Thesis

PROGRESS TOWARDS THE IMPROVED SYNTHESIS OF FK228 AND ANALOGS; AND THE TOTAL SYNTHESIS OF LARGAZOLE-AZUMAMIDE HYBRID

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY ANN E. TROUTMAN ENTITLED PROGRESS TOWARDS THE ENHANCED SYNTHESIS OF FK228 AND ANALOGS, AND THE TOTAL SYNTHESIS OF LARGAZOLE-AZUMAMIDE HYBRID BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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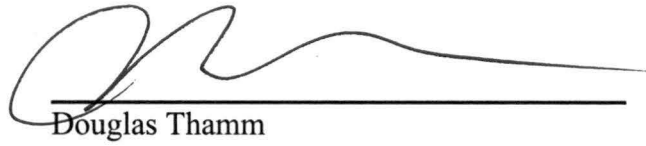

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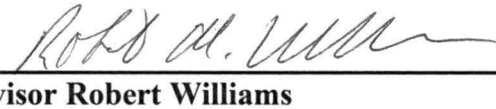

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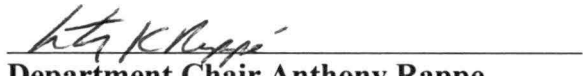

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Abstract of Thesis

Progress Towards the Enhanced Synthesis of FK228 and Analogs, and the Total Synthesis of Largazole-Azumamide Hybrid

Progress towards an improved synthesis of HDAC inhibitor FK228 and work towards the completion of FK228 and Largazole Azumamide hybrids are presented.

Through investigation of previous syntheses of FK228, a synthesis aimed at improving the overall yield and efficiency was planed out. After overcoming initial synthetic hurtles, a synthesis was attempted with moderate success. Further optimization would be needed for completion.

Interest in creating Class specific HDAC inhibitors has gained popularity due to results of recent studies. The potent biological activity of FK228 and Largazole made them ideal candidates for modification. We were interested in developing these analogs by attaching side chains of other known HDAC inhibitors onto each macrocyle core, and in doing so we hoped to increase the reactivity of each molecule.

Synthesis of the FK228-Azumamide hybrid began well, but hit several synthetic obstacles that have yet to be overcome. In contrast, the synthesis of Largazole-Azumamide hybrid proceeded smoothly, and was completed in thirteen steps and 11% yield. We are currently waiting for results of the biological activity studies.

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Chapter 1

Introduction

1.1 Background and Significance

Histone deacetylases (HDACs) are zinc metalloenzymes that catalyze the hydrolysis of acetylated lysine residues on histones. This returns the lysine side chains to their protonated state and results in the tight packaging of DNA into nucleosomes. This is due to the interaction of negatively charged DNA and these positive charges of the lysine residues. In this way, HDACs are involved in the regulation of gene expression and play an important role in the replication of DNA in eukaryotic cells; additionally, reversible lysine acetylation is an important regulatory process for non-histone proteins.

The first histone deacetylase proteins were discovered in the laboratory of Stuart Schreiber in 1996 while studying the effects of Trapoxin (**1**) and Trichostatin (**2**) in cultured mammalian cells.[1] Two nuclear proteins were isolated and identified as HDAC1 and HDAC2. Further studies by Schreiber led to the discovery of three more related deacetylases, HDAC4, HDAC5 and HDAC6.[2] To date, there are eighteen known HDACs, separated into four classes based on sequence homology to yeast counterparts. Studies by Schreiber and others have led to the realization that inhibition of HDACs causes arrest of the cell cycle in the G_1 and G_2 phases. For this reasons HDACs are recognized as promising targets for therapies intended to reverse the epigenetic states linked with cancer.[3] Experimental evidence has shown that some non-malignant diseases, such as rheumatoid arthritis[4], psoriasis[5], spinal muscle atrophy[6] and

Huntington's disease[7], might also benefit from these treatments. However, side effects of HDAC inhibitors observed in preclinical trials have limited their potential. These side effects emphasize the need for selective treatments.

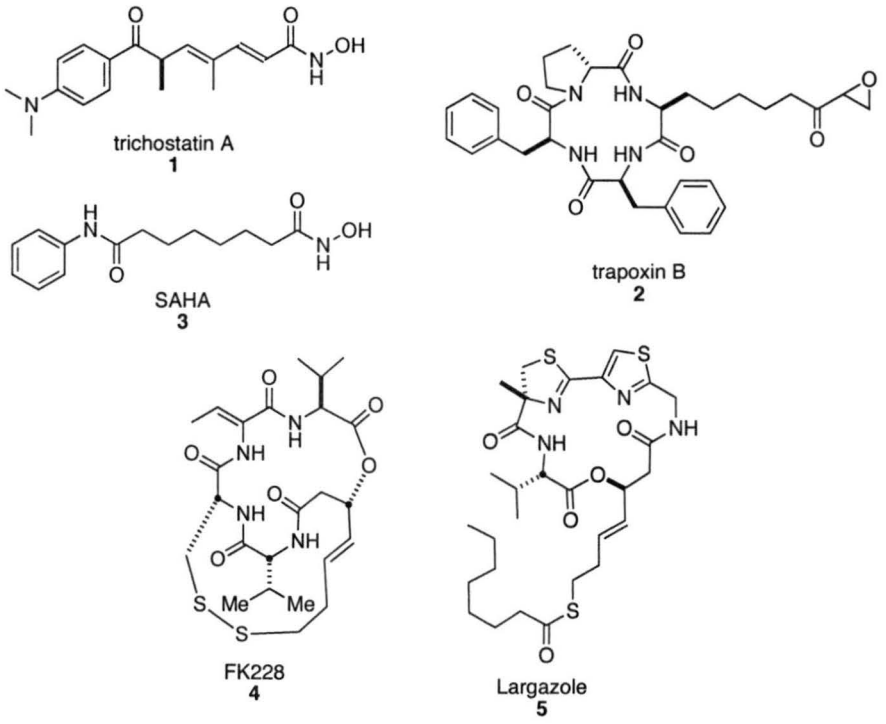


Figure 1. Structure Several Known HDAC Inhibitors

The main function of HDACs is to regulate transcription through histone deacetylation and chromatin condensation.[8] HDACs have numerous non-histone protein substrates which regulate many cell functions such as metabolism and cell death. In turn, HDAC inhibitors (HDACi) have been shown to alter gene expression, induce cell cycle and growth arrest, and cell death. However, the mechanism of action of HDACi has not yet been completely elucidated, and these varied responses are currently all overlapping and cannot yet be individually selected. It is believed that access to a specific response

pathway could increase the applicability of HDACi therapies. Development of HDACi's that specifically target a HDAC subclass are a new target of interest, as it has been seen that differences in the classes correlates with distinct functions (Figure 2)[9].

Recently, it was revealed that Class II HDACs play a critical role in cardiac growth and regulators of gene expression in the heart.[10, 11] Therefore, inhibition of Class II HDACs can stimulate cardiac growth. In contrast to these results, inhibition of Class I HDACs results in repression of cardiac growth, and normalizes cardiac gene expression.[12] Because of the lack of specificity to class in currently known HDACi's, these two responses are not effectively separated.

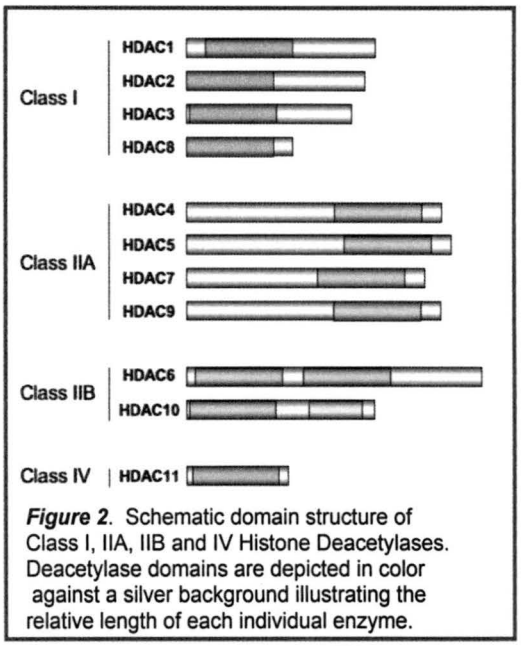


Figure 2. Schematic domain structure of Class I, IIA, IIB and IV Histone Deacetylases. Deacetylase domains are depicted in color against a silver background illustrating the relative length of each individual enzyme.

Most of the known HDACi's are HDAC class indiscriminate. SAHA, marketed as Zolinza by Merck & Co., is a nonspecific inhibitor of all HDAC classes. Because of the success of SAHA, it was first assumed that all HDAC classes were equally important. However, recent discoveries show that Class I HDACs are most clinically important in cancer therapies[12,13] as shown in studies using siRNA to knock out HDAC1 and HDAC3 in HeLa cells. The results provided more evidence that Class I HDACs are critical in cancer cell survival and growth. Similar studies where Class II HDACs were inhibited resulted in no change in the growth or survival of the cancer cells. This is still quite controversial, but this recent evidence could raise questions about the efficacy of broad spectrum treatments such as SAHA.

1.2 Structures and Mechanism

The structure of HDACi's mimic the natural HDAC substrates, N-acetyl-L-lysine. HDACi's have the general structure illustrated in Figure 3: a cap group, linker and zinc-binding motif. These components are often modified in hopes of increasing selectivity for Class I HDACs. Studies suggest that by shortening and/or eliminating the linker portion of the molecule, increased selectivity for inhibition of Class I HDAC8. This is thought to be because it mimics the shallow binding pocket of HDAC8.[14]

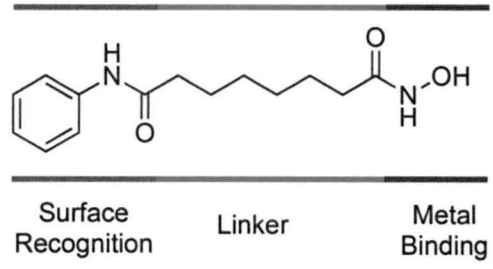


Figure 3. General Structure characteristics of HDAC inhibitors

Discriminating between HDAC1 and HDAC2 has so far proven very difficult because of the similarities between the two proteins. There are many zinc-binding motifs among the know HDAC inhibitors. The hydroxyamic acid of SAHA is thought to be the strongest binding group, but may be unfavorable for toxicology and stability reasons. The epoxy ketone of Trapatoxin is unsuitable for a drug candidate because it acts as a suicide inhibitor, resulting in irreversible inhibition. A few viable zinc-binding groups include the carboxylic acid of Azumamide E, carboxamide of Azumamide A (see Figure 2, page 35), and alkyl thiol in the active forms of FK228 and Largazole. FK228 in its

inactive form is hydrophobic and stable as the disulfide. The disulfide bond is reduced in the cell to afford the active alkyl thiol (Figure 4.) [15] As shown in Figure 4 the reduction of the disulfide bond also allows the molecule to mimic the natural substrate.

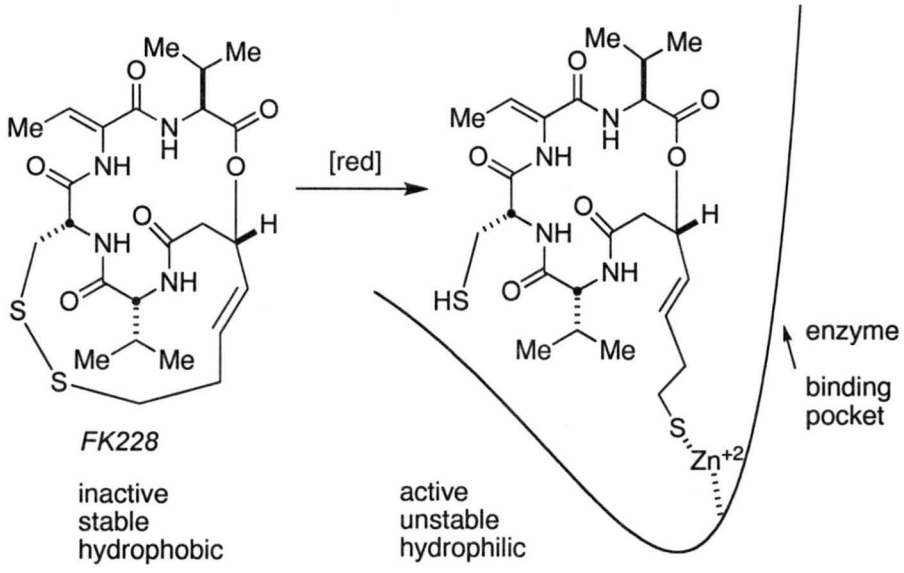


Figure 4. HDAC inhibitor binding mode

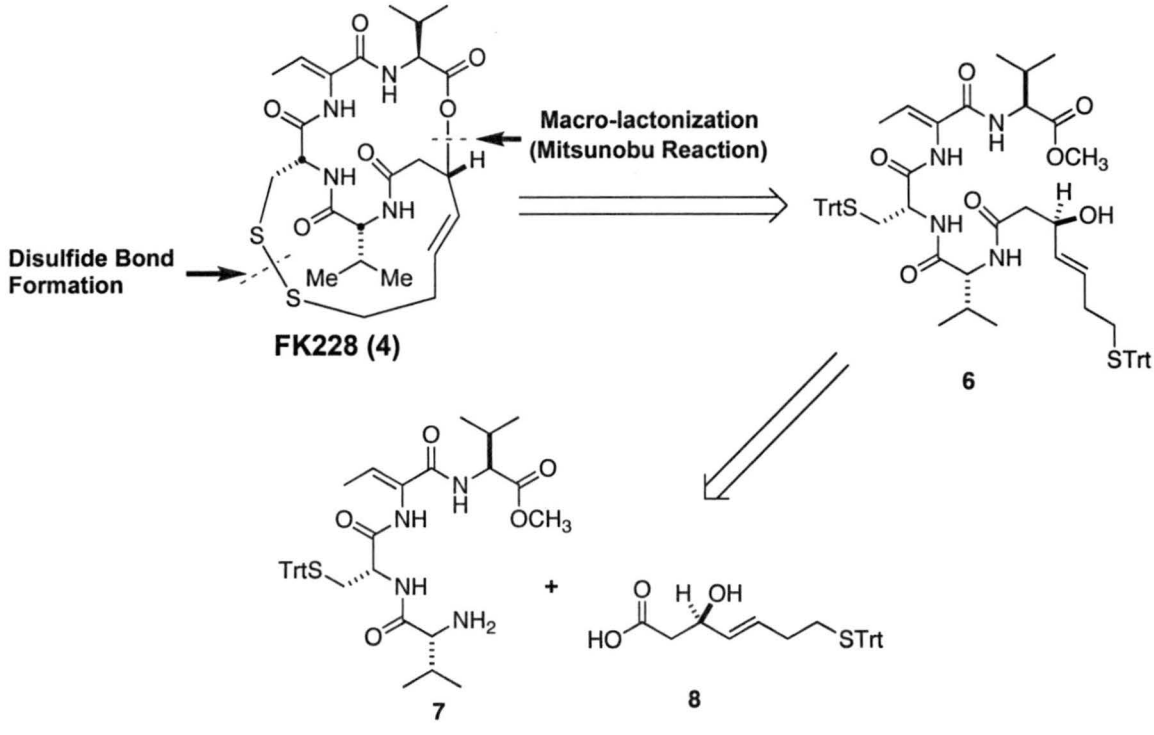
1.3 FK228 Isolation and Previous Syntheses

FK228 was isolated by the Fujisawa Pharmaceutical Co., Ltd., from the fermentation broth of *Chromobacterium violaceum*. [16] FK228 is one of several naturally occurring cyclic HDAC inhibitors. FK228 has several structural features that differentiate it from the rest of the cyclic HDAC inhibitors, such as the Azumamides, and Trapoxin shown in Figure 1. The first of said differences is macrocycle size. FK228 is a 16 membered depsipeptide macrocycle. Trapoxin and the Azumamides are made of 12 and 13 membered rings, respectively. The second difference is the length of the linker region. The natural substrate, N-Acetyl-L-lysine, is seven atoms long from the histone

backbone. Both Tapoxin and the Azumamides contain a seven-atom long side chain, mimicking the natural substrate. FK228 however, has a 5-atom side chain from the depsipeptide macrocycle. This makes the FK228 side chain (6.41 Å) significantly shorter than the natural substrate length at 7.32 Å.

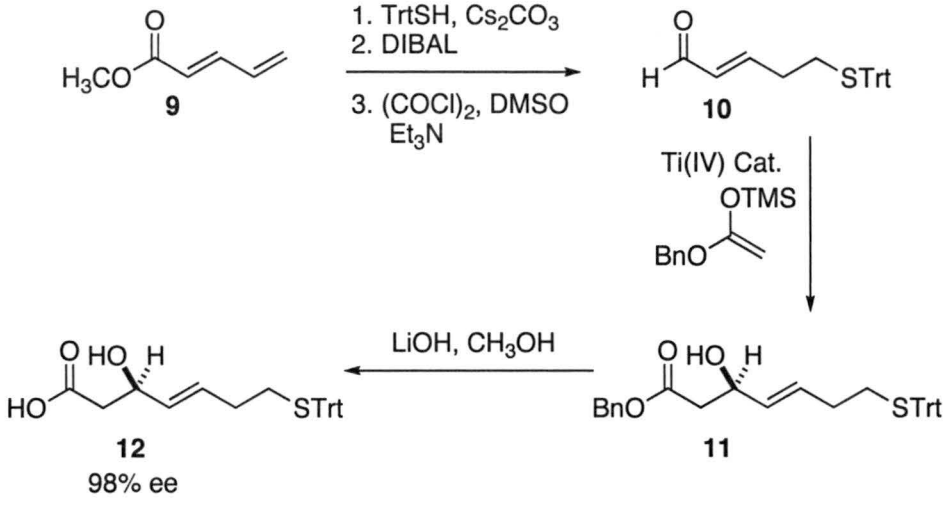
FK228 was first synthesized by Simon in 1996.[17] There have been two additional syntheses since Simon's, by the Williams group[18], and most recently by Ganesan[19] in 2008. There were two major challenges in the synthesis of FK228: (1) formation of the 16 membered cyclic depsipeptide, (2) construction of the asymmetric hydroxy mercapto heptenoic acid.

1.3.1 Simon's synthesis of FK228



Scheme 1. Retrosynthesis of Simon's Synthesis

To construct the mercapto β- hydroxy acid, a conjugate additon of methyl 2,4-pentadienoate **9** and cesium triphenylmethyl thiolate anion gave the unsaturated methyl ester. This was treated with excess Cs_2CO_3 , and then DIBAL reduction followed by Swern oxidation yielded unsaturated aldehyde **10**. An asymmetric aldol reaction using Ti-(IV) catalyst, and *O*-benzyl, *O*-TMS ketene acetal afforded the protected acid **11** in 98% ee. Hydrolysis of the benzyl ester provided the desired mercapto β- hydroxy acid **12** in 52% overall yield.

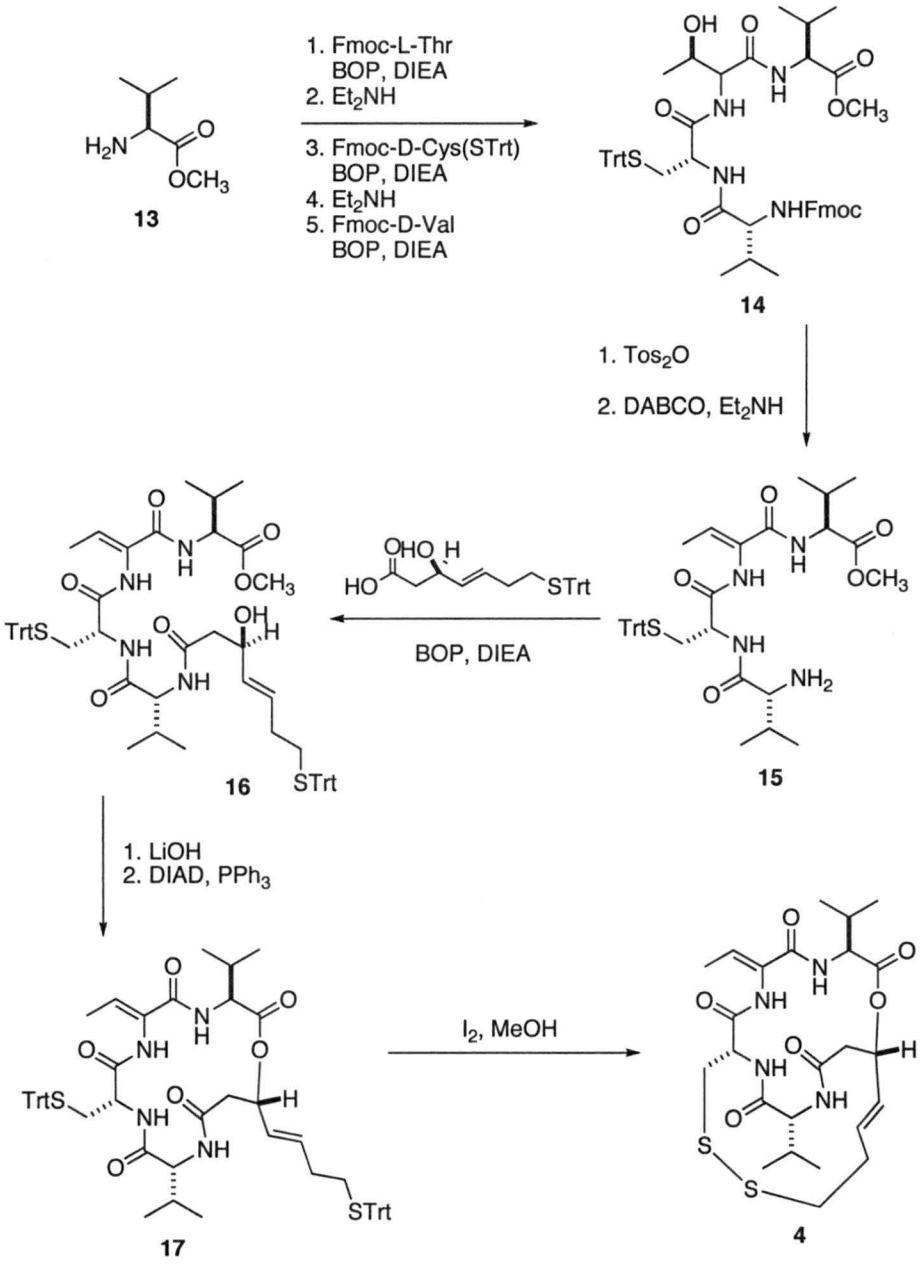


9 **10** **11** **12**

98% ee

Scheme 2. Simon's Synthesis of the Mercapto β-hydroxy Acid 12

The peptide portion was assembled using standard peptide coupling methods shown in Scheme 3. L-valine methyl ester was coupled to *N*-Fmoc-L-threonine **13**, followed by Fmoc deprotection using Et_2NH. The free amine was coupled to *N*-Fmoc-D-cysteine- (S-triphenylmethyl) to give the protected tripeptide. Fmoc deprotection of the tripeptide followed by coupling to *N*-Fmoc-D-valine afforded tetrapeptide **14**.



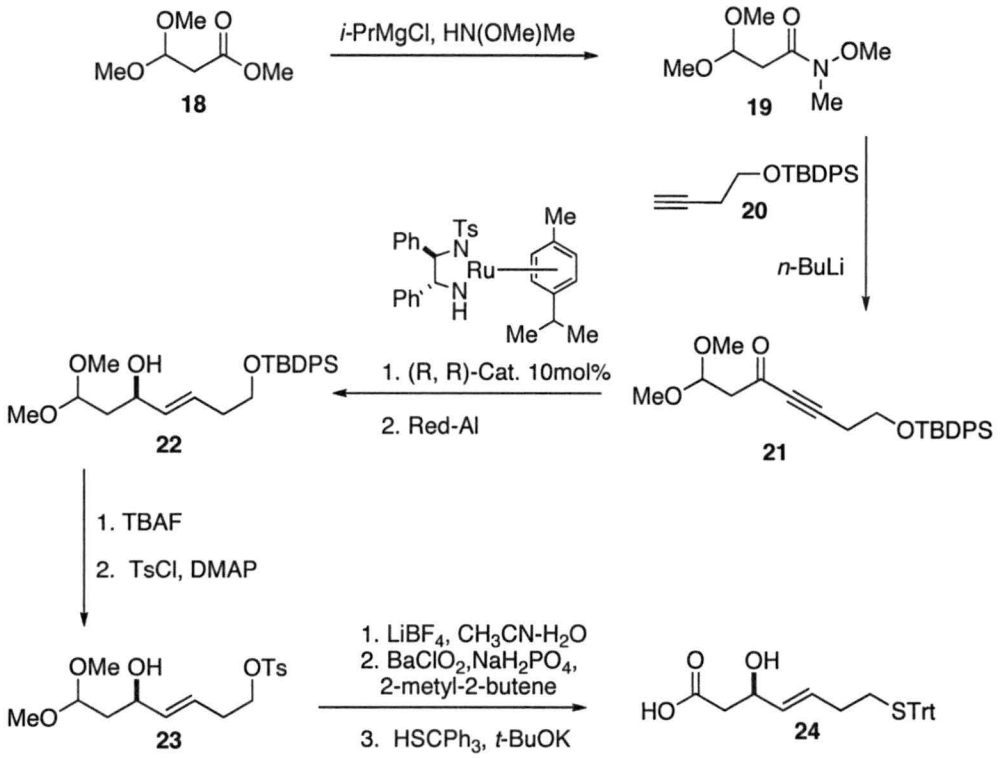
Scheme 3. Simon's Synthesis of FK228

The threonine residue was then dehydrated using tosic anhydride and DABCO, followed by Fmoc deprotection to give **15** in 74% yield. The mercapto β-hydroxy acid (**12)** was then coupled to **15** using BOP and DIEA to give hydroxy methyl ester **16.** Saponification of the methyl ester followed by a Mitsunobu reaction afforded marcrocycle **17** in a

reported 62% yield. The disulfide bond was formed with iodine and methanol to give FK228 in a reported 18% overall yield. This synthesis has proven to be difficult to reproduce at the reported yields, particularly for the mercapto β- hydroxy acid, and the Mitsinobu macrocyclization.

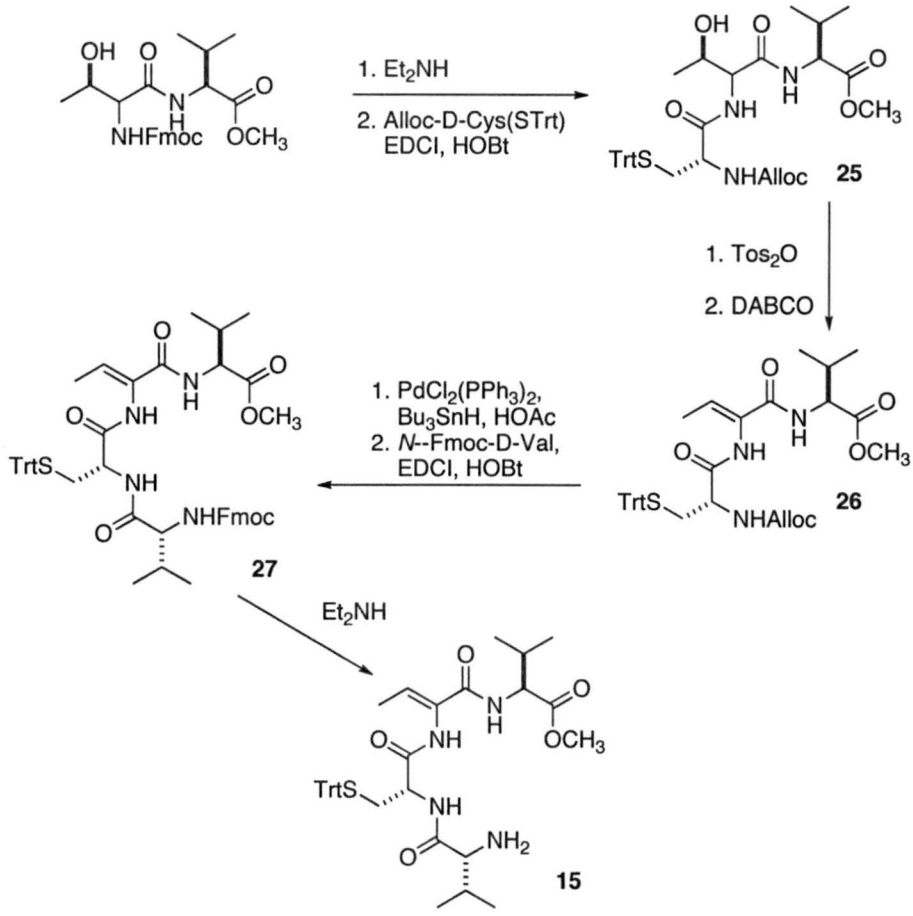
1.3.2 Williams Synthesis

The Williams group synthesis is similar to Simon's, except for the formation of the mercapto β-hydroxy acid. Our synthesis of this critical portion of the molecule was accomplished in nine steps and 13% yield. This is longer then Simon's but much more reliable and does not require the chromotagraphic separation of diastereomers of other methods.[20] To begin the β-hydroxy mercapto acid synthesis, methyl 3,3-dimethoxypropionate **18** was converted into Weinreb amide **19** using standard conditions. The propargylic ketone (**21**) was formed through the addition of lithium acetylide **20** to amide **19.** The propargylic ketone (**21**) was reduced to give (*R*)-propargylic alcohol in 99:1 er, utilizing Noyorie's asymmetric hydrogen transfer conditions. This was then treated with sodium bis(2-methoxyethoxy)aluminum hydride to selectively give (*E*)-alkene **22**. This silyl ether was then converted to the tosylate **23**, and treated with $LiBF_4$ to hydrolyze to the aldehyde. The aldehyde was immediately oxidized to the carboxylic acid using Pinnick oxidation conditions. Finally, the β-hydroxy mercapto acid **24** was completed by introduction of the trityl mercaptan in a 13.3% overall yield.



Scheme 4. Williams' synthesis of mercapto β-hydroxy acid

From this point, the synthesis of tetrapeptide **15** was modified slightly to improve yields. Specifically, *N*-Alloc-D-cysteine(STrt) was prepared and used in place of the *N*-Fmoc-D-cysteine(STrt), and coupled with L-threonine-L-valine-OMe to give the tripeptide **25** in 67% yield. An additional change to Simon's synthesis was the dehydration of the threonine residue earlier, at the tripeptide stage, instead of the tetrapeptide to give **26.** From there, deprotection of *N*-Alloc and coupling to *N*-Fmoc-D-valine gave protected tetrapeptide **27**. Finally, Fmoc deprotection of the terminal nitrogen gave **15** in 53% overall yield.



Scheme 5. Williams' Synthesis of Tetrapeptide 15

With tetrapeptide **15** and β-hydroxy mercapto acid **24** successfully completed, the final steps of Simon's synthesis were followed to complete FK228. However, the 62% yield of the Mitsunobu macrolactonization was never realized; the synthesis of depsipeptide **17** was accomplished in 24% yield. The overall result was an improved synthesis of FK228, with the highlight being the scaleable synthesis of the β-hydroxy mercapto acid.

FK228 has shown potent anitproliferative effects and progressed well in clinical trials. Unfortunately, during phase II studies serious cardiac events occurred, and enthusiasm for this agent has diminished.[21,22] Because of this, the Williams group is also

interested in analogs of FK228 to potentially reduce side effects and increase HDAC selectivity. To date the Williams groups has successfully completed an FK228 amide isostere analog.

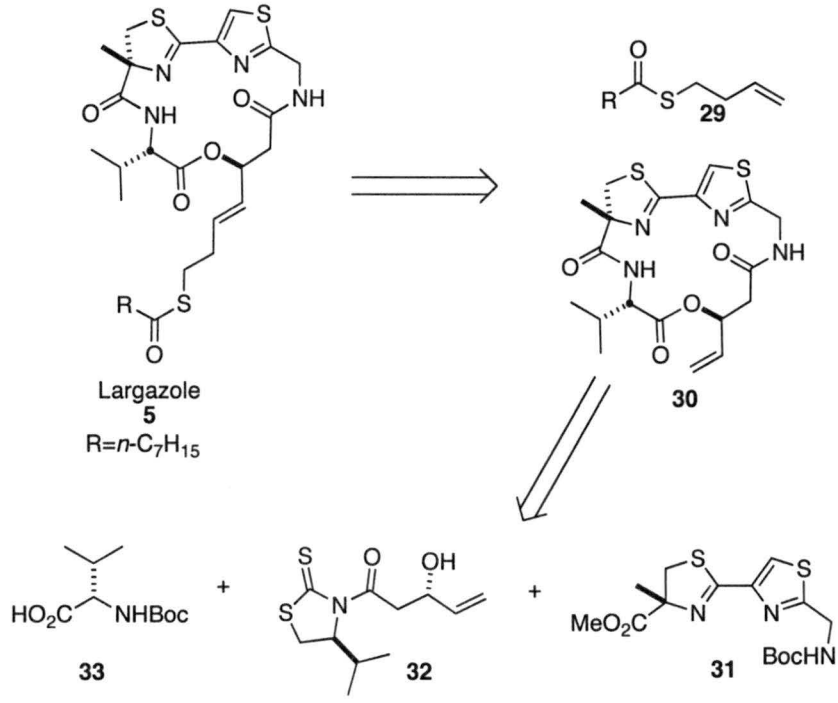
1.4 Largazole: Isolation, Activity, and Syntheses .

