Chapter 1

Introduction

1 Introduction

1.1 Fungal Infections – Prevalence, Treatment

Fungal infections are one of the major causes of mortality in immunocompromised individuals, including Acquired Immunodeficiency Syndrome (AIDS), chemotherapy and organ transplant patients. The reduced effectiveness of the patient's immune system allows opportunistic fungal infections to take hold. Fungi like *Candida albicans*, which are a part of the normal human oro-intestinal flora, can thrive in the mouths of these patients producing infectious lesions (Figure 1.1) that make swallowing extremely painful and may lead to malnutrition and severe weight loss.[1] More severe fungal infections can spread to the larynx, oesophagus and lungs leading to forms of pneumonia and meningitis which often are the immediate cause of death in these patients.[2]

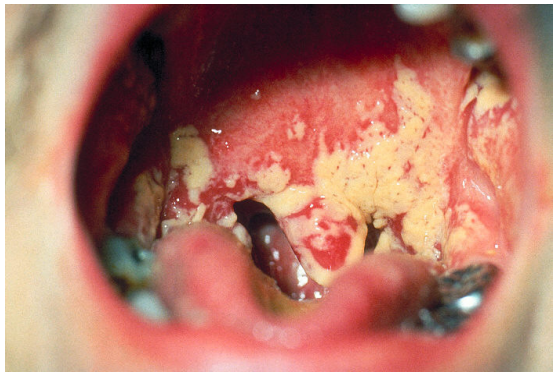


Figure 1.1 *Candida albicans* infection in the mouth of an AIDS patient.[3]

The immunocompromised population worldwide is rapidly increasing due to advances in medical techniques which have led to an increased number of patients taking immunosuppressants while undergoing organ transplantation and chemotherapy, and also due to the spread of AIDS. The World Health Organisation (WHO) released figures in 2003 revealing that 40 million people worldwide had AIDS or were infected with human immunodeficiency virus (HIV), the virus leading to AIDS. In 2003, 5 million people were newly infected with HIV

and 3 million people died from AIDS.[4,5] These alarming figures stress the importance of the search for new anti-fungal drugs.

Existing azole drugs, itraconazole[6] and fluconazole[7] (Figure 1.2) have been used extensively to treat *Candida* infections however chronic use has led to the appearance of azole-resistant strains.[8,9,10] Many of these strains are cross-resistant to all azole drugs emphasising the need for new fungicidal compounds with different modes of action. Amphotericin B (Figure 1.3) is a potent broad-spectrum anti-fungal drug which has a different mode of action to the azoles but targets the same biosynthetic pathway.[10] It's use is limited due to severe side effects[11] and nephrotoxicity.[12]

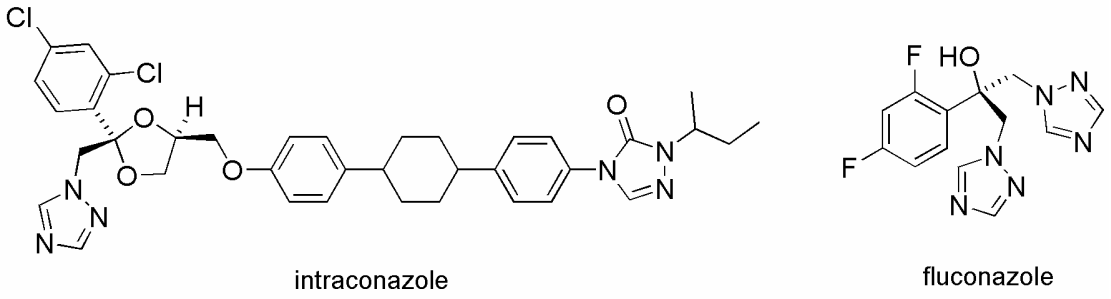


Figure 1.2 Intraconazole and fluconazole.

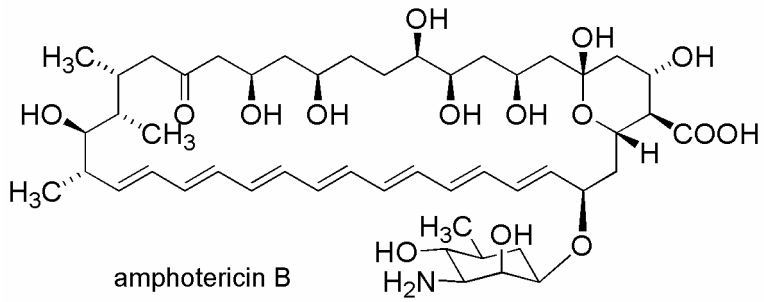
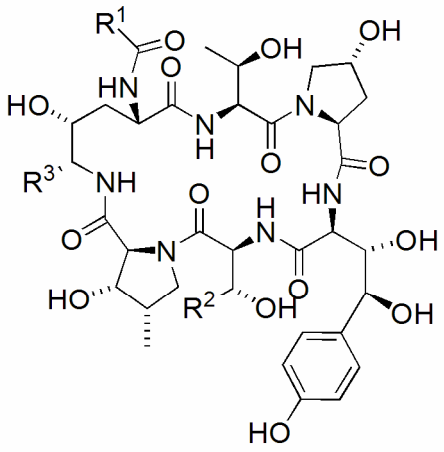


Figure 1.3 Amphotericin B.

More recently, several complex cyclic peptides (Figure 1.4) including echinocandins[13] and pneumocandins[14] have been shown to possess potent fungicidal properties against pathogenic fungal strains such as *Pneumocystis*, *Aspergillus* and *Candida*. Merck & Co have developed several semi-synthetic analogues of the echinocandins and a specific analogue, caspofungin acetate, was approved by the US Food and Drug Administration for clinical use in 2001 and is marketed as "cancidas".[15]



echinocandin B	$R^1 = C_{17}H_{31}$	$R^2 = CH_3$	$R^3 = OH$
pneumocandin A_0	$R^1 = C_{15}H_{31}$	$R^2 = CH_2CONH_2$	$R^3 = OH$
"cancidas"	$R^1 = C_{15}H_{31}$	$R^2 = CH_2CH_2NH_2$	$R^3 = NHCH_2CH_2NH_2$

Figure 1.4 Echinocandin B, pneumocandin A_0 and cancidas.

