SYNTHESIS OF THE 16-MEMBERED BCD RING OF COMPLESTATIN AND

HALOGENATION OF THE TRYPTOPHAN INDOLE

BY

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ABSTRACT OF THE THESIS

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THESIS DIRECTOR:

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Complestatin is a natural product that has demonstrated inhibition activity against HIV-1 integrase and initiation of the complement pathway. Though its biological activity is proven, the total synthesis of the molecule is challenging in many ways.

In this thesis the synthetic strategy taken for the partial synthesis of the BCD ring fragment of Complestatin is described. Part of the synthetic studies on the BCD ring involves using the mild conditions to limit isomerization of the sensitive phenylglycine subunits. This was achieved by using DEPBT and HATU as coupling reagents that limit epimerization during condensation.

Oxidative phenol coupling is used to form a biaryl ether bond between the L-tyrosine and 4-hydroxyphenylglycine subunits to synthesize the BCD ring. Previous syntheses to form this bond require the functionalization of the subunits. It is our goal to limit the synthesis by decreasing the amount to steps necessary to form the BCD ring, which is accomplished using this method. Our synthesis of the BCD ring is 6 steps from 4-hydroxyphenylglycine, with approximately 10% overall yield.

The investigation into the synthesis of Complestatin evolves around the synthesis of 6-bromotryptophan. In order to limit the amount of steps in the total synthesis, the project for the facile synthesis of 6-bromotryptophan was undertaken. It was found that the Boc protecting group on the amino group provided efficient protection of the 2-position on the indole, leading to the formation of   5-bromotryptophan.

The synthesis of the TMC-95A southern fragment was also accomplished through a series of peptide couplings.

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I would like to thank Ahalya from Jimenez's group for all the help. Our jokes and fun moments have lightened many stressful days.

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LIST OF ABBREVIATIONS

^{o}C degree celcius

$AgNO_3$ silver nitrate

$AgBF_4$ silver tetraborofluorate

$AgCO_3$ silver carbonate

Boc   tert-butoxycarbonyl

DCM   dichloromethane

DMF   N,N-dimethylformamide

DMSO   dimethyl sulfoxide

EDC.HCl   1-Ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride

Et   ethyl

EtOAc   ethyl acetate

HOBt   1-hydroxy-benzotriazole

Hz   hertz

m/z   mass to charge ratio

Me   methyl

MeOH   methanol

min	minutes
ml	milliliter
Mol	moles
NBS	N-bromosuccinimide
NMR	Nuclear Magnetic Resonance
Pd/C	palladium on carbon
Phg	phenylglycine
ppm	parts per million
^{t}BuOH	tert-butyl alcohol
^{t}BuBr	tert-butyl bromide
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran

CHAPTER 1:

COMPLESTATIN BCD RING

1.1 Introduction

The immune system is a highly developed system designed to protect us from disease causing agents such as, viruses and bacteria. Generally, products isolated from bacteria would elicit an immunological response. However, the natural product complestatin, isolated from *Streptomyces lavendulae* species actually inhibits an immune response, by the binding of an alternative complement protein, hence the name complestatin (**1**)[1,2].

Furthermore, **1** has been found to be an inhibitor of HIV gp120-CD4 receptor binding, which is an essential reaction in the entry of the HIV-1 virus into cells. [2,3,4] It was found that **1** primarily interacts with cells and inhibits viral adsorption and adsorption of affected cells to adjacent cells. [2] The use of **1** in combination with other HIV-1 drugs i.e., AZT, ddI, ddC, and nevirapine, can prove to be a potent cocktail in the fight against the spread of HIV. [2,5]

Complestatin (**1**) was first isolated by Kaneko *et al*. from *Streptomyces lavendulae*. [1] Singh *et al.* in an isolation of complestatin identified chloropeptin,[3,6] and complestatin A and B, all of which are biologically active macrocyclic polypeptides (fig. 1, table 1). They have been shown to inhibit the viral spread of HIV-1 (cytopathic effect), as well as binding to HIV- 1 integrase either when DNA is coupled to protein or in solution during strand transfer. These bicyclo hexapeptides are comprised entirely of aromatic peptides; phenylglycine, tyrosine, and tryptophan. The difference between these natural products occurs in the DEF ring at the tryptophan moiety.
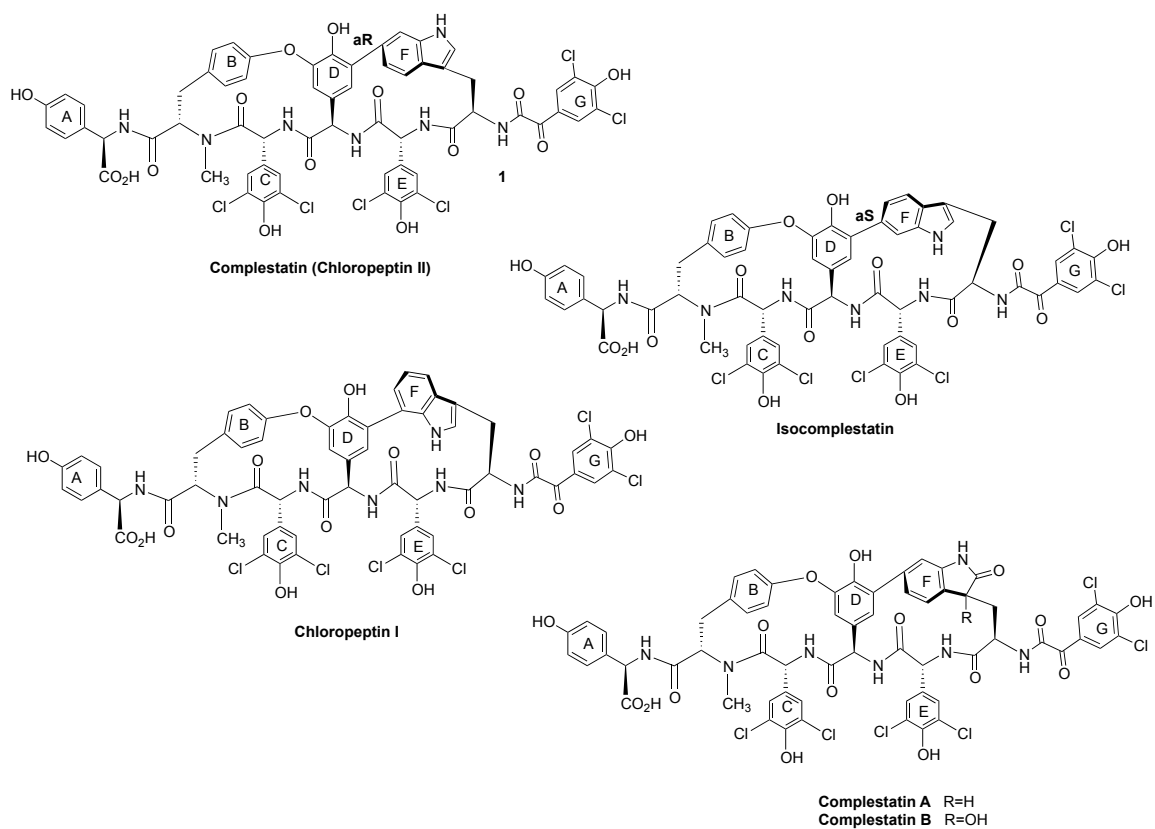
Fig. 1 Complestatin and other isolated products

		HIV-I cytopathic effect	gp120-CD4 binding	HIV-I Integrase	
	Complement Inhibitor			coupled	strand transfer
Complestatin	.5-2	1.7	2	0.2	4
Chloropeptin I		1.6	1.3	0.4	5
Complestatin A				0.8	12.5
Complestatin B				1.7	12.5

Table 1: Biological Activites of Complestatin Family of IC_{50} in μM ranges.[1,3,6]

The BCD ring in the complestatin family remains a constant; we have decided to investigate the most efficient synthesis for this ring fragment. It was found that the bicyclic pentapeptide is the pharmacophore necessary to achieve biological activity. [2,4,6] Work done by Smith *et al.* have determined that **1** binding with the CD4 receptor is assisted by multiple hydrogen-bonding through the phenolic oxygen's. Also, it was found that the Phe-43 side chain of CD4, most likely rotates into the hydrophobic pocket created by the diaryl ether BCD ring, thus increasing **1**'s binding affinity. [4]

1.2 Previous Synthesis

1.2.1 Ullmann Biaryl Ether Synthesis

Hoveyda *et al.* was the first group to attempt a total synthesis of complestatin. It was their synthetic exploration of **1** that led to the correct designation of the atropisomers in **1** and isocomplestatin [7]. The synthesis of the BCD ring was accomplished by a Cu-mediated biaryl ether synthesis. The synthetic route by Hoveyda is outlined in Scheme **1** of the biaryl ether. Hoveyda *et al.* found that using typical Cu conditions only afforded a 15-20% yield. This was solved by the use of excess MeOH to obtain 98% conversion of **7**. [8] The overall yield of BCD ring was 27% after 8 linear steps from **2**. [8]
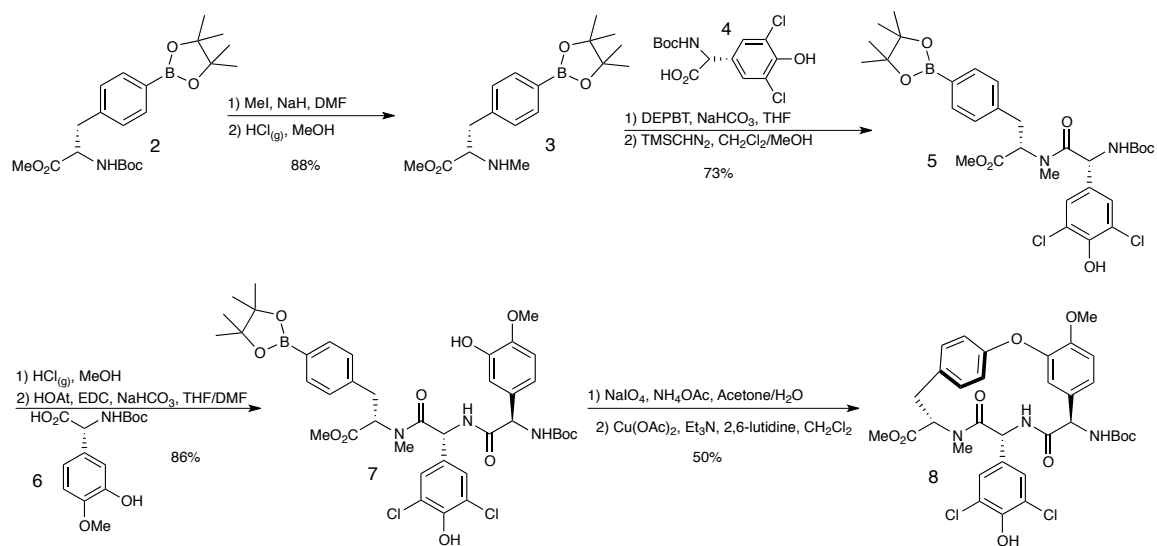
Scheme 1: Hoveyda BCD ring synthesis [8]

The major drawback from Hoveyda's synthesis arises from the need to functionalize both the B and D rings. In this synthesis, the D ring, **6**, must be synthesized separately in a five step sequence from **9** with a high enantiomeric excess. (Scheme 2) This is accomplished by using a chiral ligand in the presence of a metal catalyst, Ti(Oi-Pr)$_4$, with an overall yield of 49% after 5 steps from **9**. [8]
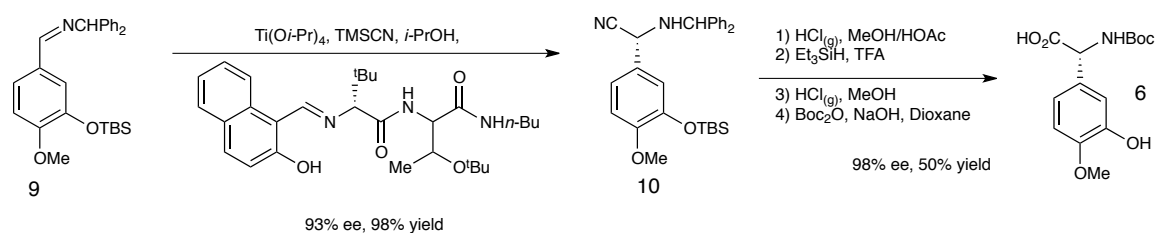
Scheme 2: Hoveyda's synthesis of the D ring [8]

