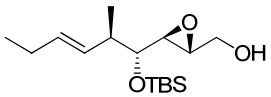
4.4.3 (2*E*,4*R*,5*R*,6*E*)-4-[(*t*-Butyldimethylsilyl)oxy]-5-methylnona-2,6-dien-1-ol (154)

A solution of the acetylenic alcohol (**153**) (1.00 g, 3.54 mmol) in anhydrous Et_2O (5 mL) was added dropwise to solution of Red-Al (1.67 mL, 5.68 mmol) in anhydrous Et_2O (3.0 mL) taking care to maintain the temperature <5°C. The reaction was allowed to attain rt and after 2.5 h once again cooled to 0°C and quenched by the careful addition of H_2O (5 mL). The

layers were separated and the aqueous layer extracted with EtOAc (3×25 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$) and evaporated. Column chromatography of the crude product using hexane-EtOAc (95:5→1:1) as eluant gave the allylic alcohol (**154**) (0.82 g, 81%) as an oil. R_f = 0.61 (hexane-EtOAc 1:1)

δ_H 5.72 (ddt, 1H, $^3J_{2,3}$ 15.4, $^3J_{1,2}$ 5.5, $^4J_{2,4}$ 1.0, H-2)
5.62 (ddt, 1H, $^3J_{2,3}$ 15.4, $^3J_{3,4}$ 5.8, $^4J_{1,3}$ 1.2, H-3)
5.43 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{7,8}$ 6.2, $^4J_{7,5}$ 0.9, H-7)
5.30 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{5,6}$ 7.4, $^4J_{6,8}$ 1.2, H-6)
4.11 (br s, 2H, H-1)
3.94 (ddd, 1H, $^3J_{3,4}$ 5.8, $^3J_{4,5}$ 5.3, $^4J_{2,4}$ 1.0, H-4)
2.18 (dddt, 1H, $^3J_{5,methyl}$, 6.8, $^3J_{4,5}$ 5.3, $^3J_{5,6}$ 7.4, $^4J_{5,6}$ 0.9, H-5)
1.94 - 2.02 (m, 2H, H-8)
1.44 (b, 1H, -OH)
0.92 (t, 1H, $^3J_{8,9}$ 7.4, H-9)
0.88 (d, 3H, methyl)
0.86 (s, 9H, *t*-butyl)
–0.07 (s, 6H, $SiMe_2$)

δ_C: 133.18D (C-3), 132.14D (C-7), 131.22D (C-6), 129.48D (C-2), 76.68D (C-4), 63.24T (C-1), 43.44D (C-5), 25.84Q (*t*-butyl CH_3), 25.66T (C-8), 18.19S (*t*-butyl C), 15.62Q (CH_3), 13.86Q (C-9), –4.41Q ($SiMe_2$).



4.4.4 (2*R*,3*S*,4*R*,5*R*,6*E*)-4-[(*t*-Butyldimethylsilyl)oxy]-2,3-epoxy-5-methylhex-6-en-1-ol (155)

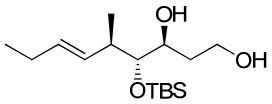
A mixture of powdered molecular sieves (4Å, 97 mg) and DCM (6 mL) under argon was cooled to −20 °C. A solution of $Ti(O^iPr)_4$ (0.15 g, 0.53 mmol) in DCM (2 mL) and (*S,S*)-DIPT (162 mg, 1.63 mmol) in DCM (1 mL). The allylic alcohol (**154**) (0.30 g, 1.05 mmol) in DCM (3 mL) was added next. The catalyst complex was left to age for 20 min before the dropwise addition of TBHP in toluene (5.6M in toluene, 0.38 mL, 2.11 mmol). The reaction mixture was stirred at −20 °C for 6 h and left in the freezer at −15°C for a further 120 h. The mixture was then allowed to warm to 0 ºC and immediately poured into a solution of iron(II) sulfate (384 mg) and tartaric acid (104 mg) in deionized water (4.88 mL) at 0 ºC. The two-phase system was stirred at rt for 30 min and filtered through celite. The layers were separated and

the aqueous layer extracted with Et_2O (3×10 mL) and the combined organic solutions dried ($MgSO_4$) and filtered. The solvent was removed by evaporation at 35 ºC.

