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Synthesis of chiral intermediates by deritivization of monosaccharides

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Synthesis of chiral intermediates by derivatization of
monosaccharaides

A Thesis

Submitted to the Graduate Faculty of the
University of New Orleans
In partial fulfillment of the
Requirements for the degree of

Master of Science
In
Chemistry

By

Sanjeeva Reddy Dodlapati

August 2011

Dedicated to:

My mother, Suguna
My father, Saidi Reddy
My uncle, Madhava Reddy

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Abstract

Conformationally constrained bicyclic amino acids are invaluable in the synthesis of natural products and peptidomimetics. Aeruginosins contain novel bicyclic amino acid, 2-carboxy-6-hydroxyl octahydrindole (Choi) as the core structure. Aeruginosins are tetrapeptide serine protease inhibitors isolated from marine sponges and cyanobacterial water blooms. Rigid bicyclic amino acid (Choi) is an essential core structure, which strongly influence biological activity of aeruginosin family members. Aeruginosins showed promising inhibitory activity against thrombin, trypsin, and factor VIIa. Thrombin and factor VIIa play a major role in blood clotting cascade; excessive coagulation lead to thrombosis and other cardiovascular diseases. Several research groups have reported a number of synthetic aeruginosin analogs. In this thesis, some of the synthetic methodologies of bicyclic amino acid core of aeruginosins are presented. Importance of bicyclic amino acids in peptidomimetic synthesis and drug designing is presented. Mainly, syntheses of ring oxygenated Choi analogs starting from glucose and mannose are presented.

Key words: Bicyclic amino acid, 2-carboxy-6-hydroxyl octahydrindole (L-Choi), ring oxygenated carboxy octahydrindole (O-Choi), aeruginosins, thrombin and peptidomimetics.

Chapter 1: Literature review on bicyclic amino acids

Introduction:

Conformationally constrained bicyclic compounds have been invaluable in medicinal chemistry. Plenty of biologically significant natural products and their synthetic analogs possess various bicyclic scaffolds as the core structure. Especially, bicyclic amino acids are prominent building blocks in the synthesis of peptidomimetics and natural product analogs. Aeruginosin family members are natural product tetrapeptides comprised of 2-carboxy-6-hydroxyoctahydrindole (Choi) or analogs of Choi. Promising anti-thrombin activity of aeruginosin family members made them attractive new class of small molecule anticoagulant drug targets. Several research groups have been pursuing synthesis of aeruginosin analogs and their core structure; some of the significant efforts will be discussed in this chapter. Conformationally constrained bicyclic amino acids provide many advantages such as preventing metabolic degradation of small molecule peptides. Hence bicyclic amino acids have been continuously receiving considerable attention from synthetic community. However, it is not possible to discuss all classes of bicyclic amino acids, mainly octahydrindole derivatives and azabicyclo amino acid analogs will be discussed.

Bicyclic amino acids in peptidomimetics

Peptidomimetic compounds are molecules whose essential elements (pharmacophores) mimic a natural peptide or protein in three-dimensional space and which retain the ability to interact with the biological target and produce the same biological effect. Peptide motifs are present in many biologically active molecules.¹⁻³ Drug design often involves the examination of protein-protein interactions associated with disease, followed by the design of small molecules that can bind to one of the interacting proteins. Often, the biological activity of proteins stems from a small-localized region of a protein surface created by secondary structural elements such as beta strand. Despite their remarkable activities in enzymatic or receptor-based assays, peptides suffer from a number of disadvantages that compromise their use as drugs.⁴⁻⁶ Linear peptide molecules possess greater flexibility to adopt random conformations and compromise their biological activity. Peptide molecules are prone to rapid metabolic degradation; they are rapidly excreted through the liver and kidney, which contribute to their exclusion as drug substance in clinical practice.

A synthetic peptidomimetic that is not subject to cleavage while maintaining the biological activity of the original peptide may have distinct advantages as a potential drug substance. Conformationally pre-organized or fixed into a shape that is recognized by a receptor can have higher affinity for that receptor, also have reduced entropy. Successful chemical modification approaches are involved in the restriction of conformations by the cyclization of peptides or the incorporation of conformationally restricted building

blocks.^{7, 8} Naturally occurring amino acids in a potential inhibitor molecule can be replaced by conformationally constrained monocyclic and bicyclic unnatural amino acids to avoid metabolic degradation and maintain its biological activity. It may also exhibit enhanced potency over their parent sequences, and more importantly, it avoids undesired side effects from byproducts of peptide degradation.⁹⁻¹¹

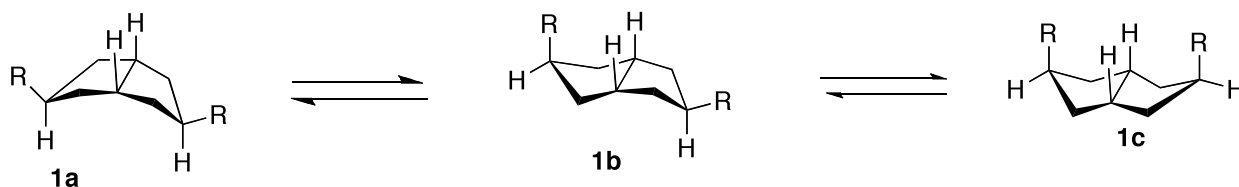


Figure 1.1. Possible conformations of bicyclo (3.3.0)octane

Bicyclic skeletons like bicyclo(3.3.0)octane (**Fig. 1**) is interesting because it has a rigid ring junction as well as conformationally flexible ends.¹² Depending upon the nature of substituents, they can adopt any one of the possible conformations in a given circumstances. Sugar derived amino acids with 5,5- and 6,5- bicyclic scaffolds have been reported as β -turn inducers.^{13, 14} For example, as shown below, bicyclic sugar derived amino acid (SAA) **2** was incorporated to induce the bioactive conformation in small cyclic RGD (Arg-Gly-Asp) peptide analog **3**, which acts as selective antagonist of $\alpha v \beta 3$ integrins expressed on GM 7373 cells.¹⁵

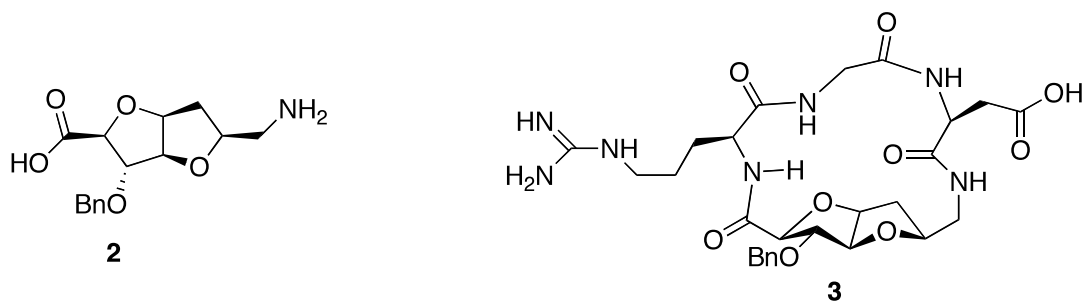
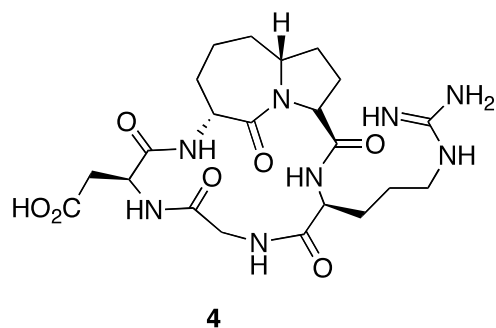
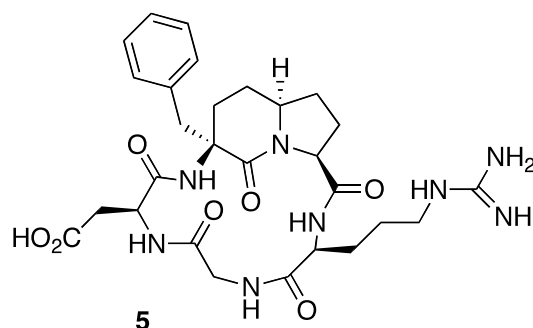


Figure 1.2. Cyclic peptide **3** consists sugar amino acid derivative bicyclic scaffold **2** as a core structure

Integrins are a large family of α/β heterodimeric transmembrane receptors comprise of glycoproteins that attach cells to extracellular matrix (ECM) proteins. Primary functions of integrins are attachment of the cell to the extracellular matrix, and signal transduction from the ECM to the cell. Many cells have multiple types of integrins on their surface, which involve in, along with fundamental cellular processes, various disease states such as tumors, immune, and inflammatory disorders. Constrained RGD analogs, with rigid bicyclic amino acid as an inducer of the desired conformation, have been reported as inhibitors of integrin receptors. Peptidomimetic RGD analog **4** with pyrroloazepinone core structure is a potent nonselective inhibitor of $\alpha v \beta 3$ ($IC_{50} = 3.7$ nM, $K_i = 3$ nM) and $\alpha v \beta 5$ ($IC_{50} = 1.4$ nM).¹⁶



$\alpha\text{v}\beta 3/\alpha\text{v}\beta 5$ inhibitor $\text{IC}_{50} = 3.7/1.4 \text{ nM}$



$\alpha\text{v}\beta 5$ inhibitor $\text{IC}_{50} = 4.1 \text{ nM}$

Figure 1.3. Cyclic peptidomimetics **4** and **5**, $\alpha\text{v}\beta 5$ inhibitors

Subsequent replacement of the pyrroloazepinone by (3R, 6R, 9S) 3-benzyl-indolizidine-2-one provided $\alpha\text{v}\beta 5$ inhibitor **5** ($\text{IC}_{50} = 4.1 \text{ nM}$).¹⁷ Constrained azabicycloalkanone amino acids such as **6** -**13** have been reported as useful in the synthesis of peptidomimetics to induce beta turns.¹⁸

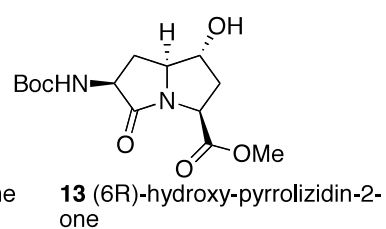
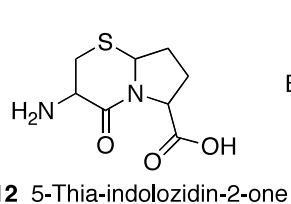
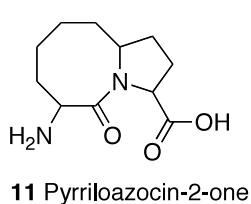
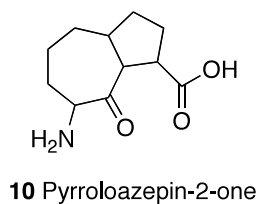
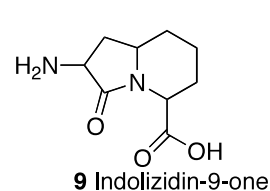
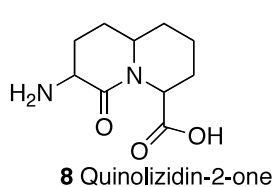
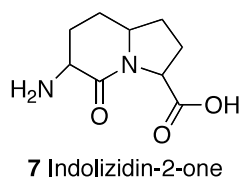
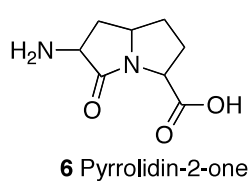


Figure 1.4. Various conformationally constrained azabicycloalkanone amino acids, potential inducers of desired conformations in peptidomimetics

A cyclic vinyl amide scaffold **16** has been designed to mimic the segment Phe43-Leu44 of CD4 **15**. CD4 is a co-receptor of a T-cell receptor, which is responsible for recognition of antigens bound to major histocompatibility complex (MHC) molecules.¹⁹ Its main function is to amplify the signal generated by the TCR by recruiting the enzyme known as tyrosine kinase lck, which is essential for activating many molecules involved in the signaling cascade of an activated T cell.

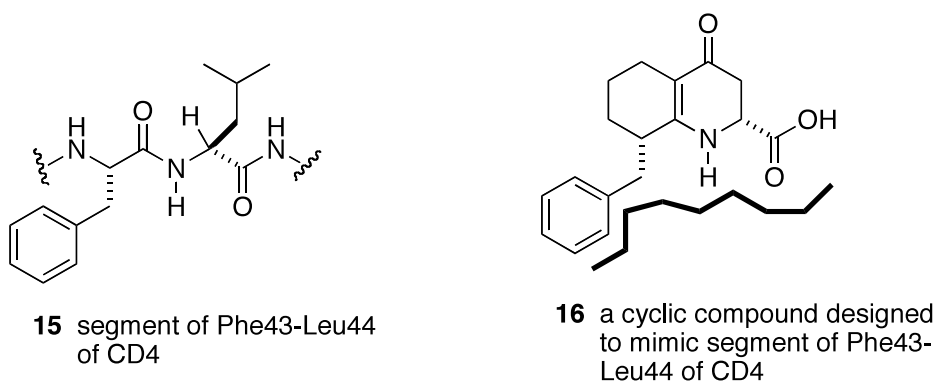


Figure 1.5. Mimicry of the Phe43-Leu44 segment of CD4 by compound **16**

The bold line indicates the region of mimicry where constraints are made from a single side.

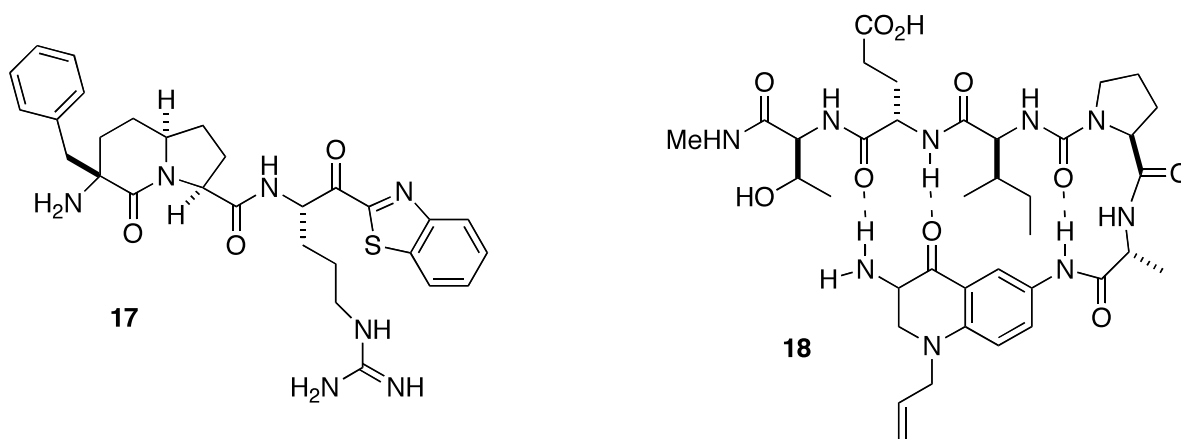


Figure 1.6. Schematic representation of hydrogen bonding in **18**

Bicyclic compound **17** is an example of beta strand mimetic. X-ray crystal structure of thrombin complex with compound **17** revealed an anti-parallel beta strand hydrogen bonds between the carbonyl oxygen and the amino nitrogen atom of bicyclic scaffold and the active site Ser214-Gly216 of thrombin's S₂ subunit.²⁰ Hetero bicyclic compounds have also been used as β -strand inducers. In tripeptide derivative of the bicyclic 3,6-diaminoquinolone **18**, hydrogen bonding scaffold induced a rigid β -strand conformation in attached amino acids.²¹ A hydrogen atom was required on the amino group at position 3 of the quinone scaffold for the formation of one of the necessary hydrogen bonds of the β -strand conformation.

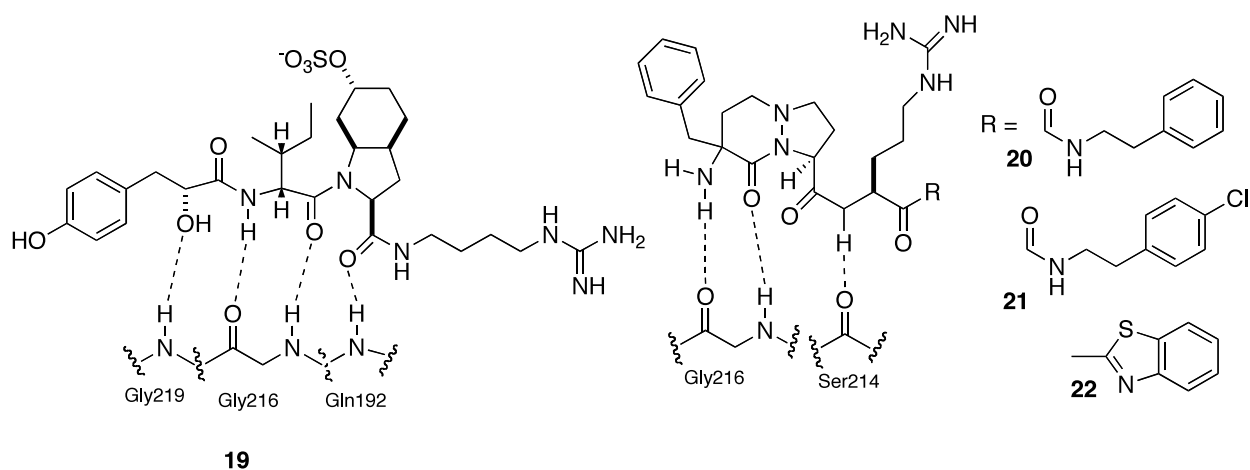


Figure 1.7. a) Schematic representation of anti-parallel β -strand hydrogen bonding between Aeruginosin 98B (**19**) and trypsin. b) Schematic presentation of the hydrogen bonding between **20-22** and thrombin.