1.2.2 Intramolecular Nucleophilic Aromatic Substitution

Another common method used by synthetic chemists to form biaryl ethers is intramolecular nucleophilic aromatic substitution. In these syntheses the tyrosine

derivatives have to be functionalized with the activator, NO_2 group, which directs the addition at the ortho position.[9] (scheme **4**) The investigation of S_NAr on macrocycles has allowed for mild conditions, which is necessary, when dealing with the sensitive phenylglycine's, which are prone to epimerization. [9]

Smith *et al.* tried a ruthenium-promoted intramolecular S_NAr reaction, which gave a 60% yield with a mixture of diastereomers and rotamers, where **17** the natural diastereomer, (SRR), was the least favored (Scheme **3**). The BCD ring fragments were synthesized in 7 steps with a total of >35% for the natural diastereomer from **11**.[4]
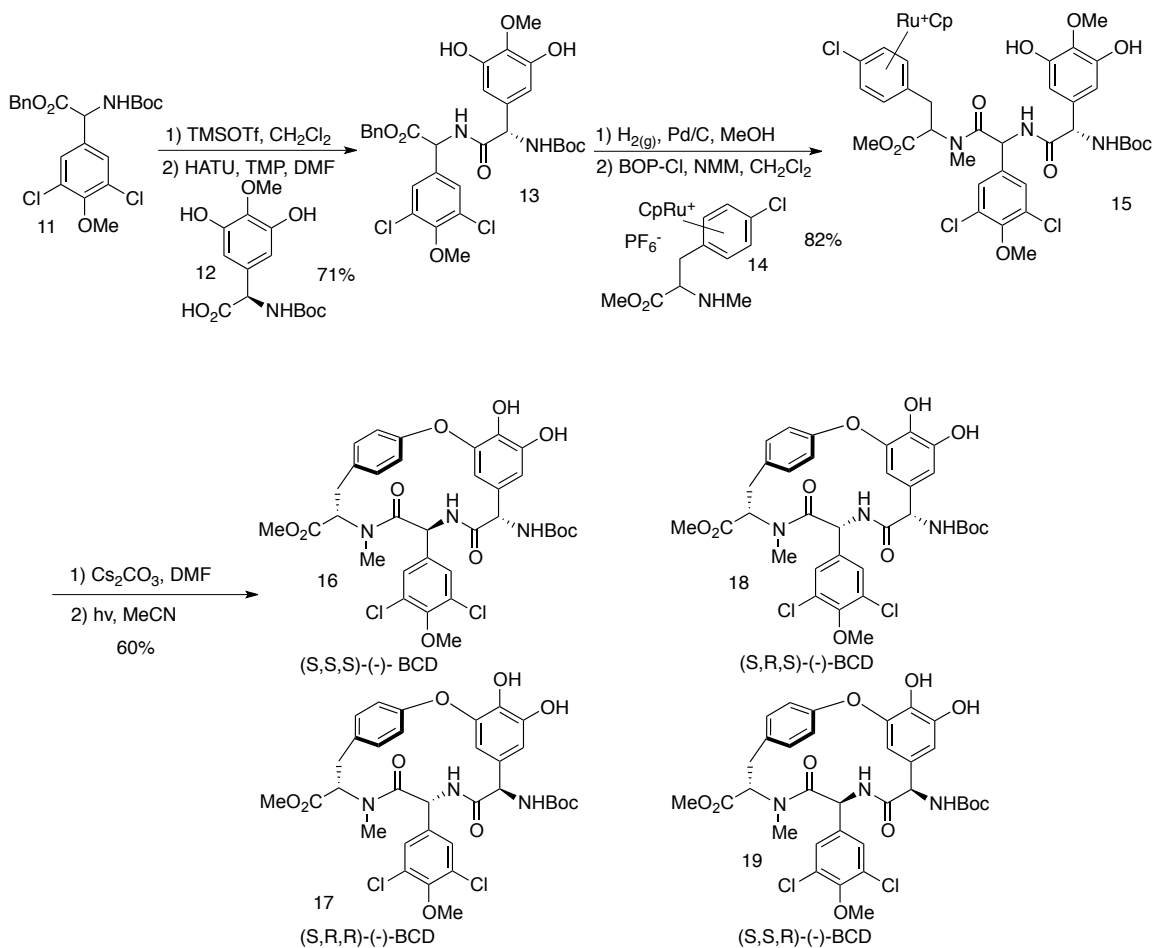
Scheme 3: Smith's synthesis of the BCD ring fragment [4]

Zhu's intramolecular S$_N$Ar reaction proceeded to achieve a single atropismer, see Scheme **4**. He also found that the cyclization was regioselective since the 14- and 17- membered rings were not observed. The BCD ring was synthesized in 4 steps from **20** and **4** with a total overall yield of 28%. [9]
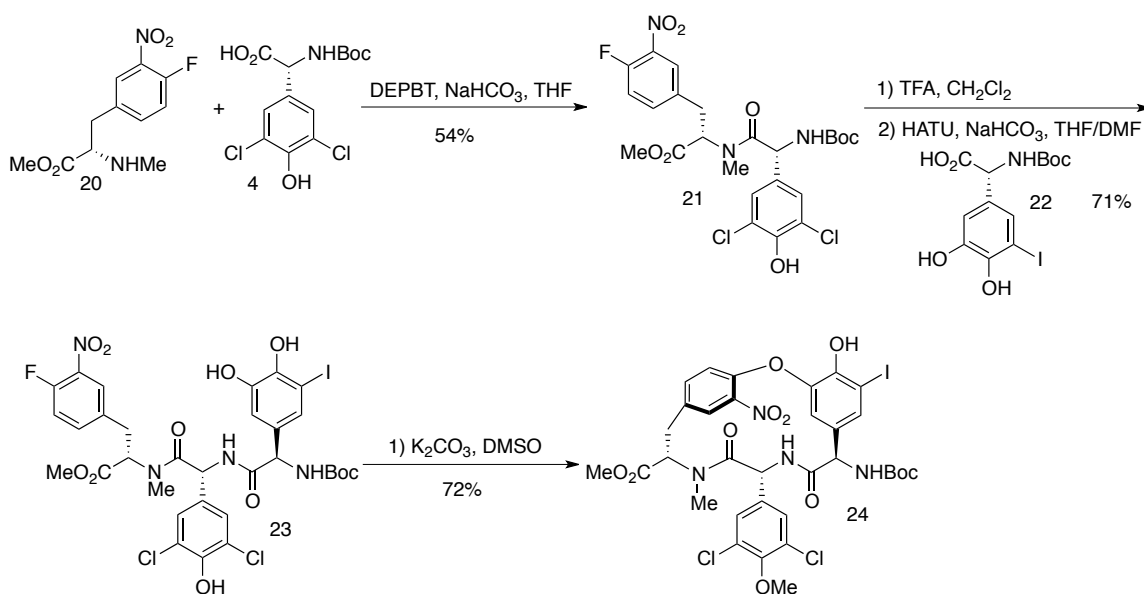
Scheme 4: Zhu's synthesis of the BCD ring fragment [9]

The major drawback to this approach is having to functionalize both the B and D rings. As shown in Scheme 5, ring B is synthesized after 2 and 5 steps with 86% and 43% overall yields from **25** and **26,** respectively. [4, 10] In Scheme 6, ring D was synthesized with overall yields of 29% for Smith's approach from **29** and 21% for Zhu's approach from **32**.[11,12]
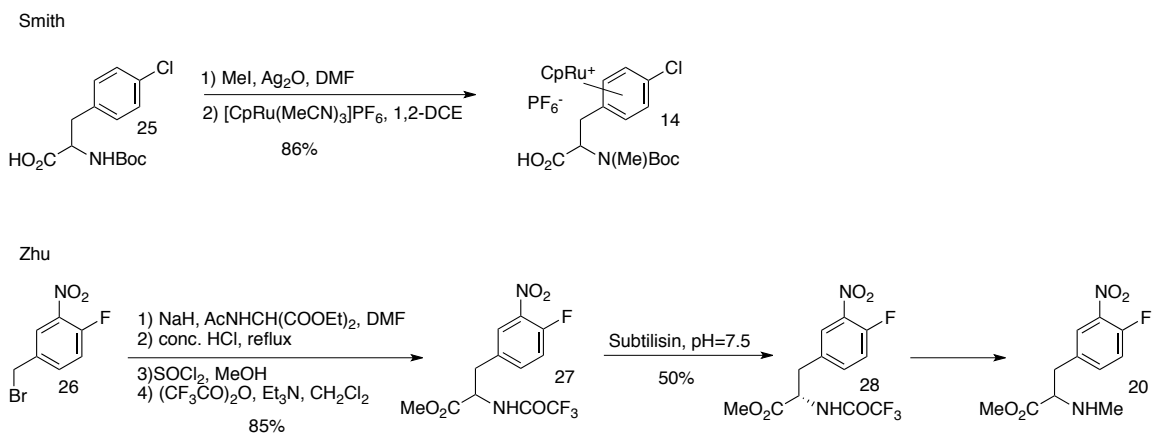
Smith

Scheme 5: Synthesis of Ring B [4,10]

Smith

Zhu
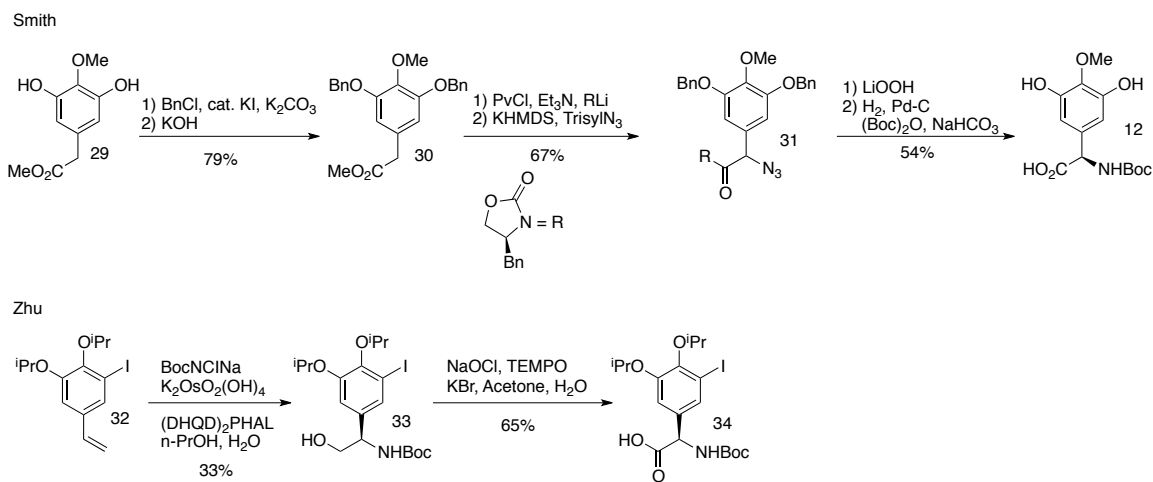
Scheme 6: Synthesis of Ring D [11, 12]

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CHAPTER TWO

SYNTHESIS OF COMPLESTATIN BCD RING

2.1 Retrosynthesis Analysis

Complestatin, **1**, is a bicyclo hexapeptide with the western macrocycle, **BCD**, containing a biaryl ether bond and the eastern macrocycle, **DEF**, containing a biaryl bond. As proposed in the retrosynthesis, the western subunit of **1** is a 16-membered ring that contains three aromatic amino acids and the three stereocenters in the macrocycle are defined by starting with L-tyrosine and 4-hydroxy-D-phenylglycine. (scheme 1) The synthetic challenges present in this macrocycle are defined by the racemization-prone chlorinated arylglycines and the difficulties associated with the construction of strained macrocycles through a biaryl ether bond. As well as overcoming the challenges, the other objectives are to reduce the amount of steps in the sequence and to apply novel chemistry.