Largazole (**5**) is a 16 membered cyclic depsipeptide HDAC inhibitor that was isolated by Luesch in 2008 from the cyanobacterium *Symploca.* In the short time since its discovery, there have been seven published syntheses of Largazole the first by Luecsh[23], followed soon after by Williams[24], Ghosh[25], Phillips[26], Numajiri[27], Ren[28], and Cramer[29]. In addition to these total syntheses, Luecsh also published the synthesis of several Largazole analogs.[30] Luesch's preliminary biological activity studies showed that Largazole has nanomolar antiproliferative activity and functions as a pro-drug similar to FK228. Williams has also investigated the biochemical activity of Largazole, and its active metabolite Largazole thiol **(28)** against HDACs 1, 2, 3, and 6. The results of these studies showed that Largazole thiol (the reduced form of Largazole), is an extremely potent Class I HDAC inhibitor.[24] The fact that Largazole thiol was much more active then Largazole, indicates that it functions as a pro-drug which is activated by the removal of the octanoyl residue of the side chain to liberate the free thiol.

1.4.1 Luesch's Largazole synthesis

To begin, Luesch envisioned installation of thioester **29** through olefin cross-metathesis of cyclic depsipeptide **30**, and formation of the depsipeptide from the three quickly prepared subunits **31, 32, 33** (Scheme 6). The thiazoline-thiazole subunit **(31)**

was formed through a condensation of **34** with (*R*)-2-methyl cystine methyl ester hydrochloride.

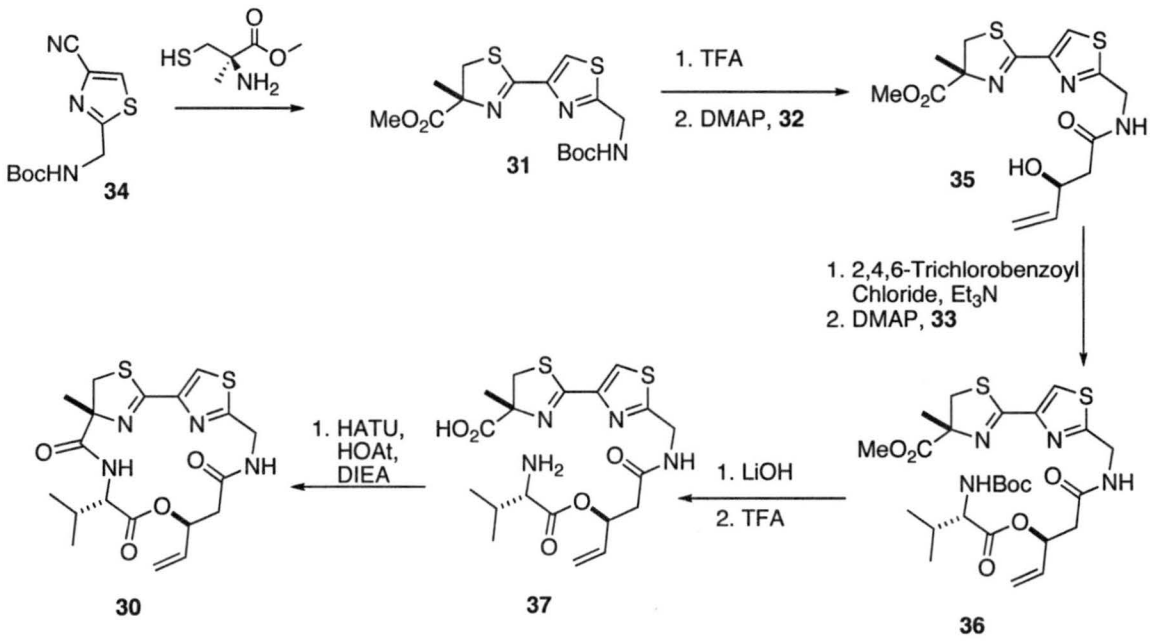


Scheme 6. Luesch Largazole Retrosynthesis

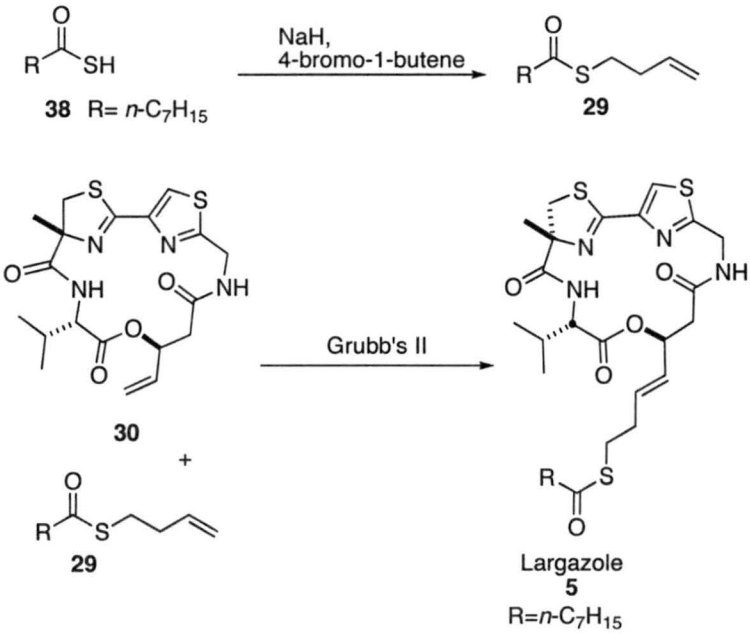
Subunit **31** was coupled after *N*-Boc deprotection to the amine **32**, which was prepared through an aldol reaction of acrolein and *N*-acyl thiazolidinethione to give **35** in 94% yield. Esterfication of **35**, with *N*-Boc-L-val **33**, afforded the acyclic depsipeptide precursor **36.** Hydrolysis of **36**, followed by *N*-Boc deprotection gave the deprotected acyclic precursor **37** in good yield. HATU mediated cyclization of **37** resulted in the cyclized depsipeptide core **30** in 64% yield (Scheme 7).

Formation of thioester **29** was accomplished in one step from the known thioacid **38**.[31] The thioacid was coupled with 4-bromo-1-butene to provide thioester **29** in 81% yield. With the thioester and depsipeptide core in hand, formation of Largazole was

accomplished through olefin cross metathesis using Grubbs II catalyst. The total synthesis was accomplished in eight steps and 19% overall yield.

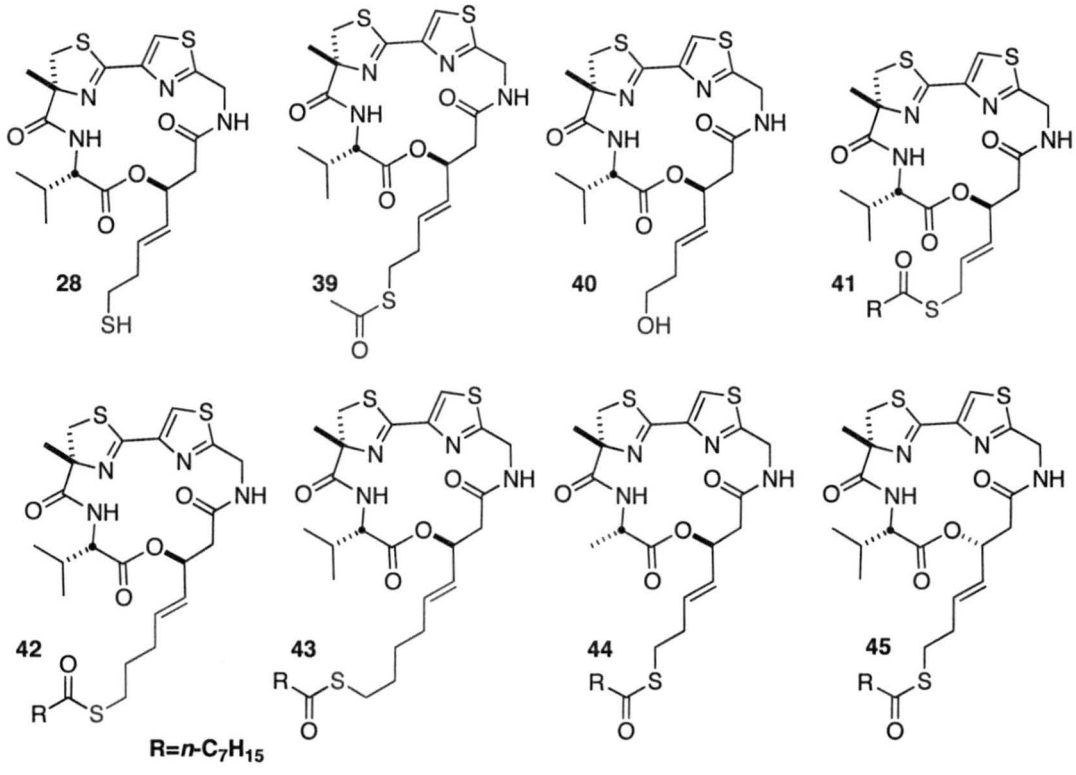


Scheme 7. Luesch Synthesis of Largazole Depsipeptide Core



Scheme 8. Thioester Formation and Completion of Largazole

Luesch then prepared several analogs in an attempt to test the structural activity relationship (Scheme 9). A largazole thiol analog **28**, acetyl analogue **39**, hydroxyl analog **40**, three linker length analogs **41**, **42**, **43** (five to seven atom linkers respectively), an alanine analog (**44**), and a C17 epimer analog (**45**) were made and their activity tested.



Scheme 9. Analogs of Largazole

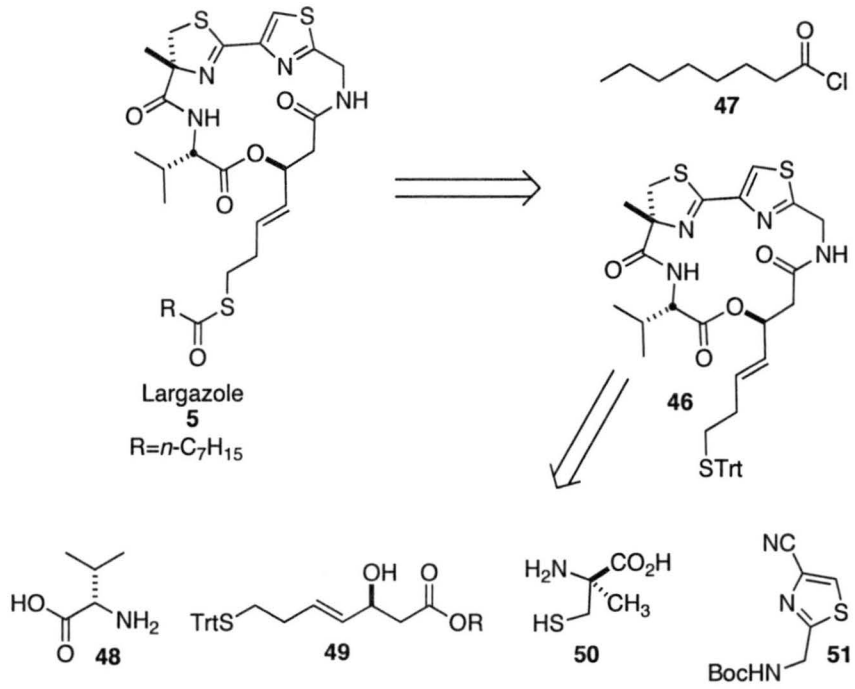
The thiol analog (**28**) was found to be the most active, confirming that Largazole is a pro-drug that will be reduced in the cell to give the free thiol. The acetyl analog (**39**) showed some activity for HDAC inhibition, but lower then that of **28**. The hydroxyl analog (**40**), did not show any real HDAC inhibition. Analog **41**, with a one carbon shorter linker than **5**, showed no activity, whereas analogs **42** and **43**, with increased linker lengths of one and two methylene units, showed a reduction of a few orders of

magnitude compared to **5.** This indicates Largazole contains the optimal linker length for highest HDAC inhibition. The alanine analogue **44** showed only a 2-fold decrease in activity in comparison to Largazole, indicating that the valine residue is essentially replaceable; however, this change did decrease its selectivity for HDAC1 vs. HDAC6. The C17 epimer analog **45**, showed very weak inhibitory activity. These results showed that linker length and configuration at C17 of the macrocycle are critical for powerful HDAC inhibitory activity.

1.4.2 Williams' Largazole Synthesis

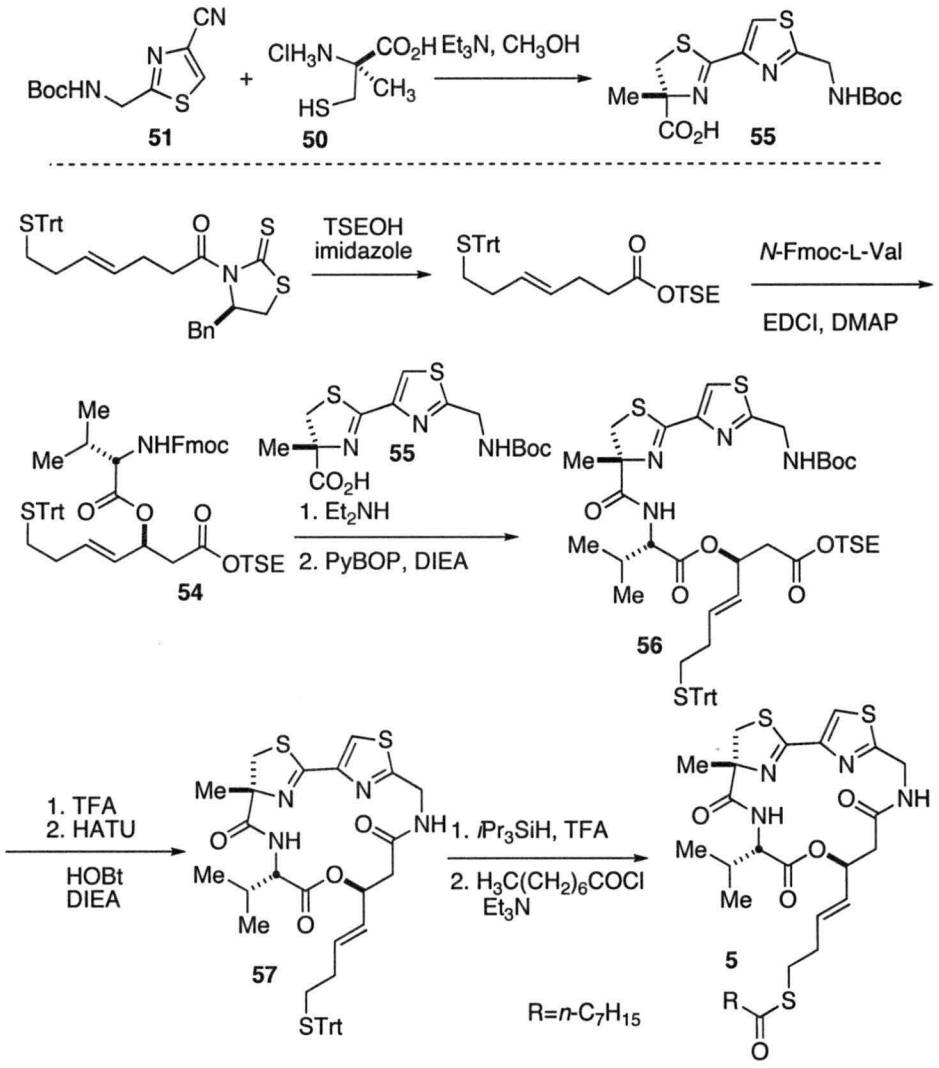
The main differences between Williams' synthesis and Luesch's are the formation of the zinc-binding side chain and the macrocyclization of the depsipeptide core. Williams' Largazole synthesis began with marcrocycle formation from four subunits that are easily made through previously published methods (Scheme 10). The thioester portion was installed through acylation of the free thiol of **28**, rather than through cross metathesis as done in Luesch's synthesis.

To begin, the β-hydroxy mercapto acid **49** was produced following a synthesis by Ganesan.[32] The protected acid **53** was made by treating thiazolidinethione **52** with 2-(trimethylsilyl)ethanol. This was coupled to *N*-Fmoc-L-valine to give **54** in good yields using an excess of the amino acid.



Scheme 10. Williams' Largazole Retrosynthesis

The thiazoline-thiazole **55** was prepared in high yields through a condensation of α-metylcysteine **(50)** with known nitrile **51**. *N*-Fmoc deprotection of **54** followed by a PyBop mediated coupling to thiazoline-thiazole **55** afforded acyclic precursor **56**. TFA deprotection of the TMS ethyl ester, and *N*-Boc group, followed by macrocylicization with HATU and HOBt gave the marcrocyle **57** in 77% yield for two steps. Removal of the trityl protecting group with iPr$_3$SiH and TFA gave Largazole thiol **28**, which was then acylated with octanoyl chloride to provide Largazole (**5**) in ten linear steps and 34% overall yield.

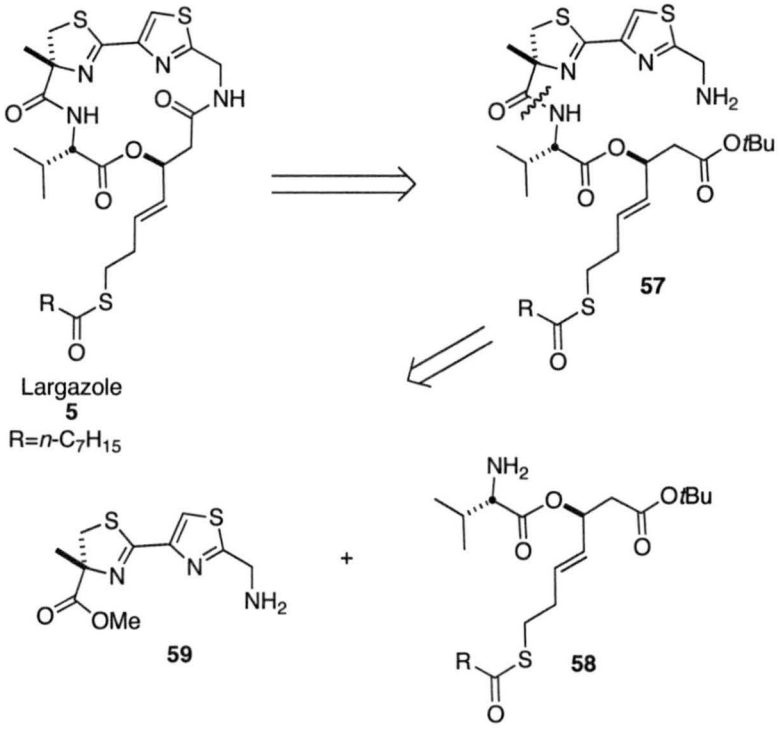


Scheme 11. Williams' Largazole Synthesis

The biological activity studies done by Williams of both Largazole, and Largazole thiol against HDAC1, 2, 3, and 6 show that Largazole, and in particular Largazole thiol, are extremely potent Class I HDAC inhibitors. They also have shown that Largazole is a pro-drug, and the octanoyl portion of the molecule allows for better cell permeability than the free thiol.

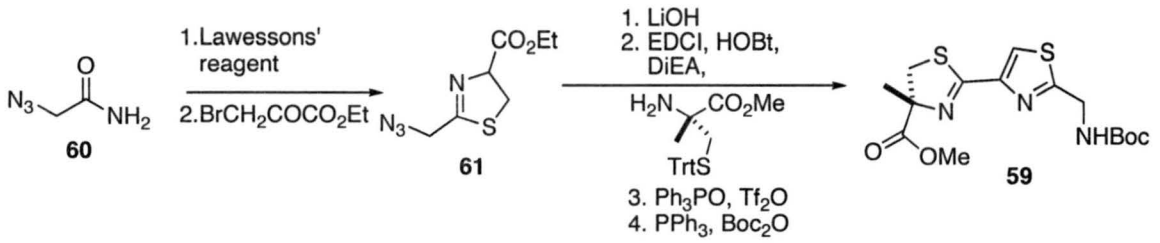
1.4.3 Ghosh's Largazole Synthesis

Ghosh's synthesis of Largazole differs from both Williams' and Luesch's in that the sensitive thioester is installed much earlier in the synthesis. The formation of the 16 membered macrocycle is the last step in the synthesis. A retrosynthetic study of Ghosh's synthesis is shown in Scheme 12, displaying how linear molecule **57** can be realized from two subunits **58** and **59**.



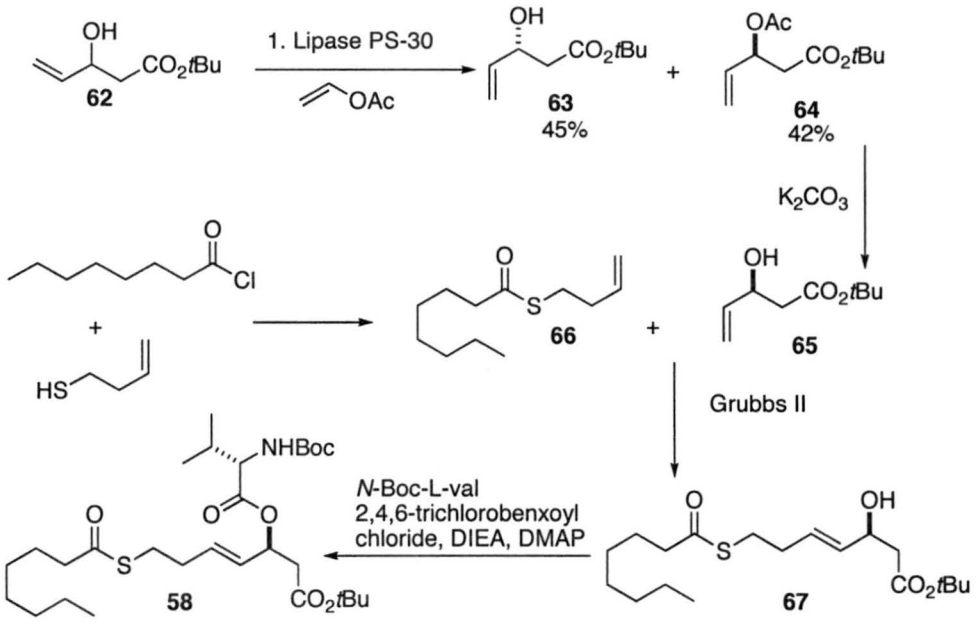
Scheme 12. Retrosynthesis of Ghosh's Largazole synthesis

The thiazoline-thiazole **59** was prepared in much the same way as the Williams synthesis; via coupling of thiazole **61** from known azido amide **60** with α-methylcysteine (Scheme 13). The azido amide was treated with Lawessons's reagent, then reacted with ethyl bromopyruvate to give thiazole **61** in 82 % yield. Saponification followed by coupling with α-methyl cysteine, and treatment with 3 equivalents of triphenylphosphine oxide and Tf_2O provided thiazoline-thiazole **59**.



Scheme 13. Formation of Thiazoline-thiazole Subunit

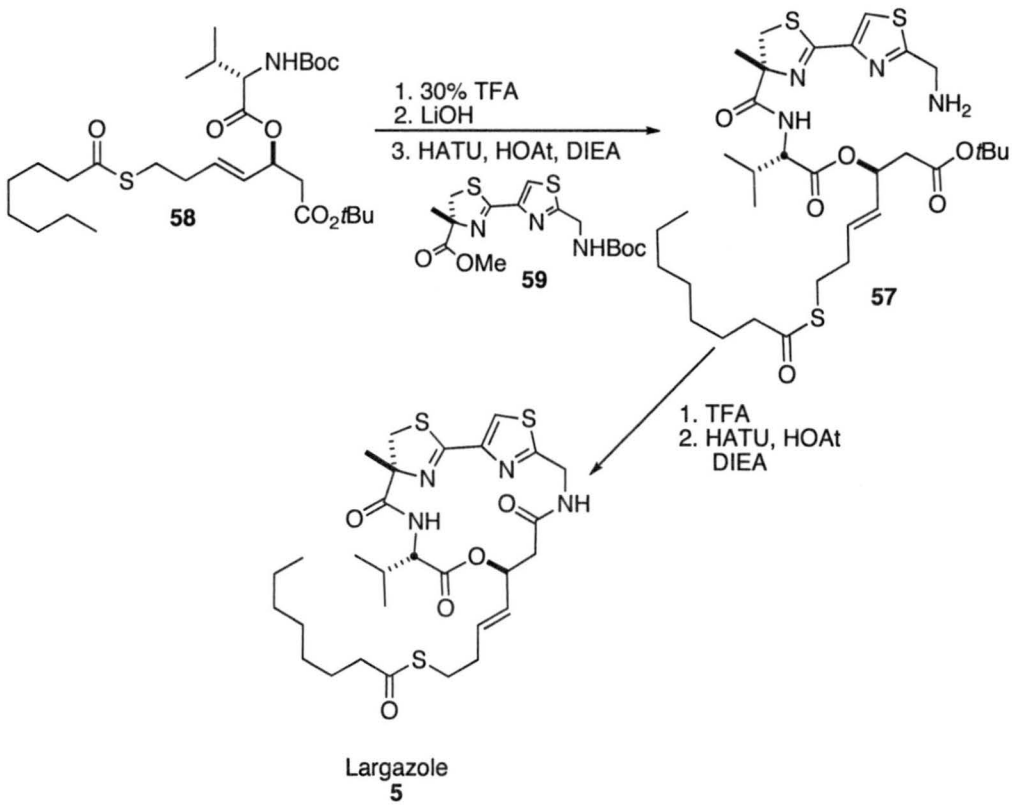
Subunit **58** was formed coupling of *N*-Boc-valine and the β-hydroxy ester **68** shown in Scheme 14. The β-hydroxy ester **67** was formed by treatment of **62** with lipase PS-30 to provide enantio-enriched alcohol **63** and acetate derivative **64.**



Scheme 14. Synthesis of Subunit 58

Treatment with K_2CO_3 selectively removed the acetate to give **65.** Cross metathesis using Grubb's 2nd generation catalyst with thioester **66** gave the β-hydroxy ester **67** in

good yield. Coupling of β-hydroxy ester **67** with *N*-Boc-L-valine provided subunit **58** in 91% yield (Scheme 14).



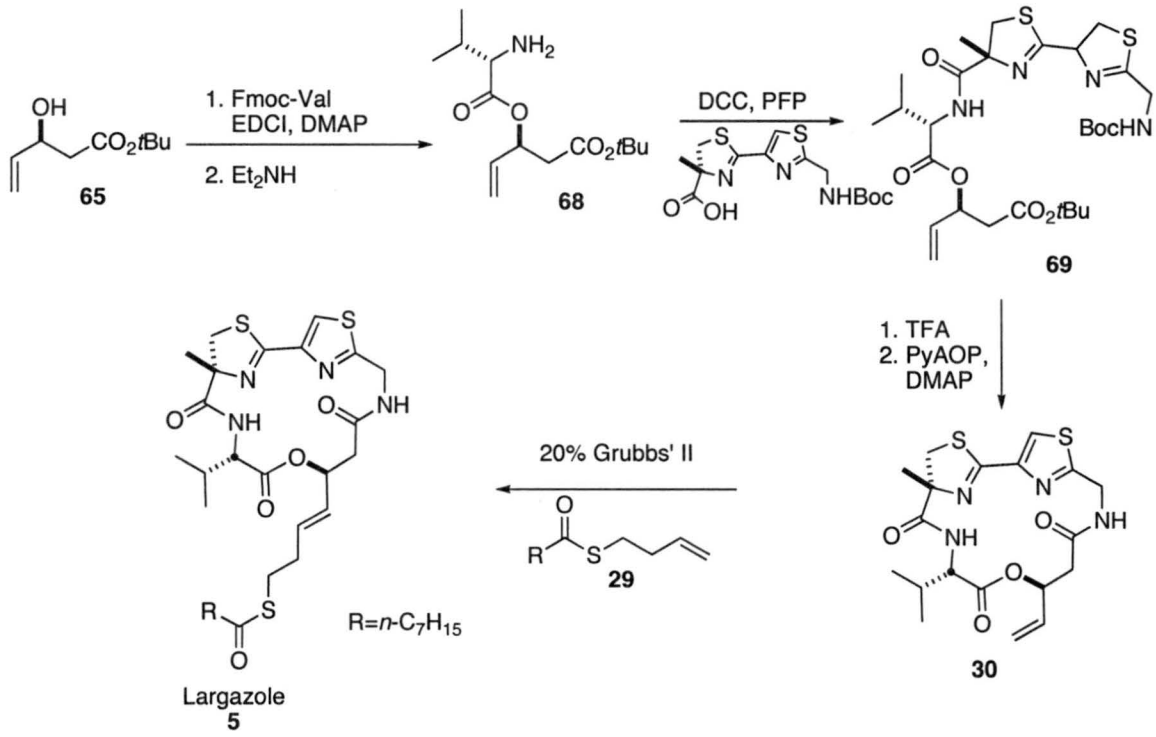
Scheme 15. Ghosh's Largazole Synthesis

Finally, completion of Largazole was accomplished through *N*-Boc deprotection of **58** and coupling with thiazoline-thiazole **59** to afford the uncyclized product in 66% yield. Deprotection of the *N*-Boc and *tert*-butyl groups, followed by treatment with HATU and HOAt provided Largazole, in 8 steps and 2% overall yield.

1.4.4 Phillips' Largazole Synthesis.

Phillips' synthesis began by coupling β-hydroxy ester **65**, formed using the same method as Ghosh's synthesis, with *N*-Fmoc-L-valine, followed by Fmoc deprotection

using Et_2NH to provide **68** in 62% yield (Scheme 16). DCC coupling of **68** to thiazoline-thiazole **59** gave the acyclic precursor (**69**) in moderate yields. Cyclization of the depsipeptide was accomplished with TFA mediated deprotection, followed by PyAOP, and DMAP to give **30** in 50% yield over two steps. The synthesis was completed with olefin metathesis of **30** with **29** to afford Largazole in ten steps and 2% overall yeild.

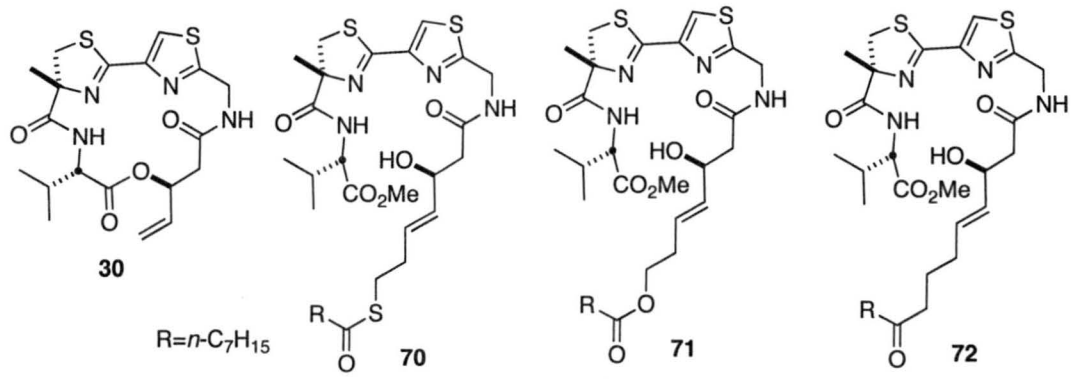


Scheme 16. Phillips' Largazole Synthesis

Phillips' synthesis, similar to Luesch's synthesis, installs the thioester as the last step through cross coupling metathesis, but it should be noted that the macrocylization occurs at a different position.

Phillips also did biological testing on Largazole, depsipeptide core **30**, and three analogs: *seco*-ester **70**, ester **71** and ketone **72** (Scheme 17) against MDA-MB231 cells

and non-transformed human mammary epithelial cells (HME). These tests showed that only Largazole inhibited growth of MDA-MB231 cells, but had little effect on the HME cells.



Scheme 17. Philips' analogs

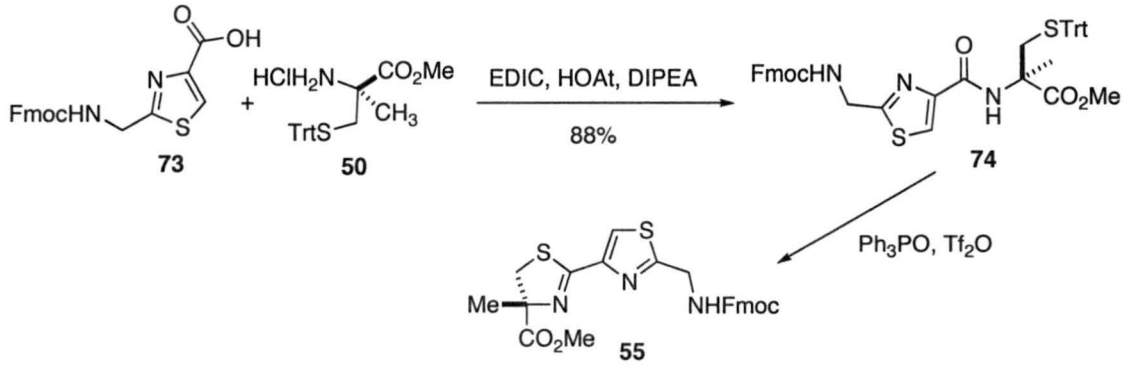
These results indicate a few areas of structural importance. Lack of activity for analog **70** shows the importance of the conformation of the depsipeptide core. Similarly, the low activity of analogs **71** and **72** highlights the importance of the zinc-binding portion of the molecule and supports the theory that Largazole is a pro-drug.

1.4.5 Numajiri's Largazole Synthesis

Numajiri's Largazole synthesis is very similar to the Williams' synthesis. The main differences are in the formation of the thiazoline-thiazole subunit and the chiral auxiliary used for the asymmetric aldol reaction of the β-hydroxycarbonyl subunit. Also the yields and methods of formation for several of the key subunits were different and less concise.