Octahydrindole scaffold in the natural product tetrapeptide orientates its pharmacophores in proximity to the active site of protease enzyme trypsin. X-ray crystal structure of trypsin enzyme complex with the compound **19** revealed an extended H-

bonding pattern that is illustrative of anti-parallel beta strand binding.²² Compounds **20-22** have diazabicyclic scaffold also show extended H-bonding with the active site of thrombin.²³ Short peptide compounds **23-27**, which contain 5,6-fused bicyclic amino acids with multiple hetero atoms as core structure, have been reported as excellent peptidomimetic inhibitors of serine proteases: trypsin, and thrombin in the nano-molar range.²⁴⁻²⁸

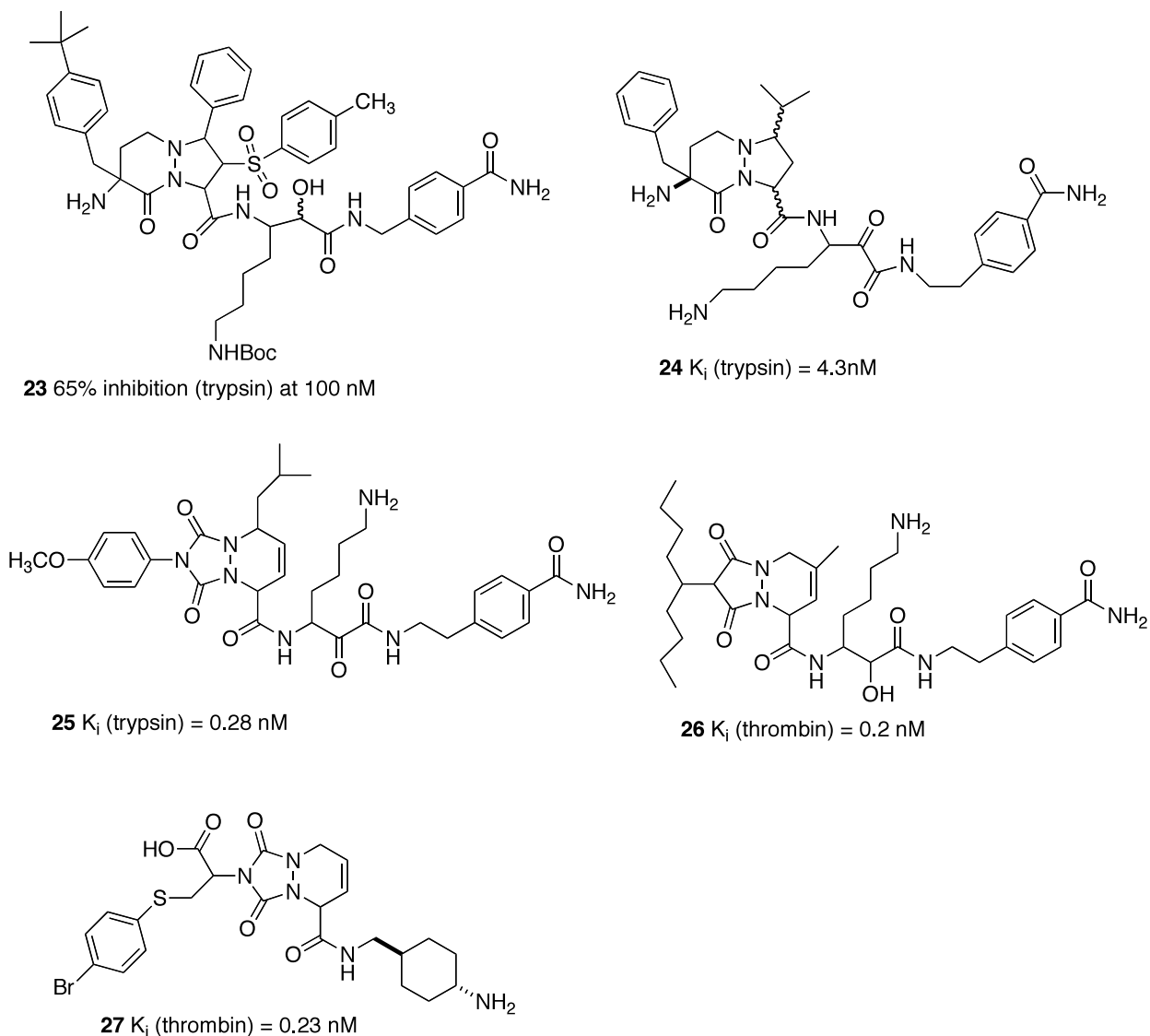


Figure 1.8. Compounds contain 5,6-fused bicyclic amino acid core structures with multiple heteroatoms

Bicyclic amino acids in drug design and development

Broad range of biological activities of bicyclic amino acids is being explored. Various bicyclic amino acids and their derivatives showed biological activity against inflammatory disorders, autoimmune disorders, cell proliferative disorders, epilepsy, faintness attacks, hypokinesia, cranial disorders, neurodegenerative disorders, depression, anxiety, panic, pain, arthritis, neuropathological, and sleep disorders. Bicyclic amino acids have also been used as core structures of drug molecules. For example, ACE inhibitors (26-29) Trandolapril and Prindopril contain the bicyclic octahydrindole 2-carboxylic acid as a core structure, Quinapril contains tetrahydroisoquinolin-3-carboxylic acid, and Ramipril has a 5,5 fused bicyclo amino acid.

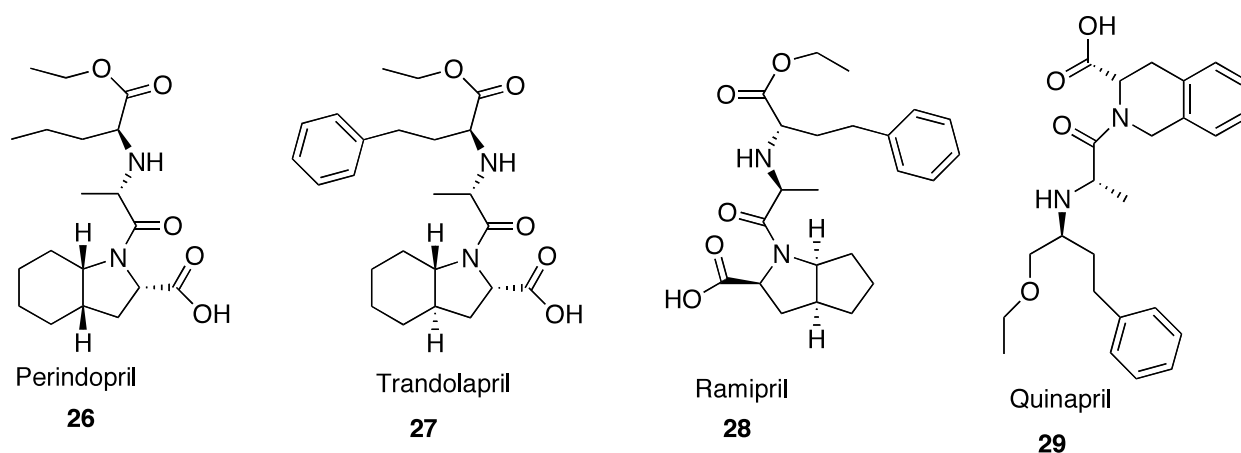
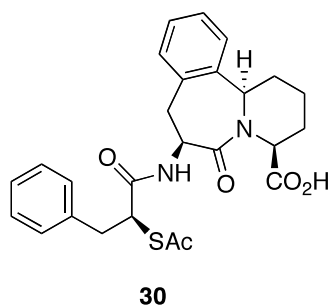


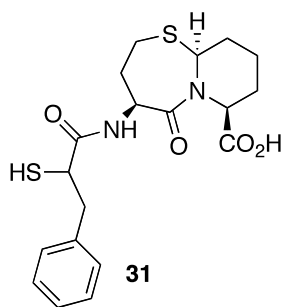
Figure 1.9. Some of the drug molecules that have constrained bicyclic amino acid as core structure

Azabicycloalkane amino acid analogs **30-39** have been reported as angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) inhibitors. ACE is an exopeptidase that catalyzes the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor; also, degrades bradykinin, a potent vasodilator. These enzymes play an important role in blood pressure regulation, body fluid homeostasis, and cell growth. Hence inhibition of these enzymes is a main goal in the treatment of conditions such as heart failure, high-blood pressure, diabetic neuropathy, and type-2 diabetes mellitus. Analogs of **32** (**35-38**), which have oxygen or methylene group in place of sulfur also 5 membered ring in **36** and **38**, showed equivalent potency to **32** against ACE and NEP. Among all these azabicycloalkane amino acid analogs, compound **30** showed higher potency against both ACE and NEP. Compound **39** showed decent potency against ACE, but its activity against NEP is not that impressive.

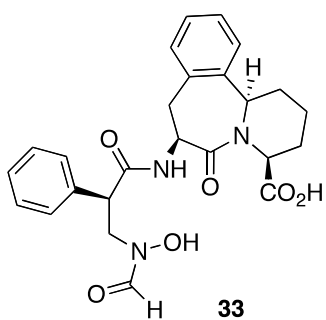
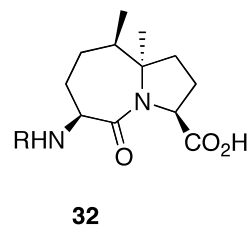
Azabicycloalkane amino acids **40- 46**, diazabicyclo alkane amino acids **47- 54**, and thia-aza bicyclo alkane amino acids **55- 58** showed inhibitory activity against serine protease thrombin. Compound **40** showed decent activity against both thrombin and trypsin, with $K_i = 0.85$ nM and 0.23 nM respectively, but obviously selectivity was a big concern. Compound **43** with tetrahydro naphthyl sulfate as R group showed 6 fold higher potency against thrombin compared to its counter parts **44** and **45**.³³⁻³⁵ Compound **46** showed significant potency against thrombin with $K_i = 9$ nM, also exhibited 20-fold selectivity over trypsin.³⁶ Since **41** and **46** are slightly different, regarding substituents on bicyclic scaffold, it would be interesting to know the comparative selectivity in favor of thrombin over trypsin, but data are not available for trypsin.³⁴



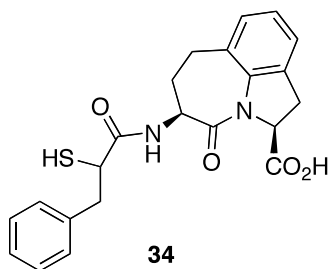
ACE IC₅₀ : 0.11 nM
NEP IC₅₀ : 0.08 nM



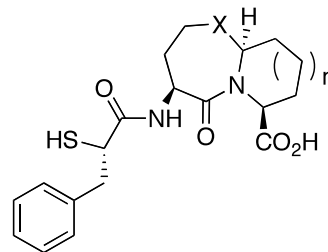
ACE IC₅₀ : 6 nM
NEP IC₅₀ : 9 nM



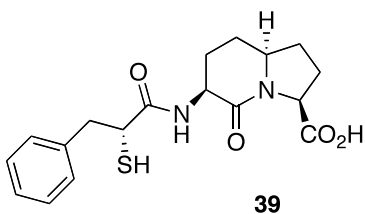
ACE IC₅₀ : 3.4 nM
NEP IC₅₀ : 1.8 nM



ACE IC₅₀ : 48 nM
NEP IC₅₀ : 40 nM

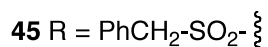
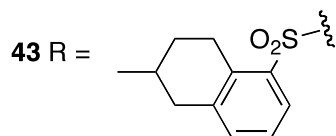
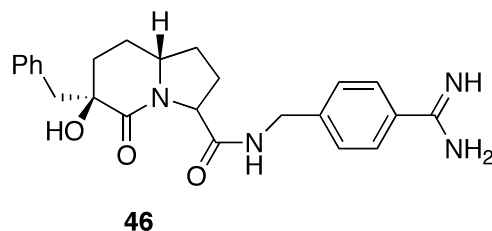
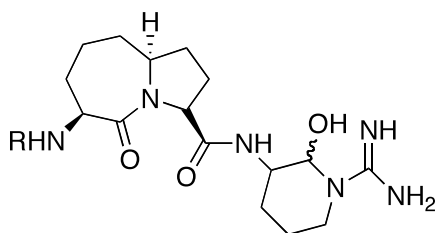
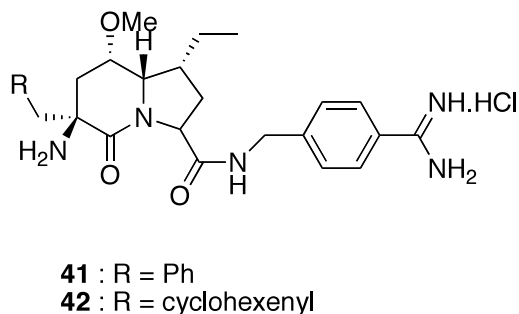
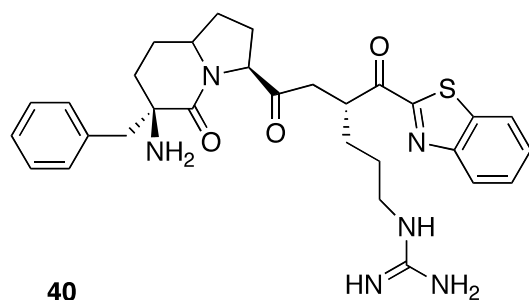


compound	ACE	NEP
35 X = O, n = 1	8	9
36 X = O, n = 0	7	26
37 X = CH ₂ , n = 1	11	25
38 X = CH ₂ , n = 0	5	18



ACE IC₅₀ : 0.87 nM
NEP IC₅₀ : 512 nM

Figure 1.10. Bicyclic amino acid ACE and NEP inhibitors



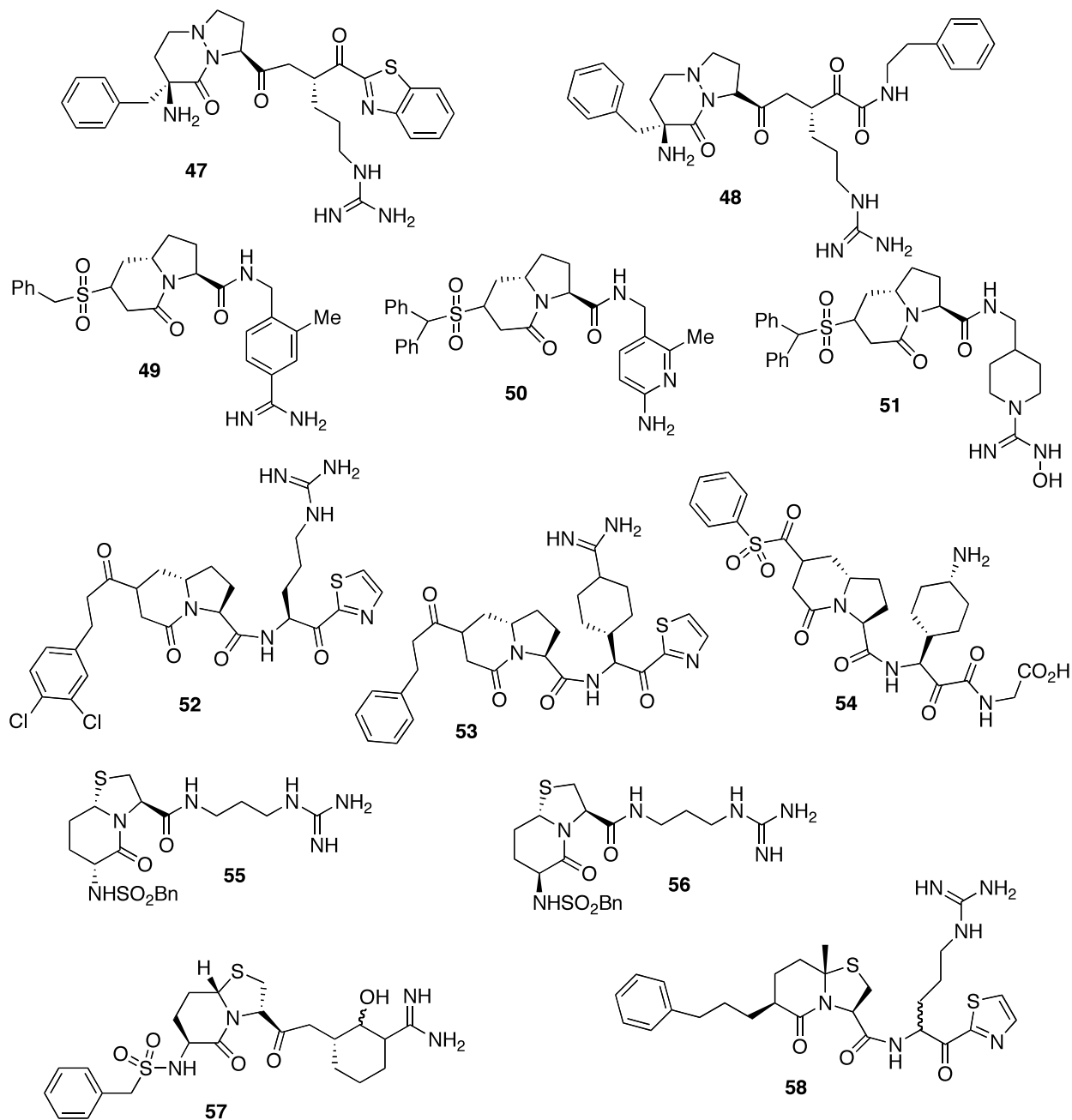
		40	41	42	43	44	45	46
Thrombin		0.85	210	170	18	110	110	9
Trypsin		0.23	-	-	100	-	-	~ 185

(40 & 46 *K_i* in nM, and 41 to 45 *IC₅₀* in nM)

Figure 1.11. Azabicyclo amino acid derivative protease inhibitors

Compounds **47** and **48** showed good potency against both thrombin and trypsin, but failed to show any selectivity.³³ Compounds **49** – **52** and **58** showed decent inhibitory activity against thrombin, but biological activity data against trypsin is not available.^{37-44, 46} Compounds **55** and **56**, which differ with respect to the stereochemistry at C6 position, appear to have some selectivity towards thrombin, but exhibiting moderate

potency.⁴⁵ Conformationally constrained azabicycloalkane amino acids **59- 63** exhibited nanomolar range inhibitory activity against caspases1 and caspase3. 6-aza-piperdinoazipinone-aspartate- α -aldehydes **59** and **61** are the most potent among all with $K_i = 1\text{nM}$ for Caspase1.^{47, 48} Compound **61** inhibited both Caspase1 and Caspase3 with $K_i = 1\text{nM}$ and 10 nM respectively.⁴⁹ Compound **62** selectively inhibited caspase1 with $\text{IC}_{50} = 36\text{ nM}$, and compound **63** selectively inhibited caspase3 with $\text{IC}_{50} = 18\text{ nM}$. Caspase1 belongs to a family of cystine proteases that always cleave peptide bonds following an aspartic acid residue. Caspase3 has been identified as a mediator of apoptosis in mammalian cells. Over expression of both caspase1 and 3 would kill cells indiscriminately. Inhibitors of caspases have been useful in the treatment of diseases such as rheumatoid arthritic, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and Parkinson's disease.



	47	48	49	50	51	52	55	56	57	58
Thrombin	0.65	0.071	7	10	1.2	0.11	145	111	16.4	2
Trypsin	0.64	0.027	-	-	-	-	5100	7900	11.6	-

(47 - 57 K_i in nM, and 58 IC_{50} in nM)

Figure 1.12. Some of the compounds that showing significant protease inhibitory action

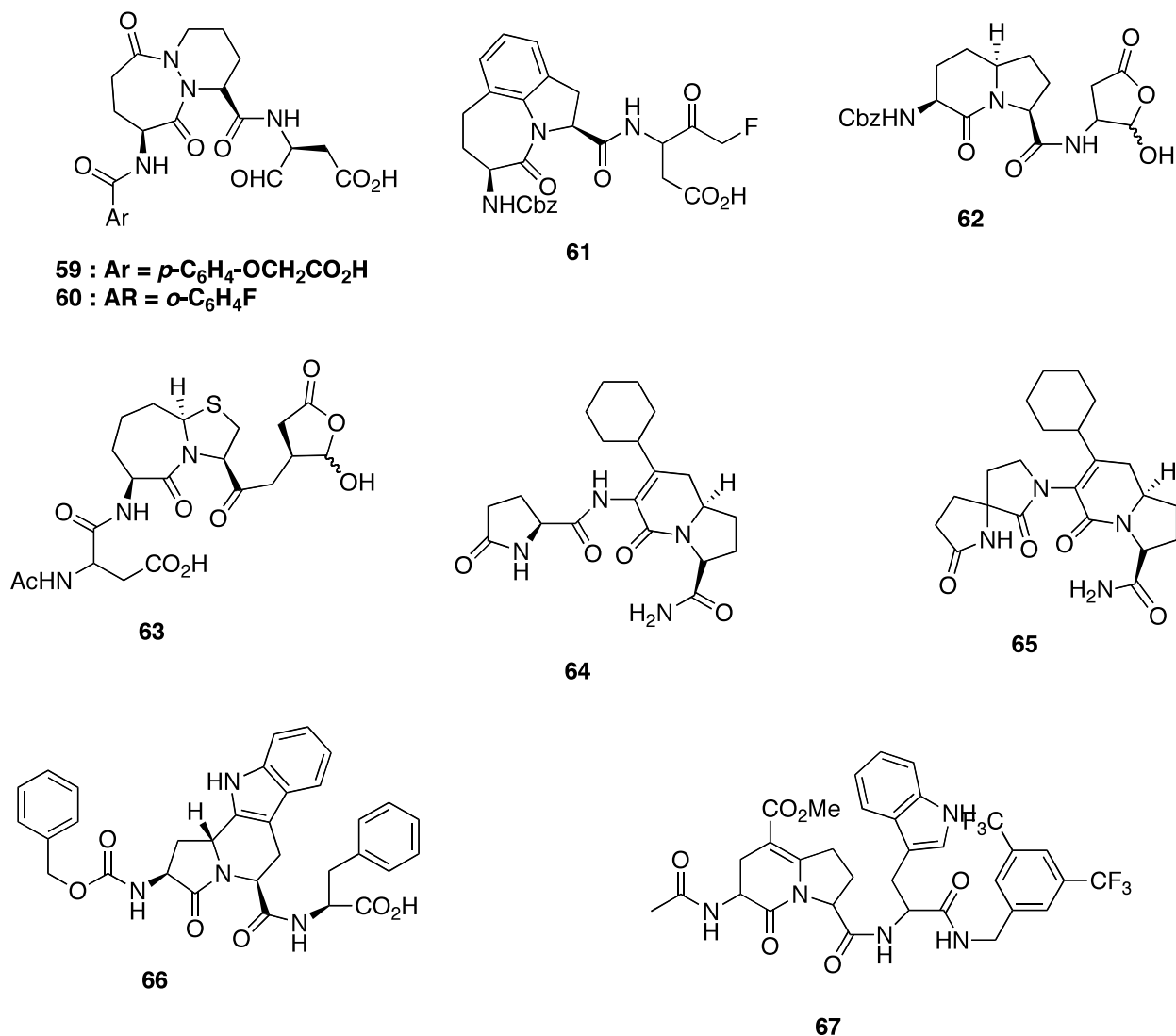


Figure 1.13. Various GPCR ligands

Various constrained unnatural bicyclic amino acid analogs have been explored as G protein-coupled receptor (GPCR) ligands. G protein-coupled receptors comprised of a large protein family of transmembrane proteins that receive chemical signals from outside the cell, and activate inside signal transduction pathways and cellular responses.