The use of commercially available L-tyrosine and 4-hydroxy-D-phenylglycine whose phenols remains unprotected throughout the sequence allow for a reduction in the total amount of steps. As with the previous synthesis of **BCD** ring, the tyrosine **B** and **D** phenylglycine ring have required further functionalization in order to form the biaryl ether bond. In our synthesis, the biaryl ether bond is formed through oxidative phenol coupling facilitated by potassium hexaferrocyanate,[1] which can be done immediately on the tripeptide, **2**, formed by coupling L-tyrosine, 3,5-dichloro-4-hydroxy-phenylglycine, and 4-hydroxyphenylglycine.
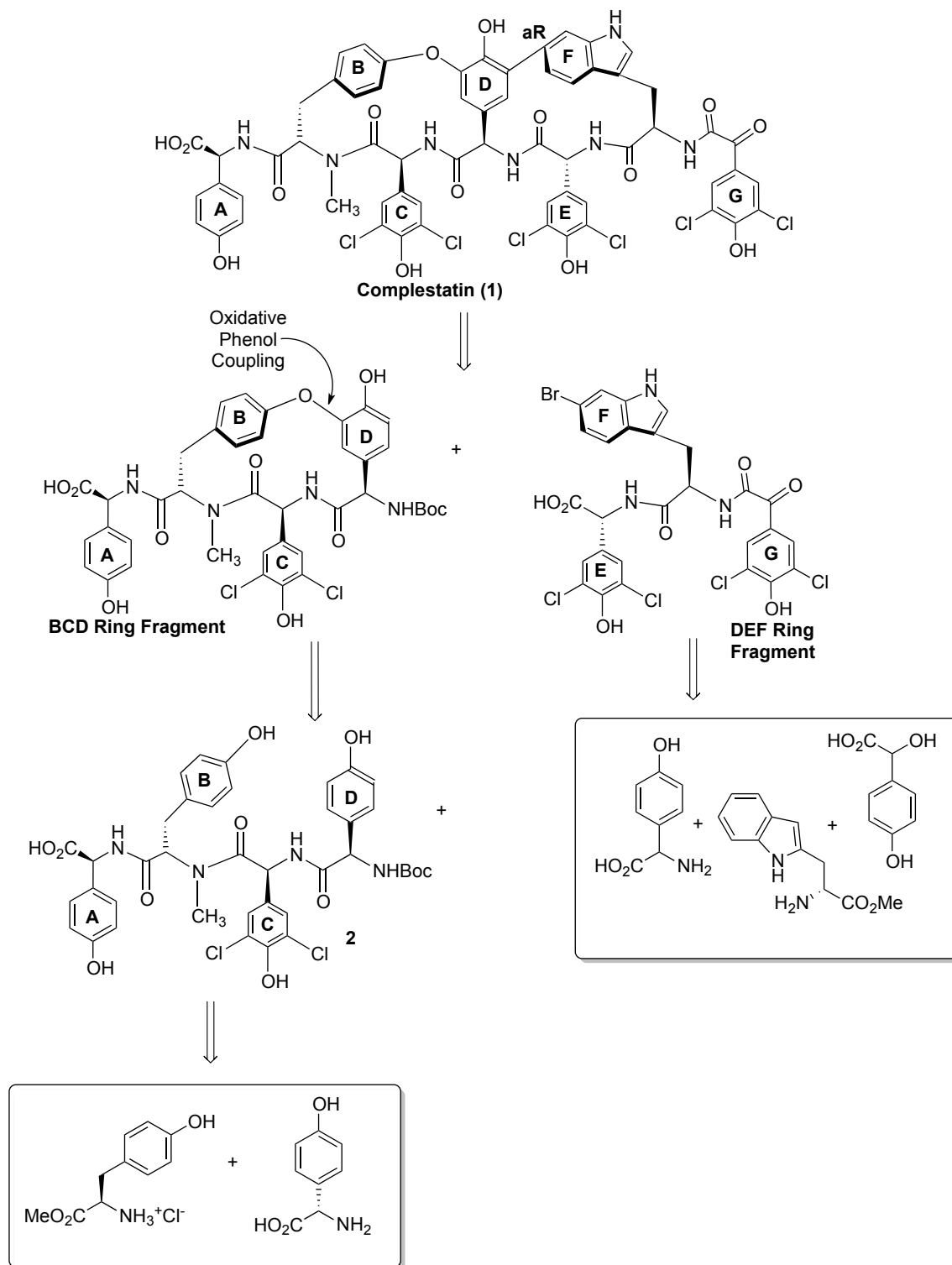
Scheme 1: Retrosynthesis of Complestatin

2.2 Model System study

The potassium hexacyanoferrate phenol oxidation utilized to form the biaryl ether

bond is a more biomimetic reaction than the Ullmann ether synthesis or the S_NAr

reaction. It is easy to believe that the enzyme used to form this natural product uses some

type of oxidation (scheme 2). For the purposes of this project, the pathway leading to

product **a** is optimal. The work done by Eickhoff *et al.* utilizing this method to make

macrocycles of tyrosine, have determined that when R is small and R_1 is electron

withdrawing that product **a** is formed preferentially.[1]
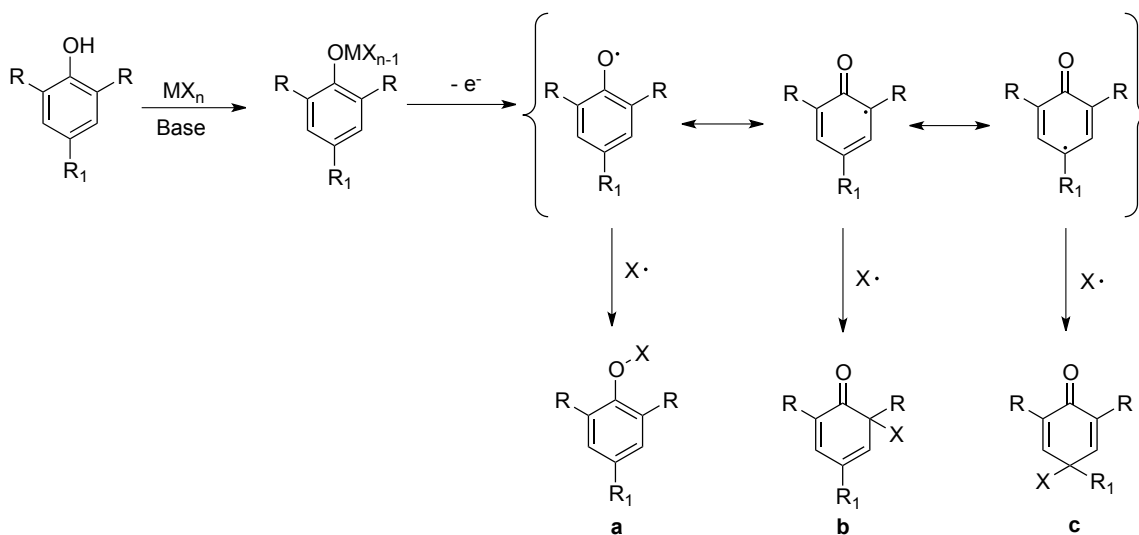
Scheme 2: $K_3[Fe(CN)_6]$ phenol oxidation scheme

To determine if this would work in the synthesis of the BCD ring the model

tripeptide, **3**, was synthesized (scheme 3). The tripeptide begins with the formation of N-

Me-Tyr-OMe, **4**, through a series of imine condensations with benzaldehyde and

formaldehyde, respectively.[2] The imines formed are reduced using $NaBH_3CN$. The

benzyl group on tyrosine is removed using $H_{2(g)}$ in the presence of Pd/C. Then **4** is

coupled with 4-hydroxy-D-phenylglycine using DEPBT and NaHCO$_3$ in THF,[3] and

again to yield **3**.
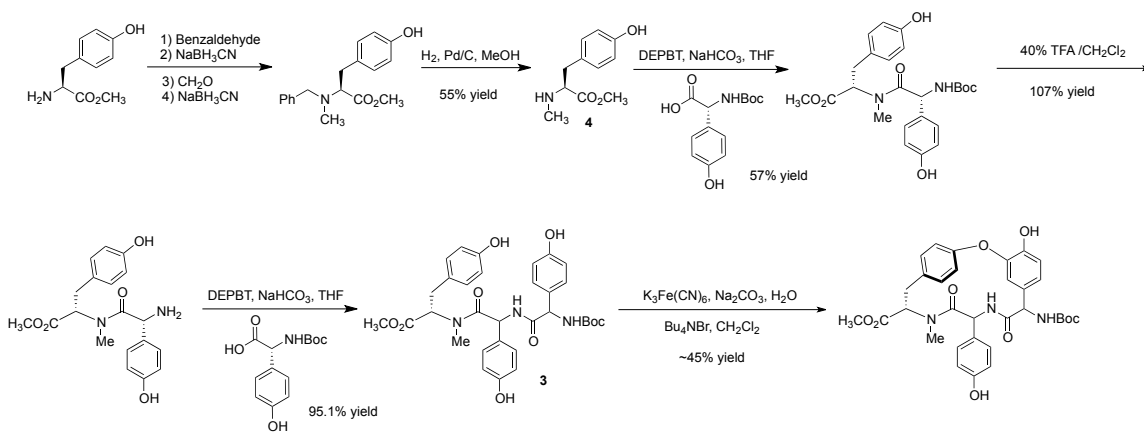
Scheme 3: Synthesis of model system

The oxidation of **3** was carried out in a biphasic system using, K$_3$Fe(CN)$_6$,

Na$_2$CO$_3$, Bu$_4$NBr, H$_2$O, and DCM.[1] The first run of the reaction was a success, with the

macrocycle having a higher Rf value than the tripeptide, in 9:1 DCM:MeOH solvent

system. The reaction was purified by column chromatography and the NMR spectrum of

the macrocycle contained all the necessary peaks, leading us to believe that the reaction

was a success.