The promising activity displayed by these echinocandin analogues[16] has given renewed impetus to the development of novel anti-fungal treatments based on other cyclic peptide leads such as the microsclerodermins[17,18,19] which display potent fungicidal properties.

1.2 Microsclerodermins: Anti-fungal Cyclic Peptides

Microsclerodermins A – I (Figure 1.5) were isolated and identified by Faulkner and co-workers from marine sponges of the *Microscleroderma* and *Theonella* species of the Lithistida order. These sponges are found in the waters around Palau and Philippines. All these peptides display potent anti-fungal activity against a range of pathogenic fungal species including *Candida albicans*. Microsclerodermins F – I were tested for cytotoxicity against the human colorectal carcinoma cell line, HCT-116, and were found to have low IC_{50}'s ranging from 1.0 μg per ml to 2.4 μg per ml indicating their potential as anti-cancer agents.[17,18,19] Unfortunately microsclerodermins are isolated in very small amounts from the natural sponges (0.001 – 0.19% dry weight). This severely limits the development of anti-fungal and anti-cancer drugs and has led to synthetic investigations of these promising compounds.

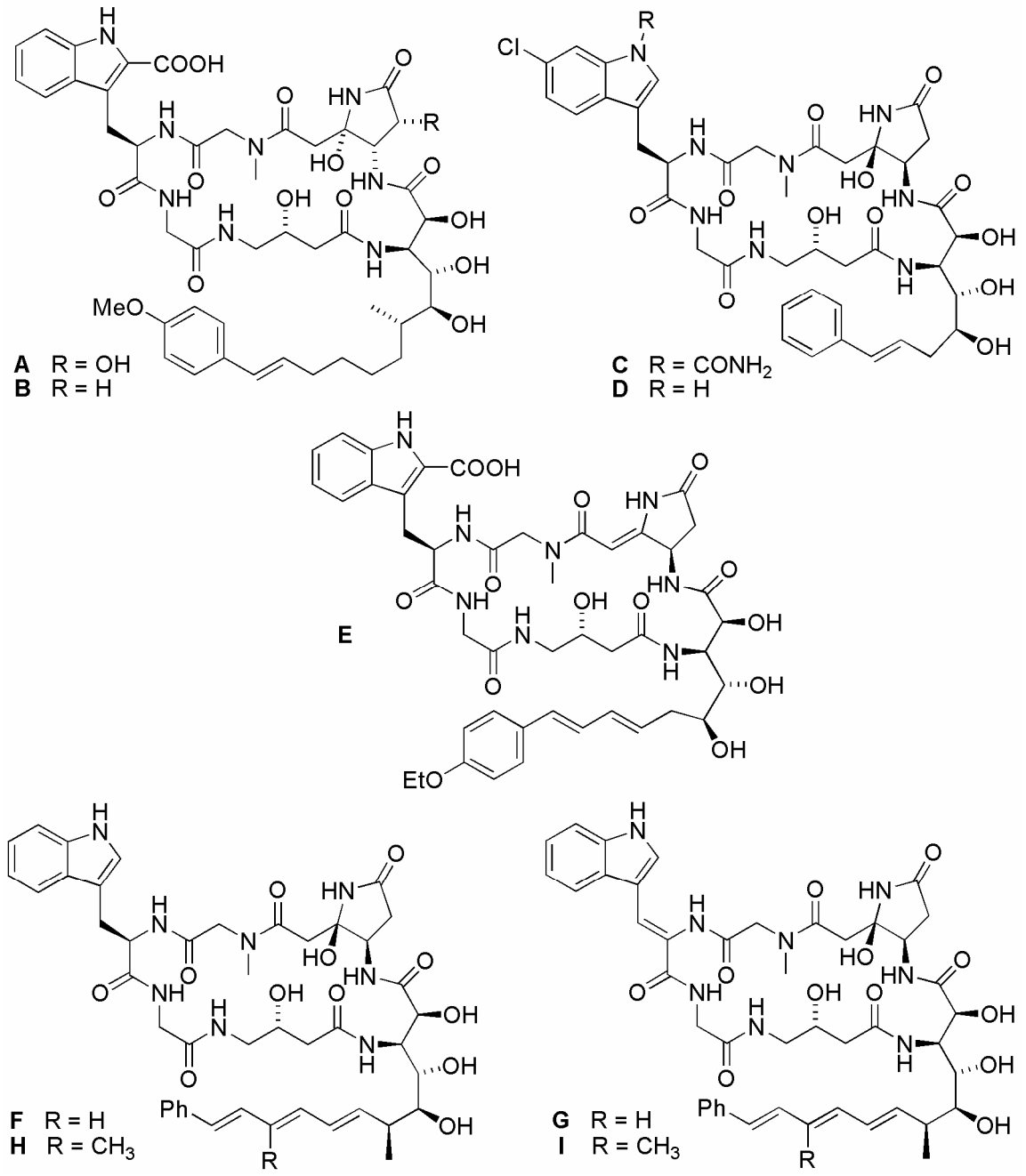


Figure 1.5 Microsclerodermins A – I.

Microsclerodermins all possess a 23-membered cyclic core constructed from six amino acid residues, three of which – glycine, *N*-methyl glycine and (3*R*)-4-amino-3-hydroxybutyric acid (GABOB) – are common to all members of the family. The various members of the microsclerodermin family differ in the structure of the three other amino acid residues; a functionalised tryptophan residue, an unusual 3-aminopyrrolidone-4-acetic acid moiety, and an ω-aryl-3-amino-2,4,5-trihydroxyacid residue as shown for microsclerodermin C (Figure 1.6).