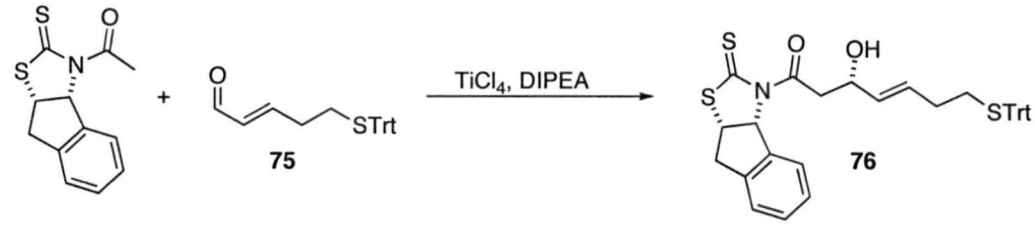
To begin the synthesis, Numajiri and co-workers started with the formation of the thiazoline-thiazole subunit **73** (Scheme 18). Starting with the thiazole-containing amino

acid and coupled that to α-methyl-Trityl protected cystine **(50)** using standard peptide coupling conditions to give **74**. Thiazoline formation was then accomplished through Kelly's method[33] of treatment with Ph_3PO and Tosic anhydride to provide Thiazoline-thiazole **(55)** in 90% yield.



Scheme 18. Numajiri's Thiazoline-thiazole synthesis

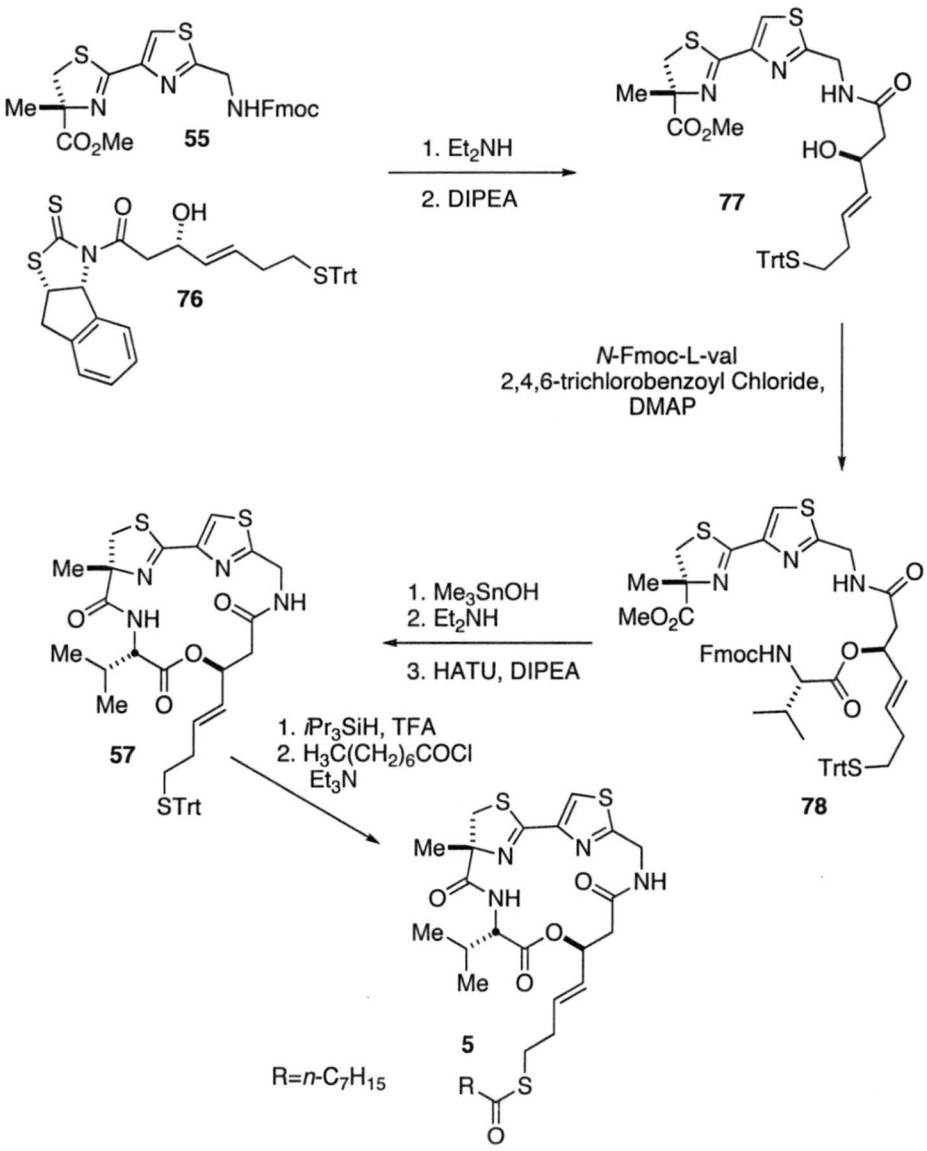
An asymmetric aldol reaction, using a modified Nagao axillary, with the known enal **(75)** completed the formation of the β-hydroxycarbonyl subunit **76** in 94% yield and 94% ee (Scheme 19).



Scheme 19. Numajiri's β-hydroxycarbonyl Synthesis

The completion of the Largazole synthesis was accomplished through N-Fmoc deprotection of the thiazoline-thiazole and coupling to the β-hydroxycarbonyl subunit to provide **77**. Esterification of the hydroxy group of **77** with Fmoc-L-valine afforded the ascylic precursor **78**. Selective saponification of the methyl ester using Me_3SnOH,

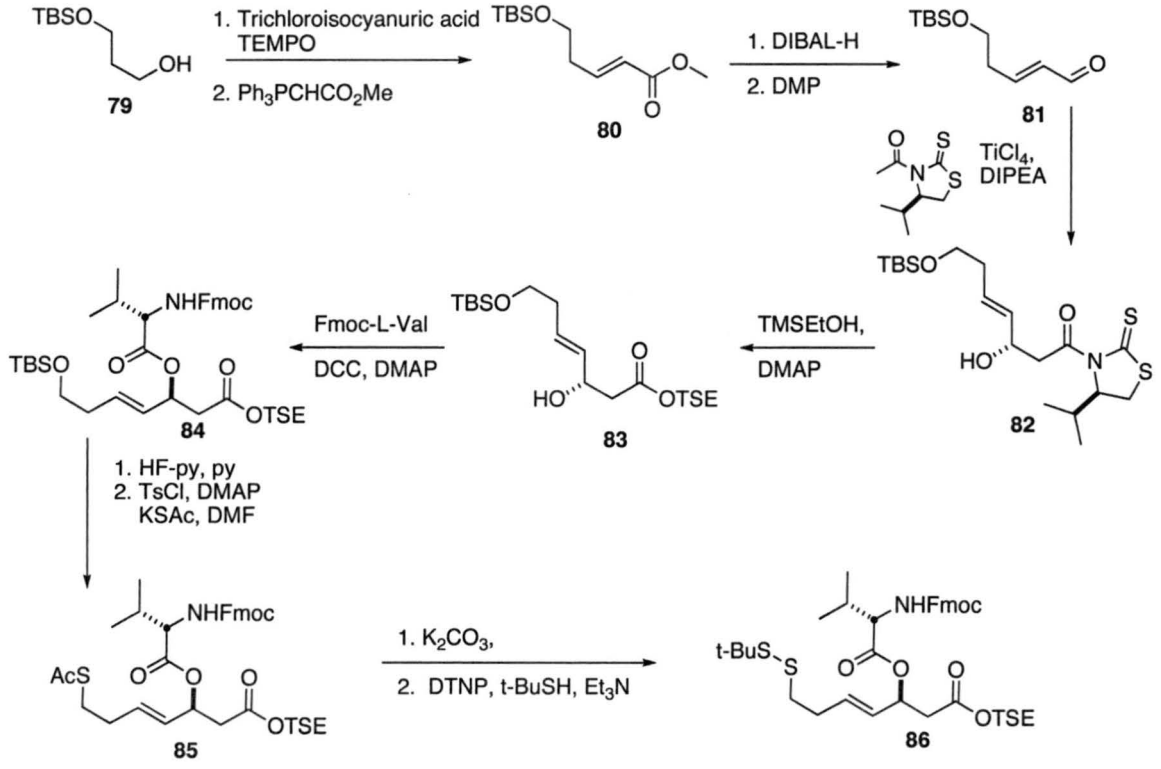
followed by N-Fmoc deprotection and HATU mediated macrolactamization provided **57** in 65% yield. Completion of Largazole (**5**) was finally accomplished in the same manner as Williams (scheme 20).



Scheme 20. Numajiri's Largazole Synthesis

1.4.6 Ye's Largazole Synthesis

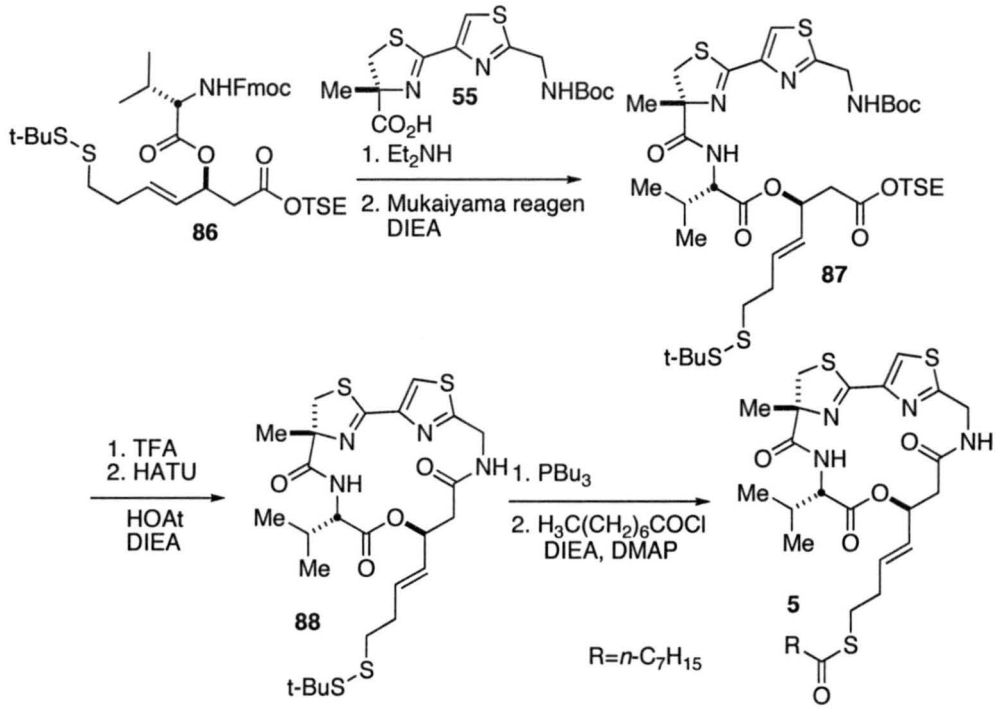
Ye's Largazole synthesis was accomplished in the same manner as the Williams synthesis, with the main differences being in the synthesis of the β-hydroxy acid, and the use different thiol protecting groups. To begin the synthesis of the β-hydroxy acid, 3-(*Tert*-butyldimethylsilyloxy)-1-propanol **(79)** was oxidized with TEMPO and trichloroisocyanuric acid, and finally reacted with (carbethoxymethylene)triphenylphosphorane to give the unsaturated ester **80** in 91% yield. DIBAL-H reduction followed by Dess-Martin oxidation gave enal **(81)**.



Scheme 21. Ye's Synthesis of the Valine Coupled Subunit

An asymmetric aldol reaction using Nagao's chiral auxiliary afforded the desired stereochemistry of the hydroxy group of **82** in 14:1 dr. The chiral auxiliary was removed

with TMS-ethanol to give the protected β-hydroxy acid **83**. Esterification of the allyl alcohol with *N*-Fmoc-L-valine was accomplished using DCC and DMAP to give the coupled product **84**. This was then converted to the disulfide **86**, by converstion of the TBS group to a tosyl group, followed by displacement with potassium thioacetate to give **85**. Formation of the free thiol with potassium carbonate, followed by treatment with 2,2'-dithiobis(5-nitropyridine) and *t*-BuSH gave the desired product **86** in 50% yield over three steps (Scheme 21).



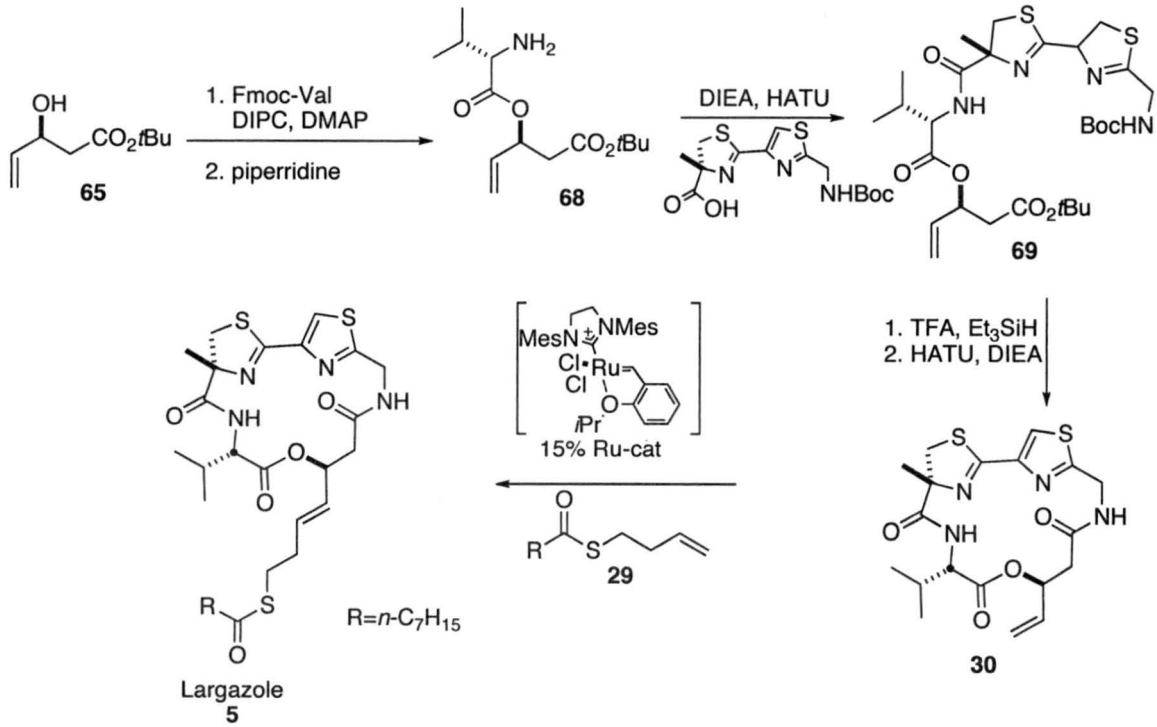
Scheme 22. Ye's Completion of Largazole

With the disulfide in hand, this was coupled to thiazoline-thiazole **(55)**, portion made in the same method utilized by the William's group, to give the acyclic precursor **87**. Global deprotection was accomplished with TFA, and followed by depsipeptide

formation with HATU and HOAt. Finally completion of the Largazole synthesis was accomplished through tributylphosphine-promoted cleavage of the disulfide to give the thiol that was then reacted with octanoyl chloride to afford Largazole in 5% overall yield in 14 steps.

1.4.7 Cramer's Largazole Synthesis

Cramer's Largazole synthesis follows the same major disconnections as Phillip's synthesis, but uses different methods to form the major subunits. The alcohol **65**, was prepared through an enzymatic resolution, followed by mild hydrolysis to give the allylic alcohol with >95% ee. Esterification of the alcohol with *N*-Fmoc-L-valine was accomplished through treatment with N,N'-diisopropylcarbodiimide (DIPC) and DMAP.



Scheme 23. Cramer's Largazole Synthesis

Removal of the Fmoc protecting group with piperidine gave the free amine 68, in 96% yield. The thiazoline-thiazole portion was made using the same methods utilized by William's and Ren. These two pieces were then coupled using HATU and DIEA to provide the acyclic precursor 69 in 96% yield. Global deprotection was accomplished through treatment with TFA and triethylsilane, followed by HATU mediated depsipeptide formation gave the macrocyclic core 30, in 77% yield. Optimized cross coupling metathesis of 30 and 29 was accomplished using a Ruthenium catalyst developed by Grela and Co-workers[34] to complete the synthesis of Largazole in nine linear steps and 19% yield.

1.4.8 Summary of Largazole Syntheses

With seven total synthesis of Largazole to date, a summary of all of the synthesis has proven helpful. Table 1. below lists the number of steps and overall yield of each synthetic approach.

Laboratory	# of Steps	Overall % Yield
Luesch	8	19
Williams	8	37
Ghosh	8	2
Phillips	10	2
Numajiri	9	34
Ye	14	5
Cramer	9	18

It is clear from this table that the Williams synthesis is by far the most successful. Its quick and clean synthesis proves to be very high yielding.

1.5 Research Objectives

The original intent of my research was to investigate ways to improve upon our synthesis of FK228 and create analogues of FK228. To start improving our synthesis of FK228 we wanted to explore closing the macrocycle through a macrolactamization rather than the previously used and very low yielding Mitsunobu macrolactonization. We were also interested in developing analogs of FK228 by implementing the side chain of other cyclic HDAC inhibitors. It was decided that the Azumamides would be a good target side chain because of the apparent ease of synthesis and ability to modify the zinc-binding warhead. Through the investigation of the FK228-Azumamide hybrid, we considered that it might be possible to make a Largazole-Azumamide hybrid as well.

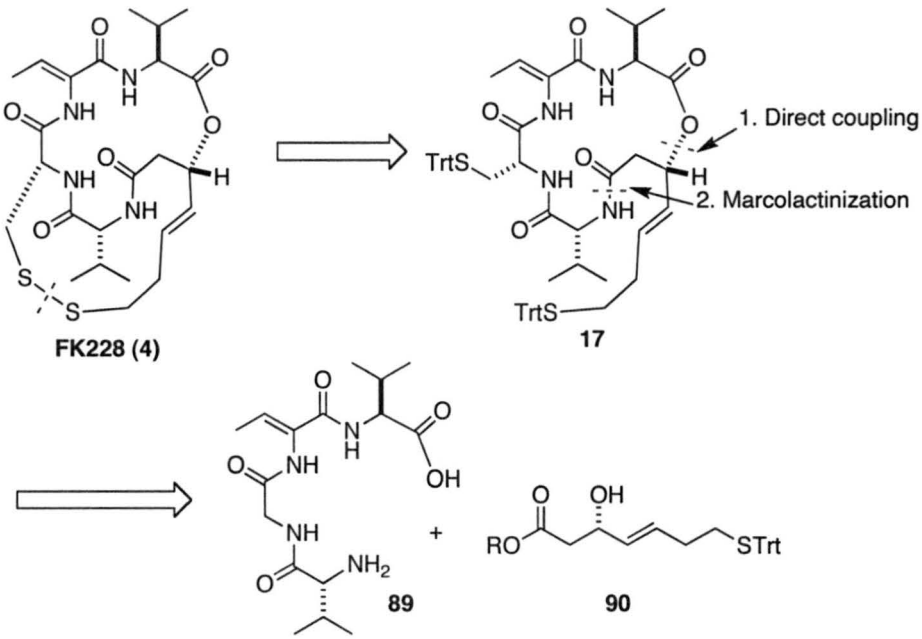
Chapter 2

Progress Towards the Improved Synthesis of FK228

2.1 Retrosynthetic Analysis

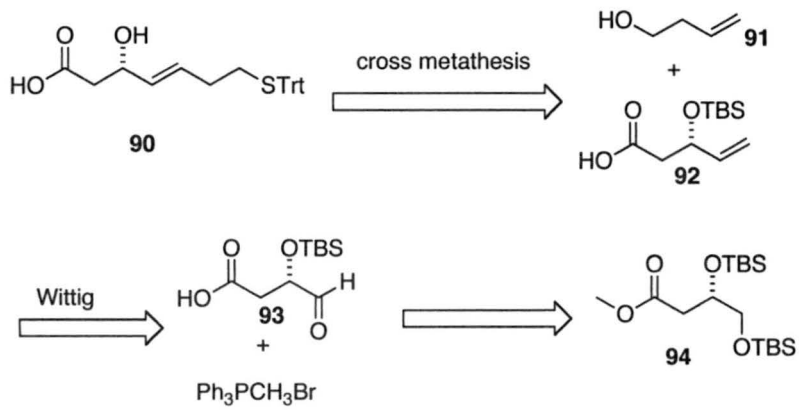
FK228 is one of the most potent HDAC inhibitors known. Due to its challenging structure, only three syntheses of the molecule have been completed since its discovery in 1996. The first synthesis by Simon[17] does not provide the compound in sufficient yield for biological study. Several of the key steps of the synthesis, namely the formation of the β-hydroxy acid portion and the Mitsunobu macrolactonization, have proven to be unreliable and difficult to reproduce. To this end, the Williams group has already completed one synthesis of FK228, where in we debut a new route utilizing Noyori's asymmetric reduction to form the β-hydroxy acid portion in nine steps, with improved enantioselectivity.[18] However, the Mitsunobu macrolactonization was extremely unreliable and low yielding. When the reaction was successful, purification of the product was tremendously difficult.

Due to persistent problems with the yield of the Mitsunobu macrolactonization, we endeavored to improve our synthesis FK228 by directly coupling the β-hydroxy acid **90** to the carboxylic acid portion of tetrapeptide **89** before macrolactamization (Scheme 24). It is our belief that these changes will complete the synthesis with higher yields.



Scheme 24. Retrosynthetic Analysis of Improved FK228 Synthesis

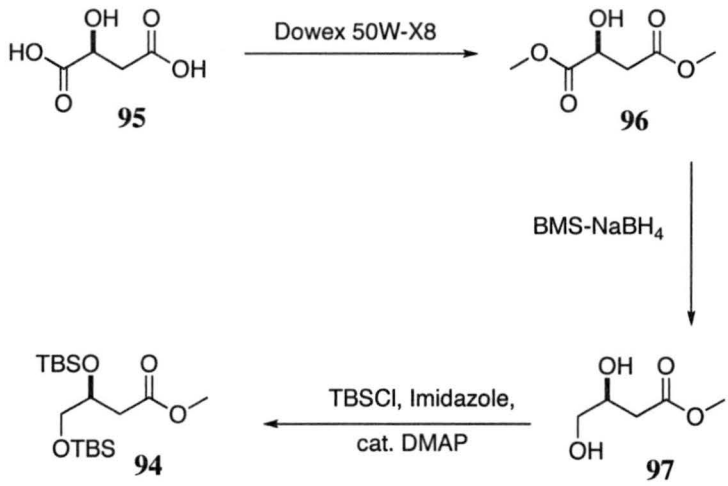
Preparing the tetrapeptide in the same manner as our previous synthesis, but we would be required to synthesize β-hydroxy acid **90** with the opposite stereochemistry of the previously synthesized acid. It was thought that **90** could be accessed through a cross metathesis of **91** and **92**, where **92** was formed from a Wittig reaction of **93**, and **93** could be accessed from diol **94** (Scheme 25).



Scheme 25. Retroxynthetic Analysis of β-hydroxy Acid Synthesis

2.2 Synthesis

Progress towards our improved synthesis began with the formation of the new β-hydroxy acid **90**. The first attempts began with the formation of optically pure dimethyl-(S)-(-)-malate **(96)** from optically pure L-malic acid (Scheme 26).[35] This reaction was very successful, and produced **96** with complete retention of stereochemistry. This product was selectively reduced to give diol **97**, and subsequently TBS protected to give **94** in good yields.



Scheme 26. Formation of Diol 94

A selective removal of the primary TBS protecting group followed by a Swern oxidation was planned to give aldehyde **93.** Unfortunately, all attempts to remove the protecting group were unsuccessful (Scheme 27), resulting in either removal of both the primary and secondary protecting groups, or no reaction and recovery of starting material.

Reaction conditions attempted

1. PPTS, MeOH, 0°C
2. PPTS, MeOH, rt
3. TsOH, MeOH, 0°C
4 TsOH, MeOH, rt
5. Acetic Acid, MeOH, 0°C
6. Acetic Acid, MeOH, rt
7. CSA, MeOH, 0°C

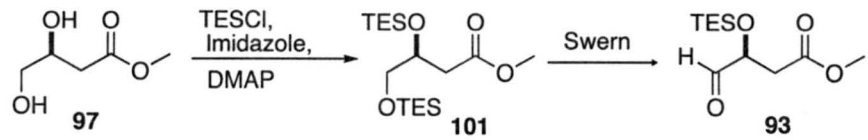
Scheme 27. Selective Deprotection of Primary Alcohol Conditions

In light of these unfavorable results, a new approach to the formation of **93** was attempted. To avoid the issue of selective deprotection, diol **97** was cyclized to lactone **99**. This was followed by TBS protection of the alcohol to give **100**. It was then hoped that opening the lactone with methanol would give aldehyde **93** (Scheme 28). Formation of **99** and **100** were very quick, high-yielding reactions. Unfortunately, the conversion of **100** to aldehyde **93** proved problematic. When protected lactone **100** was subjected to PPTS at room temperature, no reaction was seen. Likewise, when the same reaction was performed at reflux for 1.5 hours, some decomposition occurred in conjunction with recovery of starting materials. Stronger acids were tried, but led to decomposition.

Scheme 28. Second Attempt to Form Aldehyde 93

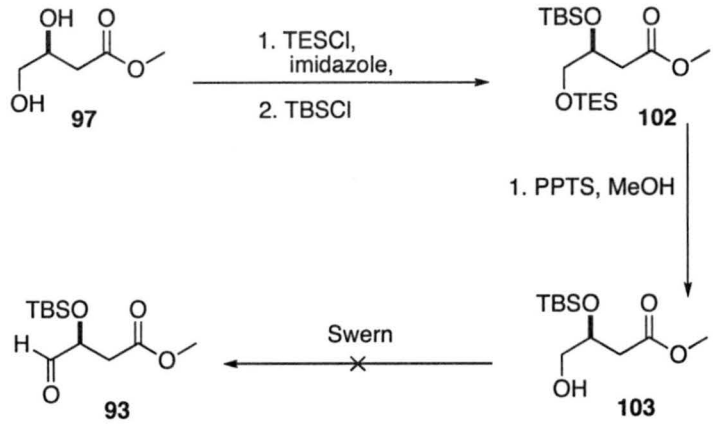
A literature search for **93** led to a paper which detailed the selective oxidation of primary silyl ethers in the presence of secondary silyl ethers in good yields.[36] This

sounded promising, as protection of diol **97** was easily accomplished. To start, diol **97** was protected as the bis-triethylsilyl ether to give **101** in good yields (Scheme 29). Swern oxidation of **101** to **93** appeared to work, but provided a mixture of products that needed to be separated. This would not have been an issue except for concerns of epimerization of the stereocenter of **93**; furthermore, decomposition was observed after chromatography. Due the fragile nature of this aldehyde, the next reaction was run using the crude product and carried on without purification to the Wittig reaction. Unfortunately, the product of the Wittig reaction was never isolated. Purification of the product proved very difficult, and it was very difficult to determine if the product had even been formed from the crude NMR.



Scheme 29. Selective Oxidation of Primary Silyl Ethers

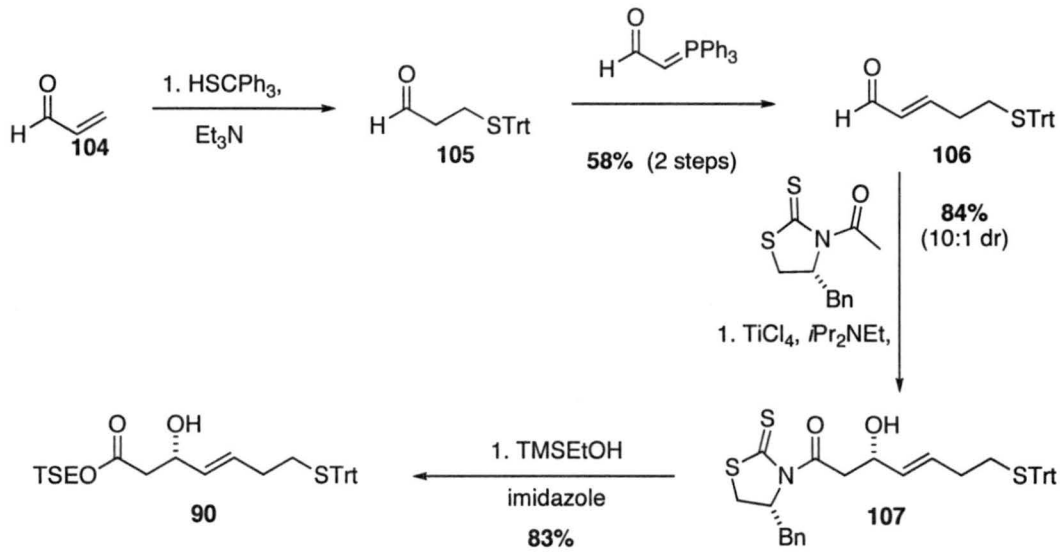
Another attempt to form aldehyde **93** involved taking advantage of the increased reactivity of the primary alcohol of **97** to selectively protect with TESCl, followed by protection of the 2° alcohol as the TBS ether to form **102**. Because TES groups are more labile, it was possible to arrive at **103**. A Swern oxidation of **103** was attempted but was never successful (Scheme 30).



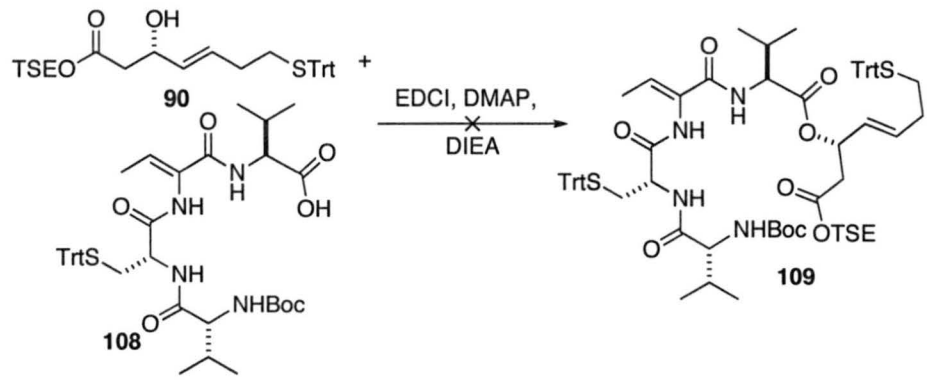
Scheme 30. Another Attempt to Form Aldehyde 93

Finally, a solution to the β-hydroxy acid problem was found with a synthesis of thiazolidinethione **107** by Ganesan (Scheme 31).[32] To begin this synthesis we started with acrolein **104** and triphenylmethanethiol to give **105** in good yields. Then a Wittig reaction with (triphenylphosphoranylidene)acetaldehyde installed the desired E-olefin of (**106**). An asymmetric aldol reaction provided **107** in 84% yield and 10:1 diastereomeric ratio. The thiazolidinethione was easily converted to TSE-protected acid **90** after treatment with 2-trimethylsilylethanol and imidazole.

With this key piece in hand, a direct coupling to *N*-Boc protected FK228 tetrapeptide **108** to form FK228 acyclic precursor **109** was attempted (Scheme 32). The tetrapeptide **108**, was formed from the stock of already prepared tetrapeptide *N*-Fmoc methyl ester. All attempts to couple these pieces failed; we therefore elected to construct the tetrapeptide in a stepwise fashion (Scheme 32).