2.3 Synthesis of BCD ring

The synthesis of the BCD ring begins with the chlorination of 4-hydroxy-

phenylglycine to give 3,5-dichloro-4-hydroxy-phenylglycine, **5**, using SO$_2$Cl$_2$.[4] This is

followed by the protection of the amino group with a Boc group. With N-Boc-3,5-

dichloro-4-hydroxyPhg in hand, it was coupled with DEPBT and NaHCO$_3$ in DMF with

a 40% yield from **5**. The reagent DEPBT is used in this case because it reduces

epimerization of the chiral center in **5** during peptide couplings.[5] The dipeptide was

deprotected with TFA and then coupled with N-Boc-4-hydroxy-phenylglycine using

HATU and NaHCO$_3$ in a 1:3 DMF/THF mixture with a 70% yield from the dipeptide.[6] In

the case of the last coupling, the reagent HATU is used because it efficiently couples

phenylglycines with little to no epimerization at the chiral centers.[7] The yield of the

coupling reactions might improve if protecting groups were used on the phenol

oxygen's.[6]
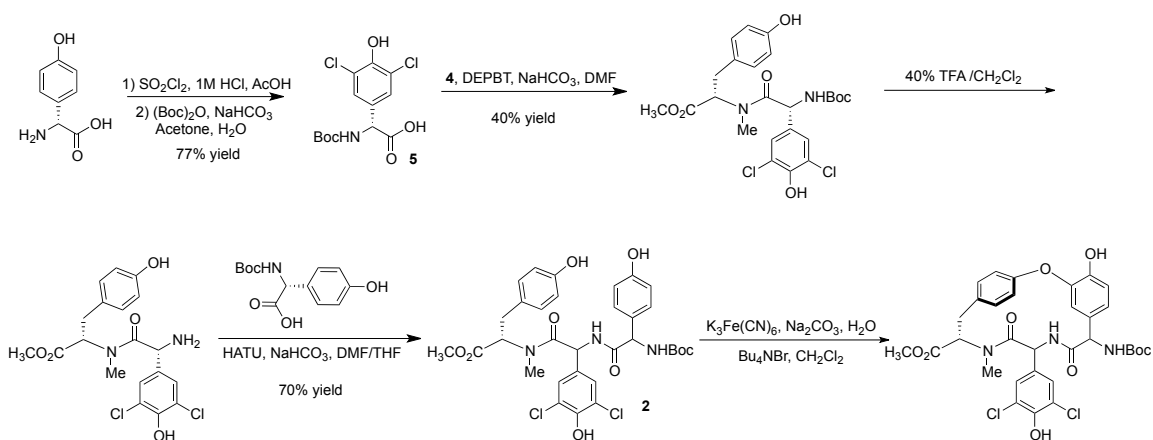
Scheme 4: Synthesis of BCD ring

Once the tripeptide was obtained, the oxidative coupling reaction proceeded and

two yet-to-be-identified spots were detected by tlc. Different reaction conditions were

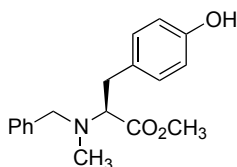explored; K$_3$Fe(CN)$_6$, Na$_2$CO$_3$, in MeOH and K$_3$Fe(CN)$_6$, NaOH$_{(aq)}$, MeOH,[8] both led to

what seems to be deprotected **2**. Further investigations into the solvents and bases used in

the sequence for optimum yield, is necessary.

2.4 Experimental Section

General. All reactions were carried out under regular atmospheric conditions unless otherwise stated. 1H and 13C nuclear magnetic resonance (NMR) spectra were recorded with a Varian VN-MRS 300, 400 or 500 MHz spectrometer. NMR chemical shifts are given in parts per million (ppm) and referenced to the internal solvent tetramethylsilane (TMS). Melting points were determined on a Thomas-Hoover capillary apparatus and were not corrected. Elemental analyses were performed by Quantitative Technologies Inc. (Whitehouse, NJ). Mass spectra were obtained either from the University of California, Riverside Mass Spectrometry facility or Finnigan LCQ-DUO mass spectrometer from Chemistry Department, Rutgers University, New Brunswick, NJ. **Materials**. Unless otherwise specified, all materials were purchased from commercially available sources and were used without further purification. Reactions were carried out in ACS grade solvents. ACS grade methanol was distilled over sodium hydride under nitrogen atmosphere. Flash column chromatography was performed with Sorbent Technologies 230x400 mesh silica gel (SiO_2). Reactions were monitored by thin layer chromatography (TLC) using Whatman 250 micron aluminum backed silica gel plates. Bulk solvents were used as eluents for chromatography unless otherwise stated.
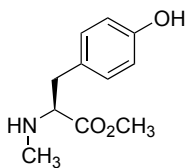
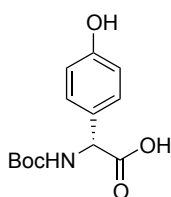**N-benzyl-N-methyl-(L)-Tyrosine methyl ester:** (L)-Tyr-OMe·HCl was dissolved in sat. NaHCO$_3$ and extracted with EtOAc 3 times. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, filtered and

concentrated to obtain (L)-Tyr-OMe (2.82 g, 14.4 mmol). The free amine was dissolved

in MeOH (146 ml, 0.1M soln.) and benzaldehyde (1.70 mL, 15.02 mmol) was added to

the flask and stirred at room temperature for 2 hr. Then NaCNBH$_3$ (965.3 mg, 15.12

mmol) was added to the reaction and stirred overnight. The next day, powdered

formaldehyde (1.57 g, 17.3 mmol) was added to reaction. Once the solution became clear

NaCNBH$_3$ (992.7 mg, 15.12 mmol) was added to the flask and stirred overnight. The

reaction solution was concentrate *in vacuo* and reconstituted in EtOAc creating a slurry,

then filtered through celite and evaporated *in vacuo*. The product was purified by flash

column chromatography using DCM and 1% methanol to give 3.22 g (108 mmol) of N-

Bn-N-Me-Tyr-OMe in 75% yield. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 2.39 (3H, s), 3.01

(1H, dd, J=6.4 Hz), 3.08 (1H, dd, J=8.8 Hz), 3.65 (1H, t, J=6.8 Hz), 3.68 (3H, s), 3.85

(2H, s), 6.83 (2H, d, J=8.4 Hz), 7.06 (2H, d, J= 8.4 Hz), 7.36 (5H, m).

N-methyl-Tyrosine methyl ester (4): In a three-necked RBF, under N$_{2(g)}$ N-Bn-N-Me-

Tyr-OMe (381.5 mg, 1.27 mmol) was dissolved in MeOH (anhyd., 12

mL) and 10% Pd/C (28.8 mg) was added to flask. Then the flask was

fitted with a H$_{2(g)}$ balloon by a 3-way connector. The flask was sealed

off and suctioned and filled with H$_2$, three times. The reaction was left to react overnight

at rt. The solvent was filtered thru celite and evaporated *in vacuo*. The product was

purified by flash column chromatography using DCM and 10% MeOH to give 156 mg

(0.746 mmol) of **4** in 58% yield. ^{1}H NMR (400 MHz, CDCl$_3$) δ = 2.83 (3H, s), 2.92 (2H,

m), 3.46 (1H, t, J=6.4 Hz), 3.69 (3H, s), 6.66 (2H, d, J=8.4 Hz), 6.97 (2H, d, J=8.4 Hz).

N-Boc-4-Hydroxy-(D)-Phenylglycine: In a round bottom flask,

D-4-hydroxyphenylglycine (702.2 mg, 4.19 mmol) was dissolved in Acetone:H_2O (1:1), then $NaHCO_3$ (1.109 mg, 5.03 mmol) was added to solution, followed by the addition of di-(tert-butyl)-carbonate (602.4 mg, 6.28 mmol) and left to stir at rt for 16 hr. The reaction was stopped and acidified to pH=3 using 5% citric acid. The solution was then extracted with EtOAc three times. The combined organic layers were washed with brine, dried over $NaSO_4$, filtered, and concentrated *in vacuo* giving 852 mg of product, 76% yield. ^{1}H NMR(400MHz, CD$_3$OD): δ = 1.43 (9H, s), 5.06 (1H, bs), 6.75 (2H, d, J=11.6 Hz), 7.20 (2H, d, J=11.2 Hz). ^{13}C (500 MHZ, CD$_3$OD): δ = 174.7, 155.3, 147.2, 134.9, 127.1, 121.4, 58.7, 28.6, 27.8.

(N-Me-Tyr-OMe)-(N-Boc-4-OH-Phg): N-Me-Tyr-OMe (66.4 mg, 0.317 mmol) was dissolved in THF (5 mL) and N-Boc-4-OH-Phg (152.5 mg, .476 mmol) was added and the flask was placed in an ice bath at 0°C. Then $NaHCO_3$ (29.5 mg, .317 mmol) and DEPBT (159.3 mg, 0.508 mmol) were added to the flask and left to react at rt overnight. The solvent was evaporated *in vacuo*, then reconstituted in EtOAc and washed with saturated $NaHCO_3$ solution and extracted with EtOAc twice. The combined organic layers were washed in brine, dried over Na_2SO_4, and concentrated *in vacuo*. The product was purified by flash column chromatography using DCM and 10% MeOH. A total of 83.3 mg of dipeptide was obtained in 57.4% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ = 1.41 (9H, s), 2.75 (3H, s), 3.29 (2H, dd, J=4.4 Hz), 3.75 (3H, s), 4.86
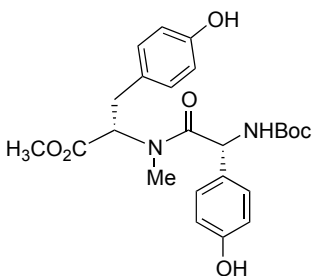
(1H, d, J=9.6), 5.08 (1H, dd, J=4.5 and 4.2), 6.59 (6H, m), 6.84 (2H, d, J=10.8 Hz). M/Z= 457.3

(N-Me-Tyr-OMe)-(4-OH-Phg)-(N-Boc-4-OH-Phg) (3): Dipeptide (81.3, .177 mmol) was dissolved in 40% TFA/DCM (1.5/2.5 mL) and stirred for 3 hr at rt. The solution was evaporated *in vacuo*. The solid obtained was dissolved in THF (5 mL) and N-Boc-4-OH-Phg was added to the flask and cooled to 0°C. Then NaHCO$_3$ (15.2 mg, 0.181 mmol) and DEPBT (84.6 mg, 0.283 mmol) were added to the flask and left to react at rt. overnight.
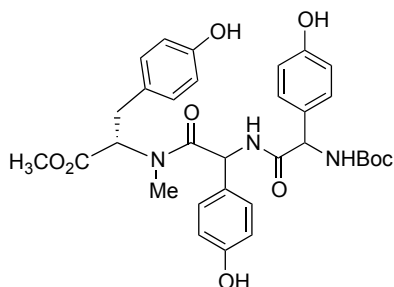
The solvent was evaporated *in vacuo*, then reconstituted in EtOAc and washed with saturated NaHCO$_3$ solution and extracted with EtOAc twice. The combined organic layers were washed in brine, dried over Na$_2$SO$_4$, filtered, and concentrated *in vacuo*. The product was purified by trituration with DCM. A total of 85.5 mg of **3** was obtained in 95% yield. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 1.43 (9H, s), 2.70 (3H, s), 3.30 (2H, dd, J=4.2 Hz), 3.67 (1H, s), 3.78 (3H, s), 5.02 (1H, s), 5.71 (1H, dd, J=4.2 and 4.5 Hz) 6.48-6.86 (8H, m), 6.98 (2H, d, J=9 Hz), 7.96 (2H, d, J=6.9 Hz).

Macrocycle: Tripeptide, **3** (16.3 mg, 0.025 mmol) was dissolved in DCM/H$_2$O (1.5/1 ml) and Bu$_4$NBr (42.4 mg, 0.125 mmol) was added, followed by Na$_2$CO$_3$ (57.3 mg, 0.494 mmol) and allowed to react at rt for 20 min. Then K$_3$Fe(CN)$_6$ (24.8 mg, 0.075 mmol) was added to the solution and allowed to react at rt for 36 hr. The solution was washed with H$_2$O, then brine, dried over Na$_2$SO$_4$, filtered, and concentrated *in vacuo*. The product was
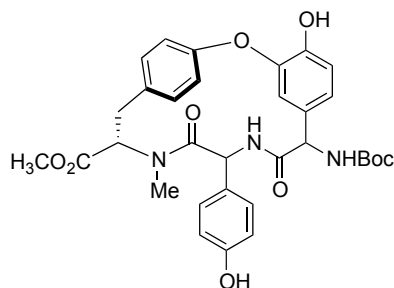
purified by flash column chromatography using DCM and 10% MeOH, A total of 8 mg

of macrocycle was obatined with 53% yield. MS= 603 m/z.