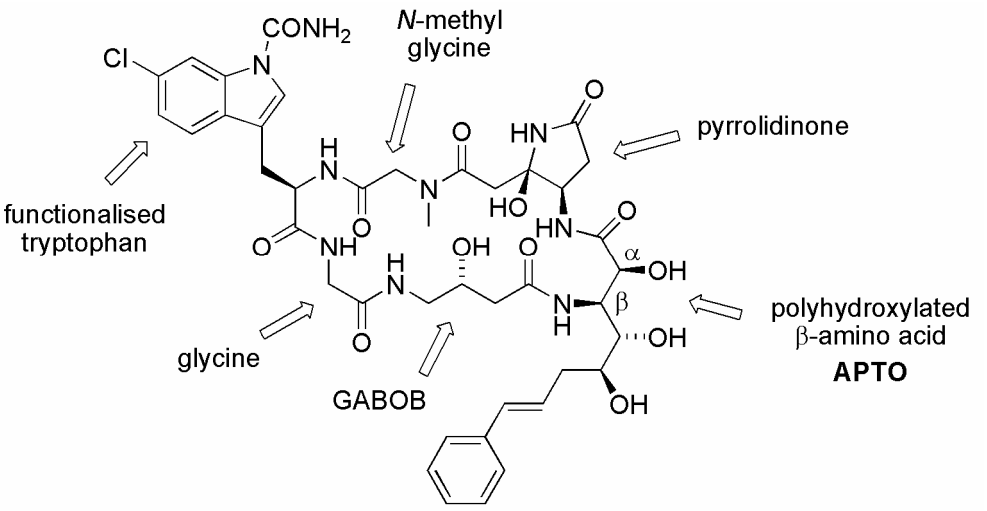


Figure 1.6 Constituent amino acids of microsclerodermin C.

The aim of the microsclerodermin project is to carry out a "versatile" total synthesis of microsclerodermin C through peptide coupling of the constituent amino acids and to apply the designed synthesis to the preparation of other members of the family. To do this, access to the constituent amino acids is required. Glycine (CAS number: 56-40-6) and *N*-methyl glycine (CAS number: 107-97-1) are both widely commercially available.[20] A short enantio-selective synthesis of a protected form of GABOB, using a substrate controlled asymmetric aminohydroxylation (AA) reaction as the key step, has recently been reported by other members of the McLeod group.[21] This synthesis is more direct than that reported previously by Sasaki, Hamada and Shioiri.[22] The tryptophan residue differs between family members: *D*-tryptophan (microsclerodermin F, H) is commercially available, and 2-carboxytryptophan (microsclerodermin A, B, E) has been previously prepared.[22,23,24] It is envisaged that the functionalised tryptophan from microsclerodermin C, D could be formed by urea formation under near neutral conditions[25] at the indole nitrogen of 6-chlorotryptophan.[26] Studies into the synthesis of the pyrollidinone residues of microsclerodermin A, B[22,23] and E[24] have been made and an alternative synthesis is being developed by other members of the McLeod group. The focus of the work presented here is the synthesis of the poly-hydroxylated β-amino acid residue APTO, from microsclerodermin C and the application of the same synthetic strategy to the synthesis of some of its analogues from the other family members.

1.3 APTO and Its Analogues

The complex functionality of these chiral poly-hydroxylated β-amino acid subunits of microsclerodermins presents a significant challenge to the synthetic organic chemist. All the analogues (for full names refer to Figure 1.7 and for structures refer to Figure 1.8) share a common "head" portion consisting of the 3-amino-2,4,5-trihydroxy acid (C_1-C_5) and contain different aryl-terminating "tails" of various chain length, saturation and substitution (≥C_6).

Microsclerodermin	Acronym	Full name
A , B	AMMTD	(2*S*,3*R*,4*S*,5*S*,6*S*,11*E*)-3-amino-12-(4-methoxyphenyl)-6-methyl-2,4,5-trihydroxydodec-11-enoic acid
C , D	APTO	(2*S*,3*R*,4*S*,5*S*,7*E*)-3-amino-8-phenyl-2,4,5-trihydroxyoct-7-enoic acid
E	AETD	(2*S*,3*R*,4*S*,5*S*,7*E*,9*E*)-3-amino-10-(4-ethoxyphenyl)-2,4,5-trihydroxydeca-7,9-dienoic acid
F , H	AMPTD	(2*S*,3*R*,4*S*,5*S*,6*S*,7*E*,9*E*,11*E*)-3-amino-6-methyl-12-phenyl-2,4,5-trihydroxydodeca-7,9,11-trienoic acid
G , I	10-methyl-AMPTD	(2*S*,3*R*,4*S*,5*S*,6*S*,7*E*,9*E*,11*E*)-3-amino-6,10-dimethyl-12-phenyl-2,4,5-trihydroxydodeca-7,9,11-trienoic acid

Figure 1.7 Full names of APTO and its analogues.