A solution of the crude product in Et_2O (5 mL) was cooled to 0 ºC and added to a pre-cooled (0 ºC) solution of NaOH (32 mg, 0.807 mmol) in brine (110 µL). The two-phase mixture was stirred vigorously for 1h with continued cooling. The layers were separated, the aqueous phase was separated and extracted with Et_2O (3×10 mL).The combined organic solutions were dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography using hexane-EtOAc (95:5→1:1) as eluant to yield the epoxy alcohol (**155**) (0.25 g, 79%) as a mixture of enantiomers in a (2*R*,3*S*):(2*S*,3*R*) ratio of 4.7:1 as a colourless oil. R_f = 0.61 (hexane-EtOAc 1:1).

δ_H: 5.48 (ddt, 1H, $^3J_{6,7}$ 15.3, $^3J_{7,8}$ 6.3, $^4J_{5,7}$ 1.0, H-7)
5.37 (ddt, 1H, $^3J_{6,7}$ 15.3, $^3J_{5,6}$ 8.0, $^4J_{6,8}$ 1.0, H-6)
3.89 (dd, 1H, $^2J_{a,b}$ 12.8, $^3J_{1a,2}$ 2.3, H-1a)
3.61 (dd, 1H, $^3J_{3,4}$ 3.8, $^3J_{4,5}$ 3.8, H-4)
3.54 (dd, 1H, $^2J_{a,b}$ 12.8, $^3J_{1b,2}$ 4.3, H-1b)
3.08 (dt, 1H, $^3J_{2,3}$ 4.3, $^3J_{1,2}$ 2.5, H-2)
2.33 (dddq, 1H, $^3J_{5,methyl}$ 7.0, $^3J_{4,5}$ 3.8, $^3J_{5,6}$ 8.0, $^4J_{5,7}$ 0.5, H-5)
1.99 (ddt, 2H, $^3J_{7,8}$ 6.3, $^3J_{8,9}$ 7.5, $^4J_{6,8}$ 1.0, H-8)
1.01 (d, 3H, $^3J_{5,methyl}$ 7.0, 5-methyl)
0.88 (s, 9H, *t*-butyl)
0.06 (s, 6H, $SiMe_2$)

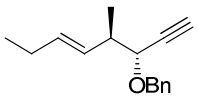
δ_C: 132.86D (C-7), 129.89D (C-6), 73.74D (C-4), 61.38T (C-1), 55.80D (C-2), 56.62D (C-3), 41.58D (C-5), 26.11T (C-8), 25.83Q (*t*-butyl CH_3), 20.84S (*t*-butyl C), 16.76Q (CH_3), 14.11Q (C-9), –4.63Q ($SiMe_2$).



4.4.5 (3*S*,4*R*,5*R*,6*E*)-4-[(*t*-Butyldimethylsilyl)oxy]-5-methylnon-6-ene-1,3-diol (156)

Red-Al (0.22 mL, 0.73 mmol) was added dropwise to a solution of the epoxy alcohol (**155**) (0.20 g, 0.67 mmol) in THF (4 mL) under an atmosphere of argon at 0 °C. The reaction was stirred for 3 h at rt (TLC control) and then quenched by the addition of H_2O (2 mL). The aqueous layer was extracted with EtOAc (3×15 mL) and the combined organic solutions were washed with brine (20 mL) and dried ($MgSO_4$). The crude consisted of the diol and deprotected triol (**173**) and it was decided not to investigate further.

4.5 ROUTE 1B



4.5.1 (3*R*,4*R*,5*E*)-3-Benzyloxy-4-methyloct-5-en-1-yne (175)

4.5.1.1 Neutral conditions

A mixture of Dudley's reagent (**181**) (2.55 g, 7.29 mmol), and anhydrous MgO (0.294 g, 7.29 mmol) (vacuum dried) and the acetylenic alcohol (**147**) (0.504 g, 3.65 mmol) in benzotrifluoride (7.6 mL, 62.0 mmol) was heated at 80-85˚C for 24 h. The reaction mixture was cooled to rt and filtered through Celite. The filtrate was concentrated under reduced pressure to produce a dark orange crude product which was purified by column chromatography (hexane-EtOAc (95:5)) to give the *O*-benzyl derivative (**175**) (0.48g, 58%) as an oil. R_f = 0.73 (hexane-EtOAc 1:1); $[\alpha]_D$ +22.4 (*c* 1.0, $CHCl_3$); ν_{max} 2867, 2930, 2957, 3023, 3064, 3285 cm^{-1}.