3,5-dichloro-4-hydroxyphenylglycine: In a three-necked RBF, 4-OH-Phg (5.03 mg,

29.9 mmol) was suspended in AcOH (100 mL), through one neck $HCl_{(g)}$ was bubbled

through the solution and exited the system into a $NaHCO_3$ bath. The central neck was

fitted with a dripping funnel and contained SO_2Cl_2 (7 ml, 36.7 mmol) in AcOH (25 mL)

added dropwise to the flask over 1 hr. The reaction was heated to $70^{\circ}C$ for 2 hr, then

cooled to rt and reacted overnight. The solution was neutralized using saturated

NH_4HCO_3 solution, the solid was filtered and 5.530 g was collected with 78% yield. ^1H

NMR (500 MHz, $CDCl_3$): δ= 6.43 (1H, s) and 7.83 (2H, s).

N-Boc-3,5-dichloro-4-hydroxyphenylglycine (5): 3,5-diCl-4-OH-Phg (200.4 mg, 0.847
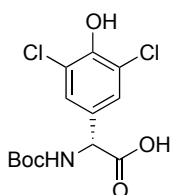
mmol) was dissolved in MeOH (5 mL) and NEt_3 (0.150 mL, 1.02 mmol).

Then Boc anhydride (107.5 mg, 1.27 mmol) was added to the solution

and left to react overnight. The reaction was concentrated *in vacuo* and

purified by flash column chromatography using DCM and 15% MeOH. Carbonic acid **5**

was obtained in quantitative yield. ^1H NMR (500 MHz, $CDCl_3$) δ= 1.48 (9H, s), 6.34

(1H, s), 7.36 (2H, s). ^{13}C (500 MHz, $CDCl_3$) δ= 188.6, 171.3, 153.1, 130.6, 130.0, 122.4,

66.1, 29.9, 15.5.

(N-Me-Tyr-OMe)-(N-Boc-3,5-diCl-4-OH-Phg): To a 25 mL round bottomed flask **4**
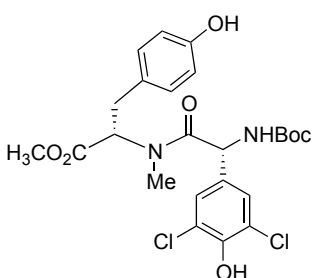
(514.8 mg, 2.46 mmol) and **5** (987.9 mg, 2.95 mmol) were

dissolved in DMF and cooled to $0^{\circ}C$. Then DEPBT (1.081 g,

3.69 mmol) was added to the solution, followed by $NaHCO_3$ (515.8 mg, 4.92 mmol), reacted at 0^oC for 2 hr and rt for 16 hr. The solvent was evaporated *in vacuo*, then reconstituted in EtOAc and washed with saturated $NaHCO_3$ solution and extracted with EtOAc twice. The combined organic layers were washed in brine, dried over Na_2SO_4, and concentrated *in vacuo*. The product was purified by flash column chromatography using 9:11 petroleum ether: EtOAc. A total of 518.8 mg was obtained with 40% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ= 1.41 (9H, s), 2.77 (3H, s), 2.86-3.00 (2H, m), 3.77 (3H, s), 4.87 (1H, d, J=12.8 Hz), 5.09 (1H, dd, J=4.5 and 4.2 Hz) 6.61 (2H, d, J=10.8), 6.88 (2H, d, J=10.4), 7.08 (2H, s).

(N-Me-Tyr-OMe)-(3,5-diCl-4-OH-Phg)-(N-Boc-4-OH-Phg) (2): Dipeptide (47.5 mg,

0.09 mmol) was dissolved in 40% TFA/DCM (1/3 mL) and stirred for 8 hr at rt. The solution was evaporated *in vacuo*. The solid obtained was dissolved in DMF/THF (1/2 mL) and N-Boc-4-OH-Phg (42.5 mg, 0.135 mmol) w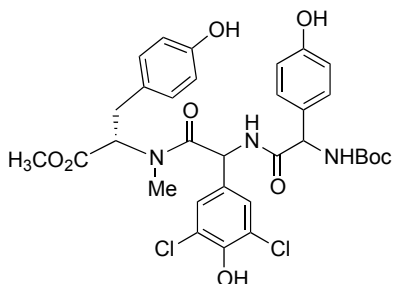as added to the flask and cooled to 0^oC. Then $NaHCO_3$ (76.2 mg, 0.91 mmol) and HATU (76.5 mg, 0.135 mmol) were added to the flask and left to react at rt. overnight. The solvent was evaporated *in vacuo*, then reconstituted in EtOAc and washed with saturated $NaHCO_3$, H_2O, and 0.1N HCl solution and extracted with EtOAc twice. The combined organic layers were washed in brine, dried over Na_2SO_4, and concentrated *in vacuo*. The product was purified by flash column chromatography using DCM and 7% MeOH. A total of 42.5 mg of **2** was obtained as a mixture of rotamers in 70% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ (major rotamer)= 1.39 (9H, short s), 2.72 (3H, s), 2.91-3.24

(2H, m), 3.51 (1H, s), 3.76 (3H, s), 4.92, (1H, s), 5.11 (1H, d, J=4.2 Hz), 6.56 (2H, d,

J=6.8 Hz), 6.80 (2H, d, J=7.2 Hz), 6.96 (2H, m), 7.03 (2H, d, J=6.4 Hz), 7.07 (2H, s),

7.46 (1H, bs). ^{13}C NMR δ= 207.0, 170.4, 169.4, 156.5, 154.8, 148.3, 129.4, 129.2, 128.5,

128.1, 127.7, 127.5, 121.6, 116.1, 115.9, 115.7, 115.5, 53.4, 52.6, 33.6, 30.9, 29.7, 28.2.

BCD Ring: Tripeptide **2** (18.2 mg, .026 mmol) was dissolved in DCM/H$_2$O (1.3/1.3 ml)

and Bu$_4$NBr (37.0 mg, 0.111 mmol) was added,

followed by Na$_2$CO$_3$ (47.5 mg, 0.444 mmol) and

allowed to react at rt. Then 20 min later, K$_3$Fe(CN)$_6$

(26.7 mg, .0666 mmol) was added and left to react at rt

for 36 hr. The solution was washed with .1N HCl, then

brine, dried over Na$_2$SO$_4$, and concentrated *in vacuo*. The product was purified by flash

column chromatography using DCM and 7% MeOH. The 2 spots have to be further

identified.

2. 5 References:

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CHAPTER THREE:

HALOGENATION OF TRYPTOPHAN INDOLE

3.1 Introduction

Tryptophan is the largest and least common of the genetically encoded amino acids. [1] This amino acid is a common precursor to a wide range of biologically active compounds such as, complestatin, celogentin, and TMC-95A. (fig. 1) Bromotryptophans in particular are prominent in the natural products of marine organisms. [2] Complestatin and the celogentin/moroidin alkaloids are examples of bicyclic peptide natural products, which contain a tryptophan residue. The macrocycle in these natural products are connected by a carbon-carbon bond formed at the 6- position of the indole sidechain. (fig. 1) Derivatizing the indole ring of L-Trp directly would greatly increase efficiency by reducing the number of reactions.
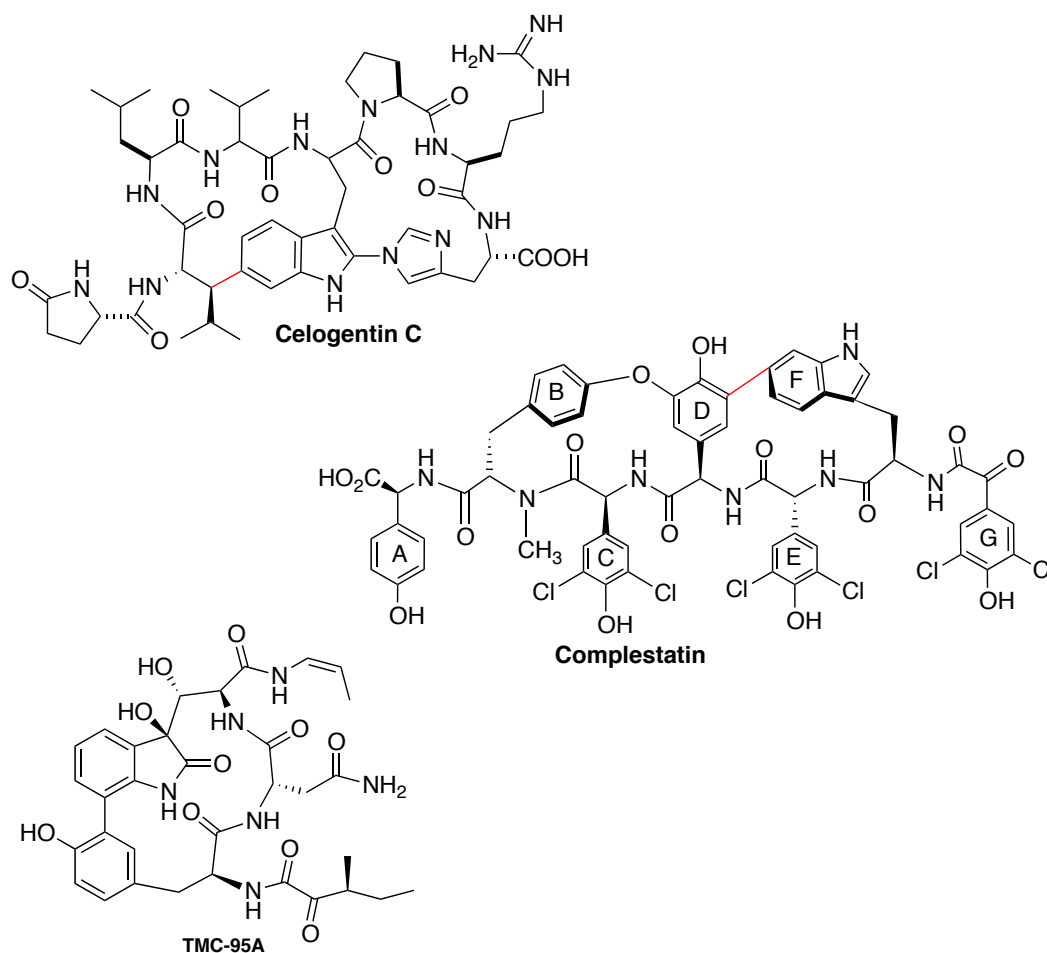
Figure 1: Natural Products containing Trp

The preparation of 6-Bromotryptophan has been previously made by condensing 6-bromoindole with serine, followed by an enzymatic resolution with D-aminoacylase.[3] (scheme 1) The unnatural tryptophan derivative is produced in 20.6% overall yield from **1** with 99% enantiomeric excess. Another method used to synthesize 6-halotryptophan is through the intermediate 6-nitrotryptophan. [4] The nitro group is then reduced, diazotized and displaced with I_2 utilizing Sandmeyer conditions.
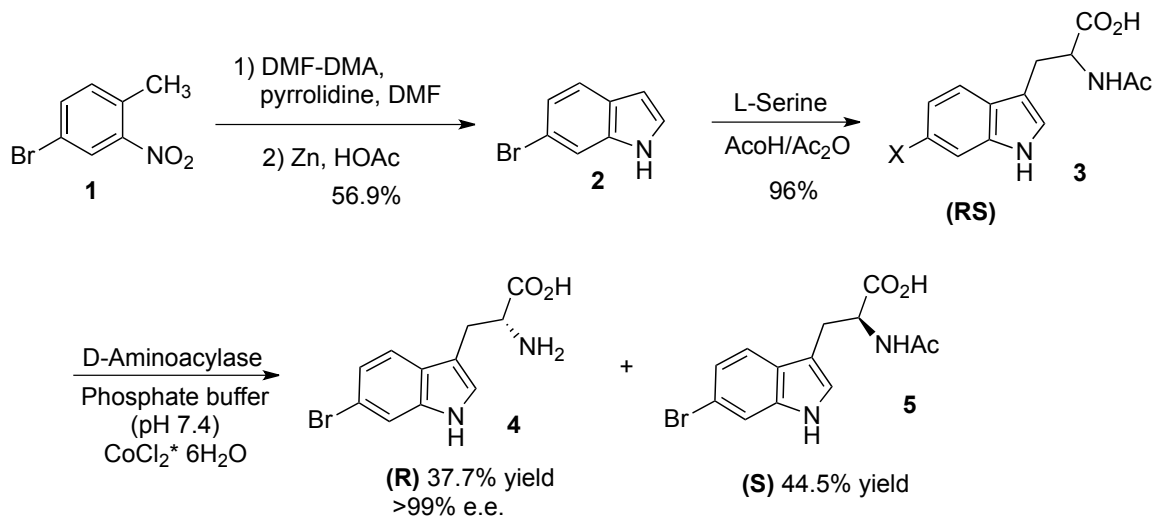
Scheme 1: 6-bromotryptophan by enzyme resolution

All of these syntheses involve multi-step reactions in which most of the material gets lost along the way. The hope is to reduce the bromination of the indole ring down to one step, thus increasing the efficiency from starting material.