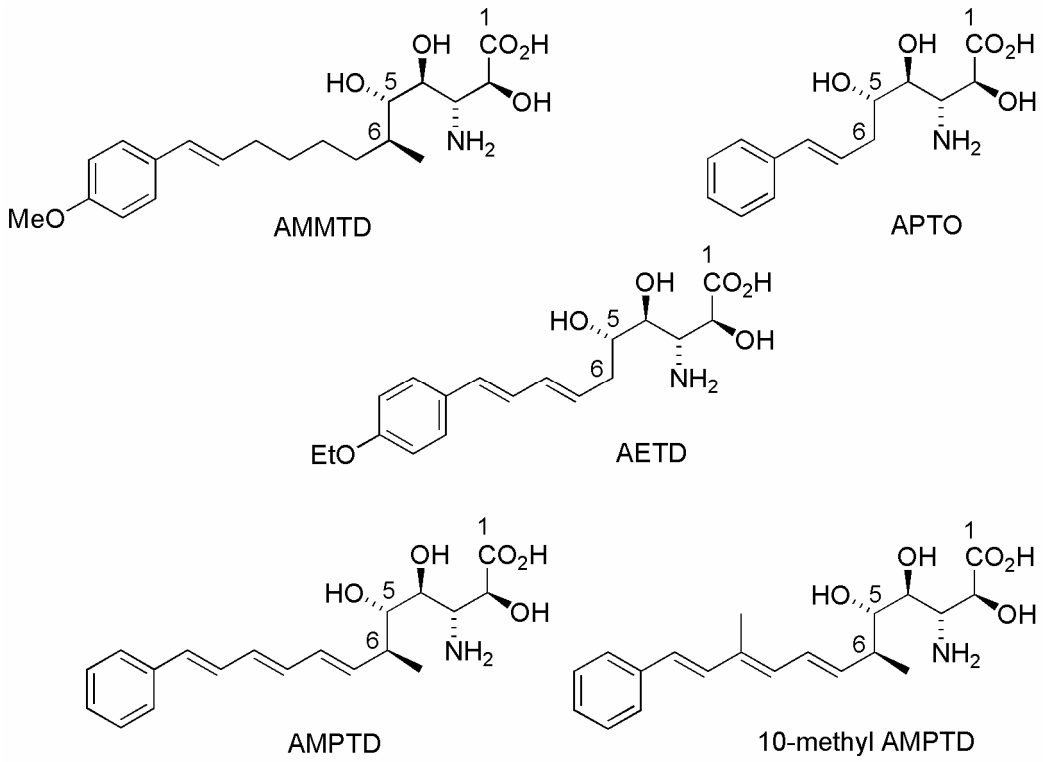


Figure 1.8 Structures of APTO and its analogues.

An ideal synthesis of these compounds should be short, efficient and highly diastereo-selective, while being flexible enough to allow easy access to all the analogues *via* common late stage precursors. To date, syntheses of two analogues of APTO have been published.

1.4 Previous Syntheses

The first synthesis of a protected form of the analogue AMMTD (**6** in Figure 1.9) was published in 1999, by Shigekazu Sasaki, Yasumasa Hamada and Takayuki Shioiri.[27] The synthesis starts with a protected form of commercially available 2-methylpropionate **7** which contains the C_6 chiral methyl group of the target. The key steps in the synthesis are a Sharpless asymmetric dihydroxylation (AD) reaction on alkene **8**, a palladium catalysed addition of anisole to alkyne **9**, a Paterson *anti*-aldol reaction to install the new diol in **10** and a nucleophilic substitution with azide to install the nitrogen in **11**. There are thirty one steps in the longest linear sequence and the final product is made with an overall yield of 2.2%. This first synthesis requires the use of chiral starting materials and the

installation of key functional groups often takes multiple steps leaving plenty of room for improvement. The strategy is specific to AMMTD with side-chain introduction in the earliest stages in the synthesis making it difficult to adapt to the other analogues.

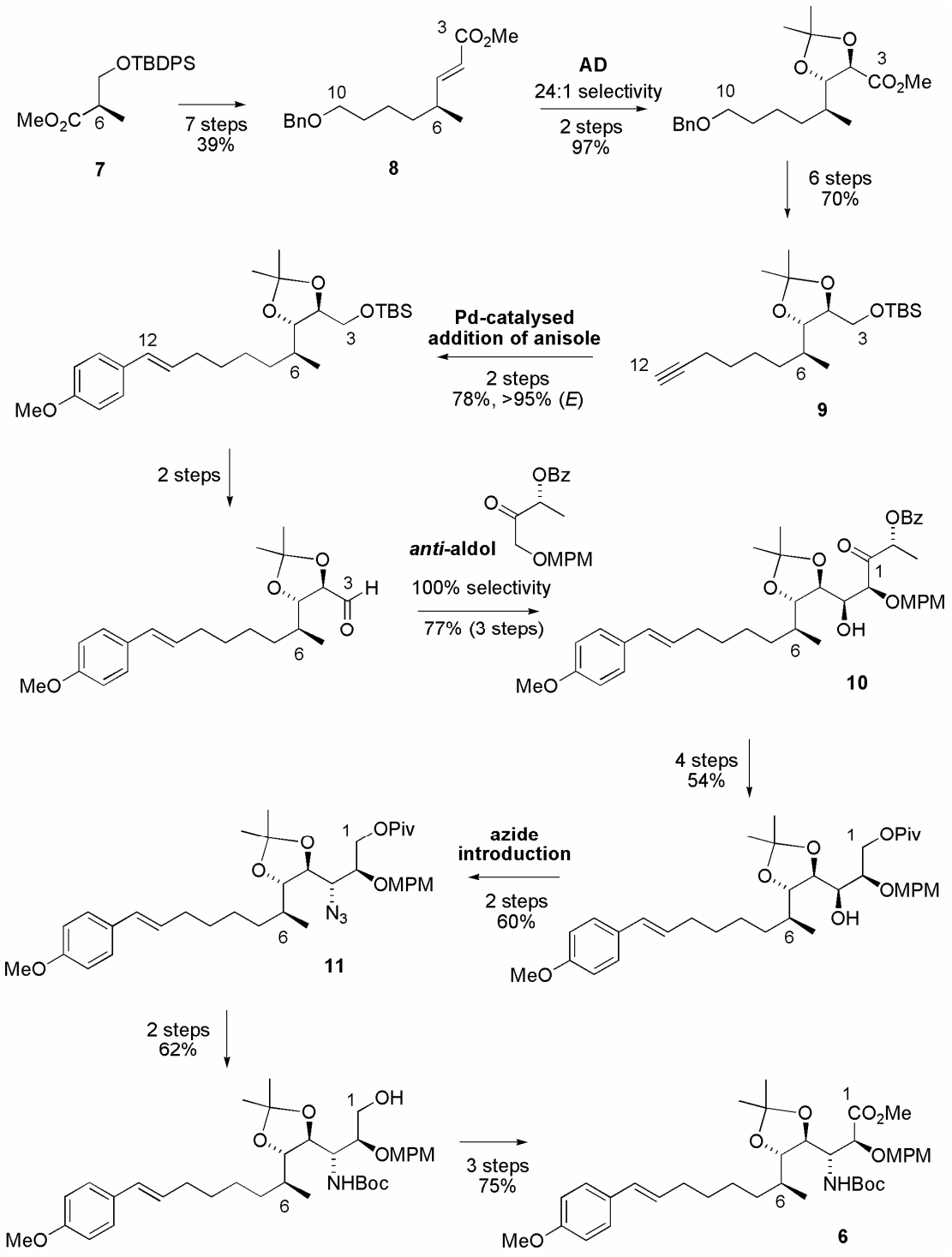


Figure 1.9 Sasaki, Hamada and Shioiri synthesis of protected-AMMTD **6**.