δ_H: 7.33 - 7.36 (m, 5H, aromatic protons)
5.53 (ddt, 1H, $^3J_{5,6}$ 15.4, $^3J_{6,7}$ 6.2, $^4J_{4,6}$ 1.0, H-6)
5.38 (ddt, 1H, $^3J_{5,6}$ 15.4, $^3J_{4,5}$ 7.5, $^4J_{5,7}$ 1.4, H-5)
4.81 (d, 1H, $^2J_{a,b}$ 12.1, benzylic CH_2)
4.50 (d, 1H', $^2J_{a,b}$ 12.1, benzylic CH_2)
3.94 (dd, 1H, $^3J_{3,4}$ 7.9, $^4J_{1,3}$ 2.1, H-3)
2.50 (m, 1H, $^3J_{3,4}$ 7.9, $^3J_{4,methyl}$ 6.8, $^3J_{4,5}$ 7.5, $^4J_{4,6}$ 1.0, H-4)
2.45 (d, 1H, $^4J_{1,3}$ 2.1, H-1)
2.02 (m, 2H, $^3J_{7,8}$ 7.4, $^3J_{6,7}$ 6.2, $^4J_{5,7}$ 1.4, H-7)
1.11 (d, 3H, $^3J_{4,methyl}$ 6.8, 4-methyl)
0.97 (t, 3H, $^3J_{7,8}$ 7.4, H-9)

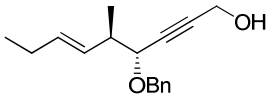
δ_C: 133.12D (C-6), 130.50D (C-5), 127.82 (aromatic carbons), 81.46S (C-2), 74.69D (C-1), 72.945D (C-3), 70.45T (4 –CH_2), 41.24D (C-4), 25.66T (C-7), 15.78Q (Me), 13.76Q (C-8).

ESI-MS: *m/z* 228 $[M+H]^+$. Exact mass: Calculated for $C_{16}H_{20}O$, 228.1514; Found, 228.1506.

4.5.1.2 Acidic conditions

Trifluoromethanesulfonic acid (125 µL, 1.43 mmol) was added slowly by micropipette to a solution of benzyl 2,2,2-trichloroacetimidate (**174**) (5.12 g, 20.3 mmol) and the alkyne (**147**) (1.40 g, 10.1 mmol) in a 7:1 mixture of cyclohexane (22.2 mL) and DCM (3.2 mL) under argon. After 48 h at rt the reaction was diluted with water (15 mL) and hexane (15 mL) and

stirred for an additional 3 h at rt. A solution of satd $NaHCO_3$ solution (15mL) was added and the reaction left to stir for another 15 min. The formed trichloroacetamide was filtered off and the layers were separated and the aqueous layer separated and extracted with hexane (3× 45 mL). The combined organic layers were washed with brine (90 mL), dried ($MgSO_4$) and evaporated to yield an orange crude product. The product was purified by column chromatography [hexane-EtOAc (95:5)] to give a colourless liquid (**175**) (1.69 g, 73 %). $[\alpha]_D$ +22.4 (*c* 1.0, $CHCl_3$),



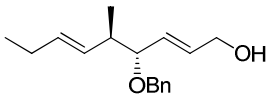
4.5.2 (4*R*,5*R*,6*E*)-(4-Benzyloxy)-5-methylnon-6-en-2-yn-1-ol (176)

n-BuLi (4.79 mL, 7.66 mmol) was added dropwise to a stirred solution of the *O*-Bn ether protected alkyne (**175**) (0.833 g, 3.65 mmol) in THF (5 mL) at −78 °C. The reaction was stirred for 30 min and allowed to reach 0 ºC. Anhydrous ground paraformaldehyde (0.328 g, 10.9 mmol) was added and the reaction stirred at 30-35 °C for 16 h. The reaction was quenched with satd NH_4Cl soln (2.5 mL). The reaction mixture was vigorously shaken and the aqueous layer separated and extracted with EtOAc (3×10 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$) and concentrated. The yellow crude product was purified by column chromatography with hexane-EtOAc (9:1) as eluent to produce the acetylenic alcohol (**176**) (0.75 g, 80 %). R_f = 0.38 (hexane-EtOAc 1:1); $[\alpha]_D$ +17.8 (*c* 1.0, $CHCl_3$).