GPC receptors involve in several diseases, and have become targets for almost 30% of the medicinal drugs that are currently being used in the treatment of various diseases. When a ligand binds to the GPCR, it causes a conformational change in the GPCR, and activates G protein. Further effect depends on the G protein that involved in signal transduction or disease. Bicyclic scaffolds **64** and **65** act as TRH-R1/2 partial agonists with K_i 1.4 μ M and 1.5 μ M, respectively.^{50, 51} Compound **66** has shown antagonist activity against the Cholecystokinin (CCK1) receptor. Dihydroindolizidinone tripeptide mimic **67** acted as a specific neurokinin (NK1) receptor antagonist with K_i = 79 nM.^{54, 55}

Aeruginosins

The novel bicyclic amino acid 2-carboxy-6-hydroxyocta-hydrindole (Choi) is important motif commonly shared among aeruginosins. Aeruginosins are linear tetrapeptides isolated from microcystic aeruginosa, which is the most common toxic cyano bacterium in eutrophic fresh water.^{37, 57} Cyanobacteria are prokaryotic photosynthetic microorganisms that are a rich source of a variety of structurally novel bioactive nitrogen compounds. Among the wide range of serine protease inhibitors found in cyanobacteria, aeruginosin 298 A, the first member of the family, was isolated by Murakama and co-workers in 1994.⁵⁶ In the span of a decade, continuous search for biologically active natural products resulted in isolation of 20 new aeruginosins that are structurally closely related. They were isolated from geographically different locations from different sources having no clear relationship to the Microcystis waterbloom.^{37, 56-66} Regardless of diverse origin, all aeruginosin family members are comprised of the distinctive *cis*-fused 2-

carboxyperhydrindole as the core structure. With few exceptions, they exhibited varying degree of inhibitory activity against one or more serine proteases. Almost all aeruginosins showed in vitro activity mainly against serine proteases: Thrombin, Trypsin, and Factor VIIa. Some of the aeruginosins have also been evaluated against other enzymes, such as plasmin, chemotrypsin, elastase, and cystine protease papain. For simplification, generalized structure of aeruginosins virtually can be divided into four common portions: a C-terminal guanidine containing group P1, a 2-carboxyperhydrindole core P2, a bulky hydrophobic amino acid P3 and an N-terminal hydroxy or acidic group P4.

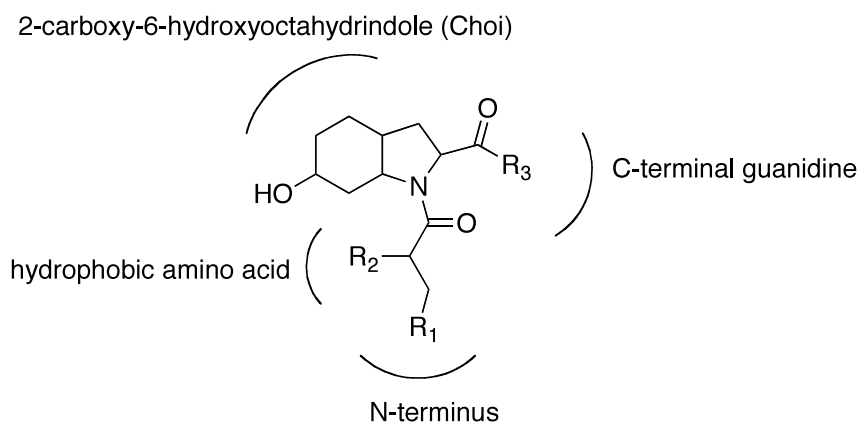


Figure 1.14. Generalized structure of the aeruginosins

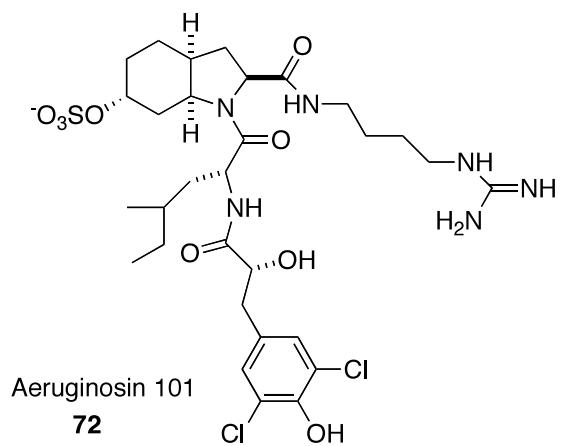
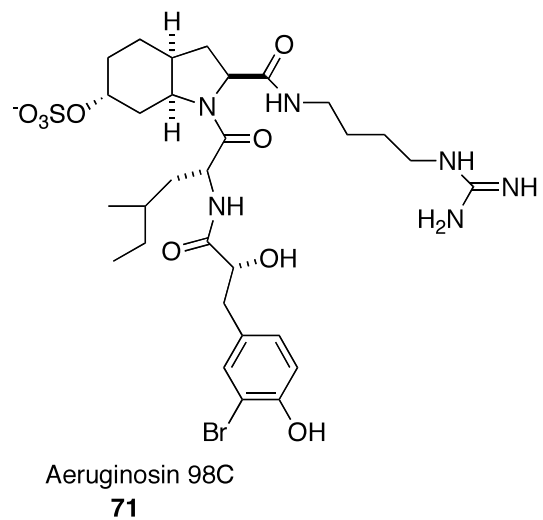
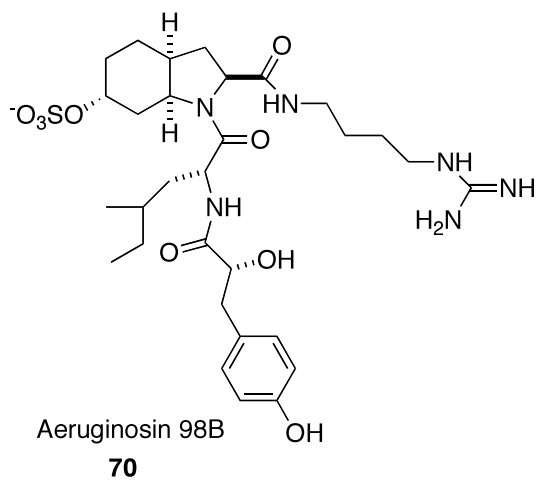
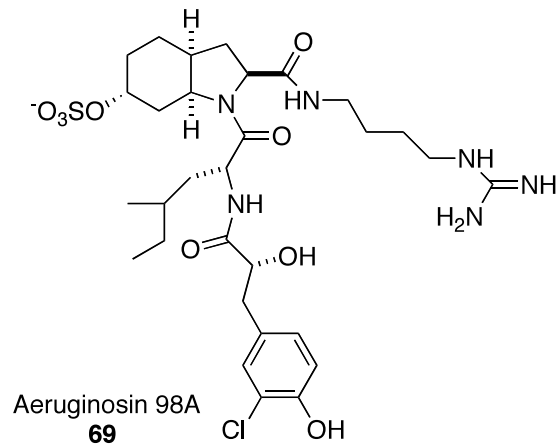
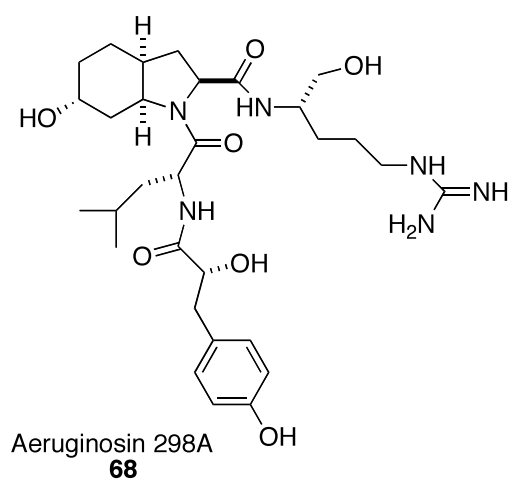


Figure 1.15. Natural product aeruginosins

Aeruginosin 98A (**69**), aeruginosin 98B (**70**), aeruginosin 98C (**71**), and aeruginosin 101 (**72**) have three common subunits: P1 basic guanidine group, O-sulfate L-Choi as P2, and D-allo-Ile as P3 subunit. The fourth subunit is D-Hpla with different substituents on the aromatic ring. They showed significant potency against thrombin, but they did not show any selectivity to thrombin over trypsin. Whereas, aeruginosin 98A and aeruginosin 98B showed moderate selectivity in favor of trypsin. Presence of halo substituents on the aromatic ring of D-Hpla seems to be causing loss of selectivity.

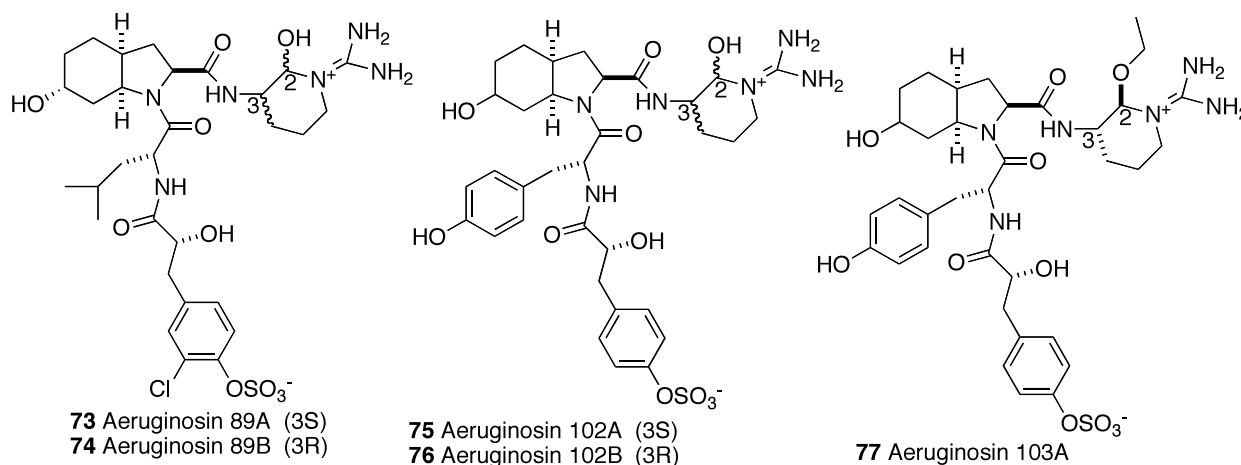


Figure 1.16. Aeruginosins that differ at P₃ residue

Compounds **73-77** contain argal group in cyclic form (6 membered) as P1 moiety, and O-sulfated D-Hpla P4 residues as common subunits. Aeruginosins 89A and 89B have D-Leu, while other three compounds: aeruginosins 102A, 102B, and 103A have D-Tyr as hydrophobic residue P3. Except 103A, in which hydroxy group is protected as an ethyl

ether, all other four compounds showed excellent activity against both thrombin and trypsin. Above details indicate that modifications in P3-P4 portion don't have much impact on potency.

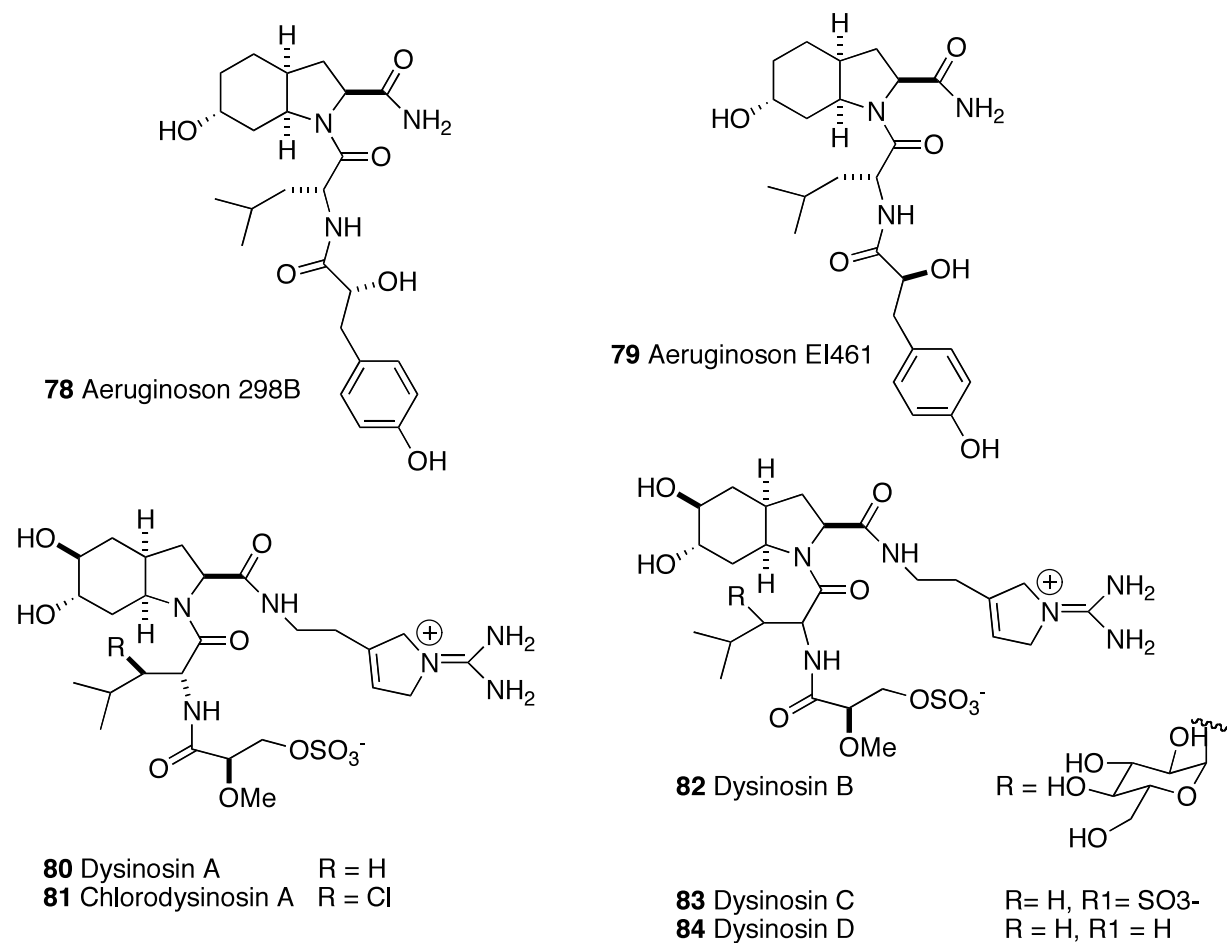


Figure 1.17. Aeruginosin 298 B, aeruginosin EI461, and dysinosins with cyclic arginine analog.

Aeruginosin 298B and aeruginosin EI461, without P1 subunit, lost their biological activity. It clearly indicates that P1 basic unit with guanidine group is essential for activity. P1 basic subunit and O-Me-O-SO₃⁻ D-lysine acid influencing biological activity in compounds dysinosin A (**80**), Chlorodysinosin A (**81**), and dysinosin C (**83**). Particularly,

Chloro substituent in Chlorodysynosin A appears to be responsible for high activity. Aeruginosin 205 B has 2-chloro substituted D-Leu showed significant potency. Hanessian and group have explained this phenomenon as chlorine effect.

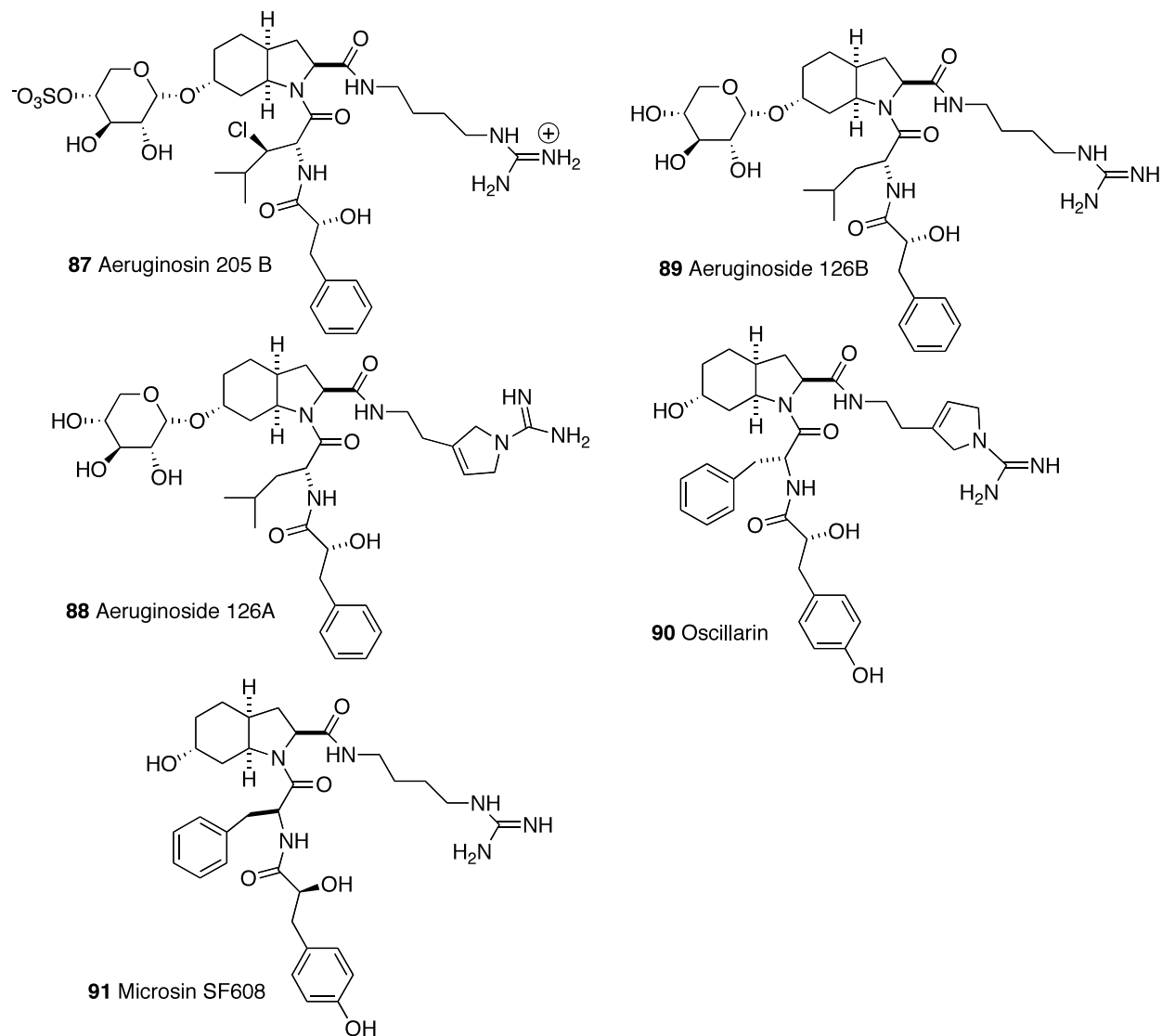


Figure 1.18. Comparison of the compounds with variations in P1, P2, P3 and P4 subunits.