3.2 Bromination of Trp. indole

The investigation began with findings of Suarez-Castillo *et al.*, that methyl 2-(1H-indol-3-yl)acetate in the presence of 2 equivalents of NBS in anhydrous DCM gave the 2,6-dibromo compound. [5] These reactions conditions were used on N-Boc-Trp-OMe (**6**), scheme 2, and it was found that the 2-position on **6** was left untouched by NBS. This means that the Boc protecting group on **6** was sterically blocking the 2- position and as a result the next most reactive position on the indole, the 5-position was brominated preferentially. The identity of 5-bromotryptophan was verified by 1D-NOE experiments. The overall yield of the reaction is 43% for the 5-bromo analog. The loss of material in

anhydrous DCM is possibly due to the cleavage of the Boc group, classified by the yellow oil left behind in the flask after workup.
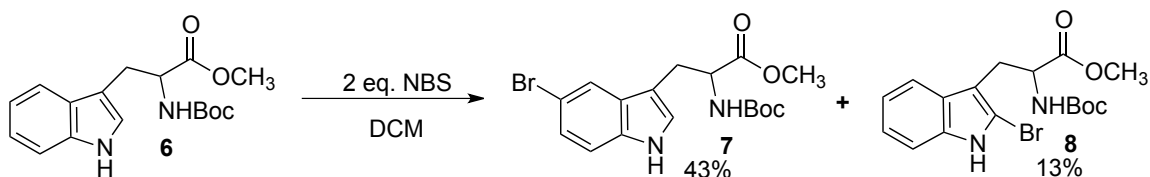
Scheme 2: Synthesis of 5-bromo-tryptophan

After the discovery that protection of the nitrogen group blocks the 2-position, further protection were done on the indole nitrogen. The idea was that the protecting group on the indole would change the electronics of the ring and the 6-position would become more reactive. It was found that when R was Boc or CO$_2$Me, it appeared the 2-position was brominated (scheme 3). These products were placed in DBU and MeOH to cleave the proctecting group on indole nitrogen, but these conditions proved to be ineffective in both cases.
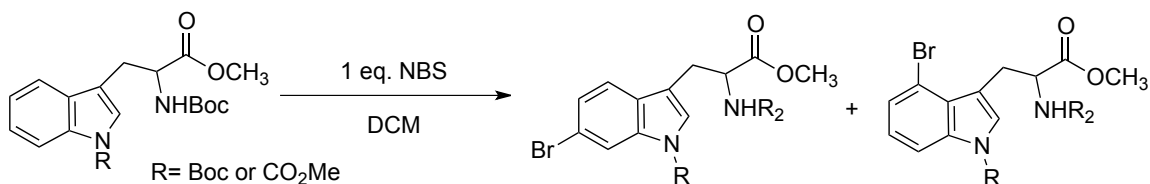
Scheme 3: Indole N-protected brominated reactions

The next set of reactions were done in acidic solvents. It was determined by David Horne *et al*. that dibromination of tryptophan methyl ester in AcOH-HCO$_2$H led to the formation of 2,5-dibromo and 2,6-dibromo tryptophan methyl ester.[6] The nitration of

N^a,N^1-bis(trifluoroacetyl)-L-tryptophan methyl ester using nitric acid in trifluoroacetic

acid led to the formation of nitro group at the 6-position. [1] (Scheme 4)
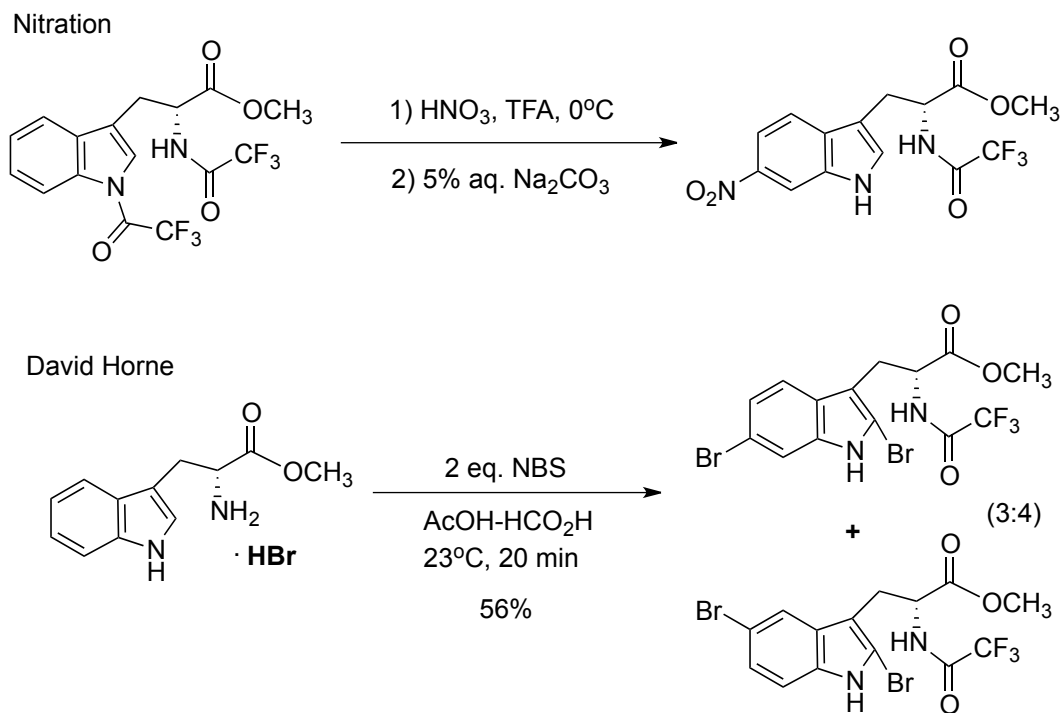
Scheme 4: Reactions of Tryptophan in acidic solvents

Utilizing this knowledge, N-trifluoroacetyl-Trp-OMe, **9**, was synthesized using

ethyl-trifluoroacetate and NEt_3 in MeOH. With **9** in hand the bromination reactions

previously mentioned were run. As scheme **5** shows, the reaction run in TFA gave a

mixture of two yet-to-be-identified derivatives. The bromination with NBS in acetic acid

provided the 2-bromotryptophan derivative.
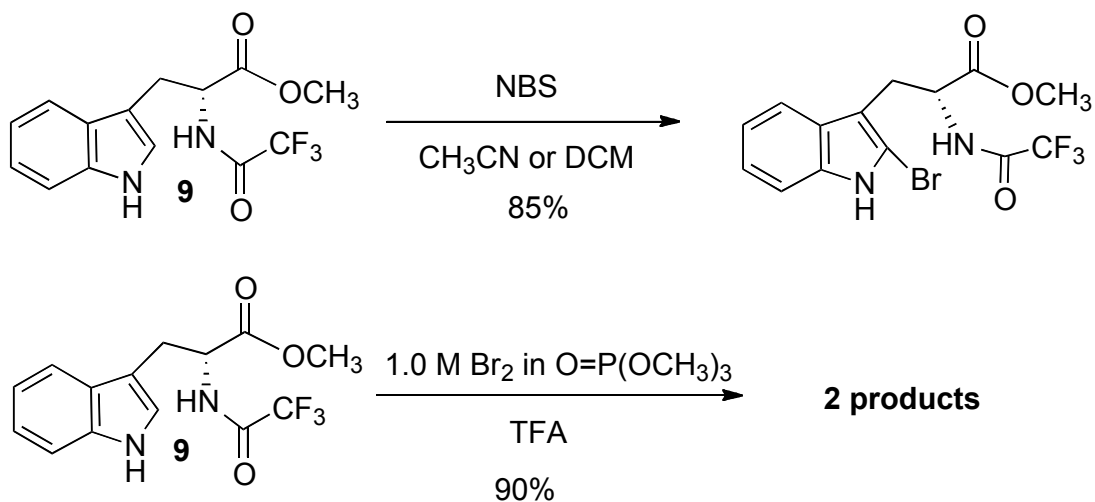
Scheme 5: N-trifluoroacetyl-Trp-OMe bromination reactions

The simplicity of these procedures compares favorably with the overall yields obtained from multistep synthetic routes to 6-halotryptophans from tryptophan derivatives.[3, 4] A savings in terms of time and materials is also realized.

3.3 Experimental Section

General. All reactions were carried out under regular atmospheric condition unless otherwise stated. 1H and 13C nuclear magnetic resonance (NMR) spectra were recorded with a Varian VN-MRS 300, 400 or 500 MHz spectrometers. NMR chemical shifts are given in parts per million (ppm) and referenced to the internal solvent tetramethylsilane (TMS). Melting points were determined on a Thomas-Hoover capillary apparatus and were not corrected. Elemental analyses were performed by Quantitative Technologies Inc. (Whitehouse, NJ). Mass spectra were obtained either from the University of California, Riverside Mass Spectrometry facility or Finnigan LCQ-DUO mass spectrometer from Chemistry Department, Rutgers University, New Brunswick, NJ.

Materials. Unless otherwise specified, all materials were purchased from commercially available sources and were used without further purification. Reactions were carried out in ACS grade solvents. ACS grade methanol was distilled over sodium hydride under nitrogen atmosphere. Flash column chromatography was performed with Sorbent Technologies 230x400 mesh silica gel (SiO_2). Reactions were monitored by thin layer chromatography (TLC) using Whatman 250 micron aluminum backed silica gel plates. Bulk solvents were used as eluents for chromatography unless otherwise stated.

N-Boc-5-bromo-Trp-OMe: To a solution of N-Boc-Trp-OMe (201.7 mg, .628 mmol) in anhydr. DCM (12 mL), N-bromosuccinimide (169.13 mg, .942 mmol) was added and left to react at rt for 14 hrs. The reaction solution was evaporated *in vacuo*, then reconstituted in EtOAc, washed with $NaHCO_3$. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Obtained 105 mg of 5-bromo analog in 40% yield.

^{1}H NMR (400 MHz, CDCl$_3$): δ = 1.45 (9H, s), 3.25 (2H, d, J=4 Hz), 3.71 (3H, s), 4.65 (1H, bs), 5.08 (1H, d, J=7.6 Hz), 7.01 (1H, s), 7.22 (1H, d, J=8.8 Hz), 7.27 (1H, d, J= 6 Hz), 7.66 (1H, s), 8.13 (1H, bs). ^{13}C NMR (500 MHz, CDCl$_3$) δ = 172.6, 134.9, 125.3, 124.2, 121.8, 113.2, 113.8, 80.2, 54.4, 52.5, 29.9, 28.6, 28.2.