δ_H: 7.33 (m, 5H, aromatic protons)
5.53 (ddt, 1H, $^3J_{6.7}$ 15.3, $^3J_{7,8}$ 6.3, $^4J_{5,7}$ 1.0, H-7)
5.38 (ddt, 1H, $^3J_{6,7}$ 15.3, $^3J_{5,6}$ 7.5, $^4J_{6,8}$ 1.5, H-6)
4.77 (d, 1H, $^2J_{a,b}$ 12.0, benzylic CH_2)
4.50 (d, 1H', $^2J_{a,b}$ 12.0, benzylic CH_2)
4.33 (dd, 2H, $^3J_{1,\text{-OH}}$ 6.3, $^5J_{1,4}$ 1.8, H-1)
3.98 (dt, 1H, $^3J_{4,5}$ 6.0, $^5J_{1,4}$ 1.8, H-4)
2.48 (m, 1H, H-5)
1.97 (m, 2H, H-8)
1.89 (d, 3H, $^3J_{5,\text{ methyl}}$ 6.8, 5-methyl)
0.96 (t, 3H, $^3J_{8,9}$ 7.5, H-9)

δ_C: 133.10D (C-7), 130.14D (C-6), 127.91 (aromatic carbons), 84.95S (C-3), 83.58 (C-2), 73.58D (C-4), 70.58T (4 –CH_2), 51.91T (C-1), 41.38D (C-5), 25.64T (C-8), 15.91Q (5-Me), 13.81Q (C-9)

ESI-MS: *m/z* 258 $[M+H]^+$. Exact mass: Calculated for $C_{17}H_{22}O_2$, 258.1620; Found, 258.1615.

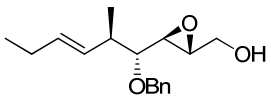


4.5.3 (2*E*,4*R*,5*R*,6*E*)-4-(Benzyloxy)-5-methylnona-2,6-dien-1-ol (177)

A solution of the acetylenic alcohol (**176**) (0.70 g, 2.71 mmol) in dry Et_2O (1.5 mL) was added dropwise to a cooled solution of Red-Al (1.28 mL, 4.34 mmol) in dry Et_2O (3 mL) while maintaining the temperature at 5 °C. After 2.5 h at rt the reaction was quenched by the careful addition of H_2O (2.5 mL). The aqueous layer was separated and the extracted with EtOAc (3×15 mL). The combined organic layers were washed with brine (30 mL) and dried ($MgSO_4$). The solvent was evaporated and the product purified by column chromatography [hexane-EtOAc (95:5→1:1)] to give the allylic alcohol (**177**) (0.65 g, 92%). R_f = 0.52 (hexane:EtOAc 1:1); $[\alpha]_D$ +39.5 (c 1.0, $CHCl_3$).

δ_H: 7.24 - 7.32 (m, 5H, aromatic protons)
5.78 (ddt, 1H, $^3J_{2,3}$ 15.7, $^3J_{1,2}$ 5.2, $^4J_{2,4}$ 0.8, H-2)
5.60 (ddt, 1H, $^3J_{2,3}$ 15.7, $^3J_{3,4}$ 7.8, $^4J_{1,3}$ 1.4, H-3)
5.47 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{7,8}$ 6.2, $^4J_{5,7}$ 0.7, H-7)
5.37 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{5,6}$ 7.4, $^4J_{6,8}$ 1.2, H-8)
4.56 (d, 1H, $^2J_{a,b}$ 12.1, benzylic CH_2)
4.36 (d, 1H', $^2J_{a,b}$ 12.1, benzylic CH_2)
4.16 (dd, 1H, $^3J_{1,2}$ 5.4, $^4J_{1,3}$ 1.4, H-1)
3.58 (ddd, 1H, $^3J_{3,4}$ 7.8, $^3J_{4,5}$ 6.1, $^4J_{2,4}$ 0.8, H-4)
2.34 (dddq, 1H, $^3J_{5,methyl}$ 6.8, $^3J_{4,5}$ 6.1, $^3J_{5,6}$ 7.4, $^4J_{5,7}$ 0.7, H-5)
1.99 - 2.08 (ddq, 2H, $^3J_{8,9}$ 7.4, $^3J_{7,8}$ 6.2, $^4J_{6,8}$ 1.2, H-8)
0.96 (t, 3H, $^3J_{8,9}$ 7.4, H-9)
0.95 (t, 3H, $^3J_{5,methyl}$ 6.8, 5-methyl)

δ_C: 133.77D (C-2), 132.23D (C-7), 131.16D (C-6), 130.27D (C-3), 127.29-128.17 (aromatic carbons), 83.38D (C-4), 70.16T (benzylic), 63.05T (C-1), 41.33D (C-5), 25.64T (C-8), 16.39Q (C-9), 13.88Q (Me)