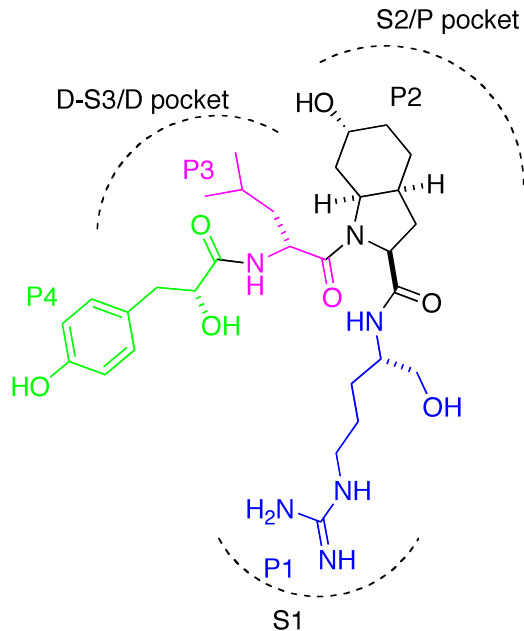


Figure 1.19. Schematic representation of thrombin active site bind to aeruginosin 298A

X-ray crystal structures of the enzyme in complex with several aeruginosin family members provided valuable information about enzyme–inhibitor interactions.⁵⁶ Most of the aeruginosin family members interacted with the active site of thrombin enzyme in a similar way and revealed the most important regions of the thrombin active site for inhibition. As shown in the above figure subsites S1, S2, and S3 are crucial for the biological activity. The S1 subsite, also known as the specificity pocket, can recognize and engage in ionic interactions with inhibitors containing C-terminal guanidine group P1. Carboxy hydrindole core structure P2 residue fits into S2 packet. Hydrophobic amino acid residue P3 and hydroxyphenyllactic acid (Hpla) P4 interact with S3 subsite.

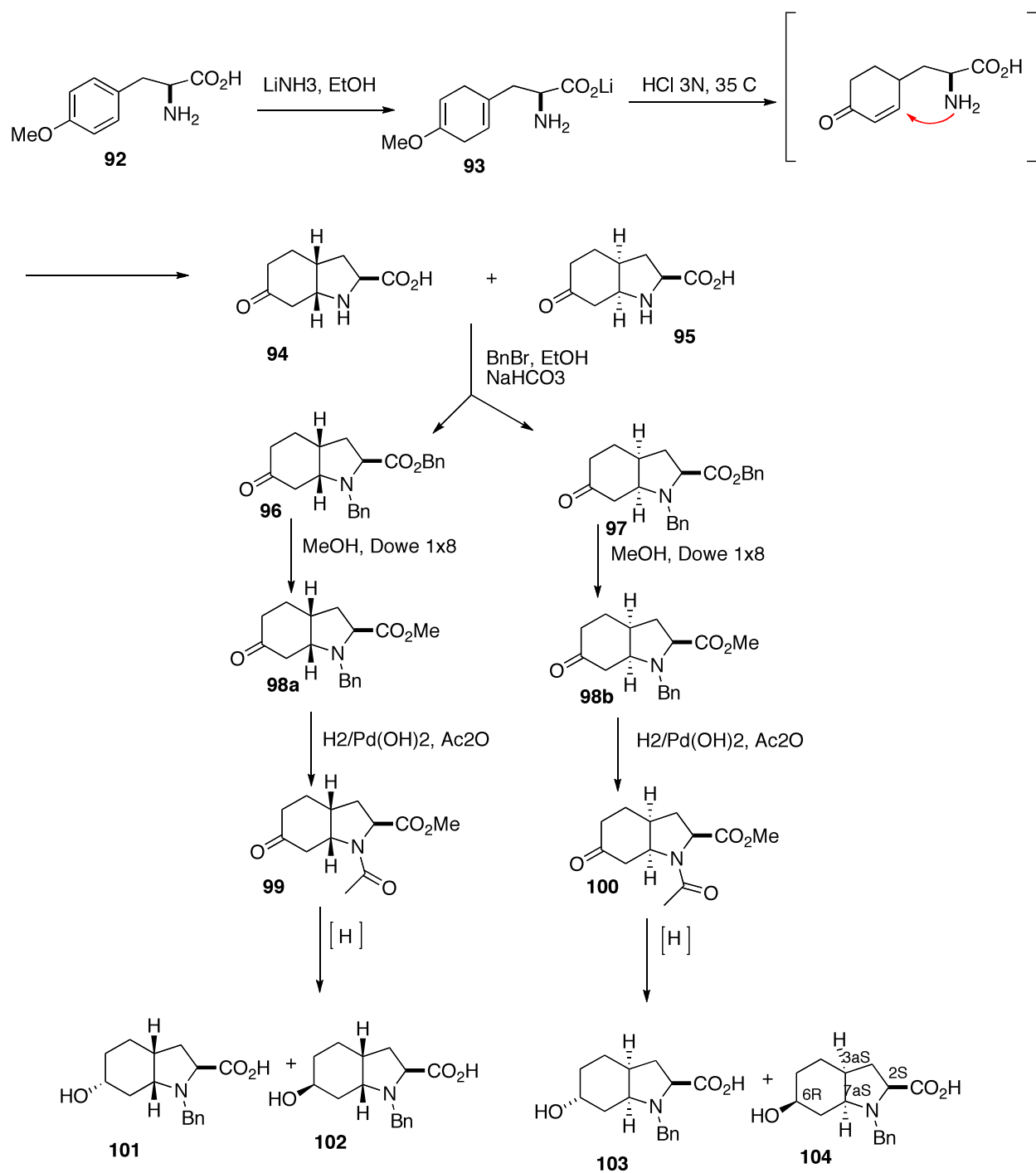
So far, X-ray crystal structures of thrombin enzyme complex with Aeruginosin 298A, dysinosin A, oscillarin, and chlorodysinosin A have been reported. All of them revealed similar binding modes in the active site, with slight variations in orienting polar substituents.⁶⁸⁻⁷¹ there are no hydrogen bonds between the hydroxy groups of the octahydrindole and thrombin active site is evident. Therefore, we can conclude that hydroxy groups on bicyclic core structure have no influence on biological activity. Sandler and co-workers have reported X-ray crystal structure of trypsin complex with aeruginosin 98B. They speculated that the selectivity of 6-O- sulfate aeruginosin 98B to trypsin over thrombin might be due to the sulfate group that was projected into the hydrophobic pocket of thrombin active site. Whereas in case of trypsin the sulfate group of aeruginosin 98B was projected in to the solution present out side the active site.⁷² Bicyclic amino acid core structure is essential to bring rigidity and stability to aeruginosins.

Synthesis of bicyclic amino acid core structure of aeruginosins:

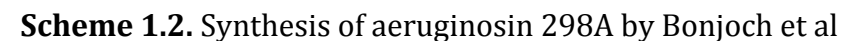
Thrombosis and related complications are major causes of potentially fatal cardiovascular and cerebrovascular disease throughout the world.⁷³ Current anticoagulation therapies, such as the administration of heparins and coumarins, are limited by narrow therapeutic windows, severe side effects, and/or the need for parenteral administration.⁷⁴ Intensive efforts are being made to develop new anticoagulants relying on direct inhibition of coagulation enzymes.⁷⁵⁻⁷⁸

The central role of thrombin in the blood coagulation cascade has made it an attractive target for the development of antithrombotic drugs.⁷⁹⁻⁸⁴ Thus, aeruginosins, which exhibited inhibitory potency against blood coagulation factors, have become attractive small-molecule targets in the search for new anticoagulants. For some of the members of aeruginosin family, stereochemistry assignments were not fully established. Several members were revised, and still being revised. A structural revision of aeruginosin 205B has been reported in 2010.⁹²

Synthesis of constrained bicyclic amino acid core structure, L-Choi, achieved by Bonjoch et al. Absolute stereochemistry of L-Choi (104) has also been established as (2S, 3aS, 6R, 7aS)-6-hydroxy octahydrindole-2-carboxylic acid. The azabicyclic core unit synthesis commenced from aromatic amino acid **92**. Birch reduction followed by acidic cleavage of the enol ether, and Michael-type addition of the pendant amine nucleophile provided a mixture of isomers **94** and **95**. After benzylation of acid and amine groups, benzyl ester was converted to methyl ester on treatment with methanol in the presence of acid resin. On treatment with concentrated acid, **98a** would be converted to more stable and desired bicyclic intermediate **98b**. Benzyl deprotection and acetyl protection of secondary amine followed by reduction of keto function group yielded desired bicyclic core structure **104 (scheme 1.1)**.⁸⁵

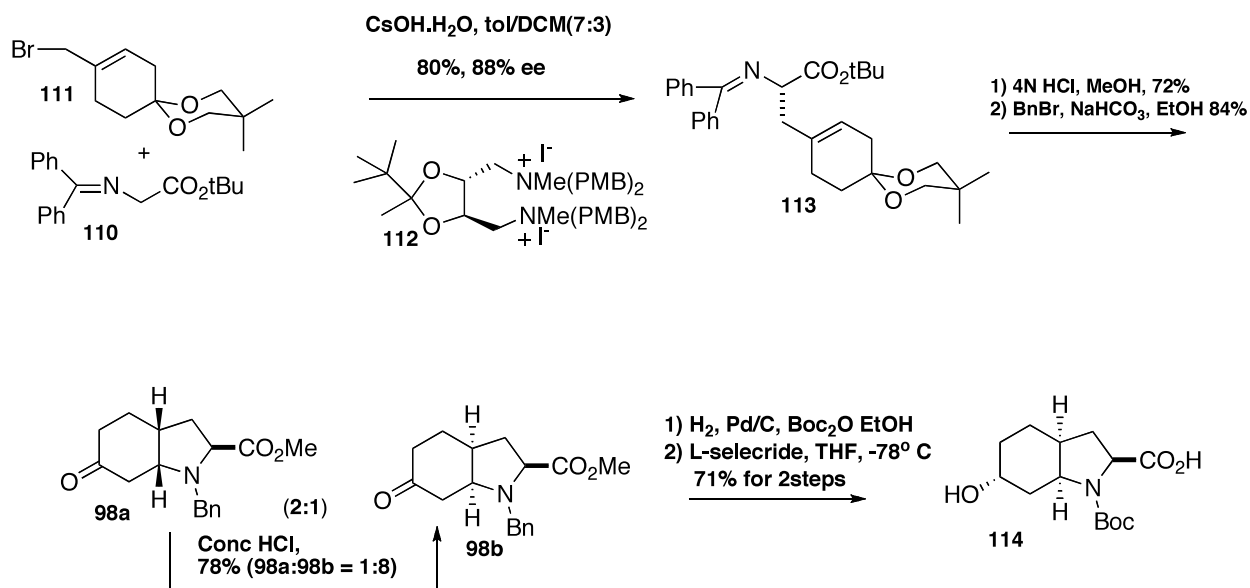


Scheme 1.1. Synthesis of methyl 6-hydroxy-*cis*-octahydrindole-2-carboxylate derivatives by Bonjoch et al



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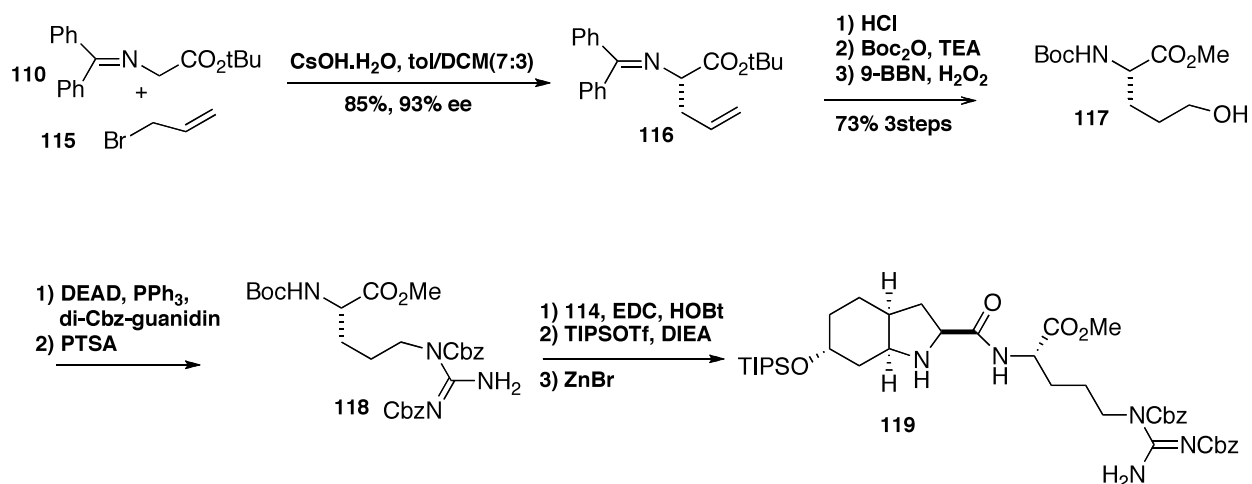
OH resulted in dipeptide **107**. Following the same protocol, coupling of protected hydroxy phenyllactic acid with intermediate **107** resulted in tripeptide **108**. Hydrolysis of methyl ester, and coupling with L-Arg (NO₂)-OMe.HCl followed by removal of the protecting groups by hydrolysis afforded aeruginosin 298A.



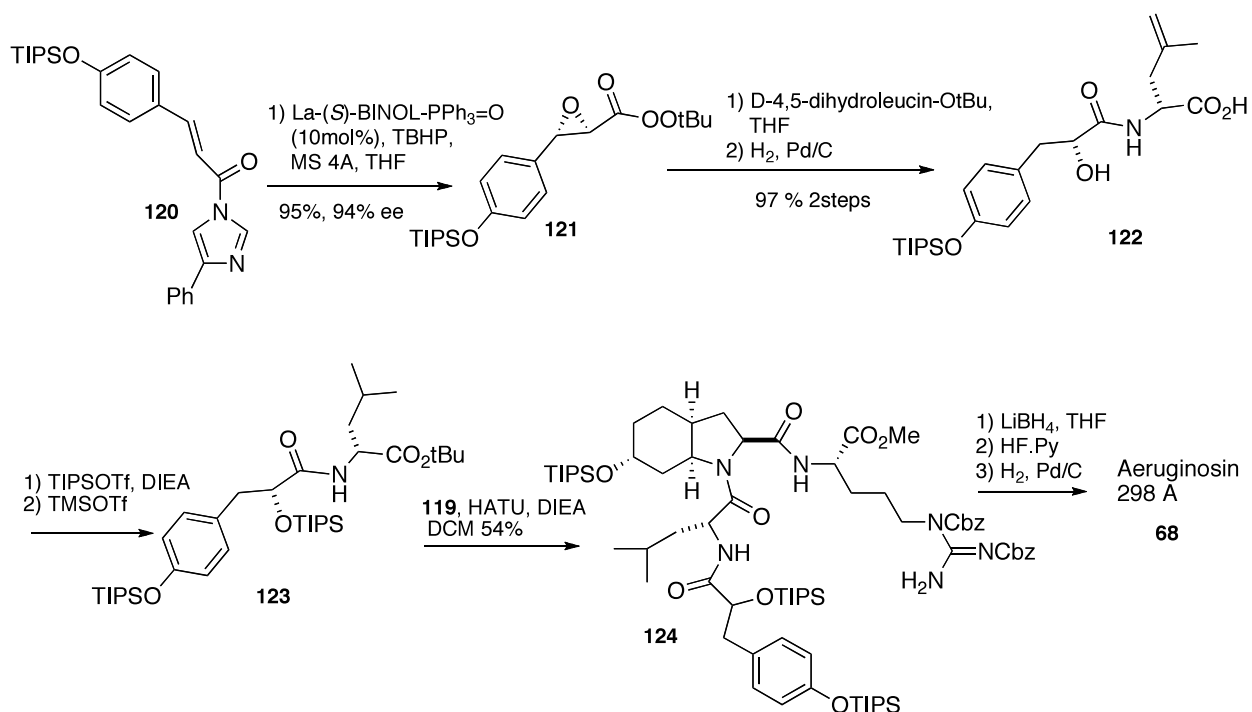
Scheme 1.3. Synthesis of bicyclic core structure by Shibasaki et al

In 2004, Shibasaki and group reported a versatile synthetic process for aeruginosin 298A as well as several analogs. Stereochemistry of all the centers was controlled by catalytic asymmetric phase-transfer reaction promoted by two-center asymmetric catalysts **112a** & **112b**. Asymmetric epoxidation promoted by a Lanthanide-BINOL complex.⁸⁹⁻⁹⁰ Synthetic strategy for bicyclic amino acid core structure **114** and then aeruginosin 298A was commenced from compound **111**. Reaction between enolate of **110** and allyl bromide derivative **111** in the presence of 10 mol% of **112a** as a catalyst to give **113**

(80% yield and 88% ee). Ketal deprotection and subsequent intramolecular Michael-type addition afforded bicyclic undesired ketone **98a** and desired ketone **98b** in 2:1 ratio. Then, acid treatment of **98a**, following the method previously reported by Bonjoch and co-workers, yielded **98b** in 78% yield.



Scheme 1.4. Synthesis of **119** by Shibasaki et al



Scheme 1.5. Synthesis of aeruginosin 298A by Shibasaki et al

Allylglycine precursor **116** was obtained from compound **111** and allyl bromide **115** by utilizing asymmetric phase-transfer alkylation in the presence of **112b**. Functional group deprotection, Boc protection, hydroxylation by asymmetric hydroboration followed by oxidation yielded **117**, which further transformed into guanidine intermediate **118**. Peptide coupling with **114** followed by Boc removal afforded dipeptide L-Choi-Argal intermediate **119** in 72% yield. Asymmetric epoxidation of **120** gave **121**, and then converted to amide **122** on coupling with D-4, 5-dehydroleucine-OtBu. Reduction of the double bond by catalytic hydrogenation, protection of alcohol, and cleavage of the t-butyl ester gave Hpla-Leu fragment **123**. Coupling with **119** in the presence of HATU yielded **124** and followed by methyl ester removal afforded aeruginosin 298A.

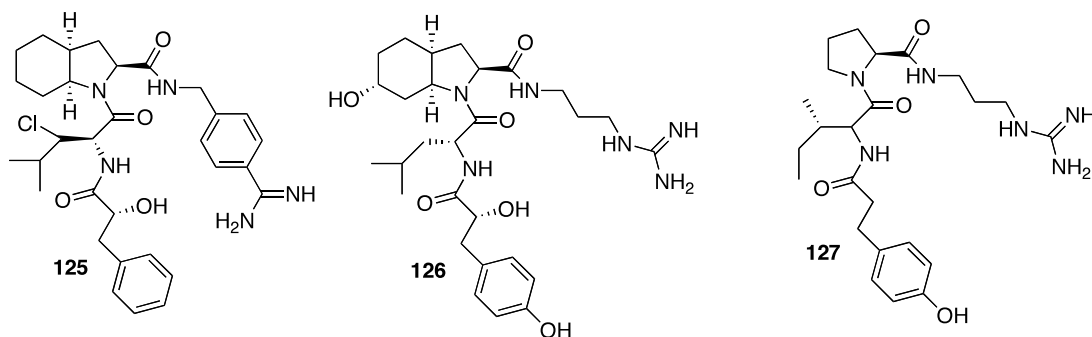
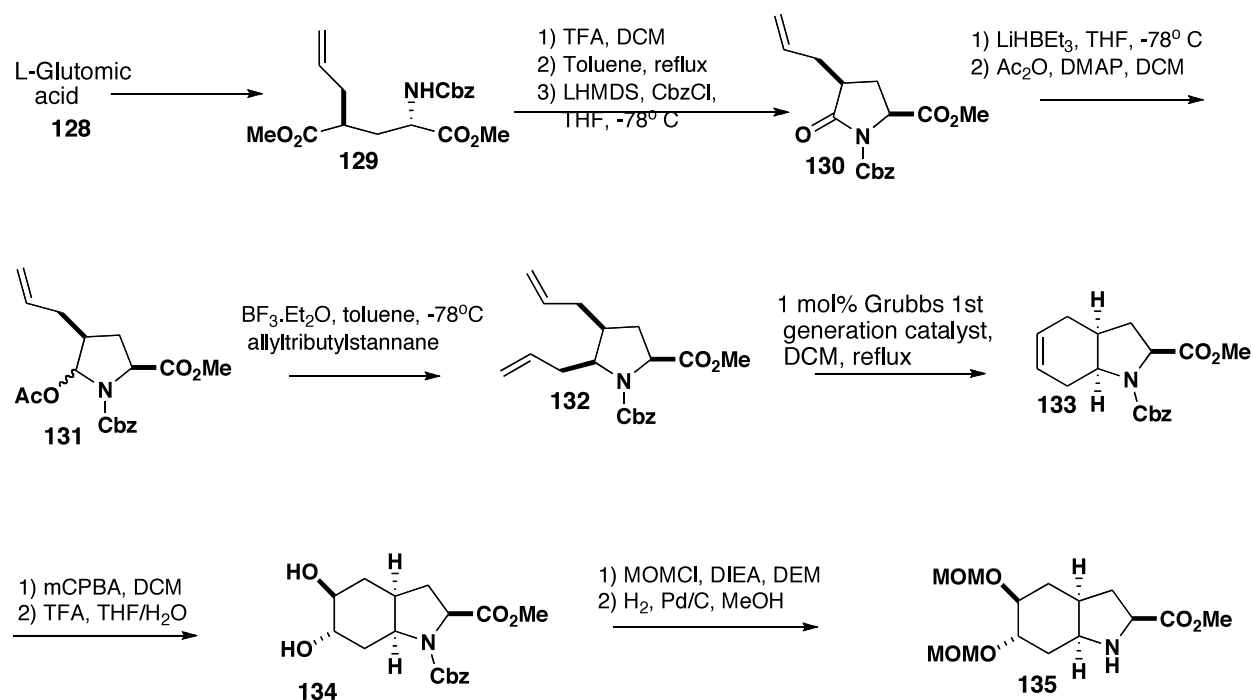


Figure 1.20. Some of the synthetic aeruginosin analogs

Total synthesis of other members of aeruginosin family also being pursued by several research groups. Hanessian and co-workers via ring-closing metathesis achieved first synthesis of a potent thrombin and factor VIIa inhibitor dysinosin A. This method conformed the structure of dysinosin A and provided an entry into the novel 5-hydroxy L-

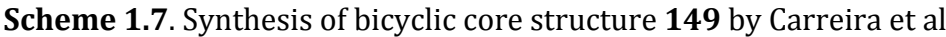
Choi and Adc structural motifs. Allylglutamate precursor **129**, which is readily available from L-glutamic acid in high yields, converted to pyroglutamate **130**, followed by reduction of the lactam function group with super-hydride (LiHBEt₃) and O-acetylation of the intermediate provided **131** in 85% yield. Alkylation of the N-acyl iminium ion intermediate was achieved in the presence of allylbutyl stannane and BF₃.Et₂O to give **132**, and then treatment with Grubb's second-generation catalyst in refluxing dichloromethane yielded bicyclic alkene **133**. Trans dihydroxylation and protecting group manipulation gave 5-hydroxy L-Choi derivative **135** in 44% overall yield from **129**.