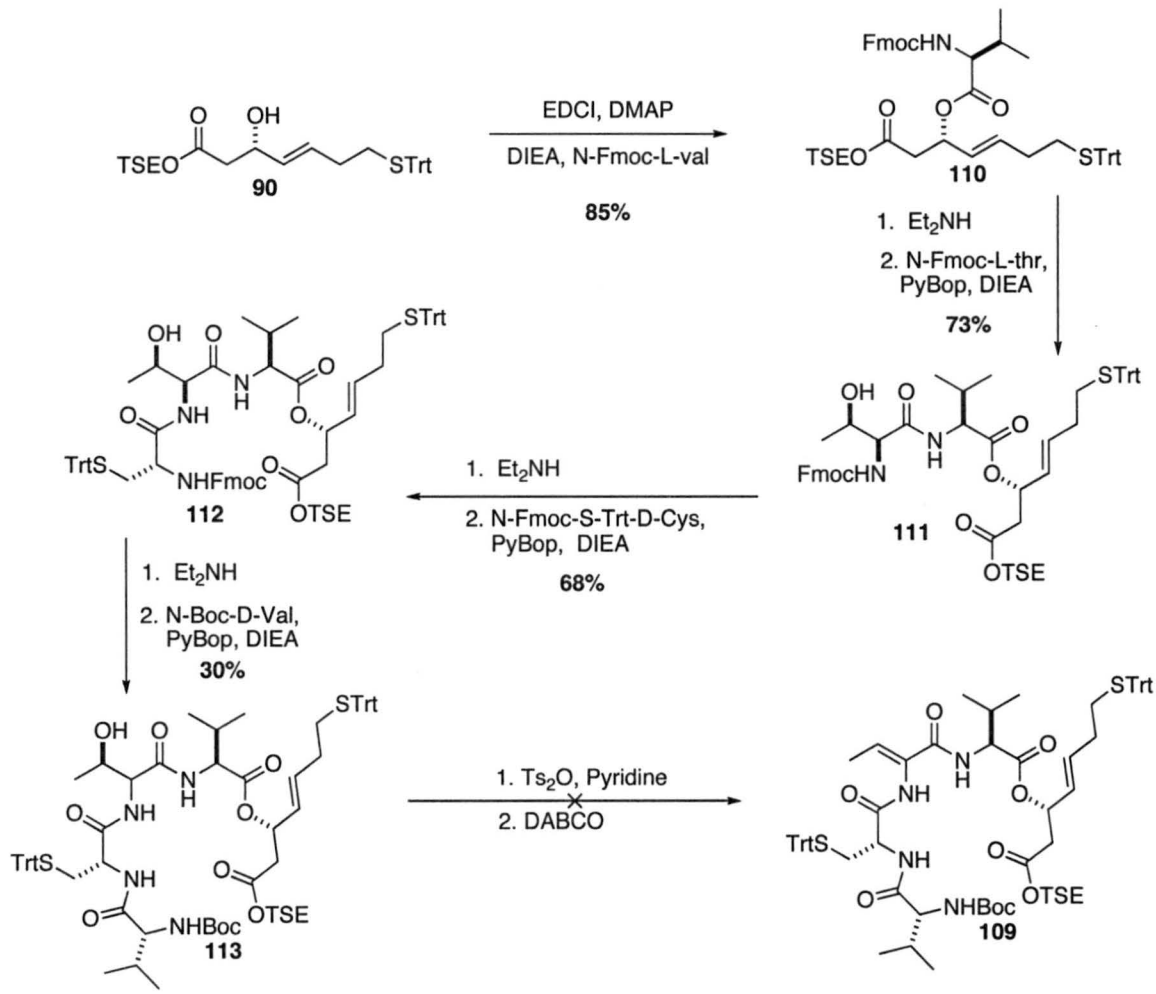
Scheme 31. Formation of β-hydroxy Acid 90



Scheme 32. Attempt to Form Acyclic FK228 Precursor

The stepwise assembly of the macrocycle began with an EDCI-mediated direct coupling of the protected β-hydroxy acid **90** to *N*-Fmoc-L-valine to provide **110** in 85% yield. Standard Et_2NH mediated *N*-Fmoc deprotection followed by peptide coupling to *N*-Fmoc-L-threonine, gave **111** in 73% yield over two steps. Again, *N*-Fmoc deprotection followed by peptide coupling with *N*-Fmoc-D-cysteine gave **112** in 65% yield. In the same manner **112** was deprotected and coupled to *N*-Boc-D-valine to give

113, albeit in a disappointing 10% yield. Still, enough product was recovered to attempt the dehydration of the threonine residue. Unfortunately, this resulted in decomposition and no product was recovered.



Scheme 33. Formation of the FK228 Acyclic Precursor

2.3 Future Investigations

Unfortunately, an FK228 synthesis following the same methods, and using the same macrolactamization strategy was recently published by Ganesan.[19] Therefore, futher investgation into this route would not be worthwhile. However, if this were not

the case, this improved synthesis could be completed by trying a variety of peptide coupling conditions to improve the formation of **113**. This improvement would supply material to again try the dehydration of the threonine residue, and hopefully finish the acyclic precursor **109**. That reaction was only tried once due to lack of material, but could be successful with optimization. Finally, an attempt could be made to cyclize **109** to **17**, followed by removal of the trityl protecting group and formation of the disulfide bond through previously established methods to give FK228 (**4**) in improved yield.

The main difference between our attempted synthesis of FK228 and that published by Ganesan, is the formation of acyclic precusor **109**. Ganesan encountered the same difficulties with coupling β-hydroxy acid **90** to the tetrapeptide. Their solution was to couple β-Hydroxy acid **90** to *N*-Fmoc-L-valine to give the coupled product **110**. They then created the tripeptide through standard peptide coupling conditions. These two pieces were easily coupled to give acyclic precursor **109**. From that point, HATU macrolactamization followed by trityl deprotection and disulfide bond formation were accomplished to give FK228.

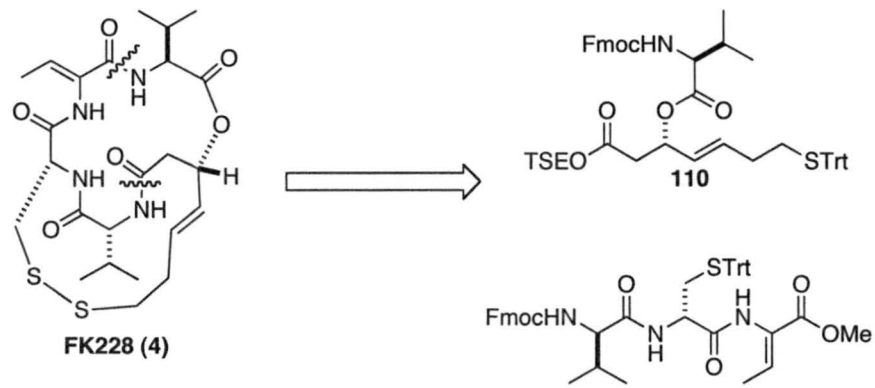


Figure 3. Ganesan's FK228 Retrosynthes

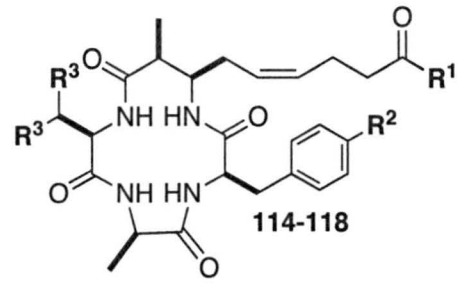
Chapter3

Progress Towards the Synthesis of an FK228 Hybrid

3.1 Background Information

Another goal of this project was to develop several analogs of FK228 by attaching new side chains of other cyclic HDAC inhibitors onto the tetrapeptide core. By doing this, we hope to increase the reactivity by changing both the linker portion conformation, but also the "warhead" that binds to the zinc in the active site. After researching other HDAC inhibitors, Azumamide E side chain was chosen because of the straightforward synthesis, and the ability to easily modify the zinc binder.

Azumamides A-E (**114-118**) were isolated from the marine sponge *Mycale izuensis* by Fusetani and coworkers in 2006 [37]. This side chain was chosen because the carboxylic acid is thought to be a stronger zinc-binder [38] then the thiol of FK228. The Azumamides have considerably weaker biological activity then FK228[38]; however this may be due to the differences in the peptide backbones of the two molecules, and not the zinc binder. A hydroxamic acid analogue of Azumamide E showed similar biological activity to FK228.[38] For these reasons once the Azumamide E side chain is completed, we hope to modify it to the amine of Azumamide A, and potentially add the very active hydroxamic acid moiety as well. The molecule could then be tested to probe the impact of this change.

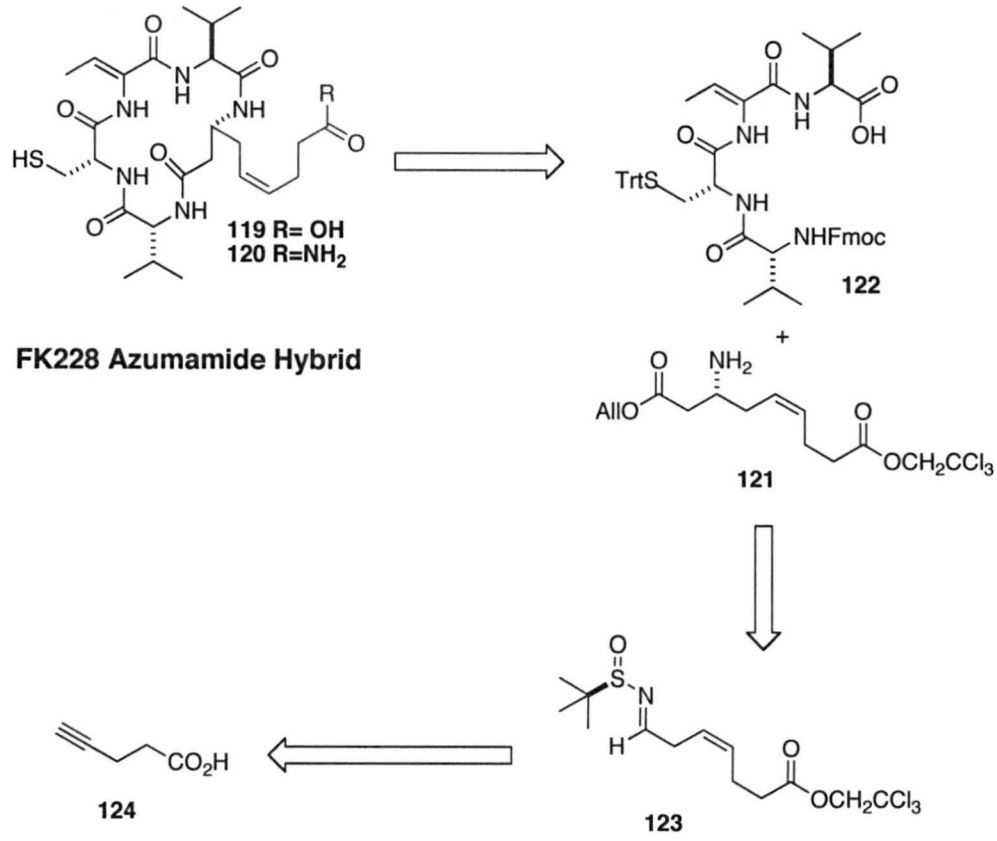


Azumamide	R^1	R^2	R^3
A (114)	NH_2	H	Me
B (115)	NH_2	OH	Me
C (116)	OH	OH	Me
D (117)	NH_2	H	H
E (118)	OH	H	Me

Figure 2. The Azumamides

3.2. Retrosynthetic Analysis

There are several total syntheses of Azumamides A and E already published. [39, 40] From these syntheses it appears the Azumamide side chains should easily add onto the tetrapepide portion in a similar fashion as the FK228 thiol side chain (Scheme 34). FK228 hybrid **119** could arise from the coupling of the *N*-Fmoc protected tetrapeptide **122** and the slightly modified Azumamide side chain **121**. The amine **121** could be prepared from 4-pentynoic acid **124** following an established procedure.[38]

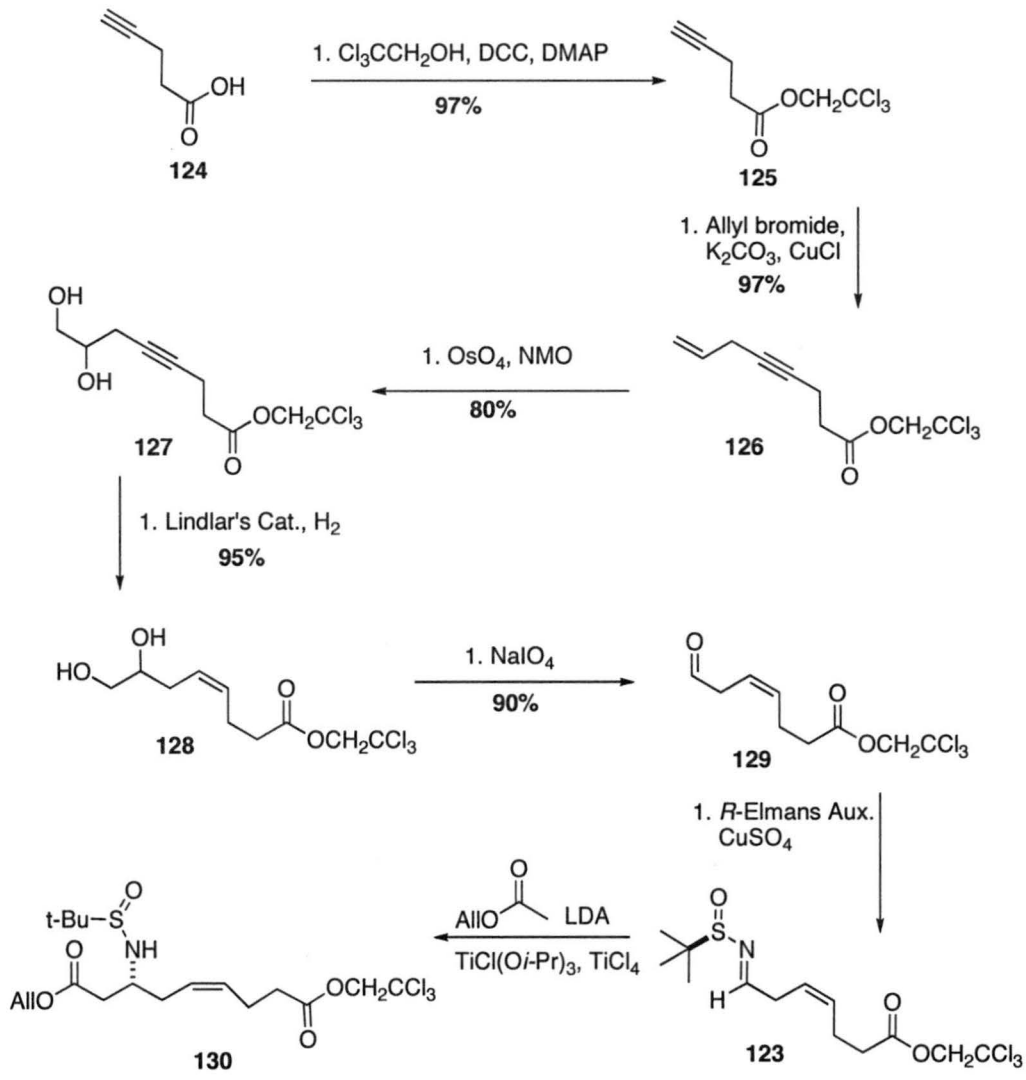


Scheme 34. Retrosynethesis of FK228 Azumamide Hybrid

3.3 Synthesis

To accomplish the synthesis of the Azumamide E side chain, 4-pentynoic acid (**124**) was protected as the trichloroethyl ester (**125**), and then coupled with allyl bromide to install the desired olefin of **126** in 80% yield. Diol formation was accomplished using osmium tetraoxide to give **127.** Reduction of the alkyne with Lindlars catalyst gave the desired (*Z*)-olifin of **128** in excellent yield. Finally treatment of **128** with sodium periodate formed aldehyde **129**. The aldehyde was then treated with Ellman's (*R*)-auxillary to create sulfinylimine **123** in 45% yield. This low yield was disappointing but not unexpected as Ganesan reports a 56% yield. However, this product proved difficult to purify through column chromatography. A Mannich reaction with the sulfinylimine

123 and allyl acetate was attempted, but the protected amine product **130** was never recovered, only decomposition was seen (Scheme 35).



Scheme 35. Formation of Azumamide side chain

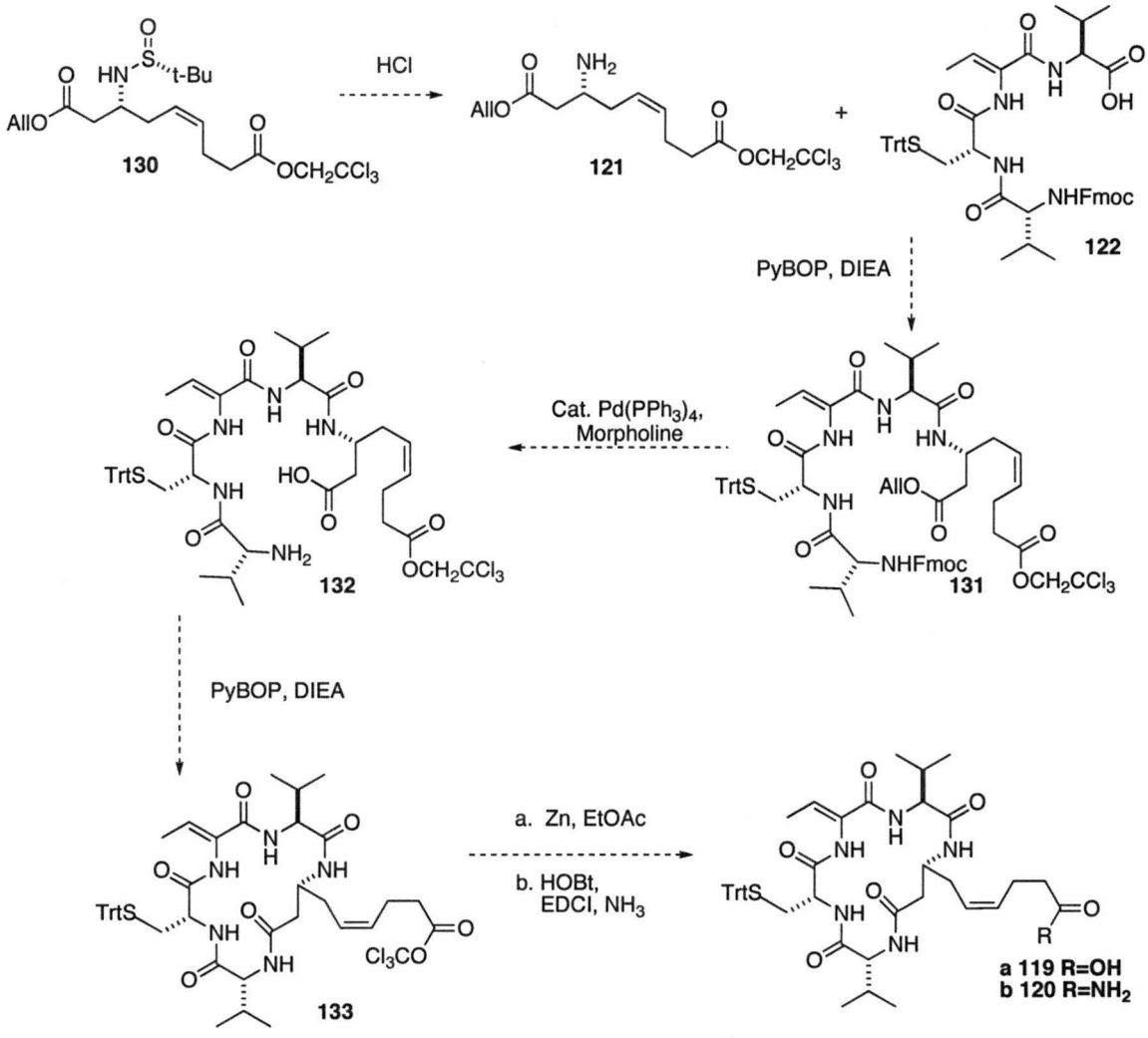
Allyl acetate was used instead of *p*-methoxybenzyl propionate because it was readily available; also we required a different protecting group from what Ganesan used in order to remove both the acid and amine protecting groups in one step before cyclization. In addition we did not require the methyl group in our finished analog that

would have been supplied by the propionate group. We considered that after successful completion of the first analog it would be a simple step to switch out allyl acetate for allyl propionate to create another analog.

3.3 Future Investigations

To continue this synthesis, the yield and purification of sulfinylimine **123** could be improved by forming aldehyde **129** and immediately carrying it on to sulfinylimine formation. This would improve yield and purification by reducing the chance of double bond migration to the conjugated α-β unsaturated aldehyde. With more product available, optimizing the Mannich reaction would be possible. From Mannich product **130**, completetion of the synthesis should proceed in a straightforward manner (Scheme 36).

The *tert*-butylsulfinyl group could then be cleaved to give the free amine **121**. This would then be coupled with Fmoc-protected FK228 tetrapeptide **122** to give **131** . Removal of the allyl and Fmoc protecting groups would give the carboxylic acid and the free amine acyclic precursor **132**, followed by macrolactamization to give the protected product **133**. Finally, removal of the trichloroethyl ester using zinc dust gives Azumamide E hybrid **119.** From there it can be imagined that Azumamide A hybrid **120** could be made through a carbodiimide-mediated reaction with ammonia.



Scheme 36. Plan for Completion of FK228 Azumamide Hybrid

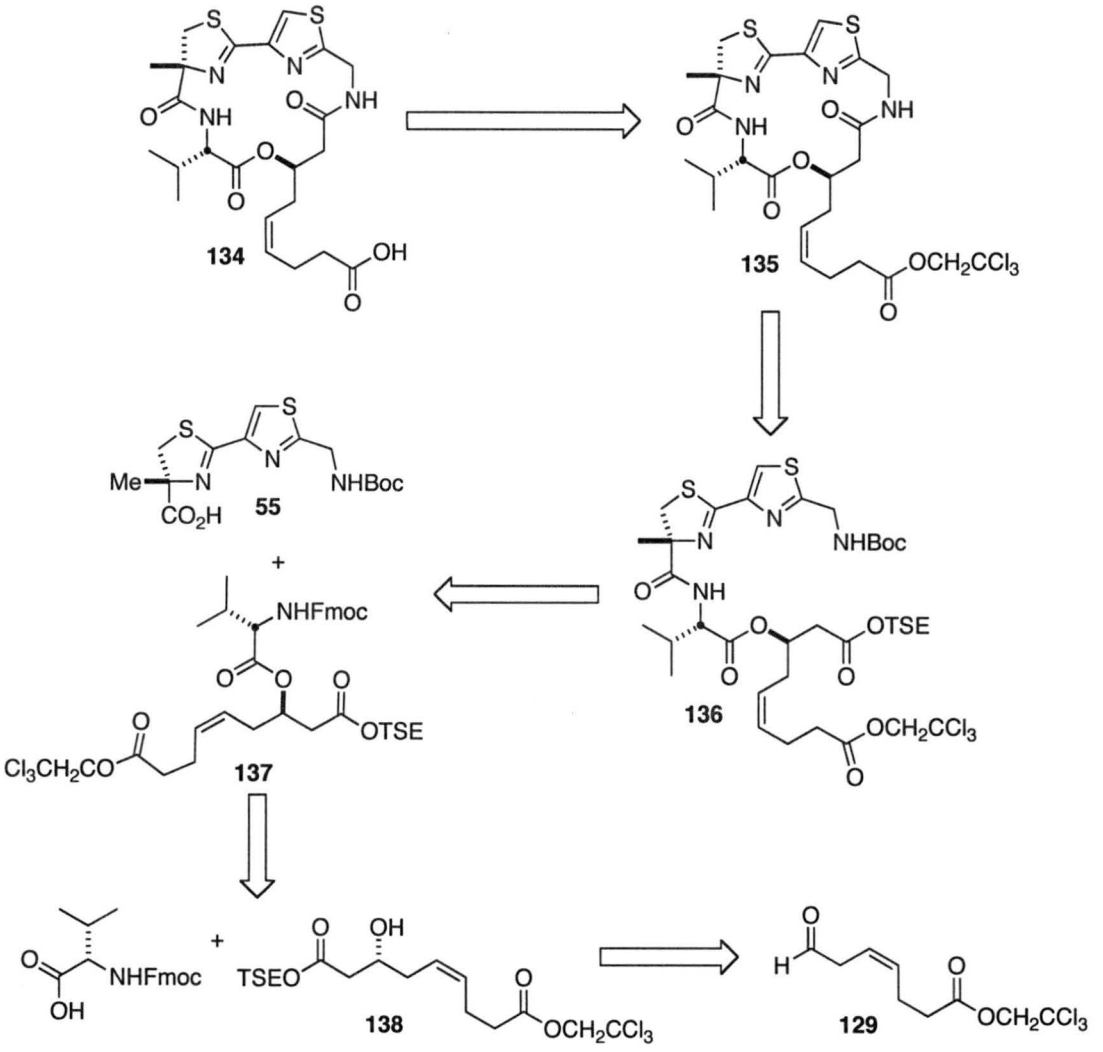
Chapter 4

Synthesis of Largazole-Azumamide E Hybrid

4.1 Retrosynthetic Analysis

Recently, the Williams group published a concise total synthesis of Largazole.[24] Due to the potent biological activity and easily modified synthesis of this molecule, analogs of Largazole quickly became targets for our synthetic efforts. Because of the work already done towards the synthesis of the FK228 Azumamide hybrid, it was natural to try to incorporate our already formed Azumamide side chain into a Largazole-Azumamide hybrid.

Formation of the Largazole-Azumamide E hybrid **134** was envisioned to proceed in a very similar fashion to our original Largazole synthesis, replacing β-hydroxy mercapto acid **49** with protected β-hydroxy acid **138**, derived from the synthesis of the Azumamide side chain. The β-hydroxy acid would be coupled to *N*-Fmoc-L-valine to give coupled product **137**. This piece would then be treated the same way as in the original synthesis and coupled to the thiazoline-thiazole **55**, to give the hybrid acyclic precursor **136**. This would then be deprotected and cyclized to afford protected Largazole hybrid **135**. Deprotection of the trichloroethyl ester would afford the desired Largazole-Azumamide hybrid **134** (Scheme 37).

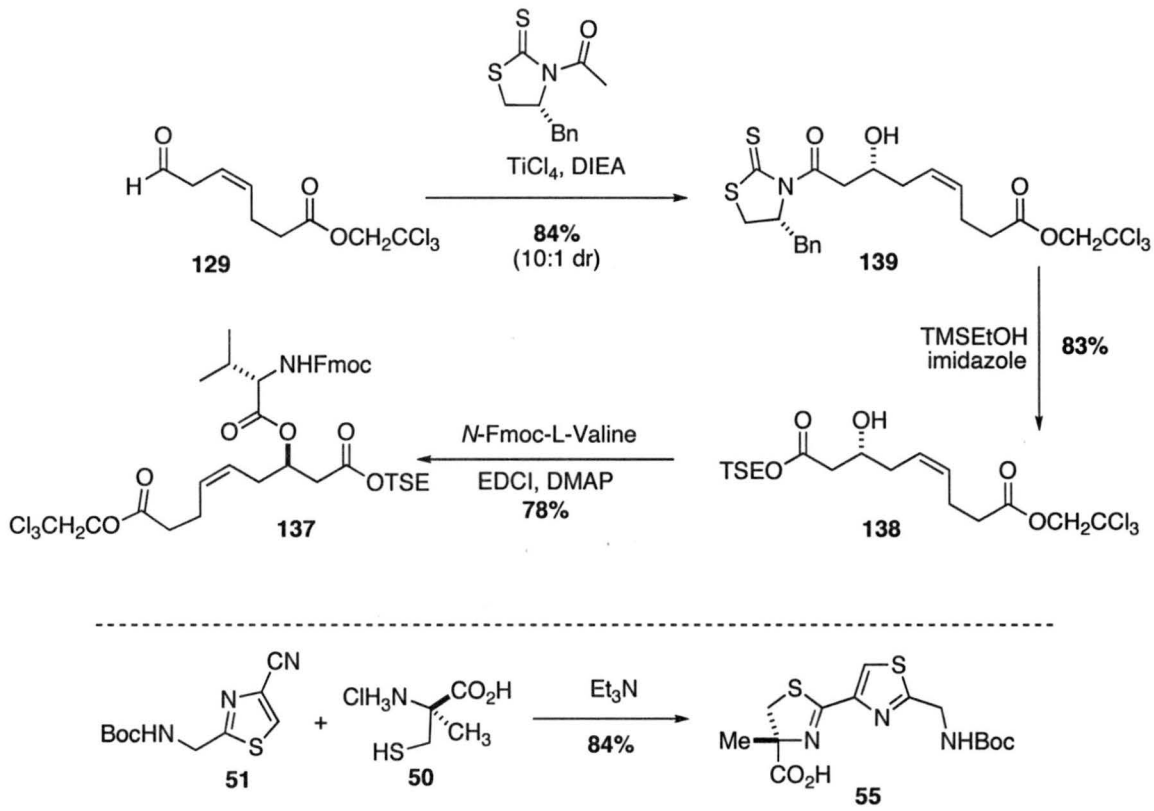


Scheme 37. Retrosynthetic Analysis of the Largazole-Azumamide Hybrid

4.2 Synthesis

To begin the Largazole hybrid synthesis, β-hydroxy acid **138** had to be formed. The same procedure used to make the Azumamide side chain[38] was followed, but the original synthetic approach was used only to access aldehyde **129**. To provide the correct stereochemistry of the hydroxyl group of the β-hydroxy acid, a stereoselective aldol reaction was done to give **139** in 84% yield and 10:1 diastereomeric ratio. The chiral auxiliary was then easily removed with 2-TMS ethanol to give protected β-hydroxy acid

138 in 83% yield. The protected acid was coupled with N-Fmoc-L-valine to give coupled product **137**. Finally, production of thiazoline-thiazole **55** was accomplished by condensing known nitrile **51** with α-methyl cysteine **50** (Scheme 38).



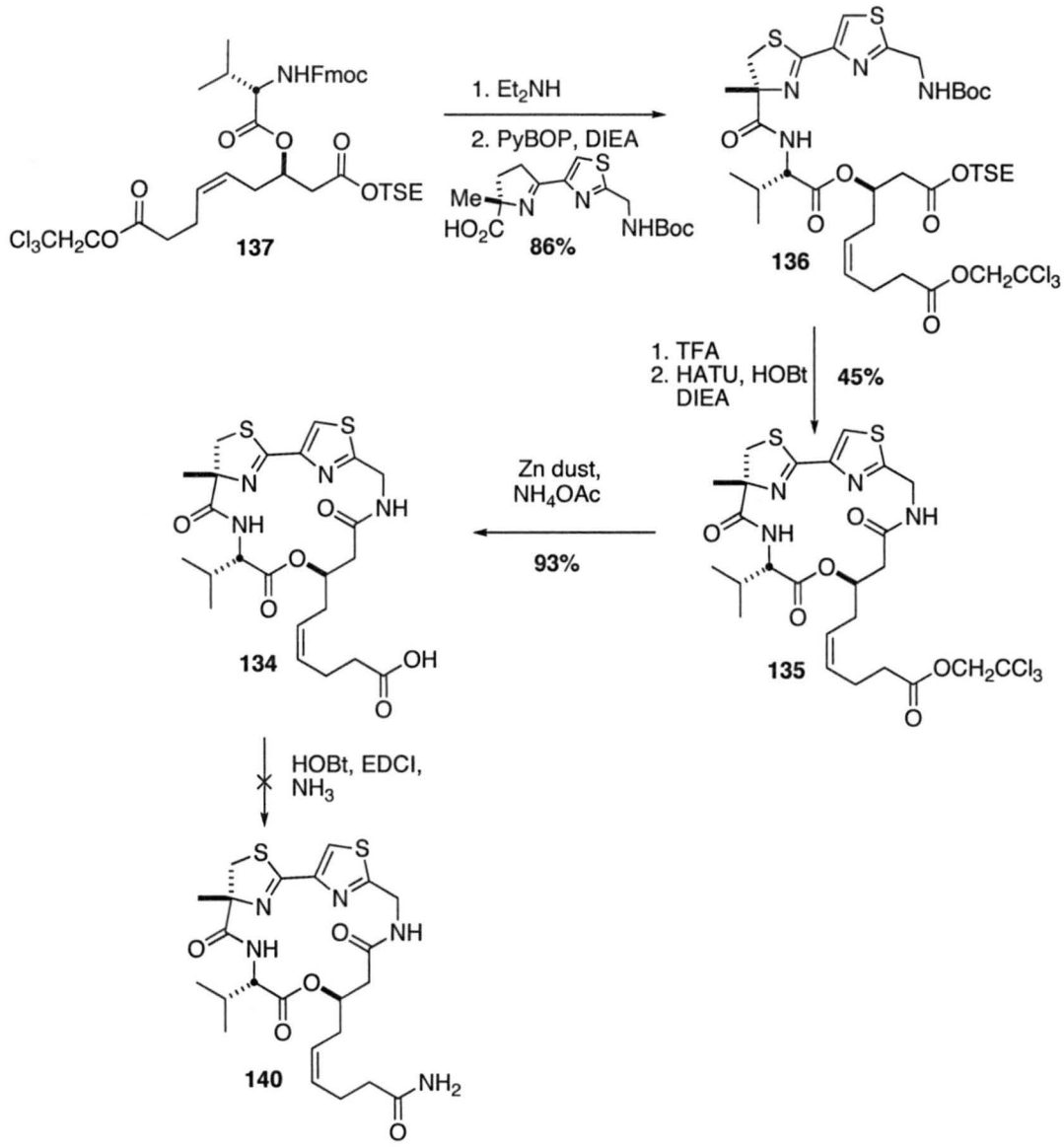
Scheme 38. Synthesis of the Largazole Azumamide hybrid side chain

Diethylamine mediated *N*-Fmoc deprotection of **137,** followed by PyBOP mediated peptide coupling to thiazoline-thiazole **55** gave protected acyclic precursor **136** in good yields. Treatment of **136** with TFA overnight removed the *N*-Boc and TMS ethyl ester protecting groups to afford the crude amino acid. The crude product was cyclized using HOBt and HATU to give protected Largazole analog **135** in a 45% yield. Purification of this macrocycle proved difficult initially, and was ultimately

accomplished by following the product with a UV light on the chromatotron with very slow elution. This reaction actually produced higher yields on larger scale (200 mg or more), than the initial attempts (50 mg or less). Finally, removal of the trichloroethyl ester was accomplished with treatment with zinc dust and ammonium acetate overnight to give the desired Largazole-Azumamide E hybrid (**134**) in 93% yield (Scheme 39).

Purification of this acid was accomplished through preparatory thin layer chromatography because of the polarity of the product. This method was used to ensure the best purification. The compound did not stain easily in any of the common TLC dips we had prepared, and it was not as highly UV active as the protected hybrid. This method of purification also allow for easy recovery of the product as the band could be cut as soon as separation was seen. For this reason the reaction was never attempted on more than 50 mg of material. However, purification of larger quantities of material could most likely be accomplished with the chromatotron, in the same manner as was done for the protected analog **135**.