4.5.4 (2*R*,3*R*,4*R*,5*R*,6*E*)-4-(Benzyloxy)-2,3-epoxy-5-methylnon-6-en-1-ol (178)

A mixture of powdered molecular sieves (4Å, 100 mg) and DCM (6 mL) under argon was cooled to –20 °C. A solution of $Ti(O^iPr)_4$ (0.17 g, 0.58 mmol) and (*S,S*)-DIPT (0.33 g, 1.61

mmol) in DCM (1 mL). The allylic alcohol (**177**) (0.30 g, 1.15 mmol) in DCM (3 mL) was added next. The catalyst complex was left to age for 20 min before the dropwise addition of TBHP in toluene (5.6M in toluene, 0.43 mL, 2.30 mmol). The reaction mixture was stirred at −20 °C for 6 h and left in the freezer at −15°C for a further 96 h. The mixture was then allowed to warm to 0 ºC and immediately poured into a solution of iron(II) sulfate (0.38 g) and tartaric acid (0.17 g) in deionized water (5.5 mL) at 0 ºC. The two-phase system was stirred at rt for 30 min and filtered through celite. The layers were separated and the aqueous layer extracted with Et_2O (3×25 mL) and the combined organic solutions dried ($MgSO_4$) and filtered. The solvent was removed by evaporation at 35 ºC.

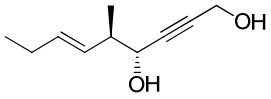
A solution of the crude product in Et_2O (5 mL) was cooled to 0 ºC and added to a pre-cooled (0 ºC) solution of NaOH (78.0 mg, 1.70 mmol) in brine (0.26 mL). The two-phase mixture was stirred vigorously for 1h with continued cooling. The layers were separated, The aqueous phase was separated and extracted with Et_2O (3×5 mL).The combined organic solutions were dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography using hexane-EtOAc (95:5→1:1) as eluant to yield the (2*R*,3*R*) epoxy alcohol (**178**) (100% dr) (0.23 g, 72%) as colourless oil. R_f = 0.43 (hexane-EtOAc 1:1); $[\alpha]_D$ +7.8 (*c* 1.0, $CHCl_3$).

δ_H: 7.31 (m, 5H, aromatic protons)
5.53 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{7,8}$ 6.3, $^4J_{5,7}$ 1.0, H-7)
5.43 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{5,6}$ 7.9, $^4J_{4,6}$ 1.3, H-6)
4.62 (d, 1H, $^2J_{a,b}$ 12.0, benzylic CH_2)
4.52 (d, 1H, $^2J_{a,b}$ 12.0, benzylic CH_2)
3.81 (m, 1H, H-1)
3.51 (m, 1H, H-1)
3.27 (dd, 1H, $^3J_{4,5}$ 4.1, $^3J_{3,4}$ 4.7, H-4)
3.11 (dt, 1H, $^3J_{2,3}$ 4.6, $^3J_{1,2}$ 2.5, H-2)
2.97 (dd, 1H, $^3J_{2,3}$ 4.6, $^3J_{3,4}$ 4.7, H-3)
2.46 (dddq, 1H, $^3J_{4,5}$ 4.1, $^3J_{5,methyl}$ 7.0, $^3J_{5,6}$ 7.9, $^4J_{5,7}$ 1.0, H-5)
2.01 (ddq, 2H, $^3J_{7,8}$ 6.3, $^3J_{8,9}$ 7.4, $^4J_{6,8}$ 1.3, H-8)
1.79 (t, 1H, -OH)
1.07 (t, 3H, $^3J_{5,methyl}$ 7.0, 5-methyl)
0.96 (t, 3H, $^3J_{8,9}$ 7.4, C-9)

δ_C: 133.09D (C-7), 129.82D (C-6), 128.28-127.56 (aromatic carbons), 80.87D (C-5), 73.18T (benzylic), 61.42T (C-1), 56.56D (C-2), 55.95T (C-3), 40.12D (C-4), 25.59T (C-8), 16.96Q (methyl), 13.04Q (C-9)

ESI-MS: *m/z* 276 $[M+H]^+$. Exact mass: Calculated for $C_{17}H_{23}O_3$, 276.1725; Found, 276.1738.