Scheme 1.6. Synthesis of Choi analog **135**

So far, limited number of total syntheses of natural product aeruginosins has been achieved. Quite a few numbers of synthetic aeruginosin analogs were reported. Considerable amount of variations made to P1, P3 and P4 residues, but very limited number of efforts have been made to synthesize aeruginosin analogs, with various synthetic octahydrindole analogs as a core structure. Compounds **125**, **126**, and **127** are the representative members of aeruginosin analogs with different bicyclic scaffold. Among all the natural product aeruginosins and their synthetic analogs, compound **125** is the most potent thrombin inhibitor reported to date. Several research groups have also reported the synthesis of various Choi analogs and their improved synthetic methodologies. Some of the significant methodologies have been discussed below.

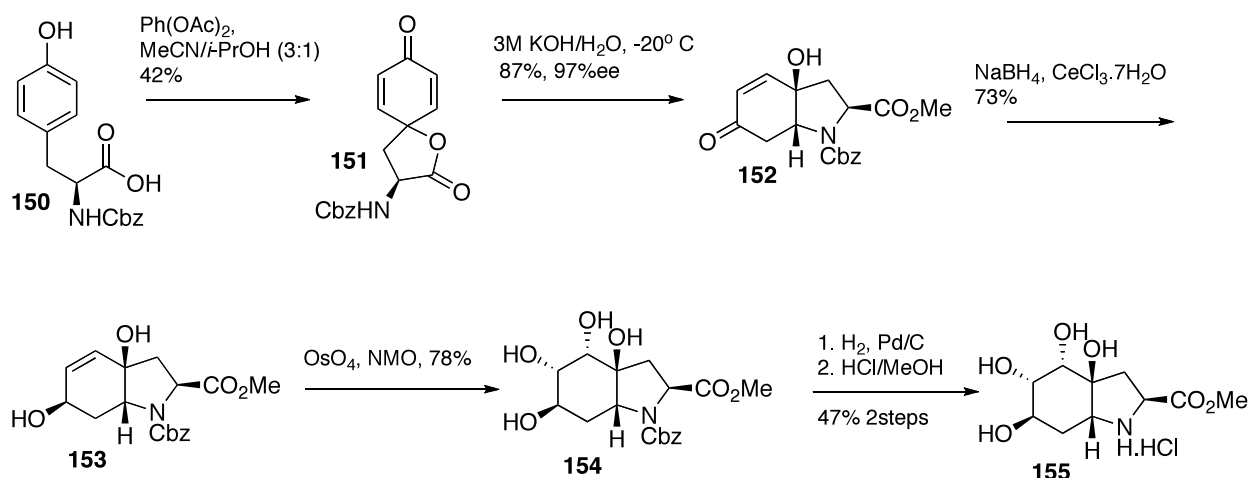
Tandem catalysis has been utilized to perform Diels-Alder reaction between bromoacrolein and furan followed by Mukaiyama Aldol reaction affording bromo intermediate **139**, with decent stereo selectivity (dr. 5:1, ee. 86:14).⁹¹⁻⁹³ Then treated **139** with potassium tertiary butoxide to obtain intermediate **140**. Compound **140** was treated with m-CPBA to form epoxide, then H₂/Pd reducing conditions were employed to reduce α , β - unsaturation, followed by K₂CO₃ treatment provided lactone intermediate **141**.



Azalactone **143** was obtained by treating **141** with bulky base and **142**. Lactone of **143** was opened, and the resulting azide was reduced to amine and protected using CbzCl. Epoxide was opened selectively using low-valent Ti reagents followed by resulting 2° alcohol was protected selectively as acetate, then 3° alcohol was removed by following the previously reported procedure. Deprotection of Cbz group followed by TMSOTf mediated nucleophilic opening yielded dihydroxy octahydrindole **149**, the core structure of MicrocineSF608.⁹³

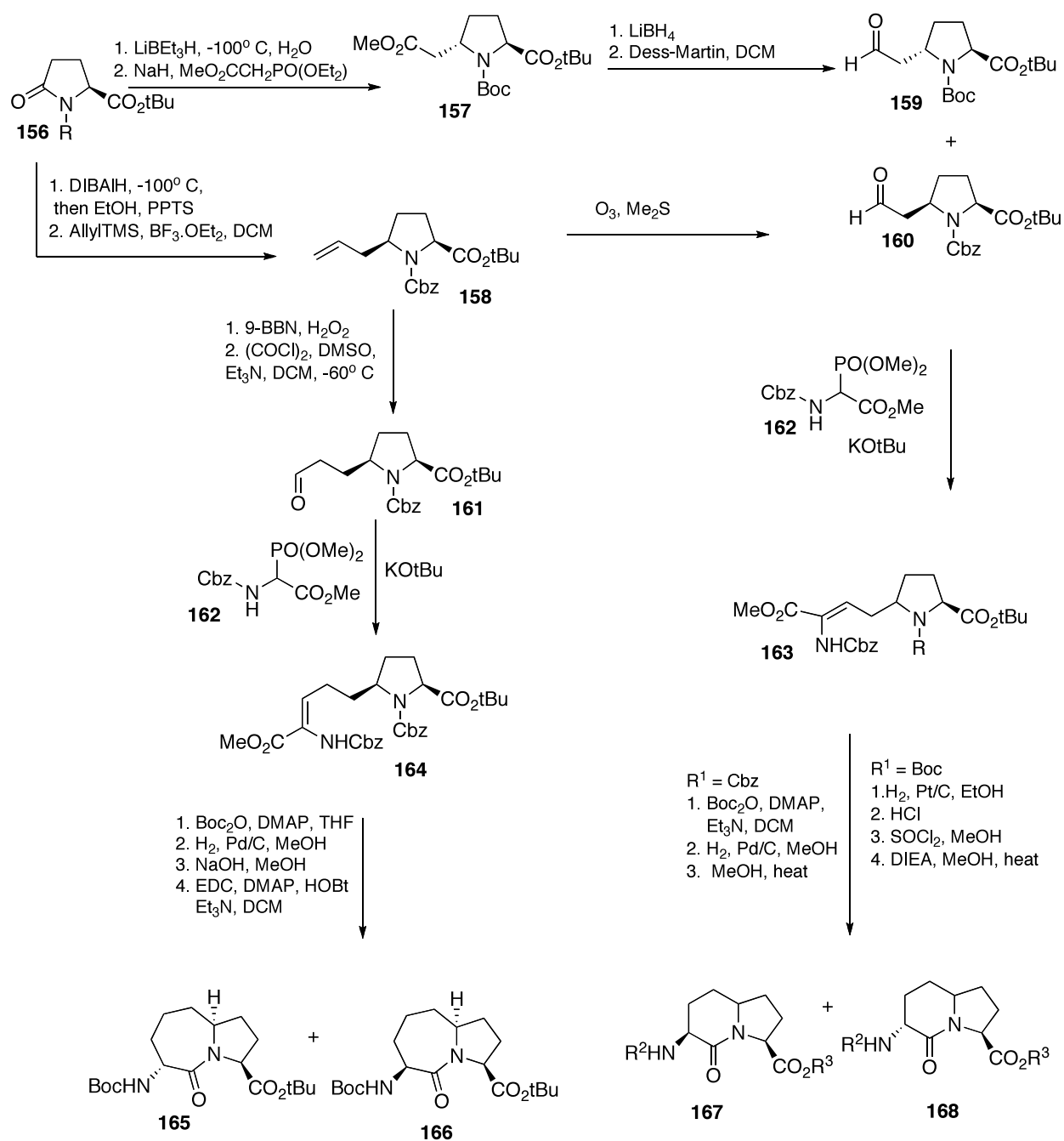
Synthesis of other bicyclic amino acids

Many other bicyclic scaffolds that closely resemble Choi analogs, like tetrahydroxy octahydrindole **155**, consist in natural product alkaloids. Some of the hydroxy indolizidine alkaloids noteworthy to mention are: lentiginosine, an amyloglucosidase inhibitor; swainsonine, an α -mannosidase inhibitor; castanospermine, an α -glucosidase inhibitor. Synthesis of tetrahydroxy octahydrindole **155** and other prominent bicyclic amino acid analogs, mainly azabicyclo amino acids and bicyclic scaffolds with more than one heteroatom, have been discussed below.⁹⁴

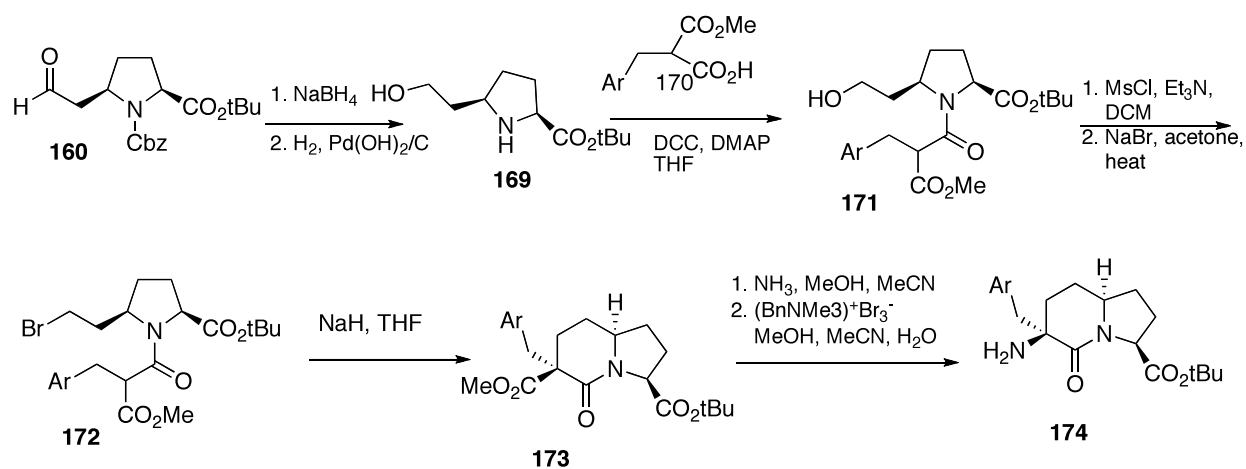


Scheme 1.8. Synthesis of tetrahydroxy octahydrindole **155**

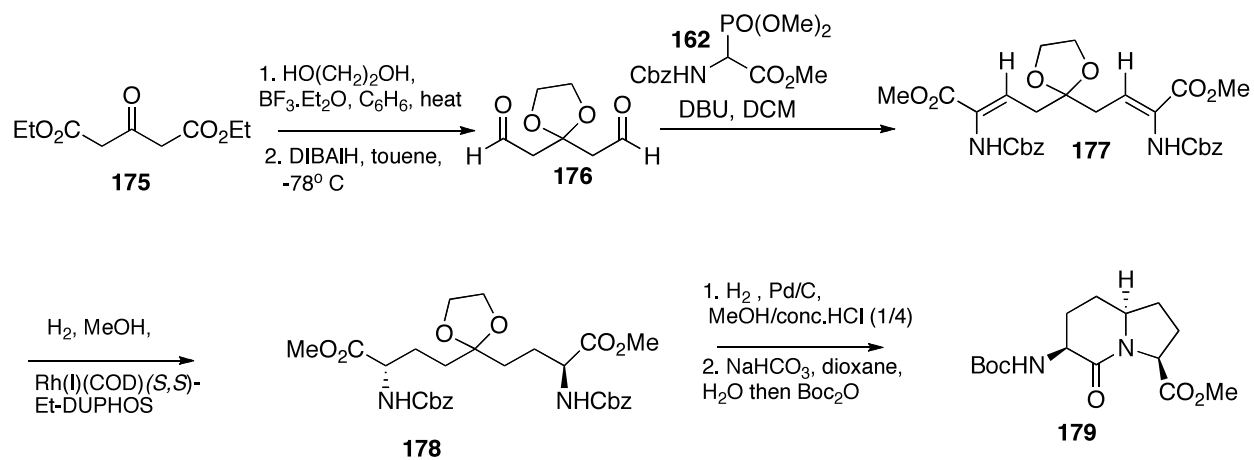
Azabicyclo amino acid analogs **165-168** can be obtained from a common starting material pyroglutamate **156**. DIBAL-H reduction of **156**, followed by reaction with allyltrimethylsilane provides intermediate **158**. Terminal hydroxylation using 9-BBN and Dess-Martine periodinane oxidation of resulting primary hydroxy group gives aldehyde intermediate **161**. Reduction of unsaturation of **164** followed by saponification and peptide coupling afford azabicyclo amino acids **165** and **166**.⁹⁵ Following the similar reaction conditions, **167** and **168** can also be obtained from their respective starting materials **159** and **160**.⁹⁶ Another azabicyclo amino acid **174**, with aryl substitution, obtained from **160** in 8 steps starting from **160**, as shown in **scheme 10**.⁹⁷



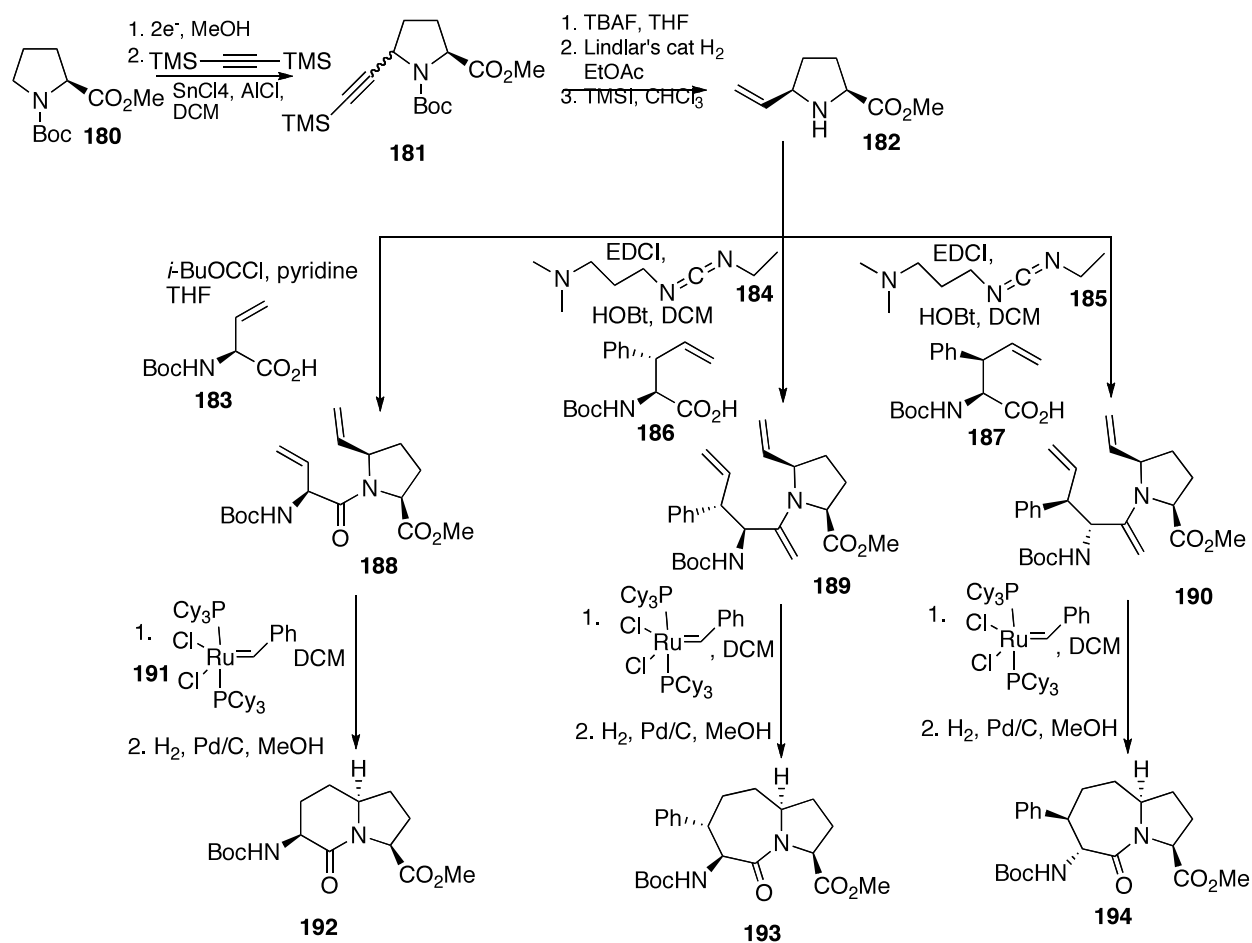
Scheme 1.9. Synthesis of various azabicyclo amino acids **165-168** starting from **156**