With the Largazole-Azumamide E hybrid in hand, attempts were made to convert the carboxylic acid to the carboxamide using HOBt, EDCI and NH_3. Unfortunately, this reaction was never successful, and resulted in a still unidentified product.

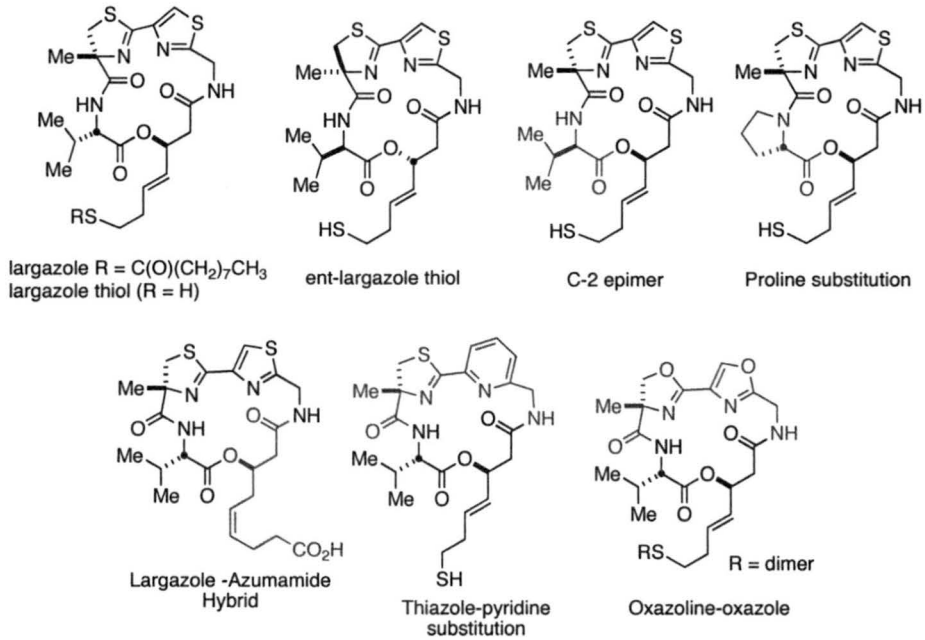


Scheme 39. Completion of the Largazole Azumamide hybrid

4.3 Future Investigations

Acid **134** was produced in 13 steps and 11% yield. This product was subjected to biological testing, along with several other Largazole analogs made by the Williams group. The results in figure 3 show that the Largazole-Azumamide E hybrid

unfortunately showed a significant decrease in potency compared to Largazole thiol and all of the other Largazole analogs.



Biochemical inhibiton of human HDACs (IC_{50}, μM).

Compound	HDAC1	HDAC2	HDAC3	HDAC6
Largazole thiol	0.0012	0.0035	0.0034	0.049
Enantiomer	1.2	3.1	1.9	2.2
C-2 epimer	0.030	0.082	0.084	0.68
Proline substitution	0.11	0.80	0.58	13
Largazole-Azumamide hybrid	> 30	> 30	> 30	> 30
Thiazole-pyridine substitution	0.00032	0.00086	0.0011	0.029
Oxazoline-oxazole	0.00069	0.0017	0.0015	0.045

Figure 3. Largazole Analog Biological Activity

However, despite these disapoining results, future work on this product should involve further attempts to synthesize the carboxamide Azumamide A analogue and the hydroxamic acid analog. These would be of great interest, as modification of the zinc-binder could have dramatic effects on the biological activity.

Chapter 5

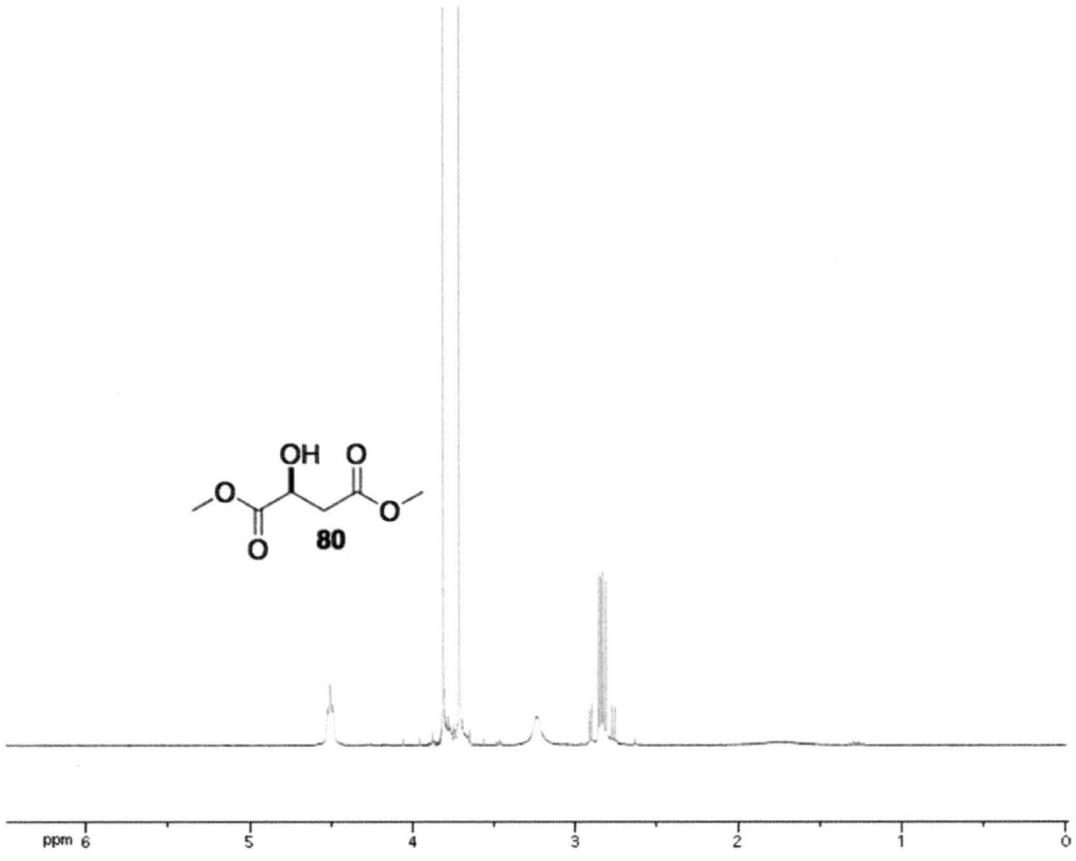
Experimentals

General Methods.

Unless otherwise noted, all materials were obtained from commercial sources and used without purification. All reactions requiring anhydrous conditions were performed under a positive pressure of argon using flame-dried glassware. Dichloromethane, tetrahydrofuran, methanol, dimethylsulfoxide, triethylamine, and acetonitrile were degassed with argon and dried through a solvent purification system (J.C. Meyer of Glass Contour). Flash chromatography was performed on Merck silica gel Kieselgel 60 (230-400 mesh) from EM science with the indicated solvent. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 300 or 400 MHz spectrometers as indicated. Mass spectra were obtained at the Colorado State University CIF on a Fisons VG Autospec.

Dimethyl (S)-(-)-Malate 96

Following a literature procedure[41] for the synthesis of optically pure Dimethyl (S)-(-)-Malate. L-(-) malic acid (100g, 0.746 mol), and Dowex 50W-X8 ion exchange resin (7g) were added to a solution of MeOH (100 mL) and $CHCl_3$ (160 mL). The solution was heated under reflux through a Soxhlet Thimble with $MgSO_4$ (80g). There was not sufficient solvent to fill the Soxhlet Extractor, so additional MeOH (100 mL) and $CHCl_3$ (160 mL) were added and the reaction was allowed to run to for 14 hours. The drying agent was changed twice during the reaction time. The solution was then cooled to room temp, filtered, and solvents removed. The resulting oil was purified by vacuum distillation, to give Dimethyl (S)-(-)-malate (37.49g, 30% yield, $[\alpha]^{24°C}_{D}$ -6.88 (neat, *l* =1dm) ^{1}H NMR ($CDCl_3$) δ 2.80 (dd, 2H, *J*= 4.5, 81 Hz), 3.7 (s, 3H), 3.8 (s, 3H), 4.5 (t, 1H, *J*=5.4 Hz).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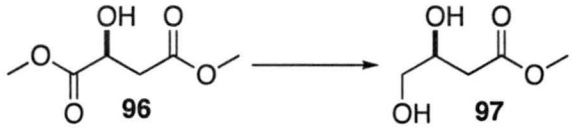
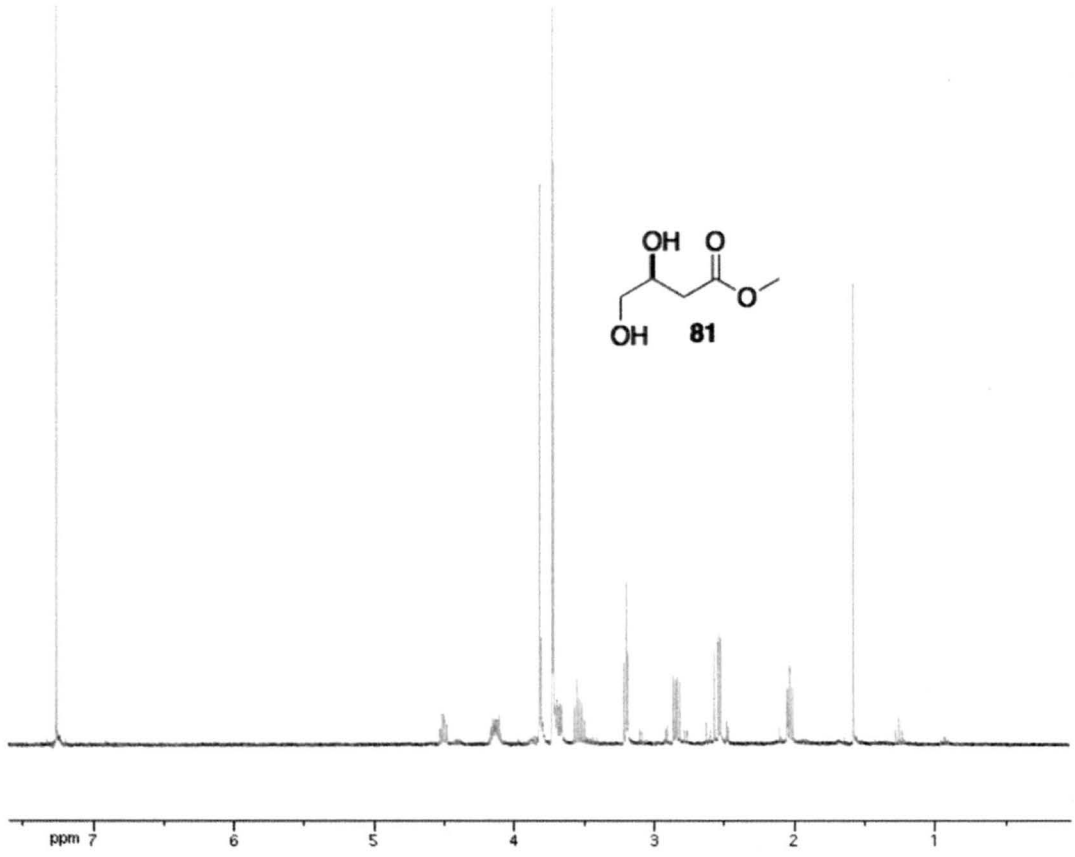


(*S*)-methyl 3,4-dihydroxybutanoate 97

Following a literature procedure[42] to a solution of Dimethyl (S)-(-)-malate (1.9457g, 12mmol) in dry THF (25 mL), BMS (6 mL, 12 mmol) was added and the mixture was stirred at room temperature for 30min. $NaBH_4$ (0.0227g, 0.6 mmol) was added to the solution and allowed to stir for 1.5 hours, before MeOH (8 ml) was added and stirred for an additional 30 min. The solvents were evaporated and the resulting oil was run through a plug of silica gel (EtOAc) to afford the alcohol product (1.3067g, 81%) ^1H NMR matched liturature values ($CDCl_3$) δ 2.55 (m, 2 H), 3.23 (m, 1H), 3.53 (m, 1 H), 3.67 (m, 1H), 3.73 (s, 3 H).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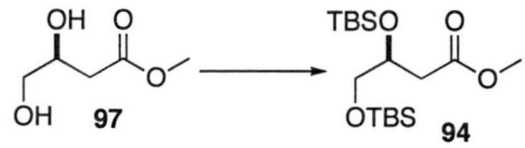
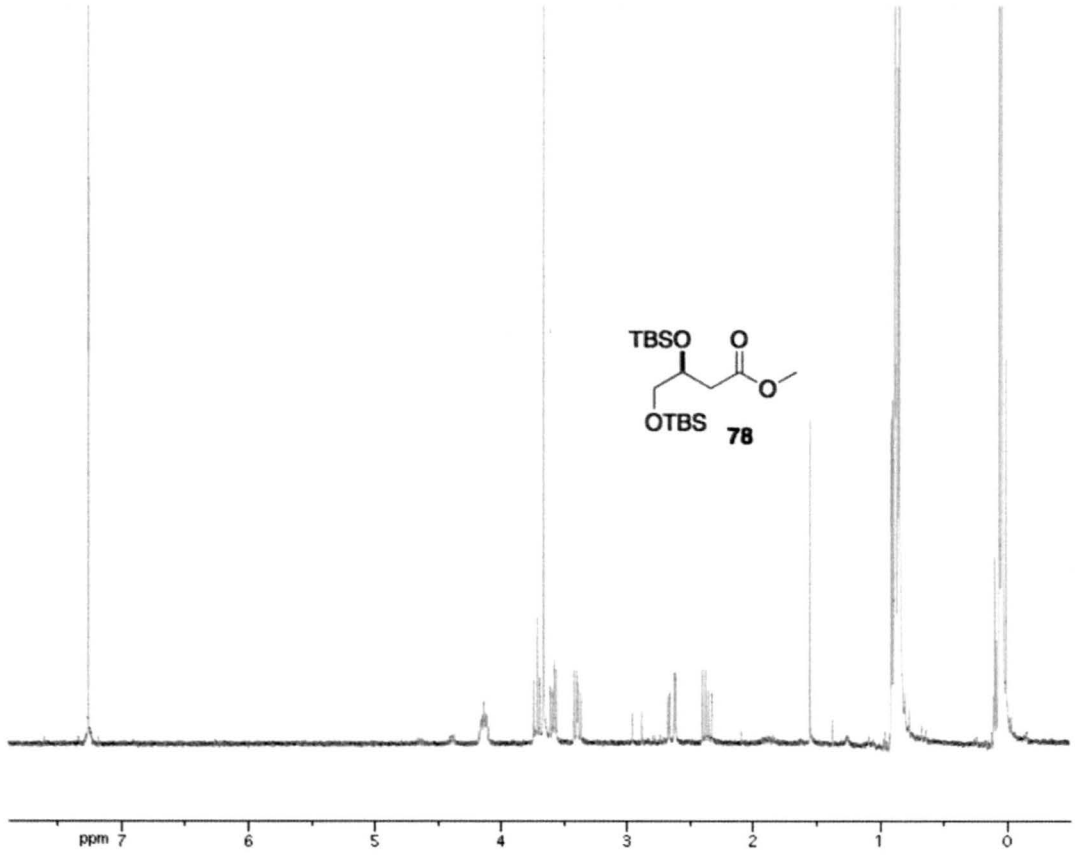
2
1



(*S*)-methyl 3,4-bis(*tert*-butyldimethylsilyloxy)butanoate 94

To a solution of (*S*)-methyl 3,4-dihydroxybutanoate (1.3067g, 9.75 mmol) in DMF (19.5 mL), 3 equivalents of imidazole (1.99g, 29.25mmol) were added followed by 2.2 equivalents of TBSCl (3.2175g, 21.45mmol), and 5 mol% DMAP (0.0596g, 0.4875 mmol). The solution was allowed to stir overnight, before quenching with H_2O and extracting with Hexanes. The organic layer was dried with $NaSO_4$, filtered, and solvents removed to provide a clear yellowish oil (*S*)-methyl 3,4-bis(*tert*-butyldimethylsilyloxy)butanoate (2.70g, 79%). ^{1}H NMR ($CDCl_3$) δ 0.1 (s, 12 H), 0.9 (s, 18 H), 2.38 (dd, 1 H, *J*= 8.1, 14.7 Hz), 2.65 (dd, 1 H, *J*=3.9, 14.7 Hz), 3.40 (dd, 1 H, *J*= 7.2, 9.9), 3.58 (dd, 1H, *J*=5.1, 9.6), 3.66 (s, 3 H), 4.14 (m, 1 H).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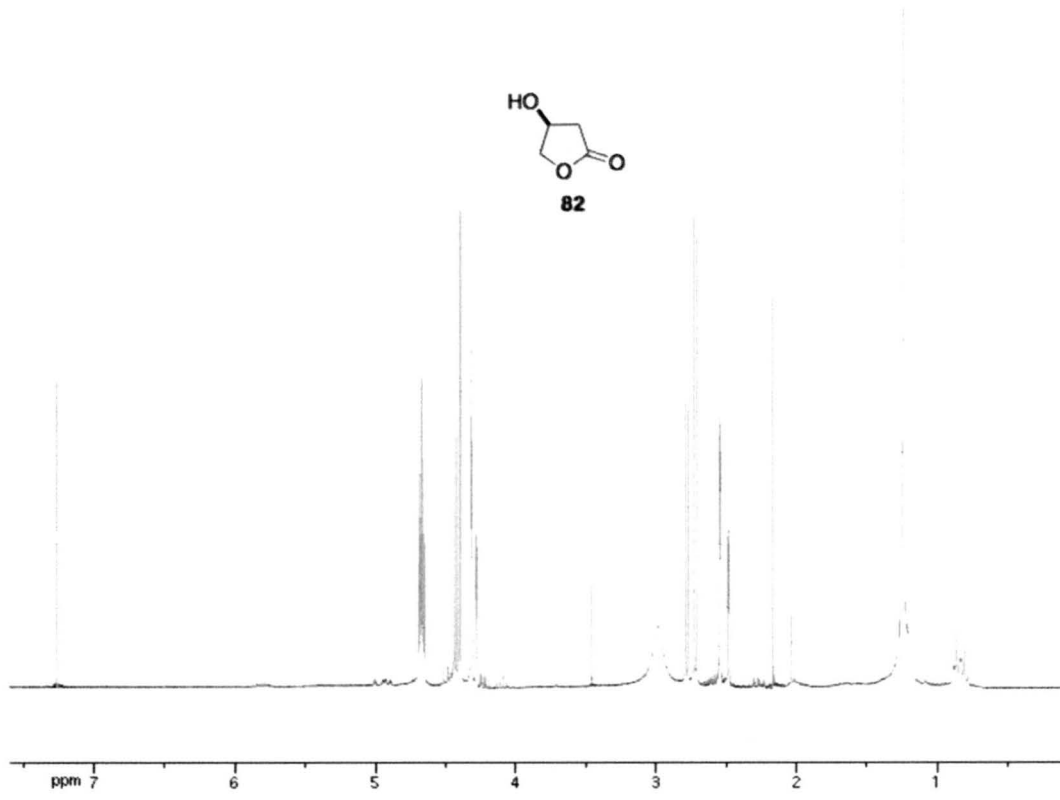
O
O
OTBS
78
ppm 7
6
5
4
3
2
1
0

97 99

(*S*)-4-hydroxydihydrofuran-2(3*H*)-one 99

From literature procedure [43] to a solution of (*S*)-4-hydroxydihydrofuran-2(3*H*)-one (0.536g, 4 mmol) in CH_2Cl_2 (5.0 mL) TFA (0.1 mL) was added and allowed to stir for 24 hours. The solution was then concentrated, and treated with CH_2Cl_2 (5 mL) and more TFA (0.16 mL). This was allowed to stir for 24 hours before solvents were removed and purified by column chromatography to afford the lactone (13) as a clear oil.

^{1}H NMR (300 MHz $CDCl_3$) δ 2.5 (dt, 1 H, *J*=0.9, 17.1 Hz), 2.75 (dd, 1H, *J*=6.0, 18.0 Hz), 3.0 (s, 1 H), 4.3 (dt, 1 H, *J*=1.2, 10.2 Hz), 4.4 (dd, 1 H, *J*=4.2, 10.2 Hz), 4.67 (m, 1 H).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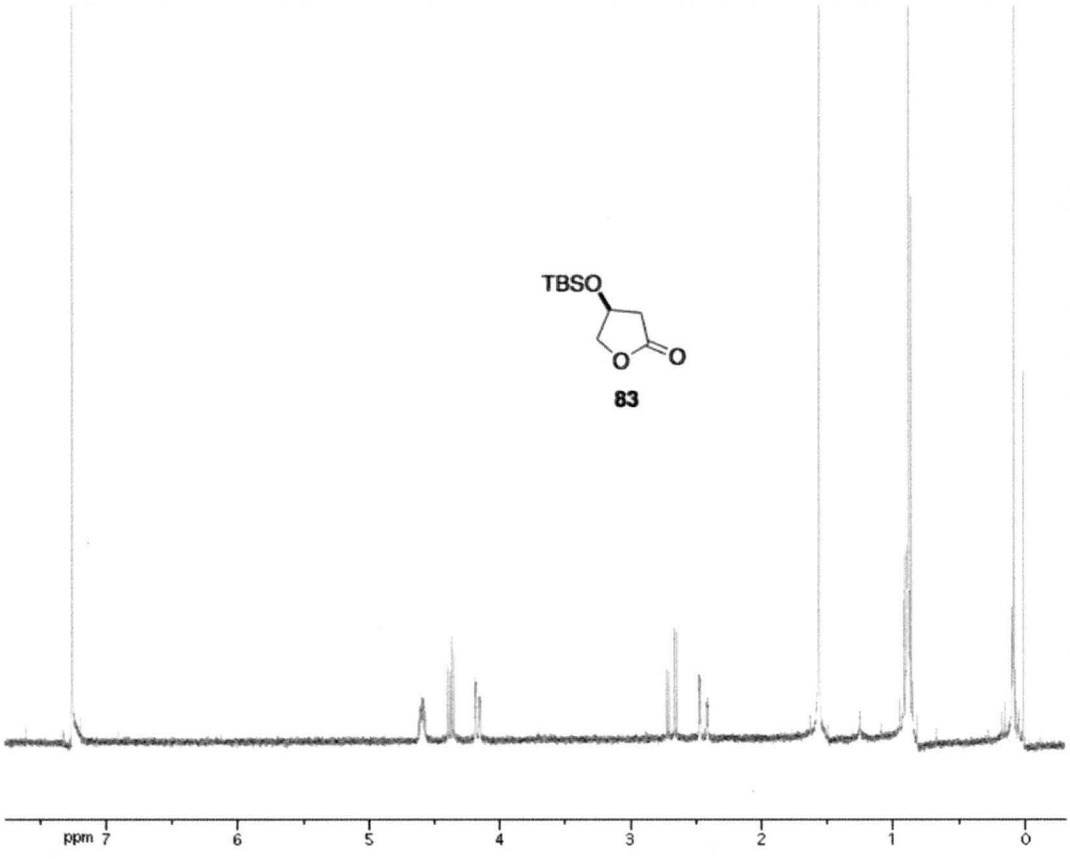
O
O
82
ppm 7
6
5
4
3
2
1

99 **100**

(*S*)-4-(*tert*-butyldimethylsilyloxy)dihydrofuran-2(3*H*)-one 100

To a solution of (*S*)-4-hydroxydihydrofuran-2(3*H*)-one (0.1920g, 1.88 mmol) in DMF (3.76 mL) two equivalents of imidazole (0.257g, 3.76mmol) was added followed by 1.1 equivalents of TBSCl (0.3118g, 2.068mmol), and finally 5 mol% DMAP (0.0114g, 0.094mmol). The solution was stirred overnight and worked up by the addition of H_2O and Hexanes. The organic layer was extracted, washed and dried with $NaSO_4$, before being filtered and solvents removed to provide (*S*)-4-(*tert*-butyldimethylsilyloxy)dihydrofuran-2(3*H*)-one as a clear oil. The product does not stain easily, but works best in $KMnO_4$. ^1H NMR (300 MHz $CDCl_3$) δ 0.1 (s, 6 H), 0.9 (s, 9 H), 2.44 (dd, 1 H, *J*=2.7, 17.4 Hz), 2.68 (dd, 1 H, *J*=6.3, 17.7 Hz), 4.17 (dd, 1H, *J*=2.4, 9.9 Hz), 4.38 (dd, 1 H, *J*=4.8, 9.6 Hz) 4.60 (m, 1 H)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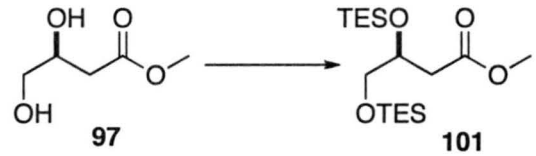
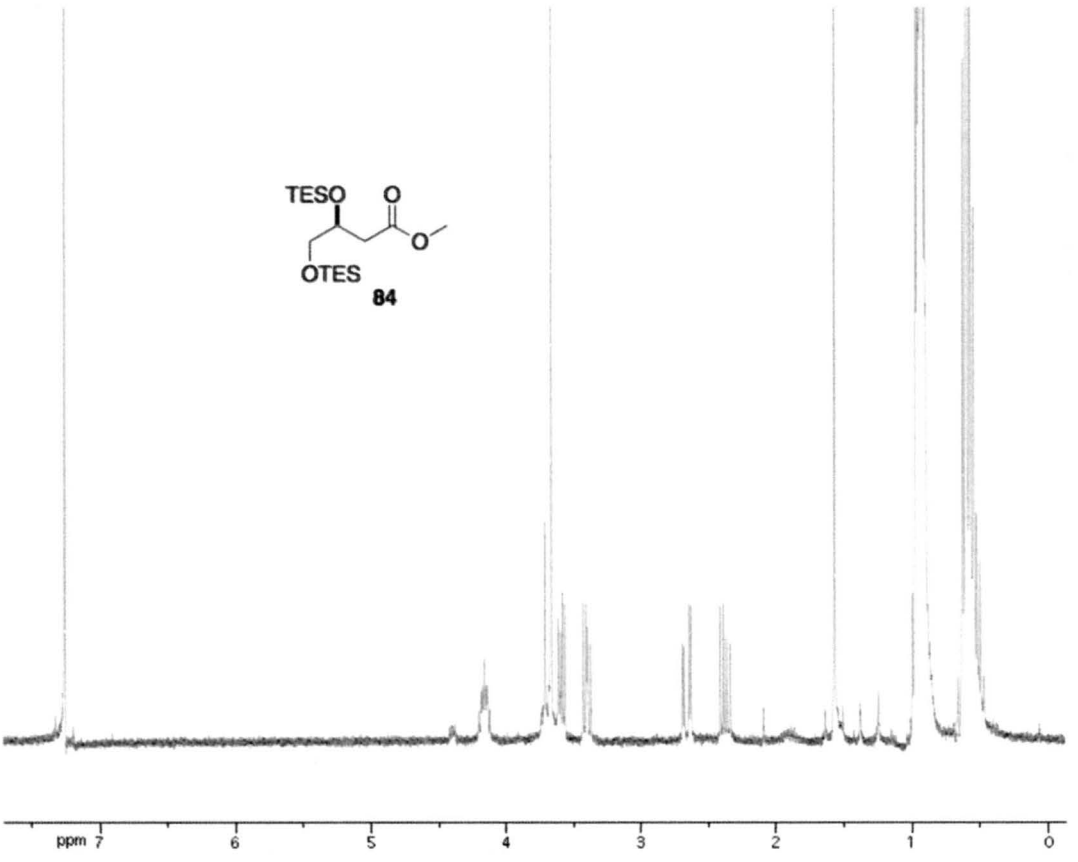


97 101

(*S*)-methyl 3,4-bis(triethylsilyloxy)butanoate 101

To a solution of (*S*)-methyl 3,4-dihydroxybutanoate (0.2031g, 1.49mmol) in DMF (3mL) three equivalents of imidazole (0.304g, 4.47mmol) was added followed by 2.1 equivalents of TESCl (0.53 mL, 3.129mmol) and 5 mol% of DMAP (0.0091g, 0.0745mmol). The solution was allowed to stir overnight, and the reaction was worked up by addition of H_2O and Hexanes. The organic layer was extracted and dried with $NaSO_4$, filtered, and solvents removed to give (*S*)-methyl 3,4-bis(triethylsilyloxy)butanoate (0.508g, 94%). ^{1}H NMR (300 MHz $CDCl_3$) δ 0.58 (m, 12 H), 0.93 (m, 18 H), 2.37 (dd, *J*= 7.8, 15 Hz, 1 H), 2.66 (dd, *J*= 4.5, 15 Hz, 1 H), 3.40 (dd, *J*= 7.2, 9.6 Hz, 1 H), 3.59 (dd, *J*= 5.4, 9.9 Hz, 1 H), 3.67 (s, 3 H), 4.16 (m, 1 H).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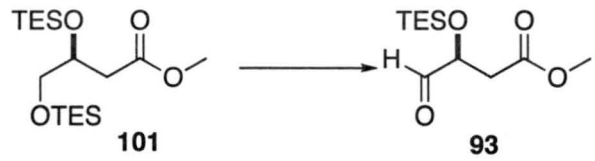
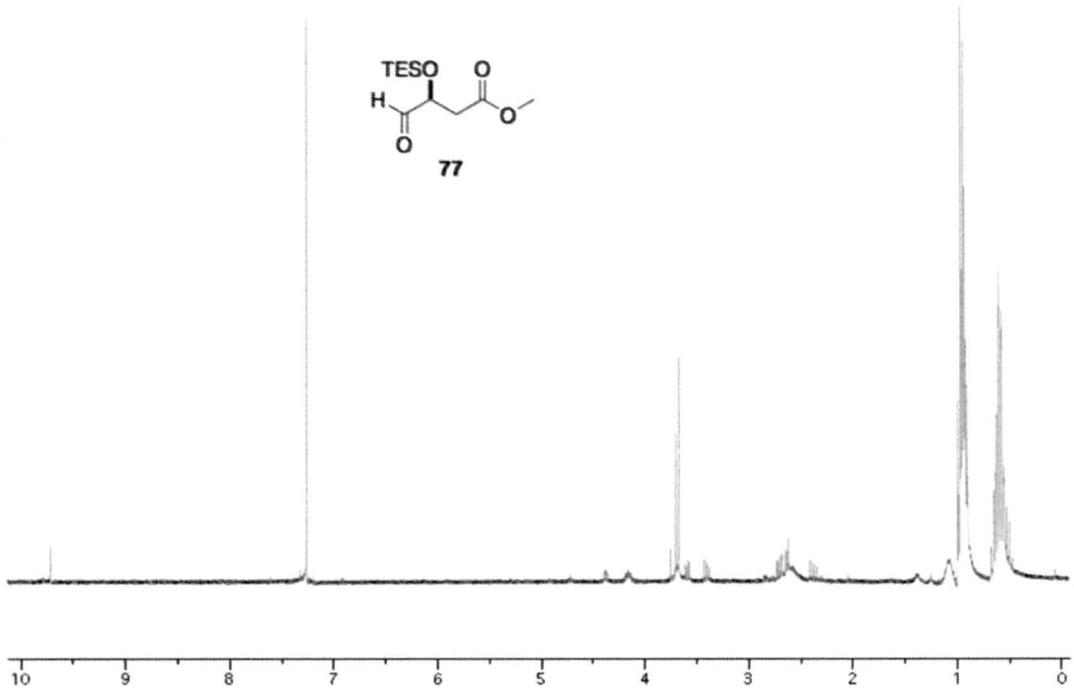