4.6 ROUTE 2

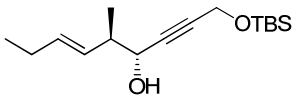


4.6.1 (4*R*,5*R*,6*E*)-5-Methylnon-6-en-2-yn-1,4-diol (183)

n-BuLi (1.6M in hexanes, 9.50 mL, 15.2 mmol) was added dropwise to a stirred solution of the acetylenic alcohol (**147**) (1.00 g, 7.24 mmol) in THF (10 mL) at −78 °C. After 30 min the reaction was allowed to warm to 0°C and dried paraformaldehyde (0.63 g, 21.7 mmol) was added. The reaction was stirred at 30-35 °C for 16 h and then quenched at −78°C with satd NH_4Cl soln. (5 mL). The reaction mixture was diluted with EtOAc (50 mL) and the aqueous layer extracted with EtOAc (3×50 mL). The combined organic layers were dried ($MgSO_4$) and the solvent evaporated. The crude product was purified by column chromatography using hexane-EtOAc (1:1) as eluent to yield the acetylenic diol (**183**) (0.86 g, 71%) as colourless liquid. R_f = 0.24 (hexane-EtOAc 1:1); $[\alpha]_D$ +8.0 (*c* 1.0, $CHCl_3$); ν_{max} 3390 (broad) cm^{-1}.

δ_H: 5.59 (ddq, 1H, $^3J_{6,7}$ 15.3, $^3J_{7,8}$ 6.5, $^4J_{5,7}$ 1.0, H-7)
5.31 (ddq, 1H, $^3J_{6,7}$ 15.3, $^3J_{5,6}$ 7.8, $^4J_{6,8}$ 1.8, H-6)
4.28 (d, 2H, $^4J_{1,4}$ 1.5, H-1)
4.15 (dt, 1H, $^3J_{4,5}$ 6.5, $^4J_{1,4}$ 1.5, H-4)
2.36 (m, 1H, $^3J_{5,6}$ 6.8, $^3J_{5,methyl}$ 6.8, $^4J_{5,6}$ 7.8, 1.0, $^4J_{5,7}$ 1.0, H-5)
2.02 (m, 2H, $^3J_{8,9}$ 7.3, $^3J_{7,8}$ 6.5, $^4J_{6,8}$ 1.8, H-8)
1.07 (d, 3H, $^3J_{5,methyl}$ 6.8, 5-methyl)
0.96 (t, 3H, $^3J_{8,9}$ 7.3, H-9)

δ_C: 135.05D (C-7), 129.43D (C-6), 85.19 (C-2), 83.91S (C-3), 66.40D (C-4), 50.87T (C-1), 43.46D (C-5), 25.63T (C-8), 15.96Q (CH_3), 13.71Q (C-9).



4.6.2 (4*R*,5*R*,6*E*)-1-[(*t*-Butyldimethylsilyl)oxy]-5-methylnon-6-en-2-yn-4-ol (184)

Et_3N (1.75 mL 12.4 mmol) and DMAP (55 mg, 0.45 mmol were added to a solution of the alkyne diol (**183**) (1.90 g, 11.3 mmol) in anhydrous DCM (57 mL). The solution was cooled to −15°C in an ice-bath and TBS-Cl (1.87 g, 12.4 mmol) was slowly added. The reaction was stirred at rt for 16 h. The reaction was quenched by the addition of H_2O (30 mL). The aqueous

phase was extracted with DCM (3×80 mL) and the combined organic layers washed with brine (160 mL), dried ($MgSO_4$) and evaporated. The crude product mixture was separated column chromatography (hexane-EtOAc 95:5) to give the primary *O*-TBS ether (**184**) (1.84 g, 67%) as an oil. R_f = 0.64 (hexane-EtOAc 1:1); $[\alpha]_D$ +21.5 (*c* 1.0, $CHCl_3$);

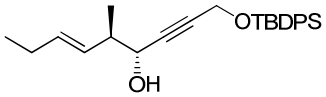
δ_H: 5.60 (ddt, 1H, $^3J_{6,7}$ 15.5, $^3J_{7,8}$ 6.4, $^4J_{5,7}$ 1.0, H-7)
5.34 (ddt, 1H, $^3J_{6,7}$ 15.5, $^3J_{5,6}$ 7.9, $^4J_{6,8}$ 1.5, H-6)
4.33 (d, 2H, $^5J_{1,4}$ 1.7, H-1)
4.14 (dt, 1H, $^3J_{4,5}$ 6.5, $^5J_{1,4}$ 1.7, H-4)
2.37 (dddq, 1H, $^3J_{5,6}$ 7.9, $^3J_{4,5}$ 6.5, $^3J_{5,methyl}$ 6.8, $^4J_{5,7}$ 1.0, H-5)
1.99 (ddq, 2H, $^3J_{7,8}$ 6.4, $^3J_{8,9}$ 7.5, $^4J_{6,8}$ 1.5, H-8)
1.08 (d, 3H, $^3J_{5,methyl}$ 6.8, 5-methyl)
0.97 (t, 3H, $^3J_{8,9}$ 7.5, H-9)
0.89 (s, 9H, *t*-butyl)
0.10 (s, 6H, $SiMe_2$)