More recently Jidong Zhu and Dawei Ma released a total synthesis of microsclerodermin E in which they report the synthesis of the protected AETD – GABOB dipeptide **12** (Figure 1.10).[24]

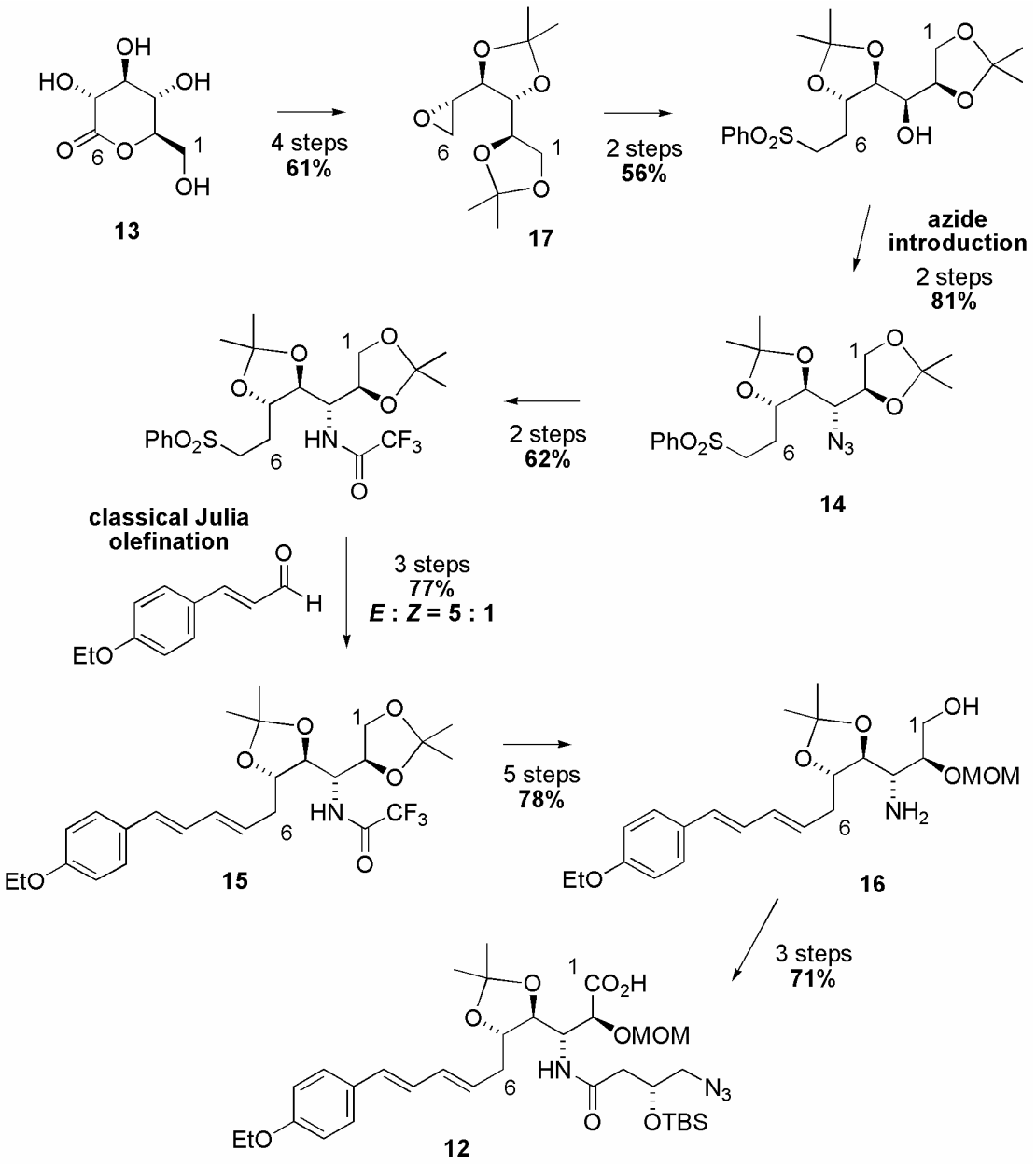


Figure 1.10 Ma's synthesis of the AETD – GABOB fragment.

The synthesis commences with commercially available δ-gluconolactone **13** which provides all of the stereochemistry in the product. The key steps in the synthesis are a nucleophilic substitution with azide to install the nitrogen in phenylsulfone **14** and a classical Julia olefination to install the conjugated tail in **15**. AETD precursor **16** is coupled to a precursor of GABOB before the terminal

alcohol is oxidised to afford acid **12**. There are twenty one steps in the longest linear sequence and the final dipeptide is made with an overall yield of 6.1% with respect to the AETD residue. This synthesis is based on a simple strategy however it involves numerous protecting group manipulations. The strategy may be limited to AETD and APTO because C_6 is present in the starting material and all the other analogues contain a chiral methyl group at this position. Use of epoxide **17** as a key intermediate in the synthesis would make installation of such a group difficult.

Both the published synthetic procedures mentioned above involve excessive protecting group manipulations and are specific to the analogues that they make. In this thesis, a versatile alternative method to synthesise these amino acid residues is reported, with APTO as the initial target.

1.5 Retro-Synthetic Analysis of APTO

In planning a synthetic strategy of APTO, all the analogues were kept in mind in order to reach a synthesis that would allow easy access to all the analogues *via* common, late-stage precursors. The aim of the work presented in this thesis was to provide a concise first synthesis of APTO and an improved synthesis of AETD. The devised approach to the synthesis of APTO involves a short, convergent and flexible strategy (Figure 1.11).