(*S*)-methyl 4-oxo-3-(triethylsilyloxy)butanoate 93

DMSO (0.31ml) in 2 mL of CH_2Cl_2 was cooled to -78°C before Oxalyl chloride $(COCl)_2$ (0.192 mL) in 1.1mL CH_2Cl_2 was added drop wise and allowed to stir for 15 min under Argon. Then (*S*)-methyl 3,4-bis(triethylsilyloxy)butanoate **15** (0.188g, 0.5 mmol) in 2 mL of CH_2Cl_2 was added and stirred for 20 min at -78°C and then warmed to -40 °C for 20 min. The solution was then cooled back down to -78°C and Triethylamine (1.05mL, 7.5 mmol) was added, and the reaction was allowed to warm to room temperature. The reaction was then diluted with H_2O and extracted with CH_2Cl_2. The organic layer was separated and washed with brine and dried with Na_2SO_4, filtered and solvents removed. The NMR showed starting material and product.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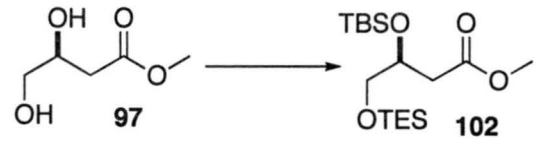
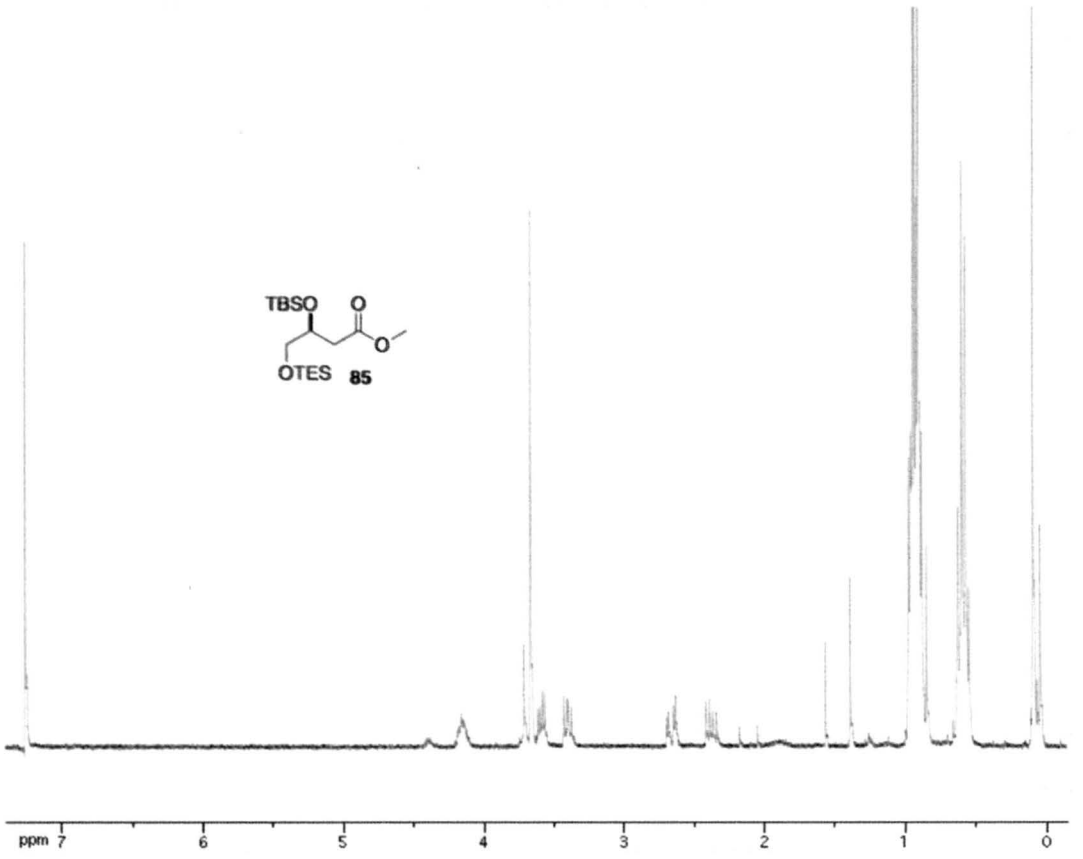


(*S*)-methyl 3-(*tert*-butyldimethylsilyloxy)-4-(triethylsilyloxy)butanoate 102

To a solution of (*S*)-methyl 3,4-dihydroxybutanoate (11) (0.2052g, 1.53mmol) in CH_2Cl_2 (3.06 mL) three equivalents of Imidazole (0.3221g, 4.59 mmol) were added and the solution cooled to 0°C and one equivalent of TESCl (0.26 mL, 1.53mmol) was added. The reaction was followed by TLC until all of the starting material appeared to be gone. Then one equivalent of TBSCl (0.2307g, 1.53mmol) was added to the solution and the reaction was allowed to warm to room temperature. The reaction was quenched with saturated sodium bicarb solution, and extracted with CH_2Cl_2. The organic layer was separated, and dried with Na_2SO_4, filtered and solvents removed. The resulting oil was purified with column chromatography to provide (*S*)-methyl 3-(*tert*-butyldimethylsilyloxy)-4-(triethylsilyloxy)butanoate **16** as a light yellow oil. ^{1}H NMR (300 MHz $CDCl_3$) δ 0.7 (s, 6 H), 0.58 (m, 9 H), 0.92 (m, 18 H), 2.38 (dd, 1 H, *J*= 8.1, 15.3 Hz), 2.65 (dd, 1 H, *J*=4.2, 14.7 Hz), 3.40 (dd, 1 H, *J*=7.2, 9.9 Hz), 3.59 (dd, 1 H, *J*= 5.1, 9.9 Hz), 3.68 (s, 3 H), 4.15 (m, 1 H).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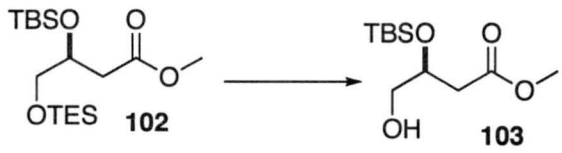
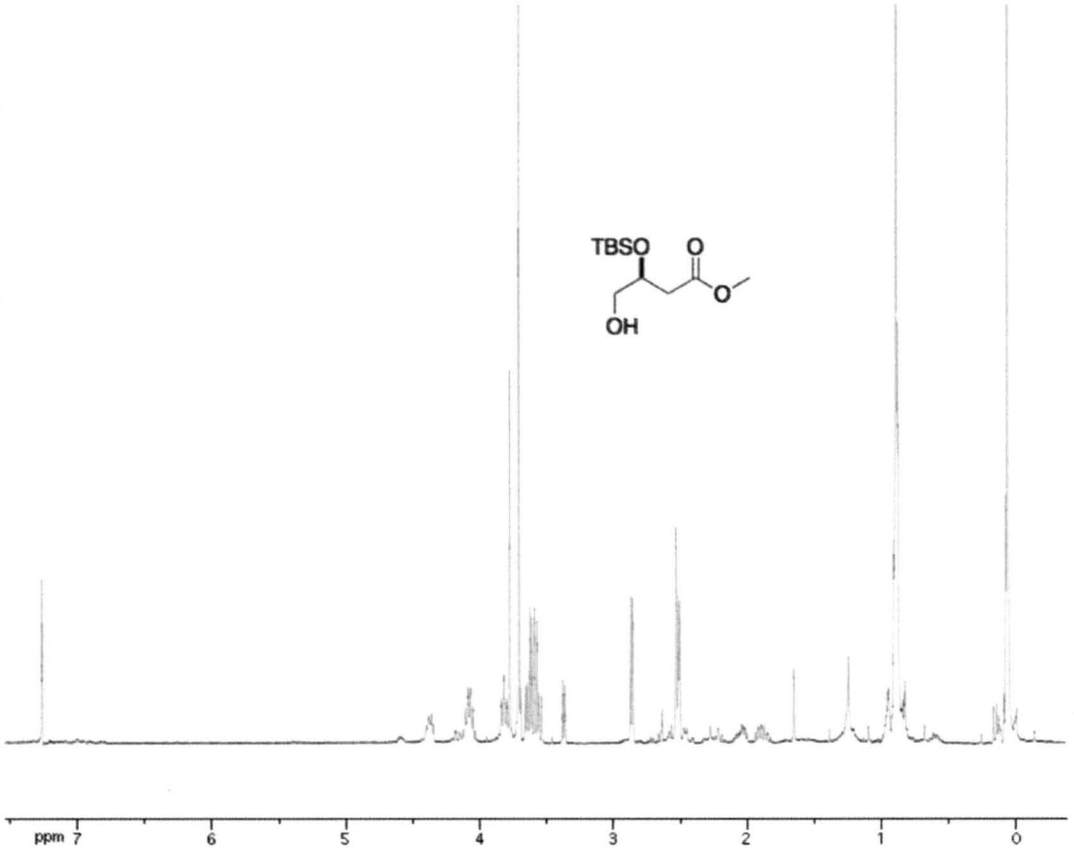


(*S*)-methyl 3-(*tert*-butyldimethylsilyloxy)-4-hydroxybutanoate 103

A solution of (*S*)-methyl 3-(*tert*-butyldimethylsilyloxy)-4-(triethylsilyloxy)butanoate **85**(0.2200g, 0.607mmol) in MeOH (6.07mL, 0.1M) was cooled to 0 C before the addition of 10 mol % of PPTS (0.0153g, 0.0607mmol). The reaction was allowed to stir for 15 min and then followed by TLC. The reaction was quenched by saturated sodium bicarb and extracted with EtOAc. The organic layer was dried with Na_2SO_4, filtered and solvents removed, and purified through a silica plug, to yield a yellow oil (0.0898g, 59.6%) ^{1}H NMR (300 MHz $CDCl_3$) δ 0.05 (s, 6 H), 0.9 (s, 9 H), 2.50 (d, 1 H, *J*= 3 Hz), 2.83 (d, 1 H, *J*=4.8 Hz), 3.60 (m, 2 H), 3.74 (s, 3 H), 4.09 (m, 1 H).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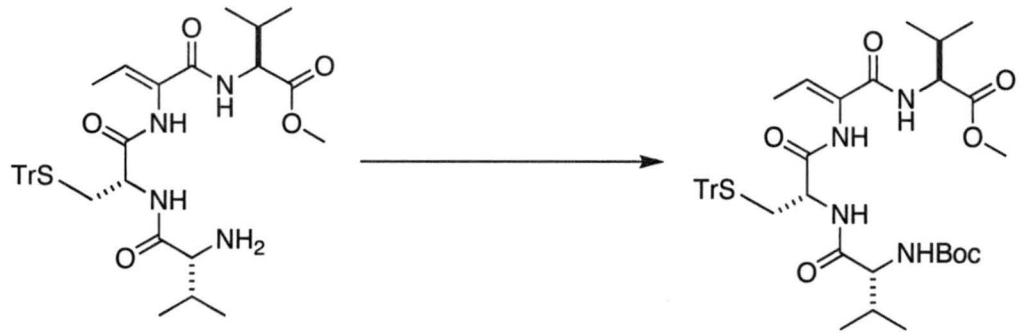
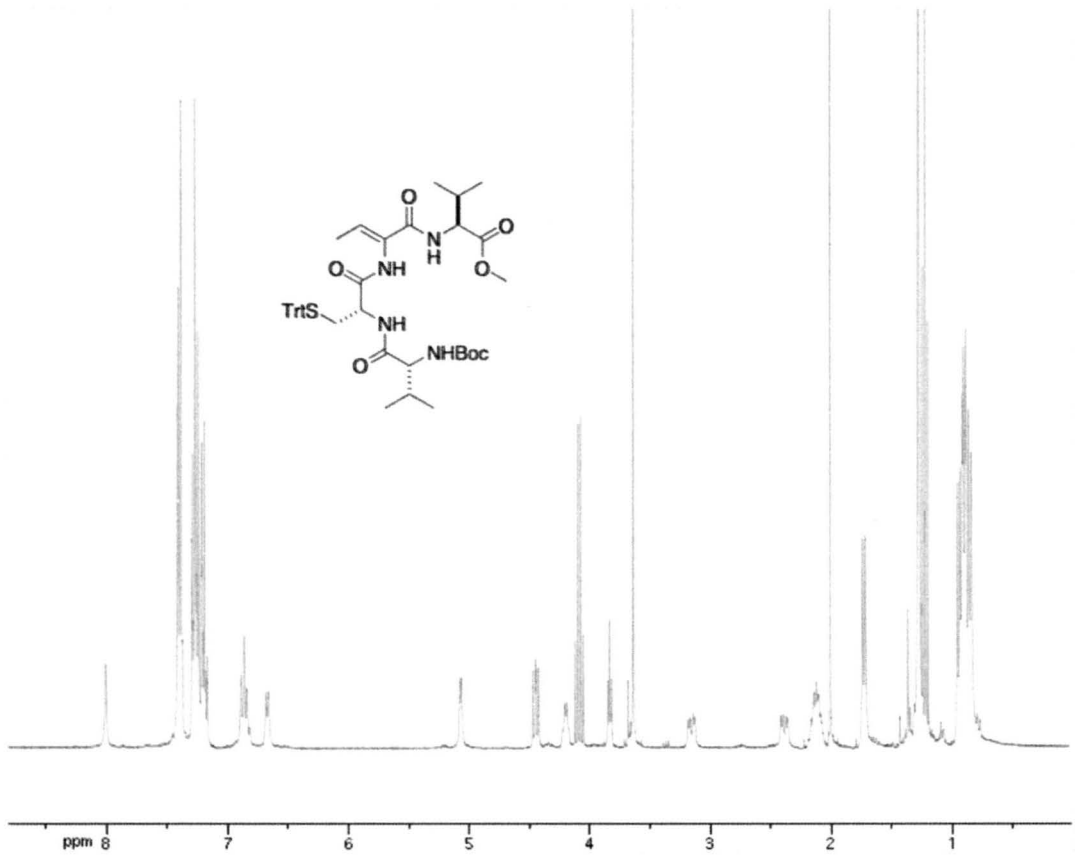


N-Boc protected FK228 tetrapeptide methyl ester

The free amine tetrapeptide (216 mg, 0.328 mmol) was dissolved in CH_2Cl_2 (3.28 mL). To this solution was added Et_3N (91μL, 0.656 mmol) and Boc_2O (78.7 mg, 0.3608 mmol). This was allowed to stir for 8 hours and followed by TLC, and quenched with Sat. aq NH_4Cl, aqueous phase was extracted with EtOAc, and the combined organic layers were condensed to give the product as a white solid. Column chromatography to purify. ^1H NMR (300 MHz $CDCl_3$) δ 0.9 (m, 12 H), 1.3 (s, 9 H), 1.73 (d, 3 H, *J*= 7.2 Hz), 2.12 (m, 2H), 2.38 (dd, 1H, *J*= 5.1, 12.3 Hz), 3.15 (dd, 1 H, *J*= 4.5, 12.3 Hz), 3.63 (s, 3 H), 3.83 (t, 1 H, *J*= 4.8 Hz), 4.2 (m, 1 H), 4.45 (m, 1H), 5.07 (d, 1H, *J*=4.5 Hz), 5.07 (d, 1 H, *J*= 4.5Hz), 6.68 (d, 1 H, *J*=6.3 Hz), 6.88 (t, 2 H, *J*=7.8), 7.24 (m, 9 H), 7.4 (m, 9 H), 8.1 (s, 1 H).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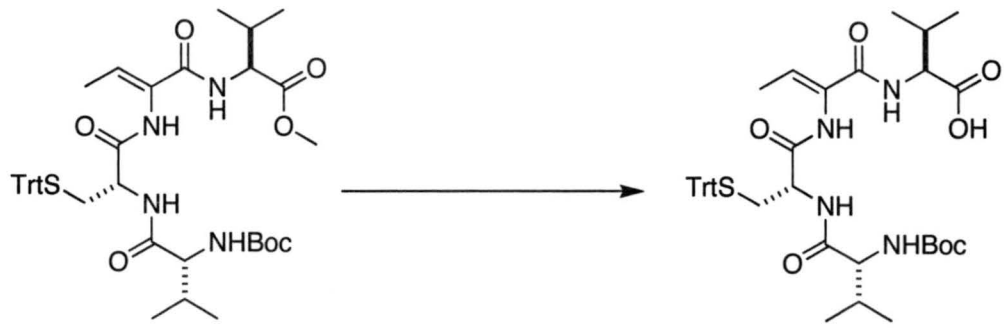
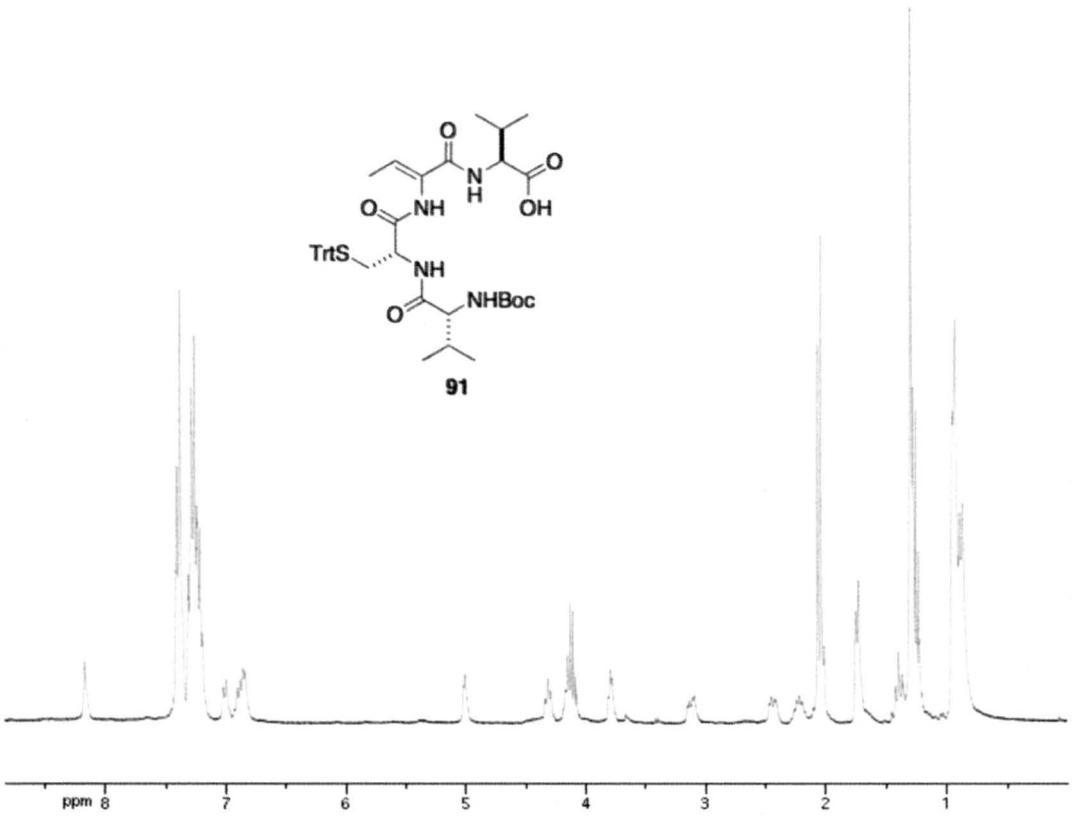


108

Saponification of the N-Boc protected FK228 tetrapeptide 108

The methyl ester (189 mg, 0.249 mmol) was dissolved in dry THF (0.5 mL) and cooled to 0°C under argon. Then LiOH-H_2O (18 mg, 0.747 mmol) was dissolved in H_2O (0.12 mL). This solution was added to the ester solution and allowed to stir for 2 hours at 0°C before quenching by pouring the reaction mixture into a solution of EtOAc (3.05 mL) and 2M aq HCl (0.61 mL). The aqueous layer was extracted with EtOAc and the combined organic layers were dried with Na_2SO_4, filtered and concentrated. The product was a white solid in 65% yield ^{1}H NMR (300 MHz $CDCl_3$) δ 0.9 (m, 12 H), 1.25 (s, 9 H), 1.74 (d, 3 H, *J*=7.2 Hz), 2.23 (m, 1 H), 2.44 (dd, 1 H, *J*= 5.1, 12.9 Hz), 3.11 (dd, 1 H, *J*= 6.1, 12.6 Hz), 3.8 (m, 1 H), 4.32 (t, 1 H, *J*= 8.1), 5.0 (s, 1 H), 6.85 (m, 2 H), 7.0 (d, 1 H, *J*= 7.5), 7.25 (m, 9H), 7.40 (m, 6 H), 8.17 (s, 1 H).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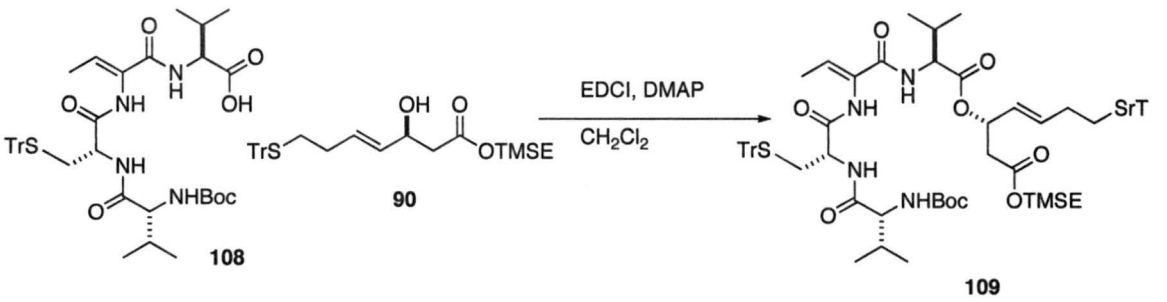
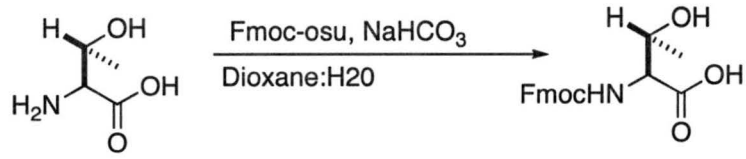


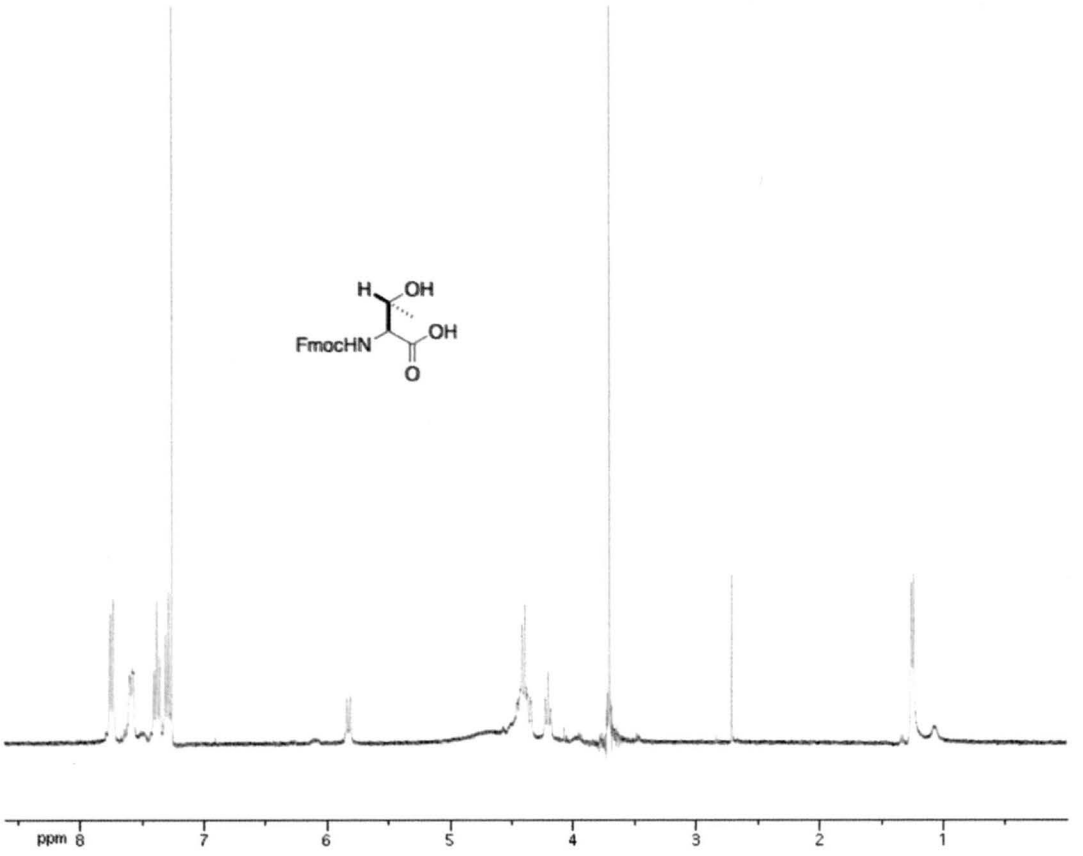
Direct Coupling of N-Boc protected Tetrapeptide to protected acid

The Boc protected tetrapeptide (120 mg, 0.162 mmol) and protected acid 4 (16.6 mg, 0.032 mmol) were dissolved in dry CH_2Cl_2 (0.58 mL) and cooled to 0°C. To this, EDCI (37 mg, 0.192 mmol) and DMAP (0.0004g, 0.003 mmol) dissolved in CH_2Cl_2 (0.145 mL) were added to the cooled reaction mixture. This was followed by iPr$_2$NEt (33μL, 0.129mmol). The reaction was allowed to warm to rt and stirred overnight. The reaction was concentrated and passed through a plug of silica gel. However no product was formed.



Formation of N-Fmoc protected Thronine

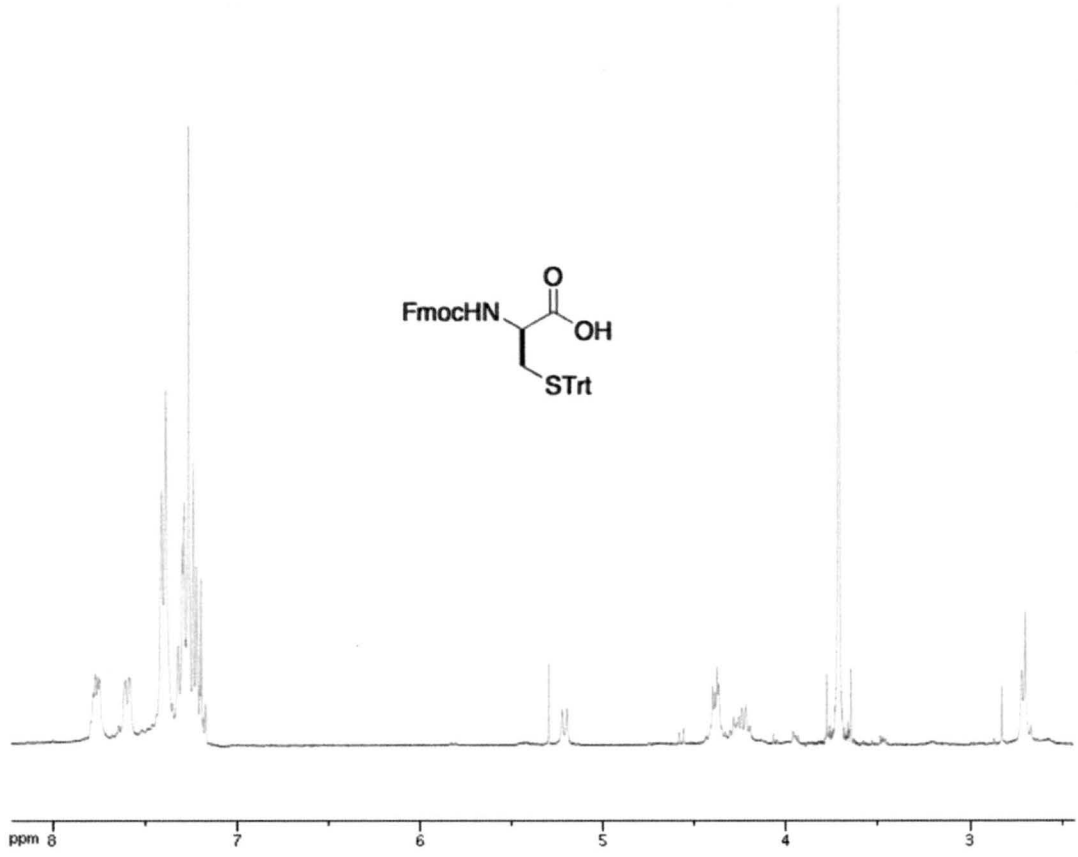
The Threonine (55.5 mg, 0.466 mmol) was dissolved in a 1:1 Dioxane:H_2O (6.21 mL), followed by $NaHCO_3$ (39 mg, 0.466 mmol), and Fmoc-osu (157 mg, 0.466 mmol). This was allowed to stir overnight, then cooled to 0°C before 1 M HCl was added till pH=4-5, then the aqueous layer was extracted with CH_2Cl_2. The product was recrystalized from EtOAc/Hexanes. ^{1}H NMR (300 MHz $CDCl_3$) δ 1.24 (d, 3 H, *J*= 6.3 Hz), 4.2 (t, 1 H, *J*= 6.9 Hz), 4.4 (m, 4 H), 5.8 (d, 1 H, *J*= 9.0 Hz), 7.38 (m, 4 H), 7.58 (d, 2 H, *J*=7.8 Hz), 7.75 (d, 2 H, *J*= 7.8 Hz).

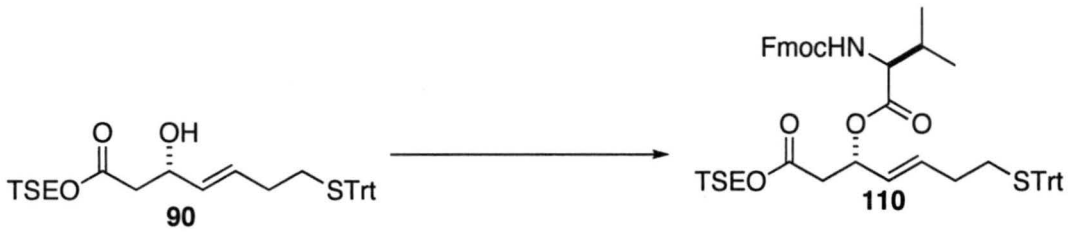


Formation of N-Fmoc Protected S-Trt-D-Cystine

The S-Trt-D-Cystine (169.5 mg, 0.466 mmol) was dissolved in a 1:1 Dioxane :H_2O (6.21 mL), followed by $NaHCO_3$ (39 mg, 0.466 mmol), and Fmoc-osu (157 mg, 0.466 mmol). This was allowed to stir overnight, then cooled to 0°C before 1 M HCl was added till pH=4-5, then the aqueous layer was extracted with CH_2Cl_2. The product was re-crystallized from EtOAc/Hexanes. ^1H NMR (300 MHz $CDCl_3$) δ 2.71 (d, 2 H, J= 6.3

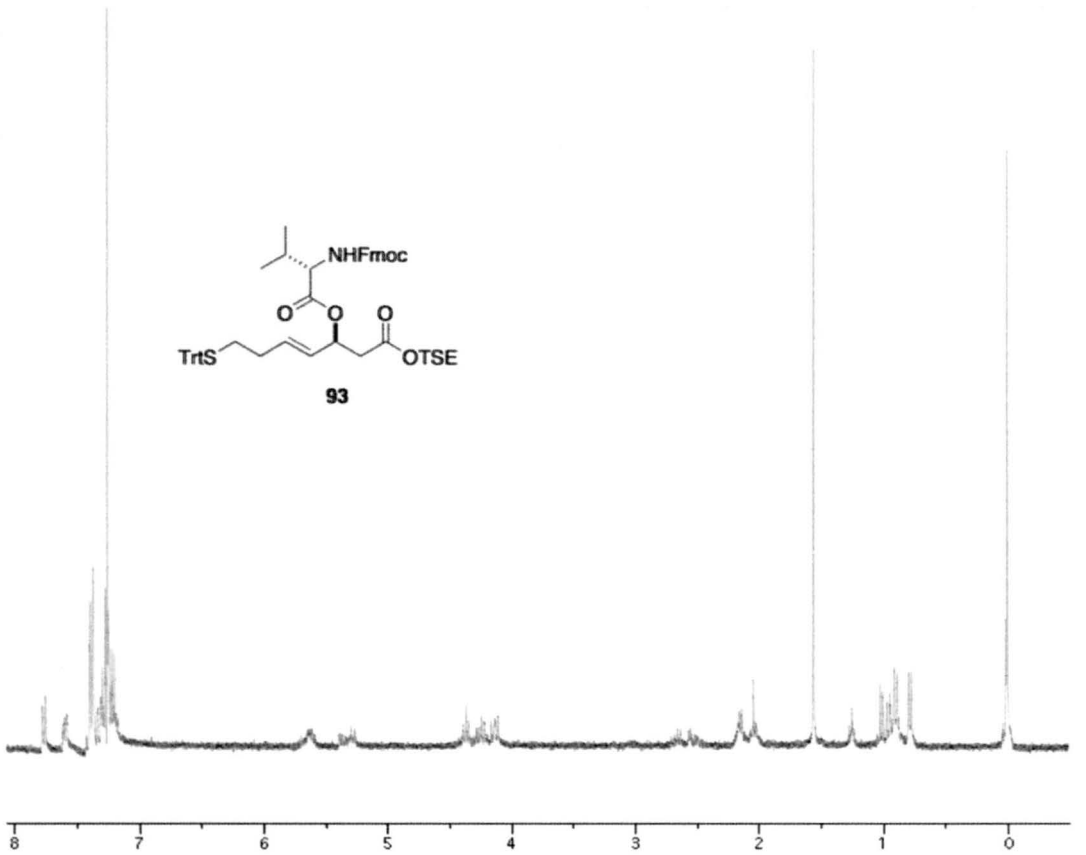
Hz), 4.30 (m, 3 H), 5.23 (d, 1 H, *J*=7.8 Hz), 7.30 (m, 18 H), 7.60 (d, 2 H, *J*= 6.0 Hz), 7.76 (m, 2 H).