N-Boc-Trp(CO$_2$Me)-OMe: To a solution of N-Boc-Trp-OMe (205.6 mg, .628 mmol) in CH$_3$CN (1 mL) was added NEt$_3$ (.2 mL, 1.256 mmol) and DMAP (76.3 mg, .628 mmol) at rt. Then methyl chloroformate was added to reaction and left to stir overnight at rt. The reaction solvent was evaporated, then solid was washed with H$_2$O and extracted with DCM three times. Combined organic layers were washed with brine, dried over Na$_2$SO$_4$, and concentrated *in vacuo*. Obtained 211.1 mg with 89% yield. ^{1}H NMR (400 MHz, CDCl$_3$) δ =1.43 (9H, s), 3.24 (2H, dd, J=14 and 14.4 Hz), 3.69 (3H, s), 4.02 (3H, s), 4.66 (1H, d, J=6.4 Hz), 5.11 (1H, d, J=7.6 Hz), 7.26 (1H, t, J=7.2 Hz), 7.34 (1H, t, J=7.6 Hz), 7.42 (1H, s), 7.51 (1H, d, J=7.6 Hz), 8.15 (1H, d, J=5.6 Hz).

N-Boc-2-bromo-Trp(CO$_2$Me)-OMe: The a solution of fully protected Trp. (20 mg, .0536 mmol) in CH$_3$CN (5 mL) was covered with foil at rt. Then NBS (21.1 mg, .0797 mmol) was added to reaction flask and left to react at rt. The solution was quenched with H$_2$O and extracted three times with DCM. The combined organic layers were washed with brine, dried over Na$_2$SO$_4$, and concentrated *in vacuo*. Obtained 22.1 mg of 2 bromo analog with 90% yield. ^{1}H NMR (400 MHz, CDCl$_3$) δ= 1.40 (9H, s), 3.12-3.28 (2H, m), 3.76 (3H, s), 3.92 (3H, s), 4.62 (1H, s), 7.16 (1H, d, J=7.6 Hz), 7.28-7.40 (3H, m).

N-trifluoroacetyl-D-tryptophan methyl ester: In a reaction flask, D-Trp-OMe (200.1 mg, ,785 mmol) was dissolved in MeOH (2 mL). The NEt$_3$ (.2 mL, 1.57 mmol) was added, followed by the addition of ethyl-trifluoroacetate (.8 mL, 6.28 mmol) and was stirred at rt. overnight. The solvent was evaporated *in vacuo* and product purified by column chromatography using 7:3 Petroleum ether: EtOAc. Obtained product as white crystals, 216.4 mg with 88% yield. ^{1}H NMR (500 MHz, CDCl$_3$) δ= 3.42 (2H, d, J=5 Hz), 3.74 (3H, s), 4.91-4.95 (1H, m), 6.84 (1H, bs), 6.98 (1H, d, J=2.5 Hz), 7.13 (1H, t, J= 7.5 Hz), 7.21 (1H, t, J=8 Hz), 7.38 (1H, d, J=8 Hz), 7.49 (1H, d, J=8 Hz), 8.09 (1H, bs). [13]C (500 MHz, CDCl$_3$) δ= 27.3, 53.1, 53.6, 109.0, 111.6, 116.9 (q, J$_{CF3}$= 287 Hz), 118.5, 119.3, 120.2, 122.7, 122.9, 123.1, 136.3, 157.0 (q, J$_{CF3}$= 37.7 Hz), 170.9.

N-trifluoroacetyl-2-bromo-D-tryptophan methyl ester: In a reaction flask covered with foil, N-CO$_2$CF$_3$-Trp-OMe (35.5 mg, .113 mmol) was dissolved in anhydrous DCM (2 mL). NBS (36.5 mg, .1695 mmol) was added to flask and left to react at rt. overnight. The solvent was evaporated *in vacuo* and reconstituted in Et$_2$O. Organic layer was washed with NaHCO$_3$, H$_2$O, and brine. Then dried over Na$_2$SO$_4$ and concentrated *in vacuo*. The product was purified by column chromatography using 7:3 Petroleum ether: EtOAc. Obtained product as yellow oil, 34.6 mg with 77% yield.

3.4 References:

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CHAPTER FOUR

SYNTHESIS OF TMC-95A SOUTHERN FRAGMENT

4.1 Introduction

The proteasome is a multicatalytic protease that is in a variety of biologically important processes. Proteasome inhibition has a high potential for treating inflammation, autoimmune diseases, and inhibitors are excellent targets for cancer treatment.[1] The selective inhibition of proteasomes may be a means for controlling these essential pathways.[2] Komatsubara and co-workers came across TMC-95A-D(**1**), which are shown to be proteasome inhibitors with IC_{50} values in the low nanomolar levels.

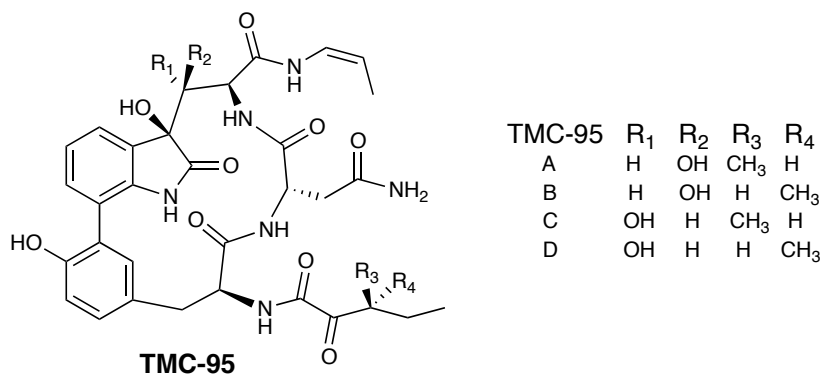| TMC-95	R_1	R_2	R_3	R_4
A	H	OH	CH_3	H
B	H	OH	H	CH_3
C	OH	H	CH_3	H
D	OH	H	H	CH_3

Fig 1: Diastereomers of TMC-95[2]

The TMC-95 family interacts with the proteasome by binding to the active site through hydrogen bonds. The biological studies shown in Table 1, depict the inhibition of the TMC-95 family towards chymotrypsin-like (CT-L), trypsin-like (TL), and post-glutamyl peptide hydrolytic (PGPH).[3,4]

| Compound	IC$_{50}$ Values (nM)		
	CT-L	TL	PGPH
TMC-95A	5.4	200	60
TMC-95B	8.7	490	60
TMC-95C	360	1400	8700
TMC-95D	270	9300	3300

Table 1: Biological activity of the TMC-95 family

As shown in Table 1, TMC-95A proves to be the most potent inhibitor in the family. Structurally, TMC-95A is a cyclic peptide with two side chains. The 17-membered macrocycle consists of a highly oxidized L-trytophan moiety, L-tyrosine, and L-asparagine and two side chains. One of the side chains, 3-methyl-2-oxopentanone is connected to the amine on tyrosine by an amide bond, while the other side, a Z-1-propenylamine is attached to the acid tryptophan by an amide bond.

4.2 Retrosynthesis

The southern fragment of TMC-95A, **1**, consists of three major stereocenters all provided by L-tyrosine, L-asparagine, and L-isoleucine. The challenges involved in the construction of the southern fragment involve achieving the tripeptide in least amount of steps possible and maintaining all the stereocenters of the peptides.

As shown in scheme 1, the northern fragment and southern fragment would be joined, initially through peptide coupling between the tryptophan amine and the asparagine acid, followed by an intermolecular biaryl coupling. A fellow lab mate, Ahalya Ramanathan, supplies the northern fragment and preceeding reactions. The southern fragment is formed through a series of peptide bonds between L-Tyr, L-Asp and 2-hydroxy-3-methyl-pentanoic acid. The hydroxyl group on the modified   L-Ile would

be oxidized to the ketone after the macrocycle has been constructed, thus avoiding

isomerization of the α-methyl group.
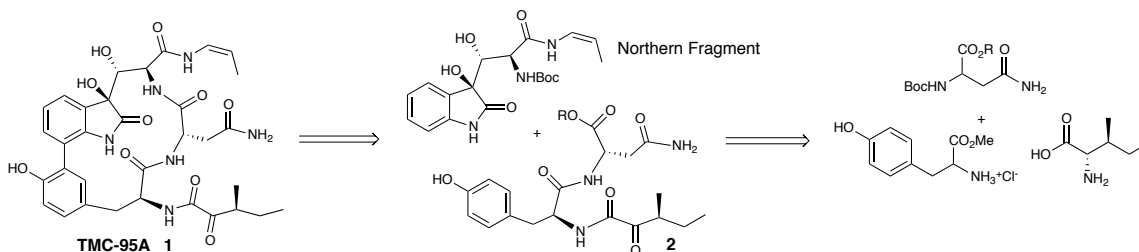
Scheme 1: Retrosynthetic Plan

The protecting group utilized on asparagine is crucial in allowing the synthesis of

1 to be short. If a tert-butyl ester is formed on asparagine the deprotection for both the

north and south fragments can be achieved in acidic medium. (Scheme 2) Thus, we

underwent the investigation into the facile synthesis of tert-butyl esters.
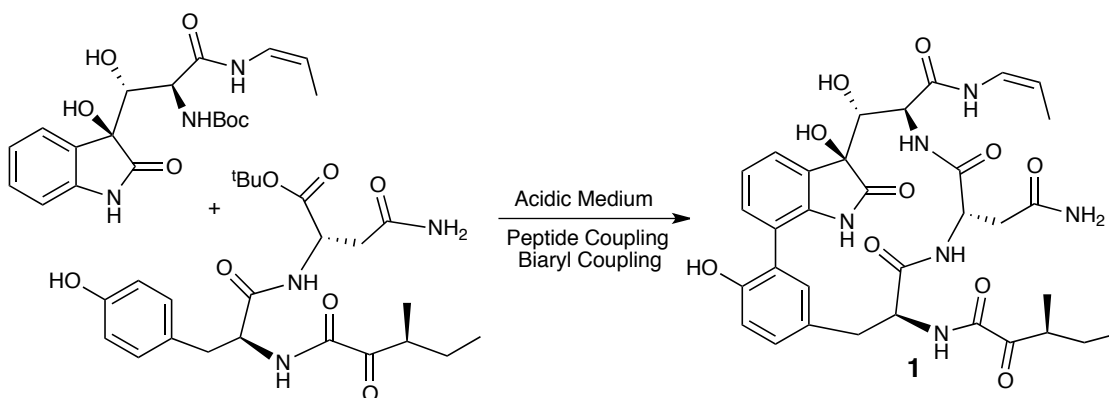
Scheme 2: TMC-95A synthesis using ^{t}Bu protecting groups

4.3 Efforts towards N-Cbz-Asp-O^{t}Bu

The methods used to form the tBu ester on asparagine range from the common

Fischer esterification to insitu formation of isobutylene. As summarized in table 2, all the

reactions were unsuccessful. The various bases used and reagents explored did not

produce the desired ester. Entries 1 and 8 utilize the usual Fischer esterification

principles. Entries 4-7 involve silver salts, which were used to generate isobutylene

insitu.

| Entry	Compound	Catalyst	Reagent	Base	Yield
1[5]	Cbz-L-Asp	70% $HClO_4$	^tBuOAc		NR
2[6]	Cbz-L-Asp	BTEAC	^tBuOAc	K_2CO_3	NR
3	Cbz-L-Asp		^tBuBr	$CsCO_3$	NR
4[7]	Cbz-L-Asp	$AgBF_4$	^tBuBr		NR
5	Cbz-L-Asp		^tBuBr	$AgCO_3$	NR
6	Cbz-L-Asp	AgBF4	^tBuBr	$CsCO_3$	NR
7	Cbz-L-Asp	$AgNO_3$	^tBuBr	DBU	<5%
8[8]	Cbz-L-Asp	H_2SO_4	^tBuOH	$MgSO_4$	50%

Table 2: Summary of esterification reactions.

These esterification reactions always led to inconclusive results. However, entry 8

proved to be successful on the small scale. When scaled up the reactions never provided

reproducible yields and were inconsistent. As a result, N-Boc-Asp-OBn ester was used in

the southern fragment.