Scheme 1.10. Synthesis of **174** from proline derivative **160**

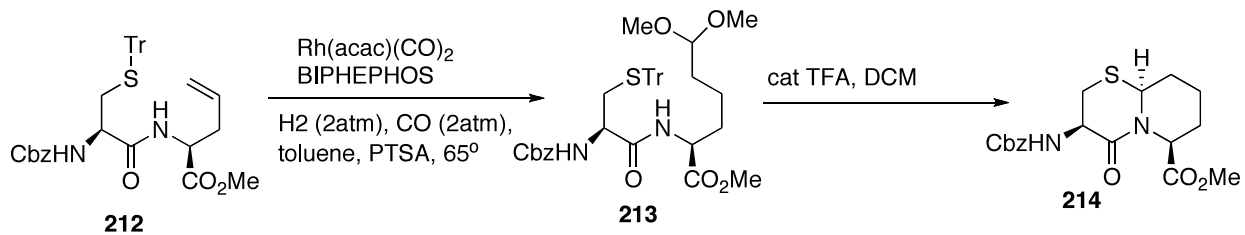
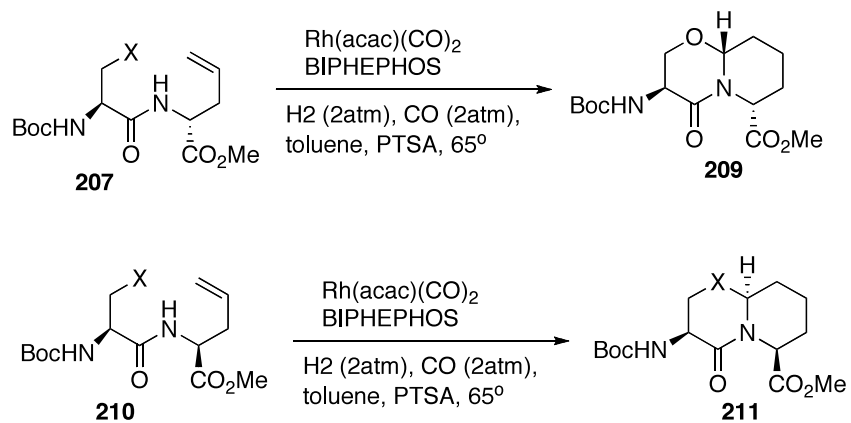
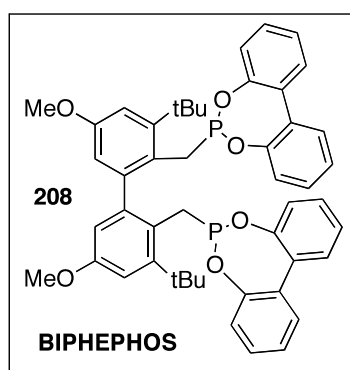
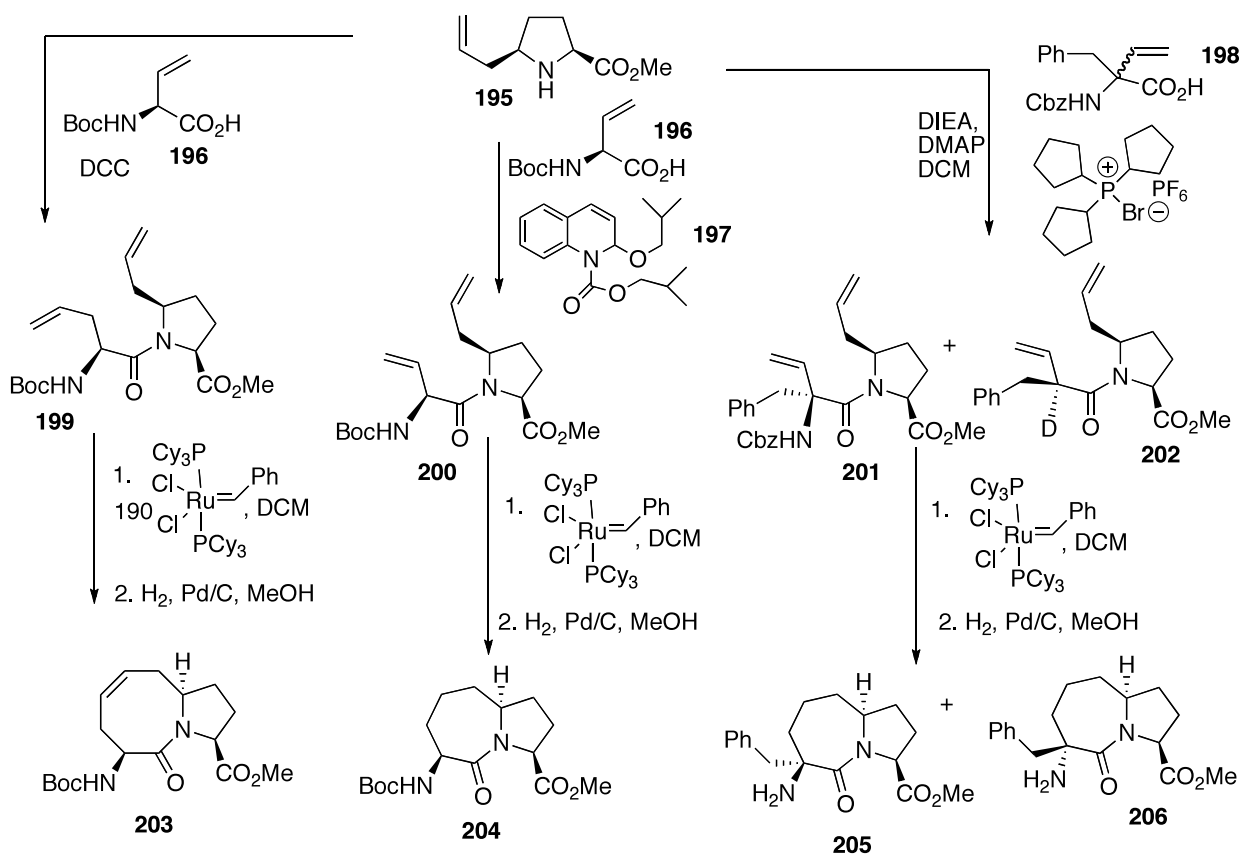


Scheme 1.11. Synthesis of **175** from proline derivative **179**



Scheme 1.12. Synthesis of azabicyclo amino acids **192- 194** from proline via ring cycloalkene metathesis

Synthesis of bicyclic amino acid **179** from keto diester **175** in 6 steps has been reported. Protection of keto function group using ethylene glycol followed by reduction of ester function groups in the presence of mild reducing agent DIBAL-H at low temperatures afford dialdehyde intermediate **176**, then let it react with 2 equivalents of **162** and undergo reduction to give **178**. Deprotection of the amine group and keto group followed by cyclization under mild basic conditions yielded **179**.⁹⁸



Scheme 1.13. Synthesis of various bicyclo amino acids

Bicyclic amino acids **192-194** can be obtained from proline derivative **180**. Cis-vinyl proline (5R) **182** was synthesized by oxidation of **180** and addition of bis(trimethylsilyl)acetylene followed by Lindlar's reduction provides **182**. Diene **188** was obtained from compound **182** by treating with compound **183**. Then, ring closing metathesis conditions were employed to compound **188** to yield bicyclic scaffold **192**. Following similar reaction conditions, azabicyclic amino acid analogs **193** and **194** have been synthesized from compound **182**, as shown in scheme 12.⁹⁸

Azabicyclo compounds **203-206** have been obtained from a homo allyl proline derivative **195**, which is a homologue of compound **182**.^{98, 99} Similar reaction conditions as used in **scheme 1.12** were employed to compound **195** to obtain corresponding products **199, 200, 201**, and **202**. Finally, ring closing metathesis of dienes **199-202** using reagent **190** yields their corresponding bicyclic scaffolds **203-206**, as shown in **scheme 13**.

Synthesis of hetero bicyclic amino acid analogs oxa-quinolidine ester **209** and aza-quinolidine ester **211** were obtained from Boc-serinyl-allylglycine methyl ester **207**. Thia-quinolidine ester **214** was obtained from S-(trityl)-N-(Boc)cysteinyl-allylglycine methyl ester **212** in excellent yield.¹⁰⁰

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Chapter 2: Synthetic process towards bicyclic amino acids

Introduction

Conformationally constrained bicyclic amino acids and their derivatives are prominent biologically active compounds in the treatment of many diseases.^{1, 2} Various bicyclic and hetero bicyclic amino acids are essential core structures of many biologically active natural products and peptidomimetics.³⁻⁵ Rigid bicyclic amino acid, 2-carboxy-6-hydroxyl octahydrindole (L-Choi) or its close derivative (**Fig. 2.1**), is a core structure of aeruginosin family members. Aeruginosins are the tetrapeptide natural product serine protease inhibitors isolated from the marine sponges and cyanobacterial water blooms. Almost all the members of aeruginosin family showed promising inhibitory activity against thrombin, trypsin, and some of them also showed significant activity against factor VIIa.⁶⁻¹⁶

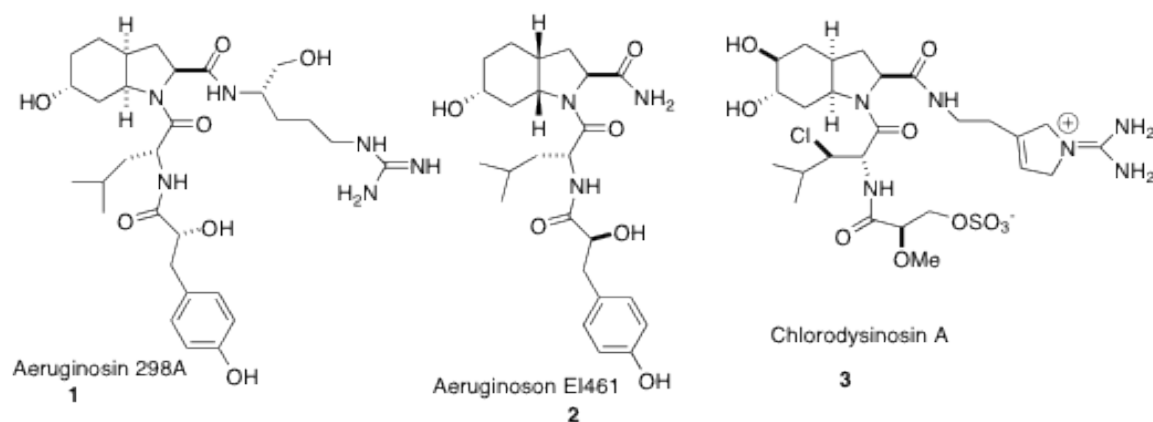


Figure 2.1. Aeruginosin family members having different bicyclic core structures

Thrombin and factor VIIIa play crucial role in blood clotting cascade; Thrombin converts soluble fibrinogen to fibrin and favors the formation of cross-linked bonds between fibrin molecules to form a mesh that stabilizes blood clot, also activates several coagulation factors.¹⁷ Excessive generation of fibrin due to activation of the coagulation cascade leads to thrombosis; hence, inhibition of thrombin is essential in the prevention and treatment of cardiovascular and cerebrovascular diseases. According to American heart association statistics, 36.2 % of people are suffering from cardiovascular or its related diseases; each year 831,000 people are succumbed to death. Current major anticoagulants have many disadvantages, such as narrow therapeutic windows, side effects, and need for parenteral administration. For example, direct thrombin inhibitors such as hirudin, dabigatran, and melagatran are suffered from expeditious clearance by kidneys, and accumulation of these drugs leads to renal impairments.¹⁷ There is a greater urgency to develop new class of anticoagulants that overcome disadvantages of currently available drugs.

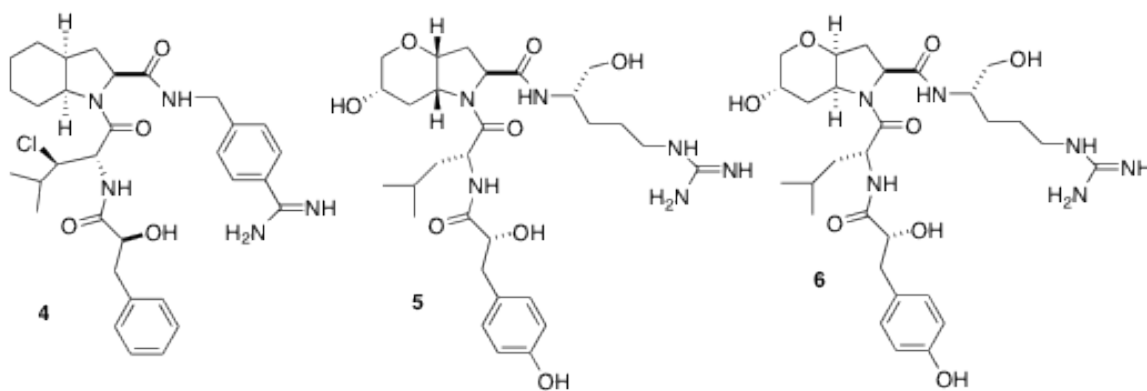


Figure 2.2. Some of the synthetic aeruginosin analogs

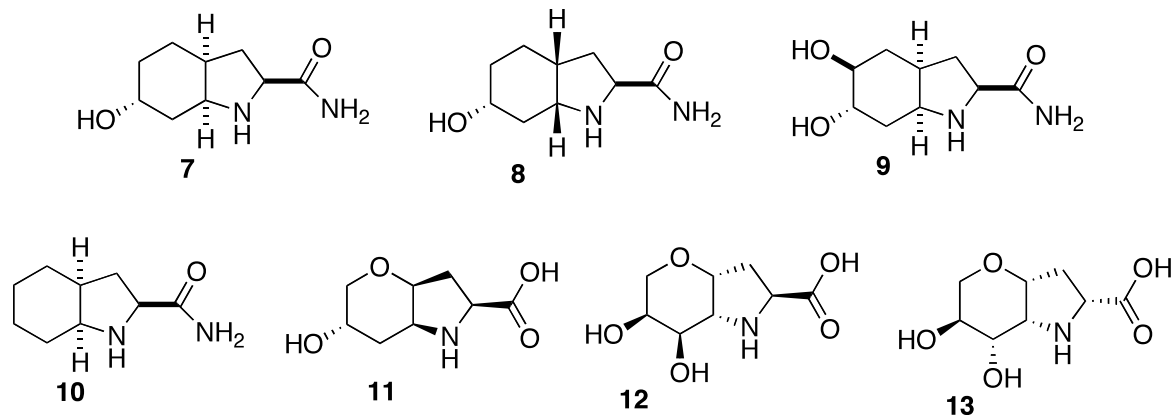


Figure 2.3. Different core structures of natural product aeruginosins **7- 9** and synthetic Choi analogs **10 – 13**

Promising thrombin inhibitory activity of aeruginosins made them attractive small molecule drug targets. Constrained cis-fused bicyclic amino acid core structure is vital to keep the pharmacophores in appropriate position for maximum interactions with active site of protease enzyme. Several research groups have been pursuing synthesis of various synthetic aeruginosin analogs (**Fig. 2.2**) and their core structure L-Choi analogs (**Fig. 2.3**) to improve efficiency and selectivity.¹⁸⁻²⁴ Our research group has been pursuing synthesis of bicyclic amino acid core structure Choi analogs with oxygen substituent in 6-membered ring, and also with varying number and position of hydroxy groups on pyrano ring.²⁵⁻²⁸

Generalized structure of aeruginosins comprised of 4 common portions: a C-terminal guanidine containing group P1, a 2-carboxyperhydrindole core P2, a bulky hydrophobic amino acid P3 and an N-terminal hydroxy or acidic group P4 (**Fig. 2.4a**).

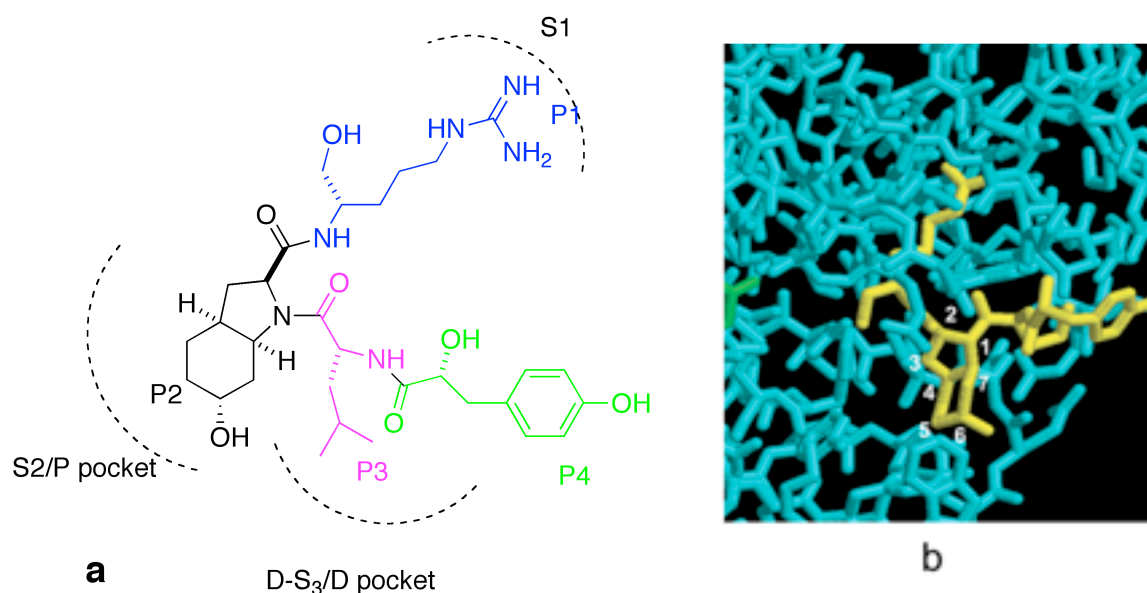


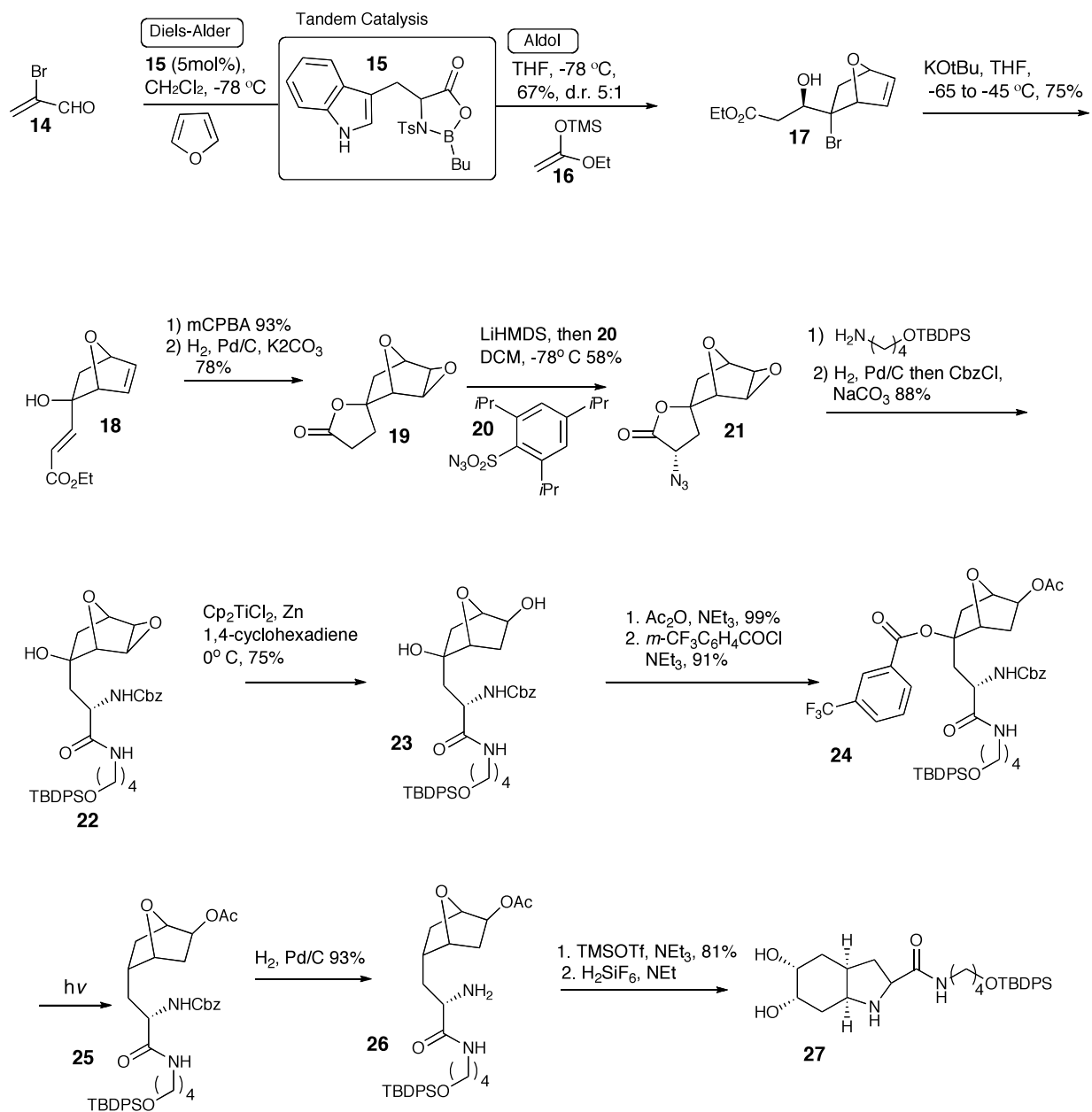
Figure 2.4. X-ray crystal structure of aeruginosin 298 A in complex with thrombin.²⁹

X-ray crystal structures of the enzyme in complex with several aeruginosin family members provide valuable information about enzyme–inhibitor interactions. Aeruginosins interact with the active site of thrombin enzyme in a similar way, and reveal the most important regions of the thrombin active site for inhibition are the S1, S2, and the d-S3 subsites, as shown in the above (**figure 2.4a**). **Figure 2.4b** represents X-ray crystal structure of aeruginosin 298 A in complex with thrombin. The S1 subsite, also known as the specificity pocket, can recognize and engage in ionic interactions with inhibitors that contain C-terminal guanidine group. Carboxy hydroxyindole core structure residue P2 fit into S2 packet. Hydrophobic amino acid P3 residue and hydroxyphenyllactic acid (Hpla) accommodate into S3 subsite.