Formation of (*S*,*E*)-2-(trimethylsilyl)ethyl 3-((*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyloxy)-7-(tritylthio)hept-4-enoate (110)

Fmoc-L-Valine (42 mg, 1.24 mmol), and protected acid **90** (128 mg, 0.248 mmol) were dissolved in dry CH_2Cl_2 and cooled to 0°C. Then a mixture of EDCI (285 mg, 1.488 mmol) and DMAP (0.003g, 0.0248 mmol) in CH_2Cl_2 (1.13 mL) were added followed by $i$pr$_2$NEt (260 μL) to the reaction mixture. This was allowed to warm to rt and stirred overnight. The reaction was condenced and purified with column chromatography (4:1) hexane/ EtOAc.) ^{1}H NMR (300 MHz $CDCl_3$) δ 0.01 (s, 9 H), 0.79 (d, 3 H, J= 6.9 Hz), 0.9 (d, 3 H, J=6.9 Hz), 1.0 (m, 4 H), 2.05 (m, 2 H), 2.16 (d, 2 H, J= 6.9), 2.54 (m, 1 H), 2.68 (m, 1 H), 4.3 (m, 7 H), 5.32 (m, 2 H), 5.65 (m, 2 H), 7.3 (m, 19 H), 7.6 (d, 2 H, J= 6.0 Hz), 7.75 (d, 2 H, J=7.8).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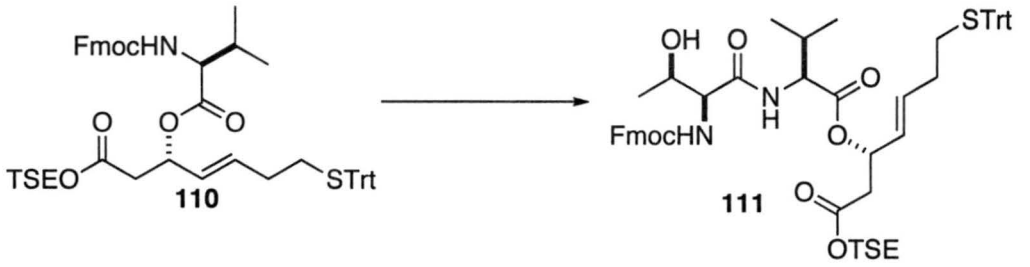
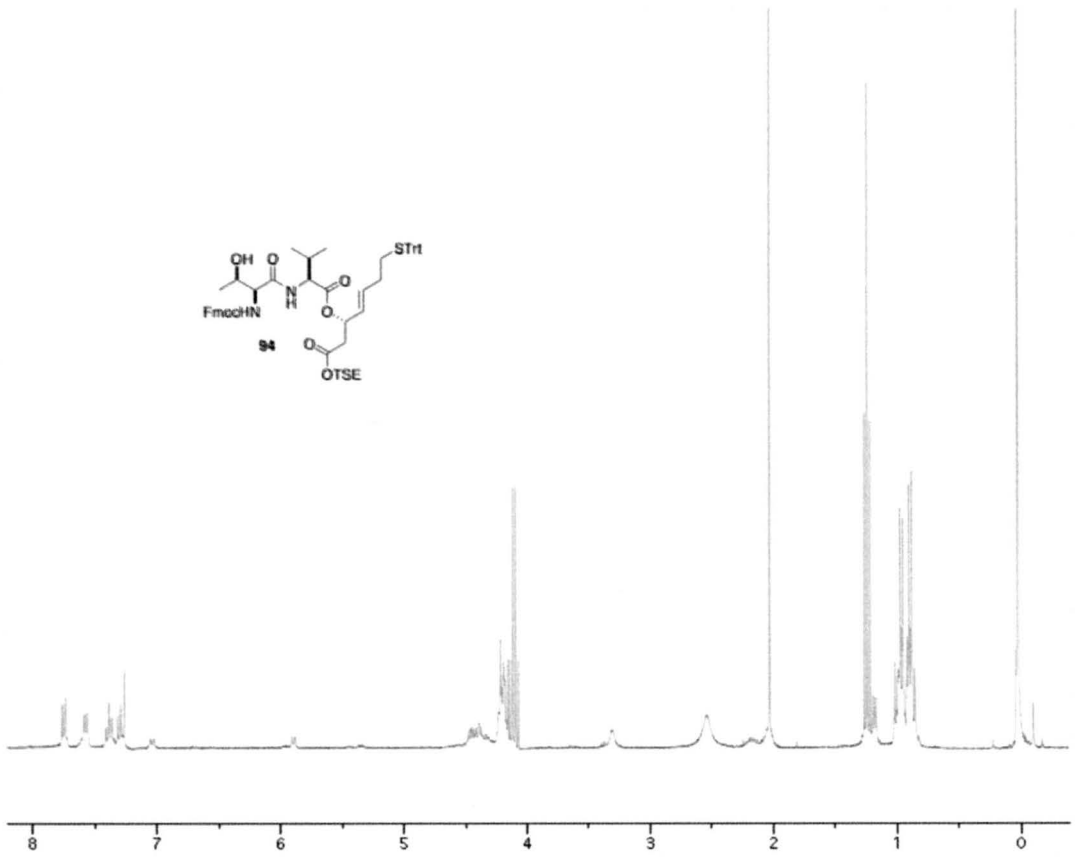


Formation of (3*S*,*E*)-2-(trimethylsilyl)ethyl 3-((2*S*)-2-(2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-hydroxybutanamido)-3-methylbutanoyloxy)-7-(tritylthio)hept-4-enoate (111)

Compound **110** (130 mg, 0.154 mmol) was dissolved in CH_3CN (7.7 mL) followed by Et_2NH. This was monitored by TLC till all starting material was gone, then concentrated and re-diluted in EtOAc and concentrated again to remove all the excess Et_2NH.

Fmoc-Thr (57 mg, 0.169 mmol) was dissolved in CH_2Cl_2 (2.8 mL) followed by PyBop (160mg, 0.308 mmol) and iPr$_2$NEt (0.08 mL) and allowed to stir for 5 min. Then the crude amide dissolved in CH_3CN (1.4 mL) was added and allowed to stir for about 2 hours. The reaction mixture was concentrated and subjected to column chromatography, to give the product as a white solid. ^{1}H NMR (300 MHz $CDCl_3$) δ 0.01 (s, 9 H), 0.8 (d, 2 H, J=6.9 Hz), 0.9 (m, 10 H), 1.24 (m, 4 H), 1.4 (d, 3 H, J=5.4 Hz), 1.5 (d, 2 H, J=6.0 Hz), 2.15 (m, 5 H), 2.6 (m, 3 H), 4.15 (t, 2 H, J=7.5 Hz), 4.37 (m, 1 H), 4.47 (m, 1 H), 5.35 (m, 1 H), 5.65 (m, 2 H), 7.3 (m, 21 H).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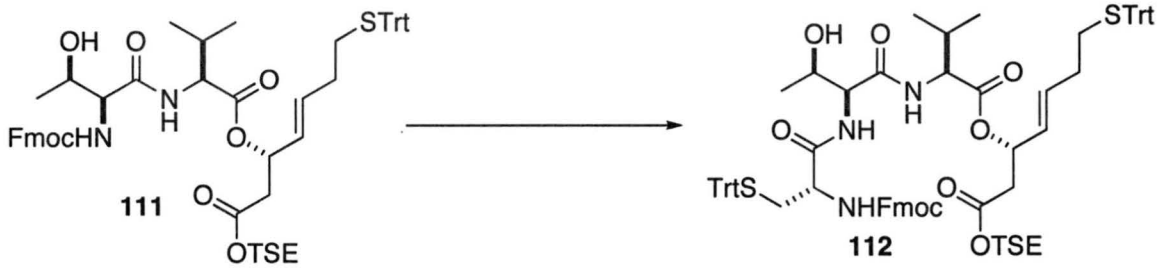
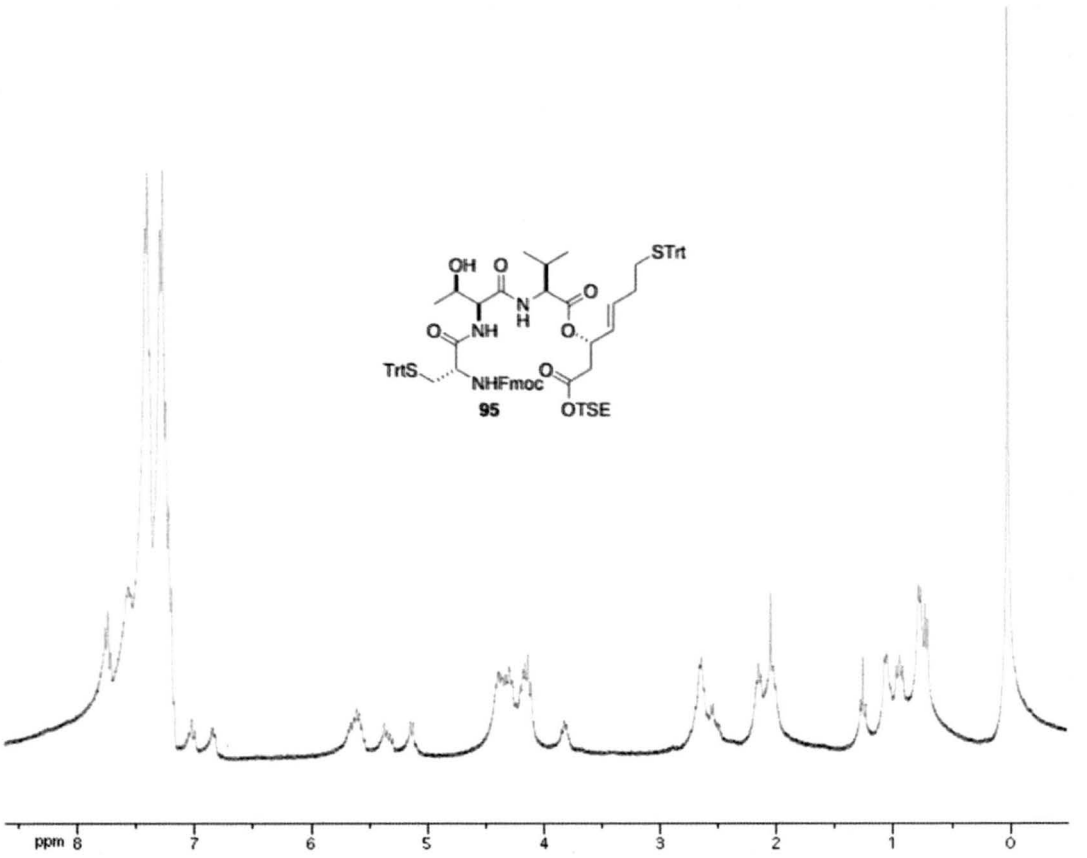


Formation of (5*R*,8*S*,11*S*)-((*S*,*E*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)-7-(tritylthio)hept-4-en-3-yl) 1-(9*H*-fluoren-9-yl)-8-((*R*)-1-hydroxyethyl)-12-methyl-3,6,9-trioxo-5-(tritylthiomethyl)-2-oxa-4,7,10-triazatridecane-11-carboxylate (112)

Compound **111** (40 mg, 0.045 mmol) was dissolved in CH_3CN (2.25 mL) followed by Et_2NH (0.225 mL). Reaction was monitored by TLC till all starting material was gone, then concentrated and re-diluted in EtOAc and concentrated again to remove all the excess Et_2NH.

STrt-NFmoc-D-Cys (29 mg, 0.049 mmol) was dissolved in CH_2Cl_2 (0.81 mL) followed by PyBop (48 mg, 0.09 mmol) and iPr$_2$NEt (24 μL) and allowed to stir for 5 min. Then the crude amide dissolved in CH_3CN (0.41 mL) was added and allowed to stir for about 2 hours. The reaction mixture was concentrated and subjected to column chromatography. ^{1}H NMR (300 MHz $CDCl_3$) δ 0.01(s, 9H), 0.75 (m, 6H), 0.95 (t, 3 H, J= 8.4), 1.08 (m, 3 H), 1.26 (t, 1 H, J=6.9), 2.0 (m, 3 H), 2.15 (m, 2 H), 2.53 (m, 2 H), 2.65 (m, 2 H), 3.8 (m, 1 H), 4.12 (m, 3 H), 4.35 (m, 5 H), 5.13 (d, 1 H, J= 7.5), 5.35(m, 1 H), 5.6 (m, 2 H), 7.0 (m, 1 H), 7.5 (m, 38 H).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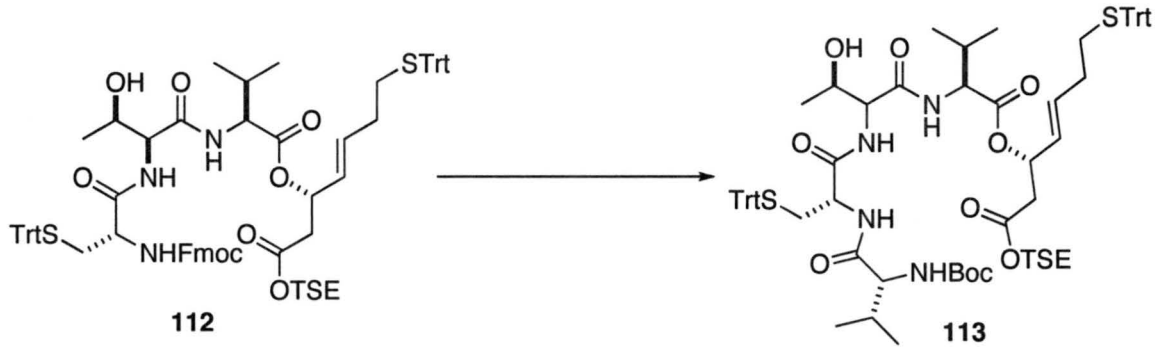
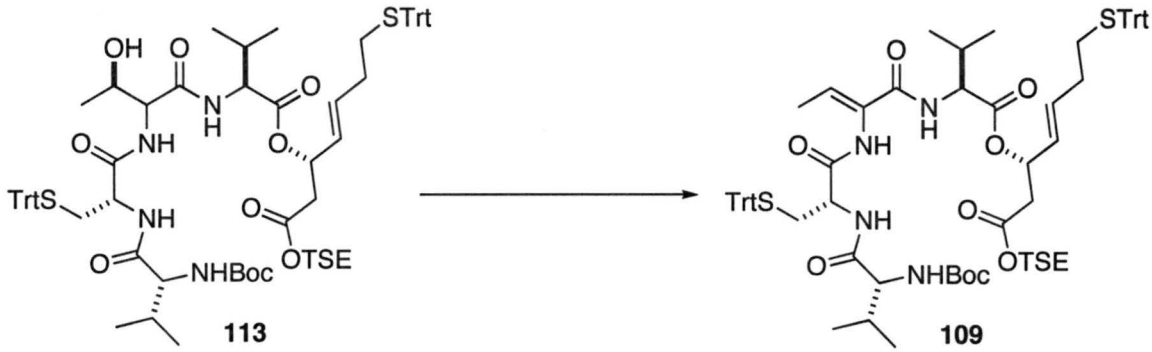


Formation of, (6*R*,9*S*,15*S*)-((*S*,*E*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)-7-(tritylthio)hept-4-en-3-yl) 12-((*R*)-1-hydroxyethyl)-6-isopropyl-2,2,16-trimethyl-4,7,10,13-tetraoxo-9-(tritylthiomethyl)-3-oxa-5,8,11,14-tetraazaheptadecane-15-carboxylate (113)

Compound 112 (72 mg, 0.056 mmol) was dissolved in CH_3CN (2.8 mL) followed by Et_2NH (0.28 mL). The reaction was monitored by TLC till all starting material was gone, then concentrated and re-diluted in EtOAc and concentrated again to remove all the excess Et_2NH.

NBoc-D-Val (13.5 mg, 0.0619 mmol) was dissolved in CH_2Cl_2 (1.03 mL) followed by PyBop (58 mg, 0.09 mmol) and iPr$_2$NEt (15 μL) and allowed to stir for 5 min. Then the crude amide dissolved in CH_3CN (0.512 mL) was added and allowed to stir for about 2 hours. The reaction mixture was concentrated and subjected to column chromatography.



Attempted formation of (6*R*,9*S*,15*S*,*Z*)-((*S*,*E*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)-7-(tritylthio)hept-4-en-3-yl) 12-ethylidene-6-isopropyl-2,2,16-trimethyl-4,7,10,13-tetraoxo-9-(tritylthiomethyl)-3-oxa-5,8,11,14-tetraazaheptadecane-15-carboxylate (109)

To a solution of 113 in pyridine was added tosylanhydride at 0°C for 40 min, then quenched with Sat. *aq*. $NaHCO_3$. This was then extracted with EtOAc, and washed with 1M HCl six times. The organic layer was dried with Na_2SO_4, filtered and concentrated. The crude product was disolved CH_3CN was added DABCO at room temperature. This was stirred for 18 hours, then concentrated and rediluted with EtOAc and 1 M aqueous HCl. Aqueous layer was extracted with EtOAc, and the combined organic layer was washed with brine, dried over Na_2SO_4 , filtered and concentrated. Unfortunately decomposition was observed.

Azumamide side chain synthesis was accomplished by following Ganesan's synthesis

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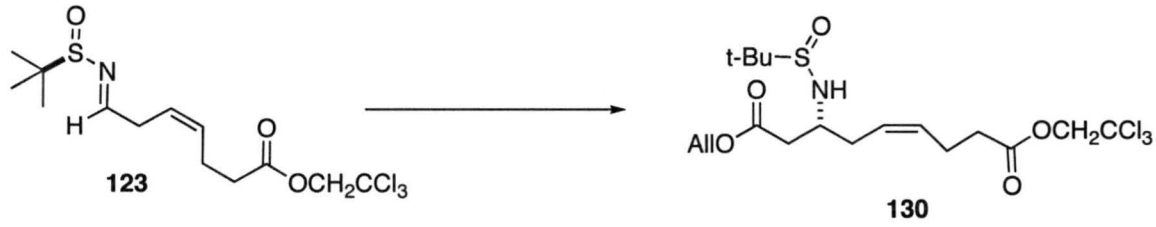


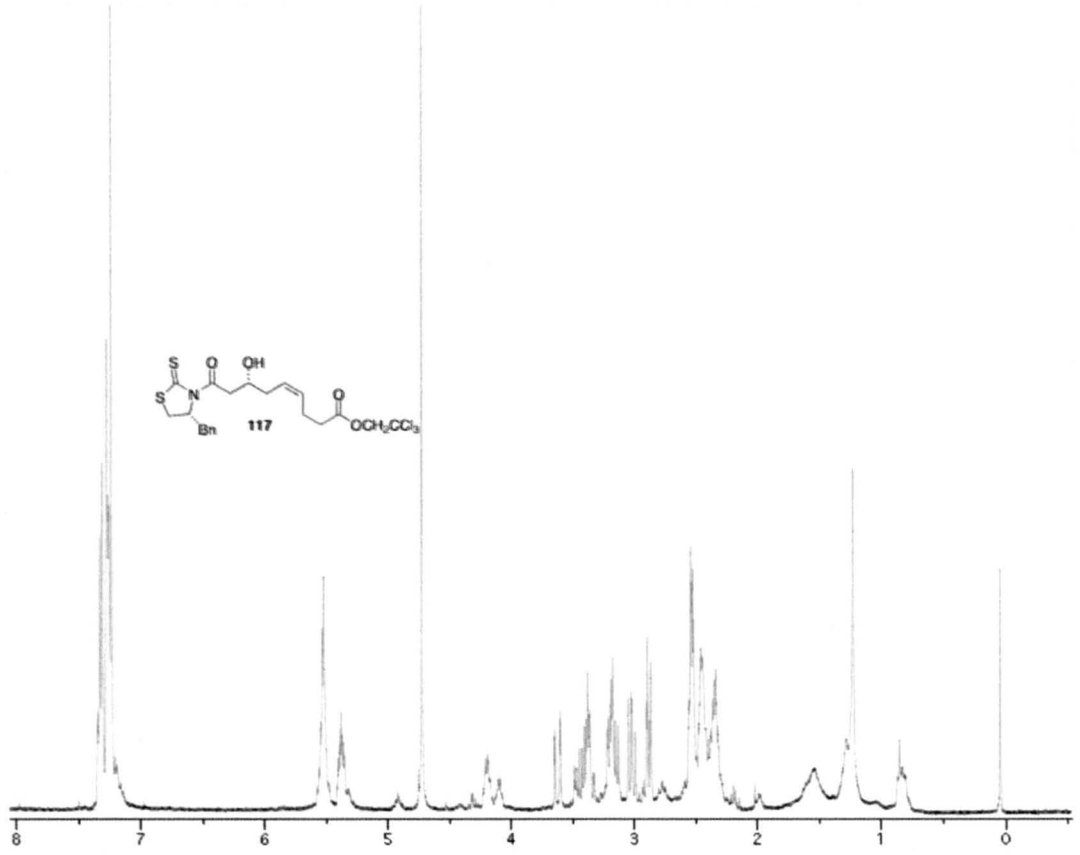
Attempted formation of (7*R*,*Z*)-9-allyl 1-(2,2,2-trichloroethyl) 7-(1,1-dimethylethylsulfinamido)non-4-enedioate 130

A solution of diisopropylamine (0.25 mL) in THF (5 mL) was cooled to 0°, then n-BuLi (2.2 mL of 1.6 M) was added and stirred for 30 minutes. The solution was then cooled to -78° and a solution of allyl acetate (0.16 mL, 1.49 mmol) in THF (1.5 mL) was added and stirred for 30 minutes.

A solution of $TiCl_4$ and $TiCl(o\text{-}Pr)_3$ was made by taking $TiCl(o\text{-}Pr)_3$ (2.98 mmol, 0.776 g) in THF (2.98) and $TiCl_4$ (0.299 mmol, 30μL) in an ice bath and stirred for 20 min. Then the LDA solution was slowly added, and stirred for 60 min.

A solution of **106** (0.088 mmol, 0.05g) in THF (0.14 mL) was slowly added to about 2 mL of the LDA solution. This was allowed to stir for 3.5 hours, then quenche with Sat. aq. NH_4Cl and extracted with EtOAc. The combined organic layers were washed with Sat. aq. NH_4Cl, and brine, then dried with Na_2SO_4 and concentrated. Only decomposition was seen.

(*R*,*Z*)-2,2,2-trichloroethyl 9-((*R*)-4-benzyl-2-thioxothiazolidin-3-yl)-7-hydroxy-9-oxonon-4-enoate. 139 A solution of the chiral auxiliary (887mg, 3.53 mmol) in CH_2Cl_2 (28.5 mL) was cooled to 0°C, followed by addition of $TiCl_4$ (0.47 mL, 4.39 mmol). The reaction was allowed to stir for 5 minutes then cooled to -78°C, before *i*Pr_2NEt (0.76 mL, 4.37 mmol) were slowly added and stirred for 2 hours. The aldehyde was dissolved in CH_2Cl_2 (2.2 mL) and added drop wise to the auxiliary solution, then stirred for 1.5 hours. The reaction was quenched with saturated *aq* NH_4Cl, and diluted with CH_2Cl_2, and warmed to room temperature. The reaction was extracted with CH_2Cl_2, then washed with brine and dried over Na_2SO_4, filtered and condensed. Purification was accomplished with silica gel chromatography (30% EtOAc/Hex) to afford yellow oil. ^1H ^1H NMR (400 MHz $CDCl_3$) δ 0.83 (m, 1H), 1.23 (s, 1H), 2.35 (m, 2 H), 2.45 (m, 2H), 2.53 (m, 2H), 2.88 (dd, 3.2, 11.6 Hz, 1 H), 3.02 (dd, 10.4, 13.2 Hz, 1 H), 3.17 (m, 2 H), 3.42 (m, 2 H), 3.62 (dd, 2.8, 17.6 Hz, 1 H) 4.15 (m, 2 H), 4.73 (s, 2H), 5.37 (m, 1 H), 5.52 (m, 2 H), 7.30 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$) d 22.8, 29.8, 32.2, 33.8, 34.3, 34.5, 36.9, 45.1, 45.5, 67.7, 68.2, 68.4, 68.5, 95.1, 126.8, 127.4, 129.1, 129.6, 130.5, 136.5, 169.1, 171.6, 173.1, 173.7. $[a]_D$ = -74.2 (c 2, $CHCl_3$)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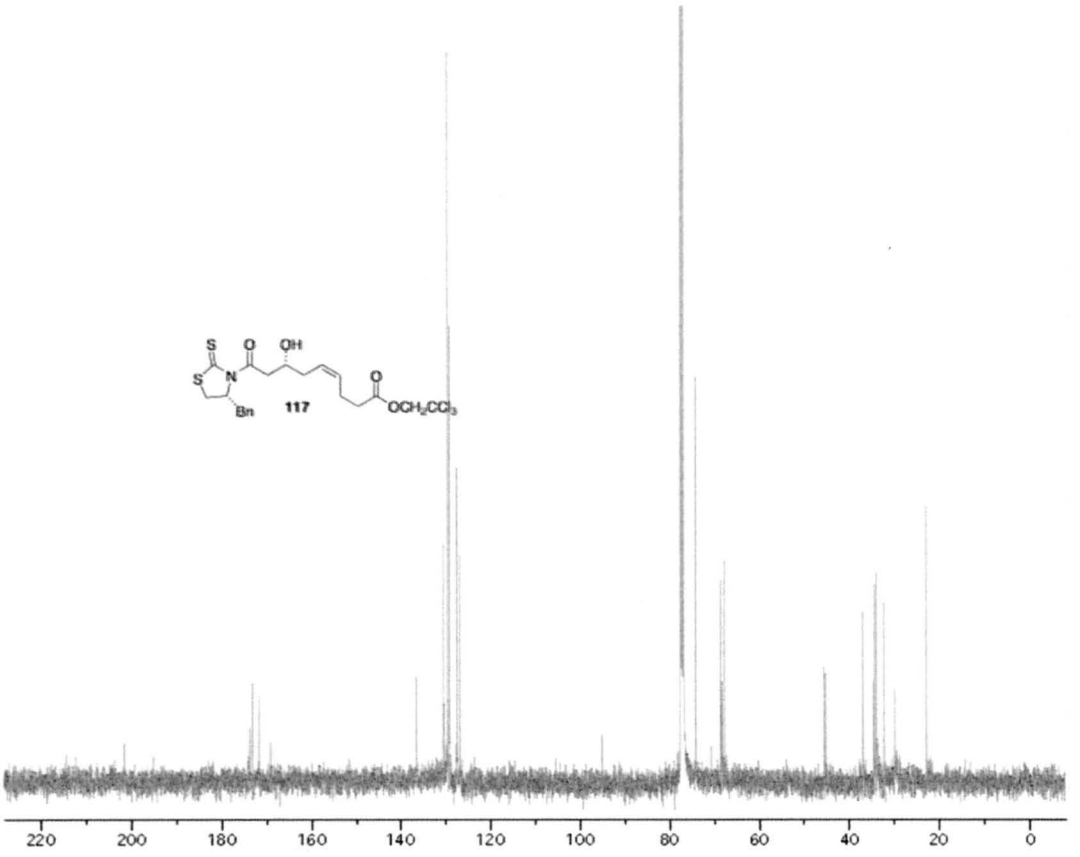
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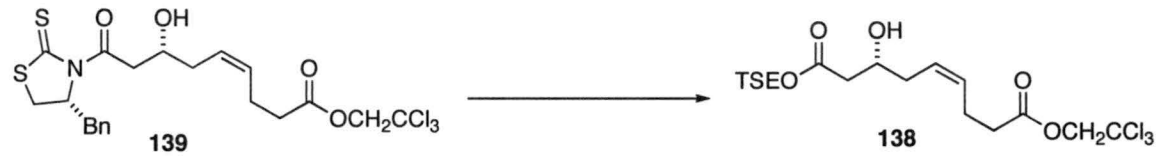
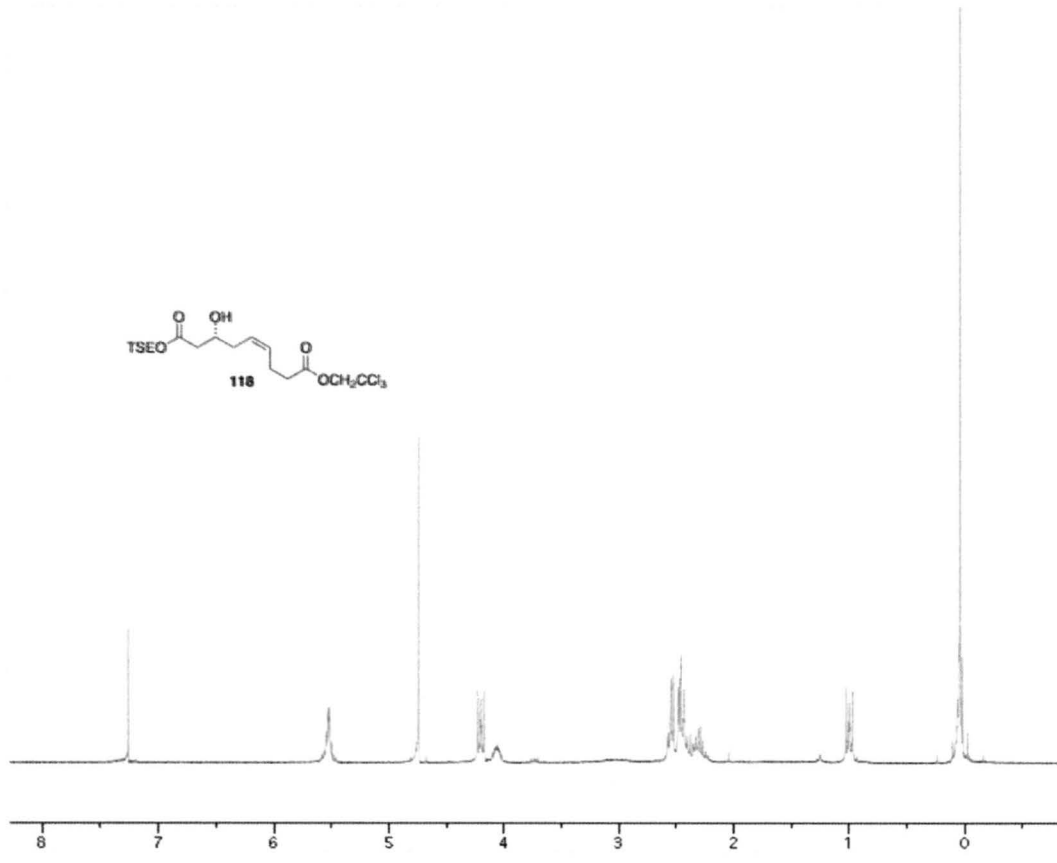