It was envisaged that APTO **1** could be afforded from protected precursor ***E*-18** by global deprotection. Protected APTO ***E*-18** could itself be derived by coupling aldehyde **19** with either phosphonium salt **20** in a Wittig olefination or with hetero-aryl sulfone **21** in a modified Julia olefination. Aldehyde **19** could be made from diol **22** in three simple steps: protection of the C_4-C_5 diol as the N,O-acetal, deprotection of the *p*-methoxyphenyl protecting group and oxidation of the resulting alcohol to the aldehyde. It was envisaged that diol **22** could be formed highly diastereo-selectively from (*E*)-alkene ***E*-23** *via* a Sharpless Asymmetric Dihydroxylation (AD). Incorporation of the *p*-methoxyphenyl protecting group into the substrate was expected to improve its interaction with the chiral ligand $(DHQ)_2PHAL$, leading to high facial selectivity in the addition. It was proposed that (*E*)-alkene ***E*-23** could be made by coupling aldehyde **24** with either hetero-aryl

sulfone **25** or **26** in a modified Julia olefination. Aldehyde **24** could be prepared from β-amino alcohol **27** in three steps: protection of the C_3-C_2 amino alcohol as an oxazolidine, de-protection of the *p*-methoxybenzoyl protecting group and oxidation of the resulting alcohol to the aldehyde.

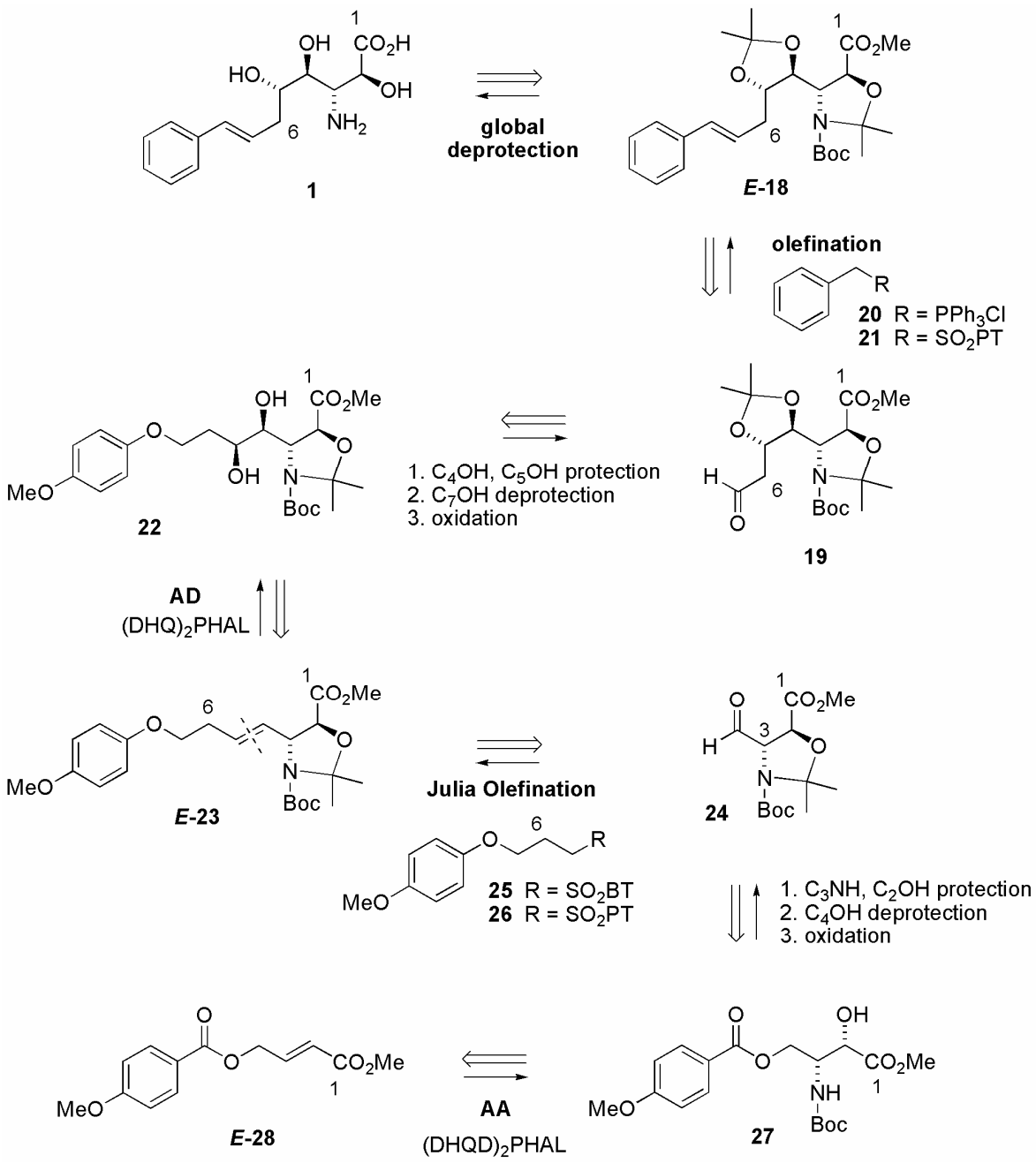


Figure 1.11 Retro-synthetic analysis of APTO.

It was envisaged that β-amino alcohol **27** could be made highly enantio-selectively from (*E*)-alkene ***E*-28** *via* a Sharpless Asymmetric Aminohydroxylation (AA). This time, incorporation of the *p*-methoxybenzoyl protecting group into the

substrate was expected to improve its interaction with the chiral ligand $(DHQD)_2PHAL$ enhancing both the facial and regio-selectivity of the addition. The syntheses of hetero-aryl sulfones **21**, **25** and **26**, phosphonium salt **20**, and alkene ***E*-28** could be completed in simple steps from commercially available starting materials.

It was envisaged that the strategy could easily be applied to AETD (Figure 1.12). The common precursor aldehyde **19** could be coupled with phosphonium salt **29** to afford protected AETD ***E*-30**. To access other family members containing the C_6-methyl group (Figure 1.13) an alternative Julia olefination reagent **31** containing the chiral methyl could be used to couple to common precursor aldehyde **24** to form alkene **32** containing the methyl group. This could be elaborated in an identical fashion to reach aldehyde **33** which upon coupling to an appropriate phosphonium salt **34** or **35** would afford analogues AMPTD ***E*-36** and 10-methyl-AMPTD ***E*-37** respectively.