δ_C: 135.07D (C-7), 129.97D (C-6), 84.70S (C-3), 84.45S (C-2), 66.85D (C-4), 52.06T (C-1), 43.75D (C-5), 26.12Q (*t*-butyl CH_3), 31.92S (*t*-butyl C), 25.99T (C-8), 16.16Q (CH_3), 14.07Q (C-9), –4.81Q ($SiMe_2$).

When the reaction was carried out at 0 °C, the di-*O*-TBS ether (**189**) (1.34 g, 30%) formed as a byproduct and was obtained as a light yellow oil; R_f = 0.69 (hexane-EtOAc 1:1).

δ_H: 5.48 (ddq, 1H, $^3J_{6,7}$ 15.3, $^3J_{7,8}$ 6.5, $^4J_{5,7}$ 0.9)
5.31 (ddq, 1H, $^3J_{6,7}$ 15.3, $^3J_{5,6}$ 7.8, $^4J_{6,8}$ 1.8)
4.32 (d, 2H, $^5J_{1,4}$ 1.6, H-1)
4.12 (dt, 1H, $^3J_{4,5}$ 6.0, $^5J_{1,4}$ 1.6, H-4)
2.30 (dddq, 1H, $^3J_{5,6}$ 7.9, $^3J_{4,5}$ 6.0, $^3J_{5,methyl}$ 6.8, $^4J_{5,7}$ 0.9, H-5)
1.99 (ddq, 2H, $^3J_{7,8}$ 6.3, $^3J_{8,9}$ 7.5, $^4J_{6,8}$ 1.6, H-8)
1.05 (d, 3H, $^3J_{5,methyl}$ 6.8, 5-methyl)
0.95 (t, 3H, $^3J_{8,9}$ 7.5, H-9)
0.89 (s, 9H, *t*-butyl)
0.88 (s, 9H, *t*-butyl)
0.11 (s, 6H, $SiMe_2$)

δ_C: 132.76D (C-7), 130.72D (C-6), 85.08S (C-3), 83.41S (C-2), 67.53D (C-4), 51.78T (C-1), 43.93D (C-5), 31.63S (*t*-butyl C), 25.80Q (*t*-butyl CH_3), 25.68T (C-8), 15.60Q (CH_3), 14.11Q (C-9), –5.06Q ($SiMe_2$).

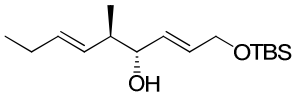


4.6.3 (4*R*,5*R*,6*E*)-1-(*t*-Butyldiphenylsilyl)oxy]-5-methylnon-6-en-2-yn-4-ol (187)

Et_3N (0.67 mL 4.77 mmol) and DMAP (21 mg, 0.17 mmol were added to a solution of the alkyne diol (**183**) (0.73 g, 4.34 mmol) in anhydrous DCM (22 mL). The solution was cooled to 0°C in an ice-bath and TBDPS-Cl (1.19 g, 4.34 mmol) was slowly added. The reaction was stirred at rt for 3 h (TLC control). The reaction was quenched by the addition of H_2O (10 mL). The aqueous phase was extracted with DCM (3×40 mL) and the combined organic layers washed with brine (80 mL), dried ($MgSO_4$) and evaporated. The crude product mixture was separated column chromatography (hexane-EtOAc 95:5) to give the primary *O*-TBDPS ether (**187**) (0.80 g, 69%) colourless liquid. R_f = 0.65 (hexane-EtOAc 1:1); $[\alpha]_D$ +9.0 (*c* 1.0, $CHCl_3$).