(*R*,*Z*)-1-(2,2,2-trichloroethyl) 9-(2-(trimethylsilyl)ethyl) 7-hydroxynon-4-enedioate. The alcohol (764 mg, 1.45 mmol) dissolved in CH_2Cl_2 (2.9 mL) was treated with imidazole (148mg, 2.175 mmol) followed by the addition of TMS Ethanol (2.08 mL, 14.5 mmol). The reaction was stirred overnight, then condensed and purified by silica gel chromatography (30% EtOAc/ Hex) to give the protected β-hydroxy acid as yellow oil. ^{1}H NMR (300 MHz $CDCl_3$) δ 0.04 (s, 9 H), 0.99 (m, 2H), 2.30 (m, 2 H), 2.45 (m, 4 H), 2.54 (m, 2 H), 4.06 (m, 1H), 4.20 (m, 2 H), 4.73 (s, 2H), 5.52 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) d -.127, 17.5, 22.8, 33.8, 34.5, 41.0,63.3, 67.9, 74.1, 95.1, 126.8, 130.3 171.7, 173.2. $[a]_D$ = -1.7 (c 2, $CHCl_3$)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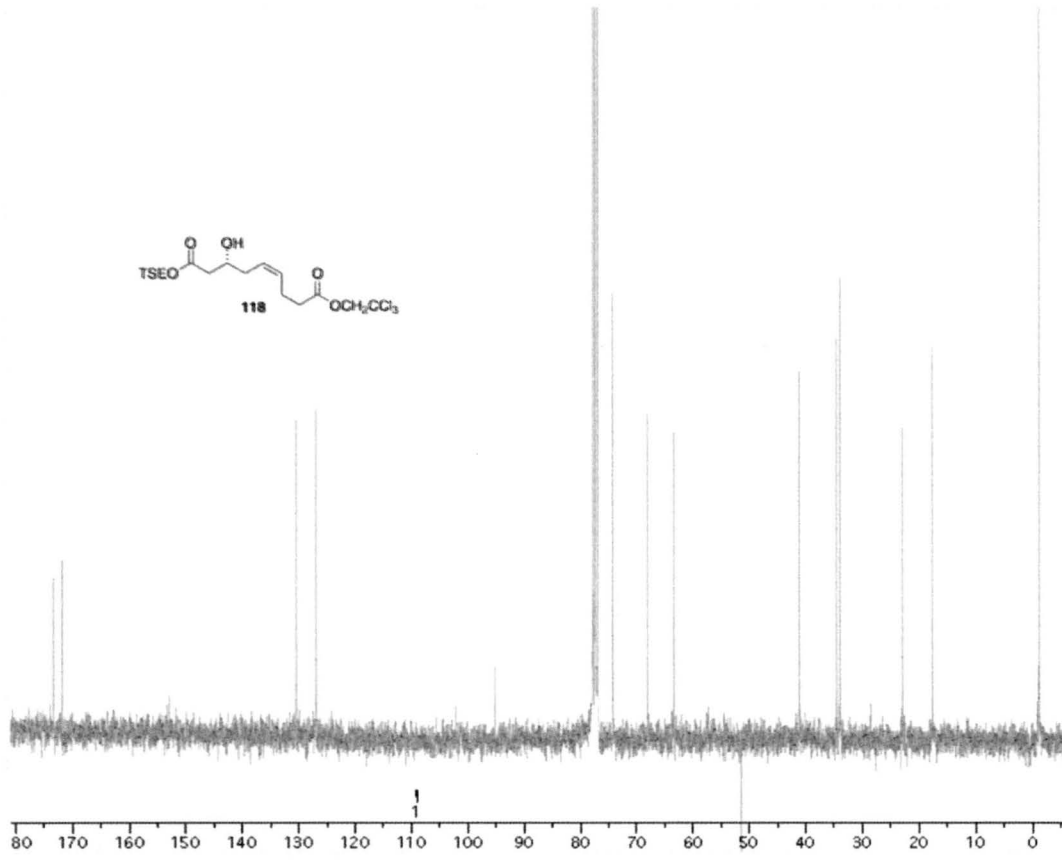
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118
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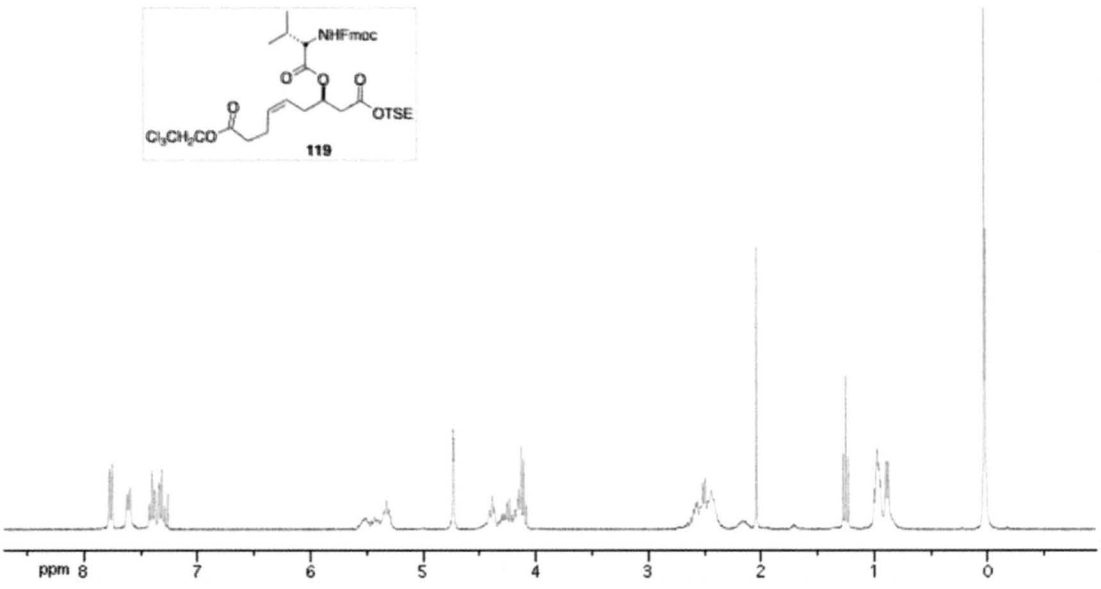
(*R*,*Z*)-1-(2,2,2-trichloroethyl) 9-(2-(trimethylsilyl)ethyl) 7-((*S*)-2-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3-methylbutanoyloxy)non-4-enedioate. The protected acid (97mg, 0.224 mmol) and N-Fmoc-L-Val (380mg, 1.123 mmol) were dissolved in CH_2Cl_2 (4.08 mL) and cooled to 0°C. EDCI (258 mg, 1.347 mmol) and DMAP (2.7mg, 0.0225 mmol) were dissolved in CH_2Cl_2 (1.02 mL) and added to the cooled reaction followed by the slow addition of iPr$_2$NEt (0.23 mL, 1.347 mmol). The reaction was allowed to warm to room temperature and stirred over night, then condensed and purified with column chromatography (30% EtOAc/ Hex) ^{1}H NMR (300 MHz CDCl$_3$) δ 0.03 (s, 9 H), 0.88 (d, 3 H), 0.97 (m, 5 H) 2.15 (m, 1 H), 2.43 (m, 3 H), 2.50 (d, 2 H), 2.59 (m, 3 H), 4.25 (m, 3 H), 4.38 (m, 2 H), 4.73 (s, 2H), 5.32 (m, 2 H) 5.43, (m, 1 H), 5.53 (m, 1 H), 7.35 (m, 4 H), 7.60 (d, 2 H), 7.76 (d, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) d - 1.27, 17.5, 19.2, 22.8, 31.4, 31.7, 33.7, 38.7, 47.4, 59.1, 63.3, 67.2, 71.5, 71.6, 74.1, 95.1, 120.2, 125.1, 125.3, 127.2, 127.9, 131.1, 131.2, 141.5, 143.9, 144.1, 156.3, 156.4. (M+Na$^=$) calcd for $C_{36}H_{46}Cl_3NO_8Si$ 753.20627, found 776.19549. $[a]_D$ = 0.0 (c 2, $CHCl_3$)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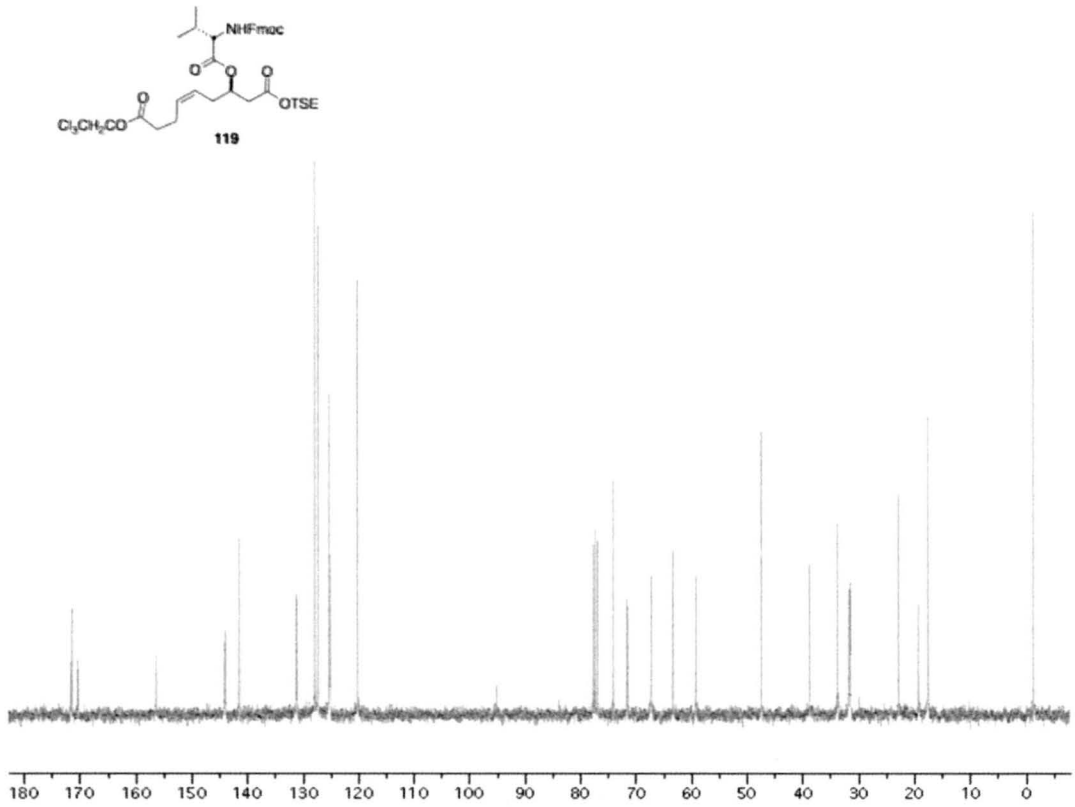
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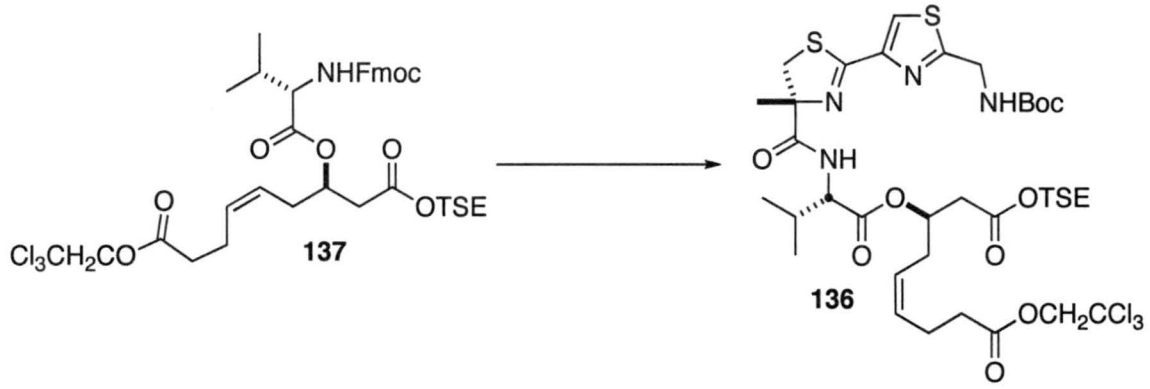
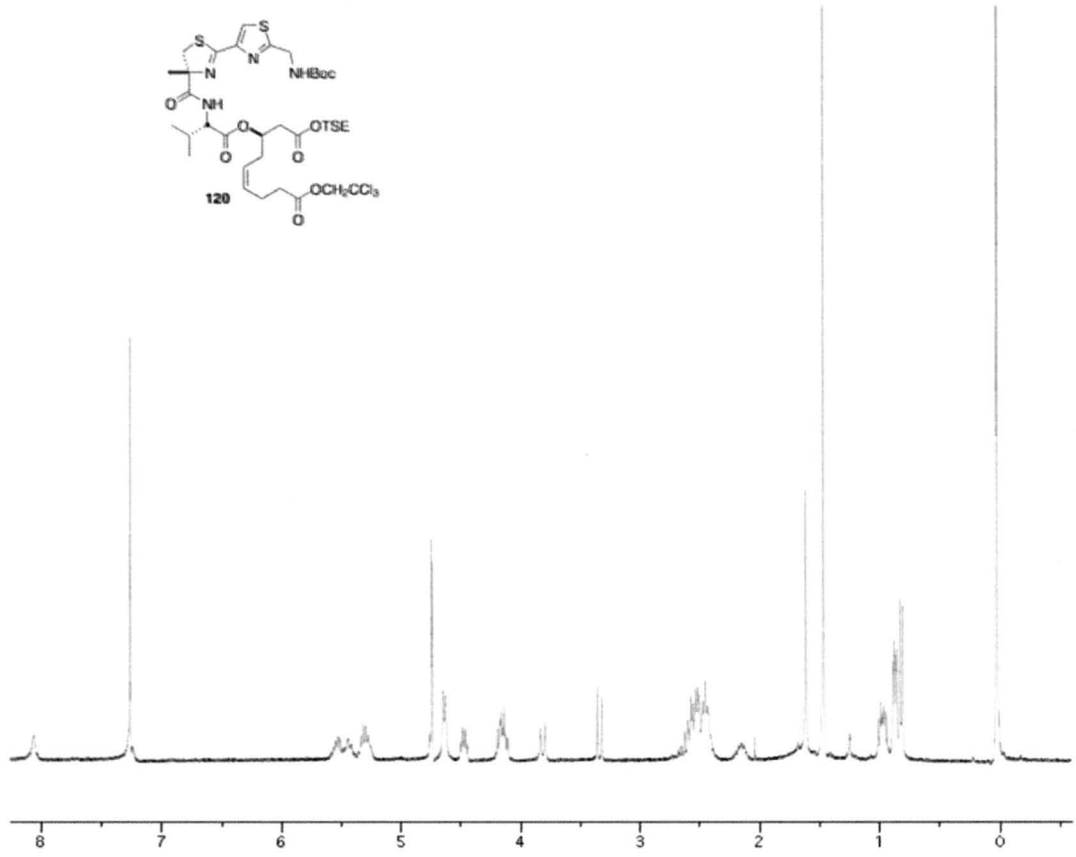
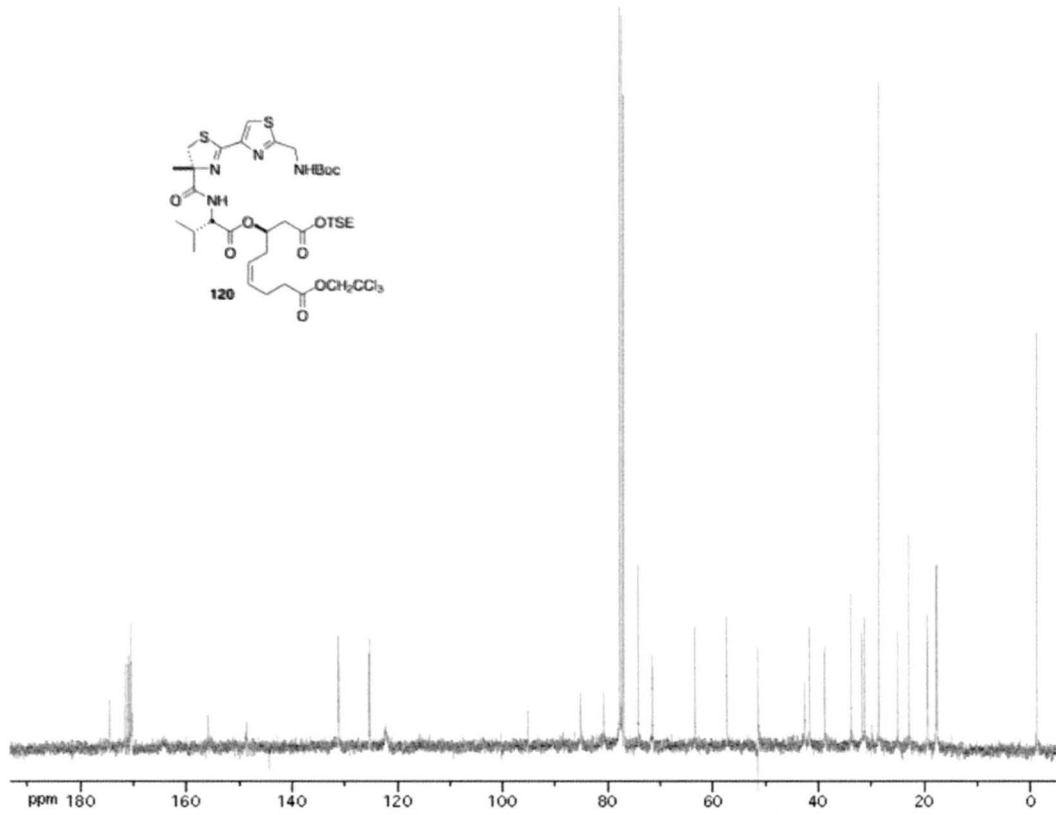


(*R*,*Z*)-1-(2,2,2-trichloroethyl) 9-(2-(trimethylsilyl)ethyl) 7-((*S*)-2-((*R*)-2-(2-((*tert*-butoxycarbonylamino)methyl)thiazol-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxamido)-3-methylbutanoyloxy)non-4-enedioate.

The protected amine (118mg 0.157 mmol) was dissolved in CH_2Cl_2 (7.85 mL) and treated with Et_2NH, then allowed to stir for 2 hrs. The reaction was concentrated, then taken up in EtOAc and re-concentrated again to remove any left over Et_2NH. The thiazolinethiazole (61.7 mg, 0.172 mmol) and PyBOP (164mg, .471 mmol) were dissolved in CH_2Cl_2 (2.87 mL) and treated with $i Pr_2NEt$ and allowed to stir for 5 minutes. Then the crude amine in CH_3CN (1.42 mL) was slowly added to the PyBOP solution and allowed to stir overnight. The reaction was then condensed and purified with column chromatography (30-50% EtOAc/ Hex). ^1H NMR (300 MHz $CDCl_3$) δ 0.03 (s, 9 H), 0.82 (d, *J*= 3 H), 0.88 (m, 3 H), 0.97 (m, 2 H), 1.47 (s, 9 H), 1.62 (s, 3 H), 2.15 (m, 1 H), 2.45 (m, 3 H), 2.57 (m, 5 H), 3.34 (d, *J*= 11.4 1 H), 3.81 (d, *J*= 11.4, 1 H), 4.15 (m, 2 H), 4.48 (m, 1H), 4.63 (d, *J*= 6.3 Hz, 2 H), 4.74 (s, 2 H), 5.31 (m, 2 H), 5.43 (m, 1 H), 5.54 (m, 1 H), 8.06 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) d -1.3, 17.5, 17.7, 19.3, 22.8, 24.9, 28.5, 31.2, 33.7, 38.7, 38.8, 41.6, 42.6, 51.4, 57.3, 63.3, 71.5, 74.1, 80.7, 85.0, 95.1, 125.1, 125.3, 131.0, 131.1, 148.5, 155.9, 170.3, 170.4, 170.8, 170.9, 171.4, 171.5, 174.4.

(M+H^{-}) calcd for $C_{35}H_{53}Cl_3N_4O_8S_2Si$ 870.21108, found 871.21836. $[a]_D$ = -19.86 (c 2, $CHCl_3$)

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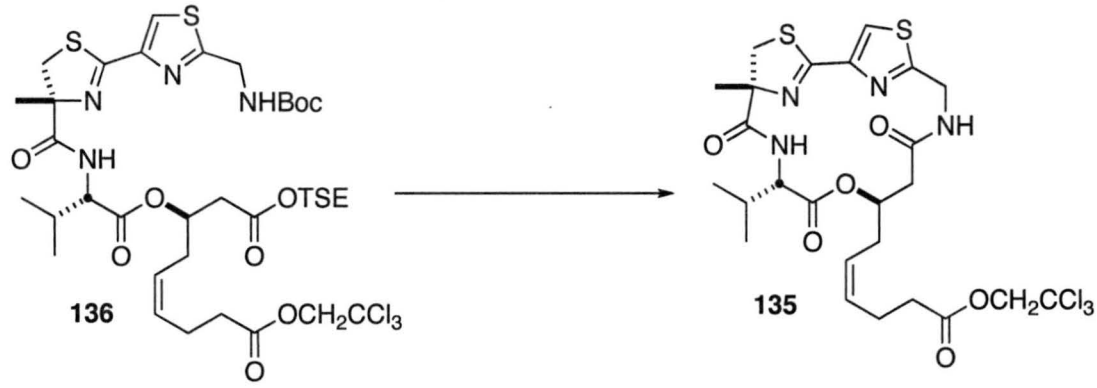
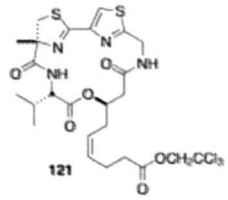


Formation of the Trichloroethyl ester protected Largazole Azumamide hybrid. The acyclic precursor (77mg, 0.088 mmol) was dissolved in CH_2Cl_2 (2.95 mL) and cooled to 0°C. TFA (0.15 mL) was slowly added to the cooled solution. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was then condensed, and re-dissolved in toluene and condensed again to remove any excess TFA. The crude amino acid was dissolved in CH_2Cl_2 (4.4 mL) then cooled to 0°C and treated with *i*Pr_2NEt (0.093mL, 0.53 mmol) and stirred for 30 min. In a separate flask HOBt (23 mg, 0.177 mmol), HATU (67 mg, 0.177 mmol) were dissolved in CH_3CN (88.7 mL) and treated with *i*Pr_2NEt (0.093 mL, 0.53 mmol). The crude amino acid solution was then added via syringe pump addition to the HATU solution in a 10-hour addition. The reaction was allowed to stir for an additional 6 hours before solvents were removed and purified with silica gel chromatography (30%-50% EtOAc/ Hex). ^1H NMR (300 MHz $CDCl_3$) δ 0. 47 (d, *J*= 6.9 Hz, 3 H), 0.69 (d, *J*= 8.7 Hz, 3 H), 1.24 (s, 1H), 1.87 (s, 3 H), 2.13 (m, 1 H), 2.43 (m, 3 H), 2.52 (d, *J*= 6.6 Hz, 2 H), 2.72 (m, 4 H), 3.29 (d, *J*= 12.3 Hz, 1 H), 4.05 (d, *J*= 11.4 Hz, 1 H), 4.27 (dd, *J*= 3, 17.7 Hz, 1 H), 4.64 (dd, *J*= 3, 9.3 Hz, 1 H), 4.75 (s, 2 H), 5.25 (m, 2 H), 5.40 (m, 1 H), 5.53 (m, 1 H), 6.33 (m, 1 H), 7.10 (d, *J*= 9.3 Hz, 1 H), 7.78 (s, 1 H) ^{13}C NMR (75 MHz, $CDCl_3$) d 14.4, 16.5, 18.5, 18.7, 19.3, 22.8, 24.1, 24.9, 29.4, 31.2, 32.7, 33.7, 33.9, 34.3, 35.5, 39.2, 41.2, 43.4, 43.7, 57.8, 59.7,

60.6, 72.5, 74.1, 83.8, 95.1, 110.5, 118.4, 124.9, 125.1, 127.3, 131.2, 146.8, 167.0, 168.7, 169.5, 170.6, 171.5, 173.2. $[a]_D = -0.8$



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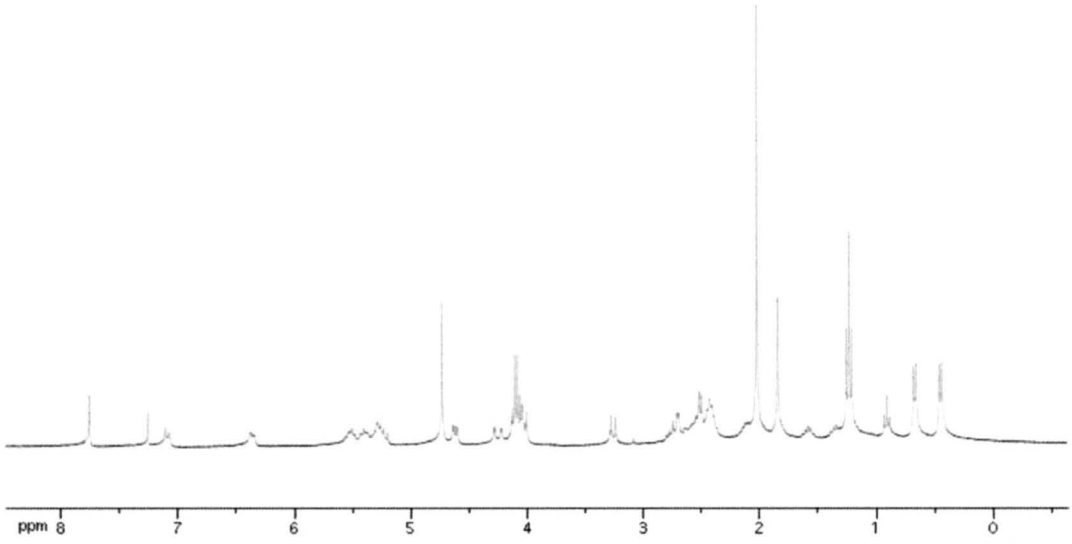

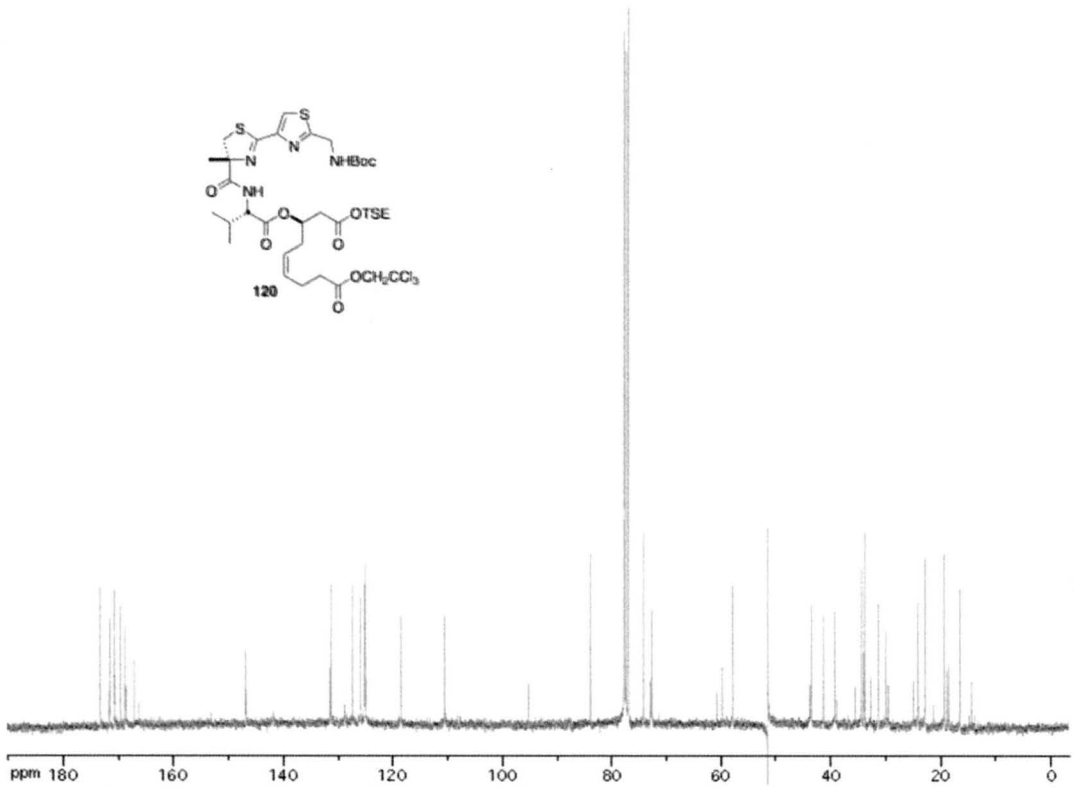
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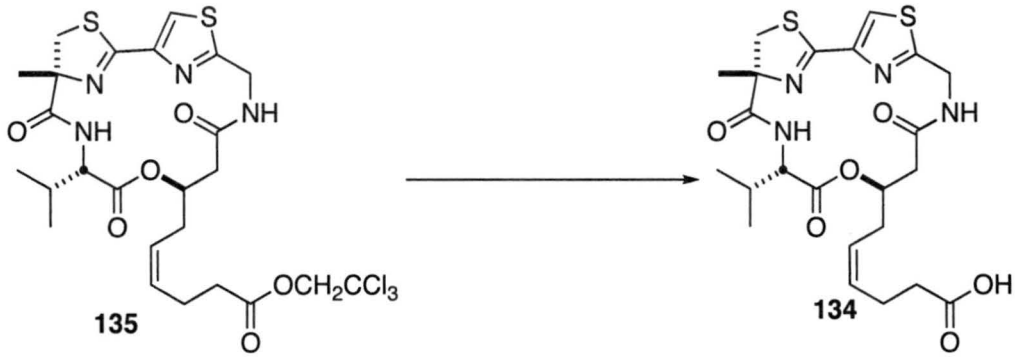
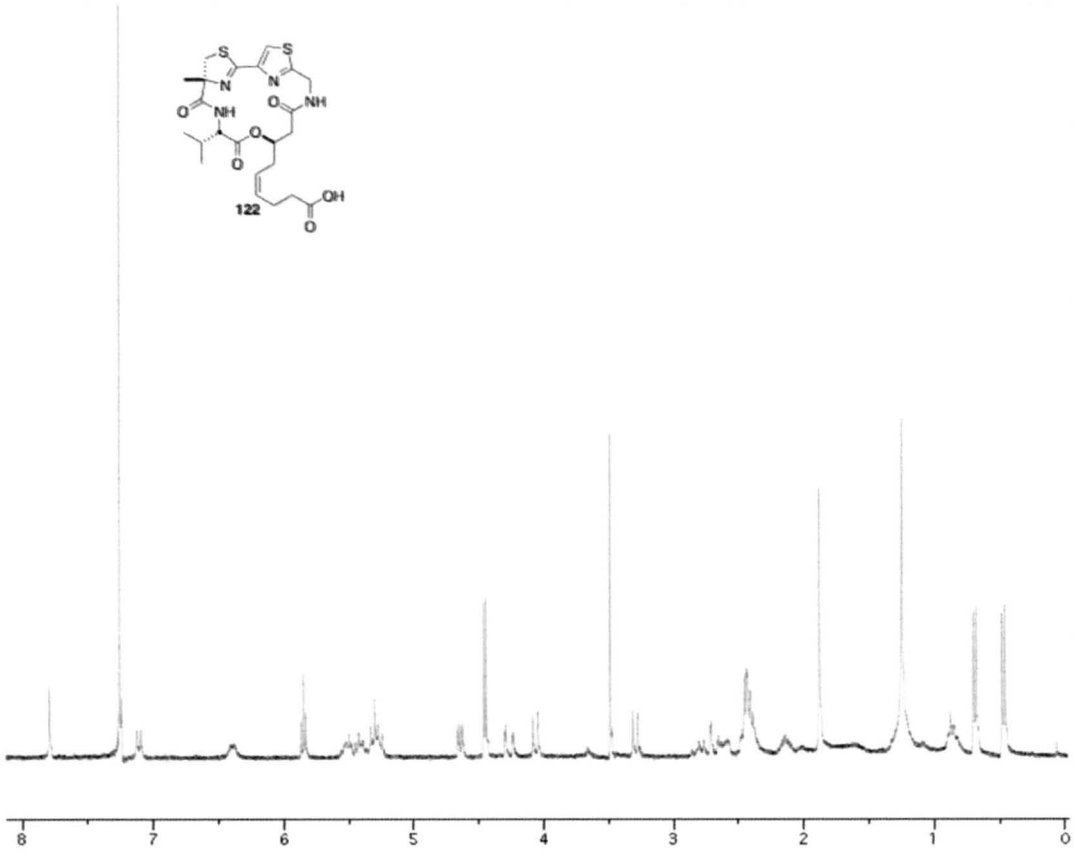


Largazole Auzumamide E Hybrid

The macrocycle (40) (9.8 mg, 0.015 mmol) was dissolved in dry THF (0.5 mL, 0.03M) and vigorously stirred. Then Zn dust (35mg, 36 mmol) was added to the solution followed by 1M NH_4OAc (0.083 mL, .18M) and allowed to stir for 24 hours under argon. The reaction was then filtered and taken up in EtOAc and washed with 5% aq $KHSO_4$ (2x 2 mL) and Brine (2x 2 mL) then dried over Na_2SO_4, filtered and solvents removed. Purification by PTLC (MeOH / CH_2Cl_2 10%). ^1H NMR (300 MHz $CDCl_3$) δ 0.47 (d, *J*= 6.9 Hz, 3 H), 0.69 (d, *J*= 6.9 Hz, 3 H), 0.85 (m, 2 H), 1.88 (s, 3 H), 2.14 (m, 1 H), 2.41 (m, 5 H), 2.71 (m, 4 H), 3.29 (d, *J*= 11.4 Hz, 1 H), 4.06 (d, *J*=11.4 Hz, 1 H), 4.27 (dd, *J*= 3.3, 17.4 Hz, 1 H), 4.45 (d, *J*= 6.0 Hz, 2 H), 4.64 (dd, *J*= 3.0, 9.3 Hz, 1 H), 5.29 (m, 2 H), 5.42 (m, 1H), 5.51 (m, 1 H), 5.85 (t, *J*= 6.0 Hz, 1 H), 6.40 (m, 1 H), 7.11 (d, *J*= 9.3 Hz, 1 H), 7.79 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) d 14.4, 16.6, 19.2, 21.8, 24.2, 29.9, 31.1, 33.7, 39.3, 41.3, 43.5, 57.8, 60.6, 68.4, 68.6, 72.4, 124.8, 131.4, 168.2, 169.6, 170.2, 172.1, 173.6. ($M-H^=$) calcd for $C_{23}H_{30}N_4O_6S_2$ 521.1534, found 521.15292. $[a]_D$ = +10.107 (c2,$CHCl_3$)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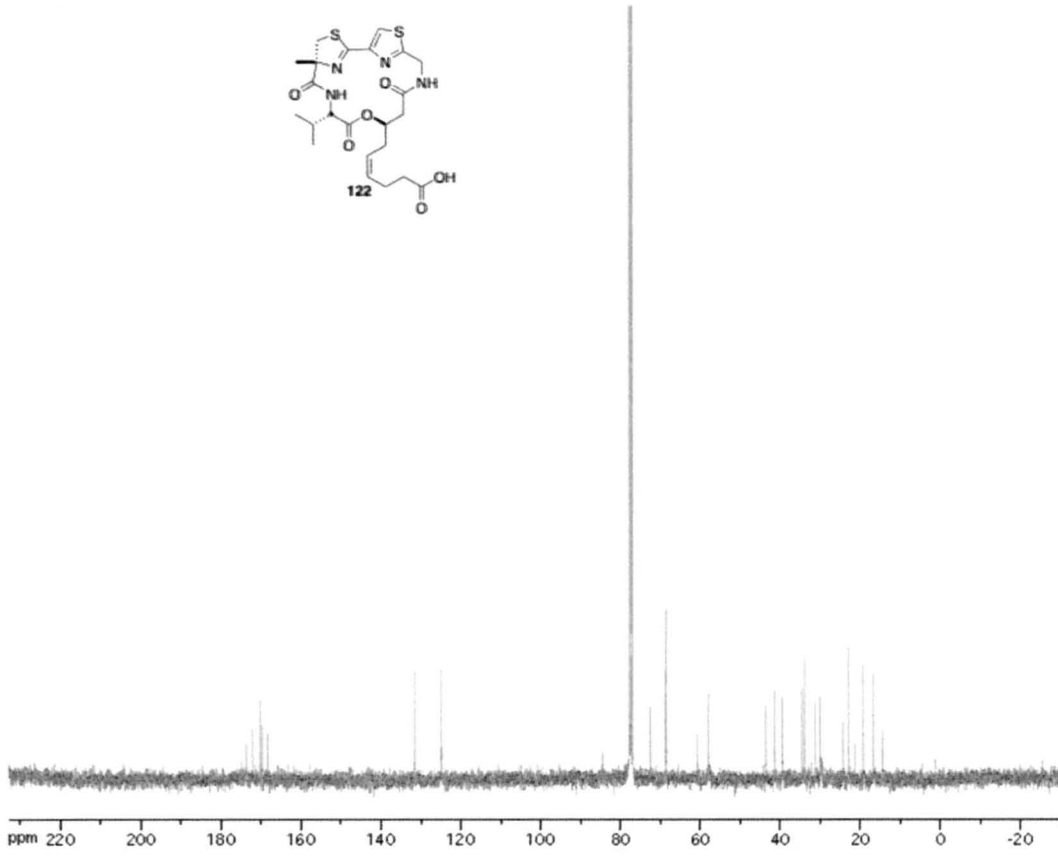
122
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