Since both thrombin and trypsin belong to the same protease family (serine protease), their binding sites closely resemble with slight variations. Because of this proximity of binding sites, structurally closely related aeruginosin members inhibit both thrombin and trypsin without any distinction. Inhibition of thrombin renders disease curative effect, but inhibition of trypsin does not have any positive medication effect. Further, it might disrupt normal functions of trypsin, might lead to unnecessary side effects.

So far, limited number of total syntheses of natural product aeruginosins has been achieved. Quite a few synthetic analogs of aeruginosin family members were reported with variations in P1, P3 and P4 residues, but a limited number of efforts have been made to synthesize aeruginosin analogs with various synthetic octahydrindole analogs as a core structure. Compounds **4** - **6** are the representative members of aeruginosin analogs with different bicyclic scaffold.^{6, 26} Wang and group have reported synthesis and biological activity evaluation of compounds **5** and **6**. Compound **5**, which has opposite stereochemistry at ring junction of bicyclic core structure compared to naturally isolated aeruginosin members, however, showed reduced inhibitory activity against thrombin (24 $\mu\text{g/L}$), its selectivity to thrombin is remarkable; showed no inhibitory activity has shown against trypsin. Among all the natural product aeruginosins and their synthetic analogs, compound **4**, reported by Hanessian and the group, is the most potent thrombin inhibitor reported to date. They have claimed that its activity is due to the presence of chlorine substitution on P3 residue, and they have termed it as chlorine effect. Several research

groups also reported the synthesis of various Choi analogs and their improved synthetic methodologies. Some of the significant methodologies have been discussed below.



Scheme 2.1. Synthesis of Choi analog **27** by Carreira et al

Eric M. Carreira and group have reported the synthesis of dihydroxy octahydrindole **27**, which is the core structure of MicrocineSF608.²⁴ Tandem catalysis, has been utilized to perform Diels-Alder reaction between bromoacrolein and furan. Followed by Mukaiyama Aldol reaction afforded bromo intermediate **17** with decent stereo selectivity (dr. 5:1, ee. 86:14). Then treated compound **17** with potassium tertiary butoxide to obtain intermediate **18**. Compound **18** was treated with *m*-CPBA to form epoxide, then H₂/Pd reducing conditions were employed to reduce α,β -unsaturation, then resulting compound was treated with K₂CO₃ to obtain lactone intermediate **19**.

Azalactone **21** was obtained by treating compound **19** with bulky base and compound **20**. Lactone of intermediate **21** was opened, and the resulting azide was reduced to amine and protected using CbzCl. Epoxide was selectively opened using low-valent Ti reagents followed by resulting 2° alcohol was protected as acetate. Then 3° alcohol was removed by following a previously reported procedure. Deprotection of Cbz group followed by TMSOTf mediated nucleophilic opening yielded dihydroxy octahydrindole **27**, the core structure of MicrocineSF608.

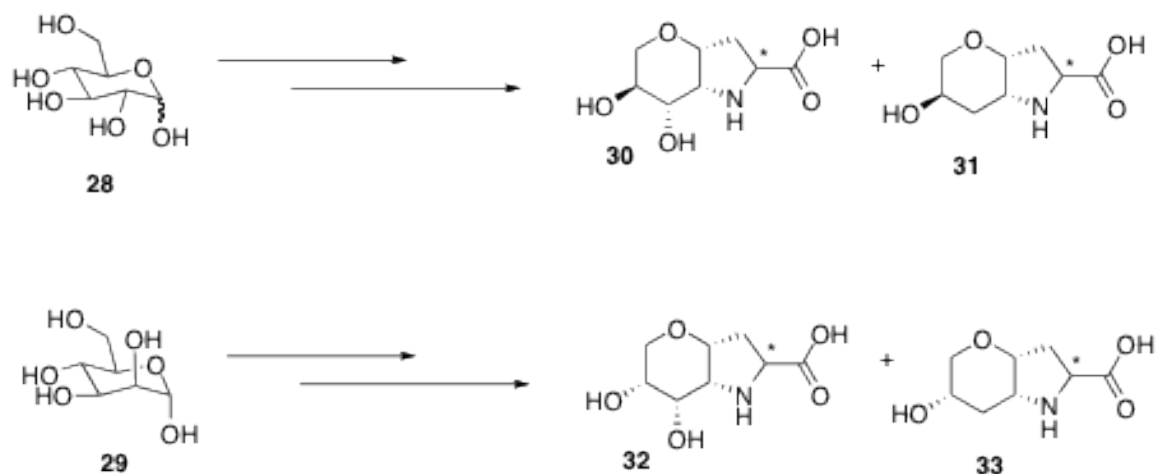
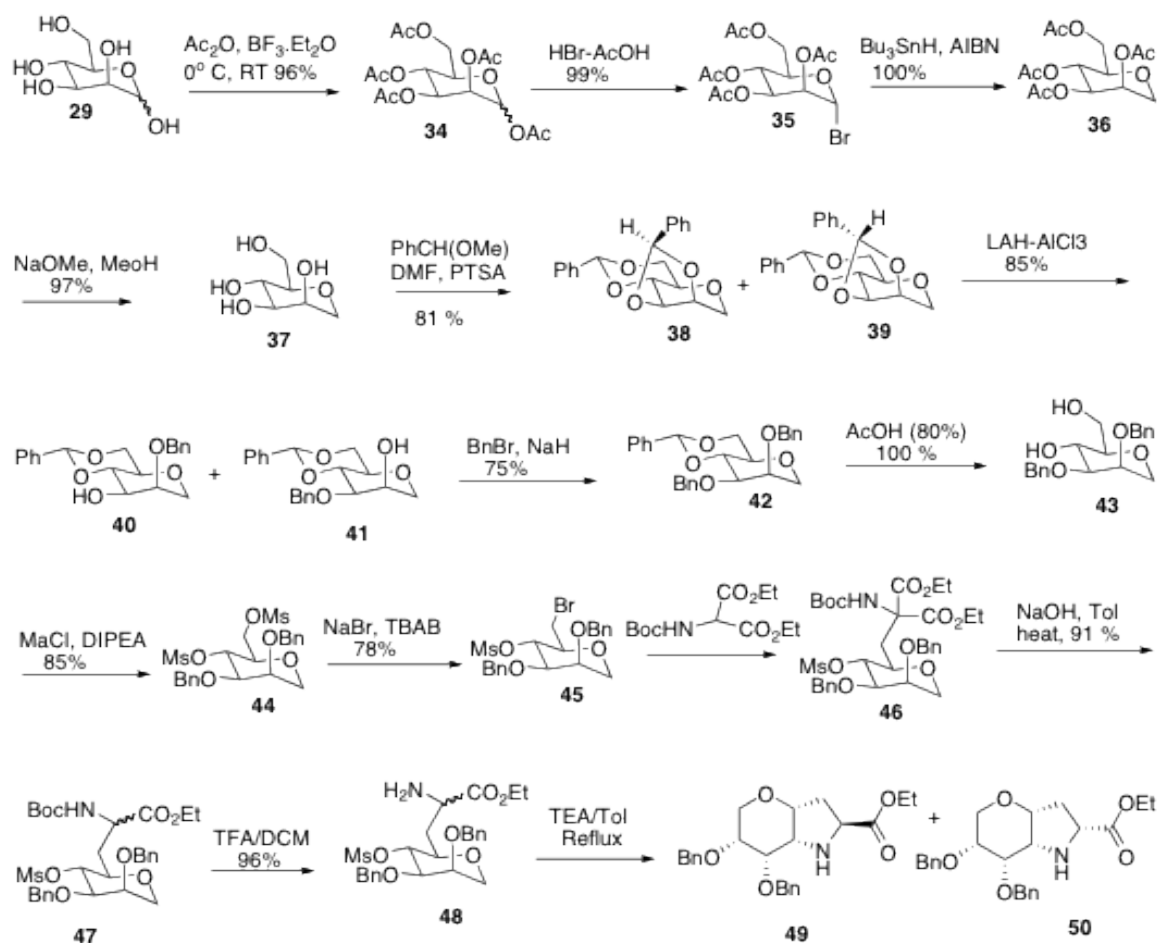


Figure 2.5. Synthesis of O-Choi analogs **30** and **31** achieved from glucose **28**, and **32** and **33** achieved from mannose **29**.

G Wang and coworkers have previously reported the synthesis of ring oxygenated Choi analogs **30** – **33** (**fig 2.5**) from D-glucose or D-mannose as starting material.²⁵⁻²⁸ As shown in scheme 2.2, O-Choi analogs **49** and **50** were synthesized starting from D-mannose **28**.²⁷ Protection of all the five hydroxy groups by treating D-mannose with acetic anhydride, then removal of anomeric acetyl group, followed by deprotection of hydroxy groups under basic reaction conditions yields tetrahydroxy sugar derivative **37**.



Scheme 2.2. Synthesis of O-Choi variants **49** and **50** from D-mannose **29**

Spatially closer C2, C3 hydroxy groups of intermediate **37** were protected as 5 membered cyclic acetal, and C4, C6 hydroxy groups were protected as 6 membered cyclic acetal. Less stable 5 membered cyclic acetal was opened under acidic conditions, resulting mixture of regio-isomers **40**, and **41** were reacted with benzyl bromide to afford intermediate **42**. Benzylidene protection was removed under acidic conditions and then again protected as mesylates. Primary mesylate was displaced with bromine to give intermediate **43**. Compound **45** reacted with N-Boc dimethylaminomalonate in the

presence of basic conditions, and then treated with sodium hydroxide, followed by refluxed conditions were employed to yield ring oxygenated bicyclic amino acids **49** and **50**.

Results and discussion

We aimed to synthesize O-Choi analog **60** starting from α -methoxy glucopyranose **51**, as shown in **fig 6**. Retro synthetic strategy for the synthesis of O-Choi analog **60** is shown above. Final target compound **60** can be achieved from 6-bromo-sugar intermediate **59** by coupling with glycine. Intermediate **59** can be achieved from *edo*-epoxide sugar intermediate **54** by reduction of epoxide followed by protection of resulted hydroxy group and acetal deprotection. Intermediate **54**³⁰ can be obtained from α -methoxy glucopyranose **51** in 3 steps. We planned to remove C₃ hydroxy group in final compound **60** by making epoxide intermediate and then subjected to reductive opening. Epoxide intermediate **54** can be obtained in quantitative yields without any purification required.

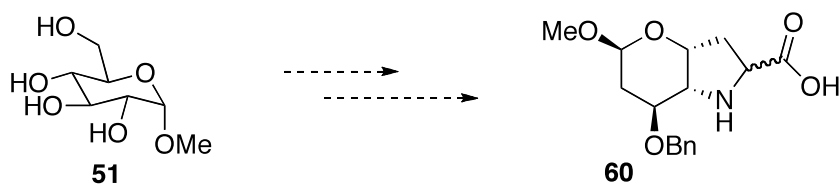
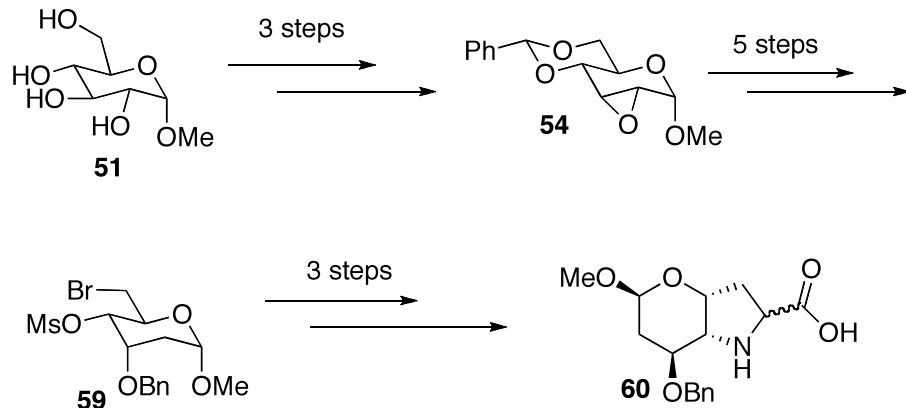
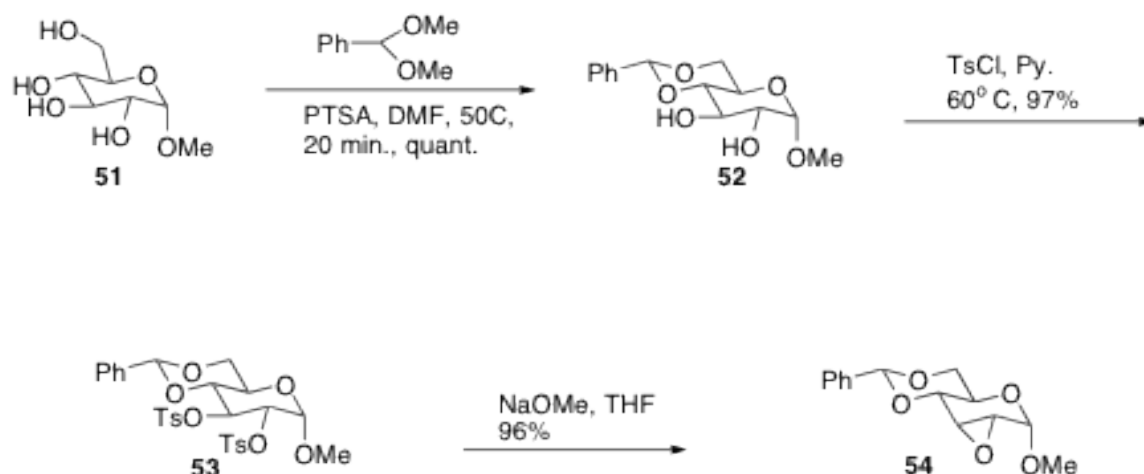


Figure 2.6. Strategy for the synthesis of **60** from D-glucopyranose



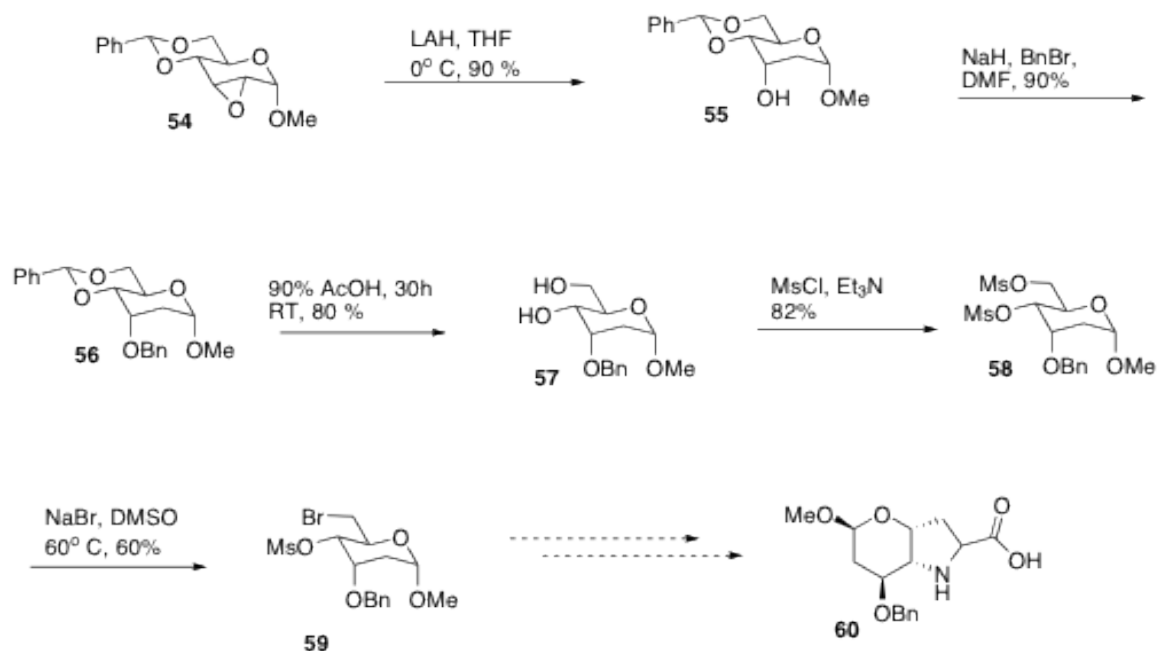
Scheme 2.3. Strategy for the synthesis of **60**

Synthesis of **60** commenced from α -methoxy glucopyranose **51** as the starting material. Hydroxy groups at C4, C6 were protected as benzylidene acetal by reacting with dimethyl benzylidene acetal in the presence of catalytic acidic conditions. 7 mol % of PTSA is enough to catalyze the reaction and complete in 20 min of time. Both the C2 and C3 hydroxy groups were protected as tosyl groups, and in the following step, treatment of ditosyl compound **53** with sodium methoxide yields endo-2, 3epoxide **54** in quantitative yield.³⁰



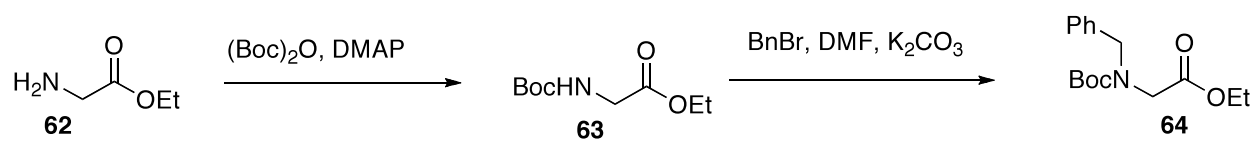
Scheme 2.4. Synthesis of *endo*-epoxide intermediate **54**

Thoroughly dried epoxide **54** was subjected to reduction with lithium aluminum hydroxide under dry conditions to epoxide at C2 from axial position, then resulting C3 axial hydroxy group was protected as benzyl ether by treating with benzyl bromide in the presence of sodium hydride. Benzyl protection of intermediate **56** was removed under acidic conditions (95% acetic acid) at room temperature to obtain compound **57**.^{31, 32} In the following step, both the hydroxy groups were protected as good leaving groups (mesylates) by treating with mesylchloride in the presence of triethyl amine. Compound **58** was treated with sodium bromide to displace primary mesylate C6 position with bromine.³³