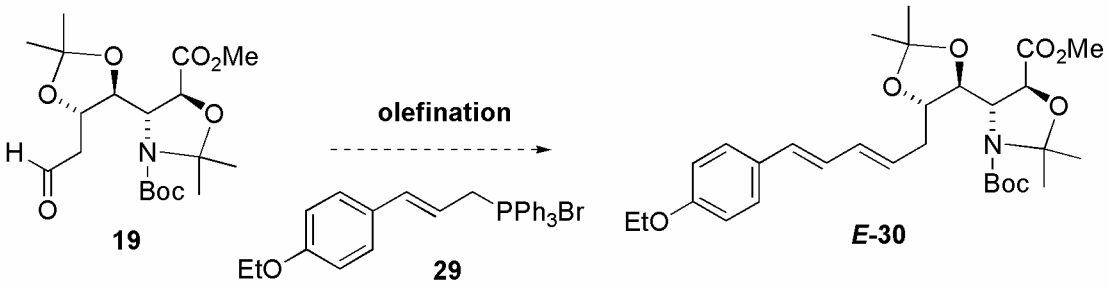


Figure 1.12 Proposed synthesis of AETD.

The proposed syntheses of protected APTO ***E*-18** and protected AETD ***E*-30** have a longest linear sequence of fourteen steps involving minimal protecting group manipulations. Specific protecting groups were chosen to not only protect reactive functional groups but to direct the regio- and stereo-control in the AA and AD reactions. By building up the head portion first, elaborating the middle of the compound and installing the tail at the end, this scheme should allow relatively easy access to the other analogues AMPTD and 10-methyl AMPTD.

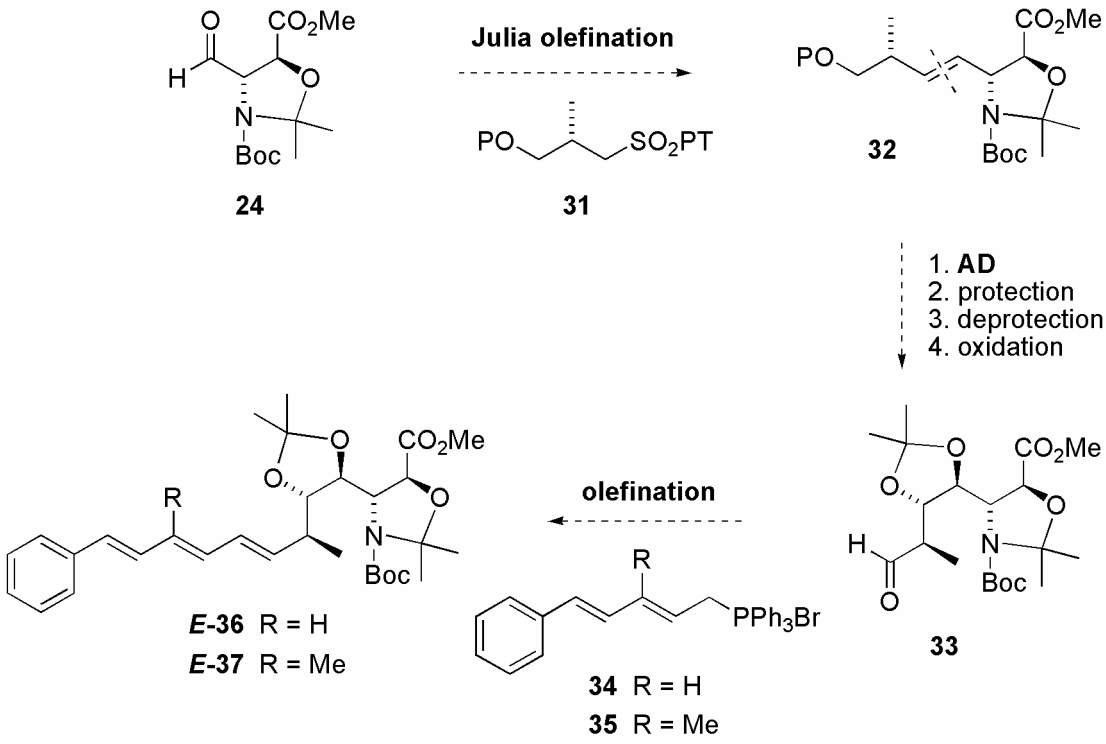


Figure 1.13 Proposed synthesis of AMPTD and 10-methyl-AMPTD.

1.6 Initial Studies

Initial studies (Figure 1.14) into the synthesis of amino-alcohol **27** were performed by Hung Duong, a summer scholar who visited the McLeod group in 2002. Commercially available *p*-anisic acid **38** was reacted with oxalyl chloride to form the acid chloride which was immediately reacted with allyl alcohol to afford allyl ester **39**.[28] Cross-metathesis, using Grubbs second generation ruthenium catalyst, of allyl ester **39** with methyl acrylate **40** afforded (*E*)-alkene ***E*-28** in good yield and formation of (*Z*)-alkene **Z-28** was not detected. AA using chiral alkaloid ligand $(DHQD)_2PHAL$ afforded desired amino alcohol **27**. Purification of amino alcohol **27** was complicated by a difficult separation from residual un-reacted *tert*-butyl carbamate and the yield was not determined at this stage.