## 4.4 Towards the synthesis of TMC-95A southern fragment

As previously mentioned, the southern fragment of TMC-95A consists of L-

tyrosine, L-asparagine, and 3 methyl-2-oxopentanone side chain and all of them are

connected through a peptide bond. Tyrosine and asparagine were bought in the

commercially available form, L-tyrosine methyl ester hydrogen chloride and N-Boc-L-asparagine. The 3-methyl-2-oxopentanone side chain was synthesized from L-isoleucine.(Scheme 3)
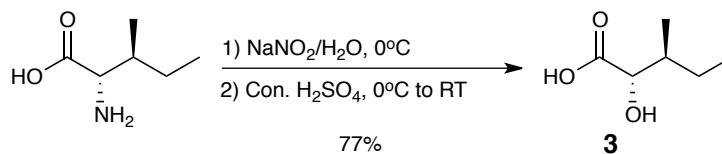


Scheme 3: Synthesis of 3-methyl-2-oxopentanone side chain

The stereochemistry of the side chain 3-methyl-2-oxopentanone is crucial in the biological activity of TMC-95A.[9] By starting with L-isoleucine the α-methyl on the side chain is already set in place. L-isoleucine was subjected to diazotization conditions in scheme 3[10], to produce the hydroxy acid that will later be turned into a ketone when the macrocycle is built.
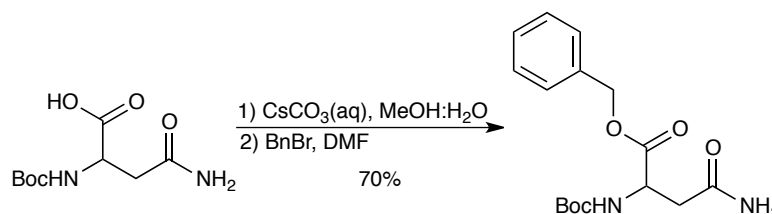
Scheme 4: Synthesis of Benzyl protected Asp.

Once the 2-hydroxy-3-methyl-pentanoic, **3**, acid is made, it is then coupled with L-tyrosine methyl ester in the presence of EDC·HCl and HOBt in DMF to give amide, **4**. The carboxylic acid of N-Boc-L-asparagine was protected with benzyl in the presence of benzylbromide in DMF, after the Cs salt was formed. (scheme 4) With the fully protected asparagine in hand, the Boc group was removed under acidic conditions and was coupled

to the hydrolyzed dipeptide, which was accomplished by the use of an aqueous lithium

hydroxide solution to give the free amine. (scheme 5). The protected southern fragment,

was stored for later use with the northern fragment.
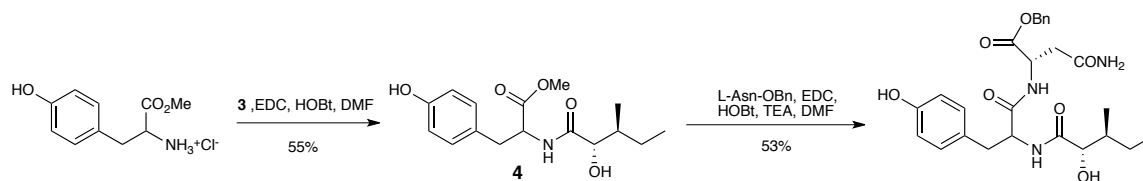
Scheme 5: Synthesis of TMC-95A

4.5 Experimental Section

General. All reactions were carried out under regular atmospheric condition unless otherwise stated. 1H and 13C nuclear magnetic resonance (NMR) spectra were recorded with a Varian VN-MRS 300, 400 or 500 MHz spectrometers. NMR chemical shifts are given in parts per million (ppm) and referenced to the internal solvent tetramethylsilane (TMS).. Elemental analyses were performed by Quantitative Technologies Inc. (Whitehouse, NJ). Mass spectra were obtained either from the University of California, Riverside Mass Spectrometry facility or Finnigan LCQ-DUO mass spectrometer from Chemistry Department, Rutgers University, New Brunswick, NJ.

Materials. Unless otherwise specified, all materials were purchased from commercially available sources and were used without further purification. Reactions were carried out in ACS grade solvents. ACS grade methanol was distilled over sodium hydride under nitrogen atmosphere. Flash column chromatography was performed with Sorbent Technologies 230x400 mesh silica gel (SiO_2). Reactions were monitored by thin layer chromatography (TLC) using Whatman 250 micron aluminum backed silica gel plates. Bulk solvents were used as eluents for chromatography unless otherwise stated.

**2-hydroxy-3-methyl-pentanoic acid (3):** L-isoleucine (3.23 g, 24.625 mmol) was
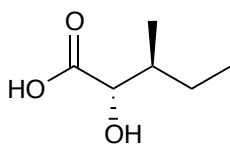
dissolved in 22 ml of 1.25M sulfuric acid in a 3-neck round bottom flask (100 ml) and was cooled to 0°C. A reflux condenser was attached to one of the necks of the flask and the condenser's other end was connected to a water aspirator. In a beaker, sodium nitrite (2.56g, 37.101 mmol)

was dissolved in 13 ml of deionized water and cooled to 0°C. The cold sodium nitrite

solution was added dropwise (0.5 drop/min) to the isoleucine solution while the suction

was mildly turned on to remove nitrogen oxide. The reaction was carried out at 0°C for 3

hours and then the suction was turned off and the reaction was slowly brought to room

temperature. After reacting overnight at room temperature, the aqueous solution was

extracted 5x with diethyl ether. The combined ether layers were washed once with brine,

dried over anhydrous sodium sulfate and the solvent removed *in vacuo*. The product was

purified by recrystallizing in chloroform and hexane to give 2.24 g (18.96 mmol) of **3** in

77% yield; ^{1}H NMR (400 MHz, CDCl$_3$): δ = 0.93 (3H, t, J=7.2 Hz), 1.03 (3H, d, J=6.8

Hz), 1.28-1.32 (1H, m), 1.39-1.47 (1H, m), 1.90 (1H, br s), 4.18 (1H, d, J=3.6 Hz).

N-Boc-L-Asn-OBn: In a 25 mL round bottom flask, N-Boc-L-Asn (232.1 mg, 0.999
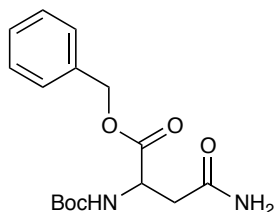
mmol) was dissolved in MeOH: H$_2$O (10:1 mL) mixture and 50

drops of 20% CsCO$_3$ aqueous solution was added to round

bottom flask to obtain pH of 7. The solution was then evaporated

in vacuo to dryness after 3 hr. The salt obtained was dissolved in

DMF (7 mL) and BnBr (0.2 mL, 0.999 mmol) was added to reaction flask and reacted

overnight. The reaction was concentrated *in vacuo* and reconstituted in EtOAc. The

organic layer was washed with H$_2$O, brine, dried over Na$_2$SO$_4$, and concentrated *in*

vacuo. The crude material was recrystallized in EtOAc/Hexanes (2:6) mixture, 248 mg

was obtained with 77% yield. ^{1}H NMR (500 MHz, CDCl$_3$) δ= 1.42 (9H, s), 2.18 (1H, t,

J=8 Hz), 2.73-2.75 (1H, m), 3.02-3.05 (1H, m), 4.23 (1H, s), 5.20 (1H, s), 7.23-7.32 (5H,

m).

**(L-Tyr-OMe)-(3-methyl-2-hydroxy-pentanoic acid)**: In a 50 mL round bottom flask,
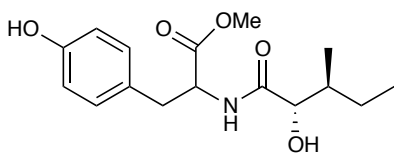
L-Tyr-OMe (4.12 g, 17.5 mmol) was dissolved in DMF and cooled in an ice bath to 0°C. Then EDC·HCl (2.85 g, 14.6 mmol) and HOBt (2.09 g, 14.6 mmol) were

added to flask. 3-methyl-2-hydroxy-pentanoic acid (3.87 g, 29.2 mmol) was dissolved in

DMF (2 mL) and transferred to reaction flask, followed by 4-methylmorpholine (1.6 mL,

14.6 mmol). The reaction stayed at 0°C for 3 hrs and reacted at rt overnight. The reaction

was concentrated *in vacuo*, then reconstituted in EtOAc and washed with sat. NaHCO$_3$.

The aqueous layers was extracted with EtOAc three times. The combined organic layers

were washed with brine, dried over Na$_2$SO$_4$, and concentrated *in vacuo*. The crude

material was purified by flash column chromatography using DCM and EtOAc (1:1).

4.52 g of dipeptide was obtained with 50% yield. ^{1}H NMR (300 MHz, CDCl$_3$) δ= 0.797

(3H, t, J=7.5 Hz), 0.910 (3H, d, J=6.9 Hz), 1.79-1.89 (1H, m), 2.98 (2H, qd, J=5.4 and 7.2

Hz), 3.70 (3H, s), 3.83 (1H, bs), 3.98 (1H, s), 4.85 (1H, q, J=6.3 Hz), 6.74 (2H, d, J=8.1

Hz), 6.98 (2H, d, J=8.4 Hz), 7.22 (1H, d, J=8.4 Hz), 7.76 (1H, bs). ^{13}C NMR (500 MHz,

CDCl$_3$) δ=8.69, 27.7, 28.6, 45.4, 58.7, 79.2, 84.6, 121.9, 127.1, 127.2, 134.6, 147.7,

155.3, 174.9.

Southern fragment (2): To a solution of L-asparagine benzylester.TFA (2 g, 9.04
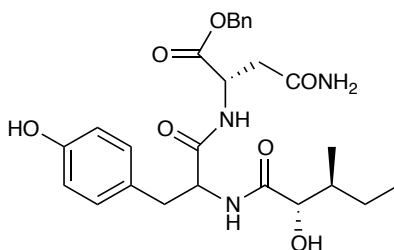
mmol)) in 16 ml DMF at 0 $^\circ$C was added 0.9 ml of triethylamine and then 1.67 g (5.66 mmol) of dipeptide-Li salt. Additionally, 1.08 g (9.04 mmol) of EDC.HCl and 0.9 g (9.04 mmol) of HOBt was added

and the reaction was slowly brought to room temperature. The solution was stirred for another 3 hours and the solvent was evaporated in vacuo. The crude solid was dissolved in DCM and washed with deionized water and with brine. The organic layer was dried over anhydrous sodium sulfate and evaporated in vacuo. The product was purified by flash column chromatography using DCM and 10% methanol to give 1.47 g (2.94 mmol, 52% yield) of white solid; 1H NMR (500 MHz, CDCl3): δ = 0.77 (3H, t, J=7.5 Hz), 0.84 (3H, d. J=7 Hz), 0.97-0.12 (2H, m), 1.71 (1H, br s), 2.58 (1H, br s), 2.72-2.74 (2H, m), 2.83-2.87 (1H, m), 3.00-3.04 (1H, m), 3.83-3.84 (1H, m), 4.68-4.79 (2H, m), 5.11 (1H, d, J=5 Hz), 5.14 (2H, s), 6.23 (1H, br s), 6.69 (2H, d, J=8.5 Hz), 6.99 (2H, d, J=8.5 Hz), 7.07 (1H, br s), 7.28-7.36 (4H, m), 7.88 (2H, d, J=8 Hz); 13C NMR (500 MHz, CDCl3) δ = 16.9, 20.5, 28.0, 41.9, 42.3, 43.2, 54.3, 58.3, 71.8, 80.9, 120.4, 132.1, 133.0, 133.2, 133.5, 135.3, 140.6, 161.1, 175.8, 176.4, 176.9, 179.0.

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