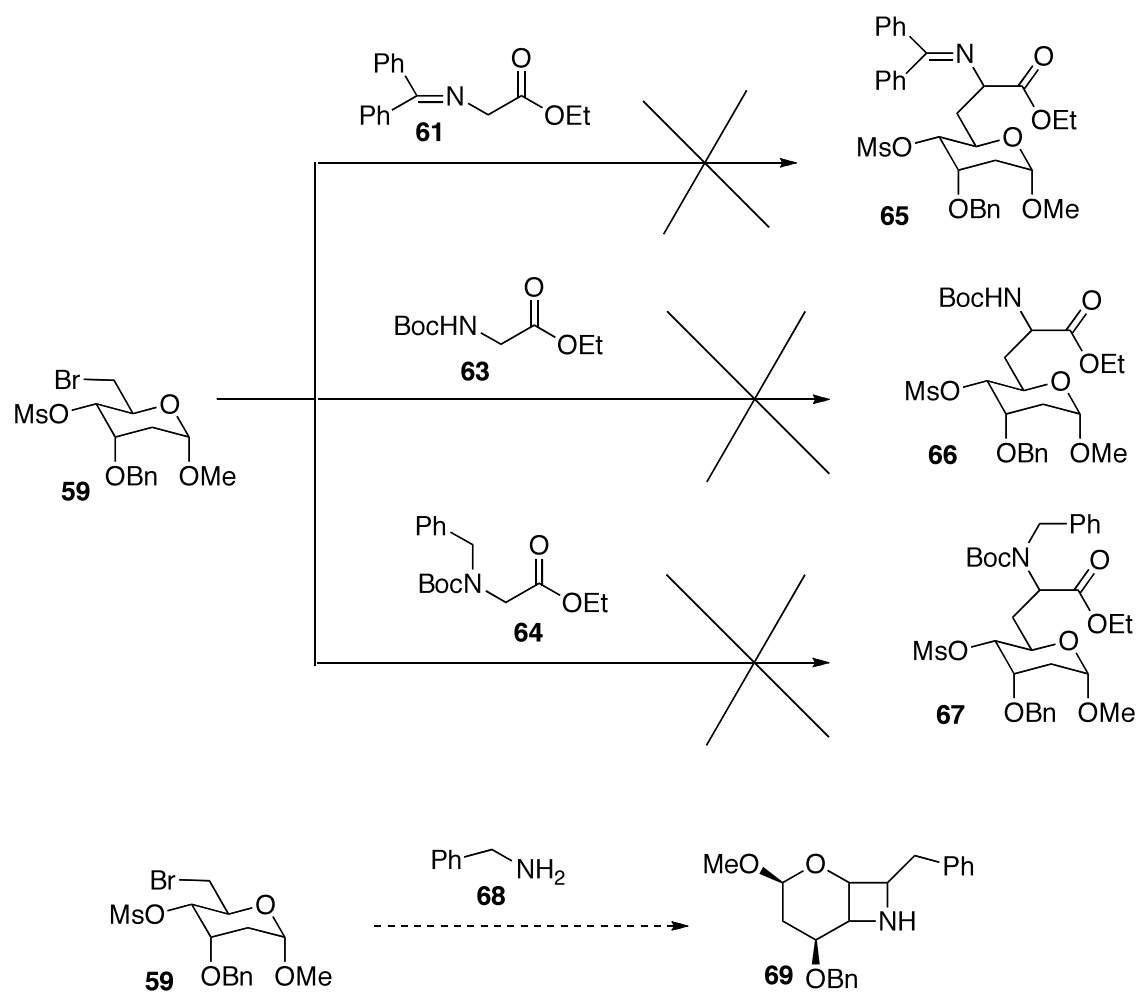


Scheme 2.5. Synthesis of bromo intermediate **59**

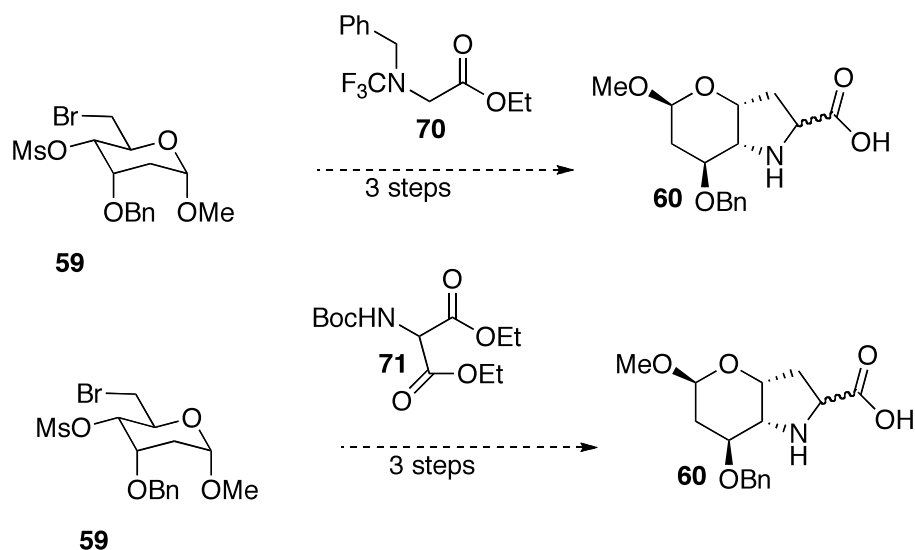
Several attempts were made to get cyclized product **60** from bromo intermediate **59**, but they were unsuccessful. Attempted to couple **59** with N-(diphenylmethylene) ethyl glycinate in the presence of non-nucleophilic bases. Sodium hydride as a base in combination with toluene as a solvent was also unsuccessful at various temperature (room temperature to reflux) conditions (**Scheme 7**). At room temperature, starting material was remained unreactive, and at higher temperature SM was slowly consumed but decomposed into several compounds. Potassium tertiary butoxide in t-butanol was used as a milder base for a couple of times, but it was not fruitful. Cyclization step was also pursued with Boc-protected ethylglycinates **63** and, but failed to cyclize. Finally, tried with benzyl amine to get 6, 4- fused bicyclic compound **66** was attempted once, but unsuccessful.



Scheme 2.6. Synthesis of ethylglycinate derivative **64**



Scheme 2.7. Attempts for alkylation reaction at different conditions



Scheme 2.8. Alternative strategies for the alkylation, which were not tried

In conclusion, epoxide intermediate 54 and bromo intermediate 59 have been synthesized in decent yields. In future, different reaction conditions (as shown in **scheme 2.8**) can be employed to achieve final target 60.

Experimental section

General method: reagents, instruments used

For the purification of compounds, fine silica gel was used. Vacuum pump has been used to dry compounds. Varian 400 MHz NMR machine was used to record ^1H and ^{13}C NMR of compounds.

α - methoxy 4,6-benzylidine 2,3-ditosylate glucopyranose 52.

15 g of **52** was taken in 500 mL of round bottom flask, 1.2 eq of benzaldehyde dimethyl acetal and 7mol % of PTSA was added to the round bottom flask. Directly put on rotary evaporator at 50 °C and started removing methanol formed in the reaction. As reaction progressing, white solid starting material increasingly dissolved and methanol continuously removed. After 25 min reaction was done, and quenched with sodium bicarbonate. Diluted the reaction mixture with 500 mL of water product was extracted into 600 mL of ethyl acetate by washing water phase with three equal portions of ethyl acetate. Collective organic phase was dried over sodium sulfate and concentrated on rotary evaporator, then kept under nitrogen flow for 4 hrs to remove DMF as much as possible. Product was recrystallized from ethanol. White solid needle like crystals, with MP 166-167 °C, obtained in quantitative yield.

^1H NMR (400 MHz, CDCl_3) δ 7.49 (m, 2H), 7.37 (m, 3H), 5.53 (s, 1H), 4.79 (d, 1H, $J = 3.9$ Hz), 4.29 (m, 1H), 3.92 (t, 1H, $J = 9.2$ Hz), 3.77 (m, 2H), 3.62 (td, 1H, $J = 9.0, 3.9$ Hz), 3.47 (m, 4H), 2.85 (s, 1H), 2.36 (d, 1H, $J = 9.2$ Hz). ^{13}C NMR (400 MHz, CDCl_3) δ 134.5, 126.7, 125.8, 123.7, 99.4, 97.2, 78.4, 70.3, 69.2, 66.4, 59.8, 53.0

α - methoxy 4,6-benzylidine 2,3-ditosylate glucopyranose 53.

15 g of **53** was dissolved in 40 mL of pyridine, 2.5 eq of TsCl was added and reaction mixture was stirred at 60 C for 36 h. Reaction mixture was diluted with 500 mL of water, product was extracted into 700 mL of dichloromethane by washing water phase with three equal proportions, then collected organic phase was washed with brine

followed by dried over sodium sulfate. After concentrating the organic phase on 15 g of **52** was taken in 500 mL of round bottom flask, 1.2 eq of benzaldehyde dimethyl acetal and 7mol % of PTSA was added to the round bottom flask. Directly put on rotary evaporator at 50 °C and started removing methanol formed in the reaction. As reaction progressing, white solid starting material increasingly dissolved and methanol continuously removed. After 25 min reaction was done, and quenched with sodium bicarbonate. Diluted the reaction mixture with 500 mL of water product was extracted into 600 mL of ethyl acetate by washing water phase with three equal portions of ethyl acetate. Collective organic phase was dried over sodium sulfate and concentrated on rotary evaporator, then kept under nitrogen flow for 4 hrs to remove DMF as much as possible. Product was recrystallized from ethanol. White solid needle like crystals, with MP 166-167 °C, obtained in quantitative yield., product was kept under nitrogen flow for 4 h to remove pyridine as much as possible, then product was recrystallized from ethyl acetate and dried on vacuum pump for 10 h. White solid product with MP 125-126 °C was obtained in quantitative yield. ¹H NMR (400 MHz, CDCl₃) δ 7.81 (d, 1H, *J* = 8.2 Hz), 7.61 (d, 1H, *J* = 8.3 Hz), 7.30 (m, 8H), 6.91 (d, 1H, *J* = 8.1 Hz), 5.27 (s, 1H), 5.10 (t, 1H, *J* = 9.5 Hz), 5.00 (d, 1H, *J* = 3.6 Hz), 4.43 (dd, 1H, *J* = 9.5, 3.6 Hz), 4.23 (dd, 1H, *J* = 10.4, 4.8 Hz), 3.84 (td, 1H, *J* = 9.9, 4.8 Hz), 3.65 (t, 1H, *J* = 10.3 Hz), 3.50 (t, 1H, *J* = 9.6 Hz), 3.36 (s, 3H), 2.41 (s, 3H), 2.23 (s, 4H). ¹³C NMR (400 MHz, CDCl₃) δ 145.0, 144.1, 136.3, 134.2, 132.0, 130.4, 129.4, 128.7, 128.3, 128.2, 126.6, 102.5, 98.1, 79.3, 77.6, 76.4, 68.6, 62.3, 56.1, 21.0.

α - methoxy 4,6-benzylidine 2,3-epoxy glucopyranose 54.

15 g of **54** was taken in 1 L round bottom flask, 150 mL of THF was added and stirred for 5 min, then cooled it to 0 °C. 3 eq of sodium methoxide dissolved in methanol was added slowly for 5 min. After 30 min of stirring, THF was removed on 15 g of **52** was taken in 500 mL of round bottom flask, 1.2 eq of benzaldehyde dimethyl acetal and 7mol % of PTSA was added to the round bottom flask. Directly put on rotary evaporator at 50 °C and started removing methanol formed in the reaction. As reaction progress, white solid starting material increasingly dissolved and methanol continuously removed. After 25 min reaction was done, and quenched with sodium bicarbonate. Reaction mixture was diluted with 500 mL of water, and product was extracted into 600 mL of ethyl acetate by washing water phase with three equal portions of ethyl acetate. Collective organic phase was dried over sodium sulfate and concentrated on rotary evaporator, then kept under nitrogen flow for 4 hrs to remove DMF as much as possible. Product was recrystallized from ethanol. White solid needle like crystals, with MP 166-167 °C, obtained in quantitative yield.

¹H NMR (400 MHz, CDCl₃) δ 7.51 (dd, 1H, J = 6.8, 2.7 Hz), 7.37 (m, 3H), 5.58 (s, 1H), 4.90 (d, 1H, J = 2.7 Hz), 4.25 (dd, 1H, J = 10.1, 5.0 Hz), 4.09 (td, 1H, J = 9.8, 5.0 Hz), 3.97 (dd, 1H, J = 9.1, 1.2 Hz), 3.69 (t, 1H, J = 10.3 Hz), 3.51 (m, 5H). ¹³C NMR (400 MHz, CDCl₃) δ 134.5, 126.7, 125.8, 123.7, 99.4, 97.2, 78.4, 72.3, 69.2, 66.4, 59.8, 53.0

 α - methoxy 4,6-benzylidine 3-hydroxy glucopyranose 55.

5 g of compound **55** was dissolved in 50 mL of THF in 500 mL round bottom flask, cooled to 0 °C, 3 eq of lithium aluminum hydride (LAH) was added carefully and stirred reaction for 6 h at 0 °C. Excess of LAH was initially quenched by 150 mL of ethyl acetate,

and then sodium sulfate hepta hydrated was added and stirred for 5 h until reaction mixture turned to white. Filtered the mixture through filter paper and repeatedly washed the solid residue with excess of ethyl acetate for several times. Product was purified by column chromatography using 15% of ethyl acetate in hexane. White solid product with MP 120-121 °C was obtained in 90% of yield, then dried the product on vacuum pump for 8 h and proceeded to next step. ^1H NMR (400 MHz, CDCl_3) δ 7.47 (dt, 1H, J = 7.1, 1.8 Hz), 7.29 (m, 3H), 5.52 (d, 1H, J = 1.4 Hz), 4.66 (d, 1H, J = 3.9 Hz), 4.24 (ddd, 1H, J = 8.7, 5.2, 2.6 Hz), 4.14 (td, 1H, J = 9.9, 5.3 Hz), 4.04 (td, 1H, J = 3.6, 1.6 Hz), 3.68 (td, J = 10.1, 1.4 Hz, 1H), 3.46 (dt, 1H, J = 9.5, 2.0 Hz), 3.29 (s, 2H), 3.13 (dd, 1H, J = 6.8, 1.5 Hz), 2.05 (m, 1H), 1.86 (m, 1H). ^{13}C NMR (400 MHz, CDCl_3) δ 137.8, 129.2, 128.4, 126.8, 102.1, 98.7, 79.8, 69.5, 65.0, 58.3, 55.5, 35.7

α - methoxy 4,6-benzylidine 3-benzyl glucopyranose 56

5 g of **56** was dissolved in 10 mL of DMF, cooled to 0 °C, and 1.5 eq of sodium hydride was added, followed by 1.5 eq of benzyl bromide was added drop wise. Reaction was done in 6 h at 0 °C, then reaction mixture was diluted with 250 mL of dichloromethane followed by 250 mL of water, water phase was washed with two more times with 150 mL of dichloromethane each. Collective organic phase was dried over sodium sulfate and concentrated on rot vap, and then kept under nitrogen flow for 4 h to remove DMF as much as possible and the impurities were removed by column chromatography using 8 % of ethyl acetate in hexane. White solid with MP 80-81 °C product was obtained in 89 % of yield. ^1H NMR (400 MHz, CDCl_3) δ 7.52 (m, 2H), 7.36 (m, 8H), 5.57 (s, 1H), 4.84 (q, 1H, J = 12.8 Hz), 4.73 (d, 1H, J = 4.6 Hz), 4.47 (td, 1H, J = 10.0, 5.3

Hz), 4.347 – 4.30 (dd, 1H, $J = 10.3, 5.3$ Hz), 3.98 (q, 1H, $J = 3.1$ Hz), 3.72 (m, 2H), 3.42 (s, 3H), 2.22 (m, 1H), 1.93 (ddd, 1H, $J = 14.9, 4.6, 3.5$ Hz). ^{13}C NMR (400 MHz, CDCl_3) δ 136.4, 135.1, 126.3, 125.6, 125.4, 124.8, 124.46, 123.6, 99.5, 95.2, 77.8, 69.4, 67.6, 66.9, 55.5, 52.9, 31.8

α -methoxy 3-benzyl, 4, 6-dihydroxy glucopyranose 57.

5 g of starting material 57 was dissolved in 30 mL of 95% of acetic acid, stirred for 36 h at room temperature, then diluted the reaction mixture with 250 mL of water and started neutralizing acetic acid by adding sodium bicarbonate slowly until no froth is formed. Product was extracted into ethyl acetate. Organic phase was purified by column chromatography using 30% ethyl acetate in hexane. Colorless semisolid product was dried on vacuum pump for 8h. The product yield is 75%. ^1H NMR (400 MHz, CDCl_3) δ 7.34 (m, 5H), 4.80 (d, 1H, $J = 11.3$ Hz), 4.73 (d, 1H, $J = 4.5$ Hz), 4.38 (d, 1H, $J = 11.6$ Hz), 3.87 (m, 4H), 3.60 (ddd, 1H, $J = 10.6, 9.7, 3.7$ Hz), 3.36 (s, 3H), 2.70 (d, 1H, $J = 10.5$ Hz), 2.33 (ddd, 1H, $J = 15.2, 2.8, 1.2$ Hz), 2.24 (m, 1H), 1.84 (s, 1H), 1.76 (ddd, 1H, $J = 4.5, 3.4, 0.9$ Hz), 1.71 (m, 1H). ^{13}C NMR (400 MHz, CDCl_3) δ 135.8, 126.3, 125.8, 125.7, 95.2, 70.6, 68.2, 66.0, 65.3, 60.8, 53.1, 29.0

α -methoxy 3-benzyl, 4, 6-dimesylate glucopyranose 58.

3 g of **58** was dissolved in 20 mL of THF, cooled to 0 °C, 3eq of methane sulfonile chloride and 3 eq of triethyl amine added, and reaction mixture was stirred for 8 h at 0 °C. THF was removed on rotary evaporator, and then solid residue was diluted with 250 mL of water and 200 mL of dichloromethane. Product was extracted into dichloromethane by

washing water phase two more times. Collective organic phase was concentrated, and the product was purified by column chromatography using 12% of ethyl acetate in hexane and dried on vacuum pump. Colorless semisolid product was obtained in 82% of yield. ^1H NMR (400 MHz, CDCl_3) δ 7.34 (m, 5H), 4.73 (ddd, 1H, J = 12.7, 6.0, 2.8 Hz), 4.47 (m, 4H), 4.13 (q, H, J = 3.5 Hz), 3.36 (s, 3H), 3.05 (s, 2H), 2.99 (s, 2H), 2.24 (ddd, H, J = 15.1, 3.8, 1.4 Hz), 1.86 (ddd, 1H, J = 15.0, 4.8, 3.2 Hz) ^{13}C NMR (400 MHz, CDCl_3) δ 138.2, 128.6, 128.1, 128.0, 98.3, 75.0, 71.9, 71.6, 68.4, 64.0, 56.0, 39.0, 37.7, 32.3

α - methoxy 3-benzyl, 4-mesylate 6-bromo glucopyranose 59.

2 g of starting material **59** was dissolved in 6 mL of DMSO, 6 eq of sodium bromide salt was added and stirred for 24 h at 60 °C, and then the reaction mixture was diluted with 200 mL of water and 150 mL of dichloromethane. Water phase was washed two more times with 100 mL of dichloromethane each time. Collective organic phase was concentrated on rotary evaporator, and then the product was purified by column chromatography using 15% ethyl acetate in hexane. Colorless semi solid product was dried on vacuum pump for 10h. Product was obtained in 60 % of yield. ^1H NMR (400 MHz, CDCl_3) δ 7.34 (m, 5H), 4.88 (m, 1H), 4.73 (m, 14H), 4.56 (d, 1H, J = 11.9 Hz), 4.45 (ddd, 1H, J = 8.7, 5.5, 2.6 Hz), 4.12(q, 1H, J = 3.6 Hz), 3.67 (dt, 1H, J = 11.2, 3.5 Hz), 3.57(m, 1H), 3.41(s, 3H), 3.15 – 2.96 (s, 3H), 2.32 – 2.18 (ddd, 1H, J = 14.9, 4.1, 1.5 Hz), 1.89 (ddd, 1H, J = 15.0, 4.9, 3.4 Hz). ^{13}C NMR (400 MHz, CDCl_3) δ 138.7, 128.6, 128.1, 128.0, 98.2, 77.9, 72.6, 71.62, 65.0, 56.0, 39.2, 33.5, 32.3

***N*-Boc-ethyl glycinate 63.**

500 mg of ethyl glycinate was dissolved in 5 mL of acetonitrile, cooled to 0 °C, then 1.05 eq of Boc anhydride and 2 eq of DMAP were added. After 6 hrs, reaction mixture was diluted with 50 mL of water and 50 mL of dichloromethane, and product was extracted into organic phase by washing water phase with 3 equal portions of dichloromethane. Collective organic phase was dried over sodium sulfate and concentrated on rota vap. Product was purified by column chromatography using 10 % of ethyl acetate in hexane. Colorless viscous liquid product was obtained in 85 % of yield. ¹H NMR (400 MHz, CDCl₃) δ 5.00 (s, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.89 (d, *J* = 5.7 Hz, 2H), 1.44 (s, 9H), 1.27 (t, *J* = 7.1, 0.8 Hz, 3H). ¹³C NMR (400 MHz, CDCl₃) δ 168.2, 154.0, 59.2, 40.3, 26.2, 12.0

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