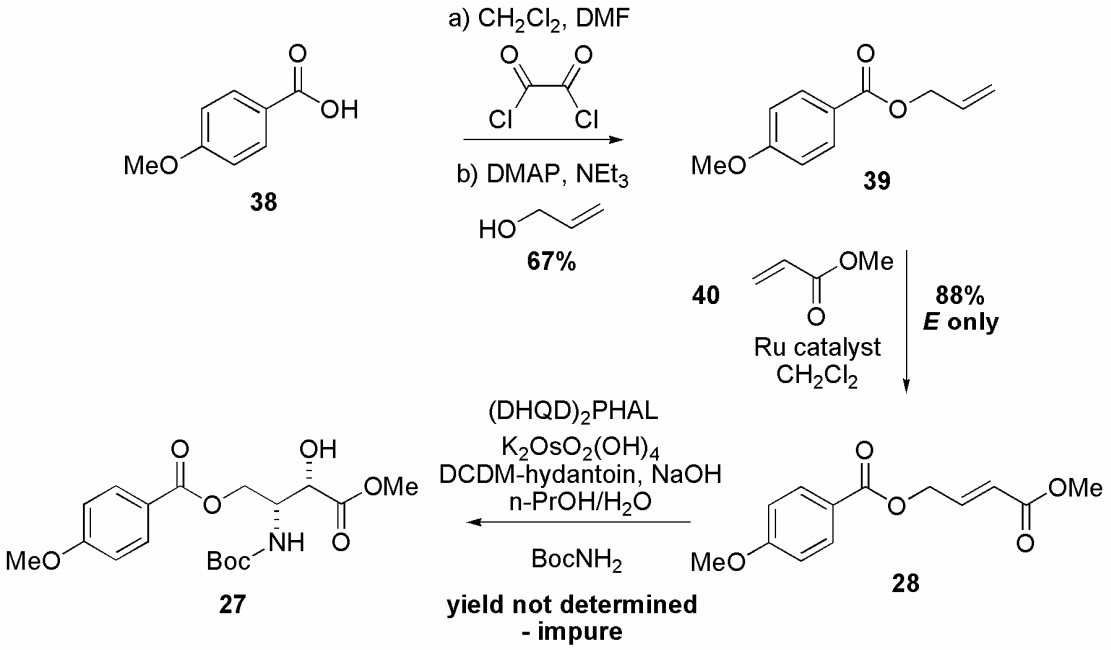


Figure 1.14 Duong's studies

In an attempt to facilitate purification and confirm the regio-chemistry, the product mixture was acetylated using acetic anhydride under standard conditions (Figure 1.15). The separation was still difficult and purification was achieved using high performance liquid chromatography (HPLC) to afford pure acetate **41** in 40% yield over the two steps. Comparison of the 200 MHz ^{1}H NMR spectra of impure amino alcohol **27** and purified acetate **41** supported the regio-chemical assignment. The signal due to C_2-H in acetyl derivative **41** was shifted downfield 0.84 ppm and was simplified to a doublet which is consistent with the alcohol group positioned at C_2 but not at C_3 in AA product **27**.

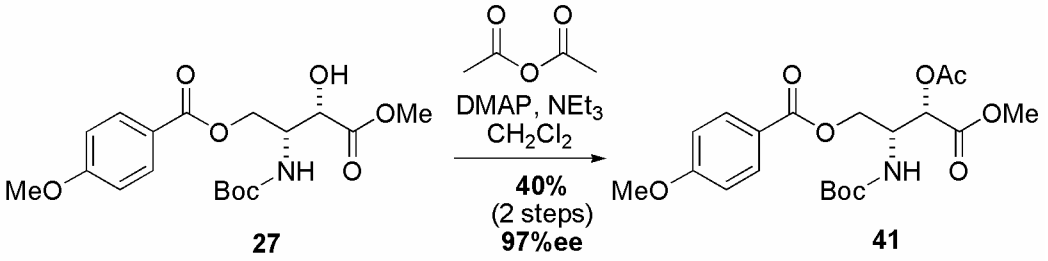


Figure 1.15 Acetylation of amino alcohol **27**.

The absolute stereochemistry was not proven but was based on the Sharpless mnemonic (see Chapter 2 for a full explanation). The enantiomeric excess (% ee) of AA product **27** was determined to be 97% by chiral HPLC of acetate **41**. The

identity of the minor enantiomer ***ent*-41** was confirmed by reference to an authentic standard synthesised under similar reaction conditions using the pseudo-enantiomeric ligand $(DHQ)_2PHAL$ (Figure 1.16). The % ee of enantiomeric AA product ***ent*-27** was determined to be 98%.

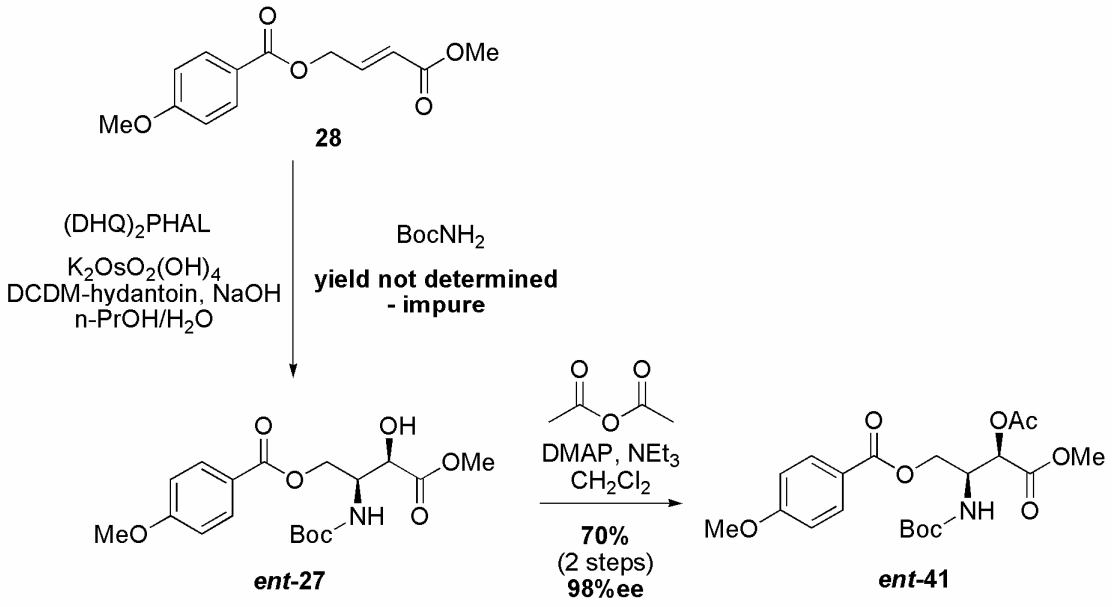


Figure 1.16 Preparation of minor enantiomer.

These early studies indicated the potential of the synthetic strategy as an effective means of synthesising APTO and led to more in-depth investigations.