δ_H: 7.34 – 7.72 (m, 10H, aromatic protons)
5.58 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{7,8}$ 6.3, $^3J_{5,7}$ 1.0, H-7)
5.31 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{5,6}$ 7.8, $^3J_{6,8}$ 1.5, H-6)
4.37 (d, 2H, $^5J_{1,4}$ 1.7, H-1)
4.08 – 4.11 (m, 1H, $^3J_{4,5}$ 4.3, $^5J_{1,4}$ 1.7, H-4)
4.32 (dddq, 1H, $^3J_{5,6}$ 7.8, $^3J_{4,5}$ 4.3, $^3J_{3,\text{methyl}}$ 7.9,$^4J_{5,7}$ 1.0, H-5)
2.02 (ddq, 2H, $^3J_{7,8}$ 6.3, $^3J_{8,9}$ 7.5, $^4J_{6,8}$ 1.5, H-8)
1.07 (s, 9H, *t*-butyl –CH_3)
1.05 (d, 3H, $^3J_{3,\text{methyl}}$ 7.9, 5-methyl)
0.95 (t, 3H, $^3J_{8,9}$ 7.5, C-9)

δ_C: 134.73D (C-7), 131.51D (aromatic carbons), 129.77D (C-6), 84.66 (C-3), 83.95S (C-2), 66.47D (C-4), 52.68T (C-1), 43.33D (C-5), 26.66S (*t*-butyl), 25.65T (C-8), 18.99Q (*t*-butyl –CH_3), 15.80Q (methyl), 13.73Q (C-9).

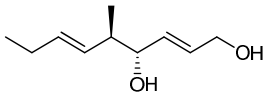


4.6.4 (2*E*,4*R*,5*R*,6*E*)-1-[(*t*-Butyldimethylsilyl)oxy]-5-methylnona-2,6-dien-4-ol (185)

A solution of the acetylenic alcohol (**184**) (1.00 g, 3.54 mmol) in dry Et_2O (5 mL) was added dropwise to a cooled solution of Red-Al (1.67 mL, 5.66 mmol) in dry Et_2O (3 mL) while maintaining the temperature at 0 °C. After 4 h at rt the reaction was quenched by the careful addition of H_2O (5 mL). The aqueous layer was separated and the extracted with EtOAc (3×15 mL). The combined organic layers were washed with brine (30 mL) and dried

($MgSO_4$). The solvent was evaporated and the product purified by column chromatography [hexane-EtOAc (3:1)] to give the minor product, the allylic alcohol (**185**) (0.15 g, 15%) as a colourless oil. R_f = 0.53 (hexane:EtOAc 1:1).

as well as the allylic diol (**190**) (0.44 g, 73%) as a light yellow liquid. R_f = 0.60 (hexane:EtOAc 1:1); $[\alpha]_D$ +12.0 (*c* 1.0, $CHCl_3$).



δ_H: 5.85 (ddt, 1H, $^3J_{2,3}$ 15.5, $^3J_{1,2}$ 5.3, $^4J_{2,4}$ 1.0, H-2)
5.68 (ddt, 1H, $^3J_{2,3}$ 15.5, $^3J_{3,4}$ 6.8, $^4J_{1,3}$ 1.5, H-3)
5.60 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{7,8}$ 6.3, $^4J_{5,7}$ 0.9, H-7)
5.33 (ddt, 1H, $^3J_{6,7}$ 15.4, $^3J_{5,6}$ 8.4, $^4J_{6,8}$ 1.6, H-6)
4.15 (ddd, 2H, $^3J_{1,2}$ 5.3, $^3J_{1,OH}$ 1.5, $^4J_{1,3}$ 0.6, H-1)
3.78 (ddd, 1H, $^3J_{4,5}$ 15.4, $^3J_{3,4}$ 6.8, $^4J_{1,3}$ 0.6, H-4)
2.15 (dddq, 1H, $^3J_{4,5}$ 15.41, $^3J_{5,6}$ 8.40, $^3J_{5,methyl}$ 6.8, $^4J_{5,7}$ 0.9, H-5)
2.03 (dddq, 2H, $^3J_{7,8}$ 6.3, $^3J_{8,9}$ 7.4, $^4J_{6,8}$ 1.6, H-8)
1.64 (b, 1H, $^3J_{1,OH}$ 1.5, -OH)
0.97 (t, 1H, $^3J_{8,9}$ 7.4, H-9)
0.95 (d, 3H, $^3J_{5,methyl}$ 6.8, 5-methyl)

δ_C: 134.89D (C-7), 132.06D (C-3), 131.52D (C-2), 130.34D (C-6), 75.58D (C-4), 63.02T (C-1), 43.63D (C-5), 25.65T (C-8), 16.65Q (CH_3), 13.82Q (C-9).